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Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: a review

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Abstract

This review provides an account of recent developments arising from a database that defined human variability in phase I metabolism (CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, hydrolysis, alcohol dehydrogenase), phase II metabolism (*N*-acetyltransferases, glucuronidation, glycine conjugation, sulphation) and renal excretion. This database was used to derive pathway-related uncertainty factors for chemical risk assessment that allow for human variability in toxicokinetics. Probe substrates for each pathway of elimination were selected on the basis that oral absorption was >95% and that the metabolic route was the primary route of elimination of the compound (60–100% of a dose). Intravenous data were used for compounds for which absorption was variable. Human variability in kinetics was quantified for each compound from published pharmacokinetic studies (after oral and intravenous dosing) in healthy adults and other subgroups of the population using parameters relating to chronic exposure (metabolic and total clearances, area under the plasma concentration–time curve (AUC)) and acute exposure (C_{max}) (data not presented here). The pathway-related uncertainty factors were calculated to cover 95%, 97.5% and 99% of the population of healthy adults and of each subgroup.

Pathway-related uncertainty factors allow metabolism data to be incorporated into the derivation of health-based guidance values. They constitute an intermediate approach between the general kinetic default factors (3.16) and a chemical-specific adjustment factor. Applications of pathway-related uncertainty factors for chemical risk assessment and future refinements of the approach are discussed. A knowledge-based framework to predict human variability in kinetics for xenobiotics showing a threshold dose below which toxic effects are not observed, is proposed to move away from default assumptions.

Keywords: Human variability; Pharmacokinetics; Metabolism; Pathway-related uncertainty factors; Chemical risk assessment

Abbreviations: ADI, acceptable daily intake; BMD, benchmark dose; CSAF, chemical-specific adjustment factor; LOAEL, low observed adverse effect level; NOAEL, no observed adverse effect level; RfD, reference dose; TDI, tolerable daily intake; AUC, area under the plasma-concentration–time curve; C_{max}, maximum plasma concentration; CYP, cytochrome P450; NP, non-phenotyped subjects; EM, extensive metabolisers; PM, poor metabolisers; FA, fast acetylators; SA, slow acetylators; GM, geometric mean; Ratio GM, ratio of geometric means; GSD, geometric standard deviation; CV, coefficient of variation; CV_{LN}, coefficient of variation (lognormal distribution); N_c, number of compounds; N_s, number of studies; N_p, number of publications; n, number of subjects

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1. Introduction

Non-genotoxic compounds, such as food additives, pesticides and many environmental contaminants, show a threshold dose below which toxic effects are not observed. Over the last 40 years, health agencies around the world have been estimating levels of exposure for such compounds that would be "without appreciable health risk" in humans by dividing a surrogate for the threshold by a standard default uncertainty factor (Dourson and Stara, 1983; Renwick, 1993; Dourson et al., 1996; Veirmeire et al., 1999).

The no observed adverse effect level (NOAEL) is the most commonly used surrogate for the threshold, and is usually derived from chronic or sub-chronic animal studies using the most sensitive species (mouse, rat, rabbit or dog) and a dose–response relationship that includes levels producing statistically significant adverse effects (WHO, 1987). The benchmark dose (BMD) concept was proposed as an alternative to the NOAEL by Crump (1984) and was defined as a lower statistical confidence limit (e.g. 95th centile) for the dose, corresponding to a predefined low level of increase in adverse effects over the background. The BMD can be calculated from continuous or quantal data and unlike the NOAEL, it takes into account the whole of the dose–response curve (Crump, 1984, 1995; Slob, 1999).

Depending on the regulatory body, the safe level of human exposure may be described as the acceptable daily intake (ADI) (Truhaut, 1991), the tolerable daily intake (TDI), or the reference dose (RfD). In most cases an uncertainty factor of 100-fold is used to convert the NOAEL/BMD from an animal study into the safe human intake (Lehman and Fitzhugh, 1954; Dourson and Stara, 1983; Renwick and Walker, 1993). This default uncertainty factor represents the product of two separate 10-fold factors that allow for interspecies differences and human variability (WHO, 1987; Renwick and Lazarus, 1998). These factors have to allow for both toxicokinetic and toxicodynamic differences. One problem with the use of uncertainty factors is that they do not allow chemical-specific metabolic or mechanistic data to contribute quantitatively to risk assessment. The 10-fold factors were therefore subdivided into toxicokinetic and toxicodynamic aspects (Renwick, 1993), and values of $10^{0.6}$ (4) and a $10^{0.4}$ (2.5) respectively were proposed for species differences, and equal values of $10^{0.5}$ (3.16) for human variability (WHO, 1994, 1999). The aim of the subdivision of the 10-fold factors was to allow the incorporation of suitable compound-specific data for one particular aspect of uncertainty (e.g. animal or human toxicokinetics for a compound under assessment) to replace the relevant part of the overall default uncertainty factor. The product of the chemical-specific value and the remaining default uncertainty factors would give a chemical-specific adjustment factor (CSAF) (WHO, 2001 see WHO website).

More recently, human variability in kinetics and dynamics have been reviewed for a range of metabolic pathways and mechanisms (Hattis et al., 1987; Hattis and Minkowitz, 1996; Naumann and Weidemann, 1995; Renwick and Lazarus, 1998; Silverman et al., 1999; Suh et al., 1999; Hattis et al., 1999; Veirmeire et al., 1999; Dorne, 2001; Dorne et al., 2001a,b, 2002, 2003a,b, 2004a,b; Naumann et al., 1997, 2001). Renwick and Lazarus (1998) analysed data on therapeutic drugs subject to a range of metabolic and elimination pathways and showed that the current 3.16 kinetic factor could not cover human variability in the case of genetic polymorphisms (differences between extensive and poor metabolisers) and the differences between healthy adults and some subgroups of the population (such as preterm infants).

These observations led to a proposal for the generation of pathway-related uncertainty factors (Renwick and Lazarus, 1998) that would constitute an intermediate option between default uncertainty factors and CSAFs. These uncertainty factors could be applied to chemicals for which the metabolic fate was known in humans, but for which chemical-specific toxicokinetic data were not available (Fig. 1). The development of pathway-related uncertainty factors required the quantification of toxicokinetic differences related to differences in metabolic pathways for both interspecies and human variability (Fig. 1). Depending on the particular endpoint and the regulatory agency, the pathway-related uncertainty factors can then be generated to cover a particular proportion of the population (e.g. to the 95th centile).

The proposal of Renwick and Lazarus (1998) has been developed using analyses of metabolism and pharmacokinetic data for probe substrates of phase I and

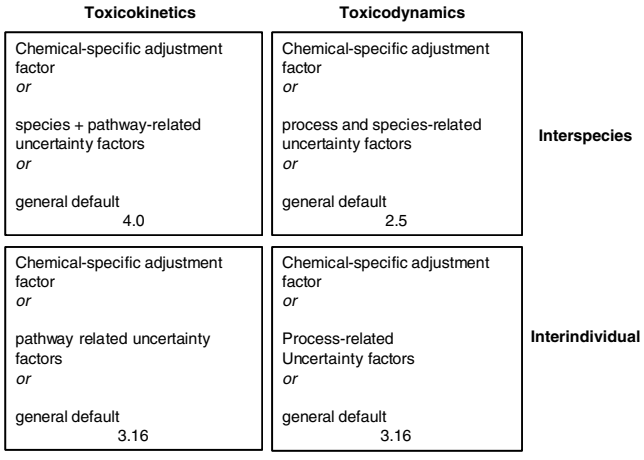

Fig. 1. Uncertainty factors, chemical-specific adjustment factors (CSAF), pathway-related uncertainty factors and the general default uncertainty factors (based on Renwick and Lazarus, 1998).

phase II metabolic pathways and renal excretion for interspecies differences and human variability (Dorne, 2001; Dorne et al., 2001a,b, 2002, 2003a,b, 2004a,b; Walton et al., 2001a,b, 2004). The analyses of human variability assessed critically the adequacy of the general 3.16 default kinetic factor, and calculated sets of pathway-related uncertainty factors for each metabolic route to cover the 95th, 97.5th and 99th centile of the general healthy adult population and available subgroups of the population respectively (Dorne, 2001; Dorne et al., 2001a,b, 2002, 2003a,b, 2004a,b).

Analysis of species differences in the toxicokinetics of compounds eliminated by a single major metabolic pathway in humans indicated that pathway-related factors for different species could be derived for some pathways, such as CYP1A2 (Walton et al., 2001a), glucuronidation (Walton et al., 2001b) and renal excretion (Walton et al., 2004), but for most compounds the pathway of elimination in humans did not reliably predict the pathway in animals (Walton et al., 2001c). In consequence, there would be considerable uncertainty in using a species-specific pathway-related factor, unless there were detailed data for both humans and the animal species, in which case a compound-specific value would be derived.

This review provides an account of recent developments arising from a database that defined human variability in different routes of metabolism and was used to generate pathway-related uncertainty factors for chemical risk assessment. These approaches allow metabolism data to be incorporated in the derivation of health-based guidance values for chemicals. The pathway-related uncertainty factors are reviewed for healthy adults and subgroups of the population, and further work is suggested to improve the use of science-based uncertainty factors.

2. Methods

2.1. Analysis of kinetic data

The methods used to review the literature (MEDLINE, PUBMED and TOXLINE from 1966 to 2001/2002/2003 depending on the pathway) and analyse pharmacokinetic data have been published previously for each individual pathway (Dorne et al., 2001a,b, 2002, 2003a,b, 2004a,b). Probe substrates were selected on the basis that the oral absorption was complete (>95%), and that a single pathway (phase I, phase II metabolism and renal excretion) was responsible for the elimination of the compound (60–100% of the dose). Intravenous data were used for compounds for which absorption was variable. The pathway was identified on the basis of quantitative biotransformation data from in vitro (microsomes, cell lines and primary cell cultures) and in vivo (urinary and faecal excretion) studies.

Human variability in kinetics was quantified for each probe substrate from published pharmacokinetic studies in general healthy adults and other subgroups of the population using parameters relating primarily to chronic exposure (metabolic and total clearances, area under the plasma concentration–time curve (AUC)) and to acute exposure (C_{max}) (data not shown). All parameters were analysed using the assumption that the data were log-normally distributed and that kinetics were linear at the doses studied. Ranking of the parameters was carried out as described previously so that no individual would contribute more than once to each analysis. Analysis of subgroups of the population included the influence of ethnicity and age.

Data for general healthy adults (16–70 years of age) were mostly from Caucasian individuals or where the ethnicity was not defined in the individual publication. Interethnic differences were investigated using mostly data for Asian, South Asian and African individuals. Variability in kinetics was analysed in different age groups and these included the elderly (healthy adults older than 70 years), children (>1 year to <16 years), infants (>1 month to <1 year) and neonates (<1 month). Differences in internal dose between each subgroup and healthy adults for each kinetic parameter were calculated based on the geometric mean ratio and on the variability ratio between general healthy adults and each subgroup; the ratios were expressed such that a value >1 indicated a higher internal dose and greater variability in the subgroup.

Individual studies reporting data for the same kinetic parameter, compound and subgroup of the population were combined using the weighted mean method described previously (Dorne et al., 2001a,b) after converting the published data, reported as an arithmetic mean and standard deviation (normal distribution) into a geometric mean (GM) and geometric standard deviation (GSD) (lognormal distribution) (Dorne et al., 2002, 2003a,b, 2004a,b). The overall coefficients of variation (CV_{LN}) for each parameter/subgroup of the population were then combined for all probe substrates for a particular pathway as an average on the log-scale to define the pathway-related variability. The ratios of internal dose and variability between healthy adults and subgroups of the population were also averaged on the log-scale to define pathway-related differences in internal dose and variability.

2.2. Derivation of pathway-related uncertainty factors

Pathway-related uncertainty factors to cover the 95th, 97.5th and 99th centiles of the healthy adult population were calculated using the overall pathway-related variability derived from the weighted mean

analysis for each kinetic parameter. When data on more than one kinetic parameter were available (i.e. clearance adjusted to body weight, clearance and AUC), the pathway-related uncertainty factors for each parameter were combined as weighted means. Pathway-related uncertainty factors for subgroups of the population were derived using a similar calculation and included the pathway-related ratio of internal dose and the pathway-related variability for each subgroup (Dorne, 2001; Dorne et al., 2002, 2003a,b, 2004a,b). The pathway-related factors were calculated as the difference between each percentile (95th, 97.5th and 99th centiles) of the subgroup compared with healthy adults, without taking into account the incidence of the subgroup in the overall population. These values assume that higher circulating concentrations of the parent compound would result in increased risk, i.e. that the parent compound is the toxic chemical species.

3. Results

The human database comprised data for phase I metabolism (CYP isoforms, ADH, hydrolysis), phase II metabolism (*N*-acetylation, glucuronidation, glycine and sulphate conjugation) and renal excretion. Phase I and phase II were divided into functionally monomorphic and polymorphic enzymes, but this subdivision is provisional because genetic polymorphism has been shown for most phase I and phase II routes of metabolism. Elimination pathways such as CYP1A2, CYP2E1, CYP3A4, ADH, esterases (hydrolysis), glucuronidation, sulphation, glycine conjugation for which no kinetic data were available in phenotyped individuals were considered as monomorphic pathways in the analyses; future publications on pharmacogenetic differences may change this subdivision. Esterases (hydrolysis) constitute a family of enzymes, and probe substrates for specific esterase enzymes were not identified. The analysis provides an overall picture of human variability in esterase activities. Paraoxonase (PON1) is an important polymorphic esterase that is involved in the metabolism of several organophosphorous insecticides and nerve agents (Costa et al., 2003); however no kinetic data were available for a probe substrate which fulfilled the criteria used for other pathways.

3.1. Pathway-related uncertainty factors in healthy adults

Interindividual differences for largely monomorphic phase I (CYP1A2, CYP2E1, CYP3A4, ADH and hydrolysis) and phase II (glucuronidation, glycine and sulphate conjugation) pathways and renal excretion, expressed as coefficients of variation (CVs), ranged between 21% and 32%, the only exception being CYP3A4 with a CV of 46%. The resulting pathway-related uncertainty factors which would cover at least 99% of the general healthy adult population were between 1.6 and 2.2 for most of the pathways and 2.8 for CYP3A4 metabolism (Table 1). CYP3A4 is involved in the bioactivation of a number of pesticides and insecticides such as azinphos-methyl, chlorpyrifos, diazinon, parathion and carbaryl (Sams et al., 2000; Tang et al., 2001, 2002; Buratti et al., 2003). Moreover, CYP3A4 both oxidises and reduces the insecticide imidacloprid at the imidazolidine and nitroimine moieties (Schulz-Jander and Casida, 2002).

The physiological/molecular basis of the CYP3A4 variability (for the oral route) has been discussed (Dorne et al., 2003a) and is related to the expression of CYP3A4 in the intestine as well as in the liver. Additional sources of variability may arise from the existence of allelic and protein variants revealing a polymorphism (for which the clinical significance is not well characterised) (Eseilt et al., 2001), and competition in the gastrointestinal tract between CYP3A4 and P-glycoprotein due to their co-localisation and overlapping substrate specificities (Wacher et al., 1995; Suzuki and Sugiyama, 2000; Sata et al., 2000; Eseilt et al., 2001).

In contrast, the data for human variability in polymorphic phase I (CYP2D6, CYP2C19, CYP2C9) and phase II (*N*-acetyltransferases) pathways (Table 1) have demonstrated that the general 3.16 kinetic default factor would be inadequate. CYP2D6 has been shown to bioactivate some organophosphorothioates, such as chlorpyrifos, parathion and diazinon to the corresponding toxic phosphate esters or ''oxons'', which are potent acetylcholinesterase inhibitors (Sams et al., 2000). CYP2C19 has also been shown to activate chlorpyrifos and metabolise methoxychlor (Tang et al., 2001; Stresser and Kupfer, 1998), while *N*-acetyltransferase metabolises potential procarcinogens such as aromatic amines (Land et al., 1989). Pathway-related uncertainty factors of up to 26 (CYP2D6), 52 (CYP2C19) and 5.2 (*N*-acetyltransferases) would be necessary to cover the 99th centiles of poor metabolisers (PMs) (CYP2D6 and CYP2C19) and slow acetylators (*N*-acetyltransferases) (assuming the parent compound was the proximate toxicant). The large variability within extensive metabolisers (EMs) and the difference in internal dose between EMs and PMs for the CYP2D6 pathway (Dorne et al., 2002) is related to the number of copies of the CYP2D6 enzyme present in an individual, or the existence of an enzyme with an altered or absent catalytic activity. Two studies have shown the presence of multiple copies of CYP2D6, with up to 13 copies present in ultra-rapid metabolisers, whereas true poor metabolisers would not possess any copies (Dahl et al., 1995; Dalen et al., 1998, 1999). The same concepts also apply to CYP2C19 metabolism (Wedlund, 2000) and *N*-acetylation (NAT2* gene) for which 8 and 14 alleles respectively

Table 1
Pathway-related uncertainty factors for general healthy adults

Pathway	N_c	N_s	n	CV_{LN}	Ratio GM	Pathway-related uncertainty factors		
						95th	97.5th	99th
Phase I								
Monomorphic pathways								
CYP1A2	4	30	379	30		1.6	1.8	2.0
CYP2A6	3	18	181	29		1.6	1.7	1.9
CYP2E1	2	20	263	26		1.5	1.7	1.8
CYP3A4	12	107	1381	46		2.1	2.3	2.7
ADH	1	15	281	24		1.5	1.6	1.8
Hydrolysis	4	22	166	28		1.6	1.7	1.9
Polymorphic pathways								
CYP2C9 (NP)	3	41	481	32		1.7	1.9	2.1
CYP2C9 (*1/*1, *1/*2, *1/*3)	2	3, 3, 3	15, 13, 15	12–25	1.1–1.7	1.3–2.1	1.4–2.1	1.5–2.2
CYP2C19 (NP)	3	7	91	43		2.0	2.3	2.6
CYP2C19 (EM)	3	7	56	60		2.5	3.1	**3.8**
CYP2C19 (PM)	3	4	21	20	31	**45**	**48**	**52**
CYP2D6 (NP)	8	41	520	63		3.0	**3.7**	**4.7**
CYP2D6 (EM)	9	24	192	66		**3.5**	**4.4**	**5.8**
CYP2D6 (PM)	7	13	74	29	9.0	**21**	**24**	**26**
Phase II								
Monomorphic pathways								
Glucuronidation	15	87	906	29		1.6	1.8	2.0
Glycine conjugation	2	21	205	21		1.4	1.5	1.6
Sulphation	3	13	97	26		1.5	1.7	1.8
Polymorphic pathways								
NAT (FA)	2	15	191	32		1.7	1.8	2.1
NAT (SA)	2	16	472	22	3.1	**4.4**	**4.8**	**5.2**
Renal excretion								
Renal excretion	6	48	444	21		1.4	1.5	1.6

The values relate to AUC and clearance data following oral administration, except for CYP2A6 for which IV data were used (based on Dorne et al., 2001a,b, 2002, 2003a,b, 2004a,b).

N_c: number of compounds in the database, N_s: number of studies in the database, n: number of subjects in the database, CV_{LN}: mean coefficient of variation, Ratio GM: ratio of geometric means between EMs and PMs (CYP2C9, CYP2C19 and CYP2D6) and FAs and SAs (NAT), NP: non-phenotyped healthy adults, *1,*2,*3 are genetic variants of CYP2C9, EM: extensive metabolisers, PM: poor metabolisers, FA: fast acetylators, SA: slow acetylators.

have been reported in some Caucasian subjects respectively (Lin et al., 1993). Data for the different CYP2C9 genotypes are given in Table 1 only for the homozygous (*1/*1) and heterozygous (*1/*2 and *1/*3) extensive metabolisers, who would constitute the major proportion of the human population at 68%, 18% and 11% (Kirchheiner et al., 2002). Data for poor metaboliser phenotypes are not reported because very few individuals have been studied for the *2/*2 ($n = 6$), *2/*3 ($n = 3$) and *3/*3 ($n = 3$) alleles (1%, 1% and 0.8% of the population). Based on these limited data, the 3.16 kinetic default factor would not be adequate for individuals carrying the *3/*3 alleles and a CYP2C9-related uncertainty factor of 6.5 would be necessary to cover 99% of this subgroup (but such a factor would cover 99.99% of the general population). More data are required to characterise the difference in internal dose and variability between the CYP2C9 phenotypes since poor CYP2C9 metabolisers appear to be more susceptible to adverse reactions (data based on warfarin and phenytoin) particularly at the start of therapy (Lee et al., 2002). Moreover, both CYP2C9 and CYP2C19 constitute important isoforms representing 20% of all CYPs in the liver (Miners and Birkett, 1998).

Finally, it should be recognised that the uncertainty factors for each polymorphic pathway in Table 1 represent worst-case scenarios because they were calculated for compounds handled primarily by the polymorphic enzyme in EMs and fast acetylators. Lower uncertainty factors would be appropriate for a compound that is only partially eliminated by a polymorphic enzyme. Exponential relationships have been demonstrated between the extent of CYP2D6 and CYP2C19 metabolism in EMs and the CYP2D6- and CYP2C19-related uncertainty factors (Figs. 2 and 3). These relationships are critical for the estimation of uncertainty factors because the 3.16 kinetic default uncertainty factor would cover PMs for substrates that were metabolised up to 25% by CYP2D6 and CYP2C19 in EMs (Dorne et al., 2002, 2003b). More quantitative metabolism data

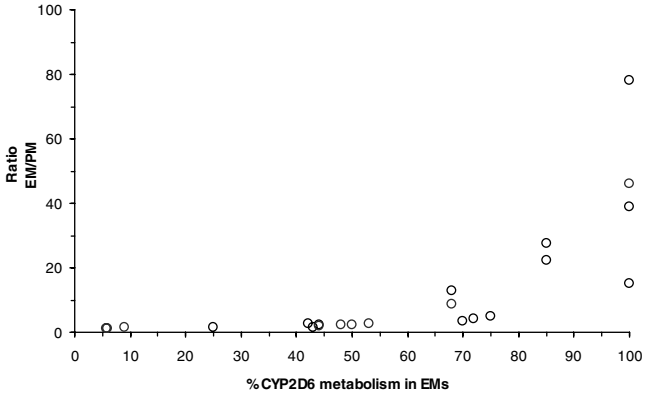

Fig. 2. Relationship between the percentage of CYP2D6 metabolism of probe substrates after oral administration in extensive metabolisers and the ratio of clearances (ml/min and ml/min/kg) between extensive and poor metabolisers. Each point represents a different compound.

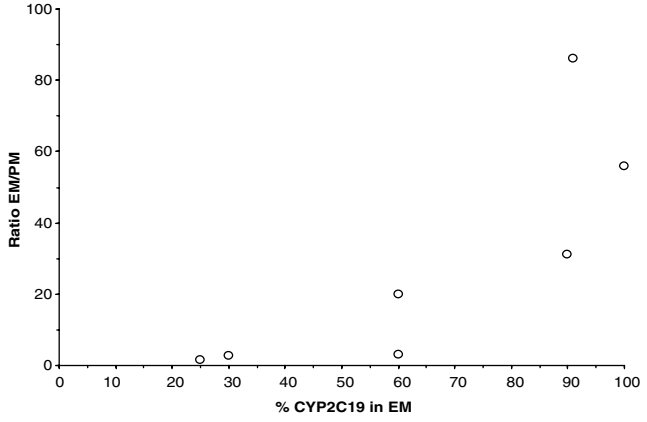

Fig. 3. Relationship between the percentage of CYP2C19 metabolism after oral administration in extensive metabolisers and the ratio of clearances (ml/min) between extensive and poor metabolisers. Each point represents a different compound.

describing the kinetics of substrates that undergo partial metabolism by CYP2C9 and NAT-2 would be required to determine whether similar relationships hold between the extent of metabolism and the uncertainty factors for these pathways.

3.2. Pathway-related uncertainty factors for interethnic differences

Pathway-related uncertainty factors calculated using data for healthy adults from different ethnic origins (African, Asian and South Asian healthy adults) are summarised in Table 2. Most uncertainty factors were below the 3.16 kinetic default for monomorphic pathways, a situation comparable to general healthy adults (Table 1). Exceptions were CYP3A4 metabolism (99th centile) in Black Africans (3.8), South Asians (4.8) and Mexicans (7.5), and metabolism via hydrolysis in Asians (3.9 based only on 12 subjects and 1 compound). These interethnic differences in CYP3A4 activity have been discussed (Dorne et al., 2003a) and can be attributed to a lower hepatic metabolism in both South Asians (Rashid et al., 1995) and Africans compared to general healthy adults (Johnson, 2000). Interethnic differences between Africans and Hispanics have been shown for nifedipine and ciclosporin and have been suggested to involve gut metabolism and/or P-glycoprotein (Lindholm et al., 1992; Johnson, 2000). More studies are required to characterise the involvement of hepatic metabolism, gut wall metabolism, P-glycoprotein and genetic polymorphisms in the observed inter-ethnic differences for CYP3A4 metabolism.

Data for polymorphic pathways in different ethnic groups raised the same concerns as those in general healthy adults, because most of the subgroups would not be covered by the 3.16 default factor. Asian healthy adult EMs and PMs for CYP2D6 were an exception to this with a higher clearance in Asian EMs than general adult EMs, and virtually no difference in clearance between Asian PMs and general healthy adult NPs. The frequency of PMs in Asian populations is 2% compared with 8% in Caucasians, and therefore the 99th centile of PMs would represent only 0.02% of an Asian population but 0.08% of a Caucasian population. The same consideration needs to be given to *N*-acetyltransferase (NAT) and the observed differences between general healthy adult FAs and Asian FAs and SAs, because the frequency of SAs in Caucasian populations (40–70%) is higher than in Japanese and Chinese populations (10–20%) (Meyer, 1994).

In contrast to CYP2D6 metabolism, the variability in CYP2C19 metabolism was higher in healthy Asian EMs than that in general healthy adult EMs, and the frequency of PMs in Asian populations is much higher than in Caucasian populations (14–21% compared with 3% respectively) (Wedlund, 2000). A very high frequency of CYP2C19 PMs (61%) has also been shown in inhabitants of Pacific islands (Vanuatu) (Wedlund, 2000). CYP2C19-related uncertainty factors for general healthy adult PMs (Table 1) were higher than that for Asian PMs, due to the larger difference in internal dose between general healthy adult EMs and PMs compared to Asian PMs (but this conclusion is based on 21 subjects). Exponential relationships between the extent of CYP2C19 metabolism of a substrate in EMs and the uncertainty factors (95th–99th centiles) (Fig. 4) were similar for Asian healthy adults to those demonstrated for healthy adults (Fig. 3). Data suggested that the kinetic default factor would cover Asian PMs for substrates for which CYP2C19 was responsible for 15–20% of the metabolism in EMs. For compounds metabolised 60% or more by CYP2C19 in EMs an uncertainty factor of 28 would cover PMs to the 99th centile in contrast with a value of 52 for general healthy adult PMs.

Table 2
Pathway-related uncertainty factors for inter-ethnic differences

Pathway	N_c	N_s	n	CV_{LN}	Ratio GM	Pathway-related uncertainty factors		
						95th	97.5th	99th
African								
CYP1A2	1	1	16	27	1.6	2.5	2.7	3.0
CYP2A6	1	1	40	28	1.0	1.6	1.7	1.9
CYP2C9	1	1	16	32	1.4	2.3	2.6	2.9
CYP2D6 (NP)	1	1	10	64	1.1	2.9	**3.5**	**4.3**
CYP2D6 (EM)	1	1	18	120	1.8	**8.2**	**11**	**15**
CYP3A4[a]	1	1	10	30	1.3	2.1	2.3	2.6
CYP3A4	2	2	21	42	1.5	2.9	**3.3**	**3.8**
Asian								
CYP2A6	1	1	37	44	1.1	2.2	2.5	2.9
CYP3A4	4	8	85	30	1.0	1.6	1.7	2.0
ADH	1	5	154	21	0.87	1.2	1.3	1.4
Hydrolysis	1	1	12	43	2.0	**3.8**	**4.3**	**5.0**
CYP2C19 (EM)[b]	2	6	40	63	1.7	**4.4**	**5.3**	**6.6**
CYP2C19 (PM)[b]	2	6	34	27	15	**24**	**26**	**28**
CYP2D6 (NP)	3	4	70	69	0.80	2.1	2.6	**3.2**
CYP2D6 (EM)	4	6	60	36	0.27	<1	<1	<1
CYP2D6 (PM)	2	2	16	41	1.2	2.1	2.4	2.7
Sulphation	1	1	12	35	0.71	1.2	1.4	1.6
NAT[c] (FA)	1	1	33	34	1.8	3.1	**3.4**	**3.9**
NAT[c] (SA)	1	1	5	39	2.8	**5.2**	**5.9**	**6.7**
Renal excretion	3	3	22	22	0.80	1.2	1.3	1.4
South Asian (Indian)								
CYP3A4	4	8	89	40	1.9	**3.7**	**4.2**	**4.8**
Renal excretion	1	1	8	11	0.64	1.0	1.1	1.2
Mexican								
CYP3A4	2	2	23	41	3.0	**5.7**	**6.5**	**7.5**

The factor allows for differences in mean and variability compared with the equivalent general healthy adults in Table 1. The values relate to AUC and clearance data following oral administration, except for CYP1A2, CYP2A6 for which IV data were used (based on Dorne et al., 2001a, 2002, 2003a,b, 2004a,b).

N_c: number of compounds, N_s: number of studies, n: number of subjects; CV_{LN} mean coefficient of variation, Ratio GM: ratio of geometric means between subgroup and healthy adults (for polymorphic pathways, NP individuals are compared with NP healthy adults and EM, PMs, FAs and SAs are compared with NP/EM and FA healthy adults), NP: non-phenotyped healthy adults, EM: extensive metabolisers, PM: poor metabolisers, FA: fast acetylators, SA: slow acetylators.

[a] North African.
[b] Chinese, Japanese, Korean.
[c] Japanese.

3.3. Pathway-related uncertainty factors for the elderly

The pathway-related uncertainty factors derived for the elderly subpopulation are summarised in Table 3 and were mostly similar to those derived for general healthy adults. Most uncertainty factors for monomorphic pathways at the 99th centile were below a value of 3.16 with the exceptions of CYP3A4 metabolism (4.9) and renal excretion (4.2). The physiological bases for these differences have been well characterised as old age affects both hepatic and renal functions (Durnas et al., 1990; Le Couteur and McLean, 1998). Age-related differences for the elimination of CYP3A4 substrates have been associated with secondary changes in liver blood flow, drug binding and distribution (Hunt et al., 1992).

The differences demonstrated for the polymorphic pathways between the elderly and general healthy adults were also consistent with reduced hepatic function in old age (Durnas et al., 1990; Le Couteur and McLean, 1998). Pathway-related uncertainty factors for CYP2D6 (non-phenotyped), CYP2C19 (non-phenotyped) and *N*-acetylation (slow acetylators) were all above the 3.16 kinetic default, with 99th centile values of 8.4, 4.3 and 7.6 respectively.

The data for the elderly related to CYP2D6 and CYP2C19 metabolism were mostly available for non-phenotyped healthy adults; no data were available for elderly PMs, but such individuals would be expected to have lower clearances than healthy adult PM because of age differences in CYP activity (Le Couteur and McLean, 1998).

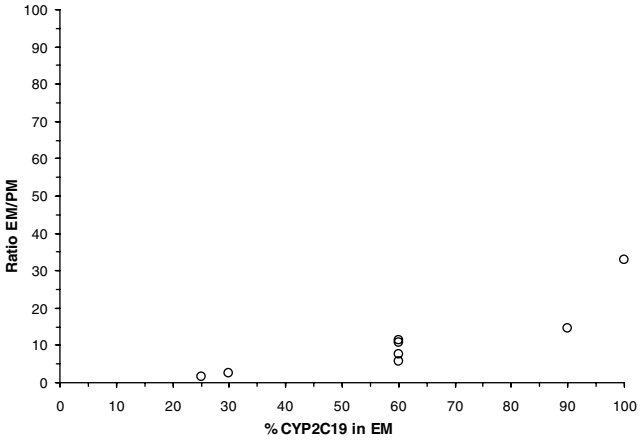

Fig. 4. Relationship between the percentage of CYP2C19 metabolism after oral administration in Asian extensive metabolisers and the ratio of clearances (ml/min and ml/min/kg) between Asian extensive and poor metabolisers. Each point represents a different compound.

3.4. Pathway-related uncertainty factors for neonates and children

Data describing kinetic variability in neonates compared to healthy adults were available for five pathways (CYP1A2, CYP3A4, glucuronidation, glycine conjugation and renal excretion) and all pathway-related default uncertainty factors were above the 3.16 default with values of 14, 12, 12, 28 and 3.4 respectively at the 99th centile (Table 4). A number of studies have shown that these enzymes are immature in neonates (Aranda et al., 1980; Pons et al., 1988; Kraus et al., 1993; Cazeneuve et al., 1994; Cresteil, 1998; Sonnier and Cresteil, 1998; Leeder et al., 2000; Ginsberg et al., 2004).

Immunodetectable CYP1A2 protein and enzyme activity were absent in foetal and neonatal human livers, increased in infant tissues between 1 and 3 months, and reached 50% of the adult level after a year. Neonates have been shown to eliminate caffeine and theophylline mainly via renal clearance, with some limited metabolism via CYP3A7, a CYP3A isoform specific to foetal liver tissue (de Wildt et al., 1999a), and CYP1A2 (Aranda et al., 1980; Pons et al., 1988; Kraus et al., 1993; Cazeneuve et al., 1994; Cresteil, 1998). In contrast, other CYP isoforms (CYP3A7, CYP2C, CYP2D6 and CYP2E1) are expressed to a much greater extent immediately post-natally (Sonnier and Cresteil, 1998; Leeder et al., 2000). Quantitative age-related differences in glucuronidation in vitro have been reported and hepatic glucuronidation in the human neonate has been shown to be relatively immature at birth; in contrast, neonatal hepatic sulphation is considerably more mature (Miners and McKenzie, 1991; de Wildt et al., 1999b; Gow et al.,

Table 3
Pathway-related default uncertainty factors for the elderly

Pathway	N_c	N_s	n	CV_{LN}	Ratio GM	Pathway-related uncertainty factors		
						95th	97.5th	99th
Phase I								
CYP1A2	2	3	27	37	1.1	1.4	1.6	1.8
CYP2A6	1	1	20	24	1.3	1.9	2.1	2.3
CYP2C9	1	1	12	30	0.74	1.2	1.3	1.5
CYP2C19	1	1	10	39	1.8	**3.4**	**3.8**	**4.3**
CYP2D6 (NP)	5	7	69	88	1.4	**5.0**	**6.3**	**8.4**
CYP2D6 (EM)	1	1	6	74	0.50	1.5	1.8	2.3
CYP2E1	1	2	22	26	1.3	1.9	2.1	2.3
CYP3A4	10	15	163	46	1.8	**3.6**	**4.2**	**4.9**
ADH	1	2	29	43	1.2	2.4	2.8	**3.2**
Hydrolysis	2	4	31	35	0.66	1.2	1.3	1.5
Phase II								
Glucuronidation	10	14	140	28	1.4	2.3	2.5	2.7
Glycine conjugation	1	3	19	26	1.0	1.6	1.8	2.0
Sulphation	3	3	24	23	1.1	1.6	1.7	1.9
NAT (FA)	2	4	56	37	1.3	2.3	2.5	2.9
NAT (SA)	2	4	105	29	3.9	**6.3**	**6.9**	**7.6**
Renal excretion								
Renal	6	8	105	33	2.0	**3.3**	**3.7**	**4.2**

The factor allows for differences in mean and variability compared with the equivalent general healthy adults in Table 1. The values relate to AUC and clearance data following oral administration, except for CYP2A6 and sulphation for which IV data were used (based on Dorne et al., 2001a,b, 2002, 2003a,b, 2004a,b).

N_c: number of compounds, N_s: number of studies, n: number of subjects, Ratio GM: ratio of geometric means between the elderly and healthy adults (lognormal distribution), CV_{LN}: mean coefficient of variation, Ratio GM: ratio of geometric means between elderly and healthy adults (for polymorphic pathways, NP individuals are compared with NP healthy adults and EM, PMs, FAs and SAs are compared with EM and FA healthy adults), NP: non-phenotyped healthy adults, EM: extensive metabolisers, FA: fast acetylators, SA: slow acetylators.

Table 4
Pathway-related default uncertainty factors for children after chronic[a] oral exposure and neonates after chronic[a] intravenous exposure

Pathway	N_c	N_s	n	Mean CV	Ratio GM	Pathway-related uncertainty factors (lognormal distribution)		
						95th	97.5th	99th
Children								
CYP1A2	1	12	195	34	0.82	1.4	1.6	1.8
CYP2C19	1	1	25	86	1.6	**5.4**	**6.9**	**9.0**
CYP2D6	1	2	173	140	4.0	**22**	**31**	**45**
CYP3A4	3	3	16	45	0.70	1.4	1.6	1.8
Hydrolysis	3	3	43	40	0.80	1.5	1.7	2.0
Glucuronidation	5	13	131	23	0.86	1.3	1.4	1.5
Glycine conjugation	1	1	20	27	0.98	1.5	1.6	1.8
NAT	1	1	25	37	1.1	2.0	2.2	2.5
NAT	1	1	25	13	1.8	2.2	2.3	2.4
Renal excretion	6	9	126	30	0.70	1.2	1.3	1.5
Neonates								
CYP1A2	2	7	251	35	6.2	**11**	**12**	**14**
CYP3A4	2	5	35	65	3.0	**8.1**	**9.7**	**12**
Glucuronidation	4	14	94	50	3.9	**8.6**	**10**	**12**
Glycine conjugation	2	1	10	16	19	**25**	**26**	**28**
Renal excretion	7	33	656	32	1.7	2.8	3.0	**3.4**

The factor allows for differences in mean and variability compared with the equivalent general healthy adults in Table 1. The values relate to clearance data following oral administration in children, except for CYP1A2 and renal excretion for which IV data were used (based on Dorne et al., 2001a,b, 2002, 2003a,b, 2004a,b). For neonates, all data was from the IV route (based on Dorne et al., 2001a,b, 2004a,b).
N_c: number of compounds, N_s: number of studies, n: number of subjects, CV_{LN}: mean coefficient of variation, Ratio GM: ratio of geometric means between children/neonate and healthy adults.
[a] The term chronic relate to clearances reflecting steady state kinetics.

2001). The capacity of the immature liver or kidney to detoxify through glycine conjugation (to form hippuric acid from benzoate) has been shown in premature newborns for the intravenous route ($n = 10$) (LeBel et al., 1988). However, these data may not be relevant to oral kinetics in term-neonates because glycine conjugation has been recognised to be mature in neonates, but it is a highly saturable metabolic pathway (Gow et al., 2001). Finally, glomerular filtration rate has been shown to increase as a function of post-conceptional age until adult values are achieved by approximately 7 months of age (Besunder et al., 1988). This would explain the lower clearances observed in neonates compared to healthy adults for xenobiotics excreted via the kidney.

Data describing the variability in kinetics for polymorphic pathways in neonates were only available for CYP2D6 and showed a much higher internal dose in two PM neonates (19- and 33-fold difference compared with adults) (Ito et al., 1998). However, these very limited data lead to concerns for CYP2D6 metabolism in neonates, which when taken together with the data for children (see below) and the data for healthy adults (Table 1), indicate that neonates would be the most susceptible subgroup when exposed to compounds handled by CYP2D6 metabolism.

The data for children (Table 4) showed higher clearances compared to healthy adults for a number of metabolic pathways. Most pathway-related factors were below the default of 3.16 (including *N*-acetylation) with the exception of the polymorphic CYP2D6 and CYP2C19 isoforms. Data for CYP2D6 and CYP2C19 pathways were available only for non-phenotyped individuals. The large number of subjects for CYP2D6 were for a single drug, desipramine, and differences in internal dose and variability compared with adults were very high in children (4-fold and 140%) and in consequence an uncertainty factor of 45 would be necessary to cover 99% of the subgroup.

4. Application of pathway-related uncertainty factors to the risk assessment of chemicals showing thresholded toxicity

The main application of these analyses is to provide risk assessors with sets of pathway-related uncertainty factors (95th, 97.5th or 99th centile) relevant to the particular fate of the chemical under assessment in order to allow the replacement of the general default kinetic uncertainty factor of 3.16. The main advantage of this approach is that risk assessors can now provide advice based on these percentiles (or others percentiles if requested) rather than using a single value (Tables 1–4) that may not be appropriate for the substrate and population group under consideration.

The application of pathway-related factors requires knowledge on the major route of elimination in humans for the compound under evaluation. Metabolism data

are frequently available for a small group of healthy adults given a low dose of the compound, and such data can define the roles of metabolism and excretion in the elimination of the compound. Because kinetic processes are first-order at low doses, it is possible to derive meaningful metabolic data from these volunteer studies using very low or tracer doses. Enzyme and isoenzyme-specific data are also required, and these can be obtained from in vitro studies using liver microsomes, heterologously expressed enzymes and/or enzyme inhibitors (Venkatakrishnan et al., 2001). Alternatively, the in vivo fate in humans can be predicted from a PBPK model based on in vitro data using human tissues combined with human physiological data. The main advantage of the data reviewed in this paper is that they will allow regulators to move away from default uncertainty factors without the need for extensive in vivo toxicokinetic studies in humans.

The 3.16-fold default factor would be too conservative to cover healthy adults to the 99th centile for all monomorphic pathways, and this default could be replaced by the relevant pathway-related values (Table 1). In contrast polymorphic pathways (CYP2D6, CYP2C19 and NAT) are of more concern because the 3.16 kinetic default factor is insufficient to cover the majority of the general healthy adult population, let alone subgroups of the population. The pathway-related factors in Tables 1–4 would be appropriate if the parent compound were the proximate toxicant because PMs and SAs would be at excessive risk. On the other hand, EMs and FAs (92% of Caucasians for CYP2D6, 97% for CYP2C19 and 30–60% for *N*-acetylation) would be more at risk if the metabolite were the proximate toxicant. Therefore the application of pathway-related uncertainty factors would need to take into account both the enzyme involved in the metabolism and the toxicological consequences of metabolism.

Application of pathway-related data can be illustrated by the hypothetical example of a particular pesticide which is metabolised primarily by CYP2D6 and PMs would constitute the potentially susceptible subgroup in the healthy adult population because the parent compound is the active form. In such a case, pathway-related uncertainty factors developed from the database could be applied under two sets of circumstances:

(i) *The percentage of an oral dose eliminated by CYP2D6 in EMs is not known (only in vitro data are available)*. Risk assessors could assume that 60% or more was eliminated by CYP2D6 (because this would a conservative assumption) and apply the CYP2D6-related uncertainty factors developed for the PMs (Table 1) with values of 21, 24 and 26 to cover the PM group to the 95th, 97.5th or 99th centiles.

(ii) *The percentage of an oral dose eliminated by CYP2D6 in EMs is known (using in vitro–in vivo extrapolations or in vivo data)*. Regulators could then use the relationship between the extent of CYP2D6 metabolism and the uncertainty factors for PMs (see Dorne et al., 2002 and Fig. 2) and derive a CYP2D6-related uncertainty factor for the appropriate extent of CYP2D6 metabolism. For example, if 40% of an oral dose of the chemical is handled by CYP2D6 metabolism, an uncertainty factor of 5.1 would be required to cover PMs to the 99th centile.

The current default kinetic uncertainty factor of 3.16 would not be conservative enough to cover all subgroups of the population (due to variability and differences in internal dose) for compounds handled by highly variable monomorphic pathways (CYP3A4) or polymorphic pathways; examples include CYP2C19, and CYP3A4 metabolism in Asian populations, CYP2D6, CYP2C19, NAT, CYP3A4 in the elderly and CYP2D6 and CYP2C19 in children.

Overall, neonates would be the most susceptible subgroup for all of these pathways because elimination processes are immature at and soon after birth (Renwick et al., 2000; Cresteil, 1998; Gow et al., 2001). Pharmacokinetic data in neonates have been analysed before and authors have discussed whether neonates would need an extra factor compared to healthy adults (Renwick, 1998; Renwick et al., 2000). Our database supports the need for an enhanced toxicokinetic factor for neonates, but not for infants and children; the magnitude of the difference between healthy adults and neonates has been defined for only four metabolic pathways (CYP1A2, CYP3A4, glucuronidation and glycine conjugation) and for renal excretion. Importantly, if data from a multi-generation study were available and if the critical endpoint were determined in neonatal animals, the interspecies comparison would include and be based on immature animals. The interspecies comparison would take into account a significant proportion of the immaturity of elimination, such that the human uncertainty factor would not be required to cover the full spectrum of age-related differences between human adults and neonates. However, when species differences in the ontogeny of an enzyme have been shown, there would be a need to assess these differences, because the age-related difference in the animal might not reflect quantitatively the magnitude of the immaturity of the enzyme in the human neonate.

Important questions are "How should risk assessors apply the pathway-related uncertainty factors developed for potentially susceptible subgroups: should a factor for either the 95th, 97.5th and 99th centiles of the subgroup be calculated, or should it be a percentile of the combined total population?" For example, if a factor covering PMs to the 99th centile were used, and this subgroup represented 1% of the total population, then the risk assessment would cover 99.99% of the general population. Such an approach would lead to more stringent

regulation for substrates of polymorphic pathways, compared with monomorphic pathways. However, if the 99th centile of the total population were used the 1% not covered would be PMs, who could vary by an order of magnitude from the 99th centile estimate.

When a specific intake estimate is available for a subgroup such as children, risk characterisation could be based on a comparison of the group-specific intake with a specific health-based guidance value determined using the pathway-related factor for the appropriate percentile of the subgroup.

5. Conclusions and future refinements to the use of uncertainty factors

Analyses of human variability in kinetics for the main metabolic pathways have enabled the derivation of pathway-related uncertainty factors for healthy adults and subgroups of the population, which can be used to replace the general kinetic default factor of 3.16. Fig. 5 summarises the approach and its applications for chemical risk assessment. The level of refinement of the uncertainty factor for any compound would depend on the extent of the data available. The worst-case scenario would be that no data describing the metabolism or kinetics of the compound were available and under these circumstances the general kinetic default of 3.16 would be used. In an ideal setting, databases describing the metabolism and/or toxicokinetics of a particular chemical in humans would be used to define the internal body burden, or to build a physiologically-based pharmacokinetic model. In this situation, the data would be used to derive a chemical-specific adjustment factor (CASF). Chemical-specific adjustment factors, integrating polymorphism data (CYP2C9 and PON1) and in vitro metabolism data (V_m, K_m), have been recently developed for warfarin and parathion using physiologically-based pharmacokinetic modelling combined with Monte Carlo models (Gentry et al., 2002).

Pathway-related uncertainty factors constitute an intermediate approach and the kinetic default could be replaced by the appropriate uncertainty factor when the pathway of metabolism of the compound and the particular enzyme(s) are known. Readily available in vitro techniques can be used to identify the various CYP isoform(s) and/or phase II enzymes responsible for the metabolism of a particular compound. It will be increasingly important to incorporate into regulatory procedures, data describing at least in vitro metabolism of the compound under assessment to allow identification of the enzyme(s) involved in its metabolism, as well as the consequences of metabolism. This is particularly

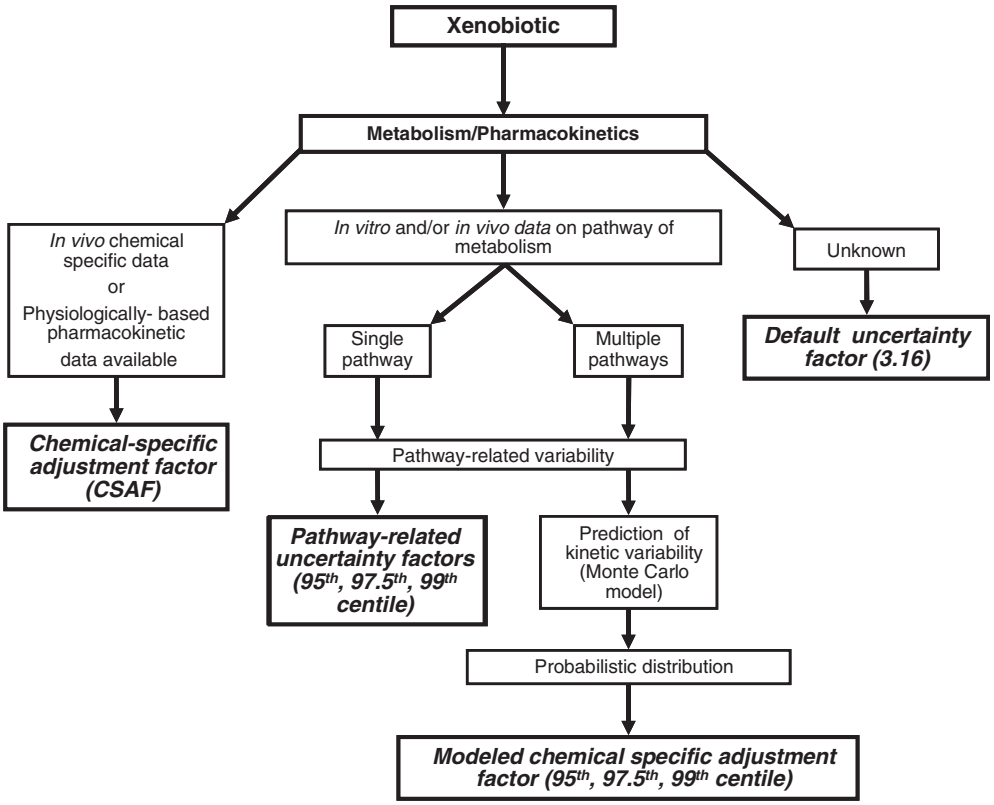

Fig. 5. Future refinements of toxicokinetic uncertainty factors.

relevant to polymorphic metabolism with high human variability such as CYP2D6 and CYP2C19 (because either EMs or PMs could be the more sensitive subgroup), and because the uncertainty factor (UF) may be higher than 3.16 if the compound is solely handled via this route. However, if there are other major pathways involved in the metabolism of the compound it is unlikely that the UF will exceed 3.16; as shown in Figs. 2 and 3, a UF of 3.16 would be adequate for compounds for which up to 25–30% of the dose is handled by CYP2D6 or CYP2C19.

Another important step forward would be the analysis of human variability in toxicodynamics to develop categorical uncertainty factors related to different mechanisms of toxicity. Renwick and Lazarus (1998) have shown that the overall variability for pharmacodynamics was 51% (based on drug effects) resulting in overall defaults of 2.2, 2.6 and 3.1 to cover 95th, 97.5th and 99th centile of the general healthy adult population. It is accepted that the dynamic effects analysed by Renwick and Lazarus (1998) were for therapeutic rather than toxic effects, but in reality suitable toxicodynamic data for humans are not available or very scarce (Hashemi et al., 2002).

Regulators throughout the world have recognised the potential advantage of moving away from default factors and point estimates to probabilistic models (Slob and Pieters, 1998; Swartout et al., 1998; Edler et al., 2002). Probabilistic multiplication of the distributions of the pathway-related defaults available in the database combined with the distribution of the dynamic default derived from Renwick and Lazarus (1998) can be used to derive combined probability distributions to replace the 10-fold uncertainty factors for human variability.

Finally, it should be recognised that the pathway-related uncertainty factors have been derived for compounds handled by a single major route of elimination (>60%), however this scenario is unusual and many environmental contaminants are eliminated by several pathways. This could be addressed by the use of approaches, such as Monte Carlo modelling, to combine the variability for different pathways. Validation of the approach would require the identification of probe substrates handled by several pathways for which the metabolism has been well characterised (based on urinary excretion data and the identification of specific enzyme isoforms identified). Monte Carlo models that predict the overall variability in kinetics, have been used and validated for compounds that are eliminated by multiple pathways (Dorne and Renwick, 2003; Dorne et al., in preparation-a, in preparation-b). These models that are based on knowledge of metabolism (in vitro and/or in vivo data) and pathway-related variability can be represented as probability distributions describing the variability in kinetics for the compound of interest and these can be used to generate appropriate modelled chemical-specific adjustment factors (WHO, 2001).

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