EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

22 April 2010
EMA/CHMP/EWP/125211/2010
Committee for Human Medicinal Products (CHMP)

Guideline on the Investigation of Drug Interactions

Draft

Draft Agreed by Efficacy Working Party	April 2010
Adoption by CHMP for release for consultation	22 April 2010
End of consultation (deadline for comments)	31 October 2010

This guideline replaces guideline CPMP/EWP/560/95

Comments should be provided using this template. The completed comments form should be sent to ewpsecretariat@ema.europa.eu

Keywords	*Interaction, guideline, metabolism, inhibition, induction, transport, absorption, food, distribution, herbal, SmPC, SPC*

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu
An agency of the European Union

© European Medicines Agency, 2010. Reproduction is authorised provided the source is acknowledged.

Guideline on the Investigation of Drug Interactions

Table of contents

Executive summary

The potential for interactions between new medicinal products and already marketed drugs should be evaluated. This applies both to 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 interaction potential is usually investigated through *in vitro* studies followed by *in vivo* studies. In addition, studies in other species may be relevant for studies of pharmacodynamic drug-drug interactions. The results of interaction studies are used to predict a number of other interactions based on the mechanism 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 as providing recommendations on all these issues. General recommendations are also provided for herbal medicinal products.

1. Introduction (background)

Drug-drug interactions are a common problem during drug treatment and give rise to a large number of hospital admissions within the EU. The aim of this guideline is to ensure that sufficient knowledge has been gained regarding potential drug interactions with medicinal products and furthermore, that the prescriber receives clear information on the interaction potential as well as practical recommendations on how the interactions should be handled 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, scientific progress has made it possible to predict clinically relevant pharmacokinetic drug interactions based on a limited number of *in vitro* and *in vivo* studies. In the last decade, knowledge has been gained in the areas of enzyme induction and drug transport which has opened up the possibility to better predict interactions via these mechanisms. However, in the area of drug transport, the knowledge about clinical consequences of drug-drug interactions is still limited and our understanding needs to be increased.

The aim of the interaction studies performed on new medicinal products under development is to gain knowledge on how the new medicinal product affects other medicinal products and *vice versa*. The interaction potential should be taken into account in the risk-benefit evaluation of the drug.

The potential for interactions is mainly investigated before marketing of a drug. Additional studies may be needed post-marketing as follow up measures/commitments or to support variation applications, e.g. for new indications or new dose recommendations. There may also be a need to perform additional studies due to newly gained knowledge as science develops or due to indications of 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 guideline aims to give recommendations and advice on which drug-drug interaction and food-drug interaction studies to perform for medicinal products. The guideline also aims at giving advice on study design, presentation of study results and translation of these results to treatment recommendations in the labeling of the drug. *It should be remembered that if justified, other approaches may be used than the ones recommended in this document. The interaction studies performed should be driven by science and by the expected clinical consequences of the interaction.*

Interactions with specific foods and herbal medicinal products may occur and should be included in the labeling if clinically relevant interactions are expected. General recommendations are presented in the guideline. Interactions with therapeutic proteins, pharmaceutical drug-drug interactions related to

physiochemical properties and impact of drugs on clinical chemical laboratory tests are not discussed in this guideline.

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 and herbal medicinal products and how to translate results of these evaluations to satisfactory treatment recommendations in the labelling. The guideline mainly addresses studies needed during development of new medicinal products. However, the need for new interaction studies should be considered during the whole life cycle of a drug based on the scientific development in the field.

3. Legal basis

This guideline has to 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 (Notice to applicants, Vol 3C, C3a, 1987)
- 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)
- Rules governing medicinal products in the European Union Volume 2C Notice to applicants; A guideline on summary of product characteristics (SmPC) September 2009
- Guideline on reporting the results of population pharmacokinetic analyses (CHMP/EWP/185990/06)
- Reflection paper on the use of pharmacogenetics in the pharmacokinetic evaluation of medicinal products EMEA/128517/2006
- ICH harmonised tripartite guideline: Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals M3 (R2)
- General Considerations for Clinical Trials (ICH topic E8, CPMP/ICH/291/95)
- Guideline for Good Clinical Practice (ICH E6 (R1), CPMP/ICH/135/95)
- Structure and Content of Clinical Study Reports (ICH E3, CPMP/ICH/137/95)

4. Pharmacodynamic interactions

The potential for pharmacodynamic interactions should be considered for drugs which compete with each other at the receptor level and/or have similar or opposing pharmacodynamic (therapeutic or adverse) effect. If such drugs are likely to be used concomitantly, pharmacodynamic interaction studies should be considered. However, many of these interactions can be predicted based on the pharmacological effects of each drug. The 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. When similar mechanisms

and/or effects are found in animals and in humans and a valid biomarker is available for animal use, animal *in vivo* studies can be used to characterise a potential interaction. Extensive pharmacological and toxicological knowledge about the drug is important for the planning of a pharmacodynamic interaction study. In general, the pharmacodynamic interaction profile of a drug can be best described by using both *in vitro* studies and *in vivo* human studies together.

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, 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 designs of these studies 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 or pharmacodynamics of the other drug.

Although not mentioned in every subsection of this document, the potential effects of metabolites on the pharmacokinetics of other drugs as well as effects of other medicinal products on the exposure of active metabolites should always be considered. Depending on the metabolite to parent exposure ratio, the effect of such metabolites on the pharmacokinetics of other drugs should be investigated (see section 5.3.3).

Furthermore, the risk of clinically relevant pharmacokinetic interactions through altered formation or elimination of metabolites should be investigated if it cannot be excluded that an altered metabolite exposure may result in an altered efficacy or safety (target and "off-target" effects) *in vivo*. The contribution of metabolites to the *in vivo* pharmacological effects of a drug is evaluated taking into account unbound drug and metabolite exposure, the *in vitro* or *in vivo* pharmacological activities, and, if available, data on parent drug and metabolite distribution to the target site. Human *in vivo* PK/PD information on metabolite contribution may be very useful.

If an investigational drug is developed for use in combination with another drug pharmacokinetic interaction studies with the combination should be considered if there are indications that the interaction profile may not be adequately predicted from the interaction data obtained with the separate drugs. If the investigational drug should only be used as combination it is recommended that the drug interaction studies are performed with the combination treatment unless the interaction potential only resides in one of the drugs.

If the investigational drug belongs to a class of substances where mechanistically unsuspected, clinically relevant drug interactions have been reported, it is recommended to perform *in vivo* interaction studies with commonly combined drugs having a relatively narrow therapeutic window.

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 ensure optimal dosing recommendations in the phase II and III clinical studies.

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 is nonlinear, it is recommended to investigate the effect of food on the highest and lowest dose of the therapeutic range. In general, recommendations regarding time of drug intake in relation to food should aim at minimising variability and obtaining target exposure. If a clinically significant effect of food is found, 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 lighter meals are recommended (See Appendix I). 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 co-administration is recommended with a meal or specific food and the drug is indicated in the paediatric population, it should be specified whether this is relevant for paediatric use (especially newborns and infants) whose diet is different (100 % milk 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 subsection 6 of this document.

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

The effects of other medicinal products on the pharmacokinetics of the investigational drug should preferably have been investigated before introducing the investigational product to patients phase II and is generally required before starting phase III. The extent of data needed at different stages of the clinical drug-development is decided case by case based on the possibility of excluding potentially interacting medicines, the pharmacokinetic characteristics of the investigational drug and the tolerability of the drug at exposures higher than the target exposure in the planned study.

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.

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 intrinsic or extrinsic factors. The studies which should be considered include food interaction studies as well as studies of the effect of increased gastrointestinal pH, sequestration and decreased or increased intestinal active transport. 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 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. Interactions affecting solubility

If the solubility of the drug is markedly pH dependent in the physiological pH range, the potential effect of drugs which increase gastric pH, such as proton pump inhibitors, should be discussed. If an effect on absorption cannot be excluded, it is recommended that the potential for interaction is investigated *in vivo*. If indicated by the physicochemical properties of the drug, it may be necessary to investigate the potential for sequestration *in vitro* and an *in vivo* study could be considered.

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 or increased Cmax either due to a primary increase in absorption or secondarily due to decreased availability of drug to intestinal drug metabolising enzymes (e.g. CYP3A). It is recommended to investigate the role of transport proteins in drug absorption if there are indications that transporters may be involved in the absorption process and the consequences of modulating this transport may be clinically relevant. Pharmacokinetic indications of clinically relevant transporter involvement in drug absorption include low bioavailability, erratic or dose-dependent absorption, or CYP3A catalysed intestinal drug metabolism as well as unexplained *in vivo* interactions with effects on intestinal absorption as a possible mechanism. Caco-2 cell *in vitro* assays are usually used for these investigations *in vitro* but other systems expressing human transport proteins may also be used. Detailed recommendations on how to study transporter involvement *in vitro* is given in appendix II.

When a candidate transporter has been identified, an *in vivo* study with a potent inhibitor is recommended if known inhibitors are registered as medicinal products in the EU. *In vivo* studies in subjects of certain genotypes giving rise to markedly altered expression or activity of the transporter may be useful for the identification of the transporter involved and may predict potential for pharmacokinetic interactions via inhibition (or induction) of the transporter.

5.2.2. Distribution

Interactions affecting distribution include displacement interactions and interactions through modulation of active uptake or efflux transport of the drug. Distribution interactions due to an alteration in drug transport are not 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

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 interactions should be discussed in light of available data on the tissue specific expression of the transporter, indications from data on distribution in preclinical species, 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) is recommended. Both target organs for the clinical effect and potential target organs for safety should be considered. Little is presently known about distribution interactions due to transporter induction. As several transport proteins and enzymes are co-regulated, the possible risks and consequences of altered drug distribution during treatment with enzyme inducers could be discussed as far as reasonable.

B. Displacement interactions

In general, the risk of clinically relevant interactions via displacement from plasma protein binding sites is considered low. Nevertheless, the possibility of displacement interactions of drugs known to be markedly protein bound should be considered. In particular, this applies to highly bound drugs (fu<3%) which

I) has a narrow therapeutic window , direct PK/PD relationship and a very small volume of distribution (<10L/70kg), or

II) has a high hepatic extraction ratio and is administered i.v. or

III) has a high renal extraction ratio

If indicated, the risk of interaction should be addressed by *in vitro* displacement studies. In case a clinically relevant interaction is suspected, an *in vivo* study could be performed.

5.2.3. Metabolism

The investigations of how the metabolism of the investigational drug is affected by other drugs, usually includes studies of how the investigational drug is eliminated as well as which enzymes are catalysing the main systemic and pre-systemic metabolic pathways. If there are metabolites significantly contributing to the pharmacological effects (target and off-target effects) *in vivo*, the main enzymes catalysing the formation and further inactivation of these metabolites should be identified. Furthermore, if a main elimination pathway is catalysed by an enzyme which is absent or has low activity in some patients due to genetic polymorphism, the major elimination pathway(s) in patients of such subgroups should be identified.

The characterisation of the major enzymes involved in drug metabolism is initiated by *in vitro* studies. The *in vitro* studies should be performed before starting phase I to enable exclusion of subjects of certain genotypes if relevant, and for the extrapolation of preclinical safety data to man. In addition, metabolites found *in vitro* could then be screened early for pharmacological activity and the pharmacokinetics of active metabolites could be investigated as early as possible in phase I. Guidance on the *in vitro* investigations of which enzymes are involved in the metabolism, as well as information on mass-balance studies is given in Appendix III.

The *in vivo* involvement of enzymes found *in vitro* to catalyse metabolism pathways which are important *in vivo*, should be confirmed and quantified. In general, enzymes involved in metabolic

pathways contributing to ≥ 25% of the oral clearance should if possible be verified *in vivo*. Similarly, if there are metabolites estimated to contribute to more than 50% of the *in vivo* target or off target pharmacological activity, enzymes contributing to > 25% of the formation or elimination of these metabolites should if possible be quantified. It should be remembered that 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. The contribution of an enzyme *in vivo* may be determined either through an interaction study with a strong inhibitor (see Appendices IV and V) or through investigating the effect of pharmacogenetics on the pharmacokinetics of the drug.

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).

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 potent and moderate inhibitors, aiming at providing the basis for 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.

In addition, there may be situations where it is expected that co-administered drugs will inhibit more than one elimination pathway 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 potent inhibitor of both pathways is recommended if the pathways together represent ≥ 25% of the oral clearance of the investigational drug and the interaction is expected to be clinically relevant. The evaluation of the effect of potent enzyme inducers on the pharmacokinetics of the investigational drug may also be required.

A) <u>Interaction studies with inhibitors of cytochrome P450 enzymes</u>

If cytochrome P450 enzymes are identified as candidate enzymes involved in the main elimination pathways of the drug (or in major formation or elimination pathways of clinically relevant active metabolites), evaluation of the pharmacokinetics of the investigational drug with and without concomitant administration of a strong specific enzyme inhibitor (see Appendices IV and V) is recommended to verify and quantify the involvement of a specific enzyme in the investigational drug elimination. If possible the inhibitor should be specific not only regarding effects of enzymes, which are able to catalyse the metabolism of the drug, but also regarding transporters involved in the disposition of the drug. For more information on design issues see section 5.4.

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 (known to inhibit 50% to ≤ 80% of the enzyme activity) is recommended in order to support the evaluation of the need for specific treatment recommendations for other inhibitors of the enzyme.

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 potent and moderate inhibitors of that particular enzyme. In this case, *in vitro* studies should be considered investigating the inhibitory effect of commonly co-administered drugs on that particular enzyme. The need of 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 and potent specific inhibitors are not available for *in vivo* use, the potential for drug interactions should be discussed in light of published literature. If suitable inhibitors are available for *in vivo* use or if there are genetic poor metabolisers, it is recommended to verify the contribution of the candidate enzyme *in vivo* and to investigate potentially clinically relevant interactions in accordance with the recommendations for drugs metabolised by cytochrome P450 enzymes.

C) Interaction studies with inducers

The effect of potent enzyme inducers on the pharmacokinetics of the investigational drug also needs consideration. Unless the effects are highly predictable and likely to result in a contraindication, an interaction study with a potent inducer is recommended if drug elimination is mainly catalysed by inducible enzymes as well as when several minor inducible pathways contribute to drug elimination and it may not be excluded that enzyme induction will affect drug exposure to a clinically relevant extent.

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 there are clinically relevant pharmacologically active metabolites of the drug, or if induction is likely to markedly increase the contribution of a usually clinically non-relevant but active metabolite, the effect of a potent inducer on the pharmacokinetics of the metabolite should be investigated.

5.2.4. Active uptake and secretion in drug elimination

As inhibition of OATPs has been reported to result in marked increases in the systemic exposure of drugs transported by this subfamily and as involvement of these transporters may be present without any indications from the *in vivo* pharmacokinetic information, the possible involvement of OATP uptake transport should be investigated *in vitro* for non-cationic drugs with ≥ 25% hepatic elimination. 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.

In line with the requirements of enzyme identification, if renal and biliary secretion account for more than 25% of systemic clearance, 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). Dependent on the information at hand, it may be difficult to estimate the quantitative importance of biliary secretion to total elimination. The importance of biliary secretion should be based on the mass balance data, available interaction data, available pharmacogenetic information, data on hepatic impairment, data on Caco-2 cell permeability etc. Data on bioavailability or i.v. mass balance data can provide important information in quantifying the importance of biliary secretion. If a large fraction of an oral dose is recovered as unchanged drug in faeces, a mass-balance study or bioavailability study should be considered.

In vitro studies investigating drug transport with and without inhibitor, or with and without expression of the transporter, are usually the first steps of the identification process. It is recommended to use a eukaryote system where the physiological functions are preserved. The concentrations of investigational drug should be relevant to the site of transport. If possible, the study should involve controls (as specific substrates as possible confirmed via the absence and presence of the transporter) verifying presence of transporter activity. The choice of controls and inhibitors should be justified by the applicant.

When a candidate transporter has been identified, an *in vivo* study with a potent 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 altered expression or activity of a certain transporter may be useful in the identification of the transporter involved 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. If relevant and possible, inclusion of PD markers is recommended in the *in vivo* studies.

Interactions with *in vivo* inhibitors, and inducers if applicable, 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.* 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 in general not be directly extrapolated to specific subpopulations that have a markedly different contribution of the affected enzyme to the elimination of the investigational drug. Such subpopulations may include genetic subpopulations such as poor metabolisers, patients of a genotype causing significantly altered transport protein activity, patients with impaired organ function, young pediatric patients (< 2 years) and patients treated with other interacting drugs.

The effect of a potent enzyme inhibitor on the exposure of an investigational drug metabolised by the inhibited enzyme 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 renal impairment. 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. Moreover, in case a very important enzyme for active substance elimination or formation is subject to genetic polymorphism, the enzymes involved in the parallel pathways should be identified. It should be considered that the genetic subgroup may have a completely different set of drug interactions. Worst case predictions of the interaction effects and resulting exposure in such predicted sub populations should be performed. An *in vivo* study investigating the interaction in the subpopulation is recommended if the interaction is likely to lead to a negative risk-benefit of the treatment in the subpopulation. In case a study is not possible, the worst case estimation will serve as basis for the treatment recommendations.

The possibility to extrapolate drug-drug interaction results from adults to children should be discussed by the applicant. However, if a drug combination is common and there is a need for a dose recommendation in paediatric patients, an *in vivo* study could be considered. 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). If an interaction study is needed, a sparse sampling and population pharmacokinetic approach could be considered if satisfactorily performed. 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

Data on the effects of the investigational 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 affected can be avoided in these studies. The information is required before starting phase III. I*n vitro* information is often sufficient at this stage. If *in vitro* data indicate that there may be a clinically relevant interaction with a drug that cannot be excluded from the phase II or III studies, it is recommended to perform *in vivo* interaction studies with these drugs prior to phase II or III. Investigational drugs which exhibit dose-dependent- pharmacokinetics 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 time-dependent inhibitor. (The phenomenon 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 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, 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. The investigation of the effect of an investigational drug on active transport of other drugs is further discussed in the Elimination subsection below. 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.

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 saturable binding site, the risk of displacement of other drugs known to be subject to clinically relevant displacement interactions should be evaluated *in vitro*. If a clinically relevant interaction is predicted based on *in vitro* data an *in vivo* study 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 directly *in vivo*, e.g. by the use of cocktail studies (See section 5.4.2).

The *in vitro* studies should include at a wide range of concentrations of the investigational drug. It is recognised that obtaining high concentrations may in some circumstances not be possible 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*.

A. Enzyme inhibition – *in vitro* 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.

The potential inhibitory effects of metabolites on the common drug metabolising enzymes should also be considered. As an arbitrary rule, a metabolite present in the circulation in molar total or unbound concentrations (AUC) at least as high as 1/5 of the concentration of the parent compound should be investigated for enzyme inhibitory potential. In addition, 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.

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 under linear substrate metabolism conditions. 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. 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 influence of investigational drug metabolism (decreasing concentrations) on the Ki estimation. If this is not possible, the concentration of investigational drug needs to be monitored and the degradation taken into account in the calculations. Known potent inhibitors should be included as positive controls in the study. The concentration range of the investigational drug should be sufficiently high for detecting clinically relevant inhibition. The range suitable depends on the potential site of enzyme inhibition, mode of administration and formulation as well as systemic exposure. 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, the free fraction in the incubate should be determined and used in the calculation of Ki. For drugs which are bases, it is recommended to use the estimated or determined unbound drug concentration in the *in vitro* system. As the actual concentration of drug near the enzyme is unknown, the Ki may vary between systems, and concentrations at the portal vein during absorption generally are higher than Cmax in plasma after oral administration, a safety factor is added in the estimations. Due to the variability connected with estimating the unbound drug fraction for highly protein bound drugs and due to the more pronounced effect of drug-protein dissociation for these drugs, a higher safety margin is applied for drugs which are > 99.0% protein bound. Below, recommendations regarding concentration ranges are given for different situations. If the incubations made indicate that Ki will be markedly higher than the concentrations given below, Ki does not need to be determined.

Intestinal exposure

If the drug is orally administered and the enzyme studied has pronounced intestinal expression, the concentration range should be sufficient for determining a Ki ≤ 10-fold the maximum dose taken at one occasion/250 ml, or alternatively,

≤ 50-fold the maximum concentration predicted in the enterocyte using the equation below where Qent is enterocyte blood flow, f_a is the fraction absorbed, k_a is the absorption rate constant.

Eq.1.

$$[\mathrm{I}]_{\mathrm{gut}} = \frac{f_{\mathrm{a}} \times k_{\mathrm{a}}(\mathbf{I}) \times \mathrm{Dose(I)}}{Q_{\mathrm{ent}}}$$

Hepatic (and renal) exposure

If the enzyme studied is mainly available in the liver, kidney or another organ with main drug input from the circulation, the concentration range should allow determination of a Ki which is ≤ 50-fold the unbound Cmax obtained during treatment with the highest dose, or

≤ 250-fold the unbound Cmax if the protein binding is > 99.0 %

Time-dependent inhibition

If enzyme inhibition is found, it should be evaluated whether the inhibition is increased by pre-incubation. If the inhibition is enhanced by pre-incubations, time-dependent inhibition (TDI) may be present. In this situation k_{inact}(maximum inactivation rate constant) and K_I (the inhibitor concentration producing half the maximal rate of inactivation) should be determined.

When time-dependent inhibition is observed, further investigations of the mechanism of the time-dependency are encouraged. If it is shown that the time-dependency 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).

Evaluation of the need for an in vivo study

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 unbound concentration near the enzyme during clinical use. An inhibition *in vivo* cannot be excluded and an *in vivo* interaction study with a sensitive probe substrate is recommended if the conditions below are fulfilled.

Orally administered drug if the enzyme has marked abundance in the enterocyte (eg CYP3A):

Ki < 10-fold the maximum dose taken at one occasion/250 ml, or,

50-fold the maximum concentration predicted in the enterocyte using equation 1.

Drugs inhibiting enzymes present in the liver, kidney or other organs:

Ki < 50-fold the unbound Cmax obtained during treatment with the highest dose or,

250-fold the unbound Cmax for drugs with a plasma protein binding > 99.0 %

If a well performed *in vivo* interaction study with a 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 the intestinal abundance of CYP3A and the higher concentrations of drug present in the intestine after oral administration, lack of inhibition *in vivo* may not be extrapolated from other enzymes to intestinal CYP3A for orally administered drugs.

II) Time-dependent inhibition

If time-dependent inhibition has been observed *in vitro*, the fold reduction in CL may be calculated as Eq. 2

$$\frac{k_{deg} + \frac{[I] \times k_{inact}}{([I] + K_I)}}{k_{deg}}$$

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. The degradation constant may be taken from the scientific literature. If possible, the constant should be based on high quality *in vivo* data. If ≥ 30% inhibition is obtained using the drug concentrations presented above, *in vivo* inhibition may not be excluded and a multiple dose *in vivo* interaction study is recommended (see Section 5.4.4).

Simulations of the interaction potential

Simulations may also be used to evaluate the *in vivo* relevance of inhibition observed *in vitro*. In such a case, the scientific basis of the simulations (equations, literature references on physiological parameters etc) should then be presented. Furthermore, extensive data on the validation needs to be shown to support the ability to quantitatively predict drug-drug interaction via inhibition of the specific enzyme. The validation set of drugs should include a large number of inhibitors and be well justified. The *in vitro* data used needs to be of high quality and any parameter estimated needs to be justified and subject to a sensitivity analysis. As above due to the limited ability to predict the actual concentration at the enzyme, a safety factor should be applied on the concentration reaching the enzyme. The safety-factor is 50 in all cases except for highly protein bound drugs (fu > 99.0%), where it is 250. If the simulation with the safety factor predicts an inhibition of > 30%, a significant interaction *in vivo* cannot be excluded and it is recommended to perform an *in vivo* study.

B. Enzyme inhibition – *in vivo* studies

If reversible inhibition has been observed *in vitro*, the pharmacokinetics of a probe drug (see Appendix VI) should be investigated after administration of a single-dose of the probe drug alone and at the steady state concentrations of the investigational drug obtained with the highest usual recommended dose. 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.

C. Enzyme induction or downregulation– *in vitro* studies

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

Cultured hepatocytes is the preferred *in vitro* system for these studies. However, well validated cell-lines with proven inducibility via the regulation pathways of interest (see below) may be used. If this approach is taken, the choice of *in vitro* system has to be scientifically very well justified.

Due to the inter-individual and cell batch variability in induction response, it is recommended to use hepatocytes from at least 3 different donors. Incubations are performed with daily addition of the investigational drug. The duration of the incubation should be well justified. A number of enzymes could be investigated. The enzymes CYP3A, CYP2B6 and CYP1A2 should always be included. It is recommended to measure the extent of enzyme induction as enzyme activity. Additional measurement

of mRNA could also be included and is mandatory for the interpretation of study results if inhibition of the studied enzyme may not be excluded at the concentrations used or if a down-regulation is suspected based on the activity assay. Potent 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, phenobarbital (0.5-1 mM) and CITCO (≤100 nM using mRNA expression) for CAR, omeprazole (25-50μM) for the Ah-receptor and dexamethasone (50μM) for GR. It is acknowledged that at present there is no selective positive control for CAR used for investigations of induction at enzyme activity level. In the future, more selective controls may be available for use.

Knowledge about the actual concentration of drug in the system is important for the *in vitro-in vivo* extrapolation. Unless *in vitro* drug metabolism or degradation of drug during culture conditions has been shown to be negligible, the concentration of parent drug in the medium should be measured at several time points the last day of the incubation for determining the actual drug exposure surrounding the cells. 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 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, i.e. range of average concentration in the media (Cavg), should cover the concentrations given above. A. If this is not possible and the study is judged inconclusive, induction (of CYP3A, CYP2B6 and CYP1A2) should be studied *in vivo*.

The viability of the cells should be determined at the beginning and end of the incubation period at the highest concentration level to certify that cell toxicity is not influencing the induction response. The cell viability should be at least 80% at baseline and >50% in the end of the incubation. If toxicity is observed, influence on the study results should be discussed in the study report and *in vivo* studies should be considered.

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 *in vitro* study is considered negative for enzyme induction if incubations with the investigational drug at the concentrations given in the inhibition part of this section give rise to a less than 50% increase in enzyme activity. However, to certify adequate sensitivity of the assay, any increase in activity or mRNA observed at the given concentrations also has to be less than 20% of the response to rifampicin 20 μM or, for Ah-receptor activation, omeprazole 25-50 microM.

If both activity and mRNA are measured, activity results prevail unless enzyme inhibition is indicated. A positive or inconclusive *in vitro* result should be confirmed *in vivo*.

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

D. 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 vivo*. In such a study, the pharmacokinetics of a probe drug (see Appendix VI) is determined after a single dose administration alone and after multiple dose administration 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

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 clinically relevant induction is observed *in vivo*, it is likely that the investigational drug also affects other enzymes, or transporters, regulated though the same regulatory pathway. However, it is difficult to extrapolate the effect quantitatively to the co-regulated proteins, which may be induced to a lesser extent. Therefore, if the investigational drug is verified to be an inducer *in vivo*, the inducing effect on co-regulated enzymes should preferably be quantified *in vivo*. Which enzymes that are at risk of induction and have been chosen for further studies should be discussed based on the available scientific literature.

5.3.4. Transport

A. 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, OATP1B1, OATP1B3, OCT2, OCT1, OAT1, OAT3 and BCRP). The transporter BSEP should also preferably be included for detecting pharmacodynamic interactions as well for adequate safety monitoring during drug development. The knowledge about transporters and their *in vivo* importance is evolving fast. 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*.

It is recommended to use a eukaryotic *in vitro* system where the physiological functions of the transporter are preserved. The effect of different concentrations of the investigational drug on transport of a substrate for the specific transporter should be investigated and Ki calculated. The *in vitro* study should include potent inhibitors as positive controls. The choice of substrates and inhibitors should be justified by the applicant.

The study should be performed over a concentration range of the investigational drug expected to be relevant for the site of interaction (see section 5.3.3.A). However, for intestinally expressed transporters like Pgp, the highest concentration studied should be sufficient for determining Ki ≤ the maximum expected concentration in the intestinal lumen (10-fold the maximum dose on one occasion/250 ml).

In vivo inhibition of intestinally expressed proteins such as Pgp can be excluded if the observed Ki value is ≥ 10-fold the maximum dose/250ml. For systemic transporter inhibition, the Ki values should be compared with the highest expected unbound Cmax as described above. It has been observed that the estimated Ki may be different in separate systems. Therefore, if there is an uncertainty in the Ki estimation, an additional *in vitro* study with another cell system should be considered. If *in vivo* inhibition may not be excluded, 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, inhibition of intestinal and renal inhibition can be determined using digoxin AUC and renal clearance.

B. 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 potential inducing effect on other transporters regulated through the same pathways should also be considered. If the investigational drug often will be combined with a drug eliminated through active transport by a PXR or CAR regulated transporter, an interaction study with that drug is recommended to enable treatment recommendations for that specific drug combination.

5.4. Design of in vivo studies

The design of the *in vivo* interaction study is adapted to the aim of the study. However, some general considerations are found below. An *in vivo* interaction study usually is of cross-over design. Parallel group design may be used when the potential inhibitor or inducer has a very long elimination half-life, but, due to the wide inter-individual variability in responses, it is generally not recommended. In case it may be suspected that compliance with study treatment may be reduced eg due to a long treatment duration or due to adverse effects, compliance should be checked regularly during the study. Comparisons with historical controls are generally not acceptable. 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 reasons, patients could be 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 (subject to 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 all intrinsic and extrinsic factors known 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 of the subjects are generally encouraged. If the pharmacokinetics of the drug is significantly affected by genetic polymorphism and it is expected that patients of a certain genotype have a larger interaction effect, it is recommended that interaction is evaluated separately in that subgroup. 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 catalysing metabolism of the parent drug, its contribution to total

clearance should be very small (<10%). 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 VI. Other drugs than the listed ones may be used if justified. Marker reactions, i.e. metabolic reactions known to be catalysed by only one enzyme may sometimes be used (see below).

The probe drug for CYP3A4 should be subject to both marked intestinal and hepatic 3A4 catalysed metabolism. The use of orally administered midazolam is recommended. if the drug is very 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 is approach is chosen, appropriate safety precautions should be made.

It is possible to use so called "cocktail studies" investigating the inhibitory or inducing effect of an investigational drug on several enzymes in one *in vivo* study. In this case, 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. Use of metabolite to parent drug concentration ratios in plasma or urine is not recommended. If satisfactorily performed, the results of the cocktail studies can be extrapolated to other drugs and be used to support treatment recommendations of the SmPC.

If well-documented probe drugs are lacking, it may be chosen to study clearance through a specific pathway as marker for the enzyme catalysing that pathway. If this is chosen, 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 divided by AUC ($\Sigma Ae_{0-\infty}/AUC_{0-\infty}$). If this approach is used, it needs to be verified that parallel pathways are not affected by the investigational drug. Another approach is to recalculate the effect on the enzyme using the fraction of the clearance catalysed by the enzyme if the fraction is well supported by literature data.

5.4.3. Dose, formulation and time of administration

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

The systemic exposure of the drug thought to affect the pharmacokinetics of the other drug should generally be the exposure obtained with the highest generally recommended dose under therapeutic (steady state) conditions. If the highest usually expected exposure is not studied, this should be well justified. 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 the full plasma concentration-time course (sampling period) of the victim drug (see also section 5.4.4 for time-dependent interactions). If the perpetrator drug is the investigational drug and a dose-range is recommended for the perpetrator drug, studying more dose levels should be considered if a significant effect is found using the highest dose.

B. <u>The victim drug</u>

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).

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 faster if this is possible from a safety point of view.

If a mutual (2-way) interaction is expected, both drugs should be administered until steady state and compared with steady state pharmacokinetics of the separate drugs administered alone.

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 with other formulations as needed of the interactions observed in earlier studies.

D. Relative time of administration

In all *in vivo* interaction studies, the time between administrations of the two drugs should be specified. Usually the drugs are administered simultaneously but sometimes, the most marked interaction is obtained when the drugs are administered at separate time-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.

5.4.4. Time dependencies

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

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 takes place simultaneously. The required duration of treatment depends on how precisely the interaction effect needs to be determined. If the study aims at investigating whether an investigational drug is an inducer or 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 if possible be presented. At present, a range of k_{deg} or enzyme half-life values are reported in the literature. If available, use of reliable *in vivo* estimations is preferred. Based on the presently available information, it appears that 80 hours is a reasonable estimation of the hepatic CYP3A4 half-life. 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 aiming 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. In case it is valuable to know the effect also 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.

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 during normal conditions do not contribute significantly to *in vivo* effects of an investigational drug, 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.

In case the investigational drug has a complex metabolism it may also be useful to measure metabolites regardless of their activity to improve understanding of the mechanism of the interaction and to extrapolate the knowledge to other drug combinations.

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 Cmin has been found to be closely related to clinical efficacy or safety, Cmin should also be investigated. 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 (eg SHBG, sex hormone binding globulin, for contraceptive steroids), it is recommended to determine both the unbound and total 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 an interaction at transporter level is investigated, or in case both a pharmacodynamic and a pharmacokinetic interaction is expected.

5.4.7. Population pharmacokinetic analysis

If conventional interaction studies *cannot be performed*, the potential for interactions may be investigated in a well performed population PK analysis on high quality data from sparse samples. This 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 used of concomitant drugs needs to be properly recorded, which includes the dose amount, 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. Further, with respect to doses, the quantification of the interaction will be dependent on the doses used and a maximum effect of the interaction may be difficult to establish due to this reason. However, the information obtained may still be used in some sense in the product information but need 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 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 5.6.2.). 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 (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.

5.5. 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 mean, standard deviation, 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 should be included in the statistical analysis. However, if a subject has dropped out from the study or has no measurable plasma concentration during a treatment period and this is unlikely to be due to the interaction, the subject can be excluded from analysis of effects related to that period. Exclusion of subjects for other reasons than the ones above should be well justified and specified in the study protocol. 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 total exposure of active species, i.e. 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 and, if possible, the calculations could be modified by the metabolite to parent target organ distribution ratio.

5.6. Translation into treatment recommendations

The consequences of an observed *in vivo* (or *in vitro*) interaction should be assessed and suitable treatment recommendations 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.

5.6.1. *In vitro* data

If positive *in vitro* studies have not been followed by *in vivo* studies, eg in cases where *in vivo* probe drugs are not available, or if an interaction of non-studied enzymes is expected based on mechanistic knowledge (eg 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.

5.6.2. *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.

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 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 patients 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 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 5.5). 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 showing that the pharmacokinetic parameters likely to be relevant for efficacy and safety does 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 on an altered relation 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.

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). This is mainly applicable if there is a well established therapeutic range. 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 carry 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 in all patients should be suitable also for the subpopulation.

In addition, combinations of drugs leading to inhibition of multiple pathways should be considered and treatment recommendations included.

5.6.3. *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 potent inhibitors or inducers (See Appendix IV). The induction results are qualitatively extrapolated to co-regulated enzymes and transporters in case 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.

5.6.4. 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 SPC. Whether such a recommendation is needed and which 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 3.1.1.1. Recommended wordings for recommendations regarding food intake are given in section Appendix VII

6. Herbal medicinal products and specific food products

Usually information about interactions between medicinal products and herbal medicinal products or specific food products such as grapefruit juice is 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. 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 could involve probe drugs if appropriate.

If there is a wish 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 combinations with other herbal medicinal products or food products.

7. Inclusion of information and recommendations in the SmPC

The guideline on summary of product characteristics (SmPC) (September 2009 - Rules governing medicinal products in the European Union Volume 2C Notice to applicants) advice on how to present information about interactions.

Information about drug interactions should be presented in the SmPC section 4.5 and 5.2 (e.g. for food-interactions) and cross-referring 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. Inside these subsections, the order of presentation should be contraindicated combinations, those where concomitant use is not recommended, followed by others.

Clear treatment recommendations should be given to the prescriber. Wording such as "caution is advised" should be avoided in favour of a recommendation on proposed actions. The need for time-specific information and recommendations should be considered. Situations when this is needed include 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 and, 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 could be summarised in section 4.5 as a mechanistic basis for the interaction information. 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*, *in* it is recommended to include the *in vitro* information in the SmPC Section 5.2 for future use. Based on the mechanism of the interaction, the results of the 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 potent inhibitor of CYP3A4 and may therefore markedly increase the systemic exposure of drugs metabolised by this enzyme such as …" or "Drug X is mainly metabolised by CYP3A4. Concomitant use of drugs which are potent inhibitors of this enzyme, such as ……, are not recommended". The most important drugs should be included in such a list to aid the prescriber.

7.2. Presentation of study results in the SmPC

The results of the study 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 eg interaction studies with probe drugs as victim drugs, should be included. even if the interaction effect is not clinically relevant for the victim drug studied. Brief, condensed, specific information on the study design relevant for the interpretation of that particular study results should be included when appropriate. Such information includes 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 S mPC. 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 VII).

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

CAR constitutive androstane receptor

Cavg average concentration

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

f_a fraction absorbed

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

1125	k_{inact}	maximum inactivation rate constant
1126	PXR	pregnane x receptor
1127	Qent	enterocyte blood flow
1128	SmPC	summary of product characteristics
1129	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 lighter meal are described below. If the pharmacokinetics is linear, i.e. the exposure of drug is dose-proportional in the therapeutic dose range, there are no special requirements with respect to the dose investigated. If the pharmacokinetics is nonlinear, it is recommended to investigate the effect of food on the highest and lowest dose of the therapeutic range.

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 lighter meal

The lighter meal could contain approximately 400-500 kcal with fat contributing to ca. 250-300 kcal.

Appendix II

In vitro investigations of involvement of transporters in drug absorption

The *in vitro* 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, obtainable through repeated changes of the receiver well), for at least four different physiologically relevant concentrations. For intestinal transport the studied range could be 0.1 to 50-fold the dose/250 ml). If systemic (post absorption) transport is investigated, the concentration range could be 0.1-fold to 50-fold unbound Cmax. If the study is not performed under sink conditions this needs to be compensated for in the calculations. If a proton gradient is used in the study, the degree of ionisation should be discussed. It is recommended that a pH of 7.4 is used on both sides when efflux is investigated. 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 impact of solubility, potential degradation and metabolism of the drug substance *in vitro* on study results should be discussed as well as the effect of organic solvents used.

In Caco-2 cell studies, the permeation of drug from the apical (A) to the basolateral (B) side of the cells are compared with the permeability of the permeation in the opposite direction (B to A). If the ratio of the A to B and B to A permeation is < 0.5 or > 2, it is concluded that there is active efflux and uptake, respectively. If active transport is concluded, the importance of the transporter for drug absorption can be estimated through a comparison of the permeability in absence and presence of the transporter in Caco-2 cells. To estimate the permeability in absence of transporters, the permeability constant is determined at concentrations high enough to completely saturate the transporters. The investigation should include a high and low permeable control (e.g. metropolol and mannitol). If the permeability in absence of transporters is high ($\geq$ 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 pronounced uptake or efflux transport is observed *in vitro*, and the permeability constant is not classified as high, attempts should be made to identify the transporter. The identification 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. It is recommended to use a eukaryote system where the physiological functions of the transporter are preserved. The concentrations of investigational drug should be relevant to the site of transport (see above). The study should include positive controls verifying presence of the specific transporter activity. The choice of controls and inhibitors should be justified by appropriate scientific references.

Appendix III

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 in *in vitro* incubations with human liver microsomes, hepatocytes, cells expressing human enzymes, liver S9 fractions etc, depending on which enzymes are investigated and access to *in vitro* systems. The *in vitro* system used should be carefully considered when interpreting study results. These are examples of *in vitro* systems for liver metabolism studies. Positive controls for each enzyme studied should be included.

- Supersomes® and other recombinant enzyme systems are a single enzyme system. Since these usually contain one single drug metabolising enzyme, they are the most sensitive to investigate whether metabolism can take place via a particular enzyme.
- Human liver microsomes (HLMs) contain major drug-metabolizing CYP and UGT enzymes. Incubations are made with HLMs from several donors either pooled or run separately. HLMs are in general robust and in general their enzyme activities are easily maintained.
- Subcellular liver fractions (S9 fraction or homogenate) contain both cytosolic and microsomal enzymes. Hence, in addition to the CYP enzymes they enable investigation of enzymes normally present in the cytosol such as sulfotransferases, glutathione transferases and other phase II enzymes, as well as aldehyde dehydrogenases, alcohol dehydrogenases.
- Intact hepatocytes (freshly isolated, cultured or cryopreserved) contain the whole complement of enzymes and may also express transporters. Hepatocytes lose their enzyme activity more easily than the other systems listed.

The *in vitro* metabolism studies should be performed at physiologically relevant concentrations under linear conditions. In multi-enzyme systems, enzyme specific inhibitors (see table 1) are added to evaluate the contribution of separate enzymes to the metabolism of the investigational drug. In cases where the inhibitor is not very specific, it is recommended to perform the study in an *in vitro* system where no other CYPs than the particular enzyme is expressed. 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. Positive controls (marker substrates) for enzyme activity (see table 2) should be included in the study. 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 1 and 2 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.

Table 1 Examples of well validated inhibitors of specific enzyme activities *in vitro*

ENZYME	INHIBITOR
CYP1A2	furafylline
CYP2B6*	ticlopidine, thiotepa
CYP2C8	montelukast
CYP2C9	sulfaphenazole
CYP2C19*	ticlopidine, nootkatone, loratadine
CYP2D6	quinidine
CYP3A4	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.

Table 2 Examples of well validated marker reactions specific enzyme activities *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
CYP3A4	midazolam 1-hydroxylation or testosterone 6β-hydroxylation, plus one structurally unrelated substance such as nifedipine, triazolam or dexamethasone

The *in vitro* data are combined with *in vivo* data such as results from an *in vivo* mass-balance study with investigational drug in order to predict which elimination pathways are the main pathways *in vivo*. The drug is administered with a radioactive label in a metabolically stable position. 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. The radiolabel should be in an as metabolically inert position as possible. In some cases two separate labelling positions have to be used to follow the fate of the investigational drug. Effort should be made to identify as much of the dose related material as possible. It is generally recommended that metabolites having an AUC ≥ 20% of parent AUC, or contributing to > 5% of the total radioactivity AUC 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 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. In case there is marked elimination of unchanged drug in faeces, additional studies may be needed to quantify the contribution of biliary excretion to drug elimination. Such studies include determination of the oral bioavailability of the formulation used in the mass-balance study or an investigation of mass-balance after i.v. administration (providing information on fecal excretion of

unchanged drug). I.v. mass-balance data may also be useful in situations with pronounced luminal metabolism to assess the contribution of metabolism pathways to systemic clearance.

If the pharmacokinetics of the drug is linear in the therapeutic dose range, the mass-balance data could 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.

Appendix IV

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 oral clearance. A drug that causes a > 5 fold increase in the plasma AUC values or ≥ 80% decrease in oral clearance is classified as strong inhibitor, 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 somewhat different. This is especially the case for substrates of the CYP3A subfamily, due to varying extent of intestinal first-pass metabolism. Therefore oral midazolam should always be used 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.

Appendix V

Table 4 Examples of strong inhibitors of specific enzyme activities *in vivo*

ENZYME	INHIBITOR
CYP1A2	furafylline
CYP2B6	
CYP2C8	gemfibrozil
CYP2C9	fluconazole*
CYP2C19*	omeprazole, fluvoxamine,
CYP2D6	quinidine, paroxetine, fluoxetine
CYP3A4	itraconazole, ketoconazole, ritonavir, clarithromycin

*moderate 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 VI

Probe drugs

A probe drug is a drug which is metabolised mainly through one enzyme *in vivo*. The enzyme contribution should have been 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.

Table 3 Examples of probe drugs

ENZYME	PROBE DRUG
CYP1A2	theophylline, caffeine
CYP2B6*	efavirenz, S-bupropion hydroxylation
CYP2C8*	amodiaquine N-deethylation, cerivastatin hydroxylation (M23 formation)
CYP2C9	S-warfarin , tolbutamide
CYP2C19*	omeprazole (single dose)
CYP2D6	metoprolol, desipramine
CYP3A4	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.

Appendix VII

Preferred 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.