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Inter-ethnic differences in CYP3A4 metabolism: A Bayesian meta-analysis for the refinement of uncertainty factors in chemical risk assessment

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ABSTRACT

CYP3A4 is the major human cytochrome P450 isoform responsible for the metabolism of more than 50% of known xenobiotics. Here, inter-ethnic differences in CYP3A4 metabolism have been investigated through a systematic review of pharmacokinetic data for 15 CYP3A4 probe substrates and parameters reflecting acute (Cmax, oral route) and chronic exposure (clearance and area under the plasma concentration-time curve, oral and intravenous route). All data were extracted in a structured database and meta-analyses were performed using a hierarchical Bayesian model in the R freeware to derive parameter, route and population-specific variability distributions for CYP3A4 metabolism. Two different approaches were applied. 1) Inter-individual differences were quantified using North American healthy adults as a reference group to compare with European, Asian, Middle East, and South-American healthy adults and with ethnicity, elderly, children and neonates. 2) Intra-ethnic–specific variability distributions were derived without comparing to a reference group. Overall, subgroup-specific distributions for CYP3A4-variability provided the basis to derive CYP3A4-related uncertainty factors (UF) to cover 95th or 97.5th centiles of the population and were compared with the human default toxicokinetic UF (3.16). The results indicate that CYP3A4-related UFs in healthy adults were higher for chronic oral exposures (2.5–3.0, UF_{95} and $UF_{97.5}$, 10 compounds) than for intravenous exposures (1.7–1.8, 2 compounds). All UFs were within the default TK UF. These distributions allow for: 1) the application of CYP3A4-related UFs in the risk assessment of compounds for which *in vitro* CYP3A4 metabolism evidence is available without the need for animal data; 2) the integration of CYP3A4-related variability distributions with *in vitro* metabolism data into physiologically based kinetic (PBK) models for quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) and 3) the estimation of UFs in chemical risk assessment using variability distributions of metabolism.

1. Introduction

Human variability in pharmacokinetic (PK), toxicokinetic (TK) or kinetic processes (namely absorption, distribution, metabolism and excretion (ADME)) and pharmacodynamics (PD) or toxicodynamic (TD) or dynamic processes are key considerations in human risk assessment of chemicals, particularly for 1) the refinement of uncertainty factors (UF) using human data, 2) the development of physiologically-based models, 3) the reduction of animal testing using quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) models. To account for the variability in kinetic and dynamic processes across and within species, a 100-fold default UF has been applied for over 60 years to sub-chronic to chronic toxicity data in test species (rat, mouse, dog, rabbit) to derive safe levels of threshold toxicants for non-cancer risk assessment. This default value

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Table 1
List of queries used for the ELS (formatted for Scopus).

Search CYP3A4 probe substrate	TITLE-ABS ("*name of probe substrate*")
Population	(TITLE-ABS (human) OR TITLE-ABS (adult) OR TITLE-ABS (adults) OR TITLE-ABS (child) OR TITLE-ABS (children) OR TITLE-ABS (infant) OR TITLE-ABS (neonate) OR TITLE-ABS (newborn) OR TITLE-ABS (newborns) OR TITLE-ABS (elderly) OR TITLE-ABS ("pregnant women") OR TITLE-ABS (men) OR TITLE-ABS (women) OR TITLE-ABS ("ethnic group") OR TITLE-ABS (caucasian) OR TITLE-ABS (asian) OR TITLE-ABS (african) OR TITLE-ABS ("genetic polymorphism*") OR TITLE-ABS ("individual susceptibility") OR TITLE-ABS ("gene environment") OR TITLE-ABS ("ethnic variability") OR TITLE-ABS ("Afro American") OR TITLE-ABS (hispanic) OR TITLE-ABS ("race difference") OR TITLE-ABS ("age difference") OR TITLE-ABS ("race differences") OR TITLE-ABS ("age differences") OR TITLE-ABS ("gender differences") OR TITLE-ABS ("gender difference") OR TITLE-ABS ("sex difference") OR TITLE-ABS ("sex differences"))
Outcomes	(TITLE-ABS (auc) OR TITLE-ABS (area under the curve) OR TITLE-ABS (area under curve) OR TITLE-ABS (half life) OR TITLE-ABS (half-life) OR TITLE-ABS (half-lives) OR TITLE-ABS (clearance) OR TITLE-ABS (cmax) OR TITLE-ABS (vmax) OR TITLE-ABS (km) OR TITLE-ABS ("michaelis constant") OR TITLE-ABS (pharmacokinetic) OR TITLE-ABS (pharmacokinetics) OR TITLE-ABS (toxicokinetic) OR TITLE-ABS (toxicokinetics))
Exclusion	(TITLE-ABS ("cell line*") OR TITLE-ABS ("cell culture*"))

TITLE-ABS: term searched only in the title and the abstract of the paper.

has been justified to allow for interspecies differences (10-fold) and human variability (10-fold) [1]. Further refinements have been proposed to subdivide both factors to allow for differences in TK and TD with two equal default UFs ($10^{0.5} = 3.16$) for the human variability [2]. Such subdivisions were introduced to allow the replacement of default UFs with chemical-specific adjustment factors (CSAF) or pathway-related (TK) or process-related (TD) UFs intermediate options [3–5].

CSAFs are derived using chemical-specific data for either or both the TK and TD dimension using physiologically-based TK (PBTK) models describing ADME processes from external to internal exposure or PBTK-TD models integrating the toxicity dose–response [6]. In order to support the use of such models, a key recommendation regards the better integration of human variability in TK, metabolism and TD when available [7,8]. This can also provide the basis for developing integrated testing strategies without the need for animal testing to move towards the use of QIVIVE [9].

Pathway-related UFs quantifying human variability in a range of metabolic pathways have also been proposed as intermediate options between default UFs and CASFs and these were first applied to CYP1A2 and glucuronidation [4,5,10–12]. Following this approach, pathway-related UFs have been published for renal excretion, a number of phase I and Phase II enzymes as well as UFs allowing for variability in pharmacodynamics [4,10–19].

Amongst the key phase I enzymes, the CYP3A isoform constitutes the most abundant CYP in the liver (29%) and intestine (70%) and has a major role in the metabolism of a large number of drugs, endogenous hormones, bile acids, fungal and plant products, including 50% of all known drugs and xenobiotics [13,20,21]. The CYP3A subfamily consists of four CYP genes: 3A4, 3A5, 3A7 and 3A43, sharing a high sequence similarity of at least 85%. The CYP3A4 isoform represents ~85% of hepatic and intestinal CYP3A. CYP3A5 is predominantly expressed in extrahepatic tissues while CYP3A7 is the main isoform in fetal liver (up to 50%) [13,22–24].

Analysis of human variability in CYP3A4 metabolism has been previously carried out by Dorne et al. [13] in order to compare healthy adults (mostly Caucasian) to various subpopulations, such as Asians, African and Mexican. In addition, CYP3A4 metabolism in various age groups, such as neonates, children and elderly was compared to adults. However, a distinction between European and North American population was not made and the paper did not include intra-ethnic variability in the subgroups. CYP3A4 related UFs were based on limited studies. Since then, considerable PK studies have been conducted with regards to CYP3A4 probe substrates and this provide a means to update knowledge on human variability for the CYPA4 pathway. In this work, a full-Bayesian approach is proposed for the meta-analysis of pharmacokinetic data using a multi-level hierarchical model to integrate quantifiable sources of variability, including inter-study, inter-ethnic, intra-ethnic and inter-individual variability for populations of different ages. In this context, inter-individual variability and related UFs are derived for each group and each pharmacokinetic parameter. Finally, a perspective on future integration of CYP3A4-variability distributions in PBPK and QIVIVE models is discussed.

2. Material and methods

2.1. Extensive literature search and data collection

An extensive literature search (ELS) was performed to identify human PK studies for CYP3A4 probe substrates in healthy adults from a range of ethnic backgrounds and in subgroups of the population: elderly, children and neonates. The ELS was performed by two independent reviewers for the period January 2002-January 2017 using PubMed and Scopus [25,26]. Probe substrates of CYP3A4 were identified from the literature as compounds that are extensively metabolised by CYP3A4 (> 60%) using *in vitro* evidence to identify relevant metabolites combined with urinary excretion profiles expressed on a dose metric basis. For each CYP3A4 probe substrate, measured PK parameters, reflecting chronic and acute exposure (AUC/clearance and Cmax, respectively), after an oral intake or intravenous injection (IV) were extracted. Table 1 provides a summary of the individual key words applied for the ELS.

Primary screening of the literature was carried out on titles and abstracts, after removal of duplicates. The following exclusion criteria were applied to peer-reviewed publications in English reporting studies that were not relevant to CYP3A4 kinetics in healthy humans: 1. other species, 2. *in vitro*, 3. development of analytical methods, 4. modelling, 5. pharmacodynamics investigations only, 6. studies for unhealthy individuals, 7. substrates other than those identified as relevant.

Articles meeting the exclusion criteria were excluded from further analysis and were not imported into the EndNote® reference software for further evaluation. Reviews and book chapters were not considered for data extraction as they do not report primary datasets. This prevents multiple inclusion of the same dataset from different references.

A second screening was performed on each full-text article to evaluate the methodological quality of the selected PK studies including design, analysis and reporting, which may lead to biased results. Here, the Klimish scoring system was not considered relevant and a specific scoring system is proposed as described in Table 2.

Table 2
Scoring system applied for the secondary screening.

Population	0 No information
	1 at least number, age and health status
	2 ethnic group and other information
Methodology	0 insufficient description
	1 inaccuracies in some points
	2 full description
Results	0 no pharma/toxicokinetics data
	1 pharma/toxicokinetics data without descriptive statistics
	2 pharma/toxicokinetics data with variability information

The scoring system was applied as follow: the required score for inclusion was 1–2 for the sections “Population” and “Methodoloy”, while a score of 2 for the “Results” section need to be fulfilled.

2.2. Meta-analysis

2.2.1. Standardisation of datasets

Data standardisation for all PK parameters collected in the database was required to perform the meta-analysis in a harmonised manner for each parameter. Body weight was expressed in kg. When available, mean body weight recorded from the study was used. Otherwise, a body weight was allocated according to the country of origin using data from Walpole et al. [27]. Dose, AUC, Cmax and Clearance were expressed in mg/kg bw, ng.h/ml/dose, ng/ml/dose and ml/min/kg bw respectively.

Data from the PK studies were mostly reported either as arithmetic means (X) and standard deviations (SD) or by geometric mean (GM) and geometric standard deviation (GSD). Since PK data are generally recognised to be lognormally distributed [5,10,28], the geometric mean (GM) and geometric standard deviation (GSD) are appropriate to summarise a lognormal distribution, all data were harmonised to GM and GSD. When these measures were not reported, they were estimated for each individual study using the following equations:

$$GM = X/\sqrt{(1 + CV_N^2)} \tag{1}$$

$$GSD = \exp(\sqrt{\ln(1 + CV_N^2)}) \tag{2}$$

where CV_N is the coefficient of variation for normally distributed data given by:

$$CV_N = SD/X \tag{3}$$

In the cases that the SD was not reported, it can be estimated from standard error SE (SEM), CV_N and 95% confidence interval of the mean according to the Eqs. (4)–(6).

$$SD = \sqrt{n}\,SE \tag{4}$$

$$SD = CV_N X \tag{5}$$

$$SD = [(UCI - LCI)/(2t_{0.975,n-1})]\sqrt{n} \tag{6}$$

where UCI and LCI refer to upper and lower bounds of confidence interval and $t_{0.975,n-1}$ is the 97.5 percentile of the t distribution with $n - 1$ degrees of freedom (we assumed that for a symmetric confidence interval, the confidence interval is constructed in the common way: $X \pm t \times SE$)).

For non-symmetric confidence intervals, it is assumed that the confidence interval is constructed around a geometric mean. According to Higgins et al. [29], the geometric standard deviation is estimated as follows:

$$GSD = \exp[(\ln(UCI) - \ln(LCI))/2t_{1-\alpha/2,n-1}]\sqrt{n} \tag{7}$$

For some studies, standard deviation was reported but not specified to be arithmetic or geometric. These were considered as GSD when reported together with a Geometric mean. The same assumption was applied to CV.

Here, it is important to highlight that estimation of variability from an interval using Eq. (6) or (7) results in overestimated variability values.

2.2.2. Bayesian hierarchical model for meta-analysis

The objective of the meta-analysis is to provide accurate information on the means (μ_j) and the inter-individual variability (τ_j) of the PK parameters for a substrate ‘j’, based on the combination of results from multiple independent studies ‘k’. For each compound and parameter, it is thus necessary to properly separate and identify the variability related to differences between studies (τ_{study}), the variability related to differences between substrates ($\tau_{substrate}$) and the variability related to differences between individuals (τ_j) by decomposing the variance of the PK parameter (clearance, AUC or Cmax). Consequently, a hierarchical model was developed based on the generic hierarchical Bayesian model for the meta-analysis of human population variability in kinetics described by Wiecek et al. [30]. The structure of the model showing the conditional dependencies among the population and the individual parameters are summarised graphically in Fig. 1.

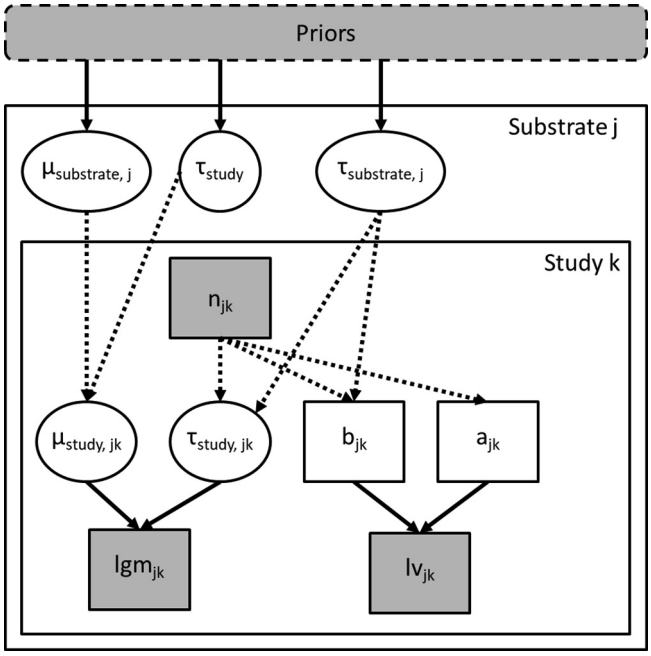

Fig. 1. Graphical representation of the hierarchical model for meta-analysis. Squares represent the known quantities: the logarithm of the geometric mean (lgm) and variance (lv) of the study k for the substrate j, the number of individuals of this study (n) and a = (n-1)/2. Circles represent unknown quantities to be updated via Bayesian inferences: the mean (μ_{jk}) and the precision (τ_{jk}) of lgm, the mean (μ_j) and the precision (τ_j) of the PK parameter for the substrate j and b = n.τ_j/2, inter-study precision (τ_{study}). Solid arrows represent a stochastic link and dashed arrows represent a deterministic link.

On the logarithmic scale, each individual value for a chosen PK parameter X_{ijk} with i = 1,2,3…,n is assumed to be independently and identically distributed according to a normal distribution of mean μ and variance σ^2 for a given substrate j in a given study k. Therefore, according to the central limit theorem, the means and the variances $\bar{X}_{jk} = \frac{1}{n}\sum_{i=1}^{n} X_{ijk}$ and $S_{jk} = \frac{1}{n_{jk}-1}\sum_{i=1}^{n}(X_{ijk} - \bar{X}_{jk})^2$ are independent conditionally to the study and the substrate and distributed according to:

$$\bar{X}_{jk} \sim Normal\left(\mu,\ \frac{\sigma^2}{n}\right) \tag{8}$$

$$V_{jk} \sim \frac{\sigma^2}{n} Chi^2(n-1) \tag{9}$$

From the literature review, the individual PK parameters X_{ijk} are not provided and only the geometric means (gm_{jk}) and the variance (v_{jk}) are available for a substrate j in a given study k. Consequently, the log of the geometric means (lgm_{jk}) and the variance (lv_{jk}) are used and modeled by:

$$lgm_{jk} \sim Normal\left(\mu_{jk},\ \frac{1}{n_{jk}\tau_j}\right) \tag{10}$$

$$lv_{jk} \sim \frac{1}{n_{jk}\tau_j} Chi^2(n_{jk}-1) \tag{11}$$

where τ_j is the precision (inverse of the variance) that describes the inter-individual variability regarding the substrate *j*. This model accounts for all the information recorded from the study under the

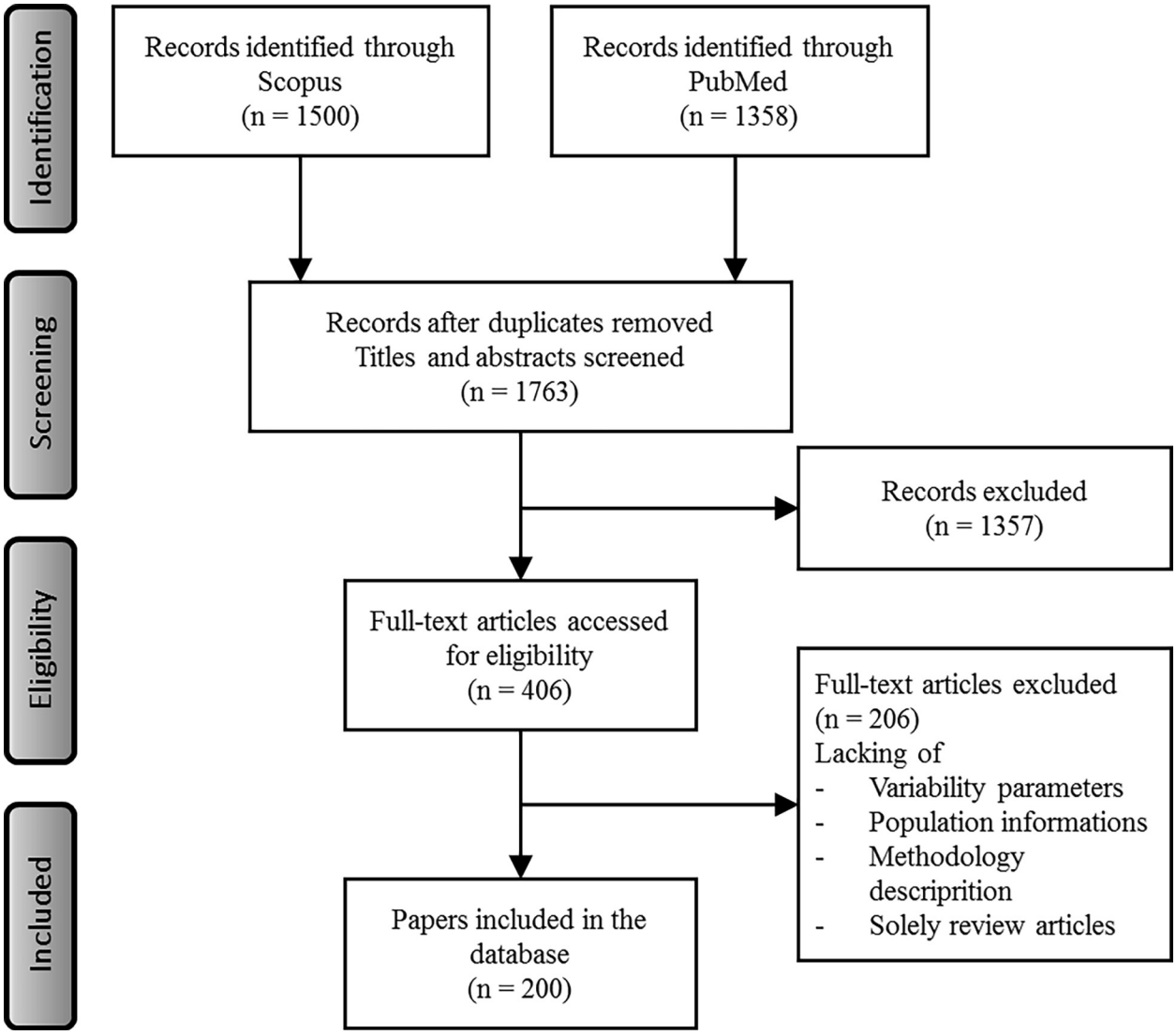

Fig. 2. Flow diagram illustrating the extensive literature search of human pharmacokinetic studies for 15 probe substrates of CYP3A4.

assumption of lognormality of data, and allows for the inference on the inter-individual variability τ_j, that is the key parameter in this work.

In order to properly describe inter-study and intra-substrate variability, a second layer in the model is required. It was built assuming that μ_{jk} is normally distributed around the substrate-specific mean μ_j with the inter-study variance τ_{study}:

$$\mu_{jk} \sim Normal\left(\mu_j, \frac{1}{\tau_{study}}\right) \quad (12)$$

Due to simplicity and to avoid identifiability issues, the inter-study variability τ_{study} was assumed to be identical for all substrates.

Bayesian inferences are used to infer on parameters of the model as it was seen as the most convenient approach to handle such a multilevel model. Since the purpose of this model is the meta-analysis of data from an extensive literature search (data published after 2002), informative priors were chosen from Dorne et al. [13] where the literature search stopped after April 2001. For the same reason, it was not consistent to look at expert knowledge to fix proper prior distributions because it may be related to data from the literature used to run the model. The JAGS software [31] is used to implement the model, the chi-square distribution being described using a Gamma distribution of parameters:

$$lv_{jk} \sim Gamma\left(\frac{n_{jk} - 1}{2}, \frac{\tau_j\, n_{jk}}{2}\right) \quad (13)$$

For each meta-analysis, 2 different Markov chains were run and convergence of the chains was assessed via Gelman-Rubin tests implemented in the Coda package of the R software [32].

2.3. Derivation of probabilistic CYP3A4-related uncertainty factors

The Bayesian hierarchical model for the meta-analyses was implemented for each PK parameter with the highest providing a distribution of inter-individual variability for each PK parameter. Uncertainty around each parameter was quantified using median values and 95% confidence intervals for each parameter estimation. The coefficient of variation was also estimated as follows:

$$CV = \sqrt{exp(\ln(\sqrt{\exp(1/\tau_j)})^2 - 1} \quad (14)$$

CYP3A4-related UFs were calculated as the ratio between the percentile of choice and the median of the distribution for each PK parameter and each sub-population with the equation (15).

$$UF_{95} = P95_{sub.pop}/P50_{ref.pop} \quad (15)$$

95th and 97.5th centiles were estimated. Higher centiles were

expected to be driven by the very end of the distribution and therefore to be very sensible and uncertain, especially because lognormal distributions were used.

The Bayesian modelling provided a distribution of values for the parameter τ_j. This makes it possible to provide a distribution of values for the uncertainty factors.

The differences in internal dose between each healthy subgroup and general healthy adults for kinetic parameters were calculated based on the μ_j ratio. This ratio reflects the differences in internal dose so that a value greater than 1 indicated a higher internal dose [33].

2.4. Software

All statistical analyses and graphical display of the data were performed using R (version 3.5). The Bayesian modelling was implemented with Jags (4.2.0) [31]. References from ELS were saved in EndNote (X8) files. All the R codes used are provided in Supplementary material C.

3. Results

3.1. Overview of data collection

A total of 2858 papers were assessed from Scopus and PubMed, dealing with 15 CYP3A4 probe substrate (alfentanil, alprazolam, budesonide, cisapride, diltiazem, felodipine, lidocaine, lovastatin, midazolam, nifedipine, nisoldipine, simvastatin, terfenadine, triazolam, zolpidem [13,34]). Fig. 2 summarises the flow of information of the ELS. The complete list of relevant articles is provided in Supplementary material A. From two independent screenings, 200 relevant papers were included in the database for extraction. 194 papers were reporting healthy adults PK data and only few reported PK data with respect to elderly, neonate and children, respectively 6, 2 and 1. A summary of all kinetic data for healthy adults is presented in Fig. 3. The full dataset of extracted information used in this review can be accessed on EFSA knowledge junction [35] or Supplementary material B.

Fig. 3 shows the raw data for each substrate and parameter of acute (Cmax) and chronic exposure (clearance and AUC) for the intravenous and oral route. As illustrated in Fig. 3, the amount of data available varied from one substrate and route to another as well as the reported geometric means (GM) for all kinetic parameters due to inter-substrate differences in kinetics. Midazolam was the most studied CYP3A4 probe substrate with 115 data points for clearance (ranging from $7.10e^{-4}$ to 11.1 ml/min/kg bw) while budesonide was the least studied (1 data points for clearance 9.2 ml/min/kg bw). Alfentanil, lidocaine, midazolam, triazolam and zolpidem, represented 25% of the database for the IV route, whereas no relevant data (oral or IV) were available for nisoldipine and terfenadine.

3.2. Inter-ethnic differences in CYP3A4 and CYP3A4-related uncertainty factors

Table 3 provides an overview of the number of substrates, number of studies with the corresponding extracted data, and individuals included in each meta-analysis.

The country of origin of the individuals in each study was indicated, while ethnic origin was not systematically spelt out. Moreover, the studies were more often carried out in a national laboratory or in a continent-wide context (US, Europe) so that results were grouped by continent. Kinetic data were available for European, East Asian, South Asian, Southeast Asian, North American, South American, Middle East and South African healthy adults. The majority of the data were from North America studies, East Asian and European studies. In order to estimate inter-ethnic differences, the North American healthy adult subgroup was used as the reference group with the highest number of CYP3A4 substrates and parameters for the oral and intravenous routes taken together.

Values from the meta-analysis of CV for inter-individual variability considering all substrates (Tables 4–7) highlight a lower inter-individual variability for the IV route compared to the oral route. The biological basis for this difference is well known and results from the fact that CYP3A4 is expressed in both the liver and the intestine [21,22]. The estimated variability for the oral route thus reflects CYP3A4-metabolism in the intestine and the liver whereas the estimated variability after IV exposure reflects only CYP3A4-metabolism in the liver [13]. Overall, inter-individual variability in kinetic parameters for healthy adults (North America) are consistent with the results of Dorne et al. [13] providing values, of 56% and 51% for the oral route (clearance/AUC and Cmax) and 43% and 31% (Clearance/AUC and Cmax) for the IV route. It is noted that the CVs for diltiazem, lovastatin and simvastatin clearance were much higher at 80%, 111% and 93% respectively, for the oral route but these were based on very limited data with only one study per substrate. CYP3A4-related UFs were estimated for the 95th and 97.5th centiles (Tables 4–7). For the oral route, the UF_{95} and $UF_{97.5}$ were 2.5–3.0, 2.3–2.7 and 1.9–2.2 for AUC, clearance and Cmax respectively.

Intra-ethnic and interethnic differences for healthy European, East Asian and Middle East adults showed similar CYP3A4-related UFs as those for healthy North American adults. However, inter-ethnic differences using the North American group as the reference group for specific substrates with limited studies, such as nifedipine, showed discrepancies with lower internal dose for AUCs (oral) and Cmax in healthy European adults (ratio of 0.7 and 0.2) and higher internal dose for healthy Middle East adults (ratio of 3.6 and 4.5).

Dorne et al. [13] found a two-fold internal dose difference between healthy South Asian adults and healthy caucasian with a similar variability compared with other ethnic groups. In the present work, CYP3A4-related UFs allowing for intra-ethnic differences in healthy South Asian adults were the lowest estimated (1.4–1.5 for AUC and Cmax, UF_{95} and $UF_{97.5}$ centile respectively) with overall CVs of 22% and 20% (4 compounds). CYP3A4-related UFs for interethnic differences were slightly higher for AUC and Cmax (3 compounds), 2.4–2.6 and 2.0–2.2 respectively, mainly due to simvastatin studies for which internal dose was 3.3 times higher than in healthy North American adults. It is noted that in this case, the interval of confidence (95%) was very large, from 0.3 to 48 for simvastatin AUC after oral administration (1 study).

Regarding healthy Southeast Asian, South African and South American adults, the number of studies and therefore the number of data was much lower than for other populations. The uncertainty in the results for those populations is thus high and have to be taken with caution. No new data were found for healthy Mexicans and sub-Saharan Africans since the previously published meta-analysis [13]. However, in this previous analysis the estimated internal dose differences allowing for inter-ethnic differences between Caucasian, Mexicans and sub-Saharan Africans for CYP3A4 probe substrates were estimated to be 3-fold (2 compounds, 2 study) and 1.5-fold (2 compounds, 3 study) respectively.

3.3. Kinetic data for the elderly, children and neonates

The number of papers reporting kinetic data for the elderly, children and neonates was very limited in both our ELS and the one conducted previously [13]. Therefore we combined kinetic data from those two databases. Thus, non-informative priors were used in the Bayesian meta-analyse.

In comparison with healthy North American adults, elderly showed a higher internal dose after oral administration (AUC and clearance). The estimated variability was similar to that of healthy North American adults with 52, 57 and 53% respectively for AUC, clearance and Cmax (Table 8). The difference in studied substrates, intravenously administered, between healthy North American adults and elderly did not allow

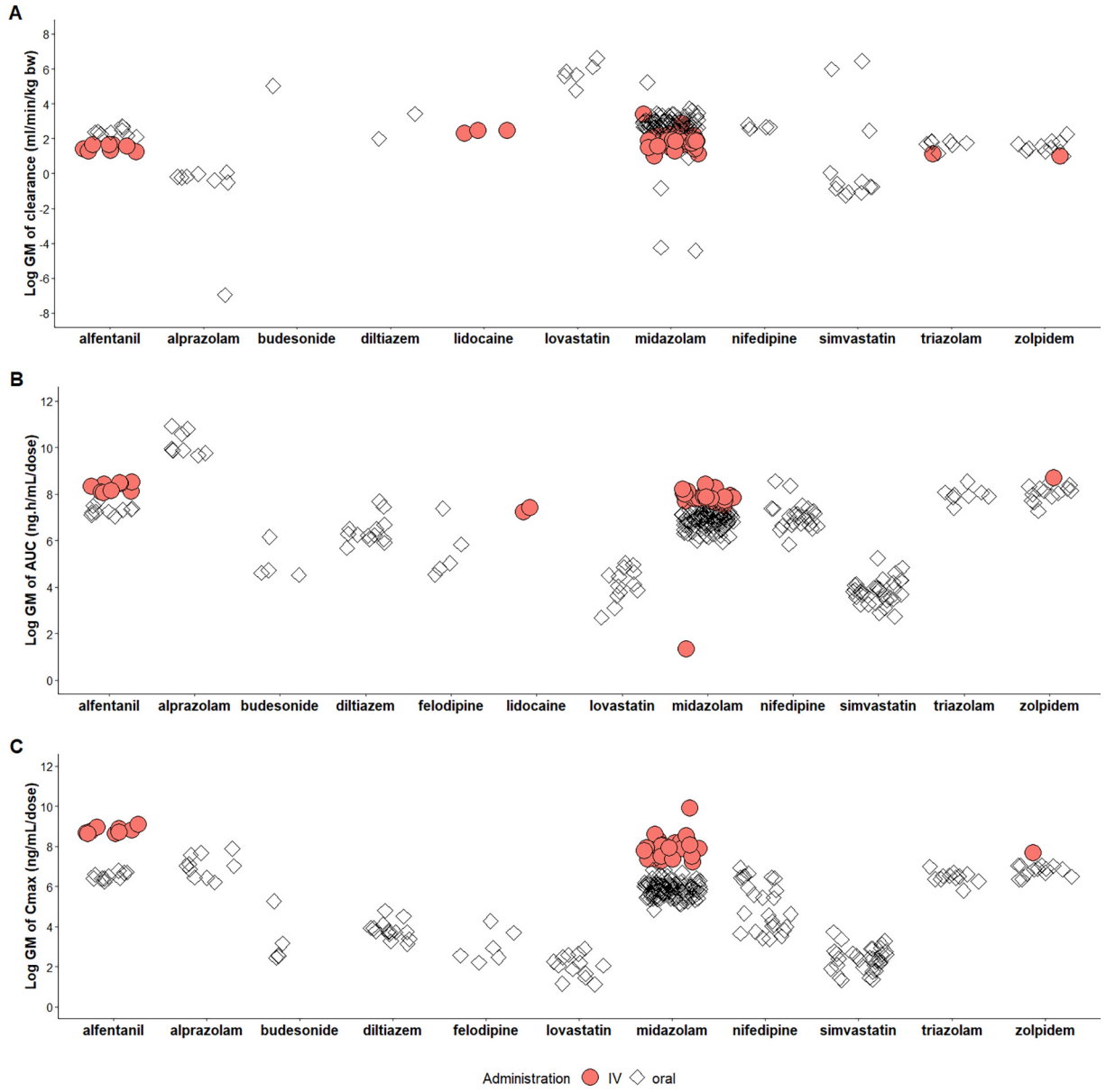

Fig. 3. Log geometric mean of extracted kinetic parameters from the included papers after standardization. A: clearance ; B: AUC ; C: Cmax. Squares: oral exposure ; red circles : IV exposure.

Table 3
Summary of the number of CYP3A4 substrates, pharmacokinetic studies and individuals in the meta-analyses

	N substrate	ns	n
Oral administration			
AUC (ng.h/ml/dose)	11	199	2921
Cl (ml/min/kg bw)	10	134	1603
Cmax (ng/ml/dose)	12	221	3211
Intravenous administration			
AUC (ng.h/ml/dose)	4	40	577
Cl (ml/min/kg bw)	6	50	734

Nsubstrate: number of CYP3A4 substrates, ns: number of studies, n: number of individuals

to compare those populations accurately. The UF after oral administration (clearance) was above the default kinetic factor, 3.9 and 4.9 for the UF_{95} and $UF_{97.5}$ respectively.

Because of the low number of studies available, the uncertainty for UFs of children after oral administration are very high and have to be taken with caution (Table 8). However, UFs of 3.6 and of 3.8 would be required in order to cover 95% and 97.5% of the children (AUC after intravenous administration, 2 compounds).

Only one new paper with neonates kinetic data was found. The studied compound was cisapride given orally to 3 groups of neonates. The variability was higher than for adults in most kinetic parameters and ranged from 45% to 68%, 58% to 82% and 44% to 58% for AUC, clearance and Cmax respectively. Neonates would require UFs of 6.9 and 7.6 for the 95th and 97.5th centiles after oral administration (Cmax). After an intravenous administration of midazolam, the estimated CV was of 86% and the corresponding UFs was also higher than the default TK factor. Due to a limited number of study and individuals, there is a high uncertainty around those UFs (Table 8).

4. Discussion and conclusions

This meta-analysis provides a quantitative account of inter-ethnic and intra-ethnic differences in CYP3A4 metabolism using markers of

Table 4
Inter-individual differences in the AUC (ng.h/ml/dose) of CYP3A4-probe substrates in healthy adults after oral administration: comparison with healthy North American adults

Ratio					Intra-ethnic				Interethnic			
Drug	ns	n	CV	GM	GM	UF95 (95% CI)		UF97.5 (95% CI	UF95 (95% CI)		UF97.5 (95% CI)	
North America												
Alfentanil	10	144	55	1533		2.3	[2.0–3.0]	2.7	[2.2–3.6]			
Alprazolam	6	54	35	18,300		1.7	[1.5–2.3]	1.9	[1.6–2.7]			
Diltiazem	1	14	76	527		3.1	[1.8–10]	3.9	[2.0–16]			
Lovastatin	5	43	78	99		3.2	[2.1–6.5]	3.9	[2.4–9.4]			
Midazolam	27	451	48	840		2.1	[1.9–2.4]	2.4	[2.1–2.9]			
Nifedipine	1	25	45	1118		2.0	[1.5–3.9]	2.3	[1.6–5.0]			
Simvastatin	2	51	87	55		3.5	[2.3–7.0]	4.4	[2.7–10]			
Triazolam	6	84	46	3328		2.1	[1.7–2.8]	2.4	[1.9–3.4]			
Zolpidem	6	73	57	3126		2.4	[1.9–3.5]	2.8	[2.1–4.5]			
Overall			51			2.3	[1.6–5.9]	2.7	[1.7–8.2]			
Europe												
Budesonide	2	24	52	105		2.2	[1.6–4.9]	2.6	[1.7–6.6]			
Diltiazem	5	33	35	452	0.86	1.8	[1.4–2.7]	2.0	[1.5–3.2]	2.0	[1.5–2.8]	2.3	[1.7–3.2]
Midazolam	13	182	40	1018	1.21	1.9	[1.7–2.2]	2.1	[1.8–2.6]	2.3	[1.9–2.8]	2.6	[2.1–3.3]
Nifedipine	8	164	59	745	0.67	2.4	[2.0–3.1]	2.9	[2.3–3.9]	2.3	[1.9–2.8]	2.5	[2.1–3.0]
Simvastatin	6	63	56	39	0.71	2.4	[1.8–3.6]	2.8	[2.1–4.5]	2.1	[1.6–2.7]	2.2	[1.7–3.0]
Zolpidem	1	24	60	3839	1.23	2.5	[1.7–5.8]	3.0	[1.9–8.1]	3.1	[1.7–8.5]	3.7	[1.9–12]
Overall			52			2.2	[1.5–4.1]	2.5	[1.7–5.4]	2.3	[1.6–5.2]	2.5	[1.8–6.6]
East Asia												
Alprazolam	3	19	21	47,995	2.62	1.4	[1.2–2.1]	1.5	[1.3–2.4]	3.8	[2.4–6.9]	4.1	[2.5–7.7]
Diltiazem	4	61	35	838	1.59	1.8	[1.5–2.4]	2.0	[1.6–2.8]	2.9	[1.9–4.4]	3.2	[2.1–5.1]
Felodipine	1	30	50	341		2.2	[1.6–4.0]	2.6	[1.8–5.3]				
Lovastatin	7	59	58	34	0.34	2.4	[1.8–3.9]	2.9	[2.1–5.1]	4.1	[2.8–5.8]	4.3	[3.0–6.3]
Midazolam	35	342	59	977	1.16	2.5	[2.1–2.9]	2.9	[2.4–3.6]	2.9	[2.3–3.6]	3.4	[2.7–4.4]
Nifedipine	9	325	40	1375	1.23	1.9	[1.7–2.1]	2.1	[1.9–2.4]	2.3	[1.8–3]	2.6	[2.0–3.4]
Simvastatin	14	257	64	39	0.71	2.6	[2.2–3.2]	3.2	[2.6–4.1]	2.1	[1.7–2.6]	2.2	[1.8–2.8]
Triazolam	2	15	54	2296	0.69	2.4	[1.5–7.4]	2.8	[1.7–11]	1.8	[0.9–3.6]	1.9	[0.9–3.8]
Zolpidem	6	61	42	3190	1.02	2	[1.6–2.7]	2.2	[1.7–3.2]	2.0	[1.4–3.2]	2.3	[1.5–3.7]
Overall			50			2.1	[1.3–3.7]	2.4	[1.4–4.8]	2.6	[1.3–5.3]	2.9	[1.4–5.7]
South Asia												
Diltiazem	3	36	23	697	1.32	1.4	[1.3–1.9]	1.6	[1.3–2.1]	2.0	[0.2–15]	2.1	[0.3–16]
Felodipine	1	24	15	1597		1.3	[1.2–1.6]	1.3	[1.2–1.8]				
Nifedipine	2	15	20	1644	1.47	1.4	[1.2–2.3]	1.5	[1.2–2.7]	1.9	[0.4–6.4]	2.0	[0.4–6.7]
Simvastatin	1	14	47	185	3.36	2.1	[1.5–6.4]	2.5	[1.6–9.2]	6.1	[0.3–39]	7.0	[0.3–48]
Overall			22			1.4	[1.2–3.8]	1.5	[1.2–5.0]	2.4	[0.3–30]	2.6	[0.3–36]
Southeast Asia												
Nifedipine	1	9	51	1031	0.92	2.2	[1.4–4.8]	2.5	[1.5–6.6]	1.4	[0.1–4.9]	1.5	[0.1–5.4]
Simvastatin	2	27	47	32	0.58	2.1	[1.5–3.9]	2.4	[1.7–5.2]	2.4	[0.3–6.2]	2.6	[0.3–6.9]
Overall			49			2.1	[1.5–4.6]	2.4	[1.6–6.2]	1.9	[0.2–5.6]	2.1	[0.2–6.2]
South America												
Budesonide	1	42	28			1.6	[1.4–2.1]	1.7	[1.4–2.4]				
Simvastatin	1	44	118	58	1.05	4.7	[2.8–12]	6.4	[3.4–19]	6.3	[0.1–72]	8.2	[0.1–102]
Overall			44			2.4	[1.4–9.8]	2.9	[1.5–15]				
Middle East												
Felodipine	1	10	49	126		2.1	[1.4–4.4]	2.5	[1.5–5.9]				
Lovastatin	1	14	8	84	0.85	1.1	[1.1–1.4]	1.2	[1.1–1.5]	1.4	[0.1–5.7]	1.5	[0.1–6.1]
Nifedipine	1	6	27	4015	3.59	1.5	[1.2–2.8]	1.7	[1.2–3.4]	5.7	[0.3–30]	6.3	[0.3–35]
Simvastatin	3	70	53	63	1.15	2.4	[1.9–3.5]	2.8	[2.1–4.5]	2.7	[0.4–8.4]	3.1	[0.4–8.9]
Overall			38			1.8	[1.1–3.7]	2.0	[1.1–4.7]	2.6	[0.2–22]	2.9	[0.2–26]
South Africa (caucasian)													
Felodipine	1	12	41	164		2	[1.4–6.0]	2.2	[1.4–8.4]				

ns: number of studies, n: number of individuals, CV: coefficient of variation (lognormal distribution), GM: geometric mean (lognormal distribution), ratio GM: ratio of geometric mean between subgroup and healthy adults from north America (lognormal distribution).

acute and chronic exposure for oral or intravenous routes. Historically, meta-analysis of human kinetic data has been using the inverse variance method using weighted geometric means corrected for study sample sizes and weighted averages of the variability for normal and lognormal data assuming fixed effect models [16]. Such inverse variance method does not provide a full account of the variability structure particularly to quantify inter-study variability and allowing for attributing relative weights according to heterogeneity of the datasets using random effect models. This is particularly relevant to pharmacokinetic studies with small sample size ($n < 10$) making inverse variance methods difficult to implement. Recently, refined approaches to meta-analysis for healthcare and risk assessment from a Bayesian perspective have been investigated [36–38]. Indeed, Bayesian inference is particularly adequately associated with hierarchical models to account for inter-study variability, or to discount information from various types of studies. Here, such a hierarchical Bayesian model was proposed for the meta-analysis of the CYP3A4-related kinetic data and allowed to account for different sample sizes of studies and their heterogeneity as well as inter-study variability so that strength can be borrowed from one study to another.

Table 5
Inter-individual differences in the clearance (ml/min/kg bw) of CYP3A4-probe substrates in healthy adults after oral administration: comparison with healthy North Americans adults

					Ratio GM	Intra-ethnic				Interethnic			
Drug	ns	n	CV	GM		UF95 (95% CI)		UF97.5 (95% CI)		UF95 (95% CI)		UF97.5 (95% CI)	
North America													
Alfentanil	9	134	59	11		2.5	[2.0–3.2]	2.9	[2.3–4]				
Alprazolam	5	57	44	0.33		2.0	[1.6–2.9]	2.3	[1.8–3.5]				
Diltiazem	1	14	80	32		3.3	[1.8–13]	4.2	[2–21]				
Lovastatin	1	10	111	302		4.4	[2.0–16]	5.7	[2.2–25]				
Midazolam	30	524	47	13		2.1	[1.9–2.3]	2.4	[2.1–2.8]				
Nifedipine	1	18	64	12		2.7	[1.7–9.6]	3.3	[1.9–15]				
Simvastatin	1	40	93	0.32		3.7	[2.4–8.1]	4.7	[2.8–12]				
Triazolam	7	97	47	5.4		2.1	[1.7–2.7]	2.4	[1.9–3.3]				
Zolpidem	6	73	69	4.6		2.8	[2.1–4.5]	3.4	[2.4–6.0]				
Overall			56			2.5	[1.7–9.3]	3.0	[1.9–14]				
Europe													
Budesonide	1	12	53	155		2.4	[1.5–9.7]	2.8	[1.6–15]				
Midazolam	10	129	44	14	0.93	2.0	[1.7–2.5]	2.3	[1.9–3.0]	2.2	[1.4–3.4]	2.5	[1.6–3.9]
Overall			46			2.1	[1.6–6.6]	2.4	[1.7–9.6]				
East Asia													
Alprazolam	3	19	26	0.71	0.46	1.5	[1.2–2.7]	1.7	[1.3–3.2]	3.4	[1–12]	3.7	[1.1–14]
Diltiazem	1	12	9	7.4	4.32	1.2	[1.1–1.5]	1.2	[1.1–1.6]	31	[4.1–128]	45	[5.9–185]
Lovastatin	5	23	40	336	0.90	1.9	[1.4–3.7]	2.1	[1.5–4.8]	2.2	[0.7–7.4]	2.5	[0.8–8.8]
Midazolam	33	324	45	14	0.93	2.0	[1.8–2.3]	2.3	[2.0–2.7]	2.2	[1.5–3.3]	2.5	[1.7–3.8]
Nifedipine	3	28	53	14	0.86	2.3	[1.6–4.5]	2.7	[1.8–6.1]	2.8	[0.8–11]	3.3	[0.9–14]
Simvastatin	9	10	64	1.02	0.31	2.6	[2.1–3.4]	3.1	[2.5–4.3]	8.3	[3.8–18]	10	[4.5–21]
Triazolam	1	12	60	6.6	0.82	2.5	[1.5–5.2]	2.9	[1.7–7.0]	3.0	[0.3–15]	3.6	[0.4–18]
Zolpidem	5	49	82	4.7	0.98	3.3	[2.2–6.5]	4.1	[2.6–9.2]	3.5	[1.2–11]	4.3	[1.4–16]
Overall			48			2.1	[1.1–4.7]	2.4	[1.1–6.3]	3.1	[0.8–13]	3.5	[0.9–16]
Southeast Asia													
Nifedipine	1	9	66	18	0.67	2.6	[1.5–6.9]	3.2	[1.7–9.7]	3.4	[0.1–64]	4.1	[0.1–87]
Simvastatin	1	9	53	18	0.02	2.4	[1.4–18]	2.8	[1.5–32]	81	[0.3–13e2]	95	[0.3–18e2]
Overall			59			2.5	[1.4–11]	3.0	[1.6–17]	16	[0.1–1e3]	20	[0.1–12e2]

ns: number of studies, n: number of individuals, CV: coefficient of variation (lognormal distribution), GM: geometric mean (lognormal distribution), ratio GM: ratio of geometric mean between healthy adults from north America and subgroup (lognormal distribution)

Inter-individual variability for the oral route for healthy adults averaged 51% (AUC), consistent with a previous study [13]. In a more recent meta-analysis of inhibition (grapefruit juice) and induction (St John's wort) of CYP3A4 metabolism in humans [39], inter-individual variability and UF_{95} were determined for 57 and 64 compounds (Cmax and AUC or clearance) respectively, for full and partial probe substrates of CYP3A4. Inter-individual variability was 56% for Cmax and 51% for AUC and Clearance and the corresponding UF_{95} and UF_{99} were 2.2–3.0 for acute exposure and 2.3–3.4 for chronic exposure, which is fully consistent with our meta-analysis.

Overall, the CYP3A4 related UFs for healthy adults were consistent with the study from Dorne et al. [13] and below the default kinetic factor (3.16) for at least 97.5% of healthy adults when considering the median value. However, our analysis by a Bayesian model taking into account the uncertainty around the estimation of the UF shows that, given the available data (number of studies and number of individuals per study), it may be that the default factor does not cover all possible cases. Indeed, the upper bound of the confidence interval is higher than 3.16. Data gaps were identified for specific ethnic groups (central and South American, Southeast Asian and African) with very few studies available and did not allow to make conclusions.

It appears that a factor of 3.16 would not cover 95% of populations like elderly, children and neonates. The lowered clearance observed in elderly can be explained by a decrease in hepatic volume and blood flow will aging and morphological changes (decrease of the muscle mass and increase of adipose tissue mass) that will impact distribution [21]. The estimated UFs were of the same range than in Dorne et al. [13] for the clearance after oral administration (4 compounds).

CYP3A7 is the main isozyme in fetal liver and represent around 32% of total CYP content [22]. An *in vitro* study of the efficiencies of CYP3A isoforms towards organophosphorothionate pesticides indicate that the 3A7 isoform is less efficient (measured as intrinsic clearance) than CYP3A4 [20]. A transition between those two isoforms will occur a few months after birth [22]. A greater variability was estimated for neonates than for adults as previously observed [13]. Therefore, neonates would require a higher UF in comparison with healthy adults, more kinetic data regarding CYP3A4 probe-substrates metabolism would thus be needed to estimate precisely UFs. For children, except to midazolam, a low variability was observed. In the literature, the clearance for midazolam in children is higher compared to adults [22] nevertheless our results showed the opposite. This might be due to discrepancies in the reported studies [40,41].

An important aspect of human variability in CYP3A is the impact of polymorphisms on polymorphic genotypes on inter and intra-ethnic differences in kinetics, however, few studies provide these type of data and currently, it is not possible to link allelic frequencies and estimated interethnic differences quantitatively. There are at least 40 allelic variants described for the CYP3A4 gene [42]. CYP3A4*1B is considered the most common genetic polymorphism in CYP3A4 and also the most extensively studied; being reported in 0.50–0.82 of Africans/African Americans, whereas it is absent in Japanese and Chinese populations and has a low frequency (0.03–0.05) in Caucasians [24,43,44]. However, its clinical significance is not yet clear due to contrasting results regarding its impact on enzymatic activity. Among all other known CYP3A4 variants, the vast majority fall in the category of rare polymorphisms, showing a frequency between 0.01 and 0.03 [24,44,45]. In contrast, CYP3A5 is expressed in extrahepatic tissues with more than 25 allelic variants [42] with CYP3A5*3 allele as the most common, which leads to the loss of CYP3A5 activity due to the disruption of the correct splicing of CYP3A5 transcripts. It has been reported in 0.77–0.96 of

Table 6
Inter-individual differences in the Cmax (ng/ml/dose) of CYP3A4-probe substrates in healthy adults after oral administration: comparison with healthy North Americans adults

					Ratio GM	Intra-ethnic				Interethnic			
Drug	ns	n	CV	GM		UF95 (95% CI)		UF97.5 (95% CI)		UF95 (95% CI)		UF97.5 (95% CI)	
North America													
Alfentanil	10	144	42	692		1.9	[1.7–2.4]	2.2	[1.8–2.8]				
Alprazolam	7	75	30	793		1.6	[1.4–2.0]	1.8	[1.5–2.3]				
Cisapride	1	15	42	527		2.0	[1.4–4.7]	2.2	[1.5–6.3]				
Diltiazem	1	14	37	43		1.8	[1.3–4.3]	2.1	[1.4–5.7]				
Lovastatin	5	49	53	9.1		2.3	[1.7–3.6]	2.7	[1.9–4.6]				
Midazolam	31	507	46	337		2.0	[1.9–2.3]	2.3	[2.1–2.7]				
Nifedipine	1	25	40	227		1.9	[1.5–3.4]	2.2	[1.6–4.3]				
Simvastatin	2	51	72	13		2.9	[2.1–5.2]	3.6	[2.4–7.1]				
Triazolam	9	167	38	680		1.8	[1.6–2.2]	2.1	[1.8–2.5]				
Zolpidem	6	73	36	831		1.8	[1.5–2.3]	2.0	[1.6–2.6]				
Overall			43			1.9	[1.4–3.8]	2.2	[1.5–4.9]				
Europe													
Budesonide	3	36	56	19		2.4	[1.7–4.3]	2.8	[1.9–5.6]				
Diltiazem	5	33	40	50	1.16	1.9	[1.5–3.1]	2.2	[1.6–3.8]	2.3	[1.2–4.7]	2.5	[1.4–5.7]
Midazolam	17	237	43	327	0.97	1.9	[1.7–2.3]	2.2	[1.9–2.7]	1.1	[0.9–1.5]	1.2	[0.9–1.5]
Nifedipine	7	155	57	51	0.22	2.4	[2.0–3.1]	2.8	[2.3–3.8]	7.1	[4.7–11]	7.8	[5.1–12]
Simvastatin	6	63	57	11	0.85	2.4	[1.8–3.6]	2.8	[2.0–4.7]	1.8	[1.1–3.0]	1.9	[1.2–3.2]
Zolpidem	1	24	36	1213	1.56	1.9	[1.4–3.5]	2.2	[1.5–4.4]	3.1	[1.1–9.4]	3.5	[1.1–11]
Overall			50			2.1	[1.5–3.5]	2.5	[1.7–4.4]	2.2	[1.0–9.3]	2.4	[1.0–10]
East Asia													
Alprazolam	3	19	31	2151	2.71	1.7	[1.3–3.0]	1.8	[1.4–3.7]	4.7	[2.3–11]	5.1	[2.5–13]
Diltiazem	7	76	32	43	1.00	1.7	[1.4–2.1]	1.8	[1.6–2.4]	1.7	[1.1–2.7]	1.9	[1.2–3.1]
Felodipine	1	30	53	19		2.3	[1.7–4.3]	2.7	[1.8–5.7]				
Lovastatin	7	59	61	5.8	0.64	2.5	[1.9–4.0]	3.0	[2.1–5.2]	2.1	[1.3–3.4]	2.2	[1.4–3.6]
Midazolam	39	372	48	379	1.12	2.1	[1.9–2.4]	2.4	[2.1–2.9]	2.4	[1.9–3.0]	2.7	[2.2–3.5]
Nifedipine	12	349	41	242	1.07	1.9	[1.7–2.2]	2.2	[1.9–2.5]	2.0	[1.5–2.8]	2.3	[1.6–3.2]
Simvastatin	14	257	63	8.9	0.68	2.6	[2.2–3.2]	3.1	[2.5–4.0]	2.2	[1.6–3.0]	2.3	[1.7–3.2]
Triazolam	2	15	48	516	0.76	2.1	[1.5–3.8]	2.4	[1.6–4.7]	1.6	[0.6–3.8]	1.6	[0.7–4.0]
Zolpidem	6	61	38	925	1.19	1.8	[1.5–2.5]	2.0	[1.6–2.9]	2.2	[1.3–3.7]	2.4	[1.4–4.3]
Overall			46			2.0	[1.4–3.5]	2.3	[1.6–4.3]	2.2	[1.0–6.4]	2.4	[1.1–7.3]
South Asia													
Diltiazem	2	18	19	39	0.91	1.4	[1.2–2.0]	1.5	[1.2–2.3]	1.6	[0.2–5.7]	1.8	[0.2–6.4]
Felodipine	1	24	11	74		1.2	[1.1–1.4]	1.3	[1.1–1.5]				
Nifedipine	2	15	20	258	1.14	1.4	[1.2–2.6]	1.5	[1.2–3.1]	1.4	[0.3–5.0]	1.5	[0.3–5.3]
Simvastatin	1	14	48	42	3.23	2.2	[1.5–6.8]	2.5	[1.6–9.7]	6.2	[0.3–35]	7.2	[0.3–43]
Overall			20			1.4	[1.1–4.0]	1.5	[1.2–5.2]	2.0	[0.2–26]	2.2	[0.2–31]
Southeast Asia													
Nifedipine	1	9	73	274	1.21	2.9	[1.6–8.4]	3.5	[1.7–12]	3.8	[0.4–16]	4.7	[0.4–22]
Simvastatin	2	27	53	6.5	0.50	2.3	[1.6–4.8]	2.7	[1.8–6.4]	2.7	[0.4–6.3]	2.9	[0.4–6.8]
Overall			59			2.5	[1.6–7.5]	2.9	[1.5–11]	3.1	[0.4–14]	3.4	[0.4–19]
South America													
Budesonide	1	42	40	127		1.9	[1.5–2.8]	2.1	[1.6–3.4]				
Simvastatin	1	44	53	7.9	0.61	2.3	[1.7–3.7]	2.7	[1.9–4.8]	2.1	[0.1–20]	2.3	[0.1–21]
Overall			46			2.1	[1.6–3.4]	2.4	[1.7–4.3]				
Middle East													
Felodipine	1	10	60	12		2.5	[1.5–5.7]	2.9	[1.6–7.7]				
Lovastatin	1	14	13	18	1.98	1.2	[1.1–1.7]	1.3	[1.1–1.9]	2.2	[0.1–10]	2.3	[0.1–10]
Nifedipine	1	6	38	1020	4.49	1.8	[1.2–4.3]	2	[1.3–5.3]	7.9	[0.3–52]	9	[0.3–65]
Simvastatin	3	70	71	14	1.08	2.9	[2.1–4.7]	3.5	[2.5–6.2]	2.9	[0.3–9.6]	3.4	[0.4–10]
Overall			49			2.1	[1.1–4.8]	2.4	[1.2–6.2]	3.3	[0.2–36]	3.7	[0.2–45]
South Africa (caucasian)													
Felodipine	1	12	22	21		1.5	[1.2–2.9]	1.6	[1.2–3.5]				

ns: number of studies, n: number of individuals, CV: coefficient of variation (lognormal distribution), GM: geometric mean (lognormal distribution), ratio GM: ratio of geometric mean between subgroup and healthy adults from north America (lognormal distribution).

Caucasians, in 0.66–0.78 of Asians and in 0.12–0.50 of Africans/African Americans [24,42,45,46]. The differences in the prevalence of CYP3A5*3 alleles in different ethnic groups reflects a biological basis of the marked differences in drug metabolism of for CYP3A5 substrates [47]. This may explain the very high variability in the kinetics of lovastatin and simvastatin, two CYP3A substrates interacting with the P-glycoprotein transporter [34]. In a recent pharmacokinetic study investigating the PK of simvastatin after dosage in different East Asian population (Koreans, Chinese and Japanese) and in Caucasian healthy adults, the authors did not find differences in AUC values among east Asians but found a significant increase in AUC in Caucasians [48]. Moreover, Kim et al. [49] studied the effect of CYP3A5 polymorphism on simvastatin PK and concluded that CYP3A5*3/*3 was significantly correlated to the internal dose of simvastatin (significant decrease in clearance). Further work on the impact of CYP3A5 polymorphism on xenobiotic metabolism is therefore needed.

The aim of this work was to derive pathway related UFs, specifically for CYP3A4. This provides an intermediate option between a chemical-

Table 7
Inter-individual differences in the AUC (ng.h/ml/dose) and clearance (ml/min/kg bw) of CYP3A4-probe substrates in healthy adults after intravenous administration: comparison with healthy North Americans adults

					Ratio GM	Intra-ethnic				Interethnic			
Drug	ns	n	CV	GM		UF95 (95% CI)		UF97.5 (95% CI)		UF95 (95% CI)		UF97.5 (95% CI)	
AUC (ng.h/ml/dose)													
North America													
Alfentanil	9	134	40	3899		1.9	[1.6–2.3]	2.1	[1.8–2.7]				
Midazolam	19	304	27	1923		1.6	[1.4–1.7]	1.7	[1.6–1.9]				
Overall			32			1.7	[1.5–2.2]	1.8	[1.6–2.6]				
Europe													
Lidocaine	2	14	40	1496		1.9	[1.4–5]	2.2	[1.5–7]				
Midazolam	3	46	24	3407	1.77	1.5	[1.3–1.8]	1.6	[1.4–2.1]	2.6	[1.8–4.1]	2.8	[1.9–4.5]
Zolpidem	1	24	41	5938		1.9	[1.5–3.6]	2.2	[1.6–4.5]				
Overall			29			1.6	[1.3–3.9]	1.7	[1.4–5.1]				
East Asia													
Midazolam	6	55	24	2472	1.29	1.5	[1.3–1.8]	1.6	[1.4–2.0]	1.9	[1.5–2.6]	2.0	[1.6–2.9]
Cl (ml/min/kg bw)													
North America													
Alfentanil	9	134	37	4.4		1.8	[1.6–2.1]	2.0	[1.7–2.5]				
Midazolam	25	411	29	6.6		1.6	[1.5–1.7]	1.7	[1.6–1.9]				
Triazolam	1	21	29	3.1		1.6	[1.3–2.7]	1.8	[1.4–3.2]				
Overall			31			1.7	[1.4–2.3]	1.8	[1.4–2.7]				
Europe													
Lidocaine	3	24	37	11		1.8	[1.4–3.1]	2.0	[1.5–3.9]				
Midazolam	4	53	25	4.4	1.50	1.5	[1.3–1.8]	1.6	[1.4–2.1]	1.7	[1.3–2.4]	1.8	[1.3–2.4]
Zolpidem	1	24	45	2.8		2.0	[1.5–4.0]	2.3	[1.6–5.3]				
Overall			34			1.7	[1.3–3.3]	1.9	[1.4–4.2]				
East Asia													
Midazolam	7	67	39	6.3	1.05	1.9	[1.6–2.5]	2.1	[1.7–2.9]	1.2	[0.9–1.6]	1.2	[0.9–1.6]

ns: number of studies, n: number of individuals, CV: coefficient of variation (lognormal distribution), GM: geometric mean (lognormal distribution), ratio GM: ratio of geometric mean between healthy adults from north America and subgroup (lognormal distribution) (1/ratio for AUC).

specific adjustment factor (CSAF) and the default UF (when no data are available) [3,50–52]. The proposed methodology and modelling can be applied to other metabolic pathways of interest to assess human inter-individual variability in TK in a boarder context.

Non-invasive *in vitro* techniques are now available to provide metabolism data from human cell lines [9,53]. Combining accurate inter-individual information from human data, as shown here, with such *in vitro* data is very useful for quantitative *in vitro* to *in vivo* extrapolation (QIVIVE). Indeed, the estimated CV can be applied to an extrapolated clearance from QIVIVE, then a lognormal distribution for clearance would be integrated in a PBK model with Markov-Chain Monte Carlo instead of a single deterministic mean value and allow for sound QIVIVE modelling.

The use of PBK modelling is increasingly recommended in chemical risk assessment [8,51,52,54,55] together with approaches to better account for inter-individual variability. Indeed, applying a PBK model with parameter specific distributions integrating variability in a Bayesian framework [56] would allow a better prediction of internal dose and decrease uncertainty in estimates. Such approaches would avoid the use of default factors and allow to apply, on a case by case basis, either CSAFs or pathway-related UFs that may be below or above these default values [57,58]. Modelling inter-individual kinetic variability with PBK models would also require taking into account variation in physiological parameters (*i.e.* organ volume, cardiac output). For this purpose, the use of the PopGen free web application may be very useful since it is able to easily generate a virtual population with outputs readily applicable for QIVIVE [59].

Inter-individual variability in internal dose may also differ for co-exposure scenarios and PBK modelling can provide a powerful tool when dealing with mixtures or multiple chemical exposure particularly in the case of TK interactions [60,61]. Desalegn et al. [60] recently reviewed the current state-of-the-art of PBK models for chemical mixtures and evaluated their applications with an emphasis on their role in chemical risk assessment. Focusing on CYP3A4 metabolism, Quignot et al. [39], proposed CYP3A4-related UFs taking into account either inhibition (grapefruit juice) or induction (St. John's wort) and these can be integrated in PBK models for mixture risk assessment.

Finally, the CYP3A4-substrates in this database have short half-lives (hours) and further analysis would need to be performed for environmental contaminants as CYP3A4 substrates that are more persistent using for example biomonitoring results. Overall, it is foreseen that in the future these CYP3A4-related variability distributions can be used along other pathway-related variability distributions in generic human PBK models and QIVIVE models integrating isoform-specific metabolism information for chemical risk assessment. Here, this approach has been explored as part of a multi-center collaborative project between EFSA, ANSES, ISS, the University of Utrecht and the University of Bretagne: "modelling human variability in toxicokinetic and toxicodynamic processes using Bayesian meta-analysis, physiologically-based modelling and *in vitro* systems".

Case studies for regulated compounds and contaminants exploring the integration of human variability for a wider range of phase I enzymes, phase II enzymes and transporters and isoform specific human *in vitro* data are underway to illustrate the practical use of these new tools in the food safety area.

Declaration of Competing Interest

Mr Keyvin Darney, Dr Emanuela Testai, Dr Franca M. Buratti, Dr Emma Di Consiglio, Mrs Emma E.J. Kasteel, Dr Nynke Kramer, Dr Laura Turco, Dr Susanna Vichi, Professor Alain-Claude Roudot and Dr Camille Béchaux all report the grant GP/EFSA/SCER/2015/01 from the European Food Safety Authority under which this study was funded and conducted. No conflicts of interests were identified.

Table 8
Pharmacokinetics of compounds eliminated via CYP3A4 metabolism in elderly, children and neonates after oral and intravenous administration: comparison with healthy North Americans adults

Drug	ns	n	CV	GM	ratio GM	UF95 (95% CI)		UF95 (97.5% CI)	
Elderly									
Oral administration									
AUC (ng.h/ml/dose)									
Diltiazem	1	16	12	430	0.82	1.7	[0.8–4.8]	1.9	[0.8–4.8]
Felodipine	1	10	47	266					
Midazolam	7	52	50	866	1.03	2.3	[1.4–4]	2.6	[1.6–5]
Nifedipine	1	6	30	1921	1.72	2.8	[1–7.9]	3	[1–9.1]
Nisoldipine	3	25	69	74					
Triazolam	2	21	76	3351	1.01	3.1	[1.3–7.5]	3.6	[1.5–7.6]
Zolpidem	3	24	74	4958	1.59	4.7	[2.1–10.6]	5.7	[2.4–14.2]
Overall			52			2.5	[0.6–8.2]	2.9	[0.6–10.4]
Clearance (ml/min/kg bw)									
Diltiazem	1	11	46	19.9	1.60	12.8	[4.6–36]	18.6	[6.6–52]
Midazolam	8	58	53	14.7	0.88	2.6	[1.5–4.6]	3	[1.8–5.8]
Triazolam	2	21	63	5	1.08	3.1	[1.5–6.5]	3.7	[1.8–7.9]
Zolpidem	2	16	73	2.8	1.64	4.9	[2.1–11.6]	6.2	[2.6–14.6]
Overall			57			3.9	[1.6–23.5]	4.9	[1.9–34]
Cmax (ng/ml/dose)									
Diltiazem	1	16	13	47.2	1.10	1.4	[0.7–2.7]	1.4	[0.8–2.9]
Felodipine	1	13	48	22.2					
Midazolam	8	58	54	358	1.06	2.5	[1.6–4.2]	2.9	[1.9–5.3]
Nifedipine	1	6	42	246	1.08	2.1	[0.8–6.5]	2.3	[0.9–8.3]
Nisoldipine	3	25	87	20.5					
Triazolam	2	21	53	725	1.07	2.4	[1.3–4.9]	2.8	[1.4–6.1]
Zolpidem	3	24	46	1347	1.73	3.6	[2–6.8]	4.1	[2.2–8.2]
			53			2.3	[0.9–5.8]	2.7	[1–7.1]
Intravenous administration									
AUC (ng.h/ml/dose)									
Alprazolam	1	13	26	24,507					
Diltiazem	1	12	13	1283					
Midazolam	2	24	42	2422	1.26	2	[0.4–5.8]	2.3	[0.4–7.2]
Nifedipine	1	5	30	3440					
Nisoldipine	1	10	42	2048					
Clearance (ml/min/kg bw)									
Alfentanil	2	25	46	3.9	1.13	1.4	[0.9–2.2]	1.5	[0.9–2.4]
Alprazolam	1	13	47	0.7					
Diltiazem	2	20	19	12.7					
Midazolam	6	70	56	5.1	1.29	1.5	[1.1–2]	1.5	[1.2–2.1]
Nifedipine	1	5	32	4.8					
Nisoldipine	1	10	52	8.3					
Overall			44			1.5	[0.9–2.1]	1.5	[1–2.2]
Children									
Oral administration									
AUC (ng.h/ml/dose)									
Alprazolam	1	11	26	6271	0.34	1.4	[0.1–23.8]	1.4	[0.1–25]
Cmax (ng/ml/dose)									
Alprazolam	1	9	17	279	0.35	1.8	[0.1–17.2]	1.8	[0.1–17.8]
Triazolam	1	11	41	187	0.28	2.4	[0.1–27]	2.5	[0.1–28]
Overall			26			2.1	[0.1–22.6]	2.1	[0.1–23.4]
Intravenous administration									
AUC (ng.h/ml/dose)									
Alfentanil	3	17	11	2164	0.56	3.7	[2.4–6]	4.3	[2.8–7]
Midazolam	2	24	67	2299	1.20	3.3	[1.5–6.9]	3.8	[1.7–7.3]
Overall			23			3.6	[1.7–6.6]	3.8	[1.7–7.2]
Clearance (ml/min/kg bw)									
Alfentanil	1	8	16	4.4	1.00	1.4	[0.2–3.8]	1.4	[0.2–4.2]
Budesonide	1	6	34	23.3					
Midazolam	2	24	86	7.6	0.87	3.8	[0.5–11.7]	4.7	[0.6–16]
Overall			36			2.2	[0.2–10.2]	2.5	[0.3–13.9]
Neonates									
Oral administration									
Cmax (ng/ml/dose)									
cisapride	3	32	59	131	0.25	6.9	[3.1–14]	7.6	[3.4–16]
Intravenous administration									
Clearance (ml/min/kg bw)									

(*continued on next page*)

Table 8 (*continued*)

Drug	ns	n	CV	GM	ratio GM	UF95 (95% CI)		UF95 (97.5% CI)	
midazolam	1	10	86	1.9	3.47	4.3	[0.1–59]	4.4	[0.1–61]

ns: number of studies, n: number of individuals, CV: coefficient of variation (lognormal distribution), GM: geometric mean (lognormal distribution), ratio GM: ratio of geometric mean between healthy adults from north America and subgroup (lognormal distribution) (1/ratio for AUC and Cmax).

Dr. Dorne works as member of staff at the European Food Safety Authority and the design of the study as part of the grant proposal. No conflicts of interests were identified.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.comtox.2019.100092.

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