01 August 2013
EMA/CHMP/EWP/125211/2010
Pharmacokinetics Working Party (PKWP)

Overview of comments received on 'Guideline on the Investigation of Drug Interactions' (EMA/CHMP/EWP/125211/2010)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	Amgen
2	Biopharmaceutical Research Technologie Servier
3	Merck Sharp & Dohme (Europe), Inc.
4	Mundipharma Research Ltd
5	Robert Hermann, MD, FCP, Clinical Research Appliance, Radolfzell, Germany Oliver von Richter, PhD, FCP, Merck Serono, Darmstadt, Germany Amin Rostami Hodjegan, PhD FCP, University of Manchester, UK
6	TNO Netherlands - Innovation for Life
7	Absorption Systems
8	Actelion Pharmaceuticals Ltd
9	Association of the European Self-Medication Industry (AESGP)
10	Bayer Schering Pharma AG
11	Dr Thomas Parke Consulting (Aclides Pharma Services)
12	European Federation of Pharmaceutical Industries and Associations (EFPIA)
13	Mr. Masoud Jamei, University of Sheffield
14	Novo Nordisk A/S
15	Orion Corporation
16	F. Hoffmann - La Roche Ltd.
17	Working Party on Pharmacotherapy and Drug Information ('WFG') of the Royal Dutch Association for the Advancement of Pharmacy (KNMP)

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1. General comments – overview

Stakeholder no.	General comment (if any)	Outcome (if applicable)[1]
1	1. The Guideline is very comprehensive but lacks relevant literature support. We also suggest to add more specific and actionable information (decision trees, etc.) that outline the essential elements of in vitro DDI studies (build out appendices) and how this information is used to select and design essential clinical DDI studies. 2. In pages 1-28, there is frequent inference that sequential human clinical studies are needed to cover many eventualities. However, there are no citations supporting clinically significant label-driving studies as examples, nor is there reference to clinically significant drug interactions of concern. This part of the Guideline is overly long and not actionable. The guidance should focus more on defining the core studies that will drive dose adjustment and patient selection parts of the label. 3. In contrast to Pages 1-28, the Appendices are very general and the in vitro studies on which a rational clinical plan is predicated are not adequately described. Actionable decision trees and parameters with a concise rationale and literature support would be better suited to defining potential clinically significant events in the overall population. Caveats for the science-driven, case-by-case exploration of outliers or data anomalies would enable a rational approach to be developed through consultation with regulators. 4. The protein-binding sections would be more relevant in a PK	1. Partly accepted. The CHMP guidelines do not generally include a great number of scientific references. The support of the recommendations consists of literature as well as our experience from data in submissions over the last decade. Decision trees have been added. 2. In the guideline, studies that “should be performed” forms the core studies needed. Some studies are needed under certain conditions only and when so, this is stated. The “clinically significant label-driving studies” are tailored to the therapeutic situation and there is no need to give specific examples. 3. Partly accepted. The appendices now contain more precise information and decision trees have been added 4. Acknowledging that displacement interactions are rare,

[1] N/A = not applicable

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	Guideline. 5. The food effects sections would be more relevant in a PK/bioavailability/bioequivalence Guideline. 6. The suggestions regarding the need for, and the design and conduct of IV and oral human radiolabel ADME studies would be more relevant in a different Guideline. 7. To facilitate global drug development, the document could be significantly more aligned with the next version of the FDA CDER Draft DDI Guideline that is in preparation and with the International Transporter Consortium (ITC) paper (that will guide elements of the forthcoming CDER draft guidance).	we still wanted to give some brief advice in this document. 5. Not accepted. A food effect is a drug interaction but with food. The investigation of food interactions is very relevant for new investigational drugs, and there is a need to give precise recommendations regarding the timing, design and interpretation of these studies. 6. Not accepted. The ADME studies add information that is important for the DDI study program. (Acknowledging that the information is also used for other parts of the clinical development, the information is given here.) 7. Agreed. Efforts have been made to harmonize key parts of the FDA and EMA guidelines. There were no major deviations between the ITC and EMA recommendations. The draft guideline contained two additional transporters (BSEP and OCT1), giving rise to safety concerns and distribution effects, respectively. The inclusion of BSEP is not related to PK. OCT1 and the MATEs are now included in a list “for consideration”.
2	In the guidelines relative to the investigation of drug interaction, you recommend to use 3 different donors of plated hepatocytes to study enzyme induction or down regulation. What do you think about using pool of cryopreserved hepatocytes (as now available in some providers) to perform theses induction experiments?	Accepted. Both fresh and cryopreserved hepatocytes may be used. This has now been specified in the document.
3	The draft Guidance includes recommendations for conducting in vitro and in vivo transporter interaction studies under a variety of circumstances. However, it's not clear if the proposed clinical studies can always be conducted or interpreted in a definitive way as appropriate substrates and selective inhibitors/inducers of the	Not accepted. As the knowledge in this area is growing fast, we do not want to recommend substrates or inhibitors. The list would soon be out of date. For some transporters, sensitive in vivo probes and selective strong inhibitors are presently lacking. This is the reason for in vivo studies to be

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	transporters are not available. We suggest adding an Appendix to the Guidance with recommended probe substrates, inhibitors and inducers for in vitro and clinical use for the various transporters discussed in the Guidance.	recommended if good substrates/inhibitors are available. The applicant needs to consult the scientific literature.
4	1. Mundipharma Research Ltd welcomes the update of the guidance document to reflect scientific advances. The guidance is comprehensive, however the wealth of information does not aid the readability of the guidance. We would propose incorporation of a "Guidance-Tree" to ease identification and suggested timing of the key drug interaction studies and the necessary decisions by the applicant during the various stages of development. 2. The guidance update does not specifically deal with non-clinical drug interaction studies. For consistency between development phases the stakeholder would like clarification on the expected minimum non-clinical drug interaction study requirements. 3. The guidance update does not distinguish between therapeutic areas. Mundipharma Research Ltd would welcome specific information relating to oncology. There are many specific challenges associated with oncology drug interaction trials due to the significant number of concomitant therapies and co-morbidities in addition to the practicalities and ethics of PK sampling from patients with prolonged and terminal conditions. We would welcome specific comment in the form of an Appendix, a guidance addendum or simply a body text sub-section to deal with the mitigating circumstances.	1. Accepted. Decision trees have been added. The timing recommended is in some cases depending on several drug and indication-related factors and therefore, we have chosen to describe this in text, where the information given may be more extensive. 2. Not accepted. We interpret the non-clinical studies mentioned in the comment as studies in preclinical species, *in vivo, ex vivo* or *in vitro*. This guideline only relates to the human development program and thus contains advice on human *in vivo* studies and studies in human biomaterial. 3. Not accepted. Some general information in present in the introduction. However, more information may be found in the clinical guidelines related to specific indications. The guideline related to oncology (EMA/CHMP/205/95/rev.4) is presently under revision.

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5	1. Undoubtedly the new guidance is a major improvement and it considers various advancements in the dynamic area of research into assessment of drug-drug interactions (DDI).	
	2. The scope of this guideline has been expanded to include also advice for drug-food interactions besides drug-drug interactions. Also general recommendations are now provided for herbal medicinal products. These additional objectives and topics of the GL, however, are currently not reflected in the title of the document. For clarity and transparency regarding the scope, contents, and objectives of the guidance document a revision or amendment of the title of the GL should be considered.	2. Not accepted. These topics can be included under the present title.
	3. The overall structure, the scope and level of detail and extent of guidance regarding transporter-based drug interactions appears to be much better than current FDA guidance expressed in the Whitepaper published by the International Transporter consortium. Although it is realized that transporter-based drug interactions represent an evolving area, it is felt that at this point in time some additional structured guidance should be possible (for details please refer to the specific comments).	N/A
	4. The paragraph on combining in vitro data and results from an *in vivo* mass-balance study is very well written and contains helpful guidance on the use and interpretation of mass balance data. The focus on mass balance/metabolite identification trials as a key element in determining the major elimination route(s) for an investigational drug and guiding the rational design of *in vitro* studies and trials elucidating effects of medicinal products on the PK of the investigational drug is appreciated.	N/A
	5.The inclusion of the total exposure of active species, i.e. the sum of	N/A

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	the unbound exposure of pharmacological equivalents in combination with an assessment of the distribution to the active site is excellent guidance to quantify the interaction on drugs with active metabolite(s).	
7	1. Decision trees would be helpful for the assessment of interactions with both transporters and metabolic enzymes, by making it clear what steps to follow under different circumstances. 2.Appendix II: Another approach to identifying transporters involved in drug disposition is the use of stable cell lines in which the expression of naturally expressed transporters has been knocked down genetically (e.g., Zhang W et al., Drug Metab Disp 2009 Apr; 37(4):737-44; Darnell M et al., Drug Metab Disp 2010 Mar; 38(3):491-7).	1. Accepted. Decision trees have been included. 2. Accepted. The initial text was intended to cover also these methods. This has now been clarified in the text.
8	1. The criteria for the need of performing clinical DDI studies to assess a drug's effect on a specific transport protein are mostly based on biochemical inhibition data and the use of safety factors. Based on available literature data, most DDIs involving transport proteins are limited in magnitude – with the exception of DDIs with OATPs. We therefore suggest to include additional factors such as safety and other development criteria into the decision which transport studies are indeed required. 2. The current draft guideline is not explicit on whether the proposed timing of transporter studies relates to parent drug only or also to its metabolites. The completion of metabolite studies before the end of phase II is considered difficult. We would appreciate the inclusion of	1. Not accepted. We agree that the cutoff of 25% contribution to drug elimination is low, both for enzyme and transport involvement. Inhibition of such a pathway will lead to a 30% increase in AUC, which is rarely clinically significant. However, we have set this cutoff to enable prediction of drug exposure when multiple pathways are affected. The criteria for performing studies with a drug as a perpetrator are the same as for enzyme inhibition (basic model). The cutoff may be changed in the future if suggested by the available data. 2. Partly accepted. Although metabolites may very well affect transporters, we have chosen not to ask for transporter inhibition data for metabolites. Testing of the pharmacological effect of metabolites should be performed

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	criteria on the extent and timing of metabolite testing.	as early as possible and this is reflected in the guideline text.
	3. Threshold criteria for metabolite structure identification: The respective threshold in the draft guideline is 20% of parent drug exposure. We would like to point out that this threshold is difficult to meet for highly metabolized compounds and would cross-refer to a recent clarification in the ICH guideline on the safety testing of drug metabolites.	3. Accepted. The wording has been harmonized. Data on the enzyme inhibitory potential is generally needed for metabolites with an AUC that is larger than 25% of the AUC of the parent drug and (at the same time) having an AUC larger than 10% of the total AUC of drug related material.
	4. Modelling and simulation: The draft guideline (lines 574-585) states that simulations may be used to evaluate the clinical relevance of in vitro inhibition. However, the safety factors of 50 or even 250, for compounds with an unbound fraction < 0.01, appears excessive. The justification that the actual concentration at the enzyme is difficult to predict can be counteracted with the need for extensive validation. Software packages such as SimCYP use physiology-based pharmacokinetic modelling (PBPK) for this purpose and are usually quite successful in quantitatively predicting clinical outcome of DDI studies and hence also the actual concentration at the enzyme. Applying 50 or 250 times the expected dose to cover the recommended safety factor appears excessive, in particular as this is linked with an inhibition threshold of only 30 % to indicate a clinical study. We would like to emphasize that PBPK-based simulations are currently the only way to realistically assess the potential clinical relevance of in vitro findings, particularly when	4. Partly accepted. A safety factor was included both to compensate for the uncertainty in the Ki estimate (shown as rather high inter-study/lab variability in the estimate) as well as the fact that at present, the concentration in the hepatocytes can not be well predicted. Validation does not take care of this problem, as the situation is drug- and dataset dependent. However, the safety factor has been removed and replaced with adequate sensitivity analyses.

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	multiple pathways and simultaneous inhibition/induction mechanisms are involved. 5.Marker substrates for P450 inhibition studies: For CYP2D6, the draft guideline only recommends bufuralol. We suggest to also include dextromethorphan to maintain consistency with the respective FDA guideline. For CYP3A4, midazolam OR testosterone and another specific marker like nifedipine are recommended. Midazolam and testosterone are described to address different CYP3A4 binding sites and should therefore both be used as markers as recommended in the current FDA guideline. 6.Clinical relevance of UGT inhibition: A literature search aiming was performed to identify marketed drugs causing clinical DDIs as a result of UGT inhibition. In a second step, the effect on those drugs on statin pharmacokinetics was investigated as statins represent a class of drugs with a narrow safety margin. Both search mainly identified HIV protease inhibitors (atazanavir/ritonavir, lopinavir/ritonavir, nelfinavir). In case mechanistic information was available, inhibition of transport proteins (OATP, MRP2, BCRP and/or P-gp) rather than glucuronidation was identified as the underlying cause. In the absence of convincing evidence that UGT inhibition alone represents a clinically relevant concern, we would therefore recommend against a systematic in vitro testing. 7. Criteria for inhibition studies with intestinal transporters: The draft guideline indicates the necessity to perform clinical DDI studies on intestinal transporters when the drug concentration in the intestine	5. Not accepted. It is not possible to include all in vitro substrates/reactions that may be used in these studies. Only examples are given. Different markers may be used if justified based on literature. The proposed change regarding CYP3A4 substrates has been made. 6. Not accepted. We do not agree that the performed search, based on DDIs with one class of drugs, would indicate that there are no clinically relevant interactions due to UGT inhibition. The guideline does not state that UGT inhibition should be tested for all drugs. Only the drugs with main elimination through metabolism catalysed by enzymes of this family are recommended to be studied in this respect. 7. Partly accepted. This is already outlined in the text.

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	exceeds the Ki value by a factor of 10. Since many drugs have limited solubility, we recommend to modify the criteria to either use the concentrations achieved when the oral dose is dissolved in a volume 250 mL or the solubility in biorelevant media. 8. P450 induction: The criteria for the classification of a candidate drug as a 'non-inducer', i.e. 1.5-fold increase over vehicle at a clinically relevant concentration, are considered too stringent in light of existing experimental variability. It is not unusual to have standard deviations of 0.1-0.4 between individual experiments. Moreover, it is known that basal CYP3A expression is prone to large inter-individual variability resulting in variable induction response. We therefore suggest to quantify the inductive effect of a candidate drug relative to a positive control like rifampicin tested in parallel. 9. Criteria for CYP2B6 induction: Literature data indicate that all drugs known to induce CYP2B6 are also inducers of CYP3A. Regulation of both P450 enzymes involves PXR as well as CAR. Consequently, only if CYP3A4 is induced by a given drug co-induction of CYP2B6 is likely. We therefore propose to perform CYP2B6 in vitro induction studies only in cases when CYP3A induction is manifest.	8. Partially accepted. The parallel method is in fact included in the guideline to assure adequate sensitivity. The measurement of activity as proposed in the draft has been changed to mRNA and the cutoff is 2-fold. The use of mRNA will increase the ability of separating mild to moderate induction from experimental variability. Additionally, for CYP3A induction, the RIS correlation method and the mechanistic static model may be used. 9. Partly accepted. There are drugs which act more on CAR than on PXR and thereby give rise to a more pronounced CYP2B6 induction than 3A4 induction. Therefore CYP2B6 should be investigated.
9	1. The list of relevant transporters should be harmonised with the ITC publication (Membrane Transporters in Drug Development", Nature Rev., Drug Discovery, 2010, 9, 215-236, Giacomini et al.).	1. Partly accepted. The list on transporters which needs to be investigated for inhibition has been harmonized. BSEP is proposed for hepatic safety reasons and not actually PK reasons (stated). Investigating the MATEs and OCT1 should be considered.

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	2. Referring to drug transporters please use IC50 instead of Ki, because it is very difficult to exactly and accurately assess the Ki of drug transporters. This would also be in line with the argumentations of the "ITC publication" 3. Please add a list of recommended “strong” and “moderate” inhibitors of P-gp (and of other transporters) to the appendix of the document. 4. Please add a list of recommended “strong” and "moderate" inhibitors of the most frequently encountered P450 enzymes to the appendix of this guideline. 5. In terms of herbal medicinal products, we welcome the differentiation made in the text of this chapter, i.e. that the potential of interactions should be investigated for new herbal preparations, whereas for traditional and well-established herbal preparations such a potential should be clarified if reports point to clinically relevant interactions in humans.	2. Not accepted. We prefer Ki as it is less affected by study conditions. The text has been changed to include that IC50 may be used under some conditions in case Ki is difficult to obtain. 3. Not accepted. As the research in the field of transporters is extensive at present and a lot of information is expected to be gained over the coming years, such lists are likely to be out of date very fast. Therefore such lists are not included. 4. Not accepted. There is a risk that such a list would be used without checking the recent literature and SmPCs. Thus, we chose not to include such lists.
10	1. We welcome the revision of the Guideline on the Investigation of Drug Interactions and the opportunity to comment on the draft guideline. Note: These comments refer to the corrected draft guideline and corresponding line numbers.	N/A
11	1. A list of high-level references might be helpful for preparation and interpretation of experiments and studies.	1. Not accepted. We prefer not to cite specific references. The applicant is advised to follow the scientific literature.

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	2. Decision trees in the Appendix could be included as a supportive piece of information both for applicants and regulators.	2. Accepted. Decision trees have been included
	3. The listings of examples for in vivo drugs in the Appendix should be extended and a table clearly structured by CYP and substrate, inhibitor or inducer might be more convenient for the reader. Subcategories (mild, moderate, strong inhibitors or inducers) and relevant drug transporters might be included as well.	3. Not accepted. See comment no 2 and 3 from stakeholder no 9.
	4. Recommendations on DDI and labelling across the ICH regions are currently not very consistent (i.e. the concept of 'sensitive substrates' and 'substrates with narrow therapeutic range' might be included in the revised Guideline).	4. Not accepted. The concept sensitive substrates may be seen as similar to probe drugs. Otherwise, in terms of predicting which DDIs that will be of highest clinical relevance, the magnitude of the interaction effect and the relationship between concentration and efficacy/safety needs to be taken into account. Therefore these expressions are avoided.
	5. Investigation of food effects other than of high-caloric meals (e.g. of calcium-rich diets) should be considered when appropriate. Sections 5.1 or 6 could be amended accordingly.	5. Accepted. This was already included but the text has been clarified in this respect.
	6. Though only in rare cases leading to clinically relevant interactions, CYP2A6 and CYP2E1 should be mentioned.	6. Not accepted. These enzymes are not part of the standard battery of enzymes studied for inhibition. The rationale is that there are very few examples of drugs mainly metabolized by these enzymes.
12	1. EFPIA welcome the release of this draft guidance by EMA as the document provides insight on the current and future directions with respect to drug-drug interactions. It is clear that a mechanistic understanding of observed drug-drug interactions is very important	1. Not accepted. This concept is already included.

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	for EMA, and that computer simulations may help to provide additional information on the presence or absence of interactions. However, a clear definition on the objective of a DDI study in vivo would be very helpful: for example, “A specific objective of an in vivo drug-drug interaction study is to determine whether the interaction is sufficiently large to necessitate a dosage adjustment, or whether therapeutic monitoring would be required.” In addition to comments and proposed revisions to the different sections of the draft revised guideline, EFPIA have the following major issues that would need further consideration:	
	2. A series of thresholds and/or reference values are different between FDA guideline and the EMA draft proposal. These criteria should be harmonized whenever possible between the two guidelines. Examples are: Safety factors applied to determine the need for an in vivo interaction study, fu vs total drug concentration, % systemic clearance needed for phenotyping experiments. We would like to propose to add references at the end with corresponding numbers in the text so the reader can access the publication that gives more information about the scientific rationale for the recommendation.	2. Partially accepted. Harmonization discussions have been taking place. Inclusion of references is not completely appropriate as some of the thresholds etc are based on the large number of files assessed during the last decade. We hope that data driven harmonization work can take place in the future.
	3.The following lists should be added to the guideline as an appendix: A list of relevant drug transporters, which should be harmonised with the ITC publication (Membrane Transporters in Drug Development", Nature Rev., Drug Discovery, 2010, 9, 215-236, Giacomini et al.); a list of the respective model substrates, the pharmacokinetics of	3. Not accepted. See comments on issue 1 and 2 from stakeholder 9 Lists are not appropriate in areas which are going through major changes. Background for the cutoffs is mainly experience from submitted applications and IVIVC knowledge on enzyme inhibition.

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	which might be affected to a clinically relevant extent by transporter modulation; a list of recommended “strong” and “moderate” inhibitors of P-gp and other transporters; a general list of acceptable probe substrates and inhibitors for enzymes including UGTs (will EMA accept, e.g. β-estradiol for UGT1A1 and zidovudine for UGT2B ? It would be desirable to have a harmonized method, especially taking into account the pronounced albumin effect for UGT2B7 and the general difficulties for in vitro/in vivo extrapolation for UGTs); a list of recommended “strong” , "moderate" and “weak” inhibitors of the most frequently encountered enzymes. In general, no rationale is given for margins 10x, 50x or 250x for highly protein bound compounds and the alternative use of model-based approaches as state-of-art in simulation approaches to assess drug-drug interactions and food effects are not adequately considered. It should be recognized that computer-based simulations, based on well defined mathematical models and integrating quality in vitro and in vivo data, should be an integral component in quantitative assessment of DDI potential. Throughout the document, many studies are recommended to be conducted during Phase I and prior to Phase II, but at this time in development the therapeutic dose/range may not be known. The recommendation is to enlarge the duration on these studies during Phase II. It is unlikely that all in vitro and in vivo assessments of transporter DDI potential or data confirming the relevance of metabolites in humans will be available prior to Phase III Performing in vivo studies in order to specifically investigate transporter/enzyme interplay will be extremely difficult due to lack of	Tables with CYP probe substrates and inhibitors have been included. However, please note that the tables just give examples and that others may be acceptable if well justified scientifically. For UGTs corresponding tables are not included for the same reasons as for the transporters. The timelines as well as the lack of data on enzyme-transporter interplay is reflected in the text. However, some clarifications have been made.

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	specific probe substrates (especially for drug transporters). Any in vivo attempt will be very complex and is not all clearly defined in current literatureaccording to our knowledge. This should be considered and acknowledged.	
	It would be preferable to place more emphasis on the known clinical relevance of the interactions. For instance, transporter substrate phenotyping is vague (the Caco-2 in vitro assessment recommended in Appendix II implies that we should look for any and every GIT transporter, and the assertion that >25% should drive investigation of uptake mechanisms for kidney and liver opens up a lot of investigation which could be very open-ended. No specific transporters are recommended for substrate investigation which is in contrast to the guidance for transporter inhibition.	Transporter investigations at an intestinal level are recommended when there are indications that interactions at this level may be clinically relevant. At a systemic (drug elimination) level, the recommendation is the same for enzymes and transporters. We do not wish to specify which transporters as this is a growing field.
	Of specific concern were the following thresholds with respect to drug metabolizing enzymes: Ki<50-fold for intestinal enzyme inhibition Ki<50-fold (or 250-fold for highly bound compounds) of unbound concentrations (rather than < 10 fold of total concentration as proposed by the FDA) for inhibition of enzymes in elimination organs 1/5 of the parent concentration for driving additional investigations on metabolite potential for DDI	The present difficulty in assessing in vivo relevance of uptake transport just based on the in vitro result for drugs found in transported by OATPs is acknowledged. We hope that future research and potentially scientific consensus discussions will be helpful setting requirements on in vitro systems and methodology on which to base these decisions. The data gained in the coming years may serve as a basis for a more data-driven cutoff.
	For in vitro induction: an individual donor value for an inducer that is >50% of baseline enzyme activity (rather than >40% of the positive control or alternatively EC50 value for enzyme activity proposed by the FDA) combined with the requirement for a non-inducer to have an individual donor value ≤20% of the respective positive control value for enzyme activity or mRNA.	The *in vitro* induction text has been markedly revised. Still a conservative cutoff is used for the basic model evaluation due to the small data set available and the varying quality of the *in vitro* induction studies submitted.

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13	These comments are solely focus on the assessment of transporters drug-drug interaction and comments on other aspects have been submitted by other colleagues.	
15	The general impression of the document is not very consistent. Some parts are written in very detailed manner while others are at a very general level. The main scientific weakness is the lack of references. Some issues, e.g. clinical relevance of transporters and applicability of simulations in DDI predictions, are not uniformly accepted in the scientific community. Therefore, it would be of utmost importance to document the literature on which this guideline is based. Only after that can detailed discussion on scientific issues continue. The most crucial thing to discuss, to our opinion, is the limits which trigger the necessity of clinical interaction trials. If this is set too stringent there will be a lot of extra, possibly unnecessary, work to be conducted.	The transporters outlines for the inhibition screening studies are generally the same as proposed in the ITC white paper, which could be called “uniformly accepted in the scientific community”. As stated in the document, the components of the list may change as science develops. The cutoffs are based on our experience with applications submitted. The cut-off regarding pathway contribution comes from the aim to predict the outcome of effects on multiple elimination pathways (e.g. RI + DDI). Some scientific references have been added.
	Moreover, we would like to see the basis for Strong emphasis on UGTs in DDIs True significance of plasma protein binding and how it is studied Modelling and simulations: Current equation based suggestions in the guideline will unavoidably yield very many false positives. EMA should clearly state which kinds of models (with references) are accepted as evidence of non-existing interaction potential. This could also be an EMA-industry joint effort to produce adequate methods for early clinical phases to ensure safety until Proof-of-Concept	Not accepted. We do not think there is a strong emphasis on UGTs in the document. DDIs at an UGT level have been observed but we are lacking knowledge about this enzyme family at the moment. Information on modeling and simulation has been added but no specific method/program has been specified.
	The structure of the document is in principle logical but in practice very hard to follow since e.g. transporters and enzymes are discussed in many different places and it is not easy to get the big picture behind the recommendations, let alone to find out which studies are required or recommended at which stage. In other words, the readability and “user experience” of this document is poor. Our	Not accepted. The logics of the guideline is to divide effects on the new drug by others drugs, from effects by the new drug on others. If this is not performed and all information on eg transporters is put in one place, the text becomes more difficult to read. Accepted. Multiple decision trees have been added.

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	suggestion is that the authors build clear decision trees that describe different steps in DDI studies. It should be made very clear which parts are mandatory and which not (and at which stages). We think that this approach would clarify the document also to the authors. Still we emphasise that the decision tree must not be too rigid since different molecules and indications need different approches. There can be chronic or acute diseases requiring different duration of medication.	
	In addition, many other criteria in legislation are different for life threatening and non-life threatening diseases. Therefore, a generic template with rigid (and not even well scientifically justified) rules may be unappropriate.	Partly accepted. If well justified, other approached may be taken than the ones outlined in the guideline. Thus, in some particular situations, studies may be performed post approval provided risk mitigation strategies (such as SmPC recommendations) are put in place to make the use of the medicine sufficiently safe awaiting the study results. However, the lack of information and potential safety consequences is included in the benefit-risk assessment.
	Unambiguous wording is requested: what do “should”, “is recommended” and “is required” mean.	The guideline gives guidance on which drug interaction evaluations the CHMP expect to see. Other approaches may be adequate if well justified (see above). The wordings “should” and “is required” means that according to the guideline, studies needs to be performed. The wording “is recommended” is weaker and means as stated that we recommend the applicant to make the studies as we believe they are appropriate, but according to the guideline there is no general requirement that these studies should be performed.
	We encourage the authors to rethink how this guideline will help	Not accepted. As science develops, the number of studies

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	develop more efficient medicines in a reasonable time frame without overwhelmingly excessive costs. This guideline places heavy emphasis on clinical confirmatory studies on PK and DDI in very early clinical development. The evidence power of in vitro experiments is low according to this guidance. Phase I and II clinical trials are mainly conducted to obtain proof-of-concept (and of course basic safety and PK info). These studies are relatively small and patient population and, to our opinion, the possible interaction data that in vitro experiments show, can in many cases be avoided in study design. There is no rationale in putting significant amount of resources in studying possible DDIs in the phase where there is no certainty of the actual efficacy of NCE, the most crucial parameter of all in drug development. In addition, it is not very ethical to expose significant number of healthy volunteers to the study molecule AND to perpetrator/victim model compounds at this stage. And last but not least, the guideline discusses continuously about “clinically relevant concentrations”. There is no way to know what the clinically relevant concentration (or dosing range, interval or even the final formulation) in human is until PoC is achieved. Therefore, early DDI studies are most probably conducted at incorrect plasma levels and will probably need to be repeated in later phases. We agree to the authors in that there should be more emphasis to understand enzymes and transporters in clinical phases. However, before PoC, this should be mainly following the information that in vitro data has given (e.g. correlating CL values to possible polymorphism and excluding patients with strong perpetrator drugs as co-medications).	needed is likely to be increased even if some aged studies will be removed from the requirements. However, this will lead to safer medicines, which is in the interest of everyone. We do not agree that the guideline puts too much emphasis on studies early in development. The recommendations are there to ensure safety of the included patients. Furthermore, there is no recommendation to perform early DDI studies if the DDI risk may be managed through other measures, e.g. exclusion criteria or monitoring. The recommendations have been explained in more detail in the final guideline text.

Stakeholder no.	General comment (if any)	Outcome (if applicable)[1]
	The authors are encouraged to compare this guideline to recently published FDA guidance “Metabolites in safety testing”. Are the recommendations and requirements in line with these requirements.	The guideline text on metabolites has been harmonized with ICH M3.
16	This guidance is scientifically comprehensive, well written and covers many aspects relating to the conduct of drug-drug interaction studies. The document is also easy to navigate. We would like to propose to add references at the end with corresponding numbers in the text so the reader can access the publication that gives more information about the scientific rationale for the recommendation.	Partially accepted. Some references on new approaches proposed have been added in the text but in general we want to keep the number of references low.
	Addition of an appendix with a list of recommended substrates and inhibitors for the main transporters would be helpful. Also a table with names of drugs that are strong, moderate and weak inhibitors of various CYP enzymes (similar to Tables 5 and 6 of FDA guidance), as well as a table for inducers. Appendix A Table 1 in the FDAs draft guidance (September 2006) is extensive but this CHMP draft only caters for CYP enzymes and not for transporters.	Not accepted. Lists of substrates and inhibitors are included in the guideline. If adding lists of weak, moderate and potent inhibitors of various enzymes, the lists become outdated and may give a false security if not noticing that new drugs should be included. The transporter area is not sufficiently mature for lists to be possible as this document should be valid also in the future.
	In general, no rationale is given for margins 10x, 50x or 250x for highly protein bound compounds and the alternative use of model-based approaches as state-of-art in simulation approaches to assess drug-drug interactions and food effects are not adequately considered. It should be recognized that computer-based simulations, based on well defined mathematical models and integrating quality in vitro and in vivo data, should be an integral component in quantitative assessment of DDI potential. When model simulations for probe substrates/inhibitors have been verified against a number of clinical studies they can then be used to	Partially accepted. For cutoffs, se above. More information on PBPK modeling and simulation has been added.

Stakeholder no.	General comment (if any)	Outcome (if applicable)[1]
	predict, with reduced uncertainty, quantitative outcomes for investigational drugs (Chien, Curr Drug Metab. 4(5):347-56, 2003). Furthermore, by incorporating known sources of variability into the models, simulations can be used to explore likely outcomes in individuals lying at the edges of the normal population and in special populations such as poor metabolizers (Rostami-Hodjegan, Nat Rev Drug Discov. 6(2):140-8, 2007). Modeling and simulation should be used, together with thorough in vitro and in vivo studies and well established physiological datasets, to provide a rational basis for risk assessment. This would be clearly preferable to the arbitrary safety factors recommended in the Guideline since when using model-based approaches, uncertainties are based on the model assumptions, account for the number and diversity of the examples used in the validation set and consider the accuracy of the simulated DDIs for those verified examples. The Guideline should recommend the benefits of a model-based approach to forecasting drug-drug interactions for novel investigational medicines such as have been shown in several recent studies. (Kanamitsu et al, 2000), (Shitara, 2006), (Rowland-Yeo, 2010). A decision tree (like the one in the FDA guidance) would be very useful. It is recommended that the guidance provides current regulatory thinking on DDI of biotherapeutics	Not accepted. In the area of therapeutic proteins, the present state of knowledge does not allow detailed advice. General advice is given in the European Medicines Agency. Guideline on the Clinical Investigation of the Pharmacokinetics of Therapeutic Proteins (CHMP/EWP/89249/2004).

Stakeholder no.	General comment (if any)	Outcome (if applicable)[1]
17	If the SPC is updated, the information in the Scientific Discussion should be updated accordingly. Sometimes recent information is to be found in a separate document 'Procedural steps taken and scientific information after the authorisation'. This can be confusing, because it's easy to overlook the fact that the 'old' data in the Scientific Discussion are overruled by later documents (example: Cholestagel and it's interaction with ciclosporine).	This is not in the scope of this guideline but we have noted your comment.
	Problems the WFG encounters up until now with SPC's: • information is too general ('rifampicin decreases the plasmalevel') • extrapolation; if there's evidence for one drug, a whole group of drugs gets the same 'treatment' while the reason is not clear • intervention 'avoid' or 'not recommended' very often is not realistic in daily clinical practice: in (geriatric) real life, patients do need multiple (interacting) medicines; this kind of warnings with the main purpose of legal safeguarding are not implemented by the WFG, and thus will not generate a signal in the computerized database • the manufacturer has more useful data on file, but it's not in the SPC	Partially accepted. The information given in the SmPC is mainly regulated in “Rules governing medicinal products I the European Union Volume 2C Notice to applicants; A guideline on summary of product characteristics (SmPC) September 2009”. We agree that the magnitude of the interaction effect should be given if data is available or should otherwise be communicated generally “a marked effect is expected.” etc. The problem with polypharmacy is not easily handled and except for some specific scenarios of multiple interactions, this may not be solved in this guideline. The problem that general warnings may not be sufficiently translated into practical recommendations, is acknowledged. This is one of the reasons, extensive lists of inhibitors are proposed for inclusion in the SmPCs. The manufacturer should submit all studies for assessment and, where relevant, the information be included in the SmPC.

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
60-62	5	Comment: The flow of statements in the Executive Summary appears ambiguous to some extent. In sentence 2 it is referred to drug-drug interactions (DDIs), in sentence 3 then it is referred to food-drug interactions ("Furthermore the effect of concomitant food intake needs to be investigated"). Sentence 3 is then followed by the general statement "The interaction potential is usually investigated through in vitro studies followed by in vivo studies. Proposed change: Consider to specify sentence 4 in the Executive Summary as follows: "The drug-drug interaction potential is usually investigated through in vitro studies followed by in vivo studies when necessary.	Accepted
64	5	Comment: "...based on the mechanism involved..." As there might be more than just one mechanism for a DDI, consider to use plural instead of singular. Proposed change (if any): "...based on the mechanisms involved..."	Accepted
66-67	5	Comment: There appears to be a typo ("This document aims **as** providing recommendations..."	Accepted.

[2] N/A = not applicable

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change: This document aims **at** providing recommendations..."	
66-77	17	Treatment recommendations are developed based on the clinical relevance of the interactions and the possibility to make dose adjustments or treatment monitoring. Comment: in our experience in most cases the groundwork (based on what evidence) for treatment recommendations in the SPC are not clear, because the clinical relevance is not clear. Proposed change (if any): add evidence on which treatment recommendations are based	Not accepted. The available data on efficacy and safety, as well as, when available data on the PK/PD relationship is taken into account. Sufficiently covered with present wording.
69-70	5	Comment: Refers to the statement "...give rise to a large number of **hospital admissions** within the EU." The primary public and individual health concerns about DDIs are thought to be serious and sometimes fatal adverse events. Another less recognized adverse consequence of DDIs might be the abolishment of efficacy without any concomitant adverse events (so called "silent" DDIs). Also the economic burden of DDIs is not mentioned. Hence, hospital submissions, which are mentioned here as the only adverse consequence of DDIs are deemed to represent a single secondary outcome of a fraction of DDIs rather than a comprehensive description of adverse DDI outcomes.	Partially accepted and included.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any): "Drug-drug interactions are a common problem **of** drug treatment and give rise to a large number of **medically important, sometimes serious or even fatal adverse events, and hospital admissions** within the EU, **and represent a significant economic burden**. It is also important to note that drug-drug interactions may not always lead to adverse events but can result in abolishment of efficacy without displaying any adverse events. "	
70-71	5	Comment: It is not clearly stated at which point of development sufficient knowledge on DDIs should have been obtained. Proposed change: The aim of this guideline is to ensure that sufficient knowledge has been gained **at the time of regulatory approval (or market access)**..."	Partially accepted. The timing of obtaining data is specified later in the document as far as possible. Information is needed not only at the time of approval but also during clinical drug development when performing studies in patients.
70-102	14	Comment: We would like to suggest that a brief clarification of the wording "medicinal products" used in the context of this guideline, is included in the introduction section. It is for instance not fully clear whether the guideline also applies to peptides/proteins and chemically modified peptides/proteins as the special issues which one might have when testing DDI's for this type of compounds, are not mentioned in the guideline. Proposed change (if any):We suggest to add the	Partially accepted. It is specified in the Introduction that the document does not include advice on therapeutic proteins. General advice is given in the European Medicines Agency. Guideline on the Clinical Investigation of the Pharmacokinetics of Therapeutic Proteins (CHMP/EWP/89249/2004).

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		following sentence in the introduction section: as a starting point the wording "medicinal products" in this guideline refers to NCE's. Assessment of the possibility for DDI's for peptides/proteins and chemically modifies peptides/proteins should be studied and designed on a case by case basis.	
75	3	Proposed change (if any): "...based on the **mechanisms** involved..."	Accepted
92-97	12	**Comment:** EMA should clarify or provide examples in relation to the wording "…other approaches" (line 95).	Not accepted. It is not possible to appropriately reflect the "other approaches" and when these may be satisfactory.
106-107	5	Comment: The content of the last sentence of this chapter (i.e. the message that DDI-studies should be considered during the whole life cycle of a drug) appears redundant as this issue is already detailed in the introduction section of the document (lines 86 to 89). Proposed change: Delete last sentence of Chapter 2 (i.e. lines 106/107) **or** delete respective statements in Chapter 1 (i.e. lines 86 to 89).	Accepted
131	5	Comment: The used term "receptor level" may be too specific in the present context, as there may be other pharmacological targets addressed by new molecular entities rather than just "receptors" (e.g. ion channels or enzymes) Proposed change: Replace the term "**receptor** level"	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		with "**target** level".	
133-135	5	Comment: From the overall context it appears unclear/ambiguous, which specific advice to the reader should be conveyed with the following statement: "However, many of these interactions can be predicted based on the pharmacological effects of each drug." Proposed change: The statement appears to require some subsequent clarification, i.e. guidance which studies should be considered in case that pharmacological effects of a drug would suggest DDI-potential.	Partly accepted. The text has been changed.
136-141	5	Comment: The last section of the PD-DDI Chapter appears not entirely consistent and concise in its overall advice directions, and also appears content-wise not entirely conclusive and informative. First, it is suggested that animal studies may provide sufficient experimental evidence to characterise a potential interaction when similar mechanisms and/or effects are found in animals and in humans and a valid biomarker may be available for animal use. However, the challenging issues how such trial outcomes could be translated to the human situation in case that the employed biomarker may not be also validated in humans, or how animal trial data can generally be extrapolated in quantitative terms, is not addressed at all. Also the fact that other (e.g. unknown) inter-species differences may confound or complicate the	Not accepted. Only general advice is suitable here.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		translation of pre-clinical PD-trial outcomes to the clinical situation is not considered in the present guidance text. Although not a single statement/advice on human PD-DDI studies is provided in the respective Chapter, it is concluded at the end that "In general, the PD interaction profile of a drug can be best described by using both in vitro studies and in vivo human studies together." Proposed change (if any): It is recommended to carefully revise the entire PD-DDI Chapter along the lines as detailed above, with the aim to increase its overall clarity in advice and usefulness to the reader. Specifically the current impression, that animal studies may generally be considered sufficient to waive human in vivo PD-studies should be subjected to a careful reconsideration.	
154/155	5	Comment: Synonymously to the term "victim drug" in the scientific literature often the term "object drug" is used. Proposed change (if any): Consider to introduce both terms into the GL text.	Not accepted. This may make the guideline less clear.
156/157	5	Comment: This Chapter exclusively refers to PK-DDIs. Proposed change (if any): Consider to remove the statement "...or pharmacodynamics..." from the last	Accepted

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		sentence of the paragraph.	
160-171 300-301 306-309 490-494	1	**Comments:** Metabolite synthesis and metabolite DDI investigations are being requested, plus new thresholds germane to safety testing of drug metabolites (i e, plasma metabolite as > 20% of parent, > 25% of CLPO, 30% of activity, > 25% of formation or elimination of a metabolite). These requests are out of scope for DDI Guidelines. **Proposed change (if any):** Refer to ICHM3 R2 or the CDER STDM Guideline and remove. Justify new thresholds.	Partly accepted. The recommendations have been harmonized with ICHM3.
160-171	12	**Comment:** It is not specified what the metabolite / parent ratio should trigger. There is also a potential inconsistency with the requirements in the ICH M3 document. Agency should also clarify the sentence (line 167) on “target and off target” since it can be very difficult to prove lack of altered efficacy or safety.	Partially agreed. Specific advice is given in the section referred to in the sentence. However, it has been specified that what is expected is data on enzyme inhibition. Regarding the ICH document, see above.
160-163	15	Comment: Define “active” metabolite. What level of activity in preclinical models yields a metabolite “active”	This is specified in the subsection referred to.
165-171	17	Comment: in our opinion, PK/PD information is not just 'very useful' but it is essential to assess the relevance of a pharmacokinetic interaction. PK/PD information is lacking in most SPC's. Sometimes some data on PK/PD can be found in the Scientific Discussion, but even	Partly agreed. PK/PD information is important but it is often not available. However, in the absence of these data, there may be other sources of information such as dose-safety and dose-exposure which may be used in combination with dose-PK for this evaluation.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		there it's hard to retrieve. The term 'clinically relevant pharmacokinetic interactions' can only be used when human in vivo PK/PD information on parent drug/metabolite contribution are avaliable. Proposed change (if any):	
172-177	12	**Comment:** Agency should clarify if this statement refers to in vitro or in vivo DDI studies.	Accepted. This has now been specified.
175-177	16	Please clarify what is meant by “interaction potential only resides in one of the drugs”.	Accepted. Reworded.
176-178	3	Comment: The definitions of the terms "mechanistically unsuspected" and "narrow therapeutic index" are not clear. Proposed change (if any): Please clarify what "mechanistically unsuspected" means and provide a definition of the term "narrow therapeutic index". Especially for labelling purposes, broad definitions that require interpretation should be avoided.	Partially accepted. Exemplified naming a field (HIV). No further examples are needed here.
176-178	9	Comment: The sentence is not really clear. What is meant by “class of substances”? It may be unlikely that you have a class of drugs (> 1), but mechanistically unexplained drug interactions. Are general DDI studies with digoxin and warfarin or other NTIs meant when they are expected to be commonly co-prescribed with the investigational compound? An	Partly accepted. General DDI studies with digoxin and warfarin are not expected as these interactions are mechanistically predictable. The aim is commonly co-prescribed drugs with a relatively narrow therapeutic index. The paragraph has been revised. See also comment above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		example could be helpful.	
178-180	1	**Comments:** Classes of "mechanistically unsuspected" DDI for clinical study are vague and not actionable. Proposed change (if any): Cite examples or delete.	See above.
178	10	Comment: How is "class" defined? By chemical class? This requires clarification. Proposed change: Class = "chemical class with significant structural similarity".	Not accepted. By class we mean therapeutic class. This may become clearer with our added example.
178-180	12	**Comment:** Agency should specify what is meant by class of substances (Clinical? Pharmacological? Other?) The need for *in vivo* studies should be based on *in vitro* data of the investigated drug rather than on properties of other compounds of a substance class, since substances of a class can vary considerably in their DDI profile.	Not accepted. See above.
181-208	1	**Comments:** Food effects – no reference to BCS class, which should influence which drugs are affected; out of scope for DDI Guidelines. **Proposed change (if any):** Put food effect studies in a PK Guideline.	Not accepted. We do not have sufficient experience to make a non-arguable link between BCS classification and food effects. The food interaction is a type of interaction with an extrinsic factor and can remain in this guideline.
184-192	5	Comment: In lines 184 to 186 it is correctly stated that the extent of food-interactions may be a function of the formulation characteristics or dosage form (i.e. of a specific pharmaceutical product not just the	Accepted. Information on this has now been added in section 5.1.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		investigational drug). However, in the following this important consideration is no longer considered in a consistent fashion. For example, in the following statements on drugs with non-linear PK, it is just referred to the dose administered, but not to different dose-strengths, which may be qualitatively and/or quantitatively not similar. Proposed change: Consider to emphasize consistently that different dosage forms that are qualitatively and/or quantitatively not the same, may have to be investigated separately. To keep the section short, it may be cross-referenced for further details regarding that biopharmaceutical matter to the current EMA Bioequivalence GL (CPMP/QWP/EWP/1401/98 Rev. 1).	
184-194	12	**Comment:** The recommendation to investigate the effect of food on the highest and lowest dose of therapeutic range in case of non – linear PK seems very stringent and needs further elaboration from the Agency. Agency should also clarify and provide criteria on what constitute a “significant” food effect.	Not accepted. If the nonlinearity is giving rise to a more than dose proportional AUC when increasing the dose, the food effect is likely most pronounced at the highest dose. Thus, if there is no or little food effect on this dose, there is no need to investigate the lowest dose. If there is a clinically relevant food effect on the highest dose, the same food recommendation may be applied to all doses, if this is chosen by the applicant in order to have clear treatment recommendations. If the exposure is less than dose proportional when increasing the dose, the food effect may be highest both at the low and high dose range. Thus, both dose extremes need to be studied.
188 –	4	Comment:	Partly accepted. The cross-reference is present but no specific

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
Section 5.1		We welcome discussion on the effects of food intake on PK, but would like to extend this to include specific reference to the effects of concomitant alcohol consumption with modified release products. Proposed change (if any): Addition of the following text preceding lines 186-188 and if necessary to cross-reference the Modified-Release and Transdermal Guidance update when available. "If the formulation.....may be needed. In particular for modified release products it may be appropriate to conduct further studies into the effect of alcohol consumption on the PK of the investigational drug."	recommendations are given as this is related to a certain kind of formulation alone.
189-194	12	**Comment:** In Phase I, a sponsor may not know the therapeutic dose range. The PK could be non-linear based on Phase I dose ranging studies, but further evaluation may lead to the therapeutic dose being within a linear range. **Proposed change (if any):** Since the therapeutic range is not known in Phase I and the formulation may change, it should be clarified that doing food effect studies in Phase I is not the expectation, but rather these studies should be conducted on a case-by-case basis.	Not accepted. Food interaction studies should be performed to support the efficacy and safety assessment of drug dosing regimens both in phase II and III unless administration close to food intake may be avoided.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
190	1	**Comment:** The reason for testing both the highest and lowest strengths for drugs with non-linear PK is not provided. The statement "Further studies are recommended" is open ended and vague. **Proposed change (if any):** Harmonize with language in EMEA Guideline on the Investigation of Bioequivalence 2010.	Not accepted. See below. The food interaction recommendations and how the results are assessed in an NCE application are different from the abbreviated application. No harmonization is needed.
190-191	3	Comment: A recommendation is made to investigate the effect of food on the highest and lowest doses in the therapeutic range if a PK nonlinearity is present. However, the effect of food would not be likely to change unless the nonlinearity is at the level of absorption. Proposed change (if any): Recommend providing caveats for when it is not necessary to conduct a food effect study at the highest and lowest doses even when the PK is nonlinear.	Not accepted. The most pronounced differences most probably take place in case of nonlinear absorption or nonlinear first pass. However, also nonlinear elimination may lead to a more pronounced food effect at the highest dose if the food effect leads to a higher bioavailability.
191-192	15	Comment: The therapeutic range of a compound is an uncertain estimate in phase I and not established until in phase II. Proposed change (if any): Suggested to investigate effect of food at one dose level (the highest dose level planned into phase IIa).	Not acceptable. The development program should be adapted to which information is needed at a certain stage of the development program. Furthermore, if the phase II highest dose is higher than the chosen phase III highest dose, it will likely not be a crucial problem to have studied a too high dose per see.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
193-194	15	Comment: Which effect is clinically significant may be difficult to evaluate before phase II efficacy and safety results are known. Proposed change (if any):	Partly accepted. This may be true but the decision needs to be taken based on the available (phase I and II) data when going into phase III.
195	9	Comment: In general one and not several studies with a light meal as defined in Appendix I should be sufficient.	Not accepted. This is a case by case decision. Usually the food interaction studies with different types of meal are performed to clarify exposure in the clinical trials and support the food recommendation aimed at in the SPC. See also above.
195	12	Comment: In general one and not several studies with a light meal as defined in Appendix I should be sufficient.	See above
195-202	12	Comment: Significant practical issues are expected to the study execution in establishing the time interval before and after a meal. Following your recommendation a time interval before and after the meal should be given in the label. However, can we really assume that drug administration will be avoided during this interval under practical conditions? This can be established with the current approach to evaluate the drug performance in fed and fasted state.	Not accepted. We do not follow how this could be established without directed studies. The feasibility of the regimen needs to be taken into account when choosing food recommendations.
201-204	5	Comment: Here it is referred to co-administration of the investigational drug/product with a meal or specific food in the paediatric population. Thereby it is specifically referred to newborns and infants, however, without mentioning that in most cases specific	Not accepted. This is already covered by the general text regarding additional food interaction studies for different formulations.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		pediatric formulations may have to be developed for these populations. Proposed change: Consider to adding a respective statement on the need for pediatric formulation development for very young children and the potential need for separate food-drug interaction studies of these formulations.	
209-220 Section 5.2	4	Comment: We welcome specific guidance on the effects of medicinal products on the PK of the investigational drug, but where combinations are inevitable (e.g. oncology) we would value specific input or further clarification. Proposed change (if any): Addition of further Appendix, or subsequent Addendum relating to the investigation of drug interactions for oncology therapeutic area.	Partly accepted. The requirements for combinations have been clarified. The requirements for oncology products depend on whether studies are possible in healthy volunteers or not. However, even if it is only possible to perform studies in patients, this does not mean that no DDI studies should be performed. These products and also the concomitantly used drugs are usually very potent and appropriate treatment recommendations needs to be available supported by appropriate studies. More information may be available in indication specific guidelines.
209-216	12	**Comment:** A complete assessment of the transporter mediated effect of other drugs on the PK of the inv. drug is not feasible prior to the phase II programme because the therapeutic dosages are still undefined. Agency should clarify and evaluate this criteria because of 1) current limitations of available in vitro methods to study DDI 2) requirement to combine *in vitro* and *in vivo* data 3) use of pharmacogenomics to identify transporters	Partly accepted. We agree that a complete assessment is not possible before mass-balance data is available. However, some transporters may be considered early based on absorption characteristics, a determined low bioavailability, prior knowledge about drugs of the same class, etc. In absence of in vivo DDI data, the study protocols should be adjusted accordingly and if possible, blood could be drawn for genotyping or genotyping applied in phase II.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		which impact the PK of inv drug.	
211-213 441-443	1	**Comment:** Results from in vitro tests should be used to justify the phase 2 study design because conducting many in vivo DDI studies prior to obtaining proof of efficacy from phase 2 studies is not practical. **Proposed change (if any):** Enable the formulation of an in vitro data-driven clinical plan that is adjusted case by case for compound-specific pharmacology and rational co-therapy.	Partly accepted. The wording has been clarified. The kind of data needed depends on the possibility to exclude drugs likely to interact based on *in vitro* metabolism data. Regarding effects on other drugs, the guideline already states that in vitro information on enzyme inhibition is sufficient in most cases when starting phase III.
211	10	Comment: “Other medicinal products” will generally be investigated in vitro during Phase III. Before Phase II, likely co-medication and mechanistic co-medication (e.g. CYP inhibitors) need to be characterized (see also line 348 ff). Proposed change: “The effects of other medicinal products relevant co-administered drugs in the target indication and mechanistic drugs on the pharmacokinetcs….”	Not accepted. If the *in vitro* studies on the inhibitory/inducing effects on enzymes are not available before phase III, proper restrictions on concomitant medication is likely not possible due to the number of drugs needs to be excluded.
211-216	15	Comment: In vivo drug interaction studies during phase I are hampered by that we do not yet know what would be the relevant pharmacologically active dose to test at. There are also ethical concerns in exposing volunteers to an investigational drug before proof of concept.	Partly accepted. The text has been clarified.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		However, in vitro drug interaction potential is usually known at this time. Proposed change (if any): In Phase IIa instruct investigators to avoid concomitant medication which based on in vitro drug interaction data may pose a risk for clinical drug interaction. Conduct in vivo drug interaction studies after phase IIa.	
212	10	Comment: Typo error. Proposed change: before introducing the investigational product to patients in phase II …	Accepted.
222-236	12	**Comment:** Agency should clarify the meaning of “sequestration”. Also GI motility should be considered as a key factor influencing absorption. Agency should elaborate the statement “..is MARKEDLY pH dependent” and the “physiological pH. **Comment:** Range” as well as what physiochemical properties of drug should be flagged and pursued (line 235).	Accepted for sequestration. Not accepted regarding marked pH dependency. We do not have data or experience enough to set a certain limit here. No elaboration needed regarding physiological pH range.
223-224	5	Comment: Here it is referred to potential GI-tract effects that may alter the absorption of the investigational drug such as increased gastrointestinal pH, sequestration and decreased or increased intestinal active transport. However, alterations of the gastric emptying time, which may also result in altered	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		drug absorption are not specifically mentioned. Proposed change: Consider to also refer to altered gastric emptying in the listing of GI-tract functions that may alter drug absorption.	
223-225	16	Transit time/gastrointestinal motility as factors influencing absorption need to be considered as well.	Accepted
225-228	5	Comment: This Chapter refers to effects of other medicinal products on the pharmacokinetics of the investigational drug. As concomitant drugs administered by virtually any route (e.g. i.v., s.c., etc.) may have potential to alter the GI-tract motility (e.g. intravenously administered erythromycin) and other GI-tract functions, it appears not entirely clear, why it is referred in this section to orally administered and orally/nasally inhaled products only. Proposed change: Rephrase the last sentence of this paragraph (line 227/228) accordingly (e.g. “However, interactions should be considered also for products administered by any route (e.g. i.v., s.c.) in case that they that might have potential to alter GI-tract functions based on their systemic exposure.	Not accepted. The text refers to characteristics of the investigational drug (victim in this section). Thus, it is relevant to mention orally administered drugs.
230-231	5	Comment: If the solubility of the drug in fact is markedly pH dependent, then it appears mandatory that the effects of an increased gastric pH (e.g. by proton pump inhibitors or antacids) need to be investigated in vivo and should not be merely	Accepted

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		discussed. Proposed change: Consider to rephrase: “If the solubility of the drug is markedly pH dependent in the physiological pH range, the potential effect of drugs which increase gastric pH, such as proton pump inhibitors, H2-receptor antagonists or antacids, should be investigated in vivo, unless it can be excluded with sufficient confidence otherwise.”	
231-236	17	A. Interactions affecting solubility If the solubility of the drug is markedly pH dependent in the physiological pH range, the potential effect of drugs which increase gastric pH, such as proton pump inhibitors, should be discussed. If an effect on absorption cannot be excluded, it is recommended that the potential for interaction is investigated in vivo. If indicated by the physicochemical properties of the drug, it may be necessary to investigate the potential for sequestration in vitro and an in vivo study could be considered. Comment: an example of incomplete information is SPC Ellaone (ulipristal): concomitant administration of drugs that affect the stomach-pH (e.g. protonpump inhibitors, antacids and H2-antagonists) can decrease the plasmaconcentration of ulipristalacetate and thus the efficacy. Concomitant administration is not recommended. The absorption of ulipristalacetate is	Accepted. This should be investigated *in vivo*. The wording has been strengthened.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		dependent on the pH. The manufacturer states by email that the dissolution is delayed at higher pH values, and that no in-vivo-study has been done to investigate the influence of drugs that increase the stomach pH. WFG: the release of ulipristal from the tablet is delayed, this suggests that the formulation of the concomitant administration is the cause of the problem, and not the absorption of the active ingredient itself. Proposed change (if any):	
333-338	17	If cytochrome P450 enzymes are identified as candidate enzymes involved in the main elimination pathways of the drug (or in major formation or elimination pathways of clinically relevant active metabolites), evaluation of the pharmacokinetics of the investigational drug with and without concomitant administration of a strong specific enzyme inhibitor (see Appendices IV and V) is recommended to verify and quantify the involvement of a specific enzyme in the investigational drug elimination. Comment: information such as 'ketoconazole increases the plasmalevel of drug A' is insufficient. To assess the consequences of an interaction we need more data, such as 'ketoconazole 500 mg for 1 week increases the plasmalevel from xx to yy mg/ml.'	Agreed. We believe the document reflect this.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Not all manufacturers are willing to provide us with this information.	
234	5	Comment: It might not be entirely clear to everybody, to which phenomena the term “sequestration” may exactly refer to. Proposed change: Specify the term sequestration, e.g. by providing examples.	Accepted.
237-257 294-296	1	**Comment:** Sweeping recommendations are given for clinical investigations of transport aspects of drug disposition. The ITC white paper did not include recommendations for certain transporters due to lack of evidence of clinical significance. **Proposed change (if any):** Limit to transporters and transporter genetic variants that lead to significant clinical DDIs (and cite references) and for which genetic variants are not rare. Make congruent with ITC and CDER draft Guideline.	Not accepted. There are some examples of altered distribution due to reduced transporter activity. However, the examples are presently quite few. We think the text well reflects the current level of knowledge and opens up for more knowledge to be gained.
241	5	Comment: The mechanistic explanation “...secondarily metabolising enzymes (e.g. CYP3A)” appears not entirely clear. Proposed change: Consider to rephrase “...secondarily due to an increased intracellular drug-concentration in the enterocyte leading to an increased fraction of dose	Not accepted. The explanation could be increased concentration in the enterocyte and nonlinearity making more drug escape the enzyme. However, it could also be less time for the drug at the site of the enzyme due to less cycling in the intestine. A less specific wording is preferred.

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		escaping intestinal drug metabolism (e.g. mediated by CYP3A)".	
244-252	12	**Comment:** Caco-2 is a variable and lab- dependent system. Important point is that clinical relevance of intestinal P-gp transporter mediated DDI is not clearly established. The Agency should consider that it would be very difficult to define the CYP3A4 – catalyzed intestinal metabolism as a key factor.	Not accepted. There are examples, such as dabigatran, of clinically very relevant Pgp inhibition.Caco-2 cells are subject to variability and therefore needs to be standardized/controlled. In addition, a second *in vitro* system is recommended in parallel. The comment on 3A4 is not understood. The degree of intestinal metabolism can be calculated from iv and oral data if full absorption is assumed. It may also be estimated by a well designed grapefruit juice study, or potentially by a comparison of the effects of a potent 3A4 inhibitor on AUC as compared to half-life.
244-246	12	Comment: Which effect is clinically significant may be difficult to evaluate before phase II efficacy and safety results are known. Proposed change (if any):	Agreed. The phase I studies may give indications though as well as knowledge from similar drugs, if available.
251	3	Proposed change (if any): We recommend to modify the sentence to "…transporter has been identified as having a major role..".	Not accepted. This may be difficult based on in vitro data and still the clinical relevance needs to be taken into account. Also, if later in development, it is understood that a transporter may be relevant, the applicant needs to perform studies to identify the transporter. We prefer the present wording.
251-252	5	Comment: The sentence suggests that there are known inhibitors (potent and specific) of ABCB1 and ABCG2 that are registered as medicinal products in the EU. We are not aware that such medicinal products exist. Could you please specify which of the currently	The document does not say selective, only potent. There are quite potent inhibitors of ABCB1 (Pgp) and some inhibitors seems to be quite potent ABCG2 (BCRP) inhibitors based on in vitro data. However, with the CYP inhibitor classification

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		registered medicinal products you consider as potent and specific (in order to predict the potential for pharmacokinetic interaction via inhibition or induction of the transporter) inhibitors of intestinal efflux transporters ABCB1 and ABCG2. Proposed change (if any):	they would not be classified as “strong”. This may also be related to the few specific, sensitive, substrates presently known.
251-255	9	Comment: It is not justified to conduct an *in vivo* study just because a transporter has been identified *in vitro*. For some compounds the actual contribution of active transporters may be too small to be of relevance. Additional data from *in vitro* studies or *in vivo* (genotype PK-correlations, dedicated analysis of human PK characteristics, dose-proportionality etc.) are required to assess the contribution of active transport processes to the absorption of a compound.	Not accepted. We are not asking for data without *in vivo* indications of important transporter involvement.
251-252	13	Comment: This is a broad statement which requires in vivo study whenever transporters are involved irrespective of whether their effects are clinically relevant or not and should be qualified. Proposed change (if any): When a candidate transporter has been identified, if interactions through inhibition are likely to be clinically relevant, an *in vivo* study with a potent inhibitor is recommended if known inhibitors are registered as medicinal products in the EU.	Accepted. The basis of the in vivo investigation is that transporter involvement seems to be of clinical importance. However, the text has been changed to make this clearer.
251-254	16	Active uptake is only known for very few transporters (e.g. PEPT1/2, MCT). Are *in vivo* studies with potent	Not accepted. The guideline needs to be open for future knowledge both on transporters and their genetic differences.

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		inhibitors of these transporters feasible since specific inhibitors are not well characterized. The recommendation of *in vivo* studies using selected genotypes seems of limited use as few examples of transporter polymorphisms affecting absorption exist. Furthermore, the complex interaction of permeability, metabolism, solubility, dissolution, and active uptake may limit the usefulness of studying small populations with specific transporter polymorphisms. We would suggest to remove this sentence.	
252-255	5	Comment: *In vivo* studies in subjects of certain genotypes may be in particular useful in cases (i.e. for transporters) for which no approved specific and potent inhibitors are registered as medicinal products in the EU. This important consideration should be explicitly mentioned. Proposed change (if any): Consider to adopt the statement above.	Agreed. The concept is already included.
252	9	Please add a list of known and accepted inhibitors of drug transporters listed in 668 to the appendix of this document.	Not accepted. (See above)
253-254	7	Exceptions should be provided when drug-specific factors such as high solubility and high intrinsic permeability make it unlikely that transporters are a major mechanism limiting absorption. "For example, the bioavailability of [BCS] Class 1 or [BDDCS] Class 1 NMEs…may not be significantly affected by a co-	Partly accepted. The text has been changed to reflect an influence of permeability. According to the present theory, BCS class II drugs may be affected by intestinal transporters. However, there are presently few known examples why we have abstained from mentioning this in the guideline.

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		administered drug that is a P-gp inhibitor…" (Zhang L et al., AAPS J 2009 Jun; 11(2):300-6). Proposed change (if any): When a candidate transporter has been identified, an *in vivo* study with a potent inhibitor is recommended if known inhibitors are registered as medicinal products in the EU, unless drug-specific factors such as high solubility or high intrinsic permeability make it unlikely that transporters are a major mechanism limiting absorption.	
253-257	12	**Comment:** The Agency may want to elaborate the meaning of "relevant inhibitors and on genotypes" since *in vivo* testing of interaction at transporter is unspecific. Please consider that it may not be justified to conduct an *in vivo* study because a transporter has been identified *in vitro*. For some compounds the actual contribution of active transporters may be too small. Also consider that a clinically significant impact on absorption due to alterations in transporter activities is only likely for compounds where active transport exceeds the contribution of passive diffusion. With the exception of narrow therapeutic index drugs, *in vitro* investigation of the potential impact of active transport on absorption may be warranted where absorption of the investigational drug is ≤50% and as such the clinical impact on absorption could be in the region of 2-fold. *In vivo* studies to evaluate impact of inhibitors	Not accepted. The investigation on transporter involvement is initiated when significant elimination through active transport is indicated or when there are signs of clinically relevant transporter involvement in drug absorption.

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		of transporters shouldn't be necessary for drugs that are highly permeable and extensively metabolized.	
253-254	15	Comment: What potent inhibitors are known Proposed change: EMA will collect a definitive table of clinically significant inhibitors that are accepted for such studies. If something else is found and scientifically justified, the sponsor can negotiate on using something outside the table.	Not accepted. A list of transporter inhibitors would be counterproductive as science is likely to evolve fast increasing our knowledge regarding inhibitors.
257	5	Comment: please state more precisely Proposed change (if any): Interactions affecting distribution include plasma protein displacement interactions and…	Accepted
259	5	Comment: Please consider rewording this sentence. The current version does not correctly reflect the published knowledge on changes in plasma concentrations mediated by changes in drug distribution exerted by drug transporter(s) Proposed change: Distribution interactions due to an alteration in drug transport are in most cases not reflected by changes in plasma concentrations alone. It is also advisable to indicate the masking effect of the concomitant protein-binding displacement and enzyme inhibition where the total drug concentration remains	Not accepted. It is not suitable to include such a specific case, where one interaction effect is counteracted by another effect. It becomes too much detail and if doing this, several other combined DDI mechanisms needs to get into the text. These complex scenarios should be taken into account only when indicated.

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		the same but free drug in plasma could be higher (see Orlando et al 2009). If such effects are suspected then *in vivo* studies should considered measuring the free drug levels (see later sections)..	
259-278	12	**Comment:** It is not fully known how to study DDI involving distribution (and available data from literature are limited). Plus DDI data due to protein binding displacement may not be conclusive. There are only very limited data on the expression of transporters in various human tissues except for the liver (only limited data on the intestine and blood-brain barrier). The available data are mostly based on a few samples only and show marked variability. Therefore, it is currently hardly possible to quantitatively assess effects of drug transporters on the distribution of a drug in a more general sense. In addition there are very limited cases when a protein binding changes may be clinical important. Therefore results may be difficult to interpret. **Comment:** Based on the available literature, animal species exhibit marked differences of expression of drug transporters in various tissues (in addition, transporters in animals may exhibit different properties due to different protein structure) therefore, data of animal experiments are of very limited value and may sometimes be misleading.	Partially accepted. There is limited data but this area is developing. More cases may be known in the future and the document should be open for this. Also, the limited data available, e.g. on Pgp and brain, OATP and liver as well as OCT-1 and target cell distribution needs to be considered. It is agreed that animal studies are not always predictive. Still effects by a transporter inhibitor/knockouts on distribution is an indication that should be followed by human *in vitro* data and if positive, *in vivo* data. Not accepted. In general, we encourage studies on transporter distribution and effects of drug distribution. This is an area under development and the present text reflects our recommendations.

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		The Agency should also clarify if it's recommending a better thorough understanding of transported tissue distribution (examples?). Agency should clarify the meaning of the following sentence "….could be discussed as far as reasonable".	
264-276	5	Comment: In this section recommendations on investigations are given, if the investigational drug is a substrate for transport proteins. However, the transporter substrate characteristic of a drug is just one (and often minor) co-determinant of its tissue distribution; the intrinsic permeability characteristics (acc. to BCS) of a compound may be even more important in that matter, as high intrinsic permeability may completely shadow or supersede the role of active transport in distribution of a particular compound. The requirement to study transporter-based interactions as described in this section merely based on the fact that compounds are transporter-substrates are likely to result in many unnecessary studies and investigations. Proposed change: Consider to specify that the required investigations would be in particular applicable to compounds with low permeability (acc. to BDDCS criteria) and which are substrates to transport proteins as outlined in Appendix II.	The text sets little firm requirements. The potential for interactions leading to altered distribution should be discussed and if indicated and feasible in vivo studies are recommended. The discussion should if scientifically possible include a discussion based on which process is rate limiting for the particular organ.
265-278	1	**Comment:** The discussion of distribution-related drug-drug interactions due to altered transport is ambiguous.	Partially accepted. There is limited knowledge in this field at the moment with the exception of some information about the CNS (as indicated by the comment). The text on induction has

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any): Cite specific references to clinically significant examples for relevant tissues, especially for transporter induction, and especially for tissues other than CNS.	been removed from the distribution section.
266-267	9	Comment: There are only very limited data, mostly indirect data based on mRNA levels, on the expression of transporters in various human tissues except for the liver (only limited data on the intestine and blood-brain barrier). The available data are mostly based on a few samples only and show marked variability. Therefore, it is currently hardly possible to quantitatively assess effects of drug transporters on the distribution of a drug in a more general sense. Proposed changes: The section should be re-phrased to take this into account.	We agree but feel that the text is reflecting the present lack of knowledge.
		Comment: Based on the available literature, animal species exhibit marked differences of expression of drug transporters in various tissues (in addition, transporters in animals may exhibit different properties due to different protein structure) therefore, data of animal experiments are of very limited value and may sometimes be misleading. Proposed changes: Re-phrase text: ".....from data on	Accepted. The uncertainty in the extrapolation of preclinical data is known and reflected in the text. However, the text has been reworded to be more clear.

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		distribution in preclinical species provided that species differences of transporter characteristics and tissue expression are well understood and are taken into account."	
274	10	Comment: Typo error. Proposed change: replace ‘is’ by ‘are’.	Corrected.
278	5	Comment: It is appreciated that the EMA guideline unlike guidance from other regulatory bodies contains a paragraph on drug displacement interactions. We however, would like to suggest highlighting the need to measure the free unbound plasma concentration instead of total plasma concentration to capture possible DDIs based on protein displacement. In this context we'd like to highlight the findings by Orlando et al. (Irreversible CYP3A Inhibition Accompanied by Plasma Protein–Binding Displacement: A Comparative Analysis in Subjects With Normal and Impaired Liver Function. Clinical Pharmacology & Therapeutics (2009); 85, 3, 319–326 doi:10.1038/clpt.2008.216) that underlines the need to measure free plasma concentration if effects on protein displacement are to be monitored.	Accepted. This information was included in the “Study design” section and is now also inserted here.
279-289 467-469	1	Comment: Protein displacement drug interactions are being suggested but are rarely, if ever, clinically significant. Proposed change (if any):	Partially accepted. The text has been shortened due to the small number of known examples. Examples will not be included in the guideline.

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		Delete or refine scope and cite clinically significant example that appears in a drug label.	
279-289	16	While it had been hypothesized that increases in unbound drug concentrations will lead to increases in drug effect, there are very limited cases when protein-binding changes may be important clinically as noted in a review by Benet and Hoener (Clin Pharmacol Ther. 2002 Mar;71(3):115-21). Depending on PK parameters and the intrinsic clearance of the drug, certain PK parameters will change with protein binding but others will not. For drugs with low hepatic extraction ratio, regardless of route of administration, total exposure is independent of protein binding and no dosing adjustments will need to be made for real or anticipated changes in unbound fraction (fu). Only high extraction ratio drugs given IV and oral drugs eliminated by non-hepatic high extraction routes will exhibit changes in unbound drug exposure when protein binding changes. Benet and Hoener considered a list of 456 drugs and found that of these, only 25 may be impacted by protein binding, resulting in changes in clinical drug exposure. Furthermore, of these 25, IV administered lidocaine may be the only drug of relevant concern due to its small therapeutic window and rapid equilibration time. Roche is in agreement with the low risk of clinically relevant interactions via displacement from plasma protein binding sites (line 280), however we would like	See comment above.

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		to raise two important points where we disagree with the guidance, as written. Firstly, for consideration of the potential for a clinically meaningful displacement interaction, highly bound drugs must possess a narrow therapeutic index, small volume of distribution AND (not OR as written, line 285) exhibit a high hepatic extraction ratio and be administered IV or exhibit a high renal extraction ratio. As written, the guidance indicates that if any one of the three criteria is met (lines 284-287) then there is a cause for concern. Secondly, we disagree with the suggestion to conduct in vitro displacement studies to decide if in vivo studies should be performed, especially in the context of section 5.3.2 Distribution, lines 468-472. *In vitro*, the potential for displacement of one drug by another is simply a function of binding affinity for the representative protein(s). In other words, while two drugs might have similar Kd values and would thus compete for binding protein sites in vitro, this in vitro result would most often not translate into a clinically meaningful effect on PK (yielding a false positive in vitro result). Therefore an in vitro result could be misleading, especially considering the lack of clinically relevant examples as discussed above. Overall, given the lack of clinically relevant examples and the risk of identifying false positives *in vitro*, it is our position that the guidance should not recommend	

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		in vitro or in vivo studies to evaluate drug displacement interactions (i.e., delete lines 288 and 289 and section 5.2.2). It should be added that for compounds binding primarily to human serum albumin, the concentrations in blood should be relatively high, more than 70 µM (i.e. the possibility to occupy about 10% of albumin in blood). For compounds binding primarily to AAG protein, the concentration should be more than 2 µM.	
284-285	5	Comment: The fact that changes in plasma protein binding affect the clearance of drugs that exhibit a high extraction ratio and are administered i.v. or have a high renal extraction ratio is derived from the concept of clearance by Benet et al.(Changes in plasma protein binding have little clinical relevance. Clin Pharmacol Ther. 2002 Mar; 71(3):115-21). The title of this publication is misleading however, given that only changes on drug clearance are discussed. It needs to be noted that small changes in the fraction unbound of a drug can have marked effects altering the distribution of drugs.	Not accepted. It is not understood why this is not mainly a function of Cu. Not introduced at present.
282	12	**Comment:** Agency should clarify if the definition of "…a highly bound drug is really fu < 3%'. Please provide rationale of it.	The figure is arbitrarily set and has been reduced to approx. <1%.
286-287	15	Comment: What is considered high hepatic and renal extraction ratio? If extraction ratio is high, the fu plays typically	The definitions of Rowland and Tozer are applicable. These are well known and need not to be defined.

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		less role than if the hepatic extraction ratio is low. Proposed change (if any): Please suggest a value for high hepatic and renal extraction ratio, respectively. Comment on molecules that are not affected by PPB in liver	
291-293	9	Comment: The text about off-target effects of metabolites requires some explanation. Are these known off-target effects of the parent drug or off-target effects in a general case? It may be hard to achieve off-target effects of all metabolites.	Partially accepted. The text has been reworded.
291-298	12	**Comment:** Conducting studies as suggested is not a trivial matter as it would require synthesis of the metabolites (could be many) and then testing for both off target and on target activity. Studies should only be considered for major metabolites and where it is technically feasible. **Proposed change:** We suggest amending lines 295-298 as follow: "should be identified <u>where possible for major circulating metabolites</u>".	Not accepted. Text clarified. It is important to know the pharmacological activity of the metabolites as early as possible to be able to follow the right substances in the clinical PK studies. This is mainly discussed for target effects in the revised guideline text although off target effects are also taken into account when of importance as indicated by other information.
291-305	12	**Comment:** We support the guideline on active metabolites and genotype effect, particularly the need to define routes which dominate in genetically impaired individuals and defining the role of metabolites in pharmacological activity. However, we are concerned both by the fact	See above regarding metabolites. The timing requirement has been reworded. The knowledge on which enzyme, based on in vitro data, that seems to be the main metabolizing enzyme is of importance if the enzyme is subject to genetic polymorphism as then poor metabolisers may need to be excluded or to have a lower dose. However, as this is not a

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		that it appears that these studies are mandatory and also as noted in the general comments about the potential timing of such studies. **Proposed Change:** Could the Agency consider modifying the guideline to clarify that these studies are advisable rather than mandatory. Could the Agency clarify that characterisation of the major enzymes will not normally be required before phase I since the relevance of the associated metabolites can only be evaluated and confirmed in humans towards the end of phase I or during phase II. The information on metabolites is not usually considered as critical for inclusion/exclusion criteria in the early clinical phases. In addition, in general it may well be advantageous to have a clear understanding of the clinical dose range and exposure levels of the investigational drug before initiating clinical interaction studies.	pharmacogenetics guideline, the wording on timing has been removed for enzyme identification.
293-295	12	**Comment:** The text about off-target effects of metabolites requires some explanation. Are these known off-target effects of the parent drug or off-target effects in a general case? It may be hard to achieve off-target effects of all metabolites.	See above.
297	12	**Comment:** Please provide criteria of “major elimination pathway”.	Accepted. A clarification has been introduced in Appendix III.
297	12	**Comment:**	See above.

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		Characterization of the major enzyme pathway is typically not done (or unnecessary) prior to phase I volunteer studies. Timing of these studies should rather be driven as development progresses.	
301-305	12	**Comment:** Pharmacological screening of metabolites identified *in vitro* may not be meaningful in terms of pharmacological activity, if the concentrations of these metabolites is low *in vivo*.	Agreed. However, the screening may give early information on which substances to follow *in vivo*.
305-306	5	Comment: The general recommendation that "enzymes contributing to ≥25% of the oral clearance of an investigational compound should be verified *in vivo*", appears meaningless in itself, as the decision criterion to which it is referred to (i.e. oral clearance) can only be assessed by the actual conduct of *in vivo* studies. Further, any alteration of the oral clearance observed in *in vivo* studies, must not necessarily reflect a single pathway inhibition of the metabolic enzyme of interest. It rather may be a composite outcome of various mechanisms. Hence, it remains largely unclear from a methodological perspective, how the decision criterion "relative contribution the overall oral clearance" can be accurately assessed for a single metabolic enzyme/pathway. Therefore, please specify the methodology on how enzymes contributing to >25% of oral clearance shall be identified Also the proposed generally applicable cut off value for the alteration of oral clearance of ≥25% appears	Agreed. A description of how % contribution is estimated has been included in appendix IV. The rationale behind the cut-off value is discussed in the beginning of this document.

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		arbitrary and overly conservative, as a decrease in oral clearance of 25% would translate into a modest total exposure increase of about 33%, a value that would hardly call for any dose-adjustment recommendations, unless a product might be a narrow therapeutic index drug. Proposed change: Consider to refer to *in vitro* based decision criteria and other applicable criteria for defining the general requirements of *in vivo* studies. Re-consider the proposed general cut-off value of ≥25% and consider instead proposing the acceptability of flexible cut-off values depending on the therapeutic margin of a given product. It might be stated, that appropriate cut-off values should be proposed and justified by the Sponsor based on the overall product characteristics.	
306	9	Comment: It is not clear how to calculate 25 % of a metabolism pathway to oral clearance. As the absolute amount of a metabolite formed in the body is generally unknown, the clearance of the metabolite cannot be easily calculated from its plasma AUC. Proposed changes: Use 25 % of the AUC of the parent compound after oral dosing as a qualifier.	See above.
306-311	12	**Comment:** It can be very difficult, if not impossible to assess the	Partly accepted. The comment is acknowledged. However, a cut-off is considered needed in the guidance and the

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		contribution of a given metabolite to the pharmacological target or off-target activity if the effect compartment is not the blood compartment. Therefore, a defined threshold of X-% as a qualifier for additional investigations is not justified.	recommended cut-off is considered reasonable.
307	9	Comment: It can be very difficult, if not impossible, to assess the contribution of a given metabolite to the pharmacological target or off-target activity if the effect compartment is not the blood compartment. Therefore, a defined threshold of X-% as a qualifier for additional investigations is not justified.	See above.
307-311	12	**Comment:** The Agency should clarify and justify the rationale of >= 25% oral clearance. The absolute amount of a metabolite in the body is generally unknown; the clearance of the metabolite is not easily calculated from its plasma AUC. In addition, the Agency should be aware that it would be difficult to determine 50% pharmacological activity on or off target.	See above and in the beginning of the document.
307; 328	16	The in vivo involvement of enzymes found in vitro to catalyse metabolism pathways which are important in vivo, should be confirmed and quantified. In general, enzymes involved in metabolic pathways contributing to ≥25% of the oral clearance should if possible be verified in vivo. This recommendation seems arbitrarily low as in cases of drug-drug interactions with perpetrator drugs, only	Rationale given in the beginning of the document.

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		modest effects are predicted for pathways with ≤ 75%. In addition, often it is impossible to quantify the contribution of individual pathways to the overall oral clearance early-on in a project. The more individual pathways are involved, the more difficult it is to quantify the individual ones. For CYP-mediated processes fm can be reliably determined in vitro, this process is not established for transporter-mediated clearance processes. The same rationale applies for testing inhibition of single or multiple pathways contributing to ≥25% of oral clearance.	
313-315	16	Contribution of a metabolic pathway in vivo may also be determined by a mass balance study with supporting in vitro data, ie if a metabolite is known to be formed by a specific enzyme only, recovery of this metabolite and its subsequent metabolites could be used to calculate the overall flux through that enzyme.	Agreed. This is the intention.
323-329	5	Comment: This Chapter refers to metabolism-based interactions. Therefore the example provided to exemplify the issue of a dual-pathway inhibition (i.e. CYP3A and P-gp inhibition) appears not entirely appropriate, as it represents a combination of a metabolism- and transporter-based DDI. Further, the proposed cut-off value of ≥25% alteration in oral clearance for the sum of two metabolic pathways appears conceptually inconsistent, as it is essentially similar to the cut-off value proposed for the	Partly accepted. The mentioning of CYP3A4 and Pgp is important as it is common situations of dual inhibition by one drug that is the aim is this text. This has now been clarified.

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		relative contribution of a single pathway. Practically, this requirement appears overly conservative and hardly feasible as it would call for *in vivo* dual-pathway inhibitor studies for a pair-wise combination of single pathways, each of which may contribute to the overall oral clearance of a compound as less as 12.5%. As a minimum requirement for the relative contribution of a single pathway to the ≥25% requirement for the sum of two pathways is not defined, it might even be the case that the dual-inhibition of a pathway that contributes less than 10% to the oral clearance of a compound together with a pathway that might contribute to about 20% must be addressed by *in vivo* studies. Also the currently proposed approach might result in the requirement of more than one dual-pathway inhibitor studies, e.g. in case that perhaps 5 to 6 different enzymes each might contribute with about 10% to 20% to the overall oral clearance of a compound and various pair-wise combinations of these pathways might sum up to clearance alterations of ≥25%. Proposed change: Consider to replace the current example of a dual-pathway inhibition with another one, exemplifying a metabolic dual-pathway inhibition (e.g. CYP 3A and CYP2D6). Carefully reconsider the proposed cut-off value for the	

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		requirement of dual-pathway inhibitor studies, and make this requirement conceptually consistent with the requirements for single-pathway inhibitor studies. Define minimum criteria for the relative contribution of metabolic enzymes/pathways that would call for their consideration in the design and conduct of *in vivo* dual pathway inhibitor studies.	
325	1	Proposed change (if any): We advocate integrating a comment that protein displacement, the determination of changes in free concentration, should be considered in addition.	Accepted. This has now been included (in 5.2.2.B). It was mentioned in another section.
325-327	5	Comment: The general recommendation that in the described cases “an interaction study with a drug should be conducted that is a potent inhibitor of both pathways if the pathways together represent ≥ 25% of the oral clearance of the investigational drug and the interaction is expected to be clinically relevant”, appears in various aspects problematic and difficult to follow. First, the availability of approved inhibitors which are similarly potent and also specific for two metabolic pathways might be questioned for most of the conceivable combinations of dual pathways. If these would be available, however, the effects of a dual pathway inhibition can be predicted based on the relative contributions of each pathway and application of “worst case” assumptions (i.e. lack of metabolic switching) and the additional need for the conduct of a	See above. The % contribution is based on in vivo mass-balance data. See above for the discussion on dual inhibition.

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		dual pathway inhibition may be questionable. Proposed change (if any): Carefully reconsider the overall approach and requirements for dual-pathway inhibitor studies, and the applicability of recommended decision criteria for *in vivo* studies. Consider to state that for dual-pathway inhibitor studies depending on the availability or non-availability of potent and specific dual inhibitors, the employment of more than just one potent perpetrator drug (e.g. combination of a potent CYP3A- and a potent CYP2D6-inhibitor) might become necessary.	
325-331	12	**Comment:** The Agency should clarify if studies to investigate 2 + pathways must be done separately or what? The Agency should provide guidance on suitable probes.	Partly accepted. The wording on inhibition of dual pathways has been somewhat changed. Suitable probes have been listed in appendices.
327-329	12	**Comment:** Please consider defining how the ≥ 25% oral clearance can be determined as well as the elimination pathways that should be considered, e.g. hepatic, biliary, renal elimination.	Accepted. This has been included in Appendix IV.
331-338	5	Comment: As this section refers to CYP P450 based metabolic DDIs, the repeatedly used term "elimination" pathways instead of metabolic pathways of the drug or metabolites might be confusing or misleading.	Not accepted. In the guideline, the contribution of a pathway to the full elimination is of interest. If talking about metabolism pathways, there is no information on the contribution to full elimination, i.e. is of importance for drug exposure. "Clearance pathway" would also be satisfactory, but we prefer "elimination".

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		Proposed change (if any): In the context of metabolism-based DDIs the term “elimination” should be avoided and either the more specific term “metabolic pathways” or the more general term “clearance pathways” might be used.	
333-345	12	**Comment:** What is the view of the agency concerning genetically polymorphic enzymes? Is comparison between poor versus extensive metabolizers, rather than conduct of an interaction study accepted to quantify the involvement of a given enzyme and/or transporter in the total clearance?	If it is known that poor metaboliser has the same (or worse) decrease in enzyme activity than the most potent in vivo inhibitor available, this would be appropriate (as long as up-regulation of another participating enzyme does not take place in the genetic subgroup.)
341-345	1	**Comment:** Strong inhibition in initial clinical data leads to implied requirement for moderate inhibitor clinical study. **Proposed change (if any):** Multiple layers of clinical DDI studies are not practical. Such studies should be conducted as case by case. Alternatively, examples where drug labels were differentiated by strong and medium clinical inhibition studies should be cited.	Not accepted. It is not uncommon for recommendations to be separated for potent and moderate inhibitors. We agree that there is no default requirement to study a moderate inhibitor once a study with a potent inhibitor has been performed. The need for an additional study depends on the magnitude of the effect of the potent inhibitor, the resulting treatment recommendations, and whether a study with a moderate inhibitor is needed to give treatment recommendations for common concomitant therapy, such as erythromycin for a CYP3A4 substrate. PBPK simulations may also be used depending on how well the interaction effect needs to be estimated.
341-342	9	Comment: The definition of a moderate inhibitor of an enzyme is very narrow, given the high variability of *in vivo* drug-drug interaction studies. As the extent of inhibition *in vivo* will be a function of the administered	Partly accepted. We agree that extent of inhibition is a matter of dose. However, the classification is based on the results with the highest dose. This information has been added in Appendix VIII.

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		dose, even "weak" (based on the inhibitory potency defined by Ki) inhibitors may have marked *in vivo* effects if the dose is high. This point requires additional consideration and re-phrasing. Proposed changes: Please add a list of recommended "moderate" inhibitors of the most frequently encountered P450 enzymes to the appendix of this guideline.	
341-345	12	**Comment:** The Agency should clarify / quantify the meaning of “strong and “moderate” inhibitor. The definition of a moderate inhibitor of an enzyme is very narrow, given the high variability of *in vivo* drug-drug interaction studies. As the extent of inhibition *in vivo* will be a function of the administered dose, even "weak" (based on the inhibitory potency defined by Ki) inhibitors may have marked *in vivo* effects if the dose is high. This point requires additional consideration and re-phrasing. The impact of additional weaker inhibitors may be assessed by modeling	See above
346-351	12	**Comment:** Instead of conducting an *in vivo* study with a moderate inhibitor, we propose that it should be acceptable to use simulations to evaluate the need for dosing recommendations.	Accepted. This has now been included as an alternative when suitable.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change: The guideline should allow simulations to evaluate dosing recommendations for other inhibitors once an *in vivo* study has been conducted with a strong inhibitor.	
353-359	1	**Comment:** Requisites for assessment of DDIs of non-CYPs are too general. **Proposed change (if any):** List which clinically significant non-CYP interactions should be evaluated based on clinical data and drug labels.	Not accepted. The text needs to be general as a number of different enzymes, more or less studied, are included in this group.
354-359	12	**Comment:** The Agency should be aware that selective inhibitors of these non-CYP enzymes are very limited to in-vivo administration. Please clarify.	Partly accepted. We agree that in vivo inhibitors may not be known. We feel the text reflects this.
360-372	1	**Comment:** There are few details on induction. Positive control inducers, approaches, and predictive in vitro tools should be recommended. **Proposed change (if any):** Put details in an Appendix. Align with CDER draft Guideline.	Not accepted. This section concerns effects of other drugs on the investigational drug. In vitro studies are not generally part of this evaluation.
360-365	17	Interaction studies with inducers - The effect of potent enzyme inducers on the pharmacokinetics of the investigational drug also needs consideration. Unless	Not accepted. There is sufficient knowledge to state that the effect of a potent inducer (eg rifampicin) will be marked if the affected drug is to a large extent metabolized by a very

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		the effects are highly predictable and likely to result in a contraindication, an interaction study with a potent inducer is recommended if drug elimination is mainly catalysed by inducible enzymes as well as when several minor inducible pathways contribute to drug elimination and it may not be excluded that enzyme induction will affect drug exposure to a clinically relevant extent. Comment: the passage 'unless the effects are highly predictable, an interaction study with a potent inducer is recommended' implies that you don't have to investigate to confirm the predicted effect. This seems strange! What about evidence-based medicine? Example of sparse information, SPC Ellaone: CYP3A4-inductors (such as rifampicin, phenytoïn, etc) can decrease the plasmaconcentration of ulipristalacetate and decrease the efficacy. Combination is not recommended. Additional information by email by the manufacturer: 'we can not make a conclusion about the efficacy, because too few patients on enzyme inducers were included during phase 3 studies.	inducible enzyme (e.g. CYP3A4). If this lack of effect is likely to result in a contraindication, there are no requirements for the interaction to be investigated.
361-362	5	Comment: Consider to replace the term “elimination” with the term “clearance”. Proposed change (if any): See above (included in the comment)	Not accepted. We see no reason to change the wording.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
361-365	12	**Comment:** Please clarify both the meaning and implication of "predictable induction" in the guideline.	See above.
364 to 370	5	Comment: It needs to be emphasized that potent induction of the formation of metabolites might become toxicologically or safety relevant even for metabolites that are not pharmacologically active. Also it needs to be emphasized for these reasons, that proper dose-adjustment recommendations in case of potent enzyme induction, can rarely be based on single-dose standard PK-assessments of the investigational product alone, but may require the comprehensive assessment of parent-drug and metabolite exposure pattern, together with safety assessments after repeat-dose treatment with the recommended/proposed adjusted dose of the investigational drug when it is coadministered with the potent enzyme inducer. Proposed change (if any): See above (included in the comment)	Partly accepted. We agree that if there are unknown pharmacologically active metabolites, and the exposure of these are increased during induction, this could be of safety concern, and even more so if an increase of the dose is proposed based on reduced exposure of the parent compound. However, a multiple-dose DDI study is a very limited safety material and the likelihood to find a safety signal is small unless acute and serious effects are observed. The text has been expanded on the importance of metabolites, but no requirement to study the DDI as a combined multiple dose regimens will be included due to the reasons stated above.
372-375	3	Comment: It is recommended that OATP uptake transport should be investigated *in vitro* for non-ionic drugs with ≥ 25% hepatic elimination. Proposed change (if any): We suggest specifying which members of the OATP family should be studied and recommend OATP1B1 and -1B3 as these are most well	Naming OATP1B1 and 1B3 accepted. We agree that it would be good if there could be studies in e.g. hepatocytes, with controls for a standardized expression of the OATP, where the likely relative importance of active uptake to drug permeation into the cell, could be determined. We discuss this approach in the final document (Appendix III).

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		characterized. We also suggest that transporter studies are needed only in cases where uptake transport is determined to be rate limiting in hepatic elimination. Characteristics of the investigational drug we recommend that should be taken into account in addition to the charge of the molecule are membrane permeability, active uptake into human hepatocytes, and high liver accumulation compared to other organs in preclinical species and/or humans. Finally, clarifying whether "≥ 25% hepatic elimination" refers to parent drug only would be helpful.	
372 ff	5	Comment: The Chapter 5.2.4 instantly embarks without any general overview or introduction into the very specific case of OATP-mediated hepatic uptake of xenobiotics. In the following, merely selected items of the topic are covered, leaving other important aspects unaddressed. It is also not explicitly stated for which transporters exactly, the transporter substrate characteristics of an investigational product need to be determined. A suitable structured guidance on the role of specific transporters in drug absorption, organ distribution and renal elimination is unfortunately missing. Similarly there is no specific guidance on differential considerations that may be applicable for efflux transporters versus uptake transporters. There is also no reference made to coupled / cooperative functions of transporters to enable vectorial active transport	Not accepted. Including a list of transporters of interest is not suitable as there is still much to learn in this field. This probably also applies to sites of expression and relative importance in different processes. Therefore we refer the applicant to the literature and to the individual PK characteristics of their investigational drug.

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		across particular cells (i.e. emphasis that active tubular secretion requires substrate-uptake from blood stream into the renal tubular cells and subsequent efflux into the tubular lumen, unless the compound is subject to glomerular filtration). Generally, the Chapter appears to require major revisions to improve its overall structure and content. Proposed change (if any): Consider to display in the introduction section a list of transporters of interest such as P-glycoprotein, OATP1B1, OATP1B3, OCT2, OCT1, OAT1, OAT3 and BCRP. Define for which of these transporters the substrate characteristics of each investigational compound should be generally characterized *in vitro*. Establish criteria, for which compounds and/or in which clinical or PK-scenarios the substrate characteristics for the remaining transporters need to be characterized.	
374-379	12	**Comment:** The Agency should provide a rationale for the 25% cut off. Please consider that factors other than transporter process may result in high liver concentration. **Comment:** The Agency should clarify why OATP studies are limited to non-cationic drugs.	Partly accepted. See above for the rationale of the cutoff. The reference to non-cationic drugs has been removed.
374	17	As inhibition of OATPs....	Agreed. Included in the list of abbreviations.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Comment: please explain what OATP means (or has that happenend earlier in the document?)	
375	5	Comment: The term “hepatic elimination” appears to require further specification. It appears unclear whether elimination of unchanged compound into bile/faeces is meant (in line 378 the term biliary secretion is used) or whether it is referred to hepatic extraction calculated as follows $E_H = CL_{blood}/Q_H$ with CL_{blood} being the blood clearance [CL_{blood} = CL/(Blood/Plasma concentration ratio] and Q_H being the liver blood flow? Proposed change (if any):	Accepted. Hepatic elimination includes metabolism as well as biliary excretion. This has now been clarified in the document.
377	16	Why is there a restriction to non-cationic drugs, as OATP does transport also cationic drugs (like digoxin), even though seems less prominent.	See above
377, 380	16	… identification of transporters involved in active renal and biliary secretion if >25% of systemic clearance. Multiple, parallel and serial transport processes might be involved in both kidney and liver. In addition metabolic steps might be included. E.g. active uptake into hepatocytes (OATPs) followed by conjugation (UGTs) and export (MRP2) into bile. Although in such cases it is possible to identify the different processes involved, it is extremely difficult (probably not possible) to quantify their contribution to overall biliary excretion and the define which one represents the	Not accepted. Unless there are pharmacologically active metabolites, focus is on the primary elimination pathways of the parent drug contributing to 25% of the elimination.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		rate-limiting step.	
378-379	3	Comment: The statement that "….account for more than 25% of systemic clearance…" is difficult to interpret. Proposed change (if any): We suggest that the "25%" refers to renal or biliary secretion, and that it only takes into account parent drug.	Accepted to count separate contribution. Not accepted to ignore transporters of importance for active metabolite, exposure.
378-380	9	Comment: The extent of biliary excretion in human is indeed difficult to assess precisely. Proposed changes: The importance of i.v. bioavailability and mass balance data for this purpose should be emphasised more strongly.	Partly accepted. The information is already present in the document. If possible, it is recommended to determine absolute bioavailability or iv mass-balance. However, it remains optional to submit the data. If the data is lacking, worst case estimations will be made and PK studies/SPC recommendations will be required based on this.
380 - 382	10	Comment: "and biliary" should be deleted as the extent of biliary excretion is usually not determinable. Proposed change: …, if renal secretion of unchanged drug accounts for…..	See above. A worst case calculation based on the mass-balance data is recommended, unless the bioavailability is known or iv mass-balance is available.
380-382	12	**Comment:** The Agency should clarify how to interpret / quantify the statement regarding "If renal and biliary secretion account for more than 25% of systemic clearance".	See above
385-387 1243-1244 1249-1250	1	**Comment:** An IV mass balance or absolute bioavailability study is inferred as necessary for fecally excreted drugs. Mass balance after a single radiolabeled dose at cold drug steady state is suggested.	See above. The information given on mass-balance studies relates to the DDI evaluation and is not presented in any guideline, therefore the information is given here.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any): Human ADME studies are out of scope for a DDI Guideline and warrant a more detailed discussion elsewhere. Omit and place in a different Guideline	
386-387	9	Comment: Please clarify if this statement is related to human data? What is meant by “large”?	As pathways contributing to ≥25% of CL/F should be characterized, and the studies suggested would clarify whether there is such biliary excretion, the 25% cutoff could be used.
386-389	12	**Comment:** The Agency should clarify the meaning of “ LARGE fraction of an oral...” Please acknowledge that estimation of biliary clearance in humans is difficult and based on many assumptions regarding active hepatic uptake, passive diffusion, active efflux and active biliary elimination.	See below.
388-389	16	Typically, parent drug determination in feces is done in mass-balance studies only; if a large fraction of parent drug is recovered in feces following oral administration, is there an expectation to get parent drug recovery data in feces following iv administration?	See above. If possible to perform, such a study is of great value and should be considered. Without the data, the excretion will be evaluated as potential biliary excretion.
390	3	Comment: The draft Guidance states that "a eukaryote system where the physiological functions are preserved" should be used. Since many transporters are orphan transporters for which the physiological substrates are not known, we suggest that the recommendation is made more practical.	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any): Recommend that validation data should support that the characteristics of the transporter in the *in vitro* expression system should be in accord with published literature and demonstrate to correlate with *in vivo* data in cases where this is possible.	
390	5	Comment: Please specify what is meant with "physiological function" of a drug transporter. For many transporters their "physiological" function is unknown Proposed change (if any): Use "**transport function**" instead of "**physiological function**"	Partly agreed. What we mean is transporter function as present *in vivo* in man. This has been clarified.
390 and following	10	Comment: It might not be feasible to determine the concentration at the site of transport. Proposed change: The concentrations of the investigational drug should cover the range of clinically relevant concentrations.	Partially accepted. The text has been expanded. It is likely not to be feasible to determine the concentration but it may be estimated and a range put around the estimation. This is very important. Too high concentrations here may potentially mask the involvement of a transporter.
391-392	12	**Comment:** The Agency should be aware that eukaryote systems may not be the most helpful / practical option (ie. Membrane vescicles could be preferred).	Accepted. The text has been reworded. The function is addressed through validation.
392-393	12	**Comment:** The Agency should clarify the following sentence “the concentration of investigational drug should be relevant to the site of transport” since it could be very difficult to know such concentration at that site. The	Accepted. Additional information has been added to explain what we would like to see. It is important not to have too high concentrations as this may saturate transporters working under *in vivo* conditions.

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		concentration at that site and use of such concentrations could lead to technical problems in the assay particularly as the study design needs to be appropriate for Km determination. Unless in vivo expression levels of transporters are known *in vitro/in vivo* extrapolation is precluded.	
393	15	Comment: Relevant concentrations not known before Phase II results Proposed change (if any):	See above.
397	16	... performing in vivo transporter inhibition studies if drug is found to be transported in vitro. Currently only few "selective" inhibitors are available for in vivo transporter inhibition studies; in particular considering the point that transporters should be specifically inhibited at the site of interest and not systemically. In addition the multiple binding sites described for several transporters (MDR1, MRP2, OATP1B1, ...) complicates the conclusion from such inhibition studies and limits the possible extrapolation of effects to other substrates/inhibitors of the same transporter (class effects as for CYPs).	Partially accepted. Potent and enough selective *in vivo* inhibitors may presently not be known. Pharmacogenetic information may be used instead if suitable genetic subgroup(s) are available with reduced function. The present text reflects the lack of information.
399-404	15	Comment Subjects with specific genotypes for many transporters/enzymes can be difficult to find in early phases	Agreed. This may be true depending on the allele frequency. Data should be gathered when possible.
403-404	12	**Comment:**	Not accepted. This is not possible as this is completely drug-

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		It would be of help if the Agency would clarify what PD markers are recommended for *in vivo* studies.	dependent.
410411	5	Comment: As this appears to be a general statement that does not just refer to metabolism-based DDIs the exclusive reference to the "...markedly different contribution of the affected **enzyme**..." appears not entirely sufficient. Proposed change (if any): Consider to rephrase ""...markedly different contribution of the affected **enzymes and/or transporters**..."	Agreed
411	5	Comment: Consider to replace the term "elimination" with "clearance". Proposed change (if any): See above (included in the comment)	Not accepted
411	16	Disease-drug interactions could have been mentioned here	Partially agreed. It is to some extent (renal impairment).
411-438 + 715-733	17	5.2.5. Special populations + 5.4.1. Study population Comment: in this passage the elderly population is not mentioned, can you add this? When it's expected that a drug will be used by elderly people, with multiple morbidity and multiple drug use, the study population should be a reflection of that. Thus, the study should be performed with elderly people, because then the results can be extrapolated	In the conventional in vivo DDI study, concomitant medications are excluded to isolate the interaction effect without interference. It is not possible to perform a study taking into account spread multiple drug use. This is a deficiency but nevertheless, the assessment may not include a large number of potentially interacting drugs. Elderly as a population with impaired renal function may present a problem that is more spread in the population. This should be taken into account, especially if the target population is elderly. This is mentioning in the final guideline.

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		to the 'real life' situation in daily practice.	
412	5	Comment: The term “genetic subpopulations” is not very well defined and may be confounded with genetic differences across different ethnicities. Also it is felt that extensive metabolisers should also be mentioned in this particular context. Proposed change (if any): Such subpopulations may include carriers of genetic variants of metabolizing enzymes (e.g. poor/extensive metabolisers)...”	Accepted.
422	5	Comment: The term "genetic subgroup" could be perceived as discriminating. Proposed change (if any): It should be considered the subpopulations carrying variant alleles of the enzymes/ transporters involved in the investigational drug clearance may have a completely different set of drug interactions	Not accepted. We do not understand why it would be discriminating. It is always possible to divide the population in subgroups. In this context it is of benefit for the subgroup. However, the text has been expanded.
427	5	Comment: The statement “In case a study is not possible, the worst case estimation will serve as basis for the treatment recommendations” does not explicitly emphasize that e.g. a contra-indication in the concerned subpopulation may be considered. Proposed change (if any): Consider to rephrase/amend: “In case a study is not possible, the worst case estimation will serve as basis for the treatment_recommendations**, special**	Not accepted. The expression “treatment recommendations” includes these recommendations.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		warnings or contra-indications in the subpopulation concerned."	
430	5	Comment: The statement "...an in vivo study **could** be considered..." appears too soft to reflect a reasonable regulatory requirement. Proposed change (if any): Consider to rephrase: "...an in vivo study **should** be considered..."	Accepted.
441-446	12	**Comment:** Please note that having data on the investigational drug on the PK of other drugs before phase II studies may lead to irrelevant data or variable data that may not be conclusive. Please note it is unlikely that a complete *in vitro* and *in vivo* assessment of the DDI potential of the investigational drug and any relevant human metabolite will be available prior to initiating Phase III studies. Indeed, it would be preferable that evaluation of *in vitro* DDI interactions involving transporters were driven more by clinical need up to phase III.	Partly accepted. The guideline states that if concomitant administration of potentially interacting drugs can be avoided in phase III in vitro information is sufficient at this stage. Regarding transporters, at present, lack of in vitro data on a certain transporter may be "solved" by excluding known substrates from the study. In the future, if a larger number of in vivo substrates are known, this may be more difficult. The text has been expanded to reflect this.
442	5	Comment: Regarding the statement "...*in vitro* information is often sufficient at this stage" it is unclear to which of the stages stage contained in the preceding sentence it is referred to (i.e. "before starting phase II studies" or "before phase III studies".)	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any):	
441-443	3	Comment: The draft Guidance states that data on the effects of the investigational drug on the PK of other drugs should preferably be available before starting Phase II. However, the clinical dose of the investigational drug may not be known prior to Phase II. Therefore, the magnitude of any observed interaction (and also whether or not an interaction is observed at all) may not be relevant to the clinical situation if the dose of the investigational drug was too high or low. This could lead to having to conduct multiple DDI studies to ensure data are available at the clinical dose. In many cases, it may be wiser to defer conducting these DDI studies until after the clinical dose is known. Proposed change (if any): Recommend acknowledging and addressing the issues with conducting DDI studies to investigate the effect of investigational drugs on the PK of other drugs in the absence of knowledge of the clinical dose of the investigational drug.	See above.
455- 456	12	**Comment:** The Agency should clarify at what point we should study the “gastric emptying”.	Accepted. These investigations are mainly relevant when there are indications that the new drug affects gastric emptying. This has been clarified.
447	16	Non-linear kinetics can also be born out of enzyme saturation	We agree but when this happens, the enzyme activity is inhibited competitively.

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463	6	Comment: it is stated: “discussed in the Elimination subsection below”. Please indicate where this subsection can be found. Section 5.2.4 deals with elimination, but this subsection is included prior to the reference. Proposed change (if any):	Accepted.
465 - 470	5	Comment: Please consider to mention the requirement to measure the free drug concentration to enable the detection of effects based on protein displacement Proposed change (if any):	Accepted.
465-466	12	**Comment:** The Agency should elaborate the meaning of “complex binding” and what decrease or increase in gastric pH is deemed significant.	Not accepted. A clarification is not considered useful or possible.
468-472	12	**Comment:** Drug interaction due to protein binding displacement is rare and may be unwarranted.	Consequence of comment not clear.
474-475	5	Comment: “However, it is also possible to study the effects directly *in vivo*, e.g. by the use of cocktail studies.” It may be not instantly clear to the reader to which drug the statement refers to. Proposed change (if any): Consider to rephrase/amend: “However, it is also possible to study the effects **of the investigational product** directly *in vivo*, e.g. by the use of cocktail studies.” Consider also referring to the applicable EMA GL in	Partly accepted. The guideline will replace the cocktail study document and therefore no cross-reference is needed.

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		which cocktail studies are further detailed (EMA/618604/2008 Rev. 2).	
478	16	It is important here (and is noted in the FDA guidance) that regardless of how wide the range, what is critical is that physiological concentrations of the drug be tested.	Not accepted. The requirements are already specified.
484-491	12	**Comment:** The guideline recommends the study of inhibition of UGT's known to be involved in drug interactions, including UGT1A1 and UGT2B7, if one of the major elimination pathways of the investigational drug is direct glucuronidation'. Currently, as documented in the literature this requirement is difficult to achieve (1) in terms of quantifying the contribution of UGT"s to the metabolism of a drug and (2) in performing the in vitro inhibition assay due to the lack of specific and selective inhibitors as positive controls. **Comment:** Considering that generally at least two structurally unrelated substrates are recommended to be used for CYP3A inhibition studies in vitro, please advise on which impact this may have on a following *in vivo* study if different results are observed with the different substrates.	Not accepted. The knowledge on UGTs is not as detailed as for the CYP family. Nevertheless, knowledge has been gained and in vitro studies investigating inhibition are possible. There is some information in vivo but as stated, known selective in vivo substrates are quite few. However, the requirements are set low and we believe it is time to start asking for these data. Furthermore, the data is quite often provided in drug applications. Accepted. This information has been included. For in vivo studies, midazolam is presently recommended for potency comparative purposes. However, it is agreed that this is not completely consistent with rationale of using different in vitro substrates.
491 to 493	5	Comment: In this statement the term concentrations instead of exposure is linked to AUC, which might be confusing or ambiguous. Also, the basis/reference-points or criteria for the	Accepted. Wording has been changed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		assessment which metabolites should be investigated regarding the enzyme inhibitory potential should be carefully reconsidered. For instance, ICH-M3-R2 refers regarding the proposed cut-off value for metabolite toxicology testing requirements to the **total drug-related exposure** rather than to **parent-drug exposure**. Some harmonisation in this respect may be desirable, and the pros and cons of each of the possible approaches need to be carefully balanced. It also appears desirable from a scientific point of view, that the GL-text clearly states, that metabolite exposure estimates should be based on unbound drug and metabolite concentrations. Proposed change (if any): Replace the term "concentrations (AUC)" with "total exposure (AUC)", because "total exposure" is the correct descriptor for the PK parameter AUC. Consider whether the metabolite exposure estimates should refer to either **total drug-related exposure**. Consider to clearly require the application of unbound drug and metabolite concentrations for the calculation of the metabolite exposure estimates.	The reason for accepting total concentrations here is that it is considered it to be too high requirements (in case parent drug binding is high) for the protein binding or all metabolites to be determined. However, a comparison of unbound concentrations is preferred.
490-496	9	Comment: The threshold of 1/5 is too low, 1/2 is more appropriate, this would also be in-line with a current PMDA guideline.	Not accepted. In our opinion ½ is too generous as a metabolite having 50 % of the exposure would need a halved Ki to be equally potent as inhibitor. This difference in in vitro potency is quite small. A 5-fold increased Ki (corresponding to a 1/5 exposure) seems more reasonable.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
491	9	Comment: Please delete "or unbound"	Accepted. Now clarified in text.
492-498	4	Comment: The stakeholder understands the background relating to the identified metabolite investigation, however, this entails a significant resource implication if all metabolites with systemic exposures greater than 20% of the parent, not just pharmacologically active metabolites are to be studied. Proposed change (if any): N/A	Accepted. The cutoff has been changed. If appropriately designed, *in vivo* cocktail studies may replace the *in vitro* characterizations.
492-498	12	**Comment:** The threshold of 1/5 of the molar concentrations (AUC) of the parent seems too low unless there are data to support this value. This requirement should be in line with ICH Guidance M3 of June 2010. This will lead to significantly higher cost to obtain metabolite standards and run studies, for circulating entities in less abundance than parent drug.	Accepted. This has now been changed to be in line with ICH M3.
493-495	3	Comment: The draft Guidance states that metabolites circulating at concentrations at least as high as 1/5 that of the parent compound should be investigated for enzyme inhibitory potential. However, this may not be practical (and may not be clinically relevant) for drugs that are extensively metabolized to many different metabolites, with no clear major metabolite. Proposed change (if any): Recommend acknowledging and addressing the issue with extensively metabolized	Accepted. See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		investigational drugs.	
493	12	**Comment:** Please delete "or unbound".	Partly accepted. See above.
494	10	Comment: The percentage in relation to parent is not consistent with the definition of a major metabolite in the ICH M3 (R 2) guidance. Proposed change: …as 1/5 of the concentration of total drug related material…..	Accepted. See above.
494 Also for 1230-1235	16	Although the arbitrary number of 1/5 appears reasonable and probably has been used as a cutoff in practice, it is recommended different physiological/pharmacokinetic scenarios be discussed. The metabolite/parent drug concentration ratio in circulation does not necessarily represent the ratio at sites of enzyme inhibition by the metabolite. Drugs that are metabolized in liver may have higher metabolite/parent drug ratio in hepatocyte than in circulation. Uncertainties usually exist around metabolite distribution after being formed in hepatocytes. This may be to a greater extent for drugs with high first pass effect. On the other hand, if a metabolite is formed in circulation, it is quite common that the metabolite has more polarized structure, altered (most likely decreased) permeability, and thus possibly higher metabolite/parent in circulation than in tissues.	The wording has been changed (see above).

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		The guidance to characterize metabolites (DDI and structural) is different from that proposed in the EMEA ICH M3 (and MIST) guidance. Recent ICH guideline states that nonclinical characterization of a human metabolite is only warranted when that metabolites is observed at exposures greater than 10% of the total drug related exposure. It would be helpful to have consistent wording across guidances'.	
501-503	12	**Comment:** The Agency should in addition describe mechanism based inhibition and the need to determine KI, Kinact.	Partly accepted. This has already been included.
502	16	This section refers to the measuring only of Ki. Does this mean that IC50 values calculated are not sufficient? Often times IC50 experiments are carried out first and only if below a certain value (eg < 30µM) is a full Ki experiment conducted.	Partly accepted. See guideline text. Ki should be determined as it is less dependent on the in vitro conditions.
508-509	12	**Comment:** Positive control inhibitors may not be needed for *in vitro* studies, since the probe substrate turnover is a proper control	Not accepted. This is only true the incubations are run under linear conditions and the substrate used is metabolized extensively by one enzyme. However, data on linearity is usually not presented in the study report. Hence, positive controls are recommended in the document.
514-516	1	**Comment:** Statement that unbound concentrations are used in vitro only for basic drugs. **Proposed change (if any):** Provide literature citations that support this.	Accepted. We refer to Gao *et al* 2010. However, we have changed the line asking for information on unspecific binding for all drugs regardless of charge, not to miss any bound drug among the acids.
515-516	5	Comment: Here it is referred to the fact that portal	Accepted

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		vein concentrations during the absorption of orally administered drugs might be generally higher than C_{max} plasma-concentrations in the systemic circulation. However, it is just said "C_{max} in plasma" without reference to the systemic circulation. Proposed change (if any): Rephrase accordingly by amending the term "systemic circulation" in order to be clear about the compartment to which it is referred to.	
523-530	9	Comment: The approach of calculating the intestinal concentration of a drug by dividing dose by 250 ml does not take limited solubility into account. Proposed changes: Please add "For compounds of low solubility the maximum possible saturating concentration in the GI tract at a pH covering the range within the GI tract should be applied." Comment: There is generally no accepted and defined standard parameter for the enterocyte blood flow in the literature. Proposed change: Please add a value or accepted standard reference for the enterocyte blood flow.	Accepted. The solubility aspect was already covered by the text. However, the information has been included in more places of the text, for clarity. Accepted.
524-532; 682-690	16	In vitro conditions to trigger in vivo studies …. Ki < 10-fold the maximum dose in 250 ml. A dose of 125 mg in 250 ml => 1 mM (for a Mw 500 compound); hence for Ki values < 10 mM in vivo studies would have to be considered. For most compounds the solubility will be limiting to perform the	Accepted. The solubility aspect was already covered by the text. However, the information has been included in more places of the text, for clarity. The cutoff for intestinal inhibition has been altered.

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		respective in vitro study, likely also in vivo such high local concentrations will never be reached. This condition would trigger multiple DDI studies with many/most of our compounds and should be established/backed-up by respective literature data. The proposed threshold is **100-fold more conservative** as compared to the typically used approach in literature for I/IC50 > 10 for considering a DDI potential (this would lead to a threshold of an IC50 of 100 µM with above dose) (Giacomini et al., 2010, Nature Reviews). We would like to propose to take into account the known solubility characteristics of the drug, i.e. use of the highest solubility observed in a relevant aqueous medium (FESSIF, FASSIF). The highest solubility should be limited to the dose dissolved in 250 mL water.	
525 - 527 and 555	10	Comment: The formula is unclear, it suggests that for a drug with a molecular weight of 400 and a 10 mg dose, the resp. concentration would be 100 µM. According to the formula given, Ki determination would have to use concentrations up to 1000 µM? For most drugs it will not be possible to test up to these high concentrations due to solubility limitations. Proposed change: Delete sentence (line 555) as inhibitor concentrations to be used for Ki determination in vitro are dependent on the inhibitory potency of the	See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		inhibitor.	
525-532	12	**Comment:** The approach of calculating the intestinal concentration of a drug by dividing dose by 250 ml does not take limited solubility into account. Proposed changes: Please add “For compounds of low solubility the maximum possible saturating concentration in the GI tract at a pH covering the range within the GI tract should be applied.” **Comment:** There is no generally accepted and defined standard parameter for the enterocyte blood flow in the literature. **Proposed change:** Please add a value or accepted standard reference for the enterocyte blood flow. **Comment:** The Agency should be aware that the calculation appears to be overly conservative such that in vitro studies will not be feasible for many compounds *in vivo*. Studies would be needed for most compounds. Also calculations recommended are not validated and require reference.	See above. See above
528	5	Comment: The average value of Q_{ent} of 18 L/h should be stated. Also, please specify the assumption that the inhibitor is not subject to extensive first pass metabolism itself.	See above

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any):	
530-531	1	**Comment:** Is the equation for [I]gut or [I]enterocyte or [I]portal vein? **Proposed change (if any):** Correct as appropriate.	The equation has been removed. The possibility of using Igut is present in the Mechanistic static model alternative.
530-532	12	**Comment:** Equation needs literature reference to support.	See above
531 and following	10	Comment: Assuming that the free fraction of a drug would be 10% then a 50 fold safety margin for Ki would equal a plasma concentration of 5 fold Cmax. Based on current knowledge, no effect of a drug has been observed when I/Ki <0.1 Proposed change: replace '…a Ki which is ≤ 50-fold the unbound…' by '…a Ki which is ≤ 10-fold the unbound Cmax ….'	Not agreed. The cut-off is based on experience of NCE applications during the last decade. However, the guideline now also includes a mechanistic static and a dynamic (PBPK) approach partly based on the paper of Fahmi et al 2009.
532-533	5	Comment: It should be specified what exactly is meant with the statement "...organ with main drug input from the circulation", as it unclear whether "circulation" should refer in this context exclusively to "systemic circulation" or also includes the "portal vein blood flow" of the liver. Proposed change (if any):	Accepted. This has been clarified.
533-537	1	**Comment:** Ki of < 50-fold the Cmax u, or < 250-fold the Cmax u	The cutoff based on unbound Cmax (50-fold margin) has been used in the EU over the last decade. Until data is

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		(if fu > 99%) is too stringent. **Proposed change (if any):** In the absence of clinical data, use in vitro data to define Ki to more reasonable multiples of intended target exposure.	available showing that another cutoff should be used, this threshold will be used. The 250-fold unbound Cmax for highly bound drugs has been replaced by a forced use of fu=1% in case a protein binding >99% has been determined (forced due to the uncertainty in the fu estimate). See also above for other approaches.
534-535	9	Comment: Required concentrations *in vitro* may not be applicable for low solubility compounds. Proposed changes: Please add: "unless such *in vitro* concentrations cannot be attained due to limited solubility of the test compound"	Partly accepted. General statement already included, but now this is further specified. See above.
534-537	12	Also calculations recommended are not validated and require reference.	Not accepted. Calculations based on Cmax unbound have been used over a decade in our assessment of in vitro studies in NCE applications. Reference is given to Fahmi *et al* (2009) for the Igut equation.
539–542	12	**Comment:** We interpret this paragraph as follows: if you don't see any reversible inhibition; there is no need to test for time-dependent inhibition (TDI). However, time-dependent inhibition might occur even when there is not an increase in pre-existing reversible inhibition. Instead, it could be a separate phenomenon. It may be best to screen for TDI in all cases. In the case of CYPs, the screen should be conducted in the presence of the necessary cofactors, NADPH. **Proposed change:**	Accepted. The text has been changed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		"If inhibition is enhanced by pre-incubations (with NADPH, in the case of CYPs), time-dependent inhibition (TDI) may be present."	
539–546	12	**Comment:** The observed increase in inhibition caused by time-dependent inhibition may be due to a formation of a metabolite that is a direct inhibitor of the enzyme or due to mechanism based inhibition – only if it is mechanism based Kinact can be determined, this is not clear in the text. **Proposed change:** "If the inhibition is enhanced by pre-incubation, further investigations of the mechanism of the time-dependency may be performed. Mechanism based inhibition may be present. In this situation Kinact (maximum inactivation rate constant) and KI (the inhibitor concentration producing half the maximal rate of inactivation) should be determined. If it is shown that the time-dependency is due to formation of a metabolite which directly and reversibly inhibits the affected enzyme, this has consequences for the in vivo relevance assessment as well as for the in vivo study design (See section 5.4.4)."	Accepted.
543-546	12	**Comment:** Defining the mechanism of time-dependent inhibition although scientifically interesting may provide little benefit for clinical DDI study design. If time-	Not accepted. Often the steady state of the perpetrator drug is reached before the new steady state of the affected enzyme. Then, the design required for TDI investigations includes longer treatment of the perpetrator. Also, the

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		dependency is established, a clinical study would be designed to achieve steady-state plasma concentrations of test compound before administration of probe compound, and this would be independent of the time-dependent mechanism. **Proposed change:** There is no clear clinical relevance for investigation of the TDI mechanism, therefore, we would suggest removing this section.	mechanism affects which parameters are relevant to determine in vitro.
543	16	Defining the mechanism of time-dependent inhibition although scientifically interesting may provide little benefit for clinical DDI study design. If time-dependency is established, a clinical study would be designed to achieve steady-state plasma concentrations of test compound before administration of probe compound, and this would be independent of the time-dependent mechanism. We would suggest to remove this section, or to make it clearer -> reversible versus irreversible time-dependent.	See above.
547	15	**Comment** This chapter should include the simple equation for evaluating the extent of DDI e.g. dAUC=1+I/Ki, preferably with modification for fraction metabolised via certain metabolic pathway.	Not accepted. However, the mechanistic static model has been introduced.
552-557	5	Comment: The arbitrary safety margin of 50-fold or higher (whilst the concentration is assessed based on free level) is deemed to be inappropriately too high leading to many unnecessary clinical studies. It was	See above.

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		assumed that the safety factor of 10 could serve as a good conservative measure ensuring protection against individual variability (when the latter is not simulated using stochastic PBPK models) as well as any experimental uncertainties and this is also in line with the typical toxicity margins applied for extrapolation from animal to human (10-fold for PK and 10-fold for PD). As it stands, for highly bound drugs the EMA guideline may be less conservative than FDA guideline however for drugs with less extensive binding it requires clinical studies in cases based on the 50-fold higher concentration margin where the actual potential for DDI are very limited. Proposed change (if any):	
554	12	**Comment:** A definition of “marked abundance” would be helpful - is this just CYP3A, or CYP2C /UGTs also?	Accepted. Based on the present knowledge, this would concern CYP3A. In the future, other, probably non-CYP enzymes may be an issue why we prefer to write “eg” CYP3A.
554-559	12	Comment: The criteria for initiating clinical drug interaction trials in humans are not substantiated by science and there is no rationale provided for the specific safety factors suggested for risk assessment from in vitro data. It would be most useful if these can be rationalized/supported based on evaluation of their performance characteristics using published primary in vitro databases (e.g., Brown HS et al., Clinical	See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Pharmacokinetics 45: 1035-1050, 2006; Obach RS et al., Journal of Pharmacology and Experimental Therapeutics 316:336-48, 2006) and published reports of clinical DDI studies. It is also unclear why 99% is suggested as the cut-off for high protein binding. The criteria as suggested are somewhat arbitrary and it is recommended that the revision of the guideline include data on the performance characteristics of these criteria. It is also recommended that alternate metrics of [I] including calculated unbound hepatic inlet concentration of the inhibitor be explored as part of evaluation of performance characteristics of the proposed criteria. We would also suggest replacing “Highest maximum dose taken at one occasion” with “highest expected clinical dose taken at one occasion”. Concentrations of 10 times dose/ 250 mL may not be achievable in in vitro studies if the dose is high enough and solubility is low. For example, if dose is 100 mg, the required concentration would be 4 mg/mL (10 mM for a drug with molecular weight of 400) which in many cases would be in excess of the aqueous solubility of typical lipophilic investigational agents. Please consider qualifying as “to the extent permitted by solubility of the investigational agent”.	Partly accepted. Inlet concentration is now included as a part of the mechanistic static model. Partly accepted. The solubility issue was already included but has now been included on more places. The cutoff has been changed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		There is a disconnect between FDA and EMA guidance on whether to use “total” or “unbound” concentrations in the calculations which change depending on what value is used. Although there is some overlap between FDA and EMA as to when an in vivo study would be required, there are many incidences where a study would be required for one agency, but not the other. For example, for a drug such as diclofenac, a study would be required by FDA, but not by EMA. EMA would require a study for a drug that has low protein binding (fu ca 0.95) and Ki such that I*fu,p/Ki is ca 0.1. We would, therefore, recommend harmonising the guidance with other regulatory agencies so that either “total” or “unbound” plasma concentrations are used consistently in the calculations.	Partly accepted. The basis of the cutoffs used is the experiences of the two agencies using the separate cutoffs. Until sufficient data is available supporting another cutoff, the EMA “basic model” requirements for inhibition of liver enzymes will be unchanged. However, the document has been changed allowing the “mechanistic static model” in addition to PBPK in these estimations.
554	16	Definition of “marked abundance” would be helpful - is this just CYP3A or CYP2C /UGTs also?	See above.
557-559	12	**Comment:** Please consider using I/Ki or I/IC50 to estimate the *in vivo* importance of an inhibition and when an *in vivo* study is required (*see the article “Predicting inhibitory drug-drug interactions and evaluating drug interaction reports using inhibition”, Bachmann and Lewis 2005, http://www.ncbi.nlm.nih.gov/pubmed/15886285*). Also please consider that if the *in vivo* study for the lowest Ki do not have any clinical relevance, it is not necessary to perform studies for enzymes with higher	See above This has already been included. However, it has now been clarified that to use this approach the Ki needs to have been estimated in the same study.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Ki. If IC50s are used, it may be divided into I/IC50 for competitive inhibition and I/(IC50/2) for non-competitive inhibition.	
558, 559	16	The choice of unbound drug concentrations as compared to total drug concentrations is appreciated but is a different approach as used by the FDA where total drug concentrations is being used as a basis. As a global company we need to obey both guidances' (CPMP and FDA). There could be situations for drugs with fu <0.2, the CPMP guidance would not require an in vivo study, but when based on the FDA guidance an in vivo study would be required. The same holds true for drugs with fu >0.2. Therefore, a higher number of interaction studies is needed to comply with both guidances'. A harmonization of the DDI guidance between CPMP and FDA would therefore be appreciated.	Partly accepted. Changes to harmonize the requirements of the agencies have been undertaken. Further, data driven, harmonization may be performed in the future.
564-568	9	Comment: There is only few data, largely estimations, on the degradation constant of P450 enzymes *in vivo*. Proposed change: Please provide a standard reference for this parameter that is accepted as "high quality data" by EMA. It should be considered that the degradation constant e.g. of CYP3A4 in the liver and intestine is likely to differ substantially.	Not accepted. Due to the presently limited knowledge, it is considered preferable not to cite a standard reference. The applicants are recommended to follow the scientific literature.
565-575	12	**Comment:** Using modelling for TDI but not for reversible inhibition is inconsistent. We would suggest to add a model	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		(equation) for reversible inhibition and to propose a mechanistic model for time-dependent inhibition. **Comment:** The appropriate value for kdeg is currently undergoing debate. There is only few data, largely estimations, on the degradation constant of P450 enzymes in vivo. Does “high quality in vivo data” run to using the kdeg that best predicted DDI due to TDI (i.e. Wang, 2010 Drug Metab Dispo 38 1094-1104 where 0.03 h-1 was used) or a more conservative value as suggested by Yang et al 2008 (Curr Drug Met, 9 , 384-393). **Proposed change:** Please provide a standard reference for this parameter that is accepted as "high quality data" by EMA. It should be considered that the degradation constant e.g. of CYP3A4 in the liver and intestine is likely to differ substantially.	See above.
565-575	12	**Comment:** It is not clear whether the inhibitor concentration [I] refer to total (bound + unbound) or unbound concentration. Currently there is a tendency to over predict time dependent inhibition, yet the use of [I] (instead of binding-adjusted [I]) will likely cause even greater over prediction and additional *in vivo* studies.	Accepted. This has been clarified. Unbound concentration is relevant for hepatic enzyme inhibition (as for competitive inhibition). In the intestine 0.1*dose/250 ml is the relevant concentration.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		We would recommend that [I] be adjusted with a binding term in the equation.	
570	5	Comment: k_{deg} and the variability associated with this parameter are not clearly specified. Later on (line 822) $t_{1/2}$ CYP3A4, liver of 80h is proposed for use but recent evidance (Rowland-Yeo et al 2010 EJPS) shows that a turn over of 44h might be a more predictive value if inactivation parameters are obtained tradition two stage *in vitro* studies. The guideline allows the user to incorporate the variability in this parameter and perform sensitivity analysis, although it would be useful if an acceptable range is provided. Also, no information on the intestinal k_{deg} is presented in the guideline and this should be included. There is a general consensus that $t_{1/2}$ CYP3A4, gut is app 24h (Gertz et al., 2008; Yang et al., 2008). Proposed change (if any):	Accepted. The set figure for k_{deg} has now been removed.
572-573	16	The appropriate value for kdeg is currently undergoing debate. Does “high quality in vivo data” run to using the kdeg that best predicted DDI due to TDI (i.e. Wang, 2010 Drug Metab Dispo 38 1094-1104 where 0.03 h-1 was used) or a more conservative value as suggested by Yang et al 2008 (Curr Drug Met, 9 , 384-393)	Partly accepted. The applicant is advised to consult the literature, attempting to find a well-supported k_{deg} and to justify the choice to the evaluating agencies. In case there are variable results for the same enzyme and site, a conservative approach is recommended. If using PBPK, a sensitivity analysis may be appropriate.
574-585	3	Comment: The draft Guidance recommends a safety factor of 50 for all drugs and of 250 for highly protein bound drugs. In principle, we agree that this is a	Partly accepted. The approach for highly bound drugs is accepted. The simulations situation is covered by the guideline text and may be reasonable provided that the effect

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		reasonable approach if no clinical data are available to validate a simulation but too conservative if the simulation has been validated in clinical studies. Proposed change (if any): We recommend that if the simulation predicted an interaction correctly in clinical studies that no additional "safety factors" are needed for other inhibitors of the same enzyme. We also suggest that for compounds that are highly protein bound and for which protein binding cannot be measured reliably, to assume an unbound fraction of 1% (or the lowest reliable measurement), and a safety factor of 50 be applied, as in other cases.	of the studied and also the “other inhibitors of the same enzyme” on a probe drug is well predicted.
574-585	9	Comment: This section should be more precise with respect to the mentioned items like: "extensive data", "validation sets of drugs", "large number of inhibitors" etc. It is unclear whether "> 30 % inhibition" relates to the pathway under consideration or to the overall clearance of the drug. General Comment: This section reads very much in favour of a single commercial supplier of simulation services. Such promotional text in a guideline of official bodies is felt to be inappropriate and should be rephrased.	Partly accepted. The text has now been reworded. However, it is not possible to be specific and still to cover all situations. The >30% inhibition relates to inhibition of a pathway or clearance catalyzed completely by the enzyme in question. However, the wording and cut-off has changed. The text does not relate to a certain supplier. It specifies the requirements. However, the text has been changed.
576-587	12	**Comment:** This section should be more precise with respect to the	See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		mentioned items such as "extensive data", "validation sets of drugs", "large number of inhibitors" etc. It is unclear whether "> 30 % inhibition" relates to the pathway under consideration or to the overall clearance of the drug?	
577-587	12	**Comment:** The draft Guidance recommends a safety factor of 50 for all drugs and of 250 for highly protein bound drugs. Incorporation of such safety factors into simulations are unnecessary. The recommended approach is overly cautious and does not promote the use and utility of simulation to guide the need for DDI studies. We recommend that the incorporation of safety factor of 50 for all drugs is not required for simulations. For highly protein bound drugs **(fu < 1.0 %,** protein binding > 99.0 %), we suggest that if there is uncertainty in the determination of the free fraction, then a simulation should be performed using fu = 0.01 and/or incorporating a sensitivity analysis on fu in the simulation. We recommend that if the simulation predicted an interaction correctly in clinical studies that no additional "safety factors" are needed for other	The requirements have been changed and the uncertainty “solved” by repeated Ki estimations and sensitivity analyses.

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		inhibitors of the same enzyme. If the sponsor is able to provide scientific data and a rationale on the concentration reaching the enzyme as an alternative, the sponsor's estimated concentration can be used instead of the proposed safety factors in the draft guidance.	
579-580	12	**Comment:** the sentence on "extensive data on validation" may be problematic. The data could consist of successful PBPK simulation of PK in e.g. healthy subjects, extensive and poor metabolizers, elderly, etc. When predictability of the simulation model has been demonstrated, the model may form the basis for simulation of drug interaction outcomes in e.g. rare subpopulations. Even if simulation results have been successful for 2 drugs, it may not be the case for another drug. Therefore it may often be more important to make the validation for each compound at a time. Please consider to add this to the text.	Accepted. A new PBPK section has been added. The qualification data set required is dependent on what the model is used for and it is difficult to set specifications that are valid in all scenarios.
580	5	Comment: There is inconsistency in the application of the dilution step (Ghanbari 2006 CDM), so please comment that the *in vitro* data for irreversible inhibition need to be of high quality and should be more specific in terms of actual requirements. Parameters need to be corrected for nonspecific binding, in particular as the two-step dilution methods generally relies on the use of high initial protein	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		concentrations which increases potential non-specific binding of inhibitors. Proposed change (if any):	
586-587	12	**Comment:** It is unclear whether "> 30 % inhibition" relates to the pathway under consideration or to the overall clearance of the drug, 30% inhibition seems quite arbitrary Please include rationale for recommending a clinical DDI study, since a significant interaction would not be expected based on 30% inhibition of a single pathway.	See above
586	16	30% inhibition seems quite arbitrary and it is not explained what would be needed to provide this number, ie if it is 30% change in enzyme activity? See also general comments.	Not accepted. The requirement is roughly in line with the 30% limit for characterizing enzyme/transporter involvement in elimination pathways. However, new wording and somewhat different threshold has been introduced.
588-593	12	**Comment:** We would like to propose to allow also for a single dose administration of the investigational drug, as long as exposures achieved are similar to the steady-state concentrations. This is applicable to drugs with no appreciable increase in exposure following multiple dosing as compared to a single dose. Similarly, the single dose may be adjusted to achieve exposures similar to those achieved at steady-state.	Accepted. This has now been included for investigations of competitive inhibition in vivo.
588-593	16	We would like to propose to allow also for a single dose administration of the investigational drug, as long	See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		as exposures achieved are similar to the steady-state concentrations. This is applicable to drugs with no appreciable increase in exposure following multiple dosing as compared to a single dose. Similar, the single dose may be adjusted to achieve exposures similar to those achieved at steady-state.	
594 & 647	12	**Comment:** Enzyme down-regulation is mentioned at both in vitro and in vivo sections, but there is no further discussion or guidance regarding this through the document. The criteria for making judgment on down-regulations should be elaborated.	Not accepted. This is not possible at present due to the very limited knowledge.
594-599	12	**Comment:** The current introduction is confusing and misleading. Not all induction studies would give an indication of down regulation. However the way it is written states that whichever study is done it will detect enzyme down-regulation. We would suggest a different structure for this section introduction. Briefly describing the different types of assays (nuclear receptor, mRNA and activity) and mentioning the pros and cons of all assays.	Partly accepted. The text has now been changed recommending cultured hepatocytes for these investigations. Other methods are considered supportive only. In this system, in vivo relevant down regulation has been observed. However, due to limited knowledge, directed positive controls are not proposed and there is no certainty that all primary down-regulation will be observed.
594-599	16	Studies should be performed to investigate whether the investigational drug induces enzymes and transporters via activation of nuclear receptors or, if relevant, other drug regulation pathways. These studies will also detect enzyme down-regulation. Usually, this is initially investigated in vitro followed by	See above.

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		in vivo studies if indicated by the in vitro results. However, it is also possible to investigate induction directly in vivo Find the current introduction confusing. For example not all induction studies would give you an indication of down regulation. I would suggest a different structure for this section introduction. Briefly describing the different types of assays (nuclear receptor, mRNA and activity) and mentioning the pros and cons of all assays.	
595-596	12	**Comment:** Transporter induction is mentioned alongside enzyme induction assessment, however no further recommendations on what to do with such information are provided, in contrast to the extensive guidance for CYPs.	The transporter area is not as mature. There is in vitro knowledge on transporter induction/regulation but human in vivo knowledge presently mainly concerns Pgp. Thus, it is difficult to give more precise wordings that will be adequate also in the future. The applicant is recommended to follow the available literature.
594-599	16	The current introduction is confusing and misleading. Not all induction studies would give an indication of down regulation. However the way it is written states that whichever study is done it will detect enzyme down-regulation. We would suggest a different structure for this section introduction. Briefly describing the different types of assays (nuclear receptor, mRNA and activity) and mentioning the pros and cons of all assays.	See above
596	16	A minor point: AHR is not a nuclear receptor, but the Guidelines do mention AHR specifically. We would recommend to rephrase the sentence for better clarity.	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
609-614	9	Comment: GR activation by glucocorticoids leading to CYP induction can be monitored in course of standard PXR/CAR induction assays (e.g. CYP3A, 2B). Mechanism of GR mediated induction well described in the literature. Proposed change (if any): Delete passage. No need for separate GR activation assay.	Not accepted. As there are indications that GR activation takes place through up-regulation of PXR, this regulation of PXR needs to be present and as such a separate positive control for this is needed.
595-596 697-704	1	**Comment:** Regarding statement that preclinical induction studies must look at transporter induction - Which ones and what are thresholds for a positive response? The statement that PXR or CAR induction mechanisms trigger an in vivo study on substrates of co-regulated transporters is open ended and vague. **Proposed change (if any):** Cite examples of clinically significant transporter induction that drove label and PK changes and were predicted in vitro.	This is related to in vivo studies. If relevant in vivo induction of enzyme(s) has been observed, co-regulated transporters may also be induced in vivo and in vivo studies should be considered. One example is the St John´s wort – digoxin interaction. However, it is not considered needed to add examples in the guideline.
597-599	12	**Comment:** Please clarify the wording "These studies will also detect enzyme down-regulation." Followed by wording "this" (next sentence) makes it appear that "this" refers back to the previous noun, which is "down-regulation". We assume that "this" is actually referring to something other than down-regulation.	Accepted. Text reworded.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change: We propose replacing lines 597-598 with the following: "Usually, detection of enzyme induction or down-regulation is initially investigated *in vitro* followed by *in vivo* studies if indicated by the *in vitro* results.	
600	12	**Comment:** Cultured hepatocytes and validated cell-lines with proven inducibility are recommended but the option of using cryopreserved hepatocytes is not discussed in the document. Cryopreserved hepatocytes are supplied characterised as "inducible" prior to the investigation. Therefore a negative result in this system (n=1 donor) should not require confirmation in further donors for early risk identification.	Partly accepted. Cryopreserved or fresh hepatocytes may be used. More than one donor is needed for the "Basic method" but for the "Mechanistic static method" it is sufficient with one donor/batch. This is now specified in the guideline.
602	12	**Comment:** We would appreciate clarification on what is exactly meant by 'scientifically very well justified.' **Proposed change:** Please replace by 'scientifically justified'	The text has been revised for another reason.
604	12	**Comment:** The number of CYPs assessed in hepatocyte induction studies should be harmonised with FDA recommendations.	Partly accepted. The requirements are harmonized.
605	12	**Comment:** Since incubation times are limited by hepatocyte culture viability and maintenance of metabolic capacity, we would appreciate clarification on what is	Partly accepted. Usually, 72 hours incubation is considered appropriate. However, this may not always be possible and the applicant may have data to show that a shorter duration is sufficient to induce a response.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		meant by the statement 'the duration of the incubation should be well justified. '	
607-610	12	**Comment:** The draft guidance suggests: "…mRNA could also be included and is mandatory for the interpretation of study results if inhibition of the studied enzyme may not be excluded at the concentrations used or if a down-regulation is suspected based on the activity assay." **Proposed change:** "Protein expression and/or mRNA can be included as additional end points for the interpretation of study results if inhibition of the studied enzyme may not be excluded at the concentrations used or if a down-regulation is suspected based on the activity assay."	Accepted. The guideline has been revised to recommend evaluation of mRNA as this increase the sensitivity of the system.
610-611	12	**Comment:** We would suggest that positive control need not be included to verify functioning regulation of GR for investigational drugs with glucocorticoid activity because there is sufficient published evidence that a GR inducer would also be a PXR/CAR ligand. **Proposed change:** Delete sentence. No need for separate GR activation assay.	See above.
612-614	3	Comment: It is recommended to use a rifampicin concentration of 20 µM in induction studies. We	The rationale of stating a concentration is that we relate the sensitivity of the assay to a certain positive control response.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		suggest that the Guidance is less prescriptive in recommending the drug concentration to be used as long as assay conditions have been validated. Proposed change (if any): Provide ranges of drug concentrations of positive controls that can be used in induction experiments (for recommendations see Chu et al., 2009, Drug Metab Dispos 37, 1339-1354).	Unless E_{max} has been reached, the concentration is important.
614-616	12	**Comment:** For omeprazole and phenobarbital as positive controls, a range of acceptable concentrations is given, but for rifampicin only the 20 µM concentration is specified. With justification, other (lower) concentrations of rifampicin should be acceptable. We would suggest that the Guidance be less prescriptive in recommending the drug concentration to be used as long as assay conditions have been validated. **Proposed change:** We suggest allowing a range of rifampicin concentrations, e.g. 10-20 µM. For recommendations see Chu et al., 2009, Drug Metab Dispos 37, 1339-1354.	Partly accepted. The range has been replaced by a set concentration. The reason is given above.
617-621	3	Comment: The draft Guidance recommends that the drug concentration in *in vitro* induction experiments should be measured at several time points. We consider that these measurements will not aid in the interpretation of the experimental data.	Partly accepted. The wording has been changed. Measurements are encouraged. In our opinion, knowledge about the actual concentration in the medium is very important in order to extrapolate the result to plasma concentrations.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any): Recommend that regular medium changes should be made during induction experiments to ensure that cells are exposed to drug (and potentially metabolites) over the course of the induction experiment.	
618-621	9	Comment: Intracellular concentrations of drug candidate are decisive for induction and can differ strongly from culture medium concentrations (e.g. active transporter, intracellular protein binding). Hepatic uptake, distribution and metabolism can be regarded comparable in hepatocytes *in vitro* and *in vivo*, thus similar conditions/concentrations should result in comparable intracellular exposures. Proposed change (if any): Omitt concentration testing. *In vitro* assay concentrations should be selected according to expected therapeutic *in vivo* conc. (Cmax) plus a reasonable safety factor, also accounting for metabolic depletion during incubation (e.g. 10-50x C_{max}).	Partly accepted. See above and new guideline text.
619-	1	**Comment:** Assessing parent remaining and protein binding at end of hepatocyte incubation and using Cav unbound is not practical. **Proposed change (if any):** Justify as case by case or remove.	See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
620 - 623	10	Comment: Delete the sentence: “Unless….actual exposure surrounding the cells.” since hepatocytes in vitro are a metabolically competent system mimicking the in vivo situation in the liver regarding drug metabolism and drug clearance.	Not accepted. The text has been reworded for other reasons but as the aim is to use medium concentrations as a reflection of plasma concentrations, it is important to know the actual medium concentrations studied in vitro.
620-623	12	**Comment:** The draft Guidance recommends that the drug concentration in *in vitro* induction experiments should be measured at several time points. The measurement of media concentrations of test compound for induction studies may be misleading in the interpretation of induction potential and should not be recommend as a general parameter to be measured. Due to various factors including hepatocyte uptake, non-specific binding to biological components, and non-specific binding to assay components the measurement of media concentration would be very misleading. If there is sufficient evidence of rapid disappearance of the unchanged drug from an *in vitro* metabolism study, we would recommend to measure induction at the maximum feasible concentrations in the *in vitro* induction study. We disagree with the necessity of measuring drug concentrations in hepatocyte incubations. By	Partly accepted. Several of the factors mentioned are reasons for actually measuring the concentration instead of using the every 24-hour applied concentration. However, the guideline wording has been changed (see above).

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		replenishing daily (or twice daily for fast metabolized compounds) with new culture media containing the study drug, should make it unnecessary to carry out this measurements.	
620	16	The measurement of media concentrations of test compound for induction studies may be misleading in the interpretation of induction potential and should not be recommend as a general parameter to be measured. Due to various factors including hepatocyte uptake, non-specific binding to biological components, and non-specific binding to assay components the measurement of media concentration would be very misleading. If requirement for measuring drug in media remains please provide guidance on how to incorporate this data into assessment of induction. For example, if drug is metabolized quickly should any reference to in vivo CL be made, if induction is observed when parent drug is cleared quickly should metabolites be investigated, should we adjust in vitro study design if *in vitro* CLint is high?	See above. The use of the measured concentrations in the medium is relevant for the comparison with Cmax. However, we agree, the cell concentrations is very relevant for detailed analysis, such as PBPK.
628-632	9	Comment: Unlike in hepatocyte suspensions, percentaged viability assessment at beginning and end of incubation period is not feasible in adherent cultures. Proposed change (if any): A suitable viability assessment (e.g. MTT-/WST-assay) at the end of the	Partly accepted. It is not recommended to adjust the absolute mRNA expression on the basis of viability. We do not completely understand why it is not possible to determine viability in the cultures mentioned. As always, deviations from the guideline are acceptable if adequately justified.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		incubation period should be performed to identify cytotoxic effects of test item and to correct enzyme activities for differences in viability. Culture quality should be by verified and documented by absolute enzyme activities (controls) and cell morphology.	
630	10	Comment: Viability determination is only needed at the end of the incubation period, it is not needed at the start because only viable cells are able to show attachment. Proposed change: delete “beginning and”.	Not accepted.
630-634	12	**Comment:** Unlike in hepatocyte suspensions, percentaged viability assessment at beginning and end of incubation period is not feasible in adherent cultures. **Proposed change:** A suitable viability assessment (e.g. MTT-/WST-assay) at the end of the incubation period should be performed to identify cytotoxic effects of test item and to correct enzyme activities for differences in viability. Culture quality should be verified and documented by absolute enzyme activities (controls) and cell morphology.	See above.
630	16	Please provide suggestions as to how to determine viability in cultured hepatocytes. Many labs will obtain activity and mRNA data from the same cells preventing cell counts/viability assay. LDH leakage is a gross	Partly accepted. Some information, but still quite general, has been included.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		marker and should be compared relative to control cells (which could also be losing viability).	
633-639	9	Comment: 50% increase over control in enzyme activity as threshold criteria for induction is not acceptable. >50% variability relative to control commonly observed, especially for highly inducible CYPs (e.g. CYP3A) and does not necessarily reflect relevant induction. >20% of positive control threshold considered too low, inconsistent with >40% threshold recommended by FDA. Proposed change: Testing of multiple (5-6) concentrations to detect concentration dependency of potential effect (e.g. EC50). >40% of positive control threshold to apply for both enzyme activity and mRNA.	Partly accepted. Concentration dependency is assumed but this has now been clarified. The text has also gone through a major revision.
633-639	12	**Comment:** 50% increase over control in enzyme activity as threshold criteria for induction is not acceptable. >50% variability relative to control commonly observed, especially for highly inducible CYPs (e.g. CYP3A) and does not necessarily reflect relevant induction. >20% of positive control threshold considered too low, inconsistent with >40% threshold recommended by FDA. **Proposed change:** Testing of multiple (5-6) concentrations to detect concentration dependency of potential effect (e.g.	See above

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		EC50). >40% of positive control threshold to apply for both enzyme activity and mRNA.	
635-639	1	**Comment:** Worst-case scenarios (highest induction magnitude donor) set action levels. **Proposed change (if any):** Use average response of a multiple donor experiment, and align positive-control-related thresholds with CDER draft guidance.	Not accepted. Although in vivo induction is also variable, in vitro induction response is likely to vary more due to culturing related issues and therefore the most responding cells may be representative for the in vivo situation in a large part of the patients.
637-641	12	**Comment:** Clarity is needed around all the numbers outlined in this section as it is not clear what are they based on. We are concerned that the thresholds selected to demonstrate that an investigational drug is not an inducer are extremely conservative and not harmonised with those of the FDA. Since the *in vitro* thresholds selected by the FDA are designed to be conservative and generate a considerable number of false positives *in vitro* we are concerned that adoption of the EMA thresholds will inevitably lead to a high number of unnecessary clinical interaction studies providing negative results. Also, for small responses, it would be prudent to define how "response" relative to positive control is determined. Is the vehicle control subtracted out? It makes a huge difference for small responses. Finally,	Partly accepted. The threshold has been changed (100% increase of mRNA as compared to vehicle response) This is probably very conservative but the approach is less conservative that the presently applied qualitative approach. Well performed in vitro induction studies are lacking in the applications. Thus, the basis for the cut-off is limited. Therefore we need to be conservative. However, we have included the RIS correlation method as an alternative based on the supportive data available. The approach is an attempt to harmonize with the present view of the FDA.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		the draft FDA guidance on Drug-Drug Interactions (dated September 2006) indicates that an induction response is negative if the increase is <40% of the positive control (theoretically maximal) response, rather than the 20% in this guideline. **Proposed change:** We suggest aligning definition of negative response with FDA guidance.	
637-641	12	**Comment:** Basal CYP activity is known to be markedly variable between hepatocytes from different donors. However, maximum CYP induction is much more consistent. For example, it is not uncommon to observe induction of CYP 1A2 by a B-naphthoflavone range from 10 to more than 100-fold increase in basal activity due to differences in basal activity. Therefore, more consistent and meaningful results may be obtained if the induction potential of a new compound is expressed as a percentage of positive control, representing maximum induction. **Proposed Changes**: It is proposed to express induction of a new compound as a percentage of positive control and to define significance if it reaches a certain percentage relative to the positive control. A percentage adopted by other regulatory agencies has been 40% of the positive	Partly accepted. The relation to positive control has benefits but also pitfalls. We have chosen to base the sensitivity assessment on the response of the positive control. Furthermore, response of positive controls is applied in the RIS correlation method.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		control – therefore a new compound is defined to be an inducer if it reaches a 40 % level relative a defined positive control.	
637-641	12	**Comment:** The *in vitro* study is considered negative for enzyme induction if incubations with the investigational drug at the concentrations given in the inhibition part of this section give rise to a less than 50% increase in enzyme activity. This statement might not be applicable for some NCEs that behave as inhibitor/inducer. Some molecules could behave as potent inhibitors and still be inducers (Xenobiotica. 2000 May;30(5):441-56). So activity could be similar to vehicle values or even lower and still be potent inducer. In these instances, mRNA information becomes a relevant part in interpretation of the data.	Accepted. That was the reason why it was stated that mRNA should be measured in these cases. However, the section has been reworded and mRNA set as default marker instead of activity. The primary reason is to increase sensitivity but of course, this may also simplify the interpretation of the results due to absence of influence of inhibition.
637-639	16	The in vitro study is considered negative for enzyme induction if incubations with the investigational drug at the concentrations given in the inhibition part of this section give rise to a less than 50% increase in enzyme activity. This statement might not be applicable for some NCEs that behave as inhibitor/inducer. In our experience and as referenced (Xenobiotica. 2000 May;30(5):441-56), some molecules could behave as potent inhibitors and still be inducers. So activity could be similar to vehicle values or even lower and still be potent inducer. In these instances, mRNA information becomes a	See above

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		relevant part in interpretation of the data.	
639	5	Comment: Units for describing micro-molar drug concentrations are abbreviated inconsistently. Proposed change (if any): Units should be used in a consistent format throughout teh document (e.g. µM)	Accepted.
639-641	16	Setting the threshold for a positive result in the *in vitro* induction assay at 20% of positive control is arbitrarily low. Variability within the assay may approach this 20% threshold and result in numerous experiments with a single positive result in single donor. This may lead to many unnecessary *in vivo* DDI studies. A threshold should be backed-up by literature data showing an in vitro to in vivo correlation. We suggest to refer to the threshold used by FDA as > 40% positive control.	See above.
640	5	Comment: The section refers to enzyme-induction related issues, therefore the reference to enzyme-inhibition appears not mandatory. Also the meaning of the phrase "...is indicated" appears not entirely clear in this context. Proposed change (if any): Consider to delete "..unless enzyme inhibition is indicated" and clarify that enzyme activity measure are the pivotal read-out from enzyme induction studies whereas mRNA and protein measurements only provide further insights in the mechanism of the induction, i.e. changes in	Not accepted. See above. The text has changed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		transcription rate and amount of protein. Please also consider that protein and mRNA measurements are required to clearly discern the effects of enzyme inhibition from suppression of enzyme transcription, the latter being an important mechanism for cytokines.	
644-646	1	**Comment:** Unknown induction mechanisms imply a clinical DDI study on the effect of potential teratogens on contraceptive steroids, regardless of in vitro induction results. **Proposed change (if any):** Give a clinically significant literature example that drove a label change and/or delete the implied requirement.	Not accepted. If the drug has teratogenic potential, an interaction study with an oral contraceptive is generally recommended if the drug will be used in women of child bearing potential. This study is crucial for which recommendations on contraception that may be given in the SPC.
644-646	12	**Comment:** We would question whether it is really needed to test *in vivo* the effect of a teratogenic substance on oral contraceptive if absence of induction in hepatocytes has been shown. We would welcome clarification as to why a negative study would not valid in this situation. **Proposed change:** Remove this point, use *in vitro* hepatocyte results to decide on clinical studies as for other investigational drugs.	See above. The rational is given in the guideline. Enzyme regulation is complex. All induction pathways may not be known yet. Therefore an in vivo DDI study is needed.
647	5	Comment: The sentence "...an *in vivo* study should be performed investigating the effect on that specific	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		enzyme *in vivo*" appears to contain redundant statements, i.e. to express the requirement unnecessarily verbose and complicated. Proposed change (if any): Consider to rephrase: "...an *in vivo* study on that specific enzyme should be performed."	
648-649	12	**Comment:** The impact of *in vitro* (apparent) down regulation of CYP mRNA is not well understood, and thus, some guidance regarding the specifics of *in vivo* work to investigate the effects of down-regulation would be appreciated.	Partly accepted. See above. Unfortunately, the knowledge is not extensive. Positive in vitro results should be followed by more extensive in vitro investigations and thereafter relevant in vivo studies. The text has been slightly expanded.
650-652	12	**Comment:** We would suggest to add in those lines or in section 5.4 (Design of *in vivo* studies) that when assessing the induction component, the probe substrate and the investigational drug should not be given on the same day, otherwise there is a risk of missing the induction effect (eg. J Clin Pharmacol. 2004 Mar;44(3):215-23).	Accepted. We have added this information although in another section as it is relevant for rifampicin, being an OATP1B1 inhibitor.
653-658	16	We would suggest to add in those lines or in section 5.4 (Design of in vivo studies) that when assessing the induction component, the probe substrate and the investigational drug should not be given on the same day, otherwise there is a risk of missing the induction effect (eg. J Clin Pharmacol. 2004 Mar;44(3):215-23).	See above.
654-656	9	Comment: Can this be conducted within a "Cocktail Study"?	Yes, provided the probe drugs used are selective also during induction, i.e. has no or very limited metabolism by co-

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
			regulated enzymes.
659-665	1	**Comment:** A positive in vivo induction study implies subsequent clinical induction studies on co-regulated enzymes and represents an open-ended implied requirement. **Proposed change (if any):** Multiple layers of clinical DDI studies are not practical. Requirements should be case by case, based on drug-specific factors.	Not accepted. Co-regulated enzymes and transporters are at risk of induction. It is recommended for the effect to be quantified for as many of these as possible. Lack of data will be reflected in the SmPC.
659	12	**Comment:** Down-regulation might need to be mentioned in this sentence **Proposed change:** Please consider rephrasing as follows: "If clinically relevant induction or down-regulation is observed *in vivo*..."	Partly accepted. The text as been reworded. In the very few cases of down-regulation we have seen, it has been difficult to predict which other enzymes would be affected due to co-regulation. However, this is likely due to the still limited knowledge on enzyme regulation.
660	10	Comment: Typo error Proposed change: Replace "though" by "through"	Accepted.
661	5	Comment: The word "that" appears out of context in this sentence. Proposed change (if any): Consider to delete "that".	Accepted.
666-674	3	Comment: The draft Guidance recommends that inhibition by the investigational drug of eight drug transporters and preferably BSEP is studied. As establishing the clinical relevance of inhibition of	Not accepted. The recommendation is written based not only on the known important substrates but also on the likely contribution of transporters to the PK of other drugs.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		transporters for most transporters is difficult due to the lack of selective probe substrates, we recommend that such studies should be done on an issue driven basis. For example, inhibition of OATP1B1 and MDR1 Pgp is important to assess the potential for DDIs with statins or digoxin, respectively, for investigational drugs likely to be co-administered with these compounds. Studying the inhibition of OAT1+3 would be important for drugs co-administered with methotrexate. Proposed change (if any): Instead of recommending studying the inhibition of a range of transporters for all investigational drugs, recommend that testing this should depend on the therapeutic class, and drugs likely to be co-administered with the investigational drug. We also suggest adding an Appendix to the Guidance with suggestions how IC_{50} values should be interpreted relative to clinical exposures, and recommendations for probe substrates and inhibitors for each transporters which can be used *in vitro* and/or in clinical DDI studies (for suggestions see Giacomini et al., 2010, Nat Rev Drug Discov 9, 215-236).	
666-668	9	Comment: It is currently difficult to define appropriate transporter substrates to conduct meaningful *in vivo* interaction studies. Proposed changes: Please add a list of the respective	Not accepted. It is agreed that for some of the transporters, optimal in vivo probe drugs remains to be found. Therefore, a list of model substrates based on today's knowledge would soon be outdated. The applicant is recommended to follow the literature.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		model substrates, the pharmacokinetics of which might be affected to a clinically relevant extent by transporter modulation.	
666-674	9	Comments: The list of relevant transporters should be harmonised with the ITC publication (Membrane Transporters in Drug Development", Nature Rev., Drug Discovery, 2010, 9, 215-236, Giacomini et al.). For example, OCT1 and BSEP are not recommended to evaluate in the decision tree of the current ITC publication. Proposed changes: Please describe the rationale to select OCT1 and BSEP in addition to ITC transporters.	Accepted. See above. BSEP inhibition has been associated with hepatic safety issues and if indicated, monitoring is advised in the clinical studies. The rational for including OCT-1 is the effect of OCT-1 polymorphism on the distribution of imatinib to the target site. In addition, an association with metformin distribution to the liver has been observed. The list has been revised.
666	12	**Comment:** There is a lack of acknowledgement in this draft guidance that transporter knowledge is still very much developing. In many cases translation of *in vitro* data to the *in vivo* situation is not fully worked out. We would like to see this emphasized more in the document.	Accepted.
666	12	**Comment:** It is not clear if calculations discussed in the 'Metabolism' section also apply to the 'Transport' section, since no detailed equations are presented in this section.	Partly accepted. This has been clarified.
666-674	12	**Comment:** The list of relevant transporters should be harmonized with the ITC publication (Membrane Transporters in	See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Drug Development", Nature Rev., Drug Discovery, 2010, 9, 215-236, Giacomini et al.). For example, OCT1 and BSEP are not recommended to evaluate in the decision tree of the current ITC publication. **Proposed change:** Please describe the rationale to select OCT1 and BSEP in addition to ITC transporters.	
666-674	12	**Comment:** The draft Guidance recommends that inhibition by the investigational drug of eight drug transporters and preferably BSEP is studied. As establishing the clinical relevance of inhibition of transporters for most transporters is difficult due to the lack of selective probe substrates, we recommend that such studies should be done on an issue driven basis. For example, inhibition of OATP1B1 and MDR1 Pgp is important to assess the potential for DDIs with statins or digoxin, respectively, for investigational drugs likely to be co-administered with these compounds. Studying the inhibition of OAT1+3 would be important for drugs co-administered with methotrexate. **Proposed change:** Instead of recommending studying the inhibition of a range of transporters for all investigational drugs, we recommend that testing this should depend on the therapeutic class, and drugs likely to be co-	See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		administered with the investigational drug. We also suggest adding an Appendix to the Guidance with suggestions how IC_{50} values should be interpreted relative to clinical exposures, and recommendations for probe substrates and inhibitors for each transporters which can be used *in vitro* and/or in clinical DDI studies (for suggestions see The International Consortium. 2010. Membrane transporters in drug development. Nature Reviews/Drug Discovery. 9:215-236).	
674-676	12	Comment: For in vivo studies we recommend that the positive control inhibitors have some established clinical precedent, as the guidance fails to make any mention of clinically relevant transporter inhibitors.	Not accepted. We prefer not to make a list of inhibitors as such a list is likely to soon be out of date.
679	16	Would IC50 be acceptable for transporter inhibition studies? Since under appropriate experimental conditions (low substrate concentration and linear transport conditions) IC50 values are close to Ki values. We therefore recommend to change accordingly throughout the manuscript.	Not accepted. Ki is the preferred parameter as it is less dependent on study conditions. Unless it is impossible to determine Ki, this is the parameter recommended. A possibility of using EC50 in case Ki may not be determined has been included. However, factors affecting the EC50 estimate must be considered.
668	5	Comment: Please consider using the official nomenclature for ABC transporters P-glycoprotein (ABCB1) and BCRP (ABCG2). For further information please refer to: http://www.genenames.org/genefamily/abc.html#table1.	Accepted. This has been added.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any): Consider to delete “that”.	
668-670	5	Comment: It is initially stated that the transporter-inhibitor characteristics of the investigational product should be investigated for a total of 8 listed transporters. Thereafter it is referred to an additional transporter (BSEP) and stated: “The transporter BSEP should also preferably be included for detecting pharmacodynamic interactions as well for adequate safety monitoring during drug development.” The statement appears largely unclear regarding the investigations that should be considered or are expected in this regard. Also no criteria are provided which product characteristics would call for consideration of BSEP. Proposed change (if any): Consider to rephrase and to propose criteria on product characteristics that would call for BSEP investigations, i.e to advise for close monitoring of serum bile salt levels if in vitro experiments indicate a potential for BSEP inhibition.	Accepted.
668 and following 679 and following 684 and	10	Comment: It is not clear if calculations discussed in the ‘Metabolism’ section also apply to the ‘Transport’ section since no detailed equations are presented in this latter section. Comment: Usually the determination of the IC50 is sufficient to evaluate the inhibitory potential towards transporters.	See above. Not accepted. See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
following		Proposed change: replace 'Ki' by 'Ki or IC50' Comment: A concentration of 10-fold the maximum dose in 250 ml seems to be a very conservative estimate for the intestinal concentration and would result in many false positives. Most drugs will not reach this concentration due to solubility limitations. Proposed change: ...the maximum dose on one occasion/250 ml Overall proposed change: *In vivo* inhibition of intestinally expressed proteins such as Pgp can be excluded if the observed IC50 or Ki value is ≥ 0.1-fold the maximum dose/250ml (as proposed by Fenner et al. 2009)	Accepted. Threshold changed based on available data. The solubility issue was already covered but has now been further clarified. Accepted based on available data.
675-684	9	Comment: It is very difficult to exactly and accurately assess the Ki of drug transporters. Almost all literature data of Ki for transporters are actually IC50 data that were then re-calculated as Kis based on certain (mostly unproven) assumptions. Hardly any literature Ki data were assessed properly by doing a large number of experiments over a range of inhibitor and substrate concentrations. Proposed changes: Use IC50 instead of Ki, this would then also be in line with the argumentations of the "ITC publication"	Partly accepted. A possibility to use EC_{50} has been added in case it is not possible to determine Ki. See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
677	3	Proposed change (if any): We suggest changing the recommendation to determine a "K_i" to a "K_i or IC50" as determining a reliable K_i is often not possible for substrates of efflux transporters like for instance Pgp due to the limited solubility of clinical candidates.	See above.
677	5	Comment: Please provide guidance for the calculation of the Ki for transporters that have been shown not to follow Michaelis-Menten kinetics (e.g. ABCB1). Proposed change (if any):	Partly accepted. Information has been added on the need to follow the literature on this matter. A possibility of using EC_{50} in case Ki may not be determined is now present. However, the assumptions behind the determination of the EC_{50} estimate must be considered.
677-681	12	**Comment:** On the same theme as above, *in vitro* assays should be validated against known clinical inhibitors to help contextualise the in vitro output.	Partly agreed. The guideline states that positive control inhibitors should be used. However, it is difficult to compare the in vitro effect - in vivo effect relation as this depends on the PK including distribution to target site and also the in vivo characteristics of the probe used.
677-681	12	**Comment:** It is very difficult to exactly and accurately assess the Ki of drug transporters. Almost all literature data of Ki for transporters are actually IC50 data that were then re-calculated as Kis based on certain (mostly unproven) assumptions. Hardly any literature Ki data were assessed properly by doing a large number of experiments over a range of inhibitor and substrate concentrations. **Proposed changes:** We suggest changing the recommendation to determine a "K_i" to a "K_i or IC50" as determining a	See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		reliable K_i is often not possible for substrates of efflux transporters like for instance Pgp due to the limited solubility of clinical candidates. This would then also be in line with the argumentations of the "ITC publication"	
680-681	5	Comment: The statement "...concentration range of the investigational drug expected to be relevant for **the site of interaction**..." appears very "implicit" and hence may carry the risk that the essence of this recommendation is not realized by everybody. Proposed change (if any): Consider to amend some explanation that the concentrations to which cellular efflux transporters on the one hand and uptake-transporters on the other hand are exposed to, may be different, depending on factors governing the establishment of an concentration-equilibrium between extracellular and intracellular compartments (e.g. the intrinsic permeability of compounds). Consider further to explain, that systemic C_{max} concentrations in principle are believed to well reflect the concentrations at uptake-transporters, but may carry for particular drugs (e.g. low permeability) some additional uncertainty regarding the estimation of intracellular drug concentrations, which are relevant for efflux transporters.	Accepted. This has been clarified.
680	12	**Comment:** The choice of (transporter) substrates and (positive control) inhibitors should be justified ...	Partly accepted. It is in our opinion too early to set lists of preferred substrates and inhibitors in the guideline. We advise the applicant to consult the literature and to make the best

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Model substrates for many transporters are rather polar and might behave different from more drug-like substrates. In addition the multiple binding sites described for several transporters (MDR1, MRP2, OATP1B1, ...) complicates the conclusion from such inhibition studies and limits the possible extrapolation of effects to other substrates/inhibitors of the same transporter (class effects as for CYPs).	possible choice of substrate/controls and data interpretation.
680	16	The choice of (transporter) substrates and (positive control) inhibitors should be justified ... Model substrates for many transporters are rather polar and might behave different from more drug-like substrates. In addition the multiple binding sites described for several transporters (MDR1, MRP2, OATP1B1, ...) complicates the conclusion from such inhibition studies and limits the possible extrapolation of effects to other substrates/inhibitors of the same transporter (class effects as for CYPs).	See above.
682-688	12	**Comment:** A concentration of 10-fold the maximum dose in 250 ml seems to be a very conservative estimate for the intestinal concentration and would result in many false positives. Due to solubility and the dose of most drugs, Ki determination at concentrations 10 times the dose/250 mL will be technically challenging, as the GI concentrations will be well in excess of 1 mM under this condition. Further, the guidance states a DDI study for intestinal transporters can be excluded only if	See above. This has been changed. We refer to Agarawal et al 2012

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		the dose/250 mL is 10 times lower than the Ki. However, using this rationale, even a moderate in vitro Pgp inhibitor will require clinical investigation if dose exceeds 15 mg. As available clinical data to date suggest that drug efflux interactions in the GI tract are modest at best, it appears that this guidance is too stringent. **Proposed change:** …(the maximum dose on one occasion/250 ml) **Proposed change:** *In vivo* inhibition of intestinally expressed proteins such as Pgp can be excluded if the observed *IC50 or Ki value is ≥ 0.1-fold the maximum dose/250ml* (as proposed by Fenner et al. 2009).	
682-683	15	Comment: Scientific rationale on this estimation of concentration relevant to site	Partly accepted. This has been clarified.
685-686	3	Comment: The draft Guidance recommends that an inhibition of intestinally expressed proteins such as Pgp can be excluded as the Ki ≥ 10-fold the maximum dose/250 mL. We consider this as too conservative. Proposed change (if any): Suggest for transporters expressed in the gut to use a K_i ≥ 0.1-fold the maximum oral dose/250 mL, or an I_2/IC_{50} or K_i ≤ 10 (where I_2 is the maximum oral dose/250 mL) as	See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		recommendation that an interactions is less likely (see Zhang et al., 2008, Xenobiotica 38, 709-724).	
685-686	9	Comment: The approach of calculating the intestinal concentration of a drug by dividing dose by 250 ml does not take limited solubility into account. Proposed changes: please add: For compounds of low solubility the maximum possible saturating concentration in the GI tract at a pH covering the range within the GI tract should be applied.	See above.
685-686	12	**Comment:** The approach of calculating the intestinal concentration of a drug by dividing dose by 250 ml does not take limited solubility into account. **Proposed changes:** Please add: For compounds of low solubility the maximum possible saturating concentration in the GI tract at a pH covering the range within the GI tract should be applied.	See above.
685-586	13	Comment: With the same justification applied to the intestinal enzymes inhibition, Eq. 1 should also be applicable for intestinal transporters. Proposed change (if any): *In vivo* inhibition of intestinally expressed proteins such as Pgp can be excluded if the observed Ki value is ≥ 10-fold the maximum dose/250ml. Or alternatively (for efflux	Not accepted. The text has been changed. The I_{gut} option no longer exists in the basic approach.

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		transporters), ≥ 50-fold the maximum concentration predicted in the enterocyte using the equation below (as a surrogate for enterocytic concentration) where Qent is enterocyte blood flow, fa is the fraction absorbed, ka is the absorption rate constant. Eq. 1. $[I]_{gut} = \frac{fa(I) \times k_a(I) \times Dose(I)}{Q_{ent}}$	
685	16	In vitro conditions to trigger in vivo studies …. Ki < 10-fold the maximum dose in 250 ml A dose of 125 mg in 250 ml => 1 mM (for a Mw 500 compound); hence for Ki values < 10 mM in vivo studies would have to be considered. For many compounds the solubility will be limiting to perform the respective in vitro study, likely also in vivo such high local concentrations will never be reached. This condition would trigger multiple DDI studies with many/most of our compounds and should be established/backed-up by respective literature data. The proposed theshold is **100-fold more conservative** as compared to the typically used approach in literature for I2/IC50 > 10 for considering a DDI potential (this would lead to a threshold of an IC50 of 100 µM with above dose)	See above.
687-688	12	**Comments:** This provides when an *in vivo* inhibition of transporter	There are situations when *in vitro* data is positive but where an in vivo study may not be performed. In such cases,

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		studies can be excluded but it is not clear when a DDI study for transporter is required. **Proposed change:** Clarify that for drugs exhibiting an $[I]_1/Ki > 0.1$ or $[I]_2/Ki > 10$, a clinical interaction study with a P-gp substrate (digoxin) is recommended for investigational drugs likely to be co-administered with digoxin.	inhibition may not be excluded and this will be reflected in the SmPC.
688	5	Comment: There is uncertainty in the K_i estimation of each transporter given that passive permeability at least for ABC-transporters is the important covariate determining the access of inhibitor and "victim" drug to the active site. Therefore, additional in vitro studies will not help to solve this issue. Instead we recommend to advice that when studying different transporters one and the same expression system should be used in order to exert control over the influence of passive permeability and keep this covariable constant. Modelling approaches that delineate the influence of passive permeability when calculating K_i-values should be encouraged. Proposed change (if any):	Partly accepted. We acknowledge the problem. So called inside out vesicles may be advantageous here as the drug has direct access to the transporter. This has been included. In the future, the knowledge gained may allow for other routes to be taken. The ongoing discussion on how to determine Ki is reflected in the guideline text.
689-691	12	**Comment:** The guideline states that it is known that an estimated Ki value can vary from one in vitro model system to another and it recommends an additional study with another cell system if there is uncertainty in the	Partly accepted. We agree that it may be difficult to know if there is any uncertainty in the Ki estimate. The text has been changed to an encouragement to consider using an additional in vitro system for transporters inhibited. However, for Pgp a recommendation of using two different systems have been

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		estimated Ki from the original model. It is unclear when a secondary or tertiary system would be necessary and we would appreciate clarification about the sources of uncertainty in the estimation of Ki would instigate further studies with different in vitro model systems. **Proposed change:** We suggest clarifying that a second system would only be considered when a Ki or IC50 for Pgp inhibition cannot be determined in a system due to technical problems or high variability.	added due to the high inter-lab variability observed.
690	10	Comment: The uncertainty of the results can be reduced by the proper use of controls which allow a classification of the drug. Proposed change: 'Therefore, if there is uncertainty in the Ki/IC50 estimation, appropriate controls should always be included.'	Partly accepted. This is recommended.
692	5	Comment: Consider rephrasing "For-P-glycoprotein, inhibition of intestinal and renal inhibition can be determined using digoxin and renal clearance" Proposed change (if any): "For-P-glycoprotein, inhibition of intestinal and renal transport can be determined using digoxin and renal clearance"	Partly accepted (renal CL for systemic inhibition). Digoxin has a too high oral bioavailability to be a good intestinal Pgp inhibition probe.
694-695	12	**Comment:**	Accepted. The text has been reworded.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		It is not clear what this sentence means we would suggest rephrasing as shown below. **Proposed change:** For P-glycoprotein, intestinal and renal inhibition can be determined….	
696 - 704	10	Comment: The knowledge on the induction of transport proteins is very limited. It is currently unclear if an induction of PXR translates into a clinical interaction. Proposed change: Delete lines 696-704.	Not accepted. There is CYP3A4 induction via PXR it is not unlikely that Pgp is also induced. This has been shown in vivo. There are also other transporters which appear inducible but human in vivo data is presently lacking.
697-698	9	Comment: Please change the sentence "If PXR and/or CAR mediated induction is observed *in vivo*, a study investigating the *in vivo* induction of Pgp mediated transport is recommended." Proposed Change: "If PXR and/or CAR mediated induction is observed *in vitro*, a study investigating the *in vivo* induction of Pgp mediated transport is recommended."	Not accepted. A study of in vivo induction of Pgp is recommended if PXR mediated induction of eg CYP3A4 has been observed in vivo. CYP3A4 is considered more sensitive for induction that Pgp and thus, if no induction is seen in vivo of CYP3A4, a Pgp induction study is not needed.
697-704	12	**Comment:** The knowledge on the induction of transport proteins is very limited. Little clinically significant evidence for drug-drug interactions mediated through drug transporter induction exists. We believe that the need for *in vivo* induction studies	Not accepted. The intention here is not that the mechanism needs to be clarified but if in vivo induction of PXR/CAR regulated enzymes is observed, it is recommended to perform a Pgp induction study.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		to determine P-gp effects if PXR or CAR are involved is excessive, the impact of the induction of P-gp should be thought through and then only if the likely impact could be significant should an *in vivo* study be undertaken.	
697-704	15	Comment: Why are nuclear receptors discussed here but not mentioned in enzyme induction? Can this be studied in vitro?	Not accepted. Nuclear receptors are mentioned in the enzyme induction subsection. See also above.
697	16	Little clinically significant evidence for drug-drug interactions mediated through drug transporter induction exists. The exception is the renal elimination of digoxin. Additionally, the degree of induction of transporters is far less than when compared to that of CYPs. If significant induction is noted *in vitro* and the investigational drug will be potentially co-administration with digoxin or other renally secreted drugs through renal MDR1, then a *in vivo* drug-drug interaction study would be recommended.	See above.
699-700	16	How would these in vivo induction studies for transporters be conducted? Some clear guidance/recommendations would be helpful. Induction of transporters may be associated with induction of enzymes, and it may be difficult to tease out the effects.	Partly agreed. We agree that the probe drug needs to be well chosen. This is the case also for CYP induction.
704	9	"The design of the *in vivo* interaction study is adapted to the aim of the study." Comment: Please include here a general statement	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		that with regard to several factors (pharmacokinetic and pharmaco-dynamic drug characteristics, safety aspects, clinical condition to be investigated) many different study designs may be considered (tailored approach).	
706	12	**Comment:** It is stated that "The design of the *in vivo* interaction study is adapted to the aim of the study." We would welcome a general statement, that with regard to several factors (pharmacokinetic and pharmaco-dynamic drug characteristics, safety aspects, clinical condition to be investigated) many different study designs may be considered (tailored approach). It is recommended to add a sequential study design option in the guidance. The sequential study design (substrate as first dose followed by the combination of the inhibitor and substrate) is particularly helpful for substrates with a long elimination half-life, or when the inducer of inhibitor has a long half-life. The design maintains the possibility for a within-subject interaction assessment, avoids carry-over in a cross-over study, and also avoids a very long study duration and potential subject drop-out.	Accepted.
707	12	**Comment:** The advantage of a parallel design if the potential inhibitor / inducer has a very long half-life is unclear. When a substrate with a short half-life is used then the	Accepted. The wording has changed.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		total duration of the study is determined by the time needed to reach steady state. This will be the same, whether a 1- way cross-over or a parallel design is used. A parallel design seems more logical if the substrate has a long half-life.	
709	5	Comment: The Chapter is about *in vivo* PK studies. Hence, the compliance issue is anyhow inherently addressed by measurement of plasma concentration – time profiles and regular quantification of trough concentrations in course of repeat-dose studies. Hence, it appears questionable whether an explicit statement on compliance checks appears mandatory and helpful in this context. It appears also unclear, which kind of additional compliance checks could be more sensitive than regular exposure measures. In case that absolute certainty on 100% test-drug intake appears desirable, then it should be rather recommended that each and every dosing-event needs to be conducted under the supervision of suitably qualified study personnel. Proposed change (if any): Consider to adopt the considerations as detailed above.	Accepted. This was the intention. The text has been clarified.
712	12	**Comment:** It is stated that comparisons with historical controls are generally not acceptable. It should be mentioned that this could be acceptable in situations such as DDI studies conducted in patients for oncology (drug	Not accepted. We agree that there are rare situations where a crossover/sequential design is not possible to apply. However, this is a case by case decision. No general statements on therapeutic areas are applicable.

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		combination) where it is difficult to obtain PK data for drug alone within the same study.	
712	16	It is stated that comparisons with historical controls are generally not acceptable. It should be mentioned that this could be acceptable in situations such as DDI studies conducted in patients for oncology (drug combination) where it is difficult to obtain PK data for drug alone within the same study.	See above.
724-725	12	**Comment:** This recommendation is not practically feasible in therapeutic areas like oncology when dealing with investigational agents that cannot be dosed in healthy volunteers, necessitating conduct of DDI studies in patients. In this instance it should be sufficient to match for major known sources of variability (e.g., ensure that both groups of subjects are of EM genotype when evaluating effect of a CYP2D6 inhibitor on the PK of an investigational agent metabolized by CYP2D6).	See above.
727-728	12	**Comment**: Logistical recruitment problems are expected in genotype subpopulations with low frequency. Therefore, a separate evaluation in a specific study would be difficult and will also not result in a sufficiently powered study. Alternative approaches such as evaluation through a population pharmacokinetic screen should be considered in such low frequency genotypes, or having genotype	Partly accepted. Population PK analysis is not a recommended approach here. If the genotype is of low frequency, stratified inclusion may be chosen. More detailed information is given about this later in the document.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		subpopulations with low frequency enrolled in well controlled Phase-I studies taking into account safety considerations.	
727-728	12	**Comment:** Although genotyping is a powerful tool, without additional information on the major clearance mechanisms, routine genotyping of volunteers is of limited value, unless clinical trials are large, or designed to investigate a specific mechanism. There are unlikely to be statistically relevant cohorts of any given genotype in small study populations to support conclusions on clearance mechanisms in early clinical programs. Genotyping information is of most tangible value in exploring DDI study outputs (e.g. extensive v/s poor metabolisers), or in larger studies (i.e. PhII or later).	Partly accepted. It is agreed that unless the study population is stratified for certain genotypes, quantification of the interaction effect will be difficult. Thus, stratification should be considered. Otherwise, genotyping information may still be helpful to clarify an interaction mechanism etc. Furthermore, if an interaction is investigated which has a mechanism of inhibition or induction of a polymorphic enzyme, the interaction effect needs to be quantified in EMs for that enzyme.
728-731	12	**Comment:** Conduct of special genetic subpopulation DDI studies can be challenging. Specifically, for drugs that can't be dosed in healthy volunteers (e.g., in the oncology therapeutic area), recruitment of patients with defined genotypes for DDI studies can be impractical. For estimating the effect of a DDI in genetically defined subgroups (e.g., PM subjects), the use of appropriately qualified PB-PK model-based simulations should be permitted in lieu of a clinical DDI study, provided a DDI study has been performed in the main group (e.g., EM subjects) and it has been verified that the	Accepted. The text has been changed including this alternative, when needed. However, genotyping in the DDI studies performed is still recommended in these situations.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		model adequately predicts the observed clinical DDI.	
729-730	5	Comment: The requirement "...to exclude subjects lacking the enzyme potentially inhibited in an interaction study should preferably be excluded from the study unless their inclusion serves to clarify the mechanism of an interaction" and in particular the exemption to which it is referred to (i.e. clarifying the mechanism of an interaction) appears not entirely complete. It needs to be considered that inhibitors often are not specific to a single enzyme and may indeed alter the capacity of several metabolic pathways in parallel, which may become in particular important in poor-metabolizers of the primary clearance pathway of the investigational drug. Also the investigation of safety aspects of the respective drug combination may be a reason to explicitly exclude PM-subjects from mechanistic DDI-studies. Proposed change (if any): Consider to adopt the suggestions as detailed above.	Party accepted. We agree in general to the comment made but think the text reflects this already.
734-735	12	**Comment:** It would be helpful if a recommended list of suitable transporter probe drugs could be provided. By their very nature, it is quite feasible that at least one uptake and one efflux transporter will be engaged by a drug substance. Additionally, thus far it has been challenging to identify good probes that do not also interact with other transporters or enzymes.	Not accepted. It is considered too early to include probe drug lists for transporters. The applicant is recommended to follow the scientific literature.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
735-736	5	Comment: The statement "If a second enzyme or transporter is catalysing metabolism of the parent drug…" is misleading, as transporters are not catalyzing any drug metabolism. Proposed change (if any): Consider to rephrase accordingly (e.g. If a second enzyme or transporter is catalyzing metabolism **or transport** of the parent drug…")	Accepted.
738-739	12	**Comment:** It would be helpful to clarify whether the 10% value is a cut-off or an expectation. What if an uptake and an efflux transporter are required to facilitate e.g. systemic clearance into the liver and then clearance from the liver into the bile?	The 10% cutoff-is an approximate expectation. Sometimes, such good probe drugs have not been found, as shown in appendix VIII. For transporters, as indicated, the situation is complex as it may be more a question about which transporter is the rate limiting one. The text has been changed to reflect this.
741	12	**Comment:** With regards to probes it would be helpful if a recommended list of transporter probe substrates could be provided.	See above.
743	5	Comment: It appears that there is a typo (i.e. "if" should be written capitalized at the beginning of the sentence). Proposed change (if any): Consider correction.	Accepted.
744-749	1	**Comment:** If drug is very likely to be administered with IV CYP3A4 substrates and oral midazolam clinical study is positive, Guideline infers a requirement for an IV	Not accepted. In the case described, an iv midazolam DDI study should be considered. However, the revised text opens up for well performed PBPK estimations.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		midazolam study. **Proposed change (if any):** Multiple layers of clinical DDI studies are not practical. Delete and recommend case-by-case approach.	
744-749	12	**Comment:** Please consider using either CYP3A or CYP3A4/5, not CYP3A4, for consistency.	Accepted
749	12	**Comments:** It would be helpful if the suggested safety precautions to be used when conducting an interaction study with IV midazolam could be described.	Subject safety should be assured. This may include immediate access to appropriate monitoring equipment and clinical expertise for these types of studies.
748-756	5	Comment: Regarding methodological details and points to consider for of cocktail studies other pertinent European guidelines may be cross-referred. Proposed change (if any): Consider to cross-refer to the Q&A guidance document "CHMP efficacy working party therapeutic subgroup on pharmacokinetics (EWP-PK); EMA/618604/2008 Rev. 2."	This guideline will replace the cocktail study part of that document.
749	10	Comment: Typo error. Proposed change: Delete first 'is'.	Accepted.
754-758	3	Comment: The draft Guidance recommends estimating effects on oral clearance (*i.e.,* CL/F) when conducting probe drug and cocktail studies. However, one needs to be careful about interpretation of oral clearance since this could be confounded by effects on	Partly accepted. In section 5, AUC has been added.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		bioavailability. Proposed change (if any): Recommend estimating effects on AUC rather than oral clearance when conducting probe drug and cocktail studies.	
755	10	Comment: The AUC ratio of metabolite to parent drug is considered to be one of the most sensitive parameters for detecting an interaction Proposed change: The sentence 'Use of metabolite to parent drug concentration ratios in plasma or urine is not recommended.' should be deleted.	Not accepted. Metabolite to parent ratios are influenced by the elimination pathway of the metabolite and also the ratio may in most cases not be translated to a quantitative effect on the enzyme activity (clearance).
755	12	**Comment:** The draft Guidance recommends estimating effects on oral clearance (*i.e.,* CL/F) when conducting probe drug and cocktail studies. However, one needs to be careful about interpretation of oral clearance since this could be confounded by effects on bioavailability. **Proposed change:** We recommend estimating effects on AUC rather than oral clearance when conducting probe drug and cocktail studies.	See above.
755	12	**Comment:** It is stated that use of metabolite to parent drug concentration ratios is not recommended. This needs to be further clarified (i.e., the ratio at a specific time point, ratio of AUC also known as metabolic ratio).	See above. If metabolite elimination is unaffected and AUC ratios are used, we agree that this is a very sensitive parameter. However, to make a translation into effects on the AUC of other substrates, the effect on AUC (i.e. CL or CL/F) is needed.

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		Most cocktail studies use metabolic ratios (e.g., hydroxymidazolam to midazolam for CYP3A activity). Molecular weight between parent and metabolites can be different (though often marginal) but this ratio gives a good indication over time if it is modified as opposed to only the parent drug. We consider that the AUC ratio of metabolite to parent drug to be one of the most sensitive parameters for detecting an interaction. **Proposed change:** The sentence 'Use of metabolite to parent drug concentration ratios in plasma or urine is not recommended.' should be deleted.	
755	16	It is stated that use of metabolite to parent drug concentration ratios is not recommended. This needs to be further clarified (ie, the ratio at a specific time point, ratio of AUC also known as metabolic ratio). Most cocktail studies use metabolic ratios (eg, hydroxymidazolam to midazolam for CYP3A activity).	See above.
768-769	9	Comment: DDI trials are usually performed in healthy subjects. Therefore the highest exposure based on the clinical use of the drug may represent an undue risk to the volunteers. Proposed changes: Re-phrase: "….should be well	Partly accepted. The text has been expanded to include recommendations for situations when the highest dose may not be safely administered to healthy volunteers.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		justified (e.g. safety aspects)"	
761-763	16	Clarification needed on the calculation of the fractional metabolic clearance.	Accepted.
768-769	12	**Comment:** DDI trials are usually performed in healthy subjects. Therefore the highest exposure based on the clinical use of the drug may represent an undue risk to the volunteers. **Proposed change:** Re-phrase: "….should be well justified (e.g. safety aspects)".	See above.
770	5	Comment: Not only the duration of the perpetrator-drug treatment needs to be considered but also the employed treatment regimen (i.e. posology) to ensure exposure to adequate perpetrator-drug concentrations over the full period of victim-drug PK-sampling (e.g. ketoconazole once daily would not be sufficient, due to its short terminal plasma disposition half life). Proposed change (if any): Consider to rephrase: "The duration **and treatment regimen (i.e. posology)** of the treatment with the perpetrator drug should be long enough.	Accepted. This has been included.
770-771	9	Comment: Is it really necessary to administer the perpetrator drug during the whole PK-sampling-period of the victim drug? Would it not be sufficient to cover	Not accepted. We need a well estimated AUC. During the blood sampling for its estimation, the perpetrator drug needs to be administered. When deciding the sampling duration a

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		the main part of the elimination phase of the victim drug (e.g. 2 or 3 half-lives)? This would reduce the subjects' exposure to the perpetrator drug and thus increase the safety of healthy volunteers, and still obtain the relevant information from the clinical trial.	potential prolongation of the half-life also needs to be taken into account.
772-773	12	**Comment:** We would question whether it is really necessary to administer the perpetrator drug during the whole PK-sampling-period of the victim drug . Would it not be sufficient to cover the main part of the elimination phase of the victim drug (e.g. 2 or 3 half-lifes)? This would reduce the subjects´exposure to the perpetrator drug and thus increase the safety of healthy volunteers, and still obtain the relevant information from the clinical trial.	See above.
774-776	12	**Comment:** The purpose to studying more dose levels of an investigational perpetrator drug, if a significant effect is found using the highest dose is unclear. An alternative approach is to use a qualified PB-PK model-based simulation to estimate the expected DDI at a different dose level provided the model adequately predicts and reproduces the observed interaction at the dose used in the first DDI study. Such an approach should be acceptable in lieu of conducting multiple DDI studies.	Partly accepted. This alternative has been included in the guideline.
783 to 785	5	Comment: It may be carefully reconsidered whether	Not accepted. The aim is to ensure companies that this

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		discussion of specific study design details that do not affect the overall outcome or interpretability of data (i.e. loading dose for steady state studies), should become part of a guidance document. Proposed change (if any): Consider to omit this statement.	approach may be taken.
783-784	12	**Comment:** Where a DDI study specifically aims at excluding a relevant interaction a single dose/single dose design should be acceptable as a “screening trial”. Only if this screening trial showed an unexpected relevant interaction this would call for an additional “confirmatory” DDI trial with multiple dose design.	Accepted. A single dose may be used if no-time-dependent phenomena was suspected and the exposure will be sufficiently long over the whole AUC of the victim drug.
786-787	9	Comment: Where a DDI study specifically aims at excluding a relevant interaction a single dose/single dose design should be acceptable (“screening trial”). Only if this screening trial showed an unexpected relevant interaction this would call for an additional “confirmatory” DDI trial with multiple dose design. Proposed change: Please add a respective statement.	See above.
801-804	17	D. Relative time of administration 'In all in vivo interaction studies, the time between administrations of the two drugs should be specified. Usually the drugs are administered simultaneously but sometimes, the most marked interaction is obtained when the drugs are administered at separate time-	N/A

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		points.' Comment: we support the statement 'In all *in vivo* interaction studies, the time between administrations of the two drugs should be specified'. Alas, this information is often lacking. Example: the SPC Cholestagel states that colesevelam decreases the AUC of ciclosporine 34% and the Cmax 44%. Information about the time between administrations is lacking. The manufacturer has provided us with additional information by email about the study design, the plasmalevels and AUC of ciclosporin with and without colesevelam (Cmax 607.4 resp.1039.9 ng/ml; AUC 2871.3 resp. 4328.7 hr.ng/ml). Study design: single dose ciclosporin, wash-out 14 days, followed by concomitant ciclosporin+colesevelam single dose. WFG: we'd like to see the above additional information in the SPC, this is very helpful.	
803-804	11	Comment: It should be clearly explained, how drugs should be administered at different time points, if not given simultaneously. “Usually the drugs are administered simultaneously but sometimes, the most marked interaction is obtained when the drugs are administered at separate time-points.”	Not accepted. This is dependent on the mechanism of the potential interaction, where it takes place, route of administration etc. We prefer not to specify.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any): Amend "… to ensure that times to achieve peak plasma concentrations of both drugs will nearly coincide"	
809-833	1	**Comment:** Discussion of simulation of time-dependent induction and inhibition needs more detail. **Proposed change (if any):** Supply a referenced table of half-lives of important P450s that can be used for simulation.	Not accepted. This is not suitable as we may have better/more agreed numbers in the future.
809-833	12	**Comment:** Please consider using the wording mechanism-based inhibitor when this applies, otherwise it becomes confusing. Kinact do only apply to mechanism-based inhibitors, not to other time-dependent inhibitory effects that e.g. are caused by a metabolite being a direct inhibitor. **Proposed change:** Please consider rephrasing as follows: *"For mechanism based inhibitors, the course of inhibition is also dependent on the inactivation rate constant (*Kinact*)."*	Accepted. This has been clarified.
809-811	17	5.4.5. Time dependencies 'For time-dependent interactions, i.e. induction or "time-dependent" inhibition, the study should aim at investigating the interaction effect at the time-point where it is at or near its maximum.'	N/A

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		Comment: again, we support this statement, because this kind of information on study design is often lacking.	
822	3	Comment: The draft Guidance recommends using a k_{deg} for CYP3A4 of 80 hours. Our internal experience, however, suggests that for modeling of CYP3A4 TDI, a shorter k_{deg} is needed to properly describe clinical TDI data. Proposed change (if any): Recommend that sponsors need to defend their choice of the k_{deg} value used for any modeling that is conducted with a clinical candidate. If a value is included in the Guidance, we suggest providing a range (see also Wang et al., 2010, Drug Metab Dispos 38, 1094-1104).	Accepted.
822-824	12	**Comment:** There is no consensus on the estimate of the half-life of hepatic CYP3A4. A range of 24-72h has been proposed previously (Venakatakrishnan and Obach 2007) which is in-line with the Pharma/FDA initiative on DDI modeling (start with 36h and range from 24-72h) whereas some reports estimate a half-life of approximately 72 hours (*Magnusson et al., Clin Pharmacol Ther 84: 52-62, 2008*), shorter half-life estimates of approximately 28 hours (*Zhang X et al., Drug Metab Dispos 37: 1587-1597, 2009; Quinney et al., Drug Metab Dispos 38: 241-248, 2010*) have also been reported. Given such uncertainty in this	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		parameter, a value of 80 hours cannot be assumed with confidence. **Proposed change:** We suggest allowing for other values to be used as long as they are justified.	
824-827	5	Comment: It may be carefully reconsidered whether discussion of specific study design details that do not affect the overall outcome or interpretability of data (i.e. loading dose for steady state studies), should become part of a guidance document. Proposed change (if any): Consider to omit this sentence.	See above
836-843	12	**Comment:** The wording "complex metabolism" is not clear. If metabolism is complex, it usually means that there are many metabolites formed, thus it would not be feasible to measure 25 to 50 different compounds and to determine their activity. In addition, is this referring to circulating metabolites or to all metabolites found in excreta? Many more metabolites are usually found in the excreta than those found in plasma. **Proposed change:** We propose deleting lines 836 (starting with “Moreover”) to 843.	Partly accepted. This can be interpreted in several ways. Expression removed.
829-831	5	Comment: The requirement to consider achievement of metabolite steady-state was emphasized previously	Partly accepted. The other place where this was repeated has been deleted. We agree about the difficulties in investigating

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		in the document and appears therefore redundant. Furthermore, it needs to be acknowledged that in most cases the information on when steady state of a metabolite is reached is not/ cannot be investigated properly, given that PK data after i.v. administration are needed to determine the apparent half-life of a metabolite, is rarely if ever gathered for a single metabolite or several metabolites since this does not reflect current standard procedures and requirements in drug development during. Proposed change (if any): Consider omission of that statement	metabolite half-life. However, this is only a design issue when the metabolite has ERL elimination. In that case, the half-life observed after oral administration provide an estimate and may be used for the steady state calculations. If this parameter has not been determined, the AUC of radioactivity of the ADME study may be used as a worst case scenario unless the metabolite is a very minor metabolite in whole blood/plasma.
839-841	9	Comment: Please define in more detail the term "complex metabolism".	Partly accepted. Expression deleted.
847	12	**Comment:** As a general rule a single dose of the victim drug is considered sufficient for evaluating a DDI. However, in line 847 and several other places in the document, it is recommended to evaluate the effects on Cmin, which can only be done in steady state. For evaluation of Cmin, we would suggest that it is sufficient to simulate the steady state profiles.	We do not particularly recommend evaluating the effect on Cmin unless the drug is subject to TDM or Cmin has been shown to be closely related to efficacy and/or safety. Here, a steady state study would be needed. Simulations may be sufficient on a case by case basis depending on how well the half-lives have been determined, how much the drug accumulated during multiple-dose conditions etc.
848-851	12	**Comment:** The value of measuring in clinical DDI studies unbound drug concentrations in addition to total drug concentrations is unclear.	Not accepted. There are reasons such as concentration-dependent binding, or a possibility of displacement, for determining unbound drug concentrations in DDI studies.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		We would recommend to measure only total drug concentrations and calculate unbound concentrations using in vitro plasma protein binding data	
853-855	12	**Comment:** This may not be feasible in a standard DDI study prior to Phase II in Healthy Volunteers. Does the agency have any pharmacodynamic markers in mind when referring to transporter interactions?	The markers chosen is a case by case decision based on the pharmacodynamics of the drug and the potential effects of having altered distribution of the drug to a transporter expressing organ.
856-895	1	**Comment:** It is not clear when a population PK DDI analysis is acceptable in lieu of conventional DDI studies. **Proposed change (if any):** Provide clarification.	Accepted. It is clarified in the new version when a population PK analysis may be acceptable.
857	12	**Comments:** We believe that the statement: “if conventional interaction studies cannot be performed” is too restrictive. A well powered, well designed population PK analysis is as relevant as a Phase I study to determine the clinical relevance of DDI.	Partly accepted. It is agreed that a population analysis performed based on rich data may be as relevant as a conventional phase I DDI study analysed in the traditional way, but a conventional non-compartmental analysis will probably also be required in addition. The use of a population analysis describes the situation when rich sampling cannot be performed and this has been clarified (See above)
863-865	9	Comment: The detail of information can hardly be collected for more than just a few (2-3) drugs of interest in Phase III trials, especially if various formulations (extendand vs. instant release) or posologies of the same drug are applied. Proposed Change: …concomitant drugs need to be properly recorded, which includes the date and day	Partly accepted. It is agreed that it may be difficult to collect all this detail for many concomitant drugs. Therefore, it is in the investigators interest to decide prior the study for which medicinal products this effort should be made (a sentence has been added). We are reluctant to delete information concerning dose, since this may be meaningful information to have for interpretation of the estimated effect.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		time of administration to ensure that the patient has been on the concomitant drug for a sufficient time period at the time of blood sampling.	
865-868	12	**Comment:** The detail of information can hardly be collected for more than just few (2-3) drugs of interest in Phase III trials, especially if various formulations (extended vs. instant release) or posologies of the same drug are applied. **Proposed Change:** …concomitant drugs need to be properly recorded, which includes the date and day time of administration to ensure that the patient has been on the concomitant drug for a sufficient time period at the time of blood sampling.	See previous comment.
874-875	9	Comment: Sample size will often be dependent from the required precision (width of CI) rather than the minimum effect size. Proposed change: Please include “width of CI”.	It is agreed that sample size is dependent on the precision required in the estimate but also on the effect size to be possible to identify. The power analysis in this case has not the same aims as when estimating the sample size in a bioequivalence study where confidence limits to fall within are predefined. The sentence has been slightly changed. **Changed text:** A power analysis can be performed a priori to estimate the minimum effect size that is likely to be detected with acceptable precision in a study using a given number of patients on a concomitant drug.
874-875	12	**Comment:**	See previous comment.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Sample size will often be dependent from the required precision (width of CI) rather than the minimum effect size **Proposed change:** Please include “width of CI”	
876	16	There is a link for effect size to Wikipedia. It may be more appropriate to either explain in a footnote or to reference an accepted citation.	The link to Wikipedia was a mistake and has been deleted.
885-886	17	Usually, the effects of concomitant drugs on oral clearance (CL/F) are identified. Comment: sometimes the SPC mentions a decrease in Cl/F.This parameter Cl/F should be avoided because it provides non-information; it could mean a decrease in Cl but also an increase in F (example: SPC Mycamine).	We agree that the effect of an interaction should be expressed in terms of changes in systemic exposure (AUC and/or C_{max}). However, this relates to that the effect is usually estimated solely for CL/F and not for parameters affecting Cmax (like absorption rate constants and volume of distribution). The text has not been revised.
876	12	**Comment:** There is a link for effect size to Wikipedia. It may be more appropriate to either explain in a footnote or to reference an accepted citation.	The link to Wikipedia was a mistake and has been deleted.
888-909	9	Comment: "95% confidence interval" (888) versus "90% confidence interval" (909) Proposed change: Please explain rationale.	The confidence interval mentioned on line 890 of draft guideline refers to the estimate of uncertainty in the PopPK model parameter(s) describing the interaction effect. The confidence level (95%) is commonly used in reporting uncertainty in parameter estimates but is essentially arbitrary. The 90% confidence interval described in the next section

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			refers to the geometric mean ratio in the case the evaluation will be performed as for a bioequivalence study, i.e. if the 90% confidence interval falls within 80-125% a lack of interaction may be concluded. Similarly, the confidence level is arbitrary, but 90% is generally used when reporting ratio of geometric means.
888	12	**Comment:** I It is not clear why 95% confidence intervals are recommended instead of 90% confidence intervals. See also line 911.	See previous comment.
897-902	5	Comment: The description and related requirements on how the data should be presented, in particular the graphical presentation (e.g. mentioning of box-whisker and spaghetti-plots) appears too detailed for a guidance document. Proposed change (if any): Consider to shorten the Chapter by removing unnecessary methodological detail.	Not accepted. This level of detail is needed to ensure that reports include sufficient information for a secondary review.
898-899	12	**Comment:** There is a need to clarify that the summary statistics on the pharmacokinetic parameters should be geometric means and % Coefficient Variation instead of mean and Standard Deviation for AUC and Cmax. The geometric mean and %CV are more appropriate for log normal distributions such as both AUC and C_{max}.	Not accepted. This level of detail is not needed. Botharithmetric mean plus SD and geometric mean plus CV are acceptable.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed Change: Use geometric mean and %CV for AUC and Cmax.	
901	9	Comment: With the term "spaghetti plot", is it really meant the comparison of single PK parameters and not the comparison of complete plasma concentration-time profiles?	Yes, we mean one specific parameter. Each individual´s parameter with and without co-treatment is usually illustrated.
902-903	5	Comment: The statement “All subjects or patients who have been included in the study should be included in the statistical analysis” appears not entirely appropriate for DDI studies with PK and/or PD outcomes, and is therefore followed by a lengthy explanatory statement to relativize this initial requirement. Proposed change (if any): Consider to rephrase: All subjects or patients who have been included in the study **and who have received their study treatments per protocol and who have evaluable data-sets of PK and/or PD readouts** should be included in the statistical analysis”.	Accepted.
905-906	9	Comment: Agree strongly to exclude only values of doubtful period, not the entire subject	N/A
908-909	12	**Comment:** Exclusion of subjects from analysis should be well justified but should it be specified in the protocol since in some situations it cannot be foreseen at the time of protocol writing. At best it should be attempted in the SAP (Statistical Analysis Plan) and updated when the	Not accepted. There are few cases where a subject´s results would not be included in the statistical analysis. In case something occurs in the study and one subject should not be included, such as a suspicion of poor compliance, statistical analysis with and without the subject needs to be shown.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		study is completed and database closed/locked.	
909-910	9	Comment: What is meant by 95% prediction interval - conditional on estimated values for interaction effect and its variability, or incorporating uncertainty (Bayes)? Proposed change: Please clarify	A “95% prediction interval” is an estimate of an interval in which future observations will fall, with a probability of 0.95, given what has already been observed.
910-912	12	**Comment:** The utility of a 95% prediction interval is not clear. Typically the ratios of geometric mean values of AUC and Cmax together with the respective 90% confidence intervals are presented. Use of 95% prediction intervals should not be required.	See above.
910-912	12	**Comment:** AUC and Cmax should be log-transformed and analyzed using a statistical model that can account for correlations. Least square means and 90%CI from the model should be back-transformed to ratio of geometric means and this should be used to draw conclusions about the presence or absence of an interaction. (Reference: Schulmann, D.J. 1987. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. Journal of Pharmacokinetics and Biopharmaceutics, 15:657-680.) **Proposed Change:** We recommend changing the statistical methods to	Not accepted. We assess the change of the mean as well as individual changes in the important PK parameters. We have nothing against the proposed approach but see no need to include this in the text.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		those listed above in the comments.	
916	5	Comment: It is unclear how a metabolite to parent target organ distribution ratio could be determined as part of a standard clinical development program for most of the investigational products. Also for many drugs the target is not just expressed in a single organ, but abundantly expressed in various tissues. Hence the overall feasibility and usefulness of this recommendation appears unclear. Further, as already outlined above, the volume of distribution of a metabolite cannot be exactly determined unless the metabolite itself is administered intravenously, which however, does not reflect current standard procedures and requirements in drug development. Proposed change (if any): Consider to omit this statement	Not accepted. This relates to situations where the target is a specific organ. There may be data available from other species and, rarely, from man (e.g. from PET studies). Sometimes, lipophilicity and permeability may be included in the discussion on how to interpret the data. These approaches are not at all as exact as we would wish, but there is rarely PK-PD data available for the metabolites separated from parent drug.
927-928	12	**Comment:** Please consider also mentioning the possibility to use simulations. **Proposed change:** Consider rephrasing as follows: *"... the potential implications should be discussed based on available scientific literature and if relevant simulations can also be taken into consideration..."*	Partly accepted. This possibility has been given earlier in the guideline and is thus included in the in vivo relevance estimations. If simulations are performed to estimate effects on enzymes not studied in vivo, and this should replace in vivo studies at the time of marketing, such simulations need to be performed in a very careful manner on a case by case basis.
939-945	17	Treatment recommendations should ensure that	N/A

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		patients receive drug treatment which is effective and safe. The evaluation should be based on information available on the relationship between exposure and efficacy/safety. If possible, a well justified target range for relevant exposure parameters should be presented for the investigational drug specifying what change in exposure would justify a posology adjustment. If the target range is based on drug exposure in patients and the interaction study was performed in healthy volunteers, potential differences in the pharmacokinetics between patients and healthy volunteers needs to be considered. Comment: we support this statement, because it's essential information and unfortunately often lacking.	
943-944	5	Comment: The requirement of a particular graphical presentation of exposure data (e.g. box-whisker plots) appears to be over-detailed and must not be part of a guidance text. Proposed change (if any): Consider to delete the text in brackets.	Not accepted. We want to be clear in order to get the presentation needed for the assessment.
944-945	5	Comment: It appears unclear whether the statement "...the frequency of patients with lower as well as higher exposure than the target range..." actually refers to the exposure data observed in the clinical patient population or to the exposure data in healthy adult subjects derived from the DDI study.	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any): Rephrase to clarify.	
951-952	5	Comment: The requirement of a particular graphical presentation of dose-adjusted exposure data (i.e. plasma concentration-time curves) besides the presentation of dose-adjusted primary PK parameters (i.e. AUC, C_{max}) appears to be over-detailed and must not be part of a guidance text. Proposed change (if any): Consider to delete the specific requirement for the presentation of dose-adjusted plasma concentration-time curves.	Not accepted. We agree that this is detailed information and that sometimes this illustration of the results may be seen as pure repetition of the results presented as numbers. However, this is a good way to illustrate the resulting exposure if preferring graphic illustration and it is important that the results are presented as clear as possible, and in different ways, to the individual assessor.
956-958	5	Comment: The statement that dose adjustment recommendations for the investigational compound in case of concomitant administration of enzyme inducing drugs could be merely justified based on theoretical discussions of the potential consequences of exposure increase of the parent drug and pharmacologically active metabolites appears quite a bit too relaxed and PK-minded. Proposed change (if any): As in this case the investigational compound would be the “victim drug” PK- and safety-data from a prototypical inducer study would only be available from a single-dose administration of the investigational compound in healthy adult subjects, which hardly allows to generate any robust safety information, as the safety of any	Partly accepted. We agree that increased metabolite exposure or altered distribution of drug and metabolites may give rise to safety problems. This has been observed, although the detection has been rare. The safety concern will not be observed in a single dose DDI study. However, even a multiple dose DDI study will not give much information on safety unless the combination results in very severe and frequent adverse effects. A much larger and longer study would be needed to adequately evaluate safety. (The situation is the same in organ impairment, where metabolite exposure may get very high.) We would like for the applicant to measure the concentrations of metabolites, in particular in vivo active metabolites or metabolites suspected to have pharmacological effects (target and off-target) if reaching an increased exposure. It is considered that a multiple dose study to be too much to ask for considering the limited

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		recommended dose-increment in the presence of the concomitant inducer actually has not been investigated/established in this case. Hence, no repeat-dose safety assessment of the increased dose-recommendations of the investigational compound when co-administered with the enzyme inducing drug would be available, in particular not in the targeted patient population. Therefore it should be concluded, that dose-adjustment recommendations for investigational compounds that are displaying significant exposure alterations upon concomitant administration of potent inducer drugs, should not be based merely on PK data derived from a single prototypical inducer-study. Rather the safety of a repeat-dose regimen of the recommended dose-adjustment of the investigational compound along with concomitant treatment of the inducer should be assessed in a subsequent study and form the pivotal basis for any dose-adjustment recommendation.	information gained. The information of the importance of metabolite analysis has been expanded.
961-965	4	Comment: The stakeholder seeks clarification where adjustment of daily dosage is required due to a lack of appropriate strengths. This relies on correlation of PK parameters to efficacy and safety and in some cases this may not be possible. Proposed change (if any): NA – seek general further clarification of Lines 961-965	We agree. This is the intention of the text. There will be situations when this is not possible due to lack of proper support.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
977-978	9	Comment Please change the sentence "This is mainly applicable if there is a well established therapeutic range". Proposed Change: "This is only applicable if there is a well established therapeutic range for narrow therapeutic index drugs with low intra-individual variability"	Not accepted. Explained further in next sentence.
978-980	9	Comment: The meaning of this sentence is unclear. Could you explain in more details this additional use of TDM? Does it refer to a DDI setting with drugs that usually do not require TDM? Proposed Change: Delete the sentence "However, TDM may also be used to aid dose adjustment of drugs for which the target concentration differs between individuals, setting the individual baseline concentration (prior to the interaction) as target concentration".	Not accepted. In some cases, some anti epileptic drugs may be given as examples here, where TDM is applied but the actual concentration window used is very wide. Inside this window, the patient will have an individual target range which is much narrower. This range is found through dose titration. In an interaction situation, TDM using the large window is less valuable than trying to normalize the plasma concentration to the concentration the patient had before starting treatment with the interacting drug.
979-980	12	**Comment:** We would recommend to make the sentence ". This is mainly applicable if there is a well established therapeutic range" more specific. **Proposed Change:** "This is only applicable if there is a well established therapeutic range for narrow therapeutic index drugs	See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		with low intra-individual variability"	
980-982	12	**Comment:** The meaning of this sentence is unclear. This additional use of TDM should be explained and detailed, for example does it refer to a DDI setting with drugs that usually do not require TDM, or the sentence deleted. **Proposed Change:** Delete the sentence " However, TDM may also be used to aid dose adjustment of drugs for which the target concentration differs between individuals, setting the individual baseline concentration (prior to the interaction) as target concentration"	See above.
996 to 1006	5	Comment: All of the topics and issues addressed in this section already have been explained in detail in previous sections of the document and appear therefore redundant. Proposed change (if any): Consider to shorten the section by cross-referring to relevant statements in previous sections	Not accepted. Some of the topics are presented in previous chapters but more detail is given in this section and we prefer to have this frame presented on how to perform this evaluation.
998-1002	17	Interactions studied with the probe drugs are mainly intended for the evaluation of the extent of inhibition or induction of an enzyme or transporter by the investigational drug. The data is used to predict interactions with other drugs which are substrates for the same enzyme or transporter. The clinical relevance	Not accepted. The information is very valuable if presented in its right context, i.e. including information on what this means in terms of expected quantitative effects on other drugs. The classification “weak”, “moderate”, “strong” does not give sufficient information. A moderate inhibitor may still give rise to a several fold increase of the AUC of a substrate, and the

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		of the effect on exposure of the probe drug per se is evaluated, but more focus is often put on absence or presence of an effect and the magnitude of the mean effect. Comment: this kind of information can lead to 'non-information' for the healthcare professional. Suggestion: mention this only in one sentence in the SPC and refer to the Scientific Discussion. Example: SPC Ilaris/RoActemra/Arcalyst: Another aspect to be taken into account is that the expression of hepatic CYP450 enzymes may be suppressed by the cytokines that stimulate chronic inflammation, such as IL-1 beta. Thus, CYP450 expression may be reversed when potent cytokine inhibitory therapy, such as canakinumab, is introduced. The normalisation of inflammatory activity induced by treatment may cause an increase of CYP that could be relevant for CYP substrates. This is clinically relevant for CYP450 substrates with a narrow therapeutic index where the dose is individually adjusted. On initiation of canakinumab in patients being treated with this type of medicinal product, therapeutic monitoring of the effect or of the active substance concentration should be performed and the individual dose of the medicinal product adjusted as necessary.	range of potencies inside the “moderate” class if large. Interactions with therapeutic proteins are not covered by this guideline.
1003-1004	12	**Comment:** We would appreciate if similar recommendations for	Not accepted. This is not yet possible as the recommendations would be outdated fast.

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		transporters could be provided.	
1015-1044	9	In terms of herbal medicinal products, we welcome the differentiation made in the text of this chapter, i.e. that the potential of interactions should be investigated for new herbal preparations, whereas for traditional and well-established herbal preparations such a potential should be clarified if reports point to clinically relevant interactions in humans. This is in line with our opinion that the long-term and safe use of well-established and traditional herbal medicinal products should be taken into account when assessing the safety of the products. Therefore new investigations on interactions shall only be required if there are sound reasons (i.e. case reports) for the occurrence of interactions in humans.	N/A
1017-1046	12	**Comment:** Given the possible high number of food compositions we would recommend the use of modelling and simulation if there is a good mechanistic basis for the expected medicinal drug- food interactions.	Partly agreed. This could be a way forward if the mechanisms are completely known. However, we are not sure the knowledge at present allows this.
1017-1046	12	**Comment:** We disagree with the general statement that for new herbal preparations, the potential for interactions should be investigated. If it has been shown that a NMP is susceptible for interactions with e.g. CYP3A inhibitors, this information will be captured in the product label with appropriate precautions for the co-administration of herbal products known to interact	Partly accepted. This guideline generally describes what studies the applicant for marketing authorization of a medicinal product / herbal medicinal products, is recommended to perform. The text has been somewhat altered in order for this to be clear.

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		with those pathways. If a manufacturer markets a new herbal product it should be the responsibility of the manufacturer to provide guidance on affinity of a new herbal product on known major drug metabolizing enzymes or transporters alike the responsibility of a sponsor to provide information on the affinity of the NMP for drug metabolizing enzymes/transporters. **Proposed change:** For new herbal preparations, the potential for interactions should be investigated "*by the manufacturer"*. For traditional and well-established herbal preparations the potential for interactions should be clarified "*by the manufacturer of the herbal preparations"* if…	
1023-1025	9	Comment: Change "For traditional and well-established herbal preparations the potential for interaction should be clarified if reports point to clinically relevant interactions in humans". Proposed Change: "For herbal preparations commonly taken simultaneously, the potential for interaction should be clarified if reports point to clinically relevant interactions in humans"	Not accepted. However, we agree that the data available will be likely commonly used combination together with drugs monitored by TDM, for which an interaction is more visible.
1024-1027	16	We disagree with the general statement that for new herbal preparations, the potential for interactions should be investigated. If it has been shown that a NMP is susceptible for interactions with e.g. CYP3A	See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		inhibitors, this information will be captured in the product label with appropriate precautions for the coadministration of herbal products known to interact with those pathways. If a manufacturer markets a new herbal product it should be the responsibility of the manufacturer to provide guidance on affinity of a new herbal product on known major drug metabolizing enzymes or transporters alike the responsibility of a sponsor to provide information on the affinity of the NMP for drug metabolizing enzymes/transportes. Proposed change: For new herbal preparations, the potential for interactions should be investigated "*by the manufacturer"*. For traditional and well-established herbal preparations the potential for interactions should be clarified "*by the manufacturer of the herbal preparations"* if…	
1060-1062	17	Clear treatment recommendations should be given to the prescriber. Wording such as "caution is advised" should be avoided in favour of a recommendation on proposed actions. The need for time-specific information and recommendations should be considered. Comment: we support this statement, because it's essential information for the healthcare professional.	N/A
1079	5	Comment: There appears to be a typo.	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any): Consider to delete the word "in" printed in italic behind the comma.	
1081 (possible 1181)	9	Comment: Would it be possible to give the drug class instead of the single drugs in certain cases (e.g. azol-antimycotics, HIV-protease-inhibitors)? Proposed changes: Re-phrase: "… to include a list of drugs or drug classes likely to be affected…"	Not accepted. In general it is of more value to cite drug substances as there may be drugs in a class which has less or no interaction potential and which the prescriber could find if only the inhibitors considered are listed.
1083-1084	12	**Comment:** We would recommend the drug class instead of the specific drugs be listed in certain cases (e.g. azol-antimycotics, HIV-protease-inhibitors)? **Proposed changes:** Re-phrase: "… to include a list of drugs or drug classes likely to be affected…"	See above.
1084-1087	12	**Comment:** We understand that the co-med list for prescribers should be as extensive as possible; however, more importantly it needs to be relevant to patients and clinically practical. This needs to tie safety and efficacy as related to PK changes, eg, NTI drugs, so that the list is more meaningful for prescribers as well as patients.	We agree and think this is reflected in the document.
Lines 1085 - 1092	4	Comment: Mundipharma Research Ltd welcomes the clarification in the guidance document related to instances when an investigational drug affects important drug metabolising enzymes and considers this to be a	Accepted. By "most important" we mean the ones for which the clinical consequences are the worst.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		pragmatic approach. However we seek clarity on how to define 'the most important drugs' which should be included in the list to aid the prescriber. Proposed change (if any): NA – seek general further clarification of 'the most important drugs'.	
1087-1091	11	Comment: Section 7.1 describes label information for non-studied interactions derived from mechanistic information. In that case, it will not be possible to select drugs for inclusion in the listing on the basis of clinical consequences as proposed. Proposed change (if any): "*In this case, drugs should be selected for inclusion based on the severity of the clinical consequences*" might be amended to "*In this case, frequently described drugs should be selected for inclusion as representative examples*". The proposed label text "*Drug X is a potent inhibitor of CYP3A4 and may therefore* ***markedly*** *increase the systemic exposure of drugs metabolised by this enzyme such as …*" should be deleted and the alternative statement used, only "*Drug X is mainly metabolised by CYP3A4. Concomitant use of drugs which are potent inhibitors of this enzyme, such as ……, are not recommended*" or the word "markedly" deleted as the extent of increase in systemic exposure is not known.	Not accepted. It is important to give the prescriber the background information supporting the recommendation of not using the drugs together.
1095	5	Comment: There appears to be a typo.	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any): Consider to replace the full stop behind the word “included” by a comma.	
1108	5	Comment: It appears, that not all abbreviations used in the document, are contained in the list of definitions/abbreviations as yet (e.g. TDM) Proposed change (if any): Check list for completeness.	Accepted.
1134-1135	12	**Comment:** Requiring a 10 hr fasting period for the reference condition in food effect studies may be impractical for compounds that can only be studied in patients with advanced disease (e.g., cytotoxic oncology drugs), where dosing on an empty stomach (e.g., no food intake for 1 hr prior to and 2 hr after dosing) should be an acceptable alternative for the reference condition in food effect studies.	Not agreed. It is not understood why fasting is not possible for patients. However, if this is impossible for a specific reason, a shorter fasting could be applied. However, the proposed periods are all too short.
1149	12	**Comments:** The lighter meal (400-500 kcal with fat contributing to ca. 250-300 kcal) is different from the standardized moderate meal (15% protein, 55% carbohydrate, 30% fat diet of 500-700 calories). **Proposed change:** We suggest that the standardized moderate meal (15% protein, 55% carbohydrate, 30% fat diet of 500-700 calories) be considered as the "lighter meal".	Not accepted. We have not been able to find a generally accepted reference “moderate meal”. No reference was added to the comment. We have reduced the fat content of the moderate meal recommended in the guideline. The main reason is for this meal to differ more from the high-fat one.
1150	12	**Comment:**	Accepted. A short introduction describing this has been

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		Caco-2 cells are somewhat variable from lab to lab and strain to strain. Although they are more representative of the GIT than a transfected cell line, it can be challenging to identify the major transporter involved. A more reliable way to explore this would be to use cell lines which overexpress specific human transporters (of known clinical relevance) and/or to employ known (specific) inhibitors. The concentrations recommended for these studies will likely be way beyond what are technically feasible, posing issues of solubility and integrity/toxicity to the cells. Overall this appendix is too prescriptive and leaves little room for alternative approaches, which is in contrast to the assertion in the body of the document which acknowledges tools other than Caco-2 are both available and useful.	inserted. There is focus of Caco-2 cells in this section as this is the main studies submitted for these transporters. Also, here, the permeability constant determinations are being described. Detailed information is given, whereas for other cell-lines (in Appendix III) the information is more general. The recommendations in the Caco-2 section (Appendix II) may be translated to other cell-lines where relevant.
1150-1151	5	Comment: "The permeability of the drug should be investigated in both directions" although not explicitly stated, implies the use of cellular monolayer assays. We oppose this contention, because a) there are in vitro-assay formats other than monolayer assays that have been shown to perform equally well or better, b) there's data showing the e.g. for ABCB1 monolayer efflux assays the in vitro-in vivo correlation is poor (Fenner et al. Drug-drug interactions mediated through P-glycoprotein: clinical relevance and in vitro-in vivo correlation using digoxin as a probe drug. Clin Pharmacol Ther. 2009 Feb;85(2):173-81.).	The sentence relates to caco-2 cell experiments. This does not apply to other assays where transport in one direction only is determined. We hope this is clear with the revised text.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any):	
1152-1155	1	**Comment:** At least 4 physiologic concentrations are required from transport studies. **Proposed change (if any):** Align with ITC recommendations	Not accepted. The guideline already recommends at least 4 concentrations.
1152	16	Transporter studies need to be performed under well-controlled conditions … Conditions for transporter studies can be well-controlled, however more importantly they are far from being standardized across different labs. Different cell lines/expression systems, assay conditions, positive and negative controls, etc. are used. Although this might not impact the diagnostic use of the tools (is a transporter involved in elimination of or inhibited by a test drug?), it probably highly impacts any quantitative comparison and in vitro to in vivo extrapolation.	We agree completely. However, at present, as stated, there is no consensus and also no in vivo expression data for all transporters. Therefore, we use these studies mainly qualitatively.
1153-1155	6	Comment: It is stated that “sink condition is obtainable through repeated changes of the receiver well”. According to us another way to create a sink condition is applicable, i.e. to add plasma protein (e.g. albumin) to the receiver well, in order to establish a low free concentration of compound. Proposed change (if any): “..obtainable through repeated changes of the receiver well, or addition of	Not accepted. By adding albumin to the receiver well, the permeability rate becomes overestimated. This is not recommended and we would not trust such results.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		plasma protein to the receiver well."	
1153 and following	10	Comment: To obtain sink conditions, the repeated change of the receiver well is in most cases not necessary. Comment: To test the permeability of a drug, three different concentrations, if properly chosen, should be sufficient for most drugs. Proposed change:…at least 3 different physiologically relevant concentrations….	See above.
1153	12	Comment: The rationale for the requirement of at least four concentrations of the investigational drug to determine its permeability is unclear. We consider that two-three concentrations testing should be appropriate in most cases. Proposed change: Please consider rephrasing as follows: "...at least two different physiologically relevant concentrations."	The four concentrations mentioned relates to the investigation of transporter involvement. The permeability rate constant in absence of transporters can be determined at fewer concentrations. Absence of transporter involvement needed in such experiments is certified by a ratio of >0.5-<2. For transported drugs, these conditions could be produced through transporter saturation or inhibition.
1155-1158	12	Comment: Recommendations to use a "concentration range of 0.1 to 50-fold of the dose/250 ml and use of sink conditions" are experimentally not feasible with most investigational drugs due to solubility limitations across such wide concentration range. We would recommend to consider qualifying as this "to the extent permitted by solubility of the investigational agent".	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
1156	10	Comment: For most drugs it will not be feasible to reach concentrations of 50-fold the dose for in vitro studies due to solubility limitations. Proposed change:….transport the studied range could be 0.01 to 1-fold the dose/250 ml.	See above.
1158-1159	12	**Comment:** We would appreciate clarification on how these calculations should be performed and whether scientific references could be provided.	The equation used would be $dC_r(t)/dt=(\mathbf{P_{app}}*A*(C_d(t)-C_r(t)))/V_r$ Palm K et al J Pharmacol Exp Ther 1999, 291(2),435-43
1158-1160	12	**Comment:** It is not clear whether transport studies are to be done under both proton gradient and under iso-pH conditions for all compounds as a general rule or not. We would appreciate this point to be clarified.	A proton gradient should be applied if needed for the specific transporter. Otherwise, the pH should be the same on both sides of the membrane not to influence the study results e.g. by ion-trapping.
1162	10	Comment: The impact of the factors described is only relevant if the recovery is too low. Proposed change: ….in vitro on study results should be discussed if the recovery is less than 80%.....	Not accepted. Some of the factors could also affect the permeation of drug through the cells.
1162-1164	12	**Comment**: It is unclear why metabolism needs to be discussed in an over-expressed transporter system in which the background cell type, e.g. Chinese hamster ovary cells, is known not to be primarily involved in metabolism. It would also be helpful if EMA can provide guidance on	Not accepted. This is not the intention of the text. There is no need to check for metabolism in cells not expressing any enzyme metabolizing the drug. The permeability assessment should be done comparing the test agent to a positive reference drug (eg with high

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		an acceptable permeability range for various positive control values (high and low permeability control compounds in the study). **Proposed change:** We would suggest that a general statement such as the following be used: “Scientifically valid protocol with proper positive controls and well controlled conditions should be used in the *in vitro* investigations of involvement of transporters in drug absorption.”	permeability). This is in line with the present text.
1163-1168	9	Comment: The sole use of efflux ratios and comparison to a fixed parameter (here < 0.5 or > 2) is not suitable for the assessment of active transport. An internal control experiment using well-established transporter substrates is required. Because *in vitro* efflux ratios are largely affected by expression level of transporter(s). Furthermore, for CaCo-2 cells such experiments are largely limited to P-gp, see also page 221 of the "ITC publication". Proposed changes: Please include a statement to the text. The use of specific inhibitors (e.g. zosuquidar) for the assessment of active transport in CaCo-2 cells and importance of supplemental efflux ratios of well-known transporter substrates as internal standard should be clearly mentioned in the text in addition to the use of saturation experiments.	Accepted. This information has been added.
1165-1170	12	**Comment:**	Accepted. See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		The sole use of efflux ratios and comparison to a fixed parameter (here < 0.5 or > 2) is not suitable for the assessment of active transport. An internal control experiment using well-established transporter substrates is required. Because *in vitro* efflux ratios are largely affected by expression level of transporter(s). Furthermore, for CaCo-2 cells such experiments are largely limited to P-gp, see also page 221 of the "ITC publication". **Proposed change:** The use of specific inhibitors (e.g. zosuquidar) for the assessment of active transport in CaCo-2 cells and importance of supplemental efflux ratios of well-known transporter substrates as internal standard should be clearly mentioned in the text in addition to the use of saturation experiments.	
1165-1175	12	**Comment:** Typically, B>A/A>B ratios are measured not A>B/B>A as suggested. We would also recommend to re-word the text as follows for clarity. **Proposed change:** Propose the following wording: "In polarized directional studies (CaCo-2 or cell line over-expressing particular transporter of interest), the permeation of drug from the apical (A) to the basolateral (B) side of the cells is compared with the permeability of the	Partly accepted. This is the more commonly used ratio (for efflux). This was a mistake in the text.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		permeation in the opposite direction (B to A). If the ratio of the B to A and A to B permeation is > 2, it is concluded that there is active efflux. If active transport is concluded, the importance of the transporter for drug absorption can be estimated through a comparison of the permeability in the presence and absence of transporter. In Caco-2 models, the permeability constant is determined at concentrations high enough to completely saturate the transporters. In polarized models with the over-expression of a transporter of interest, the permeability in the absence of transport can be determined in the parental cell line without transporter over-expression or in the presence of a strong inhibitor for the over-expressed transporter."	
1166-1168	15	Comment: At which concentration the observation of polarisation is significant? Any concentration or clinically relevant concentration	At a clinically relevant concentration. A range needs to be tested.
1168-1169	5	Comment: We welcome taking into account the (passive) permeability constant to be taken into account when assessing the importance of a transporter. However, we oppose the contention that passive permeability can be determined at concentrations high enough to completely saturate the transporters because a) it is unclear which transporters are to be saturated using a cellular system such as Caco-2 cells containing multiple transporters and b) if	Not accepted. We agree that the concentration gradient will affect the passive permeability. However, looking at the equation, Co may be removed and the outcome is thus not dependent on the concentration used.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		experiments are to be conducted under sink conditions as noted in line 1151-1152, it needs to be kept in mind that passive permeability according to Fick's first law of diffusion is directly dependent on the concentration gradient, i.e. the concentration used in the assay. As it stands, the current recommendation in the draft will yield a high number of (false positive) high permeability constants also contrasting the recommendation to study at least four different physiologically relevant concentrations. Proposed change (if any):	
1170	5	Comment: We do not entirely agree that using a high and low permeable control (e.g. metoprolol and mannitol) are sufficient controls to validate a determination of (passive) permeability constant. This is because a) the large inter-laboratory variability reported for permeability constants determined in Caco-2 monolayers and more importantly b) the steep increase in passive permeability constants when analysing compounds with a low and high intestinal permeability (determined in vivo) and/or fraction absorbed. (for review please cp. Figure 5 in Artursson P. et al. Caco-2 monolayers in experimental and theoretical predictions of drug transport. Adv Drug Deliv Rev. 2001 Mar 1;46(1-3):27-43). We suggest to recommend the use of artificial membrane permeability assays (PAMPA) for	Not accepted. In the guideline document, the method is only used qualitatively, ie may the drug be qualified as having high permeability drug (having permeability equal to or higher than metoprolol) or not. For this use, the method appears appropriate. If determining the permeability rate quantitatively, it is possible that more controls and also the use of permeability rate reference values for the controls may be needed. It is also possible that other methods may be more predictive. The experience with PAMPA in drug applications is very limited. The reference cited does not supply enough information to support its recommendation.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		determination of the (passive) permeability coefficient instead. (please cp. Sugano K et al. Coexistence of passive and carrier-mediated processes in drug transport. Nat Rev Drug Discov. 2010 Aug;9(8):597-614.) Proposed change (if any):	
1170-1171	7	Comment: The suggestion "to estimate the [*in vitro*] permeability in the absence of transporters" by determining the permeability constant "at concentrations high enough to completely saturate the transporters" seems impractical. Solubility limitations make this impractical for many, if not most, drugs. In addition, for many drugs, the cell lines used to determine permeability *in vitro* will not tolerate concentrations high enough to saturate transporters. In our experience, better approaches are to inactivate transporters through the use of a cocktail of chemical inhibitors (multiple transporters) or genetic knockdowns (one transporter at a time). Proposed change (if any): To estimate the permeability in the absence of one or more transporters, the permeability constant should be determined in the presence of chemical inhibitor(s) or in a cell line in which the expression of a transporter has been knocked down genetically (in parallel with the parental cell line expressing the transporter of interest).	Accepted. Application of inhibitors has been added and other ways of removing transporter activity generally appear satisfactory if there is data showing that the cells otherwise are intact.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
1170-1171	12	**Comment:** Unless the drug under test has the ability to interact closely with the transporter (e.g. has high passive permeability and/or high lipophilicity) it is likely that no saturation of transport may be achieved at the higher concentrations tested. This is the only methodology described for transporters and represents one approach for absorption only. No reference is made to other methods for e.g. hepatic uptake, transporter phenotyping, biliary efflux, transporter inhibition etc. It is suggested to make this more general around strategy/approaches to take when considering transporter mechanism and inhibition potential: possible systems and considerations for experimental design, accepting that with the current state of the art around transporters this is a very rapidly evolving field.	See above.
1170-1171	15	Comment: What if solubility in vitro is limited and saturating concentration is not achieved in cellular assay	The text has been changed, see above.
1171	10	Comment: For some compounds it is not possible to saturate the transporter due to solubility limitations in physiological buffers. Proposed change: … concentrations high enough to completely saturate the transporter(s) or up to a concentration equivalent to dose/250 ml.	See above
1171-1172	12	**Comment:**	Partly accepted. It is possible that there are other controls

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		We would appreciate if a rationale for using metoprolol as high permeability positive control could be provided.	which may be adequate if well validated. This is included "The investigation should include a well validated, high and low permeable reference substance (e.g. metroprolol and mannitol)."
1172-1174	7	Comment: Metoprolol should not be specified as the high-permeability marker for in vitro permeability; other compounds are equally or more suitable. Contact Absorption Systems (ihidalgo@absorption.com) regarding our validation of a better high-permeability marker. Proposed change (if any): If the permeability in the absence of transporters is high (≥ the permeability constant of a well-absorbed drug whose use as an *in vitro* high-permeability marker has been well validated)…	See above.
1182-1183	6	Comment: Suggestion to add that the study should not only include a probe substrate as a positive control, but also an inhibitor of the investigated transporter as a control. Proposed change (if any): The study should include positive controls verifying presence of the specific transporter activity, as well as an inhibitor of the investigated transporter.	Accepted.
1182-1184	12	**Comment:** We would appreciate if some examples of clinically precedented inhibitors/substrates for transporters	Not accepted. See earlier responses to comments raised.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		could be provided. There is enough information available to provide some guidance on currently accepted (or most suitable) probes. It would also be valuable to highlight where there is a current lack of a suitable specific probe for a given transporter.	
1183-1203	3	Proposed change (if any): Some of the language in this section could be adjusted. Line 1187, the word "liver" should be added to "cells expressing human liver enzymes" to be consistent with the statement that these are examples of *in vitro* systems for liver metabolism studies (line 1189-90). The second and third bullets refer to subcellular fractions. The microsomal fraction and S9 are subcellular fractions since microsomes are the membranes and S9 is the post-mitochondrial fraction. A homogenate is composed of the complete cellular materials. Line 1202; Suggestion to state that cultured hepatocytes may loose their enzyme activity over the course of days. In our experience hepatocytes in suspension are similar to recombinant enzymes and subcellular fractions since the loss of activity occurs over the course of hours.	The text on different in vitro systems has been removed.
1191	9	Comment: Text mentions a trademark of a single commercial supplier, which should be avoided. Proposed changes: Re-phrase: Most recombinant enzyme systems are single enzyme systems.	Accepted. The text has been reworded.
1192	12	**Comment:**	Not accepted. This is recommended due to the possibility of

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Having a positive control for each CYP would require multiple analytical methods, which while not impossible, would be logistically difficult and cost-prohibitive. **Proposed change:** We suggest deleting this requirement.	degraded/nonfunctional enzyme. Wording has been changed from “should” to “is strongly recommended”. The set of studies performed to investigate which enzymes are involved in the metabolism partly is the choice of the applicant. More studies may be needed if negative results needs confirmation and underestimation of *in vitro* intrinsic clearance, may affect potential PBPK analyses.
1193	12	**Comment:** The text mentions a trademark of a single commercial supplier, which should be avoided. **Proposed change:** Re-phrase: Most recombinant enzyme systems are single enzyme systems.	See above.
1193	15	Comment: The guideline should not advertise one manufacturer of recombinant enzymes. Please remove “Supersomes”	See above.
1206-1207	12	**Comment:** “Physiologically relevant” should be changed to “therapeutically relevant”. **Proposed change:** Change to the following wording: “The *in vitro* metabolism studies should be performed at therapeutically relevant concentrations of the investigative drug under linear conditions.”	Accepted.
1213-1214	12	**Comment:** With regard to the use of positive controls for each	See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		enzymatic activity, when reaction phenotyping are performed, this will results in a much more comprehensive study if it means that these controls should be included in each experimental set-up. It seems appropriate to add the supplier information about this characterization, when human liver microsomes are used, as it also is mentioned in the guideline that human liver microsomes are robust systems. Also if no metabolism occurs in the assay it would be a normal procedure to follow up on this finding, e.g. repeat study or include controls. **Proposed change:** Please consider rephrasing as follows: "*Positive controls (marker substrates) for enzyme activity (see table 2) should be included in the study or in case human liver microsomes are used, the characterization from the supplier should be available.*"	
1214	16	One in vitro system may be enough for enzyme ID c.f. FDA draft guideline from 2006: 2 different methodologies requested.	Accepted. If the main enzymes involved in the *in vitro* metabolism are identified, one *in vitro* system may be enough for this investigation. However, it is generally recommended to verify the results by performing studies in another *in vitro* system.
1219	1	**Comment:** Provide recommended concentrations for substrate and inhibitor that are appropriate for reaction phenotyping study (fm). Inhibitory monoclonal antibodies to CYPs are an alternative tool for reaction phenotyping.	Accepted. This has partly been provided. Substrates are mainly provided for enzyme inhibition studies. Of course, these substrates may be used but there are a lot of other reactions as well that may be followed. Here we wish not to make any list. The possibility of using antibodies has been

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any): Supply concentrations, and recommend antibody alternative	added.
1221	10	Comment: More inhibitors are used, and for CYP2C19 a specific inhibitor is known. Proposed change: Please consider adding these additional inhibitors: Fluvoxamine for CYP1A2, Benzylphenobarbital for CYP2C19 (specific), Fluoxetine for CYP2D6, Azamulin for CYP3A4	Not accepted. The table just provides examples and can never cover all well documented and suitable inhibitors.
1221	16	Table 1 has mix of TDI and competitive inhibitors. A-NF and benzylphenobarbital?	Partly accepted. Yes, some of the inhibitors proposed are TDI. See above for benzylphenobarbital.
1224	10	Comment: There are more CYP specific marker reactions used. Proposed change: Please consider adding: Tacrine hydroxylation for CYP1A2, Nirvanol formation (S-mephenytoin N-demethylation) for CYP2B6, Dextromethorphan O-demethylation for CYP2D6	Partly accepted. These are only examples.
1224	10	Proposed change: Please consider adding midazolam **plus** testosterone as selective CYP3A4 substrates **or** one other structurally unrelated substrate.	Accepted. The wording has been simplified/corrected to midazolam and testosterone as the only choices listed.
1224	12	**Comment:** The structure of the wording around the CYP3A4	Accepted. See above.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		marker reactions makes it difficult to discern the proper meaning. We assume that the wording is meant to summarize the conclusions reached in Kenworthy KE, Bloomer JC, Clarke SE, Houston JB. CYP3A4 drug interactions: correlation of 10 *in vitro* probe substrates. Br J Clin Pharmacol. 1999 Nov;48(5):716-27. In that paper, they suggest that midazolam and testosterone ARE structurally unrelated substrates that can cover the potential inhibition of CYP3A from different angles. Midazolam AND testosterone would thus be an ideal set of 2 unrelated substrates. **Proposed change:** Please clarify the wording surrounding the CYP3A4 probe reactions. Proposed wording for CYP3A4: "midazolam 1-hydroxylation and testosterone 6β-hydroxylation; or midazolam 1-hydroxylation or testosterone 6β-hydroxylation plus one substance structurally unrelated to the chosen reaction such as nifedipine, triazolam or dexamethasone."	
1224 (Table 2)	16	Can dextromethorphan be used as a marker for 2D6? For CYP3A4; midazolam OR testosterone AND another probe are specified. Typically midazolam AND testosterone are used. The Kenworthy et al 1999 paper that classified CYP3A4 probe types suggests looking at the benzodiazapines, the macrolides AND nifedipine not benzodiazapines OR the macrolides AND nifedipine. Please provide information as to why common practice	Dextrometorphan O-demethylation to dextrorphan is sometimes used as a marker. However, the metabolite if further metabolized by CYP3A4, why inhibition of this enzyme could affect the ratio. Regarding CYP3A4 inhibitors, see above.

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		is changing.	
1231-1232	3	Comment: The draft Guidance recommends that metabolites having an AUC ≥ 20% of parent AUC, or contributing to > 5% of the total radioactivity AUC are structurally characterized. We consider the recommendation of > 5% as too conservative as it would require characterization of significantly more metabolites than is currently customary. Proposed change (if any): We recommend that the Guidance is consistent with the recent ICH-M3 guidance, which recommends characterization of metabolites accounting for ≥ 10% of total radioactivity (as opposed to the 5% of radioactivity or 20% of parent threshold recommended in the draft Guidance).	Accepted. The text has been changed.
1231-1235	12	Comment: Levels appear different from ICH guidance on metabolite safety testing (ICH M3(R2)) and requiring a full characterization is unnecessarily rigorous. Having different thresholds in this document from that in ICH will only create confusion. In the ICH guidance a sponsor can qualify a metabolite without a full characterization if the metabolite is found in animals. Phase II metabolites should not be held to the same rigor of testing as Phase I metabolites. Some metabolites are unstable and/or difficult to synthesize which would be needed to conduct the studies required in this guidance. There could be 20 different	See above.

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		metabolites each ca 5% that would need to be isolated, characterized, synthesized and tested, yet each pathway could be completely blocked with no clinical consequences. Metabolites with long half lives will be "false positives". Proposed change: We recommend that the Guideline is consistent with the recently updated ICH-M3 guidance.	
1233	16	It is generally recommended that metabolites having an AUC >=2% of parent AUC or contributing >5% of the total radioactivity AUC should be structurally characterised. These are different thresholds from current FDA/Phrma guidances	See above
1234	3	Proposed change (if any): Suggest the word "scheme" rather than "schedule".	Accepted.
1236-1244	12	**Comments:** The mass balance studies are frequently conducted with an oral solution formulation and not on the oral market formulation. Oral bioavailability studies are conducted with an IV and the “to be marketed” formulation. Requiring bioavailability studies with radio-labelled drug used in the mass balance studies when excretion is primarily faecal is unwarranted and exposes subjects to additional radioactivity with little gain in how the drug will be used therapeutically.	Partly accepted. A possibility to discuss differences has been added. However, such may be difficult depending on other information available.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed Change: We suggest deleting lines 1239 to 1244.	
1237-1240	5	Comment: The determination of oral bioavailability does not help to assess the amount of dose excreted unchanged into faeces, given that oral bioavailability is a composite of the fraction absorbed, and the fraction metabolised in the intestine and liver upon first-pass. Please bear in mind that biliary excretion of unchanged compound into the intestine and subsequent re-absorption can obscure the determination of oral bioavailability. Therefore, please consider to state more clearly that only an i.v. mass balance trial (which would be an additional trial to the early development of an orally administered investigational drug program) will provide the information that will allow to judge to which extent hepatobiliary elimination contributes to the overall elimination of the investigational drug. Please also note, that marked elimination of unchanged drug into faeces does not necessarily reflect hepatobiliary elimination but could also represent active gut-wall secretion into the intestine as an alternative route of elimination (that would involve transporters on the basolateral and apical membrane of enterocytes which may be different from hepatic uptake and canalicular transporters) Proposed change (if any):	Accepted. By the help of clearance (if hepatic), the hepatic extraction ratio may be calculated and thus, the contribution of hepatic extraction to oral F may be estimated. However, if there is renal elimination, or intestinal metabolism, eg by CYP3A4 or other intestinal enzymes, this estimation is not possible. This has been reflected in the guideline. We acknowledge that iv mass-balance data is also very useful as it provides more precise data on contribution of different pathways to total clearance, unaffected by first pass metabolism. Accepted. It is presently unknown how often this may be important elimination pathway. The possibility has been included in the text.
1241	5	Comment: Please consider to state more precisely.	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any): I.v. mass-balance data may also be useful in situations with pronounced metabolism **in the intestinal lumen** to assess the contribution of metabolism pathways to systemic clearance.	
1246-1252	12	**Comment:** The guideline suggests a mass balance study after a single radiolabelled dose at steady state conditions. Evaluation of a single radiolabel dose at steady state achieved using a nonlabelled material would not be fully informative about drug related material (radioactivity) saturating various compartments of the body, i.e. at saturated stage the clearance of the radiolabel may be faster and may not be representative of cumulative condition of the material in the body. It is also important to highlight that it still is a single dose 14C-compound that is measured in the study and that the steady state contribution from non-labelled parent drug and metabolites are not included. Therefore the suggested mass balance study can give information about time dependent pharmacokinetics, but not any information about steady state of the metabolites.	If there is dose or time dependent elimination of a drug, the contribution of different pathways may be different at steady state than at single-dose conditions. In most cases, the steady state situation resembles the therapeutic situation and is therefore the one of interest. Thus administering a radiolabelled single dose at steady state with unlabelled drug will show what happens with one dose at steady state. As the AUC0-∞ of metabolites observed at steady state will reflect AUC0-τ of the metabolite, there is no need to estimate the latter parameter. This is not the aim of the study.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change: We would suggest that the recommendation is reviewed order to accommodate the different solutions " and investigation of the exposure of metabolites at steady state could be considered."	
1247 – 1250	5	Comment: In order to prevent repeated mass-balance trials that are lengthy, costly and, in case of conventional high ^{14}C dose trials, subject healthy volunteers to an unnecessary radiation burden, please consider to recommend the investigation of mass balance conditions at steady state in line with the current FDA guidance on safety testing of drug metabolites. Proposed change (if any):	Not accepted. A mass-balance study using radio-labelled drug is the way detection of quantitatively important metabolites may be assured. Therefore this is the recommended approach. A mass-balance study with radio-labelled drug administered at steady state is a recommended approach.
1258-1265	12	**Comment:** We would recommend to allow the use of other orally administered CYP3A sensitive substrates than Midazolam for the classification of inhibitors and inducers. Please consider the use of other CYP3A probe substrates that showed low intestinal-first pass metabolism.	Not accepted. Optimally, the effect on intestinal and hepatic enzyme would be separated through a midazolam study investigating the effect on orally and iv administered drug. If only one situation is to be studied, it should include intestinal enzyme, serving as a worst case scenario.
1263-1265	12	**Comment:** Categorizing drugs that produce up to a 50% decrease in oral midazolam AUC as mild inducers is not adequate. Clinically significant inducers like St. John's wort have been reported to produce decreases in oral midazolam AUC ranging from 41% to 79% across	Not accepted. It is important to separate the classification from potential effects on drugs with a rather narrow therapeutic window. Any classification becomes a rough measure as the variability within a class can be large and as the label may not be translated to a clinical relevant label that fits all interactions with the drug. Therefore, when

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		individual studies. In the study where only a 41% decrease in midazolam AUC was noted (*Hall SD et al., Clin Pharmacol Ther 74:525-35, 2003*), breakthrough bleeding was noted in ~60% of women taking oral contraceptives indicating a clinically meaningful extent of induction of drug-metabolizing enzymes by St. John's wort despite a <50% mean decrease in oral midazolam AUC. Therefore, a more appropriate cut-off to consider is 30% decrease in oral midazolam AUC below which an inducer may be considered as a mild inducer. For example, pioglitazone produces a 26% decrease in midazolam exposure and is not considered a clinically significant inducer from a standpoint of DDI risk, supporting a cut-off of 30%. Please consider adding the possibility for a compound to be a non-inducer.	communicating a potency classification it is important that the potential effect of the AUC of sensitive substrates is given and also information on interactions predicted from the results. It should also be noted that an interaction study with oral contraceptives would likely have been required for a "mild inducer" if given to women of child bearing potential, as available knowledge indicate that an interaction is likely.
1269	12	**Comment:** We recommend including fluvoxamine as an acceptable *in vivo* CYP1A2 inhibitor as furafylline is usually used in vitro and not in vivo and is not available in all regions in a DDI study.	Partly accepted. Furafylline has been removed. Fluvoxamine, although being a potent 1A2 inhibitor, is not specific enough to be recommended. However, of course, if involvement of enzymes co-inhibited by fluvoxamine may be excluded based on other data, then a fluvoxamine study may be used to quantify 1A2 involvement.
1269	12	**Comment:** Please consider adding ticlopidine as a strong CYP2B6 inhibitor *in vivo*. Ticlopidine at 250 mg BID produces ~90% inhibition of CYP2B6 activity reflected by a decrease in hydroxy bupropion/ bupropion ratio (*Turpeinen M et al., Clin Pharmacol Ther 2005;77:553-*	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		9)	
1277 (Table 3)	3	Proposed change (if any): We suggest adding repaglinide, pioglitazone and rosiglitazone as recommended CYP2C8 probe substrates.	Not accepted. Two probe drugs are enough as examples. Others may be used if well supported by science and justified in the application,
1279	10	Comment: Cerivastatin is no longer available. Proposed change: 'cerivastatin hydroxylation (M23 formation)' should be deleted and replaced by 'repaglinide'. Under 'CYP3A4', additional model substrates, e.g. 'nifedipine' should be mentioned.	Accepted. Midazolam is preferred for CYP3A4 in vivo studies. It is a very sensitive probe drug and it is useful to have one probe drug in order to make rough comparisons of different inhibitors potencies.
1279	12	**Comment:** Caffeine is not a selective CYP1A2 probe for in vivo induction, due to its multiple metabolic pathways, some of which are catalyzed by enzymes other than CYP1A2, e.g. 3A4. In general, to use a reaction pathway, instead of a parent drug probe would give more accurate assessment of induction.	Partly accepted. This may be valid for more drugs in the table. A general statement has been added.
1279	12	**Comment:** This table lists cerivastatin hydroxylation as a measure for effects on CYP2C8, but cerivastatin was withdrawn from the market. Therefore, this reaction does not seem to practically useful. **Proposed change:** Remove cerivastatin and please consider including repaglinide, pioglitazone and rosiglitazone as recommended CYP2C8 probe substrates.	Accepted. Repaglinide has been added.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
1279	12	**Comment:** Please consider including simvastatin and nifedipine as CYP3A4 probe substrates.	Not accepted. We prefer the use of midazolam to enable (between-study) comparisons. (See above)
1283-1393 Appendix VII	4	Comment: With reference to the previous stakeholder comment regarding investigation of alcohol interactions (Section 5.1), we would wish to propose update of Appendix VII to include the preferred wording from the CHMP following the Article 31 referral (EMEA/H/A-31/1232). Proposed change (if any): To be in-line with the final Commission decision following the referral.	As this mainly relates to a specific formulation, recommendations on this will be handled by another guideline.
1283-1393 Appendix VII	4	Comment: The stakeholder suggests a cross reference to the current Guideline on Summary of Product Characteristics Rev 2 Sept 2009 be included. We also propose that the Eu Annotated QRD template be updated under section 4.5 to include reference to an appendix containing the preferred wording for recommendations regarding food intake, in line with, for example, pregnancy and lactation statements.	Not accepted. A general reference is present in section 7. Comment noted. This is a separate procedure.
1257	5	Comment: Please consider to change the term "probe drug" by "object drug" in this context. Proposed change (if any):	Not accepted. We prefer “probe drug”.
1258	5	Comment: Please consider to delete the imprecise term "somehwat"	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome[2]
		Proposed change (if any):	
1267	5	Comment: Furafylline is not a specific CYP1A2 inhibitor in vivo, given its equipotent and time-dependent inhibition of CYP2C19 (cp. von Richter et al. Effect of fluvoxamine on the pharmacokinetics of roflumilast and roflumilast N-oxide. Clin Pharmacokinet. 2007;46(7):613-22). Please consider adding this information to the footnotes of the table. Proposed change (if any):	Accepted. Furafylline has been replaced by enoxacin.
1274	5	Comment: Check grammar (tense) of statement. Proposed change (if any): Statement should read “The **relative** contribution of **individual** enzymes to **the oral clearance of probe drugs should be** supported by well performed *in vivo* studies.	Accepted.
1277	9	Comment: Would you accept simvastatin, which is more commonly used than midazolam, as a model substrate of CYP3A4 as well? Proposed changes: please include simvastatin in the table.	We recommend midazolam. See above.