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Keyvin Darney

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L'UNIVERSITE
DE BRETAGNE OCCIDENTALE

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Par

Keyvin DARNEY

**Towards next generation risk assessment of chemicals:
Bayesian meta-analysis of Human variability in metabolism and
transporters and application for the derivation of pathway-related
uncertainty factors**

Thèse présentée et soutenue à Maisons-Alfort, le 26 février 2020

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List of abbreviations

ABC	ATP Binding Cassette
AChe	Acetylcholinesterase
ADME	Absorption, Distribution, Metabolism, Excretion
AUC	Area Under the Curve
BChE	Butyl Cholinesterase
BCRP	Breast Cancer Resistant Protein
BMD	Benchmark Dose
BMI	Body Mass Index
BMR	Benchmark Response
BSEP	Bile Salt Export Pump
CaE	Carboxyl Esterase
CI	Confidence Interval
Cmax	Maximum Observed Concentration
CSAF	Chemical Specific Adjustment Factor
CYP	Cytochrome P450
DCCA	3-(2,2 Dichlorovinyl)-2,2-Dimethyl-(1-Cyclopropane) Carboxylic Acid
EFSA	European Food Safety Agency
ELS	Extensive Literature Search
ENNS	French National Nutrition and Health Survey (Etude National Nutrition Santé)
FAO	Food and Agriculture Organization
InVS	French Institute for Public Health Surveillance (Institut De Veille Sanitaire)
IPCS	International Programme on Chemical Safety
IV	Intra Venous
LOD	Limit of Detection
LOQ	Limit of Quantification
MATE	Multidrug and Toxic Compound Extrusion
MCMC	Markov Chain Monte Carlo
MDR	Multiresistant Drug Protein
MRL	Maximum Residue Limit
MRP	Multidrug Resistance-Associated Protein
NAM	New Approaches Methodologies
NTCP	Sinusoidal Sodium-Taurocholate Cotransporting Polypeptide
OAT	Organic Anion Transporter
OATP	Organic Anion Transporter Polypeptide
OCT	Organic Cation Transporters
OECD	Organisation for Economic Co-Operation and Development
OP	Organophosphorus
P	Percentile
PBK	Physiologically Based Kinetic
P-gp	P-Glycoprotein
PD	Pharmacodynamic
PK	Pharmacokinetic

PON	Paraoxonase
QIVIVE	Quantitative <i>In Vivo to In Vitro</i> Extrapolation
QSAR	Quantitative Structure–Activity Relationship
RAC	Raw Agricultural Commodities
SLC	Solute Carrier
SNP	Single Nucleotide Polymorphism
TD	Toxicodynamic
TK	Toxicokinetic
UF	Uncertainty Factor
UGT	UDP-Glucuronosyltransferase
WHO	World Health Organization

1. General introduction

1.1. Human risk assessment of chemicals

In the modern world, humans are exposed to a wide range of chemicals throughout their life. Such chemicals occur in daily life and include pharmaceuticals mostly as therapeutic drugs, consumer products (cosmetic ingredients) and chemicals that are intentionally added to food (food additives, flavourings, food contact materials), raw commodities (pesticides/biocides: herbicides, fungicides, insecticides, etc.) or in the feed of farm animals as veterinary drugs. In addition, contaminants in the food chain constitute another source of chemical exposure for humans and include man-made contaminants such as process contaminants (acrylamide, furans), environmental pollutants (brominated flame retardants, dioxins, PCBs, perfluoro-alkyls), metals (as a result of human activity) as well as natural toxins (mycotoxins, plant alkaloids, marine biotoxins) (Dorne et al., 2009). With such a wide range of chemicals, human risk assessment of chemicals is of considerable public health importance and provides means to derive safe levels of acute and chronic exposure for subgroups of the human population including neonates, children, elderly and populations of different geographical ancestry (inter-ethnic differences) and genetic polymorphisms.

Indeed, risk assessment is a central component of risk analysis and provides a scientific basis for risk management on decisions and measures that may be needed to protect human health and for risk communication to allow an interactive exchange of information between risk assessors, managers, news media, stakeholders, and the general public (FAO/WHO, 2018) (Figure 1). The four steps of chemical risk assessment are hazard identification, hazard characterisation, exposure assessment and risk characterisation.

1. *Hazard identification* has been defined as follows “the identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system, or (sub)population. Hazard identification is the first stage in hazard assessment and the first of four steps in risk assessment” (IPCS, 2004). Toxicological endpoints can be identified from animal-based toxicity studies or from *in vitro* toxicity assays (Barlow et al., 2002; Smith, 2002). In practice, a review of studies regarding the mode of action, the toxicokinetics (the processes of absorption, distribution, metabolism and excretion (ADME) of a toxicant) and the toxicodynamics (the actions and interactions of the toxicant within the organism and describes processes at organ, tissue, cellular, and molecular levels) is performed (Dorne et al., 2011; Faustman and Omenn, 2008). From now on, toxicokinetics (“TK” or pharmacokinetics “PK”)

and toxicodynamics (“TD” or pharmacodynamics “PD”) will be referred to as kinetics and dynamics in this thesis.

2. *Hazard characterisation* is “the qualitative and, wherever possible, quantitative description of the inherent properties of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose–response assessment and its attendant uncertainties” (IPCS, 2004). Kinetic data can be implemented into hazard characterisation since they provide quantitative information about inter-species and interindividual differences and can be used in the extrapolation from high to low dose and from animal to human (Dybing et al., 2002; OECD, 2010).
3. *Exposure assessment* is the “evaluation of the exposure of an organism, system, or (sub)population to an agent (and its derivatives)” (IPCS, 2004). The objectives of exposure assessment are to determine the sources, the exposure pathways, the amounts and the duration of exposure that are experienced or anticipated with the chemical of interest by the population (Faustman and Omenn, 2008). Moreover, exposure scenarios are used in order to take into account specific populations that may be at higher exposure (U.S. EPA, 2011).
4. *Risk characterisation* is “the qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system, or (sub)-population, under defined exposure conditions” (IPCS, 2004). With regard to the definition of the risk (“the probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to an agent” (IPCS, 2004)), risk characterisation is thus the integration of both hazard identification and characterisation, leading to a health-based guidance value, with the estimated exposure assessment. It provides an estimation of the potential risk of adverse health effects in humans under different exposure scenarios (Dorne et al., 2011; IPCS, 2009).

Beyond the four pillars of risk assessment, regulatory agencies have to rely on the mechanistic assumption of whether a chemical is genotoxic or non-genotoxic. Toxic responses such as development of cancer after exposure to genotoxic carcinogens are considered to be linear at low doses over a chronic exposure with no threshold and thus having no dose without risk (Dorne, 2010).

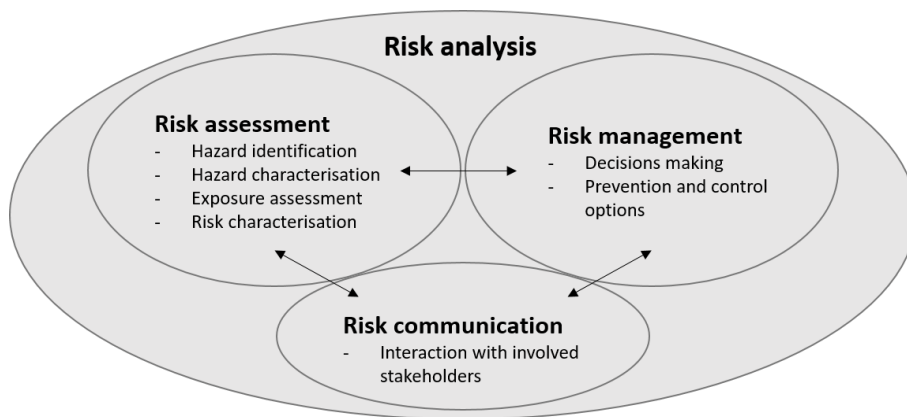


Figure 1. Risk analysis paradigm (adapted from (FAO/WHO, 1997))

Non-genotoxic chemicals are considered to show a threshold dose or concentration below which no toxic effect would be observed. Agencies worldwide have estimated health-based guidance values, such as the acceptable/tolerable daily intake (ADI, TDI), or the reference dose (RfD), derived from experimentally determined dose, the point of departure (POD), at which there was no statistically or biologically significant indication of the toxic effect of concern. Traditionally, this dose is referred as the "no-observed-adverse-effect level" (NOAEL) from animal toxicology studies. This method has limitations such as dependency on the dose selection, dose spacing, sample size and in the end ignore the shape of the dose-response curve (SCHER/SCCP/SCENIHR, 2009). The Benchmark Dose (BMD) approach has been proposed as a preferred alternative since it takes into account all of the dose-response data to estimate the shape of the dose-response curve for the toxic effect (Crump, 1984; EFSA Scientific Committee et al., 2017; U.S. EPA, 2012). The lower confidence limit of the Benchmark Dose (BMDL) provides a quantitative estimation of the quality of the dataset, resulting in a more protective POD (Figure 2) and is therefore in accordance with the precautionary principle.

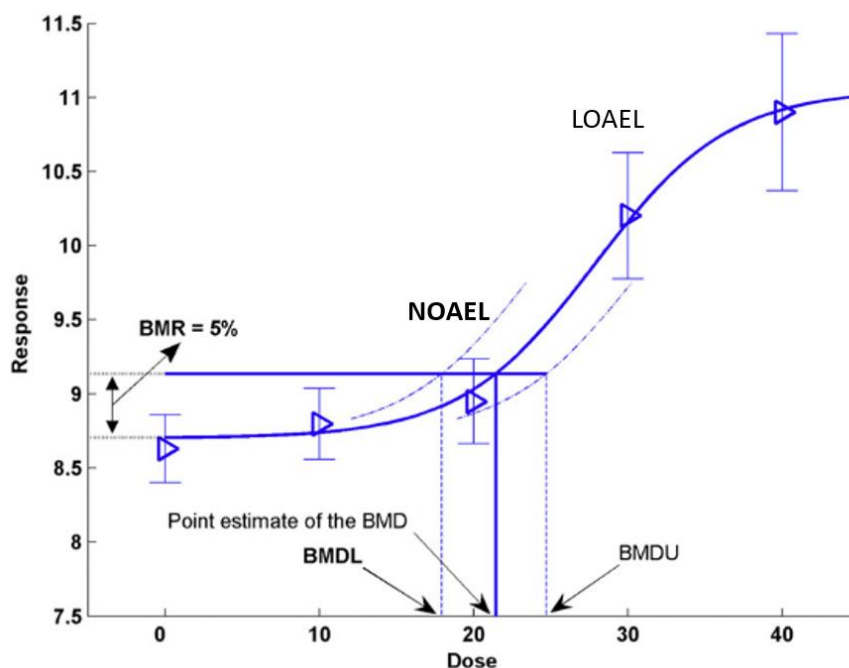


Figure 2. BMD approach in comparison with the NOAEL (fitted dose-response model on experimental data, from EFSA Scientific Committee et al. (2017))

Genotoxic and carcinogenic chemicals have the potential to directly and irreversibly interact with DNA and to cause cancer. Three major methods are used to perform risk assessment of genotoxic compounds, the linear extrapolation, the Threshold of Toxicological Concern (TTC) and the Margin of Exposure (MOE). The linear extrapolation approach involves modelling of dose-response data from laboratory animal bioassay tumour data to exposure levels consistent with human exposures. The recommended POD is the BMDL that is representative of the lower end of the observed range of tumour incidences (SCHER/SCCP/SCENIHR, 2009). The TTC approach is a screening and prioritization tool for the safety assessment of chemicals that can be used in the absence of chemical-specific toxicity data and if human exposure can be estimated. Toxicological data from other chemicals sharing structural similarities are used in order to establish levels of human exposure that would not represent safety concern for human health (EFSA/WHO, 2016). While the traditionally used principle ALARA (As Low As Reasonably Achievable) does not provide quantitative comparison between genotoxic chemicals and thus cannot be used to compare risks from different substances, the MOE allows to inform the risk managers about the magnitude of risks from genotoxic and carcinogenic substances. The MOE is defined as the ratio between a defined point of the dose-response curve and the human exposure. It has been recommended that the POD is determined with the BMD approach, using the BMDL calculated from the Benchmark response (BMR) of 10% (BMDL10) (EFSA, 2005).

The usual way to derive health-based guidance values is to divide the determined POD by uncertainty factors (UFs) which are meant to describe interspecies and interindividual differences. In cases where exposures exceed health-based guidance values, the risk characterisation does not provide risk managers with advice on the possible extent of the risk for people exposed to these higher levels. A first consideration should be that health-based guidance values themselves incorporate UFs (IPCS, 2009).

1.2. Uncertainty factors in chemical risk assessment

A 100-fold UF has been introduced 60 years ago by Lehman and Fitzhugh (1954) to account for interspecies and interindividual differences to determine health based guidance in humans from animal studies. This value is the product of two factor 10 that allows interspecies differences and human variability (IPCS, 1987). However, these default 10-fold factors do not consider metabolic data or mechanistic background quantitatively in risk assessment. The interspecies and interindividual UFs were therefore subdivided into kinetics and dynamics aspects (Renwick, 1993). Then values of $10^{0.6}$ (4.0) and $10^{0.4}$ (2.5) were proposed for interspecies differences in kinetic and dynamic. For human variability in both kinetics and dynamics the default factor of 10 is divided into $10^{0.5}$ (3.16) (IPCS, 1994). It has been demonstrated that when assessing human variability using therapeutic drugs that underwent a range of metabolic pathways, kinetic and dynamic default UFs would not cover human variability specifically for polymorphic pathways or specific populations like neonates (Renwick and Lazarus, 1998). These default kinetic and dynamic UFs can be refined by using chemical specific adjustment factors (CSAFs) (IPCS, 2005) or metabolic pathway-related UFs (Dorne, 2010; Dorne et al., 2005).

Metabolic pathway-related UFs can be applied when the metabolic fate of a chemical is known in humans, which can be assessed from *in vitro* experiments using subcellular fractions (e.g. isolated recombinant human enzymes or human liver microsomes or cytosol). The development of metabolic pathway-related UFs requires the quantification of inter-individual differences in kinetics. Parameters reflecting acute exposure (C_{max}) and chronic exposure (AUC, clearance) were used from human pharmacokinetic studies (Figure 3). Pathway-related UFs have been estimated since the end of the 90s, they are derived from analyses of pharmacokinetic data for probe substrates of phase I and phase II enzymes and renal excretion (Dorne et al., 2001a; Dorne et al., 2001b; Dorne et al., 2003a; Dorne et al., 2003b; Dorne et al., 2004a; Dorne et al., 2005; Dorne et al., 2002; Walton et al., 2001a; Walton et al., 2001b). They are considered as an intermediate option between the use of default UFs and CSAF (Figure 4).

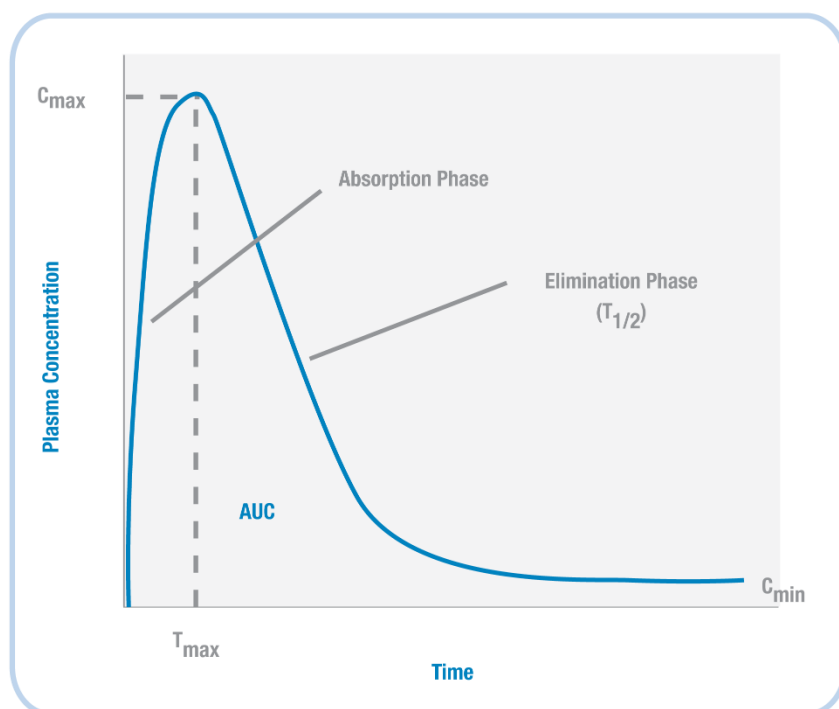


Figure 3. Pharmacokinetic parameters (C_{max}: maximum serum concentration; AUC: area under the curve; clearance = AUC/dose)

When chemical-specific data are available, a CSAF can be derived to replace the relevant default subfactor, either in kinetics and/or dynamics. Relevant kinetic data could be derived from *in vivo* studies that defined the kinetics of the chemical under the experimental conditions in animals and in humans at the anticipated human exposure dose or concentration. *In vitro* measurements of critical processes (e.g., enzyme activity) can be used to estimate interspecies differences, especially when incorporated into a physiological based kinetic (PBK) model. CSAFs to allow for inter-individual differences in dynamics have been derived from *in vitro* studies, *in vivo* studies or from *ex vivo* experimentation in which the kinetics components have been excluded (IPCS, 2005; Renwick and Lazarus, 1998).

Kinetic	Dynamic	
CSAF <i>or</i> Species + pathway-related UFs <i>or</i> Default factor 10 ^{0.6}	CSAF <i>or</i> Species + process-related UFs <i>or</i> Default factor 10 ^{0.4}	Interspecies
CSAF <i>or</i> Pathway-related UFs <i>or</i> Default factor 10 ^{0.5}	CSAF <i>or</i> Process-related UFs <i>or</i> Default factor 10 ^{0.5}	Interindividual

Figure 4. Uncertainty factors: default values, pathway-related uncertainty factors and chemical-specific adjustment factors (based on Renwick and Lazarus (1998))

1.3. Physiologically based kinetic models

PBK models are mathematical descriptions simulating the kinetics of chemicals in the body in relation to key physiological parameters (e.g. tissue blood flows and volumes), biochemical and physiochemical parameters (e.g. excretions rates and tissue/blood partition coefficients) (IPCS, 2010). These models are based on compartmental approaches, describing the body as compartments corresponding to realistic organs or tissues that reflects the determinants of the kinetics of the chemical to simulate concentration time-curves in blood or specific tissue (Bois et al., 2010; Clewell et al., 2008; Paini et al., 2019). They are traditionally used in order to perform extrapolations from route to route of exposure (e.g. intravenous to oral exposure), between different species or between sub-groups (e.g. healthy adults to patients or children). PBK models can also be used in a reverse way to estimate the exposure of a population to chemicals in comparison to biomonitoring data (Caldwell et al., 2012; Verner et al., 2009).

PBK models may present different degrees of complexity, considering the number of organs or tissues and whether they are described as homogeneously well-mixed (perfusion-limited) compartments, the tissue barrier presents no barrier to distribution, or diffusion-limited compartments, a permeability coefficient is applied. However, it is generally recognised by risk assessors that the simplest model possible is preferred while complex models would be used when necessary and sufficient input data are available (Bois et al., 2010; Paini et al., 2019).

Human PBK models have been applied to address drug development, drug-drug interactions (or drug-food, drug-herbal product) and safety assessment of food, cosmetics and environmental contaminants (Madden et al., 2019). These applications of PBK models in food safety have been reviewed by the European Food Safety Authority (EFSA, 2014). In its notes of guidance for the testing of cosmetic ingredients and their safety evaluation, the Scientific Committee on Consumer Safety recognise the use of PBK models for quantitative risk assessment (SCCS, 2018). Recently, PBK models have also been used to assess the safety of nanomaterials (Lamon et al., 2019).

1.4. Hierarchical Bayesian models for the meta-analysis of kinetic data

Previous meta-analyses on human variability in kinetics for different metabolic pathways were based on weighted geometric means assuming fixed effect models with inverse variance weights. This approach allows to derive human variability in kinetic parameters, but it did not address the relative contribution of the variability across subgroups to the overall variability in the datasets, leading to uncertainty in the parameter estimates (Dorne et al., 2005).

Recently, meta-analysis methods have been developed using Bayesian approaches in the health-care and risk assessment areas and allow for the quantification of variability and uncertainty (Rigaux et al., 2013; Sutton and Higgins, 2008). In a bayesian context, a prior distribution is set either based on expert knowledge or using evidence from the literature. These distributions are then updated taking into account available new data, leading to a posterior distribution (Figure 5) (Micallef et al., 2005). Bayesian estimation provides a distribution of credibility of the parameter values and a representation of parameter uncertainty that can be directly interpreted through the posterior distribution. Posterior distributions are estimated by generating a huge random sample of representative parameter values from the prior distribution using Markov chain Monte Carlo (MCMC) method. Consequently, it describes how uncertainty change when taking account new data (Kruschke and Vanpaemel, 2015). The Bayesian approach is ideally suited for multi-level models. Hierarchical models are used when the probability of a parameter is dependent on the value of another parameter leading a chain of dependencies among parameters (Kruschke and Vanpaemel, 2015). These models allow 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 and are useful to quantify the variability among different populations (Shao et al., 2017). In the case of kinetic parameters, intra-substrate variability is dependant of the estimated inter-study variability, which supposed two levels, with prior information applied to the “substrate” level.

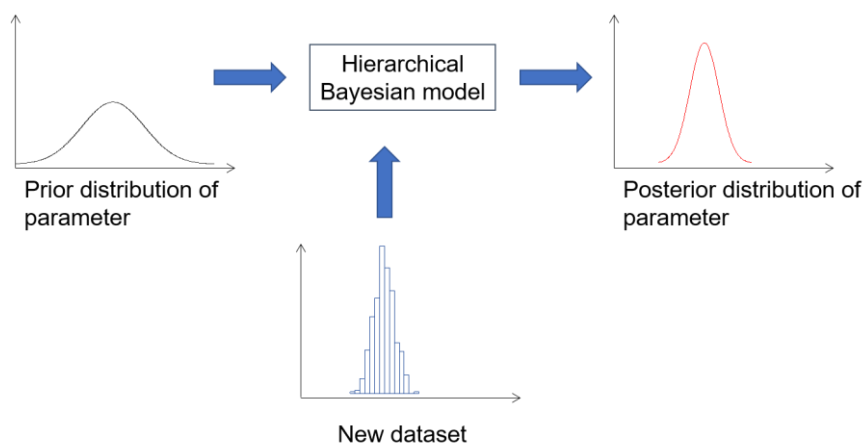


Figure 5. Principle of Bayesian statistics

In that respect, human variability in kinetic parameters of acute and chronic exposure can be derived from meta-analysis of human pharmacokinetic studies using a hierarchical Bayesian approach. Therefore, including multiple compounds that are specific of the same pathway in a hierarchical Bayesian model would then allow to refine the pathway-related UF.

1.5. Enzymes and transporters involved in ADME processes

ADME processes describe the disposition of a chemical within the body and include inter-related processes namely absorption, distribution, metabolism and excretion (Figure 6). The toxicity of a chemical is dependent on its mode of action which includes kinetics ADME processes and dynamic processes (Meek et al., 2014). Chemicals can enter the human body via oral route, dermal contact or inhalation, etc. After absorption, the chemical enters the blood stream, where it may be distributed towards organs, including the target organ or tissue where it produces damage (Lehman-McKeeman, 2008).

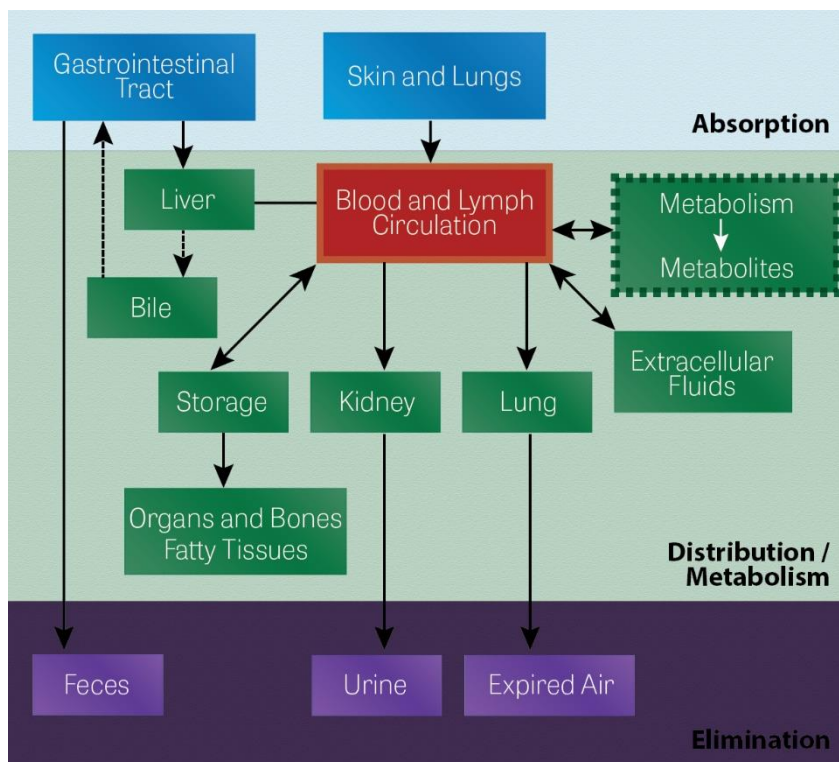


Figure 6. Absorption, Distribution, Metabolism and Elimination (Image Source: National Library of Medicine)

Xenobiotic metabolism or biotransformation is a series of enzymatic processes that transforms parent compounds into metabolites that are more hydrophilic that are easier to excrete through urinary or bile elimination. In many cases, the toxicity of a xenobiotic can be either mediated by the parent compound so that metabolism result in detoxification or through its reactive metabolites and metabolism results in bioactivation (e.g bioactivation of acrylamide to glycidamide by CYP2E1). While the intestine and the liver contain the highest enzyme concentrations, they are also widely distributed in other tissues such as kidneys which express several enzymes that actively eliminate xenobiotics into urine. Xenobiotic metabolising enzymes are classified as phase I and phase II enzymes according to their function such as hydrolysis, reduction, oxidation and conjugation respectively (Parkinson and Ogilvie, 2008). Transporters of xenobiotics are involved either in uptake or efflux processes and are consequently classified as phase 0 or phase III respectively (Doring and Petzinger, 2014).

Phase I metabolism

Phase I enzymes metabolise xenobiotics to make them more water soluble, either by hydrolysis, reduction or oxidation. Cytochromes P450 (CYPs) represents the major enzyme family oxidising 70-80% of pharmaceutical drugs in phase I drug metabolism. They are highly expressed in the liver, located into microsomes, but are also present in extra-hepatic tissues like small-intestine (Gundert-Remy et

al., 2014; Zanger and Schwab, 2013). In general, xenobiotic metabolism involves multiple CYPs but can also be isoform-specific in the case of specific probe substrates. 57 CYPs have been identified in humans but only the vast majority of xenobiotic metabolism is performed by a dozen isoforms including CYP3A4, CYP1A2, CYP2E1, CYP2D6, CYP2C9 and CYP2C19. CYP enzymes illustrate very well the importance to determine the balance between activation and detoxification. In humans, hydrolysis of coumarin by CYP2A6 into 7-hydroxycoumarin in the liver is the main detoxifying pathway (Lewis et al., 2006; Scientific Opinion of the Panel on Food Additives, 2008). On the other hand, CYPs in the liver and extrahepatic tissues play important roles in the activation of xenobiotics to toxic metabolites, such as organophosphate compounds which are metabolised by CYPs into their active form (Buratti et al., 2011). It has been estimated that CYP3A4 and CYP2D6 metabolise around 50% of pharmaceuticals (Zanger and Schwab, 2013). While CYP3A4 is the major CYP in the liver and the intestine (CYP3A4/5: 82% of small-intestine CYPs content), CYP2D6 represent only 2% and 1% of human CYPs in the liver and small-intestine respectively (Gundert-Remy et al., 2014) (Figure 7). The occurrence and frequency of polymorphic variation varies between populations from different ancestry origins and has been shown to affect drug response, genetic polymorphism is thus an important factor of variability. For example, the CYP2D6 poor metaboliser (PM) genotype is common in Caucasians but not in Asian populations, leading to differences in internal exposure of such polymorphic enzymes probe substrates (Dorne et al., 2002). This can also have an impact on the development of diseases when considering polymorphism of CYP2A6, which metabolise nicotine and cotinine, impacting internal dose of nicotine and therefore smoking habits (Lopez-Flores et al., 2017; Raunio et al., 2001). Environmental factors can also introduce variability in CYPs metabolism, as for instance CYP3A4 activity which can be inhibited by food products such as grape fruit juice or on contrary be activated by herbal agents like St. John's wort (Quignot et al., 2019).

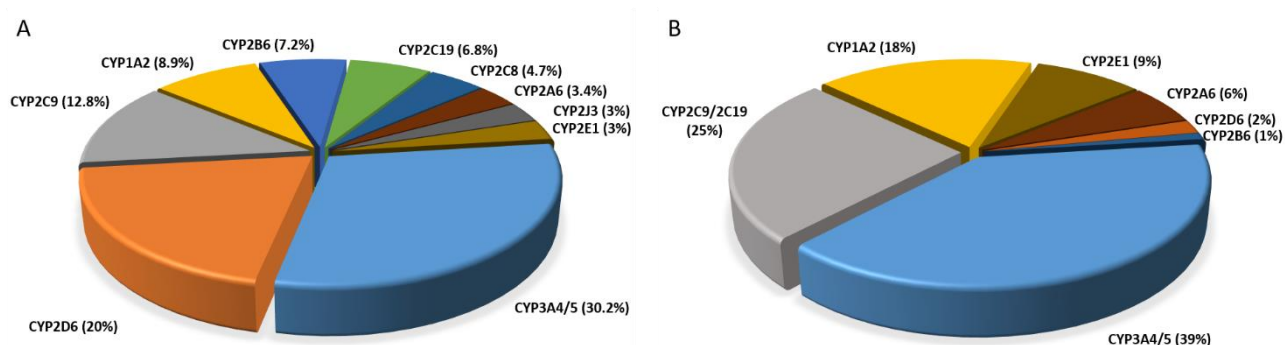


Figure 7. A: Fraction of clinically used drugs metabolized by cytochrome P450 isoforms (adapted from Zanger and Schwab (2013)) and B: distribution of the major cytochrome P450 isoforms in the human liver (adapted from Gundert-Remy et al. (2014))

One of the major hydrolytic enzyme family is the family of paraoxonases. Human paraoxonase 1 (PON1) hydrolyses a range of organophosphate compounds (OPs) but also aromatic carboxylic acid esters or nerve gas agents such as sarin and soman (Furlong et al., 2016a). It is a high-density lipoprotein associated serum enzyme. PON1 activity is influenced by its genetic polymorphism with the most common polymorphisms being in the coding region and one in the promotor region, namely L55M, Q192R and C-108T. Polymorphism in PON1 represent a major source of variability in its activity leading to differences in internal doses of toxic metabolites from OPs exposure. Regarding this point, people considered as poor metabolisers would be more susceptible to OP damage (Dardiotis et al., 2019).

Phase II metabolism

Phase II metabolism refers to conjugation reactions which include glucuronidation, sulfonation, acetylation, methylation, conjugation with glutathione and conjugation with amino acids. Glucuronidation is a major pathway of xenobiotic biotransformation in mammalian species and it is catalysed by uridine-diphosphate (UDP) glucuronosyltransferase (UGT) isoforms (Tukey and Strassburg, 2000). UGTs are a superfamily of membrane bound enzymes that catalyse the conjugation of glucuronic acid to a nucleophilic substrate, which are classified in three subfamilies: UGT1A, 2A and 2B. As for CYPs, they are predominantly expressed in the liver but some UGTs are highly expressed in kidneys (UGT1A6, 1A9, 2B7) or in the small-intestine (UGT1A1, 1A5, 1A6, 1A10, 2B7, 2B17) suggesting extra-hepatic glucuronidation (Figure 8). Although glucuronidation is recognised as a detoxification mechanism, it can result in bioactivation, such as an increase analgesic activity of morphine after glucuronidation by its metabolite morphine-6-glucuronide (Court et al., 2012; Fisher et al., 2001; Lv et al., 2019).

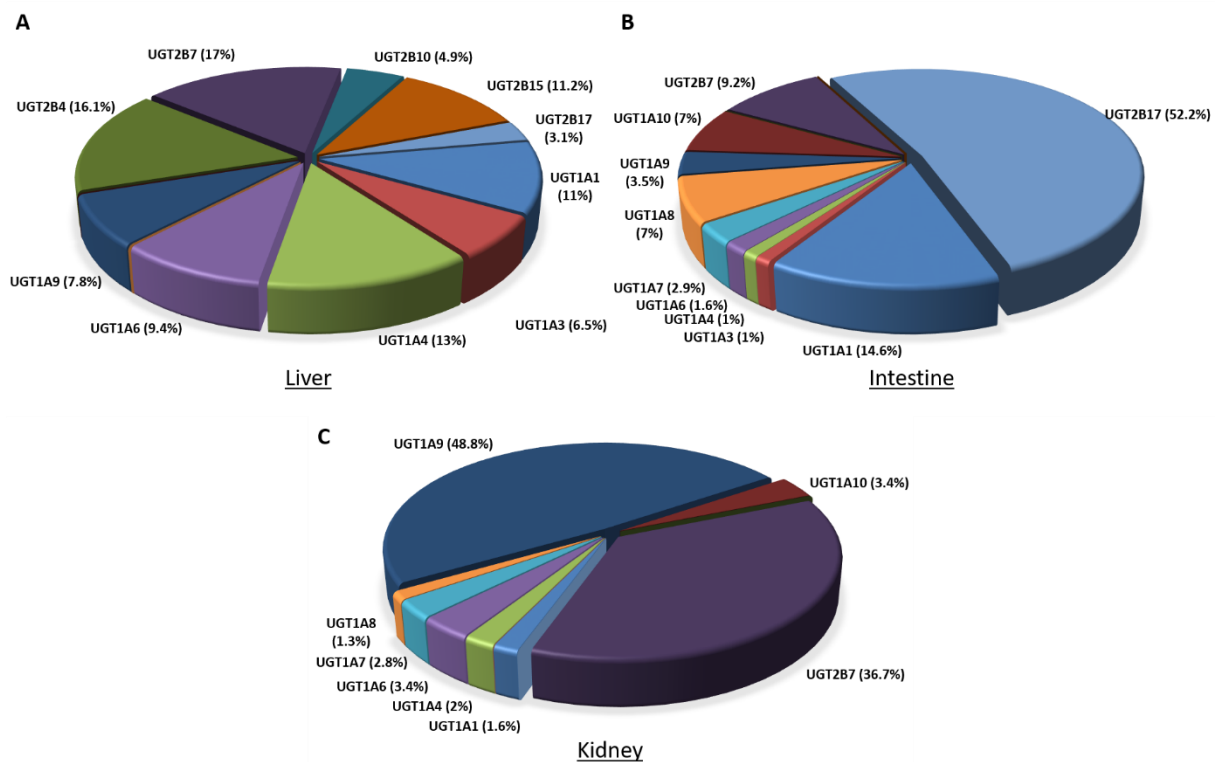


Figure 8. Distribution of the major UDP-glucuronosyltransferase isoforms in human liver (A), intestine (B) and kidney (C) (Lv et al., 2019)

Transporters

Transporters mediate the influx (phase 0) or efflux (phase 3) of xenobiotics. Transport of xenobiotic involve two superfamily of transporters. ATP-binding cassette (ABC) transporters are mainly efflux transporters that plays an important role in excretion of a wide range of xenobiotic into the bile or urines (leiri, 2012). P-glycoprotein (P-gp) is the most studied ABC transporter, it is encoded by the ABCB1 gene, also called multidrug resistance protein (MDR1). It plays an important role in the excretion of xenobiotics and endogenous substrates via the canalicular membrane of hepatocytes into bile, the apical side of enterocytes and proximal tubules into the gut lumen and urines respectively (Kim, 2002a). Another ABC transporter is the bile salt export pump (BSEP) which is involved in efflux transport of endogenous substrates via the canalicular membrane of hepatocyte (Chedik et al., 2018). Breast cancer protein (BCRP), encode by the ABCG2 gene, is highly expressed in various tissues such as intestine, liver and kidney and share the same localisation as P-gp. BCRP and P-gp both plays a protective role in the blood brain barrier (leiri, 2012). Another subfamily of ABC transporter is the multiresistant drug protein (MRP) with MRP1 the first identified MRP from drug resistant-cancer cells (nine identified MRPs in human) (Chedik et al., 2018). These are also efflux pumps and MRP2 and MRP3 are particularly important in the efflux of conjugated xenobiotic metabolites (Lehman-McKeeman,

2008). ABC transporters are therefore involved in reducing the intracellular accumulation of xenobiotics and thus in reducing their toxicity (Guéniche et al., 2019).

The second superfamily of transporters is the solute carrier (SLC), they mediate either facilitated diffusion or active transport and may act as bidirectional transports. The main SLC transporters handles organic cations (OCT), with OCT1 being expressed in hepatocytes and OCT2 on the basal side of kidney proximal tubules and both involved in the uptake of xenobiotics. The organic anion transporters (OAT) are particularly important in the renal uptake. The organic anion transporting peptides (OATP) mediates the sodium-independent transport of xenobiotics and are mostly involved in the hepatic uptake by OATP 1B1 and OATP1B3 while OATP2B1 is expressed in enterocytes (Clerbaux et al., 2019; Kim, 2002b). The sinusoidal sodium-taurocholate cotransporting polypeptide (NTCP) are also key in sodium-dependant uptake of bile acids in hepatocytes (Kim, 2002b). Contrary to most of SLCs transports, multidrug and toxin extrusion (MATE) proteins (MATE1 and MATE2-K) acts as efflux pumps at the apical site of hepatocytes and kidney proximal tubular cells where they will act similarly to P-gp and BCRP (Chedik et al., 2018; Guéniche et al., 2019). Figure 9 present the implication human transporters in absorption and eliminations processes.

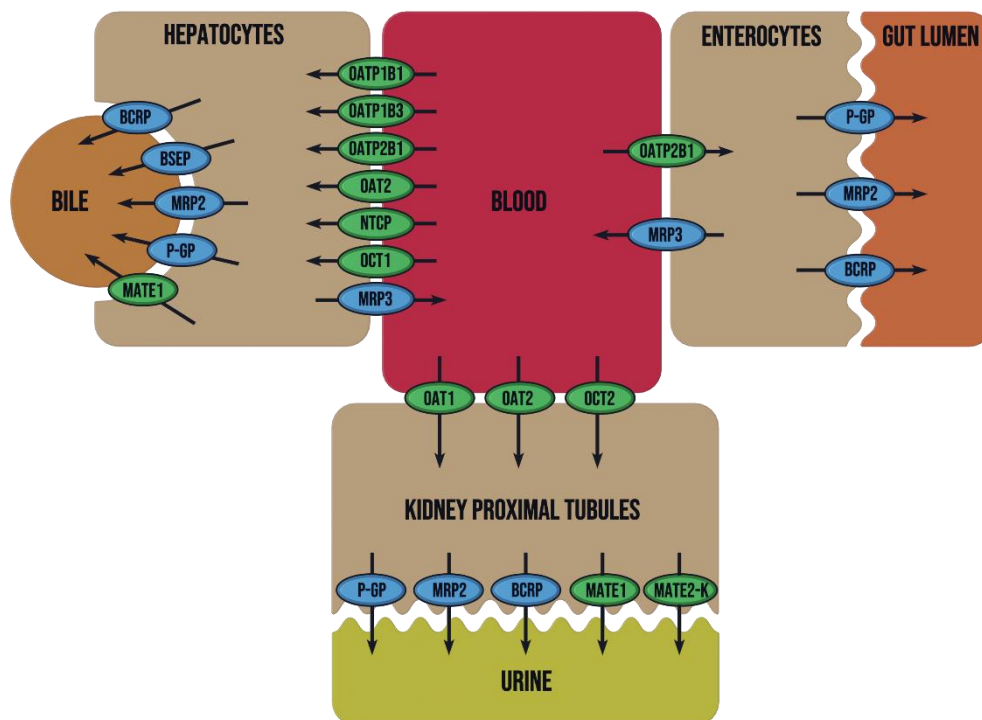


Figure 9. Membrane transporters in the human liver, kidney and intestine (green: SLC transporters, blue, ABC transporters)

1.6. Needs for research and implementation of kinetic models in risk assessment

Human kinetic data provides a rich data source to integrate quantitative ADME data in hazard assessment particularly with regards to interindividual differences in phase I and phase II enzymes as well as transporters (EFSA, 2014). The use of human kinetic data to simulate plasma and tissue concentrations of chemicals has progressed mostly in the field of pharmaceuticals and still relatively limited in the food safety area (Punt, 2018). However, kinetic data is of considerable relevance and importance in other regulatory fields with the ban of animal testing for the safety assessment of cosmetic products (Regulation (EC) No 1223/2009) and the Commission Regulation (EU) No 283/2013 (2013) requires kinetic data for active substances of plant protection products and their metabolites.

Improvement of risk assessment methods includes new approaches methodologies (NAMs) including the refinement of UFs to determine safe level of exposure as well as *in silico* models incorporating kinetics, such as physiologically based kinetic (PBK) models and represent a challenge to regulatory agencies since they are complex to implement and require specialised training (IPCS, 2010; Paini et al., 2019). In order to move towards the integration of NAMs in chemical risk assessment for the food and feed safety area with regards to human health, animal health and environment, several projects have been launched by the European Food Safety Authority (EFSA). These projects involve the integration of kinetic data and the development of modelling tools, the modelling of population dynamics of aquatic and terrestrial organisms, and the modelling of human variability in kinetic and dynamic processes with physiologically based models. PBK models provide a quantitative approach to address ADME processes and are therefore very useful tools in hazard assessment (EFSA, 2014). Models are needed to enable *in vitro* data on toxicological effects to be transformed into *in vivo* data which is a necessary step to make them usable for risk assessment. Therefore, quantitative predictions of *in vivo* kinetics from *in vitro* assays (QIVIVE) using human cells offer great opportunities to reduce uncertainty in human risk assessments and will facilitate the future development and regulatory acceptance of alternatives to animal testing with respect to the 3Rs (Replacement, Reduction and Refinement of animal studies) (Bessemers et al., 2015; OECD, 2010; Paini et al., 2017; Punt et al., 2017). However, *in vitro* assays usually provide mean values of kinetic parameters when extrapolated to *in vivo* parameters. Pathway-related UFs can be applied when human *in vitro* metabolism data are available for specific isoforms but no *in vivo* data, to implement variability on metabolism data to address the human population rather than a single individual. Previously published meta-analyses were based on weighted averages assuming fixed effect models with inverse variance weights and did not address the relative contribution of the variability across subgroups to the overall variability in the datasets.

Bayesian models allow to characterise variability and uncertainty in a robust way by partitioning observed variance between sampling variation, heterogeneity across studies, and other sources of variability, e.g. between subgroups of population. This approach leads to produce refined estimates across compounds and provides means to extend the inference to un-observed compounds and the integration of a range of human subgroups into simulated whole populations. Consequently, a generic model able to integrate such estimates across studies, substances and subgroups of human populations, while accounting for sample variation, is much needed in the area of chemical risk assessment. This data-driven approach is particularly relevant to investigate the impact of genetic polymorphisms on metabolism and kinetics which may illustrate large differences across subgroups of the population and consequently large UFs (Dorne et al., 2003b; Dorne et al., 2002; Gaedigk et al., 2017).

1.7. Scope and Aim of this thesis

This thesis aims to:

1. Quantify human variability by means of Bayesian meta-analysis for a range of phase I, phase II metabolic pathways and transporters (phase 0 and III) using pharmacokinetic markers of acute (Cmax) and chronic exposure (AUC, clearance) or enzyme activity data from available probe substrates.
2. Derive pathway-related variability distributions and pathway-related UFs for their future integration in PBK models for human risk assessment of chemicals (Figure 10).

The proposed methodology uses a multi-level hierarchical Bayesian model to integrate quantifiable sources of variability, including inter-study, inter- and intra-ethnic, inter-sensitive populations and/or inter-phenotypic variability. In this context, pathway-related variability and corresponding pathway-related UFs are derived for subgroups of the human population and pharmacokinetic parameter.

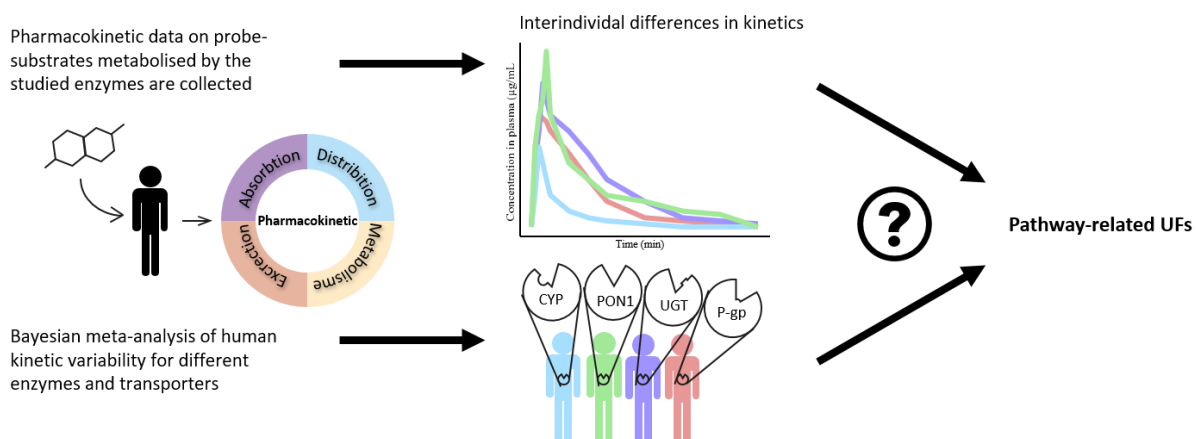


Figure 10. Graphical abstract of the aim of the thesis

Chapter 2 illustrates the implementation of PBK models in risk assessment and especially in exposure assessment by characterising aggregate exposure of the French adult population to permethrin from environmental and dietary sources.

Chapter 3 describes the meta-analysis of human pharmacokinetic data for CYP3A4 metabolism using 15 CYP3A4 probe substrates and the derivation of CYP3A4-related variability distributions and CYP3A4-related UFs with regards to a range of populations of different geographical ancestry and other specific subpopulations (i.e. neonates, infants and the elderly).

Chapter 4 focusses on another phase I enzyme, the polymorphic human serum paraoxonase 1 (PON1). In contrast to chapter 3, data collection and meta-analyses were performed on enzyme activity data resulting from human *ex vivo* measurements in blood instead of pharmacokinetic parameters. Three probe substrates have been included, each of which relate to a different binding site of the PON1 enzyme. Inter-phenotypic differences and related UFs were estimated and the incorporation of genotypic frequencies for the derivation of population PON1-related UFs are presented.

In Chapter 5, human pharmacokinetic data for the seven clinically most relevant UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7 and UGT2B15) were collected from an extensive literature search. Inter-individual differences in kinetics for these specific UGTs probe substrates were assessed using Bayesian meta-analysis to derive UGT-related variability distributions and UGT-related UFs in healthy adults.

Chapter 6 investigates human variability in the kinetics of transporter probe substrates to quantify human variability for P-gp, BCRP, MATEs, OAT1 and 3, OCTs and OATPs.

Finally, Chapter 7 provides perspectives for the implementation of pathway-related UFs in generic quantitative *in vitro-in vivo* extrapolation models and generic PBK models in chemical risk assessment. Recommendations for future work to support their implementation in the human risk assessment of chemicals and move towards the reduction of animal testing conclude.

2. Aggregate exposure of the adult French population to pyrethroids

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Toxicology and Applied Pharmacology, 351: 21-31 (2018).

Abstract

The French Nutrition and Health Survey (ENNS) reported higher biomarker levels of exposure to pyrethroids than those observed in North American and German biomonitoring studies. The authors therefore investigated aggregate exposure to permethrin as an initial case study because this compound is one of the most widely-used pyrethroid insecticides. We assessed several contamination sources—such as indoor and outdoor air, settled dust and diet—and several pathways, including oral, inhalation and dermal routes. We used permethrin exposure level estimations (computed from ENNS data) and a PBPK model calibrated with human kinetic data (from 6 individuals) to simulate an internal dose of cis- and trans-3-(2,2 dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid (cis- or trans-DCCA) in a population of 219 individuals. The urinary concentrations of cis- and trans-DCCA predicted by the PBPK model according to three permethrin exposure scenarios (“lower”, “intermediate”, and “upper”), were compared to the urinary levels measured in the ENNS study. The ENNS levels were between the levels simulated according to permethrin exposure scenarios “lower” and “intermediate”. The “upper” scenario led to an overestimation of the predicted urinary concentration levels of cis- and trans-DCCA compared to those measured in the ENNS study. The most realistic scenario was the “lower” one (permethrin concentration of left-censored data considered as 0). Using PBPK modelling, we estimated the contribution of each pathway and source to the internal dose. The main route of permethrin exposure was oral (98%), diet being the major source (87%) followed by dust (11%) then the dermal route (1.5%) and finally inhalation (0.5%).

Keywords: aggregate exposure, permethrin, PBK model, pyrethroid, diet, air and dust, DCCA

Highlights

- Assessment of the adult French population’s aggregate exposure to permethrin
- Adjustment of a PBK model of permethrin to predict urinary concentrations of DCCA
- Estimation of the contributions of each source and pathway to permethrin exposure

2.1. Introduction

Permethrin (3-phenoxybenzyl (1RS,3RS;1RS,3SR)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) is a synthetic pyrethroid insecticide. It is one of the most commonly used in France. In 2000, permethrin was not approved as an active substance for agricultural use by European regulations, but it is still approved for residential use (Regulation (EC) No 1107/2009, 2009; Regulation (EU) No 528/2012, 2012). Permethrin is widely applied through sprays or smoke bombs in households against insects such as flies or mosquitoes. Permethrin can therefore be present in various environments, including air and dust, but is rarely detected in food. Environmental exposure to permethrin of the adult French population was already assessed in a previous study (Hermant et al., 2018) using the data available from the French National Nutrition and Health Survey (ENNS) (Fréry et al., 2013). This chronic environmental exposure was evaluated considering various environmental sources (i.e. outdoor air, indoor air, and house dust) and exposure pathways (i.e. inhalation, indirect ingestion, and dermal contact). The results of this study suggested that house dust was the main environmental source of exposure, and dermal contact and indirect ingestion the major exposure pathways.

The toxicity of permethrin and its metabolites involves interactions with sodium channels, receptor-ionophore complexes and neurotransmitters. It has been suggested that oxidative stress might also be one of the toxicological mechanisms of permethrin (Wang et al., 2016). Like other Type I pyrethroids (which do not contain a cyano substituent on the α -methylene of the alcohol moiety), permethrin has elicited neurotoxic behaviour among laboratory animals referred to as T-syndrome: aggressiveness, hyperexcitability, fine tremor, prostration, increased body temperature, coma and death. This is considered to be an acute response to permethrin exposure and is dose-dependent. After dermal exposure, adverse effects include paraesthesia, a tingling to burning sensation of the skin (U.S. EPA, 2007).

In humans, permethrin is metabolized in the gastrointestinal (GI) tract and the liver. It mainly involves hydrolysis by carboxylesterases and oxidation by cytochrome P450 (CYP450), multiple CYP450 isoforms being involved (Scollon et al., 2009). Permethrin is metabolized into 3-PBA (3-phenoxybenzoic acid) and cis- or trans-DCCA (cis- or trans-3-(2,2 dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid), specific to each isomer (Willemin et al., 2015).

Based on this knowledge, physiologically-based pharmacokinetic (PBPK) models have been developed to quantify the kinetic behavior of permethrin (Tornero-Velez et al., 2012; Wei et al., 2013). This kind of model can predict the time course of a chemical and its metabolite concentrations in biological tissues according to various exposure and pharmacokinetic scenarios. Several research groups have

demonstrated the utility of PBK models in conducting reverse dosimetry. The PBK model for permethrin consists of seven essential compartments representing the lungs, fat, skin, brain, liver, rapidly-perfused tissues, and slowly-perfused tissues. Model parameters were calibrated against experimental rat data, and scaled to human anatomical and physiological parameter values.

The model predictions were compared to data from a study among rats of oral exposure to permethrin. The rats were dosed orally with 1 or 10 mg/kg permethrin in corn oil (1 ml/kg) and sacrificed at 1, 2, 3, 4, 6, 8, 12, 24, 36 or 48 h (Tornero-Velez et al., 2012). The model was used to accurately predict metabolite cis- and trans- permethrin urine concentrations during exposure, and once the exposure was stopped. Because liver clearance parameterization in humans was based on data relating to human hepatic microsomes (Scollon et al., 2009) (QIVIVE extrapolation), we instead considered human data based on cryopreserved primary hepatocytes from three donors (Willemin et al., 2015) to better estimate the clearance of cis- and trans-permethrin.

This work suggests a method for characterizing aggregate exposure beginning with contaminated environmental sources such as air, dust, and food, and leading up to individual internal doses i.e. urinary concentration levels available from the human biomonitoring section of the ENNS study (Fréry et al., 2013). This method began with the aggregate exposure assessment of the adult French population and then with the definition of a refined human PBPK model for permethrin allowing us to bridge the gap between external exposure and internal dose.

2.2. Material and Methods

Our method relating external exposure to internal dose is summarized in Figure 11. The exposure calculations required for each individual (1) to obtain levels of contamination in each environment of interest (outdoor air, indoor air, settled dust, and food), (2) human parameters such as body weight (bw) or food consumption and (3) space-time budgets. This approach focused on French data in order to obtain exposure estimates as close as possible to the real exposure of the French population and individual data, in order to best describe variability in individual exposure. The individual exposure estimates were then used as input for the PBPK model that simulated the internal dose of each individual. These models established the link between exposure (parent substance concentration) and internal dose (metabolite concentration). They described the kinetics of chemical compounds fairly realistically in each compartment—corresponding to predefined organs or tissues—irrigated by blood flow. The simulated internal doses were then compared with the internal doses measured during the ENNS study.

2.2.1. Study population and biomonitoring data

The ENNS study was conducted from 2006-2007 by the French Institute for Public Health Surveillance (InVS) (Fréry et al., 2013; Fréry et al., 2011; Saoudi et al., 2014). This cross-sectional study was designed to be representative of the French general population and included three parts: a diet study, an interview (face-to-face and self-administered questionnaires), and a clinical and biological examination. French residents aged between 18 and 74 years old were interviewed. The data collected provided a description of anthropometric, demographic and socioeconomic characteristics, dietary intake, physical activities, pesticide uses, and biological samples. First morning urine samples were collected in order to determine the cis- and trans-DCCA concentration in a sub-sample of ENNS participants (396 adults). The limit of detection was 0.03 µg/L and the limit of quantification was 0.1 µg/L (Fréry et al., 2013). Urine samples of 219 individuals were quantified and described in Table 1.

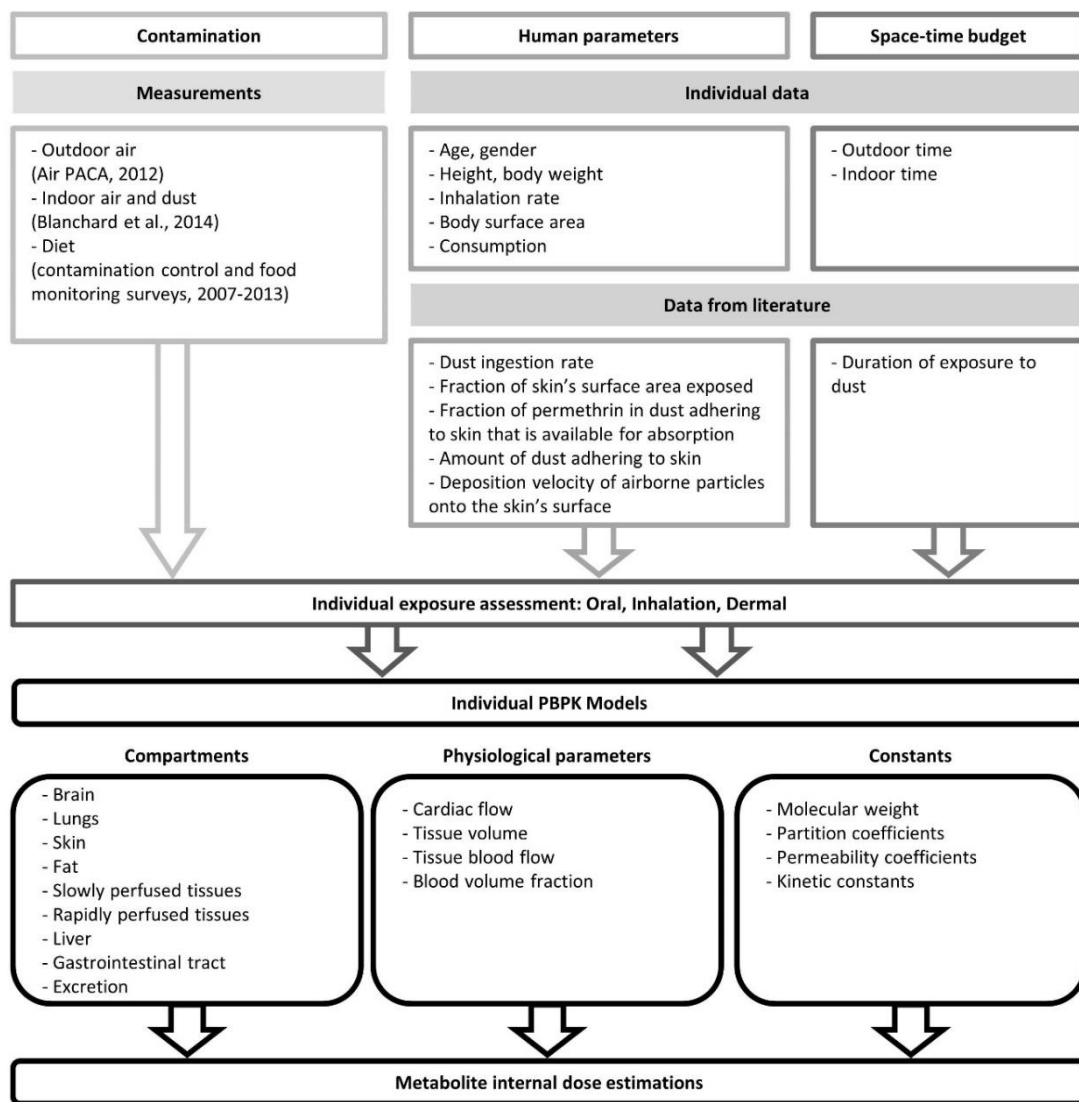


Figure 11. Diagram of the method

Table 1. Distribution of cis- and trans-DCCA urinary concentration levels in a sub-sample of ENNS participants (n = 219 adults)

	GM	95% CI	Min	P25	P50	P75	P95	Max
<i>cis-DCCA urinary concentration (μmol/g creatinine)</i>								
Total (n=219)	0.28	[0.24-0.32]	0.05	0.14	0.23	0.49	1.87	17.9
Male (n=74)	0.23	[0.19-0.28]	0.05	0.13	0.18	0.46	1.05	2.18
Female (n=145)	0.31	[0.26-0.37]	0.05	0.16	0.24	0.50	2.34	17.9
<i>trans-DCCA urinary concentration (μmol/g creatinine)</i>								
Total (n=219)	0.68	[0.59-0.79]	0.09	0.32	0.53	1.30	5.77	66.0
Male (n=74)	0.57	[0.46-0.72]	0.09	0.26	0.48	1.17	5.56	5.77
Female (n=145)	0.74	[0.61-0.90]	0.09	0.35	0.57	1.32	6.30	66.0

GM: geometric mean; CI: confidence interval; P: percentile

2.2.2. Permethrin exposure data

In this work, exposure is defined as the amount of permethrin received by an organism up to its biological barriers (respiratory epithelium, digestive mucosa or dermis) without crossing them, related to the individual's weight and duration of daily exposure (InVs, 2005).

2.2.2.1. Environmental exposure data

Environmental exposure of the adult French population to permethrin has already been assessed (Hermant et al., 2018). Inhalation, indirect dust ingestion and dermal exposure were calculated as chronic daily exposure. Table 2 presents the environmental exposure distribution according to the source of contamination and the gender of the sub-sample of 219 individuals for whom the concentration levels of urinary metabolites were quantified.

Table 2. Environmental exposure distribution (μg/kg bw/day) in an adult subgroup of the ENNS participants (n = 219)

Exposure (μg/kg bw/d)	n	Min	P25	P50	P75	Max	Mean	95% CI
<i>Daily exposure by inhalation</i>								
Total	219	7.83e-09	1.76e-07	3.04e-07	1.22e-05	2.61e-04	1.63e-05	[1.09e-05-2.18e-05]
Men	74	8.40e-09	2.12e-07	6.19e-07	1.58e-05	1.5e-04	1.28e-05	[7.56e-06-1.81e-05]
Women	145	7.83e-09	1.71e-07	2.98e-07	1.13e-05	2.61e-04	1.81e-05	[1.03e-05-2.59e-05]
<i>Daily exposure by indirect dust ingestion</i>								
Total	219	4.31e-07	1.49e-04	2.79e-04	6.82e-04	2.07e-02	1.31e-03	[8.93e-04-1.72e-03]
Men	74	1.94e-06	1.54e-04	2.83e-04	7.85e-04	2.07e-02	1.58e-03	[7.22e-04-2.44e-03]
Women	145	4.31e-07	1.49e-04	2.72e-04	6.81e-04	1.98e-02	1.16e-03	[7.15e-04-1.61e-03]
<i>Daily exposure by dermal uptake (dust and air)</i>								
Dust	219	2.20e-06	8.13e-04	1.46e-03	3.63e-03	9.22e-02	6.83e-03	[4.77e-03-8.89e-03]
Men	74	1.27e-05	8.98e-04	1.49e-03	3.79e-03	8.24e-02	8.15e-03	[4.10e-03-1.22e-02]
Women	145	2.20e-06	8.00e-04	1.46e-03	3.45e-03	9.22e-02	6.15e-03	[3.79e-03-8.50e-03]
Airborne particles	219	3.43e-08	8.71e-07	1.52e-06	6.20e-05	1.31e-03	7.98e-05	[5.33e-05-1.06e-04]
Men	74	3.97e-08	9.59e-07	3.03e-06	8.45e-05	6.52e-04	6.29e-05	[3.81e-05-8.77e-05]
Women	145	3.43e-08	8.66e-07	1.43e-06	5.82e-05	1.31e-03	8.84e-05	[5.03e-05-1.27e-04]

P: percentile; CI: confidence interval

The main pathway of exposure to permethrin was by dermal uptake, with a mean dermal exposure by contact to dust of 6.83e-03 (95% CI, 4.77e-03-8.89e-03) μg/kg bw/d and a mean dermal exposure by contact to airborne particles of 7.98e-05 (95% CI, 5.33e-05-1.06e-04) μg/kg bw/d. The second main pathway of exposure to permethrin was by indirect ingestion of dust, with a mean exposure of 1.31e-

03 (95% CI, 8.93e-04-1.72e-03) µg/kg bw/d. The last pathway was by inhalation, with a mean exposure of 1.63e-05 (95% CI, 1.09e-05-2.18e-05) µg/kg bw/d.

2.2.2.2. Dietary exposure assessment

The dietary exposure assessment combined three kinds of data: individual food consumption data, permethrin contamination levels in raw agricultural commodities (RACs) and recipes to disaggregate food into RACs.

During the ENNS study, individual food consumption data were collected with three 24-hour recalls over one year to take account of seasonal effects. Survey respondents were asked about the amount of food consumed per occasion. Sometimes food could be a RAC such as a vegetable or a fruit, but sometimes it could be a composite dish like a pizza. In order to assess permethrin exposure, all food had to be disaggregated into RACs. For example, a pizza could be initially disaggregated into a cereal-based product, vegetables, meat, and cheese. The cereal-based product could then be further disaggregated into flour (wheat), water, olive oil, salt etc. In this way, nearly 900 food items were disaggregated into RACs.

The daily intake ($C_{k,i}$) of each RAC (g/d) was calculated for each individual i from the three 24-hour recalls, with $C_{s1,k,i}$ the daily intake of RAC k from the first 24-hour recall of the week (g/d), $C_{s2,k,i}$ the daily intake of RAC k from the second 24-hour recall of the week (g/d) and $C_{w,k,i}$ the daily intake of RAC k from the 24-hour recall for the weekend (g/d).

$$C_{k,i} = \left[\left(\frac{1}{2} \times (C_{s1,k,i} + C_{s2,k,i}) \times \frac{5}{7} \right) + \left(C_{w,k,i} \times \frac{2}{7} \right) \right] \quad (1)$$

The permethrin contamination data were obtained from the contamination control and food monitoring surveys carried out by the Ministry in charge of consumption (DGCCRF), the Ministry in charge of agriculture (DGAL) and the Ministry in charge of Health (DGS), from 2007 to 2013. We selected the contamination levels of RACs identified during the disaggregation of food into RACs in the previous step, described above. A total of 35,113 samples were used, grouped into 136 different RACs (Table 3). Only 0.11% of the samples were quantified and 0.34% of all the samples had a concentration level between the limit of detection (LOD) and the limit of quantification (LOQ).

Table 3. RAC contamination data

Source	Type of data	Number of RACs and food	Number of samples
DGCCRF 2010-2013	RAC of plant origin	126	17,252
DGAL 2010, 2012 et 2013	RAC of animal origin	10	1,151

In order to assess dietary exposure, the left-censored data, i. e. contamination values below the analytical limit (LOD or LOQ), were replaced according to three scenarios, two of which complied with World Health Organization recommendations (WHO, 2013). According to these guidelines, in cases where less than 50% of the samples are quantified, a lower bound (scenario “lower”) and an upper bound (scenario “upper”) should be set. In the “lower” scenario, the lower bound is calculated by setting all non-detects and non-quantifiables to zero. In the “upper” scenario, the upper bound is calculated by setting all non-detects to the limit of detection and all non-quantifiables to the limit of quantification. A third, “intermediate”, scenario was also defined, in which the left-censored value was replaced by the median of 1,000 values sampled between 0 and the LOD or LOQ (depending on the limit available for the sample) according to a uniform distribution. This number of samples was sufficient to take into account the sampling error. For some samples, the permethrin concentration was between the LOD and LOQ, in which case the left-censored data were replaced by the median of 1,000 values randomly selected between the LOD and LOQ according to a uniform distribution. All the scenarios considered differences between and any change in analytical methods i.e. the LOD or LOQ because the left-censored value was replaced for each sample separately to take into account the fact that the level of information is not always uniform. However, the contamination level was not measured in all RACs. Either the missing value was replaced by the mean contamination level for the food group to which the missing value belonged in accordance with Foodex 2 classification (EFSA, 2015) and the three scenarios or the missing value was replaced by 0 (scenario “lower”), by the maximum residue limit (MRL) divided by two (scenario “intermediate”) or by the MRL (scenario “upper”) when the RAC was a food group such as hops.

The total daily dietary exposure ($E_{dietary,i}$) of individual i (ng/kg bw/d) was calculated by combining the daily intake ($C_{k,i}$) of RAC k (g/d) with the permethrin concentration (Q_k) of RAC k (ng/g) and then dividing by the body weight (BW_i) of individual i (kg). Dietary exposure was estimated according to the three contamination level scenarios using the SAS software package, version 9.3.

$$E_{dietary,i} = \sum_{k=1}^n \frac{C_{k,i} \times Q_k}{BW_i} \quad (2)$$

Dietary exposure varied highly according the three scenarios (Table 4). The distribution values ranged from 6.58e-04 to 0.06 and from 6.73e-02 to 1.57 µg/kg bw/d for the “lower” and “upper” scenarios respectively. However, the maximum exposure estimation according to the “upper” scenario was below the admissible daily intake (ADI =50 µg/kg bw/d). The mean values for the adult French population’s dietary exposure to permethrin were 8.68e-03 (95% CI, 7.47e-03-9.89e-03), 0.42 (95% CI,

0.39-0.44) and 0.83 (95% CI, 0.77-0.88) $\mu\text{g}/\text{kg bw}/\text{d}$ respectively for the “lower” scenario, the “intermediate” scenario and the “upper” scenario.

Table 4. Distribution of dietary intake according to gender ($\mu\text{g}/\text{kg bw}/\text{d}$)

Exposure ($\mu\text{g}/\text{kg bw}/\text{d}$)	n	Min	P25	P50	P75	Max	Mean	95% CI
Scenario “lower”	219	6.58e-04	3.42e-03	5.72e-03	9.59e-03	6.73e-02	8.68e-03	[7.47e-03-9.89e-03]
Men	74	1.02e-03	3.34e-03	5.68e-03	9.26e-03	6.73e-02	8.91e-03	[6.57e-03-1.13e-02]
Women	145	6.58e-04	3.48e-03	5.72e-03	9.82e-03	5.73e-02	8.57e-03	[7.16e-03-9.97e-03]
Scenario “intermediate”	219	0.06	0.28	0.38	0.52	1.57	0.42	[0.39-0.44]
Men	74	0.13	0.28	0.39	0.51	1.57	0.42	[0.37-0.48]
Women	145	0.06	0.28	0.37	0.52	0.98	0.41	[0.38-0.44]
Scenario “upper”	219	0.12	0.56	0.75	1.02	3.13	0.83	[0.77-0.88]
Men	74	0.25	0.55	0.77	1.01	3.13	0.84	[0.73-0.95]
Women	145	0.12	0.57	0.73	1.02	1.96	0.82	[0.76-0.88]

2.2.3. Human aggregate PBPK model

2.2.3.1. Model development and structure

In order to confront the biomonitoring results of the ENNS study with the established exposure scenarios, we developed a human aggregate PBPK model which predicts the disposition of permethrin and urinary excretion of its metabolites after oral, dermal and inhalation exposure to the parent chemical. The structure of this model is based on that of pre-existing human permethrin PBPK models (Tornero-Velez et al., 2012; Wei et al., 2013). Tissue groups/compartments were included based on consideration of the route of exposure (lungs for inhalation, skin for dermal and gastrointestinal (GI) tract for oral route), metabolism (liver), storage (fat, due to permethrin’s lipophilic properties) and neurotoxicity (brain) (Sethi et al., 2014; Shafer et al., 2005), as well as body mass balance and future expansion of the model (excretion compartment such as kidney, or poorly- and richly-perfused tissue groups). The oral route, inhalation absorption and skin absorption are described as first-order processes. The individual compartments are connected by systemic circulation. The model has distinct arterial and venous blood compartments, while tissues are described as homogeneous well-mixed compartments and diffusion-limited compartments, as described in Mirfazaelian et al. (2006).

Since we were using estimated daily exposure data for each individual, most of the formulas used by Tornero-Velez et al. (2012) and Wei et al. (2013) were adapted. We calculated the concentration of permethrin in arterial blood using an adapted formula of Ramsey and Andersen (1984). Daily inhalation exposure was used for each individual and, as permethrin has low volatility, all inhaled permethrin was assumed to be fully absorbed in lung blood, with no permethrin exhaled (Wei et al., 2013).

The skin compartment was simplified with respect to the model of Tornero-Velez et al. (2012). We chose to describe dermal absorption by the equation proposed by Wei et al. (2013). Daily dermal exposure by contact to dust and to airborne particles was used to estimate dermal absorption rate.

As for inhalation and dermal exposure, we used the daily exposure value for oral exposure, which takes into account daily dietary exposure and daily indirect dust ingestion.

The model describes metabolism by two concurrent metabolic pathways in the GI tract and in the liver, i.e. oxidative metabolism via microsomal CYP450 (Michaelis-Menten equation). To estimate hepatic metabolism, we used parameters from an *in vitro* study on human cryo-preserved primary hepatocytes of permethrin isomers (Willemin et al., 2015). We were then able to describe the formation rate of permethrin metabolites.

Since 3-PBA is a metabolite common to most pyrethroids, whereas cis- and trans-DCCA are specific to permethrin, cypermethrin, and cyfluthrin (Fréry et al., 2013; Tornero-Velez et al., 2012), we studied only people whose cis- and trans-DCCA had been simultaneously quantified in urine samples.

We had the urinary concentration levels of 219 individuals (74 men and 145 women), a population varying widely in terms of anthropometric parameters. In order to accurately describe the fate of permethrin in each one of them, the tissue volume of all the described compartments was estimated according to age, gender and body mass index (BMI). We thus simulated 219 individual urinary excretions of cis- and trans-DCCA. Cis- and trans-DCCA were described by a one-compartment PK model (Figure 12 **Erreur ! Source du renvoi introuvable.**).

Finally, the model was calibrated with human data. Healthy male and female volunteers were given permethrin, then their urinary excretion of permethrin metabolites was measured over time. The individual data used for calibration were provided by the author (Ratelle et al., 2015).

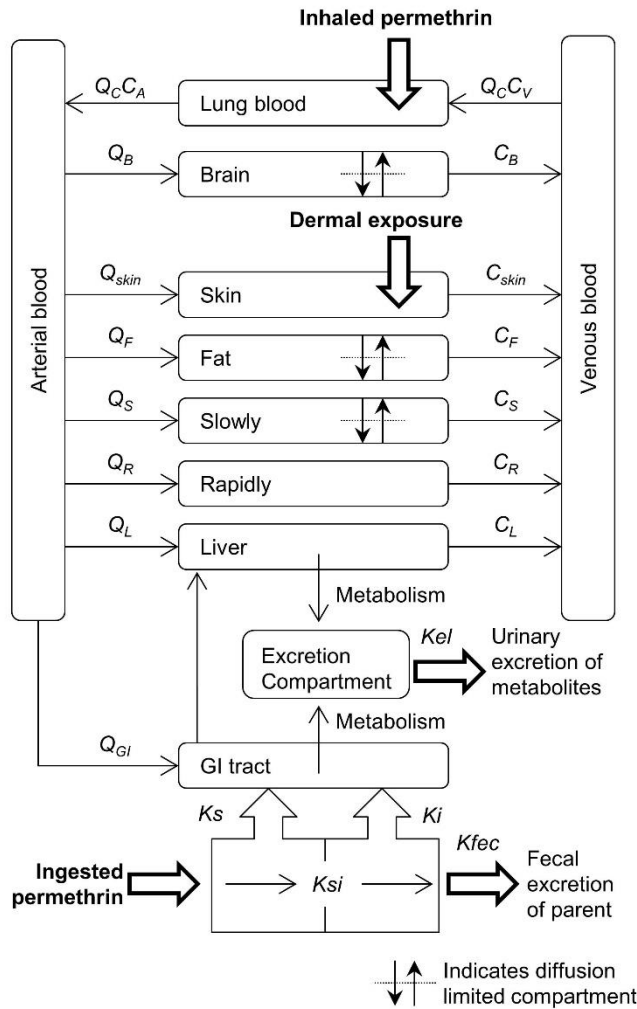


Figure 12. Human aggregate PBPK model of permethrin

Q_X : blood flow to tissue X ; C_X : concentration from tissue X ; K_s : stomach uptake ; K_{si} : stomach-intestine transfer ; K_i : intestine uptake ; K_{fec} : fecal excretion ; K_{el} : urinary excretion.

2.2.3.2. Model equations

The arterial concentration in permethrin for an individual i (CA_i) is described in the model by the adapted formula of Ramsey and Andersen (1984).

$$CA_i = \frac{BW_i \times DE_inh_i / 24 + Q_{C_i} \times CV_i \times MW \times 10^3}{QC_i} \quad (3)$$

where DE_inh_i is the daily inhalation exposure of individual i (ng/kg bw/d) (Hermant et al., 2018), BW_i is the body weight of individual i (kg), Q_{C_i} is the cardiac output (l/h), CV_i is the concentration in the venous blood ($\mu\text{mol/l}$) and MW . The inhalation rate does not appear in this equation because DE_inh_i already takes it into account. It was estimated for each individual according to the equation proposed by and recommended by the U.S. EPA Exposure Factor Handbook (U.S. EPA, 2011).

For ingested permethrin, we considered the daily food exposure and daily indirect dust ingestion of each individual (Hermant et al., 2018). The oral absorption of permethrin in the model takes into

account stomach absorption, the gastric emptying rate constant, intestinal absorption, fecal excretion and intestinal clearance.

The amount of permethrin absorbed by the skin ($dADERM_i$) is calculated as follows:

$$\frac{dADERM_i}{dt} = BW_i \times \left(\frac{DE_{dust_i} + DE_{air_i}}{M} \right) \times DABS \quad (4)$$

where DE_{dust_i} and DE_{air_i} are, respectively, the daily dermal exposure of individual i by contact with dust and with airborne particles (Hermant et al., 2018) and $DABS$ is the dermal absorption. DE_{dust_i} considers the fraction of permethrin in dust adhering to skin and available for absorption, the amount of dust adhering to skin, the permethrin concentration in house dust, the body's surface area, the fraction of the skin's surface area exposed to dust, and the daily duration of exposure to dust. DE_{air_i} considers the deposition velocity of airborne particles onto the skin's surface, the permethrin concentration in indoor and outdoor air, the body's surface area, the fraction of the skin's surface area exposed to dust, and the time spent indoors and outdoors (Hermant et al., 2018).

The single compartment model of *cis*- and *trans*-DCCA was designed to predict the urinary concentration of metabolites in order to compare the predicted results with those from the biomonitoring study. As we wished to consider daily exposure to permethrin, the model was thus designed to give us the concentration of metabolites in daily urine. This urinary concentration was normalized by the creatininuria which is quantified in the urinary samples of each participant of the ENNS study. We considered a daily urinary volume of 1.5 l and estimated daily excreted creatinine for each individual.

Physiological parameters were taken from Browne et al. (2007), except for blood volume fraction (Tornero-Velez et al., 2012) (Table 5). We decided to describe tissue volume for each individual by taking into account their respective body weight. According to the equation proposed by Deurenberg et al. (1991), we estimated the body fat volume (KVF_i) of individual i as follows:

$$KVF_i = (1.20 \times BMI_i + 0.23 \times age_i - 10.8 \times sex_i - 5.4) / (100 \times FD) \quad (5)$$

Where KVF_i is the percentage of total body weight, BMI is expressed in m^2/kg bw, age in years, and sex_i is equal to 1 if it is a man and 0 if it is a woman. FD stands for fat density.

As the sum of all tissue volume has to equal 100%, the corresponding volume of a tissue j of individual i (KVj_i) was calculated with the following equation:

$$KVj_i = (KVj / (1 - KVF)) \times (KVF - KVF_i) + KVj \quad (6)$$

With KVj and KVF the volume of tissue j and of fat respectively as given by Brown et al. (1997). Tissue volumes thereby remain consistent with individual anthropometric data and are not only influenced by body weight.

Table 5. Physiological parameters used in the permethrin PBPK model (Brown et al., 1997).

Parameter	Mean	
	Man	Woman
Cardiac flow (l / h / kg ^{0.75})	15.3	15.3
<i>Tissue volume (% of body weight)</i>		
Brain (KVB)	2	2
Fat (KVF)	21.4	21.4
Skin (KVSK)	3.7	3.7
Liver (KVL)	2.6	2.6
GI tract (KVGI)	1.7	1.7
Rapidly-perfused (KVR)	9.6	9.6
Slowly-perfused (KVBS)	59	59
<i>Tissue blood flow (% of cardiac output)</i>		
Brain	12	12
Fat	5	8.5
Skin	5	5
Liver		
Arterial	6	6
Portal	19	21
Rapidly-perfused	25.5	25
Slowly-perfused	27.5	22.5
<i>Blood volume fraction (% of tissue)</i>		
Brain	4 ^a	4 ^a
Fat	2 ^a	2 ^a
Slowly-perfused	1 ^a	1 ^a

a: Tornero-Velez et al. (2012)

The partition and permeability coefficients were mostly taken from Tornero-Velez et al. (2012). Since the authors did not provide the GI tract: blood partition coefficient, it was assumed by structural analogy that deltamethrin partition coefficients could be used by default (Mirfazaelian et al., 2006). The modifications to the skin compartment led us to use the skin: blood partition coefficient and dermal absorption from Wei et al. (2013).

The data on the hepatic metabolism of permethrin are derived from an in vitro study in which the kinetic constants V_{max} and K_m of the depletion of cis- and trans-permethrin, and the formation of 3-PBA, cis- and trans-DCCA in human primary hepatocytes were estimated (Willemin et al., 2015) (Table 6). A ratio of 2.6 was observed between the clearance (V_{max} / K_m) of cis- and trans-permethrin, a result much lower than that observed in a previous study (ratio of 12) carried out on human microsomes (Scollon et al., 2009). Moreover, the estimated clearance in this study was lower than that

calculated for human microsomes (1.6 l/h/kg bw vs. 3.1 l/h/ kg bw for cis-permethrin and 4 l/ h/kg bw vs. 38.2 l/h/kg bw for trans-permethrin). The authors studied the interaction between the two isomers. A mixture of cis:trans-permethrin (40:60) was also used, but revealed no difference in the kinetics of the parent compounds or metabolites compared to incubating the isomers separately (Willemin et al., 2015).

Parameters specific to permethrin and DCCA are summarized in Table 6. K_m and V_{max} are specific to each isoform, cis- and trans-permethrin (depletion of parent compound) and cis- and trans-DCCA (formation of metabolites). They are all taken from the study Willemin et al. (2015)

Table 6. Parameters of the PBPK model specific to permethrin (Tornero-Velez et al., 2012).

Parameter	cis-permethrin	trans-permethrin
Molar mass (g/mol)	391.3	391.3
<i>Partition coefficients (tissue:blood)</i>		
Brain	1.5	0.4
Fat	150	50
Skin	5.6 ^a	5.6 ^a
Liver	0.44	0.44
GI tract	0.44 ^b	0.44 ^b
Rapidly-perfused	0.44	0.44
Slowly-perfused	5.59	5.59
<i>Permeability coefficients (l/h)</i>		
Brain	0.003	0.003
Fat	0.1	0.1
Slowly-perfused	0.7	0.7
<i>Kinetic constants</i>		
Stomach uptake (K_s , h ⁻¹)	0.01	0.01
Intestine uptake (K_i , h ⁻¹)	0.9	0.9
Stomach-intestine transfer (K_{si} , h ⁻¹)	0.7	0.7
Fecal excretion (K_{fec} , h ⁻¹)	0.59	0.59
Dermal absorption (%)	1.5 ^a	1.5 ^a
Intestinal clearance (l/h))	0	0.78
K_m (μmol/l)	42 ^c	138 ^c
V_{max} (μmol/kg bw/h)	65 ^c	552 ^c
	cis-DCCA	trans-DCCA
Molar mass (g/mol)	209.1	209.1
<i>Kinetic constants</i>		
Urinary excretion (K_{el} , h ⁻¹)	0.06	0.06
K_m (μmol/l)*	36 ^c	36 ^c
V_{max} (μmol/kg bw/h)*	43 ^c	133 ^c

* parameter values before calibration

a: Wei et al. (2013); b: Mirfazaelian et al. (2006); c: Willemin et al. (2015)

2.2.3.3. *Sensitivity analysis*

In order to identify the influence of each of the model's parameters on the variable of interest— in this case the urinary excretion rate of cis- and trans-DCCA—we performed a sensitivity analysis. The equation used to calculate the normalized sensitivity coefficient of output i with respect to parameter j (NSC) is as follows:

$$NSC_{ij} = \frac{\Delta r_{ij}}{r_{ij}} \times \frac{\Delta p_j}{p_j} \quad (7)$$

Where p_j is the value of parameter j , Δp_j is the change in parameter j (used at 10% of p_j), r_{ij} is the corresponding model estimate for output i and Δr_{ij} is the corresponding change in in output i .

2.2.3.4. *Calibration of the model with human data*

The objective of calibration was to improve the model's prediction capability in order to describe the experimental data as well as possible. For this, one or more of the model's parameters (those identified by the sensitivity analysis described previously as being the most sensitive) were adjusted by maximizing the "maximum likelihood" function through the algorithms provided by acslX (here the Nelder-Mead algorithm seems to be the most suitable).

The study by Ratelle et al. (2015) is unique in that measurements specific to certain scenarios (time course measurements of venous blood concentration, and urine concentration levels) were available for individual subjects for controlled exposures.

The experimental data used are derived from a kinetic study on healthy volunteers who were administered oral permethrin (0.1 mg/kg bw, ratio cis:trans 40:60). The study included three men (age: 24-37 years, body weight: 78-95 kg) and three women (age: 31-36 years, body weight: 54-70 kg). Blood samples were taken at set periods over 72 h following ingestion and complete timed-urine voids were collected over 84 h post-dosing.

The predictive capacity of the model was evaluated using a Monte Carlo simulation. This method brings to draw randomly the weight of individuals in a uniform distribution between the lowest and highest weights (500 iterations between 78 and 95 kg for men and between 54 and 70 kg for women). The average age and BMI value for each gender was used.

2.2.4. *Software*

All the analyses were performed using R software (version 3.1.0, copyright 2014, The R Foundation for Statistical Computing) and Stata 14 software (StataCorp. 2015. Stata Statistical Software: Release 14.

College Station, TX: StataCorp LP). The PBK model and calibration were achieved using acslX™ software (version 3.0.0 The AEgis Technologies Group, Huntsville, AL, USA).

2.3. Results

2.3.1. Model calibration

The model was parameterized prior to testing its ability to predict the biomonitoring data which were described in detail in the ENNS study.

The sensitivity analysis showed that the parameters describing the hepatic metabolism of permethrin (depletion of permethrin and formation of DCCA, i.e. CYP450 pathway) strongly influenced the estimation of urinary cis- and trans-DCCA excretion. It was therefore important to refine the estimation of parameters K_m and V_{max} in order to improve the model's prediction using experimental human data from Ratelle et al. (2015). These data were converted into cumulative cis- and trans-DCCA to incorporate them in our model during the calibration step. Since the gender of the "simulated" individual was taken into account in the model, these parameters were calibrated for men and women separately. Table 7 shows the K_m and V_{max} (for DCCA formation) parameter values before and after the calibration phase. The new values were determined by optimization with the Nelder-Mead algorithm.

Table 7. Calibrated kinetic parameters.

Parameter	Initial value	Calibrated for men	Calibrated for women
<i>cis-DCCA</i>			
K_m ($\mu\text{mol/l}$)	36	49.2	40.0
V_{max} ($\mu\text{mol/h/kg pc}$)	43	41.9	37.0
<i>trans-DCCA</i>			
K_m ($\mu\text{mol/l}$)	36	28.8	34.1
V_{max} ($\mu\text{mol/h/kg pc}$)	133	114.4	139.9

After using Monte Carlo simulations during the calibration step, the simulated cis- and trans- DCCA excretion were compared to the experimental data obtained in the study by Ratelle et al. (2015) (Figure 13). Graphs A and C versus B and D revealed that the model tended to over-predict the urinary DCCA concentrations before the calibration phase. It was observed at all time points for male volunteers that predicted urinary excretion was higher than urinary concentration levels measured by Ratelle et al. (2015). For women volunteers, simulations were equivalent to measured urinary concentration before and after the calibration phase during the first hours after exposure but after 24h, the model over-predict the urinary DCCA concentrations before the calibration phase.

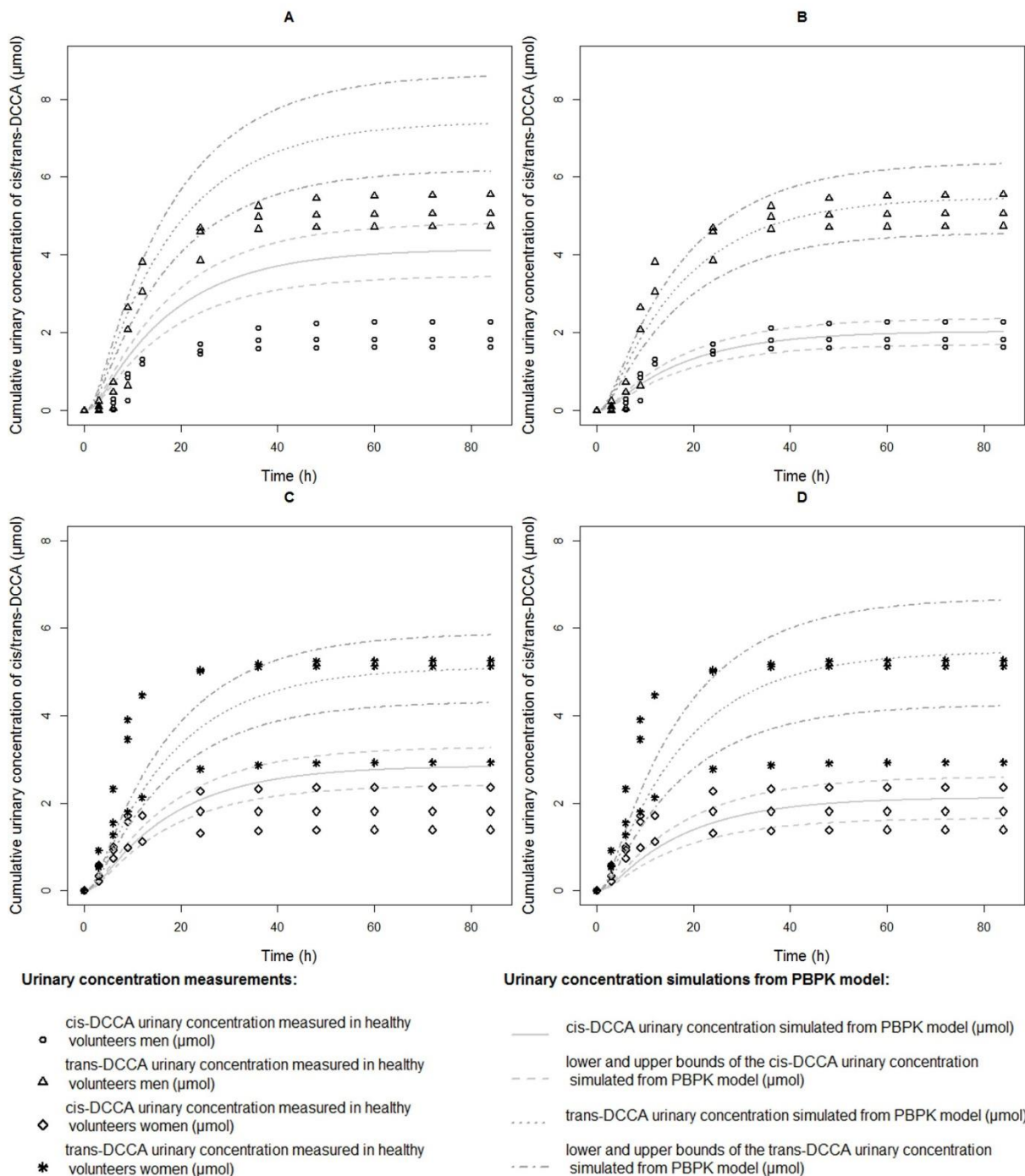


Figure 13. Modeling cumulative urinary excretion of cis- and trans-DCCA in three men (A - B) and three women (C - D) before (A - C) and after (B - D) calibration, and measured cumulative urinary concentration after 0.1 mg/kg bw of oral permethrin.

2.3.2. Simulated cis- and trans-DCCA urinary excretion

The urinary excretion of cis- and trans-DCCA was simulated with the calibrated model according to our three exposure scenarios and for the subgroup (n=219). These simulations were then compared through a paired t-test with the measurements of metabolite urinary concentrations performed on these same individuals in the ENNS study. The geometric mean of the predicted urinary concentrations

of cis-DCCA, according to the “lower”, “intermediate” and “upper” scenarios, were respectively 0.019 [0.017- 0.021] $\mu\text{mol/g}$ creatinine, 0.96 [0.88-1.05] $\mu\text{mol/g}$ creatinine and 1.91 [1.75-2.08] $\mu\text{mol/g}$ creatinine. The geometric mean of the urinary concentrations of cis-DCCA measured in the ENNS study (0.28 [0.25-0.32] $\mu\text{mol/g}$ creatinine) appeared to be located between the predicted urinary concentrations of the “lower” and “intermediate” scenarios. The statistical comparison of the urinary concentrations of cis-DCCA simulated according to our three scenarios versus the ENNS urinary concentrations of cis-DCCA was significantly unequal (all p-values < 2.2e-16). The geometric mean concentrations of the predicted urinary concentrations of trans-DCCA, according to the “lower”, “intermediate” and “upper” scenarios, were respectively 0.049 [0.043-0.055] $\mu\text{mol/g}$ creatinine, 2.51 [2.30-2.73] $\mu\text{mol/g}$ creatinine and 4.96 [4.56-5.41] $\mu\text{mol/g}$ creatinine. The geometric mean of the urinary concentration of trans-DCCA measured in the ENNS study (0.68 [0.59-0.79] $\mu\text{mol/g}$ creatinine) appeared to be located between the predicted urinary concentrations of the “lower” and “intermediate” scenarios, like for cis-DCCA urinary concentrations. The statistical comparison of the urinary concentrations of trans-DCCA simulated according to our three scenarios versus the ENNS urinary concentrations of trans-DCCA was significantly unequal (all p-values < 2.2e-16), just like for the cis-DCCA urinary concentration comparisons. The hypotheses of scenarios “intermediate” and “upper” appear to overestimate permethrin exposure in relation to DCCA urinary concentrations.

Table 8 and Table 9 present the simulated urinary excretion of cis- and trans-DCCA in men and women. The simulations from the “upper” scenario were well above both the cis-DCCA urinary concentrations measured in the ENNS population and the trans-DCCA urinary concentrations measured. These results were observed in both men and women. The comparison of mean concentrations of cis- and trans-DCCA with paired t-tests corroborated these higher concentration levels because the test results were significantly different (all p-values being less than 2.2e-16). For both cis- and trans-DCCA urinary excretions and for both men and women, the concentration measured in the ENNS study appeared to be located between the predicted urinary concentrations according to the “lower” and “intermediate” scenarios. All the comparisons between predicted concentration levels and concentration levels measured in the ENNS study were significantly unequal for both cis- and trans-DCCA concentrations and for both men and women (all p-values < 2.2e-16).

Table 8. Distribution of measured and simulated cis- and trans-DCCA urinary concentration levels ($\mu\text{mol/g}$ creatinine) after calibration for men.

Men (n=74)	GM*	95% CI	min	P25	P50	P75	P95	max
<i>cis-DCCA urinary concentration ($\mu\text{mol/g}$ creatinine)</i>								
Scenario “lower”	0.01	[0.01-0.02]	0.002	0.01	0.01	0.03	0.06	0.14
Scenario “intermediate”	0.74	[0.64-0.85]	0.20	0.49	0.67	1.10	2.20	5.48
Scenario “upper”	1.45	[1.26-1.68]	0.39	0.96	1.34	2.19	4.37	10.9
ENNS study	0.23	[0.19-0.28]	0.05	0.13	0.18	0.46	1.05	2.18

<i>trans-DCCA urinary concentration (μmol/g creatinine)</i>								
Scenario “lower”	0.04	[0.03-0.05]	0.006	0.02	0.03	0.08	0.17	0.37
Scenario “intermediate”	1.97	[1.71-2.28]	0.55	1.31	1.81	2.95	5.91	14.7
Scenario “upper”	3.91	[3.38-4.51]	1.04	2.61	3.61	5.88	11.7	29.4
ENNS study	0.57	[0.46-0.72]	0.09	0.26	0.48	1.18	5.56	5.77

*GM: geometric mean

Table 9. Distribution of measured and simulated cis- and trans-DCCA urinary concentration levels (μmol/g creatinine) after calibration for women.

Women (n=145)	GM*	95% CI	min	P25	P50	P75	P95	max
<i>cis-DCCA urinary concentration (μmol/g creatinine)</i>								
Scenario “lower”	0.02	[0.02-0.02]	0.003	0.01	0.02	0.04	0.1	0.22
Scenario “intermediate”	1.11	[1.00-1.22]	0.17	0.73	1.07	1.82	2.9	6.65
Scenario “upper”	2.19	[1.98-2.43]	0.34	1.43	2.15	3.54	5.81	13.1
ENNS study	0.31	[0.26-0.37]	0.05	0.16	0.24	0.5	2.34	17.9
<i>trans-DCCA urinary concentration (μmol/g creatinine)</i>								
Scenario “lower”	0.06	[0.05-0.06]	0.006	0.03	0.06	0.1	0.25	0.57
Scenario “intermediate”	2.83	[2.56-3.13]	0.43	1.88	2.74	4.66	7.43	17
Scenario “upper”	5.61	[5.07-6.21]	0.86	3.67	5.5	9.05	14.9	33.6
ENNS study	0.74	[0.61-0.90]	0.09	0.35	0.57	1.32	6.3	66

*GM: geometric mean

2.3.3. Contribution of sources and routes of exposure to simulated urinary concentrations of DCCA

Table 10: Contribution (percent) of sources and pathways of exposure to simulated cis- and trans-DCCA concentrations

Contribution (percent)	Scenario “lower”	Scenario “intermediate”	Scenario “upper”
Oral pathway	98 [97.6-98.4]	99.9 [99.9-100]	99.97 [99.96-99.98]
Dietary source	86.7 [84.2-89.2]	99.6 [99.4-99.7]	99.8 [99.7-99.9]
Dust source	11.3 [9-13.5]	0.38 [0.25-0.52]	0.2 [0.13-0.27]
Dermal pathway	1.51 [1.22-1.82]	0.05 [0.03-0.07]	0.03 [0.02-0.04]
Dust source	1.48 [1.18-1.78]	0.05 [0.032-0.068]	0.026 [0.016-0.035]
Airborne particles source	0.25 [0.08-0.43]	0.006 [0.002-0.01]	0.003 [0.001-0.005]
Inhalation pathway	0.49 [0.27-0.72]	0.008 [0.005-0.011]	0.004 [0.003-0.006]

Table 10 presents the contribution of the different sources and pathways of exposure. For all three scenarios, the most important route of permethrin exposure is the oral pathway, which contributes from 98% to nearly 100%. The second route is the dermal pathway, which contributes from 0.03% to 1.51%. Finally, the last route is the inhalation pathway, which contributes from 0.004 to 0.49%. Considering the sources of permethrin exposure, the main one is diet, which contributes from 86.7% to 99.8%, followed by dust (oral and dermal pathways) which accounts for 0.23% to 12.8% of permethrin exposure. The last source is air, which contributes 0.004% to 0.74% according to the different scenarios of exposure and thus appears to be a negligible source of exposure.

2.4. Discussion

This study is an initial attempt to estimate the aggregate exposure of the adult French population to pyrethroids via various sources and routes of exposure. The authors' proposed method of exposure estimation used data about environmental contamination (indoor and outdoor air, settled dust), dietary contamination and urinary concentration levels, all taking into consideration the variability of the population.

Urinary concentrations measured in the ENNS study for both cis- and trans-DCCA are between the levels of urinary excretion simulated according to the "lower" and "intermediate" exposure scenarios. The difference between these two scenarios arises from the treatment of the left-censored data for food contamination. In the "lower" scenario, left-censored data are considered as zero, whereas the "intermediate" scenario considered left-censored data as the median of values sampled between zero and the limit of detection or quantification. Aggregate exposure estimates based on the "lower" scenario appear to underestimate exposure, while the hypothesis of the "intermediate" scenario may, on the other hand, lead to an overestimation of exposure. In 2000, European regulations classified permethrin as an active substance not approved for agricultural use. Its contamination of food should thus be closer to the hypothesis formulated in the "lower" scenario, i.e. considered as zero, this scenario appearing to be more "realistic". However, its underestimation of exposure may have several causes. Due to a lack of data, some environmental exposure situations were not taken into account in the development of scenarios. This is notably the case for certain determinants which were highlighted in the ENNS study results but for which no information was available or collected during the ENNS study and which would have allowed us to build robust scenarios about the use of pesticides in indoor environments (insecticides, indoor plants or pets, occupational exposure) or outdoor environments (treatment of flower or vegetable gardens, etc.). The number of treatments performed, their duration, frequency, type or the quantity of product used (active substance) were not specified, thus making it difficult to refine the exposure scenarios integrating these kinds of activities. Yet the ENNS study showed that people treating their pets with flea treatments have a significantly higher level of metabolite concentrations than those who do not treat their animals. These treatments usually contain pyrethroids. People who treat pets can be acutely exposed during the treatment by inhalation or dermal contact but they can also be chronically exposed after the treatment due to the treatment's efficiency, prolonged over several months. This exposure is possible via the dermal route, by indirect ingestion or by inhalation during daily care and games with the animal (pyrethroids being released into the air and/or dust, hand-to-mouth contact, etc.).

This lack of data makes it difficult to evaluate the underestimation of exposure. Furthermore, DCCA compounds are not a specific metabolite of permethrin but are also common to cypermethrin and cyfluthrin, which are authorized in Europe, especially for agricultural use. The urinary concentrations measured in the ENNS study are therefore not entirely due to exposure to permethrin. Therefore, an aggregate PBPK model integrating permethrin, cypermethrin, and cyfluthrin should better describe the urinary excretion of DCCA. In addition, but to a lesser extent, the scenarios do not take into account the probable presence of DCCA in the environment or food. This molecule is more hydrophilic than permethrin (DCCA: LogP = 4, permethrin: LogP = 6.5) and once ingested will be minimally absorbed. To our knowledge, no information is available on levels of DCCA contamination in the environment, but this may not be ruled out.

Dietary exposure contributes to 86.7% of DCCA concentrations according to the “lower” scenario. However, the quantification of permethrin concentration in raw agricultural commodities (RACs) was 0.11%. Only commodities for which permethrin is quantified contribute to permethrin exposure even if they are not frequently consumed. Despite the low quantification percentage, diet is the main source of exposure. Dust is the second source of exposure, with a total contribution to urinary excretion of 12.8%, 11.3% via the oral route and 1.48% via dermal contact. Dust exposure by dermal contact is higher than dust exposure via indirect ingestion, with exposure medians of 1.4 ng/kg bw/d and 0.24 ng/kg bw/d, respectively. Nevertheless, the results of the PBPK model indicate that oral exposure contributes more to DCCA excretion. This could indicate that the skin is a substantial barrier to exposure.

PBK models for permethrin in humans have already been published (Tornero-Velez et al., 2012; Wei et al., 2013). Enhancements to the current model include using new experimentally-determined chemical-specific human parameters for model evaluation that were unavailable at the time earlier models were published. In addition, sensitivity analyses were used to determine which model inputs (parameters) were most influential for specific model responses (e.g. toxicologically relevant dose metrics or experimental measurements, for which data are or may become available). In the previous model, the scaled in vitro Vmax and Km were experimentally derived from a human hepatic microsome study (Scollon et al., 2009) with in vitro in vivo extrapolation (QIVIVE). Nevertheless, the model failed to describe the time-related pharmacokinetic data from the study by Ratelle et al. (2015). We decided to optimize metabolic parameters using the oral data for individual subjects: the use of data on human metabolism in this model limits the uncertainties related to QIVIVE that affected previous models. Obtaining gender-specific metabolic parameters reduced uncertainty about these parameters for individual men and women studied by Ratelle et al. (2015).

During the calibration phase, a sufficient number of DCCA urinary concentration measurements over time allowed us to precisely observe the kinetics of urinary excretion of metabolites during the first hours after ingestion. Before this step, the cis:trans-DCCA ratio was 1:1.79. The calibration phase produced a ratio of 1:2.60 which is closer to that measured in the ENNS study (1:2.43). However, the low number of subjects did not allow inter- and intra-individual variability of the kinetics of permethrin to be taken into account. Data on the differences between men and women with respect to carboxyl esterase (CaE) activity, which is responsible for permethrin hydrolysis and DCCA formation, are divergent. Studies have shown that in adult rats, CaE activity is lower in females (Morgan et al., 1994; Moser et al., 1998). In the study by Zhu et al. (2009), the authors concluded that gender is unlikely to be a regulatory factor of CaE activity in mouse and human liver. Butte and Kemper (1999) did not demonstrate a correlation between CaE activity and the age and/or gender of 48 human subjects either.

However, measured urinary concentrations in the ENNS study of cis- and trans-DCCA were both higher for women than for men, especially for maximal measured values which were higher than simulated ones according to the “upper” exposure scenario. Measured urinary concentrations of 3-PBA were also higher for women than for men, and seemed to be highly correlated with cis- and trans-DCCA urinary concentration. The same difference between men and women was observed for 3-PBA or 5 pyrethroids metabolites (including 3-PBA, cis- and trans-DCCA) among New York City adult population (McKelvey et al., 2013) and the Canadian general population (Canadian Health Measures Survey, Ye et al. (2016)). This difference could come from a greater exposure of women to a source that the ENNS study would not have taken into account, like indirect exposure in the workplace (Fréry et al., 2013). For instance, among the 7 individuals with the highest urinary levels of pyrethroids metabolites in the ENNS study, 3 worked in hospitals.

In risk assessment, uncertainty may be due to a lack of or limited knowledge about the routes of exposure, target population, exposure scenario, models or data used (Anses, 2016). Uncertainties remain in the proposed method, particularly because of the sensitivity of exposure models to contamination data, the analytical limits used during the contamination control and food monitoring surveys being too high. This explains why, although permethrin has been banned for agricultural use in Europe since 2000, the results from the “intermediate” scenario are higher than those measured in the ENNS study. Another uncertainty lies in the choice of the cis:trans-permethrin ratio used (40/60). This is the most frequently found ratio in literature for household products, but other ratios can be found (e.g. 25/75). Moreover, the first morning urine samples taken in the ENNS study mostly reflect exposure from the day before. It would have been better to have several samples taken at different times in order to obtain a better correlation between measured DCCA urinary concentration levels and

data from the diet study and interviews. The implementation of PBK models in risk assessment—and especially in exposure assessment—can contribute to the development of more realistic or refined exposure scenarios, particularly when using contamination data from food control surveys. The potential endocrine-disrupting effect of permethrin and its effects at low doses should prompt further investigations to refine exposures for risk characterization (Jin et al., 2012; Meeker et al., 2009).

This work is an initial step in estimating the aggregate exposure of French adults to pyrethroids, from contaminated media to individuals' simulated urinary concentrations. While this study focused on French adults, the method could be adapted to more sensitive populations or those more at risk—especially children due to the contribution of dust in exposure to pyrethroids and frequent hand-to-mouth activity. In addition, exposure scenarios considered only one compound—permethrin—but the DCCA metabolite is common to two other pyrethroid substances: cypermethrin and cyfluthrin. With additional complementary information, particularly on parameters and compounds, exposure could be better characterized and closer to that observed in the ENNS study. Moreover, the proposed method could be a baseline for a generic model of aggregate exposure to pyrethroids using 3-PBA as a biomarker. The PBK model could later be used to carry out inverse dosimetry studies, allowing us to build pyrethroid exposure scenarios based on measured urinary metabolite concentrations. This model could also help interpret biomonitoring data: our next focus will be on evaluating the toxicological implications of multi-route exposure, with a particular emphasis on how various routes of exposure contribute to internal dose metrics that could be relevant to neurotoxicity associated with exposure to permethrin in epidemiologic studies.

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Author's contribution

K. Darney: conceptualised and developed the human PBTK model, analysis of the results and drafted the manuscript. L. Bodin: assisted in the design of the study and interpretation of the results and critical review of the manuscript. M. Bouchard and J. Côté: provided human pharmacokinetic raw data and

critical review of the manuscript. J.L. Volatier: project administration. V. Desvignes: conceptualised and developed the dietary exposure assessment model, analysis of the results and drafted the manuscript.

3. 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 (C_{max} , 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 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, UF95 and UF97.5, 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.

Keywords: human variability; toxicokinetics; uncertainty factor; CYP3A4

Highlights:

- Systematic review of human kinetic parameters for 15 CYP3A4 probe substrates
- Hierarchical Bayesian meta-analysis to quantify interethnic and intra-ethnic differences
- The use of CYP3A4-specific uncertainty factors in chemical risk assessment

3.1. 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 has been justified to allow for interspecies differences (10-fold) and human variability (10-fold) (Truhaut, 1991). 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 (Renwick, 1993). 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 (Bhat et al., 2017; Dorne et al., 2001a; Renwick and Lazarus, 1998).

CSAFs are derived using chemical-specific data for either or both the TK and TD dimension using physiologically-based kinetic (PBK) models describing ADME processes from external to internal exposure or PBTK-TD models integrating the toxicity dose-response (Loizou et al., 2008). 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 (Barton et al., 2007; IPCS, 2010). This can also provide the basis for developing integrated testing strategies without the need for animal testing to move towards the use of QIVIVE (Bell et al., 2018)

Pathway-related UFs quantifying human variability in a range of metabolic pathways have also been proposed as intermediate options between default UFs and CSAFs and these were first applied to CYP1A2 and glucuronidation (Dorne et al., 2001a; Renwick and Lazarus, 1998; Walton et al., 2001b). 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 (Dorne et al., 2001a; Dorne et al., 2003a; Dorne et al., 2003b; Dorne et al., 2004a; Dorne et al., 2005; Dorne et al., 2002; Ginsberg et al., 2002; Naumann et al., 2001; Walton et al., 2001a; Walton et al., 2001b).

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 (Buratti et al., 2011; Cotreau et al., 2005; Dorne et al., 2003a). 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%) (De Wildt et al., 1999; Dorne et al., 2003a; Stevens et al., 2003; Zanger and Schwab, 2013).

Analysis of human variability in CYP3A4 metabolism has been previously carried out by Dorne et al. (2003a) 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 CYP3A4 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.

3.2. Material and methods

3.2.1. 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 C_{max}, respectively), after an oral intake or intravenous injection (IV) were extracted. Table 11 provides a summary of the individual key words applied for the ELS.

Table 11 List of queries used for the ELS (formatted for Scopus).

Search CYP3A4 probe substrate	TITLE-ABS (“ <i>name of probe substrate</i> ”)
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.

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 12.

Table 12 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 follows: the required score for inclusion was 1-2 for the sections “Population” and “Methodology”, while a score of 2 for the “Results” section need to be fulfilled.

3.2.2. Meta-analysis

3.2.2.1. 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. (2012). 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 (Dorne et al., 2001a; Naumann et al., 1997; Renwick and Lazarus, 1998), 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:

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

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

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

$$CV_N = SD/X \quad (3)$$

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 equations (4), (5) and (6).

$$SD = \sqrt{n} SE \quad (4)$$

$$SD = CV_N X \quad (5)$$

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

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. (2008), the geometric standard deviation is estimated as follows:

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

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 equation (6) or (7) results in overestimated variability values.

3.2.2.2. 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. (2019). The structure of the model showing the conditional dependencies among the population and the individual parameters are summarised graphically in Figure 14.

On the logarithmic scale, each individual value for a chosen PK parameter X_{ijk} with $i=1,2,3,\dots,n$ 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 $\overline{X}_{jk} = \frac{1}{n} \sum_{i=1}^n X_{ijk}$ and $S_{jk} = \frac{1}{n_{jk}-1} \sum_{i=1}^n (X_{ijk} - \overline{X}_{jk})^2$ are independent conditionally to the study and the substrate and distributed according to:

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

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

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:

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

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

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 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 multi-level 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. (2003a) 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 (Plummer, 2003) 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 (Plummer et al., 2006).

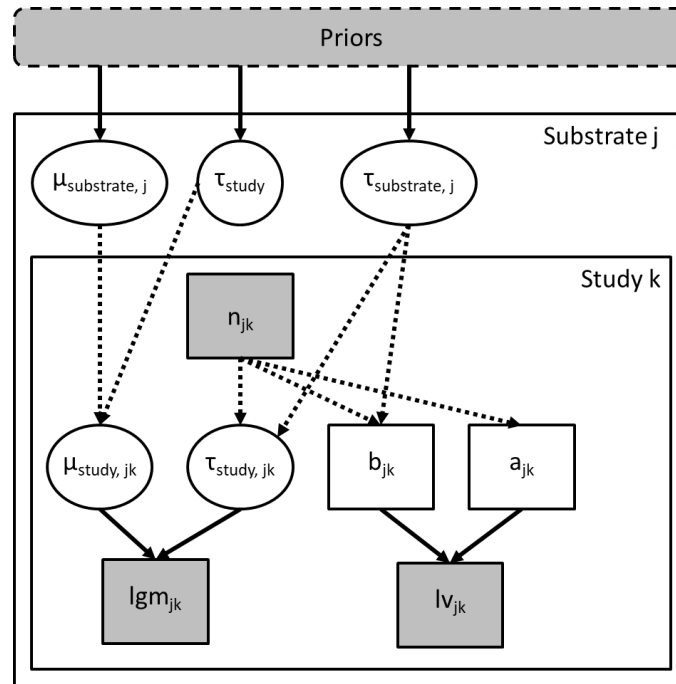


Figure 14 Graphical representation of the hierarchical model for meta-analysis.

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 $b=n.\tau_j/2$, inter-study precision (τ_{study}). Solid arrows represent a stochastic link and dashed arrows represent a deterministic link.

3.2.3. 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).

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

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 >1 indicated a higher internal dose (Dorne, 2010).

3.2.4. 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) (Plummer, 2003). References from ELS were saved in EndNote (X8) files.

3.3. Results

3.3.1. 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) (Dorne et al., 2003a; García et al., 2003). Figure 15 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 Figure 16. The full dataset of extracted information used in this review can be accessed on EFSA knowledge junction.

Figure 16 shows the raw data for each substrate and parameter of acute (C_{max}) and chronic exposure (clearance and AUC) for the intravenous and oral route. As illustrated in Figure 16, 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.

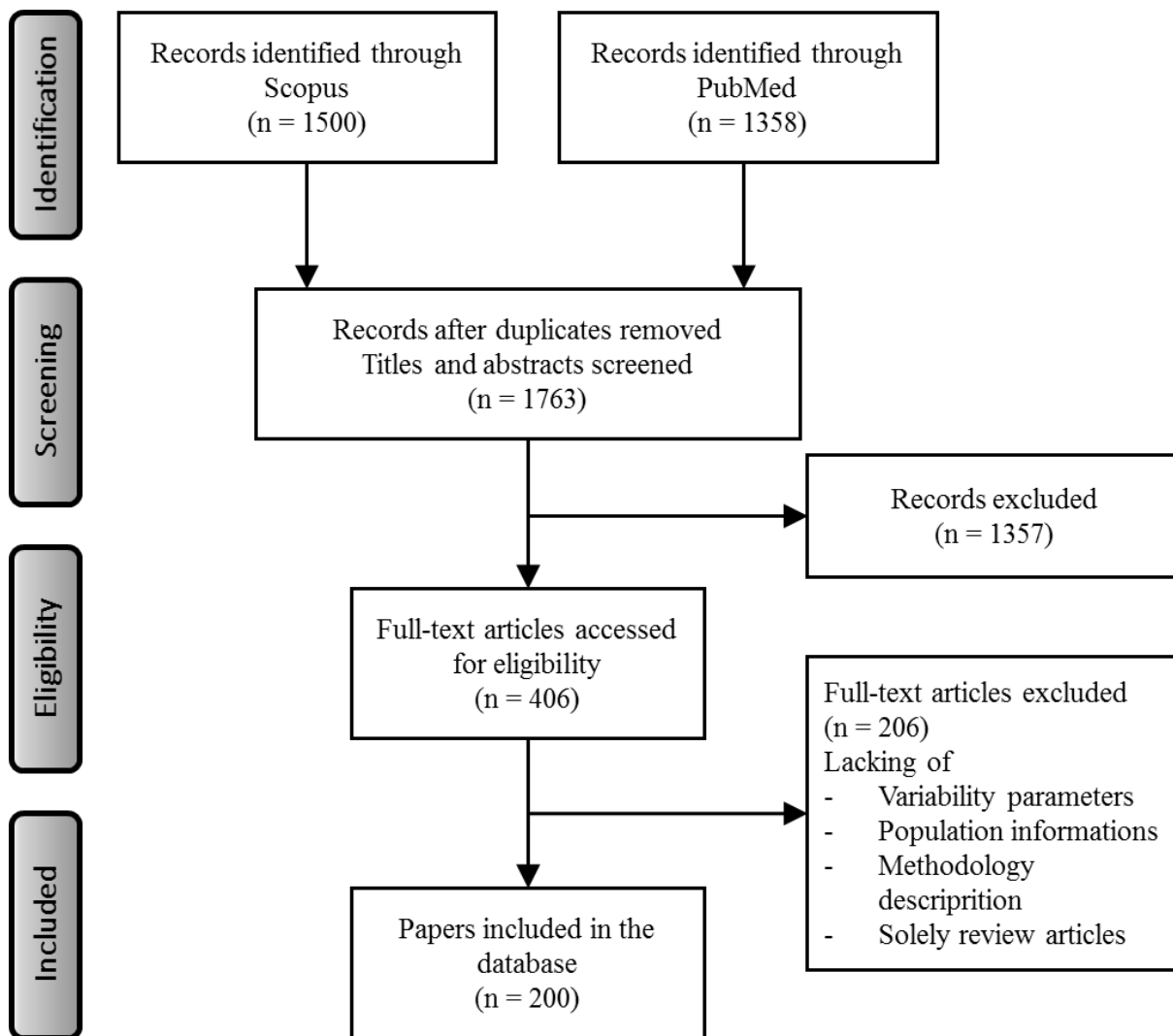


Figure 15 Flow diagram illustrating the extensive literature search of human pharmacokinetic studies for 15 probe substrates of CYP3A4

Table 13 provides an overview of the number of substrates, number of studies with the corresponding extracted data, and individuals included in each meta-analysis.

Table 13 Summary of the number of CYP3A4 substrates, pharmacokinetic studies and individuals in the meta-analyses

	Nsubstrate	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

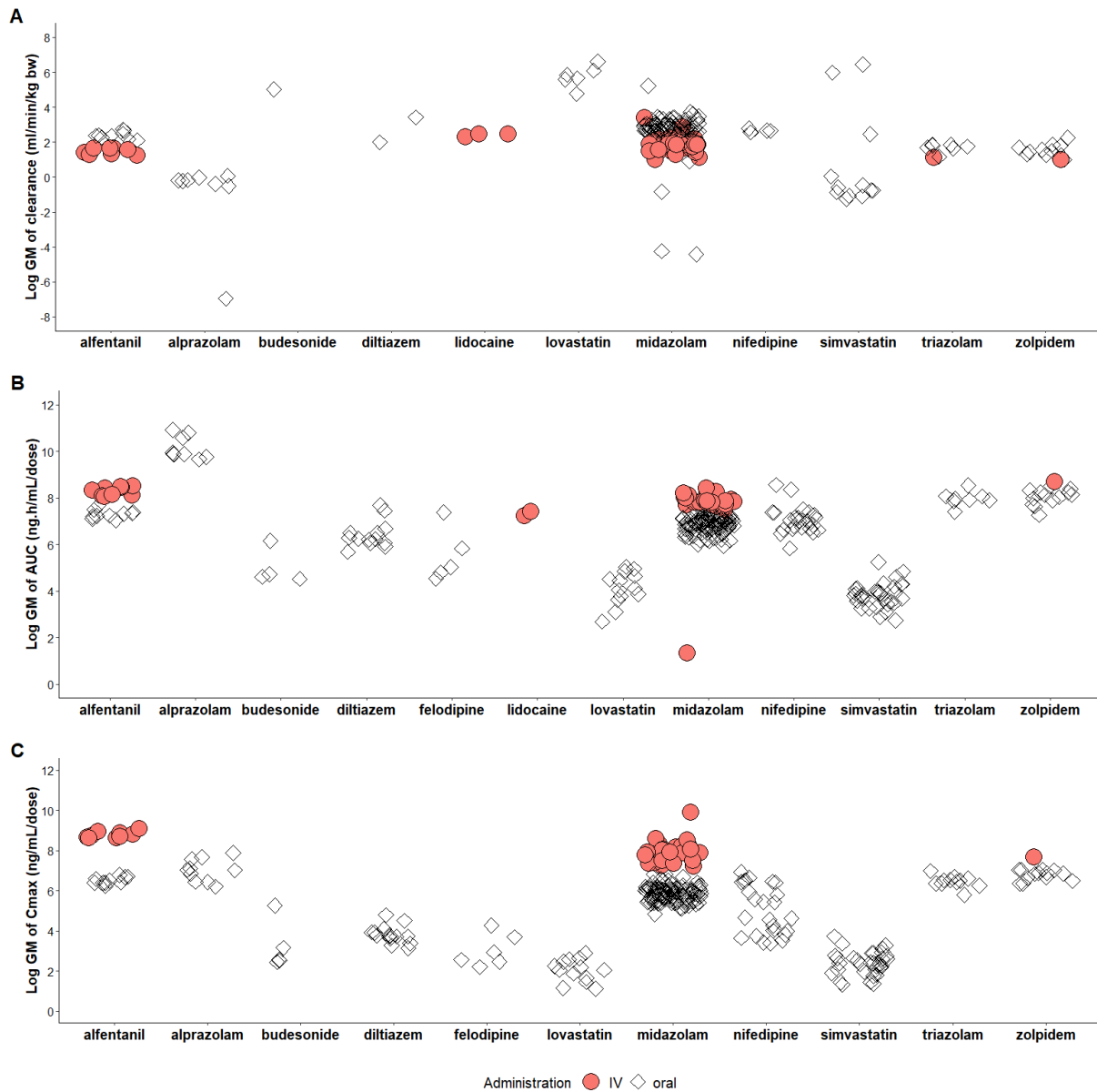


Figure 16 Log geometric mean of extracted kinetic parameters from the included papers after standardization. A: clearance ; B: AUC ; C: Cmax. Squares: oral exposure ; red circles : IV exposure.

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

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 sub-group 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 (Table 14 to Table 17) 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 (Cotreau et al., 2005; De Wildt et al., 1999). 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 (Dorne et al., 2003a). Overall, inter-individual variability in kinetic parameters for healthy adults (North America) are consistent with the results of Dorne et al. (2003a) providing values, of 56% and 51% for the oral route (clearance/AUC and C_{max}) and 43% and 31% (Clearance/AUC and C_{max}) 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 (Table 14 to Table 17). For the oral route, the UF₉₅ and UF_{97.5} were 2.5-3.0, 2.3-2.7 and 1.9-2.2 for AUC, clearance and C_{max} 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 C_{max} 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. (2003a) 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 C_{max}, UF₉₅ 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 C_{max} (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 (Dorne et al., 2003a). However, in this previous analysis the estimated internal dose differences allowing for

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)

Table 15 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

Drug	ns	n	CV	GM	Ratio GM	Intra-ethnic		Interethnic			
						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]
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]
diltiazem	1	12	9	7.4	4.32	1.2	[1.1-1.5]	1.2	[1.1-1.6]	31	[4.1-128]
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]
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]
nifedipine	3	28	53	14	0.86	2.3	[1.6-4.5]	2.7	[1.8-6.1]	2.8	[0.8-11]
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]
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]
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]
overall			48			2.1	[1.1-4.7]	2.4	[1.1-6.3]	3.1	[0.8-13]
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]
simvastatin	1	9	53	18	0.02	2.4	[1.4-18]	2.8	[1.5-32]	81	[0.3-13e2]
overall			59			2.5	[1.4-11]	3.0	[1.6-17]	16	[0.1-1e3]

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)

Table 17 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

Drug	ns	n	CV	GM	Ratio GM	Intra-ethnic				Interethnic			
						UF95 (95% CI)	UF97.5 (95% CI)	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]
CI (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)

3.3.3. 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 (Dorne et al., 2003a). 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 C_{max} (Table 18). The difference in studied substrates, intravenously administered, between healthy North American adults and elderly did not allow to compare those populations accurately. The UF after oral administration (clearance) was above the default kinetic factor, 3.9 and 4.9 for the UF₉₅ 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 18). 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 18).

Table 18 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)	UF97.5 (95% CI)		
Elderly									
<i>Oral administration</i>									
<i>AUC (ng.h/ml / dose)</i>									
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]
<i>Clearance (ml/min / kg bw)</i>									
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]
<i>Cmax (ng/ml / dose)</i>									
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]
<i>Intravenous administration</i>									
<i>AUC (ng.h/ml / dose)</i>									
alprazolam	1	13	26	24507					
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					
<i>Clearance (ml/min / kg bw)</i>									
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									
<i>Oral administration</i>									
<i>AUC (ng.h/ml / dose)</i>									
alprazolam	1	11	26	6271	0.34	1.4	[0.1-23.8]	1.4	[0.1-25]
<i>Cmax (ng/ml / dose)</i>									

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]
<i>Intravenous administration</i>									
<i>AUC (ng.h/ml / dose)</i>									
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]
<i>Clearance (ml/min / kg bw)</i>									
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									
<i>Oral administration</i>									
<i>Cmax (ng/ml / dose)</i>									
cisapride	3	32	59	131	0.25	6.9	[3.1-14]	7.6	[3.4-16]
<i>Intravenous administration</i>									
<i>Clearance (ml/min / kg bw)</i>									
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)

3.4. Discussion and Conclusions

This meta-analysis provides a quantitative account of inter-ethnic and intra-ethnic differences in CYP3A4 metabolism using markers of 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 (Dorne et al., 2005). 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 health-care and risk assessment from a Bayesian perspective have been investigated (Rigaux et al., 2013; Shao et al., 2017; Sutton and Higgins, 2008). 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.

Inter-individual variability for the oral route for healthy adults averaged 51% (AUC), consistent with a previous study (Dorne et al., 2003a). In a more recent meta-analysis of inhibition (grapefruit juice) and

induction (St John's wort) of CYP3A4 metabolism in humans (Quignot et al., 2019), inter-individual variability and UF₉₅ were determined for 57 and 64 compounds (C_{max} and AUC or clearance) respectively, for full and partial probe substrates of CYP3A4. Inter-individual variability was 56 % for C_{max} and 51 % for AUC and Clearance and the corresponding UF₉₅ and UF₉₉ 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. (2003a) 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 with aging and morphological changes (decrease of the muscle mass and increase of adipose tissue mass) that will impact distribution (Cotreau et al., 2005). The estimated UFs were of the same range than in Dorne et al. (2003a) for the clearance after oral administration (4 compounds).

CYP3A7 is the main isozyme in foetal liver and represent around 32% of total CYP content (De Wildt et al., 1999). 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 (Buratti et al., 2006). A transition between those two isoforms will occur a few months after birth (De Wildt et al., 1999). A greater variability was estimated for neonates than for adults as previously observed (Dorne et al., 2003a). 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 precisely estimate UFs. For children, except for midazolam, a low variability was observed. In the literature, the clearance for midazolam in children is higher compared to adults (De Wildt et al., 1999) nevertheless our results showed the opposite. This might be due to discrepancies in the reported studies (Malinovsky et al., 1990; Rey et al., 1991).

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 this 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 (Jarrar et al., 2016).

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 (Keshava et al., 2004; Werk and Cascorbi, 2014; Zanger and Schwab, 2013). 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 (Preissner et al., 2013; Werk and Cascorbi, 2014; Zanger and Schwab, 2013). In contrast, CYP3A5 is expressed in extrahepatic tissues with more than 25 allelic variants (Jarrar et al., 2016) 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 Caucasians, in 0.66-0.78 of Asians and in 0.12-0.50 of Africans/African Americans (Jarrar et al., 2016; Naidoo et al., 2014; Preissner et al., 2013; Zanger and Schwab, 2013). 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 (Lamba et al., 2002). This may explain the very high variability in the kinetics of lovastatin and simvastatin, two CYP3A substrates interacting with the P-glycoprotein transporter (Garcia et al., 2003). 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 (Hasunuma et al., 2016). Moreover, Kim et al. (2007) 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-specific adjustment factor (CSAF) and the default UF (when no data are available) (Bhat et al., 2017; Clewell et al., 2008; Paini et al., 2017; Paini et al., 2019). The proposed methodology and modelling can be applied to other metabolic pathways of interest to assess human inter-individual variability in TK in a broader context.

Non-invasive *in vitro* techniques are now available to provide metabolism data from human cell lines (Bell et al., 2018; Blaauboer et al., 2012). 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 (Bessemers et al., 2014; EFSA, 2014; IPCS, 2010; Paini et al., 2017; Paini et al., 2019) 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 (Bois et al., 2010) 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 (Punt et al., 2017; Yoon et al., 2015). 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 (McNally et al., 2014).

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 (Desalegn et al., 2018; Valcke and Haddad, 2015). Desalegn et al. (2018) 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. (2019), 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.

4. Bayesian meta-analysis of inter-phenotypic differences in human serum paraoxonase-1 activity for chemical risk assessment

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Abstract

Human variability in paraoxonase-1 (PON1) activities is driven by genetic polymorphisms that affect the internal dose of active oxons of organophosphorus (OP) insecticides. Here, an extensive literature search has been performed to collect human genotypic frequencies (i.e. L55M, Q192R, and C-108T) in subgroups from a range of geographical ancestry and PON1 activities in three probe substrates (paraoxon, diazoxon and phenyl acetate). Bayesian meta-analyses were performed to estimate variability distributions for PON1 activities and PON1-related uncertainty factors (UFs), while integrating quantifiable sources of inter-study, inter-phenotypic and inter-individual differences. Inter-phenotypic differences were quantified using the population with high PON1 activity as the reference group. Results from the meta-analyses provided PON1 variability distributions and these can be implemented in generic PBK models to develop quantitative in vitro in vivo extrapolation models. PON1-related UFs in the Caucasian population were above the default toxicokinetic (TK) UF of 3.16 for two specific genotypes namely -108CC using diazoxon as probe substrate and, -108CT, -108TT, 55MM and 192QQ using paraoxon as probe substrate. However, integration of PON1 genotypic frequencies and activity distributions showed that all UFs were within the default TK UF. Quantitative inter-individual differences in PON1 activity are important for chemical risk assessment particularly with regards to the potential sensitivity to organophosphates' toxicity.

Keywords: human variability; PON1 activity; polymorphism; uncertainty factor

Highlights:

- Extensive literature search of PON1 genotypic frequencies and activities in humans
- Bayesian meta-analysis to quantify inter-phenotypic and inter-individual differences
- Specific genotypes showed an exceedance of the 3.16 toxicokinetic uncertainty factors
- UFs were below 3.16 when combining genotypic frequencies and activity distributions
- Quantitative differences in PON1 polymorphisms are important for chemical risk assessment

4.1. Introduction

Human paraoxonase 1 (PON1) is a well characterised family member of high-density lipoprotein associated serum enzymes called serum paraoxonases (PONs). PONs have been identified in mammals, vertebrates and invertebrates and are involved in the detoxification process of a range of chemicals, including prodrugs like prulifloxacin, active oxons of organophosphorus (OP) insecticides as well as nerve gas agents such as sarin and soman (Costa et al., 1999; Furlong et al., 2016b). PON1 enzymes are also important in protecting the human body against vascular disease through metabolising oxidised lipids (Costa et al., 2011). In the 1960s and 1970s, human studies demonstrated that PON1 activities were polymorphically distributed and the frequency of phenotypes with low activities were variable amongst populations of different geographical or ethnic ancestry (Diepgen and Geldmacher-von Mallinckrodt, 1986).

Nearly 200 single nucleotide polymorphisms (SNPs) have been described in the literature for the PON1 gene with the two most common polymorphisms reported in the coding regions at position 55 and 192 (Gupta et al., 2011; Humbert et al., 1993; Richter et al., 2010; Shunmoogam et al., 2018). The SNP present at position 55, leucine/methionine (L55M) has been associated with altered PON1 serum concentrations, while the SNP at position 192, glutamine/arginine polymorphism (Q192R) has been associated with altered PON1 activity (Ceron et al., 2014; Shunmoogam et al., 2018). Within the promoter region of the PON1 gene, another important SNP is C-108T, affecting PON1 enzyme levels (Turgut Cosan et al., 2016). The SNPs described affect PON1 activity, but seems to be substrate dependent, since the 192R alloform hydrolyses chlorpyrifos oxon and paraoxon more rapidly than *192Q *in vitro* (Li et al., 2000). Variability in PON1 activity may not only be introduced by polymorphisms but also by age and lifestyle (Ginsberg et al., 2009; Nalcakan et al., 2016). PON1 activity is very low before birth as indicated by a 24% lower activity in premature babies (33-36 weeks of gestation) compared to babies at term (Ecobichon and Stephens, 1973). PON1 activity increases over time reaching a plateau between 6 months and a few years of age (Augustinsson and Barr, 1963; Cole et al., 2003; Ecobichon and Stephens, 1973; Holland et al., 2006; Huen et al., 2010; Smith et al., 2011).

Polymorphisms in PON1 are well described and constitute an important source of variability driving potential changes in internal dose of oxon metabolites and have been hypothesised to be involved in OP oxon resistance after OP exposure. Distributions of PON1 activities have been simulated to quantify inter-phenotypic differences while integrating genotypic frequencies and variations across a range of human populations (Ginsberg et al., 2009a). This body of evidence shows that quantification of inter-phenotypic differences across different PON1 genotypes provides a basis for the derivation of

variability distributions and PON1-related uncertainty factors (UFs) allowing for inter-individual differences in toxicokinetics (TK) (Bhat et al., 2017; Renwick, 1993; Truhaut, 1991).

This manuscript aims to investigate human variability in PON1 activities by means of extensive literature searches and hierarchical Bayesian meta-analysis for paraoxon, diazoxon and phenyl acetate as probe substrates in healthy adult populations expressing different SNPs (i.e. L55M, Q192R, and C-108T). Variability distributions for PON1 activities and PON1-related UFs have been derived while integrating quantifiable sources of inter-study, inter-individual and inter-phenotypic differences.

4.2. Material and Methods

4.2.1. Extensive literature search and data collection

Extensive literature searches (ELS) were performed to identify PON1 activity in the serum of healthy human subjects from a range of geographical ancestry or ethnic groups. Inter-phenotypic differences in relation to PON1 polymorphisms (C-108T, L55M, Q192R) were investigated for healthy adults whereas data from populations exposed to pharmaceuticals, environmental contaminants or populations with specific lifestyle or diseases were excluded. The ELS were performed by two independent reviewers (June 2019) for well-characterised PON1 probe substrates namely paraoxon, diazoxon and phenyl acetate in PubMed and Scopus (EFSA, 2010a; Quignot et al., 2015). Data reporting genotypic frequencies of PON1 in human populations from different geographical ancestry (Europe, Africa, Middle East, Asia and Oceania) were collected for the homozygous CC, QQ, LL (wild-type) and for heterozygous (CT, QR, LM) and homozygous TT, RR and MM (mutant). Each polymorphism, whether known from coding or promotor region, was associated with variability in levels of PON1 activity towards each of the three probe substrates. In order to compare different PON1 activity phenotypes, the high activity group has been considered as the reference group which varied across substrates and genotypes (Furlong et al., 2016b). The two remaining groups will be considered as sub-groups. Table 19 provides a summary of the individual keywords applied for the ELS. The complete database is available in Supplementary material.

Table 19. Keyword queries for the Extensive Literature Searches (formatted for Scopus).

General search terms	TITLE-ABS-KEY ("population distribution" OR "expression level*" OR "gene expression" OR "genetic polymorphism*" OR "individual susceptibility" OR "gene environment" OR "ethnic variability" OR caucasian OR asian OR "Afro American" OR hispanic OR "race difference" OR "age difference" OR "gender difference" OR "sex difference" OR ontogenesis OR "foetal stage" OR neonate* OR african OR children OR elderly OR "elderly people" OR adult* OR genotype)
Search terms for probe substrates	TITLE-ABS-KEY (human* W/50 (paraoxonase* OR diazoxonase* OR arylesterase* OR pon1 OR "PON1 activity"))

Exclusion	TITLE-ABS ("cell line*" OR "cell culture*")
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TITLE-ABS-KEY: term searched in the title, the abstract and the keywords of the paper.

4.2.2. Meta-analysis

4.2.2.1. Data standardisation

Data for PON1 activity were standardised to perform the meta-analysis in a harmonised manner. Activity was expressed in nmol/min/ml (paraoxon and diazoxon studies) or $\mu\text{mol}/\text{min}/\text{ml}$ (phenyl acetate studies). PON1 activities from individual studies were reported as arithmetic means (X) and standard deviations (SD) and were harmonised to geometric mean (GM) and geometric standard deviation (GSD) using the following equations:

$$GM = X / \sqrt{(1 + CV_N^2)} \quad (4)$$

$$GSD = \exp(\sqrt{\ln(1 + CV_N^2)}) \quad (5)$$

where CV_N provides the coefficient of variation for normally distributed data as:

$$CV_N = SD/X \quad (6)$$

4.2.2.2. Derivation of PON1-related variability and uncertainty factors

A Bayesian hierarchical model for the meta-analyses was implemented for PON1 activity as previously described (Darney et al., 2019) using non-informative priors. Two types of uncertainty factors were calculated: 1) UFs were calculated for the different SNPs; 2) UFs were calculated for the human population integrating PON1 activity and genotypic frequencies. Uncertainties in PON1 activities were quantified using median values and 95% confidence intervals. Coefficient of variations (CV) were also estimated as follows:

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

where τ_j is the inter-individual differences of the activity for a substrate 'j'.

Inter-phenotypic differences in PON1 activity and related UFs

PON1 activity related UFs for the reference group within a genotype were derived as ratios between given percentiles (either 95th or 97.5th centiles) and the median of the distribution. For inter-phenotypic differences, PON1-related UFs were calculated as the ratio between the percentiles of choice for the reference group and the median of the sub-group. A confidence interval around the UF is given by calculating 18000 UFs and providing the values for the median, 2.5th and 97.5th percentile.

Inter-individual differences in PON1 activity and related UFs

Distributions for PON1 activity in the whole adult population were integrated for the reference group and the two remaining sub-groups applying Monte-Carlo simulations. Genotypic frequencies collected from the literature were combined with the estimated PON1 activity distributions. PON1-related UFs were derived as described elsewhere (Wiecek et al., 2019) and illustrated in Figure 17. 1) Sampling $\alpha \times 10000$ values in the distribution for PON1 activity (α , the genotypic frequency in the population), the same pertains for the two other genotypes with the corresponding frequencies (100 iterations); 2) Calculate UFs based on the 50th and the 95th percentiles of 10^6 values; 3) Derive the distribution of UFs in the human population (18000 iterations).

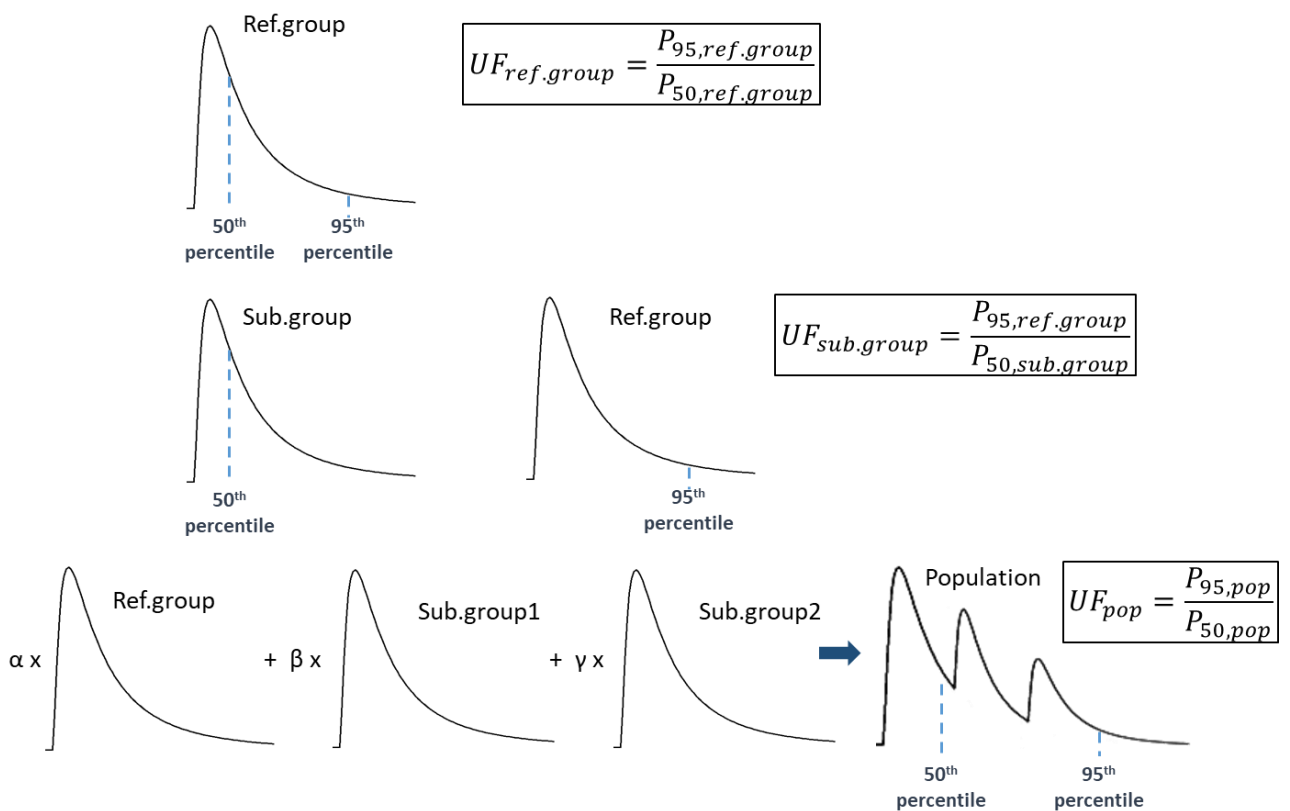


Figure 17. Population simulations for the derivation of PON1 related uncertainty factors integrating inter-phenotypic differences (reference group and sub-groups) and genotypic frequencies (α , β and γ).

4.2.3. Software

All statistical analyses and graphs were performed in R (version 3.5) and the Bayesian modelling was implemented with Jags (4.2.0) (Plummer, 2003). R codes describing the hierarchical Bayesian model for the meta-analysis have been published elsewhere (Darney et al., 2019; Wiecek et al., 2019). References from the ELS have been saved in EndNote (X8) files.

4.3. Results

4.3.1. Extensive literature searches and data collection

Results from the ELS are presented in Figure 18 as a Prisma diagram. Human PON1 activities are available for a wide range of countries worldwide. Studies focused on healthy adults (range 18-75 years) and data for both genders were equally available. Overall, 67 peer reviewed publications were selected from the ELS and these reported human PON1 enzyme activities for three SNPs (C-108T, L55M and Q192R) using paraoxon, diazoxon and phenyl acetate as probe substrates or genotypic frequencies. The L55M and Q192R SNPs were the most studied while the C-108T SNP was the least studied. Activity data were available for East Asian, European, Middle East, North Africa, North America and South America. In the collected data, PON1 activity was mostly measured in Caucasian population (5469 measurements from adults in regards to paraoxon, Q192R SNPs) while less than 1222 measurements were available for all other populations (East Asian, Middle East, Tunisia, Chile). Moreover, data retrieved from Caucasian population were the only one that cover all genotypes for all studied probe-substrates. Data gaps were identified for Central and Southern Americans, Africans as well as children and neonates.

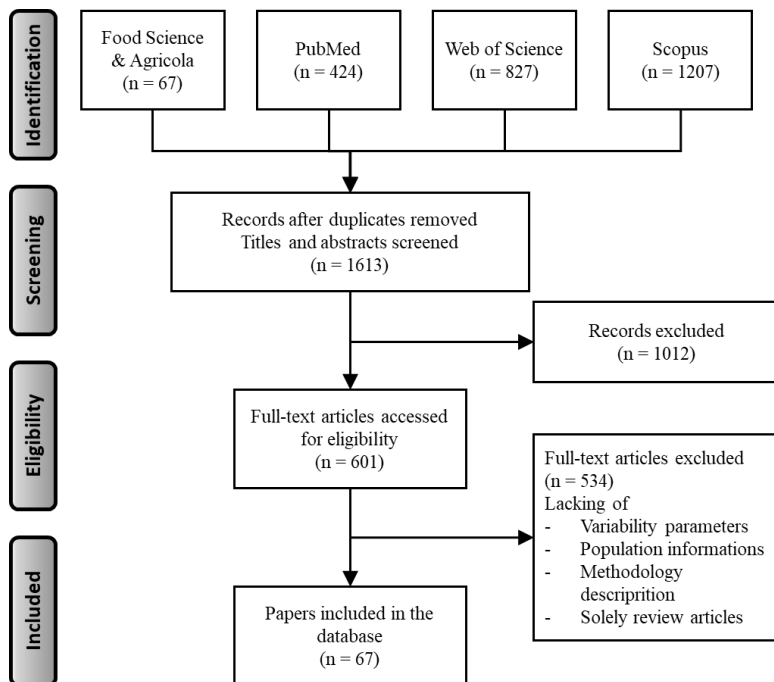


Figure 18. Flow diagram illustrating the extensive literature search of human PON1 activity studies.

PON1 genotypic frequencies were available from the literature for the three SNPs C-108T, L55M and Q192R (Figure 19). The -108CT variant was the most common in the human populations worldwide compared to the homozygous forms with the exception of the Southeast Asians and Middle Eastern populations. The 55LL genotype was dominant in Asian and Central American populations. For human

populations, the 55LL and 55LM genotypes were equally present. Overall, the frequency of the 55MM mutation was below 20% worldwide. The 192QQ genotype was predominant in most populations, except for South and Central Americans as well as East Asians for which the 192QR variant was the most common compared to the homozygous forms. In addition, the 192RR mutation was also the most common in these populations.

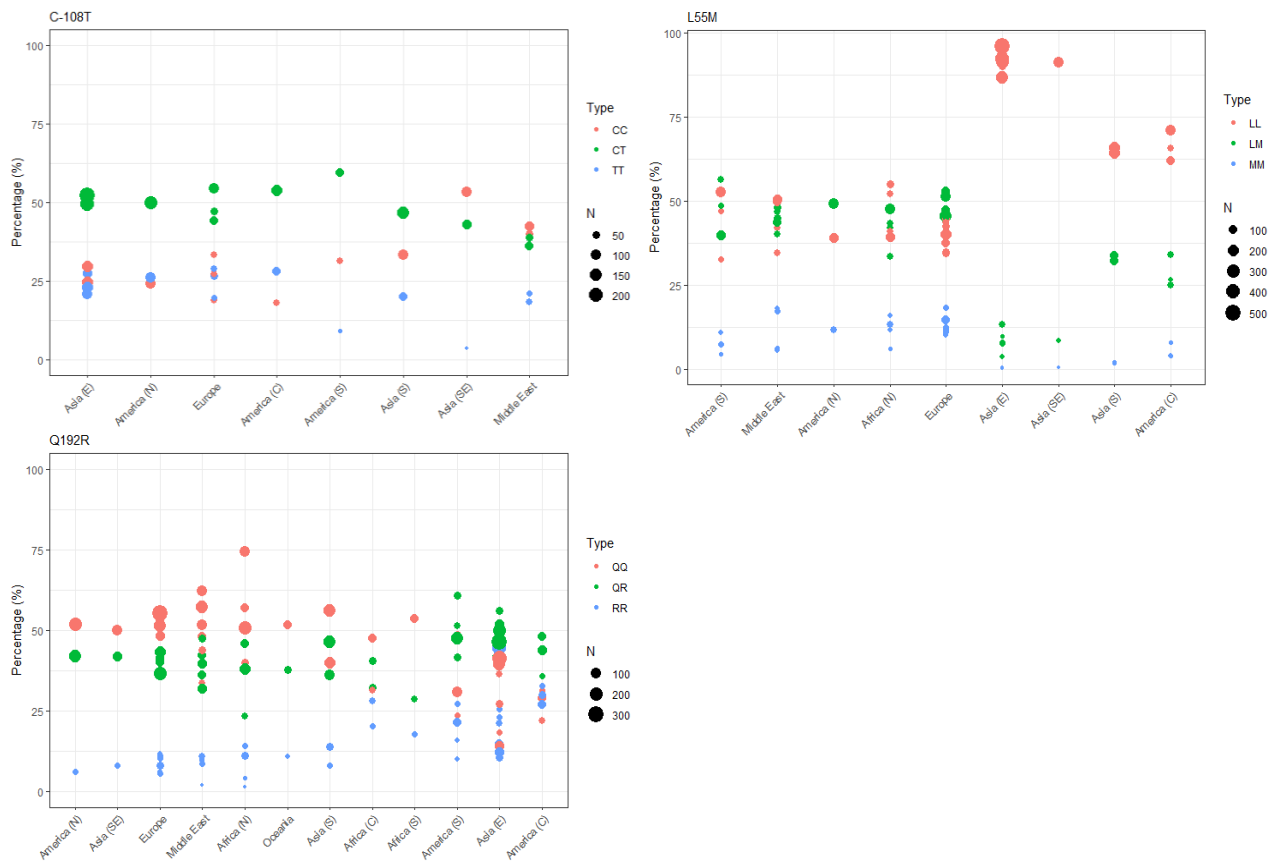


Figure 19. Genotypic frequencies for PON1 Single Nucleotide Polymorphisms in human populations worldwide (L55M, Q192R, C-108T).

PON1 activity for diazoxon and phenyl acetate substrates was the highest in the wild-type groups (CC, LL, QQ). PON1 activity towards paraoxon decreased across phenotypes in the following order CC>CT>TT for PON1 C-108T SNP, and between LL>LM>MM for PON1 L55M SNP with the exception of Q192R (Figure 20) for which the wild type QQ showed a lower activity compared to the mutant RR genotype. An important distinction needs to be highlighted for measurements of PON1 activity using paraoxon with and without salt activation of the enzyme since the latter is not recommended for measurements at the population level. Indeed, salt increases the high-activity allelic form more than other forms while amplifying the variability in the healthy population (Figure 20). All studies included in the database for diazoxon were conducted with salt activation in the assay and PON1 mean activity and its associated variability using diazoxon should be considered with caution. In contrast, salt

addition produces a decrease in PON1 activity while measuring PON1 activity using phenyl acetate and these measurements were performed without salt activation (Ceron et al., 2014).

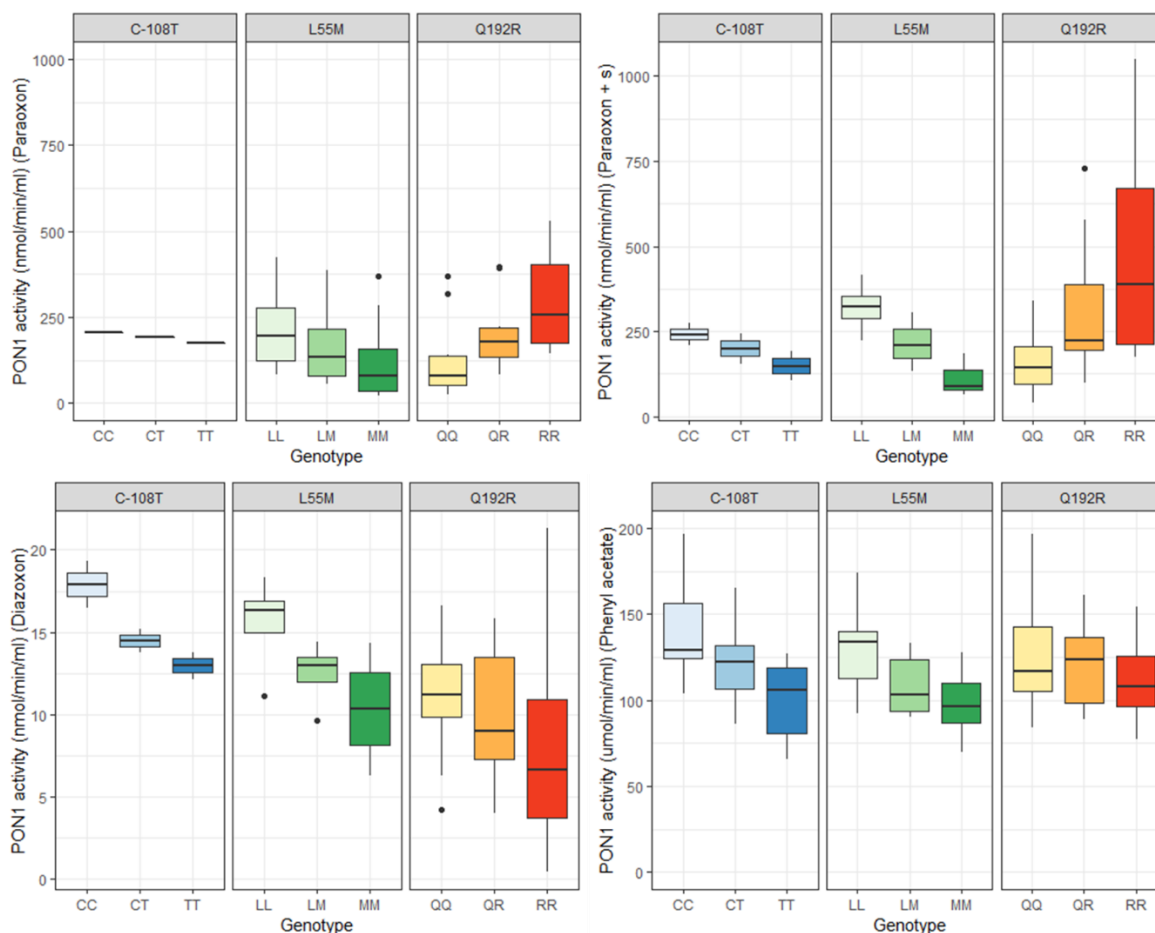


Figure 20. Inter-phenotypic PON1 activities for major human genotypes. PON1 activity characterised by paraoxon can be divided in salt activated (Paraoxon + s) and not salt activated (Paraoxon). Number of papers included: PON1 activity characterised by paraoxon C-108T (3), L55M (11), Q192R (22); PON1 activity characterised by diazoxon C-108T (2), L55M (4), Q192R (8); PON1 activity characterised by phenyl acetate C-108T (5), L55M (7), Q192R (13).

4.3.2. Inter-phenotypic differences in PON1 activity and related UFs

Inter-phenotypic differences for PON1 activity in human populations were calculated without including studies with salt activation for paraoxon for the above-mentioned reasons. Here, the meta-analyses were performed to quantify for inter-phenotypic differences across all populations. Overall, CV values from the meta-analyses highlight a larger variability in PON1 activity with paraoxon for the L55M genotypes (57-62%) (Table 20). PON1-related UFs across the SNPs also showed inter-phenotypic differences. The wildtypes 55LL and 108CC were considered as the reference group with the high PON1 activity, while the mutant 192RR was considered as the reference group for paraoxon. An exceedance of the default TK UF of 3.16 was observed for the wildtype 192QQ, and the mutants 55MM and 108TT, classified as a group with low PON1 activity, with respectively, 3, 2.4 and 1.2-fold differences compared with the reference groups. For the SNP C-108T, a single study was available for paraoxon and results

are associated with high uncertainty and have to be taken with caution. Since salt activated studies were used for diazoxon, the estimated ratio of GM and thus the corresponding UFs were most likely overestimated. All PON1 related UFs were within the default TK UF, except for the genotype -108TT (2 studies, UF97.5 of 3.7- and 1.5- fold difference with -108CC). When considering PON1 activity towards phenyl acetate, estimated CVs were within 23-35% for all SNPs. The largest inter-phenotypic differences between SNPs were 1.5-fold between -108CC and -108TT and PON1-related factors were within the default TK UF.

Table 20. Inter-phenotypic differences in PON1 activity in healthy adult for paraoxon, diazoxon or phenyl acetate probe substrates

PON1	ns	n	CV	GM	ratio GM	UF95 (95% CI)	UF97.5 (95% CI)
Paraoxon (nmol/min/ml)							
-108 CC	1	26	32	197.4		1.7 [1.4-2.6]	1.9 [1.4-3.2]
-108 CT	1	25	45	176.6	1.12	4.1 [0.8-20.0]	5.3 [1.1-25.0]
-108 TT	1	9	46	164.8	1.19	4.3 [0.8-23.0]	5.6 [1.1-30.0]
55 LL	8	1530	58	166		2.4 [2.2-2.7]	2.9 [2.6-3.2]
55 LM	8	1609	62	117.4	1.41	2.2 [1.3-3.8]	2.4 [1.4-4.1]
55 MM	8	481	57	68.4	2.43	3.8 [2.1-6.6]	4.2 [2.3-7.2]
192 QQ	10	1933	32	84.4	3.02	4.6 [2.8-7.5]	5 [3.1-8.2]
192 QR	10	1506	31	167	1.53	2.3 [1.4-2.7]	2.5 [1.6-4.1]
192 RR	10	365	26	254.7		1.5 [1.4-1.6]	1.6 [1.5-1.8]
Diazoxon (nmol/min/ml)							
-108 CC	2	55	39	16.7		1.9 [1.5-2.6]	2.1 [1.7-3.1]
-108 CT	2	81	44	13.3	1.26	2.7 [1.0-6.7]	3.1 [1.2-7.8]
-108 TT	2	54	46	11.4	1.47	3.1 [1.0-8.3]	3.7 [1.5-9.7]
55 LL	4	259	27	14.4		1.5 [1.4-1.7]	1.7 [1.5-1.9]
55 LM	4	341	21	11.9	1.21	2.1 [1.1-3.9]	2.3 [1.2-4.4]
55 MM	4	80	24	9.9	1.45	2.5 [1.3-4.8]	2.8 [1.4-5.4]
192 QQ	8	801	27	9.6		1.6 [1.5-1.6]	1.7 [1.6-1.8]
192 QR	8	699	24	8.6	1.12	1.6 [1.0-2.6]	1.7 [1.1-2.8]
192 RR	8	230	27	4.8	2	2.9 [1.8-4.6]	3.1 [2.0-4.9]
Phenyl acetate (μmol/min/ml)							
-108 CC	7	741	23	133.7		1.4 [1.4-1.5]	1.6 [1.5-1.7]
-108 CT	7	1231	26	113.9	1.17	1.4 [1.1-1.6]	1.4 [1.2-1.6]
-108 TT	7	570	35	86.7	1.54	1.8 [1.5-2.1]	1.8 [1.5-2.2]
55 LL	9	1139	26	119		1.5 [1.5-1.6]	1.7 [1.6-1.8]
55 LM	9	1289	28	101.4	1.17	1.3 [1.1-1.5]	1.4 [1.2-1.6]
55 MM	9	386	32	87.3	1.36	1.5 [1.3-1.8]	1.6 [1.3-1.9]
192 QQ	15	1523	30	118.4		1.6 [1.5-1.7]	1.8 [1.7-1.9]
192 QR	15	1425	27	113	1.05	1.2 [1.0-1.3]	1.2 [1.1-1.3]
192 RR	15	522	23	103.8	1.14	1.3 [1.1-1.4]	1.3 [1.1-1.5]

ns: number of studies, n: number of individuals, CV: coefficient of distribution (lognormal distribution), GM: geometric mean (lognormal distribution), ratio GM: ratio of geometric mean between high activity and sub-group.

4.3.3. Inter-individual differences in PON1 activity and related UFs

From the results of the ELS and meta-analysis, the reference population in the database is the Caucasian population since it is the most data rich. Genotypic frequencies were collected and integrated with inter-phenotypic differences in PON1 activity by means of simulations to derive PON1

variability distributions. The results indicate that the PON1-related UFs did not exceed the default TK UF for median values except for PON1 C-108T and L55M (paraoxon) for a UF97.5 (Table 21). Estimated inter-individual UFs were all lower than those estimated for inter-phenotypic UFs. PON1 variability distributions for Caucasian populations for each genotype are presented in Figure 21.

Table 21. PON1-related uncertainty factors for Caucasian healthy adults integrating inter-phenotypic differences for 3 SNPs (-108 CC/CT/TT: 25/50/25%; 55 LL/LM/MM: 39/48/13%; 192 QQ/QR/RR: 53/39/8%)

PON1	CV	GM	UF95 (95% CI)		UF97.5 (95% CI)	
Paraoxon (nmol/min/ml)						
-108	44	179	3.0	[1.8-14]	3.6	[2-19]
55	79	125	2.7	[2.5-3.9]	3.3	[3-4.8]
192	53	120	2.5	[1.8-4.1]	2.8	[2-4.8]
Diazoxon (nmol/min/ml)						
-108	44	14.9	2.4	[1.9-4.8]	2.8	[2.1-5.9]
55	27	12.5	1.7	[1.4-3.1]	1.8	[1.6-3.4]
192	32	8.73	1.6	[1.5-2.1]	1.8	[1.6-2.3]
Phenyl acetate (μ mol/min/ml)						
-108	32	111	1.6	[1.5-1.7]	1.7	[1.6-1.9]
55	30	106	1.6	[1.5-1.7]	1.7	[1.7-1.9]
192	28	115	1.6	[1.5-1.7]	1.7	[1.7-1.8]

CV: coefficient of distribution (lognormal distribution), GM: geometric mean (lognormal distribution), ratio GM: ratio of geometric mean between high activity and sub-group.

4.1. Discussion and Conclusion

An extensive literature search has been conducted to collect data on PON1 genotypic frequencies and activities across healthy adult world populations for three SNPs. The choice of selecting healthy human populations was related to the fact that PON1 hydrolyses phospholipid peroxides in both high-density lipoprotein and low-density lipoprotein. Bayesian meta-analysis was performed to characterise inter-phenotypic differences in PON1 activities using paraoxon, diazoxon and phenyl acetate as probe substrates and genotypic frequencies of the SNPs L55M, Q192R, and C-108T were collected and integrated to simulate PON1 variability distributions across human populations.

Inter-ethnic differences in PON1 genotypic frequencies Q192R and L55M exist, while these were not observed in the regulatory region C-108T (Ginsberg et al., 2009a). This may be related to random events or selection pressure which may have acted on PON1 polymorphism to maintain specific allele frequencies across different ethnic groups (Brophy et al., 2001; Hernandez et al., 2003). It has been observed that the 55L allele has strong linkage disequilibrium with -108C and 192R alleles respectively, indicating that high PON1 activity individuals tend to have higher PON1 enzyme levels (Koda et al., 2004; Mohamed Ali and Chia, 2008). The frequency of the *PON1* 192QQ genotype is predominantly present in African, European/North American, and Middle Eastern population, indicating that these subpopulations might be more sensitive to OP toxicity as previously suggested (You et al., 2013). In Asian, and Central/South American populations, the 192RR variant is more frequently detected, which

suggest potential decrease in sensitivity to OP toxicity. In addition, the highest PON1 activity toward paraoxon was measured in the *PON1* 192RR genotype in the Asian population (Kujiraoka et al., 2000; Li et al., 2009).

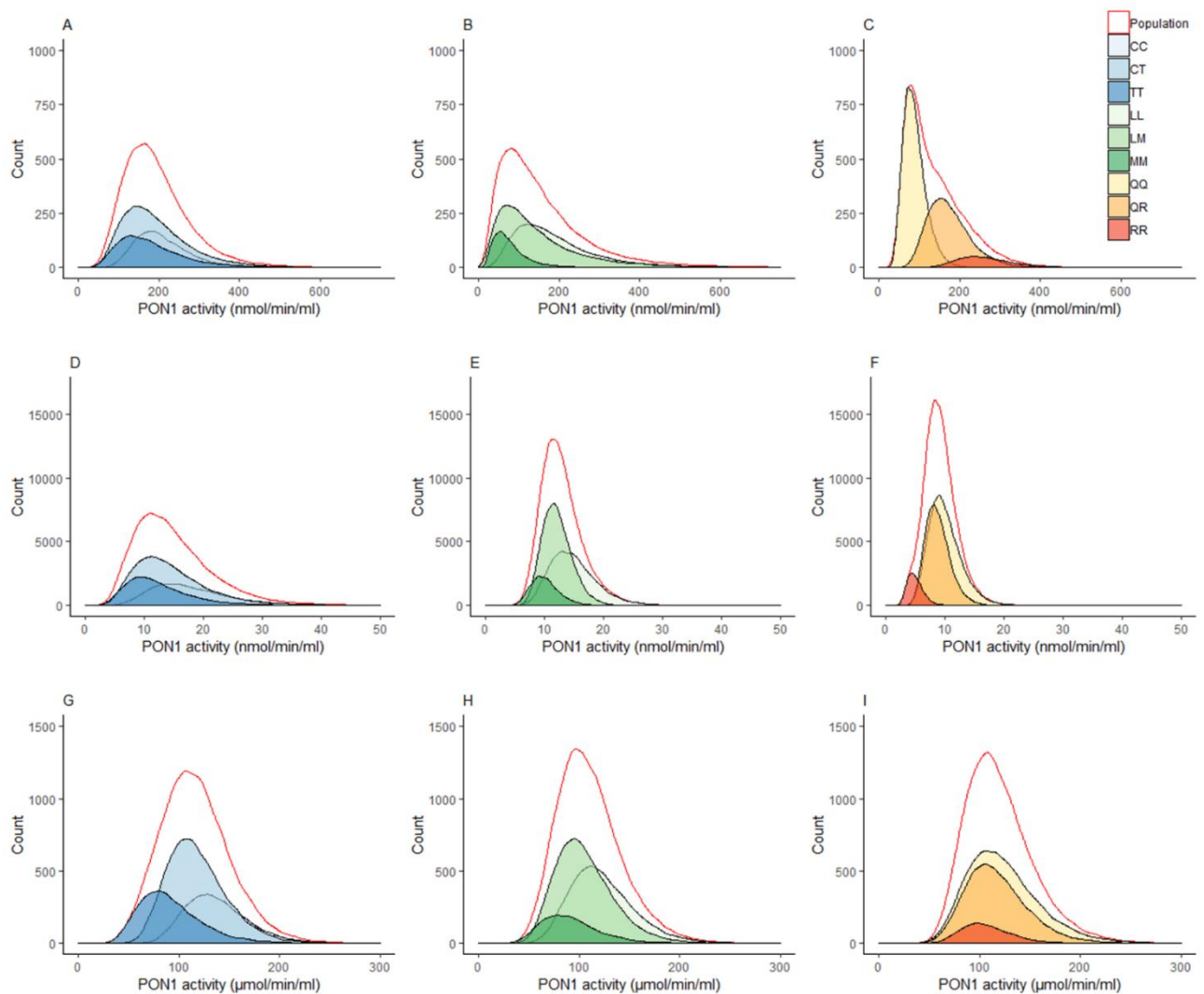


Figure 21. An example of distribution of PON1 activity for Caucasian healthy adults (out of 18000). Simulations of PON1 activity according to different genotypic frequencies (-108 CC/CT/TT: 25/50/25%; 55 LL/LM/MM: 39/48/13%; 192 QQ/QR/RR: 53/39/8%). A, B and C: PON1 activity toward paraoxon, D, E and F: PON1 activity toward diazoxon, G, H and I: PON1 activity toward phenyl acetate.

The PON1 activity data showed a high level of variation, especially for the L55M and C-108T genotypes, which has substantial consequences on the results of the Monte Carlo simulations. It is worth noting that PON1 activities were measured *ex vivo*, so the variability presented in the simulations is not directly reflecting oxon internal dose. In addition to PON1 activities, pharmacokinetic parameters reflecting acute exposure (C_{max}) and chronic exposure (AUC, clearance) would be needed to simulate the population variability in internal dose. Median values for PON1 related UFs were derived while

combining genotypic frequencies for a range of populations and PON1 variability indicated that the PON1-related UFs were generally below the default TK UF with the exception of PON1 C-108T and L55M (paraoxon UF97.5).

PON1 has been described as a good predictor of individual susceptibility to OPs toxicity (Alejo-González et al., 2018; Dardiotis et al., 2019). The meta-analysis confirmed that inter-phenotypic differences in PON1 activity have an impact on the potential susceptibility to OP toxicity in the detoxification of oxon metabolites (Costa et al., 2013). A previous meta-analysis showed that the PON1 192Q and PON1 55L alleles may increase potential susceptibility to OP toxicity for paraoxon, particularly in Caucasian populations (You et al., 2013). While our results confirm that PON1-related UFs are above the 3.16 default TK UF for the PON1 192QQ genotypes, this is not the case for the PON1 55LL genotypes. Since PON1 55MM shows a lower activity of the enzyme, susceptibility to paraoxon toxicity may increase and is indicated by an exceedance of the default TK UF. Overall, this body of evidence has been further demonstrated in previous analyses as a correlation between low PON1 activity and susceptibility to OP for a number of congeners (Costa et al., 2013). In contrast, PON1 activities for the R192Q SNP, using diazoxon as the probe substrate, did not conclude on an increase in susceptibility since the 192RR isoform displayed a lower activity toward diazoxon compared to that for the 192QQ SNP (Davies et al., 1996; Ginsberg et al., 2009a). On the other hand, phenyl acetate hydrolysis was mostly influenced by C-108T polymorphism and to a lesser extent by L55M whereas Q192R polymorphism had almost no effect and confirms its relevance as a marker of PON1 activity in human serum (Dardiotis et al., 2019). It has been hypothesised that the observed differences in PON1 activity for various substrates may arise from differences in docking sites on the enzyme. Chlorpyrifos-oxon binds similar sites as paraoxon, where the 192R and 55M alleles are the most active (Albers et al., 2010; Costa et al., 2013; Ellison et al., 2012; Ginsberg et al., 2009a). On the other hand, diazoxon share the same docking sites as sarin and soman for which individuals expressing the 192Q allele are potentially more sensitive to their toxicity (Davies et al., 1996). Based on the available data, the genotype alone is not sufficient to determine individual susceptibility to OP toxicity for a range of congeners and inter-phenotypic differences together with substrate-specific information about the specific substrate would be most appropriate to characterise such susceptibility. It is foreseen that as compound specific docking information becomes readily available, PON1 variability distributions (or UF) can be integrated to characterise susceptibility to OP toxicity bearing in mind the limitation that PON1 crystal structure remains to be elucidated (Dardiotis et al., 2019).

Although exclusion criteria have been defined to limit possible bias in the meta-analysis due to technical aspects of the PON1 assay itself or to the lifestyle of the enrolled individuals, PON1 activities have been measured *ex vivo* and can be influenced by sources of variability other than genotypes

(Ceron et al., 2014; del Carmen Xotlanihua-Gervacio et al., 2019; Ginsberg et al., 2009a). Nonetheless, 65-92% of the variability in PON1 activities can be rationalised through inter-phenotypic differences, while lifestyle contributed only for 6% to such variability (Rainwater et al., 2009). In addition, *in vivo* studies suggest, that differences in serum PON1 phenotypes are relevant for predicting the sensitivity of high chlorpyrifos-oxon concentrations (Coombes et al., 2014; Li et al., 2000). However, at environmentally relevant concentrations, reported in the nanomolar range, no significant differences were found in the hydrolysis of chlorpyrifos-oxon between PON1 192 SNPs (Coombes et al., 2014).

In addition to PON1 polymorphisms, it is suspected that variability in Acetylcholinesterase (AChE) activity, breaking down esters of choline molecules, may influence OP toxicity as they are specific inhibitors of AChE (Lionetto et al., 2013). The AChE gene is well conserved in humans and has almost no loss of function via mutations; the most frequent AChE variant being His353Asn, resulting in a phenotype with normal activity (Lockridge et al., 2016). PON1 is closely located (5.5 Mb) to the AChE gene on chromosome 7 and it has been suggested that interactions between the AChE and PON1 occur and that the two genes are regulated on the same locus region. In addition, it has been shown that individuals with high PON1 activities had lower AChE activities whereas individuals with low PON1 activities had higher AChE activities. This has been explained for scenarios of low OP exposure under which AChE is inhibited in individuals with low PON1 levels, resulting in an upregulation of AChE. In individuals with high PON1 activity the degree of AChE inhibition will be minor with no consequence on upregulation of the enzyme, resulting in low plasma levels (Akgur 1999, Bryk 2005).

For Butyl Cholinesterase (BChE), more variants lead to lower BChE activity compared to the wildtype. However, it has been shown that OP inhibition of BChE by up to 85% did not result in any clinical signs and therefore it is unlikely that BChE (and genetic variants herein) contributes to OP toxicity (Lockridge et al., 2016; Nolan et al., 1984). Overall, the observed variability in the susceptibility to OP toxicity has been shown to be based mainly on inter-individual differences in PON1, cytochrome P450, and glutathione-s transferase activities so that variability in TK processes seem to be the driving variable to the toxicodynamics (TD) of OPs (Lockridge et al., 2016). It is proposed that future research would aim at unravelling human variability in AChE inhibition of after exposure to OPs, besides the variability in baseline activity. However, it is rather difficult to measure the TD contribution alone in *in vivo* studies, since TK variability is most often accounted for. Nevertheless, variability in the reactivation of AChE after exposure to OPs has been shown and has been used to parameterise PBTK-TD models for chlorpyrifos and other OPs (Poet, 2017). Blood has been sampled and the inhibition and spontaneous reactivation of AChE has been measured *in vitro* together with variation in reactivation half-life

(Mason, 2000). Data on TD and differences in AChE binding affinity to OPs are scarce, and more data should be generated to estimate inter-individual differences in this parameter.

The results of this meta-analysis provided inter-phenotypic and inter-individual differences distributions for PON1 activities and PON1-related UFs. Variability distributions can be implemented in generic PBK models to derive internal concentrations of chemicals. This would allow to model inter-individual differences in potential sensitivity to OP toxicity for chemical risk assessment purposes. Furthermore, variability distributions can provide inputs for the calibration of human quantitative *in vitro in vivo* extrapolation (QIVIVE) models. This approach has the advantage to integrate isoform-specific metabolism information and human variability distributions for chemical risk assessment. PON1-related UFs provides an intermediate option between CSAF and the default UF when chemical-specific data are not available (Bhat et al., 2017; Clewell et al., 2008). Overall, inter-phenotypic differences in PON1 activity are important for chemical risk assessment.

5. Human variability in isoform-specific UDP-glucuronosyltransferases: markers of acute and chronic exposure, polymorphisms and uncertainty factors

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Abstract

UDP-glucuronosyltransferases (UGTs) are involved in phase II conjugation reactions of xenobiotics and differences in their isoform activities result in interindividual kinetic differences of UGT probe substrates. Here, extensive literature searches were performed to identify probe substrates (14) for various UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7 and UGT2B15) and frequencies of human polymorphisms. Chemical-specific pharmacokinetic data were collected in a database to quantify interindividual differences in markers of acute (C_{max}) and chronic (area under the curve, clearance) exposure. Using this database, UGT-related uncertainty factors were derived and compared to the default factor (i.e. 3.16) allowing for interindividual differences in kinetics. Overall, results show that pharmacokinetic data are predominantly available for Caucasian populations and scarce for other populations of different geographical ancestry. Furthermore, the relationships between UGT polymorphisms and pharmacokinetic parameters are rarely addressed in the included studies. The data show that UGT-related uncertainty factors were mostly below the default toxicokinetic uncertainty factor of 3.16, with the exception of five probe substrates (1-OH-midazolam, ezetimibe, raltegravir, SN38 and trifluoperazine), with three of these substrates being metabolised by the polymorphic isoform 1A1. Data gaps and future work to integrate UGT-related variability distributions with in vitro data to develop quantitative in vitro–in vivo extrapolations in chemical risk assessment are discussed.

Keywords: human variability, pharmacokinetics, uncertainty factor, UGT, polymorphism

Highlights:

- Extensive literature search of human kinetic parameters for UGT probe substrates
- Bayesian meta-analysis quantifying human variability in acute and chronic kinetic parameters
- UGT isoform-related uncertainty factors were below the 3.16 kinetic default uncertainty factor for most probe substrates
- Quantifying human variability in UGT polymorphisms

5.1. Introduction

Glucuronidation is an enzymatic reaction catalysed by UDP-glucuronosyltransferase (UGT) isoforms which involves the conjugation of endogenous substrates (e.g. bilirubin) and xenobiotics [e.g. pharmaceuticals (morphine), dietary chemicals (flavonoids), and environmental contaminants (mycotoxins)] with glucuronic acid (Dong et al., 2012; Lv et al., 2019). In humans, glucuronide conjugates are water soluble and readily excreted in the urine or the faeces resulting in increased elimination and most often inactivation of the compound, thereby contributing to xenobiotic detoxification (Fisher et al., 2001). Multiple UGT isoforms are often involved in xenobiotic metabolism, which, from a toxicological viewpoint, is advantageous as dysfunctionality of an isoform does not necessarily result in the impaired elimination and thus detoxification of chemicals (Lv et al., 2019). Since UGTs are ubiquitous in pharmacokinetic and toxicokinetic processes (absorption, distribution, metabolism and excretion (ADME)), their involvement in human metabolic variability is important.

The superfamily of UGT isoforms has a nomenclature which is based on similar features to that described for the cytochrome P450 (CYP) superfamily (Meech et al., 2019; Rowland et al., 2013). The subfamilies of UGT1A and UGT2B are predominantly expressed in the liver as well as in the intestine and kidney, where they mediate intestinal and hepatic first-pass glucuronidation of many phenolic compounds, including pharmaceuticals and natural phenols (Dong et al., 2012) (Figure 22). The most clinically relevant hepatic UGTs are UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7 and 2B15 (Rowland et al., 2013; Stingl et al., 2014). Other UGTs from the 2B subfamily are mainly responsible for the metabolism of endogenous compounds rather than xenobiotics, such as steroids (2B4, 2B15 and 2B17) and bile acids (2B4) (Fisher et al., 2001).

UGT isoforms are known to be highly polymorphic with more than a hundred variants described (Stingl et al., 2014). In most cases, these polymorphic variants result in lower expression levels and/or lower activity, and in some instances even complete loss of activity (Sim et al., 2013). Because of such changes in expression and/or activity, polymorphic UGT variants may cause higher plasma concentrations of (toxic) metabolites or parent compounds, resulting in chemical-induced toxicity. For example, UGT1A1 polymorphism is associated with irinotecan toxicity, while UGT2B7 gene polymorphism can affect plasma concentrations of valproic acid (Tsunedomi et al., 2017; Wang et al., 2018). For other isoforms, comparable impact of UGT polymorphisms on internal drug concentrations have been observed (Stingl et al., 2014).

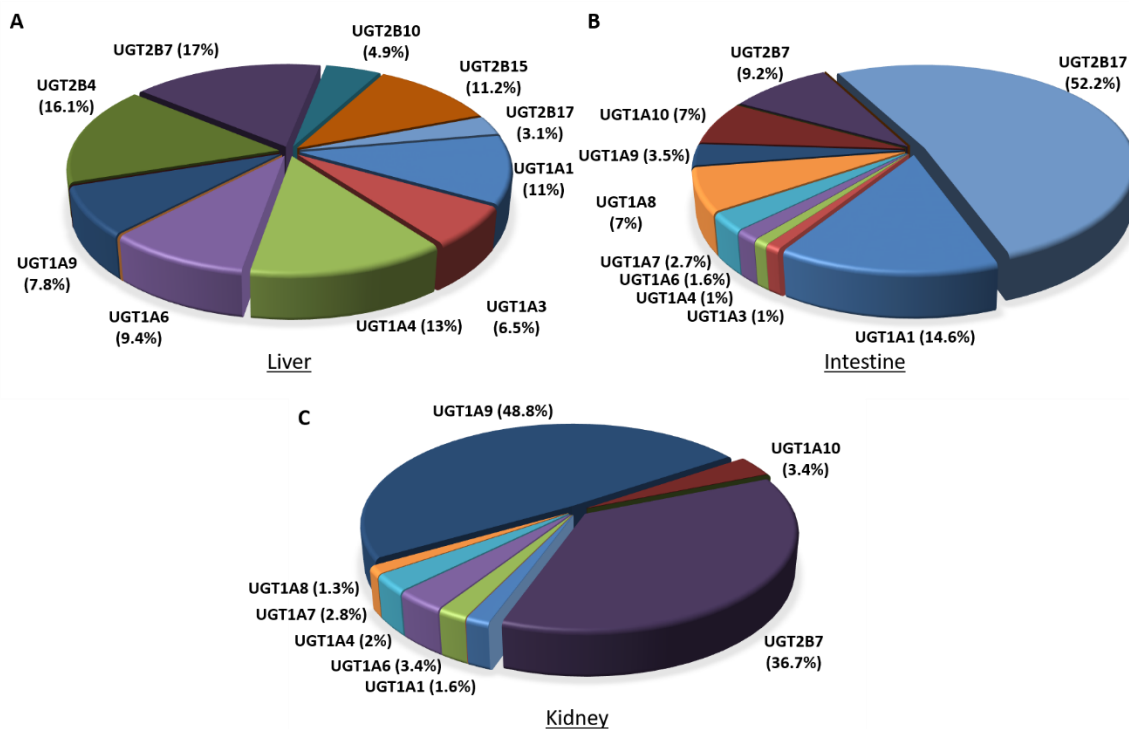


Figure 22. Average distribution of the major UDP-glucuronosyltransferase isoforms in human liver (A), intestine (B) and kidney (C) (Lv et al., 2019).

For the last 70 years, a 100-fold default uncertainty factor (UF) has been applied to derive chronic safe levels of exposure for non-cancer effects of chemicals. This default factor allows for interspecies differences (10-fold) and human variability (10-fold) to chemical exposure. In the 1990s, the 10-fold factor allowing for human variability has been refined to a composite value of two factors of 3.16 ($10^{0.5}$), accounting respectively for interindividual differences in toxicokinetics and toxicodynamics (Renwick and Lazarus, 1998). However, interindividual differences between healthy adults and potentially sensitive subgroups including neonates, elderly and poor metabolisers expressing polymorphic UGT genes may not be covered by the default kinetic factor (Dorne et al. 2001b; Renwick and Lazarus 1998). Under such circumstances, pathway-specific UFs or chemical-specific adjustment factors (CSAFs) have been proposed and can provide an option to replace such default UFs. Pathway-related UFs to account for variability have been described for CYP3A4 as well as efflux and influx transporters (Darney et al. 2019; Darney et al. 2020; Dorne et al. 2001b). Human variability in glucuronidation processes in relation to UFs has been described earlier by Dorne et al. (2001a), however, at the time, information on isoform specificity and genetic polymorphisms was very limited.

The manuscript aims to investigate human variability in UGT activity through (1) identifying isoform-specific UGT probe substrates and collecting pharmacokinetic data for intravenous and oral markers of acute (maximum concentration (C_{max})) and chronic exposure (clearance, area under the curve (AUC)) by means of extensive literature searches and meta-analyses, (2) quantifying interindividual

differences in pharmacokinetics by means of hierarchical Bayesian meta-analyses to derive UGT-related variability distributions and UGT-related UFs. Such UGT-related UFs are relevant to refine toxicokinetic UFs for risk assessment of toxicants, nutrients and environmental xenobiotics that are metabolised by UGTs, and (3) unravelling the frequencies and pharmacokinetic consequences of UGT polymorphisms in human populations. A graphical abstract is depicted in Figure 23.

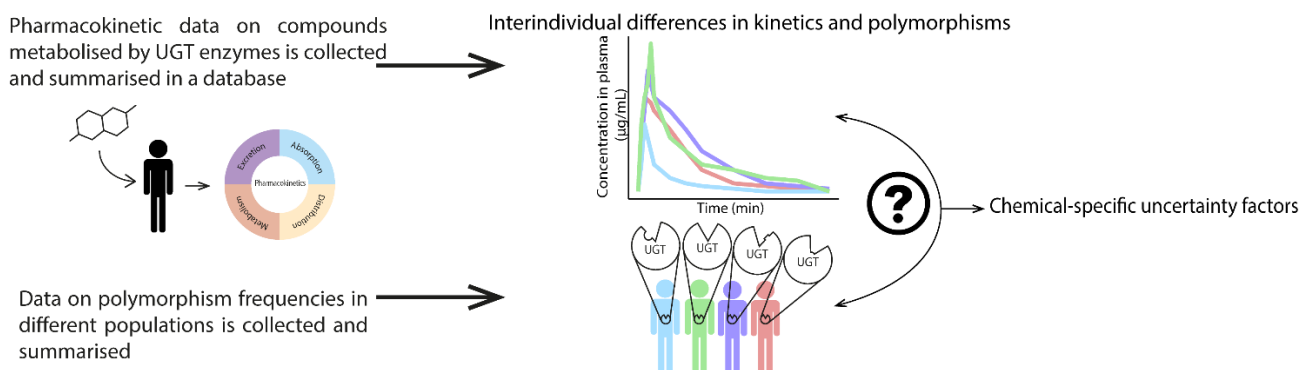


Figure 23. Graphical abstract summarizing the aim of this study.

5.2. Materials and Methods

5.2.1. Extensive literature searches (ELS) and data collection

UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7 and 2B15 were identified as the most clinically relevant UGT isoforms for xenobiotic metabolism (Rowland et al., 2013; Stingl et al., 2014). Probe substrates for these UGT isoforms were identified from the *in vitro* and *in vivo* literature as compounds metabolised by extensive glucuronidation (>60% of the dose excreted in the urine) (Lv et al., 2019; Rowland et al., 2013; Stingl et al., 2014; Yang et al., 2017).

ELS were performed using two main databases (i.e. Scopus and PubMed) to identify human pharmacokinetic (PK) studies in non-phenotyped adults for isoform-specific UGT probe substrates in adults of different geographical ancestry or ethnic background. A PK database was then computed, including intravenous and oral markers of acute (C_{max}) and chronic (clearance and AUC) exposure. Search queries for the probe substrate deferiprone are illustrated in Table 22 and queries for all other substrates are provided in Supplementary Material 1. Data reporting frequencies of UGT polymorphisms distribution and the associated PK parameters in phenotyped individuals were collected using a horizontal literature search in Google Scholar.

Table 22. Keyword queries for the Extensive Literature Searches (formatted for Scopus).

General search terms	TITLE-ABS (patient*) OR 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-
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	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")
Search terms for probe substrates	(TITLE-ABS (deferiprone) OR TITLE-ABS (ferriprox))
Search terms for pharmacokinetics	TITLE-ABS-KEY (auc) OR TITLE-ABS-KEY (area AND under AND the AND curve) OR TITLE-ABS-KEY (area AND under AND curve) OR TITLE-ABS-KEY (half AND life) OR TITLE-ABS-KEY (half-life) OR TITLE-ABS-KEY (half-lives) OR TITLE-ABS-KEY (clearance) OR TITLE-ABS-KEY (cmax) OR TITLE-ABS-KEY (vmax) OR TITLE-ABS-KEY (km) OR TITLE-ABS-KEY ("michaelis constant") OR TITLE-ABS-KEY (pharmacokinetic) OR TITLE-ABS-KEY (pharmacokinetics) OR TITLE-ABS-KEY (toxicokinetic) OR TITLE-ABS-KEY (toxicokinetics)
Exclusion	TITLE-ABS-KEY ("cell line*") OR TITLE-ABS-KEY ("cell culture*") OR TITLE-ABS-KEY (rat) OR TITLE-ABS-KEY(rats) OR TITLE-ABS-KEY (mouse) OR TITLE-ABS-KEY (mice)

TITLE-ABS-KEY: term searched in the title, the abstract and the keywords of the paper.

A two-step process was conducted to screen the retrieved studies from literature as described previously (Darney et al., 2019). This process was used to assess whether reported PK values were suitable for inclusion in the database. After removing duplicates, the following exclusion criteria were applied: 1. species other than humans, 2. in vitro studies, 3. development of analytical methods, 4. modelling studies, 5. pharmacodynamics investigations only, 6. substrates other than those identified as relevant and/or mixtures of substrates. Articles meeting the exclusion criteria were not considered for further evaluation. Furthermore, articles that were written in any other language than English or did not contain original research data (e.g. reviews) were excluded from analysis. Overall, data on non-phenotyped healthy individuals were collected and included in the meta-analysis (see “Data standardisation and meta-analyses”). The specific selection of subgroups is described in Supplementary Material 1. In a second step, the full text of the included papers was checked for PK parameter values after single-dose exposure. Repeated dosing studies and studies for which multiple formulas were administered to the same group of volunteers were excluded. However, for ethinylestradiol, data were included for both single dose and repeated dosing for 21 days, the standard regimen for contraception drugs.

5.2.2. Data standardisation and meta-analyses

Meta-analyses of PK parameter values were performed in non-phenotyped subjects for each probe substrate to derive UGT-related variability distributions and UGT-related UFs. For this purpose, each PK parameter was normalised in a harmonised manner (Cmax expressed in ng/mL; AUC ng*h/mL and

clearance in L/h/kg bw) while applying body weight correction to the applied doses (mg/kg bw). If available, the reported (mean) body weight was used, or continent specific body weights were used to normalise the dose if body weight data were not available (Walpole et al., 2012). For SN38, the dose was normalised to body surface area instead of body weight, as this is the standard measure for this compound. If body surface area data were not available, a default value of 1.79 m² was used (Sacco et al., 2010). Data from these studies were extracted mostly as arithmetic mean (AM) and standard deviation (SD), but sometimes geometric means (GM) and geometric standard deviations (GSD) were reported. Generally, PK data are recognised to follow a lognormal distribution (Dorne et al., 2001b; Naumann et al., 1997; Renwick and Lazarus, 1998). Since GM and GSD are more appropriate to summarise a lognormal distribution, all pharmacokinetic data were described as GM and GSD using the following equations:

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

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

where X is the arithmetic mean and 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 estimated from the standard error (SE, SEM) or CV using equations described previously (Darney et al., 2019).

The objective of the meta-analyses is to provide accurate information regarding interindividual differences in non-phenotyped adults of the PK parameters for a substrate expressed as distributions. Variability related to interstudy, intersubstrate and interindividual differences was analysed for each substrate and parameter and for each UGT isoform, through a decomposition of the PK parameter variance (clearance, AUC or C_{max}) 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 most compounds, except for zidovudine and oxazepam for which kinetic variability was previously meta-analysed (Dorne et al., 2001a).

The meta-analyses provided distributions describing interindividual differences for each PK parameter with quantitative uncertainty using median values and 95% confidence intervals. The coefficient of variation (CV) was also estimated as follows:

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

where τ_j is the interindividual difference of the activity for a substrate 'j'.

UGT isoform-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.

5.2.3. 2.3 Software

All statistical analyses were performed in R (version 3.5) and the Bayesian modelling was implemented in Jags (4.2.0) (Plummer, 2003). Data processing and graphical display were performed in R (dplyr and ggplot2 packages) (R Development Core Team, 2018; Wickham, 2016; Wickham et al., 2019). References of the studies used to compile the database were stored and sorted in EndNote X8.

5.3. Results and Discussion

5.3.1. Extensive literature searches and data collection

UGT isoforms can conjugate a wide variety of substrates and show a broad overlapping substrate specificity. This is advantageous when detoxifying chemicals; however, because of such overlap, identifying selective probe substrates for each isoform remains a challenge. Moreover, UGTs are also present in the gastrointestinal tract and pre-systemic conjugation occurs readily for a range of compounds. Here, to quantify isoform-specific variability in UGTs, selective probe substrates with available PK data for each isoform were selected. Moreover, differences in variability between oral and intravenous data were compared to investigate the contribution of bioavailability and pre-systemic conjugation after oral intake. A total of 14 isoform-specific UGT probe substrates covering both the UGT1A and UGT2B subfamilies were identified, namely 1-OH-midazolam (UGT1A4), codeine (UGT2B7), deferiprone (UGT1A6), entacapone (UGT1A9), ethinylestradiol (UGT1A1), ezetimibe (UGT1A1/UGT1A3), mycophenolic Acid (UGT1A9), oxazepam (UGT2B15/UGT1A9), propofol (UGT1A9), raltegravir (UGT1A1), SN38 (UGT1A1), telmisartan (UGT1A3), trifluoperazine (UGT1A4) and zidovudine (UGT2B7).

From the ELS, a total of 7173 papers were assessed from Scopus and PubMed (up to August 2019) and an extra 11 papers were retrieved from Google Scholar, for the 13 UGT isoform probe substrates and for zidovudine, 10 studies were included from a previous database (shared by Dr. N. Quinot, collated for EFSA/SCER/2014/06 project). PRISMA flow diagrams for the individual compounds are provided in Supplementary Material 2. Figure 24 provides a summary PRISMA diagram for all papers collected in the ELS (Moher et al., 2009). Overall, a total of 210 peer-reviewed publications were selected from the ELS and included in the database. Supplementary Material 1 provides the search queries for both Scopus and PubMed for the individual compounds. Table 23 illustrates the selected probe substrates, the structure of the compounds, bioavailability, percentage of glucuronidation and their site of glucuronidation.

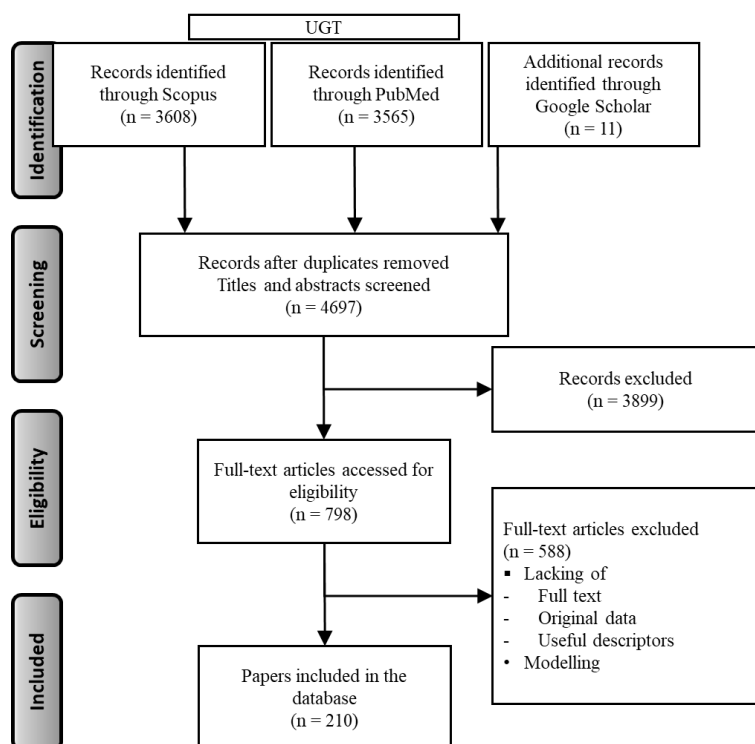
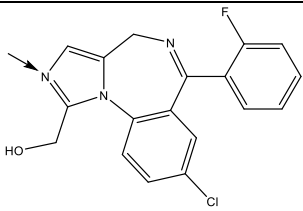
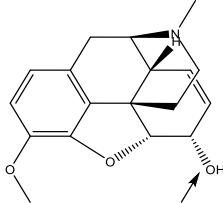
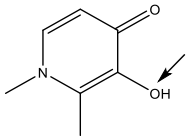
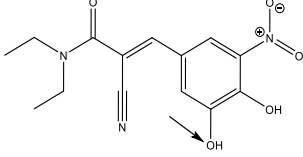
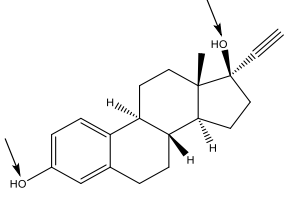
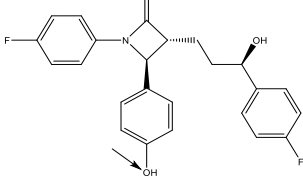
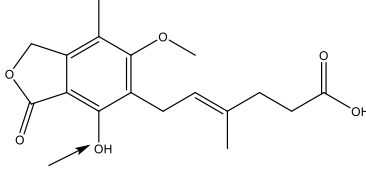
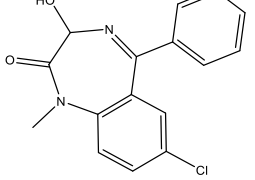
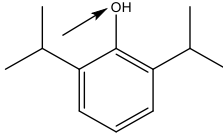
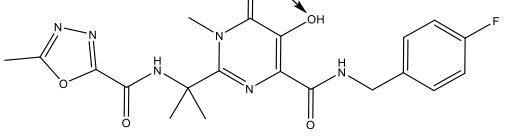
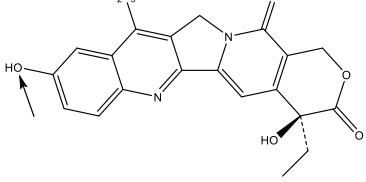
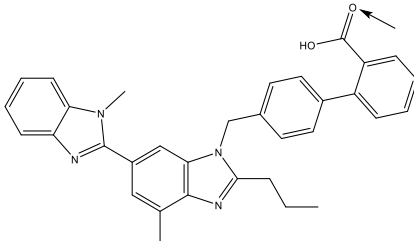
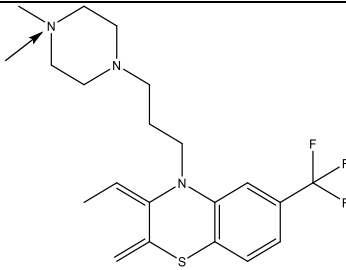
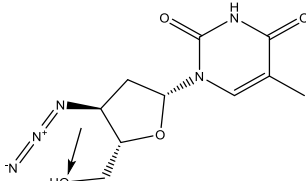


Figure 24. PRISMA diagram illustrating the extensive literature searches performed for the 13 isoform-specific UGT probe substrates (UGT1A and UGT2B subfamilies) and human pharmacokinetic studies.

Table 23. Isoform-specific UGT probe substrates. Name of probe substrate, major UGT isoform involved in glucuronidation (in bold), % bioavailability, % of dose metabolised by UGT and chemical structure are reported. Arrows indicate the main site(s) of glucuronidation.

Probe substrate	UGT isoform	Bioavailability (%)	% UGT metabolism ^a	Chemical structure
1-OH-midazolam	N-glucuronidation by 1A4 , O-glucuronidation by 2B7 ¹	31-72 ²	1-2 ³	
Codeine	2B7 ⁴	50-55 ⁵	44-62 ⁶	
Deferiprone	1A6 , other UGTs are negligible ⁷	73 ⁸	58 ⁹	

Entacapone	Mainly 1A9 , minor contribution of 1A1, 2B7 and 2B15 ¹⁰	35 ¹¹	70 ¹²	
Ethinylestradiol	Mainly 1A1 , rates by other UGTs are negligible ¹³	55 ¹⁴	~65 ¹⁵	
Ezetimibe	1A1 and 1A3 , small contribution of 2B7 and 2B15 ¹⁶	n.d. ^b	80-90 ^{c, 17}	
Mycophenolic Acid	1A9 is most efficient, small contribution of 2B7 ¹⁸	95% ^{19,d}	71 ²⁰	
Oxazepam	S-oxazepam mostly by 2B15 , R-oxazepam by 1A9 ²¹	93 ²²	>70 ²²	
Propofol (iv)	1A9 , other UGTs are negligible ²³	e	62 ²⁴	
Raltegravir	Mainly 1A1 , small contribution of 1A3 and 1A9 ²⁵	30 ²⁶	70 ²⁵	
SN38	Mainly 1A1 , small contribution of	~10 ^{28,f}	3 ²⁹	

	1A9, 1A6 and 1A3 ²⁷			
Telmisartan	Mainly 1A3 (>97%), also 1A1, 1A7, 1A8 and 1A9 ³⁰	40-60 ^{31,32}	16 ^{b, 32}	
Trifluoperazine	1A4 ³³	100 ³⁴	unknown	
Zidovudine	2B7 ³⁵	63 ³⁶	86 ³⁷	

Name of probe substrate, major UGT isoform involved in glucuronidation (in bold), % bioavailability, % of dose metabolised by UGT and chemical structure are reported. Arrows indicate the main site(s) of glucuronidation.

¹(Seo et al., 2010), ²(Heizmann et al., 1983), ³(Hyland et al., 2009), ⁴(Coffman et al., 1997), ⁵(Rogers et al., 1982), ⁶(Yue et al., 1989), ⁷(Benoit-Biancamano et al., 2009b), ⁸(Medicine, 2014), ⁹(Rodrat et al., 2012), ¹⁰(Lautala et al., 2000), ¹¹(Heikkinen et al., 2001), ¹²(Wikberg et al., 1993), ¹³(Ebner et al., 1993; Lv et al., 2019), ¹⁴(Fotherby, 1996), ¹⁵(Williams and Goldzieher, 1980), ¹⁶(Ghosal et al., 2004), ¹⁷(Kosoglou et al., 2005), ¹⁸(Picard et al., 2005), ¹⁹(Armstrong et al., 2005), ²⁰(Bullingham et al., 1996), ²¹(Court et al., 2002), ²²(Sonne et al., 1988), ²³(Seo et al., 2014), ²⁴(Favetta et al., 2002), ²⁵(Kassahun et al., 2007) fraction of dose metabolized by UGT1A1, ²⁶(Brainard et al., 2011), ²⁷(Hanioka et al., 2001), ²⁸(Furman et al., 2009), ²⁹(Slatter et al., 2000), ³⁰(Yamada et al., 2011), ³¹(Wienen et al., 2000), ³²(Stangier et al., 2000a), remainder is unchanged parent compound, ³³(Seo et al., 2014), ³⁴(Midha et al., 1984), ³⁵(Barbier et al., 2000), ³⁶(Klecker et al., 1987), ³⁷(Gallicano et al., 1999); ^a Expressed as % of the dose recovered in urine as the glucuronide, ^b n.d. = not determined. The bioavailability of ezetimibe cannot be determined, because it is insoluble in aqueous media and cannot be used for iv injection (Kosoglou et al., 2005), ^c Expressed as % of dose as glucuronide in plasma, ^d mycophenolic acid is given as a prodrug, mycophenolate mofetil, ^e no bioavailability is given for propofol, as all studies in the database are iv studies, ^f Bioavailability of irinotecan, the parent drug of SN38.

5.3.2. Interindividual differences for the kinetics of isoform-specific UGT probe substrates and related uncertainty factors in non-phenotyped adults

Results of the meta-analyses are expressed as geometric means (normalised to dose and body weight) for the 14 isoform-specific UGT probe substrates and are illustrated for markers of acute (C_{max}) and chronic (AUC/clearance) exposure after oral and intravenous administration in Figure 25. The full dataset of extracted information used can be accessed on EFSA knowledge junction. Data availability was variable for each UGT probe substrate and interstudy differences are reported for each compound. For SN38, only patient data were available and no data for healthy adult individuals were reported in the literature. For deferiprone, no clear distinction could be made between healthy adult data and patient data for all three parameters, suggesting that the condition of the individuals did not influence

the pharmacokinetics of this compound. In Fig. 5, isoform-specific interindividual differences in AUC are illustrated for world populations from different geographical ancestry or country of origin (one probe substrate per UGT isoform). These plots indicate that no clear differences in chronic exposure (AUC) can be demonstrated across world populations from different geographical ancestry and, therefore, there is indication of significant interethnic differences for these probe substrates. The same conclusion holds for other PK parameters and other probe substrates, which are illustrated in Supplementary Material 3.

Interindividual differences were higher compared to an earlier study (Dorne et al., 2001a), which included 11 probe substrates compared to 14 here; with an overlap of only two probe substrates (zidovudine (AZT) and oxazepam). In addition, Dorne et al. (2001a) mostly considered UGT2B7 probe substrates while UGT1A1-specific probe substrates were not included since they were not available at that time. Polymorphisms have the highest impact on the PK of UGT1A1 probe substrates, which may explain the larger interindividual differences in this study. Finally, the 2001 study investigated PK data for potentially sensitive subgroups including neonates, infants, children, and the elderly, but little to no data for these subgroups were available for the included probe substrates here (Dorne et al., 2001a). It is worth noting that UGT metabolism in neonates is impaired and they show a low glucuronidation activity (Allegaert et al., 2009). Data for such PK differences in markers of acute and chronic exposure are still very limited for these subgroups, but can reach 2-3-fold in comparison with healthy adults, so that the default kinetic factor may be inadequate and an extra UF may be required to cover these subgroups (Dorne et al., 2001a; Dorne et al., 2005).

UGT1A1

For UGT1A1, ethinylestradiol, ezetimibe, raltegravir and SN38 were identified as probe substrates. Besides pharmaceuticals, UGT1A1 is involved in the glucuronidation of several compounds important in (food) toxicology, including the naturally occurring food components resveratrol and several hydroxyflavones, the heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and the phytochemical ferulic acid (Brill et al., 2006; Li et al., 2011; Malfatti and Felton, 2004; Tang et al., 2010). For ethinylestradiol, only single-dose studies were used to quantify the UGT1A1 variability. PK data were available for Europeans, East Asians, South Asians, Southeast Asians, North Americans, South Americans, North Africans and Middle Eastern adults with the majority of the datasets from North American and European studies. Data gaps were identified for specific groups like Central Americans and Africans. Chemical-specific CVs ranged from 35 to 72%, while isoform-related CVs ranged from 46 to 51% (Table 24). Overall, the UGT1A1 related UFs were most often below or close to the default TK UF of 3.16 for at least 97.5% of the healthy adults when considering the median value. However, our analysis by the Bayesian model takes into account the uncertainty around the estimation

of the UF and this shows that, given the available data (number of studies and number of individuals per study), the default factor may not cover all possible cases. Indeed, the upper bound of the confidence interval is higher than 3.16. The chemical-specific data show that SN38, ezetimibe and raltegravir all have an UF_{97.5} higher than 3.16, ranging from 3.2 to 3.6 (Table 24). Ethinylestradiol is the only UGT1A1 probe substrate studied here that did not exceed the default kinetic UF for any of the parameters.



Figure 25. Log Geometric Means (GM) of extracted kinetic parameters AUC (A), clearance (B) and Cmax (C) from the included papers after standardization.
Squares: oral exposure; solid circles: IV exposure; red datapoints: healthy volunteers; blue datapoints: patients; 21d: repeated dose for 21 days.

Table 24. Pharmacokinetic parameters of UGT1A1 probe substrates in non-phenotyped adults after oral or intravenous administration.

Route	Parameter	Compound	nst	n	CV	GM	UF95	(95%CI)	UF97.5	(95% CI)
Oral	AUC (ng*h/mL/dose)	Ethinylestradiol ^a	60	1236	41	2045	1.9	[1.8-2.1]	2.2	[2.0-2.4]
		Ethinylestradiol ^b	50	974	42	1355	1.9	[1.8-2.1]	2.2	[2.0-2.5]
		Ezetimibe	11	173	44	356	2.0	[1.7-2.4]	2.3	[1.9-2.9]
		Raltegravir	6	67	60	2110	2.5	[1.9-3.8]	3.0	[2.2-4.9]
		SN38	20	139	70	8039	2.8	[2.2-3.9]	3.5	[2.6-5.0]
		Overall (n = 4)	147	2589	50			2.2	[1.7-3.6]	2.5
Oral	Clearance (mL/min/kg)	Ethinylestradiol ^a	19	324	36	6.8	1.8	[1.6-2.0]	2.0	[1.8-2.3]
		Ethinylestradiol ^b	11	135	38	6.3	1.8	[1.6-2.3]	2.1	[1.7-2.8]
		Ezetimibe	4	55	66	13.9	2.7	[2.0-4.7]	3.3	[2.2-6.2]
		Overall (n = 2)	34	514	48			2.1	[1.6-4.2]	2.5
Oral	Cmax (ng/mL/dose)	Ethinylestradiol ^a	39	1295	35	250	1.7	[1.6-1.9]	1.9	[1.8-2.1]
		Ethinylestradiol ^b	11	841	38	175	1.8	[1.7-2.0]	2.1	[1.9-2.3]
		Ezetimibe	5	173	47	25.8	2.1	[1.8-2.5]	2.4	[2.0-3.0]
		Raltegravir	5	56	72	594	2.9	[2.1-5.1]	3.6	[2.4-7.0]
		SN38	20	146	64	5.0	2.6	[2.1-3.5]	3.2	[2.5-4.5]
		Overall (n = 4)	138	2511	53			2.3	[1.7-4.1]	2.7
Intravenous	AUC (ng*h/mL/dose)	Ethinylestradiol ^b	2	24	39	3585	1.9	[1.4-3.4]	2.1	[1.5-4.3]
		Raltegravir	1	3	37	3752	1.8	[1.5-2.6]	2.0	[1.6-3.1]
		SN38	109	1407	62	67.4	2.5	[2.3-2.8]	3.0	[2.7-3.5]
		Overall (n= 3)	111	1434	46			2.1	[1.5-2.8]	2.4
Intravenous	Clearance (mL/min/kg)	Ethinylestradiol ^b	3	33	39	4.8	1.9	[1.5-3.0]	2.1	[1.6-3.7]
		Raltegravir	1	3	38	4.5	1.9	[1.2-9.2]	2.1	[1.2-13]
		SN38	6	79	68	0.32	2.8	[2.1-4.4]	3.4	[2.4-5.8]
		Overall (n= 3)	10	115	51			2.6	[1.3-5.8]	2.6

^aRepeated dosing of 21 days, ^bSingle dose. nst = number of studies, n = number of subjects

UGT1A3

UGT1A3 is a UGT isoform involved in the glucuronidation of the flavonoid icaritin and several other flavonoids (Chen et al. 2008; Wang et al. 2018a). In this study, two probe substrates were included for UGT1A3, namely telmisartan and ezetimibe. Isoform-related CVs varied from 37–62%. Highest variability was observed for telmisartan and ezetimibe clearance with CV values of 59 and 66%, respectively. It has been demonstrated previously in the literature that telmisartan shows high variability in PK parameters (Chen et al., 2013; Deppe et al., 2010; Kang et al., 2018; Stangier et al., 2000b). Overall, UGT1A3-related UFs were below the default TK UF of 3.16 (Table 25).

Table 25. Pharmacokinetic parameters for UGT1A3 probe substrates in non-phenotyped adults after single-dose oral or intravenous administration.

Route	Parameter	Compound	nst	n	CV	GM	UF95	(95%CI)	UF97.5	(95% CI)
Oral	AUC (ng*h/mL/dose)	Ezetimibe	11	173	44	356	2.0	[1.7-2.4]	2.3	[1.9-2.9]
		Telmisartan	13	225	53	2155	2.2	[1.9-2.7]	2.6	[2.2-3.3]
		Overall (n = 2)	24	398	49			2.1	[1.8-2.7]	2.4
	Clearance (mL/min/kg)	Ezetimibe	4	55	66	13.9	2.7	[2.0-4.7]	3.3	[2.2-6.2]
		Telmisartan	6	103	59	10.1	2.5	[2.0-3.5]	2.9	[2.2-4.4]

Route	Parameter	Compound	nst	n	CV	GM	UF95	(95%CI)	UF97.5	(95% CI)
		Overall (n = 2)	9	158	62		2.6	[2.0-4.2]	3.1	[2.2-5.6]
	C _{max} (ng/mL/dose)	Ezetimibe	5	173	47	25.8	2.1	[1.8-2.5]	2.4	[2.0-3.0]
		Telmisartan	9	144	38	391	1.8	[1.6-2.2]	2.0	[1.7-2.6]
		Overall (n= 2)	20	317	43		2.0	[1.6-2.4]	2.2	[1.8-2.9]
Intravenous	AUC (ng*h/mL/dose)	Telmisartan*	6	41	37	1469	1.8	[1.5-2.6]	2.0	[1.6-3.2]
	Clearance (mL/min/kg)	Telmisartan*	5	36	39	12.2	1.9	[1.5-2.9]	2.1	[1.6-3.6]

nc = number of compounds, nst = number of studies, n = number of subjects

UGT1A4

The ginsenoside 20(S)-protopanaxadiol is one of the naturally occurring probe substrates of the UGT1A4 isoform (Li et al., 2016). UGT1A4 probe substrates selected here included trifluoperazine and 1-OH-midazolam. It is important to note that 1-OH-midazolam is a metabolite of midazolam which is formed by CYP3A4 and then conjugated by UGT1A4. Variability for trifluoperazine was quite extensive, although only a limited number of publications were available, and studies were all from Canada. Large interindividual differences in PK parameters has previously been demonstrated for trifluoperazine, independent of ethnicity (Midha et al., 1988). After oral administration, 1A4 shows the highest variability regarding acute exposure (C_{max}) out of all isoforms with a CV of 62%. However, least variability was found for UGT1A4 in mRNA expression levels when compared with mRNA expression levels of UGT1A1, UGT1A3, UGT1A6 and UGT1A9 (Aueviriyavit et al., 2007). Despite this low variability in mRNA expression levels, an exceedance of the default TK UF is observed for the 97.5th percentile for 1-OH-midazolam (UF97.5: 3.3, Table 26).

Table 26. Pharmacokinetic parameters of UGT1A4 probe substrates in non-phenotyped adults after single-dose oral or intravenous administration.

Route	Parameter	Compound	nst	n	CV	GM	UF95	(95%CI)	UF97.5	(95% CI)
Oral	AUC (ng*h/mL/dose)	1-OH-Midazolam	5	67	35	308	1.7	[1.5-2.3]	1.9	[1.6-2.6]
		Trifluoperazine	7	75	64	207	2.6	[2.0-4.0]	3.2	[2.3-5.3]
		Overall (n = 2)	12	142	47		2.1	[1.5-3.7]	2.4	[1.6-4.8]
	Clearance (mL/min/kg)	Trifluoperazine	2	48	57	112	2.4	[1.8-4.0]	2.8	[2.0-5.1]
	C _{max} (ng/mL/dose)	1-OH-Midazolam	17	67	67	76	2.7	[2.0-4.3]	3.3	[2.3-5.7]
		Trifluoperazine	6	79	58	18.3	2.4	[1.9-3.5]	2.9	[2.2-4.5]
Overall (n = 2)		11	146	62		2.6	[2.0-4.0]	3.1	[2.2-5.3]	

nc = number of compounds, nst = number of studies, n = number of subjects

UGT1A6

Of the seven UGT isoforms investigated in this study, UGT1A6 has been recognised as one of the minor isoforms for glucuronidation and drug metabolism (Stingl et al., 2014). However, it is involved in the

glucuronidation of several pharmaceuticals, including acetaminophen and aspirin, and the remarkable sensitivity of cats to these analgesics is due to the lack of UGT1A6 expression in the feline liver (Shrestha et al., 2011). The natural occurring compound protocatechuic aldehyde is also metabolised by this UGT isoform (Liu et al., 2008). In this study, deferiprone was included as probe substrate for UGT1A6. Only data after oral administration were available and for all PK parameters, the CVs ranged from 36-48% (Table 27) with UGT1A6-related UFs all below the default TK UF.

Table 27. Pharmacokinetic parameters of UGT1A6 probe substrates in non-phenotyped adults after single-dose oral or intravenous administration.

Route	Parameter	Compound	nst	n	CV	GM	UF95	(95% CI)	UF97.5	(95% CI)
Oral	AUC (ng*h/mL/dose)	Deferiprone*	11	101	36	1654	1.8	[1.5-2.2]	2.0	[1.7-2.5]
	Clearance (mL/min/kg)	Deferiprone*	9	89	40	1.9	1.9	[1.6-2.4]	2.1	[1.7-2.9]
	Cmax (ng/mL/dose)	Deferiprone*	3	101	48	616	2.1	[1.7-2.8]	2.4	[1.9-3.4]

nc = number of compounds, nst = number of studies, n = number of subjects

UGT1A9

For the UGT1A9 isoform, several relevant substrates include resveratrol, several flavonols and the natural flavouring agent estragole (Brill et al., 2006; Iyer et al., 2003; Wu et al., 2011). Probe substrates for this isoform included entacapone, mycophenolic acid, oxazepam, and propofol. Overall, isoform-related CVs varied between 23 and 41%. For oxazepam, variability in PK parameters was described previously (Dorne et al., 2001a). Compared to our results, variability in Cmax and AUC was comparable, while the calculated variability was lower for clearance in our study (33% against 51%). UGT1A9-related UFs did not exceed the UF of 3.16 (Table 28).

Table 28. Pharmacokinetic parameters of UGT1A9 probe substrates in non-phenotyped adults after single-dose oral or intravenous administration.

Route	Parameter	Compound	nst	n	CV	GM	UF95	(95% CI)	UF97.5	(95% CI)
Oral	AUC (ng*h/mL/dose)	Entacapone	3	56	28	442	1.6	[1.4-2.0]	1.7	[1.5-2.3]
		Mycophenolic acid	35	837	30	3241	1.6	[1.5-1.7]	1.8	[1.6-1.9]
		Oxazepam	5	44	44	8039	2.0	[1.6-3.0]	2.3	[1.7-3.7]
	Overall (n = 3)	43	937	31		1.6	[1.4-2.6]	1.8	[1.5-3.2]	
Oral	Clearance (mL/min/kg)	Mycophenolic acid	10	140	41	3.7	1.9	[1.6-2.4]	2.2	[1.8-2.8]
		Oxazepam	10	86	33	1.4	1.7	[1.5-2.1]	1.9	[1.6-2.4]
		Overall (n = 2)	20	226	37		1.8	[1.5-2.3]	2.0	[1.6-2.7]
	Oral	Cmax (ng/mL/dose)	Entacapone	63	56	48	447	2.1	[1.7-3.1]	2.4
Mycophenolic acid			17	583	43	1818	2.0	[1.8-2.2]	2.2	[2.0-2.5]
Oxazepam			4	35	26	1243	1.5	[1.3-2.1]	1.6	[1.4-2.4]
Overall (n = 3)		24	674	41		1.9	[1.3-2.8]	2.2	[1.4-3.3]	
Intravenous	AUC (ng*h/mL/dose)	Propofol*	5	43	31	635	1.7	[1.4-2.3]	1.8	[1.5-2.7]

Route	Parameter	Compound	nst	n	CV	GM	UF95	(95%CI)	UF97.5	(95% CI)
	Clearance (mL/min/kg)	Propofol	9	79	23	24.7	1.5	[1.3-1.7]	1.6	[1.4-1.9]

nc = number of compounds, nst = number of studies, n = number of subjects

UGT2B7

UGT2B7 is a UGT isoform which conjugates natural compounds such as emodin, a Chinese traditional medicine, the natural sweetener stevioside and natural compounds from herbs such as andrographolide and estragole (Iyer et al., 2003; Tian et al., 2015; Wang et al., 2014; Wu et al., 2018). Selective pharmaceutical probe substrates included in this study were codeine and zidovudine and isoform-related CVs varied between 26 and 37% (Table 29). The PK database mainly consisted of Caucasians (North America and Europe) for both compounds. For codeine, five studies from the USA and four studies from Europe were available, and the remaining studies were from Asia or Australia. For zidovudine, six studies were available from North America, and three from South America and Europe. The variability as indicated by the CV was 26% for clearance, 28% for AUC, and 43% for the Cmax for zidovudine. While the calculated variability for clearance and Cmax is comparable to Dorne et al. (2001a), the AUC showed less variability (28%, 12 studies against 56%, 2 studies). UGT2B7-related UFs did not exceed the TK default UF.

Table 29. Pharmacokinetic parameters of UGT2B7 probe substrates in non-phenotyped adults after single-dose oral or intravenous administration.

Route	Parameter	Compound	nst	n	CV	GM	UF95	(95%CI)	UF97.5	(95%CI)	
	AUC (ng*h/mL/dose)	Codeine	18	209	29	510	1.6	[1.5-1.8]	1.7	[1.6-2.0]	
		Zidovudine	12	107	28	477	1.6	[1.4-1.8]	1.7	[1.5-2.1]	
		Overall (n = 2)	30	316	28			1.6	[1.4-1.8]	1.7	[1.5-2.0]
Oral	Clearance (mL/min/kg)	Zidovudine	8	72	26	33.3	1.5	[1.4-1.8]	1.7	[1.4-2.1]	
		Cmax (ng/mL/dose)	Codeine	11	192	33	134	1.7	[1.5-1.9]	1.9	[1.6-2.2]
			Zidovudine	9	94	43	344	2.0	[1.7-2.6]	2.3	[1.8-3.1]
			Overall (n= 2)	26	286	37			1.8	[1.5-2.5]	2.0

nc = number of compounds, nst = number of studies, n = number of subjects

UGT2B15

UGT2B15 is mostly responsible for the metabolism of endogenous compounds such as steroids (e.g. dihydrotestosterone and 17 β -diol) (Chen et al., 1993). Environmental contaminants that are metabolised by UGT2B15 include bisphenol A (Hanioka et al., 2008). Major xenobiotic substrates for UGT2B15 include the pharmaceuticals lorazepam and S-oxazepam, although lorazepam is not recommended as a probe substrate for the isoform because of the involvement of several other UGT isoforms in its glucuronidation (Lv et al., 2019; Rowland et al., 2013). Oxazepam is a benzodiazepine which is administered as a racemic mixture, with R-oxazepam being glucuronidated by UGT1A9 and S-oxazepam being glucuronidated by UGT2B15. Variability in the ratio between the R-glucuronide and

the S-glucuronide has been characterized particularly for the formation of the S-glucuronide (Patel et al., 1995). Table 28 shows that variability in oxazepam is 33% and 44% for markers of chronic exposure and 26% for markers of acute exposure and all calculated UGT2B15-related UFs are below the default TK UF. As oxazepam is the only substrate included for UGT2B15, calculated CVs and UFs for oxazepam are considered the overall isoform-specific CVs and UFs for UGT2B15.

5.3.3. Frequencies of UGT isoform polymorphisms in world populations and impact on the pharmacokinetics of probe substrates in non-phenotyped subjects

Frequencies of single nucleotide polymorphisms (SNPs) of UGT isoforms namely UGT1A1*28, UGT1A3, UGT1A4*2 (C70A), UGT1A4*3 (T142G), UGT1A6*2, UGT1A9*22, UGT2B7 C802T and UGT2B15*2 are presented in Figure 26 for world populations of different geographical ancestry. Data investigating the impact of UGT polymorphisms on in vivo PK parameters are limited and summarised in Table 30 for the probe substrates included in this study. Overall, the limited data shows that such an impact still needs to be fully characterised for endogenous substrates and xenobiotics in world populations.

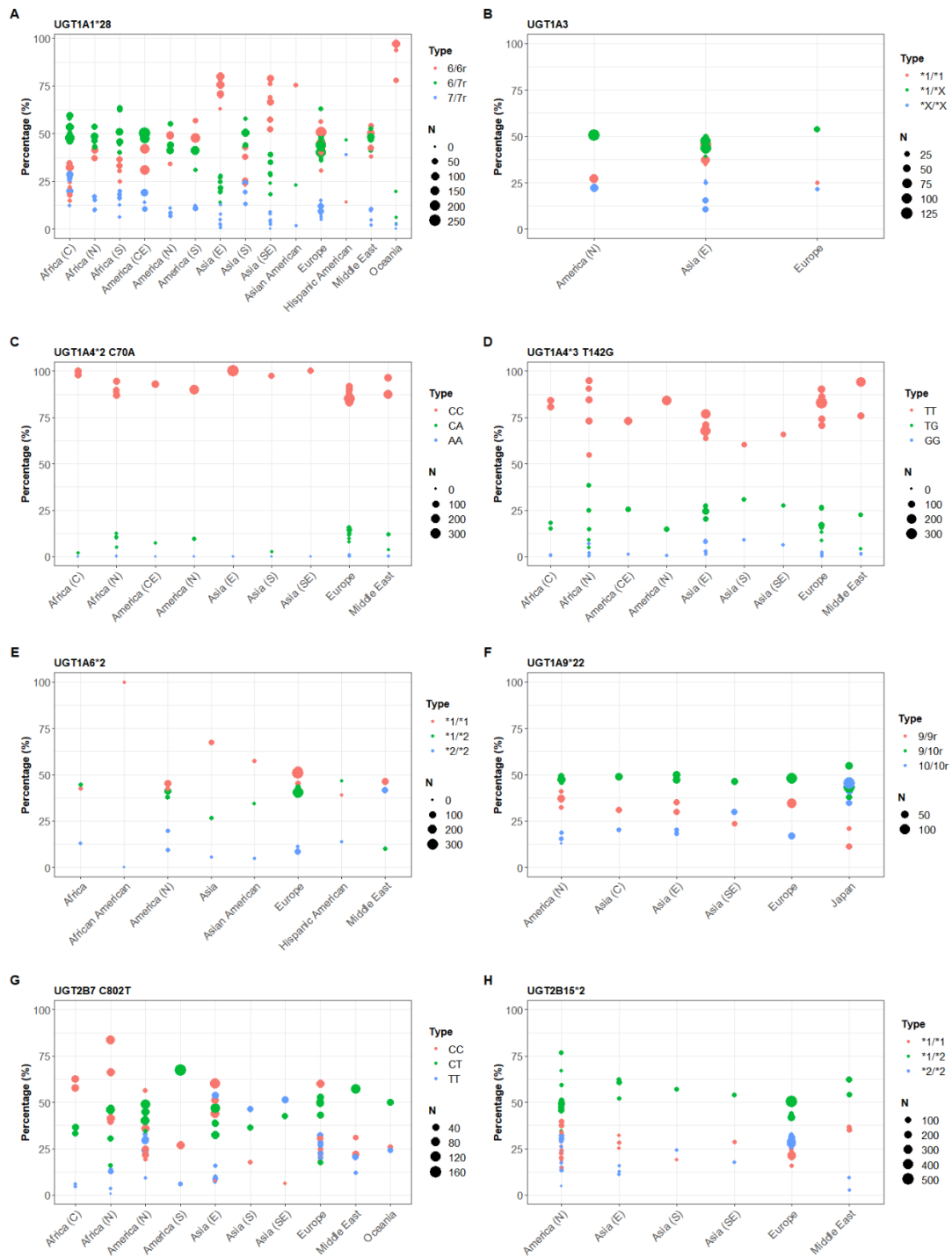


Figure 26. Frequencies of SNPs UGT1A1*28 (A), UGT1A3 (B), UGT1A4 (C70A) (C), UGT1A4 (T142G) (D), UGT1A6*2 (E), UGT1A9*22 (F), UGT2B7 (C802T) (G), UGT2B15*2 (H) Genotypes in various ethnic groups: C = Central; N = North; S = South; E = East; SE = Southeast

Table 30. Impact of UGT isoform polymorphisms on pharmacokinetic markers of chronic exposure.

Polymorphism	Ethnicity/ Geographical ancestry	Substrate	Dose	Sample size	AUC ratio to wild type (%)		Reference
					wt/var	var/var	
UGT1A1*28	Caucasian	SN38	350 mg/m ²	30/25/6	136	161	(Innocenti et al., 2004)
UGT1A1*28	Japanese	SN38	100 mg/m ²	10/7/0	337		(Fukuda et al., 2018)
UGT1A1*28	USA	SN38	125 mg/m ²	5/8/2	176	147	(Jaeckle et al., 2010)
UGT1A1*28	Japan	SN38	150 mg/m ²	41/7/3	120	261	(Satoh et al., 2011)
UGT1A1*28	Caucasian	SN38	300 mg/m ²	9/7/4	141	259	(Iyer et al., 2002)
UGT1A1*28	Korea	SN38	80 mg/m ²	69/12/0	88		(Han et al., 2006a)
UGT1A1*28	Japan	SN38	100 mg/m ²	3/3/0	401		(Hazama et al., 2010)
UGT1A1*28	Japan	SN38	50 mg/m ²	7/1/1	219	172	(Ando et al., 1998)
UGT1A1*28	Caucasian	SN38	600 mg	44/37/5	118	118	(Paoluzzi et al., 2004)
UGT1A1*28	Italy	SN38	180 mg/m ²	31/32/8	124	140	(Toffoli et al., 2006)
UGT1A1*28	USA	SN38	180 mg/m ²	9/15/5	105	209	(Denlinger et al., 2009)
UGT1A1*28	USA	SN38	20 mg/m ²	11/19/7	110	140	(Stewart et al., 2007)
UGT1A1*28	Korean	SN38	65 or 80 mg/m ²	93/14/0	85		(Han et al., 2009)
UGT1A1*28	USA	SN38	50 mg/m ²	14/7/0	91		(Bomgaars et al., 2007)
UGT1A1*28	Korean	Ezetimibe	10 mg	12/0/6		177	(Bae et al., 2011)
UGT1A1*28	Japan	Telmisartan	80 mg	43/14/0	53		(Yamada et al., 2011)
UGT1A1*28	Japan	Telmisartan	80 mg	16/3/4	39	49	(Ieiri et al., 2011)
UGT1A1*28	Caucasian	Raltegravir	400 mg	27/0/30		141	(Wenning et al., 2009)
UGT1A1*6	Japan	SN38	150 mg/m ²	41/9/9	95	214	(Satoh et al., 2011)
UGT1A1*6	Japanese	SN38	100 mg/m ²	10/10/0	125		(Fukuda et al., 2018)
UGT1A1*6	Korea	SN38	80 mg/m ²	49/26/6	111	176	(Han et al., 2006a)
UGT1A1*6	Korean	Ezetimibe	10 mg	12/0/4		97	(Bae et al., 2011)
UGT1A1*6	Japanese	Telmisartan	40 mg	10/2/0	114		(Miura et al., 2009)
UGT1A1*6	Japan	Telmisartan	80 mg	31/13/2	118	153	(Yamada et al., 2011)
UGT1A1*6	Japan	Telmisartan	80 mg	16/7/1	109	193	(Ieiri et al., 2011)
UGT1A3*2a	Japan	Telmisartan	80 mg	17/8/0	57		(Ieiri et al., 2011)
UGT1A6*2	Thailand	Deferiprone	25 mg/kg	10/8/4	72	90	(Limenta et al., 2008)
UGT1A9*22	Korea	SN38	80 mg/m ²	11/45/23	83	70	(Han et al., 2006a)
UGT1A9*22	China	Mycophenolic acid	1- 2 g	13/21/12	106	128	(Zhang et al., 2008)
UGT2B7*2	Japanese	Telmisartan	40 mg	6/6/0	103		(Miura et al., 2009)
UGT2B7*2	Japan	Telmisartan	80 mg	24/28/5	110	149	(Yamada et al., 2011)
UGT2B15*2	USA	Oxazepam	15 mg	6/20/4	147	243	(He et al., 2009)

The associated polymorphism, the predominant ethnicity (or, if not given, the country of the study), the substrate, dose, sample size and ratios of the AUC is given, relative to wild type. For the sample size, numbers of wt/wt, wt/var and var/var are given. Ratios that are significantly different from wild type according to the cited study are shown in bold and italic

Wt= wild type, var= variant, FOLFIRI= folinic acid, fluorouracil, irinotecan. Sample size: wt/wt, wt/var and var/var; wt= wild-type, var= variant

Understanding the functional role of UGT SNPs is a key aspect to quantify the relationship between their frequency distributions (Figure 26) and the pharmacokinetic consequences on UGT conjugation across world populations. Table 30 provides an account of such pharmacokinetic consequences; however, available studies from the literature are still limited. The consequences can be two-sided: an increased UGT activity would result in detoxification and a decreased UGT activity would result in an

increase in the concentration of the toxic form (parent compound). Well-known exceptions to this rule include carboxylic acid-containing drugs that are metabolised by UGTs and form acyl glucuronides, like mycophenolic acid and telmisartan. These acyl glucuronides can cause idiosyncratic drug toxicity by binding covalently to proteins (Iwamura et al., 2017). For mycophenolic acid, indeed protein adducts have been found in vivo and these can result in several adverse effects (Shipkova et al., 2002).

UGT1A1

UGT1A1 in humans is one of the most important UGT isoforms in terms of glucuronidation and is known to have multiple clinically relevant polymorphisms that can contribute to variability in PK parameters (Mehboob et al., 2017; Miners et al., 2002). Polymorphisms in UGT1A1 are extensively studied and alteration in its activity can result in Gilbert's syndrome, one of the most common syndromes in humans (Burchell and Hume, 1999; Stingl et al., 2014). Gilbert's syndrome results in hyperbilirubinaemia, as UGT1A1 is responsible for the metabolism of bilirubin. The frequency and type of polymorphisms differ between individuals from different geographical ancestry or ethnic backgrounds (Weber, 1999) and this is also apparent from the frequencies of mutations in UGT1A1 that are responsible for Gilbert's syndrome. A dinucleotide polymorphism in the TATA box promoter (UGT1A1*28) resulting in reduced UGT1A1 expression and Gilbert's syndrome is detected in only 3% of Asians and ~ 15% in Europeans, while it can be up to 36% in Africans (Beutler et al., 1998). When looking at the frequency distribution of this SNP, clear interindividual differences are indeed detected across populations of different geographical ancestry (Figure 26a, for references see Supplementary Material 4). As expected, Asian populations (especially East and Southeast Asians), as well as Oceanians, more frequently express the wild-type genotype, whereas other ethnicities predominantly express the heterozygous genotype. In Europe and Middle East, mixed frequencies in wild-type and heterozygous genotypes are observed. Another SNP in this isoform, UGT1A1*6, results in an amino acid substitution at position 71 (G71R). This mutation also causes Gilbert's syndrome and is more frequently detected in Asians (Burchell and Hume, 1999).

As mentioned earlier, data gaps for pharmacokinetics of UGT1A1 probe substrates have been identified especially for Africans and Central Americans. Distribution of genotypes for UGT1A1*28 differs for these populations compared to Europeans and this highlights that PK data in phenotyped individuals from different geographical ancestries are needed to characterise isoform-specific UGT variability and related UFs. Besides, the high variability observed for SN38 may also be rationalised by the fact that only patient data were available and included in the meta-analysis, which may bias the analysis. Moreover, internal concentrations of SN38 can be influenced by the UGT1A1*28 mutation and some PK studies included only patients with the wild-type genotype, which also results in a bias in the calculation of the variability and the UF (Ri et al., 2018).

It is striking that variability in PK for ethinylestradiol is low (35–42%; Table 24) when compared to variability in PK for raltegravir and SN38 (up to 72%, Table 24). One possible explanation is the inclusion of only females as it is used as a contraceptive, and this may reduce variability. Indeed, genetic sex differences are known to have an important effect on interindividual differences in UGT enzymes as well and this aspect is further elaborated further down in the discussion. Another possible rationale may be that the identified SNPs have a larger impact on raltegravir and SN38 metabolism than on ethinylestradiol metabolism, which could be due to differences in docking resulting in different affinities and kinetics. Unfortunately, no studies that investigated the effect of UGT1A1 polymorphisms directly on ethinylestradiol PK in vivo were available. However, both ethinylestradiol and SN38 show significantly lower in vitro rates of metabolism with UGT1A1*28 polymorphic human liver microsomes (Zhang et al., 2007).

For the UGT1A1*28 polymorphism, significantly higher values for the AUCs were reported for SN38 which corresponds with a decrease in glucuronidation capacity (Table 30). For the UGT1A1*6 polymorphism, impact on PK parameters is less clear with only one study showing a significant increase in AUC for SN38. No in vivo PK data were available for the other UGT1A1 probe substrates and the effect of either UGT1A1 polymorphism on their PK parameters.

UGT1A3

For UGT1A3 polymorphisms, the frequency distribution is rather uniform across populations of different geographical ancestry. However, data were only available for three populations (North America, East-Asia and Europe) and the heterozygous genotype was the most represented one in all three populations (Figure 26b). A contrasting exception was the observation of slightly higher frequencies for the wild type in East Asians compared to the other two populations.

UGT1A3 polymorphisms are associated with an increase in glucuronidation rates for a range of compounds. UGT1A3*2 (nucleotide changes T31C, G81A and T140C) polymorphism is correlated with an increase in glucuronidation of atorvastatin (Cho et al., 2012). Moreover, polymorphisms in UGT1A3 have been associated with polymorphisms in UGT1A1, which is due to a linkage disequilibrium within the UGT1A locus (Cho et al., 2012; Riedmaier et al., 2010; Saeki et al., 2006).

A study on telmisartan PK reported a significant influence of the *2a and *4a variants of the UGT1A3 polymorphisms, associated with a decrease and an increase in AUC, respectively (Ieiri et al., 2011) (Table 30). Furthermore, a number of studies showed an impact of UGT1A1 and UGT2B7 polymorphisms on PK parameters of telmisartan, indicating that multiple UGT isoforms may be responsible for its glucuronidation and that multiple polymorphisms can, therefore, influence its PK parameters (Ieiri et al., 2011; Miura et al., 2009; Yamada et al., 2011).

UGT1A4

For UGT1A4, the *2 and *3 mutations are the two most common SNPs. UGT1A4*2 is a mutation at codon 24, resulting in an amino acid change from proline to threonine (P24T) because of a C70A SNP. UGT1A4*3 is a T142G SNP, resulting in an amino acid change at codon 48, from a leucine to a valine (L48V). In the frequency distribution data, no differences in C70A and T142G genotypes between populations from different geographical ancestries were observed (Figure 26c/d). Compared to the mutation and the heterozygous genotype, the wild-type genotype is predominantly detected (C70A: > 80%, T142G: > 55%).

Studies on these SNPs show contradictory results. Neither polymorphism is significantly associated with trifluoperazine glucuronidation activity in vitro (Benoit-Biancamano et al., 2009a). However, decreased activities have been reported for benzidine, β -naphthylamine, steroids and tigogenin, but increased glucuronidation has been reported for clozapine and olanzapine with UGT1A4*3 (Ehmer et al., 2004; Ghotbi et al., 2010; Mori et al., 2005). This suggests that the impact of UGT1A4 mutations on PK parameters is substrate dependent, but the associated mechanism remains to be elucidated. The UGT1A4*3 has also been associated with decreased serum levels of lamotrigine which correspond to an increase in glucuronidation rates (Gulcebi et al., 2011; Reimers et al., 2016). For UGT1A4, no studies were encountered that studied effects of polymorphisms in this UGT isoform on in vivo PK parameters of the probe substrates.

UGT1A6

For UGT1A6, the most prominent mutation is UGT1A6*2, which is the result of two substitutions: T181A and R184S (Ciotti et al., 1997). The linkage disequilibrium between these polymorphisms is very high, as they are only 11 nucleotides apart (nucleotides 541 and 552) (McGreavey et al., 2005). A linkage disequilibrium between UGT1A6*2 and UGT1A1*28 is also observed (Lampe et al., 1999). No differences are seen across world populations in the frequency distribution of this polymorphism (Figure 26e).

No impact on deferiprone PK was found in vivo for UGT1A6*2 (Limenta et al., 2008). However, an in vitro study showed that the UGT1A6*2 variant could lead to either a decrease or an increase in glucuronidation capacity for various phenolic compounds (Ciotti et al., 1997; Nagar et al., 2004). Lampe et al. (1999) showed that genetic sex had more influence on the PK parameters of deferiprone whereas polymorphism had no impact. This may be due to both the variation in UGT1A6 content and activity between males and females. Indeed, glucuronidation capacity has been shown to be higher in males with a 50% higher UGT1A6 protein content in males compared to that in females (Bock et al., 1994; Court et al., 2001).

UGT1A9

For UGT1A9, SNPs have been associated with a range of impacts on the PK of xenobiotics. T98C (UGT1A9*3) may result in a decrease in glucuronidation activity, although the reported results are contradictory (Girard et al., 2004; Jiao et al., 2008; Villeneuve et al., 2003). The T-275A SNP, which is located in the promotor region, is associated with an increase in glucuronidation rates, while in another study, the glucuronidation rate of mycophenolic acid remained unaffected (Girard et al., 2004; Jiao et al., 2008; Kuypers et al., 2005; Mazidi et al., 2013). Multiple linkage disequilibria are known for polymorphisms in UGT1A9 since SNPs in UGT1A9 are linked to SNPs in UGT1A7 and UGT1A6 (Saeki et al., 2006). The frequency distributions of these genotypes across several populations are described in Supplementary Material 5.

The SNP with the most apparent differences in frequencies between populations is a 'T' deleted at position-118 in the promotor region of the gene, UGT1A9*22 (Cecchin et al., 2009). Japanese individuals show a different distribution compared to that in other populations including other Asian populations (Figure 26f). In other world populations, the heterozygous genotype is the most occurring, while in Japan most prominent frequencies are a mix between the heterozygous genotype and the homozygous mutation. It is shown that combinations of haplotypes differ between Caucasians and Asians and this might explain the large differences in frequencies observed here (Saeki et al., 2006).

The effect of UGT1A9*22 on PK parameters remains unclear since an increased transcriptional activity has been reported, but it was not associated with an impact on mycophenolic acid PK parameters (Jiao et al., 2008; Yamanaka et al., 2004; Zhang et al., 2008). A significant decrease is demonstrated in AUC for SN38 with this mutation, although SN38 is mainly metabolised by UGT1A1 (Han et al., 2006b).

UGT2B7

For UGT2B7, the frequencies of the C802T mutation are quite comparable for the three world regions (Europe, North America, South America, Figure 26g) represented in the PK database and indeed, not much variability is observed in the PK parameters of zidovudine. The SNP C802T in UGT2B7 results in an amino acid substitution at residue 268, from histidine to tyrosine (H268Y, UGT2B7*2) at the N-terminal substrate binding site of the enzyme (Yuan et al., 2015). It is demonstrated that this variant form has the same localisation as the wild type. Moreover, it is demonstrated that UGT2B7*2 can form both homodimers and heterodimers with wild-type and other polymorphic enzymes, albeit with a decrease in affinity (Yuan et al., 2015). Coffman et al. (1998) showed that the 268Y form of the UGT was ten times more efficient in the glucuronidation of buprenorphine than the 268H form. However, no differences were detected for some other opioids, like morphine and codeine. In another study of

Coffman et al. (2003), it was demonstrated that opioids bind to amino acids 84–118 of the UGT, which implies that mutations at other places are less likely to influence the binding of opioids to UGT. However, also polymorphisms outside the substrate-binding pocket can still influence the dynamics of substrate binding by, for example, altering the packing of the enzyme and thereby influencing the active site (Rutherford et al., 2008).

It is demonstrated that UGT2B7*2 in a hetero-dimer with the wild-type enzyme has an impaired glucuronidation activity for zidovudine (Yuan et al., 2015). For other chemicals including valproic acid, tamoxifen, and lamotrigine, UGT2B7 polymorphism has been shown to affect plasma concentrations (Blevins-Primeau et al., 2009; Du et al., 2016; Petrenaite et al., 2018; Sun et al., 2015; Wang et al., 2018). Molecular docking would provide an insight into the binding of substrates to UGT2B7 and other UGTs and the effect of polymorphisms hereon. However, a complete crystal structure is not available yet for human UGTs (Dong et al., 2012). The partial crystal structure of UGT2B7 that is available does not include the N-terminal substrate-binding domain and consequently does not provide insight into substrate binding (Miley et al., 2007). No in vivo data exploring the relationship between UGT2B7 polymorphisms and PK parameters of zidovudine or codeine were available. Only two studies investigated the impact of UGT2B7*2 on telmisartan PK in Japanese adults. In both studies, no significant differences in AUC were found (Miura et al., 2009; Yamada et al., 2011).

UGT2B15

For UGT2B15, the most common polymorphism is known as UGT2B15*2 and this mutation results in the substitution of an aspartic acid with a tyrosine at position 85 (D85Y). The frequency distribution of this polymorphism is comparable for different populations (Figure 26h). In one study, different ethnicities (African-American, Hispanic-American, Chinese-American, Japanese-American and Caucasian-American) in North-America were compared and all different ethnicities showed approximately the same distribution, with the heterozygous genotype being the predominant genotype (Riedy et al., 2000).

For this polymorphism, no differences were found in relation to the metabolic and PK profile of tamoxifen (Romero-Lorca et al., 2015; Sutiman et al., 2016). However, acetaminophen total clearance was significantly influenced by this polymorphism (Court et al., 2017). Moreover, in vitro data show lower median activities for S-oxazepam glucuronidation with microsomes containing the UGT2B15*2 polymorphism and a lower intrinsic clearance of bisphenol A with this SNP (Court et al., 2004; Hanioka et al., 2011). Finally, lower systemic clearance of lorazepam is reported in Asian individuals homozygous for UGT2B15*2 and the authors suggested that this polymorphism is a major contributor to interindividual differences in lorazepam PK (Chung et al., 2005). A significant increase in AUC has

been observed for UGT2B15*2 for individuals with at least one polymorphic gene. According to the study of He et al. (2009), the polymorphism accounts for 34% of the interindividual differences in oxazepam oral clearance (Table 30).

5.4. Conclusions and future perspectives

This manuscript aimed to quantify interindividual differences in UGT isoform-specific metabolism for probe substrates. Hierarchical Bayesian meta-analyses for pharmacokinetic markers of acute (C_{max}) and chronic exposure (AUC/clearance) were performed for a total of 14 probe substrates of the seven clinically most relevant UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7 and UGT2B15). The resulting variability distributions and the UGT-related UFs showed that the default factor of 3.16 would not be exceeded for at least 97.5% of non-phenotyped healthy adults when considering the median value, with a few exceptions (1-OH-midazolam, ezetimibe, raltegravir, SN38 and trifluoperazine).

Overall, interindividual differences in kinetics for intravenous- and oral route of administration were comparable. A possible explanation for such similarities lies in the fact that UGTs are more abundant in the liver compared to the intestine, so that the impact of first-pass metabolism for the included probe substrates is low (Lv et al., 2019). In contrast, similar analysis performed for CYP3A4 probe substrates revealed larger interindividual differences for markers of oral chronic exposure compared to their IV counterparts (Darney et al., 2019). Several UGT isoforms are also expressed in the kidney, including UGT1A6, UGT1A9 and UGT2B7 (Ohno and Nakajin, 2009). This would have no influence on the first-pass metabolism, but variability estimates are likely to reflect hepatic and renal UGT metabolism for the probe substrates metabolised by these isoforms.

Overall, data gaps have been identified from this human UGT PK database for a range of non-phenotyped and phenotyped populations of different geographical ancestries as well as sensitive subgroups of the population, including neonates, children and the elderly. A typical example is the lack of PK data for the African population which shows broad genetic diversity in the frequency of UGT polymorphisms. Such PK data are needed to integrate genotype frequencies in different populations and to generate distributions to address interphenotypic differences which then allow the derivation of UGT-related UFs as well as chemical-specific adjustment factors (Campbell and Tishkoff, 2008; Gaibar et al., 2018; Novillo et al., 2018).

Indeed, different UGT polymorphisms can have (substrate-dependent) impact on interphenotypic differences in PK parameters, particularly for the UGT1A1 isoform while new polymorphisms are still being characterised (Liu et al., 2019). In this light, it is recommended to investigate interphenotypic

differences in relation to UGT polymorphisms rather than geographical ancestry, since polymorphisms are better predictors of altered PK compared to ethnicity alone (Darney et al., 2020; Wu et al., 2017b).

Although isoform-specific variability was investigated here using specific probe substrates, most often several UGT isoforms are involved in the glucuronidation of xenobiotics in a concentration-dependent manner. For example, acetaminophen glucuronidation by human liver microsomes can be mediated by multiple UGTs. Three isoforms are most active and UGT1A1 is the main contributor at toxic concentrations and UGT1A6 is the most active at low concentrations (Court et al., 2001). Besides the contribution of several isoforms to the glucuronidation of one compound, other factors could also contribute to interindividual differences in metabolism by UGTs. For example, correlations have been established between UGT abundances and their activity and variability in glucuronidation is comparable to variability in UGT protein abundance (Achour et al., 2017). In addition to interphenotypic differences, age differences have been described to impact UGT expression and activities, particularly in neonates, young infants and elderly leading to slower kinetics and elimination through a reduction of PK parameters by several folds compared to that in healthy adults (Bhatt et al., 2019; Court, 2010; Dorne et al., 2001b).

UGTs are also involved in the metabolism of large numbers of xenobiotics, other than pharmaceuticals, like environmental contaminants and naturally occurring compounds. However, for these compounds multiple UGT isoforms are often involved in their conjugation. For example, isoflavones are conjugated by multiple UGT isoforms in human liver microsomes (Tang et al., 2009). Besides the involvement of several UGT isoforms in conjugation, human kinetic data for most environmental contaminants and food-relevant chemicals are still scarce in the literature.

Taken all together, investigation of isoform-specific UGT-related age and interphenotypic differences in world population will allow the characterisation and publication of full variability distributions for human populations in an open source format (as illustrated here with the relatively limited data available). It is foreseen that such distributions can then be combined with in vitro data characterising the kinetics of UGT isoform-specific metabolism for a whole host of relevant compounds including flavourings, food additives, pesticides, mycotoxins and other contaminants to develop quantitative in vitro in vivo extrapolation (QIVIVE) models. However, human in vivo PK data and mechanistically validated in vitro assays in human intestinal, liver, and kidney cells still remain mostly unavailable. Further research and validation efforts in these areas would allow to characterise either direct isoform-specific UGT metabolism, cytochrome P450 and/or influx or efflux transport with subsequent UGT conjugation as well as differential renal or bile excretion to further develop such QIVIVE models and gain experience and confidence in their use in daily chemical risk assessment.

Authors' contribution

E. Kasteel and L. Lautz carried out the data collection, analysis of the results and drafted the manuscript with equal contribution. K. Darney performed the data analysis and assisted with data interpretation and drafting the manuscript. J.L. Dorne and N. Kramer assisted in the design of the study and interpretation of the results and critical review of the manuscript. All authors commented on previous versions of the manuscript and have read and approved the final version of this manuscript.

6. Human Variability in influx and efflux transporters in relation to uncertainty factors for chemical risk assessment

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Abstract

Transporters are divided into the ABC and SLC super-families, mediating the cellular efflux and influx of various xenobiotic and endogenous substrates. Here, an extensive literature search was performed to identify *in vivo* probe substrates for P-gp, BCRP and OAT1/3. For other transporters (e.g. OCT, OATP), no *in vivo* probe substrates could be identified from the available literature. Human kinetic data (C_{max} , clearance, AUC) were extracted from 142 publications and Bayesian meta-analyses were performed using a hierarchical model to derive variability distributions and related uncertainty factors (UFs). For P-gp, human variability indicated that the kinetic default UF (3.16) would cover over 97.5% of healthy individuals, when considering the median value, while the upper confidence interval is exceeded. For BCRP and OAT1/3 human variability indicated that the default kinetic UF would not be exceeded while considering the upper confidence interval. Although limited kinetic data on transporter polymorphisms were available, inter-phenotypic variability for probe substrates was reported, which may indicate that the current default kinetic UF may be insufficient to cover such polymorphisms. Overall, it is recommended to investigate human genetic polymorphisms across geographical ancestry since they provide more robust surrogate measures of genetic differences compared to geographical ancestry alone. This analysis is based on pharmaceutical probe substrates which are often eliminated relatively fast from the human body. The transport of environmental contaminants and food-relevant chemicals should be investigated to broaden the chemical space of this analysis and assess the likelihood of potential interactions with transporters at environmental concentrations.

Keywords: influx, efflux, transporters, human pharmacokinetics, uncertainty factors, variability, polymorphism

Highlights:

- Extensive literature review of human *in vivo* transporter probe substrate variability
- Hierarchical Bayesian analysis to quantify interethnic and intra-ethnic differences
- Data for variability in P-gp, BCRP and OATs are mostly limited to healthy adults and covered by the default kinetic UF.
- Polymorphisms are more robust to study human variability in transporters' kinetics compared to geographical ancestry alone.

6.1. Introduction

Over the last two decades, efflux and influx transport proteins, expressed in a wide range of organs in the human body, have become increasingly important due to their critical role in the pharmacokinetics (PK) and toxicokinetics (TK) of xenobiotics, potentially affecting their absorption, distribution, and excretion (ADE) along with phase I and phase II metabolism (Clerbaux et al., 2019). Significant differences in substrate specificity, tissue distribution, and relative abundance of transporters have been described between experimental animal models and humans and such knowledge bring another level of complexity to ADME processes as well as the potential to improve inter-species extrapolations for hazard characterisation and risk assessment purposes. In addition, inter-phenotypic differences in transporter expression and activities have been demonstrated and can ultimately result in further modulation of the kinetics and toxicity of chemicals (Burt et al., 2016; Harwood et al., 2019; Zhang and Lauschke, 2019).

In this context, the Adenosine Triphosphate Binding Cassette Proteins" (ABC) superfamily of efflux transporters mediate the removal of exogenous compounds, import of nutrients, transport of endogenous substances, and impact on signal transduction. ABC transporters include multidrug-resistance protein 1 (ABCB1/MDR1) also named P-glycoprotein, the multidrug resistance-associated protein (MRP) family, the bile salt export pump (BSEP/ABCB11), the multidrug and toxin extrusions (MATE1/MATE2-K) and breast cancer resistant proteins (BCRP/ABCG2). P-glycoprotein (P-gp) is extensively expressed in key organs including the liver, kidney, central nervous system, small intestine and lymphoid tissues; and is involved in the transport of a range of substrates including fats, sugars, amino acids, drugs and other xenobiotics (Calcagno et al., 2017) (Figure 27). Likewise, BCRP is present in many organs and transports xenobiotics and endogenous substrates (Heyes et al., 2018; Hira and Terada, 2018; Urquhart et al., 2008). A second superfamily of membrane transporter are solute carriers, acting mostly but not exclusively as influx or chemical uptake transporters. These include organic cation transporters (OCTs), organic anion transporters (OATs) as well as organic-anion-transporting polypeptides (OATPs).

Information and data on the kinetics of probe substrates, inducers and inhibitors of such transporters are increasingly available particularly for pharmaceuticals for potential drug-drug interactions may occur as well as food and environmental chemicals such as pesticides, mycotoxins, perfluoroalkyl compounds, flavonoids and other natural bioactive compounds (e.g. coumarins, resins, saponins, terpenoids) (Chedik et al., 2018; Chedik et al., 2019; Chedik et al., 2017; Clerbaux et al., 2019; Dewanjee et al., 2017; Fardel et al., 2012; Guéniche et al., 2019). Examples of relevance to food safety include flavonoids and curcumin as inhibitors of BCRP and capsaicin and piperine as P-gp inhibitors (Fan et al.,

2019; Kusuvara et al., 2012). Several food-drug interactions have also been described for OATPs, particularly with grapefruit juice, which inhibits OATP1A2 (Fan et al., 2008; Kalliokoski and Niemi, 2009; Oswald, 2019).

The role of phase I and phase II xenobiotic-metabolising enzymes is well documented in chemical risk assessment (Ginsberg et al., 2009), while transporters are less well characterised, although they can contribute significantly to human variability in kinetic and dynamic processes. Such quantitative understanding can contribute to the refinement of default uncertainty factors (UF) with pathway-related UFs, chemical-specific adjustment factors (CSAFs) and the development of physiologically based kinetic models (Clerbaux et al., 2018; Clerbaux et al., 2019; Ginsberg et al., 2002; Ginsberg et al., 2009b; Hattis et al., 1999; Valcke and Krishnan, 2013). Based on the WHO guidance of uncertainty in hazard assessment (IPCS, 2017), the geometric standard deviation for inter-individual variability in the human equipotent dose distribution ($\log(\text{GSDH})$) has been proposed to calculate UFs (P95/P50) (Hattis and Lynch, 2007). Next to this, meta-analysis has been conducted to derive pathway-related UFs for several phase I and phase II metabolic pathways and renal excretion (Dorne, 2010; Dorne et al., 2001a; Dorne et al., 2001b; Dorne et al., 2003a; Dorne et al., 2003b; Dorne et al., 2004a; Dorne et al., 2004c; Dorne et al., 2002) and recently, this methodology has been update using hierarchical Bayesian models (Darney et al., 2019; Quignot et al., 2019; Wiecek et al., 2019).

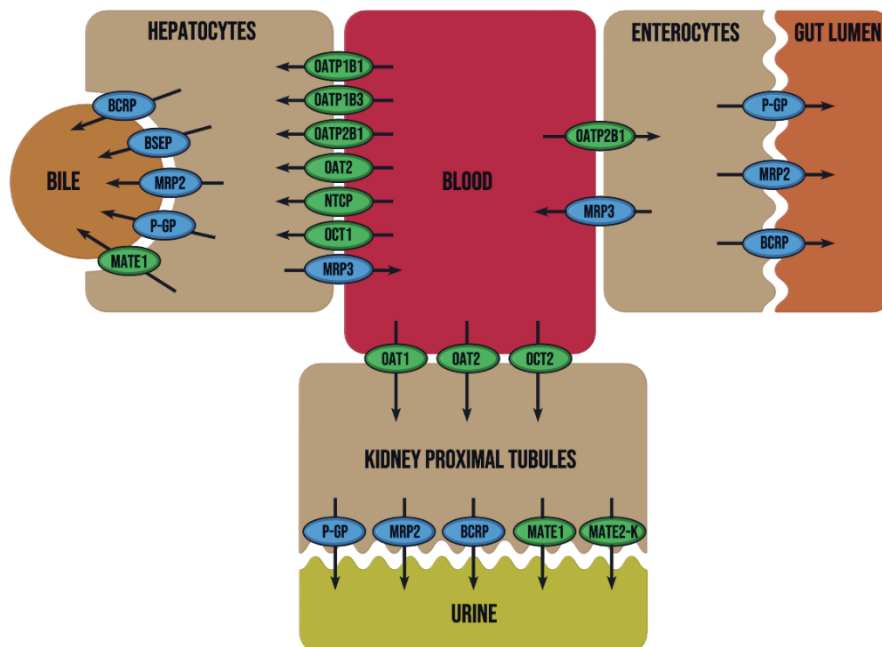


Figure 27. Membrane transporters in the human liver, kidney and intestine. (green: SLC transporters, blue, ABC transporters)

Since human variability in the kinetics of probe substrates for efflux and influx transporters has not been investigated to date, this paper aims to fill this data gap particularly for the most clinically relevant representatives of efflux and influx transporters.

This manuscript, as part of an EFSA funded project addressing human variability in metabolism and transporters aims specifically to i) quantify human variability associated with efflux and influx transporter proteins for well-characterised probe substrates of P-gp, BCRP, MATE1/MATE2-K, OAT1/3, OCTs, and OATPs using hierarchical Bayesian meta-analysis ii) derive UFs from the variability analysis and assess whether the default TK UF is sufficiently. In addition, inter-phenotypic differences for well characterised single nucleotide polymorphisms (SNPs) in the human population as well inter- and intra-ethnic differences are investigated for transporters with available data.

6.2. Material and Methods

6.2.1. Extensive literature search

In vivo probe substrates for P-gp, BCRP and OAT1/3 were identified from the FDA website and datasheets while for other transporters (OCT, OATPs, MATE1/MATE2-K), no *in vivo* specific probe substrates were available (FDA, 2017). For each probe substrate, extensive literature searches (ELS) were conducted in PubMed and Scopus (1966-June 2019) according to the EFSA guidance document using search terms related to human kinetic studies provided in Table 31 (EFSA, 2010b). Specifically, data from human kinetic studies reporting markers of oral (single) or intravenous (bolus) acute (C_{max}) and chronic exposure (area under the curve (AUC), clearance) were collected for healthy adults from different geographical ancestry or ethnic backgrounds. In addition, data for inter-phenotypic differences were investigated from the literature for three different SNPs in P-gp (3435C>T, 1236C>T, 2677G>A/T) and two SNPs for BCRP (34G>A, 421C>A).

Table 31 provides a summary of the keywords applied to the ELS. Screening of the literature was performed as previously described 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.

Table 31 List of queries used for the ELS (formatted for Scopus)

Search probe substrate	TITLE-ABS (" <i>name of probe substrate</i> ")
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 ("gender difference") OR TITLE-ABS ("sex difference*")
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 (toxicokinetic*))
Exclusion	(TITLE-ABS ("cell line*") OR TITLE-ABS ("cell culture*"))
Search genotypic data	(TITLE-ABS ("polymorphism*") OR TITLE-ABS (genotype) OR TITLE-ABS (SNP) OR TITLE-ABS (human) OR TITLE-ABS (half-life) OR TITLE-ABS ("name of transporter"))

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

6.2.2. Standardisation of datasets

Kinetic parameters collected from literature were standardised to perform the analyses in a harmonised manner for each parameter while correcting to dose and body weight namely AUC, Cmax and clearance expressed in mg/kg BW, ng.h/ml/dose, ng/ml/dose and ml/min/kg BW. Body weight correction from the parameters were performed using mean body weight (kg) recorded from the study when available or allocating them to the country of origin using data from Walpole et al. (2012). Kinetic data were often either reported as arithmetic mean (X) and standard deviation (SD) or as geometric means (GM) and geometric standard deviation (GSD). Since PK data are well recognised to follow a lognormal distribution, all PK parameters were transformed, when needed, and expressed as GM and GSD using the following equations:

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

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

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).

6.2.3. Meta-analyses

A number of meta-analyses were performed while integrating results from multiple independent kinetic studies to provide quantitative information regarding inter-individual variability of the PK parameters per chemical and results were expressed as distributions. For each substrate and parameter, variability related to inter-study, inter-substrate and inter-individual differences were analysed through a decomposition of the kinetic parameter variance (clearance, AUC or Cmax) using a hierarchical Bayesian model described previously (Darney et al., 2019; Wiecek et al., 2019). Since this paper constitutes the first comprehensive meta-analyses of kinetic variability associated to the human transporters BCRP, P-gp, and OAT1/3 using *in vivo* probe substrates, non-informative prior distributions expressed as uniform distributions were selected.

The meta-analyses provided 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:

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

where τ_j is the inter-individual variability of the activity for a substrate 'j'.

UFs related to BCRP, P-gp, and OAT probe substrates were calculated as the ratio between the percentile of choice (95th and 97.5th centiles) and the median of the distribution for each kinetic parameter (Figure 28).

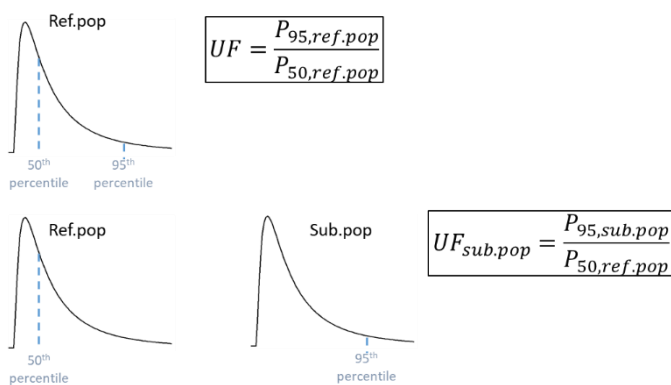


Figure 28. Different ways of calculating the variability and the coverage of default uncertainty factors (UF) from PK data for reference and sub-populations of healthy adults.

Kinetic differences in internal dose between each healthy adult subgroup and general healthy adults and other healthy adult sub-groups (inter-phenotypic and inter-ethnic differences) were derived as GM ratios so that a value >1 indicated a higher internal dose or slower elimination (Dorne, 2010).

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

6.3. Results

6.3.1. Data collection for P-gp, BCRP, and OAT1/3

2643 papers were retrieved from Scopus and PubMed for seven P-gp probe substrates (dabigatran, digoxin, fexofenadine, loperamide, quinidine, talinolol, and vinblastine) and for the OAT1 and OAT3 probe substrate adefovir and sitagliptin. For BCRP, 1115 peer reviewed publications were retrieved for sulfasalazine and rosuvastatin with 20 papers reporting PK data. 496 papers were considered eligible after the first screening while 354 were then as review articles or publications with scarce information or of poor quality. Overall, 142 papers were considered eligible and relevant for data extraction and were included in the database. A full account of the screening procedure, inclusion/ exclusion criteria and data collection is reported in Darney et al (2019b). Figure 29 summarises the flow of information for the available PK studies on P-gp, BCRP and OAT1/3 probe substrates while the full list of relevant peer reviewed publications is provided in supplementary information [A] and the full database can be accessed on EFSA knowledge junction under DOI: 10.5281/zenodo.3739015 with a Creative Commons Attribute 4.0 license.

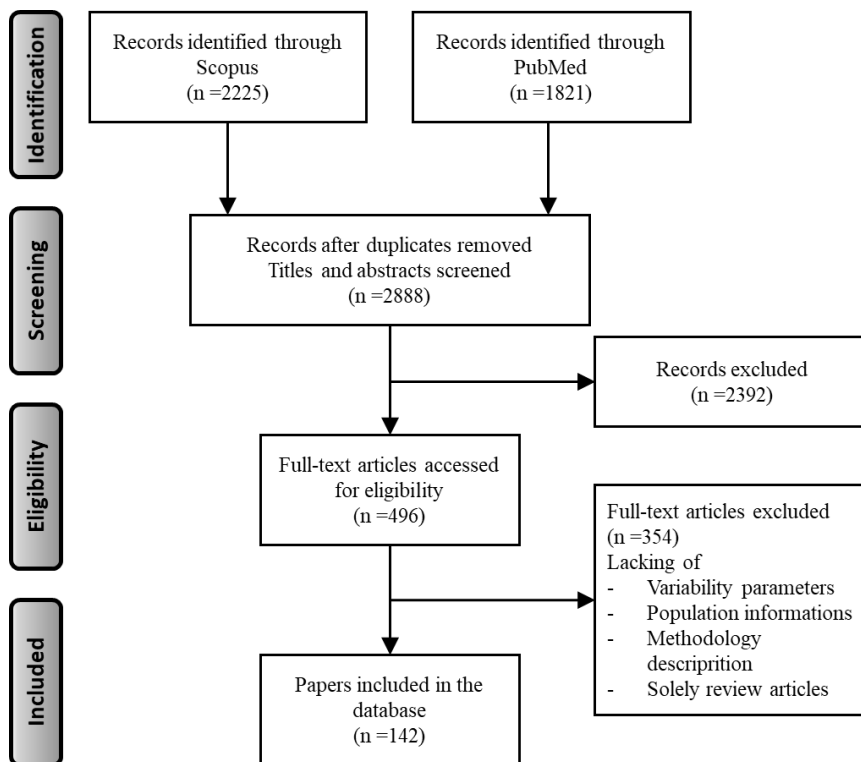


Figure 29. Flow diagram illustrating the extensive literature search of human pharmacokinetic studies for BCRP, P-gp and OAT1/3 probe substrates.

Figure 30 illustrates the raw data for each substrate and parameter of acute oral (C_{max}) and chronic exposure (clearance and AUC) after intravenous and oral dosing. 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.

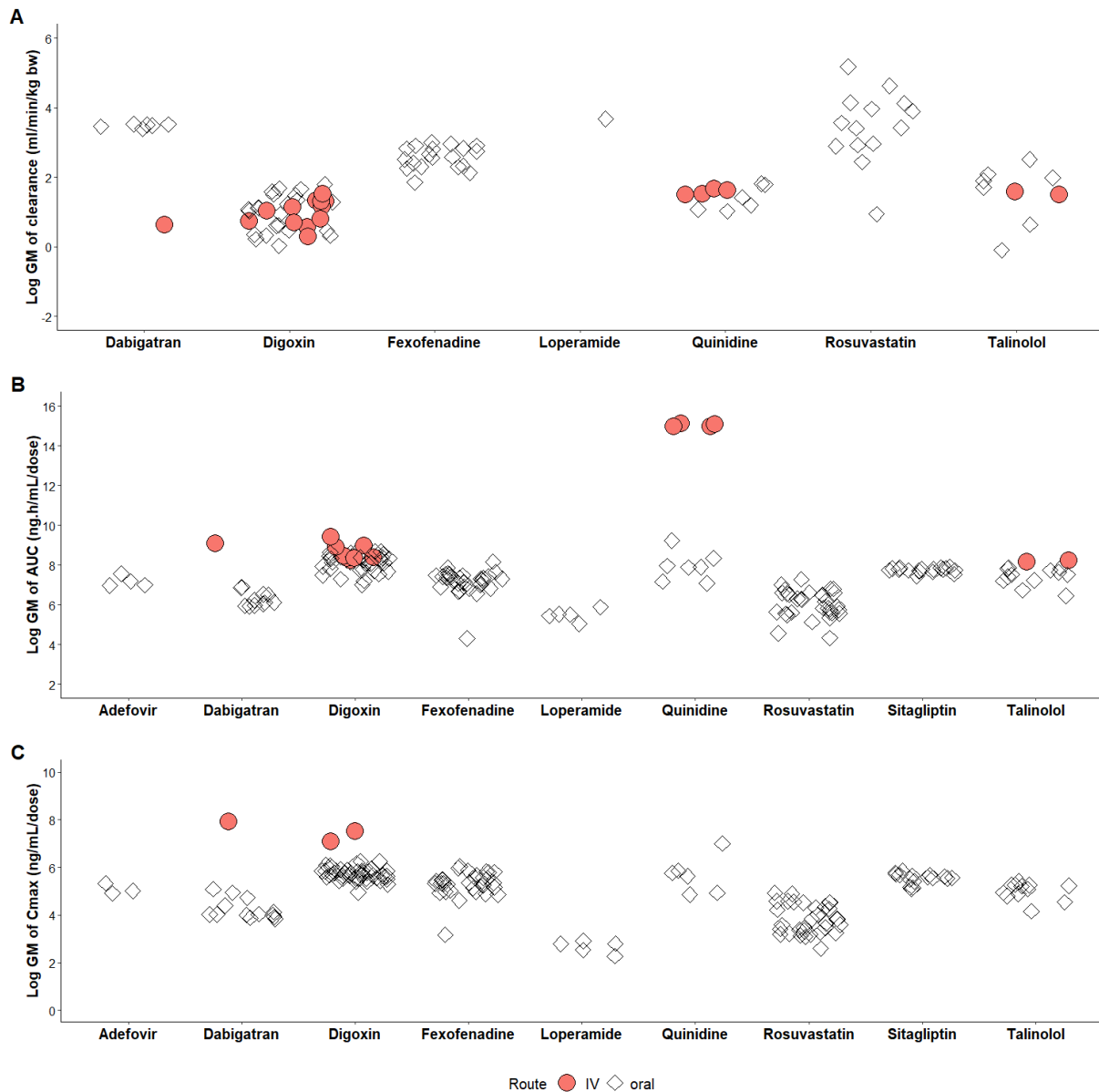


Figure 30. Log geometric mean of extracted kinetic parameters from the included papers after standardization (details in Material and Methods). A: clearance; B: AUC; C: Cmax. Squares: oral exposure; red circles: IV exposure.

6.3.2. P-glycoprotein

6.3.2.1. Data analysis

Kinetic data were available for European, East Asian, South Asian, Southeast Asian, North American and Middle East healthy adults with the majority of the datasets from North America, East Asian and European studies. In order to estimate inter-ethnic differences, European healthy adults were used as the reference group with the highest number of P-gp substrates and parameters for the oral and intravenous routes. Data gaps were available for specific groups including Central and Southern Americans, as well as North and sub-Saharan Africans.

CV values from the meta-analyses provide an account of inter-individual variability considering all substrates and highlight lower inter-individual variability for the IV route compared to the oral route (Table 32). Overall, inter-individual variability in kinetic parameters for healthy adults is around 40% for the oral route (AUC/clearance and C_{max} respectively) and 20% for the IV route (AUC/clearance). Intra-ethnic and inter-ethnic differences for healthy North American, East Asian and South Asian adults showed similar P-gp-related UFs compared to healthy European adults. However, discrepancies for specific substrates with limited data, such as talinolol were evidenced between European and North American healthy adults, with a 3.5-fold lower internal dose for AUC and clearance (oral administration) in the North American sub-group (data from a single study). P-gp-related variability taking into account inter-ethnic differences in healthy East Asian adults were highest for oral clearance (4.1-4.5 for UF₉₅ and UF_{97.5} centile respectively) (2 compounds). Overall, the default kinetic UF (3.16) would be protective of at least 97.5% of the healthy adult population when considering the median value. However, the Bayesian analysis taking into account uncertainty around the estimation of the UF shows that, given the available data (number of studies and number of individuals per study), variability may be higher than that covered by the kinetic default UF, as demonstrated by the upper bound of the 95% confidence interval.

Regarding healthy Middle Eastern, South Asian, and Southeast Asian adults, the number of studies was much lower compared to that for other populations. As a consequence, these results have to be taken with caution. Differences in AUC between healthy Middle Eastern, Southeast Asian and European adults for P-gp substrates were around 1.5-2.3 fold (3 substrates) and 2-fold (1 substrate) respectively and these inter-ethnic differences were associated with UFs of variability (95th and 97.5th centiles) of 3.3-3.7 (Middle East) and 3-3.2 (Southeast Asian) healthy adults. Results of the meta-analyses of CVs and GMs for each substrate are given in supplementary information A.

6.3.2.2. *Impact of P-gp polymorphism on variability*

An additional important aspect of the contribution of P-gp to human variability is the impact of polymorphic genotypes on kinetics, although few studies provide these types of data and it is not currently feasible to quantitatively link allelic frequencies and inter-ethnic differences. The MDR1 gene is highly polymorphic and several SNPs have been identified, among which the 3435C>T (rs1045642), 1236C>T (rs1128503), and 2677G>A/T (rs2032582) are commonly studied. The 3435C allele is associated with increased P-gp expression, while the 3435T allele is associated with decreased P-gp expression, which might lead to altered plasma levels of substrates (Hoffmeyer et al., 2000; Sipeky et

al., 2011), although results regarding the effects of SNPs in P-gp on pharmacokinetic parameters are conflicting (Wolking et al., 2015). For both 1236C>T and 2677G>A/T no conclusive findings on the functionality of P-gp could be determined (Sipeky et al., 2011).

Table 32. Differences in pharmacokinetic parameters in healthy adults after oral administration of P-gp probe substrates: comparison with healthy European adults

						intra-ethnic (UF95)		inter-ethnic (UF95)	
		ns	nc	n	CV	UF95 [95% CI]	UF97.5 [95% CI]	UF95 [95% CI]	UF97.5 [95% CI]
AUC (ng.h/ml/dose)									
	Europe	37	6	496	41.6	1.9 [1.5-6.1]	2.2 [1.6-8.4]		
	East Asia	37	5	457	31.6	1.7 [1.2-3.2]	1.8 [1.3-4.0]	2 [0.4-9.0]	2.2 [0.5-9.8]
	North America	23	5	343	40.4	1.9 [1.4-3.9]	2.2 [1.5-5.1]	2.5 [0.7-9.4]	2.8 [0.7-10]
	Middle East	5	3	66	40.5	1.9 [1.4-4.2]	2.2 [1.5-5.5]	3.3 [0.5-11]	3.7 [0.5-13]
	South Asia	5	2	104	37.5	1.8 [1.4-4.9]	2 [1.5-6.5]	2.3 [0.8-4.5]	2.4 [0.8-5.0]
	Southeast Asia	4	1	136	21.9	1.4 [1.3-1.6]	1.5 [1.4-1.7]	3 [1.6-5.6]	3.2 [1.7-6.0]
Cmax (ng/ml/dose)									
	Europe	35	5	433	37.2	1.9 [1.5-3.2]	2.1 [1.6-4.0]		
	East Asia*	29	5	361	37.5	1.8 [1.2-3.4]	2 [1.3-4.2]	1.7 [0.3-7.1]	1.8 [0.3-7.8]
oral	North America*	23	5	339	45.6	2.1 [1.5-4.6]	2.4 [1.7-6.2]	2.1 [0.9-11]	2.3 [0.9-13]
	Middle East*	5	3	66	31.5	1.7 [1.3-3.2]	1.8 [1.4-4.0]	1.4 [0.2-6.5]	1.4 [0.2-7.1]
	South Asia**	5	2	104	28.7	1.6 [1.5-2.5]	1.7 [1.4-3.0]	1.9 [1.1-4.1]	2.1 [1.2-4.9]
	Southeast Asia*	4	1	136	30.5	1.6 [1.5-1.9]	1.8 [1.6-2.1]		
Clearance (ml/min/kg)									
	Europe	20	4	239	34.7	1.8 [1.4-3.6]	2.0 [1.5-4.6]		
	East Asia*	23	3	280	33.6	1.7 [1.4-2.3]	1.9 [1.5-2.7]	4.1 [1.9-9.6]	4.5 [2.1-11]
	North America*	13	4	160	41.7	1.9 [1.4-4.6]	2.2 [1.6-6.1]	2.3 [1.2-6.2]	2.5 [1.3-8.1]
	Middle East**	3	3	42	52.5	2.3 [1.2-7.8]	2.7 [1.3-11]	1.9 [0.1-14]	2.0 [0.1-15]
	South Asia*	2	1	24	29.7	1.6 [1.3-2.6]	1.8 [1.4-3.1]		
	Southeast Asia*	3	1	103	16.5	1.3 [1.2-1.4]	1.4 [1.3-1.5]		
AUC (ng.h/ml/dose)									
	Europe	6	3	52	14.5	1.3 [1.1-2.4]	1.3 [1.1-2.9]		
	East Asia**	6	2	78	20	1.3 [1.1-2.4]	1.4 [1.2-2.8]	2.5 [1.6-3.6]	2.9 [1.9-4.3]
iv	North America**	3	2	24	20	1.4 [1.1-3.0]	1.5 [1.2-3.6]	2.7 [0.5-5.1]	3.3 [0.5-6.2]
Clearance (ml/min/kg)									
	Europe	9	3	77	16	1.3 [1.1-1.8]	1.4 [1.1-2.1]		
	East Asia**	6	2	78	19	1.4 [1.1-2.7]	1.4 [1.2-3.3]	1.4 [1.0-2.0]	1.4 [1.0-2.0]
	North America**	4	2	31	28.1	1.6 [1.3-3.1]	1.7 [1.3-3.9]	1.7 [0.9-3.4]	1.9 [0.9-3.6]

*: fexofenadine was not studied in the reference group; **: digoxin was the only common substrate with the reference group; ns: number of studies, nc: number of compounds, 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 Europe and subgroup (lognormal distribution, 1/ratio GM for AUC and Cmax).

However, it has been demonstrated that 2677A bearing subjects show higher P-gp activity for some substrates (Yi et al., 2004). The three variants show a strong linkage disequilibrium with CGC and TTT

as the most common haplotypes (Kroetz et al., 2003; Leschziner et al., 2006; Sai et al., 2003; Tang et al., 2004). There is an indication that 3435T carriers have higher drug concentrations as well as those with a TTT haplotype, which results in a higher response rate or an increased frequency of adverse effects (Wolking et al., 2015). The distributions of the genotypes for the SNPs C1236T, G2677A/T and C3435T in P-gp are shown in Figure 31. Overall, in Central and Southern Africa, the wildtype of each SNP was dominantly present. For C1236T, the genotypes frequencies were similar between North African, South American, European and Middle Eastern populations. In the Asian population, the wildtype is less frequently observed (<20%) compared to the 1236CT and 1236TT variant. For G2677A/T, similar patterns between the different ethnicities can be observed, except for the Southern African population, where the wildtype is predominantly present. The 3435CC genotype is frequently observed in Southern and Central African population, while in the Northern African population the genotypes 3435CC and 3435CT were equally observed. Overall, the homozygous 3435TT genotype in the African population was below 11%. Variability in the P-gp SNP C3435T was similar in the American, Asian, European, Middle Eastern and Oceanian population. However, larger variability in the South American population was observed.

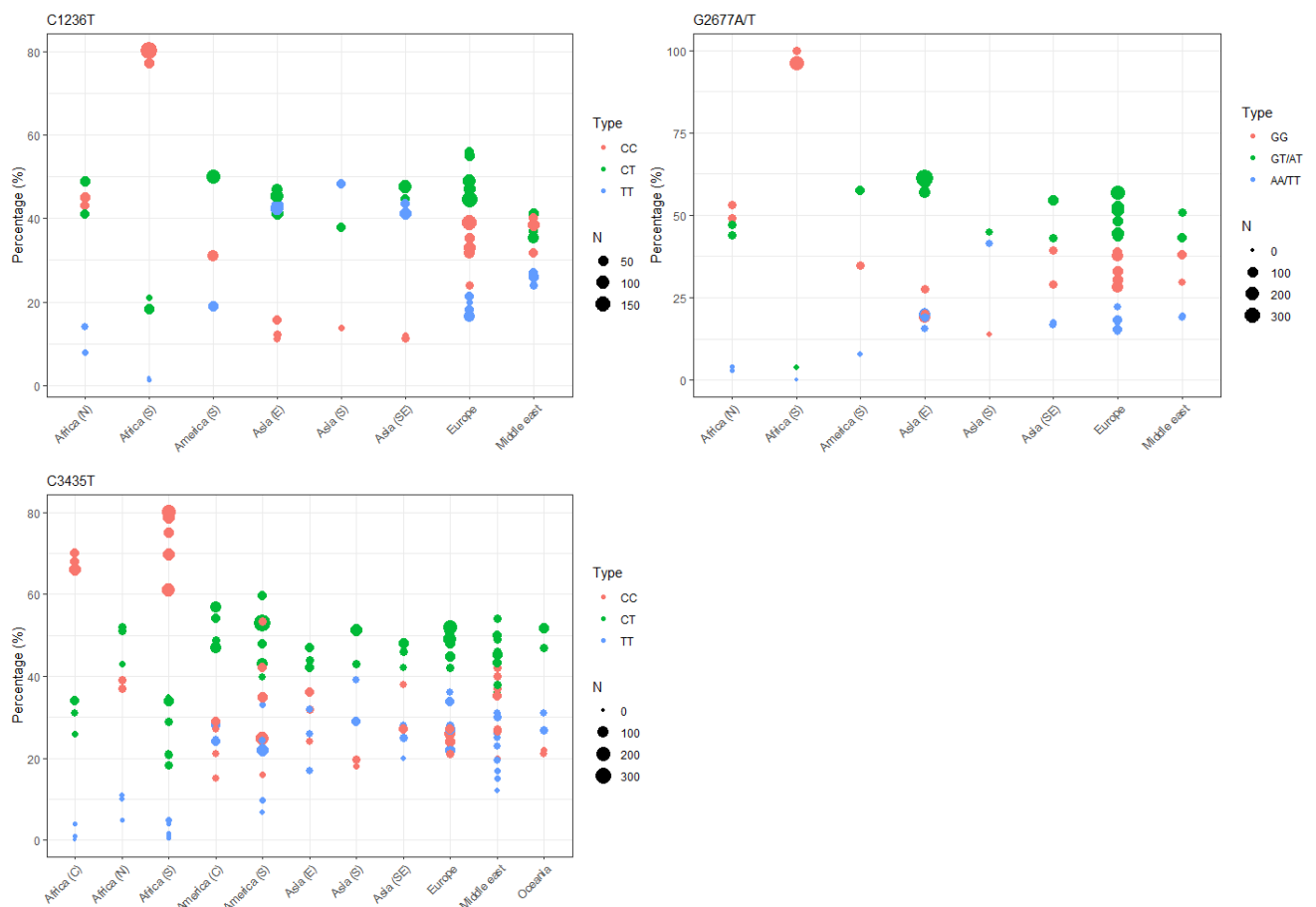


Figure 31. Frequency of SNPs in P-gp (C1236T, G2677A/T, C3435T) in various ethnic groups.

Reference C1236T: (Abuhaliema et al., 2016; Al-Mohizea et al., 2012; Bellusci et al., 2013; Bouzidi et al., 2016; Kassogue et al., 2013; Pechandova et al., 2006; Phuthong et al., 2017; Qiu et al., 2012; Sipeky et al., 2011; Swart

et al., 2012); Reference G2677A/T: (Abuhaliema et al., 2016; Al-Mohizea et al., 2012; Bouzidi et al., 2016; Brown et al., 2012; Kassogue et al., 2013; Pechandova et al., 2006; Phuthong et al., 2017; Qiu et al., 2012; Rosales et al., 2012; Sipeky et al., 2011; Swart et al., 2012) ; References C3435T: (Abuhaliema et al., 2016; Al-Mohizea et al., 2012; Ameyaw et al., 2001; Baldissera et al., 2012; Balram et al., 2003; Bellusci et al., 2013; Bernal et al., 2003; Bouzidi et al., 2016; Brown et al., 2012; Chelule et al., 2003; Cizmarikova et al., 2010; Isaza et al., 2013; Jaramillo-Rangel et al., 2018; Kassogue et al., 2013; Komoto et al., 2006; Leal-Ugarte et al., 2008; Marsh et al., 2015; Masebe et al., 2012; Miladpour et al., 2009; Ngaimisi et al., 2013; Omar and Hughes, 2013; Ostrovsky et al., 2004; Pechandova et al., 2006; Phuthong et al., 2017; Rao et al., 2010; Roberts et al., 2002; Rosales et al., 2012; Sinues et al., 2008; Sipeky et al., 2011; Swart et al., 2012; Vicente et al., 2008)

6.3.3. BCRP

6.3.3.1. Data analysis

Kinetic data were available for healthy adults after single oral exposure. PK data for rosuvastatin did not show differences between included subpopulations, so that variability and uncertainty were determined for the total human population. Values from the analysis for inter-individual variability (Table 33) highlight that the default TK UF of 3.16 would cover at least 97.5% of the healthy adult population. However, the results should be considered with caution, since only one chemical was included for the variability and UF calculation. Most PK data included in the analysis was measured in Asians, showing large variability in measured data, which may be due to polymorphisms. Less PK data was available for Caucasians and no differences between Asian and Caucasian population could be identified.

Table 33 Inter-individual differences in the rosuvastatin PK in healthy adults after oral administration

Parameter	ns	N	CV	GM	UF95 [95% CI]		UF97.5 [95% CI]	
AUC (ng.h/ml/dose)	33	445	47	411	2.1	[1.9-2.4]	2.4	[2.1-2.8]
Clearance (ml/min/kg bw)	14	134	43	1.9	2	[1.7-2.4]	2.2	[1.9-2.9]
Cmax (ng/ml/dose)	32	430	49	46.5	2.1	[1.9-2.5]	2.5	[2.2-2.9]

ns: number of studies, n: number of individuals, CV: coefficient of variation (lognormal distribution), GM: geometric mean (lognormal distribution).

6.3.3.2. Impact of BCRP polymorphism on variability

Various SNPs of the *ABCG2* gene have been identified, whereof 34G>A and 421C>A (p.Q141, rs2231142) are most commonly studied (Mao and Unadkat, 2015). 34G>A is associated with decreased BCRP activity, but studies investigating differences in drug response in relation to 34G>A are inconclusive (Niebudek et al., 2019). Variability in the frequency of G34A in BCRP between different populations is illustrated in Figure 32. While in most populations, the wildtype genotype is more frequently detected (>70%), in Asians and inhabitants of Oceania the wildtype 34GG and the homozygous mutation were less frequently observed compared to the heterozygous genotype. In East Asian populations, the wildtype genotype is more frequently observed (50-75%) compared to Southeast Asians, but is still more often observed compared to African, American, European and

Middle Eastern populations. 421C>A is associated with decreased expression of the BCRP protein (Kondo et al., 2004; Mizuarai et al., 2004), leading to altered PK parameters of several drugs (de Jong et al., 2004; Lee et al., 2015; Tanaka et al., 2015). Regarding the pattern of distributions, the homozygous mutation 421AA is detected only in <12% of the population. Nevertheless, in Southeast and East Asian populations, the genotype 421CC and 421CA are equally detected and the 421A allele is more frequently present in East and Southeast Asians compared to that in Caucasian populations. C421A SNP is considered an important BCRP variation in terms of cancer chemotherapy and drug resistance (Noguchi et al., 2009) (Table 34). Indeed, both the European Medicine Agency and the US Food and Drug Administration recommend to test for the effect of C421A SNP to take into account potentially sensitive populations (Lee et al., 2015).

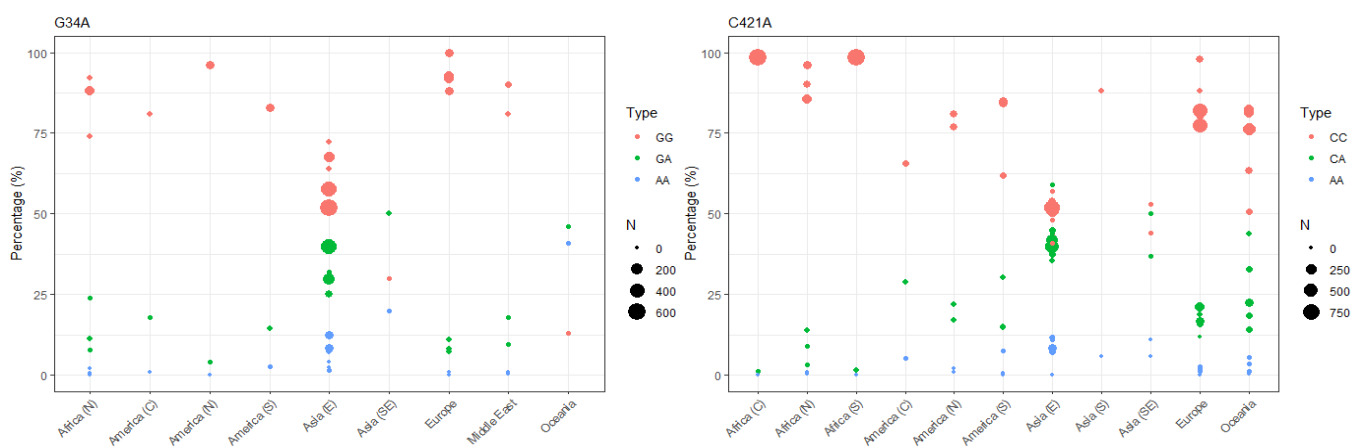


Figure 32. Frequency of SNPs in BCRP (G34A, C421A) in various ethnic groups.

References G34A: (Bosch et al., 2005; de Lima et al., 2014; Fischer et al., 2007; Kim et al., 2010; Kobayashi et al., 2005; Niebudek et al., 2019; Wan et al., 2015; Wu et al., 2015; Zamber et al., 2003); References C241A: (Andersen et al., 2009; Birmingham et al., 2015; de Jong et al., 2004; de Lima et al., 2014; El Mesallamy et al., 2014; Feher et al., 2013; Fischer et al., 2007; Genvigir et al., 2017; Hammann et al., 2012; Imai et al., 2002; Keskitalo et al., 2009; Kim et al., 2010; Kobayashi et al., 2005; Marsh et al., 2015; Niebudek et al., 2019; Oh et al., 2013; Phipps-Green et al., 2010; Soko et al., 2016; Wan et al., 2015; Wu et al., 2015; Yen-Revollo et al., 2009)

Table 34. Effects of genetic polymorphisms on the pharmacokinetics of rosuvastatin.

Population	Parameter	Ratio	Ratio	Reference
		CC/CA	CC/AA	
Finnish	AUC	0.82	0.41	Keskitalo et al. (2009)
Finnish	Cmax	0.90	0.43	
Finnish	Clearance	0.95	1.07	
Chinese	AUC	0.93	0.63	Zhou et al. (2013)
Chinese	Cmax	0.99	0.71	
Chinese	AUC	0.97	0.38	Wan et al. (2015)
Chinese	Cmax	0.92	0.31	
Chinese	Clearance	0.97	2.68	

6.3.4. Other efflux transporters

The MRP family consists of nine members, whereof MRP2 (ABCC2) and MRP4 (ABCC4) are in particular involved in the disposition of drugs and conjugates (Terada and Hira, 2015). MRP2 can transport both the parent chemical and its metabolites, unconjugated bile acids, organic anions, GSH conjugates, glucuronides, and sulphates (Kumar and Jaitak, 2019). MATE transporters are mainly expressed in the kidneys and are involved in the tubular elimination of cationic drugs and endogenous compounds. MATE1 is also expressed in the canalicular membrane of hepatocytes and shares various neutral and cationic substrates with P-gp, such as fexofenadine, levofloxacin and quinidine. Furthermore, MATE transporters are involved in the elimination of substrates which are taken up by OCTs. Examples for these substrates are metformin and cimetidine (Jetter and Kullak-Ublick, 2019; Trueck et al., 2019). However, for none of these transporters, *in vivo* probe substrates have been identified and therefore the analysis was not carried out.

6.3.5. OAT1/3: Data analysis and polymorphisms

An ELS was performed for OATs to identify human PK studies in healthy adults with different ethnic backgrounds. The literature data highlight a broad overlapping substrate specificity among different OATs that does not allow to draw conclusions clearly referred to a single transporter; the PK parameters measured are in most cases based on the net result of the actions of more than one carrier. Adefovir and sitagliptin have been identified as *in vivo* probe substrates for OAT1 and OAT3, respectively. The analysis showed that the overall variability of the OAT1/3 transporters is lower compared to P-gp and BCRP (Table 35). Variability is below the default TK UF of 3.16, but number of subjects available from kinetic studies is limited, especially regarding adefovir, and therefore results should be considered with caution. Coding regions of *OAT1* and *OAT3* have low genetic and functional diversity suggesting that coding region variants of these transporters may not contribute substantially to inter-individual differences observed in pharmacokinetics of chemicals (Yee et al., 2018).

Table 35. Differences in pharmacokinetic parameters in healthy adults after oral administration of OAT1/3 probe substrates

Drug	Parameter	ns	n	CV	GM	UF95 (95% IC)	UF95 (97.5% IC)
Adefovir	AUC (ng.h/ml/dose)	4	67	23	1323	1.5 [1.3-1.7]	1.6 [1.4-1.9]
	Cmax (ng/ml/dose)	3	43	25	169	1.5 [1.3-1.9]	1.6 [1.4-2.2]
Sitagliptin	AUC (ng.h/ml/dose)	17	219	20	2300	1.4 [1.3-1.5]	1.5 [1.4-1.6]
	Cmax (ng/ml/dose)	16	195	32	256	1.7 [1.5-1.9]	1.9 [1.6-2.2]

ns: number of studies, *n*: number of individuals, *CV*: coefficient of variation (lognormal distribution), *GM*: geometric mean (lognormal distribution).

6.3.6. OATPs

The OATP family consists of 11 members, whereof OATP1A2, OATP1B1, OATP1B3, and OATP2B1 are most extensively characterized and are involved in the disposition of drugs and xenobiotics (Konig et al., 2006; Zhang and Lauschke, 2019). For this class of carriers, the ELS evidenced that different OATP isoforms show a broad overlapping substrate specificity and in many cases transporters other than OATPs can act on the same substrate. A good example is provided by various studies on statins: as discussed above, rosuvastatin's cellular influx and efflux are mediated by OATP1B1 and BCRP, respectively and the measured PK parameters are the net result of multiple transporters. *In vivo* studies on statins PK showed that OATP1B1 polymorphisms can influence the internal concentration of rosuvastatin and other statins (Giacomini et al., 2013; Pasanen et al., 2007; Wu et al., 2017a), but the impact of OATP1B1 genotypes on drug disposition is highly compound-specific (Giacomini et al., 2013). Nevertheless, since no clear probe substrates have been established for OATP transporters *in vivo*, analysis of human variability for these transporters could not be carried out.

6.3.7. OCTs

OCTs and MATEs are transporters that transcellularly translocate cationic drugs: together they represent an essential system for renal elimination of therapeutic drugs and other xenobiotics (Ayrton and Morgan, 2008; Matsushima et al., 2009; Wang et al., 2008). These two families of carriers share several substrates and inhibitors (Motohashi and Inui, 2013; Nies et al., 2011). Metformin is recommended as a probe drug for the renal proximal tubular transporter OCT2, but it lacks specificity because excretion of metformin across the apical membrane is carried out by MATE1 and MATE2-K (Trueck et al., 2019). Furthermore, OCT1 is involved in apical transport and may mediate metformin reabsorption (Momper et al., 2016). There are indications that polymorphisms in OCT2 can influence the *in vivo* PK of metformin causing variability in drug response (Islam et al., 2018; Song et al., 2008; Wang et al., 2008; Yee et al., 2018). However, no probe substrates were available for *in vivo* OCT transporters so that analysis of pharmacokinetic variability in this transporter was not performed.

6.4. Discussion

Data for human variability in the pharmacokinetics of transporter substrates are scarce for non-phenotyped individuals, let alone polymorphisms and until now such information has not been integrated in human health risk assessment for pharmaceuticals and environmental chemicals (Clerbaux et al., 2018; Clerbaux et al., 2019). Nevertheless, several studies have indicated that variable BCRP and P-gp expression/function may determine variation in PK parameters for specific substrates. Both transporters are highly expressed at the apical membrane of enterocytes and may limit the oral bioavailability of a range of chemicals (Clerbaux et al., 2019; Harwood et al., 2019; Maliepaard et al.,

2001; Thiebaut et al., 1987). This observation is also relevant for other barrier systems such as the blood brain barrier and the placenta in which both BCRP and P-gp are highly expressed to protect the fetus. In addition, a number of variant alleles have been hypothesised as risk factors for fetal toxicity with no clear conclusions so far (Allikmets et al., 1998; Hitzl et al., 2004; Maliepaard et al., 2001; Tanabe et al., 2001).

Here data obtained by means of an extensive literature search on well characterised *in vivo* probe substrates of P-gp, BCRP and OAT1/3 were analysed to identify the associated human variability. For other transporters (e.g. OCT, OATP), no *in vivo* probe substrates could be identified and therefore the analysis of pharmacokinetic variability was not performed. The impact of polymorphisms was also analysed and in most cases, the effects of the SNPs on transporters seem to be substance specific, due to changes of the substrate-binding domain which alters substrate affinity.

Based on the available data for P-gp, limited to the adult life stage and certain ethnic groups (largely Caucasian), the calculated human variability indicated that the kinetic default UF of 3.16 would be protective of 97.5% of healthy individuals, when considering the median value, while it is exceeded when considering the upper confidence interval. The variability of kinetic parameters observed following IV injection is generally 50% lower when compared to the oral administration. This can be explained by the aforementioned expression of P-gp in the intestine, that will influence the bioavailability of orally administered chemicals (Li et al., 2017; Thiebaut et al., 1987).

Our assessment reflects the total variability related to the probe substrates. Indeed, the contribution of P-gp to the overall pharmacokinetics of drugs is unknown in most cases and dual- or multiple-transporter mediated transporting of chemicals may mask the net *in vivo* function of P-gp. Indeed, digoxin, the most frequently tested drug for P-gp, is also a substrate of a sodium-dependent transporter (Taub et al., 2011). Another P-gp probe substrate, fexofenadine, is suspected to be a multiple-substrate for the drug transporters MRP2 and OATP2B1/OATP1A2, which are all polymorphic (Ming et al., 2011). Compounds like quercetin can competitively inhibit the members of MDR family, P-gp, MRP1 and BCRP (Ofer et al., 2005) as well as CYP3A4 (Wink et al., 2012). The interplay between P-gp and CYP3A4 can be relevant in determining inter-individual differences since they share substrate affinity and are co-inducible in response to at least some xenobiotics. For this reason, P-gp potentiates CYP3A4-mediated drug disappearance during intestinal secretory detoxification for a range of compounds (Chan et al., 2004).

For BCRP and OAT1/3, human variability data were limited to healthy adults and indicated that the default kinetic UF of 3.16 was not exceeded and provides a sufficient level of protection considering the upper confidence interval (95%CI). For BCRP, some literature data indicate that rosuvastatin

plasma concentrations are significantly higher in an Asian population compared to Caucasian populations (Birmingham et al., 2015; Keskitalo et al., 2009; Lee et al., 2005). This could be attributed to the presence of the C421C > A SNP which has been reported to markedly affect the PK parameters of rosuvastatin (Table 34) (Birmingham et al., 2015; Keskitalo et al., 2009). The same polymorphism also significantly affects the PK of other drugs, such as topotecan and diflomotecan (Heyes et al., 2018; Hira and Terada, 2018; Sparreboom et al., 2004; Sparreboom et al., 2005). However, for these compounds, the contribution of metabolism to the inter-individual variability cannot be underestimated. Indeed, topotecan and diflomotecan undergo CYP3A4 metabolism, which can have an influence on the PK parameters (Graham et al., 2009; Rodriguez-Antona and Ingelman-Sundberg, 2006). For rosuvastatin only approximately 10% of the parent compound is metabolised (primarily by CYP2C9). Due to the small contribution to rosuvastatin's internal dose, and to the lack of the most common CYP2C9 variant alleles in Asian populations, it is not expected that the higher systemic exposure in Asians is based on CYP2C9-mediated metabolism (Yasuda et al., 2008). In addition, while BCRP mediates rosuvastatin excretion from the cell, another carrier, namely OATP1B1, mediates rosuvastatin uptake into the cells. This suggests that also polymorphisms in OATP1B1 may influence the *in vivo* kinetics of this substrate (Giacomini et al., 2013; Pasanen et al., 2007; Wu et al., 2017a).

We are aware, as stated above, that the contribution of transporter variability alone cannot be distinguished from other factors that can also contribute to variability of the PK parameters. However, the results suggest that based on the available data on healthy adults, inter-individual differences associated with the activity of transporters is mostly covered by the 3.16 default kinetic UF using data for pharmaceutical probe substrates. A rationale for such limited variability lies in the fact that very few probe substrates are transported by one specific carrier-mediated process, so it is reasonable to assume that the overlapping substrate specificity of transporters (from same or different classes) may reduce the variability due to possible compensation mechanisms (Chedik et al., 2018; Clerbaux et al., 2019). The involvement of multiple transporters can also influence the occurrence of chemical interactions mediated by transport processes induction or inhibition; these have been observed *in vitro* but *in vivo* evidence is mostly lacking, likely due to low exposure to environmental chemicals or food components which are generally well below the concentrations of administered therapeutic drugs (Chedik et al., 2018). Accidental exposure to very high levels or intoxication events with high peak blood concentrations may represent an exception.

The methodology and modelling presented here has been previously applied to the CYP3A4 isoform (Darney et al., 2019) and it is currently being explored for other phase I and phase II isoforms enzymes to generate variability distributions for human inter-individual differences in PK parameters (Darney et al., 2020). Here, it is foreseen that *in vitro* kinetic data and transporter variability can be integrated

in quantitative *in vitro* *in vivo* extrapolation (QIVIVE) to estimate intrinsic clearance for the human population. Non-invasive *in vitro* techniques are now available to investigate the involvement of transporters and generate chemical-specific data using human cell lines or human liver microsomes (Harwood et al., 2016; Kumar et al., 2015; Poulin, 2013; Prasad and Unadkat, 2014; Yoon et al., 2013; Zhang et al., 2019). The variability derived here for specific transporters can then be integrated in physiologically-based kinetic models with Markov-Chain Monte Carlo, allowing full probabilistic integration, instead of using a single deterministic mean value. In addition, data for protein abundance of transporters and their activity can also further support the modelling of transporter kinetics by physiologically-based kinetic-QIVIVE link models including the mechanistic modelling of chemical oral absorption as well as chemical-chemical and drug-drug interactions (Barton et al., 2013; Harwood et al., 2013; Harwood et al., 2014; Jamei et al., 2014; Neuhoff et al., 2013).

Since only healthy adults were considered in this study, due to the lack of data for other subgroups the transporter-related variability described here may not be applicable to sensitive subpopulations, such as neonates, children and elderly as well as non-healthy individuals or specific ethnic groups for which data are not available. However, almost no studies have been performed investigating transporter-dependent pharmacokinetics in children and studies in neonates are not available (Rodieux et al., 2016).

There is a current trend to replace traditional default UFs by using data-derived UFs based on a quantitative understanding of population characteristics, PK data and/or toxicodynamic data to reduce uncertainty in chemical risk assessment (Bhat et al., 2017). Although limited kinetic data on transporter polymorphisms were available, inter-phenotypic variability for probe substrates was reported, which may contribute to human variability in PK parameters, and can therefore result in exceedance of the default kinetic UF. Overall, to predict whether the kinetic portion of the intra-individual UF is protective of humans, it is recommended that genetic polymorphisms across all human groups are investigated since polymorphisms provide a better predictor in altered pharmacokinetics than ethnicity alone (Darney et al., 2020; Wu et al., 2017a).

Kinetic data were mostly available for on pharmaceutical probe substrates which are eliminated relatively fast from the human body (i.e. short half-lives). However, data on the transport of environmental contaminants and food-relevant chemicals, particularly persistent ones, are very scarce and it is not certain that the UFs derived for pharmaceuticals are applicable to these chemicals as well. Therefore, assessment of such chemicals would need to be performed on a case by case basis either using the default factor, the transporter-related UFs or chemical specific adjustment factors. The chemical-specific adjustment factors will be necessary when 1. the compound is handled

by a combination of phase I, Phase II pathways and transporters. 2. the compound is persistent with long half-lives. 3. pharmacokinetic data shows inter-phenotypic differences in the substance's specific transporter(s) handling. This suggests a need to investigate their kinetic and transport profile to broaden the chemical groups of this analysis to such persistent compounds. Relevant examples for transporters as P-gp, BCRP2, OCTs are food additives (sweeteners), organochlorines, pyrethroids such as allethrin and tetramethrin, and organophosphorus pesticides (Chedik et al., 2018; Chedik et al., 2017; Guéniche et al., 2019; Sjöstedt et al., 2017). Overall, these investigations should include environmental concentrations to investigate the likelihood of such interactions with transporters to occur.

7. General discussion

The overall objective of this thesis was to develop tools that can be used to predict human kinetic variability and pathway-related UFs for chemical risk assessment for their future integration in PBK models. An aggregated PBK model for permethrin, a synthetic pyrethroid insecticide, has been developed in order to assess exposure of the French population (Chapter 2). Sensitivity analyses have been conducted and confirm that kinetic parameters were the most influential on the output parameter of the model (urinary excretion of the metabolites) and that efforts should be conducted to refine such parameters. Therefore, a hierarchical Bayesian model for the meta-analysis of kinetic data has been developed and pathway-related variability and UFs have been calculated for two phase I enzymes, namely CYP3A4 and paraoxonase-1 (PON1) (Chapter 3 and 4). The same approach has been used for phase II enzymes, UDP-glucuronyltransferases (UGT) 1A1, 1A3, 1A4, 1A6, 1A9, 2B7 (chapter 5) and finally for influx and efflux transporters involved in present in the intestine, liver and kidneys (Chapter 6).

7.1. After almost 20 years, here we are

7.1.1. Bayesian meta-analyse of kinetic data

The quantification of human variability for a range of metabolic pathways have been proposed at the end of the 90s (Renwick and Lazarus, 1998). After which, pathway-related UFs have been published for a number of phase I, phase II enzymes and renal excretion as well as UFs allowing for variability in pharmacodynamics (Dorne et al., 2001a; Dorne et al., 2003a; Dorne et al., 2003b; Dorne et al., 2004a; Dorne et al., 2005; Dorne et al., 2002; Ginsberg et al., 2002; Naumann et al., 2001; Walton et al., 2001a; Walton et al., 2001b).

However, kinetic data from pharmacokinetic studies were limited in terms of number of studies and sample size (typically below 10). Additionally, the previously published meta-analyses were based on weighted averages assuming fixed effect models with inverse variance weights (Dorne et al., 2001b). While this approach does allow to derive parameter specific variability for different populations, it did not address the relative contribution of the variability across subgroups to the overall variability in the datasets, leading to uncertainty in the parameter estimates which is not quantified since the end result is here a single value, either a CV or an UF. Over the last decade, significant new studies have been published on the kinetics of pharmaceuticals as probe substrates of a range of selected phase I, phase II pathways and transporters. Moreover, Bayesian methods allow to quantify the variability and uncertainty and are more suitable for datasets with large heterogeneity across studies and with hierarchical structure (Shao et al., 2017). It is also important to note that Markov chain Monte Carlo

methods allow to simulate and derive posterior distributions to provide quantitative descriptors of the associated uncertainty (Kruschke and Vanpaemel, 2015), as the 95% confidence (or credibility) intervals. This may lead to large intervals with small sample size and indicating large uncertainty for the estimated parameter. Such a hierarchical Bayesian models have been described to investigate human variability and uncertainty in metabolism (chapter 3) for a range of phase I, phase II metabolism and transporters (chapter 3 to 6).

7.1.2. Pathway-related variability and uncertainty factors for chemical risk assessment

Chapter 3 provides a quantitative estimation of intra- and inter-ethnic differences in CYP3A4 metabolism. This pathway was previously studied by Dorne et al. (2003a), overall, the CYP3A4 related UFs derived in this chapter for healthy adults were consistent with those in Dorne et al. (2003a). Different populations have been considered, healthy adults from different geographical ancestry as well as other groups including neonates, infants and elderly. However, there was insufficient data regarding the genotype or phenotype of the volunteers. It has been concluded that polymorphism can be an important factor with regards to CYP3A4-related variability. However, CYP3A4 polymorphism has been classified as “low” due to rare genetic variants that are not associated with polymorphic phenotypes of demonstrated clinical relevance (Stingl et al., 2013). Other CYP enzymes present a higher degree of polymorphism and allelic variants can lead to enzyme deficiency which lead to a range of phenotypes within a population so that individuals can be classified as “poor metaboliser” (PM), “intermediate metaboliser” (IM), “extensive metaboliser” (EM) or “ultra-rapid metaboliser” (UM). The major polymorphic CYP enzymes are CYP2D6, CYP2C9 and CYP2C19 (Waring, 2019).

Human serum PON1 is another example of polymorphic phase I enzyme (Furlong et al., 2016a). However, the activity of this esterase is substrate dependent (Li et al., 2000); the phenotype is not classified as described previously but as “PON1 status” (Costa et al., 2005) since the same genotype can exhibit different level of activity toward different probe substrates (Chapter 4). PON1 has been described as a good predictor of individual susceptibility to organophosphate insecticides (OPs) toxicity (Alejo-González et al., 2018; Dardiotis et al., 2019). The conducted meta-analysis confirmed that inter-phenotypic differences in PON1 activity have an impact on the potential susceptibility to OP toxicity in the detoxification of oxon metabolites (Costa et al., 2013).

UGT isoforms are known to be highly polymorphic with more than a hundred variants (Stingl et al., 2014). In most cases, these polymorphic variants result in lower expression levels and/or lower activity, with sometimes even complete loss of activity (Sim et al., 2013). However, as for Chapter 3, there was insufficient information concerning genetic polymorphism of the volunteers (Chapter 5). Besides

different phenotypes, age is also an important factor impacting on human variability in xenobiotic metabolism, since it has been reported that neonates and young children show different expression levels of UGT compared to adults that can lead to a 5-fold difference in pharmacokinetic parameters (Bhatt et al., 2019; Court, 2010; Dorne et al., 2001b).

Next to phase I and II metabolic enzymes, transporters can contribute significantly to human variability in kinetics (Dorne et al., 2004b). While this chapter describe the first meta-analysis of kinetic variability related to specific human transporters, probe substrates have been identified only for two ABC transporters, P-gp and BCRP and two SLC transporters, OAT1 and OAT3. The effects of the genetic polymorphism on transporters seem to be substance specific, due to changes of the substrate-binding domain which alters substrate affinity. This chapter mainly covers the absorption and elimination processes with regard to P-gp and BCRP that are both highly expressed at the apical membrane of enterocytes and may limit the oral bioavailability of a range of chemicals (Clerbaux et al., 2019).

These chapters outline opportunities and challenges associated with the derivation of pathway-related variability to support chemical risk assessment with data-driven approaches to replace default UFs. Data gaps have been identified for both phase I and II enzymes and for transporters. Chapter 3 highlights the importance to consider specific subpopulations such as neonates, infants and elderly for whom the human kinetic UF was exceeding the default value of 3.16. However, these populations are rarely included in pharmacokinetic studies, mainly due to ethical considerations restricting clinical studies in specific subpopulations such as neonates and infants. Moreover, when polymorphic expression of an enzyme constitutes the a key factor impacting metabolic activity and its associated variability within and across sub-populations, phenotypic characterisation in the human volunteers would significantly support taking into account the most sensitive populations but is not systematically performed (either the PM or UM depending of the relative level of toxicity between the parent compound and its metabolites) (Waring, 2019). Furthermore, the analyses performed here conclude that it is recommended to investigate polymorphisms rather than geographical origins, since polymorphisms provide a better predictor in the alteration of pharmacokinetics compared to that for genotypic frequencies alone (Wu et al., 2017a).

Furthermore, the methodology and modelling proposed here can be applied to other metabolic pathways of interest to assess human inter-individual differences in kinetics in a broader context. *In vitro* techniques are now available to provide metabolism data from human cell lines (Bell et al., 2018; Blaauboer et al., 2012). Combining accurate inter-individual information from human data, as shown here, with such *in vitro* data can provide a very useful tool for the development of quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) models (Bell et al., 2018; Bteich et al., 2019; Poulin, 2013; Poulin and

Haddad, 2013; Yoon et al., 2013). Indeed, the estimated CV can be applied to an extrapolated clearance from QIVIVE and can be combined in a PBK model with a lognormal distribution describing human variability in clearance using Markov-Chain Monte Carlo methods instead of single deterministic mean values. This will ultimately allow to increase the confidence in such modelling approaches while providing credibility intervals (95% CI) around the derived UFs in a transparent manner.

A scientific report from EFSA (EFSA, 2014) has critically evaluated a range of approaches such as *in vitro* and *in silico* methods, integrated testing strategies, OMICs, and PBK modelling as “modern methodologies and tools for human hazard assessment of chemicals”. A key aspect to move towards the applicability of these tools in chemical risk assessment was discussed and characterised as the integration of exposure data (external dose) together with kinetic processes (internal dose) and dynamic data providing quantitative metrics for the expression of chemical-specific toxicity. Moreover, given the trend to reduce animal testing in chemical risk assessment, the challenge for the PBK modelling community is to calibrate model parameterisation increasingly or entirely on ADME properties derived from *in vitro* or *in silico*, with limited or no availability of *in vivo* kinetic data (Leonard and Tan, 2019; Madden et al., 2019; Punt et al., 2017). Currently, application of PBK models in human risk assessment are possible for the hazard characterisation of compounds with well-known kinetics, as well as for exposure assessment using reverse dosimetry modelling using inputs from biomonitoring studies from national surveys (Tohon et al., 2018). Chapter 2 has described a reverse dosimetry approach for exposure assessment of the adult French population to permethrin. Physiological variability has been taken into account in order to model urinary excretion of metabolites in the studied population. However, fix values of K_m and V_{max} were used in the model to describe the metabolism of permethrin (Willemin et al., 2015). More recently, Mallick et al. (2019) developed a life-stage PBK model to assess internal doses of pyrethroids in humans. This study displays the relative contribution of CYPs and carboxylesterases to the metabolism of *cis*-permethrin. Integration of the quantified human kinetic variability in such PBK model would allow to consider human variability in the estimation of internal dose of such insecticides and to refine their risk assessment.

7.2. Towards next generation human risk assessment of chemicals

The use of PBK modelling together with approaches to better account for inter-individual variability are increasingly recommended in human risk assessment of chemicals (Bessemers et al., 2014; EFSA, 2014; IPCS, 2010; Paini et al., 2017; Paini et al., 2019; Sachana, 2019). Indeed, applying a PBK model with parameter specific distributions integrating variability in a Bayesian framework would allow a better prediction of internal dose and decrease uncertainty in estimates (Bois et al., 2010; Chetty et al., 2018; Krauss and Schuppert, 2016; McNally et al., 2018; Tsiros et al., 2019). 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 (Punt et al., 2017; Yoon et al., 2015). 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 (McNally et al., 2014; McNally et al., 2015).

A tiered approach has been suggested for PBK modelling regarding the availability of chemical-specific information (Paini et al., 2019). Considering kinetic, *in vivo* data are limited to few extensively studied compounds and for limited species (data rich). Data poor chemicals should then be address either with one compartment model or simple PBK model. However, the derived human pathway-related variability can be used alongside *in vivo* clearance extrapolated from *in vitro* assays (Bteich et al., 2019; Louisse et al., 2019; Poulin and Haddad, 2013; Timoumi et al., 2019) to derive log-normal distributions of the parameter. This would allow to refine the input values of kinetic data for the PBK model and then to use a higher tiered model. Development and application of PBK models would furthermore benefit from the setup of ADME and chemical-specific parameters as well as data on enzyme expression and activities to support the development of QIVIVE models (Lautz et al., 2019). While there are available *in vitro* assays for liver and intestinal metabolism with relevant QIVIVE models (Clerbaux et al., 2018; Yoon et al., 2015), validated *in vitro* assays that allow to model renal excretion of chemicals still need to be developed.

Another source of chemical-specific data for the development of PBK models is increasingly arising from *in silico* tools (Madden et al., 2019). For instance, quantitative structure–activity relationship (QSAR) models allows to predict compound-specific tissue:blood partition coefficients (Hendriks et al., 2005; Huizer et al., 2012). OMICs technologies (*i.e.* transcriptomics, proteomics, metabolomics) also provide qualitative and quantitative information on expression and activity of enzymes and transporters in a range of animal species and can further support to gain insight for the characterisation of differences in ADME processes (EFSA, 2018).

7.3. Conclusion and recommendations

In order to move towards the next generation of chemical risk assessments particularly through the use of pathway-related variability, we recommend the following steps when considering data poor compounds (summarised in Figure 33):

1. Isoform-specific intrinsic clearance can be produced *in vitro* (Timoumi et al., 2019) and integrated into QIVIVE models to estimate isoform-specific *in vivo* intrinsic metabolic clearances (Bell et al., 2018; Bteich et al., 2019; Mallick et al., 2019; Poulin and Haddad, 2013).
2. Pathway-related variability can be applied to derive log-normal distributions of intrinsic clearances based on extrapolated values.
3. Further development of generic human PBK models and integration of population parameters. A virtual population, either healthy adults or a specific population (infants, elderly, etc.) can be modelled using the web application PopGen (McNally et al., 2014; McNally et al., 2015).
4. Other chemical-specific parameters can be obtained through QSAR models such as the tissue:blood partition coefficients (Hendriks et al., 2005).
5. Blood or tissue concentration of the compound or its metabolites can be derived using Monte Carlo simulations.
6. When dynamic data either from *in vitro* or *ex vivo* experiments are available (dose-response curve), a BMD can be derived (McNally et al., 2018) and risk assessment can be performed based on exposure scenarios or reverse dosimetry simulations to assess the actual population exposure and its associated risk.

Further research are required to investigate kinetic variability for specific populations specifically for neonates and infants. Moreover, when considering pharmacokinetic studies of compounds that are metabolised by polymorphic pathways, volunteers should be classified according to their phenotype to refine UFs for ultra and/or poor metabolisers. *In vitro* or *in silico* (QSAR) models should be also developed for the prediction of human renal excretion. Moreover, isoform-specific information are already produced when addressing adverse outcome pathways such as drug-induced liver injury (Vinken, 2013; Vinken, 2015) (e.g. for food safety). Such kinetic data should be requisite form the industrials when submitting new active substances for risk assessment.

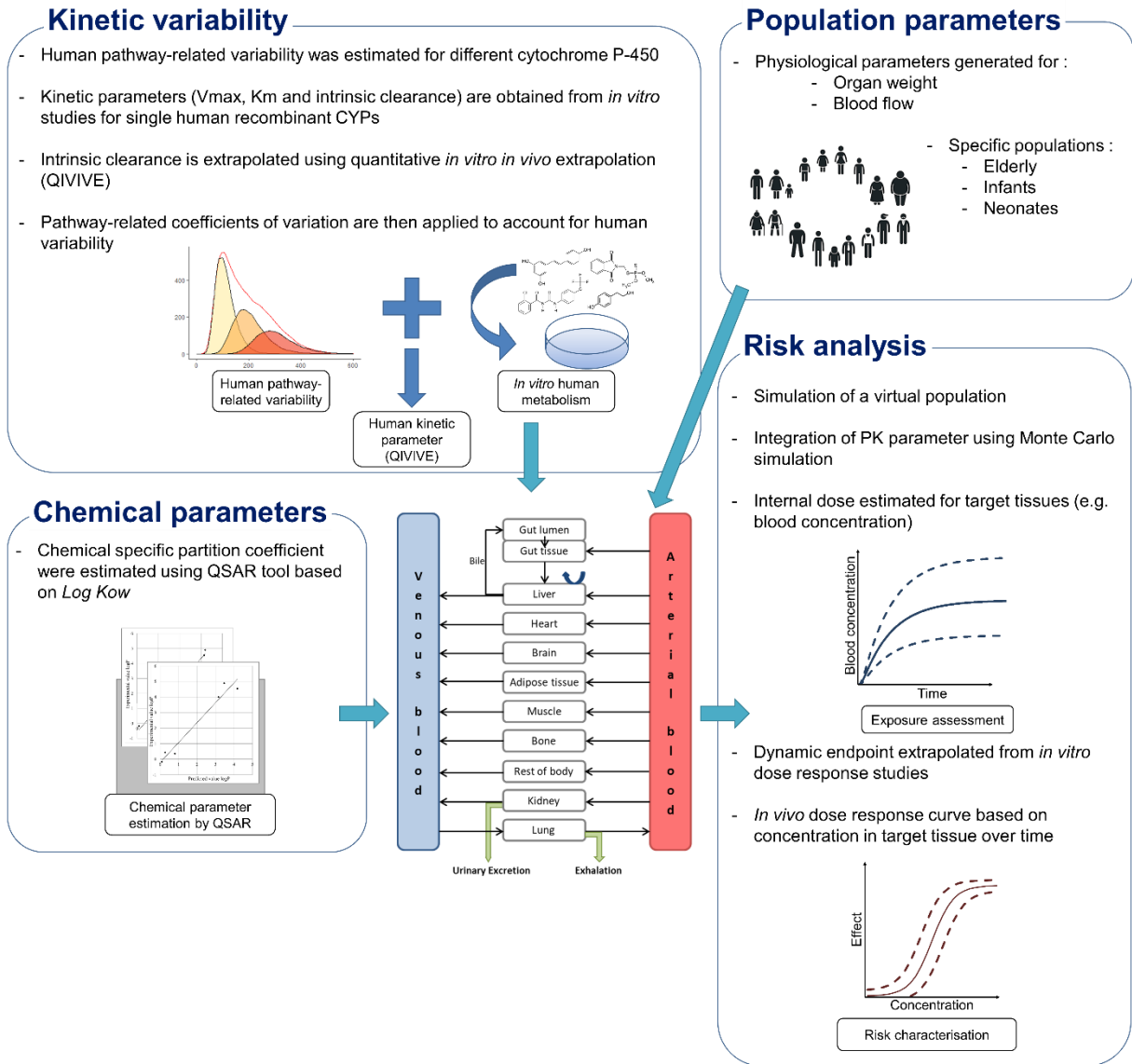


Figure 33. Integrating human variability in kinetics for the risk assessment of data poor chemicals.

8. References

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Université Grenoble Alpes – UFR de Médecine : Master 2 Méthodes de recherche en environnement, santé, toxicologie, écotoxicologie (MRESTE), Unité d'Enseignement « Modélisation en santé environnement » (10/2018 et 11/2019).

Université Paris Est Créteil – Faculté des Sciences et Technologie : Master 2 Sciences et Technologie de l'agriculture, de l'alimentation et de l'environnement parcours analyse des risques sanitaires liés à l'alimentation (ARSA), Unité d'enseignement « Analyse quantitative des risques chimiques » (10/2018 et 11/2019)

Conservatoire national des Arts et Métiers (CNAM, Paris) : Unité d'enseignement « Quantification et surveillance des risques » (05/2019).

Trainee supervisor: Master 2 student « Analyse du risque toxicologique pour le consommateur » (Université de Bretagne Occidentale), 6 months internship, « Acquisition, vérification et consolidation de données pour modèles PBPK enfants » (03-08/2018).

Résumé français :

Introduction

Evaluation des risques chimiques pour l'Homme

Dans le monde moderne, les humains sont exposés à une vaste gamme de produits chimiques tout au long de leur vie. Ces produits chimiques sont présents dans la vie quotidienne et comprennent les produits pharmaceutiques, les produits de consommation (ingrédients cosmétiques) et les produits chimiques qui sont intentionnellement ajoutés aux aliments (additifs alimentaires, arômes, matériaux en contact avec les aliments), aux matières premières (pesticides/biocides : herbicides, fongicides, insecticides, etc.) ou dans les aliments pour animaux d'élevage en tant que médicaments vétérinaires. De plus, les contaminants de la chaîne alimentaire constituent une autre source d'exposition chimique pour les humains et comprennent les contaminants d'origine humaine tels que les contaminants liés aux processus de transformation (acrylamide, furanes), les polluants environnementaux (ignifugeants bromés, dioxines, BPC, perfluoroalkyls), les métaux (résultant de l'activité humaine) ainsi que les toxines naturelles (mycotoxines, alcaloïdes végétaux, biotoxines marines) (Dorne et al., 2009). Avec un tel éventail de produits chimiques, l'évaluation des risques des produits chimiques pour les humains revêt une importance considérable pour la santé publique et permet de calculer des niveaux sûrs d'exposition aiguë et chronique pour des sous-groupes de la population humaine, notamment les nouveau-nés, les enfants, les personnes âgées et les populations d'ascendance géographique différente (différences interethniques) et de polymorphismes génétiques.

En effet, l'évaluation des risques est un élément central de l'analyse des risques et fournit une base scientifique pour la gestion des risques sur les décisions et les mesures qui peuvent être nécessaires pour protéger la santé humaine et pour la communication des risques afin de permettre un échange interactif d'informations entre les évaluateurs des risques, les gestionnaires, les médias, les parties prenantes et le grand public (FAO/OMS, 2018). Les quatre étapes de l'évaluation des risques chimiques sont l'identification des dangers, la caractérisation des dangers, l'évaluation de l'exposition et la caractérisation des risques.

Selon que la substance étudiée est génotoxique et cancérigène ou non-génotoxique, différentes méthodes sont utilisées pour effectuer l'évaluation des risques de ces composés. Traditionnellement, le point de départ ou dose critique est estimée à partir de la plus forte dose testée sans effet observé (NOAEL) dans le cas des composés non-génotoxique (SCHER/SCCP/SCENIHR, 2009). La méthode de la benchmark dose (BMD) est recommandée dans le cas des substances génotoxiques et cancérigènes (Crump, 1984; EFSA Scientific Committee et al., 2017; U.S. EPA, 2012).

La façon habituelle de calculer les valeurs sanitaires de référence est de diviser la dose critique déterminée par des facteurs d'incertitude (UFs) qui visent à décrire les différences entre les espèces et les individus. Dans les cas où les expositions dépassent les valeurs sanitaires de référence, la caractérisation des risques ne fournit pas aux gestionnaires de risques des conseils sur l'étendue possible du risque pour les personnes exposées à ces niveaux supérieurs. Une première considération devrait être que les valeurs sanitaires de référence incorporent elles-mêmes les facteurs d'incertitude (IPCS, 2009).

Facteurs d'incertitude dans l'évaluation des risques des produits chimiques

Lehman et Fitzhugh (1954) ont introduit il y a 60 ans un facteur d'incertitude de 100 pour tenir compte des différences interspèces et interindividuels afin de déterminer, à partir d'études sur les animaux, des valeurs sanitaires de référence chez l'Homme. Ce facteur 100 est le produit de deux facteurs 10 qui tiennent compte des différences entre les espèces et de la variabilité chez les humains (IPCS, 1987). Toutefois, ces facteurs 10 par défaut ne tiennent pas compte des données métaboliques ou du contexte mécanistique de façon quantitative dans l'évaluation des risques. Les facteurs d'incertitude interspèces et interindividuels ont donc été subdivisés en aspects cinétiques et dynamiques (Renwick, 1993). Les valeurs de $10^{0,6}$ (4,0) et de $10^{0,4}$ (2,5) ont ensuite été proposées pour les différences entre les espèces sur le plan cinétique et dynamique. En ce qui concerne la variabilité cinétique et dynamique chez l'Homme, le facteur par défaut de 10 est subdivisé en deux facteurs $10^{0,5}$ (3,16) (IPCS, 1994). Il a été démontré que lors de l'évaluation de la variabilité humaine à l'aide de médicaments, les facteurs cinétiques et dynamiques par défaut ne couvriraient pas la variabilité humaine spécifiquement pour les voies polymorphiques ou pour des populations spécifiques comme les nouveau-nés (Renwick et Lazarus, 1998). Ces facteurs d'incertitude par défaut cinétiques et dynamiques peuvent être affinés en utilisant des facteurs d'ajustement spécifiques aux produits chimiques (CSAF) (IPCS, 2005) ou des facteurs d'incertitude liés aux voies métaboliques (Dorne, 2010 ; Dorne et al., 2005).

Modèles bayésiens hiérarchiques pour la méta-analyse des données cinétiques

Les précédentes méta-analyses sur la variabilité cinétique chez l'homme pour différentes voies métaboliques étaient basées sur des moyennes géométriques pondérées en supposant des modèles à effet fixe avec des poids de variance inverses. Cette approche permet d'estimer la variabilité humaine des paramètres cinétiques, mais elle ne tient pas compte de la contribution relative de la variabilité entre les sous-groupes à la variabilité globale des ensembles de données, ce qui entraîne une incertitude dans les estimations des paramètres (Dorne et al., 2005).

Dans un contexte Bayésien, une distribution à priori est établie soit sur la base de connaissances d'experts, soit en utilisant des preuves tirées de la littérature. Ces distributions sont ensuite mises à jour en tenant compte des nouvelles données disponibles, ce qui conduit à une distribution à posteriori (Micallef et al., 2005). L'estimation Bayésienne fournit une distribution de la crédibilité des valeurs des paramètres et une représentation de l'incertitude des paramètres qui peut être directement interprétée par la distribution postérieure. Les distributions à posteriori sont estimées en générant un énorme échantillon aléatoire de valeurs de paramètres représentatives à partir de la distribution antérieure à l'aide de la méthode de Monte Carlo par chaîne de Markov (MCMC). Par conséquent, elle décrit comment l'incertitude change lorsqu'on tient compte de nouvelles données (Kruschke et Vanpaemel, 2015).

La variabilité humaine des paramètres cinétiques de l'exposition aiguë et chronique peut être dérivée d'une méta-analyse d'études pharmacocinétiques humaines utilisant une approche bayésienne hiérarchique. Par conséquent, l'inclusion de plusieurs composés spécifiques d'une même voie dans un modèle bayésien hiérarchique permettrait alors de préciser le facteur d'incertitude liée à la voie.

Objectifs de la thèse

Cette thèse vise à :

1. Quantifier la variabilité humaine au moyen d'une méta-analyse Bayésienne pour plusieurs voies métaboliques de phase I et phase II et des transporteurs (phase 0 et III) en utilisant les marqueurs pharmacocinétiques d'exposition aiguë (C_{max}) et chronique (AUC, clairance) ou les données d'activité enzymatique des substrats spécifiques.
2. Estimer les distributions de variabilité liées aux voies métaboliques et les facteurs d'incertitude liés à ces voies en vue de leur intégration future dans les modèles de PBK pour l'évaluation des risques des substances chimiques pour l'Homme.

La méthodologie proposée utilise un modèle Bayésien hiérarchique à plusieurs niveaux pour intégrer les sources quantifiables de variabilité, y compris la variabilité inter-études, inter et intra-ethnique, inter-populations sensibles et/ou inter-phénotypique. Dans ce contexte, la variabilité liée à la voie métabolique et les facteurs d'incertitude correspondants sont estimés pour des sous-groupes de la population humaine et du paramètre pharmacocinétique.

Modélisation physiologiques basés sur la cinétique

Les modèles physiologiques basés sur la cinétique (PBK) fournissent une approche quantitative pour traiter les processus d'absorption, distribution, métabolisme et excrétion (ADME) et sont donc des

outils très utiles dans l'évaluation des dangers (EFSA, 2014), notamment dans la réalisation de dosimétrie inverse, permettant de combler les inconnus entre dose externe et dose interne.

L'étude nationale nutrition santé (ENNS) a fait état de niveaux de biomarqueurs d'exposition aux pyréthrinoïdes plus élevés que ceux observés dans les études de biosurveillance nord-américaines et allemandes. L'exposition globale à la perméthrine a été étudiée comme première étude de cas, car ce composé est l'un des insecticides pyréthroïdes les plus utilisés. Nous avons évalué plusieurs sources de contamination - comme l'air intérieur et extérieur, la poussière déposée et le régime alimentaire - et plusieurs voies d'exposition, notamment par voie orale, par inhalation et par voie cutanée. Nous avons utilisé des estimations du niveau d'exposition à la perméthrine (calculées à partir des données de l'ENNS) et un modèle pharmacocinétique et pharmacocinétique étalonné avec des données cinétiques humaines (provenant de 6 individus) pour simuler une dose interne d'acide cis- et trans-3-(2,2 dichlorovinyl)-2,2-diméthyl-(1-cyclopropane) carboxylique (cis- ou trans-DCCA) dans une population de 219 individus. Les concentrations urinaires de cis- et de trans-DCCA prédites par le modèle PBPK selon trois scénarios d'exposition à la perméthrine ("inférieure", "intermédiaire" et "supérieure") ont été comparées aux concentrations urinaires mesurées dans l'étude ENNS. Les niveaux de l'ENNS se situaient entre les niveaux simulés selon les scénarios d'exposition à la perméthrine "inférieur" et "intermédiaire". Le scénario "supérieur" a entraîné une surestimation des concentrations urinaires prévues de cis- et de trans-DCCA par rapport à celles mesurées dans l'étude de l'ENNS. Le scénario le plus réaliste était le scénario " inférieur " (concentration de perméthrine des données censurées à gauche considérée comme nulle). A l'aide d'un modèle PBK chez l'Homme, nous avons estimé la contribution de chaque voie et source à la dose interne. La principale voie d'exposition à la perméthrine était la voie orale (98 %), l'alimentation étant la principale source (87 %), suivie des poussières (11 %), puis de la voie cutanée (1,5 %) et enfin de l'inhalation (0,5 %).

Des analyses de sensibilité des paramètres du modèle PBK ont été effectuées et confirment que les paramètres cinétiques sont ceux qui influent le plus sur le paramètre de sortie du modèle (excrétion urinaire des métabolites) et que des efforts devraient être déployés pour préciser ces paramètres. Par conséquent, un modèle Bayésien hiérarchique pour la méta-analyse des données cinétiques a été élaboré.

Différences interethniques liées au métabolisme de CYP3A4

CYP3A4 est la principale isoforme des cytochromes P450 humain et responsable du métabolisme de plus de 50 % des xénobiotiques connus. Les différences interethniques dans le métabolisme du CYP3A4 ont été étudiées dans le cadre d'une revue systématique des données pharmacocinétiques de 15

substrats de sonde du CYP3A4 et des paramètres reflétant l'exposition aiguë (C_{max}, voie orale) et chronique (clairance et AUC, voie orale et intraveineuse). Toutes les données ont été extraites dans une base de données structurée et des méta-analyses ont été effectuées à l'aide d'un modèle Bayésien hiérarchique afin de déterminer les distributions de la variabilité du métabolisme du CYP3A4 en fonction des paramètres, de la voie d'administration et de la population. Deux approches différentes ont été appliquées. 1) Les différences interindividuelles ont été quantifiées en utilisant les adultes nord-américains en bonne santé comme groupe de référence pour les comparer aux adultes européens, asiatiques, du Moyen-Orient et sud-américains en bonne santé, ainsi qu'aux personnes âgées, aux enfants et aux nouveau-nés. 2) Les distributions de la variabilité intra-ethnique ont été estimées sans comparaison avec un groupe de référence. Dans l'ensemble, les distributions de la variabilité du CYP3A4 propres aux sous-groupes ont servi de base pour calculer les facteurs d'incertitude liés au CYP3A4 pour couvrir le 95^{ème} ou 97,5^{ème} centile de la population et ont été comparées au facteur d'incertitude cinétique par défaut chez l'Homme (3,16). Les résultats indiquent que les facteurs d'incertitude liés au CYP3A4 chez les adultes en santé étaient plus élevés pour les expositions chroniques par voie orale (2,5-3,0, UF95 et UF97,5, 10 composés) que pour les expositions par voie intraveineuse (1,7-1,8, 2 composés). Tous les facteurs d'incertitude se situaient dans les limites du facteur d'incertitude par défaut de la cinétique. Ces distributions tiennent compte de : 1) l'application des facteurs d'incertitude liés au CYP3A4 dans l'évaluation des risques des composés pour lesquels on dispose de données *in vitro* sur le métabolisme du CYP3A4 sans qu'il soit nécessaire d'obtenir des données sur les animaux ; 2) l'intégration des distributions de variabilité liées au CYP3A4 avec les données sur le métabolisme *in vitro* dans des modèles cinétiques physiologiques (PBK) pour l'extrapolation quantitative *in vitro vers in vivo* (QIVIVE) et 3) l'estimation des facteurs d'incertitude dans l'évaluation des risques chimiques à l'aide des distributions de variabilité du métabolisme.

Variabilité humaine liée à la paraoxonase-1

La variabilité des activités de la paraoxonase-1 (PON1) chez l'Homme est due à des polymorphismes génétiques qui influent sur la dose interne en métabolites actifs (oxon) des insecticides organophosphorés. Ici, une revue de la littérature approfondie a été effectuée pour recueillir les fréquences génotypiques humaines (c.-à-d. L55M, Q192R et C-108T) dans des sous-groupes ayant des origines géographiques différentes et pour trois substrats spécifiques de PON1 (paraoxon, diazoxon et acétate de phényle). Des méta-analyses bayésiennes ont été effectuées pour estimer les distributions de variabilité des activités du PON1 et des facteurs d'incertitude liés à PON1, tout en intégrant des sources quantifiables de différences inter-études, inter-phénotypiques et inter-individuelles. Les différences inter-phénotypiques ont été quantifiées en utilisant la population ayant une activité PON1

élevée comme groupe de référence. Les résultats des méta-analyses ont fourni des distributions de variabilité d'activité de PON1 et celles-ci peuvent être intégrées dans des modèles PBK génériques pour élaborer des modèles d'extrapolation quantitative *in vitro* et *in vivo*. Les facteurs d'incertitude liées à PON1 dans la population caucasienne étaient supérieurs au facteur d'incertitude cinétique par défaut de 3,16 pour deux génotypes, soit le -108CC en utilisant du diazoxon comme substrat et le -108CT, le -108TT, le 55MM et le 192QQ en utilisant du paraoxon comme substrat. Cependant, l'intégration des fréquences génotypiques et des distributions d'activité des PON1 a montré que tous les facteurs d'incertitude étaient dans les limites du facteur d'incertitude par défaut cinétique. Les différences quantitatives interindividuelles de l'activité des PON1 sont importantes pour l'évaluation des risques chimiques, en particulier en ce qui concerne la sensibilité potentielle à la toxicité des insecticides organophosphorés.

Variabilité humaine des UDP-glucuronosyltransférases

Les UDP-glucuronosyltransférases (UGT) sont des enzymes qui sont couramment impliquées dans les réactions de conjugaison de phase II des xénobiotiques. Les différences dans les activités des isoformes d'UGT peuvent entraîner de grandes différences interindividuelles dans les profils cinétiques des composés glucuronidés. Dans cette étude, les différences interindividuelles des paramètres pharmacocinétiques des marqueurs de l'exposition aiguë et chronique ont été quantifiées pour quatorze composés métabolisés par sept isoformes d'UGT (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7 et UGT2B15) et les fréquences des polymorphismes de ces UGT dans les populations humaines ont été étudiées. Les paramètres pharmacocinétiques sont résumés dans une base de données et les facteurs d'incertitude liés aux UGT, pour les composés individuels et pour chaque isoforme, ont été calculés et comparés au facteur d'incertitude par défaut (3,16). L'information sur les distributions de fréquence des polymorphismes a été comparée à la base de données des paramètres pharmacocinétiques. Les résultats montrent que les données pharmacocinétiques dans la littérature sont surtout disponibles pour les populations caucasiennes et rares pour les autres ethnies. De plus, les renseignements sur les polymorphismes en relation avec les paramètres pharmacocinétiques sont rarement abordés dans les études incluses, bien que des différences soient observées dans les distributions de fréquence et que ces polymorphismes aient une grande influence sur les paramètres cinétiques. Dans l'ensemble, le facteur d'incertitude par défaut de 3,16 couvre la population adulte pour toutes les isoformes UGT incluses et pour la plupart des composés, sauf pour le 1-OH-Midazolam, l'ézétimibe, le raltégravir, le SN38 et la trifluopérazine. Le facteur d'incertitude calculé le plus élevé était de 3,6 pour le raltégravir. Les facteurs d'incertitude calculés propres aux isoformes indiquent que

le facteur d'incertitude cinétique par défaut couvrirait plus de 97,5 % des personnes en bonne santé pour toutes les isoformes d'UGT.

Variabilité humaine liée aux transporteurs : ATP Binding Cassette et transporteurs de solutés

Les transporteurs sont divisés en deux super-familles, à savoir les transporteurs ABC (ATP Binding Cassette) et les transporteurs de solutés (SLC), qui servent d'intermédiaires pour l'afflux et le reflux cellulaire de divers substrats xénobiotiques et endogènes. Ici, une revue de la littérature approfondie a été effectuée pour les substrats spécifiques *in vivo* de la P-glycoprotéine (P-gp), de la protéine résistante au cancer du sein (BCRP) et des transporteurs anioniques organiques (OAT1/3). Pour les autres transporteurs (OCT, OATP), aucun substrat spécifique *in vivo* n'a pu être identifié. Les données cinétiques humaines, telles que la C_{max}, la clairance et l'AUC, ont été extraites de 142 publications. Les analyses de données ont été effectuées à l'aide d'un modèle bayésien hiérarchique et les distributions de variabilité ainsi que les facteurs d'incertitude liés aux transporteurs ont été estimés. D'après les données disponibles, limitées à la classe d'âge adulte, à certains groupes ethniques (surtout caucasiens), pour la P-gp, la variabilité humaine calculée a indiqué que le facteur d'incertitude par défaut de la cinétique (3,16) couvrirait plus de 97,5 % des individus en bonne santé, lorsqu'on considère la valeur médiane, alors qu'elle est dépassée lorsqu'on considère l'intervalle de confiance supérieur. Pour la BCRP et l'OAT1/3, la variabilité humaine, limitée aux adultes en bonne santé, indique que le facteur d'incertitude par défaut de 3,16 n'est pas dépassé, même si l'on tient compte de l'intervalle de confiance supérieur. Bien que les données cinétiques sur les polymorphismes des transporteurs soient limitées, on a signalé une variabilité inter-phénotypique pour les substrats spécifiques, ce qui pourrait indiquer que le facteur d'incertitude par défaut actuelle pourrait être insuffisant pour couvrir ces polymorphismes. Dans l'ensemble, pour prédire si la part de la cinétique dans le facteur d'incertitude intra-individuel protège les humains, il est recommandé d'étudier les polymorphismes génétiques dans tous les groupes humains plutôt que d'utiliser l'ethnicité comme mesures substitutives des différences génétiques.

Conclusion

Afin de passer à la prochaine génération d'évaluations des risques chimiques, en particulier par l'utilisation de la variabilité liée aux voies métaboliques, nous recommandons les étapes suivantes lors de l'examen des substances pour lesquels les données sont insuffisantes :

1. La clairance intrinsèque spécifique de chaque isoforme peut être produite *in vitro* (Timoumi et al., 2019) et intégrée dans les modèles QIVIVE pour estimer les clairances métaboliques

intrinsèques *in vivo* spécifiques de ces isoformes (Bell et al., 2018b ; Bteich et al., 2019 ; Mallick et al., 2019 ; Poulin et Haddad, 2013).

2. La variabilité liée à la voie métabolique peut être appliquée pour estimer des distributions log-normales des clairances intrinsèques basées sur des valeurs extrapolées.
3. Poursuite de l'élaboration de modèles PBK humains génériques et intégration des paramètres de population. Une population virtuelle, soit des adultes en santé ou une population spécifique (nouveau-nés, personnes âgées, etc.) peut être modélisée à l'aide de l'application Web PopGen (McNally et al., 2014b ; McNally et al., 2015).
4. D'autres paramètres spécifiques aux produits chimiques peuvent être obtenus par le biais de modèles QSAR, comme les coefficients de partage tissus-sang (Hendriks et al., 2005).
5. La concentration du composé ou de ses métabolites dans le sang ou les tissus peut être calculée à l'aide de simulations de Monte Carlo.
6. Lorsque des données de dynamiques provenant d'expériences *in vitro* ou *ex vivo* sont disponibles (courbe dose-réponse), on peut calculer une BMD (McNally et al., 2018) et évaluer les risques en fonction de scénarios d'exposition ou de simulations de dosimétrie inverse pour évaluer l'exposition réelle de la population et les risques qui y sont associés.

D'autres recherches sont nécessaires pour étudier la variabilité cinétique pour des populations spécifiques, en particulier pour les nouveau-nés et les enfants. De plus, lorsqu'on envisage d'effectuer des études pharmacocinétiques de composés qui sont métabolisés par des voies polymorphiques, les volontaires doivent être classés selon leur phénotype afin d'améliorer la précision des facteurs d'incertitude pour les métaboliseurs très rapides et/ou lents. Des modèles *in vitro* ou *in silico* (QSAR) devraient également être élaborés pour la prédiction de l'excrétion rénale humaine. De plus, des renseignements propres aux isoformes sont déjà produits lorsqu'il s'agit d'étudier les voies d'effets indésirables, comme les lésions hépatiques d'origine médicamenteuse (Vinken, 2013 ; Vinken, 2015). Toutefois, peu d'efforts ont été déployés jusqu'à maintenant pour produire des données cinétiques propres aux isoformes, car elles ne sont pas requises par les évaluateurs de risques (ex. cadre réglementaire de la sécurité des aliments). De telles données cinétiques devraient être exigées des industriels lorsqu'ils soumettent de nouvelles substances actives pour l'évaluation des risques.

Titre : Vers la nouvelle génération d'évaluation des risques des produits chimiques : Méta-analyse Bayésienne de la variabilité humaine liée au métabolisme et aux transporteurs et application à la détermination des facteurs d'incertitude liés aux voies de cinétique

Mots clés : variabilité humaine ; toxicocinétique ; facteur d'incertitude ; polymorphisme ; modèle Bayésien ; modèle PBK

Résumé : Dans le monde moderne, les humains sont exposés à une vaste gamme de produits chimiques tout au long de leur vie. L'évaluation des risques des substances chimiques pour l'homme revêt une importance capitale pour la santé publique et permet de calculer des niveaux sûrs d'exposition aiguë et chronique pour des sous-groupes de la population humaine, notamment les nouveau-nés, les enfants, les personnes âgées et les populations d'origine géographique et de polymorphismes génétiques différents. L'application des données cinétiques liées aux voies métaboliques pour tenir compte de la variabilité humaine dans la quantification du danger a le potentiel de réduire l'incertitude et de mieux caractériser la variabilité par rapport

à l'utilisation traditionnelle des facteurs d'incertitude par défaut.

Cette thèse vise à : 1) Quantifier la variabilité humaine au moyen d'une méta-analyse Bayésienne pour plusieurs voies métaboliques de phase I, phase II et transporteurs en utilisant des marqueurs pharmacocinétiques d'exposition aiguë et chronique ou des données d'activité enzymatique pour les substrats spécifiques disponibles. 2) Estimer les distributions de variabilité liées aux voies métaboliques et les facteurs d'incertitudes liés à ces voies pour leur intégration future dans les modèles physiologiques basés sur la cinétique pour l'évaluation des risques des produits chimiques pour l'Homme.

Title : Towards next generation risk assessment of chemicals: Bayesian meta-analysis of Human variability in metabolism and transporters and application for the derivation of pathway-related uncertainty factors

Keywords : human variability ; toxicokinetics ; uncertainty factor ; polymorphism , Bayesian model ; PBK model

Abstract: In the modern world, humans are exposed to a wide range of chemicals throughout their life. Human risk assessment of chemicals is of considerable public health importance and provides means to derive safe levels of acute and chronic exposure for subgroups of the human population including neonates, children, elderly and populations of different geographical ancestry and genetic polymorphisms. The application of pathway-related kinetic data to address human variability in the quantification of hazard has potential to reduce uncertainty and better characterize variability compared with the use of traditional default uncertainty factors.

This thesis aims to 1) quantify human variability by means of Bayesian meta-analysis for a range of phase I, phase II metabolic pathways and transporters using pharmacokinetic markers of acute and chronic exposure or enzyme activity data from available probe substrate, 2) derive pathway-related variability distributions and pathway-related uncertainty factors for their future integration in physiologically based kinetic models for human risk assessment of chemicals.