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REVIEW

The Importance of the Human Mass Balance Study in Regulatory Submissions

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The human mass balance study is a key study in the Clinical Pharmacology package of new drug applications. This study, along with the mass balance studies in toxicology species, provides essential information on the exposure of the parent compound and metabolites. Despite current regulatory guidance and previous publications, a lack of this study, or deficiencies in the study, are still seen in regulatory submissions today. This restricts the assessment of the benefit/risk in all populations and on the potential for drug–drug interactions leading to unnecessary precautions in the label. A review of new drug applications identifies a number of examples of inadequate characterization of circulating drug-related components or of elimination pathways, with questions raised during the regulatory review. In light of this, new insight is given on what is required from the mass balance study and on how to ensure sufficient information is captured.

The human mass balance study is one of the most informative studies in the clinical pharmacology package needed for understanding the pharmacokinetic (PK) properties of a new chemical entity. This package usually consists of single-ascending and multiple-ascending dose PK studies, interaction studies (including food interaction), influence of organ impairment (e.g., renal and hepatic), PK studies in other special populations, and mass balance studies. These studies form a part of the data set reviewed in a marketing authorization application (MAA) and thus included in the risk–benefit assessment for a new medical product within the European Union.[1]

The human mass balance study, also often referred to as the absorption, distribution, metabolism, and excretion (ADME) study, has gathered much attention in the scientific literature, and it can be very work intensive and costly and thus is often performed late in the clinical pharmacology characterization of new drugs. For regulatory purposes it has the following two major objectives: identify and quantify circulating parent and metabolites and elucidate the elimination pathways of the medicinal product. The first objective should, for example, explore whether there are any metabolites contributing substantially to the safety profile of the drug substance that may warrant standalone nonclinical characterization.[2] Furthermore, it should be evaluated if any metabolite could contribute to the pharmacological activity or have a potential risk to cause drug interactions and should be investigated for enzyme inhibition or induction *in vitro*.[3] The second objective is related to understanding of the fraction absorbed (F_a) and clearance mechanism and whether further studies are needed in, for example, subjects with organ impairment and/or if any clinical drug–drug interaction (DDI) studies are warranted. The human mass balance study is most commonly performed using a radiolabeled version of the medical product to be investigated. In the rare cases where a drug is well absorbed and there is no metabolism and the parent compound is completely excreted via the kidney, then the lack of a radiolabeled mass balance studies could be considered acceptable.

Although human metabolites related to safety testing was discussed in scientific literature from a much earlier date,[4] the first regulatory guidance for the importance of metabolite identification was initially highlighted by the US Food and Drug Administration (FDA; 2008 guidance for industry).[5] In this publication, a metabolite of concern was expressed as one that had a systemic area under the plasma concentration curve (AUC) at steady state of > 10% that of the parent. This was rapidly adopted and debated by the pharmaceutical industry, but the relevance of referring to the circulating parent quantity was questioned. This was particularly of relevance to compounds that are highly metabolized, and thus the quantity of the parent itself may be low.

In 2009, the International Council for Harmonisation (ICH; M3 (R2)) guideline[2] was published that changed the focus to specify that human metabolites > 10% of total drug-related exposure required evaluation. It was recognized, however, that in the case of a daily administered dose that is < 10 mg, greater fractions of the drug-related material might be more appropriate triggers for testing. These considerations are often referred to as the metabolite in safety testing (MIST) approach.[4] In 2012, the ICH published a question-and-answer document on this guidance, including clarity on a number of aspects around metabolites.[6] The main European Union regulatory guidance for performing and interpreting the human mass balance study can be found in the guidance on the investigation of drug interaction.[3] In addition, several reviews on the conduct of mass balance studies have been published from both academia and the pharmaceutical industry.[7–10] A book chapter[11] was also published by the FDA describing regulatory aspects of the mass balance study. It is

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now nearly 10 years since the ICH M3 (R2) guideline[2] came into force at the end of 2009, and reviews of the decade of use have already been discussed in at least two articles.[12,13]

According to the European Medicines Agency (EMA) guideline,[3] the results of a mass balance study should generally be available before starting phase III clinical trials. In general, during the past 10 years, there has been a shift to conduct the human mass balance study earlier in clinical development,[11] i.e., before the demonstration of efficacy. Timing may also differ depending on the indication, e.g., within oncology[14] where the time in development may be shorter in the clinical phase when compared with other indications[15] and therefore may need to start before phase II is final. The earlier timing of the mass balance study is an advantage not only because of the efficient handling of possible metabolites that need safety testing but also it lends itself to a more complete package at the time of marketing authorization as elucidation of major elimination pathways can be time consuming and often includes clinical interaction studies.

The mass balance study is one of the most frequent areas of regulatory concern during MAA review in the clinical pharmacology field. During the scientific evaluation of a stand-alone application (new chemical entitys and known active substances), these questions are often considered essential to be resolved prior to an authorization being granted (major objection). In the present retrospective analysis, cases where questions (other concerns and major objections) have been raised related to the human mass balance study in centralized procedures during the committee for medicinal products for human use (CHMP) evaluation will be discussed. Furthermore, recommendations or best practices for some aspects of planning and performing the mass balance study will be given.

METHODOLOGY

The EMA electronic records management system was searched using the term "mass balance" for relevant documentation, including the different assessment reports generated during the evaluation of applications, to identify those that included reference to mass balance studies during the period 2010–2019. In addition, a search was used that had identified all clinical pharmacology major objections that arose in the initial assessment process of all applications for new MAAs from 2013 to 2017. Of the 472 new drug applications from 2013 to 2017, 10 had a major objection related to ADME or metabolism data. A list of drugs was compiled that had questions related to ADME characterization and the initial European public assessment report (EPAR) from the initial assessment available as of January 2019 for each accessed to collect all available data on the elimination and metabolite characterization. This search was also supplemented with input from the authors based on their personal experience.

ISSUES WITH PLASMA METABOLITE IDENTIFICATION IN DOSSIERS FOR MARKETING AUTHORIZATIONS

Knowledge of drug-related components circulating in plasma is essential to allow a good understanding of the exposure–efficacy and exposure–safety response relationships of a new drug.[16] The majority of drug-related components should be identified, and their biological activity determined. In addition, metabolites with > 10% of total drug-related exposure will need to be characterized in at least one of the nonclinical species in the toxicity evaluation as per the ICH M3 guidance.[2]

In the EMA drug interaction guidance[3] (Appendix V of the guidance), consistent with the MIST guidance, it is suggested that effort should be made to identify as much of the dose-related material as possible. It is generally recommended that metabolites contributing to > 10% of the AUC of drug-related material (i.e., radioactivity in a well-performed mass balance study) are structurally characterized to exclude the possibility of unknown significant metabolites. It is also recommended to investigate the potential inhibitory effects on the common drug metabolizing enzymes of phase I metabolites with an AUC both larger than one fourth of the AUC of the parent drug and larger than 10% of the drug-related exposure. *In vitro* studies should be performed to investigate whether the metabolite inhibits the cytochrome P450 enzymes most commonly involved in drug metabolism for both competitive and time-dependent inhibition. These presently include CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A. In addition, it is recommended to study inhibition of UDP-glucoronosyltransferases (UGTs) known to be involved in drug interactions, including UGT1A1 and UGT2B7, if one of the major elimination pathways of the investigational drug is direct glucuronidation. To interpret data from these studies, the determination of the plasma protein binding is required. If the protein binding of the parent and metabolite(s) is high (> 90%), it is recommended to determine the protein binding within the same study so as not to introduce interstudy variability. Concentrations in terms of unbound metabolite concentrations should then also be used in consideration of possible target and off-target pharmacology.

If pharmacologically active metabolites are identified, defined as one estimated based on unbound systemic exposure whose activity contributes to ≥50% of the *in vivo* target pharmacological effect, enzymes contributing to the formation pathways of these metabolites should be identified (refer to following Elimination section). This figure is calculated based on exposure of unbound parent drug and metabolite and the relative pharmacological activity. It is also recommended that these important metabolites are measured in the *in vivo* interaction studies and in special populations.

Despite the recommendations in the guidance[3] and the extensive literature reviews,[7–10,12,13,15] shortcomings in the understanding of circulating metabolites are still apparent in recent regulatory submissions and have been identified in a number of applications for new marketing authorizations during the past 10 years. The reasons for this are varied but can often be attributed to deficiencies in the knowledge normally gained from the mass balance study and range from the complete absence of a human mass balance study to poorly designed, or incomplete studies, or to unexpected findings in the study confounding the interpretation of the results, e.g., if the radiolabel is found to be in a metabolically labile position.

Table 1 shows examples of applications during the past 10-year period where regulatory concerns were highlighted as part of the drug review on characterization of plasma metabolites in a human mass balance study. These statements are taken directly from the EPARs following the initial assessment and are used as illustrative examples of issues encountered and should not be taken out of context; full details of the assessment in the EPAR should also be taken into account.

In five of the cases identified, no mass balance study was performed, e.g., cariprazine (Reagila), cladribine (Mavenclad), fexinidazole (Fexinidazole Winthrop), masitinib, and rucaparib (Rubraca). Therefore, in these cases there was a complete lack of the data that would normally be expected from such a study, including the identification of total drug-related components in plasma. Without the use of radiolabel in a study, it is very difficult to make definitive conclusions to judge that all (total recovery) circulating drug-related components have been identified, and thus it is not possible to fully assess the relevance of the nonclinical program as part of the risk assessment for the medical product. In one case (rucaparib (Rubraca)), a mass balance study was already in progress, but the drug was authorized as a conditional approval before the study was complete as it was indicated in an area of unmet medical need and the benefit of immediate availability was considered to outweigh the risk from less comprehensive data than normally required. In at least one case, a study was requested and deemed necessary prior to authorization (masitinib). In all cases, the lack of data was highlighted as a concern and triggered questions that had to be answered by the applicant during the regulatory assessment process. This did not, however, always necessitate a new study. In some cases, the available knowledge of the drug and its metabolites, combined with consideration of the difficulties in performing the study and of the indication, e.g., orphan indication or last-line treatment, meant that eventually the drugs were licensed without the study (cladribine (Mavenclad), cariprazine (Reagila), fexinidazole (Fexinidazole Winthrop). In these cases, extensive *in vitro* metabolite profiling and metabolite assays of unlabeled studies were used as supportive data (**Table 1**).

In another eight cases, significant deficiencies in the human mass balance study were noted in the characterization of the metabolites during the initial assessment (betrixaban, enclomifene, lurasidone (Latuda), neratinib (Nerlynx), opicapone (Ongentys), perampanel (Fycompa), ponatinib (Iclusig), and vintafolide (Vynfinit)). It is expected that any component that could contribute to more than 10% of the total AUC is to be identified, therefore the radioactive AUC of any peak in the radio-chromatogram is compared with the total radioactive AUC (% metabolite = $AUC_{\text{radioactivity metabolite}}/AUC_{\text{total radioactivity}} \times 100$). In cases where the parent compound accounts for more than 90% of the total radioactivity, no further metabolite profiling is generally required, although there may be exceptions to this, e.g., if there is a functional group of concern for safety. If more than 10% of radioactivity is not accounted for, the potential of an unidentified relevant metabolite cannot be excluded. This is, however, considered in terms of the available chromatographic data, e.g., if the remaining radioactivity is obviously present as a number of minor peaks. In all of the cases identified previously, it was considered that there was insufficient characterization of circulating drug-related components so that unidentified metabolites could not be excluded. In several cases, only a small proportion of the AUC of the total radioactivity had been characterized, e.g., enclomifene and pitolisant (Wakix; see **Table 1**). In most cases, this was the result of an incomplete profiling of the complete radioactivity time course profile, and in some cases, plasma was only profiled during the first 24 hours or less, whereas the radioactive half-life was much longer, e.g., lurasidone (Latuda). In a number of cases, a longer plasma half-life of radioactivity than for the parent compound was evident, e.g., opicapone (Ongentys) and ponatinib (Iclusig), indicating metabolites with slower elimination rate limited clearance (see **Figure 1** for a literature example[17]). In one case, uncertainties about levels of metabolites in later time points meant a concern over possible unidentified persistent major metabolites, e.g., opicapone (Ongentys). The incomplete metabolite identification in the plasma samples was attributed on occasions to low analytical sensitivity because of the low radioactive dose and has been attributed by the applicant to the fact that a microdose study was used, e.g., neratinib (Nerlynx). In all cases, these gaps in knowledge led to additional regulatory concerns raised to applicants and additional studies being required with additional profiling of plasma samples from the mass balance study (opicapone (Ongentys), ponatinib (Iclusig), Latuda (Lurasidone), and betrixaban) and/or from other clinical studies, e.g., unlabeled studies after dosing to steady state or to new mass balance studies being performed (neratinib (Nerlynx), enclomifene, pitolisant (Wakix), and perampanel (Fycompa)).

In other examples, although there was sufficient information on the identification of human metabolites, it was not considered that these had been adequately characterized in nonclinical safety species to support human exposure. In one case, further evidence was required in relation to a disproportionate metabolite (lesinurad (Zurampic)) to give reassurance on exposure in humans (**Table 1**). For guanfacine (Intuniv), pharmacological activity data and characterization of the elimination pathways of the major metabolites were required. In a number of cases, CYP inhibition data or plasma protein-binding data of metabolites were found to be missing, and this was often requested during the assessment process or as a postauthorization study if not available at the time of assessment completion (e.g., apremilast (Otezla) and pacritinib).

The mass balance study is generally performed in healthy male volunteers, and based on the understanding gained in this population, consideration needs to be given to the plasma exposure of drug and metabolites at the proposed posology in the patients and in other populations, e.g., different disease states or age groups. In some cases, metabolites may become more important in special populations, e.g., those subject to renal clearance in patients with kidney function impairment. An example of this is brivaracetam (Briviact). In a radioactive mass balance study in healthy male subjects, the parent drug represented 83–99% of the circulating radioactivity in plasma up to 24 hours after administration. In patients with severe renal impairment, following administration of a single dose of 200 mg brivaracetam, the AUC for major metabolites was

Table 1 Plasma metabolite identification issues in EPARs

Drug	Selection of statements related to metabolite identification in the EPAR (verbatim). These statements are taken directly from the initial assessment EPARs and are used as illustrative examples of issues encountered
Apremilast (Otezla)	ADME Study CC-10004-PK-002 characterised the pharmacokinetic profile of a single oral 20 mg suspension dose of apremilast in healthy male subjects and found that in line with *in vitro* findings, apremilast was extensively metabolised into multiple metabolites. All of the main metabolites are pharmacologically inactive. Data extracted from table 19: Apremilast (mean) 44.78% and M12 (mean) 38.74% of total radioactivity. The applicant will provide results of *vitro* studies to evaluate M12 as an inhibitor of CYP1A2, 2B6, 2C8, 2C9 and 2D6, and as an inducer of CYP2B6 and CYP1A2.
Betrixaban Refused	Lastly, as the performed mass balance study is deemed failed regarding characterization of plasma radioactivity, the lack of information regarding human major or unique metabolites according to ICH M3R(2) was further addressed during the assessment rounds. The only remaining concern for a potential poor bridge to the preclinical studies refers to the scenario if there is a human specific metabolite which is eliminated at a slower rate than the parent compound betrixaban and consequently may become a large metabolite in terms of exposure, i.e., AUC. The risk for formation of such a metabolite, with toxic properties, is however deemed low and no further information is deemed necessary. Additional factors considered in this judgement are the limited treatment duration and the fairly large population in APEX from which there are safety data collected.
Cariprazine (Reagila)	Based on the fact that a [14C] labelled human mass balance study was not performed there remains a lack of full understanding of the existence of any human unique metabolite in plasma. Although the probability that additional metabolites are formed *in vivo* in humans cannot be fully excluded, it is judged to be low. It is generally established that a radioactive human mass balance should be a part of the drug development program in order to investigate the elimination pathways as well as to enable assessment of the relevance of nonclinical species with respect to formed human metabolites. Given the potential risk for lenticular changes and cataracts in human; i.e. potential serious toxicity to a sensitive organ, the request of a radioactive human mass balance study has been reevaluated.
Cladribine (Mavenclad)	Due to the lack of a human mass balance, there is insufficient understanding of the existence of any human specific circulating drug-related material in human plasma or any major circulating metabolite. Overall, this gap was addressed by the available *in vitro* data and identification of metabolites in human plasma, which showed that cladribine is not metabolised to a meaningful extent.
Enclomifene Refused	Based on the data presented by the applicant, it is not possible to evaluate whether there are human major or unique metabolites which according to ICH M3R(2) would need to be specifically addressed. In the ADME study, when comparing AUC for total radioactivity in plasma with the sum of the AUCs for the parent compound and the metabolites (4-OH enclomifene, desenclomifene, and 4-OH desenclomifene), the latter seem to account for approximately 3% of total drug exposure. There is no apparent explanation about this discrepancy. Additional information would therefore be required, possibly from another ADME study. In addition, the available long-term safety data in nonclinical studies have not been shown to be sufficient to support the chronic treatment with enclomifene in males. This is due to the uncertainties with regard to the adequate characterisation of all the relevant human metabolites in the mass-balance study and all the relevant human metabolites should be demonstrated to be tested in the chronic toxicity studies.
Fexinidazole (Fexinidazole Winthrop)	After 120 minutes incubation with human hepatocytes, M1 was the main metabolite, while very small amounts of M2 and M3 (N-des-methyl fexinidazole sulfoxide) were detected. It is considered that the *in vitro* and nonclinical studies suggest that characterising fexinidazole, M1 and M2 accounts for nearly all the exposure observed. (See also **Table 2**.)
Guafancine (Intuniv)	The lack of a mass-balance study was considered as a significant deficiency in the dossier. The applicant identified 3-hydroxy guanfacine sulfate (M13) as a major circulating metabolite, representing a mean of 61% of the plasma radioactivity. However, it is unknown if this metabolite is active. Although in general phase II metabolites are not pharmacologically active, the applicant was asked to evaluate that this holds also for M13, which represents substantial (60%) plasma radioactivity. If the sulphate conjugate contributes to the pharmacological activity, the elimination pathway of this metabolite should be investigated and potential interactions should be discussed. These studies could be performed as a postapproval commitment.
Lesinurad (Zurampic)	All metabolites in humans were identified in the nonclinical toxicology species, with only M4 considered to be a human disproportionate urinary metabolite. M4 is formed via an epoxide intermediate (M3c) that was not detected in animals or humans. The Applicant suggested that *in vivo* M3c is rapidly hydrolyzed by microsomal epoxide hydrolase (mEH) into M4 (major metabolite in human urine) or M9 (major metabolite in monkey bile) and the documentation provided to support this hypothesis was considered sufficient by the CHMP.
Lurasidone (Latuda)	Two mass-balance studies have been performed for lurasidone and have provided information on the pharmacokinetics of lurasidone and its metabolites. Due to inadequacies in the design of the mass-balance studies and uncertainties in the interpretation of the results, the applicant was asked to submit more detail regarding the metabolism and elimination of lurasidone. The applicant has provided requested analyses and discussions based on the available data together with additional estimation of the likely exposure to the unknown metabolites providing therefore enough reassurance on the safety implications of the PK of the product. Based on the results from study D1050184, the inactive metabolites ID-20219 and ID-20220 were the main radioactive components in serum (24% and 11%) except for parent lurasidone (10.7%). The active metabolite ID-14283 contributed to 2.8% of total radioactivity (up to about 30% of parent exposure). The other identified and unknown metabolites contributed to < 10% of the total radioactivity in serum. The radioactivity data presented were based on 8-hour sampling time.

(Continues)

Table 1 (Continued)

Drug	Selection of statements related to metabolite identification in the EPAR (verbatim). These statements are taken directly from the initial assessment EPARs and are used as illustrative examples of issues encountered
Masitinib Refused	No mass balance study has been conducted. Based on *in vitro* data, masitinib is claimed to be extensively metabolised. A number of metabolites are proposed, including AB3280 which is considered to be the primary metabolite. It remains unclear to which extent metabolites have been identified in humans.
Neratinib (Nerlynx)	Plasma radioactivity was too low to profile or quantitate parent or metabolites, and plasma PK parameters are reported for the analysis of unlabeled compounds. The Applicant is conducting another mass balance study. In the event that additional major plasma metabolites are identified the Applicant will consider the evaluation of plasma protein binding and pharmacokinetic characterisation.
Opicapone (Ongentys)	An additional circulating metabolite, M10, was identified late in the procedure. This metabolite only appeared post 72 hours and over the 504 hour time period of the ADME study this metabolite accounted for a possible 32% of radioactivity however, time points were very limited. As no mass could be assigned to this metabolite in the ADME study, absolute identification was not possible. However, based on the analysis using two distinct chromatographic conditions and the pattern of human metabolism in general, it is considered highly likely that M10 represents the hydroxylated sulphate metabolite BIA 9-4588, a possible secondary metabolite of BIA 9-1103. Additional studies in rats showed that this metabolite is present in the rat, and thus this metabolite has been qualified in toxicology studies. In addition, reanalysis of samples from clinical studies suggests that this is not a major metabolite at steady state dosing in humans.
Pacritinib (Enpaxiq) Withdrawn	The major circulating component in plasma is pacritinib (72% of the radioactivity). M1 and M2 are the main metabolites with M2 being just over 10% of total radioactivity and having lower pharmacological activity. However, the plasma protein binding of M2 has not been determined. The radioactive half-life is 55 hours, slightly longer than that of pacritinib. Most of the metabolites quantified had disappeared by the last time point measured 120 hours. However, the position for M3 is not clear.
Perampanel (Fycompa)	The excretion balance study (007) was deficient for a number of reasons, most importantly because metabolic profiling was carried out on samples from single time points representing in total approximately 5% of the dose. The has highlighted data available from another study (017). The objective of the study was to evaluate the absolute bioavailability of perampanel following concomitant administration of an i.v. microdose of 14C-perampanel solution and a single oral dose of perampanel and to investigate the metabolite profile of perampanel in plasma, urine and faeces, and characterise metabolites where appropriate. These data were considered acceptable as supportive since there is reasonable evidence that the absolute bioavailability of perampanel is high with a minimal first pass effect. Study 017 provides a greater insight and is reassuring that there are no unidentified major metabolites, although the quantitative contribution of individual metabolites is not completely elucidated. Of note are two metabolites, M7 and M15, are formed from reactive intermediates. The quantitative importance of reactive metabolic pathways is unknown for perampanel because of the deficiencies in studies 007 and 017. (See also **Table 2**)
Pitolisant (Wakix)	The CHMP identified several shortcomings in the documentation provided on the pharmacokinetics of pitolisant. The Applicant has been requested to perform a new balance study after repeated dose administration in order to identify the major metabolites and characterize their PK behaviour and the mechanisms underlying their formation. This study will be conducted as a post approval measure.
Ponatinib (Iclusig)	In plasma samples from the ADME study, a long terminal half-life of radioactivity of 149 hours is seen, further profiling of these samples is required to determine what is contributing to this long half-life. The Applicant committed to evaluate plasma samples from the human ADME study in order to identify and quantify metabolites of ponatinib. The major metabolite identified in humans is AP24600, formed by amide hydrolysis of ponatinib. This metabolite was only identified during the mass balance study and was found in humans, rats, and monkeys (albeit at low levels). CYP3A4/5-mediated metabolism of ponatinib *in vitro* resulted in the formation of both AP24567 and AP24734. AP24567 (N-desmethyl metabolite) was subsequently identified as a metabolite of ponatinib in human plasma, whereas AP24734 was not observed to any significant extent in patient plasma. Plasma levels of AP24567 were approximately 1% to 2% of ponatinib plasma levels in patients.
Rucaparib (Rubraca) Conditional approval	Preliminary metabolite profiling was performed using steady state plasma samples collected from three patients in Study CO-338-010 treated with 600 mg rucaparib BID. Pharmacokinetics of the main metabolites were not described by the Applicant. Results from the mass balance study are awaited. The mass-balance study should allow to identify the contribution of the metabolites in the PK of rucaparib. The applicant is encouraged to monitor M324 and M338 metabolites plasma levels in patients after repeated administration and assess if potential for accumulation of these metabolites could be excluded. Considering the relative abundance of metabolites M324 and M338, they should be properly characterised (pharmacodynamic, pharmacokinetic, interactions).
Vintafolide (Vynfinit) Withdrawn	There is no information (pharmacological activity or pharmacokinetics) on other human metabolites than DALVBH and it is unclear which entities (parent compound and/or vinca-containing metabolites) contributed to the systemic toxicity of vintafolide. Considering the short exposure after each dose, the drug administration in cycles and the possibility to dose-adjust based on toxicity, routine risk minimisation activities were considered acceptable to handle the risk of increased exposure until further information is available through the additional pharmacovigilance activities. The CHMP recommended that the applicant performs a mass balance study to collect these data if feasible.

4-OH, 4 hydroxy; ADME, absorption, distribution, metabolism, and excretion; AUC, area under the plasma concentration curve; BID, twice a day; CHMP, committee for medicinal products for human use; CYP, cytochrome P450; EPAR, European public assessment report; ICH, International Council for Harmonisation; PK, pharmacokinetics.

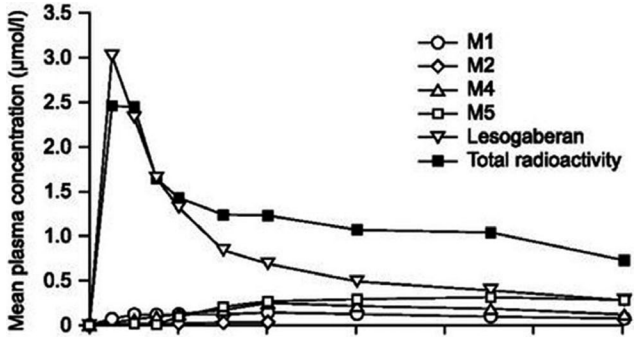


Figure 1 Literature example of metabolites (M1, M2, M4, M5) slowly eliminated in plasma. Lesogaberan and metabolite concentrations plotted against time after the final lesogaberan dose in humans, single 100-mg dose (^{14}C-labeled).[17]

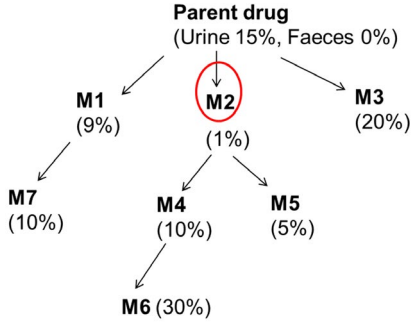
3-fold to 21.5-fold higher when compared with healthy subjects, whereas the AUC of the parent compound remained relatively unchanged (small increase of 21%). In this case, consideration needed to be given to whether metabolites had been adequately characterized in nonclinical toxicological studies and to the possible contribution of these metabolites to pharmacological activity in these patients based on the known exposure–response relationship.

ISSUES WITH THE DESCRIPTION OF ELIMINATION PATHWAYS IN DOSSIERS FOR MARKETING AUTHORIZATIONS

An adequate characterization of the elimination pathways of a new medicinal product is important to allow a good understanding of the fate of the drug. In a mass balance study, the excretion of the parent compound and its metabolites in urine and feces should be investigated. The position of the radiolabel should be in a nonlabile position, but if this is not possible expired air should be collected in the clinical study if elimination as carbon dioxide (CO_2) or other volatile products cannot be avoided. For some molecules where metabolism leads to cleavage into two significant fragments, labelng two parts of the molecule (^{14}C and ^{3}H) or performing two studies with separate labeling positions could be considered.

The European guideline on drug interaction[3] provides guidance about the identification and quantification of the elimination pathways in the mass balance study. As per the guideline, the total recovery of radioactivity in urine and feces should preferably exceed 90% of the dose, and more than 80% of the recovered radioactivity should be identified. The stability of the parent compound and metabolites in excreta should also be considered as well as the extraction efficiency from feces. As, for example, some glucuronides are unstable and are back converted to the parent compound in the gut, an investigation of the stability of glucuronides in feces should be considered to support the evaluation.

As recommended in the aforementioned guideline, the quantitative contribution of the different elimination pathways is estimated based on the amount of dose excreted as primary and secondary metabolites along specific routes. The contribution of primary pathways to total drug elimination (including



Formation of M2 is the main elimination pathway (1+10+5+30 = 46%)
Formation of M1 and M3 each contribute to less than 25%

Enzyme catalyzing formation of M2 should be identified

Figure 2 Example of a mass balance scheme showing recovery of radioactivity as percentage of dose in excreta and identification of the main elimination pathways. The metabolites are reported as M1 to M7.

first pass) should be estimated as the sum of amounts found in excreta of all metabolites originating from one primary pathway divided by the dose (**Figure 2**). The calculated fraction of dose is the preferred way for this to be expressed, as using the drug-related material in excreta could give an incorrect impression of the fraction identified. For example, if the recovery of total radioactivity in the mass balance is moderate, e.g., a recovery of 80% and 80% of the radioactivity is identified, this will result in only 64% of dose identified. In such a case, the contribution of the identified pathways to total drug elimination needs to be discussed. When reporting the data, clear tables on metabolites in excreta and their contribution in percentage of dose should be provided.

Intravenous dosing is recommended even for oral medicinal products to provide a reliable estimation of the contribution of the elimination pathways and important information in quantifying the biliary/gut wall secretion of orally administered drugs.[18] Such a study may require additional dedicated formulation and toxicology studies. Depending on the estimated F_a, different scenarios for the potential risk of DDIs can also be evaluated (**Figure 3a,b**) when the estimation of F_a is not available. For example, if a significant amount of the parent drug is found in feces and the origin is not verified, the parent drug found in feces may be entirely unabsorbed, i.e., the denominator in the calculation should be the amount of drug-related material found in excreta subtracted by the amount of unchanged drug found in feces (cf. case B in **Figure 3**). Other sources of data in addition to the oral mass balance study could be taken into account to inform about biliary/gut wall secretion, for example, food interaction data, drug interaction data, *in vitro* stability in gut preparation, data in subjects with hepatic impairment, potential pharmacogenetics data, data on Caco-2 cell permeability, and so on. If the F_a is not known, worst case scenarios for both high and low F_a may need to be taken into account, and DDI risk based on both the transporters (**Figure 4**) and enzymes involved should be evaluated. If uncertainties remain, it may lead to extensive restrictions for comedication with other medical

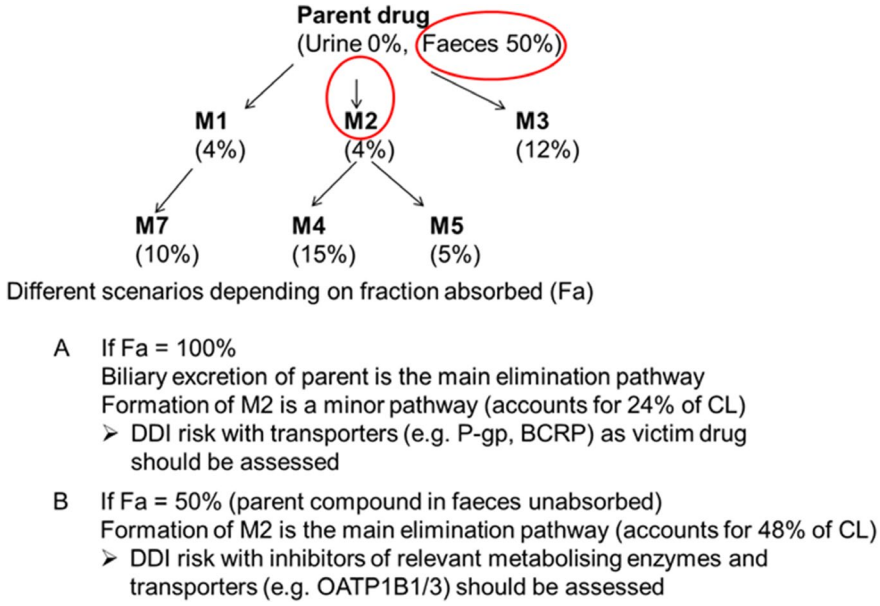


Figure 3 Illustration of potential risks of DDIs depending on fraction absorbed. BCRP, breast cancer resistance protein; M1 to M7, metabolites 1 to 7; CL, clearance; DDI, drug–drug interaction; OATP1B1/3, organic-anion-trasporting polypeptide 1B1/3; P-gp, P-glycoprotein.

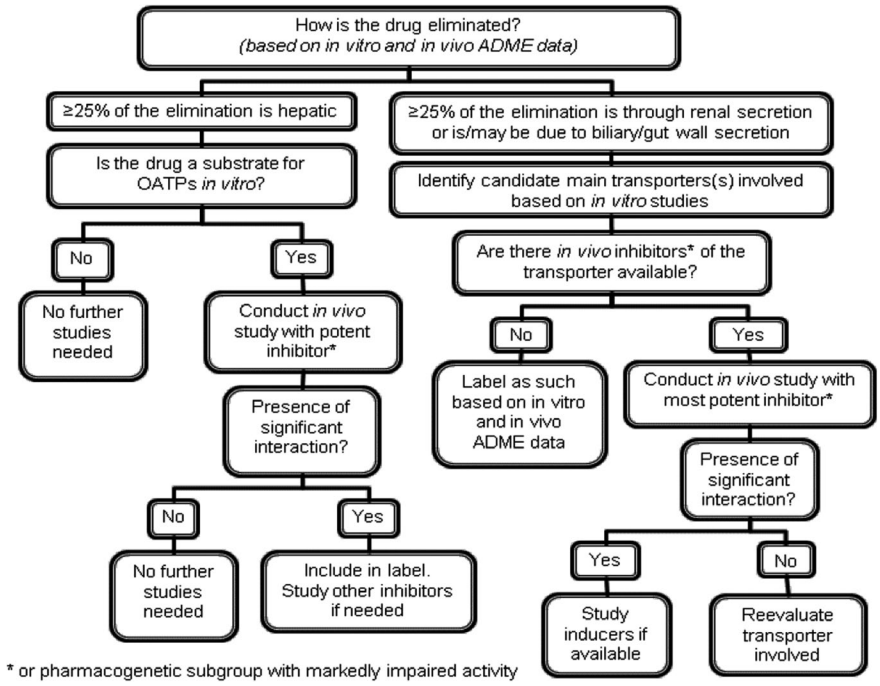


Figure 4 Investigation of transporter involvement in drug elimination (from European Medicines Agency drug–drug interaction guideline). ADME, absorption, distribution, metabolism, and excretion; OATP, organic anion-transporting protein.

products in the summary of product characteristics (SmPC) wording.

According to the guideline on drug interactions, the identification of the enzymes involved in all metabolic pathways estimated to contribute to ≥25% of drug elimination should be performed if possible. For pharmacologically active metabolites, including actives derived from prodrug, the contribution based on *in vitro* activity and estimated unbound systemic exposure needs to be considered as the fraction of the formation and elimination that needs to be characterized for enzyme involvement depends on how much the metabolite is estimated to contribute to the *in vivo* target effect. For example, if the active metabolite is estimated to contribute to 50% or 75% to the pharmacological effect, enzymes contributing

to formation and elimination pathways estimated to contribute to ≥50% and ≥33%, respectively, should be identified.

If the results of the *in vitro* and *in vivo* mass balance studies suggest that active renal, biliary, or gut wall secretion of unchanged drug is involved in a main part of the drug elimination, it is important to investigate the relevant transporters involved.

Moreover, the transporters involved in the elimination should be identified if the active secretion is the major elimination pathway of a metabolite with significant target activity.

Figure 4 shows the decision trees for the investigation of transporters involved in drug elimination from the EMA guideline on drug interaction.

Despite these recommendations in the guidance, incomplete or missing identification and quantification of the excretion pathways, as well as of the enzymes or transporters involved in the elimination process, have been highlighted as part of the scientific evaluation of the dossier.

Table 2 shows a summary of applications that have been identified during the past 10-year period that have had inadequate characterizations of the elimination pathways in a human mass balance study.

No human mass balance study was conducted initially in six of the procedures identified (e.g., rucaparib (Rubraca), guanfacine (Intuniv), biotin (Qizenday), masitinib, meropenem/vaborbactam (Vabomere), fexinidazole (Fexinidazole Winthrop)). The lack of such a study was considered a significant deficiency in the dossier as it rendered it difficult to understand the clearance pathways without these data. In all cases, the company was requested to commit to conduct a human mass balance study during the regulatory assessment process or to adequately justify the lack of these data.

In some cases (e.g., fexinidazole (Fexinidazole Winthrop), meropenem/vaborbactam (Vabomere); see **Table 2**) the lack of a mass balance study was justified through a discussion of clinical, *in vitro*, and nonclinical data and with consideration of the particular circumstances of the application. For example, the excretion data for fexinidazole and its metabolites were investigated after using nonradiolabeled single and multiple administrations of increased doses of fexinidazole. In addition, *in vitro* permeability studies and the mass balance study in rats were considered, and it was agreed plausible that a high fraction of fexinidazole would be absorbed from the gut. The lack of data to inform on interactions was handled by very strict wording in the SmPC regarding comedication of other medical products with fexinidazole. In another case (meropenem/vaborbactam (Vabomere)), clinical urinary excretion data were used to justify the lack of the mass balance study. In addition, in this case, the available *in vitro* data supported the lack of metabolism.

In at least another seven cases (ibrutinib (Imbruvica), pitolisant (Wakix), Neratinib (Nerlynx), lurasidone (Latuda), vintafolide (Vynfinit), sodium benzoate, and perampanel (Fycompa)), serious deficiencies were identified because of inadequate available mass balance data and therefore the lack of an understanding of the elimination pathways.

Despite the guideline on drug interaction recommendation to investigate the ADME properties after intravenous (i.v.) administration of the investigational drug, in a number of cases (e.g., lurasidone (Latuda), ibrutinib (Imbruvica)), only an oral mass balance study was conducted. The estimation of the elimination based on oral mass balance data is generally considered a rough approximation of elimination pathways, and this may lead to underestimations and overestimations of the importance of an elimination pathway for the clearance of a drug. As stated previously, in cases where a large fraction of the drug is found unchanged in feces, knowledge about the oral bioavailability adds important information for understanding the role of biliary and/or gut wall secretion to drug elimination. An i.v. micro dose of a radiolabeled drug has been shown to provide valuable information to inform the definitive contribution of clearance pathways in the cases of neratinib (Nerlynx) and perampanel (Fycompa).

In at least one case (canagliflozin (Invokana)), the extent of unabsorbed drug was not adequately investigated. In this example, the oral doses were excreted by approximately 60% via feces and by about 33% via kidney (**Table 2**) no human data on biliary excretion of unchanged drug are available. In at least one case (pitolisant (Wakix)), the metabolites were excreted in urine and expired air as the metabolite CO_2. Because of the uncertainties around quantitation, an additional mass balance study was considered necessary with the drug radiolabeled at a nonlabile position.

For a number of examples (pitolisant (Wakix), Neratinib (Nerlynx), lurasidone (Latuda), and perampanel (Fycompa)), an additional mass balance study was considered necessary to adequately characterize the urinary and fecal recovery of total radioactivity and provide urine and fecal samples for metabolite profiling and metabolite identification.

In some cases, the total recovery of radioactivity in urine and feces was < 90% of the dose, and < 80% of the recovered radioactivity was identified. This may be a result of the persistent metabolites[17] (**Figure 1**) or metabolites with very long half-life.[11] In at least one example (ibrutinib (Imbruvica)), the elimination of covalently bound radioactivity was very slow. After a single oral administration of radiolabeled [^{14}C]ibrutinib in healthy subjects, approximately 90% of radioactivity was excreted within 168 hours, with the majority (80%) excreted in the feces and < 10% accounted for in urine. Unchanged ibrutinib (Imbruvica) accounted for approximately 1% of the radiolabeled excretion product in feces and none in urine. Mean recovery of total radioactivity in urine and feces assigned for 7.8% and 80.6% of the dose, respectively. Negligible amounts of the parent ibrutinib (Imbruvica) and a small amount of the active metabolite PCI-45227 were detected in urine and feces. The remaining identified radioactivity in excreta was accounted to a large number of metabolites. The sum of identified drug-related material in excreta, corrected for recovery and column recovery, was 64.1% of the dose. Because almost no ibrutinib was detected in urine and feces, it can be concluded that ibrutinib is almost completely absorbed and that metabolism is the major elimination pathway.

Parent compound excreted in feces more than 48 hours after administration can be considered absorbed, and it can

Table 2 Mass balance and elimination issues in EPARs

Drug	Selection of statements related to metabolite identification in the EPAR (verbatim). These statements are taken directly from the initial assessment EPARs and are used as illustrative examples of issues encountered
Biotin (Qyzenday) Withdrawn	Due to limited duration of sampling, the elimination half-life and derived parameters (AUCinf, CL/F, Vz/F) could not be determined. The applicant should be able to characterise the elimination of the drug, especially in terms of apparent CL and terminal half-life. The applicant estimates that the percentage of recovered biotin in the urine in the 24 hours following a 100 mg dose is 31% while for a 300 mg dose it is 17%, however given the limited number of subjects included in the analysis (n = 4), these results are considered preliminary. Across human, rat and pig is almost exclusively renally excreted.
Canagliflozin (Invokana)	Oral doses were excreted by approximately 60% via faeces and by about 33% by the kidneys. It still remains to be determined to which extent unabsorbed drug, biliary excreted drug or, potentially, excreted hydrolysed (unstable during analysis procedures) glucuronide contribute to unchanged canagliflozin in faeces. The UGT2B4 contribution has not been confirmed *in vivo*, but a clinically meaningful DDI at this level is unlikely. *In-vitro* investigations indicated potential interactions at the level of P-gp, MDR1 and MRP2 transporters, CYP3A4 and CYP2C9 to be addressed in clinical DDIs.
Dextromethorphan/ quinidine (Nuedexta) Withdrawn	The Applicant's discussion of literature data was not sufficiently reassuring and it is viewed as important to have documented the quantitative impact of strong CYP3A inhibitors on DM, DX and Q for the product. As this is an important route for quinidine elimination (and is also involved to a minor extent in DM metabolism) and as quinidine is associated with a concentration-dependent increase in the QT interval, it is viewed as a potential safety concern for the product. The applicant proposes to conduct a drug-drug interaction (DDI) study of Nuedexta (DM 30 mg/Q 10 mg) with the potent CYP3A4 inhibitor ketoconazole as a post-authorisation commitment.
Dolutegravir (Tivicay)	The absolute bioavailability is not known, however the fraction absorbed from tablet formulation is estimated to be approximately 50%, based on recovered radioactivity in late faeces samples (> 72 hours after dose, major part corresponds to parent compound). It is likely that the major fraction of the DTG recovered in faeces originates from biliary excreted glucuronide conjugate, based on similar data from bile duct cannulated Cynomolgus monkeys.
Eribulin (Halaven)	Eribulin is mainly eliminated through biliary excretion of unchanged drug. This route contributes to 70% of total clearance. The transporter involved has not been identified. If the secretion is completely inhibited, it could in theory give rise to a more than threefold increase in plasma concentrations. The applicant will investigate which transporter is involved to allow predictions of potential drug interactions at transporter level. While this is investigated, the applicant will include a list of potent inhibitors of hepatic uptake and efflux transporters in the SmPC and propose adequate practically applicable treatment recommendations for situations where concomitant treatment is needed. Eribulin should not be used concomitantly with inhibitors of hepatic transport proteins such as organic anion-transporting proteins (OATPs), P-glycoprotein (P-gp), multidrug resistant proteins (MRPs) as described in section 4.5 of the SmPC.
Fexinidazole (Fexinidazole Winthrop)	Both bioavailability and total elimination pathways of fexinidazole are unknown as mass balance study was not provided. The applicant justified the lack of a mass balance study, based on available *in vitro* metabolism data for fexinidazole and metabolites, *in vivo* mass balance data in rats and excretion data in humans for fexinidazole after oral administration. The rat mass balance study indicated a plausible biliary excretion of metabolites, as well as urinary excretion of other metabolites than M1 and M2. Given the results from *in vitro* permeability studies with fexinidazole as well as the rat mass balance study, it is plausible that fexinidazole is characterized by a high fraction absorbed from the gut. The SmPC text has been updated to not recommend other concomitant medications with fexinidazole due to PK interactions, with caution suggested for CYP2D6 inhibitors and paracetamol. These adjustments to the SmPC are acceptable, given the limited knowledge regarding the metabolic pathways of fexinidazole and its metabolites.
Guafancine (Intuniv)	The lack of a mass-balance study was considered as a significant deficiency in the dossier. Based on the newly submitted mass-balance study with guanfacine prodrug, it was estimated that renal excretion is the major elimination pathway (80% of the radioactivity) with parent drug representing 30% of the urinary radioactivity. Considering that metabolism accounts for more than 50% in the drug elimination, the *in vitro* studies identifying transporters involved in hepatic uptake are necessary and the applicant committed to perform such studies post-approval. In addition, when a candidate transporter has been identified, an *in vivo* study with a strong inhibitor/inducer of the transporter at the site of interest is recommended, if feasible
Ibrutinib (Imbruvica)	In the human mass balance study, elimination of covalently bound radioactivity was slower than total radioactivity. After a single oral administration of radiolabeled [14C]-ibrutinib in healthy subjects, approximately 90% of radioactivity was excreted within 168 hours, with the majority (80%) excreted in the faeces and < 10% accounted for in urine. Negligible amounts of parent ibrutinib and a small amount of the active metabolite PCI-45227 were detected in urine and faeces. The remaining identified radioactivity in excreta was accounted to a large number of metabolites. The sum of identified drug related material in excreta, corrected for recovery and column recovery, was 64.1% of the dose. Since almost no ibrutinib was detected in urine and faeces it can be concluded that ibrutinib is almost completely absorbed and that metabolism is the major elimination pathway. The CHMP considers the following measures necessary—as part of the RMP—to address the issues: (i) a clinical drug interaction study with oral contraceptives, if feasible; (ii) further evaluation of potential DDIs with PPI; (iii) an *in vitro* DDI study to evaluate the reversible CYP3A inhibition by ibrutinib; (iv) an *in vitro* study to investigate the time dependent inhibition by ibrutinib on CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6; (v) an *in vitro* study to investigate the inhibitory potential of ibrutinib towards BCRP, OATP1B1, OATP1B3, OCT2, OAT1 and OAT3; (vi) an *in vivo* DDI study between ibrutinib and a model CYP3A4 substrate, with a large contribution of intestinal CYP3A4 metabolism to its first pass extraction; (vii) an *in vitro* study to investigate the induction by ibrutinib on CYP1A2 and CYP2B6.

(Continues)

Table 2 (Continued)

Drug	Selection of statements related to metabolite identification in the EPAR (verbatim). These statements are taken directly from the initial assessment EPARs and are used as illustrative examples of issues encountered
Lurasidone (Latuda)	Two studies, D1050184 and D1050262, examined the absorption, metabolism, and excretion of [14C]-lurasidone after a single 40 mg oral suspension dose. The results showed that between 67 and 80% of the substance was eliminated in faeces and 19 and 9% in urine resulting in a dose recovery of > 85% in both studies, respectively. Faeces samples were only analysed in one of the studies and consisted almost entirely of unchanged lurasidone. It was also concluded that the elimination of the active metabolite, ID-14283, via metabolism/active transport, should be further investigated *in vitro* due to its substantial contribution to efficacy. The applicant provided *in vitro* data to investigate the metabolism of ID-14283 and also committed to the post-authorisation measure to study ID-14283 as a substrate of OATP1B1 and OATP1B3 and to update SmPC wording accordingly.
Masitinib Refused	In the absence of a mass balance study, which represents a major gap in the understanding of the elimination pathways, further studies are needed to elucidate masitinib elimination and its potential for DDI. In the event of a proven clinical benefit with Masipro, lack of information on DDI could not be managed through the RMP or PI warnings as in the context of symptomatic treatment for a condition where patients need a range of other medications and knowledge of DDI is essential.
Meropenem/ vaborbactam (Vabomere)	After single doses the mean Ae0-24 was estimated at 2,100 mg for the 2,000 mg dose, giving a mean percent urinary excretion of 105% (2,000 mg), most of which appeared in urine in the first 8 hours. The mean percent urinary excretion fe0-8 following repeated dosing at 2 g q8h was 91.6%. These data, as well as the lack of any metabolism of vaborbactam *in vitro* support the omission of a study with radiolabelled vaborbactam.
Neratinib (Nerlynx)	Due to the low sensitivity of the assay and low radioactivity level in the body, the metabolic profiling and disposition of neratinib was not characterised in study 3144A1-1108-US. The Applicant is conducting another mass balance study. The primary objectives of this study are to determine the recovery of radioactivity, the whole blood to plasma concentration of total radioactivity, the urinary and faecal recovery of total radio activity, and provide plasma urine and faecal samples for metabolite profiling and metabolite identification.
Perampanel (Fycompa)	(See also **Table 1**). The metabolic profiling carried out in study 017 was more informative than that in study 007 since (i) three urine and three faecal samples spaced in time were used for the profiling and (ii) almost 50% of the dose was represented in these samples (5% in urine and 42% in faeces). For comparison, the standard expected for an excretion balance study is that metabolic profiling is carried out on samples representing > 80% of excreted radioactivity. Although representing a smaller % of the dose than ideal, it is reassuring that that the samples were spread over time and that the metabolite patterns were similar in the latter two sample collection intervals. CYP3A4/5 has been shown to be involved in the metabolism of perampanel, but the involvement of other enzymes cannot be ruled out and requires further investigation. While the data generally support CYP3A4 an elimination pathway, there are sufficient inconsistencies and gaps that do not exclude the possibility of other metabolic pathways that could represent a concern for DDIs. Gaps include (i) lack of data for inhibitors of enzymes other than CYP3A4 (i.e., perampanel was incubated with human liver microsomes in the presence and absence of ketoconazole and a CYP3A antibody, but not inhibitors of other enzymes), (ii) the metabolic pathway responsible for the production of the *in vivo* metabolite M5 is unknown and (iii) the quantitative importance of M5 *in vivo* is not known because of the deficiencies in studies 007 and 017.
Pitolisant (Wakix)	The main route of elimination of pitolisant is hepatic metabolism. The mass balance study showed that excretion (renal or biliary) does not play an important part in the elimination of parent drug. The metabolites were excreted in urine and presumably expired air (as CO_2). It is claimed that approximately 25% of administered radioactivity could be accounted for in expired air, however the methods, assumptions and impact on study conclusions were not detailed and therefore could not be endorsed by the CHMP. The Applicant committed to perform a new mass-balance study with the drug radiolabelled at a non-labile position. The Applicant has been requested to conduct as post-approval measures a number of PK studies in order to further elucidate the contribution of different enzyme pathways to pitolisant's metabolism and characterise the risk of drug-drug interactions.
Pomalidomide (Imnovid)	A mass balance study using radiolabelled pomalidomide was conducted in eight healthy male volunteers to quantify the rates and routes of elimination and to identify and quantify circulating and excreted metabolites. The elimination of total radioactivity in plasma mirrored that of parent drug. There were no circulating major metabolites in plasma. The mean total recovery of radioactivity was lower than ideal (88% of dose) and ranged from 64% to 110%. Metabolism is the primary mechanism of elimination of pomalidomide (< 10% of dose excreted unchanged in urine and faeces) and urine is the primary route for excretion of pomalidomide metabolites (72% of dose was recovered in urine and 15% in faeces). The predominant metabolites in excreta were the hydrolysis product, M11 (24% of dose), and two glucuronides (M12 and M13; 29% of dose) of a hydroxylated metabolite (M17). The M17 pathway (M17 + glucuronide metabolites) accounts for at least 35% of the dose. Six other minor metabolites were also detected in excreta. Given the low turnover (< 10%) during *in vitro* experiments, further evidence to confirm the enzymes responsible for the elimination of pomalidomide is required. PAM (II/0016/G)- The final study report of a study that evaluated the PK of pomalidomide administered with the CYP1A2 inhibitor fluvoxamine was submitted. Fluvoxamine co-administration resulted in an approximate doubling of exposure to pomalidomide. Sections 4.2, 4.5 and 5.2 of the SmPC were updated to instruct that if strong inhibitors of CYP1A2 (e.g., ciprofloxacin, enoxacin and fluvoxamine) are co-administered with pomalidomide, the dose of pomalidomide should be reduced by 50%.

(Continues)

Table 2 (Continued)

Drug	Selection of statements related to metabolite identification in the EPAR (verbatim). These statements are taken directly from the initial assessment EPARs and are used as illustrative examples of issues encountered
Rucaparib (Rubraca)	A mass balance study is ongoing. This study will further elucidate distribution, mean pathways of metabolism, routes of elimination and potential interactions of rucaparib and its metabolites. These data will also allow to confirm the mean absolute oral bioavailability at the 600 mg dose and to clarify the reasons of low bioavailability. The applicant is recommended to submit the results. *In vitro* data suggested slow metabolism by CYP enzymes, with CYP2D6 and to a lesser extent CYP1A2 and CYP3A4 contributing to the metabolism of rucaparib. Rucaparib was shown to be a substrate of P-gp and BCRP. If results of Study CO-338-045 (mass balance) support the *in vitro* results, the effects of strong inhibitors of CYP2D6, P-gp and BCRP on rucaparib pharmacokinetic would have to be investigated in dedicated *in vivo* drug-drug interaction studies. Additionally, the effects of strong inhibitors and strong inducers of CYP1A2 and CYP3A4 on rucaparib pharmacokinetic could have to be investigated in dedicated *in vivo* drug-drug interaction studies. CYP3A4 was less important *in vitro*, but the relative/quantitative contribution of different CYPs *in vivo* cannot be determined from *in vitro* data. CYP3A4 is highly abundant *in vivo*, and could contribute to a high degree of first-pass metabolism. From the safety data currently available, it appears that no conclusions on the safety of concomitant use of strong CYP1A2 or CYP3A4 inhibitors can be drawn. As CYP3A4 inhibition or induction can lead to very large effects on exposure of a CYP3A4 substrate, e.g. if there is a large degree of first-pass metabolism, a warning against use of strong CYP3A4 inhibitors and inducers has been included in the SmPC, awaiting data from the mass-balance study and, if necessary, further DDI studies.
Sodium benzoate (Prohippur) Withdrawn	Following a low dose of 1 mg/kg, 97% was excreted as hippuric acid in urine. It is not known what this figure is for higher doses. Elimination of hippurate is proposed to be non saturable and linear up to doses of 160 mg/kg. Given the plasma profiles it might be expected that there may be some differences in the initial rate however this is not shown. In two healthy men, a dose of 1 mg/kg (8.2 μmol/kg) of 14C-labeled benzoic acid was shown to be excreted entirely as hippuric acid: 97% of the 14C administered was excreted in the urine within 4 hours of dosing and almost 100% within 12 hours.
Vintafolide (Vynfinit) Withdrawn	The major elimination pathways and main metabolites of vintafolide and DAVLBH need to be clarified including the identification of the main metabolising enzymes and transporters. The CHMP recommended that the applicant performs a mass balance study to collect these data if feasible. If the results of the mass-balance study indicate a role of biliary excretion or if mass balance data are lacking, the applicant will study biliary transport further *in vitro*, and clarify if vintafolide and its metabolites are substrates for hepatic uptake and efflux transporters.

Ae0-24, cumulative urinary excretion from administration until 24 hours; AUCinf, area under the plasma concentration curve from adminsitration to infinite time; BCRP, breast cancer resistance protein; CHMP, committee for medicinal products for human use; CL/F, clearance/bioavailalbility; CL, clearance; CO_2, carbon dioxide; CYP, cytochrome P450; DDI, drug–drug interaction; EPAR, European public assessment report; MDR1, multi-drug resistance gene 1; MRP2, multi-drug resistant protein 2; OAT1, organic anion-trasporter 1; OAT3, organic anion-transporter 3; OATP1B1, organic anion-transporting protein 1B1; OATP1B3, organic anion-transporting protein 1B3; OCT2, organic cation transporter 2; PAM, post-authorisation measure; P-gp, P-glycoprotein; PPI, proton-pump inhibitor; q8h, drug administration every 8 hours; RMP, risk management plan; SmPC, summary of product characteristics; UGT2B4, UDP-glucuronosyltransferase 2B4; Vz/F, volume of distribution/bioavailability.

therefore be important to report the time course of the parent compound in feces. In one example, dolutegravir (Tivicay), a large fraction of the dose was found in feces as the parent compound after oral dose and the absolute bioavailability was not determined. However, the F_a was estimated to be approximately 50% based on recovered radioactivity in late feces samples (> 72 hours after dose, mainly as the parent compound).

Deficiencies were highlighted in the case of perampanel (Fycompa) during the initial assessment as the first mass balance study was conducted in elderly subjects and in addition the metabolic profiling was carried out on samples from single time points, representing in total approximately only 5% of the dose. The metabolic profiling carried out in a second study in nonelderly healthy volunteers was more informative than the previous study as almost 50% of the dose was represented in the studied samples (5% in urine and 42% in feces). Although < 80% of excreted radioactivity was recovered, in this case it was considered reassuring that the samples were spread over time and that the metabolite patterns were similar in the latter two sample collection intervals. In this case, the poor extraction in fece samples was attributed to the difficulties during the process of sample preparation or analysis.

In a number of applications (fexinidazole (Fexinidazole Winthrop), canagliflozin (Invokana), guanfacine (Intuniv), pacritinib, masitinib, pomalidomide (Imnovid), ibrutinib (Imbruvica), pitolisant (Wakix), vintafolide (Vynfinit), and perampanel (Fycompa)) *in vitro* and/or *in vivo* clinical studies were requested by the CHMP as postauthorization measures to adequately elucidate the contribution of different enzyme pathways to the drug metabolism and to characterize the potential risk of drug interactions. In some cases, metabolism accounted for more than 50% of the drug elimination, but additional *in vitro* studies were requested as postauthorization measures to identify the transporters involved in hepatic uptake. In one example (i.e., eribulin (Halaven)), the active compound was mainly eliminated through biliary excretion of unchanged drug. This route contributes to 70% of total clearance. However, the transporter involved has not been identified. If the secretion is completely inhibited, it could in theory give rise to a more than threefold increase in plasma concentrations. In some cases, an *in vivo* study with a strong inhibitor or inducer, if available, of the transporter at the site of interest was considered necessary. Prior to these data becoming available, the lack of knowledge and uncertainty in relation to DDIs leads to possible unnecessary cautionary wording and contraindications in the SmPC.

In at least one example (rucaparib (Rubraca)), the mass balance study was in progress, *in vitro* data suggested metabolism by CYP enzymes, and the parent compound was shown to be a substrate of P-glycoprotein and breast cancer resistance protein. Based on the results of the mass balance study, *in vivo* DDI studies are needed to investigate the effects of strong CYP inhibitors and inducers and transporter inhibitors on rucaparib (Rubraca) PK.

CONCLUSION

Despite regulatory guidance and literature publications on the importance of mass balance studies, guidance on how to perform these studies, and the associated metabolite identification studies, it is still relatively common to see deficiencies in these studies in new drug applications. If no mass balance study is submitted or the study is judged as inconclusive, the shortcomings are mainly related to two issues: characterization of circulating drug-related components and elucidation of elimination pathways. In the case of insufficient characterization in human plasma, it is not possible to assess the relevance of the nonclinical toxicity studies in relation to human safety or to fully understand the contribution of metabolites to the exposure response of the entity. If the study is judged as inconclusive regarding how the compound is eliminated (e.g., a large fraction of the parent recovered in feces after an oral dose and the F_a is unknown), this has led to substantial restrictions regarding concomitant administration of other medicinal products and the use of the drug in patients with organ impairment. This lack of information on elimination is not considered as significant an omission, and safety risk, as the lack of characterization of circulating material in plasma because of the fact that the lack of information can usually be handled with restrictions for coadministered medicines in the SmPC; however, this can severely limit the usage of the drug, e.g., in different populations. In both cases, the benefit/risk assessment is impacted for the proposed medicinal product, and identification of these issues during regulatory assessment leads to delays and the need for postauthorization studies or risk management plan provisions to provide clarity on drug interactions or exposure in special populations.

It is strongly recommended that a mass balance study be performed in general on all new active substances unless it can be shown that the data on circulating drug-related components in plasma and drug elimination can be described unambiguously without the use of a radiolabel. The study results and the results of linked studies, e.g., to characterize metabolites in nonclinical species and to fully understand possible interactions, should be available ideally before phase III and no later than at the time of regulatory submission for marketing authorization.

This review has highlighted a number of common issues in the conduct of mass balance studies that, if considered prior to initiation of the study, should ensure a well-designed conclusive study and prevent the need for further studies and the possible delays in the marketing authorization while further data are gathered.

Some of the key points to be considered during study planning and conduct are the following:

- The use of the i.v. route, or the inclusion of an i.v. arm, if an oral product, allows definitive information on extent of different elimination pathways, particularly if a large fraction of the parent is expected in feces.
- The position of radiolabel should be in a nonlabile position.
- The period of collection of excreta should be over a long enough time interval in the mass balance study to allow the required recovery of dose in excreta.
- The period of collection of plasma samples for metabolite profiling should be over a long enough time interval in the study to allow the required characterization of the radioactivity in plasma, considering any late appearing or persistent metabolites.
- Study samples should be kept and stored adequately so that additional analysis can be performed if necessary. If drugs are unstable or metabolized in blood, it is essential that stabilizers are added to collection tubes.
- The report should include clear presentation of recovery data for sample preparation. For excreta: Fraction of dose and fraction identified should be clearly documented. For plasma/blood: AUC and C_{max} of total radioactivity and fraction accounted for by the parent and for each of the identified metabolites should be documented. Any deviations from the guidelines should also be justified and discussed.
- It is suggested that due consideration of the importance of the mass balance study, and of the information required from the study, prior to submission of new drug applications would allow the inclusion of this important data to support the drug safety, and this would result in faster approvals and optimal availability of new drugs in all populations.

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Dedication. The authors dedicate this paper to the memory of Monica Edholm.

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