EUROPEAN MEDICINES AGENCY
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Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000

An agency of the European Union

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EMA/CHMP/ICH/652460/2022

Overview of comments received — ICH Harmonized Guideline Drug Interactions M12 (EMA/CHMP/ICH/652460/2022)

Please note that comments will be sent to the ICH M12 EWG for consideration in the context of Step 3 of the ICH process.

1. General comments – overview

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
MSD	0	0	Introduction	Throughout the document the role of the drugs involved in drug interactions are referred to as "victim" and "perpetrator". These terms are outdated and mildly offensive given the current state of the world. This guidance could help usher in a better way to talk about DDI instead of unnecessarily reminding those involved of either perpetrators or victims.	Throughout the entire document replace the word "victim" with "object". Throughout the entire document replace the word "perpetrator" with "precipitant".
Certara Integrated Drug Development	0	0	1,4	Characterization of how an in vivo relevant pharmacologically active metabolite is formed, and which enzyme(s) are catalyzing its formation, is sometimes forgotten in the Clinical Pharmacology development program, in the regulatory assessment and labelling. Hence, we propose that the need to focus also on these pathways are clearly reflected in suitable, but multiple, parts of the guideline	
Certara Integrated Drug Development	0	0	2.1.4	Please clarify what composes a positive CYP2C19 activity signal. Is an increase activity of CYP2C19 in a concentration-dependent manner, with activity ≥ 2-fold at 15 x Cmax,u adequate for basic risk assessment? Please comment on impact of using the activity as endpoint for CYP2C19in cases where inhibition by parent drug or metabolite(s) is observed in the concentration range.	
Certara Integrated Drug Development	0	0	2.1.4.2	Please include a recommendation on how to decide which type of function to use in estimating Emax and EC50.	
Certara Integrated Drug Development	0	0	2.3.2	Please clarify the concentrations of metabolite that should be used in the in vitro studies and what cutoff should be applied. Is it the same as for parent drug? If both parent and metabolite showsshow inhibitory potential, how would the in vivo relevance of the combination of exposures of inhibitory substances be assessed using the basic model? Or is the basic model not recommended in these situations? Perpetrator effects are highly unlikely for some phase 2 conjugates. Please specify if there are phase 2 conjugates which does not need screening. See CPT Pharmacometrics Syst. Pharmacol. (2016) 5, 505–515; doi:10.1002/psp4.12110	
Certara Integrated Drug Development	0	0	2.3.3	Please include information on which concentration to use in the Induction potential assessment for extrahepatically (including intestinally) formed metabolites.	
Certara Integrated Drug Development	0	0	3.1.3	Please mention the particular need for drug interaction evaluations informing use of concomitant use of common/essential comedications when the investigational drug is an established in vivo inducer.	
Certara Integrated Drug Development	0	0	3.2.1.4	Here reference could be made to PBPK approaches to estimate DDI risk based in DDI studies in the most sensitive scenario.	
Certara Integrated Drug Development	0	0	3.2.1.6	Please also propose staggering doses when the victim or perpetrator has a short half-life	
Certara Integrated Drug Development	0	0	3.2.1.7	Please add herbal supplements here.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Certara Integrated Drug Development	0	0	3.2.1.9	Please provide information on the relationship between toxicity and renal or hepatic efflux transporters. Inhibition of uptake transporters would reduce distribution to the tissue while inhibition of the efflux transporter would increase tissue concentrations and thus risk of toxicity. This is presently not clear in the guideline. Inhibition of uptake transporters could, dependent on presence of parallel elimination pathways give rise to reduced efficacy due to reduced distribution to the target organ of efficacy. Here PBPK approaches could be useful and could be recommended.	
Certara Integrated Drug Development	0	0	3.2.3	Please explain the need the address therapeutically important comedications when a new drug is found to be an inducer. In addition, please include the need for estimating perpetrator and victim DDI risk at steady state. Furthermore, note the risk that the index inhibitor may have reduced exposure, limiting the enzyme inhibition effect by that particular inhibitor. The net inhibitory effect of different enzyme inhibitors would be influenced by their sensitivity to induction. Here PBPK approaches are very useful simulating worst case scenarios.	
Certara Integrated Drug Development	0	0	3.2.4.3	The text is not sufficiently actionable. Please clarify the expectations. Please consider mentioning the need of a DDI study with systemically acting contraceptive steroids if UGT induction is suspected.	
Certara Integrated Drug Development	0	0	3.2.6	Please explain that estimating AUC is generally needed and that for some probes, measuring fractional clearance via different routes could improve the sensitivity of the probe and mechanistic understanding.	
Certara Integrated Drug Development	0	0	5.3.1	Please consider expressing the no effect boundaries also as % change from control group and not absolute values. Absolute exposure parameters are sensitive to both intra-study and intra-population exposure differences.	
Certara Integrated Drug Development	0	0	5.3.2	Strong/Moderate/Weak classification system beyond CYP enzymes. Inclusion of transporters (especially P-gp and BCRP) would allow to limit the co-medication exclusions during drug development, with a direct benefit to patients participating in clinical trials.	
Certara Integrated Drug Development	0	0	7.3.1	The mechanistic static model can be very useful for DDI predictions. Please clarify the use for induction in vivo relevance assessment also in section 2.1.4.	
Certara Integrated Drug Development	0	0	7.3.7.1	Please clarify the recommendations for how to use the mechanistic-static model to assess the in vivo relevance of an in vitro transporter inhibition signal. If wanting to have a text open for data supported applications also for presently non- qualified transporter inhibition assessment, this can still be maintained	
Certara Integrated Drug Development	0	0	7.5.1	Please include that caffeine is also a NAT substrate Please highlight that Dextromethorphan is also a CYP3A substrate. Please consider replacing dextromethorphan with metoprolol.	
Certara Integrated Drug Development	0	0	7.5.3	As this is a young area where science is developing fast, it would be suitable with a list of references (incl high level study results) to be published supporting tables 10 and 11. This allows a critical evaluation of these probes and inhibitors as needed when science develops, and comparison with new upcoming probes.	
Certara Integrated Drug Development	0	0	7.5.3.1	Table 18. Please comment on the requirements for metformin DDIs outlining what to measure for which transporter. If uncertain, please provide as clear guidance as possible.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Certara Integrated Drug Development	0	0		The draft guidance is very well written and covers most aspects of Drug Interactions. Having a harmonized, data driven and updated DDI guideline is beneficial for the pharmaceutical industry. The detailed advice on in vitro DDI study design is highly appreciated. We appreciate the advice on modelling included in the guidance and plant to cover the MIDD approaches in a separate guideline. We would like to comment on some of the recommendations made. DDIs in special populations including DDIs in renal impairment, pediatric patients and genetic (PGx) subpopulations is not covered in the present guideline. We recommend that this important topic is further discussed. Management of drug interactions in the pediatric population is recommended in the new FDA Pediatric Clinical Pharmacology guideline and in EU guidelines such as the Notice to Applicants SmPC guideline. It is uncommon with an ICH guideline in an area that is under significant scientific development. To adapt to this situation, there are parts of the guideline text that talks about future/upcoming applications. If wishing to mention this in the guideline for example to promote a fast development, the present ability to accept such approaches should be made clear. In addition, more information should be inserted on what data should be generated for regulatory acceptance. Referring to papers only and not outlining practical advice is confusing and can be misleading. Due to the foreseen fast development, it seems suitable to have a time plan outlining potential revision, keeping the guidance up to date and actionable. A final comment is that the guideline document is very long. We appreciate the detailed advice given and would propose that all suitable measures possible should be taken to make the guideline easier to read and also to allow the reader to easily know that everything has been read on a certain topic. Please consider including hyperlinks and also decision trees including references to different sections.	
WuXi Apptec, DMPK-NJ	0	0	multiple	Mixed information throughout the guidance and it is difficult for Sponsors to decide the timing to conduct the in vitro DDI studies (see below).	Clarify the timing for in vitro DDI studies. Please conceal the discrepancies and clarify if in vitro DDI data are necessary in the IND data packages to regulatory agencies, or it is okay to have the in vitro DDI data during clinical phases.

2. Specific comments on text

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Amgen	174	175	1	Original text: "The scope of the guideline is limited to pharmacokinetic interactions, with a focus on enzyme- and transporter-mediated interactions." For clarity and consistency, it is better to say metabolic enzyme, please insert "metabolic" before enzyme.	Amgen recommends the following revision: "The scope of the guideline is limited to pharmacokinetic interactions, with a focus on metabolic enzyme- and transporter-mediated interactions."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Charles River	174	181	1,3	The scope currently mentions small molecules and biologics but contains no considerations for other modalities which may have very distinct ADME(T) properties and therefore very distinct DDI risk properties. For multiple such modalities, DDI strategies are being or have been proposed by specific working groups, such as: 1) GalNac-conjugated siRNA-s where decision trees for DDI risk assessment are also proposed: Humphreys, Sara C et al. "Considerations and recommendations for assessment of plasma protein binding and drug-drug interactions for siRNA therapeutics." Nucleic acids research vol. 50,11 (2022): 6020-6037. doi:10.1093/nar/gkac456 2) A very recent update on ADCs: Beaumont, Kevin et al. "ADME and DMPK considerations for the Discovery and Development of Antibody Drug Conjugates (ADCs)." Xenobiotica; the fate of foreign compounds in biological systems, 1-44. 31 Oct. 2022, doi:10.1080/00498254.2022.2141667 3) And therapeutic peptides (manuscript in preparation by the EFPIA peptide DDI working group headed by Carolina Säll, recently submitted to CPT and presented at the 2022 DMDG oligonucleotide and peptide workshop) 4) For peptides, the FDA Drug-Drug Interaction Assessment for Therapeutic Proteins Guidance for Industry 2020 draft document also contains a decision tree for DDI risk assessment that could be adopted: https://www.fda.gov/media/140909/download Page 8, lines 238-240 The section should be amended with an additional paragraph on a wider range of modality-specific strategies as well as the reference from the evolving DDI landscape as in the FDA 2020 guidelines: https://www.fda.gov/media/134582/download Page 2, II. / 3rd Paragraph	Recommended addition to text after line 181. For new drugs beyond small chemical molecules and the therapeutic peptide aspects directly covered in these guidelines, such as oligonucleotide-based therapeutics (siRNA, ASOs, etc.), PROTACs or other new modalities, specific DDI strategies may apply which should be guided by the identification of known or suspected mechanisms for DDI that should inform subsequent experimental risk assessment workflows with absence of DDI risk based on molecular characteristics sufficiently justified. This guidance outlines a general framework for conducting in vitro experiments and interpreting in vitro study results to determine the potential for clinical DDIs. The recommendations in this guidance are based on current scientific understanding. The recommendations outlined here may be periodically updated as the scientific field of DDIs evolves and matures.
Janssen R&D	175	176	1,3	Oligonucleotides are generally considered small molecules but do not exert metabolic or transporter mediated DDIs.	These aspects in general apply to the development of small chemical molecules excluding oligonucleotide drugs.
Novo Nordisk	175	177	1,3	The scope of the guideline is mainly small chemical molecules. Suggest to add that peptides larger than 2kDa should be out of scope for this guideline. The recently submitted Cross-industry White Paper by Säll et al. states that available submission packages reveal DDI likelihood is low for peptides >2 kDa, making it reasonable to adopt a risk-based approach during drug development for larger peptides. (Säll et al. Industry perspective on therapeutic peptide drug-drug interaction assessments during drug development: a European Federation of Pharmaceutical Industries and Associations white paper. Submitted to Clinical Pharmacology & Therapeutics. October 2022.	Suggest to include that "peptides larger than 2kDa are out of scope for this guideline".
AbbVie	182	186	1,3	Improve comprehensiveness of the guidance by providing brief descriptions on other PK interactions.	Brief description of these other PK interactions, similar to therapeutic protein and ADC DDIs, would enhance the comprehensiveness of the guideline.
Bayer AG	194	194	1.4.	victim DDIs can arise from induction and/or inhibition of not just elimination processes but also absorption (GIT efflux transporters like P-gp), distribution (active liver uptake)	we suggest the following wording '... involves identification of the principal routes of the drug's absorption, distribution & elimination.'
Amgen	197	199	1	Original text: "In some instances, e.g., if a large part of the dose is found as unchanged drug in feces, an absolute bioavailability study can also be a useful complement to aid interpretation." We recommend that the guideline provide a criterion on the percentage of unchanged drug instead of large part of dose.	Amgen recommends the following revision: "In some instances, e.g., if a large part of the dose (e.g. >10-15%) is found as unchanged drug in feces, an absolute bioavailability study can also be a useful complement to aid interpretation."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Gilead Sciences	199	201	1.4	"Using data from the mass balance study, the quantitative contributions of the different elimination pathways should be estimated based on the amount of dose excreted as primary and secondary metabolites along specific routes'	The statement is not scientifically sound and can be mis-leading. Mass balance data should not be used to identify the elimination pathways for DDIs, particularly transporter DDIs. For example, atorvastatin is mainly metabolized by CYP enzymes in a Mass balance study, but the rate-determining clearance pathway is the OATP-mediated hepatic uptake that is confirmed by DDI studies.
AbbVie	203	203	1,4	Clarification. "Identify the main enzymes or transporter proteins'. Should it not be both? i.e. and/or	Identify the main enzymes and/or transporter proteins
AbbVie	207	210	1,4	Identification of DDI risk should be in a new paragraph or the 'perpetrator' piece should be moved up to the prior paragraph. Right now the clinical management seems to be associated with 'perp'	Move up in section
Immunic AG	207	210	1.4	This text is misleading because the reader may understand that a potential DDI (based on in vitro experiments) always requires a clinical DDI study.	Please add the following text to this section of the guideline: *Modeling and simulation approaches (mechanistic static or PBPK) can also be used to translate in vitro results to the clinical setting (see sections 3.1 and 7.3 of this guideline).*
WuXi Apptec, DMPK-NJ	212	213		… should be gained "as early in drug development as practically possible" to ensure… typically phase 2/3 studies.	This implies in vitro DDI studies should be performed during Phase 1 studies (before phase 2/3).
Roche	215	215		Consider removing "typically in phase 2/3" as oncology studies enroll patients in Phase I as well.	
EuropaBio/VCLS	218	219	1,4	For oncology drug development, Drug-Drug interaction are not mentioned neither in ICH S9 guideline nor in Q&A document relative to ICH S9. Does ICH M12 guidance mean that, in oncology, in vitro DDI studies (CYP and transporters) should be conducted before FIH, knowing that most often patients are included in these trials, not healthy volunteers. Considering that these patients are the most concerned by polypharmacy, ICH M12 may clarify this point.	Clarify the timelines of the in vitro and in vivo DDI studies as regards ICH S9 and ICH M3(R2) guidelines.
EuropaBio/VCLS	218	219	1,4	Considering that ICH M3(R2) mentions that DDI should be conducted before Phase 3, ICH M12 may clarify which guideline prevails to avoid any misunderstanding.	
EuropaBio/VCLS	218	219	1,4	Does it mean that CYP phenotyping should be conducted before phase 1?	
Novo Nordisk	218	225	1,4	Why is phenotyping required before phase 1 – How does information from phenotyping studies guide phase 1 studies	Please elaborate
WuXi Apptec, DMPK-NJ	218	219		In vitro data … should be obtained before starting phase 1 (first-in-human) to evaluate metabolic stability…	This states that in vitro phenotyping studies should be performed before Phase 1 studies.
EuropaBio/VCLS	221	224	1,4	"If in vitro studies suggest the possibility of clinically significant interaction with inhibitors or inducers of a metabolic enzyme, it is preferable that dedicated clinical DDI studies be conducted prior to studies in patients". "It is preferable" should be clarified. Does it mean that in vitro DDI studies on enzymes (substrate and inhibition) should be conducted before phase 2 (non-oncology) or before FIH (oncology)?	
Janssen R&D	223	224	1,4	In oncology, the FIH study is often undertaken in patients taking various concomitant medicines. As such, conducting a clinical DDI in HV prior to dosing in patients is not possible.	If in vitro studies suggest the possibility of clinically significant interaction with inhibitors or inducers of a metabolic enzyme, it is preferable that dedicated clinical DDI studies be conducted prior to studies in patients, when appropriate and feasible.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Bayer AG	226	229	1,4	moderate inhibitors should also be considered in some cases, e.g. fm close to 1	Based on results of the mass balance study and in vitro studies, clinical studies with strong (or in justified cases with moderate) index enzyme inhibitors and inducers should be considered to confirm and quantify the main metabolism pathways and define the risk for clinically significant DDIs.
EuropaBio/VCLS	226	227	1,4	"The results of the mass balance study should generally be available before starting phase 3". However, if reaction phenotyping on CYP has been made preIND, that means that another reaction phenotyping CYP study should be needed to estimate the contribution to ≥25% if the mass balance study suggests metabolism as an important elimination mechanism. Is it correct? Thanks for clarification.	
EuropaBio/VCLS	232	234	1,4	"If a drug has limited absorption or is expected to undergo significant active hepatic uptake, biliary excretion or active renal secretion as unchanged drug, the relevant transporters should be identified in vitro before clinical studies in patients to avoid protocol restrictions". Does it mean that, for oncology clinial trials, the transporter investigations should be conducted as preIND-enabling studies?	
Gilead Sciences	232	235	1.4	"to undergo significant active hepatic uptake, biliary excretion or active renal secretion as unchanged drug, the relevant transporters should be identified in vitro before initiating clinical studies in patients to avoid protocol restrictions."	The information of biliary excretion is likely not obtained from phase I studies. Need to specify if the information obtained from preclinical species is relevant. Also later, the transporters that are involved into biliary excretion should be specified, as apparently the role of MRP and MATE transporters on biliary excretion is not required to be assessed.
Bayer AG	236	238	1.4.	It is stated that information on the perpetrator potential of the investigational drug towards major drug transporters should be available before administering it to patients. It is not said, however, which are considered "major" transporters (e.g. P-gp, BCRP and OATP, or all transporters included in the guideline?).	We propose to clarify which transporters should be investiagted before administering the investigational drug to patients. E.g. UDP-glucuronosyl transferase (UGT) enzymes and transporters
Bayer AG	239	242	1,4	Not clear whether this refers to in vitro or in vivo assessment of metabolite data (or both)	Recommend to specify whether this refers to in vitro or (clinical) in vivo data
Bayer AG	249	251	2.1.1.	The sentence "If the mass-balance study suggests metabolism as an important elimination mechanism for the drug, enzymes involved in metabolic pathways which based on the mass-balance study are estimated to contribute to ≥ 25% of drug elimination should normally be identified" is overly complicated and could be shortened for better readability	Recommend to shorten, e.g.: "Enzymes involved in metabolic pathways which - based on the mass-balance study - contribute to >= 25% of total drug elimination, should normally be identified by in vitro screening"
Bayer AG	259	259	2.1.1.	CYP1A1 substrates can also be subject to victim DDIs (e.g. Riociguat)	suggest to include CYP1A1 in list of additional CYPs to investigate
Janssen R&D	259	259	2.1.1	CYP1A1 mediated DDIs recently have been reported to be of clinical significance	Other CYP enzymes, including CYP1A1, CYP2A6, CYP2E1, CYP2J2, and CYP4F2.
Amgen	263	265	2	Original text: “The most frequently evaluated, Uridine 5’-diphospho-glucuronosyltransferase (UDP-glucuronosyl transferases (UGTs)), are responsible for glucuronide conjugation of drugs and metabolites.” Edit recommended for proper use of parenthesis.	Amgen recommends the following revision: “The most frequently evaluated, Uridine 5’-diphospho-glucuronosyltransferase (UDP)-glucuronosyl transferases (UGTs)), are responsible for glucuronide conjugation of drugs and metabolites.”

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Certara Integrated Drug Development	263	263	2.1.1	For UGT phenotyping, we proposed an approach similar to CYP phenotyping where the most common hepatic UGT enzymes should be investigated first versus the proposed extensive panel of 11 isoforms. As an example, the intestinal UGT1A10 with limited representative drug substrates might add little value to the panel. Please reduce the list of UGTs to include only UGTs associated with in vivo DDIs (and significant pharmacogenetic differences). Regarding the "other phase 2 enzymes" could you please clarify the scope of phenotyping. Is identification of a Phase 2 metabolite specific to one of the enzyme family adequate or is additional work with recombinant isoforms expected, if so, which isoforms?	
Janssen R&D	265	266	2.1.1	Mainly is not quantitative enough. There can be 10 pathways of which glucuronidation is the most important pathway representing 20% of total clearance.	A phenotyping study is recommended for an investigational drug if it is mainly eliminated by direct glucuronidation represents more than 50% of the drugs elimination.
Bayer AG	275	276	2.1.1.	FDA recommends pharmacogenetic studies for highly polymorphic enzymes and a fraction metabolized of >= 80%	This is quite vague and also not reflected in 4.1. Suggestion to align with FDA
EuropaBio/VCLS	277	280	2.1.1	Does it mean that if the investigational drug is a substrate of CYP2D6 with a metabolic pathway≥25% of total elimination, a clinical study with a strong inducer of CYP2D6 will not be needed?	
WuXi Apptec, DMPK-NJ	283	284	2.1.2 & 2.1.2.2	Clarification of "Time-dependent inhibition" to be "Irreversible time-dependent inhibition" as opposed to "reversible inhibition" mentioned in (line 283 and 287)	
Bayer AG	284	285	2.1.2.	paragraph entitled '2.1.2. Drug as an inhibitor of CYP enzymes' but contains that sentence 'Investigation of potential inhibition of UGT enzymes is further discussed in Section 2.1.3. For details on the experimental 286 setup for these experiments, refer to Sections 7.1.1 and 7.1.3'	suggest to remove that sentence altogether.
Bayer AG	284	284	2.1.2.	Reference to Section 2.1.3. does not fit to the header	Remove or move under 2.1.
WuXi Apptec, DMPK-NJ	297			To do CYP inhibition DDI assessment, Cmax (Cmax at the highest recommended dose at steady state) is used in the equation (line 295)	where "the highest recommended dose" usually is obtained in phase 2/3)
Bayer AG	298	309	2.1.2.1.	We fully agree that the use of actually measured plasma protein binding data should be allowed over the mandatory use of a value of 1%, as, in our estimation, the uncertainties in the determination of fu are by no means larger compared to those for other assays such as IC50 determinations, where such standards are not applied. We agree to the use of a validated PPB assay systems for these means and we believe that it is imperative to apply rigid bioanalytical criteria. However, we consider the mandatory use of fully validated bioanalytical methods for matrices used in PPB assays as undue. To our knowledge, this is also not practice across the pharmaceutical indstry.	We propose to rephrase the section on plasma protein binding by restating more clearly what is the expectation with regard to the BA method and to generally reconsider the mandatory use of a fully validated method.
WuXi Apptec, DMPK-NJ	301	302	2.1.2.1	…the measured fu,p can be used if the accuracy and precision of measurement is demonstrated.	It implies that there is no need to demonstrate accuracy and precision of measurement if measured fu,p is > 1%.
Certara Integrated Drug Development	303	303	2.1.2.1	Line 303. Please clarify what criteria needs to be achieved to consider a plasma protein binding method acceptable as a "full validation"? Alternatively, we suggest to remove that wording: "Such a demonstration should include full validation data of the protein binding assay including a bioanalytical method with appropriate positive controls (i.e., drugs with high binding to relevant plasma proteins)."	

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EuropaBio/PTC Therapeutics Limited	310	316	2.1.2.1	For estimation of the in vitro inhibition constant (Ki) value, PTC requests clarification of whether this requires the human intestinal microsomes to be used in the assay or whether it could be assumed that the value Ki for intestinal CYP3A Ki will be equivalent to the liver CYP3A Ki.	
EuropaBio/PTC Therapeutics Limited	310	316	2.1.2.1	Lines 310 through 316 and Line 518 suggest that the maximum clinical dose/250 mL be used as intestinal concentration to predict inhibition potential of intestinal CYP3A, P-gp, and BCRP for orally administered drugs. PTC agrees this should be the ideal situation for highly soluble drugs, however, for drugs with poor aqueous solubility but with high dose, the soluble concentrations in the intestine may be much lower than estimated based on the above equation. Conversely, investigational drugs that are highly lipophilic may also undergo lipid absorption via the lymphatic system and this may further minimize the DDI potential mediated by intestinal CYP3A, P-gp, and/or BCRP.	PTC suggests the solubility of a drug product in a vehicle that simulates intestinal fluids may be used as a substitute if an investigational drug is highly lipophilic.
Certara Integrated Drug Development	312	312	2.1.2.1	Please use Kiu (ie unbound)	
EuropaBio/VCLS	312	312	2.1.2.1	Suggest mentioning that Ki is unbound in the formula	Ki,u
Janssen R&D	312	312	2.1.2.1	For intestinal inhibition Ki is proposed whereas for hepatic inhibition Ki,u is proposed. The rationale for this is unclear.	
Sanofi	312	312	2.1.2.1	The projected drug concentration in the intestine is calculated as dose/250ml, which results in very significant overestimation of the concentration for many compounds especially for low soluble compounds. Overestimation makes compounds appear to inhibit CYP significantly, which is misleading.	Propose to make the GI tract concentration estimation flexible, such as using two methods: using dose/250ml for high soluble compounds; for low solubility compounds, sponsors can measure solubility of compounds in simulated intestinal fluid, in this case, compounds are prepared in clinical formulation.
Janssen R&D	313	315	2.1.2	With every IC50 some inhibition will always be predicted.	If risk for clinically relevant (or significant) inhibition cannot be excluded using this basic method, mechanistic static and/or PBPK models can be used to interpret the in vitro experiment results (refer to Section 7.3). If in vitro data and modeling do not exclude the risk for a clinically relevant (or significant) inhibition, a clinical DDI study with a sensitive index substrate should be conducted.
EuropaBio/VCLS	329	346	2.1.2.2	In case of irreversible inhibition of intestinal CYP3A4, what is the equation and cutoff to be taken into account? Do we have to follow the equation from PMDA guidance or the EMA guidance?	Add equation and cutoff for irreversible inhibition of intestinal CYP3A4
Sanofi	331	343	2.1.2.2	Regardind Cut off values, the risk is excluded when < x value and latter in the text, it is mentionned R > x to interpret in vitro experiments	be consistent between the equation and the text: proposal "the risk of in vivo inhibition can not be excluded (…) if (kobs+kdeg)/kdeg ≥ 1.25" or the other way around
Roche	333	333		Should the "5" in the equation be "50" instead per FDA DDI guidance?	

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Amgen	334	334	2	Original text: Kobs: kobs = (kinact × 5 × Cmax,u) / (KI,u + 5 × Cmax,u). The other regulatory guidance used to have scalar 50 (e.g FDA) or no scalar (e.g. EMA). Please provide rationale for 5x multiplier applied to Cmax,u (for induction study the scaler is 10: line 435).	We recommend alignment on the scalar factor in this guideline.
AbbVie	337	337		Typo - Refer to Table 6, not Table 5	
Janssen R&D	347	347	2.1.3	Recommend providing a cut-off for when direct glucuronidation is considered a main metabolic pathway for elimination.	
Charles River	350	352	2.1.1	Should define "major elimination pathway" more clearly.	If direct glucuronidation is one of the major elimination pathways (≥ 25% of elimination) of an investigational drug, it is recommended to study in vitro whether the drug can inhibit UGTs including UGT1A1 and UGT2B7.
Janssen R&D	350	352	2.1.3	UGT2B17 is a polymorphic enzyme, and has been shown to be responsible to PK variability for several drugs. Suggest adding UGT2B17.	If direct glucuronidation is one of the major elimination pathways of an investigational drug, it is recommended to study in vitro whether the drug can inhibit UGTs including UGT1A1, UGT2B7 and UGT2B17.
WuXi Apptec, DMPK-NJ	351	352	2.1.3	"…one of the major elimination pathways…"	Please define "major"
WuXi Apptec, DMPK-NJ	351			If direct glucuronidation is one of the major elimination pathways of an investigational drug, it is recommended…	Accurate "major elimination pathway" is obtained based on Metabolite identification results of radiolabeled mass balance, usually in Phase 2/3. So the need for UGT inhibition evaluation will not be confirmed until after Phase 2/3.
Certara Integrated Drug Development	354	354	2.1.3	The guideline states that "When an investigational drug is to be used with another drug that is mainly metabolized by direct glucuronidation, it is recommended to evaluate the in vitro potential inhibitory effect of the investigational drug on the UGT isoform(s)" responsible for the elimination of the other drug. This statement seems very suitable also for the more unusual CYPs (or other drug-metabolizing enzymes). We would propose that, depending on the therapeutic window of the new drug, if a drug is mainly metabolized by a less studied enzyme, the perpetrator potential of common comedications should be considered. Please clarify the expression "is to be used". Does this relate to rather common comedication?	
EuropaBio/VCLS	354	357	2.1.3	Does it apply to combination products only, or does it mean that in case of add-on therapeutic, the potential inhibitor effect of the investigational drug should be considered on the list of other drugs already taken by the patients (in case of direct glucuronidation of these other drugs)	
Roche	355	355		Replace "used" with "frequently used" to differentiate from occassional comedication use.	
Charles River	363	365	2.1.4	Both mRNA and enzyme activity changes should be captured. While indeed, using activity endpoints, concomitant inhibition cannot be ruled out, on the other hand, mRNA level changes do not necessarily translate to changes in enzyme activity. Discrepancies between the two readouts could inform evaluation of DDI risk.	To assess the DDI liability of a drug as an inducer, studies should be performed in human hepatocytes from at least 3 individual donors and the extent of enzyme induction should be measured as changes mRNA and enzyme activity levels.
WuXi Apptec, DMPK-NJ	363	380	2.1.4	CYP2C8 and CYP2C9 are not mentioned in the text	Consider changing CYP2C to CYP2C8, CYP2C9 and CYP2C19

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Janssen R&D	375	380	2.1.4	For a drug which induces CYP3A4, it is unclear whether the clinical DDI study should include CYP2C8, CYP2C9 and CYP2C19 substrates or if a single CYP2C substrate is sufficient.	
Roche	381	382			It would be great if the threshold value for the perpetrator DDI risk of OCT2/MATE1/MATE2-K (0.02) could be clarified in the text.
Janssen R&D	383	386	2.1.4	In case of solubility limitations Emax cannot be reached but still initial slope is relevant since this represents the worst case scenario assessment for induction.	If the basic method indicates induction potential, the evaluation can continue using more quantitative approaches (e.g., correlation methods) provided it is possible to study a wide range of concentrations of the investigational drug to determine induction parameters (e.g., Emax and EC50). calculate EC50 Emax or the initial slope of induction.
Gilead Sciences	386	387	2.1.4	"For the more quantitative approaches, one well-performing, qualified batch of hepatocytes is sufficient"	The statement conflicts to the above statement (line 364 P12) of "at least 3 individual donors and the extent of enzyme induction should be measured at mRNA level"
Charles River	397	399	2.1.4.1	Current wording does not sufficiently clarify whether both provided criteria need to be met for the need for further evaluation.	In vivo induction potential cannot be excluded if the drug in hepatocytes from at least one donor meets both of the following criteria, and further evaluation of the induction potential should be conducted:
EuropaBio/VCLS	401	402	2.1.4.1	Coud you please confirm that the highest concentration to be tested in the CYP induction study should be 15x Cmax,u and that the hepatic inlet concentration is not needed anymore for CYP induction, even in the case of oral drugs?	
Janssen R&D	401	402	2.1.4.1	FDA uses Imax,u (e.g., 30x Cmax,u) to determine whether a clinical DDI study should be conducted. When protein binding is determined to be less than 1% (fu<0.01) it is indicated that a default value of 0.01 should be used in the calculation. Section 2.1.2.1 indicates that in some situations fu,p <0.01 can be measured. therefore, this sentence should be corrected to be in alignment with the statements in section 2.1.2.1.	the fold-change of CYP mRNA expression is ≥ 2-fold at 15× Cmax,u (default fu,p = 0.01, if fu,p <0.01 not experimentally determined to be < 1% as per also refer to Section 2.1.2.1).
Janssen R&D	401	402	2.1.4.1	Clarification is requested as to whether separate criteria for induction of intestinal CYP3A4 should be added versus just using the criteria used for liver CYPs (eg CYP3A4, CYP2C's, CYP1A2, CYP2B6; i.e, 2-fold at 15X Cmax,u).	
Amgen	403	407	2	Original text: "In addition, the induction potential cannot be ruled out for an investigational drug that increases CYP enzyme mRNA less than 2-fold of the vehicle control but more than 20% of the response of the positive control. Further evaluation is recommended when there is an inconclusive finding, e.g., conducting in vitro testing with hepatocyte from another donor that has .6-fold mRNA increase of the CYP enzyme by a positive control." This statement should include an exemption for the induction of CYP2Cs (2C9 and 2C19), which are very challenging to evaluate the induction potential.	Amgen recommends the following revision: "In addition, the induction potential cannot be ruled out for an investigational drug that increases CYP enzyme mRNA less than 2-fold of the vehicle control but more than 20% of the response of the positive control, except for the induction of CYP2Cs (2C9 and 2C19), which are very challenging to evaluate the induction potential. Further evaluation is recommended when there is an inconclusive finding, e.g., conducting in vitro testing with hepatocyte from another donor that has ≥6-fold mRNA increase of the CYP enzyme by a positive control."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Certara Integrated Drug Development	403	403	2.1.4.1	The concept that a negative signal needs to be also < 20% of induction signal obtained with the positive control, has been misunderstood by many over the years. Thus, we feel that this text needs to be very clear. A proposal is made below, only focusing on the 6-fold change. An example could also be added. "The positive controls shows whether their specific induction pathway is present and sufficiently active in the hepatocytes. To ensure that the hepatocyte batches are sufficiently sensitive to inducers, the positive control signal needs to be >6-fold at individual batch level. "	"The positive controls shows whether their specific induction pathway is present and sufficiently active in the hepatocytes. To ensure that the hepatocyte batches are sufficiently sensitive to inducers, the positive control signal needs to be >6-fold at individual batch level. "
WuXi Apptec, DMPK-NJ	420	421	2.1.4.2	RIS and R equations and EC50 definition (the concentration causing half the maximal effect)	Since Cmax,u is used in the RIS equation, the proper in vitro parameter for the calculation should be "EC50,u), as indicated in Section 7.1.1 lines 1329-1330. (Note Line 419 and 420 are switched)
Amgen	447	447	2	Original text: "In vitro induction studies can also detect enzyme down-regulation." We recommend indicating the criterion for enzyme down-regulation.	Amgen recommends the following revision: "In vitro induction studies can also detect enzyme down-regulation by 50% or more."
EuropaBio/VCLS	448	452	2.1.4.4	In case of a teratogen product, it is quite usual that EMA requests an in vivo study regarding its effect on contraceptive steroids, regardless of the in vitro induction results. This aspect was not covered in the draft guidance, is it intentional?	
Sanofi	448	452	2.1.4.4	Down regulation: give more insights on additional in vitro and/or clinical studies to be launched as no clear in vivo effect has been really evidenced so far	Suggest using PBPK modelng to derisk the down-regulation effect
Certara Integrated Drug Development	449	449	2.1.4.4	The draft guideline states that "If concentration-dependent down-regulation is observed in vitro and is not attributable to cytotoxicity, additional in vitro or clinical studies can be considered to understand the potential clinical consequences." It is not unusual that concentration-dependent decreases in mRNA occur. Besides down-regulation, this can be seen as a sign of toxicity which will impact the ability of induction to be detected in the assay. Thus, either it can be interpreted as downregulation or as an inconclusive in vitro study. In both cases, there is a need to follow this up with more data. These could come from in vitro studies (shorter duration, validated ligand-binding assays, or if beneficial, reporter gene assays). Please address whether an in vitro induction study having a reduction in mRNA is still seen as conclusive study for induction investigations.	
AbbVie	458	460		Cinical DDI via P-gp or BCRP inhibtion in the liver or kidney is know to have limited impact. Given that, P-gp or BCRP substrate assessment for parenteral drugs may not be necessary.	Remove the sentence "Because P-gp and BCRP are also…a major elimination pathway of the drug".
Amgen	468	470	2	Original text: "Organic anion transporter (OAT)1, OAT3, and Organic cation transporter (OCT)2 are renal uptake transporters. Multidrug and toxin extrusion protein (MATE)1 and MATE2-K are renal efflux transporters." We recommend combining the these sentences for additional clarity to indicate MATEs and OAT1/3 and OCT2 are involved in active renal secretion.	Amgen recommends the following revision: "Organic anion transporter (OAT)1, OAT3, and Organic cation transporter (OCT)2 are renal uptake transporters. Multidrug and multidrug and toxin extrusion protein (MATE)1 and MATE2-K are renal efflux transporters."
AbbVie	478	485	2.2.1	The guidance states, "Besides the above-mentioned transporters, the importance of in vitro evaluation of a drug as substrate of additional transporters can be decided on a case-by-case basis." However, there is no supporting clinical evidence and there is no guidance on how to determine this on a 'case-by-case basis.'	remove wording from guidance

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EuropaBio/VCLS	478	485	2.2.1	Mentioning that the decision to evaluate the additional transporters MRP2, OATP2B1 and OCT1 can be based on the site of action, passive permeability and knowledge about absorption and elimination pathways is too vague as elimination pathway investigation may not be available at the time of this decision. Suggest including these transporters in the substrate studies.	
AbbVie	481	482	2.2.1	Text suggests OAT2B1 is "responsible for absorption of certain drugs", which is a misleading statement.	Consider revising to state “may participate in” instead of “is responsible for”
Janssen R&D	481	481	2.2.1	Expression and functionality of OATP2B1 is being investigated (Pharmacol. Ther. 2019 Apr;196:204-215. doi: 10.1016/j.pharmthera.2018.12.009.) OATP2B1 also expressed in liver (similar expression level as for OATP1B3) and may contribute to hepatic elimination of some drugs.	OATP2B1 is an uptake transporter present in the liver and intestines, and is responsible for absorption of certain drugs;…
AbbVie	493	493	2.2.1.1	The guidance states, "For uptake studies, if there is significant uptake of a tested drug in transporter-expressed cells relative to the vehicle control-transfected cells (e.g., ≥2-fold than controls) and the uptake in transporter-expressed cells can be inhibited by more than 50% by a known inhibitor of the transporter, the tested drug can be considered a substrate of the transporter examined." However, the cut-off of '>2-fold' than controls should be removed because cut-offs are discussed on lines 501-503 and the Sponsor may choose different cut-offs based on experience and justification of alternative methods.	remove the (e.g. >2-fold than controls).
Janssen R&D	497	497	2.2.1.1	In the recent FDA in vitro DDI Guidance use of either the net flux ratio or the efflux ratio is allowed. It is recommended that the rationale for only using the net efflux ratio be added to the text.	
Janssen R&D	502	503	2.2.1.1	Guidance on Include proposed cut-offs for vesicular assays would be welcome.	
Charles River	508	511	2.2.2	Inclusion of MDR3 inhibition in the consideration panel for hepatotoxicity is recommended along the same reasoning as BSEP. Yoshikado, Takashi et al. “Itraconazole-induced cholestasis: involvement of the inhibition of bile canalicular phospholipid translocator MDR3/ABCB4.” Molecular pharmacology vol. 79,2 (2011): 241-50. doi:10.1124/mol.110.067256 Aleo, Michael D et al. “Evaluating the Role of Multidrug Resistance Protein 3 (MDR3) Inhibition in Predicting Drug-Induced Liver Injury Using 125 Pharmaceuticals.” Chemical research in toxicology vol. 30,5 (2017): 1219-1229. doi:10.1021/acs.chemrestox.7b00048 MDR3 is also routinely included now in in silico hepatotoxicity simulations, such as the DILIsym platform developed by Simulations Plus. https://www.simulations-plus.com/resource/simulating-multidrug-resistance-protein-3-mdr3-inhibition-mediated-cholestatic-liver-injury-using-dilisym-x-a-quantitative-systems-toxicology-qst-modeling-platform/ Watkins, Paul B. “The DILI-sim Initiative: Insights into Hepatotoxicity Mechanisms and Biomarker Interpretation.” Clinical and translational science vol. 12,2 (2019): 122-129. doi:10.1111/cts.12629	Sponsors can consider evaluating the inhibition potential of a drug on other transporters such as BSEP (bile salt export pump, a hepatic efflux transporter responsible for excretion of bile acids and involved in bile acid homeostasis), MDR3 (phospholipid transporter essential for optimal bile formation), MRP2, OCT1, and OATP2B1 on a case-by-case basis.
EuropaBio/PTC Therapeutics Limited	508	511	2.2.2	In Lines 508-511, the investigation of the inhibition potential of a drug on additional transporters (eg, MRP2, OCT1, OATP2B1, and BSEP) have been recommended under certain conditions.	PTC would appreciate inclusion of the recommended ratio and cut-off values for those transporters into Table 1.
WuXi Apptec, DMPK-NJ	509	511	2.2.2	Inhibition potential for BSEP, MRP2, OCT1 and OATP2B1 should be evaluated on a case by case basis	the calculations and cut-off values for these transporters are not mentioned in Table 1 (line 518)
Bayer AG	518	519	2.2.2.	The cut-off value which mandates a clinical study for MATE1 and MATE2K was set to Cmax,u/IC50 <0.02. We acknowledge that this value has been derived from in vitro in vivo correlations and factors in a safety margin as the unbound plasma concentration cannot always be used as surrogate of the unbound intracellular concentration. However, we would like to point out that, for drugs which do not accumulate in renal proximal tubule cells, clincial studies become mandated already at a predicted AUCR of only 2%. This might lead to the conduct of a large number of potetially unnecessary trials. Also, based on literature reviews, for clincial relevant MATE inhibitors, such as Pyrimethamine, Cimetidine and Trimethoprim AUCRs of >200% are calculated (Elsby et al., Pharmacol Res Perspect 5(5), 2017 and Chu et al. Drug Metab Dispos 44(9) 2016).	We propose to closely monitor results of MATE1/2K DDI studies and continually to correlate them with predicted AUCRs, to investigate whether the cut-off of 0.02 might indeed be too strict. In our estimation, a cut off of 0.1 might also provide sufficient safety margins, as long as no active tubular secretion (as indicator of a potential active uptake into the cell) is apparent.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EuropaBio/PTC Therapeutics Limited	518	518	2.2.2	Lines 310 through 316 and Line 518 suggest that the maximum clinical dose/250 mL be used as intestinal concentration to predict inhibition potential of intestinal CYP3A, P-gp, and BCRP for orally administered drugs. PTC agrees this should be the ideal situation for highly soluble drugs, however, for drugs with poor aqueous solubility but with high dose, the soluble concentrations in the intestine may be much lower than estimated based on the above equation. Conversely, investigational drugs that are highly lipophilic may also undergo lipid absorption via the lymphatic system and this may further minimize the DDI potential mediated by intestinal CYP3A, P-gp, and/or BCRP.	PTC suggests the solubility of a drug product in a vehicle that simulates intestinal fluids may be used as a substitute if an investigational drug is highly lipophilic.
EuropaBio/VCLS	518	521	2.2.2	The cut-off values for BSEP, MRP2, OCT1 and OATP2B1 are missing, could you provide them?	Add cut-off values for BSEP, MRP2, OCT1 and OATP2B
EuropaBio/VCLS	518	520	2.2.2	Regarding Cmax,inlet,u, would it be possible to indicate how to calculate this concentration at liver inlet?	[I]in,max = [I]max + (ka x Dose x Fa)/Qh
Gilead Sciences	518	518	Table 1	Table 1: Recommended ratio and cut-off value for drug as inhibitor of transporters	The rationale for easing the cut-off value for uptake transporters but keeping the lower value for efflux transporters should be elaborated.
Janssen R&D	518	518	2.2.2.	Mate -- Ki or IC50 > 50 × Cmax,u (i.e., Cmax,u/ Ki or IC50 < 0.02) --> Propose to harmonize for all transporters including MATE to a margin value < 0.1	MATE1/MATE2-K: Ki or IC50 > 50 × Cmax,u (i.e., Cmax,u/ Ki or IC50 < 0.02 0.1) MATE2-K: Ki or IC50 > 50 × Cmax,u (i.e., Cmax,u/ Ki or IC50 < 0.1)
Roche	518	518		The presentation of this table may be confusing for readers. The title of the table suggested that it's showing the cutoff values for drugs with potential to be inhibitor of transporters but the second column is showing otherwise. For instance, for P-gp and BCRP, the cutoff was shown as "(Dose/250mL)/IC50 < 10" instead of (Dose/250 mL)/IC50 >/= 10	Suggest to either changing the title or the presentation of the cutoffs in the second column.
Sanofi	518	518	2.2.2	Cut-off value for MATEs transporter of 0.02 seems too stringent: leads often to false positive (50 %)	Would recommend to use the FDA 2020 threshold of 0.1

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Certara Integrated Drug Development	520	520	2.2.2	Line 520. Please use one term of unbound hepatic inlet concentrations and show the equation clearly. On line 520 the parameter (Cmax,inlet,u) is explained, but no equation is provided. Line 523. We propose to harmonize the P-gp inhibitory assessment with the EU DDI guideline. When a drug is orally administered, the risk of P-gp inhibition should not only be evaluated using intestinal concentrations. It is still of interest to know whether the drug inhibits hepatic, renal or BBB expressed P-gp. This is needed to evaluate DDIs with parenterally administered, P-gp transported, concomitant drugs. We could have a situation where the DDI risk only relates to orally administered comeds. This is important in particular at some indications where comeds often are given iv such as oncology. For OATP1B1/3 please propose what composes a suitable maximum hepatic inlet concentration for a non-orally administered drug. Please also provide guidance for hepatic uptake transporters for metabolites. Table 1: Please include information also for the transporters MRP2, OCT1 and OATP2B1 to support the in vivo relevance assessment if these transporters are studied. Based on their location, 50*Cmax,u, 10 × Cmax,inlet,u (aligned with ITC publication) and 0.1 x (Dose/250 mL), respectively could be used as cutoffs while awaiting a data driven approach. In addition, please describe when obtaining data on these transporters should be considered. Suggested reference for OCT1: Zamek-Gliszczynski et al, Transporters in Drug Development: 2018 ITC Recommendations for Transporters of Emerging Clinical Importance. Clin Pharmacol Ther. 2018 Nov;104(5):890-899. doi: 10.1002/cpt.1112.	
AbbVie	526	528	2.2.2	The guidance states, "Since the majority of the in vitro inhibitory potency data in those analyses were IC50, both IC50 and Ki values can be used when applying the basic methods above. However, if the potential for an interaction is studied further with modeling approaches, Ki should be determined and used." However there is no rationale for only using Ki values for modeling.	We recommend removing the statement about using Ki values for further modeling approaches or provide a rationale or justification its inclusion.
Sanofi	527	531	2.2.2	It is asumed that IC50 can be used instead of Ki (provied [S]<< Km) but it is recommended to determine Ki for modeling purposes	Rephrase for clarity in order that IC50 can be used instead of Ki if [S] << Km
Janssen R&D	528	528	2.2.2	Clarification is requested as to whether it is also permitted to use a conservative approach and estimate Ki as IC50/2 if substrate concentration is used below Km.	
Janssen R&D	530	531	2.2.2	This would allow the use of IC50/2 as a conservative approach before the need to embark on an elaborate mechanism of inhibition study.	Assuming competitive inhibition, the Ki of an inhibitor approaches IC50 when substrate concentration is much less than Km. Independent of the mechanism of inhibition Ki is never more than 2x smaller than IC50 if substrate is incubated close to Km.
Gilead Sciences	538	539	2.2.2	"Alternatively, the inhibition potential of a drug can be evaluated using mechanistic static models, PBPK modelling, or endogenous biomarkers."	Suggest to add potential "endogenous biomarkers" to this sentence
Janssen R&D	538	539	2.2.2	Suggest specifying which biomarkers are currently considered validated enough. At minimum suggest that CP-I be mentioned as an alternative for a statin DDI.	Alternatively, the inhibition potential of a drug can be evaluated using mechanistic static models, PBPK modeling, or endogenous biomarkers such as …..

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Sanofi	550	552	2.3.1	Clarify significant amounts? Threshold given only for metabolite as Inhibitor, same threshold for substrate? Are only metabolites formed in plasma of concern? Are non-circulating metabolites formed in hepatic system in vitro (suggested at 7.1.2.1 Metabolic Pathway Identification) and found in bile of rodent out of scope (e.g. for CYP Inh.) ?	Consider adding specific values
Sanofi	550	552	2.3.1	Could you recommend the appropriate phase for human metabolite DDI (before end of Phase 2?, after FIH (multiple dose studies)?)	Please add text with recommended drug development stage for human metabolite DDI studies
Sanofi	554	554	2.3.1	it is not clear what is the meaning of available data in "If available data indicate that change in metabolite exposure .." is it from human radiolabeled study?	a) Add specific, similar to Line 587-589 of section 2.3.3; b) Add specific in Section 5.3 interpreting DDI study results regarding metabolites; c) Add specific if metabolite studies are needed in Section 7.1
Certara Integrated Drug Development	555	555	2.3.1	Please include limits on how large fraction needs to be characterized of the formation and elimination of in vivo relevant active metabolites? A language like the one in the EU DDI guideline could be used. "As a general guidance, 50% of the elimination of a metabolite estimated to contribute 50% of the target effect may be used. If the investigational drug is a pro-drug acting through one pharmacologically active metabolite, enzymes estimated to contribute to 25% of the formation and elimination of the active metabolite should if possible be identified."	
Charles River	577	583	2.3.2	The reasoning that in case a clinical DDI study is needed for a specific CYP or transporter-mediated perpetrator interaction with the parent compound, metabolites do not need to be assessed as potential perpetrators as they will be naturally present in the system for a clinical evaluation does not consider the possibility that the metabolites may have perpetrator effects via routes completely different from those affected by the parent compound that will not be caught with the same clinical probe as the parent DDI interactions used as a starting point. This strategy should only be applied if DDI risk linked to metabolites mediated by other CYPs or transporters (where parent risk was not identified) can be ruled out.	If in vitro assessments suggest that the parent drug inhibits major CYP enzymes and transporters and clinical DDI studies are planned, in vitro assessments of metabolites as enzyme or transporter inhibitors may not be needed because the inhibition potential of metabolites would be implicitly reflected in a clinical DDI study along with the parent drug, unless clinically relevant exposures of the metabolite cannot be adequately represented in the clinical DDI study (i.e., the study duration does not allow the metabolite to accumulate). For this approach, however, DDI risk mediated by other transporters or CYP enzymes where clinical follow-up for the parent is not needed, has been sufficiently ruled out using in vitro experiments. In vitro assessments of metabolites is recommended in a similar way as conducted for the parent and can become useful in interpreting the results of DDI studies.
Amgen	584	586	2	Original text: "If in vitro assessments suggest that the parent drug alone does not inhibit major CYP enzymes/transporters or is not expected to inhibit enzymes/transporters clinically, DDI liability due to metabolites as inhibitors can still exist." We believe this text belongs in section 2.3, which describes when to assess for DDI potential of metabolites. We recommend moving this text in section 2.3 as a separate paragraph after line 551.	Amgen recommends the following revision: "2.3 DDI Potential of Metabolites […] As described below, evaluation of the DDI potential of metabolites with significant plasma exposure or pharmacological activities should be considered. If in vitro assessments suggest that the parent drug alone does not inhibit major CYP enzymes/transporters or is not expected to inhibit enzymes/transporters clinically, DDI liability due to metabolites as inhibitors can still exist. As a pragmatic rule, it is recommended to investigate the CYP enzyme and transporter inhibitory potential of metabolites that have AUC metabolite ≥ 25% of AUC parent and also account for at least 10% of drug-related material in circulation (i.e., considered as major metabolite often determined based on radioactivity data)."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
AbbVie	586	589	2.3.2	This recommendation should also apply to both victim and perpetrator determination of DDI potential for metabolites. Thefore we recommend moving this discussion to the beginning of section 2.3.2.	Recommend moving the statement on lines 586-589 to the beginning of section 2.3.2.
EuropaBio/PTC Therapeutics Limited	586	602	2.3.2	In Lines 586 through 589 and Lines 599 through 602, investigation of the CYP enzyme and transporter inhibitory potential is recommended for metabolites that have AUCmetabolite ≥25% AUCparent AND account for at least 10% drug-related material in circulation. While metabolite induction potential on CYP enzymes is recommended if the metabolite has AUCmetabolite ≥25% AUCparent.	PTC requests that the induction potential should only be investigated if both the aforementioned criteria for inhibitory are met.
Janssen R&D	587	589	2.3.2	It should be clarified whether it is acceptable to use total exposure for this assessment.	-
Amgen	599	602	2	Original text: "However, when the drug is a prodrug or a metabolite is mainly formed extra-hepatically, in vitro evaluation of a metabolite's induction potential on CYP enzymes is recommended if the metabolite is a major metabolite and has AUCmetabolite/AUCparent ≥ 25%." Amgen recommends inserting "and also account for at least 10% of drug-related material in circulation" similar to what is recommended for inhibition study for metabolites (line 588).	Amgen recommends the following revision: "However, when the drug is a prodrug or a metabolite is mainly formed extra-hepatically, in vitro evaluation of a metabolite's induction potential on CYP enzymes is recommended if the metabolite is a major metabolite and has AUC metabolite/AUC parent ≥ 25% and also account for at least 10% of drug-related material in circulation."
Roche	692	695	3.2.1.2	"It can be more informative to build a dose adjustment of the victim drug into the study to allow identification of doses that can be administered together in clinical practice". This does not seem feasible when the extent of DDI was not fully known prior to the study initation. More healthy subjects would also be needed. PBPK modeling can also be used to simulate the magnitude of DDI after dose adjustment.	Suggest to delete.
Janssen R&D	718	718	3.2.1.3	Rifampicin is not recommended anymore for clinical DDI studies by the FDA due to high levels of nitrosamines in the API global supply. Please refer to other compounds (carbamazepine/phenytoin)	
AbbVie	721	722	3.2.1.3	The line states "If the substrate demonstrates time-dependent pharmacokinetics, multiple-dose administration of the substrate and a perpetrator should be evaluated." However, some victim substrates demonstrate accumulation with repeated dosing specifically due to enzyme saturation. In such cases, single dose should be preferred to get the highest DDI. Does the guidance mean that in all the cases including ezyme saturation, is multiple dosing of the victim to be conducted? Multiple dosing of the victim during DDI studies is probably not worth it in all cases particularly, if the accumulation is due to enzyme saturation.	Suggest to modify to "multiple-dose administration can be considered if time-dependent PK is thought to be affecting DDI magnitude"
Roche	729	730		"Formulation-related differences in DDI may also occur. There are 'several' examples of excipients resulted in altered DDIs.". The two references (25 and 26) referred to the same molecule.	Suggest to change to "there was an example" or add other examples.
Janssen R&D	751	755	3.2.1.6	Rifampicin is not recommended anymore for clinical DDI studies by the FDA due to high levels of nitrosamines in the API global supply. Please refer to other compounds (carbamazepine/phenytoin)	
Roche	770	770		Discussion on the need for measuring the PK of index inhibitors (expectations, conditions where measurement is recommended, Cmax and Cmax,ss after single or multiple dosing, respectively, etc) would be appreciated	
Roche	785	788	3.2.1.9	This can be challenging even for DDI studies in patients. The long term effect of perpetrators are typically not stuided in a classic DDI study. PK comparison approach is usually used to extrapolate the PD effect.	Suggest to delete.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Roche	789	789		More specific details/examples of nested DDI studies use cases/scenario can encourage the use of this approach in the drug development as this is not a common approach.	Consider citing the following reference as an example where the DDI potential of an investigational drug was studied in the Phase I study: PMID: 34471960
Janssen R&D	811	814	3.2.2	It is unclear whether PK samples will be collected only from participants who have been administered one of the concomitant medicines being evaluated.	
Janssen R&D	821	827	3.2.2	It is unclear whether this refers to population PK analysis based on just one study or a pooled analysis across studies.	
Janssen R&D	821	827	3.2.2	In addition to population PK analysis, if a nested DDI study data could be analysed following the same way as analyzing a stand-alone DDI study data, then the word 'typically' should be changed to 'also'.	Nested DDI studies are typically also evaluated using population PK analysis, which should be performed according to well-established scientific practice using a model that is validated in relation to its purposes.
Bayer AG	836	839	3.2.3.1	moderate inhibitors should also be considered in some cases, e.g. fm close to 1	When evaluating the investigational drug as a substrate, the first clinical DDI studies should, in general, determine the effects of a strong index inhibitor and a strong index inducer on the investigational drug. Moderate index inhibitors or inducers can be used if strong index inhibitors or inducers are not available for a particular enzyme or in justified cases, e.g. with expected exposures
Roche	848	850	3.2.3.1	Should more in vitro studies be conducted before "further clinical investigations with strong inhibitors of alternative candidate enzymes should be conducted"?	Consider to change the statment to: further clinical investigations with strong inhibitors of alternative candidate enzymes should be conducted if indicated relevant by in vitro data.
Roche	857	859		A caveat is that we would not know the most extreme DDI effect and it may be challenging to decide on the most appropriate moderate inducer or inhibitor to use. An alternative option to consider is to perform DDI with the strong inhibitor and PBPK can then be used to simulate the DDI effect mediated by a moderate inhibitor/inducer.	
Roche	891	891		The text mentions that the magnitude of DDI for UGT inhibitors is relatively weak and DDI study should be conducted case-by-case. I fully agree and would like to challenge that this should also apply for non-OATP1B transporter DDIs (renal and intestinal transporters suc as OATs, OCT2 and MATEs). I would appreciate that distinction of OATP1B vs other transporters are made in the section 3.2.5.1 when discussing the factors to determine the need for clinical DDI studies.	
Bayer AG	903	904	3.2.4.1	Suggest to provide specific examples of important variants (SNPs) in UGT molecular species with genetic variation that affect pharmacokinetics, or provide supporting literature, etc. Having common recognition of important mutations (SNPs) that affect pharmacokinetics will greatly improve the evaluation in clinical trials.	Suggest to provide specific examples of important variants (SNPs) in UGT molecular species with genetic variation that affect pharmacokinetics, or provide supporting literature, etc.
Bayer AG	903	903	3.2.4.1.	We suggest adding UGT2B10 and UGT2B17 to the examples given in parentheses	UGT2B10: S. Fowler et al, J Pharmacol Exp Ther 352:358–367, UGT2B17: Y.-H. Wang et al, Clinical pharmacology & Therapeutics2012, 92, 96-102
Gilead Sciences	914	916	3.2.4.2	Investigational Drug as an Inhibitor of UGTs	In this case, it may be premature to include UGT DDI assessment on all potential coadministration with UGT substrates

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
WuXi Apptec, DMPK-NJ	917	917	3.2.4.2	…whether the drug can inhibit UGTs including UGT1A1 and UGT2B7.	The basic model calculations and cut-off values for UGT inhibition potential is not defined in this section. It is mentioned in Section 3 (Clinical Evaluation), line 917 (Section 3.2.4.2). The criterion belongs to in vitro data and should be moved to Section 2.1.3
WuXi Apptec, DMPK-NJ	917	918	3.2.4.2	…propose an alternative with justification.	Since "limited availability of data" (line 914) for UGT inhibition, justifications of an alternative criterion is challenging. Suggest provide justification of using the same criterion as CYP inhibition evaluation for UGT inhibition evaluation.
Gilead Sciences	946	946	Table 2	"When intestinal absorption is limited, or biliary excretion/active renal secretion is a major elimination pathway"	Challenge remains of how to determine biliary excretion is a major elimination pathway in human. Perhaps, some guidance can be useful here e.g., use information obtained from in vitro or preclinical species
Roche	989	991		Metabolic or PD markers "should be" included is a strong recommendation and I believe this has to be case-by-case.	Suggest to change to "may be considered".
Bayer AG	993	996	3.2.5.2	Suggest to specify for which of the endogenous substrates in the recent literature reports Health Authorities/guideline recognize potential utility. While we understand that the knowledge is continuously being updated, we expect that it would improve evaluation of endogenous substrates in clinical trials if Health Authorities/guideline indicate specific examples of endogenous substrates of which they recognize the potential utility.	Suggest to specify which of the endogenous substrates in the recent literature reports Health Auhotities/guideline recognize their potential utility.
Certara Integrated Drug Development	993	996	3.2.5.2	Please provide more information on the use of endogenous biomarkers and what is needed to validate such markers. At present, can results with endogenous substrates be considered as supportive data for modelling, for labelling or for considerations of DDI risk during early drug development?	
Janssen R&D	1011	1022	3.2.6	It will be more instructive to add details on the selection process for the cocktail drugs to ensure the drugs in the cocktail do not interact with each other.	
Janssen R&D	1028	1028	4	UGT2B17 is a polymorphic enzyme, and has been shown to be responsible to PK variability for several drugs. Suggest adding UGT2B17.	Important pharmacogenes include those that encode phase 1 (e.g., CYP2C9, CYP2C19, CYP2D6) and phase 2 (e.g., NAT2, UGT1A1, UGT2B17)
Janssen R&D	1047	1049	4	Guidance would be welcome on how many PM subjects for which plasma PK should be available in order to waive a formal DDI study.	-
Gilead Sciences	1074	1074	4.2	Therapeutic Protein DDIs	A mention ought to be made for oligonucleotide e.g. the FDA has some guidance on DDI liability for oligonucleotides
Charles River	1075	1076	4,2	It should be better defined what is considered a protein for applying this approach and assuming lower DDI risk. Based on the data communicated by the EFPIA Peptide DDI Working group at the 2022 DMDG oligonucleotide and peptide workshop (2-3 October 2022, Amsterdam) for which the manuscript has been recently submitted to CPT so publication is expected for Q1 2023, a cut-off of 2 kDa can be identified above which the frequency of in vitro DDI risk significantly decreases.	In general, the risk of pharmacokinetic DDIs is lower for proteins larger than a 2 kDa molecular size. The in vitro assays that are applicable for small molecules are generally not applicable to these proteins.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Novo Nordisk	1075	1076	4	Suggest to include peptides in this section.	Change to "peptides (>2kDa) and proteins". See comment to line 175-177.
Immunic AG	1081	1101	4.2.1	Not only therapeutic proteins, but also small molecules can elicit pro- and antiinflammatory effects. Cytokine-induced changes in gene expression of metabolizing enzymes and transporters may then cause DDIs.	Please add this information to section 4.2.1. or another section of this guideline.
Sanofi	1081	1081	4.2.1	Any considerations for in-vitro studies or specific monitoring of IL-6? Also, a growing body of literature supports IL-6 increases as the main driver for cytokine interactions. Measing IL-6 increases in combination with adequate PBPK modelling may help to de-risk cytokine DDI.	
Immunic AG	1093	1095	4.2.1	This text is difficult to understand because the drug class of cytokine modifiers is not well established.	Please give examples for the drug class of cytokine modifiers.
Sanofi	1093	1094	4.2.1.	Should explicitly allow sponsor to use PBPK model for justifying therapeutic proteins DDI effect because of cytokines up-/down-regulation on CYP expression	If the investigational drug is a cytokine or a cytokine modifier, sponsors should consider whether to perform a clinical DDI study to evaluate the effects of the investigational therapeutic protein on sensitive substrates for CYP enzymes, PBPK modelling can also be justified.
Certara Integrated Drug Development	1095	1095	4,2	PBPK approaches can be used to inform the DDI risk assessment. Please consider including this possibility in the guideline.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Charles River	1100	1101	4.2.1	After 4.2.1 Proinflammatory Cytokine-Related Mechanism, the alternate scenario for non-proinflammatory cytokine related mechanisms as also proposed in the FDA Drug-Drug Interaction Assessment for Therapeutic Proteins Guidance for Industry 2020 draft document. https://www.fda.gov/media/140909/download Page 3-4, Lines 103-130. It also contains a decision tree for DDI risk assessment that could be adopted: Page 8, lines 238-240	B. Mechanisms of DDIs Unrelated to Proinflammatory Cytokines Mechanisms unrelated to proinflammatory cytokines have been observed or postulated where the TP acts as a perpetrator (e.g., an inhibitor or inducer) or a victim of a small molecule or other TP DDI. Depending on the expected mechanism of the DDI, a TP could be evaluated as a victim or as a perpetrator. Scenarios when DDI evaluation should be considered include: • When a TP affects human physiological processes that can in turn alter the pharmacokinetic profiles of co-administered medications (e.g., GLP-1 receptor agonists such as dulaglutide and albiglutide result in delayed gastric emptying). In this case, the sponsor should evaluate the TP as a perpetrator. • Co-administered medications that impact the TP target or target-mediated disposition. In these cases, depending on the role of the TP in the DDI, the sponsor should evaluate the DDI potential of the TP either as a perpetrator or as a victim. • Co-administered medications that compromise the function of the FcRn can affect TPs which interact with the FcRn (e.g., blocking or interfering with the interaction between TPs containing an Fc region of human IgG and FcRn). In these cases, the sponsor should evaluate the DDI potential of the TP as a victim. • Co-administration of immunosuppressors with a TP whose pharmacokinetics are affected by immunogenicity (e.g., methotrexate on the clearance of adalimumab). Since immunogenicity (i.e., the formation of antibodies to TPs) can alter the clearance of some TPs, drugs that suppress immunogenicity can change the clearance of a TP. In these cases, the sponsor should evaluate the DDI potential of the TP as a victim. This type of DDI evaluation can be difficult to prospectively design, in which case a descriptive analysis can often be considered adequate.
Janssen R&D	1132	1132	5.1.2.	Recommend specifying whether PK parameters would need to be derived from population PK model parameter estimates or from parameters obtained at individual level.	
Bayer AG	1140	1140	5.2.	Currently states to report "in some situations, Cmin" as endpoint	Quite vague and could be aligned with Section 5.1.1. which states Cmin as relevant parameter for assessment at steady state after multiple dosing. In particular important for drugs where pharmacodynamic effect is related to the minimum concentration.
Certara Integrated Drug Development	1246	1246	5.3.3.1	Add something about evaluation of the risk in those complex situations to inform the drug label (especially when the study cannot be run).	
Roche	1246	1246		Recommended adding the use of endogenous biomarkers to understand complex DDI mechanisms	
Certara Integrated Drug Development	1289	1289	7.1.1	The draft guideline states that “Recombinant human CYP and UGT enzymes” can be used to evaluate the risk for enzyme-mediated interactions. . Please include “such as”... not to restrict the use of other recombinant enzymes: Recombinant enzymes, such as human CYP and UGTs.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Bayer AG	1291	1295	7.1.1	Could it be considered to reduce the suggested number of donors (at least 10) for phenotyping and inhibition experiments? It is in practice difficult to use hepatocytes pooled from 10 donors and, considering that "at least 3 individual donors" for induction experiments is recommended, the suggested number for phenotyping and inhibition experiments could be lowered as well.	For consideration - to reduce the suggested number of donors (at least 10) for phenotyping and inhibition experiments
Bayer AG	1293	1295	7.1.1.	we suggest to add hepatocytes from individual donors with well characterized enzymatic activities for phenotyping (or inhibition) experiments. Especially, enzyme activities of single hepatocyte donors are of interest for metabolizing enzymes contributing relevantly to the biotransformation of a drug candidate and its estimation of the victim DDI risk.	For phenotyping and inhibition experiments, hepatocytes pooled from at least 10 donors or hepatocytes from single donors with well characterized activities of the metabolizing enzymes is suggested....
Certara Integrated Drug Development	1319	1319	7.1.1	Please provide some practical advice. When estimating I gut, please comment on the use of FaSSIF and FeSSIF solubility. Could PBPK approaches including sensitivity analyses be utilized to estimate intestinal exposures?	
WuXi Apptec, DMPK-NJ	1322	1323	7.1.1	… a no-solvent control to evaluate potential effect of solvent on enzyme reaction.	Please clarify the interpretation of CYP inhibition data with the solvent effect data. Based on WuXi AppTec experience, commonly used organic solvents such as DMSO, methanol and acetonitrile have limited effect on CYP inhibition potential evaluation when controlled to low levels (e.g., <0.2% for DMSO and <=1-2% for acetonitrile and methanol). In this case, a no-solvent control is not necessary and adds no values to CYP inhibition potential evaluation. Non-organic solvent such as water may actually have impact on CYP activity (because buffer strength or pH changes in the final samples). Uncommon solvents (such as DMF, hexane etc) or solubilizers (such as cyclodextrin, tween-80, etc) may have much more impact on CYP inhibition evaluations. For studies with uncommon solvents/solubilizers, additional investigations are needed to understand if CYP inhibition potential can be appropriately evaluated, but this should be a case-by-case scenario. Therefore we suggest to not include "no-solvent control" in the guidance. Instead, the Sponsors should provide data to show that the test concentrations are soluble in the samples and if solubility limits the test of high concentrations, clinical studies will be warranted. In addition, the sponsor should show CYP inhibition potential is evaluated correctly with proper control data.
Amgen	1323	1326	7	Original text: "There is at present much uncertainty regarding how to interpret in vitro inhibition and induction data when sufficiently high concentrations cannot be tested; thus the general recommendation is to test the DDI potential of these compounds in vivo, unless in vitro testing is sufficiently justified." In vitro studies should be tested at highest possible concentration whenever there is solubility limitation but should not be recommended for in vivo clinical DDI studies just based on inability to test high concentration in the in vitro system since solubility limitation will be similar in vitro and in vivo.	Amgen recommends the following revision: "There is at present much uncertainty regarding how to interpret in vitro inhibition and induction data when sufficiently high concentrations cannot be tested; thus the general recommendation is to test the DDI potential of these compounds in vivo, unless in vitro testing is sufficiently justified."
WuXi Apptec, DMPK-NJ	1329	1339	7.1.1	Actual unbound concentrations of the drug in the in vitro system (e.g., incubation medium) should in general be used for extrapolating in vitro results to in vivo scenarios	Please clarify if nominal concentrations or measured drug concentrations in the medium on the last day of incubation should be used for the calculation of EC50?
WuXi Apptec, DMPK-NJ	1329	1339	7.1.1	… sponsors are encouraged to measure concentrations of the parent drug in the medium on the last day of incubation with hepatocytes and protein binding should also be considered.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Amgen	1333	1334	7	Original text: "Non-specific binding can be measured experimentally (e.g., using equilibrium dialysis or ultrafiltration) or predicted using in silico methods (43, 44)." Amgen recommends adding ultracentrifugation as a non-specific protein binding assay technique.	Amgen recommends the following revision: "Non-specific binding can be measured experimentally (e.g., using equilibrium dialysis or ultrafiltration or ultracentrifugation) or predicted using in silico methods (43, 44).
Certara Integrated Drug Development	1336	1336	7.1.1	The draft guideline states that use of actual unbound concentrations is encouraged in in vitro induction assays. We do agree that this is important as metabolism/degradation could be substantial. Please clarify, how the EC50 and Emax should be adjusted for loss of drug.	
EuropaBio/PTC Therapeutics Limited	1336	1339	7.1.1	As outlined in Lines 1336 through 1339, PTC agrees that the measurement of investigational drug concentrations in the incubation medium provides valuable information on metabolic and/or chemical stability of an investigational drug during incubation period. However, measurement of drug concentration in the medium could underestimate the cellular drug concentration if an investigational drug accumulates in hepatocytes with time, especially after repeated daily dosing.	
Sanofi	1338	1339	7.1.1	Could a threshold value be proposed when measured concentrations are substantially lower than nominal concentrations	Propose to use 80 % as set for Transporters line 1566 and 1567
Charles River	1358	1359	7.1.2.2	The FDA recommends using 2 different types of systems for phenotyping which approach should be maintained to assure data consistency, especially as currently there is no industry-wide consensus on which system is the most reliable and predictive of in vivo situations. FDA 2020 In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions https://www.fda.gov/media/134582/download Page 17-18 VII / A / 1. / b There are two widely used methods for identifying the individual CYP enzymes responsible for a drug's metabolism: (1) the first method uses chemicals, drugs, or antibodies as specific enzyme inhibitors in human liver microsomes or hepatocytes (e.g., a pool of more than 10 donors); and (2) the second method uses individual human recombinant CYP enzymes. The sponsor should consider the following recommendations when performing reaction phenotyping experiments: • The sponsor should use both methods to identify the specific enzymes responsible for a drug's metabolism.	Reaction phenotyping should be done in HLM or hepatocytes using selective enzyme inhibitors (chemicals or antibodies) and in human recombinant enzymes.
EuropaBio/VCLS	1358	1359	7.1.2.2	"Reaction phenotyping can be done either in HLM or hepatocytes using selective enzyme inhibitors or in human recombinant enzymes". Please confirm the "or" to avoid any misunderstanding when compared with FDA guidance which requested that both systems were used.	
Certara Integrated Drug Development	1365	1365	7.1.2.2	Please describe is more detail how in vitro information on enzymes able to catalyze the formation of important primary metabolites observed in the mass balance study could help to support the importance of enzymes in the elimination of a drug as well as formation and elimination of active metabolites. This methodology could be valuable both to inform cases where identifying major enzymes/transporters are particularly difficult, and be used in situations where conventional DDI studies are challenging to perform. The method can also be used to inform PBPK modelling approaches.	
Certara Integrated Drug Development	1377	1377	7.1.2.2	We propose for antibodies to be removed as an option. This method has been completely replaced by other methods.	
Certara Integrated Drug Development	1386	1386	7.1.2.2	Please add that stability issues can exist for glucuronides also in urine and plasma.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EuropaBio/PTC Therapeutics Limited	1388	1420	7.1.3	In Lines 1388 through 1420, it is suggested that an investigational drug be pre-incubated with human liver microsomes at drug concentrations that surround 10-fold or greater of its reversible half-maximal inhibitory concentration (IC50) value, followed by residual enzyme activity measurement after proper dilution (10-fold or greater) to evaluate if an investigational drug is a time-dependent enzyme inhibitor using IC50 shift approach. PTC considers this may be not practical due to solubility limitation even at proportionately increased liver microsomal protein concentrations (≥0.5 to 2 mg protein/mL depending on individual CYPs, as typically 0.05 to 0.2 mg protein/mL be used for IC50 determination). Conversely, enzyme inactivation may be also compromised due to increased protein binding of the investigational drug at such high microsomal protein concentrations.	As an alternative, PTC proposes for an investigational drug that shows low aqueous solidity and/or high protein binding potential, the IC50 shift should be evaluated within solubility range and at lower liver microsomal protein concentrations with minimal dilution after pre-incubation.
Bayer AG	1392	1392	7.1.3.		we suggest using "KI"with capital "I" for TDI
WuXi Apptec, DMPK-NJ	1392		7.1.3	… and Ki and kinact for TDI	Ki KI
Charles River	1396	1398	7.1.3	Higher number of concentrations recommended for reliable inhibition curve fitting and IC50 determination – industry practice is usually to test 6-11 concentrations depending on assay system and provider.	If clinical interaction cannot be excluded at the high concentration, lower drug concentrations should be tested to estimate the drug's IC50 or Ki value; it is recommended to examine at least six different concentrations of the investigational drug.
H. Lundbeck A/S	1407	1416	7.1.3	For determination of TDI, in the ICH M12 draft guideline it is recommended to preincubate with a higher concentration, e.g. 10 fold, of HLM and test compound, and then to dilute 10-fold when adding the probe substrate. We do not agree that this dilution procedure is preferred. When preincubating with a higher HLM concentration there is a risk for lower inhibition due to 1) compounds are more highly bound to the HLM and 2) the inhibitor may be rapidly metabolised and thus, the inhibitor concentration will decrease in the incubation. For more details see reference Parkinson et al 2011 DMD 39:1370-1387	For example, TDI can be detected by assessing a difference in IC50 curves generated with and without a pre-incubation with nicotinamide adenine dinucleotide phosphate (NADPH) (i.e., IC50 shift), decreases in enzyme activity (measurement of the pseudo first-order rate constant, kobs) or percent activity loss with the inactivator over time (also called standard dilution methods). In In the IC50 shift assay, pooled HLM should typically be pre-incubated for 30 min with the investigational drug at concentrations that surround 10-fold (or greater, depending on the dilution factor) of their reversible IC50 values with or without NADPH. The probe substrate should then be added into the pre-incubation samples The pre-incubation samples should then be diluted (10-fold or greater) into an incubation containing probe substrate (at a concentration around its Km for the reaction) and NADPH.
Janssen R&D	1407	1433	7.1.3	1) Parkinson DMD 2011 have published that a TDI approach without dilution has several advantages over the dilution approach's (lower protein concentration, less aspecific binding, less metabolism of the inhibitor over pre-incubation, etc). Propose to add in Parkinson reference at line 1407 to leave the option to screen for TDI in an assay without dilution. 2) In case of IC50 shift a follow up assay with dilution and substrate conc > Km can be used to deconvolute enzyme inactivation from eg a metabolite with reversible inhibition properties. Propose to add this text to current section.	Reference: Andrew Parkinson, Faraz Kazmi, David B. Buckley, Phyllis Yerino, Brandy L. Paris, Jeff Holsapple, Paul Toren, Steve M. Otradovec and Brian W. Ogilvie. An Evaluation of the Dilution Method for Identifying Metabolism-Dependent Inhibitors of Cytochrome P450 Enzymes. Drug Metabolism and Disposition August 2011, 39 (8) 1370-1387. Proposed change to language: Line 1407 There are various assays to identify TDI of CYP enzymes using both dilution and non-dilution methods (Parkinson, 2011). Line 1420: ... particularly at least one with a lower fold-shift (e.g. ritonavir) (52). In case of an IC50 shift a follow up assay with dilution and substrate conc > Km can be used to deconvolute enzyme inactivation from eg a metabolite with reversible inhibition properties

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Charles River	1414	1416	7.1.3	The "dilution method" using a minimum 10-fold dilution before probe and cofactor addition is not widespread among providers, less fold dilutions can be applied at this stage, only when further investigations and kinetics determinations are conducted. The approach has also been assessed by A. Parkinson et al. and the necessity of the >10-fold dilution has not been proven: Parkinson, Andrew et al. "System-dependent outcomes during the evaluation of drug candidates as inhibitors of cytochrome P450 (CYP) and uridine diphosphate glucuronosyltransferase (UGT) enzymes: human hepatocytes versus liver microsomes versus recombinant enzymes." Drug metabolism and pharmacokinetics vol. 25,1 (2010): 16-27. doi:10.2133/dmpk.25.16	The pre-incubation samples should then be diluted into an incubation containing probe substrate (at a concentration around its Km for the reaction) and NADPH.
Charles River	1416	1418	7.1.3	A cut-off range as a reference for identifying a positive shift is confusing, a single specific cut-off value would be more useful for risk evaluation.	A left shift of the IC50 curve (≥ 2-fold) from the samples pre-incubated with NADPH compared to those without, suggests a potential for enzyme inactivation by the investigational drug.
H. Lundbeck A/S	1422	1425	7.1.3	Same comment and rationale as above	When such a method is used, the test compound should be pre-incubated with pooled HLM with and without NADPH typically for 30 min, whereafter the probe substrate is added to the pre-incubation buffer. the reaction should then be diluted appropriately (10-fold or greater to dilute out the test compound).
Charles River	1441	1443	7.1.4	Both mRNA and enzyme activity changes should be captured. While indeed, using activity endpoints, concomitant inhibition cannot be ruled out, on the other hand, mRNA level changes do not necessarily translate to changes in enzyme activity. Discrepancies between the two readouts could inform evaluation of DDI risk.	It is recommended to measure the extent of enzyme induction at the mRNA as well as enzyme activity level. Measuring only the enzyme activity is usually not recommended as the induction could be masked in the presence of concomitant inhibition.
WuXi Apptec, DMPK-NJ	1447	1450	7.1.4	at least 6-fold increase for CYP1A2, 2B6 and 3A4	Consider changing the criteria for CYP2B6 to 4-fold. Based on our CYP2B6 Positive control results ~20 % results are <6-fold (n=213), only ~2.3% are <4-fold
WuXi Apptec, DMPK-NJ	1461		7.1.4	Culture quality should be verified and documented	Please clarify the agency expectation for verification and documentation of culture quality
WuXi Apptec, DMPK-NJ	1467		7.1.4	…demonstrate viability at the start of incubation	Please clarify "the start of incubation"
WuXi Apptec, DMPK-NJ	1467	1468	7.1.4	demonstrate viability at the end of incubation that deviated markedly from viability at the beginning of experiment	Define "markedly"
Certara Integrated Drug Development	1490	1490	7.1.4	Please clarify this text. Is this relevant only for situations where the "d" factor is estimated and not set to 1? Please clarify how the induction parameters should be recalculated if there is substantial degradation during the incubations.	
WuXi Apptec, DMPK-NJ	1490	1497	7.1.4	For the correlation or mechanistic static methods, sponsors can use only one hepatocyte donor.	Please explain if we can only use one "calibrated" lot of hepatocyte donor or can we continue to use 3 lots for mechanistic static method? Also, take into account the difficulty in acquiring sufficient vials of one inducible lot to "calibrate" and continue to do the testing of investigational drugs.
Charles River	1507	1508	7.2.1	Vesicles are not frequently used for MATE interaction evaluation and have very limited literature on predictive value for in vivo situations and calibration with relevant reference compounds.	Sentence should be removed.
Bayer AG	1509	1511	7.2.1.	The guideline suggests the use of non-transfeted vesicles as control experiment when conducting studies with membrane vesicles. In our estimation this control experiment is not necessary, once a control with AMP (i.e. also without active transport) is conducted.	We propose not to manate the conduct of control experiments with untransfected control vesicles.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Amgen	1546	1547	7	Original text: "Transport studies should be performed under linear transport rate conditions (probe substrate concentration used is usually below its Km for the transporter)." For monolayer cell system, which is used to determine efflux transporter kinetics parameters and IC50 value, the linear transport rate conditions may not apply. Amgen recommends clarifying the sentence.	Amgen recommends the following revision: "Transport studies should be performed under linear transport rate conditions. For monolayer cell system where linear transport rate conditions may not apply, the sponsor should add the test drug to either the apical or basolateral side of the cell monolayer and measure the amount of the drug permeating through the cell monolayers in the receiver chamber over time."
WuXi Apptec, DMPK-NJ	1563	1565	7.2.1	The experiment should include a solvent (vehicle) control, and when appropriate, also a no-solvent control.	Based on WuXi AppTec experience, organic solvents such as DMSO and methanol at ≤ 1% in our routine transporter assays have limited effect on transporter studies. In this case, a no-solvent control is not necessary and doesn't add any values to a transporter study. Suggest modifying the sentence "The experiment should include a solvent (vehicle) control, and when appropriate, also a no-solvent control." to "The experiment should include a solvent (vehicle) control. When a higher concentration (e.g. > 1%) or a nonroutine organic solvent is used, it should be compared with the control conditions with a routine organic solvent."
Bayer AG	1566	1567	7.2.1.	The guideline document states that sufficient recovery should be demonstrated in inhibitor and substrate studies and suggests that 80% recovery is sufficient. It is not clear, whether 80% of the drug need to be recovered from the assay media (i.e. had to be in solution), or whether also drug that was recovered from the plasticware (and was non-specifically bound) should be factored in for the calculation of recovery. While we acknowledge the need for the determination of recovery in substrate studies and the loss due to NSB and solubility issues in inhibitor studies, for many drugs it is not possible to achieve recovery >80% (in solution) while it is still possible to reliably determine transport characteristics.	We kindly ask for clarification, whether it is meant that 80% of the compound should be recovered from the assay media or whether the necessity to recover 80 % includes what can be recovered from plasticware after the assay. As the former would, in many cases, not be achievable, we ask to reconsider (ad state more clearly) this cut-off if it is intended to be used as a strict cut-off.
EuropaBio/PTC Therapeutics Limited	1566	1567	7.2.1	In Lines 1566-1567, PTC requests additional clarification around the statement, "For both substrate and inhibitor studies, the sponsor should demonstrate sufficient total recovery of the drugs (eg, 80%)" given investigational drug recovery is typically evaluated for transporter substrate assays only.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Janssen R&D	1566	1567	7.2.1.	The strict cut-off required (80%) does not consider confounding factors from individual experiments as part of data interpretation. For example, in IC50 determinations in an uptake assay, its likely to get lower recovery at lower concentrations because non-specific binding is a factor. But compensation calculations (i.e., measurement of actual incubation concentrations) allow data to still be used for accurate IC50 determination based on measured compound. The FDA in vitro DDI guideline gives a more comprehensive discussion to low recovery: "For both substrate and inhibitor studies, the sponsor should demonstrate sufficient total recovery of the drugs. If the total recovery falls below a pre-specified boundary set by the laboratories, the nature and extent of the effects leading to a decrease of recovery should be investigated and considered when evaluating the potential DDI risk of a test drug. The sponsor should attempt to assess the impact of the following factors: - The stability of the test drug for the duration of study - The effect of nonspecific binding of the test drug to cells/apparatus - The test drug's solubility limits - The effect of adding serum or proteins to the media" Propose to use language included in FDA guidance for recovery as it takes into account nuances such as non-specific binding into account.	For both substrate and inhibitor studies, the sponsor should demonstrate sufficient total recovery of the drugs (e.g., 80% (55)). If the total recovery falls below a pre-specified boundary set by the laboratories, the nature and extent of the effects leading to a decrease of recovery should be investigated and considered when evaluating the potential DDI risk of a test drug. The sponsor should attempt to assess the impact of the following factors: - The stability of the test drug for the duration of study - The effect of nonspecific binding of the test drug to cells/apparatus - The test drug's solubility limits - The effect of adding serum or proteins to the media.
WuXi Apptec, DMPK-NJ	1566		7.2.1	For both substrate and inhibitor studies, the sponsor should demonstrate sufficient total recovery of the drugs (e.g.,	Please clarify whether the studies are for permeation assays or uptake assays. Also when both substrate and inhibition assays are conducted, total recovery of the drug from substrate assays can represent total recovery from inhibition assays. Therefore, there is no need to measure total recovery from inhibition studies.
EuropaBio/VCLS	1573	1575	7.2.2	Would it be possible to provide support to define highest concentrations in transporter subtrate studies? Is it correct to mention that the highest concentration should be the hepatic inlet and intestinal concentrations for hepatic transporters and P-gp/BCRP respectively? It is well noted that high concnetrations may saturate transporters and that lower concentrations than the highest one should be tested.	
EuropaBio/VCLS	1573	1575	7.2.2	What would be the highest concentrations to be used for renal transporters (OAT1, OAT3, OCT2, MATE1, MATE2-K) in substrate investigation?	
Certara Integrated Drug Development	1582	1582	7.2.2	Please clearly state that the high permeability positive control must be included in the Caco-2 cell study. (ie historical comparisons are not allowed)	
Charles River	1597	1600	7.2.3	The current wording suggests a scenario where a preliminary solubility test is run and if the highest recommended test concentration can be reached, the next step is definitely in vivo. We assume this would mean a human clinical DDI assessment, that comes with a significant cost both in terms of cost and project timelines. Reaching the highest recommended concentration in vitro is most commonly an issue for assessing intestinal inhibition of BCRP and MDR1 where the recommended test concentration is 0.1 × the highest therapeutic dose/250 ml. While for all other assays, unbound concentrations are considered, but for intestinal drug levels available for transport this is more difficult to estimate, but it is likely less than the total API content of the drug product at a given timepoint and section of the intestine. To limit running unnecessary clinical DDI investigations and increasing burden on the trial population, we would recommend generating this data in vitro in all cases and considering solubility limits and compound characteristics for risk assessment in a case-by-case basis. A similar angle , in this case to handle insufficient compound recovery, is found for CYP induction in the ICH M12 draft guidelines Page 40, Line 1336-1339.	There is at present much uncertainty regarding how to extrapolate in vitro results to in vivo when sufficiently high concentrations cannot be tested. In such cases, in vitro data should be generated up to the highest soluble concentration and in case for this range, no DDI risk is identified, the need for an in vivo clinical test of the DDI potential should evaluated in a case-by-case basis, taking into account solubility limits and compound characteristics.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Charles River	1605	1607	7.2.3	Higher number of concentrations recommended for reliable inhibition curve fitting and IC50 determination – industry practice is usually to test 6-11 concentrations depending on assay system and provider.	If the test drug demonstrates inhibitory activity at the recommended cut-off concentration, the sponsor should test additional concentrations to estimate IC50 or Ki values. The sponsor should evaluate at least six concentrations of the investigational drug with the probe substrate.
EuropaBio/PTC Therapeutics Limited	1628	1631	7,3	Lines 1628-1631 indicate that "If those assessments indicate further evaluations should be conducted, they may be conducted using mechanistic static models or PBPK models, or by conducting a clinical DDI study". PTC requests clarification on whether this is a tiered approach (ie, static models testing before PBPK models) or whether either model can be exclusively tested. If the latter, PTC request clarification on which model should take priority if the results are different.	
Sanofi	1653	1700	7.3.1.3	Equation to calculate AUCR of the substrate drugs apply mostly to CYP but the equations are in the "Evaluation of The Potential for Transporter-Mediated DDIs" section, line 1659 and 1693	Move the equation in section 7.3.1.2 as most of the parameters refer to CYPs (fm, fg)
Certara Integrated Drug Development	1659	1659	7.3.1.1	The net effect model equation is outlined, but it is never fully used as a net effect model as induction and inhibition cannot be combined. This is agreed, but it would be clearer to separate the induction and inhibition parts of the equation. Please consider using separate equations	
EuropaBio/PTC Therapeutics Limited	1659	1674	7.3.1.3	PTC requests clarification to whether the equations presented to calculate the AUC ratio of the substrate drugs (Lines 1659 through 1674) are applicable to the mechanistic model of both CYP-mediated DDI and transporter-mediated DDIs	
Bayer AG	1672	1674	7.1.3.1.	Although the formulas indcated in Table 3 are well applicable for CYP enzymes, these exact formulas have, to the best of our knowledge, not been proposed for drug transporters in the scientific literature. Also, these formulas for use of the basic and static model are not included in the referenced literature (line 1656) and have never been proposed for transporters in regulatory guidelines, before. As an example, for transporters the term [I]g is not used and was (to our knowledge) never before used in literature or in regulatory guidelines.	We propose to re-evaluate the formulas in table 3 and to check whether they are actually applicable to transporters and to correct the formulas if necessary.
WuXi Apptec, DMPK-NJ	1673	1674	7.3.1.3	Ki, KI, and EC50 in Table 3	Suggest clarify that unbound or total values of Ki, KI, and EC50 should be used for DDI evaluations
Certara Integrated Drug Development	1676	1676	7.3.1	Line 1676, Please describe the equation for the unbound maximum hepatic inlet plasma concentration is a more mathematically correct and easier to interpret manner, making sure it is easy to read. (See Equation 5, Parkinson DMD (2019) 47:779-784). The draft guideline states that the results of ongoing efforts could result in a more quantitative use the mechanistic static model. It seems reasonable to apply a cautious approach to quantitative use of a static and rather crude model. The model will need substantial evaluation due to its scientific limitations. It is likely that the "most relevant drug concentrations in gut and liver will be highly drug (victim and perpetrator) dependent. Please also separate this information, if at all included, from the transporter text.	
Bayer AG	1733	1735	7.3.2.1	Suggest to mention the possibility that the PBPK model could be used to predict the extent of DDI with more intensely interacting drugs, with conditions such as "when it is expected that concomitant use with a strong inducer or inhibitor should be avoided." We expect that PBPK models may also be used to predict the extent of DDI effects with more intensely interacting drugs when a model has been already established for interactions with the indicator drug of the interacting drug.	Suggest to mention the possibility that the PBPK model could be used to predict the extent of DDI with more intensely interacting drugs, with conditions such as "when it is expected that concomitant use with a strong inducer or inhibitor should be avoided."
Bayer AG	1793	1795	7.3.2.2	The current description is vague as to what to do if negative DDI prediction can be supported.	Suggest to mention the possibility for avoidance of conducting clinical trials if negative DDI prediction can be supported.
AbbVie	1812	1812	7.4.1.1	Lines 1816-1818 states the following: "The following tables are provided to help sponsors design in vitro studies and to evaluate the interaction potential (Tables 5-7). These tables are not exhaustive, and sponsors can use other inhibitors/inducers with appropriate justification." However, the sentence only refers to Tables 5-7 and it is unclear whether or not Table 4 should also be referenced.	We recommend changing lines 1816-118 to also reference Table 4.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Janssen R&D	1819	1819	7.4.1.2	N-3-Benzylphenobarbital and benzylnirvanol should also be mentioned as selective inhibitors	CYP2C19: loratadine, ticlopidine*, N-3-Benzylphenobarbital and benzylnirvanol
WuXi Apptec, DMPK-NJ	1819		7.4.1.2	Table 5: Examples of inhibitors for CYP enzymes (in vitro studies: CYP2C19	Please add reversible inhibitor N-3-benzyl-nirvanol to the list for CYP2C19 inhibitors. Based on WuXi AppTec experience, it is a very good CYP2C19 inhibitor.
Janssen R&D	1823	1823	7.4.1.2	Typographical error: CYP2B6	CYP2B6
Bayer AG	1829	1829	7.4.2.1	We propose to add Levomedetomidine as UGT2B10 substrate	http://dmd.aspetjournals.org. doi:10.1124/dmd.108.021709.
WuXi Apptec, DMPK-NJ	1850		7.4.3	Table 11, example inhibitors OCT2: Cimetidine, Clonidine	Verapamil is a well-known OCT2 inhibitor, suggest adding verapamil to OCT2 inhibitor table.
Abbvie	1872	1872	7.5.1.1	In Table 12, why tolbutamide was not included as CYP2C9 clinical substrate	please clarify and include it as a moderately sensitive substrate
Roche	1872	1872		Consider adding tolbutamide as another CYP2C9 substrate	
Abbvie	1886	1886	7.5.1.2	In Table 13, itraconzole should also be indicated as BCRP inhibitor in the comments column for consistency as it was stated as BCRP inhibitor in Table 19	Add itraconazole as BCRP inhibitor in the comments column.
Roche	1886	1886		Consider adding clopidogrel as another CYP2C8 index inhibitor. Clopidogrel has multiple clinical DDI data supporting CYP2C8 inhibition and might be better than gembibrozil with less OATP1B confounding.	
AbbVie	1905	1905	7.5.2	Atazanavir is also an inhibitor of OATP1B	Add OATP1B to footnote. Supporting references: PMID: 21861202, 22541068, 23886114
Bayer AG	1941	1941	7.5.3.1	digoxin substrate for evaluation of the effect on renal P-gp as abs BA rel high	Footnote to the table: For P-glycoprotein, renal inhibition can be determined using renal clearance of digoxin.
Certara Integrated Drug Development	5226	5226	7,2	The draft guideline states that the transporter substrate and inhibitor studies should have sufficient recovery, giving 80% as a landmark. We fully agree that 80% recovery would be a generally suitable limit for inhibition studies. A lower recovery could also be sufficient if compensated for in the calculations but could give rise to variability if lower than ca 50%. In the transporter substrate assays, besides causing variability, we do not see the need of having such a high recovery. If concentrations are low, the drug would still be subject to drug transport	
MSD	1.2, Line 160		Introduction	Alternatively, some DDIs can reduce efficacy treatment.	Should alter statement to reflect DDI can reduce or enhance efficacy (ie., ritonavir-boosted protease inhibitors)
MSD	2.1.2.1, Lines 304-306		IN VITRO EVALUATION	Measuring PPB with multiple assays may not necessarily produce more reliable data. For example, ultrafiltration may not produce accurate data for drugs with high non-specific binding. Multiple assays would not be preferred or necessary if a validation of equilibrium dialysis assay can be demonstrated.	Recommend deleting that this practice is preferred. Recommend to change to: In some cases, demonstration of reproducible findings with different assays (e.g., ultrafiltration, equilibrium dialysis, ultracentrifugation) may increase the reliability of the fu,p measurement.
MSD	2.1.2.1; lines 301-302		IN VITRO EVALUATION	As stated, methodologies for accurate measurement of PPB have advanced. It is not clear what does a statement " in some situations" refer to and it is considered unnecessary in the context. If full validation, including accuracy and precision can be demonstrated, then results should be acceptable in all those cases.	Recommend to remove "in some situations" : Hence, the measured fu,p can be used if the accuracy and precision of measurement is demonstrated."
MSD	2.2.1, lines 458-460		IN VITRO EVALUATION	As the expression of BCRP in the human kidney cortex is below the limit of quantification (PMID: 27621205) and the	"Because P-gp and BCRP are also expressed in the liver (P-gp/BCRP) and kidneys (P-gp), in vitro study should be considered……."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
MSD	2.2.1, Lines 460-462		IN VITRO EVALUATION	The current text "can help determine whether the drug penetrates into the brain" is misleading with respect to the role Pgp has at determining brain concentrations	Suggest the sentence be edited as "In addition, if the pharmacologic target is within the brain, evaluating the drug as a substrate of Pgp or BCRP may help determine the extent to which the drug penetrates into the brain."
MSD	2.2.1, lines 483-484		IN VITRO EVALUATION	The relationship between transporter phenotyping and passive permeability is not well defined. Current statement doesn't provide a clear guidance on the use of passive permeability data to decide whether to evaluate additional transporters.	Suggested to remove "passive permeability" in line 484.
MSD	2.3.1, Lines 561-562		IN VITRO EVALUATION	Conduct of PPB for parent drug and metabolite in same experiment.	Propose to delete this. Most metabolites are identified/synthesized long after definitive PPB for parent drug is determined. Any evidence to suggest inter-study variability here?
MSD	3.2.1.3, Line 713		CLINICAL EVALUATION	2 weeks for clinical induction seems overly conservative	Recommend 7 – 10 days?
MSD	3.2.1.6, Lines 751-755		CLINICAL EVALUATION	Rifampicin is an inhibitor of both OATP1B1 and OATP1B3 (OATP1B). In addition to OATP1B and P-gp, rifampicin also inhibits BCRP (PMID: 27943276).	……For example, rifampin is an inducer of multiple enzymes and transporters, and also an inhibitor of transporters (e.g., OATP1B, P-gp, and BCRP)……. For the accuracy, please change "OATP1B1" to "OATP1B" in the text.
MSD	3.2.5.1 Lines 938-942		CLINICAL EVALUATION	Except for the limited roles of gut efflux transporters on intestinal absorption of BCS class I compounds, the relationship between passive permeability and clinically relevant transporter-mediated victim DDIs is not well established. Thus, passive permeability should not be used as one of criteria to decide whether clinical victim DDIs is warranted for transporter substrates.	Suggest to remove "passive permeability" in the text, and include the statement in Table 2 that gut P-gp/BCRP DDI studies can be waivered for BCS class I compounds.
MSD	3.2.5.1, Line 946 Table 2		CLINICAL EVALUATION	Based on Table 2, a clinical DDI study is recommended for in vitro P-gp/BCRP substrates if biliary excretion/active renal secretion is a major elimination pathway. However, there is a limited clinical DDI risk for P-gp and BCRP inhibition in the liver (P-gp/BCRP) or kidney (P-gp) according to the totality of data reported to date (PMID: 35612761). For instance, the increases in systemic exposure of P-gp substrates due to inhibition of liver and kidney P-gp are usually < 2-fold, which can be combined with the inhibition of other transporters/enzymes. Thus, clinical DDI studies due to inhibition of hepatic P-gp/BCRP, and renal P-gp should be limited to the substrate drugs with narrow therapeutic windows (e.g., digoxin) that are administered by a non-oral route. Furthermore, clinical relevance of renal BCRP on active renal secretion of drugs are not established.	Change to "biliary excretion (P-gp/BCRP)/active renal secretion (P-gp) is a major elimination pathway for drugs with narrow therapeutic windows and is administered by a non-oral route".
MSD	3.2.5.2, Lines 993-996		CLINICAL EVALUATION	Endogenous transporter substrates	Add clarity around whether or not endogenous substrate data would be acceptable in lieu of index substrate (ie., in a nested study?).
MSD	3.2.6, Line 1022		CLINICAL EVALUATION	Is there evidence that DDI findings with microdose studies do not extrapolate to therapeutic dose, or is this conjecture?	Clarify statement.
MSD	4.2, Line 1074		OTHER TOPICS	Therapeutic protein DDI	Any plan to include guidance on peptides or oligos?

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
MSD	4.2, Line 1077		OTHER TOPICS	Section is titled Therapeutic Protein DDIs, however the 2nd paragraph uses term "monoclonal antibody" several times. As monoclonal antibodies are a subset of therapeutic proteins, would be more general and consistent to use the term therapeutic proteins. Direct replacement of terms.	When evaluating the potential for a DDI between monoclonal antibodies therapeutic proteins and small molecules or between monoclonal antibodies therapeutic proteins, the mechanisms of a potential DDI should be considered, taking into account the pharmacology and clearance of the monoclonal antibodies therapeutic proteins as well as any administered medications in the patient population.
MSD	4.2, Lines 1075-1080		OTHER TOPICS	Consider including some points from line 103 of FDA 2020 guidance, on therapeutic protein DDIs around mechanisms of DDIs unrelated to proinflammatory cytokines. The only point in this ICHM12 draft that suggests DDI mechanisms other than cytokine modulation or ADCs is line 1078 which mentions "between monoclonal antibodies", but as no examples are shown it could be easily overlooked. While several of these interactions are not common, they have been observed, is good for scientific awareness. The examples are listed in the column to the right, FDA 2020 guidance also has the accompanying literature references as examples. Could add as an appendix if considered too long for main body.	1. When a therapeutic protein (TP) affects human physiological processes that can in turn alter the pharmacokinetic profiles of co-administered medications (e.g., GLP-1 receptor agonists such as dulaglutide and albiglutide result in delayed gastric emptying). In this case, the sponsor should evaluate the TP as a perpetrator. 2. Co-administered medications that impact the TP target or target-mediated disposition. In these cases, depending on the role of the TP in the DDI, the sponsor should evaluate the DDI potential of the TP either as a perpetrator or as a victim. Note: This is generally due to a PD interaction, but the PK of mAbs can be influenced by changes in target levels. 3. Co-administered medications that compromise the function of the FcRn can affect TPs which interact with the FcRn (e.g., blocking or interfering with the interaction between TPs containing an Fc region of human IgG and FcRn). In these cases, the sponsor should evaluate the DDI potential of the TP as a victim. 4. Co-administration of immunosuppressors with a TP whose pharmacokinetics are affected by immunogenicity (e.g., methotrexate on the clearance of adalimumab). Since immunogenicity (i.e., the formation of antibodies to TPs) can alter the clearance of some TPs, drugs that suppress immunogenicity can change the clearance of a TP. In these cases, the sponsor should evaluate the DDI potential of the TP as a victim. This type of DDI evaluation can be difficult to prospectively design, in which case a descriptive analysis can often be considered adequate.
MSD	7.1.4, Lines 1494-1497		APPENDICES	It adds unnecessary work and cost to include 2 positive controls when performing specific in vitro studies to model human induction DDI, namely RIS and PBPK. In either case, the concentration response of the test compound and rifampin are used in all the calculations within the model. It is not clear how one would use a second positive control in these cell models, or how to set up any criteria to judge success given it is superfluous in the model.	However, due to day-to-day variability in induction response, in some in vitro studies at least 2 of the inducers (weak and strong) of the calibration set should be included as controls when performing the in vitro study evaluating the induction potential of an investigational drug to scale the results to the calibration set of that hepatocyte batch. If using either RIS or PBPK approaches, a second positive control is not required.
MSD	7.2.1, Lines 1509-1511		APPENDICES	For vesicular inhibition assays, the assessment of uptake of probe drugs in control vesicles is not necessary. It should only be used in substrate studies. As defined in line1541-1543, evaluation of transporter inhibition in transfected-cell lines alone can be sufficient.	Change to "When membrane vesicles are used to evaluate whether a drug is a substrate of a transporter, the ATP-dependent, transporter mediated uptake of drugs in both transporter-containing vesicles and control vesicles are needed. When assessing a drug as an inhibitor of a transporter, evaluation of the uptake of a known probe substrate using transporter-containing vesicles alone can be sufficient".

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
MSD	7.2.1, Lines 1564-1565		APPENDICES	The no-solvent control is not needed for in vitro substrate or inhibition assays as the data are always normalized by vehicle-treated group, and the solvent is <1%.	Suggest to remove recommendation on adding no-solvent control.
MSD	7.2.1, Lines 1515-1517		APPENDICES	It is a bit confusing about the definition of sink conditions. For in vitro substrate assays, the transport was typically assessed by adding compounds to donor compartment and adding buffer to receiver one. For inhibition assays, the inhibitor was added to both donor and receiver compartments.	Please add clarification about whether sink conditions are for substrate or inhibition assays and add reference for 10% cutoff value.
MSD	7.2.3, Lines 1601-1604		APPENDICES	Transporter inhibition assays are generally conducted in protein free buffers over a short period. If the compounds are known to be soluble, stable and not highly bound, determining unbound concentrations in assay medium should not be necessary. A recent study (PMID: 35489778) demonstrated that correction of nonspecific binding of inhibitor drugs in a bi-directional P-gp inhibition assay did not improve the predictive performance. It is possible that unbound inhibitor concentrations measured in the incubation medium are not relevant concentrations for P-gp inhibition, as substrate binding sites of P-gp are localized intracellularly.	Change to "Sponsors are encouraged to measure unbound drug concentrations in the medium for highly bound drugs."
MSD	7.3.1.2, Line 1652		APPENDICES	Does 'confirmed' mean with a clinical study?	Clarify what is meant by "confirmed"
MSD	7.3.1.3, Lines 1696-1700		APPENDICES	It is unnecessarily strict to use bioequivalence bounds, which were never meant to be used to help verify PBPK models, as a criterion to judge the fitness of a model for a particular question. The allowable fluctuations (i.e. bounds) needed to qualify a model should be expected to be clearly stated and context dependent. In many cases, the bounds for PBPK qualification make most scientific sense when paired with information on the clinical bounds used to define efficacious doses	Change Line 1698 to: "If AUCR is outside the predetermined bounds for qualification, further evaluation may be needed ….
MSD	7.3.2, Line 1720		APPENDICES	"in some scenarios"	It would be very useful to know what these scenarios are where the agency would accept modeling data. Please include examples for reference.
MSD	7.3.2, Lines 1710-1722		APPENDICES	Consider mentioning that PBPK modeling could be used to extrapolate DDI predictions for adults to pediatric populations based on available information on the ontogeny of drug metabolizing enzymes and transporters	Propose to include extrapolation of DDI predictions from adults to pediatrics.
MSD	7.3.2.2, Lines 1793-1795		APPENDICES	Translation of in vitro inhibition data for transporters into a reliable PBPK model is not well established. Would not recommend to use PBPK modeling to support negative DDI when the drug is an inhibitor in vitro without supporting clinical data (i.e. DDI study with another substrate of the same transporter or biomarker data)	Suggest adding text to indicate that supporting clinical data would be needed.
MSD	7.4.3, Line 1848 Table 10		APPENDICES	In Table 10, creatinine was listed as in vitro substrate for OCT2/MATE1/2K. Although creatinine has been known as an endogenous substrate of OCT2/MATE1/2K, it is not a sensitive in vitro substrate of these transporters, and it is challenging to use it as an in vitro probe substrate to generate robust transporter inhibition data (PMID: 24646860; PMID: 26825641).	Suggest to remove creatinine from in vitro substrate list of OCT2/MATE1/2K.
MSD	7.4.3, Line 1850 Table 11		APPENDICES	Cyclosporin A is a frequently used in vitro inhibitor for P-gp, and encequidar is also a potent P-gp inhibitor in vitro and in vivo in gut	Suggest to include cyclosporin A and encequidar as P-gp in vitro inhibitors.