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Guideline on the investigation of drug interactions

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* The correction concerns section 5.3.4.1 (p 26) and the corresponding decision tree no. 6 (p 61) to read “if the observed Ki value is lower or equal to /…/”; Appendix VII, Table 5 to read “See section 5.4.2”.* Decision tree 4.

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Guideline on the investigation of drug interactions

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Executive summary

The potential for pharmacokinetic interactions between new medicinal products and already marketed drugs should be evaluated. This applies to both effects of the medicinal product on other drugs as well as the effect of other drugs on the medicinal product. Furthermore the effect of concomitant food intake needs to be investigated. The drug-drug interaction potential is usually investigated through *in vitro* studies followed by *in vivo* studies. The results of interaction studies are used to predict a number of other interactions based on the mechanisms involved. Treatment recommendations are developed based on the clinical relevance of the interactions and the possibility to make dose adjustments or treatment monitoring. This document aims to provide recommendations on all these issues. General recommendations are also provided for herbal medicinal products.

1. Introduction

Drug-drug interactions are a common problem during drug treatment and give rise to a large number of hospital admissions as a result of medically important, sometimes serious or even fatal adverse events. Drug-drug interactions can also cause partial or complete abolishment of treatment efficacy. The ageing European population, where polypharmacy is more frequent, increases the likelihood of such interactions and underlines the importance of a scientifically sound understanding of the potential for drug-drug interactions for all new chemical entities. A number of drugs have been withdrawn from the market as a result of drug-drug interactions that were only discovered post-marketing. The potential for drug-drug interactions is considered in the benefit-risk evaluation of a medicinal product and can negatively impact on this balance either through increased incidence of adverse events or reduced efficacy.

This guideline outlines a comprehensive, systematic and mechanistic approach to the evaluation of the interaction potential of a drug during its development and offers guidance to ensure that the prescriber receives clear information on the interaction potential as well as practical recommendations on how the interactions should be managed during clinical use.

The first CHMP interaction guideline was adopted in 1997 and this is the first revision of this guideline. During the past 20 years, considerable scientific progress has been made so that today clinically relevant pharmacokinetic drug interactions can be predicted from a limited number of well designed, mechanistically-based *in vitro* and *in vivo* studies. More recently, our understanding of enzyme induction and drug transporter-interactions has progressed so that these interactions can also be anticipated. *In vitro in vivo* extrapolation of drug transporter interaction is currently less mature and requires additional experience and continued scientific developments. Thus, the approach defined for drug-transporter interactions is likely to continue to evolve.

The aim of the interaction studies performed on new medicinal products under development is to gain knowledge of how the new medicinal product affects the safety and efficacy of other medicinal products and vice versa. The potential for interactions is mainly investigated before marketing of a drug. Knowledge about the interaction potential should be gained as early as practically possible to assure safety during clinical phase II and III studies, as well as during clinical use after approval. Additional studies may be needed post-approval to optimize drug safety and to support treatment recommendations in the labeling and variation applications, e.g. for new indications or new dose recommendations. There may also be a need to perform additional studies due to emerging science or as a result of suspected drug interactions reported post marketing. The marketing authorization holder is advised to perform and report interaction studies as needed during the full life-cycle of the medicinal product.

This document provides recommendations on the pharmacokinetic and pharmacodynamic drug-drug interaction studies as well as food-drug interaction studies to be conducted including advice on study design, presentation of study results and translation of these results to treatment recommendations in the labeling of the drug. General advice is also given for herbal medicinal products.

It is recognized that the program to adress the interaction potential of an individual drug needs to be tailored to the specific drug. Alternative approaches are acceptable if adequately justified and driven by science and the expected clinical consequence of the interaction.

2. Scope

The scope of this guideline is to provide advice and recommendations on how to evaluate the potential for drug-food and drug-drug interactions for medicinal products (including herbal medicinal products) and how to translate the results of these evaluations to appropriate treatment recommendations in the labelling.

Interactions with therapeutic proteins including peptides and oligunucleotides, pharmaceutical drug-drug interactions related to physiochemical properties and impact of drugs on clinical chemical laboratory tests are not discussed in this guideline.

3. Legal basis and relevant guidelines

This guideline should be read in conjunction with the introduction and general principles (4) of the Annex I to Directive 2001/83/EC as amended, as well as European and ICH guidelines for conducting clinical trials, including:

- Pharmacokinetic studies in man (Eudralex vol 3C C3A)
- Guideline on the role of pharmacokinetics in the development of medicinal products in the paediatric population (EMEA/CHMP/EWP/147013/2004)
- Guideline on the evaluation of the pharmacokinetics of medicinal products in patients with impaired hepatic function (CPMP/EWP/2339/02)
- Note for guidance on the evaluation of the pharmacokinetics of medicinal products in patients with impaired renal function (CHMP/EWP/225/02)
- A guideline on summary of product characteristics (SmPC) September 2009(Eudralex vol 2C)
- Guideline on reporting the results of population pharmacokinetic analyses (EMEA/CHMP/EWP/185990/2006)
- Guideline on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products. (EMA/CHMP/37646/2009)
- Guideline on the clinical investigation of the pharmacokinetics of therapeutic proteins (EMEA/CHMP/89249/2004).
- Note for guidance on Modified Release Oral and Transdermal Dosage Forms: Section 2 (Pharmacokinetic and Clinical Evaluation) (CPMP/EWP/280/96)
- Note for guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals ICH M3, CPMP/ICH/286/95
- Note for Guidance on General Considerations for Clinical Trials (ICH E8, CPMP/ICH/291/95)

- Note for Guidance on Guideline for Good Clinical Practice (ICH E6, CPMP/ICH/135/95)
- Structure and Contents on Clinical Study Reports (ICH E3, CPMP/ICH/137/95)

4. Pharmacodynamic interactions

Pharmacodynamic interactions may be caused by a large variety of mechanisms. It is therefore not possible to give detailed guidance for pharmacodynamic interaction studies. The studies needed should be determined on a case-by-case basis. The potential for pharmacodynamic interactions should be considered for drugs which compete with each other at the pharmacological target and/or have similar or opposing pharmacodynamic (therapeutic or adverse) effects. If such drugs are likely to be used concomitantly, pharmacodynamic interaction studies should be considered. Extensive pharmacological and toxicological knowledge about the drug is important for the planning of pharmacodynamic interaction studies. It is recommended that both in vitro studies and human *in vivo* studies are used to characterize the pharmacodynamic interaction profile.

5. Pharmacokinetic interactions

Pharmacokinetic interaction studies should generally be performed in humans. Preclinical studies in animals may sometimes be relevant, but due to the marked species differences, direct extrapolation of such results to humans is difficult. Therefore, the wording *in vivo* below means in humans. Similarly *in vitro* studies should be performed using human enzymes and transporters. Deviations from this approach should be well justified and supported by scientific literature.

Potential for pharmacokinetic interactions should be investigated both with respect to the effects of other drugs on the investigational drug and the effects of the investigational drug on other medicinal products. As the study designs and considerations are different, this section is divided into two subsections: "*Effects of other medicinal products on the pharmacokinetics of the investigational drug"* (section 5.2) and *"Effects of the investigational drug on the pharmacokinetics of other drugs"* (section 5.3*).* The wording "investigational drug" is here used for the drug developed by the marketing authorisation applicant or holder reading this document. Sometimes the expressions "victim drug" and "perpetrator drug" are used. The victim drug is the drug affected by the drug-drug interaction, regardless of whether it is the investigational drug or another medicinal product. The perpetrator drug is the drug which affects the pharmacokinetics of the other drug.

Although not mentioned in every subsection of this document, the effects of other medicinal products on the exposure of clinically relevant pharmacologically active metabolites should always be considered. The risk of clinically relevant pharmacokinetic interactions through altered formation or elimination of metabolites should be investigated if available data indicate that an altered metabolite exposure may result in an altered efficacy or safety ("target" as well as "off-target" effects) *in vivo* (see section 5.2.3). The contribution of metabolites to the *in vivo* pharmacological effects of a drug is evaluated taking into account human unbound drug and metabolite exposures *in vivo*, the *in vitro* or *in vivo* pharmacological activities and potencies, and, if available, physiochemical data related to target tissue distribution or data on relative parent drug and metabolite distribution to the target site. Human *in vivo* exposure-response information on metabolite contribution is usually very valuable when translating altered metabolite exposure into treatment recommendations. Finally, as metabolites may inhibit drug metabolising enzymes, the effect of metabolites with a moderate to high exposure should be investigated (see section 5.3.3).

Drug interaction studies required during drug development have a mechanistic rationale. Usually, the potential for drug interactions is investigated *in vitro* and then followed by *in vivo* studies. The *in vivo* part of the interaction documentation is usually composed of a number of interaction studies, some of

these are purely mechanistic, such as studies with strong and moderate inhibitors of an enzyme involved in drug metabolism, aiming at providing the basis for further interaction predictions. Other studies may be performed with likely interacting drugs expected to be commonly used concomitantly with the investigational drug aiming to obtain a specific dose recommendation. Studies may also be performed in order to verify the suitability of a proposed dose adjustment or to confirm a lack of interaction with a commonly co-prescribed drug in the target population. PBPK (Physiologically based pharmacokinetic) modelling and simulation may be used at different stages during drug development to inform study design, to estimate the potential for drug-interactions qualitatively as well as estimate an interaction effect quantitatively. The supporting data needed in different scenarios are presented in different subsections of the guideline.

If an investigational drug is developed for use in combination with another drug, the drug interaction potential for the combination should be addressed. Pharmacokinetic interaction studies with the combination should be considered if there are indications that the interaction profile may not be adequately predicted from *in vitro* and *in vivo* interaction data for the separate drugs.

The recommendations in this guideline are based on advances in scientific knowledge resulting in the fact that most drug-drug interaction can be predicted. However, it is acknowledged that there are remaining scientific uncertainties. In the area of HIV there have been cases of unexpected interactions. When developing a drug in such an area, *in vivo* interaction studies should be considered with commonly combined drugs having a relatively narrow therapeutic window while more knowledge is gained on the mechanism behind the unsuspected interactions in the field.

5.1. Effects of food intake on the pharmacokinetics of the investigational drug

The effect of food intake on the rate and extent of absorption of an orally administered investigational drug should be investigated as early as possible during drug development to optimize dose finding and to ensure optimal food recommendations in the phase III clinical studies and drug labelling. In general, recommendations regarding timing of drug intake in relation to food should aim at minimising variability and obtaining optimal exposure.

If the formulation is modified during the clinical development or if a new pharmaceutical form is developed, the possibility of an altered food effect should be considered and additional food interaction studies may be needed.

The effect of a high-fat meal on the absorption of the investigational drug should be investigated as worst-case scenario. The standardized procedure is presented in Appendix I. If the pharmacokinetics are nonlinear with less than dose-proportional increases in AUC when increasing the dose, it is recommended to investigate the effect of food on the highest and lowest doses of the therapeutic range. If the nonlinearity give rise to larger than dose-proportional increases, studying the dose giving rise to the most marked saturation, usually the highest dose, may be sufficient.

Regardless of dose-linearity, further strength(s) may need to be investigated in case the strengths deviate markedly in composition, the substance has poor solubility under GI conditions and a food effect has been observed on other strengths.

If a clinically significant effect of food is found and the medicinal product therefore will have a specific recommendation regarding time of administration in relation to food-intake, further food-drug interaction studies are recommended. Which studies are relevant to perform depends on whether fed conditions or fasting conditions will be recommended and on how frequently the drug will be administered. If the drug will be recommended to be taken with a meal, studies of the effects of a moderate meal are recommended (See Appendix I) and it may be useful to also investigate different

food compositions (such as a carbohydrate-rich meal). If administration is recommended under fasting conditions in the morning, studies should be performed establishing the sufficient fasting time period between drug administration and the intended meal. If the drug will be dosed on an empty stomach, either several times a day or at a time point other than the morning, studies should be performed establishing the time interval before and after a meal when drug administration should be avoided.

If physiochemical properties and *in vitro* data indicate that complex binding might become an issue *in vivo*, the need for a food interaction study with a calcium-rich meal should be considered. If co-administration is recommended with a meal or specific food due to a marked effect of food on the pharmacokinetics, and the formulation is indicated for the paediatric population, it should be addressed whether this is relevant for paediatric use (especially newborns and infants) whose diet is different (100 % milk in newborns). In newborns, this may be investigated using the population PK approach.

Recommendations regarding interaction studies with special kind of foods (e.g. grapefruit juice) are given in section 6.

Advice regarding the investigation of the effect of food or alcohol on drug release from controlled release formulations are given in guidelines specific for these formulations.

5.2. Effects of other medicinal products on the pharmacokinetics of the investigational drug

In vitro information supporting the prediction of the effects by other medicinal products on the pharmacokinetics of the investigational drug should preferably be available before introducing the investigational product to patients (phase II) and is generally required before starting phase III. The extent of data (*in vitro* or *in vivo* data) needed at different stages of the clinical drug-development is decided case by case based on the possibility of excluding potentially interacting medicines (such as strong enzyme inhibitors) giving rise to relevant interactions, the pharmacokinetic characteristics of the investigational drug, and the safety of the drug at exposures higher than the target exposure in the planned study. PBPK simulations may be of value in the DDI assessment at different stages in drug development (See section 5.5)

Interactions at the level of absorption, distribution and elimination should be considered. If a marked interaction is observed *in vivo* and the mechanism is not clear, further studies *in vitro* and *in vivo* are recommended to clarify the mechanism of the interaction and to enable the prediction of further interactions with the same or related mechanisms.

5.2.1. Absorption

The investigation of absorption interactions serves to identify situations where the solubility, dissolution or absorption of a drug is altered by extrinsic factors. Studies of the effect of increased gastrointestinal pH, complex binding, and modified intestinal active transport should be considered. In some cases, drugs modulating gastric emptying and intestinal motility may be of importance. Which studies are needed for a specific medicinal product depend on the mode of administration, bioavailability of the medicinal product and the physicochemical properties of the investigational drug. Interactions at absorption level should be investigated mainly for orally administered investigational drugs and the text below refers to orally administered formulations. However, interactions should be considered also for inhaled and nasally administered products with potential for oral absorption.

A. <u>Interactions affecting solubility</u>

If the solubility of the drug or the dissolution of the formulation 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*. If indicated by the physicochemical properties of the drug, it may be necessary to investigate the potential for complex binding *in vitro* and an *in vivo* study could be considered.

B. Interactions affecting intestinal active transport

Involvement of transport proteins (transporters) in drug absorption is evaluated to enable predictions of interactions where the absorption of the drug is altered due to inhibition or induction of these proteins. Inhibition or absence of an intestinal uptake transporter can result in decreased systemic drug exposure and/or lower Cmax. Inhibition of an intestinal efflux transporter may result in increased systemic drug exposure and/or increased Cmax either due to a primary increase in absorption and/or, secondarily, due to decreased availability of drug to intestinal drug metabolising enzymes (e.g. CYP3A).

It is recommended that the involvement of transporters in drug absorption is evaluated *in vitro* in Caco-2 cells. To evaluate the importance of active transport for drug absorption, the permeability of the investigational drug should be taken into account. If the *in vitro* transport and permeability data indicate that active intestinal transport may affect the bioavailability of the new drug, attempts should be made to identify the transporter involved *in vitro*. Detailed recommendations on how to study intestinal transporter involvement and to determine the apparent permeability constant *in vitro* is given in appendices II and III.

When a candidate transporter has been identified, and interactions through inhibition are likely to be clinically relevant, an *in vivo* study with a strong inhibitor is recommended if known inhibitors are registered as medicinal products in the EU. If the candidate transporter is subject to genetic polymorphism, *in vivo* studies in subjects of certain genotypes giving rise to markedly altered expression or activity of the transporter may be useful for investigating the involvement of the transporter *in vivo* and the estimation of the potential for pharmacokinetic interactions via inhibition (or induction) of the transporter.

5.2.2. Distribution

Interactions affecting distribution include interactions through modulation of active uptake or efflux transport of the drug, as well as displacement interactions. Distribution interactions due to an alteration in drug transport may not be fully reflected by changes in plasma concentrations alone. Therefore, the inclusion of pharmacodynamic markers to reflect altered distribution to the organs expressing the transporter should be considered whenever possible.

A. Distribution interactions due to altered transport

Little is presently known regarding distribution interactions due to transporter inhibition. Interactions at a transport protein level are expected to give rise to altered distribution of drug to organs where these transporters are expressed. If the investigational drug is a substrate for transport proteins, the potential for clinically relevant distribution interactions should be discussed in light of any available data on the tissue specific expression or *in vivo* importance of the transporter in the particular organ, data on permeability, data on distribution in preclinical species taking potential species differences into account, available clinical safety data in patients with reduced transport caused by genetic polymorphism or interactions, as well as the expected clinical consequences of an altered distribution. If indicated and feasible, *in vivo* studies investigating the effect of transporter inhibition on the pharmacokinetics as well as pharmacodynamics (including PD markers for the potential effect on the transporter expressing organ) are recommended. Distribution imaging techniques could be considered. Both target organs for the clinical effect and potential target organs for safety should be considered. As an example, inhibition of transporter mediated efflux of a hepatotoxic drug from the liver could in

theory give rise to increased hepatocyte drug exposure and therefore increase the frequency of concentration-dependent hepatotoxicity.

If the transporter potentially controlling target tissue exposure is subject to marked genetic polymorphism, investigations of the effect of a genotype giving rise to reduced transporter activity on the target organ safety (or efficacy if relevant) in phase III trials could indicate the consequences of transporter inhibition by a concomitant drug.

B. Displacement interactions

In general, the risk of clinically relevant interactions via displacement from plasma protein binding sites is low. Nevertheless, the possibility of displacement interactions of drugs known to be markedly protein bound should be considered. This may be of particular importance for highly bound drugs (arbitrary fu<1%) having a narrow therapeutic window, a high hepatic extraction ratio (if administered i.v), or a high renal extraction ratio. If indicated, the risk of interaction should be addressed by *in vitro* displacement studies using therapeutically relevant concentrations. If a clinically relevant interaction is suspected, an *in vivo* study could be performed. Unbound concentrations should be determined in such a study.

5.2.3. Metabolism

Investigations of how the metabolism of the investigational drug is affected by other drugs, usually include studies of how the investigational drug is eliminated as well as which enzymes are catalysing the main systemic and pre-systemic elimination pathways. Main enzymes catalysing the main pathways of formation and further elimination of pharmacologically active metabolites also need consideration.

In vitro metabolism studies should generally be performed before starting phase I to identify the main metabolites formed *in vitro.* These studies provide information necessary for the extrapolation of preclinical safety data to man and also allow for early screening of main metabolites found *in vitro* for target pharmacological activity.

I*n vitro* studies should also be performed to identify candidate enzymes responsible for the main metabolic pathways of the parent drug. Guidance on the *in vitro* investigations of which enzymes are involved in the metabolism is given in Appendix IV.

The mass-balance study (See Appendix V), in which unchanged drug and metabolites are followed after administration of radiolabelled drug, provides information on systemic metabolite exposure as well as data used to estimate the main elimination pathways. The results of the mass-balance study should generally be available before starting phase III. These data are then combined with the *in vitro* information on which enzymes are mainly responsible for catalysing different reactions. The *in vivo* involvement of enzymes found *in vitro* to catalyse relevant metabolism pathways, should be confirmed and quantified. This may be done through an interaction study with a potent selective inhibitor of the enzyme (See Appendix VI) or by pharmacogenetic studies if the enzyme is subject to genetic polymorphism. If the *in vivo* results do not support major involvement of the candidate enzyme, additional *in vitro* and *in vivo* studies are needed to identify the enzyme involved.

In general, enzymes involved in metabolic pathways estimated to contribute to ≥ 25% of drug elimination should be identified if possible and the *in vivo* contribution quantified. This applies to cytochrome P450 (CYP) enzymes and non-CYP enzymes.

Similarly, if there are pharmacologically active metabolites estimated based on unbound systemic exposure whose *in vitro* activity contributes to ≥ 50% of the *in vivo* target pharmacological effect, enzymes contributing to main formation and elimination pathways of these metabolites should be

identified. If the protein binding of parent and metabolite(s) is high, it is recommended to determine the protein binding in the same study so as not to introduce inter-study variability. The fraction of the formation and elimination that needs to be characterised for enzyme involvement depends on how much the metabolite contributes to the *in vivo* target effect. As a general guidance, 50% of the elimination of a metabolite estimated to contribute 50% of the target effect may be used. If the investigational drug is a pro-drug acting through one pharmacologically active metabolite, enzymes estimated to contribute to 25% of the formation and elimination of the active metabolite should if possible be identified. If there are metabolites in man which are suspected to cause adverse effects based on available nonclinical, or clinical information, major enzymes involved in formation and elimination pathways of these metabolites should also if possible be identified. It is also recommended that such metabolites are measured in the *in vivo* interaction studies.

Identification of enzymes involved in minor pathways may be needed if these pathways have a marked importance in some subpopulations due to intrinsic or extrinsic factors (see section 5.2.5). In addition, there may be situations where several drugs have *in vivo* co-inhibitory potential of two specific proteins involved in the elimination of the investigational drug, such as CYP3A inhibitors that also inhibit Pgp mediated renal or biliary excretion. In these cases an interaction study with a drug that is a strong inhibitor of both pathways may be needed. An evaluation of the effect of strong inducers of the involved proteins on the pharmacokinetics of the investigational drug may also be required (see below).

A) Interaction studies with inhibitors of cytochrome P450 enzymes

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 enzyme inhibitor (see Appendices IV and V) is recommended to verify and quantify the involvement of a specific enzyme in the investigational drug elimination. If possible the inhibitor should be specific, not affecting any other enzyme or transporter involved in the elimination of the drug. For more information on design issues see section 5.4. If there are metabolites known to contribute to the efficacy and/or safety of the investigational drug, the effect on the exposure of these metabolites should be investigated in the *in vivo* study. However, it is also recommended to measure the concentrations of metabolites which may influence efficacy or safety if their exposure is increased.

If the interaction study with the strong inhibitor results in a marked effect on the exposure of the investigational drug, potentially leading to dose adjustments, contraindications or other specific treatment recommendations, an additional study with a moderate inhibitor of the enzyme is recommended in order to support the evaluation of the need for specific treatment recommendations for other inhibitors of the enzyme. Alternatively, if a high precision of the interaction effect estimate is not needed, PBPK simulations of the effect of moderate inhibition could be used to support a general labelling statement valid for moderate inhibitors. In this case, the results of the interaction study with a strong inhibitor should be quantitatively well predicted by the model used. Additionally, results of an *in vivo* drug interaction study with a moderate inhibitor and a probe drug for the specific enzyme should be well predicted by the model (see also section 5.5.)

If the candidate enzyme is a cytochrome P450 enzyme which is relatively little studied and generally not included in the enzyme inhibition screening of drugs, there may be little information on strong and moderate inhibitors of that particular enzyme. In this case, *in vitro* studies should be considered to investigate the inhibitory effect of commonly co-administered drugs on that particular enzyme. The need for such studies is dependent on the safety at supra-therapeutic drug exposures as well as the contribution of the catalysed pathway to drug elimination.

B) Interaction studies with inhibitors of non-cytochrome P450 enzymes

If the investigational drug is metabolised by non-cytochrome P450 enzymes, it is recommended to, if possible, verify the contribution of the candidate enzyme *in vivo* through either *in vivo* drug interaction studies with potent inhibitors or studies in subjects of a "poor metaboliser" genotype. The potential for drug interactions should be discussed in light of published literature. If possible, potentially clinically relevant interactions should be investigated in accordance with the recommendations for drugs metabolised by cytochrome P450 enzymes.

C) Interaction studies with inducers

The effect of enzyme inducers on the pharmacokinetics of the investigational drug also needs consideration. If the drug is eliminated through metabolism mainly catalysed by one or more inducible enzymes, or if elimination is catalysed by CYP3A only to a limited extent, an interaction study with a potent inducer is recommended. This also applies to situations where it may not be excluded that enzyme induction will affect drug exposure to a clinically relevant extent, such as drugs mainly eliminated through secretion by inducible transport proteins. However, an interaction study is not necessary if the effects are highly predictable, such as the effect of rifampicin on a drug mainly eliminated through CYP3A catalysed metabolism, and likely to result in a contraindication. The need to investigate the effect on exposures of pharmacologically active metabolites (target or off-target pharmacological effects) should be considered. If there are metabolites known to contribute to the efficacy and/or safety of the investigational drug, the effect on the exposure of these metabolites should be investigated in the *in vivo* study. However, it is also recommended to measure the concentrations of metabolites which may influence efficacy or safety if their concentrations are markedly increased.

If concomitant treatment with a specific enzyme inducer is likely to be common and clinically needed, an *in vivo* study investigating the interaction with that particular inducer is recommended in order to establish adequate treatment recommendations. The time dependency of the induction needs to be considered in the study design (see section 5.4). If a dose adjustment is needed based on the study results, the need for a study using the proposed regimen should be considered. This is mainly applicable when there is a two-way (mutual) interaction, or when the dose of the inducer is adjusted.

In studies of the effects of potent inducers on an investigational drug, rifampicin is often chosen due to its potency. As rifampicin also inhibits the hepatic uptake transporter OATP1B1, the day(s) of blood sampling for the investigational drug should be carefully chosen if the investigational drug is transported by the inhibited protein. Different sampling days should be considered depending on the aim of the study. If the study aims at forming a basis for rifampicin co-therapy *per se* based on the net effect of transporter inhibition and the general induction, sampling for the concentrations of the investigational drug should be performed during rifampicin treatment. However, if the aim is to illustrate the effect of potent enzyme inducers, and thus to extrapolate the effect to other inducers, sampling for the investigational drug is optimally performed one day after the last rifampicin dose.

5.2.4. Active uptake and secretion in drug elimination

Information on transporters involved in major elimination processes should be gained as early as possible during drug development. The need for data at different phases is driven by the predicted magnitude of the exposure increase if the transporter is inhibited and the clinical consequences of such an increase. *In vitro* data may be sufficient before phase III provided use of potentially significantly interacting drugs may be restricted in the study protocol.

Inhibition of OATPs has been reported to result in marked increases in the systemic exposure of drugs subject to hepatic uptake transport by members of this subfamily, and involvement of these transporters may be present without any indications from the *in vivo* pharmacokinetic information.

Therefore, the possible involvement of OATP1B1 and 1B3 uptake transport should be investigated *in vitro* for drugs estimated to have ≥ 25% hepatic elimination (clearance by hepatic metabolism and biliary secretion together contributing to ≥ 25%). As scientific knowledge evolves, other hepatic uptake transporters may need screening if their inhibition generally has been observed to lead to large effects on drug elimination.

Investigations of transporters involved in drug elimination are indicated if available *in vivo* data shows that active renal, biliary or gut wall secretion of unchanged drug is involved in a main part of the drug elimination and thus modulation of the transporter involved may be of clinical relevance. In line with the requirements for enzyme identification, if renal secretion or biliary/gut wall secretion separately is estimated to account for more than 25% of drug elimination, attempts should be made to identify the transporter(s) involved in the active secretion. The importance of renal secretion is estimated by comparing total renal clearance to the renal filtration clearance (GFR*fu). Depending on the information at hand, it may be difficult to estimate the quantitative importance of biliary and gut wall secretion to total elimination. The importance of biliary/gut wall secretion should be based on the mass balance data supported by available interaction data, potential pharmacogenetic information, data in patients with hepatic impairment, data on Caco-2 cell permeability (if absolute bioavailability is unknown), etc. (See Appendix V). An i.v. mass balance study can provide important information in quantifying the importance of biliary/gut wall secretion of orally administered drugs. Data on absolute bioavailability may also inform the estimation of the extent of elimination through these elimination routes. Thus, if a large fraction of an oral dose is recovered as unchanged drug in faeces, an i.v. mass-balance study or an absolute bioavailability study is of great value and should be considered.

In line with requirements for enzyme involvement, if active secretion is the major elimination pathway of a metabolite with significant target activity (estimated contribution to *in vivo* pharmacological effect ≥50% of total effect) attempts should be made to identify the transporter(s) involved. The need to investigate transporter involvement in renal or biliary/gut wall excretion of metabolite should also be considered when available preclinical and clinical information indicate that the metabolite has a major contribution to off-target (adverse) effects.

In vitro studies usually compose the first steps of the identification process. The *in vitro* studies are further described in Appendix III. The transporters selected for *in vitro* investigation should be based on available expression data of the transporter in the eliminating organ and, if possible, whether uptake or efflux transporters or a combination are expected to be involved and be rate limiting for the elimination process.

When a candidate transporter has been identified, an *in vivo* study with a strong inhibitor of the transporter at the site of interest is recommended, if interactions through inhibition are likely to be clinically relevant and if known inhibitors are marketed within the EU. *In vivo* studies in subjects of certain genotypes giving rise to markedly reduced expression or activity of a certain transporter may be useful to verify and quantify involvement of a certain transporter and may give an indication of the pharmacokinetic consequences of transporter inhibition. However, quantitative extrapolation of such data to drug interactions with inhibitors should be justified based on the published literature. As transporter inhibition may alter drug distribution in parallel, inclusion of PD markers is encouraged in the *in vivo* studies if relevant and possible.

Interactions with *in vivo* inhibitors should be predicted based on the acquired *in vivo* information and the scientific literature. If there are commonly used drug combinations where an interaction is expected, it is recommended to investigate the interaction *in vivo.* If there are inducers of the transporter marketed within the EU, an interaction study with such an inducer is recommended. The possible effect of transporter inhibition and induction on availability of the investigational drug for

metabolism (transporter-enzyme interplay), such as the interplay observed between Pgp and CYP3A, should be discussed, and if needed, an *in vivo* study should be considered.

5.2.5. Special populations

An interaction effect may not be directly extrapolated to specific subpopulations that have a markedly different contribution of the affected enzyme and/or transporter to the clearance of the investigational drug. Such subpopulations may include carriers of certain alleles coding for an enzyme or transporter subject to genetic polymorphisms, patients with impaired renal function and young paediatric patients (< 2 years). Patients treated with inhibitors of a major elimination pathway should also be considered.

The effect of a strong enzyme inhibitor on the exposure of an investigational drug is dependent on the quantitative contribution of parallel elimination pathways. If the parallel pathway is renal excretion, the interaction effect will be different in patients with reduced renal function. If the parallel pathway is metabolism or biliary excretion, the effect of an interaction will be different in patients with reduced or abolished activity of the enzyme or transporter involved in the pathway. In case the parallel pathway is subject to genetic polymorphism, the interaction effect will be different in genetic subpopulations with altered enzyme activity as compared to “wild type”.

Moreover, it should be considered that genetic subgroups may have a completely different set of drug interactions. If a major enzyme (or transporter) for active substance elimination or formation is absent or has very low activity in a subpopulation due to genetic polymorphism, the enzymes (or transporters) involved in the parallel pathways should be identified and their contribution quantified, as these pathways will be the main elimination pathways in the genetic subpopulation.

When indicated, drug interactions relevant to the subpopulation should be investigated. An *in vivo* study exploring the interaction in the subpopulation is recommended. In case a clinical study is not possible, a worst case estimation of the effect on the active substance exposure may be performed.

It may also be acceptable to use PBPK simulations to predict the interaction effect in the subpopulation if the simulation is qualified for this purpose. This includes an adequate prediction of the relative contribution of enzymes to *in vivo* clearance. Thus, the results of potent inhibition (or polymorphism) of the separate enzymes *in vivo* should be well predicted. Also the *in vivo* effect on an adequate probe drug by the inhibitor chosen for the DDI PBPK simulation should be well predicted. PBPK simulations may serve as a basis for treatment recommendations. However, specific dose recommendations may need support by *in vivo* interaction data in the subpopulation.

If the investigational drug will mainly be used in elderly and an interaction study has been performed in young subjects, the interaction effect may be different if the parallel pathway is renal excretion. Dependent on the therapeutic window of the affected drug, the difference in interaction effect may need to be estimated and reflected in the treatment recommendations.

If the medicinal product will have a paediatric indication, the possibility to extrapolate drug-drug interaction results from adults to children should be discussed by the applicant. This is further discussed in EMEA/CHMP/EWP/147013/2004 (Guideline on the role of pharmacokinetics in the development of medicinal products in the paediatric population). An *in vivo* study should be considered if a drug combination suspected or known to interact is common in the paediatric population and there is a need for clear dosing recommendations. If an interaction study is needed, a sparse sampling and population pharmacokinetic approach could be considered. The applicant is invited to find ways of providing satisfactory supportive data, such as drug interaction simulations provided that the simulations successfully quantify the observed interaction in adults and the data on enzyme abundance and other physiological parameters in the paediatric population are reliable.

5.3. Effects of the investigational drug on the pharmacokinetics of other drugs

In vitro data on the effects of the investigational parent drug on the pharmacokinetics of other drugs should preferably be available before starting phase II studies unless all concomitant drug treatments at risk of being clinically relevantly affected can be avoided in these studies. The *in vitro* information should be available before starting phase III. If *in vitro* data indicate that there may be a clinically relevant interaction with a drug that cannot be safely managed by protocol restrictions in the phase II or III studies, it is recommended to perform *in vivo* interaction studies with these drugs prior to phase II or III. Depending on how precise the interaction effect needs to be estimated, PBPK simulations may inform the phase II or III study protocol (see section 5.5). Investigational drugs which exhibit dose-dependent- pharmacokinetics in the therapeutic dose/concentration-range, unrelated to dissolution or protein binding, are likely to inhibit an enzyme or transporter. Likewise, if a drug exhibits time-dependent pharmacokinetics, it is likely to be an inducer or mechanism-based inhibitor. (The time-dependency may also be caused by a metabolite.) The mechanism of the non-linearity should therefore, if possible, be identified. Also, if an interaction is observed *in vivo* and the mechanism is not clear, further studies *in vitro* and *in vivo* are recommended to clarify the mechanism of the interaction and to enable prediction of related interactions.

5.3.1. Absorption

If there are indications that the investigational drug affects gastric emptying or intestinal motility, it may affect the rate and extent of absorption of other drugs. This mainly affects drugs with a narrow therapeutic window, modified release formulations and drugs known to have a physiological absorption window, marked permeability limited absorption or serious Cmax related effects. The interaction potential should be considered and, if indicated, the effect should be studied on relevant drugs (e.g. paracetamol as probe substrate in case of effects on gastric emptying). It should be remembered that this is often a systemic effect that may be caused also by parenterally administered drugs. The absorption of other drugs could also be affected through inhibition of intestinal transport proteins. Advice on investigations of the effect of an investigational drug on active transport of other drugs is given in section 5.3.4. If the investigation drug increases gastric pH, the effect on other drugs sensitive to this should be predicted and the need for *in vivo* studies considered. Other mechanism of interference with drug absorption, such as complex binding should also be considered. *In vivo* investigations on the effect of the absorption of other drugs may be performed in parallel with the phase III study, unless concomitant use of drugs potentially subject to a clinically relevant interaction may not be managed by protocol restrictions in the phase II and III studies.

5.3.2. Distribution

The degree of protein binding of the investigational drug should be determined before phase I. If the investigational drug is extensively protein bound to a specific binding site and present at concentrations saturating the binding sites, the risk of displacement of other drugs known to be subject to clinically relevant displacement interactions could be evaluated *in vitro* at a time point relevant for the clinical development program. If a clinically relevant interaction is predicted based on *in vitro* data, an *in vivo* study measuring unbound concentrations could be considered.

5.3.3. Metabolism

The potential of an investigational drug to inhibit or induce the metabolism of other drugs should be investigated. Usually the investigation is initiated by *in vitro* studies and those studies are followed by

in vivo studies if the *in vitro* data show that an effect *in vivo* cannot be excluded. However, it is also possible to study the effects of the investigational drug directly *in vivo*, e.g. by the use of cocktail studies, which, if well designed may investigate both inhibitory (competitive and time-dependent) and inducing effects of both parent and metabolites (See section 5.4.2).

It is recognised that obtaining high concentrations in the *in vitro* studies may not be possible in some circumstances due to poor substance solubility or cell toxicity. In these cases, the data is assessed on a case by case basis. If the *in vitro* studies are considered inconclusive, it is recommended that the potential interaction is investigated *in vivo*.

5.3.3.1 Enzyme inhibition

A. *In vitro* enzyme inhibition studies

In vitro studies should be performed to investigate whether the investigational drug inhibits the cytochrome P450 enzymes most commonly involved in drug metabolism. These presently include CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A. In the future, more clinically important drug metabolising enzymes may be known and included in this list. In addition, it is recommended to study inhibition of UGTs 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. Likewise, if the investigational drug is mainly metabolised by an enzyme not listed above, it is recommended to study the inhibitory effect on that specific enzyme if feasible. It is also recommended to investigate the enzyme inhibitory effect of major metabolites. More information on this is found in the end of this subsection.

An *in vitro* inhibition study could be performed using human liver microsomes, hepatocytes, or other cells expressing the investigated enzyme. The enzyme activity is monitored by investigating the metabolism of a specific marker substrate (Table 4, Appendix VII) under linear substrate metabolism conditions. CYP3A4 inhibition should be investigated using both midazolam and testosterone as subsrates. The effect of a range of investigational drug concentrations are investigated and Ki (the inhibition constant i.e. dissociation constant of the inhibitor from the enzyme-inhibitor complex) is determined. The study should include an investigation of whether pre-incubation with the investigational drug alters the inhibitory potential of the drug. If the pre-incubation affects the potency, more detailed investigations are needed (see below). If the investigational drug is metabolised by the enzymes present in the incubation, the marker substrate should, if possible, have a markedly faster metabolism rate than the investigational drug to minimize the influence of investigational drug metabolism (decreasing concentrations) on the Ki estimation. If this is not possible, the concentrations of the investigational drug need to be monitored and/or the degradation taken into account in the calculations. Known strong inhibitors should be included as positive controls in the study, their Ki determined and compared to literature/reference in house values. The concentration range of the investigational drug should be sufficiently high for detecting clinically relevant inhibition and depends on the potential site of enzyme inhibition, mode of administration and formulation as well as systemic exposure. It is recommended to use the estimated or determined unbound drug concentration in the *in vitro* system. In situations where it is important to have a precise value on fu_{mic} (unbound microsomal fraction), such as estimations of inhibition or induction potential not followed by an *in vivo* study, determining the fraction (experimentally) is recommended. This also applies if there are reasons to believe that the free inhibitor concentration is markedly lower than the total concentration in the incubation, i.e. if the substance binds covalently to proteins or may adsorb to the walls of the test tube.

As the actual concentration of drug near the enzyme is unknown, there is an intra-study/systems variability in Ki, and concentrations at the portal vein during absorption generally are higher than systemic concentrations after oral administration, a safety factor is sometimes added in the estimations. Recommendations regarding concentration ranges are given for different situations below.

If the incubations performed indicate that Ki will be markedly higher than the concentrations given below, Ki does not need to be determined. However, a supportive discussion should be presented by the applicant. If Ki has been determined in several *in vitro* systems, or with several substrates for the same enzyme (e.g. CYP3A substrates), the lowest Ki adequately measured should be used in the estimations of *in vivo* relevance.

If the inhibition is enhanced by pre-incubations, time-dependent inhibition (TDI) is present. The increased inhibition over time may either be due to formation of an inhibitory metabolite or due to mechanism-based inactivation (MBI). For mechanism based inactivators, k_{inact} (maximum inactivation rate constant) and K_I (the inhibitor concentration producing half the maximal rate of inactivation) should be determined. The *in vitro* study needs to be carefully performed and factors affecting the results should be taken into account. See the scientific literature for relevant protocols. Please note the need to determine non-specific binding at the initial step due to the general use of high protein concentrations. If it is shown that the TDI is due to formation of a metabolite which 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).

Intestinal exposure

If the drug is orally administered and the enzyme studied has pronounced intestinal expression (e.g. CYP3A4) the concentration range should be sufficient for determining a Ki ≤ 0.1-fold the maximum expected dose taken at one occasion /250 ml. If it is adequately justified that sufficiently high concentrations may not be reached in the intestine due to solubility limitations in intestinal fluid, lower concentrations can be sufficient.

Hepatic (and renal) exposure

If the enzyme studied is mainly available in the liver, or the kidney/another organ with main drug input from the systemic circulation, the concentration range should allow determination of a Ki which is ≤ 50-fold the mean unbound Cmax obtained during treatment with the highest dose. In this estimation, when an estimation of fu is used, figures lower than 1% should not be used due to the uncertainties in the estimation. Thus, as an example, if the free fraction has been estimated to be 0.5% *in vitro* or *ex vivo*, a 1% free fraction should be used.

Enzyme inhibition by metabolites

The potential inhibitory effects of metabolites on the common drug metabolising enzymes should be considered. As a pragmatic rule, it is recommended to investigate the enzyme inhibitory potential of phase I metabolites with an AUC both larger than one fourth of the AUC of parent drug and larger than 10% of the drug-related exposure (radioactive moieties in the mass-balance study, see Appendix V). If data on protein binding is available, unbound concentrations should be used. However, in absence of these data, total concentrations (bound + unbound) may be used. As for the parent drug, the concentration range studied should include 50-fold the unbound Cmax of the metabolite. As an alternative to the *in vitro* investigation, a well designed cocktail study may be performed. In this case, the study needs to be designed for observing potential mechanism based inhibition at steady state concentrations of the metabolites (see section 5.4.4).

If there are indications that an observed *in vivo* drug interaction is caused by a metabolite, *in vitro* enzyme inhibition studies on selected metabolites may provide useful information for the design of future *in vivo* studies and interpretation of *in vivo* interaction study results.

Evaluation of the need for an *in vivo* study

Based on the results of the *in vitro* studies and the *in vivo* pharmacokinetics of the investigational drug, the risk of inhibition *in vivo* is evaluated. If it cannot be excluded that enzyme inhibition takes

place *in vivo*, studies *in vivo* are recommended. The assessment may be based either on a "basic model" (A:1 below) or on the "mechanistic static model" (A:2 below) (Fahmi *et al*, 2009[1]) i.e. evaluating the risk of inhibition by a worst case constant exposure of the investigational drug. Additionally, well performed and qualified dynamic, PBPK, simulations may also be used as described below (A:3).

The basic model may be used as a first screening method for this purpose. If the basic model gives a positive inhibition signal, the evaluation may optionally be followed by an evaluation using the mechanistic static or dynamic models below. The mechanistic static model may also be used for estimating the net effect of reversible inhibition and MBI.

A:1 Basic Model

I) Reversible inhibition

If reversible inhibition (inhibition not affected by pre-incubation) is observed *in vitro*, the risk of inhibition *in vivo* is evaluated by comparing observed Ki values with a worst case estimation of the concentration near the enzyme during clinical use. In these calculations, the lowest figure on free fraction recommended is 1% due to the uncertainties in the estimation.

Inhibition by parent drug

Enzyme inhibition *in vivo* by a parent drug cannot be excluded, and an *in vivo* interaction study with a sensitive probe substrate is recommended, if the conditions below are fulfilled.

For orally administered drugs if the enzyme has marked abundance in the enterocyte (e.g. CYP3A):

[I] / Ki ≥ 10 where [I] is the maximum dose taken at one occasion/250 ml.

For drugs regardless of mode of administration and inhibition of enzymes in the liver, or in organs, exposed to the drug through the systemic circulation

[I] / Ki ≥ 0.02 where [I] is the unbound mean Cmax obtained during treatment with the highest recommended dose

Inhibition by metabolites

Enzyme inhibition *in vivo* by a metabolite cannot be excluded, and an *in vivo* interaction study with a sensitive probe substrate is recommended, if the conditions below are fulfilled.

For metabolites regardless of mode of administration and site of the enzyme

[I] / Ki ≥ 0.02 where [I] is the unbound mean Cmax obtained during treatment with the highest dose

As for parent drug, a lower fu than 1% should not be used in the calculations due to the uncertainty in the determination. If the protein binding has not been determined, the total (bound plus unbound) concentration is used.

Due to the difficulties in predicting the concentration of inhibitory metabolite at the site(s) of the enzyme, PBPK modelling and simulations are encouraged to support the evaluation. This may also suitable when predicting the net inhibition of parent and metabolite on the same enzyme.

[1] Fahmi *et al* Drug Metabolism and Disposition. 37:1658–1666, 2009

II) Mechanism based inactivation

If MBI has been observed *in vitro*, the ratio of predicted CL in the absence and presence of the inhibitor i.e. the AUC ratio with inhibitor to without inhibitor, may be calculated as

Eq. 1

$$R = (k_{obs} + k_{deg})/k_{deg} \text{ where } K_{obs} = k_{inact} \times [I]/(K_I + [I])$$

where k_{deg} is the degradation constant of the enzyme, k_{inact} is the maximum inactivation rate constant and [I] is the concentration of the inhibitor (See "Intestinal exposure" and "Hepatic (and renal) exposure" above). The degradation constant may be taken from the scientific literature. If possible, the constant should be based on *in vivo* data. Please note that enzymes which are present both in the intestine and liver, such as CYP3A, have separate degradation constants in the two tissues. If ≥ 20% inhibition is obtained, i.e.. R ≥1.25, using the drug concentrations presented above, *in vivo* inhibition may not be excluded and a multiple dose *in vivo* interaction study is recommended (see Sections 5.4.3 and 5.4.4).

A:2 Mechanistic static model

A mechanistic static model has been proposed by Fahmi et al[2] Eq. 2). This model includes the effect of reversible and time dependent enzyme inhibition, as well as enzyme induction. Thus, the mechanistic static model may be used as an aid to estimate the net effect of several interaction processes. However, due to the limited experience with this model, it is presently not recommended to use Eq 2 to estimate the net effect of simultaneous inhibition and induction.

The model may be used as an alternative or subsequent approach to the basic model. Regardless of the result of the basic model, if the mechanistic static model does not indicate in vivo inhibition, an *in vivo* study is not required. However, as this model does not include a safety factor to take into account inter-study variability in the Ki estimation or the potential for higher concentrations in the hepatocytes than in plasma, Ki needs to be verified in a second *in vitro* system and the lowest Ki observed for the specific enzyme used in the estimations. Furthermore, the potential for higher hepatocyte concentrations should be discussed. If available data indicate that the drug may accumulate in hepatocytes, this should be taken into account in the AUCR estimations.

If the model estimates a positive result, i.e. an AUCR outside 0.8-1.25, *in vivo* studies are indicated to quantify the effect *in vivo* as well as, if needed, characterise the time course of the net effect.

Eq.2

$$\mathbf{AUCR} = \left(\frac{1}{[\mathbf{A_h} \times \mathbf{B_h} \times \mathbf{C_h}] \times \mathbf{f_m} + (1 - \mathbf{f_m})} \right) \times \left(\frac{1}{[\mathbf{A_g} \times \mathbf{B_g} \times \mathbf{C_g}] \times (1 - \mathbf{F_g}) + \mathbf{F_g}} \right)$$

A, B and C in the equation denotes MBI, induction and reversible inhibition, respectively and are described in table 1 below. F_g is the fraction available after intestinal metabolism, and f_m is the fraction of systemic clearance of the substrate mediated by the CYP enzyme that is subject to inhibition/induction. If the maximum effect on hepatic metabolism catalysed by a certain enzyme is to be estimated, f_m may be set to 1. If estimating the effect of a certain medicinal product, such as orally administered midazolam, substance dependent parameters should be supported by scientific literature. The induction part of the equation (B_h and B_g) may only be used after qualifying the hepatocyte batch used for this purpose and is further discussed below in 5.3.3.2. In these calculations, the lowest figure

[2] Fahmi *et al* Drug Metabolism and Disposition. 37:1658–1666; 2009

on free fraction recommended is 1% due to the uncertainties in the estimation. The input parameters should all be discussed and if needed justified based on available data and/or scientific literature. A conservative choice of parameters is recommended.

Table 1

Time-dependent inhibition	$A_h = \frac{k_{deg,h}}{k_{deg,h} + \frac{[I]_h \times k_{inact}}{[I]_h + K_I}}$	$A_g = \frac{k_{deg,g}}{k_{deg,g} + \frac{[I]_g \times k_{inact}}{[I]_g + K_I}}$
Induction	$B_h = 1 + \frac{d \bullet E_{max} \bullet [I]_h}{[I]_h + EC_{50}}$	$B_g = 1 + \frac{d \bullet E_{max} \bullet [I]_g}{[I]_g + EC_{50}}$
Reversible inhibition	$C_h = \frac{1}{1 + \frac{[I]_h}{K_i}}$	$C_g = \frac{1}{1 + \frac{[I]_g}{K_i}}$

Subscripts “h” and “g” denote liver and gut, respectively.

[I]h is the maximal unbound inhibitor/inducer concentration in portal vein ([I]u,inlet,max), decribed as $fu,b \times ([I]max,b + F_a x F_g \times ka \times Dose/Q_H)$ (Ito et al.[3]) where F_a is the fraction of the dose absorbed, F_g is the fraction of absorbed dose escaping gutwall extraction, ka is the absorption rate constant and Q_H is the total hepatic blood flow (97 L/hr, Yang et al [4]), fu,b is the unbound fraction in blood and [I]max,b is the maximal total (free and bound) inhibitor concentration in the blood at steady state. The ka should preferably be determined but may otherwise be set, as a worst-case estimate, to 0.1/min. The applicant should show in detail and justify how ka was estimated. If there is any uncertainty in the ka estimate, a sensitivity analysis should be performed.

$[I]g = F_a \times k_a \times Dose/Q_{en}$ (Rostami-Hodjegan and Tucker)[5] where Q_{en}, is the enterocyte bloodflow is 18L/h (Yang *et al*.[6]).

d is a scaling factor determined with linear regression of the control data set (see 5.3.3.2. A:2 below)

A:3 Dynamic, PBPK, model

PBPK simulations may also be used to evaluate the *in vivo* relevance of competitive or time-dependent inhibition observed *in vitro*. In such a case, the scientific basis of the simulations (models as well as all input parameters) should be presented and justified. Furthermore, extensive data on model verification needs to be shown to support the ability to quantitatively predict drug-drug interaction via inhibition of the specific enzyme. Simulations of available PK studies of the investigational drug should be compared to the observed PK profiles. When possible, model verification should include the simulations of the effects of known interacting drugs on the PK of a probe substrate.

The *in vitro* data used needs to be of high quality, and any uncertainty in the model, for example in the parameter(s) determined *in vitro* or parameters estimated, needs to be identified and subject to a

[3] Ito *et al* AAPS PharmSci 4 (3) article 20; 2002

[4] Yang *et al*. Drug Metabolism and Disposition 35:501-2; 2007

[5] Rostami-Hodjegan A and Tucker GT, *Drug Discov. Today Technol.* 1:441–448; 2004

[6] Yang J et *al.* Curr Drug Metab. 8(7):676-684; 2007

sensitivity analysis. Ki should, if possible, be verified in a second *in vitro* system. If protein binding is high (>99%), a sensitivity analysis should be performed decreasing the binding down to 99%. The consequences of higher concentrations of the inhibitory substance at the site of the enzyme (e.g. liver) as compared to those in plasma, should be taken into account in the simulation. In both cases, because the drug PBPK model needs to maintain its ability to predict the observed plasma concentration-time curve of the interacting drug, a major part of the uncertainty could if suitable be added, i.e. sensitivity analyses performed, on the interaction parameters (e.g. Ki). The parameter range used in the sensitivity analysis should be justified, and a conservative approach is recommended. Unless well justified, negligible protein binding should be assumed in the enterocyte when estimating intestinal enzyme inhibition. Further information is given in section 5.5. If the simulation predicts an inhibition of > 20% of the clearance of the probe substrate, a significant interaction in vivo cannot be excluded and it is recommended to perform an in vivo study. If the results of the simulation with appropriate sensitivity analyses are negative and the modelling is acceptable, no *in vivo* study of the effect of the specific enzyme is required, regardless of the results of the basic model estimations or the static mechanistic model.

B. Enzyme inhibition – *in vivo* studies

When investigating whether an investigational drug reversibly inhibits an enzyme, the pharmacokinetics of a probe drug (see Appendix VII) should be investigated after administration of a single-dose of the probe drug alone and at steady state concentrations of the investigational drug obtained with the highest usual recommended dose.

If a well performed *in vivo* interaction study with a sensitive probe drug does not show enzyme inhibition, these results can be extrapolated to all enzymes observed to be reversibly inhibited *in vitro* for which an equal or higher Ki has been observed. However, due to inter-study variability, the Ki:s used in this approach should have been determined in the same study. For orally administered investigational drugs, lack of inhibition of hepatic enzymes may not be extrapolated to intestinal enzymes, such as CYP3A, as the inhibitory concentration is the enterocytic, instead of hepatocytic, concentration. If the inhibition is time-dependent, this should be reflected in the study design. More information on *in vivo* study design is given in section 5.4.

5.3.3.2 Enzyme induction and down-regulation

A. Enzyme induction or down-regulation– *in vitro* studies

Studies should be performed to investigate whether the investigational drug induces enzymes and transporters via activation of nuclear receptors, the Ah-receptor or, if relevant, other drug regulation pathways. Usually, this is initially investigated *in vitro* followed by *in vivo* studies if indicated by the *in vitro* results. However, it is also possible to investigate induction directly *in vivo*.

It should be noted that there may still be mechanisms of induction which presently are unknown. Therefore, a potential human teratogen (Definition given in EMEA/CHMP/203927/2005) needs to be studied *in vivo* for effects on contraceptive steroids if the drug is intended for use in fertile women, regardless of the *in vitro* induction study results.

The *in vitro* induction studies may also detect enzyme down-regulation. Below advice is given primarily on the investigation of the potential of an investigational drug to give induction. The experience is presently very limited regarding drug-induced down-regulation and mechanisms behind these effects. If a concentration-dependent down regulation is observed *in vitro*, additional *in vitro* (or *in vivo*) studies of the effect on other drug metabolising enzymes are recommended to investigate which enzymes are affected unless this may be predicted. Thus, the effect of the investigational drug on enzymes observed to be down-regulated *in vitro* and/or the enzymes at risk of down-regulation based on mechanistic knowledge should be studied *in vivo*.

Cultured hepatocytes (fresh or cryopreserved) are the preferred *in vitro* system for induction (and down-regulation) *in vitro* studies. Minimally derived hepatocyte lines (e.g. HepaRG), nuclear receptor binding assays, or reporter gene assays are considered as supportive data only.

Incubations are performed with daily addition of the investigational drug. The duration of the incubation is generally 3 days. Shorter durations should be well justified. The medium, containing the drug, is changed regularly. Knowledge about the actual concentration of drug in the system is important for the *in vitro-in vivo* extrapolation. Unless loss due to *in vitro* drug metabolism, degradation or lysosomal trapping of drug during culture conditions has been shown to be negligible, or if the loss has been quantified in the system prior to the induction assay and compensated for through the amount of drug added /medium change interval, measurements of concentration of parent drug in the medium are encouraged at several time points the last day of the incubation. Unless the incubations are run under serum-free conditions or degree of protein binding in human plasma is low, the degree of protein binding in the medium should be determined and unbound concentration used throughout the *in vitro* evaluation. The possibility of non-specific binding should also be taken into account.

To increase the sensitivity of the assay and for the response not to be affected by enzyme inhibition, it is recommended to measure the extent of enzyme induction at mRNA level. If induction due to protein stabilisation is suspected, induction should be measured also at activity level.

If it is not possible to reach sufficiently high concentrations (see below) e.g. due to cell toxicity and the study is judged inconclusive, the potential for induction should be studied *in vivo*.

Culture quality should be verified and documented by enzyme mRNA measurements and cell morphology. A suitable viability assessment should be performed before and at the end of the incubation period at the highest concentration level to certify that cell toxicity is not influencing the induction response. If toxicity/loss of viability is observed, influence on the study results should be discussed in the study report and *in vivo* studies be considered.

It is recommended to first evaluate the induction potential using the basic model. If the basic method indicates induction via PXR, the evaluation can continue using the mechanistic static model and/or the RIS correlation model provided it is possible to apply sufficiently high concentration of the investigational drug for E_{max} and EC_{50} to be determined. For the latter approaches, only one well performing batch of hepatocytes is needed. If the basic method indicates that the investigational drug induces drug metabolising enzymes to a great extent through CAR, it is presently difficult to use the mechanistic static or RIS correlation method due to the difficulties in performing an adequate method qualification. In the future it may however be possible to use these approaches. At present, if CAR seems to play a major role in the induction, *in vivo* data is needed to support the induction evaluation of separate CAR regulated enzymes.

A:1 Basic method

Incubations with cultured (fresh or cryopreserved) hepatocytes are performed as above. Due to the inter-individual and cell batch variability in induction response, it is recommended to use hepatocytes from at least 3 different evaluable donors for the “basic method” evaluation. If cells from a donor do not respond satisfactorily to the positive controls, if the viability of the cells is <80% at the start of the incubation, or if the viability at the end of the incubation deviates markedly from the other donors, the cells should be replaced by hepatocytes from a new donor. A number of enzymes could be investigated. The enzymes CYP3A4, CYP2B6 and CYP1A2 should always be included as markers of induction mediated via PXR/CAR (CYP3A4, CYP2B6) and the Ah-receptor (CYP1A2).

Strong inducers should be included as positive controls to verify functioning regulation pathways via PXR, CAR and the Ah-receptor (GR for investigational drugs with glucocorticoid activity). Other

receptors/transcription factors and enzymes may be added to this list as science develops. The positive controls used should be as selective as possible and be chosen based on current scientific knowledge. Currently, rifampicin (20µM) is recommended as positive control for PXR, CITCO (≤100 nM) for CAR, omeprazole (50µM) for the Ah-receptor and dexamethasone (50µM) for GR.

The investigational drug concentration range that needs to be investigated depends on enzyme studied and the *in vivo* pharmacokinetics of the drug. The studied exposure range (or range of the worst case unbound average concentration in the media (Cavg)), should cover the worst case concentrations expected in the hepatocytes *in vivo*. At present, 50-fold the mean unbound maximum plasma concentration (Cmax) obtained at steady state during treatment with the maximum therapeutic dose seems sufficient for drugs affecting enzymes in the liver. For intestinal enzymes (CYP3A4) the maximum concentration may be set to 0.1*dose/250ml. At least three different concentrations should be used.

The induction results are evaluated separately for each donor and the donor cells with the most pronounced induction effect on the specific enzyme should then be used as a "worst case" in the subsequent calculations. The levels of mRNA are compared to the control (vehicle) incubations. The *in vitro* study is considered positive for enzyme induction if incubations with the investigational drug at the concentration given above give rise to a more than 100% increase in mRNA and the increase is concentration dependent. To ensure adequate sensitivity of the assay, an observed concentration-dependent increase in mRNA of <100% can be considered as a negative finding only when the increase in mRNA is less than 20% of the response of the positive control (rifampicin 20 µM or, for Ah-receptor activation, omeprazole 50 µM).

A positive or inconclusive *in vitro* result should be confirmed *in vivo* or lack of induction potential needs to be shown in another *in vitro* study.

If a 50% decrease in mRNA is observed which may not be attributable to cell toxicity, this may indicate down-regulation of the enzyme and an *in vivo* study investigating this time-dependent phenomenon is recommended. If the *in vitro* study is inconclusive, additional studies, *in vitro* and/or *in vivo* are recommended.

A:2 RIS correlation method

Using the RIS method, a certain batch of hepatocytes is "qualified" for future induction studies. (Fahmi and Ripp, 2010[7]). A large set of inducers (n≥8) covering the full *in vivo* induction potency range and including at least 2 mild inducers, are used in the qualification process. E_{max} and EC_{50} are determined for all inducers and the correlation of the RIS (relative induction score, see Eq 3 below), vs. *in vivo* change in the AUC of a certain enzyme probe drug (e.g. midazolam) for each inducer is calculated.

Eq.3.

$$RIS = E_{max} * [I] / (EC_{50} + [I])$$

Where EC_{50} is the concentration causing half the maximal effect; E_{max} is the maximum induction effect; and [I] is the unbound maximum plasma concentration in plasma.

If this method is used, both the qualification data set/qualification report and the data on the investigational new drug should be submitted. Based on the qualification data set, the relationship between *in vitro* inducing potency (RIS) and observed *in vivo* effect on midazolam is used to set a predefined cut-off for risk of mild *in vivo* induction. The risk of induction *in vivo* is then assessed using the estimated RIS of the investigational drug.

[7] Fahmi and Ripp, 2010 (Expert Opinion on Drug Metabolism and Toxicology 2010, 6 (11):1399-1416)

The concentrations of investigational drug used for the RIS estimation should be in accordance with the mechanistic-static model estimations and where the enzyme induced is mainly located. Thus, for CYP3A induction estimations, the RIS calculations should be based on the unbound hepatic inlet plus the maximum enterocyte concentrations. As for inhibition, the possibility of a higher concentration in the hepatocytes than in plasma should be discussed and taken into account in the evaluation. Furthermore, if there are indications of pronounced metabolism of the investigational drug in the hepatocytes, likely to affect the E_{max} and EC_{50} estimation to a more marked extent than the positive control inducers used to qualify the hepatocytes, this needs to be reflected in the analysis, e.g. through a sensitivity analysis.

When performing the *in vitro* study investigating the induction potential of the investigational drug, at least 2 of the inducers of the qualification set should be included as positive controls having a predefined response range at the applied concentration to verify that the qualification is valid for the actual *in vitro* experiment.

A:3 Mechanistic static model

The mechanistic static model may be used to assess the *in vivo* inducing potency of a drug based on in vitro induction data (Fahmi and Ripp, 2010[8]). Using this method, the *in vitro* system is qualified in a similar way as the RIS correlation method (see above). The qualification is based on determinations of EC_{50} and E_{max} of a large set of inducers of different potency and how well the mechanistic static model predicts the observed effect of the inducers. A scaling factor is estimated enabling the translation of individual EC_{50} and E_{max} values into validated parameters to be included in the mechanistic static model. The approach proposed in the mechanistic static model (see table 1) includes the use of the observed *in vitro* induction parameters of the control inducers to predict the *in vivo* effects of the inducers on a specific probe drug (e.g. midazolam). The predicted effect is then compared with the observed effect of the probe drug *in vivo* and a scaling factor, d, is estimated (see table 1). The scaling factor is then used in the estimations of the *in vivo* effect by the investigational drug using the mechanistic static model and the observed EC_{50} and E_{max} of the investigational drug. In the study where IC_{50} and E_{max} is determined for the investigational drug, at least two inducers at specified concentrations needs to be included as positive controls. The acceptance range of the controls should be predefined based on the qualification results.

As when using the RIS correlation method (see above), the possibility of a higher concentration in the hepatocytes than in plasma should be considered, as well as pronounced metabolism of the investigational drug.

If aiming to estimate the exposure of a probe drug resulting from both induction and inhibition (reversible or MBI), an *in vivo* study is recommended due to the limited experience with the mechanistic static model for quantifying the interaction effect and the likely need to investigate the time course of the net interaction effect to optimize the treatment recommendations.

B. Enzyme induction or down-regulation - *in vivo* studies

If *in vitro* induction results have indicated that induction or down-regulation *in vivo* may not be excluded, an *in vivo* study should be performed investigating the effect on that specific enzyme. In such a study, the pharmacokinetics of a probe drug (see Appendix VII) is determined after a single dose administration alone and after multiple dose administrations of the highest recommended dose of the investigational drug (see section 5.4). If there are indications that the investigational drug both inhibits and induces drug metabolising enzymes, it is recommended to study the pharmacokinetics of

[8] Fahmi and Ripp, 2010 (Fahmi and Ripp, Expert Opinion on Drug Metabolism and Toxicology 2010, 6 (11):1399-1416)

the probe drug at both early and late time points during the investigational drug treatment period. The effect of reversible inhibition may be more pronounced in the beginning of the treatment and the induction may be most pronounced after ending the treatment. If screening for induction is performed *in vivo* as a replacement of an *in vitro* study, the effect on CYP3A, CYP2B6 and CYP1A2 should be included.

If induction through PXR/CAR activation is indicated *in vitro*, an *in vivo* induction study should be performed investigating the effect on CYP3A. If CYP3A is inhibited by the investigational drug, (an) other inducible enzyme(s) such as CYP2C9 and CYP2C19 should be investigated. If the *in vitro* data indicate that CAR may have a major role in the induction observed, e.g. if CYP2B6 is induced to an unusually marked extent, an *in vivo* study on a CYP2B6 probe drug should be considered.

If clinically relevant induction or down-regulation is observed *in vivo*, it is likely the investigational drug also affects other enzymes, or transporters, regulated through the same regulatory pathways. However, it is difficult to extrapolate the effect quantitatively to the co-regulated proteins, which may be induced/down regulated to a lesser extent. Therefore, if the investigational drug is verified to be an inducer *in vivo* or if down-regulation is observed, the effect on co-regulated enzymes and transporters should preferably be quantified *in vivo*. This may be investigated in a cocktail study using full AUC or, if needed, fractional metabolic clearance measurements. Enzymes and transporters at risk of induction should be discussed based on the available scientific literature.

5.3.4. Transport

5.3.4.1. Inhibition of transport proteins

In vitro inhibition studies are recommended to investigate whether the investigational drug inhibits any of the transporters known to be involved in clinically relevant *in vivo* drug interactions. Presently, these include P-glycoprotein/MDR1 (ABCB1), OATP1B1 (SLCO1B1), OATP1B3 (SLCO1B3), OCT2 (SLC22A2), OAT1 (SLC22A6), OAT3 (SLC22A8) and BCRP (ABCG2). Investigations of the inhibitory effect on OCT1 (SLC22A1), MATE1 (SLC47A1) and MATE2 (SLC47A) could also be considered. Inhibition of the transporter BSEP (ABCB11) should also preferably be investigated. If *in vitro* studies indicate BSEP inhibition adequate biochemical monitoring including serum bile salts is recommended during drug development. *In vitro* data on transporter inhibition should preferably be available before initiating phase III. The knowledge about transporters and their *in vivo* importance is evolving quickly. The choice of transporters investigated should be driven by scientific evidence, and transporters may be added to or removed from the list as science develops. In addition to the listed transporters, there may also be a need to investigate effects on other transporters to clarify the mechanism of an unexpected interaction observed *in vivo*. As science is rapidly evolving in this field, no lists on *in vitro* and *in vivo* substrates are presented as such lists may need frequent updates. The substrates/probe drugs chosen should be based on the current scientific literature.

It is recommended to use an *in vitro* system where the human *in vivo* transport functions of the transporter are preserved. The influence of passive permeability should also be taken into account when choosing an *in vitro* system. The effect of different concentrations of the investigational drug on transport of a substrate for the specific transporter should be investigated and Ki calculated. At present, the optimal way to determine Ki is under discussion. The applicant is recommended to follow the scientific literature. IC_{50} may be used only in situations where Ki is not possible to obtain and if used, linear conditions and lack of time-dependency of the inhibition should be shown. The *in vitro* study should include known inhibitors (strong and less potent) as positive controls. The choice of substrates and inhibitors should be justified.

The study should be performed over the concentration range of the investigational drug expected to be relevant for the site of interaction. The highest concentration studied should be sufficient for determining Ki ≤ the following concentration

- For intestinally expressed transporters like Pgp, the maximum expected concentration in the intestinal lumen on the apical side of the enterocytes (0.1-fold the maximum dose on one occasion/250 ml) or, if low solubility, the maximum possible concentration at the pH range of the GI tract.
- For hepatic uptake transporters, a concentration of 25-fold the unbound hepatic inlet concentration ([I]u,inlet,max) after oral administration.
- For renal uptake and efflux transporters, for hepatic efflux transporters and hepatic uptake transporters after iv administration, a concentration 50-fold unbound Cmax.

In these calculations, the unbound fraction should be set to 1% if determined to be <1%. Non-specific binding should be considered also in these systems.

In vivo inhibition of a transporter at a certain site can be excluded if the observed Ki value is higher than or equal to the concentrations given above (0.1*dose/250 ml, 25*([I]u,inlet,max, or 50* unbound $Cmax_u$, respectively).

Due to the current high inter-laboratory variability in the inhibition parameter estimation for Pgp, the use of two separate systems is recommended for this transporter. This approach could also be considered for other transporters if indicated. If *in vivo* inhibition may not be excluded based on the Ki, an *in vivo* study is recommended. If inhibition may be of relevance at several clinically relevant physiological sites, the study should if possible aim at investigating the extent of inhibition at those sites. For P-glycoprotein, renal inhibition can be determined using renal clearance of digoxin. Inhibition of intestinal Pgp may be assessed in an *in vivo* DDI study with a sufficiently specific Pgp substrate with low oral bioavailability. Dabigatran etexilate or, if no OATP1B1 or 1B3 inhibition is expected, fexofenadine, seems to be more sensitive to intestinal Pgp inhibition than oral digoxin. Therefore, these drugs, and in particular dabigatran etexilate due to the clinical relevance, are presently recommended as probes for intestinal Pgp inhibition. The applicant should follow the scientific literature in this area as better probes may become established in the future.

5.3.4.2. Induction of transport proteins

If an investigational drug has been observed to be an inducer of enzymes via nuclear receptors such as PXR and CAR, it is likely that transporters regulated through these receptors will be induced. If PXR and/or CAR mediated induction is observed *in vivo*, a study investigating the *in vivo* induction of Pgp mediated transport is recommended. The need for *in vivo* studies of the potential inducing effect on other transporters regulated through the same pathways should also be considered. If the investigational drug will often be combined with a drug which has a pharmacokinetics significantly influenced by a PXR or CAR regulated transporter, an interaction study with that drug is recommended to enable specific treatment recommendations for the combination.

5.4. Design of in vivo studies

The design of the *in vivo* interaction study is adapted to the aim of the study, the mechanism(s) of the potential interaction investigated, pharmacokinetic and pharmacodynamic drug characteristics, mode of administration, safety aspects and target patient population etc. However, some general considerations are found below. An *in vivo* interaction study usually is of cross-over or sequential design. Parallel group design is generally not recommended due to the confounding inter-individual variability. Comparisons with historical controls are generally not acceptable. However, if a crossover

or sequential design is impossible to perform, a parallel group design may be acceptable. If it is suspected that compliance with study treatment could be reduced e.g. due to a long treatment duration or due to adverse effects, compliance should be checked regularly through plasma concentration measurements during the study. An open study is satisfactory, but blinding should be considered if pharmacodynamic markers are included in the study. Simulations may provide valuable information for optimising the study design.

5.4.1. Study population

Interaction studies are usually performed in healthy adults although in some cases, e.g. for tolerability/safety reasons, patients are included. Historically, the number of subjects in interaction studies has been small. However, the number of subjects in an *in vivo* interaction study should be determined taking into account intra-subject variability (inter-subject variability in cases of parallel group design) as well as the magnitude of the effect considered relevant to detect. In some situations where it is particularly important to estimate the range of the interaction effect and where potential outliers are important for the treatment recommendations, inclusion of a large number of subjects in a crossover-study should be considered.

In a parallel group study, the subjects should be matched for intrinsic and extrinsic factors likely to affect the pharmacokinetics of the studied drug. In a cross-over study, the demographics of the subjects are not of importance unless there are indications that the interaction effect may be significantly affected by such factors. However, genotyping for genes coding for relevant enzymes and transporters are generally encouraged. If the pharmacokinetics of the drug are significantly affected by genetic polymorphism and it is expected that patients of a certain genotype have a larger interaction effect, it is recommended that the interaction potential is evaluated separately in that subgroup (see section 5.2.5.). 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.

5.4.2. Probe drugs and cocktail studies

In vivo studies performed to investigate whether the investigational drug inhibits or induces a drug metabolising enzyme or transporter *in vivo* should be performed with well validated probe drugs. A probe drug is a drug which is exclusively or almost exclusively eliminated through metabolism catalysed by one specific enzyme or eliminated through excretion by one specific transporter *in vivo*. If a second enzyme or transporter is involved in the elimination of parent drug, its contribution to total clearance should be very small. The drug should have a well characterised elimination and enzyme/ transporter contribution *in vivo* and should have linear pharmacokinetics. Examples of probe drugs for various enzymes are given in Appendix VII. Other drugs than the listed ones may be used if justified. Only probe drugs for the most commonly involved CYPs are given in appendix VII. If inhibition of other enzymes is to be studied, the applicant should base the choice or “probe drug” and parameters investigated on the scientific literature. Marker reactions, i.e. metabolic reactions known to be catalysed by only one enzyme, may sometimes be used (see below).

The probe drug for CYP3A should be subject to both marked intestinal and hepatic 3A catalysed metabolism. The use of orally administered midazolam is recommended. If the drug is likely to be administered with i.v. administered CYP3A substrates and a marked effect is found on orally administered midazolam, an interaction study with i.v midazolam should be considered to investigate the effect on systemic CYP3A catalysed metabolism, as this enables better interactions predictions. If this approach is chosen, appropriate safety precautions should be made. Alternatively, PBPK could be

helpful in estimating the effect on hepatic CYP3A4 based on the results in the oral midazolam DDi study.

It is possible to use so called “cocktail studies” to investigate the effects of an investigational drug on several enzymes and transporters in one *in vivo* study. If well designed, cocktail studies may investigate both inhibitory (competitive and time-dependent) and inducing effects. The study is usually used to investigate the effects indicated *in vitro*. *In vivo* cocktail studies may also be used to replace studies of the *in vitro* inhibition and induction potential of parent drug (and metabolites) on enzymes (and transporters).

The “cocktail” should be composed of specific probe drugs (see also appendix VIII) for each of the enzymes to be studied. The specificity of the probe drugs should have been demonstrated in DDI studies with selective inhibitors of the specific enzyme and/or in pharmacogenetic studies. It should have been demonstrated *in vivo* that the probe drugs combined in the “cocktail” do not interact with each other. The doses used should preferably be the doses used in this validation. Deviations from this should be justified. Full characterisation of the plasma concentration-time curves of the probe drug is recommended, estimating the effect on (oral) clearance or AUC. If satisfactorily performed, the results of the cocktail studies can be extrapolated to other drugs and can be used to support treatment recommendations in the SmPC.

In most cases, metabolite to parent drug ratios does not provide a true quantification of the effect on enzyme activity. Furthermore, the ratios are affected by the clearance of the metabolite. Therefore, use of metabolite to parent drug concentration ratios in plasma or urine is generally not recommended. However, in cases where characterisation of the full concentration-time curve is not feasible, the ratios may be used as a semi-quantitative screening for enzyme inhibition and induction. However, in these cases, the potential effect of the investigational drug on the clearance of the metabolite should be negligible.

If optimal probe drugs are lacking, clearance through a specific pathway may be investigated as a marker for the enzyme catalysing that pathway. It should be possible to determine the fractional metabolic clearance along this pathway. This is calculated as a ratio between the sum of all primary and secondary metabolites formed through the specific pathway and excreted in urine divided by AUC of the parent drug ($\Sigma Ae^{m}_{0-\infty}/AUC^{M}_{0-\infty}$). Another method to quantify the effect on a certain enzyme based on an effect on the AUC of a non-selective probe drug, is to back-calculate the effect on AUC observed to an effect on the pathway of interest using prior *in vivo* data on the relative contribution of the pathway to systemic clearance. If these two approaches are used, it should be verified that parallel pathways are not affected by the investigational drug.

5.4.3. Dose, formulation and time of administration

A. <u>The perpetrator drug</u>

The systemic exposure of the potential perpetrator drug should be the exposure obtained with the highest generally recommended dose under therapeutic (steady state) conditions. If a worst case estimation is aimed at, the dosing frequency of the perpetrator should be the approved/intended dosing frequency that is likely to give rise to the most marked interaction. If the highest expected exposure is not studied, this should be well justified (e.g. safety aspects). In this case, well performed PBPK simulations may be used to predict the likely effect on active substance exposure if all interaction mechanisms are included and the model predicts the results of the studied dose adequately. If a metabolite is responsible for the enzyme inhibition, steady state of the metabolite should have been reached. The duration of the treatment with the perpetrator drug should be long enough to certify that it covers at least 90% of the plasma concentration-time curve (sampling period) of the victim drug

(see also section 5.4.4 for time-dependent interactions). If the perpetrator is a strong inhibitor chosen to estimate the maximum effect on the investigational drug exposure (as well as to quantify enzyme contribution to the overall elimination of the investigational drug), the choice of perpetrator and its dosing regimen should be chosen to obtain maximum inhibition at the site(s) of the enzyme during the full plasma concentration-time course of the investigational drug. If the perpetrator drug is the investigational drug and a dose-range is recommended for the perpetrator drug, studying several dose levels should be carefully considered if a significant effect is found using the highest dose. PKPB simulations may be used for these estimations provided that the interaction effect of the highest dose is well predicted. However, dependent on the therapeutic index of the victim drug, a conventional interaction study may be needed to support a dose adjustment of the victim drug.

In some cases, alternative perpetrator drug regimens, such as a high single-dose, may be used to reach concentrations higher than the maximum steady state concentrations during the plasma concentration time-course of the probe drug. This design is not appropriate for quantifying an inhibitory effect during therapeutic use or any time-dependent phenomena, but it may be used as a first qualitative screening for competitive inhibition.

B. The victim drug

If the victim drug has linear pharmacokinetics, it is sufficient to investigate the pharmacokinetics of the victim drug after a single-dose with and without treatment with the perpetrator drug. Any dose in the linear range can be used. If the victim drug has dose-dependent pharmacokinetics, the dose used should be the therapeutic dose for which the most pronounced interaction is expected. If the dose-dependency is more pronounced at multiple-dose conditions, a steady state comparison of the pharmacokinetics of the victim drug is recommended. If the victim drug has time-dependent pharmacokinetics, this should be reflected in the study design (see section 5.4.4).

If a mutual (2-way) interaction is expected, it is recommended that both drugs are administered until steady state and compared with steady state pharmacokinetics of the separate drugs administered alone or, if both drugs show dose and time independent pharmacokinetics, a single dose of the separate drugs administered alone.

When the perpetrator or victim drugs are administered to obtain a steady state exposure, a loading dose regimen may be used to shorten the time needed to reach steady state if this is possible from a safety point of view. However, the duration of the treatment needs to be considered if a time-dependent mechanism is investigated.

The safety of the subjects in the study should always be considered. A reduced dose of the victim drug(s) may need to be considered for safety reasons.

C. Formulations

The possibility of formulation differences in interaction potential should be considered when extrapolating interaction study results between formulations. This applies particularly to differences in route of administration or substantial differences in *in vivo* rate and extent of absorption between formulations. Simulations may help in evaluating the need for additional studies. If it is likely that the interaction potential (both as victim and as perpetrator drug) is markedly different separate *in vivo* studies may be needed for specific formulations. The worst case scenario, i.e. the formulation likely to give the most marked interaction may be studied initially followed by studies as needed with other formulations.

D. Relative time of administration

The effect of administration time of the victim and perpetrator drug should be carefully considered. 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-points. Recommendations of drug administration in relation to food should be followed. If these recommendations are different for the included drugs, this should be considered in the study design.

If a large part of the interaction occurs during first-pass, the interaction may be minimised through "staggered dosing", i.e. by separating the administrations of the two drugs in time. This approach should be considered if appropriate from a compliance perspective, in particular if the victim drug does not exist in a suitable strength for making a suitable dose adjustment.

5.4.4. Time dependencies

For time-dependent interactions, i.e. induction or "time-dependent" inhibition, the study should aim to investigate the interaction effect at the time-point where the induction or inhibition effect is at or near its maximum.

The maximum effect is expected when a new steady state level of the affected enzyme has been obtained. This is dependent on the rate of enzyme turnover (k_{deg}), and on the time needed to reach steady state for the inducer/inhibitor. For time-dependent inhibitors, the course of inhibition is also dependent on the inactivation rate constant (k_{inact}). The processes leading to a new steady state level of active enzyme take place simultaneously. The required duration of treatment depends on how precisely the interaction effect needs to be determined. If the study aims to investigate whether an investigational drug is an inducer or a time-dependent inhibitor *in vivo*, determining 80% of the induction or inhibition effect is sufficient. If the interaction study will be used for dosing recommendations, a study investigating the true maximum effect is needed. The chosen duration should be justified, e.g. by simulations, and the estimated % of maximum induction/inhibition be presented. At present, a range of enzyme half-life values are reported in the literature. If available, use of reliable *in vivo* estimations is preferred. The choice of k_{deg} value should be justified based on the scientific literature. The chosen treatment duration should be justified, e.g. by simulations, where a sensitivity analysis can be made to account for the variability in the reported k_{deg} /enzyme half-lives. A loading dose regimen to reach steady state of the inducer/inhibitor faster may be used as long as the treatment duration at steady state is sufficient for the target fraction of the new steady state enzyme levels to be reached. If it is also valuable to know the effect at other time points during drug treatment, adding more determinations of the victim drug´s pharmacokinetics is recommended.

If "time-dependent" inhibition has been observed to be caused by a metabolite *reversibly* inhibiting the enzyme, the duration of the treatment with the parent drug should be sufficient for steady state of the metabolite to be reached. If the (apparent) half-life of the metabolite has not been determined, the half-life of radioactivity determined in the mass-balance study (see Appendix IV) may be used as a worst-case estimation.

When designing a study investigating the effect of an inducer on the pharmacokinetics of an investigational drug, the need to measure metabolite exposures should be considered (see 5.4.5).

5.4.5. Active metabolites

If there are active metabolites contributing to the efficacy and safety of the drug, the exposure to these metabolites should be evaluated in the interaction studies. Moreover, if there are pharmacologically active metabolites which do not contribute significantly to *in vivo* effects of an investigational drug during normal conditions, the need for determining the exposure of these metabolites should be considered as a marked increase in exposure resulting from the interaction could be clinically relevant.

Measuring metabolites, regardless of pharmacological activity, to improve understanding of the mechanism of an interaction, should also be considered. This is of particular importance where multiple elimination pathways are affected by a perpetrator.

5.4.6. Pharmacokinetic parameters

The pharmacokinetic parameters determined should be the ones relevant for the use and interpretation of the study results. Usually such parameters include Cmax, Tmax and AUC, CL and the terminal half-life. If C_{trough} has been found to be closely related to clinical efficacy or safety, C_{trough} should also be investigated or simulated based on single-dose data. Measuring renal clearance should be considered if the perpetrator may affect renal secretion of a drug. If the binding of a drug to plasma proteins is concentration dependent within the therapeutic concentration range, or if the concentrations of binding proteins may change significantly during the study (e.g. SHBG, sex hormone binding globulin for contraceptive steroids), it is recommended to determine unbound plasma drug concentrations. Unbound concentrations should also be determined when investigating potential displacement interactions.

Inclusion of a pharmacodynamic marker or a relevant clinical test is generally encouraged, especially when investigating an interaction at transporter level, or in case both a pharmacodynamic and a pharmacokinetic interaction is expected.

5.4.7. Population pharmacokinetic analysis

If conventional interaction studies with rich sampling *cannot be performed,* the potential for interactions may be investigated in a well performed population PK analysis on high quality data from sparse samples (i.e. phase 2/3 data). This is mainly appropriate when the interaction study needs to be performed in patients. The approach could also be used to detect unexpected interactions. The method is mainly used to investigate the effects of other drugs on the investigational drug.

If a population pharmacokinetic analysis is used, the analysis should be performed according to well-established scientific knowledge, the model should be qualified in relation to its purposes (e.g. predictive properties for various sub-populations and analysis of precision using adequate methods) and the analysis needs to be reported appropriately.

Further, the background information needs to be of high quality. To draw inference from a population analysis, the documentation about doses of concomitant drugs needs to be properly recorded, including the dose, timing of doses and also whether the patient has been on the concomitant drug for a sufficient time period at the time of blood sampling. This detailed information may be difficult to collect for many drugs. Based on knowledge of frequently co-administered medicinal products and suspected interaction, a well-founded decision on drugs to study could be taken prior to the study. Further, the quantification of the interaction will be dependent on the doses of the perpetrator drugs used. Thus, the maximum effect of the interaction may be difficult to establish.

The information obtained in the population PK analysis may be used in the product information but needs to be worded properly. For example, it may be stated that a population PK analysis based on phase III data indicated that concomitant treatment with drug X at a dose range y-z mg reduced the systemic exposure by on average w% (range).

A sufficient number of patients should be treated with the investigational drug and the concomitantly given drug. 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. The size of the effect that is of interest to be detected should be guided by the

therapeutic index of the investigational drug (See also section 5.7.2.). Pooling of data for different drugs, e.g. based on inhibitory potency, should in general not be performed unless the inhibitory or inducing potency is very similar. If possible, it may be advantageous to determine plasma concentration(s) of potentially interacting drugs.

Due to the sparse sampling in phase II and III studies, the absorption phase (and accordingly C_{max}) may not be properly described, and therefore the population analysis may not be sensitive to identify and quantify an interaction with large effects on Cmax. Usually, the effects of concomitant drugs on oral clearance (CL/F) are identified. Thus, for drugs where it is known that C_{max} may be related to adverse effects or efficacy, time points for PK sampling should be carefully selected, otherwise the population approach is of limited value.

To draw appropriate conclusions from the population analysis the uncertainty in the estimated interaction effects (eg. 95% confidence intervals) should be estimated by appropriate methods, i.e. preferably using methods not assuming symmetrical distribution of the confidence interval, e.g. bootstrapping or log-likelihood profiling. Such uncertainty analysis is of importance when the aim is to claim no effect of a concomitant drug, as well as when significant effects have been identified. Depending on the width of this confidence interval, the uncertainty of the conclusion (lack of an interaction and/or clinical relevance of an interaction) can be assessed. If an interaction effect is found that needs to result in a dose adjustment of the victim drug, a conventional study is usually needed to support the quantitative adjustment unless the dose may be titrated based on a clinical or PK marker.

5.5. PBPK modelling and simulation

PBPK modelling and simulations may be used at different stages of drug development. Early in drug development, PBPK modelling and simulations serve two main purposes in the interactions assessment. PBPK simulations may support the absence of *in vivo* drug-drug interaction potential, as stated in section 5.3.3.1A. If adequately qualified, it may also support the statements regarding restrictions in concomitant medications in clinical trials while planned *in vivo* drug-drug interaction studies have not been performed. During late stages of drug development, when more *in vivo* data are available, including results from pharmacokinetic studies using a variety of dosing regimens and route of administrations and drug-drug interaction studies with strong enzyme inhibitors intended to identify worst case scenarios, the information obtained can be utilized to update PBPK models established earlier. For example, *in vivo* data of the pharmacokinetic changes of an investigational drug by a strong enzyme inhibitor can be used to evaluate and update the PBPK model. Subsequently, the updated model may be used to simulate the effect of a less potent inhibitor or the effect of the same strong inhibitor under different dosing regimens. Depending on how well the interaction effect needs to be estimated, the simulation results may be used to support labelling. At all stages, PBPK modelling and simulations can be used to inform the design of *in vivo* DDI studies.

When using the PBPK modelling and simulation approach, several important issues need to be taken into account regarding both the choice of structural models and the availability of drug-dependent parameters. The report of the analysis should include detailed description of the structural models, original source and justifications for both system- and drug-dependent parameters, model assumptions and their physiological and biochemical plausibility, sensitivity analyses for relevant parameters, type of error models etc. If possible, the data files used in the simulation (preferably including drug-dependent parameters, the structure model and any simulation options chosen) should be submitted as supplementary material. The PBPK model needs to be qualified for its purpose. In general, the performance of the model needs to be supported by relevant *in vivo* data. The data needed in different situations have been specified in relevant sections in this document.

5.6. Presentation of in vivo study results in the study report

Individual data on pharmacokinetic parameters should be listed with and without co-administration of the interacting drug. Standard descriptive statistics for each treatment group, including arithmetic mean, and standard deviation or geometric mean and CV, as well as range should be provided for the pharmacokinetic parameters. The parameters representing drug exposure (e.g. Cmax and AUC) could be presented as box-whiskers-plots with and without concomitant medication. The plots should include the individual data points either overlaid or next to the boxes. A comparison of the individual pharmacokinetic parameters with and without concomitant medication should also be presented graphically e.g. as spaghetti-plots connecting the data points with and without co-administration within each individual. All subjects or patients who have been included in the study, who have received both study treatments and who have evaluable data-sets of PK and/or PD readouts should be included in the statistical analysis of PK and/or PD parameters. However, if a subject has dropped out from the study or has no measurable plasma concentration during a treatment period and this may be due to the interaction, this should be taken into account in the evaluation. If the sampling is incomplete due to an adverse even related drop out, as much data as possible should be collected to estimate the subject´s drug exposure. Exclusion of subjects for other reasons than the ones given above should be well justified and preferably be specified in the study protocol. When indicated, the interaction effect should be presented with and without the individual proposed for exclusion. The interaction effect should be calculated and the change in relevant pharmacokinetic parameters presented. Individual changes in pharmacokinetic parameters should be listed together with descriptive statistics, including the 90% confidence interval and the 95% prediction interval for the interaction effect.

If the pharmacokinetics of active metabolites has been investigated, the data should be presented in a similar way for the metabolites. If suitable, the active moiety, i.e. the total exposure of active species, estimated as the sum of the unbound exposure of pharmacological equivalents, should be presented in addition to the effects on the separate substances. However, this estimation is only correct if the distributions of parent drug and metabolite to the target site(s) are similar. The validity of this assumption should be discussed based on data on permeability/lipophilicity etc and, if possible, the calculations should be modified by the metabolite to parent target organ distribution ratio. If the protein binding of parent and metabolite(s) is high, it is recommended to determine the protein binding in the same study so as not to introduce inter-study variability.

5.7. Translation into treatment recommendations

The consequences of an observed *in vivo* (or *in vitro*) interaction should be assessed and suitable treatment recommendations or warnings given. The mechanistic information gained from the interaction studies should be used to predict other interactions and suitable recommendations should be made for the predicted interactions.

5.7.1. *In vitro* data

If positive *in vitro* studies have not been followed by *in vivo* studies although *in vitro* data indicate risk of an *in vivo* effect, e.g. in cases where *in vivo* probe drugs are not available or drug interaction studies not practically possible or have not been conducted, or if an interaction of non-studied enzymes and transporters is expected based on mechanistic knowledge (e.g. co-regulated enzymes and transporters affected by induction), the potential implications should be discussed based on available scientific literature, and if possible, translated into treatment recommendations.

5.7.2. *In vivo* effects of other drugs on the investigational drug

The clinical relevance of the effects of the studied drugs on the pharmacokinetics of the investigational drug should be assessed and the results used to predict the effects of other drugs where a similar interaction by the same mechanism can be expected. As described in section 5.2.3, if there are drugs that have a weaker effect on the investigational drug, separate studies should preferably have been performed if the expected interaction is likely to be clinically relevant. If such studies are lacking, the pharmacokinetic consequences of the interaction should, if possible, be predicted and the clinical relevance assessed. The prediction could be based on the difference in inhibition potential between the drugs and the effect of the drug with the most potent effect. Mechanistic models can be used to support these predictions.

Treatment recommendations should ensure that 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 target range, i.e. 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. The observed exposure (box-whiskers plots including individual data), should be analysed with respect to target criteria taking into account the frequency of subjects in the interaction study with lower as well as higher exposure than the target range and the clinical consequences of these deviations. For individually dose-titrated drugs, the data should be analysed with respect to relative individual increase or decrease in exposure. If the inter-individual variability in interaction effect is especially important and the interaction study is deemed too small to estimate this variability satisfactory, PBPK simulations may be performed to estimate the range of interaction effect if the model well predicts the outcome of the *in vivo* study.

If a marked interaction is observed and a dose adjustment proposed, it is recommended that the resulting relevant individual exposure parameters are estimated in support of the proposed dose adjustment and the estimated exposure is evaluated with respect to target criteria as above. Unless the drug has a large therapeutic window, it is recommended that the plasma concentration-time curves obtained with the dose adjustment are simulated.

Presence of active metabolites should be considered when proposing dose adjustments. When relevant, the active moiety can be used to develop dose adjustment (see section 5.6). However, increased exposure must also be considered from a safety perspective and the exposure of all relevant active substances should as far as possible be within a well tolerated range after dose adjustment. If dose adjusting for the effects by an inducing drug, the consequences of the potential increase in exposure of pharmacologically active metabolites formed through the induced pathway(s), should be discussed.

When an alteration in dosing frequency is considered instead of adjusting the daily dose due to the lack of appropriate strength(s) available of the pharmaceutical form, adequate support is needed to show that the pharmacokinetic parameters likely to be relevant for efficacy and safety do not deviate in a clinically relevant manner from the conditions for which satisfactory clinical efficacy and safety has been established.

If proposing a dose-adjustment based on Cmin (either during the evaluation of a general dose adjustment or if proposing a dose-adjustment within the subject based on Cmin), the possibility of an altered relationship between Cmin and AUC should be considered if the systemic elimination of the drug is changed.

If the interaction is time-dependent, the time course needs to be taken into account in the development of dosage recommendations. Different recommendations might be needed at different time points both after initiating and ending treatment with the perpetrator.

If the interaction is expected to have severe consequences and there is no possibility of normalising the exposure through dose adjustment, the drug combination should be avoided. The benefit –risk of the combination should be included in the evaluation e.g. some combinations may be necessary even at increased risk. If the consequences of the interaction are not severe and/or considered manageable through additional safety or efficacy monitoring, this should be clearly recommended in the SmPC. As clear advice as possible on the practical management of the situation should be given.

An additional solution for management of drug interactions is Therapeutic Drug Monitoring (TDM). TDM is particularly useful if there is a large variability in interaction effect by a commonly used concomitant drug/group of drugs that necessitates individual dose adjustment. This approach is mainly applicable if there is a well established therapeutic concentration window. 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. If TDM is recommended, advice on sampling days and times should be given in the SmPC. Additionally, the need for non-TDM guided dose adjustment on the first treatment days should be discussed.

Treatment recommendations should include recommendations for patients who have certain characteristics leading to a different interaction effect and who may have specific important interactions. If pharmacogenetic testing is not performed before the combination treatment is started, the recommendation for all patients should also be suitable for the subpopulation. In addition, combinations of drugs leading to inhibition of multiple pathways should be considered and treatment recommendations included.

5.7.3. *In vivo* effects of the investigational drug on other drugs

The evaluation of the effects of the investigational drug on other drugs includes:

- evaluation of results of studies investigating the effects of the investigational drug on probe drugs
- mechanism-based extrapolation of observed effects to other drugs
- evaluation of the results of studies on specific drug combinations to provide combination-specific treatment recommendations.

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 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.

In vivo enzyme inhibitors and inducers should, if possible, be classified as either mild, moderate or strong inhibitors or inducers (See Appendix VIII). The induction results are qualitatively extrapolated to co-regulated enzymes and transporters if induction of these proteins has not been quantified *in vivo*. Based on the *in vivo* inhibition and induction studies with the probe drugs, other drugs which are substrates for the enzyme/transporter and likely to be affected in a clinically significant manner should be discussed and adequate treatment recommendations presented.

5.7.4. Food effects

If food interaction studies indicate that there should be specific recommendations on how to take the drug in relation to food, clear information about this should be given in the SmPC. Whether such a recommendation is needed and what the recommendation should be depends on intra- and inter-individual variability, potential recommendations regarding concomitant food intake in the pivotal clinical phase III studies, as well as the relationship between concentration and effect of the drug. This is further described in section 5.1. Recommended wordings for recommendations regarding food intake are given in Appendix IX.

6. Herbal medicinal products and specific food products

Information about interactions between medicinal products and herbal medicinal products or specific food products such as grapefruit juice is usually based on the scientific literature and translated into general recommendations regarding use of the food products or herbal products containing a specific component. The interaction potential of one specific herbal medicinal product or food product is difficult to extrapolate to other products produced from the same raw source material. Usually, the interacting substances have not been sufficiently well identified, and therefore analysis of the product contents may not be used to make safe extrapolation of the magnitude of the interaction effect. For new herbal preparations the potential for interactions should be investigated by the applicant. For traditional and well-established herbal preparations the potential for interaction should be clarified if reports point to clinically relevant interactions in humans

Usually there are no data on the pharmacokinetics of the constituents of herbal preparations or herbal substances and consequently the *in vivo* relevance of *in vitro data* cannot be assessed. However, if available *in vivo* information indicates that herbal preparations or the constituents of the herbal preparation may give rise to clinically relevant drug-interactions, *in vitro* studies on the enzyme inhibitory potential of the constituents or the herbal preparations are encouraged as such studies may facilitate the setting of causal relationship and appropriate specifications. In case an *in vivo* interaction study is not performed with a certain herbal preparation, available information on other preparations of the herbal component(s) may be extrapolated to its labelling as a precautionary measure. To obtain product specific information in the labelling of a specific herbal product, an *in vivo* drug interaction study with that product should be considered. Such interaction studies should involve probe drugs if appropriate.

If there is a need to investigate the effect of a herbal medicinal product or a special kind of food (e.g. grapefruit juice) on the pharmacokinetics of a medicinal product in order to substantiate the information about the interaction in the SmPC, effort should be made to choose a specific herbal or food product and mode of intake of the product known to give a marked interaction effect. Inclusion of a probe drug in the study, i.e. a drug shown to interact with the herbal or food component, could be considered to verify the sensitivity of the study. The magnitude of the interaction could be given in the SmPC of the medicinal product but together with information on the difficulty to extrapolate the magnitude to other herbal medicinal products or food products based on the same components.

7. Inclusion of information and recommendations in the SmPC

The guideline on summary of product characteristics (SmPC) (September 2009 – Eudralex vol. 2C) gives advice on how to present information about interactions.

Information about drug interactions should be presented in the SmPC sections 4.5 and 5.2 (e.g. for food-interactions) and cross-referred to the sections 4.2, 4.3 or 4.4 if relevant. Section 4.5 should contain all detailed information on drug interactions and only the recommendation should be given in the cross-referred sections.

In section 4.5 interactions affecting the investigational drug should be given first, followed by interactions resulting in effects on other drugs. In these subsections, the order of presentation should be contraindicated combinations, those where concomitant use is not recommended, followed by others. The text should be as mechanistically clear as possible to enable mechanism-based prediction of interactions with drugs not mentioned in the text.

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. If a drug combination is stated to be “not recommended”, practical recommendations should be presented in case concomitant treatment may not be avoided. The need for time-specific information and recommendations should be considered. Such situations include interactions where the time interval between perpetrator and victim drug administration is of particular importance and a certain time-interval between administrations have been studied, time dependent interactions such as induction or mechanism based inhibition, drugs with long half-lives, etc. The estimated course of onset of the interaction as well as the time-course after ending concomitant treatment should be given as well as, when relevant, time-specific recommendations. If it is likely that the interaction effect would be different with another dose or at another time point than the one studied, this should be reflected in the recommendations.

Information on absence of interactions (supported by *in vivo* data) could be reported briefly if considered of interest to the prescriber.

In special circumstances, where there are very limited therapeutic alternatives due to marked interactions with most drugs of the same class, examples of less interacting drugs could be given as assistance for the prescriber.

When relevant, the interaction potential in specific populations, such as children or patients with impaired renal function, should be addressed.

7.1. Mechanistic information and prediction of non-studied interactions

Brief information about the major enzymes involved in the elimination of the drug, transporters with a major impact on absorption, distribution or elimination of the drug as well as effects of the investigational drug on enzymes and transporters should be summarised in section 4.5 as a mechanistic basis for the interaction information. Similarly, if it has been shown that the investigational drug inhibits or induces enzymes and transporters *in vivo*, this information should be presented along with the potency as inhibitor and/or inducer according to Appendix VIII. The classification should be clarified by giving an estimation of how much the systemic exposure sensitive substrates of the enzyme/transporters could be affected by the investigational product. Based on the mechanistic knowledge, the results of interaction studies should be extrapolated to other drugs. It is recommended to include a list of drugs likely to be affected to a clinically relevant extent in the SmPC to assist the prescriber. The list should be as extensive as possible and it should be indicated that the list probably does not cover all relevant drugs. In some instances such a list may be too long, such as when the investigational drug affects a very important drug metabolising enzyme (e.g. CYP3A4). In this case, drugs should be selected for inclusion based on the severity of the clinical consequences of the interaction. e.g. “Drug X is a strong inhibitor of CYP3A and may therefore markedly increase the systemic exposure of drugs metabolised by this enzyme such as …” The most important drugs from a

clinical relevance perspective should be included in such a list to aid the prescriber. If *in vitro* data indicate that a medicinal product affects an enzyme or a transporter but the available scientific knowledge does not allow predictions of interactions *in vivo*, it is recommended to include the *in vitro* information in the SmPC Section 5.2 for future use.

7.2. Presentation of study results in the SmPC

The results of relevant studies should be presented as mean effect on the most important exposure parameter. In specific cases where considered relevant for the prescriber, the variability of the effect can be given. Results of interaction studies used to predict other drug-interactions on a mechanistic basis e.g. interaction studies with probe drugs as victim drugs, should be included, even if the interaction effect is not clinically relevant for the specific victim drug studied. Brief, condensed information on the study design needed for the interpretation of those particular study results should be included if it is of value to the prescriber. Such information may include dose (in case a dose range is used for the interacting drugs or if the therapeutic dose has not been used in the study), as well as timing and duration of treatment (if a time-dependent interaction has been investigated but full induction has not been obtained). In case the interaction effect may be significantly different with a different dose or when the full time-dependent interaction has been obtained, this should be stated in the SmPC. Information on study design that is not of use to the prescriber should not be included in the text. The conclusions of *in vitro* studies indicating an effect on other drugs should be presented if no *in vivo* information is available. However, otherwise, the conclusions of *in vitro* studies should be reported in section 5.2.

The effect of concomitant food intake on the pharmacokinetics should be presented in section 5.2 and clear recommendations given in section 4.2 (see Appendix IX).

Definitions

Ae amount of parent drug excreted unchanged in urine

AhR aryl hydrocarbon receptor

AUC area under the plasma concentration-time curve

CAR constitutive androstane receptor

BCRP breast cancer resistance protein

BSEP bile salt efflux pump

CAR constitutive androstane receptor

Cavg average concentration

CV coefficient of variation

CITCO: (6-(4-chlorophenyl)imidazo[2,1-beta][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime)

Cmax peak concentration

Cmin trough concentration

CL clearance

ERL elimination rate limited (elimination)

f_a	fraction absorbed
fu	fraction unbound
GFR	glomerular filtration rate
GR	glucocorticoid receptor
k_a	absorption rate constant.
Ki	inhibition constant
K_I	the inhibitor concentration producing half the maximal rate of inactivation
k_{inact}	maximum inactivation rate constant
MATE	multidrug and toxin extrusion proteins
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCT	organic cation transporter
PEPT1	intestinal peptide transporter I
Pgp	P-glycoprotein
PXR	pregnane X receptor
Qent	enterocyte blood flow
SmPC	summary of product characteristics
TDM	therapeutic drug monitoring
Tmax	time when Cmax occurs

Appendix I

Standard procedures for food interaction studies

A single-dose of the investigational drug is administered with 240 ml of water after a 10-hour fasting period and 30 minutes after intake of a meal has been started. Except for the meal, the subjects should refrain from food for at least 4 hours after dosing and all food -intake should be standardised for at least 12 hours post-dose. The recommended composition of a high fat meal and a moderate meal are described below. In case a single dose study may not be performed in healthy subjects or patients, a multiple-dose, steady state study with administration of doses under fasting and fed conditions, respectively may be satisfactory.

The standardised high-fat meal

The high fat meal should contain 800-1000 kcal with 500-600 kcal from fat and 250 kcal from carbohydrates. A typical standard test meal can be two eggs fried in butter, two strips of bacon, two slices of toast with butter, 120 ml of hash brown potatoes and 240 ml of whole milk. Substitutions in this test meal can be made as long as the meal provides similar amounts of calories from protein, carbohydrate, and fat and has comparable meal volume and viscosity.

The moderate meal

The moderate meal could contain approximately 400-500 kcal with fat contributing to ca. 150 kcal.

Appendix II

In vitro investigations of involvement of transporters in drug absorption and determinations of the apparent permeability constant

The *in vitro* investigation of transporter involvement at an intestinal level usually begins with Caco-2 experiments. In these cells, the intestinal permeability may also be qualitatively assessed. Several transporters may be present in the Caco-2 cells. Other *in vitro* systems may be used as well to investigate transporter involvement and may have advantages depending on if specific inhibitors are available. This appendix focuses on Caco-2 cell studies, while other *in vitro* systems are discussed in Appendix III.

The *in vitro* Caco-2 cell study needs to be performed under well-controlled conditions. The permeability of the drug should be investigated in both directions, preferably under sink conditions (the concentration on the receiver side is less than 10% of the concentration on the donor side) unless the absence of sink-conditions is compensated for in the calculations. At least four different physiologically relevant concentrations should be studied. For intestinal transport the studied range could be 0.01 to 1-fold the dose/250 ml unless solubility limits the concentration range.

To verify adequate expression of Pgp in the cells, a positive control, i.e. an adequate Pgp probe substance, should be investigated and its permeability ratio found sufficiently high based on published literature. Presence of adequate expression of other transporters, such as BCRP, PEPT-1, etc could be investigated in a similar manner if there are indications based on physiochemical properties or knowledge on similar substances that these transporters could be involved.

It is recommended that a pH of 7.4 is used on both sides when efflux is investigated. If a proton gradient is needed to investigate the involvement of a certain transporter, the degree of ionisation and likely influence on the study results should be discussed. Determination of mass-balance (% recovery of the applied amount of drug in the receiver and donor side) is recommended unless the absorption predicted from the *in vitro* data is complete. The conditions on both sides of the membrane/cells should be as similar as possible. Thus if plasma protein/albumin is added, it should be added on both sides, so as not to influence the permeation of drug over the membrane. The impact of solubility, organic solvents used, potential degradation and metabolism of the drug substance *in vitro* on study results should be discussed.

In Caco-2 cell studies, the permeation of drug from the apical (A) to the basolateral (B) side of the cells is compared with the permeation in the opposite direction (B to A). If the ratio of the B to A and A to B permeation is > 2 or < 0.5, this suggests involvement of an efflux transporter, or an active uptake transporter, respectively.

If intestinal active transport is concluded, the permeability in the absence of transporters could be taken into account, estimating the importance of the transporter for drug absorption. To estimate the permeability in the absence of transporters, the permeability constant is determined at concentrations high enough to completely saturate the transporters (assessed as an B to A/A to B ratio of 0.5 – 2). If this is not possible, e.g. due to cell toxicity or insufficient solubility, inhibitors of the transporters may be used. If this approach is chosen, it should be established that the cell-layer is unaffected. The investigation should include a well validated, high and low permeable reference substance (e.g. metroprolol and mannitol). If the permeability in the absence of transporters is high (≥ the permeability constant of the highly permeable drug metoprolol), the effect of active drug transport will be negligible as compared to the passive, concentration-gradient driven, absorption of the drug.

If there are indications that the active transport may be of clinical importance, attempts should be made to identify the transporter. In the Caco-2 cell study, this is usually investigated by determining the permeability rate and ratio with and without adding a selective inhibitor to the cells.

The use of Caco-2 cell permeability is presently semi-quantitative as described above. Use of multiple controls in combination with well established permeability ranges for each control may make quantitative prediction possible in the future. It is also possible that other *in vitro* systems may be used. In these cases, the system should have been validated for its use.

Appendix III

In vitro studies identifying transporters involved in drug disposition

The identification of which transporter is involved in an identified uptake or efflux process affecting drug disposition may be done *in vitro* through transport studies intended to isolate the effect of a specific transporter.

In vitro studies investigating drug transport with and without presence of the specific transporter activity are usually the first steps in the identification process. There are several systems available as well as several ways of modulating the transporter activity. These presently include but are not limited to: (over-)expressing the transporter in vector systems and comparing to normal vectors, using selective inhibitors to inhibit one specific transporter, inhibiting transporter expression either by knocking out the gene or through the use of silencing mRNA, etc. The choice of *in vitro* system used should be justified based on scientific literature and on the physiochemical properties, such as membrane permeability of the substance. It is recommended to use an *in vitro* system where the human *in vivo* transport functions of the transporter are preserved. The concentration range of investigational drug should be relevant to the site of transport. Thus, if involvement of the transporter is possible at sites exposed to markedly different concentrations, e.g., such as Pgp potentially involved in the intestine as well as the kidneys/liver/blood-brain barrier, different concentration ranges needs to be studied. The concentration interval studied should cover a ca 100-fold difference in concentration and should have as therapeutically plausible concentration as possible in the middle of the range. The choice of concentration range should be justified. At least 4 different concentration of the investigational drug should be tested. In contrast to the transporter inhibition assays, it is important not to study too high concentrations as this may saturate transporters active at lower concentrations. To validate the assay, the study should include known substrates investigated with and without the transporter activity. The choice of controls and inhibitors should be justified by appropriate scientific references or in house data. The specificity requirements for inhibitors used are dependent on whether other transporters are present in the *in vitro* system.

The *in vitro* studies will investigate whether the investigational drug can be transported by the transport protein. However, in case there is no *in vivo* data to support the potential quantitative importance of the transporter, making a quantitative estimate of the potential influence of the transporter on the pharmacokinetics of the drug is presently difficult. For hepatically eliminated drugs potentially subject to hepatic uptake transport, an attempt to assess the *in vivo* importance of the transporter could be made by determining the uptake into hepatocytes with or without inhibitor or saturation of the transporter. In such an investigation, controls need to certify that adequate amount of the uptake transporter activity is present in the cells. In the future, this may be a possible approach but at present, the applicant is encouraged to follow the literature and to apply a scientific approach.

Appendix IV

In vitro investigations of which enzymes are catalysing the main elimination pathways

The metabolism of an investigational drug, and the formation and metabolism of clinically relevant active metabolites, is usually first investigated through *in vitro* incubations with human liver microsomes (HLMs), hepatocytes, recombinant cells expressing human liver enzymes, liver S9 fractions etc, depending on which enzymes are investigated. CYP and UGT enzymes are present in all systems mentioned (recombinant cells usually express only one single enzyme). Cytosolic enzymes such as sulphotransferases, glutathione transferases, aldehyde dehydrogenase and alcohol dehydrogenase etc are present in S9 fractions and hepatocytes. Hepatocytes may also express transporters to some extent. The *in vitro* system(s) used should be carefully considered when interpreting study results. The *in vitro* metabolism studies should be performed at therapeutically relevant concentrations under linear conditions. In multi-enzyme systems, enzyme specific inhibitors (see table 2) are added to evaluate the contribution of separate enzymes to the metabolism of the investigational drug. In cases where the inhibitor is not sufficiently specific, it is recommended to perform the study in an *in vitro* system where no other CYPs than the particular enzyme is expressed. Specific antibodies may be used instead of inhibitors if the specificity is adequately supported. The metabolism may be investigated as rate of disappearance of drug and/or as formation of metabolites. If possible, it is recommended to follow metabolite formation to enable the identification of the metabolic pathway catalysed by a particular enzyme. It is recommended to include positive controls (marker substrates) for enzyme activity in the studies. Correlation studies, correlating a certain enzyme activity (probe substrate metabolism) with the metabolism of the investigational drug, is of less value than *in vitro* studies with inhibitors due to the often seen correlation between enzyme activities of different enzymes. 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. If no or little metabolism is observed *in vitro* but is present *in vivo*, effort should be made based on structure and published data to find an *in vitro* system with which the enzyme involved may be identified. In table 2 and 3 examples of well validated specific inhibitors and marker reactions/substrates are given. Please check the available literature regarding which concentration to use in the *in vitro* incubations.

The *in vitro* data are combined with *in vivo* data such as results from an *in vivo* mass-balance study (see Appendix V) with investigational drug in order to predict which elimination pathways are the main pathways *in vivo*.

Table 2 Examples of well validated inhibitors of specific enzyme activities *in vitro*

ENZYME	INHIBITOR
CYP1A2	furafylline
*C*YP2B6*	ticlopidine, thiotepa
CYP2C8	montelukast
CYP2C9	sulfaphenazole
CYP2C19*	-(-)-N-3-benzyl-phenobarbital , nootkatone, loratadine
CYP2D6	quinidine
CYP3A	ketoconazole, itraconazole

*presently no specific inhibitor known for *in vitro* use. Listed inhibitor(s) are not specific but can be used together with other information or in a mono-enzyme system.

Appendix V

Identifying and quantifying the main elimination pathways *in vivo* using the mass balance study

The drug is administered with a radioactive label in a metabolically stable position. In some cases two separate labelling positions have to be used to follow the fate of the investigational drug. If there are no dose- or time-dependencies in the first-pass metabolism or elimination of the drug, a single dose study is sufficient. If the elimination is nonlinear, the design and degree of saturation should mimic the therapeutic situation. For drugs with dose-dependent elimination and significant accumulation under multiple-dosing, it should be considered to administer the radiolabelled single dose when steady state has been obtained with unlabelled drug. Alternatively, a supratherapeutic single dose could be used. The steady state dosing approach is also suitable if there drug has time-dependent pharmacokinetics. In the mass-balance study, the systemic exposure of parent drug and metabolites in relation to total exposure of radioactive material is obtained as well as the excretion of parent drug and metabolites in urine and faeces. A longer half-life of radioactivity than for parent compound indicate metabolites with slower, ERL (elimination rate limited), elimination. Effort should be made to identify as much of the dose related material as possible. It is generally recommended that metabolites contributing to > 10% of the AUC of drug related material (e.g. radioactivity in a well performed mass-balance study) are structurally characterised. Preferably total recovery of radioactivity in urine and faeces should exceed 90% of the dose and more than 80% of the recovered radioactivity be identified.

A likely metabolism schedule is proposed based on knowledge of possible metabolic reactions and metabolites observed in the study. The quantitative contribution of the different elimination pathways are estimated based on the amount of dose excreted as primary and secondary metabolites along specific routes. The contribution of primary pathways to total drug elimination (including first-pass) is estimated as the sum of amounts found in excreta of all metabolites originating from one primary pathway divided by the dose or total amount of drug related material found in excreta if considered more suitable. If a significant amount of unchanged drug is found in faeces and it may not be verified that this is originating from biliary (or gut wall) secretion, the denominator in the calculation should be the amount of drug related material found in excreta subtracted by the amount of unchanged drug found in faeces. This will constitute a worst case estimation from this respect of the contribution of the different (primary) elimination pathway.

The estimation above based on oral mass-balance data is a rough estimation of the importance of elimination pathways. The presence of first-pass metabolism or poor absorption may lead to both under- and over-estimations of the importance of an elimination pathway for the clearance of a drug. Information on oral bioavailability adds important information for understanding the role of biliary and/or gutwall secretion to drug elimination if a large fraction of the drug is found unchanged in faeces. (The i.v. clearance data gained may also be used to estimate the contribution of hepatic first-pass to an incomplete bioavailability.) If there are indications that the bioavailability is markedly formulation dependent, the likely bioavailability of the formulation used in the mass-balance study should be discussed.

If there is first-pass metabolism, i.v. mass-balance data can provide a true estimate of the contribution of the elimination pathways to drug clearance. Therefore, if an i.v. formulation can be produced, the added knowledge obtained by an i.v. mass-balance study should carefully be considered.

Contribution of pathways to active metabolite elimination is estimated in a similar way based on the ratio of the amount of a secondary metabolite originating from the active metabolite found in excreta divided by the total sum of secondary metabolites of the active metabolite excreted plus any active metabolite excreted unchanged. Usually, only one pathway contributes to active metabolite formation

but in some cases, more than one pathway is involved. If possible, a similar approach may be used in these circumstances.

If the pharmacokinetics of the drug is linear in the therapeutic dose range, the mass-balance data can be extrapolated from the dose of the mass-balance study to any dose administered in the range. However, in case the elimination shows dose-dependency, this should be considered when extrapolating the data to other doses than the one administered in the mass-balance study. In addition, if (oral) clearance under multiple-dose conditions is different from at single-dose conditions using the same dose, extrapolation of the results to the steady state situation should be performed with caution and investigation of mass-balance after a single radiolabelled dose at steady state conditions could be considered.

When the quantitatively important metabolism pathways have been determined, *in vitro* and *in vivo* studies will provide information supporting which enzymes or transport proteins mainly are involved in these pathways. As the ability to predict interactions affecting the investigational drug is dependent on the identification of these proteins, extensive studies may be needed in case less known enzymes are involved.

Appendix VI

Table 3 Examples of strong inhibitors of specific enzyme activities in vivo

ENZYME	INHIBITOR
CYP1A2	enoxacine
CYP2B6	ticlopidine
CYP2C8	gemfibrozil
CYP2C9	fluconazole*
CYP2C19	omeprazole*
CYP2D6	quinidine, paroxetine, fluoxetine
CYP3A	itraconazole, ketoconazole, ritonavir, clarithromycin

*moderate/weak inhibitors as no strong inhibitors are presently available or suitable for *in vivo* use. If possible, investigating the effect of pharmacogenetics may be preferable for quantifying enzyme contribution.

Appendix VII

In vitro marker reactions for specific enzymes

The investigation of enzyme inhibitory potency usually starts with *in vitro* investigations of the inhibitory effect of certain marker reactions. These reactions should be selectively catalysed by the enzyme investigated. Below are examples of well validated marker reactions.

Table 4 Examples of well validated enzyme marker reactions *in vitro*

ENZYME	MARKER REACTION
CYP1A2	phenacetin O-deethylation
CYP2B6	efavirenz hydroxylation, bupropion hydroxylation
CYP2C8	paclitaxel 6-hydroxylation, amodiaquine N-deethylation
CYP2C9	S-warfarin 7-hydroxylation, diclofenac 4´-hydroxylation
CYP2C19	S-mephenytoin 4´-hydroxylation
CYP2D6	bufuralol 1´-hydroxylation
CYP3A	midazolam 1-hydroxylation and testosterone 6β-hydroxylation*

*CYP3A inhibition should be investigated using both marker reactions

Probe drugs for *in vivo* use

A probe drug is a drug which is metabolised mainly through one enzyme *in vivo*. The relative enzyme contribution to the (oral) clearance of the probe drug should be supported by well performed *in vivo* studies. Below is a list of probe drugs for use in interaction studies. Other probe drugs may be used if justified through available scientific literature. In induction studies the importance of specificity, i.e. the risk of overestimation of the induction potential of a specific enzyme due to induction of parallel pathways, should be considered.

Table 5 Examples of probe drugs

ENZYME	PROBE DRUG
CYP1A2	theophylline, caffeine
CYP2B6*	efavirenz, S-bupropion (hydroxylation)
CYP2C8*	amodiaquine (N-deethylation), repaglinide
CYP2C9	S-warfarin, tolbutamide
CYP2C19*	omeprazole (single dose)
CYP2D6	metoprolol, desipramine
CYP3A	midazolam**

*There is no well-documented probe-drug at present but these alternatives may be used (See section 5.4.2). Well validated probe drugs of these enzymes may be established in the future and it is advisable to follow the scientific literature.

** Midazolam should be used regardless of which CYP3A4 marker reaction was associated with the lowest Ki *in vitro*. In the future, a structure based choice of probe drug and structure/correlation-based interaction predictions may be possible.

Appendix VIII

Classification of inhibitors and inducers according to potency.

Enzyme inhibitors may be classified based on their potency, i.e. magnitude of the mean effect on mean oral clearance of a probe drug for the enzyme when the inhibitor is administered at its highest therapeutic dose. A strong inhibitor causes a > 5 fold increase in the plasma AUC values or ≥ 80% decrease in oral clearance, a moderate inhibitor causes a > 2-fold increase in the plasma AUC or 50 - ≤ 80% inhibition of oral clearance, a mild inhibitor causes 1.25 to 2 fold increase in the plasma AUC or ≤ 50% inhibition of oral clearance. Depending on the probe drug used and its bioavailability, the increase in AUC may be different. This is especially the case for substrates of the CYP3A subfamily, due to varying extent of intestinal first-pass metabolism. Therefore oral midazolam is recommended when classifying a drug as a CYP3A inhibitor.

Inducers of CYP3A should be classified based on the effect on oral midazolam clearance or plasma AUC. A ≤ 50%, > 50 - ≤ 80% and > 80% reduction in midazolam AUC after oral administration classifies an investigational drug as mild, moderate and strong inducer, respectively. Induction of other enzymes, should if possible, be classified in a similar way if the effect was investigated using an orally administered probe drug metabolised practically exclusively by that enzyme.

The dose-dependency of both classifications should be remembered, and if a dose range is recommended for the investigational drug, additional studies reflecting the effect of lower doses may be needed.

The potency classification of a drug should be presented in the SmPC together with the magnitude of interaction effect observed.

Appendix IX

Preferred SmPC wordings for recommendations regarding food intake

The effect of concomitant food intake on the pharmacokinetics should be presented in section 5.2 and clear recommendations given in section 4.2. These are the preferred wordings in recommendations regarding drug intake in relation to meals:

[Medicinal product] can be taken with or without meals.

[Medicinal product] should be taken on an empty stomach, at least X hours before or X hours after a meal.

[Medicinal product] should be taken on an empty stomach 1 hour before breakfast

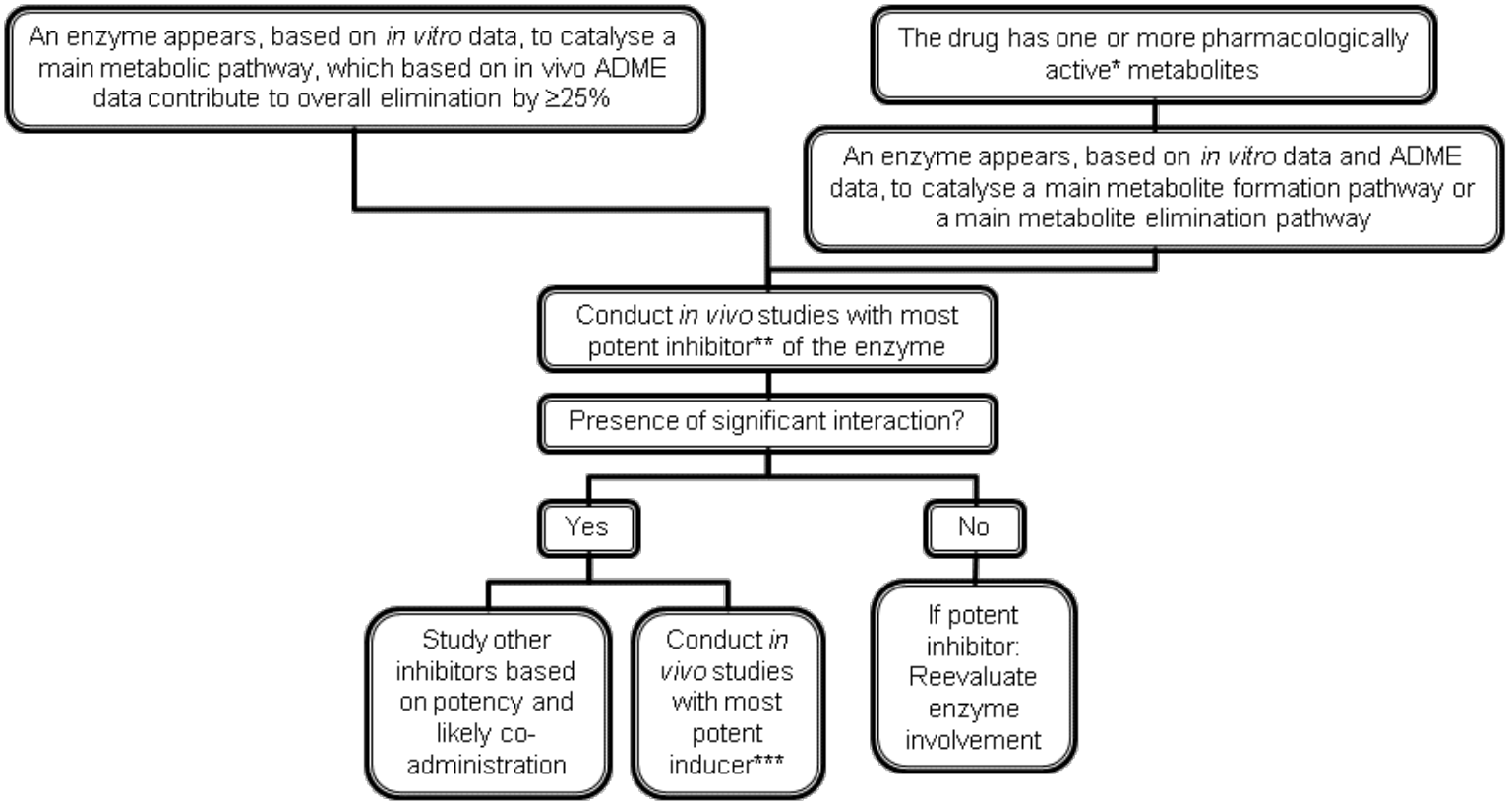
[Medicinal product] should be taken together with a meal.

[Medicinal product] should be taken with a light meal.

Appendix X

Decision trees

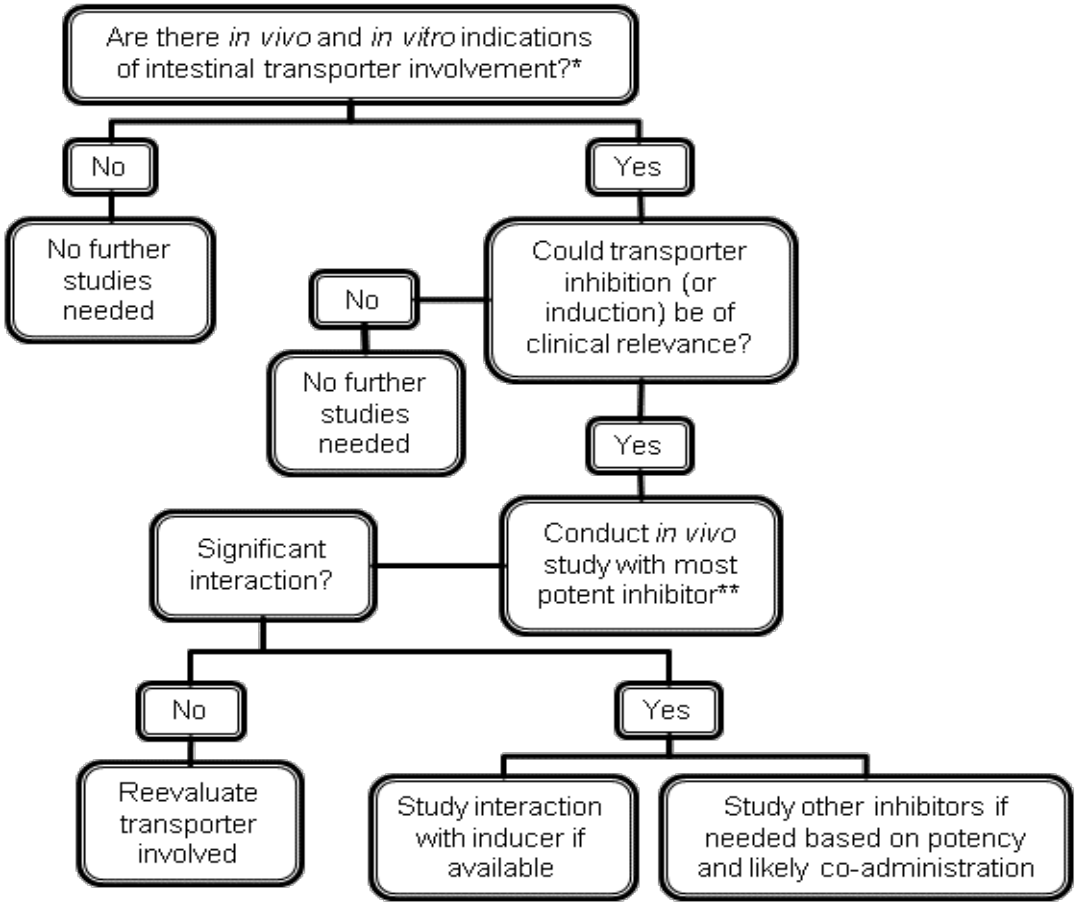
1. Investigations of enzymes catalysing drug metabolism and formation/metabolism of pharmacologically active metabolites (section 5.2.3 and Appendix IV, V and VI)



*based on *in vitro* potency and unbound metabolite concentrations
**or pharmacogenetic subgroup with markedly reduced activity
***unless the results are highly predictable and likely to result in a contraindication.

1

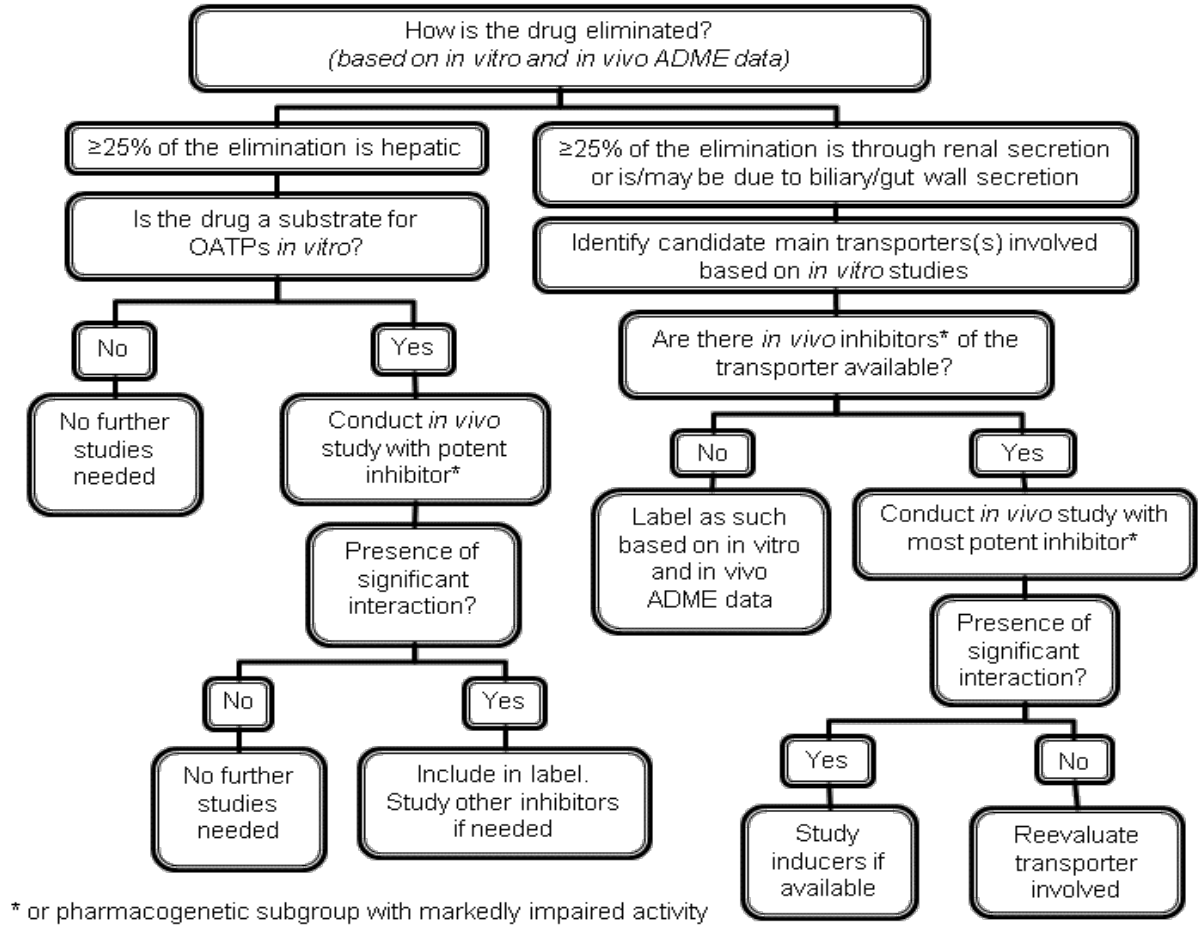
2. *In vivo* investigations of intestinal transporter involvement (Section 5.2.1., Appendices II and III)



*See guideline text
** Or pharmacogenetic subgroup with markedly reduced activity

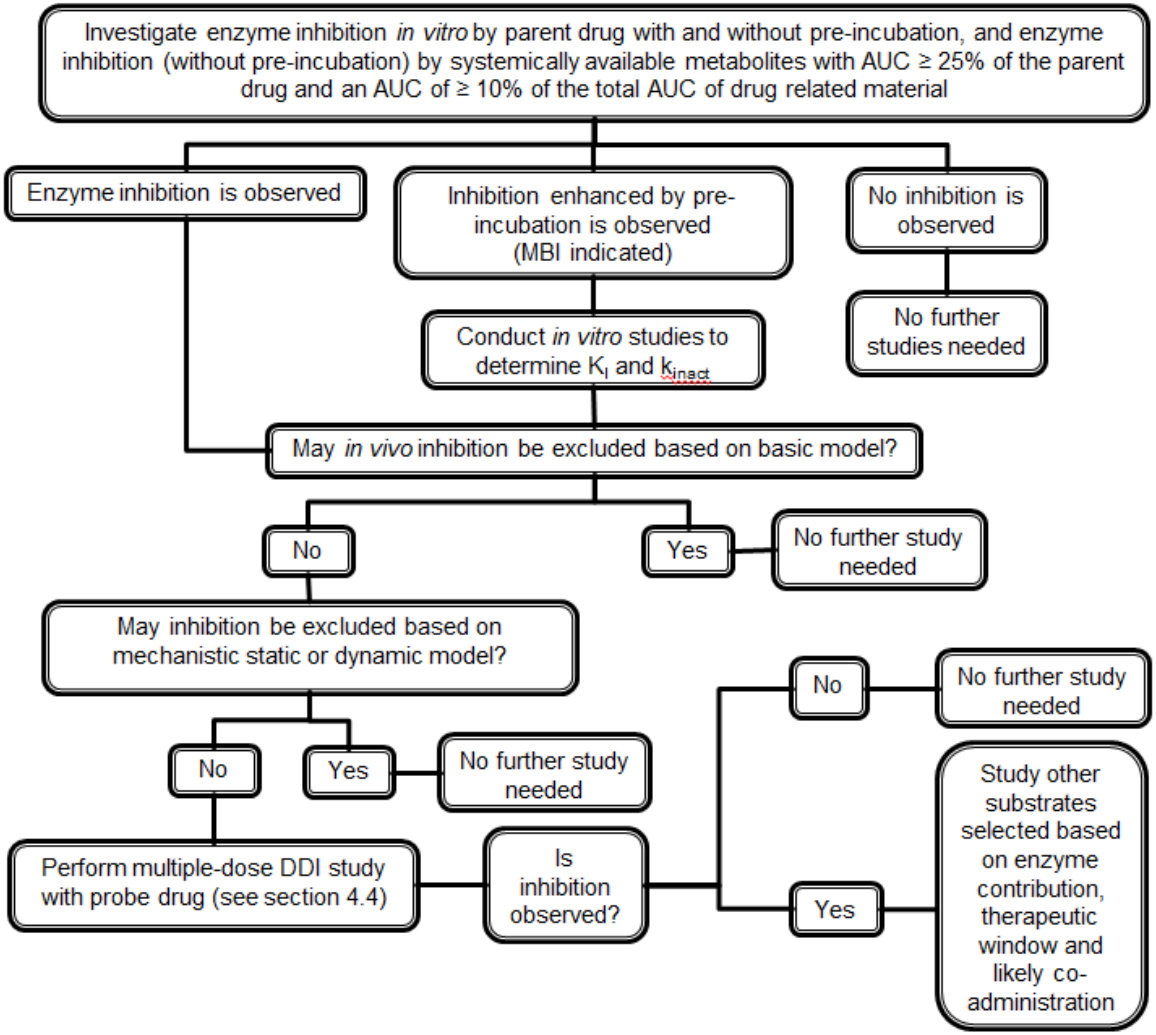
2

3. Investigations of transporter involvement in drug elimination (Section 5.2.4. and Appendix III and V)

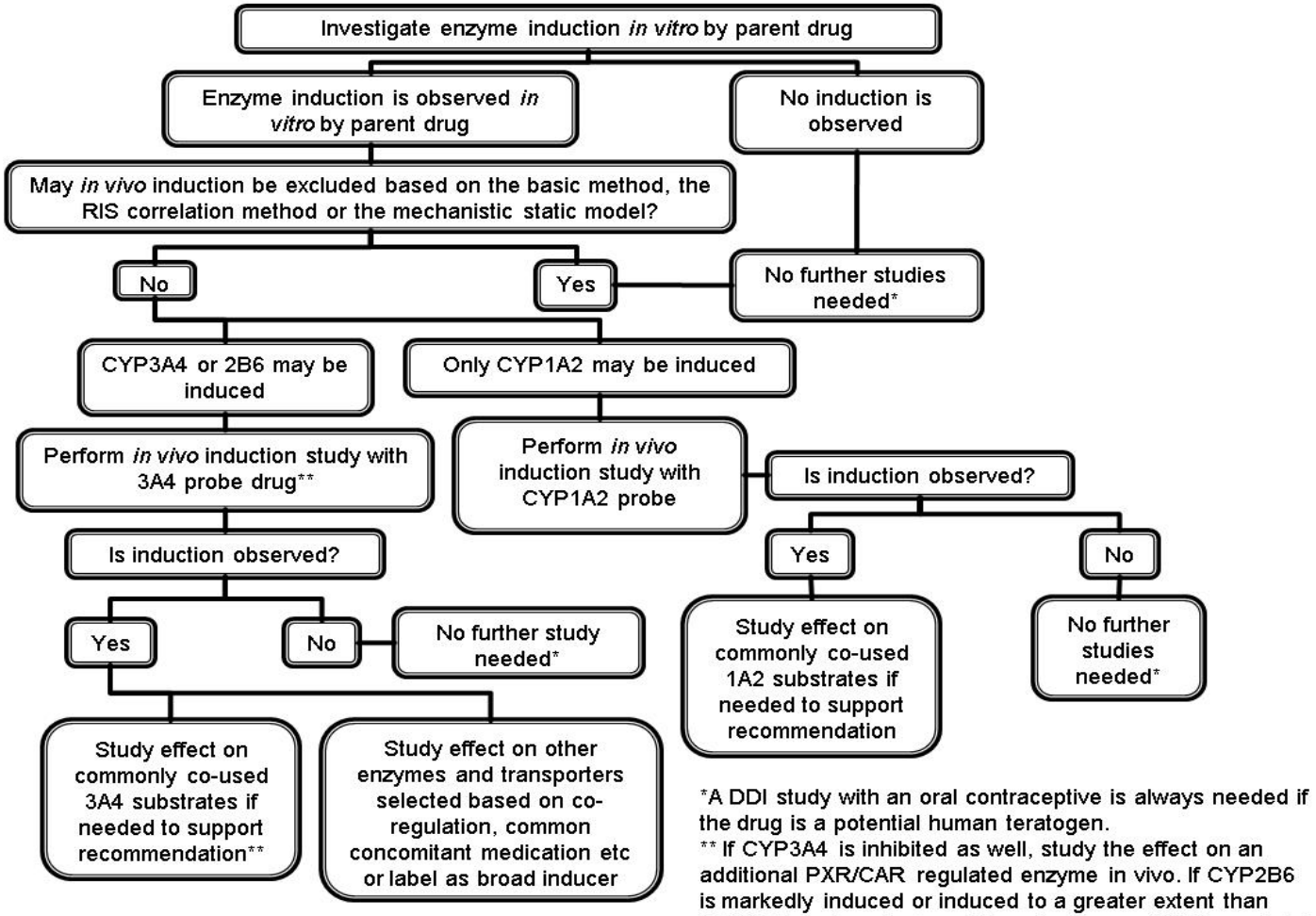


* or pharmacogenetic subgroup with markedly impaired activity

4. Investigations of the enzyme inhibitory potential of a drug (Section 5.3.3.1 and Appendices VII and VIII)



5. Investigations of the enzyme inducing potential of a drug (Section 5.3.3.2 and Appendix VII)



*A DDI study with an oral contraceptive is always needed if the drug is a potential human teratogen.
** If CYP3A4 is inhibited as well, study the effect on an additional PXR/CAR regulated enzyme in vivo. If CYP2B6 is markedly induced or induced to a greater extent than CYP3A4, in vivo studies with probe drugs of CAR regulated enzymes are recommended.

5

6. Investigation of the transporter inhibitory potential of a drug (Section 5.3.4.1)