RESPONSE DOCUMENT

INITIAL QUALIFICATION PROCEDURE –
LIST OF ISSUES SIMCYP SIMULATOR

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CONTACT: **KAREN ROWLAND YEO**
SENIOR VICE-PRESIDENT
CERTARA UK LTD
LEVEL 2-ACERO
1 CONCOURSE WAY
SHEFFIELD S1 2BJ
UK

EMAIL: KAREN.YEO@CERTARA.COM

EMA Issue 1

4 contexts of use (CoU) are defined; however, the analyses are not submitted per CoU. The CoUs are defined mechanistically while analyses were shown per enzyme for all 3 CoU together

or for all enzymes combined per mechanism of inhibition. Discuss whether pooling of the DDI data for the various CYP enzymes is appropriate for each CoU and whether DDI data for the two mechanisms of inhibition can be combined per enzyme. Consider in this discussion that a rank order approach may be considered for hepatic reversible inhibition but not for MBI (ICH-M12), the location of inhibition (only hepatic or hepatic and intestinal) and the verification of kdeg values for the various enzymes.

RESPONSE: A fundamental assumption with the use of PBPK models for predicting DDIs by reversible inhibition is that the main determinants of any DDI are the fraction of the victim dose that is cleared by the pathway that is being inhibited (fm) and the potency of the inhibitor (Ki) and the concentration of the inhibitor at the active site of the enzyme ([I]). If a victim drug is mainly cleared by a CYP enzyme and a clinical DDI study is conducted with a strong inhibitor of the enzyme, it is assumed that this will be the worst-case scenario and the DDI potential with weak/moderate inhibitors will be lower.

This concept has been demonstrated in the literature for CYP3A4 compounds based on compilation of clinical data (Hisaka *et al*., 20210). The basic assumption of the *in vivo*-based method is that the magnitude of change in AUC for various DDIs is determined by two parameters i.e., CR and IR (Figure 1). The CR is the contribution ratio of the target metabolizing enzyme to the clearance of a substrate drug after oral absorption, and the IR is the inhibition ratio of the enzyme caused by an inhibitor drug. The clinical data for CYP3A4 shown in the figures below [1] indicate that the increase in AUC is associated with a higher CR (equivalent to fmCYP3A4) for the substrate and increasing potency of the inhibitor against CYP3A4. In Figure 2, predicted fold-increases in AUC for substrates with varying contributions of inhibitable pathways (CR) in combination with inhibitors of increasing inhibitory potencies (IR) are shown.

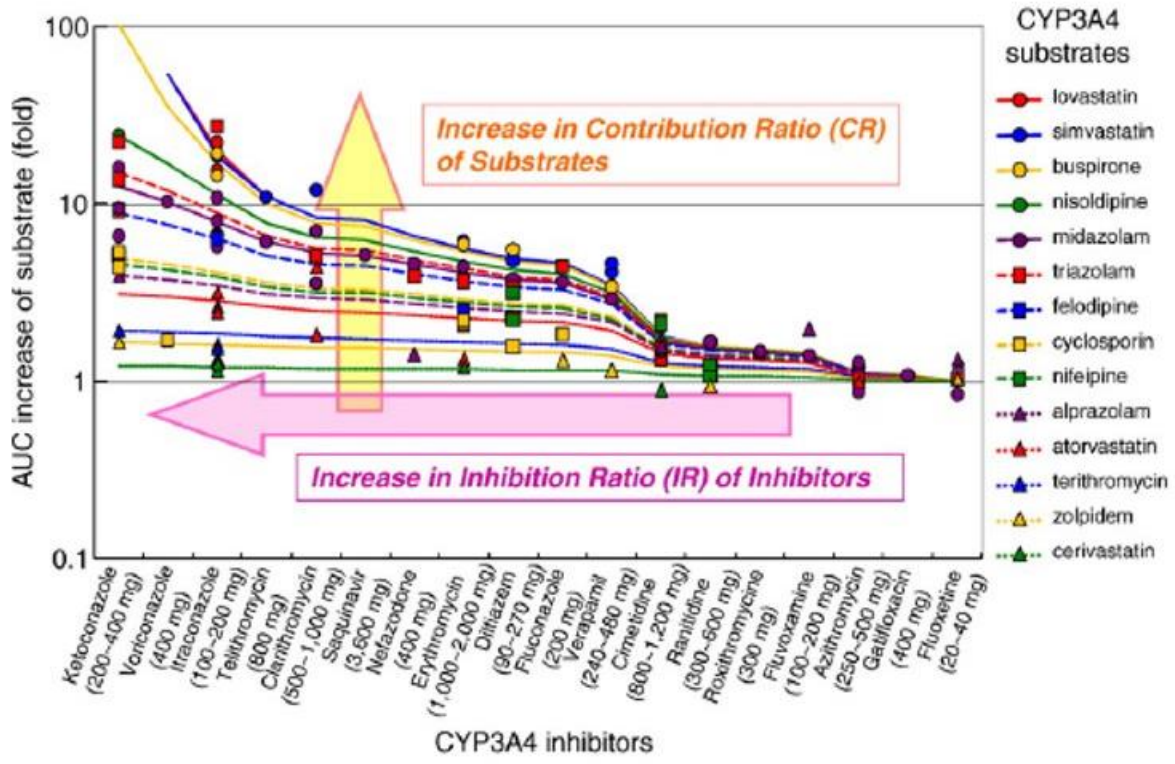


Figure 1. Impact of inhibitory potency (IR) and contribution of inhibitable pathway (fm) to the magnitude of drug interaction

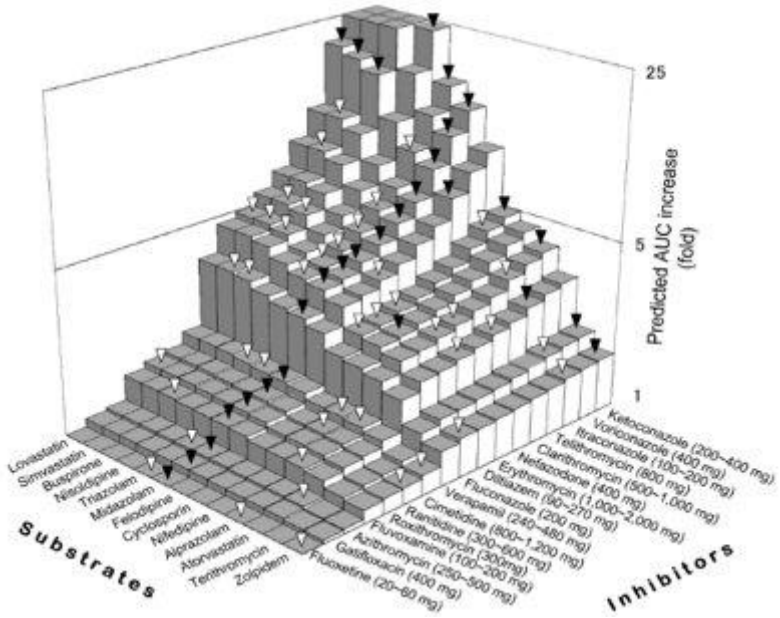


Figure 2. Predicted fold-increase in AUC for substrate and inhibitor combinations. The bars with open triangles indicate the studies that were used to construct the substrate and inhibitor matrix and the data with closed triangles were used as a verification dataset.

The basic assumption of assessing DDI risk is that the inhibitory potency is strong > moderate > weak and this holds true regardless of the enzyme involved. A similar approach is adopted in the DDI guidelines from various regulatory agencies where static models are used to predict the magnitude of competitive inhibition of any enzyme based on fm, appropriate inhibitor concentrations and inhibitor potency (FDA Guidance; EMA Guideline). No distinction is made as to the identity of the enzyme under consideration. Fundamentally, there is no reason why this cannot be the case for PBPK modelling as it is for the static model.

For any single drug, the most potently inhibited enzyme *in vitro* should also be the one most affected *in vivo*. Factors that affect our ability to quantitatively predict the magnitude of interactions from *in vitro* potency data should be equivalent irrespective of the enzyme targets are being compared. Thus, a rank order approach (ICH-M12 Guideline) should be reliable for using *in vitro* drug inhibition data in the planning of *in vivo* drug interaction studies. If a clinical study is conducted with a sensitive substrate of the enzyme most potently inhibited *in vitro*, then it should be assumed that the worst-case scenario has been determined. Indeed, in a study reported by Obach *et al*. (2005), 21 drugs with an appropriate *in vivo* DDI data set (at least 3 *in vivo* DDI studies with selective probe substrates for 3 different CYP enzymes) were used to test the rank-order approach. It was found that application of the rank order approach would have captured most of the observed interactions greater than 2-fold and drug interaction strategies would have been appropriately executed for 18 of 21 drugs using the *in vitro* inhibition data. In Table 1, we provide the rank order of *in vitro* Ki values for a series of perpetrators used by Obach *et al.* [4] in their analysis, some of which are also included in the Simcyp Simulator V19.

In such cases where a drug inhibits several CYP enzymes and *in vitro* Ki values are available, a reasonable strategy would be to conduct a clinical DDI study using a sensitive substrate for the enzyme that is most potently inhibited *in vitro*. Once a PBPK model has been developed for the drug and the clinical DDI with the sensitive substrate accurately predicted, simulations with other probe substrates could be run to determine the DDI potential of the drug *in vivo* for the other CYP enzymes using the *in vitro* Ki values. To demonstrate this, consider fluconazole with Ki values of 2.0, 10.7, and 23.0 μM for CYP2C19, CYP3A4, and CYP2C9, respectively. Simulations of fluconazole in combination with omeprazole, midazolam, or S-warfarin (references within Obach *et al., 2005*), confirm that the rank order approach applies and importantly, that the PBPK models and compound files within the Simcyp Simulator can capture the results (Table 2). Workspaces can be found within “Issue 1” in the main folder called “Final Responses”.

Table 1. Rank order of in vitro Ki values for a series of perpetrators

	In vitro rank order (Obach et al., 2005)				Simcyp				
Cimetidine	2D6	3A	2C9/1A2		2D6	3A			
Citalopram	2D6	3A/2C19	2C9						
Clarithromycin	3A	2C19/1A2	2C9		3A				
Diltiazem	3A	2C9/2D6	1A2		3A				
Erythromycin	3A	1A2/2C9			3A				
Fluconazole	2C19/3A/2C9	1A2			2C19	3A	2C9		
Fluoxetine	2D6	3A	1A2		2D6	2C19	3A		
Fluvoxamine	1A2	2C19	2D6/2C9	3A	1A2	2C19	2D6/2C9	3A	
Ketoconazole	3A	2C9/2C19	1A2	2D6	3A	2C8	2C9		
Metronidazole	3A	1A2	2C9						
Nefazodone	3A	2C9	1A2						
Paroxetine	2D6	1A2	2C19/3A	2C9	2D6	3A4		1A2	
Propranolol	1A2	3A	2C9		1A2				
Ranitidine	3A/2D6	2C9/1A2							
Risperidone	2D6	2C19	1A2						
Roxithromycin	3A	2C19/1A2/2C9							
Sertraline	2D6	3A/1A2	2C9						
Terbutaline	2D6	1A2	3A/2C9						
Ticlopidine	2C19	1A2	3A		2B6	2C19			
Troleandomycin	3A	2C19/1A2							

agrees with rank order approach
in simcyp but not the rank order approach
not in simcyp but in rank order approach

Table 2. Simulations of drug interactions with fluconazole – rank order approach

Substrate	Fluconazole Ki (μM)	Fluconazole dose	Predicted AUCr	Observed AUC ratio
Omeprazole (2C19)	2.0	100mg QD	6.76	6.29
Midazolam (3A4)	10.7	200mg QD	3.75	3.75
S-Warfarin (2C9)	23.0	300mg QD	2.27	2.27
Theophylline (CYP1A2)	>800 (DIDB); 800 used in simulation	100mg BD	1.02	1.19

For a qualified PBPK platform, it is expected that successful prediction of a clinical DDI between a victim and a strong inhibitor would allow prediction of the magnitude of interaction with a moderate and weak inhibitor of the same enzyme. For a specific CYP enzyme, demonstrating that strong, moderate and weak inhibition of a particular enzyme (e.g., CYP1A2) give correct relative changes in Cmax and AUC ratios for a sensitive substrate should qualify the platform for that particular enzyme. If the platform predicts similar changes in Cmax ratio and AUC ratio when the substrate metabolic intrinsic clearance (CLint) and inhibitor Ki are changed to reflect another enzyme (e.g., CYP2E1), this would demonstrate that the platform is behaving appropriately. The main considerations are the relative abundance in the liver (52 *versus* 61 pmol/mg protein) and intestine (in this example neither isozyme is expressed in the intestine) which for an oral drug will give different levels of AUC and Cmax ratio. However, the relationship of strong moderate and weak inhibitors should be maintained for each enzyme.

This is illustrated below for theophylline which was used as a sensitive substrate of CYP1A2 and fluvoxamine as a strong inhibitor, propranolol as a moderate inhibitor (240 mg TID) and cimetidine as a weak inhibitor of CYP1A2. The substrate model was then changed so that theophylline was now metabolised by CYP2E1, and the inhibitor files were amended so that the Ki value was now applied to CYP2E1 instead of CYP1A2. The enzyme abundance values in the liver (52 pmol/mg for CYP1A2 and 61 pmol/mg for CYP2E1) and gut (0 for both isozymes) were the default values in the simulator and were not changed. Given the similarity in CYP abundance in liver (impact on fmCYP), the CL/F of the two substrates in the absence

of inhibitor was similar (Table 3). Thus, the simulated DDIs for the two scenarios were also comparable (Table 4).

Table 3. Characteristics of the two substrates used in the CYP1A2/2E1 exercise.

	CYP1A2	CYP2E1
Fa	0.86	0.86
Fg	1	1
F_H	0.96	0.96
Cmax (ng/ml)	6654	6150
CL (L/h)	4.31	4.57

Table 4. Predicted DDIs of the two substrates used in the CYP1A2/2E1 exercise.

Enzyme	Inhibitor	Mean CmaxR	Mean AUCR
CYP1A2	Cimetidine (Weak)	1.31	1.46
CYP2E1	Cimetidine	1.38	1.56
CYP1A2	Propranolol (Moderate)	1.03	2.03
CYP2E1	Propranolol	1.02	2.07
CYP1A2	Fluvoxamine (Strong)	3.00	3.83
CYP2E1	Fluvoxamine	4.09	5.35

The concept was extended to CYP3A4 where midazolam (a sensitive CYP3A4 substrate) and both competitive and mechanism-based inhibitors (MBI) were used. With MBI there is some added complexity as the kdeg value can vary from enzyme to enzyme and for some enzymes there are less data available to define kdeg. This is only likely to affect interactions in the liver, as the same kdeg value is used for all isozymes in the gut as it is based on the turnover of the enterocyte which is faster than the turnover of the individual enzymes.

To illustrate this concept, the following simulations were run using midazolam with a strong, moderate and weak inhibitor. To simplify things, the CYP3A5 component of the midazolam file in the Simcyp Simulator V19 was removed – all other parameters were unaltered. DDIs with ketoconazole (strong reversible CYP3A4 inhibitor), clarithromycin (strong MBI), diltiazem (moderate MBI), fluconazole (moderate competitive) and cimetidine (weak competitive) were simulated. The midazolam file was then adapted so that the clearance was mediated by CYP2J2 instead of CYP3A4 (Table 5). The DDIs were run with the same inhibitors

as used previously – the CYP3A4 inhibition values (Ki or K_{inact} and K_I) were assigned to CYP2J2 instead of CYP3A4. Note in these simulations the Vmax for recombinant CYP2J2 was increased by 110-fold to account for the differences in abundance of CYP2J2 and CYP3A4. Fu_{gut} was adjusted so that the Fg in the CYP3A4 and CYP2J2 simulations were in the same range.

Table 5. Characteristics of the two substrates used in the CYP3A4/2J2 exercise.

	CYP3A4	CYP2J2
Fa	0.87	0.87
Fg	0.60	0.56
F_H	0.56	0.61
Cmax (ng/ml)	25.4	33.0
CL (L/h)	116	73

The results show that the same pattern is seen for CYP3A4 and CYP2J2 substrates and strong, moderate and weak inhibitors when the substrate has a similar CL and fm by the enzyme and the inhibitors have the same kinetics and Ki against the relevant enzyme. Clarithromycin-mediated inhibition of the CYP2J2 substrate gives a lower interaction than the CYP3A4 substrate if autoinhibition of clarithromycin due to mechanism-based inhibition of CYP3A4 is not considered in the simulation. When autoinhibition is considered (higher concentrations of clarithromycin) the effect of clarithromycin on the two substrates was similar.

Table 6. Predicted DDIs of the two substrates used in the CYP3A4/CYP2J2 exercise.

Enzyme	Inhibitor	Mean CmaxR	Mean AUCR
CYP3A4	Cimetidine	1.24	1.34
CYP2J2	Cimetidine	1.25	1.36
CYP3A4	Clarithromycin	2.59	8.96
CYP2J2	Clarithromycin	2.21	4.32
CYP2J2	Clarithromycin (inc 3A4 autoinhibition)	2.75	8.87
CYP3A4	Diltiazem	1.92	3.05
CYP2J2	Diltiazem	1.95	3.45

CYP2J2	Diltiazem (inc 3A4 autoinhibition)	1.92	3.33
CYP3A4	Fluconazole	2.11	3.57
CYP2J2	Fluconazole	2.09	3.72
CYP3A4	Ketoconazole	3.49	15.25
CYP2J2	Ketoconazole	3.40	12.94

In conclusion, we think it is entirely appropriate to combine the DDI data for the various enzymes for each CoU and also for the two mechanisms of inhibition.

References

Hisaka A, Ohno Y, Yamamoto T, Suzuki H. Theoretical considerations on quantitative prediction of drug-drug interactions. Drug Metab Pharmacokinet. 2010;25(1):48-61. doi: 10.2133/dmpk.25.48. PMID: 20208388.

US Food and Drug Administration. In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry. US Food and Drug Administration. 2020. https://www.fda.gov/regulatory-infor mation/search-fda-guidance-documents/vitro-drug-inter action-studies-cytochrome-p450-enzyme-and-transporter mediated-drug-interactions [cited 18 December 2020].

European Medicines Agency. Guideline on the investigation of drug interactions. 2012.https://www.ema.europa.eu/en/ documents/scientific-guideline/guideline-investigation drug-interactions-revision-1_en.pdf [cited 18 December 2020].

Draft ICH Guideline M12 on drug interaction studies, July 2022.

Obach RS, Walsky RL, Venkatakrishnan K, Houston JB, Tremaine LM. In vitro cytochrome P450 inhibition data and the prediction of drug-drug interactions: qualitative relationships, quantitative predictions, and the rank-order approach. Clin Pharmacol Ther. 2005 Dec;78(6):582-92. doi: 10.1016/j.clpt.2005.09.004. PMID: 16338274.

EMA Issue 2

Please discuss your views on the use of AFE and AAFE as performance metrics for assessing Simcyp's overall predictive performance and for detecting bias in specific subgroups within the DDI Qualification Matrix.

RESPONSE: These metrics have typically been used to assess the performance of PBPK models for predicting PK parameters (Margolskee A *et al*., 2016). It is perceived that each of these metrics provides a different measure and perspective on the performance of the simulations. For example, the percentage of predictions within a certain fold error provides a

measure of accuracy, while the average fold error (AFE) gives an indication of the bias of the predictions. Consider the following:

If $\boldsymbol{x}$ is a set of observations and $\boldsymbol{p_x}$ the corresponding set of model predictions, the fold error ($\boldsymbol{FE}$, a vector) and the average fold-error (AFE, a scalar) are defined as in reference (Zuegge et al., 2001):

$$\boldsymbol{FE} = log\left(\frac{\boldsymbol{p_x}}{\boldsymbol{x}}\right) \tag{1}$$

$$AFE = exp\left(\frac{\sum_1^n \boldsymbol{FE}}{n}\right) \tag{2}$$

The AFE is therefore simply the geometric average of the predictions over observation ratios. The standard display of AUC ratios from different studies for model validation plots is similar to what is shown in **Figure** . We simulated virtual DDI trials, so there are many more trials than usual to get a clearer view; the prediction trial is also larger than usual (200 subjects) for increased precision (the R script used is given in Appendix 1, Simulation script 1):

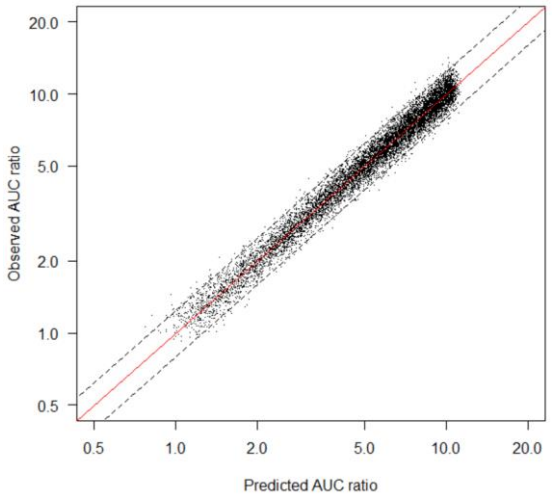


Figure 3: Observed vs. predicted AUC ratios for 10,000 virtual clinical studies. In red: identity line; Dashed lines: 1.25-fold errors limits.

It is obvious that the $\boldsymbol{FE}$ values are simply a projection of the points in **Figure** on a line perpendicular to the diagonal line. It is therefore simply a one-dimensional summary of **Figure** . **Figure** is a histogram of $\boldsymbol{FE}$ values:

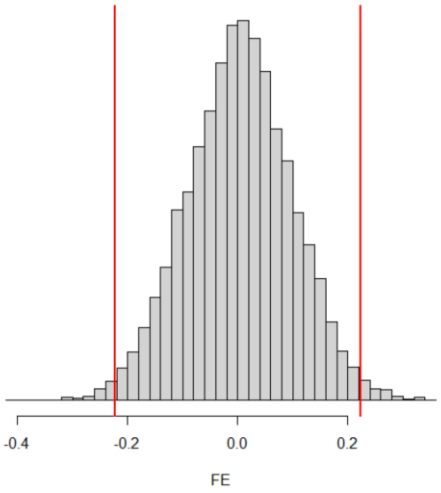


Figure 4: Histogram of the fold-errors (*FE*) in AUC ratios for 10,000 virtual clinical studies. In red: 1.25-fold errors limits. *AFE* is just the average of that.

Figure can be used to just give us the fraction of points falling outside any fold-error interval, but is in fact less informative than **Figure** , which displays the same information but with the added dimension of the AUC values. The AFE value is just the average of the values shown in **Figure** and is in fact even less informative. Any systematic bias would even be more apparent on **Figure** 3.

The absolute *FE* (*AFE*) measure just folds the left part of **Figure** (below zero) on the right part (see **Figure**) and *AAFE* gives an average of those deviations. AAFE is a second-order moment of the distribution and is not directly related to decisions about risks of over- or under-estimation of DDIs. As such, it does not seem particularly useful in decision making.

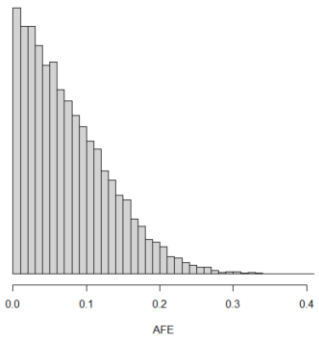


Figure 5: Histogram of the absolute fold-errors (*AFE*) in AUC ratios for 10,000 virtual clinical studies. *AAFE* is just the average of that.

Our conclusion is that evaluations based on plots like Figure , may be more informative than AFE and AAFE measures, and that for each drug the predicted/observed fold should

be put into context of the therapeutic window. However, as all other PBPK performance related studies have used AFE and AAFE as metrics, we think it is still important to present them.

References

Margolskee A et al. (2016). IMI - Oral biopharmaceutics tools project - Evaluation of bottom-up PBPK prediction success part 2: An introduction to the simulation exercise and overview of results. Eur J Pharm Sci. 2017 Jan 1;96:610-625. doi: 10.1016/j.ejps.2016.10.036. Epub 2016 Nov 2. PMID: 27816631.

Zuegge J, Schneider G, Coassolo P, Lave T. Prediction of hepatic metabolic clearance: comparison and assessment of prediction models. Clinical Pharmacokinetics. 2001;40:553–63.

EMA Issue 3

Please discuss the relevance of the proposed acceptance criteria (e.g., within 1.25-fold, within 1.5-fold, within 2-fold, within Guest criterion, misclassification rate) for the context of use considering exposure-response and therapeutic window.

RESPONSE: The predictive performance of PBPK models is commonly assessed using a comparative approach whereby the accuracy of a particular simulated scenario is represented as a ratio of predicted or simulated parameter *versus* observed parameter which is then expressed as a fold. One of the more challenging issues that has been debated over the years is what fold (predicted/observed ratio) would constitute a successful prediction? Several factors need to be considered in answering this issue, including the intended purpose of the simulation, the therapeutic index of the drug or drugs in issue, or steepness of the exposure–response relationship.

If simulations are used to support specific dosing recommendations to be tested in future clinical trials or to be incorporated in prescription drug labeling, the proposed dosing recommendations should be discussed and justified within the totality of evidence, including the context of known exposure-response relationships and the level of confidence in the PBPK model for its intended uses.

In conclusion, whilst we can provide the percentage of predictions falling within 1.25-, 1.5- and 2.0-fold of observed data, the acceptance criteria for the drug being investigated should be put into context of the dose response curve and therapeutic window.

EMA Issue 4

Please present an analysis of Observed vs. Predicted DDIs strictly adhering to the context of use.

RESPONSE: We have presented the analyses of predicted *versus* observed DDIs in a number of different ways:

- Each enzyme per mechanism and COU
- All enzymes per mechanism and COU

It should be noted that for some drug pairs, there are multiple clinical studies. After much discussion about applying potential weighting for a drug pair based on the number of subjects, it was decided that all studies should be included as different subjects were used in each case and different dosage regimens were applied in terms of periods of dosing, time of dosing and actual dose, each of which are specified in the clinical trial design sheets. Thus, no weighting was applied. The analyses including figures are presented in excel sheets listed below:
"CYP1A2-ClinicalData_TrialDesignSetting-02"
"*CYP2C8-ClinicalData_TrialDesignSetting-02"*
"*CYP2C9-ClinicalData_TrialDesignSetting-02"*
"*CYP2C19-ClinicalData_TrialDesignSetting-02"*
"*CYP2D6-ClinicalData_TrialDesignSetting-02"*
"*CYP3A4-ClinicalData_TrialDesignSetting-02"*

Word documents providing a summary of the analyses are available within each enzyme folder.

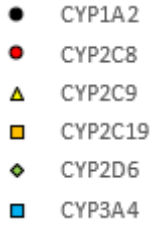
An excel sheet called *"CYP InhibitionSummaryAllData"* and a word document called *"CYP inhibition summary 202312-allCYP"* provide an overview of all the analyses and are available within the "Responses folder to submit".

Finally, an excel sheet called *"COU summary"* has been prepared to indicate how predictions for drug pairs were assigned to a specific COU.

Over the next few pages, we show how many DDIs we have now simulated for each enzyme and present predicted versus observed ratios for each mechanism (Figure 6 – Competitive Inhibition; Figure 7 – MBI).

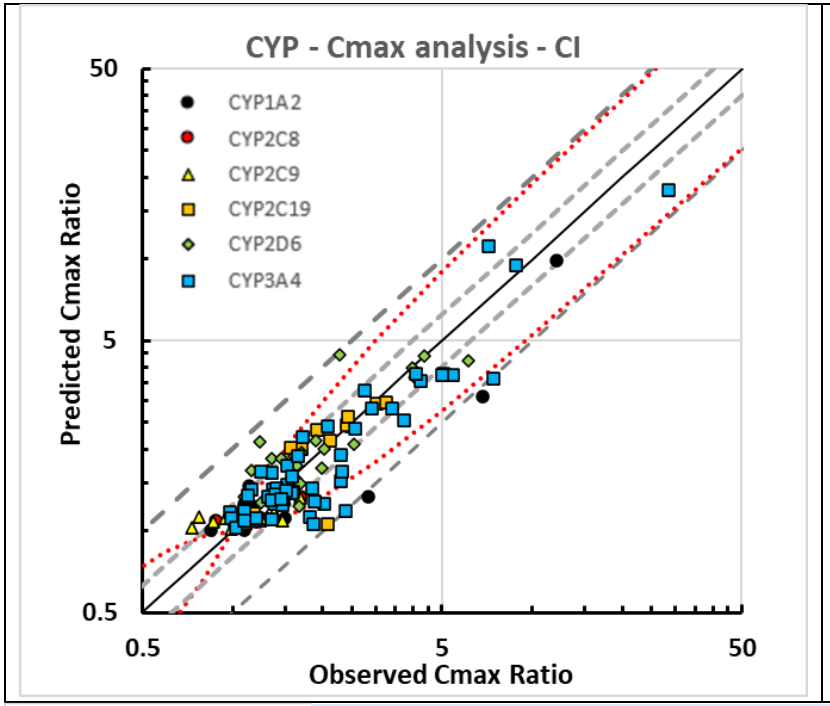
CYP-inhibition analysis

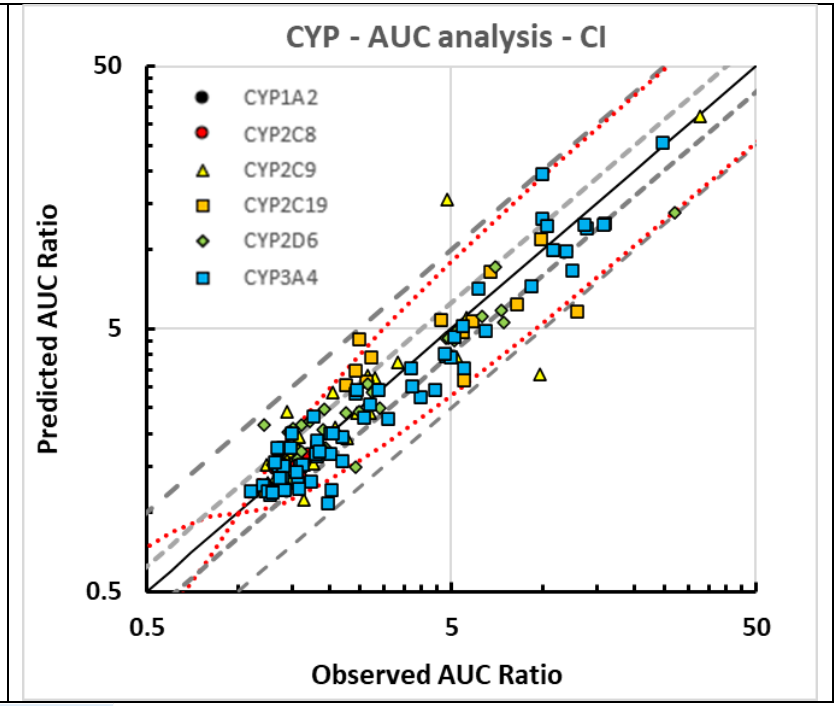
CYP1A2, CYO2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5



Enzyme	CI	MBI	ALL
CYP1A2	42	0	42
CYP2C8	7	10	17
CYP2C9	25	3	28
CYP2C19	15	13	28
CYP2D6	32	14	46
CYP3A4/5	66	28	94

1) Completive inhibition only

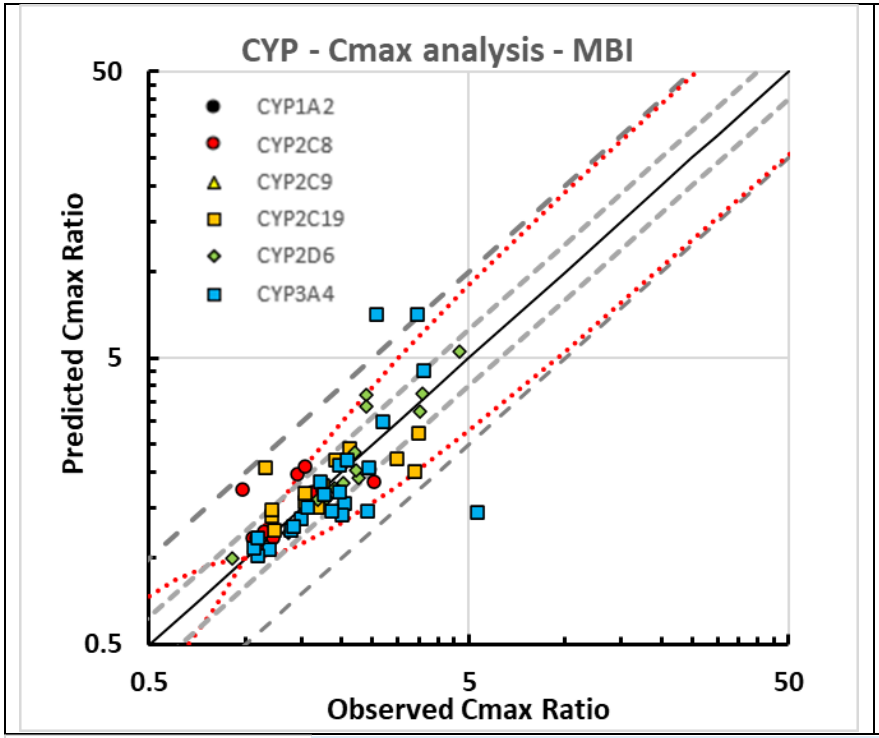


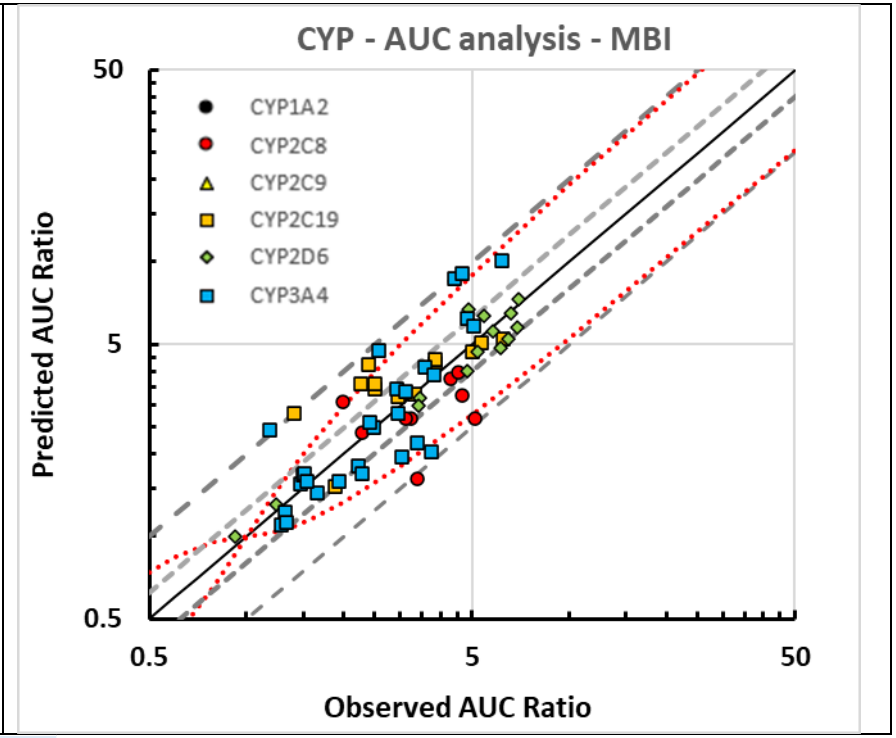


ALL - CI	V19R1 Built 96	
	Cmax Ratio	**AUC Ratio**
AFE (bias)	0.95	0.99
AAFE (precision)	1.20	1.19
Number Studies	130	187

2-fold		1.5-fold		1.25-fold		
Cmax Ratio	AUC Ratio	Cmax Ratio	AUC Ratio	Cmax Ratio	AUC Ratio	
3	**3**	**13**	**14**	**37**	**51**	NO
130	187	130	187	130	187	TOTAL
97.69	**98.40**	**90.00**	**92.51**	**71.54**	**72.73**	%

2) Mechanism-based inhibition only





ALL - MBI	V19R1 Built 96	
	Cmax Ratio	**AUC Ratio**
AFE (bias)	1.01	1.02
AAFE (precision)	1.23	1.25
Number Studies	60	68

2-fold		1.5-fold		1.25-fold		
Cmax Ratio	AUC Ratio	Cmax Ratio	AUC Ratio	Cmax Ratio	AUC Ratio	
3	**2**	**8**	**14**	**17**	**21**	NO
60	68	60	68	60	68	TOTAL
95.00	**97.06**	**86.67**	**79.41**	**71.67**	**69.12**	%

EMA Issue 5

What is the interstudy variability for the in vivo DDI studies? Please provide the interstudy variability for all contexts of use. Please discuss the option to incorporate variability of observed and predicted AUCRs in the assessment of performance.

RESPONSE: Inter-study variability is unavoidable but is usually confounded by inter-individual variability. It can be estimated from data only when several similar studies are available for the same combination of drugs, and when individual data are available, at least for some studies. We gathered such a data set (see **Figure**) (1-14). Joint estimation of the inter-study variability and inter-individual variability was performed by meta-analysis, using a multilevel model. The model was cast in a Bayesian framework and inference was performed using MCMC simulations with the *R* package *Nimble* (15) (see also code in Appendix, Inference script 1).

The prior for the inter-individual variance of AUC ratios (in log-space) was set to a vague truncated normal distribution:

$$V_{sub} \sim \mathcal{TN}(0, 0.25, 0, 1) \tag{3}$$

The same vague distribution was used for the prior on the inter-study variance (in log space also):

$$V_{stu} \sim \mathcal{TN}(0, 0.25, 0, 1) \tag{4}$$

A vague uniform prior was used for the mean chemical-pair-specific AUC ratios. For pair *i*:

$$AUC_i \sim \mathcal{U}(1, 50) \tag{5}$$

The variance of the observed mean AUC ratios for given chemical-pair *i* in study *j* is simply:

$$V_{i,j} = \frac{V_{sub}}{N_{i,j}} + V_{stu} \tag{6}$$

where $N_{i,j}$ is the number of subjects in study *j* for chemical-pair *i*.

The observed mean AUC ratios for given chemical-pair *i* in study *j* is assumed to be distributed lognormally around the interaction-pair-specific mean:

$$AUC_{i,j} \sim \mathcal{LN}\left(\log(AUC_i), V_{i,j}\right) \tag{7}$$

Finally, subject level AUC ratios, for chemical-pair *i* in study *j*, subject *k*, when available, are assumed to be also lognormally distributed, with a common inter-individual variance:

$$AUC_{i,j,k} \sim \mathcal{LN}\left(\log\left(AUC_{i,j}\right), V_{sub}\right) \quad (8)$$

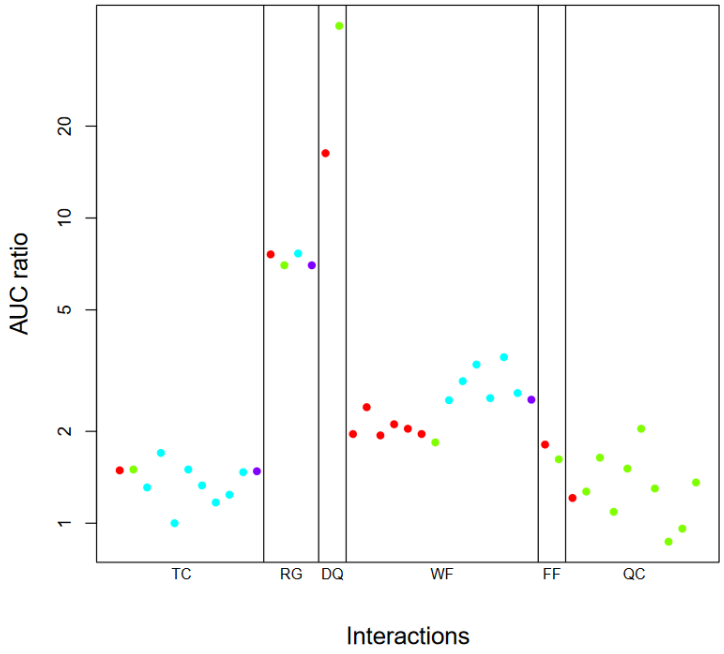


Figure 8: AUC ratios observed in different DDI studies (TC: theophylline-ciprofloxacin; RG: repaglinide-gemfibrozil; DQ: dextromethorphan-quinidine; WF: warfarin-fluconazole; FF: flurbiprofen-fluconazole; QC: quinidine-cimetidine). For each drugs pair, the results of similar design studies are color-coded. Series of points of the same color are individual values in the same study.

The MCMC sampling estimates of the model parameters of interest are given in Table . All parameters are reasonably well estimated (see also in Appendix, section MCMC posterior plots) The main parameters of interest are the inter-study and inter-individual variances (V_{stu} and V_{sub}). V_{stu} central estimate is 0.074 (on the logscale, corresponding to an arithmetic CV of 28%) and V_{sub}) central estimate is 0.041 (on the logscale, corresponding to an arithmetic CV of 20%). It appears from this analysis, with this small but diverse data set, that both variances are not very large and are approximately of the same size.

Table 7: Statistical summaries of the posterior distributions of the variance meta-analysis parameters. $\hat{R}$ is a convergence diagnostic (16), which should be close to 1, as here.

Parameter	Mean	SD	2.5th pctile	50th pctile	97.5th pctile	$\hat{R}$
V_{stu}	0.074	0.042	0.027	0.063	0.193	1.01
V_{sub}	0.041	0.013	0.023	0.039	0.075	1.00
AUC_1	1.50	0.22	1.13	1.48	2.00	1.00
AUC_2	7.53	1.05	5.69	7.46	9.87	1.00
AUC_3	27.7	5.3	18.5	27.2	39.8	1.01
AUC_4	2.35	0.34	1.76	2.32	3.11	1.00
AUC_5	1.83	0.37	1.23	1.78	2.68	1.01
AUC_6	1.38	0.28	1.03	1.32	2.07	1.00

In a separate analysis, we also tried to estimate a set of chemical-pair-specific inter-individual variances, but there are not enough data to get a stable inference on them and the inter-study variance estimates were the same (data not shown).

Our conclusion is that there are insufficient data to be able to perform an appropriate assessment of predicted *versus* observed variability.

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EMA Issue 6

Please discuss the option to perform an in-depth assessment of differences in study design such as different doses investigated and exposure time, time between administration of substrate and

inhibitor separately. These could allow achieving a better understanding of Simcyp's predictive performance for different dose levels of the same perpetrator or its effect over time.

RESPONSE: There are a number of drug combinations that have been used in simulations where multiple studies are available. All the predicted *versus* observed datasets are available for closer scrutiny.

EMA Issue 7

Please discuss whether intrinsic variability of compounds had an impact of the performance of the predictions. Please comment on separate consideration on orally administered drugs compared to IV administration (some compounds have a high inter- and intrasubject variability especially after oral administration. Intravenous administration filters some of the variability out).

RESPONSE: Except for CYP3A4, there are few drugs where both IV and oral data are available for a specific enzyme. However, to demonstrate the above point, we use midazolam as an example. The UOW Drug interaction database was searched in December 2023 for all studies that reported either intravenous (IV) or oral pharmacokinetics of midazolam– see excel file called "Midazolam PK". This gave 217 intravenous studies and 827 oral studies. Studies in patients and paediatric subjects were excluded and duplicate studies were removed. Thereafter, the mean and SD data for clearance were collated or calculated from the information provided where possible to show the variability in observed pharmacokinetics. For some studies, only mean data (6 IV studies; 23 oral studies) were reported. Where clearance was reported per kg body weight, this was corrected for the mean body weight of the subjects reported in the clinical study. If body weight was not reported an assumed body weight (usually 78 kg) was used. Thus, 101 IV studies and 211 oral studies were available for comparison (see below). Simulations of IV and Oral midazolam pharmacokinetics were conducted in a population of 1000 healthy subjects aged 20-50 and 50% female subjects. The 1000 virtual subjects were assigned to 100 trials of 10 subjects and the CL (based on AUCinf) was plotted for each trial and compared to the clinical data in the figures below.

The IV data appears to capture the observed mean of the means and the mean SD across studies, but clearly, we are missing the inter-study variance (Figure 9). In our simulations, inter-study variance can only be SD^2/N, where N is the # of subjects, here 10; so, our inter-study variance is entirely due to intrinsic inter-subject variability, when it appears that there is a separate study effect. For oral, the variability of our means (inter-study variability) appears to better match the observed inter-study variability (Figure 10a and b).

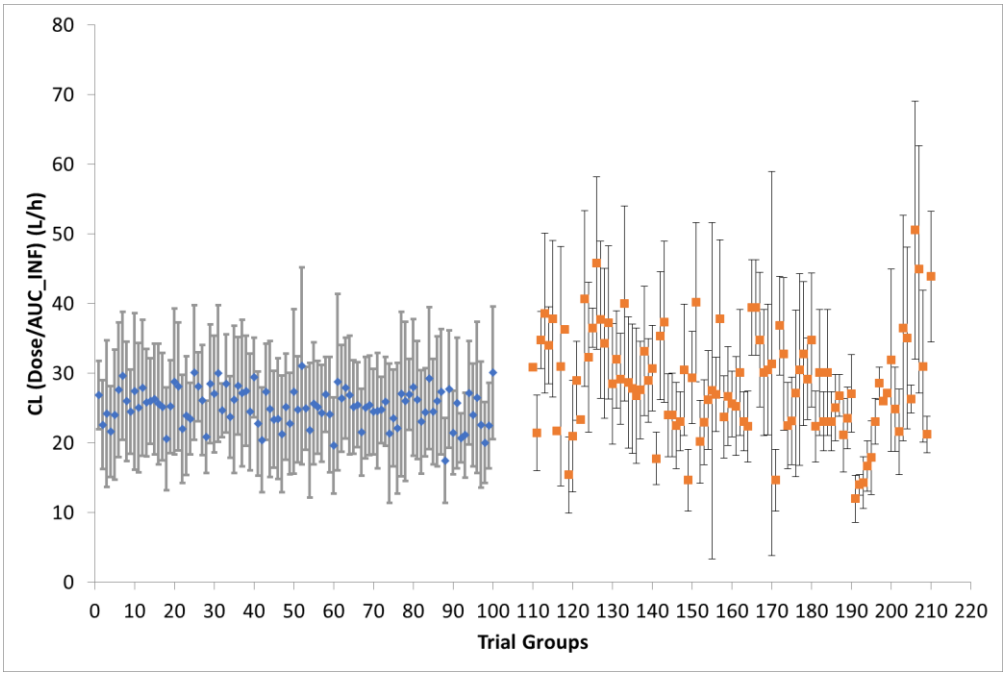


Figure 9. Simulated (blue) and observed (orange) CL (mean +/- SD) after intravenous dosing of midazolam. Each data point is an individual simulated or observed trial.

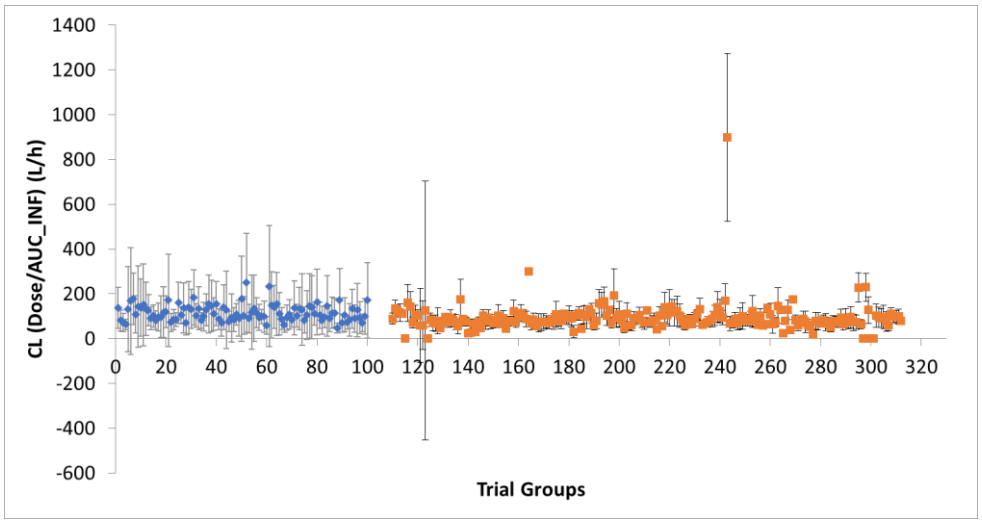


Figure 10a. Simulated (blue) and observed (orange) CL (mean +/- SD) after oral dosing of midazolam. Each data point is an individual simulated or observed trial.

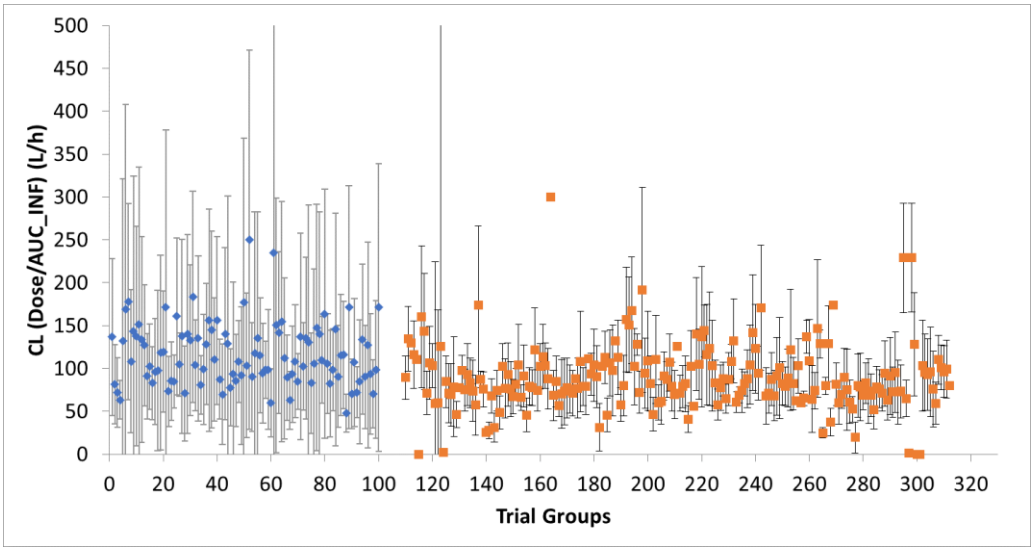


Figure 10b. Simulated (blue) and observed (orange) CL (mean +/- SD) after oral dosing of midazolam. Each data point is an individual simulated or observed trial. The y axis only shows data from 0-500 L/h.

To address the issue of intrinsic variability, we performed simulations using a population model. We can use the same simulation framework to address this issue. To be precise (see also code in Appendix, Simulation script 2):

At the subject level, the baseline AUC for drug i in subject j is assumed to be lognormally distributed around population mean μ_i (in log space) with population (between-subject variability) standard deviation Σ (in log space):

$$AUC_{i,j} \sim \mathcal{LN}(\mu_i, \Sigma) \tag{9}$$

At the first occasion (single drug administration), the AUC observed for drug i in subject j is assumed to be lognormally distributed around the subject baseline value with an occasion specific standard deviation σ_1:

$$AUC_{i,j,1} \sim \mathcal{LN}\left(\log\left(AUC_{i,j}\right), \sigma_1\right) \tag{10}$$

At the second occasion (two interacting drugs administration), the AUC observed for drug i in subject j is assumed to be lognormally distributed around the subject baseline value multiplied by a factor δ_i (the interaction effect) with an occasion specific standard deviation σ_2:

$$AUC_{i,j,2} \sim \mathcal{LN}\left(\log\left(AUC_{i,j,1} \times \delta_i\right), \sigma_2\right) \tag{11}$$

Occasion specific standard deviations σ_1 and σ_2 both include intra-subject variability and measurement uncertainty.

The observed AUC ratio for drug i in subject j is simply:

$$AUC_{r,i,j} = {AUC_{i,j,2}}/{AUC_{i,j,1}} \quad (12)$$

In the following, we show the effect of intrinsic variability (decomposed into between-subject and occasion specific variabilities) on the performance of predictions (assessed with clear visual checks).

Effect of intrinsic variability components on the performance of predictions

Figure show the effect of various levels of intrinsic variability component on predictive performance *if the model predicts correct between subject and inter-occasion variabilities*. Basically, the effect of between subject variability is removed by the study design (subject-level observations are paired). Only inter-occasion variability affects the performance by spreading the predictions, but without bias.

However, it is interesting to consider how potential approximations of the variabilities by the model affect its predictive performance. This is examined in the next section.

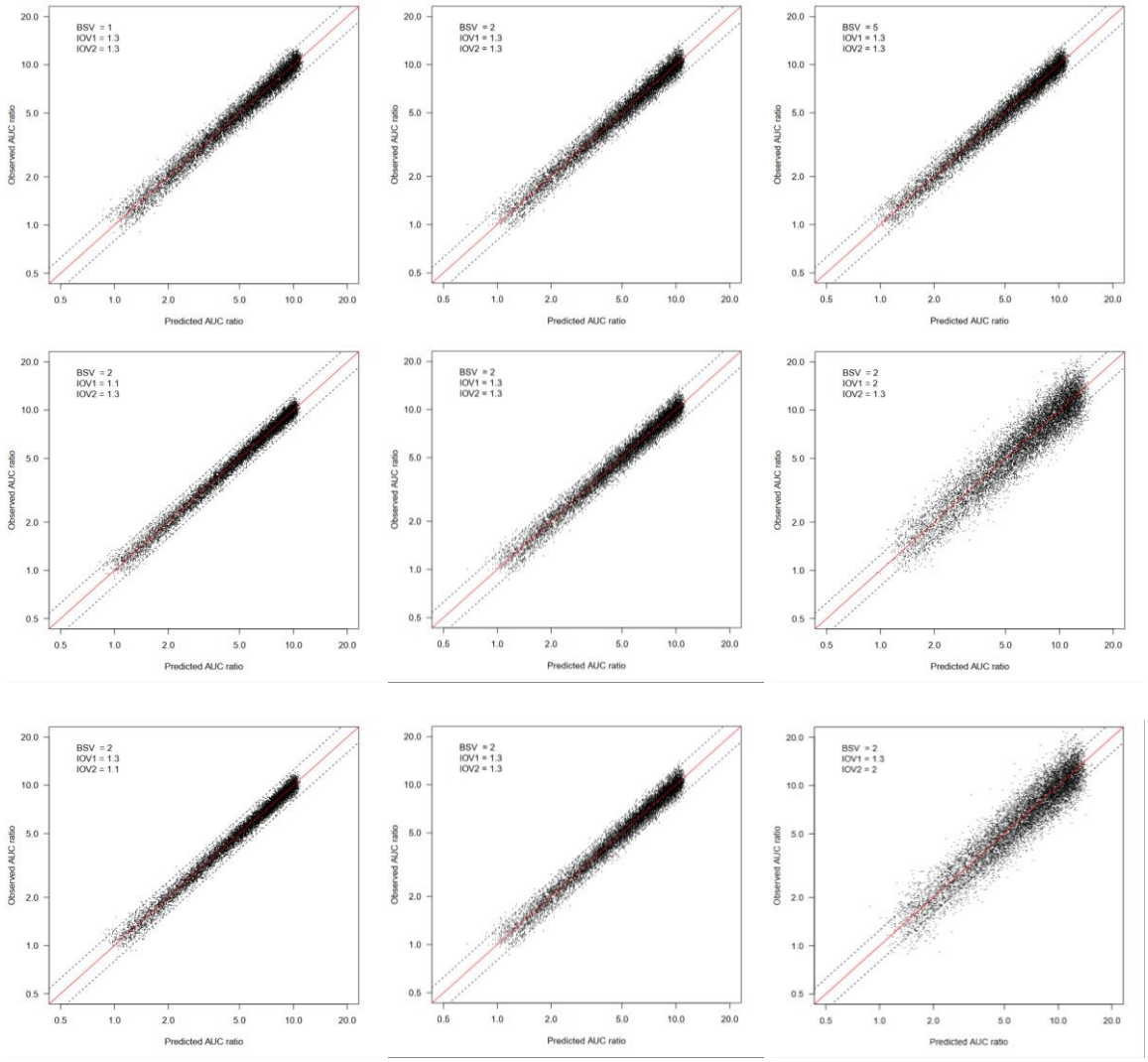


Figure 11: Effect of varying between subject variability (BSV), inter-occasion variability for the study reference arm (IOV_1) or for the test arm (IOV_2) on AUC ratio predictions.

Effect of intrinsic variability mis-estimation on the performance of predictions

Figure show the effect of various levels of *biases* affecting intrinsic variability component on predictive performance. Bias affecting of between subject variability has no impact because its effect is removed by the study design (subject-level observations are paired). Bias affecting inter-occasion variability does affects the performance by shifting the predictions away from identity line. It is therefore important that inter-occasion variability be correctly estimated.

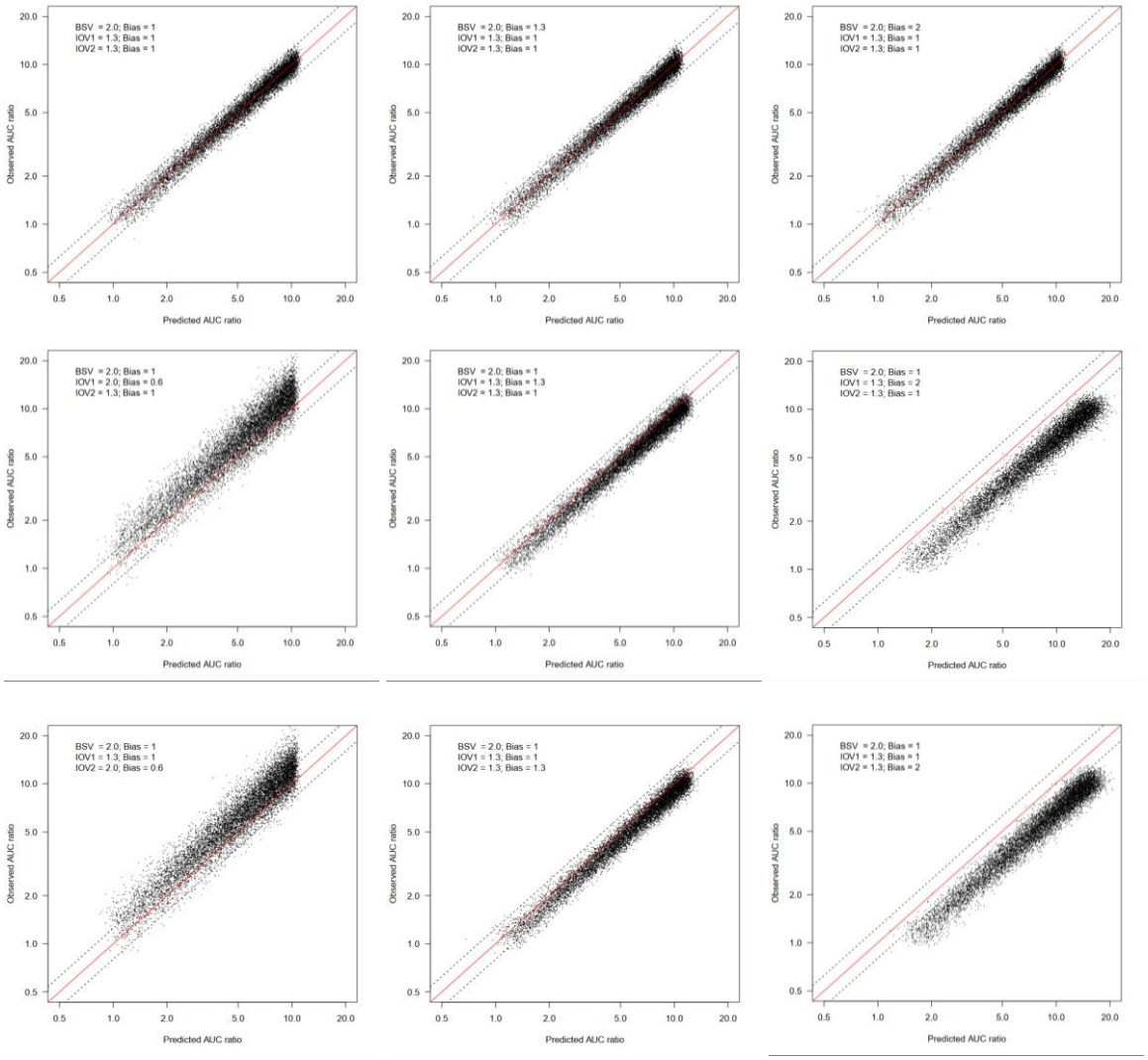


Figure 12: Effect of over- or under-estimation (biases) affecting between subject variability (BSV), inter-occasion variability for the study reference arm (IOV_1) or for the test arm (IOV_2) on AUC ratio predictions.

EMA Issue 8

Please discuss the option to include separate acceptance criteria for Cmax, AUC and for different CoU.

RESPONSE: As mentioned previously one of the more challenging issues that has been debated over the years is what fold (predicted/observed ratio) would constitute a successful prediction? We think that formally introducing separate acceptance criteria for Cmax, AUC and for COU may overcomplicate matters as appropriate acceptance criteria are likely to be dependent on the drug's therapeutic window and exposure response.

EMA Issue 9

Please discuss the model development/verification/refinement/application workflow. This information needs to be provided separately for each use case and each compound in a tabular format. It may need to be clarified for each case if the same data were used for model development and evaluation and how 'optimisation' of the model is understood.

RESPONSE: Prior to integration within the platform, a rigorous feasibility assessment is conducted for each compound to ensure that there are sufficient *in vitro* and clinical data available to develop and verify the files for their intended use i.e., quantitative prediction of CYP-mediated DDIs either as a victim and/or perpetrator. As part of this process, relevant information on physicochemical properties, cell permeability, protein and blood binding, *in vitro* metabolism and clinical PK is collated. Where multiple values for data are available, a meta-analysis approach is used as described in Howgate *et al*. (2006). to obtain a weighted geometric mean value and variance for a particular parameter. Simulations using each of the compound files aims to describe concentration-time profiles from clinical datasets based on *in vitro* data alone, at least in the initial stages. Model development is performed initially using intravenous data (if available) with a focus on the distribution and elimination parameters. Thereafter, absorption related parameters are introduced into the PBPK models for each compound to predict plasma concentration-time profiles following oral administration. At each stage, optimisation of relevant parameters is performed using clinical data, if necessary, to ensure accurate recovery of observed data.

- For a victim drug (substrate), it is important to characterise the clearance routes and demonstrate that when inhibited, the observed increase in exposures is accurately captured.
- Initially *in vitro* metabolism data are scaled to a CL value and compared against observed data. If underestimated, the hepatic intrinsic clearance (CL_{int}) can be estimated from the observed CLpo value using a retrograde model integrated within the Simcyp Simulator V19 and described by the following equation:

$$CLu_{H,int} = \frac{\frac{CL_{po}}{B:P} fa\, f_G - \frac{CL_R}{B:P}}{Uptake\, fu_b \left(1 + \frac{\frac{CL_R}{B:P}}{Q_H}\right)} \qquad (13)$$

Where B:P is the concentration ratio of drug in blood to plasma; fu_b is fraction of unbound drug in blood; Q_H is the blood flow in the hepatic vein; f_G is the fraction escaping first pass

metabolism in the gut; CL_R is the renal clearance; fa is the fraction absorbed; Uptake is a factor that accounts for any active hepatic uptake (assumed to be the default value = 1).

- Initially, the *in vitro* metabolism data are used to assign the relative contributions of the CYP enzymes (fmCYP). If the clinical DDI study with a strong inhibitor is not predicted accurately, the fmCYP is optimized to capture the observed data. Thereafter, independent clinical studies are used to verify the optimized fmCYP.
- For a perpetrator (inhibitor), it is necessary to ensure that after integration of the inhibitory parameters into the PBPK model, they lead to accurate prediction of clinical DDIs with a sensitive substrate. If not, the inhibitory parameters are optimized to capture the observed DDI. Thereafter, independent clinical studies are used to verify the optimized fmCYP.

Within each of the CYP enzyme analysis files, there is a worksheet called *"Source of fm and Ki values"*. On this worksheet, it is indicated how the fm values were derived for each of the substrates and whether/how the inhibition parameters were derived/optimised.

Reference

Howgate EM, Rowland-Yeo K, Proctor NJ, Tucker GT and Rostami-Hodjegan A. Prediction of in vivo drug clearance from in vitro data: impact of interindividual variability. *Xenobiotica* 2006; 36 (6): 473-497.

EMA Issue 10

The University of Washington Database (UW) was used by the Applicant to form the part of the DDI qualification matrix where clinical DDI studies were selected if compound files for both substrate and inhibitor were available within the SimCYP simulator. However, some of the available DDI studies in this database were still omitted by the Applicant in the qualification procedure without a precise/detailed explanation and the inclusion/exclusion criteria as discussed in the powerpoint presentation were not always consistently conducted:

a. *Cocktail studies were sometimes included in the analysis,*
b. *studies that were used for inhibitor optimization sometimes seemed to be included in the analysis (e.g. amiodarone, fluconazole, ritonavir),*
c. *Duplicates of the same interaction data seem to be included and this should be avoided e.g. Mean and gMean, AUCt and AUCinf, subgroup + total group analysis, males vs females etc*
d. *Studies with complex interactions were sometimes included in the qualification set, which is out of scope.*
e. *Please provide detailed information as why studies included in the Washington database were not included in the qualification dataset.*
f. *A table with the DDI qualification set studies is included as attachment with tentative remarks to the above observations Please comment on the*

appropriateness of inclusion of the highlighted studies in the qualification data set.

g. *the Applicant should present a tabular overview of all omitted studies/references for each pair of substrate-inhibitor where reason(s) for the omission of each individual study/reference should be clearly stated.*

RESPONSE: For CYP1A2, CYP2C8, CYP2C9, CYP2C19, and CYP2D6, UOW searches were performed to identify clinical DDI studies involving moderate to sensitive substrates according to FDA guidance and UOW criteria. Initially, studies were excluded if they were:

- Cocktail studies.
- Involved patients.

Thereafter, only studies involving Simcyp compounds (or published models that were reproducible) were retained. Then studies were excluded if they were:

- Duplicate studies.
- Involved complex interactions e.g. repaglinide/gemfibrozil.

For each enzyme, in the following excel files there is a worksheet called "UOW" that indicates which studies were selected and excluded (with clarification):

"CYP1A2-ClinicalData_TrialDesignSetting-02"

"*CYP2C8-ClinicalData_TrialDesignSetting-02"*

"*CYP2C9-ClinicalData_TrialDesignSetting-02"*

"*CYP2C19-ClinicalData_TrialDesignSetting-02"*

"*CYP2D6-ClinicalData_TrialDesignSetting-02"*

EMA Issue 11

Please justify the selection of the ADAM or the first order absorption model.

RESPONSE: Typically, the simplest absorption model is used initially especially if the compound is well behaved in terms of its absorption characteristics. If reliable *in vitro* data are available, fa (the fraction absorbed) and ka (absorption rate constant) describing first order absorption can be predicted using MDCK or Caco-2 cell data, which are preferred over PSA/HBD data. If intestinal transport needs to be considered for the drug or a complex formulation is being considered, then ADAM would be applied.

An excel file called "V19-ADAM_FO" has been provided to indicate which compounds use first order versus ADAM models.

EMA Issue 12

Fraction metabolized (Fm) reported in briefing document, publication by Kilford et al 2022 and substances files are not identical. Even though the impact on the predicted values might be small, these are compound specific characteristics and should be the same since all three are based on V19R1. Please clarify and indicate the impact.

RESPONSE: Some of the differences are due to the fact that CYP3A5 was not considered in the fmCYP contributions in the Kilford analysis (2022). In addition, some of the files were in development for later versions of the Simcyp Simulator. We present fmCYP values for all substrates run in V19 using the same conditions (same population). These data can be found in an excel file called *"fm-Fg-F-202312"* in the "Responses Folder to submit".

EMA Issue 13

The minimal PBPK model considers hepatic and intestinal metabolism. Involvement of intestinal metabolism is considered for CYP3A4, but also for CYP2D6, CYP2C9 and CYP2C19. While for CYP3A4 references were provided to demonstrate that the intrinsic activity of CYP3A4 is the same in liver and intestine but the degradation rate of CYP3A4 is different in liver and intestine no information on the intrinsic activity and kdeg for the enzymes CYP2D6, CYP2C9 and CYP2C19 was provided. Please provide this information and discuss if a rank order approach would be appropriate for intestinal CYP enzymes considering variable expression of the enzymes along the intestine from duodenum to distal ileum (or even colon).

RESPONSE: With MBI there is some added complexity as the kdeg values can vary from enzyme to enzyme and for some enzymes there are less data available. These data were summarised in a publication by Yang *et al.* (2008). The table from this publication (shown below) indicates that the half-lives of CYP2D6, CYP2C9 and CYP2C19 are 23, 104 and 26 h which translate to kdeg values of 0.031 (=0.0693/23), 0.0067 and 0.027 h^{-1}, respectively. The latter values are used in Simcyp V19.

It should be noted that the same kdeg value (0.03 h^{-1}) is used for all isozymes in the gut as it is based on the turnover of the enterocyte which is faster than the turnover of the individual enzymes (Yang *et al.*, 2008).

Table 1. Turnover Half-Lives of Human Hepatic CYPs (Updated from Ghanbari *et al.* 2006 [139]. NC = Not Clear).

Enzyme	Method	n	$t_{1/2}$ (h) *	Reference
CYP1A2	*In vitro* Method 1	1	51	[67]
	In vitro Method 2	NC	43**	[10]
	In vitro Method 2	5	36 (8-58)	[74]
	In vivo Method 1	12	39 (27-54)	[92]
	In vivo Method 3	7	105	[101]
CYP2A6	*In vitro* Method 2	2	26 (19-37)	[74]
CYP2B6	*In vitro* Method 2	1	32	[74]
CYP2C8	*In vitro* Method 2	5	23 (8-41)	[74]
CYP2C9	*In vitro* Method 2	5	104	[74]
CYP2C19	*In vitro* Method 2	3	26 (7-50)	[74]
CYP2D6	*In vitro* Method 2	4	70	[74]
	In vivo Method 2	13	51	[96, 98]
CYP2E1	*In vitro* Method 2	5	27 (7-40)	[74]
	In vivo Method 1	6	60	[91]
	In vivo Method 2	11	50 ± 19	[95]
CYP3A4	*In vitro* Method 1	1	44	[68]
	In vitro Method 2	NC	26**	[10]
	In vitro Method 2	4	79	[74]
	In vivo Method 1	15	72**	[87]
	In vivo Method 3	6	96 ± 38 (53-154)	[140]
	In vivo Method 1	7	72 (20-146)	[141]
	In vivo Method 1	3	(85-806)	[142]
	In vivo Method 1	8	(36-50)	[143]
	In vivo Method 3	13	10** (2-158)	[144]
	In vivo Method 3	35	94 (62-205)	[100]
	In vivo Method 3	7	70	[101]
	In vivo Method 3	16	85 ± 61	[145]
	In vivo Method 3	6	140 (48-284)	[146]
CYP3A5	*In vitro* Method 2	3	36 (15-70)	[74]

* $t_{1/2} = 0.693 / k_{deg}$. Values in brackets are ranges and ± indicates SD
** estimated from an analysis of the reported data

Regarding the intrinsic activity of CYP2C9, CYP2C19 and CYP2D6 in liver *versus* gut, there are no *published* data indicating that there is a relationship. However, we have extracted relevant data for each of the enzymes from a PhD thesis by Von Richter 2000. It appears that the intrinsic activity of CYP2C9 may be similar in the liver and intestine. We have shared these data in an excel file within the folder called "Issue 13".

Reference:

Yang J, Liao M, Shou M, Jamei M, Rowland-Yeo K, Tucker GT and Rostami-Hodjegan A. Cytochrome P450 turnover: regulation of synthesis and degradation, methods for determining rates, and implications for the prediction of drug interactions. *Curr Drug Metab* 2008; 9: 384-394 (2008).

EMA Issue 14

The conditions under which the fm- and Ki-values, Kapp/kinact have been optimised for the various substrate files need to be described in greater detail in order to allow adequate assessment of the use of the platform for the CoUs.

a. *The (type of) clinical data that were used to verify and/or optimise fm should be clarified. Did this include data from DDI studies with strong, moderate and/or weak inhibitors? This is a crucial point that requires clarification in order to adequately assess CoU1, since CoU1 states that clinical DDI data with a strong inhibitor will be available and can be used to verify the fm-value. Were data from moderate and weak inhibitors DDI studies used to verify and/or optimise fm values when developing the PBPK models for the substrates included in the SimCYP platform provided by the Applicant?*
b. *For each substrate compound file where a fm parameter optimization was implemented, please provide information on if independent clinical studies were available (and if so, how many), along with appropriate tables and figures of the independent verification.*
c. *The optimization of Ki-values for inhibitors cannot be followed based on the provided compound summaries for inhibitors. For each inhibitor compound file where a Ki parameter optimization was implemented, please provide information on if independent clinical studies were available (and if so, how many), along with appropriate tables and figures of the independent verification. Also provide info on in vitro Ki vs optimized Ki and effect on exposure prediction of optimization. By the end of the qualification procedure, the compound summaries should be updated accordingly.*
d. *Please provide a Table with Ki values for the inhibitors and compare these with median and range of Ki values reported in DIDB.*
e. *Provide similar information as requested in c+d for optimization of Kapp and kinact values.*

- RESPONSE: We provide a general response that captures points a-c. Initially, the *in vitro* metabolism data (and mass balance data if available) are used to assign the relative contributions of the CYP enzymes (fmCYP) and clearance routes to the elimination of the drug. If the clinical DDI study with a strong inhibitor is not predicted accurately, the fmCYP is then optimized to capture the observed data. Thereafter, independent clinical studies are used to verify the optimized fmCYP.
- For a perpetrator (inhibitor), it is necessary to ensure that after integration of the inhibitory parameters into the PBPK model, they lead to accurate prediction of clinical DDIs with a sensitive substrate. If not, the inhibitory parameters are optimized to capture the observed interaction. Thereafter, *independent* clinical studies are used to verify the optimized fmCYP.

- We have followed the above approach for the development of compound files as substrates and inhibitors. To the best of our knowledge, we have removed all clinical DDIs that have been used to optimize the fmCYP values or inhibitory parameters.
- Within each of the CYP enzyme analysis files, there is a worksheet called *"Source of fm and Ki values"*. On this worksheet, it is indicated how the fm values were derived for each of the substrates (in vitro data or clinical DDI study) and whether/how the inhibition parameters were derived/optimized (invitro data or clinical DDI study).
- Going forward we will endeavour to capture this information on the compound file summaries.

Regarding points d) and e) above, we have collated the requested data relating to the inhibitory potencies of the drugs and included two excel files in two subfolders called *"Issue 14-Ki"* and *"Issue 14-Kapp-kinact"* within *"Responses Folder to submit"*.

EMA Issue 15

Polymorphism: please provide a comparison of substrates single dose and multiple dose data for EM and PM for CYP2C9, CYP2C19, CYP2D6 (observed and predicted PK parameters) and comparison of PM with strong inhibitors. In principle, the exposures should be comparable for poor metabolisers or for EM with a strong inhibitor. Therefore, poor metabolisers could be used to verify the model. How are PM, IM, EM, UM for the various polymorphic CYP enzymes included in Simcyp?

RESPONSE: We have compiled a dataset where we show studies with metoprolol exposures in CYP2D6 PM subjects (n=9 studies) *versus* metoprolol exposures in subjects following coadministration with a strong CYP2D6 inhibitor paroxetine (n=2). In the latter 2 studies, the CYP2D6 phenotypic status of the subjects is not clear. In the second dataset, we show studies with omeprazole exposures in CYP2C19 PM subjects (n=8 studies) *versus* omeprazole exposures in subjects following coadministration with a strong CYP2C19 inhibitor omeprazole (n=8). The number of PM subjects tends to be small and the phenotypic status of the subjects in the DDI studies is not clear. In principle, this sounds like a reasonable approach; however, in practice, it may not be possible given the relatively small numbers and the lack of clarity regarding the phenotypic status of some of the subjects.

A folder *"Issue 15"* with two excel sheets indicating the data for metoprolol and omeprazole have been provided in the "Responses Folder to submit".

Regarding the second question, and how Simcyp handles phenotypic data we provide the following information. On the demographic screen of the population tab, the frequency of different phenotypes (EM, IM, UM and PM) is entered. These values are then used when setting up the population for a particular simulation; individuals are randomly assigned to the phenotypes depending up on the frequency associated with the enzyme in a population. The values of the phenotype frequency can be different for each population and can also be altered by the user to reflect those of a specific study. The values are taken from a meta-analysis of literature data where the different phenotype frequencies have been measured.

For each phenotype an associated abundance with variability is provided for the liver and intestine. These distributions are sampled for an individual once their phenotype has been defined. Thus, each individual has a phenotype and associated enzyme abundance for each relevant enzyme.

EMA Issue 16

What constitutes a "sensitive" CYP substrate? Please discuss using the ICH M12 definition.

RESPONSE: Ideally, drugs should be selected for clinical studies based on their sensitivity, specificity, safety profiles, and reported DDI studies with inhibitors. According to the ICH-M12 definition, sensitive index substrates are index drugs that demonstrate an increase in AUC of ≥5-fold with strong index inhibitors of a given metabolic pathway in clinical DDI studies. Moderately sensitive substrates are drugs that demonstrate an increase in AUC of ≥2- to < 5-fold with strong index inhibitors of a given metabolic pathway in clinical DDI studies. Where possible, we have included sensitive and moderate sensitive substrates for each enzyme.

EMA Issue 17

For several input parameters throughout the documentation (e.g., compound summaries), the Method/Reference that has been used to derive several of the input parameters cannot be followed. The Applicant should update the documentation to clarify the Method/Reference, including instances where the following terms have been used by the Applicant:

a. *The Applicant states "meta-analysis" as a reference without specifying which underlying literature references this refers to.*
b. *For some parameters the Applicant stated "optimized" without clearly specifying the reference for which study that was used to optimize the parameter.*
c. *For some parameters the Applicant stated "predicted" without specifying the method used to predict the parameter.*

RESPONSE: All this information has been collated and is logged in the form of meta-analyses within excel sheets for each compound file. We understand the need for transparency and will endeavour to capture this information within the compound file summaries. Although it does need to be recognized that even for one compound, many different references have been collated over the years and critiqued to provide even a single source. It is difficult to translate this amount of information into a summary document that can be easily interpreted.

EMA Issue 18

The model validation of the DDIs throughout the documentation (in particular, the compound summaries) provided by the Applicant are mostly in the form of Tables of the observed and predicted ratio of Cmax and AUC, respectively, as well as the ratio of the observed and predicted ratios (i.e., the "ratio of ratio"). The level of detail of this format is considered a limitation of the work since it does not allow a sufficiently in-depth assessment of CoU1. For clinical DDI studies where this is applicable, the Applicant should provide more detailed model validation, including tables with the predicted and observed Cmax and AUC, respectively (i.e. not only a Table with ratios) as well as Figures comparing the observed and predicted drug concentration vs time curves (with and without the interaction).

RESPONSE: For each of the enzymes, we have reviewed the clinical data and extracted observed data when available/possible. We have prepared overlays of the clinical data which are executable in Simcyp V19. We have rerun all the simulations, overlaid observed data (when available) and prepared word documents with the profiles, predicted and observed Cmax and AUC values and corresponding ratios. These can be found within each enzyme folder in a sub-folder called *"DDI Simulation Summaries"*.

SPECIFIC ISSUES ON CYP1A2 FOR COU1

EMA Issue 19

The Simcyp Simulator V19 R1 contains only two CYP1A2 inhibitors (ciprofloxacin and fluvoxamine) both of which are classified as strong CYP1A2 inhibitors. Therefore, there is no evidence presented by the Applicant that the Simcyp Simulator is capable of predicting DDI scenarios with different levels of CYP1A2 inhibition (i.e., there are no weak nor moderate CYP1A2 inhibitors available in the Simcyp platform). The Applicant should update/extend the compound summaries to address these issues or otherwise, based on this limitation, it is currently not considered possible to qualify the platform for CoU1 for CYP1A2.

RESPONSE: The DDI matrix has been updated to include cimetidine and propranolol as weak and moderate inhibitors of CYP1A2, respectively (see below). This information and the updated analysis can be found in the excel file *CYP1A2-ClinicalData_TrialDesignSetting-02"*.

Substrate	FDA	CDIS	fm%	F_G	F_H	F	Dose [mg]
Caffeine	Index	Sensitive	97.93	1	0.93	0.81	150
Duloxetine		Sensitive	56.22	1	0.48	0.32	60
Olanzapine		NA	36.62	1	0.8	0.76	10
Theophylline		Moderate sensitive	75.83	1	0.96	0.83	125
Tizanidine	Index	Sensitive	96.57	1	0.17	0.16	4

Inhibitor	FDA	CDIS
Cimetidine		Weak
Ciprofloxacin		Strong
Fluvoxamine	Strong index	Strong
Propranolol		Moderate

CDIS: Certara Drug Interaction Solutions.

EMA Issue 20

In addition to the lack of adequate evidence to support CoU1, as stated above, the following specific issues would also need to be resolved before CYP1A2 can be considered qualified.

A. *For ciprofloxacin, it is stated that the Ki parameter was optimised based on the reference Kim et al. (2003), while the observed data from this reference were also used to verify the predictive performance as a part of the present qualification procedure. The Applicant should discuss whether this caused biased prediction and update the documentation as necessary.*

RESPONSE: This study is still included as a trial in the excel sheet called *"CYP1A2-ClinicalData_TrialDesignSetting-02"* to indicate the trial design but the simulation results have now been removed from the CYP1A2 analysis itself.

B. *Regarding the CYP1A2 substrates the Applicant has presented only 3 substrates in total. It is also noted that tizanidine is not actually available as a compound file in the Simcyp Simulator. According to the compound summary of tizanidine: "There is limited data in the public domain with which to verify the performance of the RES-Tizanidine file and therefore the file has been made available via the Simcyp members area, rather than*

via the Simcyp simulator". The Applicant should discuss this limitation and how to mitigate it.

RESPONSE: The CYP1A2 DDI matrix has now been expanded to include two other substrates with varying contributions of CYP1A2 to the metabolic clearance (duloxetine and olanzapine) (see Table 1 above).

The main issue we have with the tizanidine file is that whilst it accurately predicts the DDI with fluvoxamine (P/O AUC ratio = 0.99) it significantly underpredicts the DDI with ciprofloxacin (P/O AUC ratio = 0.34). The study investigators themselves [1] state that *"Ciprofloxacin, at a usual dose of 500 mg twice daily, had a strong pharmacokinetic interaction with tizanidine. However, this interaction differed qualitatively and quantitatively from the recently described fluvoxamine-tizanidine interaction and quantitatively from the previously published interactions of ciprofloxacin with other drugs. Ciprofloxacin increased the AUC(0-inf) of tizanidine by 10-fold, in some subjects by up to 24-fold, and the Cmax by 7-fold, but in contrast to the effect of fluvoxamine, the elimination half-life of tizanidine was prolonged only marginally. In previous reports, ciprofloxacin has only moderately (less than 2-fold) increased the AUC of other drugs that are metabolized by CYP1A2, including theophylline, caffeine, clozapine, and ropivacaine."* As we already have the sensitive CYP1A2 substrate caffeine we could have removed it from the analysis, but we wanted to be transparent.

Reference:

1. Granfors, M.T., Backman, J.T., Neuvonen, M., Ahonen, J. & Neuvonen, P.J. Fluvoxamine drastically increases concentrations and effects of tizanidine: a potentially hazardous interaction. Clinical Pharmacology & Therapeutics 75, 331-41 (2004).

SPECIFIC ISSUES ON CYP3A4 FOR COU1

EMA Issue 21

To demonstrate that the implementations of the simulation designs are adequate, the Applicant provided spread sheets (in .xlsx format) for all CYP enzymes apart from CYP3A4. The Applicant should provide this type of spread sheet also for CYP3A4.

RESPONSE: This information has now been provided in the excel file "*CYP3A4-ClinicalData_TrialDesignSetting-02*".

EMA Issue 22

Cimetidine is stated by the Applicant to be the only weak CYP3A4 inhibitor included in the platform. According to the model validation of DDI studies with cimetidine as a perpetrator, the PBPK platform tends to under-predict the magnitude of the interactions. This is considered a concern of the qualification of CYP3A4 for CoU1, since from a safety perspective, the platform may not be considered conservative when it comes to predicting interactions with weak CYP3A4 inhibitors. The Applicant should discuss the impact of these model-misspecifications and preferably also add additional weak CYP3A4 inhibitors to the documentation and/or improve the cimetidine model.

RESPONSE: The Ki value has been modified to improve the predictions for CYP3A4-mediated DDIs involving cimetidine. An updated V19 compound file summary has been prepared and the results of the updated CYP3A4 simulations are also shown in the excel file called *"CYP3A4-ClinicalData_TrialDesignSetting-02"*.

Furthermore, cimetidine has also been used as a weak inhibitor of CYP1A2, CYP2D6 and CYP2C19. The inhibitory parameters used in the simulations for each of the enzymes are shown in the excel file called "Cimetidine source Ki values" found within "Issue 22" in the main folder called "Final Responses".

EMA Issue 23

Cyclosporin is referred to as one of the moderate CYP3A4 inhibitors. The only clinical dataset referred to is an interaction study with repaglinide, where the main mechanism of interaction appears to OATP-inhibition. In addition, the UW database classifies cyclosporin as a weak CYP3A4 inhibitor. The relevance of cyclosporin for qualification of the prediction of CYP3A4 inhibition needs to be justified.

RESPONSE: Many of the cyclosporin DDI studies cited in the DIDB were conducted in patients and therefore cannot be included in the analysis. Due to the lack of relevant DDI studies and the fact that the simulated study involves a complex DDI (OATP1B1 and CYP3A4), we have removed cyclosporin from the analysis as a moderate CYP3A4 inhibitor.

EMA Issue 24

Fluvoxamine is referred to as one of the moderate CYP3A4 inhibitors, however, the UW database classifies fluvoxamine as a weak CYP3A4 inhibitor. Several of the input parameters (including CYP Ki values) appear to be optimised based on clinical data but no details are given (see also general issue above). Validation of CYP3A4 inhibition has only been performed with two substrates (midazolam and quinidine), and for quinidine CYP2C inhibition also

appears to be of importance. It is also unclear if any of these substrates were used to optimise the CYP3A4 Ki. The relevance of fluvoxamine for qualification of the prediction of CYP3A4 inhibition needs to be justified.

RESPONSE:

The CYP3A4 Ki value was derived initially from a meta-analysis of *in vitro* HLM data using midazolam and triazolam as the CYP3A4 probe substrates. After correcting for non-specific microsomal binding, the resultant value was 7.89 µM. Yao et al. (2001) reported that on comparison of *in vitro* and *in vivo* K_i values based on unbound fluvoxamine concentrations, fluvoxamine inhibition potency is approximately 10 times greater *in vivo* than *in vitro*. Thus, a final value of 0.789 µM was used in all simulations.

Reference:

Yao C, Kunze KL, Kharasch ED, Wang Y, Trager WF, Ragueneau I, Levy RH. Fluvoxamine-theophylline interaction: gap between in vitro and in vivo inhibition constants toward cytochrome P4501A2. Clin Pharmacol Ther. 2001 Nov;70(5):415-24. doi: 10.1067/mcp.2001.119724. PMID: 11719727.

EMA Issue 25

Itraconazole is used by the Applicant as a strong CYP3A4 inhibitor to predict DDI scenarios for the SimCYP platform qualification purpose. However, itraconazole is also known as a clinical P-gp inhibitor, which do not seem to be incorporated in the SimCYP compound file V19. On the other hand, some inhibitory parameters towards transporters like BCRP and OATPs (which are less relevant for itraconazole as an inhibitor) appear to be included. Generally, it is common that CYP3A4 substrates are simultaneous P-gp substrates. The Applicant is asked to discuss this limitation of the itraconazole compound file (i.e., in terms of additional interaction mechanisms) and how this would impact its' potential use as a strong CYP3A4 inhibitor for qualification of CoU1.

RESPONSE: The focus of the current qualification exercise is to assess the performance of the Simcyp Simulator V19, for DDIs involving CYP1A2-, CYP2C8/9/19-, CYP2D6- and CYP3A4-mediated inhibition. Thus, we believe that the issue of itraconazole being a dual CYP3A4/P-gp inhibitor is out of scope for COU1.

EMA Issue 26

Ketoconazole is included in the framework as a strong CYP3A4 inhibitor. A few points are unclear regarding the inhibitor compound summary file for ketoconazole, as outlined in the following:

A. *The fuinc/fumic seems to be important for predicting all DDIs and was estimated based on in vitro data. However, the Applicant only included a brief description of this approach, and it is not possible to adequately assess this assumption. Therefore, the Applicant is asked to provide more details to support the relevance of the optimisation of fuinc/fumic. Please discuss from a mechanistic point of view, why the fuinc would be different for different CYP enzymes (according to Table 2 in the compound summary for ketoconazole).*

RESPONSE: Non-specific microsomal binding (NSMB) in *in vitro* metabolism systems leads to an underestimation of the true intrinsic metabolic clearance of compounds being studied or indeed the inhibitory potency (Gardner *et al*., 2022). Therefore *in vitro* binding needs to be accounted for when extrapolating *in vitro* data to predict the *in vivo* metabolic clearance or the *in vivo* inhibitory potency of a compound. When several sources of *in vitro* Ki are available, an individual fu_{inc}/fu_{mic} (measured or predicted at the protein concentration used to determine the Ki value) is applied to each respective value. Thus, fu_{inc}/fu_{mic} values can be different across CYP enzymes as the degree of NSMB is dependent on the protein concentration used in the *in vitro* experiment.

For example, in Yao *et al.* (2001), in vitro CYP1A2 Ki values for fluvoxamine based on total inhibitor concentrations were 177, 121 and 52 µM in human liver microsomes with 1 mg/ml protein, 0.5 mg/ml protein and 0.3 mg/ml protein, respectively. After correcting for NSMB, the corresponding in vitro values based on unbound fluvoxamine concentrations were 35, 36 and 36 µM, respectively.

References:

Gardner I, Xu M, Han C, Wang Y, Jiao X, Jamei M, Khalidi H, Kilford P, Neuhoff S, Southall R, Turner DB, Musther H, Jones B, Taylor S. Non-specific binding of compounds in *in vitro* metabolism assays: a comparison of microsomal and hepatocyte binding in different species and an assessment of the accuracy of prediction models. Xenobiotica. 2022 Aug;52(8):943-956. doi: 10.1080/00498254.2022.2132426. PMID: 36222269.

Yao C, Kunze KL, Kharasch ED, Wang Y, Trager WF, Ragueneau I, Levy RH. Fluvoxamine-theophylline interaction: gap between in vitro and in vivo inhibition constants toward cytochrome P4501A2. Clin Pharmacol Ther. 2001 Nov;70(5):415-24. doi: 10.1067/mcp.2001.119724. PMID: 11719727.

B. *Similar to itraconazole, ketoconazole is also known to inhibit P-gp which does not seem to be incorporated for SimCYP V19 for ketoconazole. In line with the concern raised for itraconazole (see above), the Applicant is asked to discuss the limitation of the lack of P-gp interactions in the ketoconazole file and how this would impact the potential use as a strong CYP3A4 inhibitor for qualification of CoU1.*

RESPONSE: The focus of the current qualification exercise is to assess the performance of the Simcyp Simulator V19, for DDIs involving CYP1A2-, CYP2C8/9/19-, CYP2D6- and CYP3A4-mediated inhibition. Thus, we believe that the issue of ketoconazole being a dual CYP3A4/P-gp inhibitor is out of scope for COU1.

C. *In the ketoconazole compound summary file, the text refers to model validation figures with plasma concentrations vs time, including interaction data from Stoch et al 2009 and Olkkola et al 1994 in Figures 10 -12. However, Figures 10 – 12 are not available in the compound summary for ketoconazole, and the Applicant should provide an updated compound summary file, include Figures 10 – 12.*

RESPONSE: An updated compound file summary has now been provided for ketoconazole in V19.

SPECIFIC ISSUES ON CYP2D6 FOR COU1

EMA Issue 27

In the compound summaries for substrates, it is stated that nebivolol is a new compound for SimCYP V20, which is confusing since the current procedure concerns SimCYP V19. The Applicant is asked to please clarify this finding and if applicable, should re-run the nebivolol model using SimCYP V19, since this is the version that is the topic of the current procedure.

RESPONSE: To clarify, whilst doing the V19 qualification analysis, nebivolol, was being developed as a new compound for V20. Thus, the same parameters that were used for the compound in V20 were applied in V19. The compound file summary that was provided was run in V19 and does not need to be re-run.

EMA Issue 28

For fluvoxamine, more details with respect to how the Ki for CYP2D6 was optimised needs to be provided before fluvoxamine can be considered acceptable to be part of the qualification of the CYP2D6 pathway (see also general issue, above).

RESPONSE:

The CYP2D6 Ki value was derived initially from a meta-analysis of *in vitro* HLM data using CYP2D6 probe substrates. After correcting for non-specific microsomal binding, the resultant value was 1.89 µM. Yao *et al.* (2001) reported that on comparison of *in vitro* and *in vivo* K_i values based on unbound fluvoxamine concentrations, fluvoxamine inhibition potency is

approximately 10 times greater *in vivo* than *in vitro*. Thus, a final value of 0.189 µM was used in all simulations.

Reference

Yao C, Kunze KL, Kharasch ED, Wang Y, Trager WF, Ragueneau I, Levy RH. Fluvoxamine-theophylline interaction: gap between in vitro and in vivo inhibition constants toward cytochrome P4501A2. Clin Pharmacol Ther. 2001 Nov;70(5):415-24. doi: 10.1067/mcp.2001.119724. PMID: 11719727.

EMA Issue 29

Several points are unclear regarding the model(s) for fluoxetine and norfluoxetine:

A. *Fluoxetine and norfluoxetine appear to have comparable PK behaviour in many other aspects and it is unclear if the fu should differ this much between these two compounds. The chosen fu for norfluoxetine should be further justified.*

RESPONSE: The protein binding data (0.079) for racemic fluoxetine came from clinical studies (Aronoff *et al.,* 1984 and Schenker *et al.,* 1988). As no corresponding data were available for norfluoxetine, an average value (0.165) for the enantiomers was used (Lutz *et al.,* 2013).

References

Aronoff, G. R., R. F. Bergstrom, S. T. Pottratz, R. S. Sloan, R. L. Wolen, and L. Lemberger. 1984. "Fluoxetine Kinetics and Protein Binding in Normal and Impaired Renal Function." Journal Article. Clin Pharmacol Ther 36 (1): 138–44.

Schenker S, Bergstrom RF, Wolen RL, Lemberger L. Fluoxetine disposition and elimination in cirrhosis. Clin Pharmacol Ther. 1988 Sep;44(3):353-9. doi: 10.1038/clpt.1988.161. PMID: 3262026.

Lutz JD, VandenBrink BM, Babu KN, Nelson WL, Kunze KL, Isoherranen N. Stereoselective inhibition of CYP2C19 and CYP3A4 by fluoxetine and its metabolite: implications for risk assessment of multiple time-dependent inhibitor systems. Drug Metab Dispos. 2013 Dec;41(12):2056-65. doi: 10.1124/dmd.113.052639. Epub 2013 Jun 19. PMID: 23785064; PMCID: PMC3834134.

B. *The y-axis limits for the validation without any interaction for repeated dosing is not acceptable and should be updated. The y-axis should be limited to a much more plausible range given the data (e.g. ~50-500 rather than 1-1000). The Applicant is asked to please update the figures accordingly.*

RESPONSE: This has been amended and an updated V19 compound file summary has been provided.

C. The DDIs are underpredicted in the inhibitor file and a strong justification or model improvement is needed in case CoU1 is to be applied with fluoxetine as the perpetrator for the CYP2D6 pathway.

RESPONSE: We are surprised by this comment as, of the 7 DDIs that have been simulated, 4 of them fall within 1.25-fold of the observed data. The results are shown below and can be found in the excel sheet called *"CYP2D6-ClinicalData_TrialDesignSetting-02"*.

				PREDICTED		OBSERVED		P/O Ratio	
		SUBSTRATE	INHIBITOR	CMAX	AUC	Cmax	AUC	Cmax	AUC
24	CYP2D6	Nebivolol, 10 mg SD on day 21	Fluoxetine, 20 mg QD days 1-21	2.39	6.92	3.38	5.80	1.41	0.84
10	CYP2D6	Desipramine HCL, 50 mg QD for 28 Days	Fluoxetine, 20 mg QD Days 8-28	4.00	4.80	3.99	4.60	1.00	0.96
25	CYP2D6	Tolterodine, 2.36 mg BID days 22-24 (5 doses)	Fluoxetine, 20 mg QD for 24 days	3.57	4.87	3.75	6.68	1.05	1.37
26	CYP2D6	Tolterodine, 2.36 mg BID days 22-24 (5 doses)	Fluoxetine, 20 mg QD for 24 days	1.36	1.24	1.22	1.31	0.89	1.06
16	CYP2D6	Dextromethorphan, 30 mg on Day 12	Fluoxetine, 20 mg SD Day 1, then 60mg QD days 2-14 (1 hour before Dextromethorphan)	-	27.21	4.94	13.80	-	0.51
8	CYP2D6	Desipramine HCL, 50 mg (3 hours after Fluoxetine)	Fluoxetine, 60 mg Day 1	1.63	2.25	1.56	2.39	0.96	1.06
9	CYP2D6	Desipramine HCL, 50 mg on Day 8 (3 hours after Fluoxetine)	Fluoxetine, 60 mg QD for 8 Days	2.54	7.43	2.09	5.27	0.82	0.71

D. The numbers differ for the prediction of the fluoxetine-desipramine DDIs between the fluoxetine and desipramine compound summary files. The Applicant is requested to clarify this issue.

RESPONSE: This has been amended and an updated V19 compound file summary has been provided.

EMA Issue 30

For quinidine, the model is under-predicting the DDI studies for quinidine as a perpetrator. This is especially true for the metoprolol scenario only including CYP2D6 extensive metabolizers (EM) (Leemann et al 1993). Furthermore, there are no CYP2D6 only substrates included in the presented DDI scenarios which is another limitation. All CYP2D6 are also CYP3A4 substrates which quinidine also inhibits, and this makes it difficult to conclude whether the CYP2D6 part of the model is inappropriate. The Applicant should address these or otherwise the quinidine model will not be viewed as a relevant compound file for supporting the qualification of CoU1 for CYP2D6.

RESPONSE: Whilst there is a tendency for underprediction of DDIs with quinidine, the P/O AUC ratios are still reasonable with values of 0.94 and 0.61 for metoprolol and 0.80 and 0.88 for dextromethorphan. However, to determine whether CYP2D6 inhibition is correctly predicted by quinidine, we have added propranolol to the analysis. CYP2D6 is the main enzyme involved in the metabolism of propranolol (55%) – there is no CYP3A4 component. P/O AUC ratios for propranolol using 3 different dosage regimens of quinidine are 1.04, 1.16 and 1.29. Thus, it appears that the CYP2D6 inhibitory component of quinidine is appropriate. The results are shown below and can be found in the excel sheet called *"CYP2D6-ClinicalData_TrialDesignSetting-02"*.

Number	CYP	Substrate Dose	Inhibitor Dose	Observed		Predicted		Predicted /Observed	
				Cmax Ratio	AUC Ratio	Cmax Ratio	AUC Ratio	Cmax Ratio	AUC Ratio
28	CYP2D6	Propranolol 10 mg SD	Quinidine 100 mg 12h before and simultaneously		2.76		2.86		1.04
29	CYP2D6	Propranolol 20 mg SD	Quinidine 200mg		2.66		3.09		1.16
30	CYP2D6	Propranolol 80mg SD	Quinidine 50mg		1.92		2.48		1.29
19	CYP2D6	Metoprolol Tartrate, 200 mg on Day 4 (2 hours after Quinidine)	Quinidine, 100 mg QD for 5 days	-	4.89	2.32	4.59	-	0.94

14	CYP2D6	Dextromethorphan, 30 mg SD	Quinidine, 50 mg (1 hour before dextromethorphan)	4.38	7.31	4.40	5.86	1.00	0.80
15	CYP2D6	Dextromethorphan, 30 mg SD	Quinidine, 50 mg (1 hour before dextromethorphan)	6.10	6.34	4.21	5.57	0.69	0.88
20	CYP2D6	Metoprolol Tartrate, 20mg IV infusion	Quinidine, 50 mg SD (12 hours before metoprolol)	-	2.43	1.01	1.48	-	0.61

EMA Issue 31

Several points are unclear regarding the model for cinacalcet:

A. *According to the validation of the no interaction data for the multiple dose scenario (Table 1 in the cinacalcet compound summary) and the interactions with dextromethorphan and desipramine for cinacalcet as a perpetrator (Table 6 in the cinacalcet compound summary) the model is not at all describing the observed data which is not acceptable. The Applicant should clarify if these issues are due to typos/errors in the compound summary for cinacalcet or if there are problems with the underlying model. The Applicant should update the model and/or compound summary file as applicable, to resolve the outlined issues.*

RESPONSE: The cinacalcet file is correct – the compound file summary has been updated to reflect that the DDIs are accurately predicted.

B. *For cinacalcet, it appears that all data are from fed conditions, whereas CoU1 specifically states that qualification is sought for the fasted conditions. The Applicant is asked to clarify this aspect and justify how data from fed conditions could be applicable to CoU1.*

RESPONSE: We have reviewed all the clinical studies to confirm the precise fasted/fed conditions of each clinical study. The compound file summary has been updated to reflect this. Only the DDI study with desipramine which was conducted in fasted conditions was used for the analysis.

C. *The only Ki listed in Table 7 is for CYP2C9 which is a suspected typo (should probably read CYP2D6) which the Applicant should correct.*

RESPONSE: The compound file summary has been updated to reflect that the Ki value should be listed for CYP2D6 not CYP2C9.

EMA Issue 32

For ritonavir, the validation of the multiple dose data without any interaction (Figure 1 in the ritonavir compound summary) there is a distinct peak at around 20 hours which is not captured by the model. Furthermore, it appears that the study (Greenblatt et al 2009) has a quite complex study design with respect to timing of all the doses. To better assess this aspect, the Applicant is asked to provide documentation to show how the study design for Greenblatt et al 2009 was implemented.

RESPONSE: As there is only one DDI study available for ritonavir, and cimetidine has now been introduced as a weak CYP2D6 inhibitor, we have decided to exclude ritonavir from the analysis.

EMA Issue 33 – COU1

The Applicant has not provided evidence that the SimCYP platform could potentially be capable of fulfilling CoU1 for CYP1A2, CYP2C8, CYP2C9 and CYP2C19 enzymes. The mentioned CYP enzymes do not have included examples of the prediction at all three levels of inhibition (i.e., there are no examples of weak, moderate and/or strong inhibition predictions). Therefore, the qualification of SimCYP platform in terms of CoU1 for the mentioned CYP enzymes do not seem feasible unless further adequate examples/evidence are provided.

RESPONSE: The DDI matrix has been extended to include weak, moderate, and strong inhibitors for CYP1A2, CYP2C8, CYP2C9 and CYP2C19. For each enzyme, this information can be found on the worksheet called "Substrate-Inhibitor Pairs" in the following excel files:

"CYP1A2-ClinicalData_TrialDesignSetting-02"

"*CYP2C8-ClinicalData_TrialDesignSetting-02"*

"*CYP2C9-ClinicalData_TrialDesignSetting-02"*

"*CYP2C19-ClinicalData_TrialDesignSetting-02"*

In addition, the information is summarised below:

Inhibitor	FDA	CDIS	ICH-M12
Cimetidine		Weak	
Ciprofloxacin		Strong	Moderate sensitive
Fluvoxamine	Strong index	Strong	Sensitive
Propranolol		Moderate	Moderate sensitive

CYP2C8 Inhibitor	FDA	CDIS
Gemfibrozil	strong	strong
Clopidogrel	moderate	moderate
Trimethoprim	weak	weak
Tucatinib	nothing on CYP2C8	weak

CYP2C9 Inhibitor	FDA	CDIS
Sulphaphenazole	NA	strong
Amiodarone	moderate	moderate
Fluconazole	moderate	moderate
Fluvoxamine	weak	weak

CYP2C19 Inhibitor	FDA	CDIS
Fluvoxamine	index	strong
Fluconazole	strong	strong
Fluoxetine	strong	strong
Ticlopidine	strong	strong
Voriconazole	moderate	moderate
Omeprazole	weak	moderate
Cimetidine	nothing on CYP2C19	weak

EMA Issue 34 – COU2

For inhibitors it is important that the pharmacokinetics of the inhibitor is well described under the conditions used in the clinical DDI studies. Please provide a Table with Cmax and AUC following single dose and multiple dose administration and indicate when steady-state had been reached for inhibitor and metabolite if applicable. Please present observed vs predicted PK values, indicate if PK of the inhibitor is time-dependent.

RESPONSE: For each of the inhibitors, dosage regimens that were used in the clinical studies have been indicated on a worksheet called "Sources of fm and Ki values" found in each of the enzyme analyses:

"CYP1A2-ClinicalData_TrialDesignSetting-02"

"*CYP2C8-ClinicalData_TrialDesignSetting-02"*

"*CYP2C9-ClinicalData_TrialDesignSetting-02"*

"*CYP2C19-ClinicalData_TrialDesignSetting-02"*

"*CYP2D6-ClinicalData_TrialDesignSetting-02"*

On that worksheet, the dosage regimens provided in the compound files summaries have also been indicated. In cases where dosage regimens used in clinical DDI studies have not been

included in the compound file summary, and data are available in publications, additional simulations have been run. With each enzyme folder, a sub-folder called “Inhibitor Profiles” contains the additional inhibitor PK information. For CYP3A4, as there are many inhibitors, a separate excel file rather than a worksheet was prepared and included in the CYP3A4 “Inhibitor profiles” folder.

EMA Issue 35 – COU2

How is CYP3A5 DDIs addressed? Differences in CYP3A4 inhibition potential may be observed in subjects with and without CYP3A5 activity. The fm values for CYP3A5 were not provided at all for all substrates. Furthermore, no information on CYP3A5 inhibition is provided or incorporated into the PBPK model. This may need to be indicated as a limitation of the PBPK model.

RESPONSE: We provide a reference indicating how CYP3A5 is addressed within the Simcyp Simulator (Cubitt *et al.,* 2011). When available, CYP3A5 metabolic data have been included for compounds including alprazolam, cyclosporin, midazolam and triazolam. Given that only 17% of the Caucasian population have CYP3A5, on average its relative contribution tends to be much smaller than that of CYP3A4. Furthermore, CYP3A5 expression in the liver and intestine is lower than that of CYP3A4 (103 versus 137 pmol/mg microsomal protein and 24.6 versus 66.2 nmol/intestine). Thus, fmCYP3A5 values for the aforementioned substrates are 4.2%, 8.4%, 8.5% and 7.1%, respectively.

In terms of inhibitory potential, if CYP3A5 data are available for inhibitors, these parameters have been included e.g., ketoconazole (competitive inhibition) and verapamil (MBI). Furthermore, in cases where it has been recognised that CYP3A5-mediated inhibition occurs, but data are not available (e.g., atazanavir), it has been assumed that the inhibitor is equipotent towards CYP3A5 and CYP3A4.

In a default Caucasian population (17% with CYP3A5), on average, the fmCYP3A5 of midazolam is about 8.54%. When co-administered with ketoconazole which inhibits both CYP3A4 (Ki=0.015 µM) and CYP3A5 (Ki=0.109 µM), the fmCYP3A5 increases to 12.07% despite being inhibited but not as much UGT1A4 whose contribution to the clearance increases from 3.35 to 35.28% (no inhibition).

Statistics	% contribution in absence of inhibitor(s)				
	CYP3A4 Liver	CYP3A5 Liver	UGT1A4 Liver	UGT1A4 Kidney	Renal

Mean	87.62	8.54	3.35	0.06	0.42
Statistics	**% contribution in presence of inhibitor(s)**				
	CYP3A4 Liver	**CYP3A5 Liver**	**UGT1A4 Liver**	**UGT1A4 Kidney**	**Renal**
Mean	49.29	12.07	35.28	0.41	2.94

References
Cubitt HE, Yeo KR, Howgate EM, Rostami-Hodjegan A, Barter ZE. Sources of interindividual variability in IVIVE of clearance: an investigation into the prediction of benzodiazepine clearance using a mechanistic population-based pharmacokinetic model. Xenobiotica. 2011 Aug;41(8):623-38. doi: 10.3109/00498254.2011.560294. Epub 2011 Mar 24. PMID: 21434772.

EMA Issue 36 – COU2

Please, provide separate figures for competitive inhibition and MBI inhibitors for individual CYP enzymes. If an inhibitor or inhibitor + metabolite is both a reversible inhibitor and MBI, it would be appreciated if it could be shown what the contribution of each inhibition pathway is. X-axis and Y-axis seem inverted for plots predicted AUC/Cmax ratio v.s. observed AUC/Cmax ratio for individual CYP enzymes.

RESPONSE: Separate figures have been provided for competitive inhibition and MBI and for each enzyme and for each COU. The figures are presented in word as summary documents in each of the enzyme folders.

EMA Issue 37 – COU2

There is limited DDI information of reversible inhibitors with weak, moderate and sensitive substrates for several enzymes. Please discuss the possibility for inclusion of more substrates and reversible inhibitors for CYP1A2, CYP2C8, CYP2C19 with a good representation of mild, moderate and sensitive/strong? In addition, how is a distinction made between CYP3A4 and 3A5 in the model?

RESPONSE: The DDI matrix has been extended to include weak, moderate and strong inhibitors for CYP1A2, CYP2C8 and CYP2C19. For each enzyme, this information can be found on the worksheet called "Substrate-Inhibitor Pairs" in the following excel files:

"CYP1A2-ClinicalData_TrialDesignSetting-02"

"*CYP2C8-ClinicalData_TrialDesignSetting-02"*

"*CYP2C19-ClinicalData_TrialDesignSetting-02"*

In addition, the information is summarised below:

CYP1A2 Inhibitor	**FDA**	**CDIS**	**ICH-M12**
Cimetidine		Weak	
Ciprofloxacin		Strong	Moderate sensitive

Fluvoxamine	Strong index	Strong	Sensitive
Propranolol		Moderate	Moderate sensitive

CYP2C8 Inhibitor	**FDA**	**CDIS**
Gemfibrozil	strong	strong
Clopidogrel	moderate	moderate
Trimethoprim	weak	weak
Tucatinib	nothing on CYP2C8	weak

CYP2C19 Inhibitor	**FDA**	**CDIS**
Fluvoxamine	index	strong
Fluconazole	strong	strong
Fluoxetine	strong	strong
Ticlopidine	strong	strong
Voriconazole	moderate	moderate
Omeprazole	weak	moderate
Cimetidine	nothing on CYP2C19	weak

We have discussed in detail how CYP3A5 is handled in the response to Issue 35.

SPECIFIC ISSUES ON CYP2C8 FOR COU3

EMA Issue 38

No sensitive substrates (fm > 80%) were included. It is thus unclear, which substrate should be used in a clinical study as a sensitive substrate according to CoU (2 and 3).

According to the draft ICH-M12 Guideline, a sensitive substrate is a drug that demonstrates an increase in AUC of ≥5-fold with a strong index inhibitor of a given metabolic pathway in clinical DDI studies. Repaglinide is the only CYP2C8 substrate (also CYP3A4 and OATP1B1) where such an increase is obtained in a clinical study with gemfibrozil. It should be noted that gemfibrozil also inhibits OATP1B1 and OAT3 (acknowledged in Table 13 of ICH-M12 Guideline) and therefore, inhibition is not exclusive to CYP2C8. Despite the fmCYP2C8 of 66%, repaglinide remains one of the most sensitive index substrates of CYP2C8 and therefore, is typically used in clinical studies. Of the remaining CYP2C8 substrates, montelukast appears to be the most sensitive substrate in that AUC ratios ranged from 3- to 4-fold when co-administered with clopidogrel or gemfibrozil.

Substrate	**FDA**	**CDIS**	**ICH-M12**	**fm%**
Repaglinide	sensitive	sensitive	Sensitive index	66.1
Rosiglitazone	moderate sensitive	moderate sensitive		56.1

Pioglitazone	moderate sensitive	moderate sensitive	64.2
Montelukast	moderate sensitive	moderate sensitive	77.65
Tucatinib	Nothing on CYP2C8	moderate sensitive	71.73

EMA Issue 39

Gemfibrozil and metabolite are inhibitors of CYP2C8 but also of OATP1B1/3. Repaglinide is a substrate for both CYP2C8 and OATP1B1. Therefore, this is considered a complex interaction which is out of scope of this qualification. Please comment.

RESPONSE: We have presented the CYP2C8 analysis with and without the repaglinide/gemfibrozil DDIs. Even in the latter case, there remain 17 DDI pairs in the CYP2C8 analysis.

EMA Issue 40

Dose – and time variant studies (Honkalammi et al., Tornio et al,) between gemfibrozil and repaglinide indicate that Mechanism based-inhibition of CYP2C8 is probably underestimated or kdeg of CYP2C8 is not correct. Inclusion of more CYP2C8 MBI inhibitors and other CYP2C8 substrates may help elucidate this issue. Please discuss.

RESPONSE: As discussed in Issue 38, we have now extended the CYP2C8 analysis to include other substrates. There are now 8 DDIs involving gemfibrozil (600 mg BID) and 4 substrates. Whilst there is a tendency to underpredict (AFE=0.84), 6 of the predicted AUC ratios are within 1.25-fold of observed data.

Study	**Substrate Dose**	**Observed**		**Predicted**		**Predicted /Observed**	
		Cmax Ratio	**AUC Ratio**	**Cmax Ratio**	**AUC Ratio**	**Cmax Ratio**	**AUC Ratio**
Karonen_2010	Montelukast, Oral 10 mg on day 3 (1 hr after Gemfibrozil)	1.53	4.54	2.09	3.96	1.36	0.87
Karonen_2012	Montelukast, Oral 10 mg on day 3 (1 hr after Gemfibrozil)	1.46	4.28	1.96	3.74	1.34	0.87

Deng_2005 (in Chinese)	Pioglitazone, Oral 30 mg on day 3 at 8 AM	1.11	3.38	1.06	1.62	0.96	0.48
Jaakkola_2005	Pioglitazone, Oral 15 mg on day 3 at 9 AM	1.06	3.22	1.17	2.69	1.11	0.83
Aquillante_2012	Pioglitazone, Oral 15 mg on day 3 at 9 AM	1.09	3.12	1.17	2.69	1.08	0.86
Aquillante_2012	Pioglitazone, Oral 15 mg on day 3 at 9 AM	1.15	4.66	1.23	3.25	1.08	0.70
Niemi3	Rosiglitazone, Oral 4 mg at 9 AM on day 3	1.22	2.29	1.17	2.38	0.96	1.04
Topletz_Erickson_2022	Tucatinib, Oral 300 mg on day 5, 8AM	1.62	3.04	1.69	3.26	1.05	1.07

SPECIFIC ISSUES ON CYP2C9 FOR COU3

EMA Issue 41

Amiodarone is the only MBI used. No independent model verification with amiodarone was performed - "developed as research files" according to the Applicant. This implies that predicted and observed values are based on the same studies. If so, the validity of the model for CYP2C9 cannot be assumed. The Applicant is invited to discuss.

RESPONSE: The inactivation parameters were based on *in vitro* data (Rougee *et al.,* 2017; McDonald *et al.,* 2012, 2015). Four clinical study designs involving S-warfarin (n=2) and oral and IV phenytoin (n=2) were included in the updated CYP2C9 analysis. A new and updated compound summary for Amiodarone is supplied with further details on the compound

development and verification (*00 V19_Amiodarone summary_FOfile_new.pdf*). No DDI study was used to optimise inhibitory parameters for the parent and metabolite files for CYP2C9.

References

Heimark, L.D. et al. The mechanism of the interaction between amiodarone and warfarin in humans. Clinical Pharmacology & Therapeutics 51, 398-407 (1992).

Nolan Jr, P.E., Marcus, F.I., Hoyer, G.L., Bliss, M. & Gear, K. Pharmacokinetic interaction between intravenous phenytoin and amiodarone in healthy volunteers. *Clinical Pharmacology & Therapeutics* **46**, 43-9 (1989).

McDonald, M.G., Au, N.T., Wittkowsky, A.K., Rettie, A. E. Warfarin-Amiodarone drug-drug interactions determinations of [I]u/KI,u for Amiodarone and its plasma metabolites. *Clin Pharm and Ther.* **91**(4): 709-717 (2012).

McDonald, M.G., et al.,.P450-Based drug-drug interactions of amiodarone and its metabolites: Diversity of inhibitory mechanisms. *Drug Metab Dispos* **43**:1661-1669 (2015).

O'Reilly, R.A., Trager, W.F., Rettie, A.E. & Goulart, D.A. Interaction of amiodarone with racemic warfarin and its separated enantiomorphs in humans. *Clinical Pharmacology & Therapeutics* **42**, 290-4 (1987).

Rougee, L. R. A., et al. The Impact of the Hepatocyte-to-Plasma pH Gradient on the Prediction of Hepatic Clearance and Drug-Drug Interactions for CYP2C9 and CYP3A4 Substrates. *Drug Metab Dispos* **45**(9): 1008-1018 (2017).

SPECIFIC ISSUES ON CYP2D6 FOR COU3

EMA Issue 42

The only inhibitor used was paroxetine, which is also an MBI of CYP3A4. However, no clinical study with a CYP3A4 substrate has apparently been included. How is ensured that CYP3A4 contribution is correctly captured by the model?

RESPONSE: We used the UOW DDI database to search for clinical DDIs involving paroxetine. Of 76 DDIs, only 1 study used a CYP3A4 substrate, ranolazine which is considered to be moderately sensitive. The search results can be found in the subfolder “Issue 42” found within the folder called “Response Folder to Submit”.

SPECIFIC ISSUES ON CYP2C19 FOR COU3

EMA Issue 43

The in vitro MBI data for (nor)fluoxetine are from Lutz et al. study. How are R- and S-isomer considered, are the data averaged?

RESPONSE: For fluoxetine, the CYP2C19 K_{app} and k_{inact} values were averaged from the R and S enantiomers (Lutz *et al.,* 2013). For norfluoxetine, the CYP2C19 k_{inact} values were the mean

of the R and S enantiomers (Lutz *et al.,* 2013); K_{app} was optimised from Vlase *et al.* (2010) using the clinical DDI study with omeprazole. Thus, the latter study was excluded from the CYP2C19 analysis.

References

Lutz JD, VandenBrink BM, Babu KN, Nelson WL, Kunze KL, Isoherranen N. Stereoselective inhibition of CYP2C19 and CYP3A4 by fluoxetine and its metabolite: implications for risk assessment of multiple time-dependent inhibitor systems. Drug Metab Dispos. 2013 Dec;41(12):2056-65. doi: 10.1124/dmd.113.052639. Epub 2013 Jun 19. PMID: 23785064; PMCID: PMC3834134.

SPECIFIC ISSUES ON CYP3A4 FOR COU3

EMA Issue 44

The MBI parameters for atazanavir are from a CDER (FDA) document containing no information on methodology. It is thus unclear, in which system / under which conditions the in vitro data were generated.

RESPONSE: These are the only data that are available in the public domain. The number of atazanavir studies is small (n=3) and can be excluded from the analysis if there is an issue with the lack of information on the methodology.

EMA Issue 45

The methodology for studying CYP3A5 inhibition by erythromycin from McConn et al. 2004 could not be evaluated as the full text article was not submitted. The reference should be provided as full text.

RESPONSE: The full reference has now been provided.

EMA Issue 46

The MBI data for ritonavir are from Kirby et al. 2011, the study was apparently conducted in HLM but without methodology description the methodology could not be assessed. Kapp provided by the Applicant (0.18 µM) differs from that by Kirby et al. (0.25 µM). The Applicant should explain. The MBI data for CYP3A5 are assumed to be the same as for CYP3A4. This should be justified.

RESPONSE: The reason for the difference is that the reported K_{app} was not corrected for NSMB. The inactivation parameters were determined at a protein concentration of 0.25 mg/mL which

translates to a fumic value of 0.71. When this is applied to the K_{app}, the resultant value is 0.18 µM.

In terms of inhibitory potential, if CYP3A5 data are available for inhibitors, these parameters are typically included. However, often these data are not available, but in cases where it has been recognised that CYP3A5-mediated inhibition occurs it is assumed that the inhibitor is equipotent towards CYP3A4.

References

Kirby BJ, Collier AC, Kharasch ED, Whittington D, Thummel KE, Unadkat JD. Complex drug interactions of HIV protease inhibitors 1: inactivation, induction, and inhibition of cytochrome P450 3A by ritonavir or nelfinavir. Drug Metab Dispos. 2011 Jun;39(6):1070-8. doi: 10.1124/dmd.110.037523. Epub 2011 Mar 15. PMID: 21406602; PMCID: PMC3100903.

EMA Issue 47

For simvastatin, fm of 88.7% is reported to be predicted using HLM data from multiple sources, but no references are specified. The references should be provided as full texts.

RESPONSE: A folder *"Issue 47– simvastatin references"* with all references has now been provided in the "Responses Folder to submit".

EMA Issue 48

For nifedipine, fm of 99.8% (as in the Briefing Document) was defined by Vmax and Km values that were obtained by a meta-analysis of published values (n=8 and 3 studies, respectively), but the respective references are not specified. The references should be provided as full texts.

RESPONSE: A folder *"Issue 48 – nifedipine references"* with all references and an excel sheet indicating the sources (see below) has now been provided in the "Responses Folder to submit".

For nifedipine, fm of 99.8% (as in the Briefing Document) was defined by Vmax and Km values that were obtained by a meta-analysis of published values (n=8 and 3 studies, respectively), but the respective references are not specified. The references should be provided as full texts.	
CYP3A4 references	**PMID**
Emoto et al 2006 Xenobiotica 36 219-233	https://pubmed.ncbi.nlm.nih.gov/16702113/
Emoto et al 2007 Xenobiotica 37 (9) 986-999	https://pubmed.ncbi.nlm.nih.gov/17896325/
Niwa et al 2003 Xenobiotica 33 (7) 717-729	https://pubmed.ncbi.nlm.nih.gov/12893521/
Galetin et al 2003 DMD 31:1108–1116	https://pubmed.ncbi.nlm.nih.gov/12920166/
Ingelman-Sundberg et al 1996 Biochem Biophys Res Com 221 318-322	https://pubmed.ncbi.nlm.nih.gov/8619853/
Carr et al 2006 DMD 34:1703–1712	https://pubmed.ncbi.nlm.nih.gov/16815964/
Williams et al 2002 DMD 30:883–891	https://pubmed.ncbi.nlm.nih.gov/12124305/
Patki et al 2003 DMD 31:938–944	https://pubmed.ncbi.nlm.nih.gov/12814972/
Shaw et al 1997 Arch Biochem Biophys 348, (1) p. 107–115	https://pubmed.ncbi.nlm.nih.gov/9390180/
CYP3A5 references	
Emoto et al 2006 Xenobiotica 36 219-233	https://pubmed.ncbi.nlm.nih.gov/16702113/
Patki et al 2003 DMD 31:938–944	https://pubmed.ncbi.nlm.nih.gov/12814972/
Williams et al 2002 DMD 30:883–891	https://pubmed.ncbi.nlm.nih.gov/12124305/

EMA Issue 49

With the exception of CYP3A4/5 a limited number of inhibitors is included. Please discuss the possibility of including additional inhibitors (and substrates) for the different CYPs.

RESPONSE: We have now added 2 extra MBI compounds – clopidogrel (CYP2C8) and mirabegron (CYP2D6). In addition, we have been able to simulate more DDIs with existing MBI compounds because of the increased number of substrates.

Enzyme	CI	MBI	ALL
CYP1A2	42	0	42
CYP2C8	7	10	17
CYP2C9	25	3	28
CYP2C19	15	13	28
CYP2D6	32	14	46
CYP3A4/5	66	51	117

Appendix

Simulation script 1

```
## ============================================================================
## Average Fold Error (AFE), Average Absolute Fold Error (AAFE) code.
##
## Interaction studies are comparative trials where the individual
## values of a PK parameter (usually some form of AUC) for subjects in
## a drug treatment group are compared to those in a test group where
## the same drug and another (interacting) one are administered.
## We can simulate such trials to replace them. The issue is "how
## good" is the model at simulating real life, and how do we measure
## that?
## The issue is obviously embedded in a population variability framework.
```

```
##
## ============================================================================

## Most interaction clinical trials are one group of subjects, each
## getting the reference treatment and then the test (two drugs)
## treatment (I. Gardner personnal communication)

## ============================================================================
## Simulate a clinical DDI trial:
## Simulate AUCs from a reference (single drug treatment) group.
## There is a population level, a subject level and an occasion level.

## Make a function
Simulate_trial = function(N.subj, AUC.pop.GM.1, AUC.pop.GSD.1,
                          AUC.occas.GSD.1, AUC.occas.GSD.2, Delta) {
  ##
  ## Subject level AUC after single drug administration
  AUC.subj.1 = rlnorm(N.subj, meanlog=log(AUC.pop.GM.1),
                      sdlog=log(AUC.pop.GSD.1))
  ##
  ## Occasion level AUC after single drug administration
  AUC.occas.1 = rep(0, N.subj)
  for (i in 1:N.subj)
    AUC.occas.1[i] = rlnorm(1, meanlog=log(AUC.subj.1[i]),
                            sdlog=log(AUC.occas.GSD.1))
  ##
  ## Subject level AUC after two drugs administration
  AUC.subj.2 = AUC.subj.1 * Delta # Delta could be randomized...
  ##
  ## Occasion level AUC after two drugs administration
  AUC.occas.2 = rep(0, N.subj)
  for (i in 1:N.subj)
    AUC.occas.2[i] = rlnorm(1, meanlog=log(AUC.subj.2[i]),
                            sdlog=log(AUC.occas.GSD.2))
  ##
  AUC_ratios = AUC.occas.2 / AUC.occas.1
  return(list(AUC.ref    = AUC.occas.1,
              AUC.test   = AUC.occas.2,
              AUC.ratios = AUC_ratios))
}
## try it:
Simulated.data = Simulate_trial(N.subj=20, AUC.pop.GM.1=50, AUC.pop.GSD.1=2,
                                AUC.occas.GSD.1=1.3, AUC.occas.GSD.2=1.3,
                                Delta=1.2)
hist(log(Simulated.data$AUC.ratios))


## ============================================================================
## Simulate studies for many different compounds (one study per compound):

N.comp = 10000

## Simulate data
N.subj = 20
AUC.pop.GM.1 = Delta = rep(1, N.comp)
Data = matrix(0, N.subj, N.comp)
for (i in 1:N.comp) {
  AUC.pop.GM.1[i] = runif(1, 10, 500)
  Delta[i] = runif(1, 1, 10)
  Data[,i] = Simulate_trial(N.subj, AUC.pop.GM.1[i], AUC.pop.GSD.1=2,
                            AUC.occas.GSD.1=1.3, AUC.occas.GSD.2=1.3,
                            Delta[i])$AUC.ratios
}
data.means = apply(Data, MAR=2, mean)
data.SDs   = apply(Data, MAR=2, sd)
## plot(data.means)

## Simulate predictions
```

```
N.subj = 200 # Note that it makes sense to simulate a large trial here.
AUC.pop.GM.pred = Delta.pred = rep(1, N.comp)
Predictions = matrix(0, N.subj, N.comp)
for (i in 1:N.comp) {
  AUC.pop.GM.pred[i] = rnorm(1, AUC.pop.GM.1[i], 1)
  Delta.pred[i] = rnorm(1, Delta[i], 0.1)
  Predictions[,i] = Simulate_trial(N.subj, AUC.pop.GM.pred[i],
                                   AUC.pop.GSD.1=2,
                                   AUC.occas.GSD.1=1.3, AUC.occas.GSD.2=1.3,
                                   Delta.pred[i])$AUC.ratios
}
pred.means = apply(Predictions, MAR=2, mean)
pred.SDs   = apply(Predictions, MAR=2, sd)

dev.new()
lims = c(0.5, 20)
plot(pred.means, data.means, las=1, log="xy", xlim=lims, ylim=lims, pch=".",
     xlab="Predicted AUC ratio", ylab="Observed AUC ratio")
x = c(0.1, 50)
lines(x, x,    lty=1, col="red")
for (fc in c(1.25)) {
  lines(x, x*fc, lty=2)
  lines(x, x/fc, lty=2)
}

## Compute AFE, which is just a summary of the above
FE = log(pred.means / data.means)
hist(FE, yaxt="n", 40, ylab="", main="")
abline(v=c(-log(1.25),log(1.25)), col="red", lwd=2)
exp(mean(FE)) # AFE

## Compute AAFE
AFE = abs(FE)
hist(AFE, yaxt="n", 40, ylab="", main="")
exp(mean(AFE)) # AAFE

## End.
```

Simulation script 2

```
## ========================================================================
## Average Fold Error (AFE), Average Absolute Fold Error (AAFE) code.
##
## Interaction studies are comparative trials where the individual
## values of a PK parameter (usually some form of AUC) for subjects in
## a drug treatment group are compared to those in a test group where
## the same drug and another (interacting) one are administered.
## We can simulate such trials to replace them. The issue is "how
## good" is the model at simulating real life, and how do we measure
## that?
## The issue is obviously embedded in a population variability framework.
##
## ========================================================================

IDtag = "_2" # version number

## Most interaction clinical trials are one group of subjects, each
## getting the reference treatment and then the test (two drugs)
## treatment (I. Gardner personnal communication)

## ========================================================================
## Simulate a clinical DDI trial:
## Simulate AUCs from a reference (single drug treatment) group.
## There is a population level, a subject level and an occasion level.

## Make a function
Simulate_trial = function(N.subj, AUC.pop.GM.1, AUC.pop.GSD.1,
```

```
                          AUC.occas.GSD.1, AUC.occas.GSD.2, Delta) {
  ##
  ## Subject level AUC after single drug administration
  AUC.subj.1 = rlnorm(N.subj, meanlog=log(AUC.pop.GM.1),
                      sdlog=log(AUC.pop.GSD.1))
  ##
  ## Occasion level AUC after single drug administration
  AUC.occas.1 = rep(0, N.subj)
  for (i in 1:N.subj)
    AUC.occas.1[i] = rlnorm(1, meanlog=log(AUC.subj.1[i]),
                            sdlog=log(AUC.occas.GSD.1))
  ##
  ## Subject level AUC after two drugs administration
  AUC.subj.2 = AUC.subj.1 * Delta # Delta could be randomized...
  ##
  ## Occasion level AUC after two drugs administration
  AUC.occas.2 = rep(0, N.subj)
  for (i in 1:N.subj)
    AUC.occas.2[i] = rlnorm(1, meanlog=log(AUC.subj.2[i]),
                            sdlog=log(AUC.occas.GSD.2))
  ##
  AUC_ratios = AUC.occas.2 / AUC.occas.1
  return(list(AUC.ref    = AUC.occas.1,
              AUC.test   = AUC.occas.2,
              AUC.ratios = AUC_ratios))
}
## try it:
Simulated.data = Simulate_trial(N.subj=20, AUC.pop.GM.1=50, AUC.pop.GSD.1=2,
                                AUC.occas.GSD.1=1.3, AUC.occas.GSD.2=1.3,
                                Delta=1.2)
hist(log(Simulated.data$AUC.ratios))


## ============================================================================
## Simulate studies for many different compounds (one study per compound):

N.comp = 10000

## Simulate data
N.subj = 20
AUC.pop.GM.1 = Delta = rep(1, N.comp)
Data = matrix(0, N.subj, N.comp)
for (i in 1:N.comp) {
  AUC.pop.GM.1[i] = runif(1, 10, 500)
  Delta[i] = runif(1, 1, 10)
  Data[,i] = Simulate_trial(N.subj, AUC.pop.GM.1[i], AUC.pop.GSD.1=2,
                            AUC.occas.GSD.1=1.3, AUC.occas.GSD.2=1.3,
                            Delta[i])$AUC.ratios
}
data.means = apply(Data, MAR=2, mean)
data.SDs   = apply(Data, MAR=2, sd)
## plot(data.means)

## Simulate predictions
N.subj = 200
AUC.pop.GM.pred = Delta.pred = rep(1, N.comp)
Predictions = matrix(0, N.subj, N.comp)
for (i in 1:N.comp) {
  AUC.pop.GM.pred[i] = rnorm(1, AUC.pop.GM.1[i], 1)
  Delta.pred[i] = rnorm(1, Delta[i], 0.1)
  Predictions[,i] = Simulate_trial(N.subj, AUC.pop.GM.pred[i],
                                   AUC.pop.GSD.1=2,
                                   AUC.occas.GSD.1=1.3, AUC.occas.GSD.2=1.3,
                                   Delta.pred[i])$AUC.ratios
}
pred.means = apply(Predictions, MAR=2, mean)
pred.SDs   = apply(Predictions, MAR=2, sd)
```

```
dev.new()
lims = c(0.5, 20)
plot(pred.means, data.means, las=1, log="xy", xlim=lims, ylim=lims, pch=".",
     xlab="Predicted AUC ratio", ylab="Observed AUC ratio")
x = c(0.1, 50)
lines(x, x,    lty=1, col="red")
for (fc in c(1.25)) {
  lines(x, x*fc, lty=2)
  lines(x, x/fc, lty=2)
}

## Compute AFE, which is just a summary of the above
FE = log(pred.means / data.means)
hist(FE, yaxt="n", 40, ylab="", main="")
abline(v=c(-log(1.25),log(1.25)), col="red", lwd=2)
exp(mean(FE)) # AFE

## Compute AAFE
AFE = abs(FE)
hist(AFE, yaxt="n", 40, ylab="", main="")
exp(mean(AFE)) # AAFE


## ============================================================================
## Simulate studies with different between subject variabilities:

N.comp = 10000

## Simulate data
N.subj = 20
Sigma  = 2   # pop GSD (BSV); (1, 2, 5)
sigma1 = 1.3 # inter-occasion GSD ref  group (IOV1) (1.1, 1.3, 2)
sigma2 = 1.3 # inter-occasion GSD test group (IOV2) (1.1, 1.3, 2)
AUC.pop.GM.1 = Delta = rep(1, N.comp)
Data = matrix(0, N.subj, N.comp)
for (i in 1:N.comp) {
  AUC.pop.GM.1[i] = runif(1, 10, 500)
  Delta[i] = runif(1, 1, 10)
  Data[,i] = Simulate_trial(N.subj, AUC.pop.GM.1[i],
                            AUC.pop.GSD.1=Sigma,
                            AUC.occas.GSD.1=sigma1,
                            AUC.occas.GSD.2=sigma2,
                            Delta[i])$AUC.ratios
}
data.means = apply(Data, MAR=2, mean)
data.SDs   = apply(Data, MAR=2, sd)
## plot(data.means)

## Simulate predictions
N.subj = 200
AUC.pop.GM.pred = Delta.pred = rep(1, N.comp)
Predictions = matrix(0, N.subj, N.comp)
for (i in 1:N.comp) {
  AUC.pop.GM.pred[i] = rnorm(1, AUC.pop.GM.1[i], 1)
  Delta.pred[i] = rnorm(1, Delta[i], 0.1)
  Predictions[,i] = Simulate_trial(N.subj, AUC.pop.GM.pred[i],
                                   AUC.pop.GSD.1=Sigma,
                                   AUC.occas.GSD.1=sigma1,
                                   AUC.occas.GSD.2=sigma2,
                                   Delta.pred[i])$AUC.ratios
}
pred.means = apply(Predictions, MAR=2, mean)
pred.SDs   = apply(Predictions, MAR=2, sd)

## plot
fname = paste0("Observed vs predicted AUC ratio, BSV=", Sigma,
               " IOV1=", sigma1, " IOV2=", sigma2, ".pdf")
pdf(fname)
```

```
lims = c(0.5, 20)
plot(pred.means, data.means, las=1, log="xy", xlim=lims, ylim=lims, pch=".",
     xlab="Predicted AUC ratio", ylab="Observed AUC ratio")
x = c(0.1, 50)
lines(x, x,    lty=1, col="red")
for (fc in c(1.25)) {
  lines(x, x*fc, lty=2)
  lines(x, x/fc, lty=2)
}
ltext = c(paste0("BSV  = ",  Sigma),
          paste0("IOV1 = ", sigma1),
          paste0("IOV2 = ", sigma2))
legend(0.5, 20, ltext, bty="n")
dev.off()


## ============================================================================
## Simulate the effect of bias in variability estimates:

N.comp = 10000

## Simulate data
N.subj = 20
Sigma  = 2   # pop GSD (BSV);
sigma1 = 1.3 # inter-occasion GSD ref  group (IOV1)
sigma2 = 2 # inter-occasion GSD test group (IOV2)
Bias.S  = 1
Bias.s1 = 1
Bias.s2 = 0.6
AUC.pop.GM.1 = Delta = rep(1, N.comp)
Data = matrix(0, N.subj, N.comp)
for (i in 1:N.comp) {
  AUC.pop.GM.1[i] = runif(1, 10, 500)
  Delta[i] = runif(1, 1, 10)
  Data[,i] = Simulate_trial(N.subj, AUC.pop.GM.1[i],
                            AUC.pop.GSD.1=Sigma,
                            AUC.occas.GSD.1=sigma1,
                            AUC.occas.GSD.2=sigma2,
                            Delta[i])$AUC.ratios
}
data.means = apply(Data, MAR=2, mean)
data.SDs   = apply(Data, MAR=2, sd)
## plot(data.means)

## Simulate predictions
N.subj = 200
AUC.pop.GM.pred = Delta.pred = rep(1, N.comp)
Predictions = matrix(0, N.subj, N.comp)
for (i in 1:N.comp) {
  AUC.pop.GM.pred[i] = rnorm(1, AUC.pop.GM.1[i], 1)
  Delta.pred[i] = rnorm(1, Delta[i], 0.1)
  Predictions[,i] = Simulate_trial(N.subj, AUC.pop.GM.pred[i],
                                   AUC.pop.GSD.1=Sigma*Bias.S,
                                   AUC.occas.GSD.1=sigma1*Bias.s1,
                                   AUC.occas.GSD.2=sigma2*Bias.s2,
                                   Delta.pred[i])$AUC.ratios
}
pred.means = apply(Predictions, MAR=2, mean)
pred.SDs   = apply(Predictions, MAR=2, sd)

## plot
fname = paste0("Observed vs predicted AUC ratio, Bias.S=",
               format(Bias.S, nsmall=1),
               " Bias.s1=", format(Bias.s1, nsmall=1),
               " Bias.s2=", format(Bias.s2, nsmall=1), ".pdf")
pdf(fname)
lims = c(0.5, 20)
plot(pred.means, data.means, las=1, log="xy", xlim=lims, ylim=lims, pch=".",
```

```
     xlab="Predicted AUC ratio", ylab="Observed AUC ratio")
x = c(0.1, 50)
lines(x, x,    lty=1, col="red")
for (fc in c(1.25)) {
  lines(x, x*fc, lty=2)
  lines(x, x/fc, lty=2)
}
ltext = c(paste0("BSV  = ", format(Sigma,  nsmall=1), "; Bias = ", Bias.S),
          paste0("IOV1 = ", format(sigma1, nsmall=1), "; Bias = ", Bias.s1),
          paste0("IOV2 = ", format(sigma2, nsmall=1), "; Bias = ", Bias.s2))
legend(0.5, 20, ltext, bty="n")
dev.off()

## End.
```

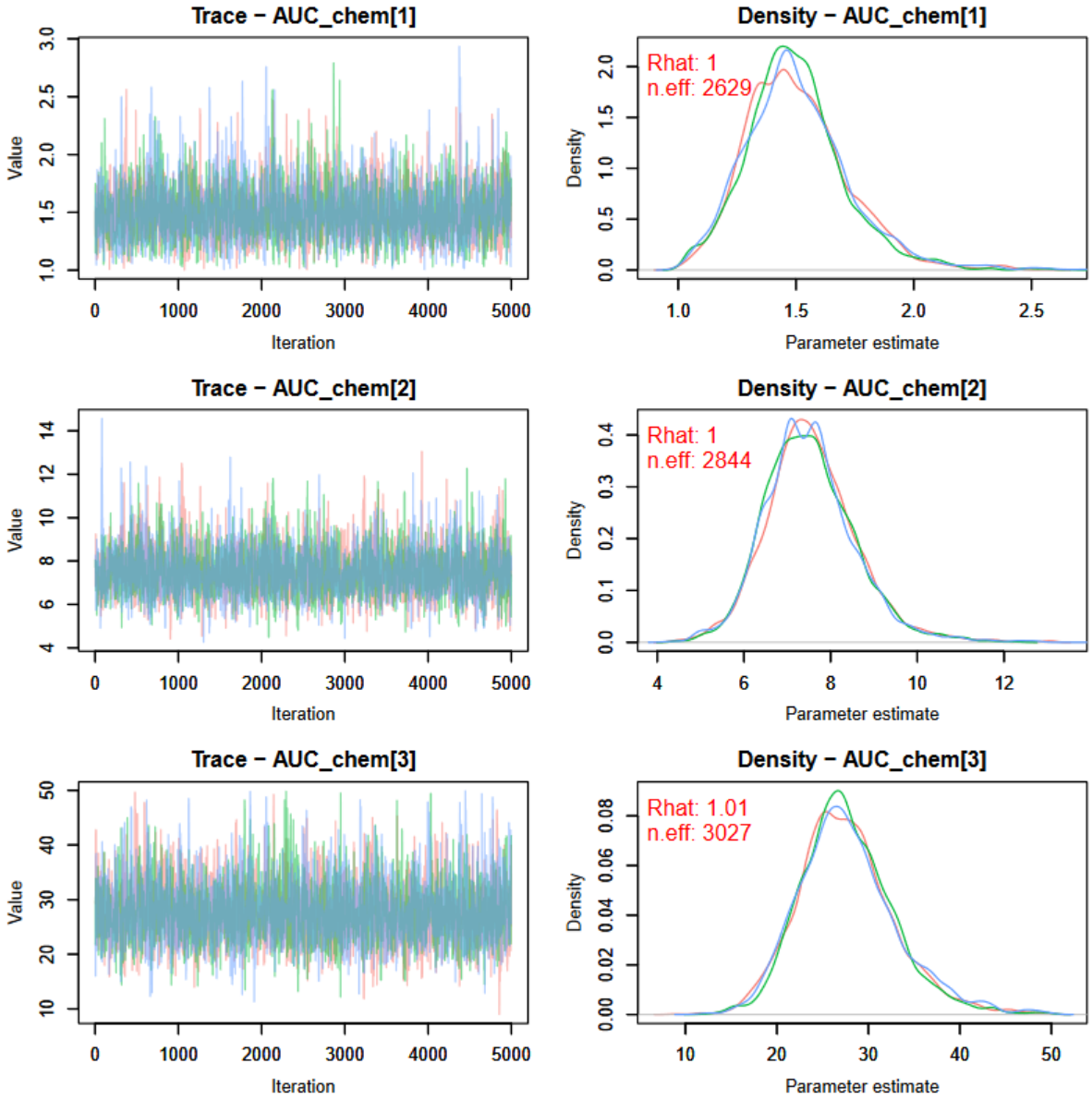
Inference script 1

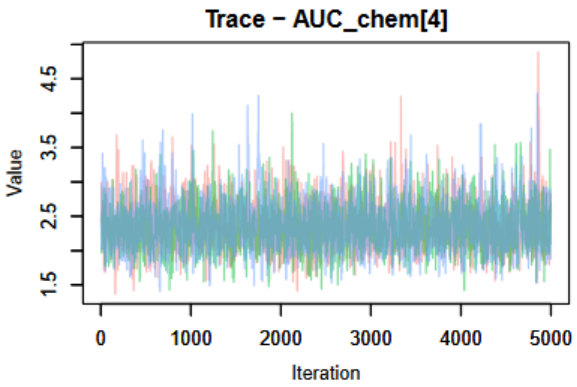
```
## Meta-analysis of theophylline-ciprofloxacin and others data to
## estimate inter-study variability
## v1: do not use individual data, skip them
## v2: improve variance priors
## v3: use individual data; one between subject variability (fixed effect)

## read the data
DDI.data = read.csv("DDI.csv")

chems = unique(DDI.data$Chems)

## nice plot
## pdf("data plot.pdf")
## places = c(11.5, 15.5, 17.5, 31.5, 33.5)
## color-code studies
## studies = unique(DDI.data$Study)
## cols = rainbow(length(studies))
## plot(DDI.data$AUC_ratio, log="y", xaxt="n", col=cols[DDI.data$Study],
##      xlab="Interactions", ylab="AUC ratio", cex.lab=1.3, pch=16)
## abline(v=places)
## mtext(chems, side=1, at=c(11, places[2]+places[1], places[3]+places[2],
##                           places[4]+places[3], places[5]+places[4],
##                           43.5 + places[5])/2, cex=0.8)
## dev.off()

## prepare for MCMC
library(coda)
library(MCMCvis)
library(corrplot)
library(nimble)

## ------------------------------------------------------------------------
## Hierarchical core Nimble (BUGS) code
myNimbleCode = nimbleCode({ ## BUGS (extended) code
  ##
  Var_subjs ~ T(dnorm(0, 0.25), 0, 1) # var in log space, rather vague
  Var_inter_studies ~ T(dnorm(0, 0.25), 0, 1)
  for (i in 1:N_chems) {
    AUC_chem[i] ~ dunif(1, 50)
    for (j in 1:N_studies[i]) {
      Var_studies[i,j]    <- Var_subjs / N_subjs[i,j] + Var_inter_studies
      AUC_chem_study[i,j] ~  dlnorm(meanlog = log(AUC_chem[i]),
                                    varlog  = Var_studies[i,j])
      for (k in 1:(N_subjs[i,j] * has.data[i,j])) {
        AUC_chem_study_subj[i,j,k] ~ dlnorm(meanlog=log(AUC_chem_study[i,j]),
                                            varlog =Var_subjs)
      }
    }
  }
```

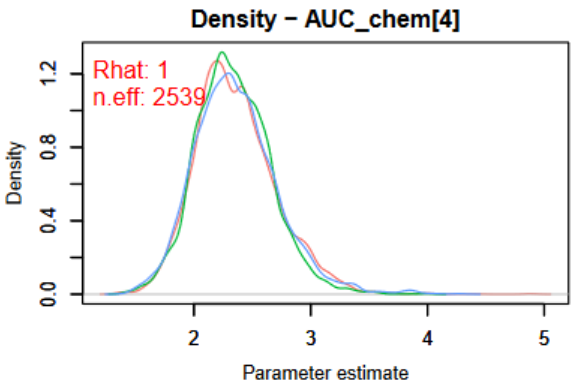
```
}) # End myNimbleCode

N_chems   = length(chems)
N_studies = vector() # number of studies per chemical
for (i in 1:N_chems) {
  N_studies[i] = max(DDI.data$Study[which(DDI.data$Chem == chems[i])])
}

## matrix of number of subjects per chemical and study
N_subjs = matrix(0, N_chems, max(N_studies))
for (i in 1:N_chems) {
  for (j in 1:N_studies[i]) {
    N_subjs[i,j] = DDI.data$N[which((DDI.data$Chem == chems[i]) &
                                    (DDI.data$Study == j))][1]
  }
}

## matrix of mean AUC ratio data per chemical and per study
AUC_chem_study = matrix(NA, N_chems, max(N_studies))
for (i in 1:N_chems) {
  for (j in 1:N_studies[i]) {
    my.index = which((DDI.data$Chem == chems[i]) & (DDI.data$Study == j))[1]
    if (is.na(DDI.data$Subject[my.index])) {
      AUC_chem_study[i,j] = DDI.data$AUC_ratio[my.index]
    }
  }
}

## indicator variable for studies with individual data
has.data = is.na(AUC_chem_study) & (N_subjs > 0)

## matrix the AUC ratios data per chemical, per study, and per subject,
AUC_chem_study_subj = array(NA,
                            dim=c(N_chems, max(N_studies),
                                  max(DDI.data$Subject, na.rm=T)))
for (i in 1:N_chems) {
  for (j in 1:N_studies[i]) {
    my.index = which((DDI.data$Chem == chems[i]) & (DDI.data$Study == j))
    l.index  = length(my.index)
    if (l.index > 1) {
      AUC_chem_study_subj[i,j,1:l.index] = DDI.data$AUC_ratio[my.index]
    }
  }
}

constants = list(N_chems   = N_chems,
                 N_studies = N_studies,
                 N_subjs   = N_subjs,
                 has.data  = has.data)

data  = list(AUC_chem_study      = AUC_chem_study,
             AUC_chem_study_subj = AUC_chem_study_subj)

inits = list()

Rmodel = nimbleModel(myNimbleCode, constants, data, inits, calculate=F)

Node.names = Rmodel$getNodeNames(includeData=T)
Rmodel$simulate(nodes = Node.names)
res = values(Rmodel, Node.names)
names(res) = Node.names
res

conf = configureMCMC(Rmodel)

Rmcmc  = buildMCMC(conf)

Cmodel = compileNimble(Rmodel, showCompilerOutput=F)
```

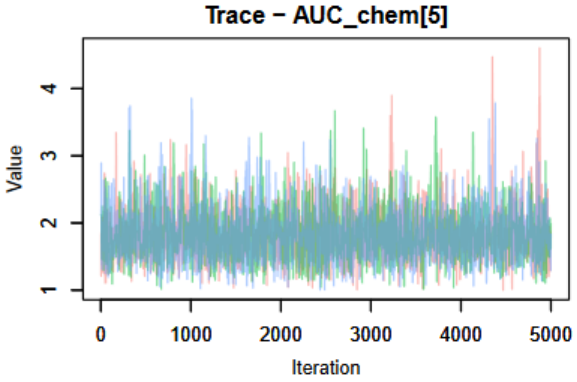
```
Cmcmc  = compileNimble(Rmcmc, project=Rmodel)

N.iter = 10000
mysamples = runMCMC(Cmcmc, niter=N.iter, nburnin=5000, nchains=3, setSeed=1)
str(mysamples)

mcmc.sum = MCMCsummary(object = mysamples, round = 5)
mcmc.sum
write.table(mcmc.sum, file="mcmc.sim_v3.txt", quote=F, sep="\t")

fname = "traces_v3.pdf"
MCMCtrace(object=mysamples, ISB=F,
          pdf=T, open_pdf=F,
          filename=fname,
          iter=5000,
          ind=TRUE, Rhat=TRUE, n.eff=TRUE)
dev.off()

End.
```

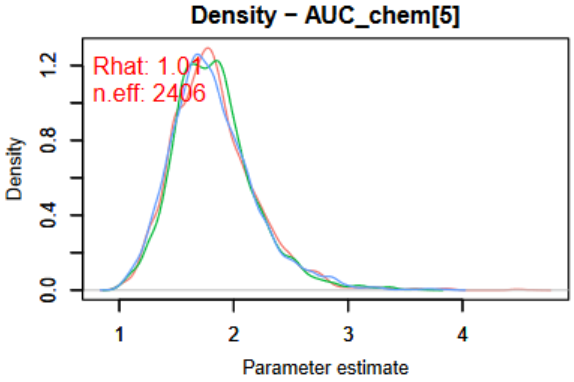
MCMC posterior plots

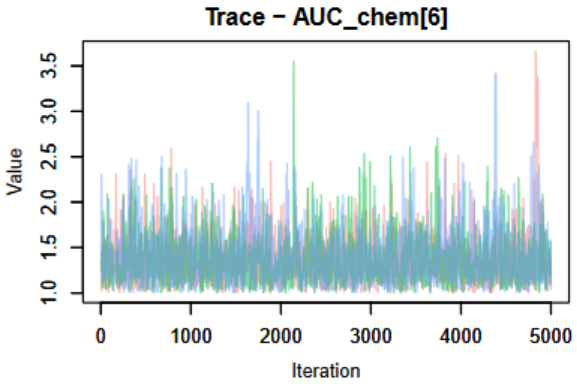


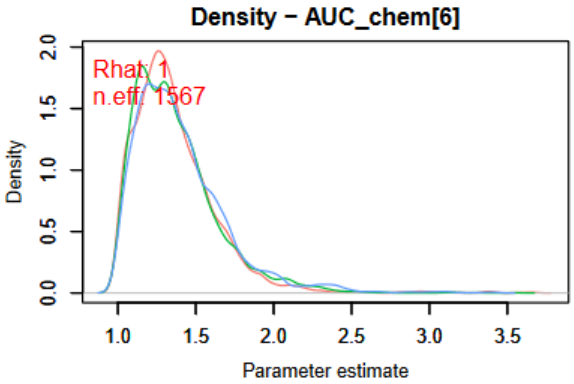

Trace – AUC_chem[4]
Value
Iteration

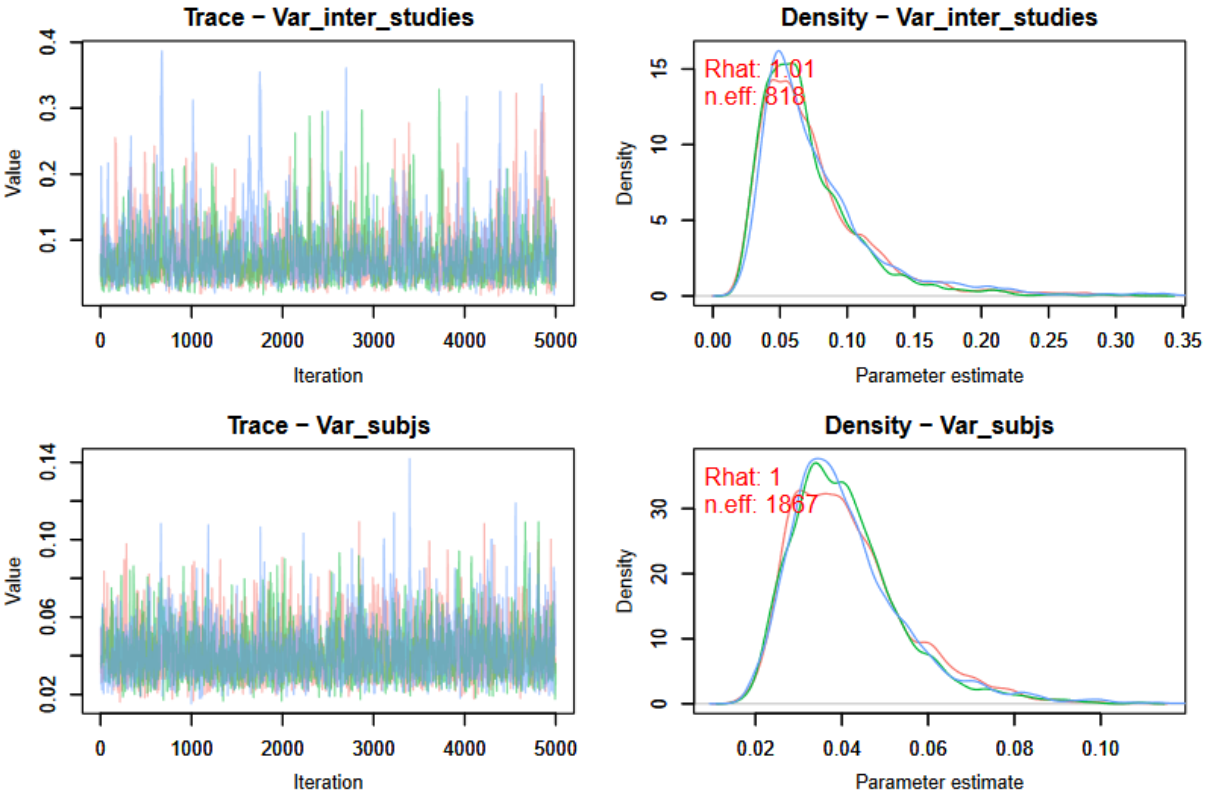

Density – AUC_chem[4]
Rhat: 1
n.eff: 2539
Density
Parameter estimate


Trace – AUC_chem[5]
Value
Iteration


Density – AUC_chem[5]
Rhat: 1.01
n.eff: 2406
Density
Parameter estimate


Trace – AUC_chem[6]
Value
Iteration


Density – AUC_chem[6]
Rhat: 1
n.eff: 1567
Density
Parameter estimate


Trace − Var_inter_studies
Value
Iteration
Density − Var_inter_studies
Rhat: 1.01
n.eff: 818
Density
Parameter estimate
Trace − Var_subjs
Value
Iteration
Density − Var_subjs
Rhat: 1
n.eff: 1867
Density
Parameter estimate