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4 **Guideline on the Investigation of Drug Interactions**
5 **Draft**

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7 This guideline replaces guideline CPMP/EWP/560/95

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9 Comments should be provided using this [template](#). The completed comments form should be sent to ewpsecretariat@ema.europa.eu

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11 Guideline on the Investigation of Drug Interactions

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57 **Executive summary**

58 The potential for interactions between new medicinal products and already marketed drugs should be
59 evaluated. This applies both to effects of the medicinal product on other drugs as well as the effect of
60 other drugs on the medicinal product. Furthermore the effect of concomitant food intake needs to be
61 investigated. The interaction potential is usually investigated through *in vitro* studies followed by *in*
62 *vivo* studies. In addition, studies in other species may be relevant for studies of pharmacodynamic
63 drug-drug interactions. The results of interaction studies are used to predict a number of other
64 interactions based on the mechanism involved. Treatment recommendations are developed based on
65 the clinical relevance of the interactions and the possibility to make dose adjustments or treatment
66 monitoring. This document aims as providing recommendations on all these issues. General
67 recommendations are also provided for herbal medicinal products.

68 **1. Introduction (background)**

69 Drug-drug interactions are a common problem during drug treatment and give rise to a large number
70 of hospital admissions within the EU. The aim of this guideline is to ensure that sufficient knowledge
71 has been gained regarding potential drug interactions with medicinal products and furthermore, that
72 the prescriber receives clear information on the interaction potential as well as practical
73 recommendations on how the interactions should be handled during clinical use.

74 The first CHMP interaction guideline was adopted in 1997 and this is the first revision of this guideline.
75 During the past 20 years, scientific progress has made it possible to predict clinically relevant
76 pharmacokinetic drug interactions based on a limited number of *in vitro* and *in vivo* studies. In the last
77 decade, knowledge has been gained in the areas of enzyme induction and drug transport which has
78 opened up the possibility to better predict interactions via these mechanisms. However, in the area of
79 drug transport, the knowledge about clinical consequences of drug-drug interactions is still limited and
80 our understanding needs to be increased.

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

84 The potential for interactions is mainly investigated before marketing of a drug. Additional studies may
85 be needed post-marketing as follow up measures/commitments or to support variation applications,
86 e.g. for new indications or new dose recommendations. There may also be a need to perform
87 additional studies due to newly gained knowledge as science develops or due to indications of drug
88 interactions reported post marketing. The marketing authorization holder is advised to perform and
89 report interaction studies as needed during the full life-cycle of the medicinal product.

90 This guideline aims to give recommendations and advice on which drug-drug interaction and food-drug
91 interaction studies to perform for medicinal products. The guideline also aims at giving advice on study
92 design, presentation of study results and translation of these results to treatment recommendations in
93 the labeling of the drug. *It should be remembered that if justified, other approaches may be used than*
94 *the ones recommended in this document. The interaction studies performed should be driven by*
95 *science and by the expected clinical consequences of the interaction.*

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

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

101 **2. Scope**

102 The scope of this guideline is to provide advice and recommendations on how to evaluate the potential
103 for drug-food and drug-drug interactions for medicinal products and herbal medicinal products and how
104 to translate results of these evaluations to satisfactory treatment recommendations in the labelling.
105 The guideline mainly addresses studies needed during development of new medicinal products.
106 However, the need for new interaction studies should be considered during the whole life cycle of a
107 drug based on the scientific development in the field.

108 **3. Legal basis**

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

- 112 - Pharmacokinetic studies in man (Notice to applicants, Vol 3C, C3a, 1987)
- 113 - Guideline on the role of pharmacokinetics in the development of medicinal products in the
114 paediatric population (EMA/CHMP/EWP/147013/2004)
- 115 - Guideline on the evaluation of the pharmacokinetics of medicinal products in patients with impaired
116 hepatic function (CPMP/EWP/2339/02)
- 117 - Note for guidance on the evaluation of the pharmacokinetics of medicinal products in patients with
118 impaired renal function (CHMP/EWP/225/02)
- 119 - Rules governing medicinal products in the European Union Volume 2C Notice to applicants; A
120 guideline on summary of product characteristics (SmPC) September 2009
- 121 - Guideline on reporting the results of population pharmacokinetic analyses (CHMP/EWP/185990/06)
- 122 - Reflection paper on the use of pharmacogenetics in the pharmacokinetic evaluation of medicinal
123 products EMA/128517/2006
- 124 - ICH harmonised tripartite guideline: Guidance on nonclinical safety studies for the conduct of
125 human clinical trials and marketing authorization for pharmaceuticals M3 (R2)
- 126 - General Considerations for Clinical Trials (ICH topic E8, CPMP/ICH/291/95)
- 127 - Guideline for Good Clinical Practice (ICH E6 (R1), CPMP/ICH/135/95)
- 128 - Structure and Content of Clinical Study Reports (ICH E3, CPMP/ICH/137/95)

129 **4. Pharmacodynamic interactions**

130 The potential for pharmacodynamic interactions should be considered for drugs which compete with
131 each other at the receptor level and/or have similar or opposing pharmacodynamic (therapeutic or
132 adverse) effect. If such drugs are likely to be used concomitantly, pharmacodynamic interaction
133 studies should be considered. However, many of these interactions can be predicted based on the
134 pharmacological effects of each drug. The interactions may be caused by a large variety of
135 mechanisms. It is therefore not possible to give detailed guidance for pharmacodynamic interaction
136 studies. The studies needed should be determined on a case-by-case basis. When similar mechanisms

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

142 **5. Pharmacokinetic interactions**

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

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

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

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

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

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

179 **5.1. Effects of food intake on the pharmacokinetics of the investigational**
180 **drug**

181 The effect of food intake on the rate and extent of absorption of an orally administered investigational
182 drug should be investigated as early as possible during drug development to ensure optimal dosing
183 recommendations in the phase II and III clinical studies.

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

187 The effect of a high-fat meal on the absorption of the investigational drug should be investigated as
188 worst-case scenario. The standardized procedure is presented in Appendix I. If the pharmacokinetics is
189 nonlinear, it is recommended to investigate the effect of food on the highest and lowest dose of the
190 therapeutic range. In general, recommendations regarding time of drug intake in relation to food
191 should aim at minimising variability and obtaining target exposure. If a clinically significant effect of
192 food is found, further food-drug interaction studies are recommended

193 Which studies are relevant to perform depends on whether fed conditions or fasting conditions will be
194 recommended and on how frequently the drug will be administered. If the drug will be recommended
195 to be taken with a meal, studies of the effects of lighter meals are recommended (See Appendix I). If
196 administration is recommended under fasting conditions in the morning, studies should be performed
197 establishing the sufficient fasting time period between drug administration and the intended meal. If
198 the drug will be dosed on an empty stomach, either several times a day or at a time point other than
199 the morning, studies should be performed establishing the time interval before and after a meal when
200 drug administration should be avoided.

201 If co-administration is recommended with a meal or specific food and the drug is indicated in the
202 paediatric population, it should be specified whether this is relevant for paediatric use (especially
203 newborns and infants) whose diet is different (100 % milk in newborns). This may be investigated
204 using the population PK approach.

205 Recommendations regarding interaction studies with special kind of foods (e.g. grapefruit juice) are
206 given in subsection 6 of this document.

207 **5.2. Effects of other medicinal products on the pharmacokinetics of the**
208 **investigational drug**

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

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

219 **5.2.1. Absorption**

220 The investigation of absorption interactions serves to identify situations where the solubility,
221 dissolution or absorption of a drug is altered by intrinsic or extrinsic factors. The studies which should
222 be considered include food interaction studies as well as studies of the effect of increased
223 gastrointestinal pH, sequestration and decreased or increased intestinal active transport. Which studies
224 are needed for a specific medicinal product depend on the mode of administration, bioavailability of the
225 medicinal product and the physicochemical properties of the investigational drug. Interactions at
226 absorption level should be investigated mainly for orally administered drugs and the text below refers
227 to orally administered formulations. However, interactions should be considered also for inhaled and
228 nasally administered products with potential for oral absorption.

229 A. Interactions affecting solubility

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

235 B. Interactions affecting intestinal active transport

236 Involvement of transport proteins (transporters) in drug absorption is evaluated to enable predictions
237 of interactions where the absorption of the drug is altered due to inhibition or induction of these
238 proteins. Inhibition or absence of an intestinal uptake transporter can result in decreased systemic
239 drug exposure and/or lower C_{max}. Inhibition of an intestinal efflux transporter may result in increased
240 systemic drug exposure or increased C_{max} either due to a primary increase in absorption or
241 secondarily due to decreased availability of drug to intestinal drug metabolising enzymes (e.g. CYP3A).
242 It is recommended to investigate the role of transport proteins in drug absorption if there are
243 indications that transporters may be involved in the absorption process and the consequences of
244 modulating this transport may be clinically relevant. Pharmacokinetic indications of clinically relevant
245 transporter involvement in drug absorption include low bioavailability, erratic or dose-dependent
246 absorption, or CYP3A catalysed intestinal drug metabolism as well as unexplained *in vivo* interactions
247 with effects on intestinal absorption as a possible mechanism. Caco-2 cell *in vitro* assays are usually
248 used for these investigations *in vitro* but other systems expressing human transport proteins may also
249 be used. Detailed recommendations on how to study transporter involvement *in vitro* is given in
250 appendix II.

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

256 **5.2.2. Distribution**

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

262

263 A. Distribution interactions due to altered transport

264 Interactions at a transport protein level are expected to give rise to altered distribution of drug to
265 organs where these transporters are expressed. If the investigational drug is a substrate for transport
266 proteins, the potential for clinically relevant interactions should be discussed in light of available data
267 on the tissue specific expression of the transporter, indications from data on distribution in preclinical
268 species, available clinical safety data in patients with reduced transport caused by genetic
269 polymorphism or interactions, as well as the expected clinical consequences of an altered distribution.
270 If indicated and feasible, *in vivo* studies investigating the effect of transporter inhibition on the
271 pharmacokinetics as well as pharmacodynamics (including PD markers for the potential effect on the
272 transporter expressing organ) is recommended. Both target organs for the clinical effect and potential
273 target organs for safety should be considered. Little is presently known about distribution interactions
274 due to transporter induction. As several transport proteins and enzymes are co-regulated, the possible
275 risks and consequences of altered drug distribution during treatment with enzyme inducers could be
276 discussed as far as reasonable.

277 B. Displacement interactions

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

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

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

285 III) has a high renal extraction ratio

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

288 **5.2.3. Metabolism**

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

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

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

306 pathways contributing to $\geq 25\%$ of the oral clearance should if possible be verified *in vivo*. Similarly, if
307 there are metabolites estimated to contribute to more than 50% of the *in vivo* target or off target
308 pharmacological activity, enzymes contributing to $> 25\%$ of the formation or elimination of these
309 metabolites should if possible be quantified. It should be remembered that if the *in vivo* results do not
310 support major involvement of the candidate enzyme, additional *in vitro* and *in vivo* studies are needed
311 to identify the enzyme involved. The contribution of an enzyme *in vivo* may be determined either
312 through an interaction study with a strong inhibitor (see Appendices IV and V) or through investigating
313 the effect of pharmacogenetics on the pharmacokinetics of the drug.

314 Identification of enzymes involved in minor pathways may be needed if these pathways have a marked
315 importance in some subpopulations due to intrinsic or extrinsic factors (see section 5.2.5).

316 The *in vivo* part of the interaction documentation is usually composed of a number of interaction
317 studies, some of these are purely mechanistic, such as studies with potent and moderate inhibitors,
318 aiming at providing the basis for interaction predictions. Other studies may be performed with likely
319 interacting drugs expected to be commonly used concomitantly with the investigational drug aiming to
320 obtain a specific dose recommendation. Studies may also be performed in order to verify the suitability
321 of a proposed dose adjustment or to confirm a lack of interaction with a commonly co-prescribed drug
322 in the target population.

323 In addition, there may be situations where it is expected that co-administered drugs will inhibit more
324 than one elimination pathway of the investigational drug, such as CYP3A inhibitors that also inhibit Pgp
325 mediated renal or biliary excretion. In these cases an interaction study with a drug that is a potent
326 inhibitor of both pathways is recommended if the pathways together represent $\geq 25\%$ of the oral
327 clearance of the investigational drug and the interaction is expected to be clinically relevant. The
328 evaluation of the effect of potent enzyme inducers on the pharmacokinetics of the investigational drug
329 may also be required.

330 A) Interaction studies with inhibitors of cytochrome P450 enzymes

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

339 If the interaction study with the strong inhibitor results in a marked effect on the exposure of the
340 investigational drug, potentially leading to dose adjustments, contraindications or other specific
341 treatment recommendations, an additional study with a moderate inhibitor of the enzyme (known to
342 inhibit 50% to $\leq 80\%$ of the enzyme activity) is recommended in order to support the evaluation of
343 the need for specific treatment recommendations for other inhibitors of the enzyme.

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

350

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

352 If the investigational drug is metabolised by non-cytochrome P450 enzymes and potent specific
353 inhibitors are not available for *in vivo* use, the potential for drug interactions should be discussed in
354 light of published literature. If suitable inhibitors are available for *in vivo* use or if there are genetic
355 poor metabolisers, it is recommended to verify the contribution of the candidate enzyme *in vivo* and to
356 investigate potentially clinically relevant interactions in accordance with the recommendations for
357 drugs metabolised by cytochrome P450 enzymes.

358 C) Interaction studies with inducers

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

364 If concomitant treatment with a specific enzyme inducer is likely to be common and clinically needed,
365 an *in vivo* study investigating the interaction with that particular inducer is recommended in order to
366 establish adequate treatment recommendations. The time dependency of the induction needs to be
367 considered in the study design (see section 5.4).

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

371 **5.2.4. Active uptake and secretion in drug elimination**

372 As inhibition of OATPs has been reported to result in marked increases in the systemic exposure of
373 drugs transported by this subfamily and as involvement of these transporters may be present without
374 any indications from the *in vivo* pharmacokinetic information, the possible involvement of OATP uptake
375 transport should be investigated *in vitro* for non-cationic drugs with $\geq 25\%$ hepatic elimination. As
376 scientific knowledge evolves, other hepatic uptake transporters may need screening if their inhibition
377 generally has been observed to lead to large effects on drug elimination.

378 In line with the requirements of enzyme identification, if renal and biliary secretion account for more
379 than 25% of systemic clearance, attempts should be made to identify the transporter(s) involved in
380 the active secretion. The importance of renal secretion is estimated by comparing total renal clearance
381 to the renal filtration clearance (GFR*fu). Dependent on the information at hand, it may be difficult to
382 estimate the quantitative importance of biliary secretion to total elimination. The importance of biliary
383 secretion should be based on the mass balance data, available interaction data, available
384 pharmacogenetic information, data on hepatic impairment, data on Caco-2 cell permeability etc. Data
385 on bioavailability or i.v. mass balance data can provide important information in quantifying the
386 importance of biliary secretion. If a large fraction of an oral dose is recovered as unchanged drug in
387 faeces, a mass-balance study or bioavailability study should be considered.

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

395 When a candidate transporter has been identified, an *in vivo* study with a potent inhibitor of the
396 transporter at the site of interest is recommended, if interactions through inhibition are likely to be
397 clinically relevant and if known inhibitors are marketed within the EU. *In vivo* studies in subjects of
398 certain genotypes giving rise to markedly altered expression or activity of a certain transporter may be
399 useful in the identification of the transporter involved and may give an indication of the
400 pharmacokinetic consequences of transporter inhibition. However, quantitative extrapolation of such
401 data to drug interactions with inhibitors should be justified based on the published literature. If
402 relevant and possible, inclusion of PD markers is recommended in the *in vivo* studies.

403 Interactions with *in vivo* inhibitors, and inducers if applicable, should be predicted based on the
404 acquired *in vivo* information and the scientific literature. If there are commonly used drug
405 combinations where an interaction is expected, it is recommended to investigate the interaction *in vivo*.
406 The possible effect of transporter inhibition and induction on availability of the investigational drug for
407 metabolism (transporter-enzyme interplay), such as the interplay observed between Pgp and CYP3A,
408 should be discussed, and if needed, an *in vivo* study should be considered.

409 **5.2.5. Special populations**

410 An interaction effect may in general not be directly extrapolated to specific subpopulations that have a
411 markedly different contribution of the affected enzyme to the elimination of the investigational drug.
412 Such subpopulations may include genetic subpopulations such as poor metabolisers, patients of a
413 genotype causing significantly altered transport protein activity, patients with impaired organ function,
414 young pediatric patients (< 2 years) and patients treated with other interacting drugs.

415 The effect of a potent enzyme inhibitor on the exposure of an investigational drug metabolised by the
416 inhibited enzyme is dependent on the quantitative contribution of parallel elimination pathways. If the
417 parallel pathway is renal excretion, the interaction effect will be different in patients with renal
418 impairment. If the parallel pathway is metabolism or biliary excretion, the effect of an interaction will
419 be different in patients with reduced or abolished activity of the enzyme or transporter involved in the
420 pathway. Moreover, in case a very important enzyme for active substance elimination or formation is
421 subject to genetic polymorphism, the enzymes involved in the parallel pathways should be identified. It
422 should be considered that the genetic subgroup may have a completely different set of drug
423 interactions. Worst case predictions of the interaction effects and resulting exposure in such predicted
424 sub populations should be performed. An *in vivo* study investigating the interaction in the
425 subpopulation is recommended if the interaction is likely to lead to a negative risk-benefit of the
426 treatment in the subpopulation. In case a study is not possible, the worst case estimation will serve as
427 basis for the treatment recommendations.

428 The possibility to extrapolate drug-drug interaction results from adults to children should be discussed
429 by the applicant. However, if a drug combination is common and there is a need for a dose
430 recommendation in paediatric patients, an *in vivo* study could be considered. This is further discussed
431 in EMEA/CHMP/EWP/147013/2004 (Guideline on the role of pharmacokinetics in the development of
432 medicinal products in the paediatric population). If an interaction study is needed, a sparse sampling
433 and population pharmacokinetic approach could be considered if satisfactorily performed. The applicant
434 is invited to find ways of providing satisfactory supportive data, such as drug interaction simulations
435 provided that the simulations successfully quantify the observed interaction in adults and the data on
436 enzyme abundance and other physiological parameters in the paediatric population are reliable.

437 **5.3. Effects of the investigational drug on the pharmacokinetics of other**
438 **drugs**

439 Data on the effects of the investigational drug on the pharmacokinetics of other drugs should
440 preferably be available before starting phase II studies unless all concomitant drug treatments at risk
441 of being affected can be avoided in these studies. The information is required before starting phase III.
442 *In vitro* information is often sufficient at this stage. If *in vitro* data indicate that there may be a
443 clinically relevant interaction with a drug that cannot be excluded from the phase II or III studies, it is
444 recommended to perform *in vivo* interaction studies with these drugs prior to phase II or III.
445 Investigational drugs which exhibit dose-dependent- pharmacokinetics unrelated to dissolution or
446 protein binding are likely to inhibit an enzyme or transporter. Likewise, if a drug exhibits time-
447 dependent pharmacokinetics, it is likely to be an inducer or time-dependent inhibitor. (The
448 phenomenon may also be caused by a metabolite.) The mechanism of the non-linearity should
449 therefore, if possible, be identified. Also, if an interaction is observed *in vivo* and the mechanism is not
450 clear, further studies *in vitro* and *in vivo* are recommended to clarify the mechanism of the interaction
451 and to enable prediction of related interactions.

452 **5.3.1. Absorption**

453 If the investigational drug affects gastric emptying or intestinal motility, it may affect the rate and
454 extent of absorption of other drugs. This mainly affects drugs with a narrow therapeutic window,
455 modified release formulations, drugs known to have a physiological absorption window, marked
456 permeability limited absorption or, serious C_{max} related effects. The interaction potential should be
457 considered and, if indicated, the effect should be studied on relevant drugs (e.g. paracetamol as probe
458 substrate in case of effects on gastric emptying). It should be remembered that this is often a systemic
459 effect that may be caused also by parenterally administered drugs. The absorption of other drugs could
460 also be affected through inhibition of intestinal transport proteins. The investigation of the effect of an
461 investigational drug on active transport of other drugs is further discussed in the Elimination
462 subsection below. If the investigation drug increases gastric pH, the effect on other drugs sensitive to
463 this should be predicted and the need for *in vivo* studies considered. Other mechanism of interference
464 with drug absorption, such as complex binding should also be considered.

465 **5.3.2. Distribution**

466 The degree of protein binding of the investigational drug should be determined before phase I. If the
467 investigational drug is extensively protein bound to a specific saturable binding site, the risk of
468 displacement of other drugs known to be subject to clinically relevant displacement interactions should
469 be evaluated *in vitro*. If a clinically relevant interaction is predicted based on *in vitro* data an *in vivo*
470 study could be considered.

471 **5.3.3. Metabolism**

472 The potential of an investigational drug to inhibit or induce the metabolism of other drugs should be
473 investigated. Usually the investigation is initiated by *in vitro* studies and those studies are followed by
474 *in vivo* studies if the *in vitro* data show that an effect *in vivo* cannot be excluded. However, it is also
475 possible to study the effects directly *in vivo*, e.g. by the use of cocktail studies (See section 5.4.2).

476 The *in vitro* studies should include at a wide range of concentrations of the investigational drug. It is
477 recognised that obtaining high concentrations may in some circumstances not be possible due to poor
478 substance solubility or cell toxicity. In these cases, the data is assessed on a case by case basis. If the

479 *in vitro* studies are considered inconclusive, it is recommended that the potential interaction is
480 investigated *in vivo*.

481 A. Enzyme inhibition – *in vitro* studies

482 *In vitro* studies should be performed to investigate whether the investigational drug inhibits the
483 cytochrome P450 enzymes most commonly involved in drug metabolism. These presently include
484 CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A. In the future, more clinically
485 important drug metabolising enzymes may be known and included in this list. In addition, it is
486 recommended to study inhibition of UGTs known to be involved in drug interactions, including UGT1A1
487 and UGT2B7, if one of the major elimination pathways of the investigational drug is direct
488 glucuronidation. Likewise, if the investigational drug is mainly metabolised by an enzyme not listed
489 above, it is recommended to study the inhibitory effect on that specific enzyme if feasible.

490 The potential inhibitory effects of metabolites on the common drug metabolising enzymes should also
491 be considered. As an arbitrary rule, a metabolite present in the circulation in molar total or unbound
492 concentrations (AUC) at least as high as 1/5 of the concentration of the parent compound should be
493 investigated for enzyme inhibitory potential. In addition, if there are indications that an observed *in*
494 *vivo* drug interaction is caused by a metabolite, *in vitro* enzyme inhibition studies on selected
495 metabolites, may provide useful information for the design of future *in vivo* studies and interpretation
496 of *in vivo* interaction study results.

497 An *in vitro* inhibition study could be performed using human liver microsomes, hepatocytes, or other
498 cells expressing the investigated enzyme. The enzyme activity is monitored by investigating the
499 metabolism of a specific marker substrate under linear substrate metabolism conditions. The effect of a
500 range of investigational drug concentrations are investigated and K_i (the inhibition constant i.e.
501 dissociation constant of the inhibitor from the enzyme-inhibitor complex) is determined. If the
502 investigational drug is metabolised by the enzymes present in the incubation, the marker substrate
503 should, if possible, have a markedly faster metabolism rate than the investigational drug to minimize
504 influence of investigational drug metabolism (decreasing concentrations) on the K_i estimation. If this is
505 not possible, the concentration of investigational drug needs to be monitored and the degradation
506 taken into account in the calculations. Known potent inhibitors should be included as positive controls
507 in the study. The concentration range of the investigational drug should be sufficiently high for
508 detecting clinically relevant inhibition. The range suitable depends on the potential site of enzyme
509 inhibition, mode of administration and formulation as well as systemic exposure. If there are reasons
510 to believe that the free inhibitor concentration is markedly lower than the total concentration in the
511 incubation, i.e. if the substance binds covalently to proteins or may adsorb to the walls of the test tube,
512 the free fraction in the incubate should be determined and used in the calculation of K_i . For drugs
513 which are bases, it is recommended to use the estimated or determined unbound drug concentration in
514 the *in vitro* system. As the actual concentration of drug near the enzyme is unknown, the K_i may vary
515 between systems, and concentrations at the portal vein during absorption generally are higher than
516 C_{max} in plasma after oral administration, a safety factor is added in the estimations. Due to the
517 variability connected with estimating the unbound drug fraction for highly protein bound drugs and due
518 to the more pronounced effect of drug-protein dissociation for these drugs, a higher safety margin is
519 applied for drugs which are > 99.0% protein bound. Below, recommendations regarding concentration
520 ranges are given for different situations. If the incubations made indicate that K_i will be markedly
521 higher than the concentrations given below, K_i does not need to be determined.

522 **Intestinal exposure**

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

526 ≤ 50-fold the maximum concentration predicted in the enterocyte using the equation below where
527 Q_{ent} is enterocyte blood flow, f_a is the fraction absorbed, k_a is the absorption rate constant.

528 Eq.1.

529
$$[I]_{gut} = \frac{f_a \times k_a(I) \times Dose(I)}{Q_{ent}}$$

530

531 **Hepatic (and renal) exposure**

532 If the enzyme studied is mainly available in the liver, kidney or another organ with main drug input
533 from the circulation, the concentration range should allow determination of a K_i which is ≤ 50-fold the
534 unbound C_{max} obtained during treatment with the highest dose, or

535 ≤ 250-fold the unbound C_{max} if the protein binding is > 99.0 %

536 **Time-dependent inhibition**

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

541 When time-dependent inhibition is observed, further investigations of the mechanism of the time-
542 dependency are encouraged. If it is shown that the time-dependency is due to formation of a
543 metabolite which reversibly inhibits the affected enzyme, this has consequences for the *in vivo*
544 relevance assessment as well as for the *in vivo* study design (See section 5.4.4).

545 **Evaluation of the need for an in vivo study**

546 I) Reversible inhibition

547 If reversible inhibition (inhibition not affected by pre-incubation) is observed *in vitro*, the risk of
548 inhibition *in vivo* is evaluated by comparing observed K_i values with a worst case estimation of the
549 unbound concentration near the enzyme during clinical use. An inhibition *in vivo* cannot be excluded
550 and an *in vivo* interaction study with a sensitive probe substrate is recommended if the conditions
551 below are fulfilled.

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

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

554 50-fold the maximum concentration predicted in the enterocyte using equation 1.

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

556 K_i < 50-fold the unbound C_{max} obtained during treatment with the highest dose or,

557 250-fold the unbound C_{max} for drugs with a plasma protein binding > 99.0 %

558 If a well performed *in vivo* interaction study with a probe drug does not show enzyme inhibition, these
559 results can be extrapolated to all enzymes observed to be reversibly inhibited *in vitro* for which an
560 equal or higher K_i has been observed. However, due to the intestinal abundance of CYP3A and the
561 higher concentrations of drug present in the intestine after oral administration, lack of inhibition *in vivo*
562 may not be extrapolated from other enzymes to intestinal CYP3A for orally administered drugs.

563 II) Time-dependent inhibition

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

565 Eq. 2

566
$$\frac{k_{deg} + \frac{[I] \times k_{inact}}{([I] + K_i)}}{k_{deg}}$$

567

568

569 where k_{deg} is the degradation constant of the enzyme, k_{inact} is the maximum inactivation rate constant
570 and $[I]$ is the concentration of the inhibitor. The degradation constant may be taken from the scientific
571 literature. If possible, the constant should be based on high quality *in vivo* data. If $\geq 30\%$ inhibition is
572 obtained using the drug concentrations presented above, *in vivo* inhibition may not be excluded and a
573 multiple dose *in vivo* interaction study is recommended (see Section 5.4.4).

574 **Simulations of the interaction potential**

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

586 B. Enzyme inhibition – *in vivo* studies

587 If reversible inhibition has been observed *in vitro*, the pharmacokinetics of a probe drug (see Appendix
588 VI) should be investigated after administration of a single-dose of the probe drug alone and at the
589 steady state concentrations of the investigational drug obtained with the highest usual recommended
590 dose. If the inhibition is time-dependent, this should be reflected in the study design. More information
591 on *in vivo* study design is given in section 5.4.

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

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

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

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

606 of mRNA could also be included and is mandatory for the interpretation of study results if inhibition of
607 the studied enzyme may not be excluded at the concentrations used or if a down-regulation is
608 suspected based on the activity assay. Potent inducers should be included as positive controls to verify
609 functioning regulation pathways via PXR, CAR and the Ah-receptor (GR for investigational drugs with
610 glucocorticoid activity). Other receptors/transcription factors and enzymes may be added to this list as
611 science develops. The positive controls used should be as selective as possible and be chosen based on
612 current scientific knowledge. Currently, rifampicin (20µM) is recommended as positive control for PXR,
613 phenobarbital (0.5-1 mM) and CITCO (≤ 100 nM using mRNA expression) for CAR, omeprazole (25-
614 50µM) for the Ah-receptor and dexamethasone (50µM) for GR. It is acknowledged that at present
615 there is no selective positive control for CAR used for investigations of induction at enzyme activity
616 level. In the future, more selective controls may be available for use.

617 Knowledge about the actual concentration of drug in the system is important for the *in vitro-in vivo*
618 extrapolation. Unless *in vitro* drug metabolism or degradation of drug during culture conditions has
619 been shown to be negligible, the concentration of parent drug in the medium should be measured at
620 several time points the last day of the incubation for determining the actual drug exposure surrounding
621 the cells. Unless the incubations are run under serum-free conditions or degree of protein binding in
622 human plasma is low, the degree of protein binding in the medium should be determined and unbound
623 concentration used throughout the *in vitro* evaluation. The investigational drug concentration range
624 that needs to be investigated depends on enzyme studied, and the *in vivo* pharmacokinetics of the
625 drug. The studied exposure range, i.e. range of average concentration in the media (C_{avg}), should
626 cover the concentrations given above. A. If this is not possible and the study is judged inconclusive,
627 induction (of CYP3A, CYP2B6 and CYP1A2) should be studied *in vivo*.

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

633 The induction results are evaluated separately for each donor and the donor cells with the most
634 pronounced induction effect on the specific enzyme should then be used as a "worst case" in the
635 subsequent calculations. The *in vitro* study is considered negative for enzyme induction if incubations
636 with the investigational drug at the concentrations given in the inhibition part of this section give rise
637 to a less than 50% increase in enzyme activity. However, to certify adequate sensitivity of the assay,
638 any increase in activity or mRNA observed at the given concentrations also has to be less than 20% of
639 the response to rifampicin 20 µM or, for Ah-receptor activation, omeprazole 25-50 microM.

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

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

645 D. Enzyme induction or down-regulation - *in vivo* studies

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

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

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

664 **5.3.4. Transport**

665 A. Inhibition of transport proteins

666 *In vitro* inhibition studies are recommended to investigate whether the investigational drug inhibits any
667 of the transporters known to be involved in clinically relevant *in vivo* drug interactions. Presently, these
668 include P-glycoprotein, OATP1B1, OATP1B3, OCT2, OCT1, OAT1, OAT3 and BCRP). The transporter
669 BSEP should also preferably be included for detecting pharmacodynamic interactions as well for
670 adequate safety monitoring during drug development. The knowledge about transporters and their *in*
671 *vivo* importance is evolving fast. The choice of transporters investigated should be driven by scientific
672 evidence and transporters may be added to or removed from the list as science develops. In addition
673 to the listed transporters, there may also be a need to investigate effects on other transporters to
674 clarify the mechanism of an unexpected interaction observed *in vivo*.

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

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

685 *In vivo* inhibition of intestinally expressed proteins such as Pgp can be excluded if the observed K_i
686 value is \geq 10-fold the maximum dose/250ml. For systemic transporter inhibition, the K_i values should
687 be compared with the highest expected unbound C_{max} as described above. It has been observed that
688 the estimated K_i may be different in separate systems. Therefore, if there is an uncertainty in the K_i
689 estimation, an additional *in vitro* study with another cell system should be considered. If *in vivo*
690 inhibition may not be excluded, an *in vivo* study is recommended. If inhibition may be of relevance at
691 several clinically relevant physiological sites, the study should if possible aim at investigating the
692 extent of inhibition at those sites. For P-glycoprotein, inhibition of intestinal and renal inhibition can be
693 determined using digoxin AUC and renal clearance.

694 B. Induction of transport proteins

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

703 **5.4. Design of *in vivo* studies**

704 The design of the *in vivo* interaction study is adapted to the aim of the study. However, some general
705 considerations are found below. An *in vivo* interaction study usually is of cross-over design. Parallel
706 group design may be used when the potential inhibitor or inducer has a very long elimination half-life,
707 but, due to the wide inter-individual variability in responses, it is generally not recommended. In case
708 it may be suspected that compliance with study treatment may be reduced eg due to a long treatment
709 duration or due to adverse effects, compliance should be checked regularly during the study.
710 Comparisons with historical controls are generally not acceptable. An open study is satisfactory, but
711 blinding should be considered if pharmacodynamic markers are included in the study. Simulations may
712 provide valuable information for optimising the study design.

713 **5.4.1. Study population**

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

722 In a parallel group study, the subjects should be matched for all intrinsic and extrinsic factors known to
723 affect the pharmacokinetics of the studied drug. In a cross-over study, the demographics of the
724 subjects are not of importance unless there are indications that the interaction effect may be
725 significantly affected by such factors. However, genotyping for genes coding for relevant enzymes and
726 transporters of the subjects are generally encouraged. If the pharmacokinetics of the drug is
727 significantly affected by genetic polymorphism and it is expected that patients of a certain genotype
728 have a larger interaction effect, it is recommended that interaction is evaluated separately in that
729 subgroup. Subjects lacking the enzyme potentially inhibited in an interaction study should preferably
730 be excluded from the study unless their inclusion serves to clarify the mechanism of an interaction.

731 **5.4.2. Probe drugs and cocktail studies**

732 *In vivo* studies performed to investigate whether the investigational drug inhibits or induces a drug
733 metabolising enzyme or transporter *in vivo* should be performed with well validated probe drugs. A
734 probe drug is a drug which is exclusively or almost exclusively eliminated through metabolism
735 catalysed by one specific enzyme or eliminated through excretion by one specific transporter *in vivo*. If
736 a second enzyme or transporter is catalysing metabolism of the parent drug, its contribution to total

737 clearance should be very small (<10%). The drug should have a well characterised elimination and
738 enzyme/ transporter contribution *in vivo*, and should have linear pharmacokinetics. Examples of probe
739 drugs for various enzymes are given in Appendix VI. Other drugs than the listed ones may be used if
740 justified. Marker reactions, i.e. metabolic reactions known to be catalysed by only one enzyme may
741 sometimes be used (see below).

742 The probe drug for CYP3A4 should be subject to both marked intestinal and hepatic 3A4 catalysed
743 metabolism. The use of orally administered midazolam is recommended. If the drug is very likely to be
744 administered with i.v. administered CYP3A substrates and a marked effect is found on orally
745 administered midazolam, an interaction study with i.v. midazolam should be considered, to investigate
746 the effect on systemic CYP3A catalysed metabolism, as this enables better interactions predictions. If
747 this approach is chosen, appropriate safety precautions should be made.

748 It is possible to use so called "cocktail studies" investigating the inhibitory or inducing effect of an
749 investigational drug on several enzymes in one *in vivo* study. In this case, it should have been
750 demonstrated *in vivo* that the probe drugs combined in the "cocktail" do not interact with each other.
751 The doses used should preferably be the doses used in this validation. Deviations from this should be
752 justified. Full characterisation of the plasma concentration-time curves of the probe drug is
753 recommended, estimating the effect on (oral) clearance. Use of metabolite to parent drug
754 concentration ratios in plasma or urine is not recommended. If satisfactorily performed, the results of
755 the cocktail studies can be extrapolated to other drugs and be used to support treatment
756 recommendations of the SmPC.

757 If well-documented probe drugs are lacking, it may be chosen to study clearance through a specific
758 pathway as marker for the enzyme catalysing that pathway. If this is chosen, it should be possible to
759 determine the fractional metabolic clearance along this pathway. This is calculated as a ratio between
760 the sum of all primary and secondary metabolites formed through the specific pathway divided by AUC
761 ($\Sigma Ae_{0-\infty}/AUC_{0-\infty}$). If this approach is used, it needs to be verified that parallel pathways are not
762 affected by the investigational drug. Another approach is to recalculate the effect on the enzyme using
763 the fraction of the clearance catalysed by the enzyme if the fraction is well supported by literature data.

764 **5.4.3. Dose, formulation and time of administration**

765 A. The perpetrator drug

766 The systemic exposure of the drug thought to affect the pharmacokinetics of the other drug should
767 generally be the exposure obtained with the highest generally recommended dose under therapeutic
768 (steady state) conditions. If the highest usually expected exposure is not studied, this should be well
769 justified. If a metabolite is responsible for the enzyme inhibition, steady state of the metabolite should
770 have been reached. The duration of the treatment with the perpetrator drug should be long enough to
771 certify that it covers the full plasma concentration-time course (sampling period) of the victim drug
772 (see also section 5.4.4 for time-dependent interactions). If the perpetrator drug is the investigational
773 drug and a dose-range is recommended for the perpetrator drug, studying more dose levels should be
774 considered if a significant effect is found using the highest dose.

775 B. The victim drug

776 If the victim drug has linear pharmacokinetics it is sufficient to investigate the pharmacokinetics of the
777 victim drug after a single-dose with and without treatment with the perpetrator drug. Any dose in the
778 linear range can be used. If the victim drug has dose-dependent pharmacokinetics, the dose used
779 should be the therapeutic dose for which the most pronounced interaction is expected. If the dose-
780 dependency is more pronounced at multiple-dose conditions, a steady state comparison of the

781 pharmacokinetics of the victim drug is recommended. If the victim drug has time-dependent
782 pharmacokinetics, this should be reflected in the study design (see section 5.4.4).

783 When the perpetrator or victim drugs are administered to obtain a steady state exposure, a loading
784 dose regimen may be used to shorten the time needed to reach steady state faster if this is possible
785 from a safety point of view.

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

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

790 C. Formulations

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

799 D. Relative time of administration

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

803 Recommendations of drug administration in relation to food should be followed. If these
804 recommendations are different for the included drugs, this should be considered in the study design.

805 If a large part of the interaction occurs during first-pass, the interaction may be minimised through
806 "staggered dosing", i.e. by separating the administrations of the two drugs in time.

807 **5.4.4. Time dependencies**

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

810 The maximum effect is expected when a new steady state level of the affected enzyme has been
811 obtained. This is dependent on the rate of enzyme turnover (k_{deg}), and on the time needed to reach
812 steady state for the inducer/inhibitor. For time-dependent inhibitors, the course of inhibition is also
813 dependent on the inactivation rate constant (k_{inact}). The processes leading to a new steady state level
814 of active enzyme takes place simultaneously. The required duration of treatment depends on how
815 precisely the interaction effect needs to be determined. If the study aims at investigating whether an
816 investigational drug is an inducer or time-dependent inhibitor *in vivo*, determining 80% of the
817 induction or inhibition effect is sufficient. If the interaction study will be used for dosing
818 recommendations, a study investigating the true maximum effect is needed. The chosen duration
819 should be justified, e.g. by simulations, and the estimated % of maximum induction/inhibition if
820 possible be presented. At present, a range of k_{deg} or enzyme half-life values are reported in the
821 literature. If available, use of reliable *in vivo* estimations is preferred. Based on the presently available
822 information, it appears that 80 hours is a reasonable estimation of the hepatic CYP3A4 half-life. The
823 chosen treatment duration should be justified, e.g. by simulations, where a sensitivity analysis can be

824 made to account for the variability in the reported k_{deg} /enzyme half-lives. A loading dose regimen
825 aiming to reach steady state of the inducer/inhibitor faster may be used as long as the treatment
826 duration at steady state is sufficient for the target fraction of the new steady state enzyme levels to be
827 reached. In case it is valuable to know the effect also at other time points during drug treatment,
828 adding more determinations of the victim drug 's pharmacokinetics is recommended.

829 If "time-dependent" inhibition has been observed to be caused by a metabolite *reversibly* inhibiting the
830 enzyme, the duration of the treatment with the parent drug should be sufficient for steady state of the
831 metabolite to be reached.

832 **5.4.5. Active metabolites**

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

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

842 **5.4.6. Pharmacokinetic parameters**

843 The pharmacokinetic parameters determined should be the ones relevant for the use and interpretation
844 of the study results. Usually such parameters include C_{max} , T_{max} and AUC, CL and the terminal half-
845 life. If C_{min} has been found to be closely related to clinical efficacy or safety, C_{min} should also be
846 investigated. If the binding of a drug to plasma proteins is concentration dependent within the
847 therapeutic concentration range, or if the concentrations of binding proteins may change significantly
848 during the study (eg SHBG, sex hormone binding globulin, for contraceptive steroids), it is
849 recommended to determine both the unbound and total drug concentrations. Unbound concentrations
850 should also be determined when investigating potential displacement interactions.

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

854 **5.4.7. Population pharmacokinetic analysis**

855 If conventional interaction studies *cannot be performed*, the potential for interactions may be
856 investigated in a well performed population PK analysis on high quality data from sparse samples. This
857 approach could also be used to detect unexpected interactions. The method is mainly used to
858 investigate the effects of other drugs on the investigational drug.

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

863 Further, the background information needs to be of high quality. To draw inference from a population
864 analysis the documentation about doses used of concomitant drugs needs to be properly recorded,
865 which includes the dose amount, timing of doses and also whether the patient has been on the

866 concomitant drug for a sufficient time period at the time of blood sampling. Further, with respect to
867 doses, the quantification of the interaction will be dependent on the doses used and a maximum effect
868 of the interaction may be difficult to establish due to this reason. However, the information obtained
869 may still be used in some sense in the product information but need to be worded properly. For
870 example, it may be stated that a population PK analysis based on phase III data, indicated that
871 concomitant treatment with drug X at a dose range y-z mg reduced the systemic exposure by on
872 average w% (range).

873 A sufficient number of patients should be treated with the investigational drug and the concomitantly
874 given drug. A power analysis can be performed a priori to estimate the minimum [effect size](#) that is
875 likely to be detected in a study using a given number of patients on a concomitant drug. The size of
876 the effect that is of interest to be detected should be guided by the therapeutic index of the
877 investigational drug (See also section 5.6.2.). Pooling of data for different drugs, e.g. based on
878 inhibitory potency, should in general not be performed unless the inhibitory or inducing potency is very
879 similar. If possible, it may be advantageous to determine plasma concentration(s) of potentially
880 interacting drugs

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

887 To draw appropriate conclusions from the population analysis the uncertainty in the estimated
888 interaction effects (95% confidence intervals) should be estimated by appropriate methods, i.e.
889 preferably using methods not assuming symmetrical distribution of the confidence interval, e.g.
890 bootstrapping or log-likelihood profiling. Such uncertainty analysis is of importance when the aim is to
891 claim no effect of a concomitant drug, as well as when significant effects have been identified.
892 Depending on the width of this confidence interval, the uncertainty of the conclusion (lack of an
893 interaction and/or clinical relevance of an interaction) can be assessed.

894 ***5.5. Presentation of in vivo study results in the study report***

895 Individual data on pharmacokinetic parameters should be listed with and without co-administration of
896 the interacting drug. Standard descriptive statistics for each treatment group, including mean,
897 standard deviation, range should be provided for the pharmacokinetic parameters. The parameters
898 representing drug exposure (e.g. C_{max} and AUC) could be presented as box-whiskers-plots with and
899 without concomitant medication. The plots should include the individual data points either overlaid or
900 next to the boxes. A comparison of the individual pharmacokinetic parameters with and without
901 concomitant medication should also be presented graphically e.g. as spaghetti-plots connecting the
902 data points with and without co-administration within each individual. All subjects or patients who have
903 been included in the study should be included in the statistical analysis. However, if a subject has
904 dropped out from the study or has no measurable plasma concentration during a treatment period and
905 this is unlikely to be due to the interaction, the subject can be excluded from analysis of effects related
906 to that period. Exclusion of subjects for other reasons than the ones above should be well justified and
907 specified in the study protocol. The interaction effect should be calculated and the change in relevant
908 pharmacokinetic parameters presented. Individual changes in pharmacokinetic parameters should be
909 listed together with descriptive statistics, including the 90% confidence interval and the 95% prediction
910 interval for the interaction effect.

911 If the pharmacokinetics of active metabolites has been investigated, the data should be presented in a
912 similar way for the metabolites. If suitable, the total exposure of active species, i.e. the sum of the
913 unbound exposure of pharmacological equivalents, should be presented in addition to the effects on
914 the separate substances. However, this estimation is only correct if the distributions of parent drug and
915 metabolite to the target site(s) are similar. The validity of this assumption should be discussed and, if
916 possible, the calculations could be modified by the metabolite to parent target organ distribution ratio.

917 **5.6. Translation into treatment recommendations**

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

922 **5.6.1. In vitro data**

923 If positive *in vitro* studies have not been followed by *in vivo* studies, eg in cases where *in vivo* probe
924 drugs are not available, or if an interaction of non-studied enzymes is expected based on mechanistic
925 knowledge (eg co-regulated enzymes and transporters affected by induction), the potential
926 implications should be discussed based on available scientific literature, and if possible translated into
927 treatment recommendations.

928 **5.6.2. In vivo effects of other drugs on the investigational drug**

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

937 Treatment recommendations should ensure that patients receive drug treatment which is effective and
938 safe. The evaluation should be based on information available on the relationship between exposure
939 and efficacy/safety. If possible, a well justified target range for relevant exposure parameters should
940 be presented for the investigational drug specifying what change in exposure would justify a posology
941 adjustment. If the target range is based on drug exposure in patients and the interaction study was
942 performed in healthy volunteers, potential differences in the pharmacokinetics between patients and
943 healthy volunteers needs to be considered. The observed exposure (box-whiskers plots including
944 individual data), should be analysed with respect to target criteria taking into account the frequency of
945 patients with lower as well as higher exposure than the target range and the clinical consequences of
946 these deviations. For individually dose-titrated drugs, the data should be analysed with respect to
947 relative individual increase or decrease in exposure.

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

953 Presence of active metabolites should be considered when proposing dose adjustments. When relevant,
954 the active moiety can be used to develop dose adjustment (see section 5.5). However, increased
955 exposure must also be considered from a safety perspective and the exposure of all relevant active
956 substances should as far as possible be within a well tolerated range after dose adjustment. If dose
957 adjusting for the effects by an inducing drug, the consequences of the potential increase in exposure of
958 pharmacologically active metabolites formed through the induced pathway(s), should be discussed.

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

964 If proposing a dose-adjustment based on C_{min} (either during the evaluation of a general dose
965 adjustment or if proposing a dose-adjustment within the subject based on C_{min}), the possibility on an
966 altered relation between C_{min} and AUC should be considered if the systemic elimination of the drug is
967 changed.

968 If the interaction is time-dependent, the time course needs to be taken into account in the
969 development of dosage recommendations. Different recommendations might be needed at different
970 time points.

971 If the interaction is expected to have severe consequences and there is no possibility of normalising
972 the exposure through dose adjustment, the drug combination should be avoided. The benefit –risk of
973 the combination should be included in the evaluation e.g. some combinations may be necessary even
974 at increased risk. If the consequences of the interaction are not severe and/or considered manageable
975 through additional safety or efficacy monitoring, this should be clearly recommended in the SmPC. As
976 clear advice as possible on the practical management of the situation should be given. An additional
977 solution for management of drug interactions is Therapeutic Drug Monitoring (TDM). This is mainly
978 applicable if there is a well established therapeutic range. However, TDM may also be used to aid dose
979 adjustment of drugs for which the target concentration differs between individuals, setting the
980 individual baseline concentration (prior to the interaction) as target concentration. If TDM is
981 recommended, advice on sampling days and times should be given in the SmPC. Additionally, the need
982 for non-TDM guided dose adjustment on the first treatment days should be discussed.

983 Treatment recommendations should include recommendations for patients who carry certain
984 characteristics leading to a different interaction effect and who may have specific important
985 interactions. If pharmacogenetic testing is not performed before the combination treatment is started,
986 the recommendation in all patients should be suitable also for the subpopulation.

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

989 **5.6.3. *In vivo* effects of the investigational drug on other drugs**

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

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

996 Interactions studied with the probe drugs are mainly intended for the evaluation of the extent of
997 inhibition or induction of an enzyme or transporter by the investigational drug. The data is used to
998 predict interactions with other drugs which are substrates for the same enzyme or transporter. The
999 clinical relevance of the effect on exposure of the probe drug *per se* is evaluated, but more focus is
1000 often put on absence or presence of an effect and the magnitude of the mean effect.

1001 *In vivo* enzyme inhibitors and inducers should, if possible, be classified as either mild, moderate or
1002 potent inhibitors or inducers (See Appendix IV). The induction results are qualitatively extrapolated to
1003 co-regulated enzymes and transporters in case induction of these proteins has not been quantified *in*
1004 *vivo*. Based on the *in vivo* inhibition and induction studies with the probe drugs, other drugs which are
1005 substrates for the enzyme/transporter and likely to be affected in a clinically significant manner should
1006 be discussed and adequate treatment recommendations presented.

1007 **5.6.4. Food effects**

1008 If food interaction studies indicate that there should be specific recommendations on how to take the
1009 drug in relation to food, clear information about this should be given in the SPC. Whether such a
1010 recommendation is needed and which the recommendation should be, depends on intra- and inter-
1011 individual variability, potential recommendations regarding concomitant food intake in the pivotal
1012 clinical phase III studies, as well as the relationship between concentration and effect of the drug. This
1013 is further described in section 3.1.1.1. Recommended wordings for recommendations regarding food
1014 intake are given in section Appendix VII

1015 **6. Herbal medicinal products and specific food products**

1016 Usually information about interactions between medicinal products and herbal medicinal products or
1017 specific food products such as grapefruit juice is based on the scientific literature and translated into
1018 general recommendations regarding use of the food products or herbal products containing a specific
1019 component. The interaction potential of one specific herbal medicinal product or food product is difficult
1020 to extrapolate to other products produced from the same raw source material. Usually, the interacting
1021 substances have not been sufficiently well identified and therefore analysis of the product contents
1022 may not be used to make safe extrapolation of the magnitude of the interaction effect. For new herbal
1023 preparations the potential for interactions should be investigated. For traditional and well-established
1024 herbal preparations the potential for interaction should be clarified if reports point to clinically relevant
1025 interactions in humans

1026 Usually there are no data on the pharmacokinetics of the constituents of herbal preparations or herbal
1027 substances and consequently the *in vivo* relevance of *in vitro* data cannot be assessed. However, if
1028 available *in vivo* information indicates that herbal preparations or the constituents of the herbal
1029 preparation may give rise to clinically relevant drug-interactions, *in vitro* studies on the enzyme
1030 inhibitory potential of the constituents or the herbal preparations are encouraged as such studies may
1031 facilitate the setting of causal relationship and appropriate specifications. In case an *in vivo* interaction
1032 study is not performed with a certain herbal preparation, available information on other preparations of
1033 the herbal component(s) may be extrapolated to its labelling as a precautionary measure. To obtain
1034 product specific information in the labelling of a specific herbal product, an *in vivo* drug interaction
1035 study with that product should be considered. Such interaction studies could involve probe drugs if
1036 appropriate.

1037 If there is a wish to investigate the effect of a herbal medicinal product or a special kind of food (e.g.
1038 grapefruit juice) on the pharmacokinetics of a medicinal product in order to substantiate the
1039 information about the interaction in the SmPC, effort should be made to choose a specific herbal or

1040 food product and mode of intake of the product known to give a marked interaction effect. Inclusion of
1041 a probe drug in the study, i.e. a drug shown to interact with the herbal or food component, could be
1042 considered to verify the sensitivity of the study. The magnitude of the interaction could be given in the
1043 SmPC of the medicinal product but together with information on the difficulty to extrapolate the
1044 magnitude to combinations with other herbal medicinal products or food products.

1045 **7. Inclusion of information and recommendations in the** 1046 **SmPC**

1047 The guideline on summary of product characteristics (SmPC) (September 2009 - Rules governing
1048 medicinal products in the European Union Volume 2C Notice to applicants) advice on how to present
1049 information about interactions.

1050 Information about drug interactions should be presented in the SmPC section 4.5 and 5.2 (e.g. for
1051 food-interactions) and cross-referring to the sections 4.2, 4.3 or-4.4 if relevant. Section 4.5 should
1052 contain all detailed information on drug interactions and only the recommendation should be given in
1053 the cross-referred sections.

1054 In section 4.5 interactions affecting the investigational drug should be given first, followed by
1055 interactions resulting in effects on other drugs. Inside these subsections, the order of presentation
1056 should be contraindicated combinations, those where concomitant use is not recommended, followed
1057 by others.

1058 Clear treatment recommendations should be given to the prescriber. Wording such as "caution is
1059 advised" should be avoided in favour of a recommendation on proposed actions. The need for time-
1060 specific information and recommendations should be considered. Situations when this is needed
1061 include time dependent interactions such as induction or mechanism based inhibition, drugs with long
1062 half-lives, etc. The estimated course of onset of the interaction as well as the time-course after ending
1063 concomitant treatment should be given and, when relevant, time-specific recommendations. If it is
1064 likely that the interaction effect would be different with another dose or at another time point than the
1065 one studied, this should be reflected in the recommendations.

1066 Information on absence of interactions (supported by in vivo data) could be reported briefly if
1067 considered of interest to the prescriber.

1068 In special circumstances, where there are very limited therapeutic alternatives due to marked
1069 interactions with most drugs of the same class, examples of less interacting drugs could be given as
1070 assistance for the prescriber.

1071 When relevant, the interaction potential in specific populations, such as children or patients with
1072 impaired renal function, should be addressed.

1073 ***7.1. Mechanistic information and prediction of non-studied interactions***

1074 Brief information about the major enzymes involved in the elimination of the drug, transporters with a
1075 major impact on absorption, distribution or elimination of the drug as well as effects of the
1076 investigational drug on enzymes and transporters could be summarised in section 4.5 as a mechanistic
1077 basis for the interaction information. If *in vitro* data indicate that a medicinal product affects an
1078 enzyme or a transporter but the available scientific knowledge does not allow predictions of
1079 interactions *in vivo*, in it is recommended to include the *in vitro* information in the SmPC Section 5.2
1080 for future use. Based on the mechanism of the interaction, the results of the interaction studies should
1081 be extrapolated to other drugs. It is recommended to include a list of drugs likely to be affected to a
1082 clinically relevant extent in the SmPC to assist the prescriber. The list should be as extensive as

1083 possible and it should be indicated that the list probably does not cover all relevant drugs. In some
1084 instances such a list may be too long, such as when the investigational drug affects a very important
1085 drug metabolising enzyme (e.g. CYP3A4). In this case, drugs should be selected for inclusion based on
1086 the severity of the clinical consequences of the interaction. e.g. "Drug X is a potent inhibitor of CYP3A4
1087 and may therefore markedly increase the systemic exposure of drugs metabolised by this enzyme such
1088 as ..." or "Drug X is mainly metabolised by CYP3A4. Concomitant use of drugs which are potent
1089 inhibitors of this enzyme, such as, are not recommended". The most important drugs should be
1090 included in such a list to aid the prescriber.

1091 **7.2. Presentation of study results in the SmPC**

1092 The results of the study should be presented as mean effect on the most important exposure
1093 parameter. In specific cases where considered relevant for the prescriber, the variability of the effect
1094 can be given. Results of interaction studies used to predict other drug-interactions on a mechanistic
1095 basis eg interaction studies with probe drugs as victim drugs, should be included. even if the
1096 interaction effect is not clinically relevant for the victim drug studied. Brief, condensed, specific
1097 information on the study design relevant for the interpretation of that particular study results should
1098 be included when appropriate. Such information includes dose (in case a dose range is used for the
1099 interacting drugs or if the therapeutic dose has not been used in the study), as well as timing and
1100 duration of treatment (if a time-dependent interaction has been investigated but full induction has not
1101 been obtained). In case the interaction effect may be significantly different with a different dose or
1102 when the full time-dependent interaction has been obtained, this should be stated in the S mPC. The
1103 conclusions of *in vitro* studies indicating an effect on other drugs should be presented if no *in vivo*
1104 information is available. However, otherwise, the conclusions of *in vitro* studies should be reported in
1105 section 5.2.

1106 The effect of concomitant food intake on the pharmacokinetics should be presented in section 5.2 and
1107 clear recommendations given in section 4.2 (see Appendix VII).

1108 **Definitions**

1109	Ae	amount of parent drug excreted unchanged in urine
1110	AhR	aryl hydrocarbon receptor
1111	AUC	area under the plasma concentration-time curve CAR constitutive androstane receptor
1112	CAR	constitutive androstane receptor
1113	Cavg	average concentration
1114	CITCO:	(6-(4-chlorophenyl)imidazo[2,1-beta][1,3]thiazole-5-carbaldehyde-O-(3,4-
1115		dichlorobenzyl)oxime)
1116	Cmax	peak concentration
1117	Cmin	trough concentration
1118	CL	clearance
1119	f _a	fraction absorbed
1120	GFR	glomerular filtration rate
1121	GR	glucocorticoid receptor
1122	k _a	absorption rate constant.
1123	Ki	inhibition constant
1124	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	Q _{ent}	enterocyte blood flow
1128	SmPC	summary of product characteristics
1129	T _{max}	time when C _{max} occurs

1130 **Appendix I**

1131 **Standard procedures for food interaction studies**

1132 A single-dose of the investigational drug is administered with 240 ml of water after a 10-hour fasting
1133 period and 30 minutes after intake of a meal has been started. Except for the meal, the subjects
1134 should refrain from food for at least 4 hours after dosing and all food intake should be standardised for
1135 at least 12 hours post-dose. The recommended composition of a high fat meal and a lighter meal are
1136 described below. If the pharmacokinetics is linear, i.e. the exposure of drug is dose-proportional in the
1137 therapeutic dose range, there are no special requirements with respect to the dose investigated. If the
1138 pharmacokinetics is nonlinear, it is recommended to investigate the effect of food on the highest and
1139 lowest dose of the therapeutic range.

1140 *The standardised high-fat meal*

1141 The high fat meal should contain 800-1000 kcal with 500-600 kcal from fat and 250 kcal from
1142 carbohydrates. A typical standard test meal can be two eggs fried in butter, two strips of bacon, two
1143 slices of toast with butter, 120 ml of hash brown potatoes and 240 ml of whole milk. Substitutions in
1144 this test meal can be made as long as the meal provides similar amounts of calories from protein,
1145 carbohydrate, and fat and has comparable meal volume and viscosity.

1146 *The lighter meal*

1147 The lighter meal could contain approximately 400-500 kcal with fat contributing to ca. 250-300 kcal.

1148 **Appendix II**

1149 ***In vitro* investigations of involvement of transporters in drug absorption**

1150 The *in vitro* study needs to be performed under well-controlled conditions. The permeability of the drug
1151 should be investigated in both directions, preferably under sink conditions (the concentration on the
1152 receiver side is less than 10% of the concentration on the donor side, obtainable through repeated
1153 changes of the receiver well), for at least four different physiologically relevant concentrations. For
1154 intestinal transport the studied range could be 0.1 to 50-fold the dose/250 ml). If systemic (post
1155 absorption) transport is investigated, the concentration range could be 0.1-fold to 50-fold unbound
1156 C_{max}. If the study is not performed under sink conditions this needs to be compensated for in the
1157 calculations. If a proton gradient is used in the study, the degree of ionisation should be discussed. It
1158 is recommended that a pH of 7.4 is used on both sides when efflux is investigated. Determination of
1159 mass-balance (% recovery of the applied amount of drug in the receiver and donor side) is
1160 recommended unless the absorption predicted from the *in vitro* data is complete. The impact of
1161 solubility, potential degradation and metabolism of the drug substance *in vitro* on study results should
1162 be discussed as well as the effect of organic solvents used.

1163 In Caco-2 cell studies, the permeation of drug from the apical (A) to the basolateral (B) side of the
1164 cells are compared with the permeability of the permeation in the opposite direction (B to A). If the
1165 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
1166 uptake, respectively. If active transport is concluded, the importance of the transporter for drug
1167 absorption can be estimated through a comparison of the permeability in absence and presence of the
1168 transporter in Caco-2 cells. To estimate the permeability in absence of transporters, the permeability
1169 constant is determined at concentrations high enough to completely saturate the transporters. The
1170 investigation should include a high and low permeable control (e.g. metoprolol and mannitol). If the
1171 permeability in absence of transporters is high (\geq the permeability constant of the highly permeable
1172 drug metoprolol), the effect of active drug transport will be negligible as compared to the passive,
1173 concentration-gradient driven, absorption of the drug.

1174 If pronounced uptake or efflux transport is observed *in vitro*, and the permeability constant is not
1175 classified as high, attempts should be made to identify the transporter. The identification may be done
1176 *in vitro* through transport studies intended to isolate the effect of a specific transporter. *In vitro* studies
1177 investigating drug transport with and without presence of the specific transporter activity are usually
1178 the first steps in the identification process. It is recommended to use a eukaryote system where the
1179 physiological functions of the transporter are preserved. The concentrations of investigational drug
1180 should be relevant to the site of transport (see above). The study should include positive controls
1181 verifying presence of the specific transporter activity. The choice of controls and inhibitors should be
1182 justified by appropriate scientific references.

1183 **Appendix III**

1184 **Investigations of which enzymes are catalysing the main elimination pathways**

1185 The metabolism of an investigational drug, and the formation and metabolism of clinically relevant
1186 active metabolites, is usually first investigated in *in vitro* incubations with human liver microsomes,
1187 hepatocytes, cells expressing human enzymes, liver S9 fractions etc, depending on which enzymes are
1188 investigated and access to *in vitro* systems. The *in vitro* system used should be carefully considered
1189 when interpreting study results. These are examples of *in vitro* systems for liver metabolism studies.
1190 Positive controls for each enzyme studied should be included.

- 1191 • Supersomes® and other recombinant enzyme systems are a single enzyme system. Since these
1192 usually contain one single drug metabolising enzyme, they are the most sensitive to investigate
1193 whether metabolism can take place via a particular enzyme.
- 1194 • Human liver microsomes (HLMs) contain major drug-metabolizing CYP and UGT enzymes.
1195 Incubations are made with HLMs from several donors either pooled or run separately. HLMs are
1196 in general robust and in general their enzyme activities are easily maintained.
- 1197 • Subcellular liver fractions (S9 fraction or homogenate) contain both cytosolic and microsomal
1198 enzymes. Hence, in addition to the CYP enzymes they enable investigation of enzymes normally
1199 present in the cytosol such as sulfotransferases, glutathione transferases and other phase II
1200 enzymes, as well as aldehyde dehydrogenases, alcohol dehydrogenases.
- 1201 • Intact hepatocytes (freshly isolated, cultured or cryopreserved) contain the whole complement of
1202 enzymes and may also express transporters. Hepatocytes lose their enzyme activity more easily
1203 than the other systems listed.

1204 The *in vitro* metabolism studies should be performed at physiologically relevant concentrations under
1205 linear conditions. In multi-enzyme systems, enzyme specific inhibitors (see table 1) are added to
1206 evaluate the contribution of separate enzymes to the metabolism of the investigational drug. In cases
1207 where the inhibitor is not very specific, it is recommended to perform the study in an *in vitro* system
1208 where no other CYPs than the particular enzyme is expressed. The metabolism may be investigated as
1209 rate of disappearance of drug and/or as formation of metabolites. If possible, it is recommended to
1210 follow metabolite formation to enable the identification of the metabolic pathway catalysed by a
1211 particular enzyme. Positive controls (marker substrates) for enzyme activity (see table 2) should be
1212 included in the study. If the main enzymes involved in the *in vitro* metabolism are identified, one *in*
1213 *vitro* system may be enough for this investigation. However, it is generally recommended to verify the
1214 results by performing studies in another *in vitro* system. If no or little metabolism is observed *in vitro*
1215 but is present *in vivo*, effort should be made based on structure and published data to find an *in vitro*
1216 system with which the enzyme involved may be identified. In table 1 and 2 examples of well validated
1217 specific inhibitors and marker reactions/substrates are given. Please check the available literature
1218 regarding which concentration to use in the *in vitro* incubations.

1219 **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

1220 *presently no specific inhibitor known for *in vitro* use. Listed inhibitor(s) are not specific but can be
 1221 used together with other information or in a mono-enzyme system.

1222 **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 6 β -hydroxylation, plus one structurally unrelated substance such as nifedipine, triazolam or dexamethasone

1223 The *in vitro* data are combined with *in vivo* data such as results from an *in vivo* mass-balance study
 1224 with investigational drug in order to predict which elimination pathways are the main pathways *in vivo*.
 1225 The drug is administered with a radioactive label in a metabolically stable position. In the mass-
 1226 balance study, the systemic exposure of parent drug and metabolites in relation to total exposure of
 1227 radioactive material is obtained as well as the excretion of parent drug and metabolites in urine and
 1228 faeces. The radiolabel should be in an as metabolically inert position as possible. In some cases two
 1229 separate labelling positions have to be used to follow the fate of the investigational drug. Effort should
 1230 be made to identify as much of the dose related material as possible. It is generally recommended that
 1231 metabolites having an AUC \geq 20% of parent AUC, or contributing to > 5% of the total radioactivity
 1232 AUC are structurally characterised. Preferably total recovery of radioactivity in urine and faeces should
 1233 exceed 90% of the dose and more than 80% of the recovered radioactivity identified.

1234 A likely metabolism schedule is proposed based on knowledge of possible metabolic reactions and
 1235 metabolites observed in the study. The quantitative contribution of the different elimination pathways
 1236 are estimated based on the amount of dose excreted as primary and secondary metabolites along
 1237 specific routes. In case there is marked elimination of unchanged drug in faeces, additional studies
 1238 may be needed to quantify the contribution of biliary excretion to drug elimination. Such studies
 1239 include determination of the oral bioavailability of the formulation used in the mass-balance study or
 1240 an investigation of mass-balance after i.v. administration (providing information on fecal excretion of

1241 unchanged drug). I.v. mass-balance data may also be useful in situations with pronounced luminal
1242 metabolism to assess the contribution of metabolism pathways to systemic clearance.

1243 If the pharmacokinetics of the drug is linear in the therapeutic dose range, the mass-balance data
1244 could be extrapolated from the dose of the mass-balance study to any dose administered in the range.
1245 However, in case the elimination shows dose-dependency, this should be considered when
1246 extrapolating the data to other doses than the one administered in the mass-balance study. In addition,
1247 if (oral) clearance under multiple-dose conditions is different from at single-dose conditions using the
1248 same dose, extrapolation of the results to the steady state situation should be performed with caution
1249 and investigation of mass-balance after a single radiolabelled dose at steady state conditions could be
1250 considered.

1251 **Appendix IV**

1252 **Classification of inhibitors and inducers according to potency.**

1253 Enzyme inhibitors may be classified based on their potency, i.e. magnitude of the mean effect on oral
1254 clearance. A drug that causes a > 5 fold increase in the plasma AUC values or $\geq 80\%$ decrease in oral
1255 clearance is classified as strong inhibitor, a moderate inhibitor causes a > 2-fold increase in the plasma
1256 AUC or $50 - \leq 80\%$ inhibition of oral clearance, a mild inhibitor causes 1.25 to 2 fold increase in the
1257 plasma AUC or $\leq 50\%$ inhibition of oral clearance. Depending on the probe drug used and its
1258 bioavailability, the increase in AUC may be somewhat different. This is especially the case for
1259 substrates of the CYP3A subfamily, due to varying extent of intestinal first-pass metabolism. Therefore
1260 oral midazolam should always be used when classifying a drug as a CYP3A inhibitor.

1261 Inducers of CYP3A should be classified based on the effect on oral midazolam clearance or plasma AUC.
1262 $A \leq 50\%$, $> 50 - \leq 80\%$ and $> 80\%$ reduction in midazolam AUC after oral administration classifies an
1263 investigational drug as mild, moderate and strong inducer, respectively. Induction of other enzymes,
1264 should if possible be classified in a similar way if the effect was investigated using an orally
1265 administered probe drug metabolised practically exclusively by that enzyme.

1266 **Appendix V**

1267 **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

1268 *moderate inhibitors as no strong inhibitors are presently available or suitable for *in vivo* use. If
1269 possible, investigating the effect of pharmacogenetics may be preferable for quantifying enzyme
1270 contribution.

1271 **Appendix VI**

1272 **Probe drugs**

1273 A probe drug is a drug which is metabolised mainly through one enzyme *in vivo*. The enzyme
1274 contribution should have been supported by well performed *in vivo* studies. Below is a list of probe
1275 drugs for use in interaction studies. Other probe drugs may be used if justified through available
1276 scientific literature.

1277 **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

1278 *There is no well-documented probe-drug at present but these alternatives may be used' (See section
1279 5.4.2). Well validated probe drugs of these enzymes may be established in the future and it is
1280 advisable to follow the scientific literature.

1281 **Appendix VII**

1282 **Preferred wordings for recommendations regarding food intake**

1283 The effect of concomitant food intake on the pharmacokinetics should be presented in section 5.2 and
1284 clear recommendations given in section 4.2. These are the preferred wordings in recommendations
1285 regarding drug intake in relation to meals:

1286 *[Medicinal product] can be taken with or without meals.*

1287 *[Medicinal product] should be taken on an empty stomach, at least X hours before or X hours after a*
1288 *meal.*

1289 *[Medicinal product] should be taken on an empty stomach 1 hour before breakfast*

1290 *[Medicinal product] should be taken together with a meal.*

1291 *[Medicinal product] should be taken with a light meal.*