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Committee for Medicinal Products for Human Use (CHMP)

Qualification Opinion for Simcyp Simulator

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Qualification opinion

The Simcyp v19 physiological based pharmacokinetics (PBPK) platform is qualified for predicting the average magnitude of interactions mediated by CYP enzymes (CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5) within the specific COU 1-3.

Context of Use (CoU)

1. To predict the average inhibitory effects (expressed as GMR) of weak and moderate CYP inhibitors on the exposure of a drug administered orally under fasted conditions or intravenously in healthy subjects when a clinical study with a strong CYP inhibitor of the same enzyme has been conducted (and used to verify the fmCYP).

2. To predict the average CYP-mediated inhibitory effect (expressed as GMR) of a drug on the exposure of other CYP substrates administered orally under fasted conditions or intravenously in healthy subjects when a clinical study with a sensitive CYP substrate of the same enzyme has been conducted (and used to verify the competitive inhibition constant (Ki)).

3. To predict the average CYP-mediated MBI effect (expressed as GMR) of a drug on the exposure of other CYP substrates administered orally under fasted conditions or intravenously in healthy subjects when a clinical study with a sensitive CYP substrate of the same enzyme has been conducted (and used to verify the inhibition constant (KI) and the rate of enzyme inactivation (kinact)).

This means that when Simcyp V19 is used per CoU and per the good practice recommendations below to support the DDI risk for a new medicinal product, its predictive performance can be referenced from this qualification in regulatory submissions.

A model-based Bayesian meta-analysis was performed to quantify the uncertainty in SimCYP predicted DDIs based on data from 220 clinical studies included in the qualification matrix. The SimCYP platform generally over-predicted the observed GMRs. The bias[3] in predicted GMR_{AUC} was +5.8 % (95% credible interval: [+1.9; +10 %]) for competitive inhibition and +4.2 % [-3.6; +13 %] for mechanism-based inhibition. The imprecision[4] in the predicted GMR_{AUC} was 18 % [14; 22 %] (CV%, natural scale) for competitive inhibition and 25 % [18; 34 %] for mechanism-based inhibition. Irrespective of the type of interaction, the SimCyp platform under-predicted[5] the between-subject variability in the individual AUC ratios (BSV_{AUC}) 2.0-fold [0.53; 7.5-fold]. For GMR_{Cmax}, based on data from 160 clinical studies respective bias and imprecision were +4.3 % [-0.34; +9.4 %] and 18 % [15; 23 %] for CI, and +6.2 [-3.3; 17 %] and 30 % [23; 40 %] for MBI. Between-subject variability in the individual Cmax ratios (BSV_{Cmax}) was under-predicted 3.1-fold [0.79; 13-fold]. As a result of the magnitude of the bias in DDI between-subject variability estimated for Simcyp, the BSV of the interaction is outside the scope of this qualification. Details on the Bayesian analysis for AUC and Cmax are provided in Annex 1.

The estimated uncertainty in the predicted GMRs should be accounted for when using the platform as per CoU to predict DDIs for regulatory decision-making.

It is the user´s responsibility to assess whether the specific clinical scenario falls within the Simcyp qualification space as defined by the new drug's clinical pharmacology, compliance to good practice recommendations, and CoU.

[3] Bias was expressed as the percentage difference between the predicted GMR and the observed GMR and was calculated from the posterior distribution for 'GMR bias' as $(e^{(\text{-GMR bias})} - 1) \times 100$ %

[4] The imprecision is expressed as the coefficient of variation (CV%) in the natural domain and is calculated from the posterior distribution for 'Between-study variances' as $\text{sqrt}(e^{\text{Between-study variance}}-1) \times 100$ %

[5] Bias in the predicted between-subject variability was expressed as the ratio of the predicted BSV over the true BSV and was calculated from the posterior distribution for 'BSV bias' as $\text{sqrt}(e^{(\text{BSV bias})})$

It is also the user's responsibility to assess that the predictive performance of Simcyp is sufficient for the intended use.

In scope

- CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 interactions.
- CYP-mediated competitive inhibition and mechanism-based inhibition (MBI) via these enzymes.
- The qualification opinion is related to the systems parameters and the compound models implemented in the platform (Simcyp Simulator (V19 R1)) for the purpose of this qualification.
- The qualification opinion is related to prediction of DDIs in a Caucasian adult healthy subject population.
- Prediction of GMR for AUC and Cmax.

Out of scope

- Platform technical verification (including implementation of software calculations and quality control).
- Non CYP enzymes (for example UGTs).
- DDIs involving transporters or induction PBPK mediated DDI predictions
- Predictions without a clinical DDI study for model parameter optimisation/validation.
- Prediction of between subject variability of the interaction.
- DDIs in non-Caucasian populations.

SIMCYP platform description

Systems models

The platform uses either a full or minimal PBPK model with various absorption models. The default Simcyp parameter values related to virtual North European Caucasian population (physiological parameters including liver volume and blood flows, enzyme abundances) are the only covered by the present qualification. Unbound concentrations of inhibitor in the liver and portal vein (or enterocyte concentrations in the case of ADAM model) are used as the driving force for inhibition of metabolism in the liver and gut, respectively. The 'well-stirred' model of hepatic clearance is used. A full description of the PBPK model and the virtual population in scope of this qualification is provided in Annex 2.

Compound models

The substrates and inhibitors models included in Simcyp are selected based on the FDA and EMA recommendations for reference index substrates and inhibitors. The respective compound models are informed by relevant information on physicochemical properties, cell permeability, protein and blood binding, in vitro metabolism and clinical PK of the concerned drugs. The relevant clinical DDI studies identified for each compound are either originating from The University of Washington Drug Interaction Database (DIDB) or scientific literature. Different clinical studies are used for model optimisation and model validation. More details on the Simcyp compounds development and validation are provided in the scientific discussion and in the respective Simcyp compound summaries. Example of compound summaries can be found in Annex 3. The compound summaries related to this qualification can be made available upon request to EMA.

Reporting the anticipated uncertainty in SimCyp-predicted GMRs for upcoming applications in accordance with the CoU

This opinion is supported by an analysis quantifying the SimCyp uncertainty associated with future DDI predictions for regulatory decision-making. The potential bias and imprecision in the Simcyp Simulator's predictions of geometric mean ratios (GMRs) is expected to be influenced by the type of inhibition (competitive vs. mechanism-based inhibition). The model supporting the meta-analysis was cast for inference in a Bayesian framework. All observed and predicted drug-drug interactions (DDIs) in the qualification matrix were analysed simultaneously, with types of inhibition as covariates. Consequently, the expected uncertainty associated with GMR predictions was derived from the posterior parameter distributions and is reported here as credible intervals. For more details the reader is referred to the scientific discussion and the annexes.

Of note, the uncertainty quantification in this qualification is based on the assumption that information can be leveraged across various CYPs and different degrees of inhibition (i.e. CYP agnostic approach to qualification). This assumption is deemed plausible due to the physiology of drug-drug interactions (DDI), the way this is captured in the PBPK platform, and the intended context of use.

It is anticipated that when using Simcyp according to Qualified CoU in regulatory submissions, applicants will provide information on the expected uncertainty related to the GMR predictions. Graphs and tables included in this opinion (shown below) allow applicants/regulators to offset predicted GMRs against the expected uncertainty associated with the predictions.

The following visualizations were derived from the model-based meta-analysis. Figures 1-4 are hypothetical examples proposed by the EMA to report and contextualize the uncertainty associated with GMR_{AUC} predictions from the SimCyp platform when the predictions are used to support regulatory decisions. Visualizations to contextualize the uncertainty associated with GMR_{Cmax} predictions are included in Annex 1. Applicants wishing to use similar visualizations are referred to Annex 1 for the Stan code of the final Bayesian meta-analysis and the R-code for constructing Figures 1-4.

It is important to note that the risk reported in the figures is based on average DDI prediction, not the risk of an individual DDI PK metrics falling outside the no-effect boundaries. While Simcyp can simulate individual DDI exposure ratios, which can be compared with no-effect boundaries to support regulatory decision-making, this application of Simcyp is beyond the scope of this qualification.

Figure 1 shows the expected uncertainty (y-axis) in true GMR_{AUC} against a hypothetical Simcyp predicted GMR_{AUC} (x-axis). The predicted GMR_{AUC} reflects the SimCYP prediction for the DDI of interest. This information is also included in tabular format (see Annex 1).

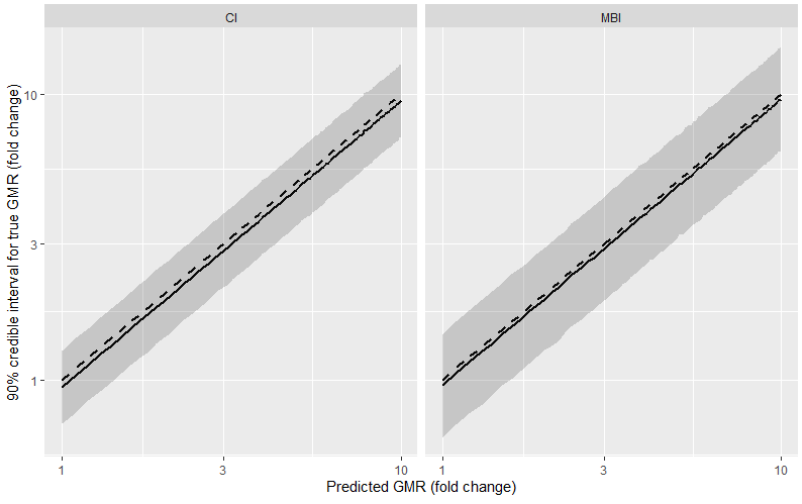


Figure 1: Credible interval for in true GMR_{AUC} vs predicted GMR_{AUC}
90% credible intervals for the true GMR (i.e., fold-changes) are shown with a grey shaded area. Type of inhibition was CI for competitive inhibition and MBI for mechanism-based inhibition. The dashed black line depicts the identity line where the predicted GMR_{AUC} aligns 100% with the true GMR_{AUC}.

Figure 2 shows the expected uncertainty for the true GMR_{AUC} vs. the Simcyp predicted GMR_{AUC} (set to a 1.5-fold predicted increase in exposure, shown as the filled blue circle) and offsets this against the no-effect boundaries of the object of the interaction (hypothetically set to 0.5 to 2-fold). The error bars denote the credible interval for the true GMR_{AUC}.

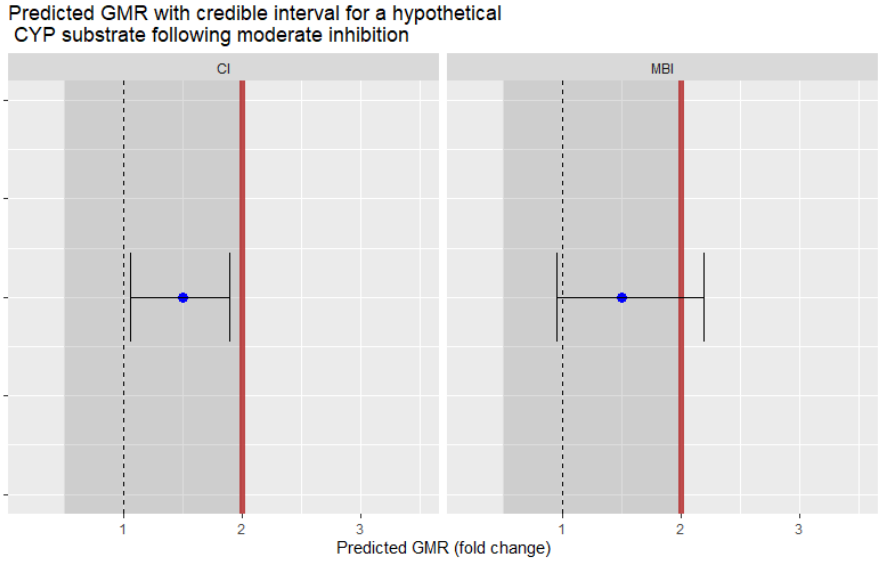


Figure 2: Predicted GMR_{AUC} following CYP inhibition for hypothetical substrate in the case of competitive inhibition (CI; left pane) or mechanism-based inhibition (MBI; right pane). The grey shaded area is defined by the no-effect boundaries of the substrate drug. The red vertical line indicates the upper boundary. The dashed vertical line indicates no inhibition. The blue dot represents the hypothetical point estimate of the GMR_{AUC} predicted by the Simcyp® platform (in this case 1.5). The error bar gives the 90% credible interval for the true GMR_{AUC}.

Figure 3 shows the probability of the true GMR_{AUC} to exceed the upper no-effect boundary versus the predicted GMR_{AUC}. Four hypothetical upper no-effect boundaries are included in Figure 3 (2-fold, 3-fold, 4-fold and 5-fold).

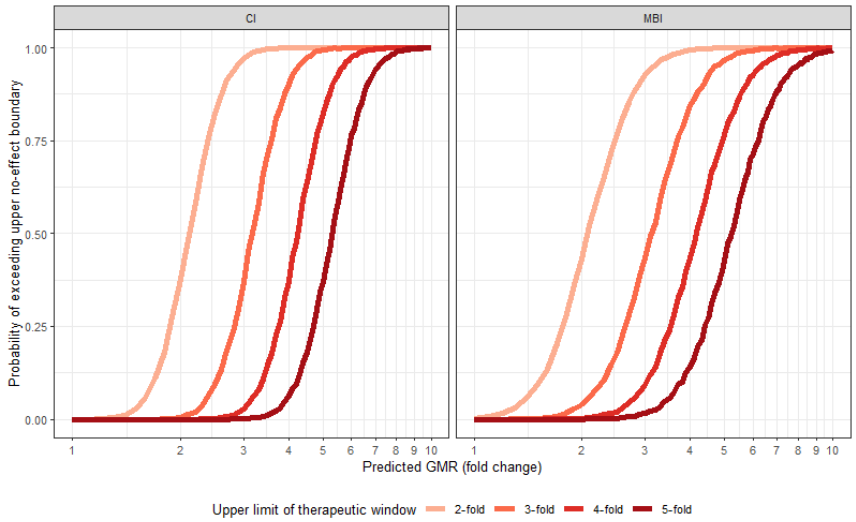


Figure 3: Probability of true GMR_{AUC} exceeding the upper no-effect boundary versus the predicted GMR_{AUC} for competitive inhibition (CI; left pane) or mechanism-based inhibition (MBI; right pane). The coloured lines represent hypothetical upper limits for the therapeutic window and are 2-, 3-, 4- and 5-fold relative to the typical exposure.

Figure 4 integrates the information contained in Figure 3 across different no effect boundaries. On the y-axis, the maximum predicted $GMR_{\mathbf{AUC}}$ associated with a risk of <5 % for the true $GMR_{\mathbf{AUC}}$ exceeding the upper no-effect boundary (x-axis, expressed relatively) is shown.

The threshold of 5% serves as an example; the chosen threshold should be justified e.g. considering the uncertainty around the no-effect boundaries, DDI BSV variability and associated model risk as defined in ICH M15.

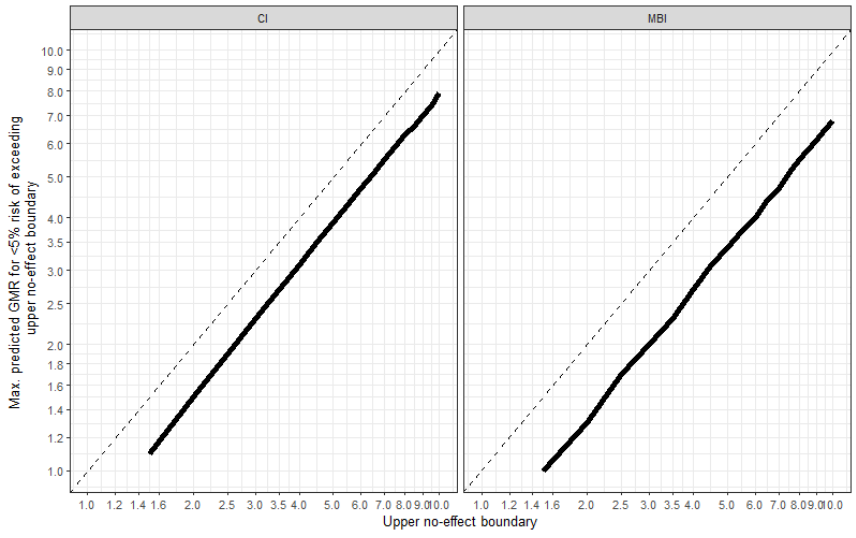


Figure 4: Maximum predicted GMR_{AUC} (y-axis) for <5% risk of exceeding the k-fold relative upper no-effect boundary (x-axis) for competitive inhibition (CI; left panel) or mechanism-based inhibition (MBI; right panel).

Regulatory submission and assessment

The application of Simcyp predictions including uncertainty to support regulatory decisions should be accompanied by a thorough discussion of clinical pharmacology aspects of the dossier (e.g. mass-balance results, DDI results with strong inhibitors/sensitive substrates, etc). Furthermore, it is essential to demonstrate in the submissions that the proposed clinical scenario falls within the Simcyp qualification space, as defined by the context of use (COU) and good practice recommendations. A justification that the Simcyp compound models used for simulations are fit for purpose is required. As stated in the good practice section below, if any relevant change is introduced in the systems, the compound models or in the qualification matrix used in regulatory submissions for DDI prediction, this may need to be accompanied by an updated uncertainty quantification analysis and graphs, see lifecycle management section below.

The applicants are encouraged to use the MIDD M15 table for assessment of MIDD evidence to support the use of Simcyp evidence in regulatory submissions. Narrowing it down to the scope of this qualification, the predicted GMR including the proposed credible intervals should be contrasted to the no-effect boundaries for the substrate drug as defined in the ICH M12 guideline. The predicted GMR including credibility intervals can be used to describe the magnitude of the interaction and to determine whether interventions such as dose adjustments should be considered. Applicants/assessors should also consider the variability of the interaction and the model risk in the final decision.

Good practice recommendations for users of the platform and assessors

The present Qualification Opinion can be used as a reference for Simcyp V19 platform DDI performance when applied in drug development and regulatory submissions as per the qualified CoU and the good practice recommendations below.

The good practice recommendations should be read in conjunction with the recommendations in ICH M12 guidance on the use of PBPK models to predict enzyme DDIs and the M15 ICH guidance on General principles for model informed drug development.

It is reiterated here that the qualification does not cover complex DDIs involving transporters/enzymes or inhibition/induction. The results of the Bayesian uncertainty analysis presented in this qualification opinion are only applicable to the Simcyp V19 systems model and compound models in scope of this qualification. The suitability of the substrate and inhibitor files for the simulations should be evaluated by the user/assessor.

Any modification to the systems or the compounds and/or the qualification matrix would require justification to support decision making and may include an updated Bayesian uncertainty quantification and generation of new related results. The data requirements and good practice steps for development and validation of a PBPK model as per the present qualification opinion are summarized in Table 1.

Table 1: Data requirements and good practice steps for development and validation of a PBPK model for a new drug and for bridging to the qualified Simcyp DDI performance when used according to COU1, 2 and 3.

	COU1- Prediction of DDIs for a new drug being a substrate	**COU2- Prediction of DDIs for a new drug being a competitive inhibitor**	**COU3- Prediction of DDIs for a new drug being a mechanism-based inhibitor**
Confirm in scope scenario	First, it must be ensured that the new drug has got a DDI potential that is simple enough to be within the scope of this qualification. For a drug as a substrate (COU1) it must be ensured that the drug is not a substrate of a transporter that is also inhibited by the inhibitor drug investigated. For a drug as an inhibitor (COU2 and 3) it must be ensured that the drug neither has inducing capacity nor is an inhibitor of a transporter that is of importance for the PK of the substrates drug investigated.		
In vitro data	Physicochemical properties, solubility data (for complex absorption), permeability data, plasma protein and blood binding, in vitro metabolism data (including reaction phenotyping) and inhibition data (if relevant) are required and should be generated using industry standard protocols. Metabolism and inhibition data should be corrected for non-specific microsomal binding (measured or predicted) at the relevant microsomal protein concentration (final incubation concentration).		
Clinical data	Pharmacokinetic studies including single (SD) and multiple dosing (MD) in healthy subjects over a range of doses under fasting conditions are required.		
	A clinical study with a strong CYP inhibitor is also required.	A clinical study with a sensitive CYP substrate is also required.	
Simulations	To ensure that the characteristics of the virtual subjects are matched closely to those of the subjects in the clinical studies, age range, ethnicity and sex ratios should be replicated in an appropriate number of simulated trials that considers variability of subject covariates (usually at least 10), based on the number of subjects in each clinical trial. In addition, the dosage regimens used in the clinical studies should be replicated.		
Drug model development	Initially, simulations based on in vitro data alone should be performed to determine whether the predicted PK parameters and concentration-time profiles are consistent with clinical datasets (SD and MD). Model development should be performed initially using intravenous data, if available, with a focus on the distribution (D) and elimination (E) parameters. Subsequently, absorption (A) related parameters (permeability and solubility) should be introduced into the PBPK model and the predicted profiles and related PK parameters should be compared to observed plasma concentration-time profiles following oral administration. Optimisation of relevant drug model parameters can be performed using clinical data, if necessary, to ensure accurate recovery of observed data (PK parameters and the shape of the concentration-time profile). For example, metabolic intrinsic clearance data can be scaled to accurately capture clearance. The volume of distribution may also need to be optimised via the Kp scalar to accurately predict the observed values if IV data are available. Usually, AUC and Cmax of predicted values after single and multiple doses are expected to be within 1.25 of the observed values.		
	Data from the mass balance study may inform renal clearance and in vivo fm for the various enzymes.		
Model validation	If available, independent datasets (not used for optimisation) should be used to assess the performance of the model with respect to single and multiple dosing.		
Model optimisation for DDI prediction	After accurate recovery of the PK parameters and concentration-time profiles, the clinical DDI should be simulated using the Simcyp Simulator file for the strong inhibitor. The model for the	After accurate recovery of the PK parameters and concentration-time profiles, the clinical DDI should be simulated using the Simcyp Simulator file for the sensitive substrate. The model for the sensitive substrate should be validated for the dosage regimen used in the clinical study, especially if there is non-linearity associated with the disposition of the drug.	

	strong CYP inhibitor should be validated for the dosage regimen used in the clinical study, especially if there is non-linearity associated with the disposition of the drug.		
	If the degree of interaction is not predicted accurately (i.e. observed/predicted AUC and/or Cmax ratio >1.25-fold different), the contribution of the primary CYP enzyme (fmCYP) involved in the metabolism can be optimized to capture the observed DDI with the strong CYP inhibitor. Ideally, both predicted changes in Cmax and AUC should be captured by the model. Optimization of the fm for one enzyme impacts the fm for the other enzymes. If fm needs to be optimised, this should preferably be validated with a DDI study for another CYP enzyme.	If the degree of interaction is not predicted accurately (i.e. observed/predicted AUC and/or Cmax ratio >1.25-fold different), the in-vitro determined Ki value for the new drug should be optimised to capture the observed DDI with the sensitive CYP substrate. Ideally, both predicted changes in Cmax and AUC should be captured by the model. If the Ki of one enzyme is optimised, and the in vitro Ki value was determined using a multiple enzymes in vitro method, e.g. microsomes, consequences for the Ki values of the other CYP enzymes should be discussed. Sensitivity analyses may resolve this issue.	If the degree of interaction is not predicted accurately (i.e. observed/predicted AUC and/or Cmax ratio >1.25-fold different), the in-vitro determined kinact or KI value for the new drug should be optimised to capture the observed DDI with the sensitive CYP substrate. It is proposed that kinact optimisation is initially performed followed by KI if needed. In order to optimise both KI and kinact, DDI data with various dosing regimen should be available. If autoinhibition is relevant for the new drug, MD data could also be used to optimise the inactivation parameters. Ideally both predicted changes in Cmax and AUC should be captured by the model.
Model application	Assessment of the effects of moderate and weak inhibitors (Simcyp Simulator compound files) of the CYP under investigation on the exposure of the new drug. The results of (graphical) uncertainty quantification should be generated and interpreted based on the intended use.	Assessment of the effects of the new drug on less sensitive substrates (Simcyp Simulator compound files) of the CYP under investigation. The results of (graphical) uncertainty quantification should be generated and interpreted based on the intended use.	

Lifecycle management

The qualification is valid for Simcyp V19R1. Lifecycle management does not include what is out of scope for V19 and does not fall within the qualified COU.

The performance defined in this Qualification does not automatically apply to newer versions of the Simcyp PBPK platform. Every time a new Simcyp version is used in regulatory submissions a de novo justification of the assumptions and methods for uncertainty quantification may not be needed if it is demonstrated that the CoU, Qualification matrix and scope complies with the V19 qualification space. However, the new version DDI prediction may require updated results, e.g. updated uncertainty quantification analysis and graphs (see scripts and methodology outlined for the qualification of V19). Assessors should ensure that the new version and applications falls within the scope of the lifecycle management defined here. The recommendations for good practices, reporting and assessment may then be applicable to the assessment of newer versions.

Scientific discussion

The qualification team's review concentrated on the following critical aspects.

A. Model development and evaluation

Systems model

Systems models development and verification is described in the submission. These include default Simcyp parameter values for creating a virtual North European Caucasian population (physiological parameters including liver volume and blood flows, enzyme abundances), selection of full or minimal PBPK model, different absorption models. Unbound concentrations of inhibitor in the liver and portal vein are used as the driving force for inhibition of metabolism in the liver and gut, respectively. The 'well-stirred' model of hepatic clearance is used. The information provided by Simcyp for the systems models and parameters is considered adequate for the contexts of use proposed.

Selection of Compound files

The compound files within the Simcyp Simulator (V19 R1) have been developed and added over the past 20 years. Substrates and inhibitors included as compound files were selected based on the FDA and EMA recommendations for reference index substrates and inhibitors. Throughout this 20-year period of development, clinical DDI studies for each compound were identified on an individual basis using The University of Washington Drug Interaction Database (DIDB) and literature searches. Each of the clinical studies were reviewed to determine whether they should be included or excluded from the development and validation of the compound file. Clinical DDI studies were included if they were randomised controlled clinical DDI studies and were excluded if they were:

- Conducted in patients
- Case studies
- Cocktail studies
- Micro-dosing studies

Development and validation of compound files within the simulator

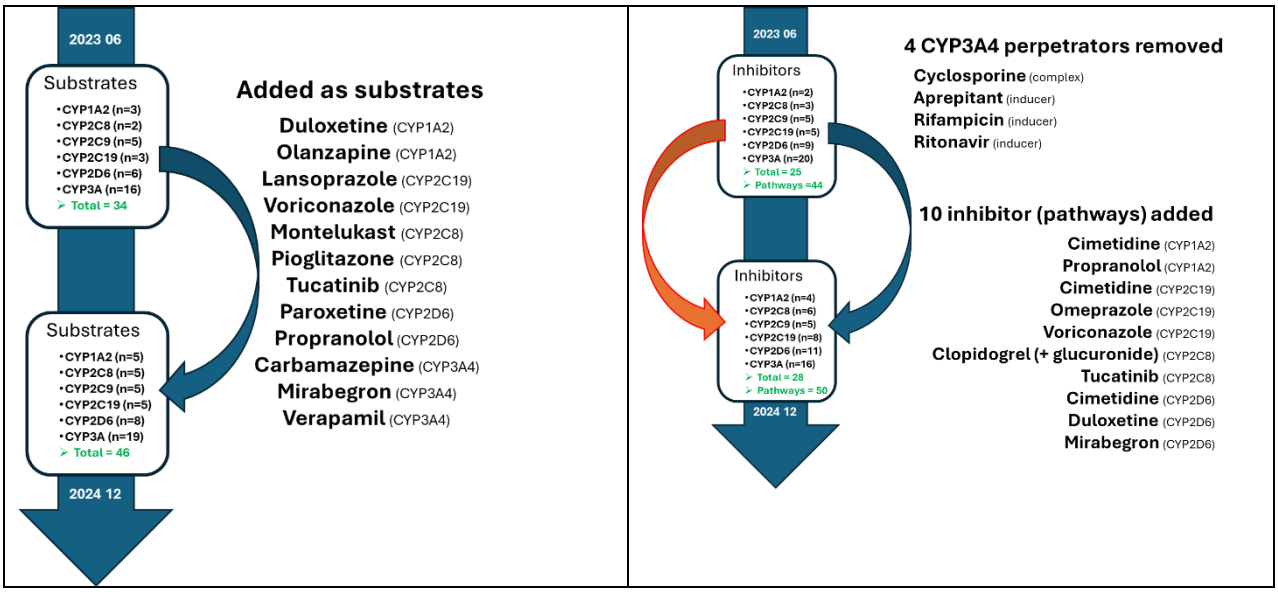
Prior to integration within the platform, a feasibility assessment is conducted for each compound to ensure that there are sufficient in vitro and clinical data available to develop and validate the files for their intended use i.e. quantitative prediction of CYP-mediated DDIs substrate and/or precipitant. 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. to obtain a weighted geometric mean value and variance for a particular parameter. Development and validation of each compound file is performed according to best practice approaches described in several publications.

Simulations using each of the compound files aim 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. Of the compounds included in the qualification matrix, a first-order absorption model was applied for most of the substrates and inhibitors. The ADAM model was used to describe the absorption of ibrutinib, flurbiprofen, ciprofloxacin, gemfibrozil, and verapamil.

At each stage, optimisation of relevant parameters is performed using clinical data, if necessary, to ensure accurate recovery of observed data. Optimised values are then verified using independent clinical data.

For a substrate, 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 precipitant (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 inhibitory parameters. All clinical DDIs that have been used to optimize the fmCYP values or inhibitory parameters are removed from the DDI qualification matrix.

The flowchart below summarises the changes in the Simcyp substrate and inhibitor files introduced after the start of the qualification procedure in response to the qualification team comments. See Annex 4 for more information.



The source of the input data, the optimization process, the method used to derive the parameters, and the clinical DDI studies for each compound, along with the level of validation performed, are all documented in a compound-specific file (Example provided in Annex) or in scientific literature for few compounds not expected to be routinely used for predicting DDI liability. These compound files should be reported in submissions to enable a thorough assessment of the DDI predictions.

The final list of substrate and inhibitor files in scope of this qualification are provided in Table 2 below. It should be noted that these files were implemented in Simcyp V19. Some of the compound files were only included in the analysis to make the qualification matrix more diverse in terms of inhibitor strength and sensitivity. Therefore, it is unlikely that a number of the compound files will be used for prediction of DDI liability in regulatory submissions. Newer Simcyp Versions may include additional or modified compound files, see lifecycle management above.

Table 2 final list of substrate and inhibitor files in scope of this qualification

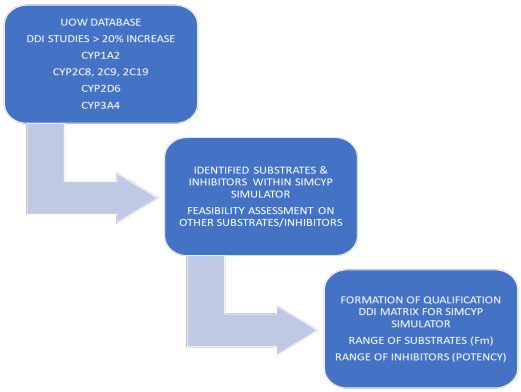
Substrates	**Inhibitors**
• Caffeine , CYP1A2 • Duloxetine , CYP1A2 • Olanzapine , CYP1A2 • Theophylline , CYP1A2 • Tizanidine , CYP1A2 • Imipramine , CYP2C19 • Lansoprazole , CYP2C19 • Omeprazole , CYP2C19 • S-Mephenytoin , CYP2C19 • Voriconazole , CYP2C19 • Montelukast , CYP2C8 • Pioglitazone , CYP2C8 • Repaglinide , CYP2C8 • Rosiglitazone , CYP2C8 • Tucatinib , CYP2C8 • Celecoxib , CYP2C9 • Flurbiprofen , CYP2C9 • Phenytoin , CYP2C9 • S-Warfarin , CYP2C9 • Tolbutamide , CYP2C9 • Atomoxetine , CYP2D6 • Desipramine , CYP2D6 • Dextromethorphan , CYP2D6 • Metoprolol , CYP2D6 • Nebivolol , CYP2D6 • Paroxetine , CYP2D6 • Propranolol , CYP2D6 • Tolterodine , CYP2D6 • Alfentanil , CYP3A4 • Alprazolam , CYP3A4 • Aprepitant , CYP3A4 • Atazanavir , CYP3A4 • Carbamazepine , CYP3A4 • Clarithromycin , CYP3A4 • Dexamethasone , CYP3A4 • Ibrutinib , CYP3A4 • Midazolam , CYP3A4 • Mirabegron , CYP3A4 • Nifedipine , CYP3A4 • Quinidine , CYP3A4 • Repaglinide , CYP3A4 • Rifabutin , CYP3A4 • Sildenafil , CYP3A4 • Simvastatin , CYP3A4 • Triazolam , CYP3A4 • Verapamil , CYP3A4 • Zolpidem , CYP3A4	• Cimetidine , CYP1A2 • Ciprofloxacin , CYP1A2 • Fluvoxamine , CYP1A2 • Propranolol , CYP1A2 • Cimetidine , CYP2C19 • Fluconazole , CYP2C19 • Fluoxetine , CYP2C19 • Fluvoxamine , CYP2C19 • Nor-fluoxetine , CYP2C19 • Omeprazole , CYP2C19 • Ticlopidine , CYP2C19 • Voriconazole , CYP2C19 • Clopidogrel , CYP2C8 • Clopidogrel acyl glucuronide , CYP2C8 • Gemfibrozil , CYP2C8 • Gemfibrozil glucuronide , CYP2C8 • Trimethoprim , CYP2C8 • Tucatinib , CYP2C8 • Amiodarone , CYP2C9 • Fluconazole , CYP2C9 • Fluvoxamine , CYP2C9 • Mono Desethyl Amiodarone , CYP2C9 • Sulphaphenazole , CYP2C9 • Bupropion , CYP2D6 • Cimetidine , CYP2D6 • Cinacalcet , CYP2D6 • Duloxetine , CYP2D6 • Fluoxetine , CYP2D6 • Fluvoxamine , CYP2D6 • Hydroxy-bupropion , CYP2D6 • Mirabegron , CYP2D6 • Nor-fluoxetine , CYP2D6 • Paroxetine , CYP2D6 • Quinidine , CYP2D6 • Amiodarone , CYP3A4 • Atazanavir , CYP3A4 • Cimetidine , CYP3A4 • Clarithromycin , CYP3A4 • Desmethyl-diltiazem , CYP3A4 • Diltiazem , CYP3A4 • Erythromycin , CYP3A4 • Fluconazole , CYP3A4 • Fluoxetine , CYP3A4 • Fluvoxamine , CYP3A4 • Hydroxy-itraconazole , CYP3A4 • Itraconazole , CYP3A4 • Ketoconazole , CYP3A4 • Nor-fluoxetine , CYP3A4 • Nor-verapamil , CYP3A4 • Verapamil , CYP3A4

B. Model clinical validation and applicability

DDI qualification matrix

The University of Washington Drug Interaction Database (DIDB) was applied to identify clinical DDI studies involving CYP1A2, CYP2D6, CYP2C8, CYP2C9, CYP2C19 and CYP3A4/5 where observed increases in plasma exposure of substrates greater than 20% (because of the DDI) were reported (Figure 5). DDI studies were flagged if both substrate and inhibitor were available as compound files within the Simcyp Simulator (V19 R1).

Figure 5. The workflow used to identify substrates and inhibitors for the DDI Qualification Matrix.



Where possible, another criterion for selection of compounds/DDI studies was to ensure the inclusion of a range of weak, moderate and strong inhibitors and substrates that were susceptible to differing degrees of inhibition. In DDI clinical studies, it is customary to use inhibitors which are known to have a strong effect. However, the inhibitory effect of a precipitant is also dependent on the metabolic characteristics of a substrate, i.e., affinity to the principal enzyme, relative contribution of a specific enzyme to overall metabolism or PK behavior of a drug, and alternative enzymatic and excretory clearance routes. Consequently, the interaction outcome of a "strong" precipitant may be strong, moderate, or weak, depending on the substrate drug. Thus, the intensity of inhibition is defined by the ICH-M12 based on the AUC change of a sensitive object drug. Strong, moderate, and weak inhibitors give rise to an increase in AUC of a victim drug by at least 5-fold, between 2- and 5-fold, and 1.25- to 2-fold, respectively.

In addition to reference substrates and inhibitors, so-called "sensitive" substrates were also included. Usually, sensitive substrates are metabolised almost completely or to a significant extent by the CYP enzyme concerned, so that the inhibition by a specific inhibitor will lead to a significant increase in the exposure of the substrate drug.

It is also noted that the phenotypic classification of subjects for polymorphic CYP enzymes in the DDI qualification matrix is unclear.

The Table below summarise the changes in the Simcyp DDI matrix introduced in response to the qualification team comments. Complex interactions i.e. involvement of transporter/enzyme such as CYP3A4/P-gp, CYP2C8/OATP1B and inhibition/induction, which were included in the first round have been deleted in the final DDI matrix. See Annex 4 for more information.

Table 3. Differences in the matrix between March 2023 and December 2024

March, 2023				December, 2023				December, 2024			
Enzyme	**CI**	**MBI**	**ALL**	**Enzyme**	**CI**	**MBI**	**ALL**	**Enzyme**	**CI**	**MBI**	**ALL**
CYP1A2	20	0	20	CYP1A2	42	0	42	CYP1A2	42	0	42
CYP2C8	3	13	16	CYP2C8	7	10	17	CYP2C8	7	10	17
CYP2C9	17	4	21	CYP2C9	25	3	28	CYP2C9	19	3	22
CYP2C19	6	5	11	CYP2C19	15	13	28	CYP2C19	15	13	28
CYP2D6	17	9	26	CYP2D6	32	14	46	CYP2D6	34	10	44
CYP3A4/5	59	52	111	CYP3A4/5	66	28	94	CYP3A4/5	64	29	93
	122	83	205		187	68	255		181	65	246

In total, 46 substrates and 28 inhibitors were identified for inclusion in the DDI matrix for qualification of CYP-mediated inhibition using the Simcyp Simulator (V19R1). There were 181 clinical studies involving competitive inhibition and 65 clinical studies involving time-dependent inhibition (MBI) and 124 unique pairs of inhibitors-substrates.

Please note that the DDI matrix and compounds discussed here focus on AUC. A similar exercise for Cmax is provided in Annex 1, but is not detailed here for the sake of brevity.

Imbalance of Simcyp compounds and DDI matrix

The uncertainty quantification in this qualification is based on the assumption that information can be leveraged across various CYPs and different degrees of inhibition (i.e. CYP agnostic approach to qualification). This assumption is deemed plausible due to the physiology of drug-drug interactions (DDI), the way this is captured in the PBPK platform, and the intended context of use (Ref. Response documents)

This said, the Qualification team identified the following limitations which were discussed in the Response document 21_12_2023.

The published interaction studies in the qualification matrix are unbalanced in terms of CYP involvement, mechanism, and degree of inhibition (weak, moderate, strong).

Table 4: Number of clinical studies in the DDI Qualification Matrix for AUCR predictions

Enzyme	CI	MBI	ALL
CYP1A2	42	0	42
CYP2C8	7	10	17
CYP2C9	19	3	22
CYP2C19	15	13	28
CYP2D6	34	10	44
CYP3A4/5	64	29	93
total	181	65	246

Small sample sizes and unclear CYP phenotypic status of subjects in DDI studies are reported. Additionally, some compound files used for qualification, such as nebivolol, are not formally part of version 19. There is sometimes limited information on the development and performance of certain compounds. Predictions for CYP3A activity are based on the combined data for CYP3A4 and CYP3A5 (CYP3A4/5) due to the lack of specific probes and inhibitors for these enzymes in vivo.

These limitations do not impede the qualification of Simcyp for the specific contexts of use (COUs), but they should be considered when using Simcyp for DDI prediction. The unbalanced dataset however makes some extra caution warranted if Simcyp is applied to enzymes or situations supported by very limited clinical data.

Simulations

To ensure that the characteristics of the virtual subjects were matched closely to those of the subjects studied in vivo, numbers, age range, ethnicity and sex ratios were replicated in 10 simulated trials and

for the number of subjects in each clinical trial. Qualification was performed based on prediction of the observed clinical interactions for the respective drug pairings.

Performance Metrics and Related Acceptance Criteria

The Applicant proposed acceptance criteria based on the ratio of the area-under-the-curve of the plasma concentration-time profile (AUC) in the absence and presence of inhibitor (AUCi/AUC, where AUCi and AUC are the AUC(0-∞) values of the substrate in the presence and absence of inhibitor, respectively). In addition, the ratio of the maximum plasma concentration (Cmax) in the presence and absence of inhibitor was also proposed. Mean Cmax and AUC ratios from 10 simulated trials were compared against the mean ratios from each clinical study included in the DDI QM. Average fold error (AFE) and absolute average fold error (AAFE) as described by Shimizu et al.[6] were used to assess the bias and precision of the predictions, respectively (Ref. Response Document 19 Dec 2023).

Table 5. Average fold error (AFE) and absolute average fold error (AAFE) reported in Response Document 19 Dec 2023

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

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

In addition, predicted AUC and Cmax ratios were compared to the observed data.

Table 6: All-CI- Percent of DDI mean predictions meeting specified fold ratios as reported in Response Document 19 Dec 2023

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	%

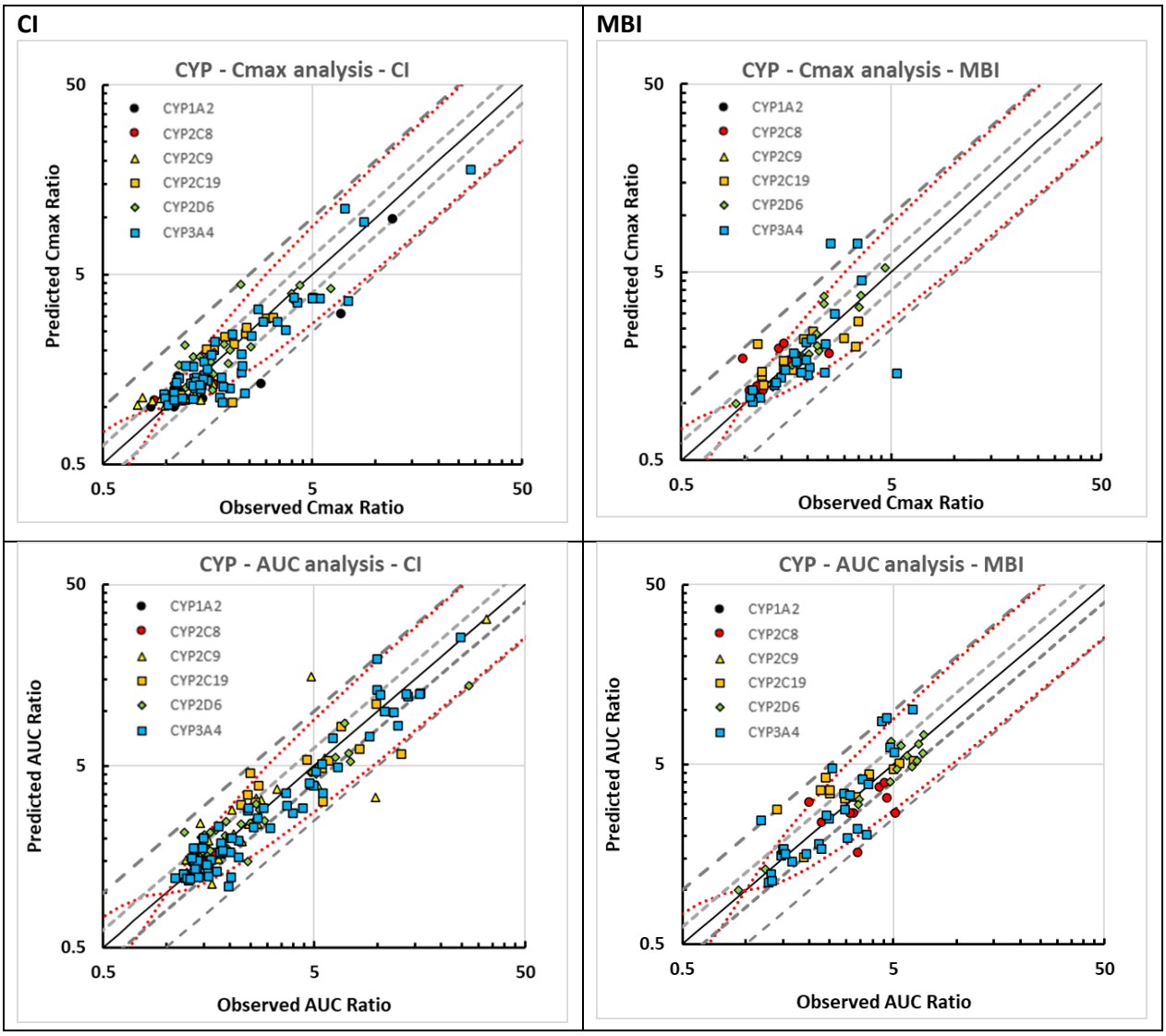
Table 7: All- MBI Percent of DDI mean predictions meeting specified fold ratios as reported in Response Document 19 Dec 2023

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**	%

[6] Shimizu H, Yoshida K, Nakada T, et al. Prediction of human distribution volumes of compounds in various elimination phases using physiologically based pharmacokinetic modeling and experimental pharmacokinetics in animals. Drug Metab Dispos.2019; 47:114-123

For the graphical comparisons, predictions were assessed as to fall within 1.5-fold of observed data. For clinical DDIs resulting in weak to moderate inhibition, the validation criteria proposed by Guest et al.[7] were proposed. For more details the reader is referred to the Response Document 19 Dec 2023.

Figure 6. Graphical comparison of observed vs simulated DDIs per mechanism.



Uncertainty quantification

In the first List of Issues, the qualification team challenged the proposed performance metrics and acceptance criteria. The QT argued that the heterogeneity in the quantity (number of subjects) and quality (uncertainty of reported point estimate of clinical DDI study) of the information contained in the DDI QM should be accounted for in the assessment of the predictive performance of the Simcyp platform. The shortcomings of the proposed performance metrics and acceptance criteria were discussed with and acknowledged by the applicant during the March '24 SAWP meeting.

In response to a related issue in the 1st LoI (EMA issue 5) the applicant reported results from a Bayesian meta-analysis. This analysis quantified inter-study variability for a subset of 6 drug pairs for which several similar clinical DDI studies were available. Inspired by this work, the QT suggested in the 2nd LoI that a similar model, fitted to the full DDI QM, could overcome some of the limitations of the

[7] Guest EJ, Aarons L, Houston JB, Rostami-Hodjegan A, Galetin A. Critique of the two-fold measure of prediction success for ratios: application for the assessment of drug-drug interactions. Drug Metab Dispos.2011; 39:170-173.

aforementioned performance metrics and could take due account of the heterogeneity and uncertainty in the clinical DDI studies in the DDI QM when evaluation the predictive performance of the Simcyp platform.

In the subsequent Response Document (dd. 26/06/2024) and July '24 SAWP meeting the applicant shared with the QT the results from a hierarchical Bayesian meta-regression model quantifying potential biases and imprecision in Simcyp GMR_{AUC} predictions. For more information the reader is referred to the respective documents. In short (as shown in Figure 7), the model bridged Simcyp predicted geometric mean ratios for AUC (GMR_{AUC}) to the observed GMR_{AUC} by a GMR bias parameter which was estimated. A similar approach was followed to bridge the Simcyp predicted between-subject variability in the AUC ratios (BSV_{AUC}) to the total observed variability, acknowledging that the total observed variability was composed of the BSV (divided by the known number of subjects in the DDI study) and the between-study variance (referred to as "imprecision" in the remainder of this discussion).

During the interactions with the applicant around the model-based approach to uncertainty quantification, it was noted that potential bias and imprecision in the Simcyp predicted GMR_{AUC} may depend on factors such as the individual CYP studied, the mechanism involved, the administration route, etc. Stratification and/or hierarchical modelling strategies were discussed as a means to explore the variability in bias and imprecision across the DDI QM. Subsequently, the QT explored different implementations of the Bayesian meta-regression model to improve the goodness-of-fit of the proposed model to the data and to challenge some of the assumptions underlying the proposed model. Recognizing that the CoU focusses on the prediction of GMR point estimates, and that GMR bias and imprecision drive the width of the credible interval for the true GMR, the QT explored factors that could explain differences in GMR bias and/or imprecision only.

For brevity, we present here a selection of the evaluated models, and in particular a comparison between the original proposed model ("Model A"; Stan code: "m201.stan"), a model including covariate effects for CYP and "type of inhibition (MBI vs. CI)" on GMR bias and between-study variability ("Model B"; Stan code: "m200.stan") and a model with "type of inhibition (MBI vs. CI)" on GMR bias and between-study variability ("Model F"; Stan code "m202.stan" in Annex 1). For more details the reader is referred to the 3rd LoI, the Appendix to the 3rd LoI (for Stan code), and the Response Documents to the 3rd LoI (dd. 16/12/2024 & 19/02/2025).

Figure 5: Directed acyclic graph representations of model A, B and F. Observations are in red; Simcyp® predictions in blue; Latent variables in grey; estimated parameters in black

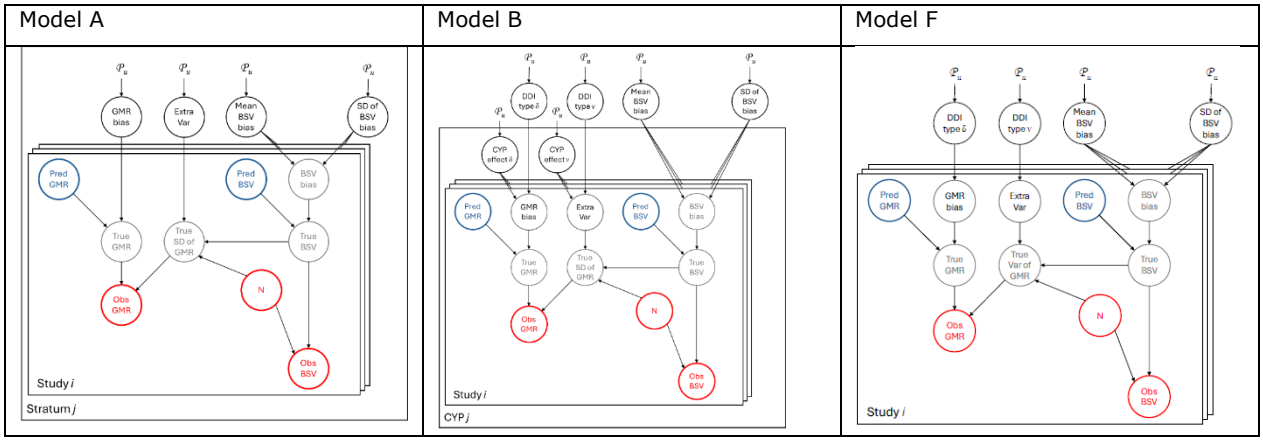


Table 8 shows a comparison between the pointwise out-of-sample prediction accuracy for model A, B and F estimated through WAIC and PSIS-LOO CV as described by Vehtari, Gelman and Gabry[8]. In addition, we present posterior predictive checks (PPC) which demonstrate the ability of the different models (A, B and F) to reproduce the observed GMR_{AUC} and between-subject variability in the GMR (BSV_{AUC}) from the DDI QM from the Simcyp predicted GMR_{AUC}, BSV_{AUC}, and the parameters in the model describing the bias and imprecision in Simcyp predictions.

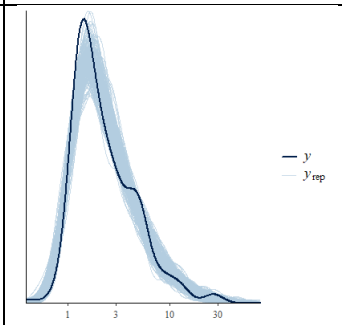
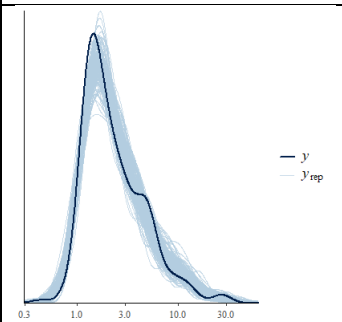
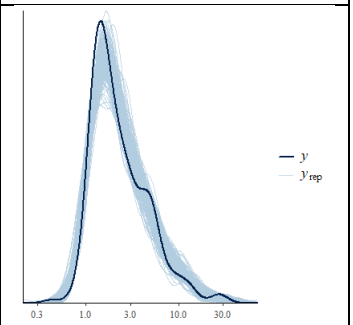
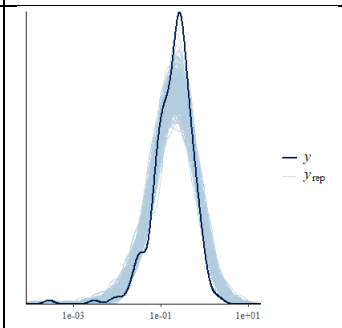
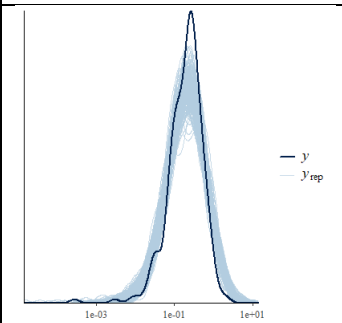
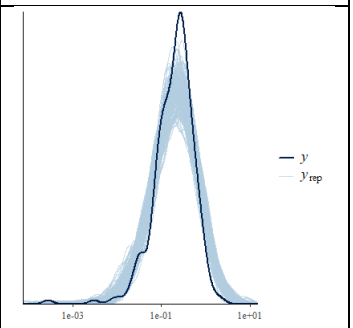
	Model A	Model B	Model F
	m201.stan / fit.m0	m200.stan / fit.m1	m202.stan / fit.m3
WAIC	-101.0 (Δ = -4.7, SE = 6.0)	-96.3 (-)	-96.9 (Δ = -0.7, SE = 5.7)
PSIS-LOO CV	-166.8 (Δ = -3.0, SE = 6.5)	-163.8 (-)	-166.1 (Δ = -2.3, SE = 6.2)
Posterior predictive check for GMR			
Posterior predictive check for BSV			

Table 8: Comparison of prediction accuracy for models A, B and F.
WAIC: Widely applicable information criterion; PSIS-LOO CV: Pareto-smoothed importance sampling leave-one-out cross-validation, Δ denotes the difference in metric compared against the best-performing model, SE is the estimate for the standard error for the difference according to Vehtari, Gelman and Gabry[6]; GMR: geometric mean ratio; BSV: between-subject variability in GMR

Table 8 shows that "Model B" has the highest expected out-of-sample predictive performance (i.e. highest WAIC and PSIS-LOO CV). The expected predictive performance for "Model A" and "Model F" is lower, albeit not significantly different from "Model B" (as shown by the high standard errors for the difference in expected log pointwise predictive density). In line with this finding, the posterior predictive checks for GMR_{AUC} and BSV_{AUC} for "Model A" and "Model F" are not different from the posterior predictive check for "Model B". A sensitivity analysis consisting of removing the hierarchical structure (mixed effects) in the model for BSV_{AUC} bias, to derive a more parsimonious model, confirmed the above findings.

[8] Vehtari, A., Gelman, A., and Gabry, J. (2017a). Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. Statistics and Computing. 27(5), 1413--1432. doi:10.1007/s11222-016-9696-4

Based on the above, the QT concluded that the data does not support a separate GMR bias or between-study variability for the different CYPs. This conclusion aligns with the assumption of the CYP-agnostic approach taken in this qualification. At the same time, the QT expected a priori that GMRAUC predictions for scenarios involving mechanism-based inhibition are likely subject to higher uncertainty compared to competitive inhibition, owing to the more complex nature of the physiological processes involved (e.g. the involvement of the dynamics of CYP enzyme turn-over). Therefore, the QT decided to select "Model F" as the final model to quantify the uncertainty in the Simcyp platform. The Stan code for "Model F" for GMRAUC can be found in annex 1. Parameter estimates for the uncertainty quantification of GMRAUC are shown below in Table 9, posterior distributions for GMR bias and imprecision are shown in Figure 8. All graphs and tables presented in this opinion are based on "Model F". "Model F" was also used to quantify the uncertainty in Simcyp predicted GMR and BSV for C_{max}. Parameter estimates for the model for GMR_{Cmax} and the hypothetical examples shown in Figures 1-4 to help contextualize the uncertainty in predicted GMR_{Cmax} are shown in annex 1.

Table 9: Parameter estimates for "Model F" for GMR_{AUC}

Parameter	**Model F**	
	Mean	SD
Mean GMR biases		
CI	-0.0568	0.0192
MBI	-0.0413	0.0404
Between-study variances		
CI	0.0321	0.0069
MBI	0.0628	0.0191
BSV* bias mean	1.4050	0.0955
BSV* bias SD	1.2889	0.0737

* BSV: Between-subject variance.

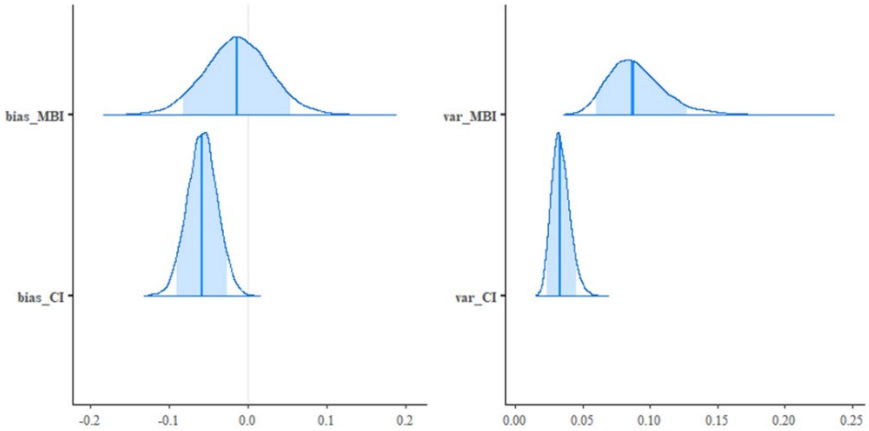


Figure 6: posterior distributions of GMR bias (bias) and imprecision (var) for competitive inhibition (CI) and mechanism-based inhibition (MBI) according to "Model F".

Annexes (published on EMA website with this draft Opinion)

https://www.ema.europa.eu/en/human-regulatory-overview/research-development/scientific-advice-protocol-assistance/opinions-letters-support-qualification-novel-methodologies-medicine-development

Annex 1: Bayesian Analysis

Annex 2: Description of Systems models and related parameters for the full and minimal PBPK models

Annex 3: Simcyp Compound Summaries (examples)

Annex 4: Evolution of DDI qualification matrix and substrates inhibitors

Annex 5: DDI Qualification Matrix

Annex 6: Interactions with QT team and key documents

Interactions	**Documents by EMA**	**Documents by Applicant**
Initial submission/ preparatory TC		EMA_qualification_CYPDDI_document_December 16 EMA Qualification – February 21 2023(2)
Start of procedure		Briefing Document – EMA_Qualification_March 13-2023 Response Document to EMA Appendix 3 – Inhibitory mechanisms UOW_Matrix_summary
1st Discussion meeting	Simcyp Simulator (102776) List of Issues	Response Document_EMA_21_12_2023 Certara - SAWP Meeting - March 6-2024
2nd Discussion meeting	Simcyp Simulator - 2nd List of issues	Response Document_EMA_26_06_2024 Certara - SAWP Meeting - July 10-2024
3rd Discussion meeting	Simcyp Simulator (102776) - 3rd List of issues Appendix to 3rd List of Issues	EMA response document - 3rd list of issues - 16-12-2024 Certara - SAWP Meeting - January 15-2025
Request for additional information	Request for additional information from Simcyp	EMA response document_19_2_2025_Items 2-4 EMA response document - 19_02_2025_Items 1 and 5 EMA response - additional information_March 18_2025 MAR2025-V19-Fluvoxamine-summary EMA response document_09_04_2025_Items 1-6 IVIVE and PBPK Proposal Version control