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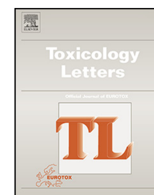

Human Variability in Carboxylesterases and carboxylesterase-related Uncertainty Factors for Chemical Risk Assessment

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HIGHLIGHTS

- Extensive literature search of human kinetic parameters and enzyme activities for *in vivo* CES-1 and CES-2 probe substrates.
- Hierarchical Bayesian meta-analysis to quantify inter-individual differences.
- Human variability in CES ranges from 30-55% for CES1 and CES2 across probe substrates.

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ABSTRACT

Carboxylesterases (CES) are an important class of enzymes involved in the hydrolysis of a range of chemicals and show large inter-individual variability *in vitro*. An extensive literature search was performed to identify *in vivo* probe substrates for CES1 and CES2 together with their protein content and enzymatic activity. Human pharmacokinetic (PK) data on Cmax, clearance, and AUC were extracted from 89 publications and Bayesian meta-analysis was performed using a hierarchical model to derive CES-related variability distributions and related uncertainty factors (UF). The CES-related variability indicated that 97.5% of healthy adults are covered by the kinetic default UF (3.16), except for clopidogrel and dabigatran etexilate. Clopidogrel is metabolised for a small amount by the polymorphic CYP2C19, which can have an impact on the overall pharmacokinetics, while the variability seen for dabigatran etexilate might be due to differences in the absorption, since this can be influenced by food intake. The overall CES-related variability was moderate to high *in vivo* (<CV 50%), which might be due to possible polymorphism in the enzyme but also to the small sample size available per chemical. The presented CES-related variability can be used in combination with *in vitro* data to derive pathway-specific distributions.

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1. Introduction

Carboxylesterases (CES) are an important class of enzymes involved in the hydrolysis of a wide range of drugs, endogenous substrates, and environmental chemicals, containing ester, thioester, carbamate, and amide groups. CES catalyse the addition of water to an ester group and such hydrolysis produces polar compounds namely carboxylic acids and an alcohols, which are more readily eliminated from the body. CES probe substrates can be prodrugs or active compounds which can be either activated or inactivated by hydrolysis respectively. For prodrugs, either a carboxylic acid or an alcohol (as the hydrolysis product), may be the pharmacologically active moiety.

Six CES isoforms (CES1 to CES6) have been identified based on the homology of amino acid sequences with the most common carboxylesterase-1 (CES1) and carboxylesterase-2 (CES2) isoforms involved in the metabolism of xenobiotics (Di, 2019; Laizure and

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Parker, 2020). CES are located in many tissues, although the highest concentrations are in the liver and small intestine for CES1 and CES2, respectively, accounting for the great majority of the overall drug metabolism associated with CES activity (Imai, 2006; Xu et al., 2002; Zhang et al., 2002). The blood is devoid of significant CES1 or CES2 activities. As a consequence, first-pass hydrolysis, catalysed by CES, is a relevant process for oral prodrugs, affecting their bioavailability prior to reaching the systemic circulation. Exposure to active metabolites of prodrugs may be affected by variability in CES1 and CES2 activities due to genetic polymorphisms which may provide relevant predictors of xenobiotic disposition and response (Oh et al., 2017; Tarkiainen et al., 2012; Tarkiainen et al., 2015a; Tarkiainen et al., 2015b; Wang et al., 2018).

Several specific substances have been identified for CES1 and/or CES2 isoforms (Laizure and Parker, 2020). A recent review describes several CES specific probe substances, including drugs, natural substances and other compounds, which can act as CES modulators (Wang et al., 2018). Generally speaking, CES1 hydrolyses ester structures, such as oseltamivir, enalapril, imidapril, clopidogrel, cocaine and heroin. CES2 hydrolyses esters with a larger alcohol group as well as a small acyl group like irinotecan, prasugrel, and capecitabine. In addition, substrate affinity for CES has been predicted using *in silico* models based on docking analyses of known substrates and molecular dynamics. These studies confirmed that optimal substrates for CES1 have smaller and polar alkyl/aryl groups and larger hydrophobic acyl moieties and that ionisation state is also an important feature (Vistoli et al., 2009; Vistoli et al., 2010). The CES hydrolysis generally unmask structural groups such as alcohol, amine, or carboxylic groups, which are then often conjugated by UDP-glucuronosyltransferases (UGT). This interplay of CES and UGT enzymes has been observed for a range of drugs and other xenobiotics, such as irinotecan, flutamide, fenofibrate, and mycophenolate mofetil (Oda et al., 2015).

Inter-individual differences in CES activity related to hydrolysis and clearance of xenobiotics have been shown to be large, due to observed variation in hydrolysis of substrates in human liver microsomes (Hosokawa et al., 1995; Xu et al., 2002). Underlying factors impacting variability in CES activity include genetic polymorphisms, enzyme induction and inhibition as well as altered activity due to diseases of the gastro-intestinal tract and the liver. Several genetic variations of potential clinical significance have been identified in the carboxylesterase genes and these provide important predictors of drug disposition and response (Laizure et al., 2013; Laizure and Parker, 2020; Tarkiainen et al., 2012; Zhu et al., 2008). A range of studies have shown that CES1 proteins tend to be expressed at higher level and associated with higher hydrolytic efficiency in females compared to males even after body weight adjustment (Di, 2019; Patrick et al., 2007; Shi et al., 2016; Vree et al., 2003). Furthermore, expressions of CES1 and CES2 are developmentally regulated and have shown an age-dependent increase in activity. Both CES protein expression levels and corresponding activities in microsomes are absent in the foetus and low in neonates (Yang et al., 2009). CES levels rapidly increase over the first few weeks after birth and its ontogenesis depends on the expression of the microsomal isoenzymes CES2 which is similar compared to that in adults after 3 weeks of age. In contrast, CES1 expression reaches levels that are only half of those in adults by approximately 7 months of age (Hines et al., 2016).

Consistently with the expression patterns, CES activity remains lower in children and gradually increases until adulthood (Boberg et al., 2017; Shi et al., 2011; Yang et al., 2009). CES activity measured in liver microsomes has been shown to be 4 to 10-fold higher in adults (>18 years) compared to that in children (0 days–10 years of age) and foetus (82–224 gestation days), respectively. The age differences in CES expression and activity can lead to higher sensitivity to adverse effects of certain CES substrates (Boberg et al., 2017; Hines et al., 2016). No significant differences across populations of different geographical ancestry have been observed (Di, 2019; Patrick et al., 2007; Shi et al., 2016; Vree et al., 2003). The observed inter-individual differences in CES isoform activities have mostly been reported in in vitro investigations and mainly in a qualitative manner.

Recently, Bayesian meta-analysis methods have been developed and applied to quantify variability and uncertainty for a range of phase I, phase II xenobiotic-metabolising enzymes as well as transporters (Darney et al., 2020a; Darney et al., 2019; Darney et al., 2020b; Kasteel et al., 2020; Quignot et al., 2019; Wiecek et al., 2019). Such Bayesian models allow to characterise inter-individual differences in enzyme activities while separating variability and uncertainty in a quantitative fashion, taking into account observed variance, such as sample size variation, heterogeneity across studies, as well as other sources of variability, i.e. subgroups of population. Consequently, such methodologies leads to more precise estimates of inter-individual differences across substrates for a given metabolic phase I, phase II pathway or a transporter. This manuscript provides a meta-analysis of human variability associated with carboxylesterase activities and kinetic parameters for well-characterised probe substrates using hierarchical Bayesian meta-analysis. It is part of an EFSA funded project, investigating human variability in phase I and Phase II metabolism as well as transporters. This approach can guide and inform the implementation of CES-related variability distributions and CES-related

Table 1
Search queries for the Extensive Literature Searches on human kinetics.

Generic population search terms	TITLE-ABS (human*) OR TITLE-ABS (subject*) OR TITLE-ABS (volunteer*) OR TITLE-ABS (adult*) OR TITLE-ABS (child) OR TITLE-ABS (children) OR TITLE-ABS (infant) OR TITLE-ABS (neonate) OR TITLE-ABS (newborn*) OR TITLE-ABS (elderly) 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 ("Afro American") OR TITLE-ABS (hispanic) OR TITLE-ABS ("ethnic variability") OR TITLE-ABS ("genetic polymorphism*") OR TITLE-ABS ("individual susceptibility") OR TITLE-ABS ("race difference*") OR TITLE-ABS ("gender difference*") OR TITLE-ABS ("sex difference*") OR TITLE-ABS (ontogenesis) OR TITLE-ABS (foetal stage) OR TITLE-ABS (genotype)
Exclusion	TITLE-ABS ("cell line*") OR TITLE-ABS ("cell culture*") OR TITLE-ABS-KEY (rat) OR TITLE-ABS-KEY (rats) OR TITLE-ABS-KEY (mouse) OR TITLE-ABS-KEY (mice)
Specific parameters for CES activity[a]	TITLE-ABS (population distribution) OR TITLE-ABS (tissue distribution) OR TITLE-ABS (tissue localisation) OR TITLE-ABS (intestine) OR TITLE-ABS (liver) OR TITLE-ABS (kidney) OR TITLE-ABS (lung) OR TITLE-ABS (expression level*) TITLE-ABS (gene environment) OR TITLE-ABS (induction) OR TITLE-ABS (inhibition) AND TITLE-ABS carboxylesterase*" OR CES1 OR CES2
Compound	TITLE-ABS ("*name of probe substrate*")
Outcome for probe substrates	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 (pharmacokinetic*) OR ABS (toxicokinetic*)

TITLE-ABS: term searched only in the title and the abstract of the paper.
In addition to the generic population search terms, specific parameters were included for the ELS for CES activity

uncertainty factors in human PBK models for substances relevant to the food safety area.

2. Material and Methods

2.1. Extensive literature search

Extensive literature searches (ELS) were performed were conducted in PubMed, Scopus and WoS (up to January 2020) according to the EFSA guidance document using search terms provided in Table 1 (EFSA, 2010). CES activity has been identified in healthy human subjects from a range of geographical ancestry together with their tissue and intracellular localisation (reported as enzyme activity, protein expression or content). In addition, *in vivo* probe substrates have been identified from the ELS as well as from available reviews on CES (Laizure and Parker, 2020; Wang et al., 2018). Specifically, data from human PK studies reported markers of oral (single) or intravenous (bolus) acute (Cmax) and chronic exposure (area under the curve-AUC, clearance).

Table 1 provides a summary of individual search queries and keywords applied to the ELS. Screening of the literature was performed as previously described (Darney et al., 2019; Buratti et al., 2021) starting with screening of titles and abstracts after removal of duplicates and application of exclusion criteria including: species other than humans, *in vitro* studies, development of analytical methods, modelling approaches, pharmacodynamic investigations, studies for unhealthy individuals, substrates other than those identified as relevant (Darney et al., 2019). Only publications written in English were considered.

2.2. Standardisation of dataset and meta-analyses

Meta-analyses were performed in non-phenotyped subjects for each probe substrate to derive CES-related variability distributions and CES-related UFs. PK parameters were normalised in a harmonised manner (Cmax expressed in ng/ml; AUC in ng.h/ml; clearance in ml/min/kg BW), while applying body weight correction to the applied doses (mg/kg bw). If

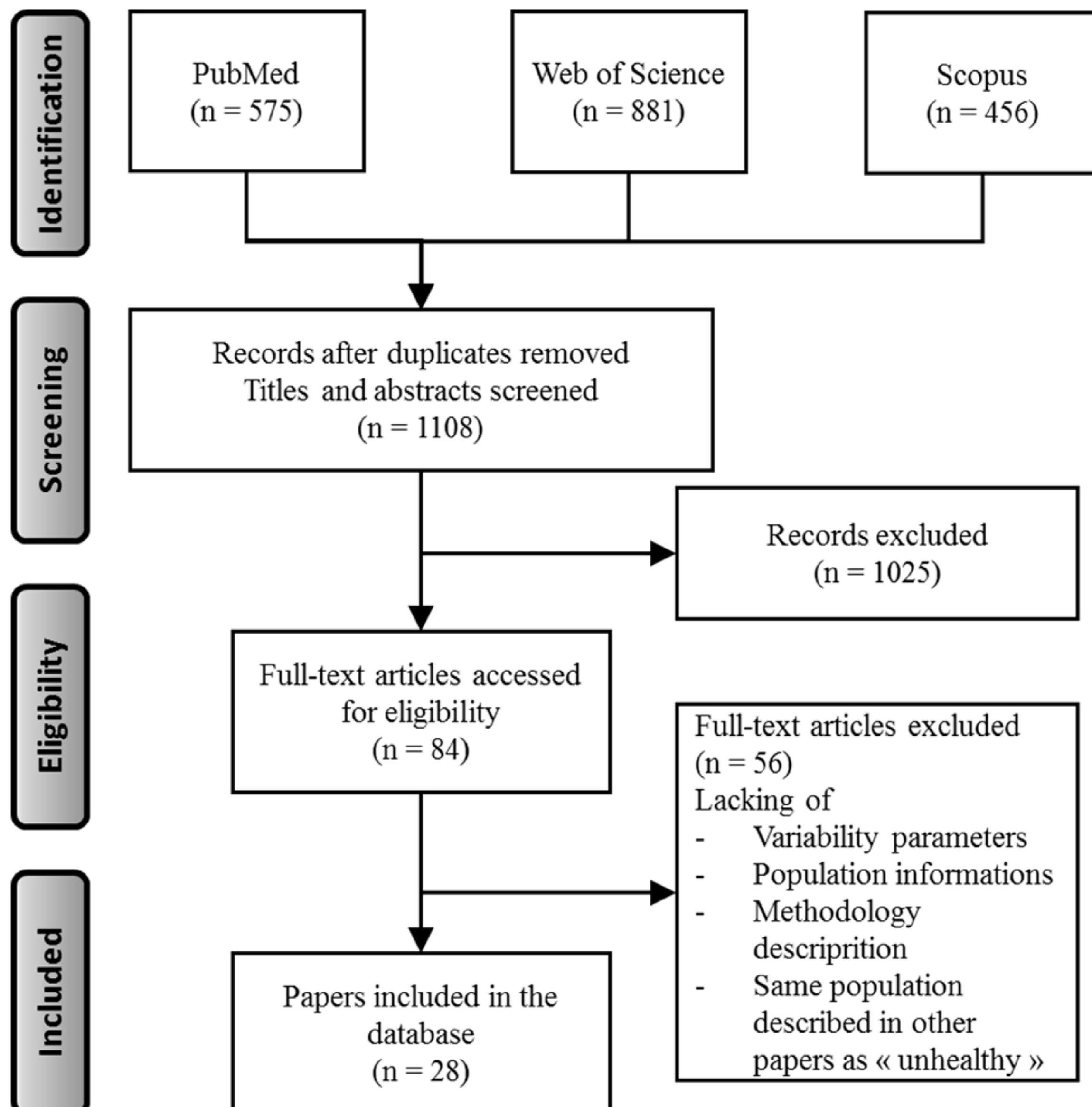

Fig. 1. Flow diagram for the extensive literature search of human variability of CES enzymatic activity.

available in the included study, the (mean) body weight was used, or continent specific body weights were applied to normalise the dose if these data were not available (Walpole et al., 2012). Data from included studies were mostly reported as arithmetic mean (AM) and standard deviation (SD), but in some cases geometric mean (GM) and geometric standard deviation (GSD) were reported. In general, PK data are recognised to follow a lognormal distribution (Dorne et al., 2001; Naumann et al., 1997; Renwick and Lazarus, 1998). All PK data were described as GM and GSD, since they are more appropriate to summarise lognormal distribution:

$$GM = \frac{X}{\sqrt{1 + CV_N^2}} \quad (1)$$

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

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

$$CV_N = \frac{SD}{X} \quad (3)$$

In some studies, SD was not reported and was derived from the standard error (SE, SEM), CV, or 95% confidence interval of the mean as described previously (Darney et al., 2019).

The meta-analyses provide more accurate information regarding inter-individual differences in non-phenotyped healthy adults of the included PK parameters for each chemical expressed as distributions. For each chemical and parameter, variability related to inter-study, inter-substrate and inter-individual differences was analysed, through a decomposition of the PK parameter variance (clearance, AUC or Cmax) using a previously described hierarchical Bayesian model (Darney et al., 2019; Wiecek et al., 2019). For the meta-analysis, non-informative prior data were selected for all chemicals expressed as uniform distributions. Overall, the meta-analyses provide probabilistic variability and uncertainty distributions describing inter-individual differences for each PK parameter using median values and 95% confidence intervals. The coefficient of variation (CV) were also estimated as follows:

$$CV = \sqrt{exp\left(\ln\left(\sqrt{\exp(1/\tau_j)}\right)\right)^2 - 1} \quad (4)$$

where τ_j is the inter-individual variability of the activity for a substrate 'j'. CES-related UFs were calculated as the ratio between the percentile of choice and the median of the distribution for each PK parameter for 95th and 97.5th centiles.

2.3. 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) (Plummer, 2003). References from the ELS were computed in EndNote (X8) files and the R codes used for the meta-analyses are previously published (Darney et al., 2019).

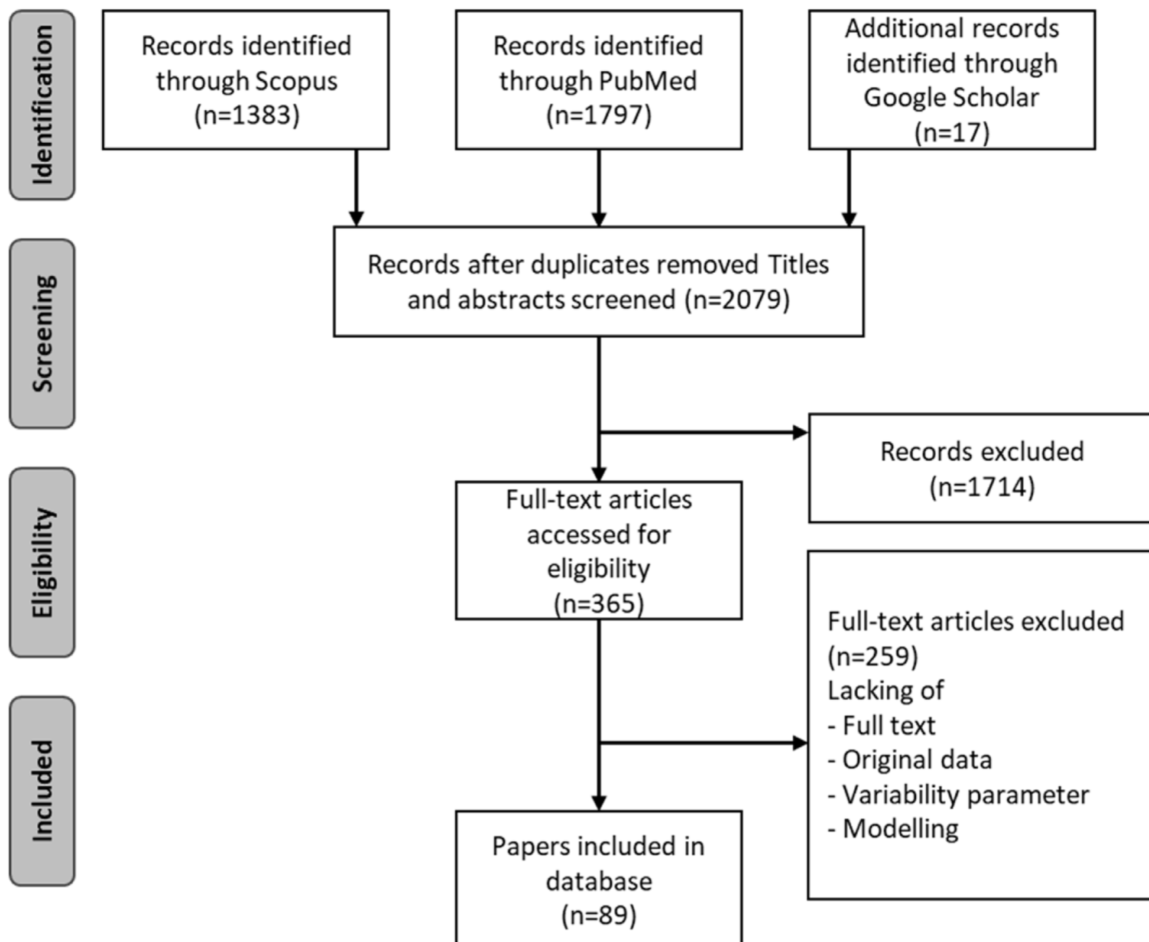

Fig. 2. Flow diagram for the extensive literature search of human pharmacokinetic studies for CES probe substrates.

3. Results

3.1. Data collection for enzymatic activity and localisation of carboxylesterases in humans

The results obtained from the ELS are summarised in Fig. 1.

Studies focused on healthy adults (range 18-75 years) from both sexes when available. Unhealthy individuals were excluded since health status can influence CES activities and distribution, as demonstrated by the secretion of CES1 into human blood in hepatocellular carcinoma (Na et al., 2009) or the decrease in CES activity in patients affected by hepatitis and cirrhosis (Yang et al., 2007). Overall, 84 peer reviewed publications were selected and their eligibility for the meta-analysis was assessed particularly for study design and methodological quality as previously reported (Darney et al., 2019). Amongst those, some papers were excluded for lacking quantitative information on variability (i.e. reported in figures or as qualitative results), demographic distribution, methodological details. Reviews, book chapters and other sources reporting primary datasets, were excluded from the data extraction to avoid multiple inclusions of the same dataset from different sources. Twenty-eight papers were then considered relevant for the extraction and included in the specific database. The database is provided in supplementary information [A] and the full database can be accessed on EFSA knowledge junction under DOI [https://doi.org/10.5281/zenodo.4943670].

Human CES activities are available for a range of countries worldwide, but most data were available for North America (50%), Europe (25%) and to a minor extent for East Asia (20%). Overall, CES activities were mostly reported for adult Caucasian population. However, many of the retrieved papers (65%) did not provide information on geographical ancestry and data gaps were identified. Scarce information was available for CES (generally reported as protein content) in adolescents and children, neonates and infants (up to 1 year). Some authors evidenced that CES protein content varied across age groups, with values 4 to 5-fold higher in adolescents and adults compared to that in infants and neonates (Boberg et al., 2017; Hines et al., 2016). In line with recent reviews, our data indicate that CES, reported as total activity, is mainly localised in the liver and gastro-intestinal tract, although a few peer reviewed publications suggested that the active enzymes are also present in the lung, nasal cavity, skin, plasma as well as in the placenta (Di, 2019). Identification of activity and protein content for both CES1 and CES2 isoforms, revealed that both are localised in the liver, while CES1 levels are higher in the microsomal fraction compared to that in the cytosol (Boberg et al., 2017; Hines et al., 2016). Interestingly, CES1 activity was measured in one study as hydrolysis of o-nitrophenyl acetate in hepatic microsomes from single individuals and showed high inter-donor variability with values ranging from 542 to 8194 nmol/mg per min (Hatfield and Potter, 2011). The presence of CES1 and CES2 in the gastro-intestinal tract has also been reported and and shown to be differentiated in different parts of the intestine (colon, duodenum, small intestine). The wide number of probe substrates and the wide variety of *in vitro* or *ex-vivo* experimental conditions for measuring CES activities, did not allow standardisation of the available data. However, most of the retrieved peer-reviewed literature focused on the use of *in vivo* drugs and reported data on PK parameters. Among such probe drugs, clopidogrel, oseltamivir, were considered relevant to inform the subsequent ELS.

3.2. Data collection for kinetic parameters of probe substrates of carboxylestserases in humans

From the ELS leading to data collection of kinetic parameters, a total of 3180 papers were assessed for 23 CES probe substrates using Scopus and PubMed and 17 additional papers were retrieved from Google Scholar. Fig. 2 provides the PRISMA diagram for the ELS. 365 papers were considered eligible from the first screening and 89 papers were considered eligible from the second screening. Data extraction was then performed for 10 of the 23 CES probe substrates and included aspirin, cilazapril, clopidogrel, dabigatran

Table 2
Inter-individual differences in Carboxyl-Esterase (CES) isoforms CES1/CES2 and CES-related Uncertainty Factors for markers of chronic oral exposure (AUC (ng.h/ml/dose kg body weight) for healthy adults of different geographical ancestry.

Group	Probe Substrate	Np	*n*	GM	CV (%)	UF95	UF97.5
Caucasian	Aspirin	3	57	878.8	28.6	1.6 [1.4; 2.0]	1.7 [1.5; 2.3]
	Cilazapril	4	56	5720.7	50.2	2.2 [1.7; 4.1]	2.5 [1.9; 4.1]
	Clopidogrel	3	412	165.5	117.7	4.6 [3.8; 5.9]	6.2 [4.9; 8.3]
	Dabigatran	9	224	428.5	64.4	2.6 [2.2; 3.3]	3.2 [2.6; 4.1]
	Enalapril	5	117	690.4	34.6	1.7 [1.5; 2.1]	1.9 [1.7; 2.4]
	Oseltamivir	14	709	128.6	32.7	1.7 [1.6; 1.8]	1.9 [1.8; 2.0]
	Quinapril	4	44	1123.4	35.4	1.8 [1.5; 2.5]	2.0 [1.6; 3.0]
	Ritalin	8	161	218.9	33.0	1.7 [1.5; 2.0]	1.9 [1.7; 2.2]
	Rufinamide	3	59	7718.3	39.2	1.9 [1.5; 2.5]	2.1 [1.7; 3.0]
	All Substrates	53	1839		37.3	1.8 [1.5; 5.1]	
East Asian	Aspirin	2	63	459.7	29.3	1.6 [1.4; 2.0]	1.8 [1.5; 2.3]
	Cilazapril	1	12	3012.5	58.6	NA	NA
	Clopidogrel	12	324	2.9	93.0	3.7 [3.0; 4.6]	4.7 [3.8; 6.2]
	Dabigatran	1	36	348.9	54.2	NA	NA
	Enalapril	2	41	936.0	39.1	1.9 [1.5; 2.8]	2.1 [1.6; 3.4]
	Oseltamivir	6	100	139.3	28.8	1.6 [1.4; 1.9]	1.7 [1.5; 2.1]
	Ritalin	1	4	149.7	68.5	NA	NA
	Rufinamide	1	10	8965	37.1	NA	NA
	All Substrates	22	528		34.6	1.7 [1.4; 4.3]	1.9 [1.5; 5.6]
Southeast Asian	Clopidogrel	1	3	6.1	15.1	NA	NA
	Oseltamivir	1	24	110.8	53.2	NA	NA
	Quinapril	1	24	853.2	38.5	NA	NA
South Asian	Enalapril	1	36	658.0	171.5	NA	NA
	Oseltamivir	1	42	67.9	22.8	NA	NA
	Valacyclovir	1	41	15502	25.0	NA	NA
Middle East	Enalapril	3	66	755.7	32.9	1.7 [1.5; 2.2]	1.9 [1.6; 2.5]

Ns: number of studies, nc: number of compounds, n: number of individuals, CV: coefficient of variation (lognormal distribution), GM: geometric mean (lognormal distribution); NA: not available.

etexilate, enalapril, oseltamivir, methylphenidate (ritalin), quinapril, rufinamide, valacyclovir. All available PK studies and the full list of relevant peer reviewed publications are provided in supplementary information [B] and the full database is available on EFSA knowledge junction under DOI [https://doi.org/10.5281/zenodo.4943670].

3.3. Human variability in CES and CES-related Uncertainty Factors

Human kinetic data were available for Caucasian, East, Southeast and South Asians, and Middle East healthy adults, while the majority of the data were available for Caucasian healthy adults. Overall, inter-individual differences in kinetic parameters for healthy adults was around 43% for the oral route and are illustrated in Tables 2 and 3 for markers of chronic exposure (AUC and Clearance respectively) and Table 4 for markers of acute exposure (Cmax). Clopidogrel showed a variability above 100% for all included parameters, regardless of the ethnicity. Variability in AUC is similar across populations, with high variability for clopidogrel, cilazapril and dabigatran etexilate. Ritalin kinetic parameters showed high variability in East Asians compared to Caucasians, which might be due to the low number of individuals included in the study., the default kinetic UF (3.16) would be protective for over 97.5% of the healthy adult population while considering the median value from the analysis (with the exception of clopidogrel and dabigatran etexilate).

Inter-individual differences in clearance values were across the different populations, with large differences for clopidogrel and dabigatran etexilate. Inter-individual differences for oseltamivir and quinapril clearances were high for Southeast Asians, which may be due to the limited number of available studies. Overall, the default kinetic UF would be protective for at least 95% of the healthy adult population when considering the median value.

Variability in Cmax was similar across populations of different geographical ancestry, with high values for clopidogrel, cilazapril, dabigatran etexilate, oseltamivir, ritalin and quinapril. Variability in ritalin Cmax showed high values in East Asians compared to that in Caucasians and again this may be due to the low sample size in the study. The default kinetic UF would be protective of at least for 97.5% of the healthy adult population when considering the median value, with the exception of clopidogrel and dabigatran etexilate, which both exceeded the UF of 3.16.

4. Discussion and Conclusion

This manuscript aimed to quantify inter-individual differences in the kinetics of CES probe substrates. The quantification of species differences in CES tissue distribution and catalytic activities is particularly challenging since data from experimental test species or *in vitro* systems are used for safety assessment (Di, 2019). The tissue distribution of CES presented here has been recently confirmed in some experimental work carried out with a range of human tissue samples (Basit et al., 2020). In addition, the subcellular localisation of CES seems to play a key role in the metabolism of a wide range of xenobiotics (Sato et al., 2012). Indeed, it has been reported that in the human liver, CES1 can interplay with Phase II enzymes, i.e. UDP-glucuronosyltransferases for the conjugation of hydrolytic products and this is favoured by the common localisation of both enzyme systems on the luminal side of the endoplasmic reticulum (Di et al., 2019).

Data from this meta-analysis represent a step forward to integrate variability distributions, reflecting inter-individual differences in metabolism, in PBK models and for the development of quantitative *in vitro in vivo* extrapolation (QIVIVE). Such models may prove useful for i) pharmaceuticals metabolised by CES and may potentially lead to an improved assessment of clinical outcomes after treatment of patients from different geographical ancestry. In addition, it can support the design for prodrugs using information on CES specific tissue distribution, and ii) risk assessment of chemicals in food and feed for which human populations are exposed via the diet or the environment.

The collected data on CES activities in healthy subjects highlighted the heterogeneous nature of the datasets as the result of a wide variability in experimental conditions (e.g. probe substrate, assays, time-points, biological matrices). In addition, uncertainties related to the limited number of enrolled individuals, particularly for specific age groups, quantification of inter-individual differences in CES activities was not feasible and would have led to relatively weak estimates. Thus, hierarchical Bayesian meta-analyses of *in vivo* kinetic parameters for a total of 10 specific probe substrates were performed using markers of acute (Cmax) and chronic exposure (AUC/clearance). The resulting variability distributions and the CES-related UFs showed that the default factor of 3.16 would be conservative for at least 97.5% of non-phenotyped healthy adults when considering the median value, with the exception of clopidogrel and dabigatran. Despite 85-90% of clopidogrel is hydrolysed by human CES1, most research has focused on the role of hepatic CYP450 metabolism as the primary source of its response variability. Indeed, even if clopidogrel metabolism involves in a minor extent CYP450, the highly polymorphic isoforms (i.e.CYP2C19) may impact the overall human variability of the kinetics for this compound (Brown and Pereira, 2018; Trenk et al., 2013). The high variability seen in dabigatran etexilate may be due to differences in its absorption,

Table 3
Inter-individual differences in Carboxyl-Esterase (CES) isoforms CES1/CES2 and CES-related Uncertainty Factors for markers of chronic oral exposure (clearances (ml/min/kg body weight) for healthy adults of different geographical ancestry.

Group	Probe Substrate	Np	n	GM	CV (%)	UF95	UF97.5
Caucasian	Aspirin	1	30	1.3	27.9	NA	NA
	Cilazapril	2	26	0.2	34.2	1.7 [1.4; 3.4]	1.9 [1.5; 3.4]
	Dabigatran	6	153	1.7	61.1	2.5 [2.1; 3.2]	3.0 [2.4; 4.1]
	Oseltamivir	13	679	8.2	42.7	2.0 [1.8; 2.0]	2.2 [2.1; 2.5]
	Ritalin	1	22	3.0	37.0	NA	NA
	All substrates	21	858		43.0	2.0 [1.4; 3.1]	2.3 [1.5; 3.1]
East Asian	Cilazapril	1	12	0.3	43.5	NA	NA
	Clopidogrel	2	42	271.8	59.1	2.5 [1.8; 6.3]	3.0 [2.0; 6.3]
	Oseltamivir	6	100	8.2	27.8	1.6 [1.4; 1.8]	1.7 [1.5; 2.1]
	Rufinamide	1	10	0.1	34.0	NA	NA
	All substrates	21	858		36.3	1.8 [1.4; 4.1]	2.0 [1.5; 5.4]
Southeast Asian	Oseltamivir	1	24	6.7	64.4	NA	NA
	Quinapril	1	24	1.0	126.1	NA	NA

dabigatran_e: dabigatran etexilate; ns: number of studies, nc: number of compounds, n: number of individuals, CV: coefficient of variation (lognormal distribution), GM: geometric mean (lognormal distribution); NA: not available.

Table 4
Inter-individual differences in Carboxyl-esterase (CES) isoforms CES1/CES2 and CES-related Uncertainty Factors for markers of acute oral exposure (Cmax (ng/ml/dose kg body weight) for healthy adults of different geographical ancestry.

Group	Chemical	Np	N	GM	CV (%)	UF95	UF97.5
Caucasian	Aspirin	3	57	812.0	36.0	1.8 [1.5; 2.4]	2.0 [1.6; 2.8]
	Cilazapril	5	68	1551.0	42.9	2.0 [1.6; 3.2]	2.2 [1.8; 3.2]
	Clopidogrel	3	412	70.2	135.2	5.4 [1.6; 3.2]	7.4 [5.7; 10]
	Dabigatran	9	240	53.7	66.0	2.7 [2.3; 3.3]	3.3 [2.7; 4.2]
	Enalapril	5	117	418.1	36.8	1.8 [1.6; 2.2]	2.0 [1.7; 2.5]
	Oseltamivir	13	670	56.1	56.7	2.4 [2.2; 2.7]	2.8 [2.5; 3.2]
	Quinapril	3	38	734.5	39.3	1.9 [1.5; 2.9]	2.1 [1.6; 3.5]
	Ritalin	8	145	37.4	31.2	1.7 [1.5; 1.9]	1.8 [1.6; 2.1]
	Rufinamide	3	59	405.3	25.8	1.5 [1.3; 1.9]	1.6 [1.4; 2.1]
	All Probe Substrate	52	1806		41.1	1.9 [1.2; 5.9]	2.2 [1.5; 8.3]
East Asian	Aspirin	2	63	346.2	39.7	1.9 [1.6; 2.5]	2.1 [1.7; 3.0]
	Cilazapril	2	36	948.7	44.7	2.0 [1.6; 4.6]	2.3 [1.7; 4.2]
	Clopidogrel	12	324	1.5	109.5	4.3 [3.5; 5.6]	5.7 [4.4; 7.8]
	Dabigatran	1	36	41.1	57.3	NA	NA
	Enalapril	3	61	591.5	34.2	1.7 [1.5; 2.3]	1.9 [1.6; 2.6]
	Oseltamivir	6	100	37.3	57.6	2.4 [1.9; 3.3]	2.9 [2.2; 4.2]
	Ritalin	1	4	41.1	48.3	NA	NA
	Rufinamide	1	10	394.0	27.1	NA	NA
	All Probe Substrate	25	584		47.6	2.1 [1.5; 5.0]	2.5 [1.7; 6.7]
Southeast Asian	Clopidogrel	1	3	0.9	13.9	NA	NA
	Oseltamivir	1	24	60.0	66.2	NA	NA
	Quinapril	1	24	676.7	49.7	NA	NA
South Asian	Enalapril	3	66	320.4	32.5	1.7 [1.4; 2.4]	1.9 [1.5; 2.8]
	Oseltamivir	1	42	21.8	29.2	NA	NA
	Valacyclovir	1	41	5011.0	35.0	NA	NA
Middle East	Enalapril	1	36	529.8	33.8	NA	NA

dabigatran_e: dabigatran etexilate; ns: number of studies, nc: number of compounds, n: number of individuals, CV: coefficient of variation (lognormal distribution), GM: geometric mean (lognormal distribution); NA: not available.

influenced by food intake, since no other enzyme beside CES1 is involved in the conversion of dabigatran etexilate to the active form dabigatran (Stangier, 2008).

Overall, PK data for CES probe substrates were relatively well represented for Caucasian and East Asian populations, however PK data for the African population, known for their broad genetic diversity in the frequency of CES polymorphisms, were scarce (Marsh et al., 2004). Hence, PK data for phenotyped individuals are needed to 1.integrate inter-genotypic frequencies from populations of different geographical ancestry and 2. generate distributions to address inter-phenotypic differences, 3. derive CES-related UFs and, when available data allows, chemical-specific adjustment factors.

CES have a critical role in the detoxification of organophosphates and pyrethroid pesticides, both bio-activation and detoxification of pro-drugs as well as in endogenous lipid metabolism, i.e. triacylglycerol, cholesteryl esters (Morris et al., 2014; Phillips and Stapleton, 2019; Ross et al., 2010). It has been reported that CES mediates at least 20% of hydrolysis reactions for marketed drugs and for about 50% of marketed pro-drugs (Di, 2019). Hence, CES1 inhibition could profoundly impact the PK of pro-drugs as well as the modulation of their pharmacodynamics, thus impacting on the efficacy. Other examples include Organophosphate ester flame retardants which inhibit CES1 and may impact on both the bioactivation of prodrugs and other xenobiotics (Phillips and Stapleton, 2019) as well as potentially alter hepatic lipid metabolism (Morris et al., 2014). *In vitro* evidence suggest that an a number of perfluoroalkyl substances also inhibit CES1 and CES2, depending on both the length of the carbon chain and the *in vivo* extrapolated plasma concentration (Qi et al., 2020). A large number of phytochemicals are also known as CES1 inhibitors including flavonoids, triterpenes and other phenolic compounds (Wang et al., 2020). Furthermore, many pesticides containing organophosphate or carbamate moieties can inhibit CES activity (Hatfield and Potter, 2011). Finally, impact of the human microbiota on the hydrolysis of chemicals has been recently investigated (Noh et al., 2017). Indeed, the gut microbiota has potential implications in drug PK, including their bioavailability, either activating drugs or decreasing their therapeutic efficacy (Flowers et al., 2020). Identifying and characterising the impact of the human gut microbiota on the kinetics of chemicals is becoming an important research area and has the potential to support individualised drug treatment.

The present analysis of human variability in the kinetics of CES probe substrates indicated moderate values (<CV 50%), which may be due to the relatively limited data available (and the small sample size in the studies). On the other hand, further work is required to investigate the presence of CES polymorphisms and its consequence on variability in CES metabolism across populations of different geographical ancestry. However, the CES-related variability distributions generated here can be used as an input for PBK and QIVIVE models which are increasingly applied in chemical risk assessment as part of the battery of New Approach Methodologies (EFSA, 2014; Bessems et al., 2014; Paini et al., 2019). Specifically, available isoform-specific data, CES-related variability distributions and variability distributions for phase I (Cytochrome P450 isoforms) and Phase II enzymes (UDP-glucuronosyltransferases, Glutathione-S-transferases etc), can be integrated to better characterise variability and uncertainty in kinetics and metabolism in humans (or other test species) for compounds of relevance to food safety and environmental health. As described previously in the literature, these approaches provide a scientific basis for integrating human variability in risk assessment, reduce animal testing and refine default uncertainty factors into chemical-specific adjustment factors or pathway-related uncertainty factors to move towards a science-based derivation of safe levels of chemicals in food and in the environment (Buratti et al., 2021; Darney et al., 2020a; Darney et al., 2019; Darney et al., 2020b; Kasteel et al., 2020; Punt et al., 2017).

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CRediT authorship contribution statement

E. Di Consiglio: Investigation, Data curation, Writing - original draft, Writing - review & editing. **K. Darney:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Visualization. **F.M. Buratti:** Investigation. **L. Turco:** Investigation. **S. Vichi:** Investigation. **E. Testai:** Writing - review & editing, Project administration. **L.S. Lautz:** Conceptualization, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration. **J.L.C.M. Dorne:** Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors report no declarations of interest.

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.2021.07.005.

References

Basit, A., et al., 2020. Characterization of Differential Tissue Abundance of Major Non-CYP Enzymes in Human. Mol Pharm 17 (11), 4114–4124.
Bessems, J.G., et al., 2014. PBTK modelling platforms and parameter estimation tools to enable animal-free risk assessment: Recommendations from a joint EPAA – EURL ECVAM ADME workshop. Regulatory Toxicology and Pharmacology 68 (1), 119–139.
Boberg, M., et al., 2017. Age-Dependent Absolute Abundance of Hepatic Carboxylesterases (CES1 and CES2) by LC-MS/MS Proteomics: Application to PBPK Modeling of Oseltamivir In Vivo Pharmacokinetics in Infants. Drug metabolism and disposition: the biological fate of chemicals 45 (2), 216–223.
Brown, S.-A., Pereira, N., 2018. Pharmacogenomic Impact of CYP2C19 Variation on Clopidogrel Therapy in Precision Cardiovascular Medicine. Journal of personalized medicine 8 (1), 8.
Buratti, F.M., et al., 2021. Human variability in glutathione-S-transferase activities, tissue distribution and major polymorphic variants: Meta-analysis and implication for chemical risk assessment. Toxicol Lett 337, 78–90.
Darney, K., et al., 2020a. Bayesian meta-analysis of inter-phenotypic differences in human serum paraoxonase-1 activity for chemical risk assessment. Environmental International 138, 105609.
Darney, K., et al., 2019. Inter-ethnic differences in CYP3A4 metabolism: A Bayesian meta-analysis for the refinement of uncertainty factors in chemical risk assessment. Computational Toxicology 12, 100092.
Darney, K., et al., 2020b. Human variability in influx and efflux transporters in relation to uncertainty factors for chemical risk assessment. Food and Chemical Toxicology .
Di, L., 2019. The Impact of Carboxylesterases in Drug Metabolism and Pharmacokinetics. Current drug metabolism 20 (2), 91–102.
Dorne, J.L.C.M., Walton, K., Renwick, A.G., 2001. Uncertainty factors for chemical risk assessment: human variability in the pharmacokinetics of CYP1A2 probe substrates. Food and Chemical Toxicology 39 (7), 681–696.
EFSA, 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 8 (6), 1637.
EFSA, 2014. Modern methodologies and tools for human hazard assessment of chemicals. EFSA Journal 12 (4), 3638.
Flowers, S.A., Bhat, S., Lee, J.C., 2020. Potential Implications of Gut Microbiota in Drug Pharmacokinetics and Bioavailability. Pharmacotherapy 40 (7), 704–712.
Hatfield, M.J., Potter, P.M., 2011. Carboxylesterase inhibitors. Expert opinion on therapeutic patents 21 (8), 1159–1171.
Hines, R.N., Simpson, P.M., McCarver, D.G., 2016. Age-Dependent Human Hepatic Carboxylesterase 1 (CES1) and Carboxylesterase 2 (CES2) Postnatal Ontogeny. Drug Metabolism and Disposition 44 (7), 959–966.
Hosokawa, M., et al., 1995. Interindividual variation in carboxylesterase levels in human liver microsomes. Drug metabolism and disposition: the biological fate of chemicals 23 (10), 1022–1027.
Imai, T., 2006. Human carboxylesterase isozymes: catalytic properties and rational drug design. Drug metabolism and pharmacokinetics 21 (3), 173–185.
Kasteel, E.E.J., Darney, K., Kramer, N.I., Dorne, J.L.C.M., Lautz, L.S., 2020. Human variability in isoform-specific glucuronidation: Markers of acute and chronic exposure, polymorphisms and uncertainty factors. Archives of Toxicology..
Laizure, S.C., Herring, V., Hu, Z., Witbrodt, K., Parker, R.B., 2013. The role of human carboxylesterases in drug metabolism: have we overlooked their importance? Pharmacotherapy 33 (2), 210–222.
Laizure, S.C., Parker, R.B., 2020. Is genetic variability in carboxylesterase-1 and carboxylesterase-2 drug metabolism an important component of personalized medicine? Xenobiotica; the fate of foreign compounds in biological systems 50 (1), 92–100.
Marsh, S., et al., 2004. Pharmacogenomic assessment of carboxylesterases 1 and 2. Genomics 84 (4), 661–668.
Morris, P.J., et al., 2014. Organophosphorus flame retardants inhibit specific liver carboxylesterases and cause serum hypertriglyceridemia. ACS chemical biology 9 (5), 1097–1103.
Naumann, B.D., et al., 1997. Use of toxicokinetic and toxicodynamic data to reduce uncertainties when setting occupational exposure limits for pharmaceuticals. Human and Ecological Risk Assessment: An International Journal 3 (4), 555–565.
Noh, K., et al., 2017. Impact of gut microbiota on drug metabolism: an update for safe and effective use of drugs. Arch Pharm Res 40 (12), 1345–1355.
Oda, S., Fukami, T., Yokoi, T., Nakajima, M., 2015. A comprehensive review of UDP-glucuronosyltransferase and esterases for drug development. Drug metabolism and pharmacokinetics 30 (1), 30–51.
Oh, J., et al., 2017. The novel carboxylesterase 1 variant c.662A&G may decrease the bioactivation of oseltamivir in humans. PLOS ONE 12 (4) e0176320.
Paini, A., et al., 2019. Next generation physiologically based kinetic (NG-PBK) models in support of regulatory decision making. Computational Toxicology 9, 61–72.
Patrick, K.S., et al., 2007. Influence of ethanol and gender on methylphenidate pharmacokinetics and pharmacodynamics. Clinical pharmacology and therapeutics 81 (3), 346–353.
Phillips, A.L., Stapleton, H.M., 2019. Inhibition of Human Liver Carboxylesterase (hCE1) by Organophosphate Ester Flame Retardants and Plasticizers: Implications for Pharmacotherapy. Toxicological Sciences 171 (2), 396–405.
Plummer, M., 2003. JAGS: A Program for Analysis of Bayesian Graphical Models Using Gibbs Sampling. In: Hornik, K., Leisch, F., Zeileis, A. (Eds.), Proceedings of the 3rd International Workshop on Distributed Statistical Computing, Vienna Austria, pp. 1–10.
Punt, A., Peijnenburg, A., Hoogenboom, R., Bouwmeester, H., 2017. Non-animal approaches for toxicokinetics in risk evaluations of food chemicals. Altex 34 (4), 501–514.
Qi, W., Clark, J.M., Timme-Laragy, A.R., Park, Y., 2020. Per- and Polyfluoroalkyl Substances and Obesity, Type 2 Diabetes and Non-alcoholic Fatty Liver Disease: A Review of Epidemiologic Findings. Toxicological and environmental chemistry 102 (1-4), 1–36.
Quignot, N., Wiecek, W., Amzal, B., Dorne, J.L., 2019. The Yin-Yang of CYP3A4: a Bayesian meta-analysis to quantify inhibition and induction of CYP3A4 metabolism in humans and refine uncertainty factors for mixture risk assessment. Arch Toxicol 93 (1), 107–119.
Renwick, A.G., Lazarus, N.R., 1998. Human Variability and Noncancer Risk Assessment— An Analysis of the Default Uncertainty Factor. Regulatory Toxicology and Pharmacology 27 (1), 3–20.
Ross, M.K., Streit, T.M., Herring, K.L., 2010. Carboxylesterases: Dual roles in lipid and pesticide metabolism. Journal of pesticide science 35 (3), 257–264.
Shi, D., Yang, D., Prinssen, E.P., Davies, B.E., Yan, B., 2011. Surge in expression of carboxylesterase 1 during the post-neonatal stage enables a rapid gain of the capacity to activate the anti-influenza prodrug oseltamivir. The Journal of infectious diseases 203 (7), 937–942.
Shi, J., et al., 2016. Association of Oseltamivir Activation with Gender and Carboxylesterase 1 Genetic Polymorphisms. Basic & clinical pharmacology & toxicology 119 (6), 555–561.
Stangier, J., 2008. Clinical pharmacokinetics and pharmacodynamics of the oral direct thrombin inhibitor dabigatran etexilate. Clin Pharmacokinet 47 (5), 285–295.
Tarkiainen, E.K., et al., 2012. Carboxylesterase 1 polymorphism impairs oseltamivir bioactivation in humans. Clinical pharmacology and therapeutics 92 (1), 68–71.
Tarkiainen, E.K., et al., 2015a. Carboxylesterase 1 c.428G&A single nucleotide variation increases the antiplatelet effects of clopidogrel by reducing its hydrolysis in humans. Clinical pharmacology and therapeutics 97 (6), 650–658.
Tarkiainen, E.K., et al., 2015b. Effect of carboxylesterase 1 c.428G & A single nucleotide variation on the pharmacokinetics of quinapril and enalapril. British journal of clinical pharmacology 80 (5), 1131–1138.
Trenk, D., Kristensen, S.D., Hochholzer, W., Neumann, F.J., 2013. High on-treatment platelet reactivity and P2Y12 antagonists in clinical trials. Thromb Haemost 109 (5), 834–845.
Vistoli, G., Pedretti, A., Mazzolari, A., Bolchi, C., Testa, B., 2009. Influence of ionization state on the activation of temocapril by hCES1: a molecular-dynamics study. Chemistry & biodiversity 6 (11), 2092–2100.
Vistoli, G., Pedretti, A., Mazzolari, A., Testa, B., 2010. Homology modeling and metabolism prediction of human carboxylesterase-2 using docking analyses by GriDock: a parallelized tool based on AutoDock 4.0. Journal of computer-aided molecular design 24 (9), 771–787.
Vree, T.B., et al., 2003. Differences between lovastatin and simvastatin hydrolysis in healthy male and female volunteers:gut hydrolysis of lovastatin is twice that of simvastatin. TheScientificWorldJournal 3, 1332–1343.
Walpole, S.C., et al., 2012. The weight of nations: an estimation of adult human biomass. BMC Public Health 12 (1), 439.
Wang, D., et al., 2018. Human carboxylesterases: a comprehensive review. Acta pharmaceutica Sinica. B 8 (5), 699–712.
Wang, L., et al., 2020. Pentacyclic triterpenoid acids in Styrax as potent and highly specific inhibitors against human carboxylesterase 1A. Food & Function..
Wiecek, W., Dorne, J., Quignot, N., Bechaux, C., Amzal, B., 2019. A generic Bayesian hierarchical model for the meta-analysis of human population variability in kinetics and its applications in chemical risk assessment. Computational Toxicology 12, 100–106.

Xu, G., Zhang, W., Ma, M.K., McLeod, H.L., 2002. Human carboxylesterase 2 is commonly expressed in tumor tissue and is correlated with activation of irinotecan. Clinical cancer research : an official journal of the American Association for Cancer Research 8 (8), 2605–2611.

Yang, D., et al., 2009. Human carboxylesterases HCE1 and HCE2: ontogenic expression, inter-individual variability and differential hydrolysis of oseltamivir, aspirin, deltamethrin and permethrin. Biochemical pharmacology 77 (2), 238–247.

Zhang, W., Xu, G., McLeod, H.L., 2002. Comprehensive evaluation of carboxylesterase-2 expression in normal human tissues using tissue array analysis. Applied immunohistochemistry & molecular morphology : AIMM 10 (4), 374–380.

Zhu, H.J., et al., 2008. Two CES1 gene mutations lead to dysfunctional carboxylesterase 1 activity in man: clinical significance and molecular basis. American journal of human genetics 82 (6), 1241–1248.