Inter-phenotypic differences in CYP2C9 and CYP2C19 metabolism: Bayesian meta-regression of human population variability in kinetics and application in chemical risk assessment

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Toxicology Letters 337 (2021) 111–120

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Inter-phenotypic differences in CYP2C9 and CYP2C19 metabolism: Bayesian meta-regression of human population variability in kinetics and application in chemical risk assessment

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HIGHLIGHTS

- Extensive literature search of kinetic parameters for CYP2C9/CYP2C19 substrates.
- Bayesian meta-regression to quantify inter-individual variability in pharmacokinetics.
- Compound-specific and pathway-related uncertainty factors were derived.

ARTICLE INFO

Article history:
Received 12 June 2020
Received in revised form 13 November 2020
Accepted 19 November 2020
Available online 21 November 2020

Keywords:
CYP2C9
CYP2C19
Inter-individual variability
Polymorphic pathways
Risk assessment
Uncertainty factors

ABSTRACT

Quantifying variability in pharmacokinetics (PK) and toxicokinetics (TK) provides a science-based approach to refine uncertainty factors (UFs) for chemical risk assessment. In this context, genetic polymorphisms in cytochromes P450 (CYPs) drive inter-phenotypic differences and may result in reduction or increase in metabolism of drugs or other xenobiotics. Here, an extensive literature search was performed to identify PK data for probe substrates of the human polymorphic isoforms CYP2C9 and CYP2C19. Relevant data from 158 publications were extracted for markers of chronic exposure (clearance and area under the plasma concentration-time curve) and analysed using a Bayesian meta-regression model. Enzyme function (EF), driven by inter-phenotypic differences across a range of allozymes present in extensive and poor metabolisers (EMs and PMs), and fraction metabolised (Fm), were identified as exhibiting the highest impact on the metabolism. The Bayesian meta-regression model provided good predictions for such inter-phenotypic differences. Integration of population distributions for inter-phenotypic differences and estimates for EF and Fm allowed the derivation of CYP2C9- and CYP2C19-related UFs which ranged from 2.7 to 12.7, and were above the default factor for human variability in TK (3.16) for PMs and major substrates (Fm >60%). These results provide population distributions and pathway-related UFs as conservative *in silico* options to integrate variability in CYP2C9 and CYP2C19 metabolism using *in vitro* kinetic evidence and in the absence of human data. The future development of quantitative extrapolation models is discussed with particular attention to integrating human *in vitro* and *in vivo* PK or TK data with pathway-related variability for chemical risk assessment.

1. Introduction

The cytochrome P450 2C (CYP2C) family includes four enzymes, namely CYP2C8, CYP2C9, CYP2C18, and CYP2C19. Of these enzymes, CYP2C9 is the most abundantly expressed in the liver, accounting for approximately 20% of total hepatic P450 protein and is also expressed in the gut (Zhang et al. 2016). After CYP3A4 and CYP2D6, CYP2C9 is one of the most important CYP isoform

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http://dx.doi.org/10.1016/j.toxlet.2020.11.016
0378-4274/

catalysing the biotransformation, mostly as oxidation reactions, for approximately 15% of all known therapeutic drugs including hypoglycemic agents, anticonvulsants, anticoagulants, nonsteroidal anti-inflammatory drugs (NSAIDs), antihypertensive, and diuretic drugs (Daly et al. 2017; Isvoran et al. 2017; Marcath et al. 2019). A number of these drugs have a narrow therapeutic index and the impact on efficacy and safety is partly explained through the quantification of inter-individual differences in CYP2C9 protein expression and activity (Daly et al. 2017). Over 60 different gene polymorphisms of CYP2C9 have been reported. The *CYP2C9*1* allele is considered the wild-type (WT) and carriers of *CYP2C9*1* are considered to be extensive metabolisers (EMs), while allelic variants produce allozymes with reduced or deficient enzyme function (EF) in poor metabolisers (PMs). The most studied variants in Caucasians are *CYP2C9*2* and *CYP2C9*3*, while in Asians these two variants are infrequent (Daly 2015; Dorji et al. 2019). Other variants such as *CYP2C9*5*, **6*, **8*, and **11* are more common amongst African descendants (Alessandrini et al. 2013; Kudzi et al. 2009). CYP2C19 accounts for only 1% of the total CYP enzymes in the liver, but is involved in the metabolism of around 10% of therapeutic drugs used in clinical practice (Hiratsuka 2016), such as proton pump inhibitors and antiepileptics. Over thirty allele variants have been described for CYP2C19 and the *CYP2C19*1* allele is considered the WT, while the alleles **2*, **3*, **4*, **5*, **6*, **7*, and **8* are associated with reduced EF and **17* is associated with an increased EF (Alessandrini et al. 2013; Brown and Pereira 2018). Polymorphisms related to changes in EF are less frequent in Caucasians (~2-5%) compared to that in Asian populations (18-23%) (Bertilsson 1995; Gardiner and Begg 2006; Goldstein 2001; Isomura et al. 2010; Nakamura et al. 1985).

Impact of CYP2C9 and CYP2C19 polymorphisms on the pharmacokinetics (PK) of a large number of therapeutic drugs is well described in the literature (Daly et al. 2017; Hirota et al. 2013; Stingl et al. 2013). These changes in PK profile of drugs can affect their efficacy and safety, including hepatoxicity and gastrointestinal bleeding after exposure to NSAIDs (Krasniqi et al. 2016), and neurotoxicity associated with changes in phenytoin concentrations (Dorado et al. 2013). Attention is also given to drugs with a narrow therapeutic index, such as the anti-coagulant warfarin, since carriers of the *CYP2C9*2* and **3* variants have been associated with a higher risk of bleeding (Higashi et al. 2002; Kawai et al. 2014). For CYP2C19, the **2* variant has been associated with ischaemic events including myocardial infarction and stent thrombosis (Kubica et al. 2011; Sibbing et al. 2009).

In the context of chemical risk assessment, human variability in toxicokinetics (TK), including inter-phenotypic differences in polymorphic CYPs, can be accounted for by the refinement of the default TK uncertainty factor (UF) (TK UF: 3.16) (Clerbaux et al. 2018; Clerbaux et al. 2019). Indeed, depending on the consequence of metabolism (detoxification or bioactivation), PMs may constitute a sensitive subgroup and may not be covered by such default TK UF. In such situations, assessment of safe levels of human exposure for xenobiotics using pathway-related UFs or chemical-specific adjustment factors (CSAFs) can provide an evidence-based option. In the past, pathway-related UFs have been derived for multiple metabolic pathways, renal excretion and variability in a range of pharmacodynamics processes (Dorne et al., 2001a, 2001b; Dorne et al. 2003a; Dorne et al. 2002; Dorne et al. 2003c; Dorne et al. 2004c; Renwick and Lazarus 1998). In this context, CYP2C9 and CYP2C19 pathway-related UFs have been derived using human PK data for CYP2C9 and CYP2C19 substrates (Dorne et al. 2003c; Dorne et al. 2004c). However, in the previous studies, data on the impact of inter-phenotypic differences on the kinetics of CYP2C9 and CYP2C19 probe substrates were limited and significant new data have been published over the last decade. Many of the previously published meta-analyses used fixed-effects approach which may not be suitable for analysing data in situations where heterogeneity is high. Recently, meta-analysis methods to quantify variability and uncertainty of kinetic parameters in various enzymes have been developed using Bayesian approaches (Darney et al. 2020; Darney et al. 2019; Quignot et al. 2019; Wiecek et al. 2019). In particular, Wiecek et al. (2019) provided a hierarchical Bayesian meta-analysis method which is applicable to such datasets and to the chemical risk assessment field. Such models make use of available PK data in a more robust manner – compared to that using a standard weighted average of reported data – through modelling reported means and population variability in an integrated manner, that is, under a single model. This can be of particular relevance in situations under which inter-individual variability may differ between population groups (here, inter-phenotypic differences in metabolism). This has been shown previously when comparing healthy adults and subgroups of the population (children, neonates, elderly) by Dorne et al. (2004b), as well as for CYP3A4 substrates after single exposure and co-exposure to grapefruit juice or St John's wort (Quignot et al. 2019). This type of meta-analysis model is referred to a meta-regression model, which uses linear regression to account for the impact of a "group" covariate (*e.g.,* "polymorphism" or "age group"), and is specific to each study arm. In addition to study arm means, dispersions are also modelled.

Here, extensive literature searches have been conducted and a database of published PK studies has been constructed for CYP2C9/CYP2C19 probe substrates. A Bayesian meta-analysis of PK parameters to investigate human variability in markers of chronic exposure to pharmaceuticals (area under the curve (AUC) and clearance (CL)) for different subgroups of human populations was performed using the model published by Wiecek et al. (2019). CYP2C9 and CYP2C19-related UFs were then derived to be further applied to TK, hence correlating (potential) findings of toxicity with a corresponding level of chemical exposure. Population distributions reflecting magnitudes of changes in internal dose across phenotypes have been derived and correlations between such magnitudes and compound-specific parameters were fitted.

2. Material and Methods

2.1. Data collection and harmonisation

Meta-analysis of kinetic data requires sample means and variations reported by individual studies, classified by compound and kinetic parameter. Such data are obtained through systematic reviews or extensive literature searches (ELS), to ensue comprehensiveness and transparency (EFSA 2010; FDA 2009). PK data for substrates of CYP2C9 and CYP2C19 were collected through an ELS, performed as published previously (Quignot et al. 2015) using online databases PubMed (www.ncbi.nlm.nih.gov/pubmed), Embase® (www.embase.com), Cochrane (all databases, www.cochrane.org), and Web of Science™ (www.webofknowledge.com) and covering literature up to 2018. An initial screening of titles and abstracts, followed by a second screening based on full texts, were performed against the inclusion criteria (healthy adults, data on polymorphic genotype/phenotype, quantitative data including statistical descriptors about PK parameters AUC and/or CL), to identify relevant peer reviewed publications. Studies in which humans were exposed to more than one compound were excluded, as well as articles published in another language than English. Substrates of CYP2C9 and CYP2C19 were identified from the literature. For each compound, the fraction metabolised (Fm) was determined using available literature, including human studies reporting excretion of major metabolites and their conjugates and/or computing the ratio of the chronic PK parameters (AUC, CL) between PMs and Ems, as well as *in vitro*

studies with recombinant enzymes identifying CYP isoforms involved. For compounds concluded as "major" substrates based on *in vitro* data but without data on Fm, a default Fm of 0.6 was applied. Compound- and enzyme-specific Fm are presented in Supplementary material 1. PK data for CYP2C9 and CYP2C19 substrates were analysed and meta-analysed as detailed below.

From a data collection perspective, aggregate data are reported using a range of measures, regardless of whether a geometric or an arithmetic scale is used. For dispersion, commonly reported statistics are standard deviation (SD), standard error, 95% confidence interval, interquartile range or min-max range. Therefore, before conducting a meta-analysis data must be converted to a common format. Kinetic data are assumed to be log-normally distributed (Dorne et al., 2001a, 2001b; Naumann et al., 1997; Renwick and Lazarus 1998), so all measures of dispersion were converted to geometric standard deviation (GSD) and all means or medians to geometric means (GM). For the purpose of modelling, all values were further transformed into logarithms to obtain normal distributions. The harmonisation method is described in (Quignot et al. 2019) and (Wiecek et al. 2019). When reported, ratios of geometric means were used for model validation.

2.2. Bayesian meta-regression model

2.2.1. Input data

Data were subdivided into AUC and CL for CYP2C9 and CYP2C19 substrates. For this analysis, the inputs were limited to EM (genotype *CYP2C*1/*1*) and PM (genotypes *CYP2C*2/*2*, **2/*3*, **3/*3*). Detailed list of studies is presented in Supplementary material 2. Due to the log-normal nature of the data, input values for the model were GM, geometric variance (GV) (or converted GM and GV) and sample sizes. Data were modelled using normal distributions, after conversion of means and variances onto the log scale.

2.2.2. Statistical model

The model used is an adaptation of the one detailed in Wiecek et al. (2019), therefore only a short description is provided below. Separate models were developed for four sets of data (each polymorphic pathway and PK parameter). The models characterised (1) the ratios of (geometric) means between EMs and PMs and, (2) inter-individual variability specific to each polymorphic group. The aim of the model is to quantify how population means and variances vary across subgroups of human populations for kinetic data. The model is concerned with behaviour of means and variances on parameter-, subgroup- and compound-specific basis. Indices i = 1,2,N are for the reported logarithms of sample geometric means, *lgm*, the reported logarithms of sample variations, *lv*, and the respective sample sizes, denoted as *n*. As mentioned above, the inputs for the model are the sample means and variations *lgm* and *lv*. These depend on true study means μ_i and true study SDs σ_i through sampling distributions. True means and log-SD's are then linear functions of compound-, study-, group-specific parameters:

$$lgm_i \sim \mathcal{N}(\mu_i, \frac{\sigma^2{}_i}{n_i}) \tag{1}$$

$$lv_i \sim \Gamma(\frac{n_i - 1}{2}, \frac{n_i - 1}{2\sigma_i^2}) \tag{2}$$

$$\mu_i = \mu^c_{c(i)} + \mu^s_{s(i)} + \log(R_i) \tag{3}$$

$$\log(\sigma_i) = \gamma^c_{c(i)} + \gamma_{g(i)} \tag{4}$$

The prior distributions for model parameters $\mu^c_{c(i)}$, $\mu^s_{s(i)}$, $\gamma_{g(i)}$ (respectively: compound-specific mean, study-specific mean, subgroup-specific variability parameter) are unchanged from the cited paper (Wiecek et al. 2019). Ratio parameter *R* is described in detail below. For the compound-specific variability parameter, $\gamma^c_{c(i)}$, a Bayesian "hyperparameter" for mean and SD was added, *i.e.*, $\gamma^c_{c(i)} \sim \mathcal{N}\left(\mu_\gamma, \sigma^2_\gamma\right)$, where both mean and the SD have distribution $\mathcal{N}\left(0, 2.5^2\right)$. This allows to estimate a pathway-specific σ value. The Bayesian model was coded and estimated with the Markov Chain Monte Carlo (MCMC) software Stan, version 2.18 (https://mc-stan.org/) using the Hamiltonian Monte Carlo approach and standard Bayesian posterior checks to ensure convergence of the MCMC procedure. Four chains with 1,000 post-warmup iterations each were used. Model code is included in Supplementary material 3.

Relationship between fraction metabolised Fm, enzyme function (EF) and probabilistic uncertainty factor UF

Metabolising phenotype (*i.e.*, EM, where EF is assumed to be 1, or PM, where EF is assumed to be 0) will lead to changes in plasma concentration of compounds metabolised by the corresponding enzymes. As shown by Gibbs et al. (2006), the ratios of GMs for PMs

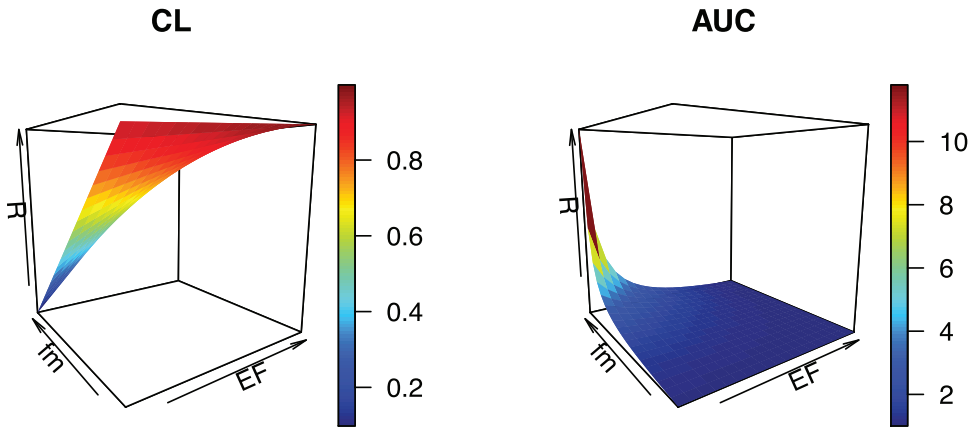

Fig. 1. Relationship between fraction metabolised Fm, enzyme function EF and geometric mean ratio R.

to EMs for CL and AUC are:

$$R_{CL} = \frac{CL_{PM}}{CL_{EM}} = EF \cdot fm + (1 - fm) \quad (5)$$

$$R_{AUC} = \frac{1}{R_{CL}} \quad (6)$$

Both relationships are illustrated in Fig. 1: when fm is high, the ratio is very sensitive to small changes in *EF*.

For CL, UF(x) is a ratio of x-th percentile in PM to the median in EM individuals. For AUC the relationship is inverted, as by convention UF is higher than 1. Under the assumption of AUC and CL following a particular distribution (in this case, log-normal):

$$UF(95)_{AUC} = \frac{Q \leq (0.50, \mu_{EM}, \sigma_{EM})}{Q(0.95, \mu_{PM}, \sigma_{PM})} \quad (7)$$

$$UF(95)_{CL} = \frac{Q(0.95, \mu_{PM}, \sigma_{PM})}{Q(0.50, \mu_{EM}, \sigma_{EM})} \quad (8)$$

where Q(x,y,z) is an x-th quantile of the log-normal distribution with mean y and scale z. Therefore, UF depends not only on the ratio of means, but also on variance parameter.

Using the model, UFs specific to each compound can be calculated using two relationships:

- $\mu_{PM} = \mu_{EM} R = \mu_{EM}(EFfm + 1 - fm)$. This relationship is both drug- and sample-specific. One possible approach is to treat R as drug-specific, but assume an extreme or "idealised" behaviour of PMs, where EF is exactly 0. This assumption will exaggerate UF.
- $\log(\sigma_{EM}) = \log(\sigma_{PM}) + \gamma_{EM}$, that is, a single parameter (γ) describes the average impact of polymorphism on variance; the "baseline" variance σ_{PM} is however drug-specific.

Using the model, UFs can also be derived for hypothetical compounds, *i.e.*, by assuming particular value for Fm and using average estimates of EF and σ within the considered population. Exact method is presented in Results section.

3. Results and discussion

3.1. Overview of data collected

Out of 1,898 studies retrieved, relevant data from 158 publications were extracted, providing 642 PK data (GMs and GSDs) for AUC and/or clearance parameters of 53 compounds, in 5,844 individuals. The summary of the PK data collected for CYP2C9 and CYP2C19 probe substrates is presented in Table 1 and Fig. 2. Fig. 2 highlights large inter-study differences, in particular for compounds metabolised by CYP2C9 (wider distribution as shown in A.). Furthermore, the relationship between inter-phenotypic differences between PMs *vs.* EMs (as GM ratios) and compound-specific Fm were larger for CYP2C19 probe substrates: the higher the Fm, the higher the inter-phenotypic differences between PMs and EMs.

3.2. Meta-regression

The meta-regression explores the relationship between compound-specific Fm for CYP2C9 and CYP2C19 and the human inter-phenotypic differences for the two pathways. Parameter prior and posterior distributions are reported in Supplementary material 4. Simulations were performed using MCMC sampling; their convergence were assessed and produced satisfactory and consistent results. Overall, the posterior distributions for Fm estimates were close to the prior distributions in most cases, which suggests that informative priors on Fm's are necessary for the statistical model to be identified. Variability in EFs across studies was large, which can be explained by presence of different genotypes. Indeed, the distribution of mean EFs in the studied human populations was found to be multimodal. Post hoc, the distribution on EFs was compared with collected CYP2C9 genotypes, however these data were not used for inference.

The results in this section are divided according to Fm (3.2.1), EF (3.2.2), inter-phenotypic differences (3.2.3) and between-subject variability (3.2.4). These four aspects are then summarised with regards to sources of variability (Section 3.2.5) and combined (Section 3.2.6) in Bayesian calculations of UFs for either specific compounds or for a "hypothetical" compound (that is, a compound with a pre-defined value of Fm).

3.2.1. Estimation of fraction metabolised

Estimates of Fm for individual compounds are presented in Supplementary material 4, together with a comparison of posterior distributions with their prior counterpart. Overall, the match between prior distributions (constructed based on Fm reports in the literature, different to the studies used for input data) and posterior distributions was good.

In some cases, the posterior provided larger values compared to that in the prior distribution, suggesting that either the prior was an underestimate of the true Fm, or that the model simulations accounted for inter-phenotypic differences with a higher uncertainty. In other few cases, the prior and posterior distributions

Table 1
Summary of the data collection for human pharmacokinetic parameters

CYP	Parameter	N_s	N_p	N_c	n	Compounds
2C9	AUC	95	43	21	1,109	acenocoumarol (-R), acenocoumarol (-S), celecoxib, chlorpropamide, diclofenac, flurbiprofen, glyburide, ibuprofen, ibuprofen (-R), ibuprofen (-S), lornoxicam, losartan, meloxicam, nateglinide, phenprocoumon (-R), phenprocoumon (-S), phenytoin, piroxicam, tenoxicam, tolbutamide, torasemide, warfarin, warfarin (-R), warfarin (-S)
2C9	CL	107	42	24	1,359	acenocoumarol (-R), acenocoumarol (-S), celecoxib, chlorpropamide, diclofenac, flurbiprofen, fluvastatin (-R), fluvastatin (-S), glyburide, ibuprofen, ibuprofen (-R), ibuprofen (-S), lornoxicam, losartan, meloxicam, nateglinide, phenytoin, piroxicam, tenoxicam, tolbutamide, torasemide
2C19	AUC	284	92	23	2,084	carisoprodol, citalopram, citalopram (-R), citalopram (-S), escitalopram, esomeprazole, gliclazide, lanzoprazole, lanzoprazole (-R), lanzoprazole (-S), mephenytoin (-R), mephenytoin (-S), moclobemide, omeprazole, omeprazole (-R), omeprazole (-S), pantoprazole, pantoprazole (-S), pantoprazole (-R), rabeprazole, rabeprazole (-R), rabeprazole (-S), voriconazole
2C19	CL	156	56	22	1,292	amitriptyline, citalopram, citalopram (-R), citalopram (-S), escitalopram, esomeprazole, gliclazide, hexobarbital, hexobarbital (-R), hexobarbital (-S), lanzoprazole, mephenytoin (-R), mephenytoin (-S), moclobemide, omeprazole, omeprazole (-R), omeprazole (-S), pantoprazole, pantoprazole (-S), pantoprazole (-R), rabeprazole, voriconazole
All	Total	642	158	53	5,844	

N_s: Number of study arms; ***N_p***: Number of publications; ***N_c***: Number of compounds; ***n***: Number of individuals

were virtually the same, suggesting that Fm for a particular compound could not be statistically identified using available data. However, in these cases, even if the estimation of Fm was uncertain, the Bayesian meta-regression model leveraged all of the available study data to estimate other model parameters (*e.g.*, EF, between-subject variability).

3.2.2. Enzyme function estimates

Substantial variability in (mean) EFs across study arms was observed (Supplementary material 4). For CYP2C9, median EF for groups categorised as EMs was 75-77%, and for groups categorised as PMs, 20-26%. For CYP2C19, EF was 84% for EMs and 11-12% for PMs for which ranges correspond to 2 different models of AUC and CL parameters. From the PM and EM distributions, a "typical" relative EF, as the ratio of EF in PM populations to EF in EM populations, was then calculated: 0.26-0.34 for CYP2C9 and 0.12-0.14 for CYP2C19. These ratios were then subsequently used for the derivation of pathway-related UFs to account for the variability in EF for PM and EM subgroups (ranging from above 0 to 1).

These estimates, obtained from a large set of human studies, are valuable findings and provide means to characterise variability in CYP2C9 and CYP2C19 metabolism across available subgroups of the human populations. In addition, *post hoc* comparisons of the estimated EFs to genotype (rather than phenotype) information, which has not been used for the meta-regression, were performed. This comparison illustrated in Fig. 3 confirmed the biological basis of the mechanistic assumption applied in the model *i.e.*, individuals categorised as PMs, those and carrying the allele **3* (particularly homozygous individuals **3/*3*) have the lower EF (Fig. 3).

The calculation of inter-phenotypic differences in PM and EM subgroups depends not only on the EF ratio calculated above, but also on the proportions of PMs and EMs in the population. The proportion of PMs in the Caucasian population is on average 4-6% for CYP2C9 and CYP2C19 (Goldstein 2001). Consequently, it was assumed that the median for PMs lies within the top 5% of the population distribution and that the "typical" EF ratio can be used for calculating the ratios in Section 3.2.3 and 3.2.5.

3.2.3. Estimates of inter-phenotypic differences between extensive and poor metabolisers for specific CYP2C9 and CYP2C19 substrates

Inter-phenotypic differences in PK parameters for probe substrates of the CYP2C9 and CYP2C19 isoforms were calculated as the ratios of geometric means for EM vs PM and assessed as functions of Fm and EF. These are presented in Fig. 4. For the calculation, a "typical" relative EF from Section 3.2.1 was assumed and Fm values were the posterior distributions from the meta-regression model.

The results showed adequate predictions of inter-phenotypic differences expressed as geometric mean ratios between EMs and PMs for probe substrates of CYP2C9 and CYP2C19 (Fig. 4). Overall, variability from one compound to another was low (<30%). Major substrates for CYP2C9 (flurbiprofen, celecoxib, meloxicam, tolbutamide, torasemide, warfarin (-S)) and CYP2C19 (escitalopram, mephenytoin, moclobemide, omeprazole, pantoprazole) were associated with higher inter-phenotypic differences as well as larger variability.

3.2.4. Inter-individual variability estimates

Inter-individual variability (σ parameter of the log-normal distribution) was estimated by the model, both in terms of an average pathway-specific value and in terms of inter-phenotypic differences to quantify the impact of CYP2C9 and CYP2C19 polymorphism on PK parameters. Inter-compound variability was also accounted for and is reported in Supplementary Material 4. Overall, the estimated average pathway- and parameter-specific variability ranged from 26-31%, with a mean CV of 25% for CYP2C9

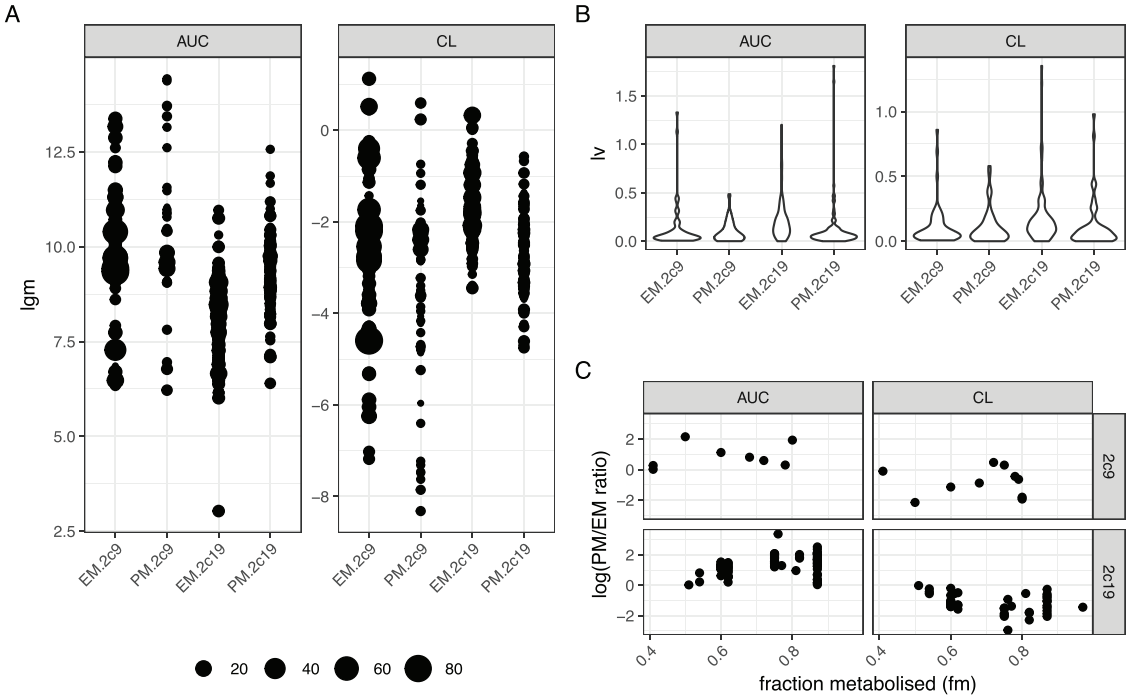

Fig. 2. Distribution of input data (A. logarithms of sample geometric means, lgm; B. logarithms of sample variations, lv; C. ratios of geometric means between poor metabolisers PMs and extensive metabolisers EMs, according to Fm).

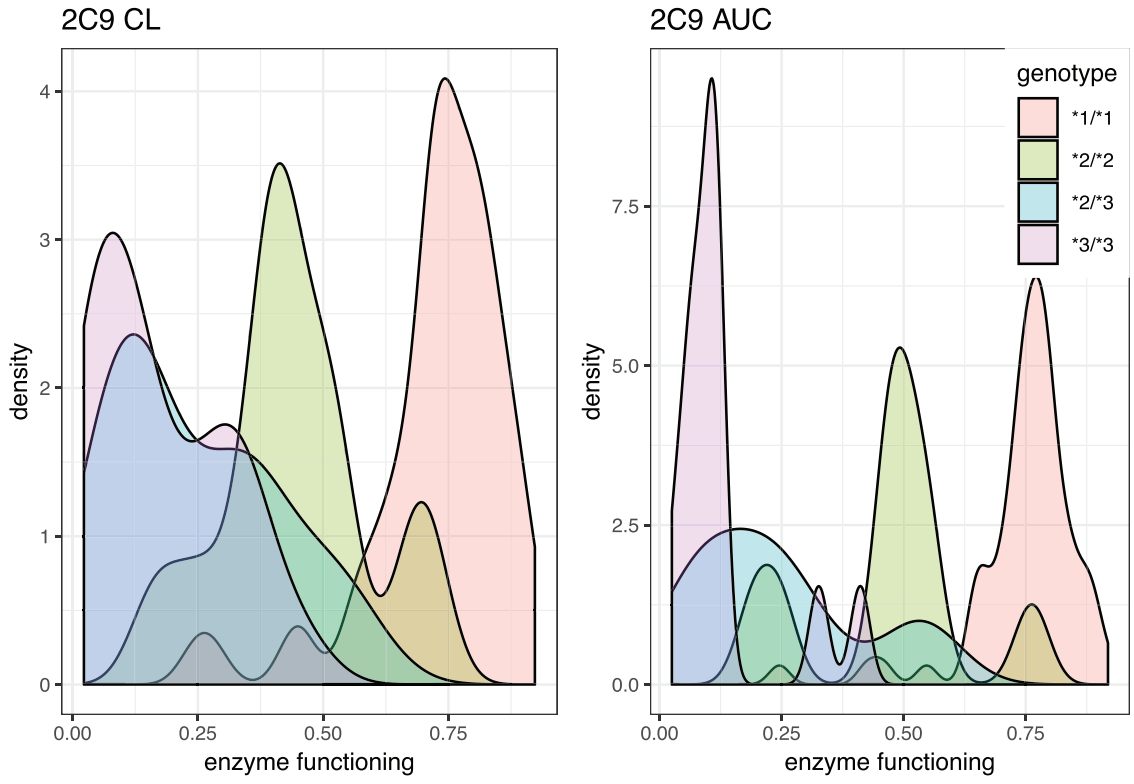

Fig. 3. Distributions of mean EFs in human subpopulations for CYP2C9 clearance and AUC parameters.

and 40% for CYP2C19. However, uncertainty in the estimates was significant, suggesting that, 1) variances are difficult to estimate using the available data and 2) individual compounds have a considerable impact on variability. Higher CV values ranging between 60-70% were common for both pathways, with typically higher values observed for the CYP2C19 pathway. Overall, CV estimates were in agreement with previous meta-analyses, for which CV overall mean values of 20% and 44 to 50% were derived for CYP2C9- and CYP2C19-related variability, respectively, in healthy adults (Dorne et al. 2003b; Dorne et al. 2004a). Substantial and significant variability differences were observed for all four models between PM and EM groups, with variability for the EMs on average 14% to 47% higher than for the PMs (*i.e.,* CYP2C19 AUC), depending on the model (see the table detailing γ^c parameter/ 'gamma_group' in Supplementary material 4). These inter-individual differences were also partly compound-dependent.

3.2.5. Sources of variability

Variability within the EM population and within compounds for the CYP2C9 and CYP2C19 pathways can be partly related to the fraction of the compound metabolised by the isoform, *i.e.*, amount of the dose metabolised by the CYP2C enzyme as well as the impact of liver blood flow for compounds with high clearances. For lower clearances, liver blood flow has a more limited influence on inter-individual variability in PMs compared to that for EMs. Furthermore, inter-individual differences within the EM phenotype can be also explained by a greater homogeneity in the enzyme activity compared to the PM phenotype. Indeed, in this work, individuals with genotypes associated with decreased or non-functional activity were considered as PMs. Additionally, due to weak or even absence of activity of CYP2C9/CYP2C19 in PMs, metabolism of CYP2C substrates can be carried out by other CYP450 isoforms. If this CYP substitution pathway is monomorphic, variability is likely to be lower. Finally, protein binding and differences in binding affinity to the isoforms and enzyme-substrate reaction also provide rationales for the observed inter-individual differences in kinetics between compounds.

3.2.6. Derivation of pathway-related uncertainty factors (UFs)

Pathway-related UFs were derived by combining outputs of inference on Fm (section 3.2.1), mean EFs (section 3.2.2), inter-phenotypic differences in PK parameters (section 3.2.3) and patient variability within studied groups (section 3.2.4) for each set of parameters (AUC, CL) and pathway (CYP2C9, CYP2C19).

3.2.6.1. Compound-specific UFs. Compound-specific UFs were also calculated for each parameter and both CYP2C9 and CYP2C19 pathways. The full set of results is provided in Supplementary material 5. Overall, mean estimates for compound-specific UFs to cover the 95th centile of the population (UF95) were within the range or above the default TK UF of 3.16 with UFs between 2.1-7.9 (compounds handled by CYP2C9) and 2.6-12.8 (CYP2C19). Unsurprisingly, the highest UFs were found for PMs of CYP2C9 (7.9) and CYP2C19 (12.8).

Since the calculated values for the compound-specific UFs are also Bayesian and therefore variable and uncertain, uncertainty on some UFs was substantial, especially when ratios were large and Fm was not known precisely. For example, for CYP2C19 clearance of omeprazole (R), the 95% uncertainty interval ranged from 7.7 to 24.1.

Both theoretical (see section 2.2.3) and observed data (see Supplementary material 5) showed that the 95% UF can often be exceeded due to combination of high (mean) EF, high Fm, and inter-individual differences. Hence, major CYP2C substrates would

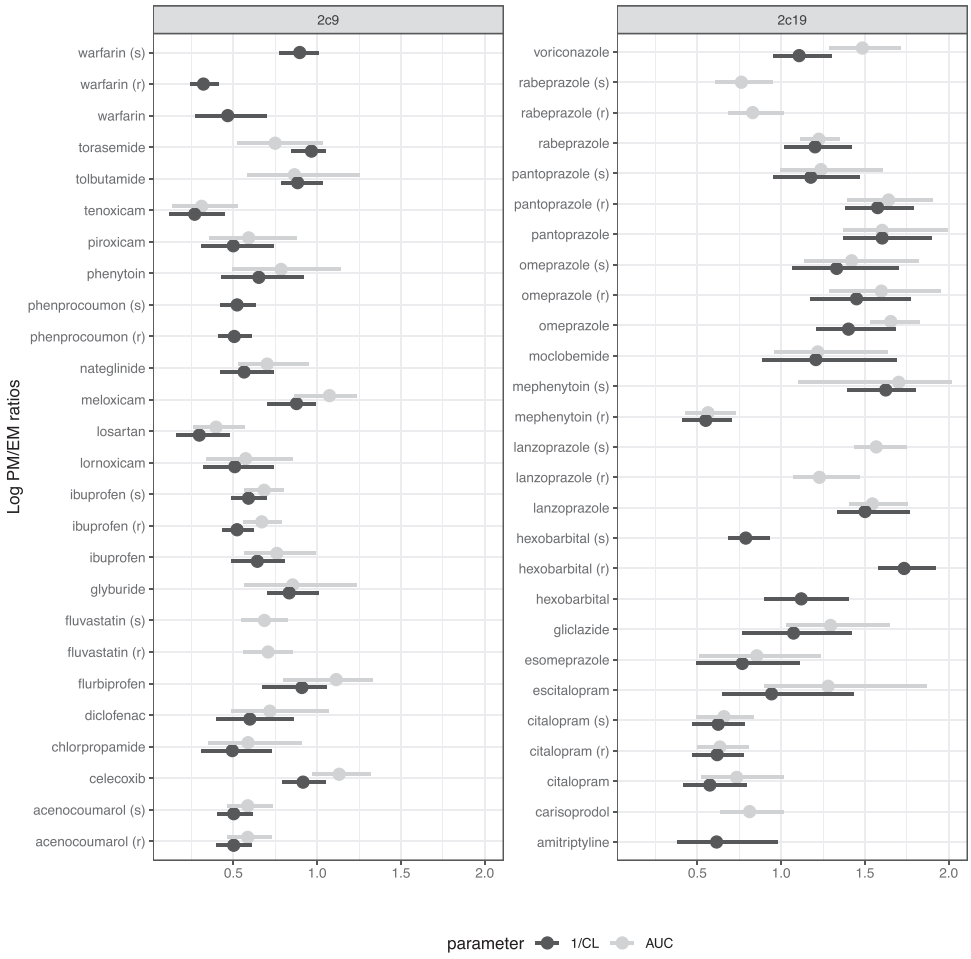

Fig. 4. Inter-phenotypic differences in the pharmacokinetics of CYP2C9 and CYP2C19 probe substrates for markers of chronic exposure (clearance, CL and AUC) expressed as ratios of geometric means, on log scale, between poor metabolisers PMs and extensive metabolisers EMs. To allow for comparison across CL and AUC, reciprocal of CL was used.

require higher UFs to cover such inter-phenotypic differences compared to minor CYP2C substrates.

3.2.6.2. Pathway-related UFs. Pathway-related UFs were also derived as a function of EF and Fm (combined into a ratio of means) and variability. The following assumptions were made:

1 For Fm (contribution of the pathway), hypothetical 60% and 90% values were considered.
2 For EF, the ratio derived in Section 3.2.2, *i.e.*, the ratio of 5th percentile in PMs to the median in EMs (specific to each pathway and parameter), was used.
3 For the variability analysis, the values were based on model inference on σ across studies. The value of 0.3 was used as a default, considering the typical (average) σ in 0.26-0.31 range estimated by the model. A value of 0.6, which would correspond to high variability, observed for some compounds, was also considered.

These three values were then used to derive UFs according to the overall formula presented in Methods. Average pathway-related UFs covering the 95th percentile of the population for CYP2C9 and CYP2C19 were derived (Table 2). When considering hypothetical compounds with Fm of 60%, UFs were within the range or slightly above the default TK UF of 3.16: ranges of 2.7-4.8 (CYP2C9 pathway) and 3.4-5.7 (CYP2C19 pathway). When considering compounds with Fm of 90%, UFs were above the default TK UF of 3.16, and up to 12.7 for the CYP2C19 pathway, when considering the high variability scenario.

Table 2
Pathway-related UFs

	2C19 AUC	2C19 CL	2C9 CL	2C9 AUC
Fm 60%, low sigma	3.5	3.4	2.7	2.9
Fm 60%, high sigma	5.7	5.5	4.4	4.8
Fm 90%, low sigma	7.7	7.1	4.0	4.9
Fm 90%, high sigma	12.7	11.7	6.6	8.0

Overall, when the Fm of a compound by CYP2C9 or CYP2C19 metabolism was low, CYP2C9 and CYP2C19-related UFs were consistently lower and logically can be associated with a limited impact of inter-phenotypic differences for the two isoforms. A CYP2C-related uncertainty factor of 13 could be proposed to cover 95% of the population regardless of the compound, provided it is metabolised by the isoforms. This is above the range of the previously estimated CYP2C9-related UF95 ranging from 1.3 to 5.9 for healthy adults (all phenotypes) (Dorne et al. 2004a) and the previously estimated CYP2C19-related UF95 ranging from 2.0 to 4.7 for healthy adults non-phenotyped, EMs and slow extensive metabolisers (SEMs), but is below the CYP2C19-related UF95 of 45 for healthy adults PMs (Dorne et al. 2003b).

4. Conclusions

This manuscript describes extensive literature searches and meta-regressions for 158 human PK studies to quantify inter-phenotypic differences (EMs and PMs) in markers of chronic exposure for CYP2C9 and CYP2C19 probe substrates. Using the whole dataset, compound-specific Fm and subpopulation-specific EF, a Bayesian meta-regression model provided a range of quantitative estimates as the basis to derive compound- and pathway-related UFs for risk assessment. Broadly speaking, this study supports the trend to replace the traditional default or categorically-based UFs using either chemical specific adjustment factors or data-derived UFs taking into account population characteristics, dose metrics, exposure scenarios, TK and/or TD, as well as inter-individual variability to reduce uncertainty in chemical risk assessment (Bhat et al. 2017). Specifically, the model developed and the results thereof illustrate the integration of human variability in CYP2C9 and CYP2C19 metabolism and CYP2C9- and CYP2C19-related UFs for chemical risk assessment. Results showed that up to 8.0 and 12.7 (instead of the default kinetic factor of 3.16) would be required to cover up to 95% of individuals, respectively.

From a mechanistic point of view, the integration of enzyme specific parameters (*i.e.,* EF and CYP2C Fm) in the model allowed the prediction of PK changes between EMs and PMs for a wide range of CYP2C9 and CYP2C19 pharmaceutical substrates, while minimising confounding factors. Inclusion of functional consequences for each isoform accounted for the activity and stability of the enzyme. In this analysis, the authors focused on markers of chronic exposure, namely AUC and CL parameters, since those parameters are appropriate to investigate on Fm and its impact on inter-phenotypic differences in CYP2C9 and CYP2C19, in contrast with markers of acute exposure which are strongly dependent on bioavailability (Quignot et al. 2019). The dataset is of direct relevance for the assessment of PK variability for pharmaceuticals with a narrow therapeutic index since relatively small alterations in concentrations may have a direct impact on internal dose, pharmacodynamic and consequently likelihood of toxicodynamic consequences. Likewise, the dataset is also of relevance to assess human variability in TK for emerging designer drugs with phenotypic dependent toxicity as well as residues of chemicals in food (e.g. pesticides, food additives, contaminants). An important aspect of this analysis is the assumption that PMs may be the most susceptible group compared to EMs. This assumption is relevant when the parent compound is the toxic moiety so that the consequence of metabolism would be detoxification and an increase in internal dose would increase the risk of adverse effect. In contrast, if the consequence of CYP2C9/CYP2C19 metabolism is bioactivation, PMs would have lower internal dose of the toxicant and EMs would be at higher risk with the most susceptible group being ultra-rapid metabolisers (URMs). For a potential use of this approach in drug safety or chemical risk assessment, this study has been limited to healthy adults because of the available data and the predicted inter-phenotypic differences and consequently the pathway-related UFs are likely to be slightly different in other human subpopulations such as children or neonates (Dorne et al. 2003b), patients prescribed with various co-medications (Xie et al. 2016) as well as patients with impaired renal or liver function (Almazroo et al. 2017).

Broader application of this approach in chemical risk assessment requires data for *in vitro* and *in vivo* kinetic characteristics, isoform-specific metabolism and toxicodynamics, pathway-related variability and inter-phenotypic differences. It is foreseen that the integration of isoform-specific *in vitro* data within generic quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) models will allow to test the performance of predictions for inter-phenotypic differences in polymorphic metabolism for compounds with no available human kinetic data. Moreover, as *in vitro* data become more available, these models will provide the basis to develop Quantitative Structure Activity Relationship (QSAR) models for the prediction of TK properties for a broader range of xenobiotics (EFSA 2014b). Here, the main TK determinant presented in this study is CYP2C9/CYP2C19 metabolism and this approach can be applied to relevant xenobiotics in food or the environment for which *in vitro* evidence is available (*e.g.*, flavourings, pesticides, food additives, contaminants, etc.), while integrating information on efflux/uptake transporters and other phase I and phase II enzymes (Darney et al. 2020; Darney et al. 2019; Kasteel et al. 2020). In the same manner, available information or predictions for other kinetic properties including plasma protein binding and systemic clearance relative to liver blood flow may be considered to inform such QIVIVE models and allow calibration and model evaluation.

Non-invasive *in vitro* techniques using human cell lines or liver microsomes from donors are now available to generate metabolism data and can provide quantitative information including EF and Fm for specific isoforms (Basketter et al. 2012; Bell et al. 2018; Blaauboer et al. 2012). In addition, the use of physiologically-based kinetic (PBK) model is increasingly recommended in chemical risk assessment (Bessems et al. 2014; EFSA 2014a; Paini et al. 2019; WHO 2010) and quantification of inter-individual differences allows for sound development of these models. Combining distributions quantifying inter-individual variability and uncertainty from PK studies from the pharmaceutical database with isoform-specific *in vitro* data is a promising QIVIVE approach to reduce uncertainty in chemical risk assessment. With regards to CYP2C9/CYP2C19-related variability, distributions can be integrated with human *in vitro* data within PBK models with Markov-Chain Monte Carlo. Applying a PBK model with distributions for each parameter in a Bayesian framework, as previously done by Bois et al. (2010), allows a better prediction of internal dose and decreases the uncertainty during risk assessment. This type of approach avoids the use of default UFs and allows the derivation of appropriate UFs covering the extent of human variability in TK processes for the chemical under assessment (Punt et al. 2017). Overall, it is foreseen that the analysis and model presented here can be applied and/or combined with other metabolic pathways and toxicodynamic endpoints to develop open source generic human PBK models to inform chemical risk assessment using higher tier approaches depending on the level of knowledge (Einolf 2007; EMA 2012; FDA 2012; Jamei 2016; Zhuang and Lu 2016). For given compounds metabolised by multiple CYP isoforms, phase II enzyme isoforms such as UDP-glucuronysyl-transferases (UGTs) and transporters, individual isoforms can already be identified using available *in vitro* methods. Using the approach described here, the relative contribution of each isoform, expressed as Fm, can be simulated using MCC, with available human variability distributions for each CYP, UGT isoforms or transporters (including inter-phenotypic differences when relevant). Finally, the relative contribution of each isoform to the overall variability of the outcome population distributions can be assessed through global sensitivity analysis and used as a basis to derive compound-specific UFs quantifying human variability in TK (Darney et al. 2020; Darney et al. 2019; Kasteel et al. 2020).

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Full publication list is provided in Supplementary materials

Code availability

Code is available in Supplementary materials

Funding

This work has been co-financed by the European Food Safety Authority (EFSA) under contract CFT/EFSA/EMRISK/2012/01 and Certara.

Transparency document

The Transparency document associated with this article can be found in the online version.

CRediT authorship contribution statement

N Quignot: Conceptualization, Project administration, Writing - original draft, Writing - review & editing. **W Więcek:** Software, Visualization, Writing - original draft, Writing - review & editing. **LS Lautz:** Writing - review & editing. **JLCM Dorne:** Writing - review & editing, Project administration. **B Amzal:** Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

This work has been co-financed by the European Food Safety Authority (EFSA) under contract CFT/EFSA/EMRISK/2012/01 and Certara. The authors would like to thank Katarzyna Miernik, Iwona Kuter, Mateusz Nikodem, Agnieszka Zyla from Analytica LASER, a Certara company, and Camille Béchaux from EFSA, for data collection and analysis. The views expressed in this manuscript are the authors only and do not reflect the views of the European Food Safety Authority.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxlet.2020.11.016.

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