Review

Metabolism, variability and risk assessment

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A R T I C L E   I N F O

Article history:
Received 29 July 2009
Received in revised form 26 October 2009
Accepted 3 November 2009
Available online 20 November 2009

Keywords:
Variability
Toxicokinetics
Uncertainty factors
Risk assessment
Mixtures
Human
Ecology

A B S T R A C T

For non-genotoxic carcinogens, “thresholded toxicants”, Acceptable/Tolerable Daily Intakes (ADI/TDI) represent a level of exposure “without appreciable health risk” when consumed everyday or weekly for a lifetime and are derived by applying an uncertainty factor of a 100-fold to a no-observed-adverse-effect-levels (NOAEL) or to a benchmark dose. This UF allows for interspecies differences and human variability and has been subdivided to take into account toxicokinetics and toxicodynamics with even values of 10^3 (3.16) for the human aspect. Ultimately, such refinements allow for chemical-specific adjustment factors and physiologically based models to replace such uncertainty factors.

Intermediate to chemical-specific adjustment factors are pathway-related uncertainty factors which have been derived for phase I, phase II metabolism and renal excretion. Pathway-related uncertainty factors are presented here as derived from the result of meta-analyses of toxicokinetic variability data in humans using therapeutic drugs metabolised by a single pathway in subgroups of the population. Pathway-related lognormal variability was derived for each metabolic route. The resulting pathway-related uncertainty factors showed that the current uncertainty factor for toxicokinetics (3.16) would not cover human variability for genetic polymorphism and age differences (neonates, children, the elderly). Latin hypercube (Monte Carlo) models have also been developed using quantitative metabolism data and pathway-related lognormal variability to predict toxicokinetics variability and uncertainty factors for compounds handled by several metabolic routes. For each compound, model results gave accurate predictions compared to published data and observed differences arose from data limitations, inconsistencies between published studies and assumptions during model design and sampling.

Finally, under the 6th framework EU project NOMIRACLE (http://viso.jrc.it/nomiracle/), novel methods to improve the risk assessment of chemical mixtures were explored (1) harmonisation of the use of uncertainty factors for human and ecological risk assessment using mechanistic descriptors (2) use of toxicokinetics interaction data to derive UFs for chemical mixtures.

The use of toxicokinetics data in risk assessment are discussed together with future approaches including sound statistical approaches to optimise predictability of models and recombinant technology/toxicokinetics assays to identify metabolic routes for chemicals and screen mixtures of environmental health importance.

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1. Introduction

For one country is different from another; its earth is different, as are its stones, wines, bread, meat, and everything that grows and thrives in a specific region.

Paracelsus (1493–1541)

Living organisms are exposed to a plethora of chemicals in their environment and food. To cite but a few, these chemicals include contaminants such as persistent organic pollutants (dioxins, polychlorinated biphenyls, brominated flame retardants), perfluoroalkyl acids, pharmaceuticals, agricultural contaminants (mycotoxins, plant toxins, marine biotoxins), process-related contaminants (acrylamide, furans, polyaromatic hydrocarbons) [Dorne et al., 2007a,b, 2009], chemicals intentionally added to food/the environment i.e. food additives, flavourings and food contact materials or resulting from intentional treatment of raw commodities i.e. pesticides/biocides (herbicides, fungicides, insecticides, etc.) [Dorne et al., 2009]. Through biological evolution, the major kingdoms, animalia, fungi, planta, protista, bacteria and archaea have developed a core machinery of enzymes, transporters and excretory pathways for the bioactivation/nutritional use and/or detoxification/excretion of such chemicals. In humans, these metabolic pathways have been classified into phase I enzymes (cytochrome P-450 (CYP), esterases, alcohol dehydrogenase), phase II enzymes (amino acid conjugation; UDP-glucuronyl-transferases, sulpho-transferases, methyl-transferases, N-acetyltransferases, glutathione-S-transferases, etc.), phase 0 (transporters such as P-glycoprotein and Organic Anion Transporter Proteins (OATPs) and renal excretion.

The quantification of human variability in these metabolic and elimination pathways (pharmacokinetics/toxicokinetics) has been central to develop strategies for individualized drug therapy and to characterise and include potential susceptible subgroups of the population in the chemical risk assessment process. Implementation of metabol/toxicokinetic variability in chemical risk assessment constitutes the main topic of this review with particular focus on the replacement of default uncertainty factors for non-genotoxic carcinogens with pathway-related uncertainty factors and predictive Monte Carlo models. Uncertainty factors are also addressed within the context of metabolic/toxicokinetic interactions for binary mixtures and the potential harmonisation of ecological and human risk assessment within the integrated 6th framework EU project NOMIRACLE (NOvel Methods for Integrated Risk Assessment of Cumulative stressors in Europe). Future directions to include toxicokinetic data in chemical risk assessment data will conclude.

2. Chemical risk assessment: genotoxic versus non-genotoxic carcinogens

Qualifying and quantifying hazard and risk are the corner stones of the risk assessment paradigm. According to regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 “Hazard” is defined as a biological, chemical or physical agent in, or condition of, food and “Risk” is defined as a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard (EC, 2002). The International Program on Chemical Safety (IPCS) of the WHO within the project for the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals has defined hazard as “the inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub)population is exposed to that agent” and risk as “the probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to an agent” (WHO, 2008).

Historically, the application of the precautionary principle, and the four pillars of risk assessment namely hazard identification, hazard characterisation, exposure assessment, risk characterisation; have enabled scientists and public health agencies to protect consumers from adverse health effects that may result from acute and chronic chemical exposure (Svendsen et al., 2008). In practice, once the amount of a chemical has been measured in food through validated analytical techniques, its toxicological effects (dose–response) characterized, a health-based guidance value can be derived and related to the exposure so that the risk to human health can be characterised (Dorne et al., 2009). Beyond classes of chemicals and the risk assessment pillars, regulators have relied upon two basic mechanistic differences to assess human health risks related to chemical exposure i.e. whether the chemicals are genotoxic carcinogens or non-genotoxic carcinogens. Such classification constitutes the basis for the derivation of health-based guidance values in humans.

3. Genotoxic carcinogens

Genotoxic carcinogens and their metabolites are assumed to act via a mode of action that involves a direct and potentially irreversible DNA-covalent binding with a linear dose–response relationship over a chronic to life time exposure with no threshold or dose without a potential effect (Dorne et al., 2009). Traditionally, risk managers have applied the ALARA (As Low as Reasonably Achievable) principle. However, this approach is limited since it does not provide a quantitative comparison between different carcinogens and consequently does not provide a measure of risk. Nowadays throughout the world, three major approaches are used to deal with such genotoxic carcinogens namely, (1) linear extrapolation from high animal dose studies to low exposures in humans, (2) threshold of toxicological concern and (3) margin of exposure approach and these have been recently reviewed elsewhere by Pratt et al. (2009).

(1) Linear extrapolation (LE) has been used by the US EPA, Norway and in the European Union for the risk assessment of industrial chemicals for non-thresholded carcinogens and for carcinogens for which the mode of action is unclear. LE often involves modeling of dose–response data from high dose carcinogenicity studies in animals using the lower end of the observed range of tumour incidences so that a risk estimate of cancer for low dose life time exposure in humans (1 in 10⁷ or 10⁸) can be derived. Typically, LE has been using the lower 95% confidence interval of the Bench Mark Dose (BMD) producing a 10%, 5%, 1% increase in tumour incidence compared to background incidences (BMDL10, BMDL05, BMDL01) from mostly 1.1 to 1.5.
animal data or in rare occasions human epidemiological data when available. In Europe for industrial chemicals, the T25 has been applied as the dose corresponding to a 25% increase tumour type, although ideally the BMDL is preferred because the T25 does note address statistical and model uncertainties in the observations. Overall, LE provides estimates of the possible range of cancer risk associated with lifetime exposure to a particular concentration of a genotoxic carcinogen in food, air or from other exposure routes (e.g. a risk of 0–1 in a million). LE has limitations in the fact that the potency of the carcinogen in animals is assumed to directly relate to the potency in humans and such assumptions are still not supported by substantive data (Pratt et al., 2009).

(2) The Threshold of Toxicological Concern (TTC) was originally proposed by Cramer et al. (1978) to establish exposure thresholds predicted to be without adverse effects based on the toxicity of structurally related compounds. One of the main advantages of the TTC approach is that low exposure risk can be evaluated without the need for chemical-specific data from animal toxicity studies. Kroes et al. (2004) proposed a TTC decision tree using a database excluded high potency carcinogens, metals, proteins and polyhalogenated rings structured compounds such as dioxins. The exclusion criteria were based on the facts that relevant toxicity data were not available to derive TTC values and that the uncertainty factors used would not allow for marked species differences in elimination for polyhalogenated ring-structured compounds. From this analysis, threshold values for three groups of chemicals were proposed according to their toxicity in relation to human exposure and expressed in μg/kg bw/day for a 60 kg adult with group I (9) (low), group II: 3 (intermediate) and group III: 1.5 (high) (Kroes et al., 2004; Pratt et al., 2009). For genotoxic carcinogens or compounds with a structural alert for genotoxicity, a TTC of 0.15 μg/kg bw/day has also been suggested by Kroes et al. (2004) based on LE of bioassay data (cancer risk of 1 in 10^5) for structurally related substances. However, for more potent genotoxic carcinogens such as Aflatoxin-like, azoxy- and nitroso-compounds a practical TTC could not be established (Renwick, 2005).

(3) The margin of exposure (MOE) approach has recently been recommended by the scientific committee of the Joint Food and Agricultural Organization of the United Nations/WHO (FAO/WHO) Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) to advice risk managers about the nature and the magnitude of the risks associated with the exposure to genotoxic and carcinogenic substances in food (EFSA, 2005; Barlow et al., 2006). These recommendations came about after an international conference organized by the International Life Science Institute (ILSI), EFSA and the WHO to critically review and discuss the ALARA, TTC and MOE approaches since at the time there was no consensus on the evaluation of genotoxic carcinogens (Barlow et al., 2006; O'Brien et al., 2006). The MOE is determined as the ratio of a defined point on a dose-response curve for adverse effects obtained in animal experiments (in absence of human epidemiological data) and human intake data. Two reference points describing the dose–response relationship have been proposed namely the preferred BMD and BMDL or the T25. Overall, the Scientific Committee of EFSA considered that an MOE of 10,000 or more, based on a BMDL10 derived from animal cancer bioassay data and taking into account the uncertainties in the interpretation, “would be of low concern from a public health point of view and might reasonably be considered as a low priority for risk management actions” (EFSA, 2005). Recent risk assessments performed by the JECFA and EFSA using this approach have included Aflatoxins, Ethyl carbamate, polycyclic aromatic hydrocarbons (FAO/WHO, 2006; EFSA, 2008a,b).

Recently, some experimental data have shown the existence of a threshold for some genotoxic carcinogens such as N-nitrosodimethylamine for which adducts and altered foci in rat liver were observed at lower doses than the threshold for carcinogenicity (Waddell et al., 2006). However, such data need to be substantiated to assist regulators to base risk assessments on the concept of thresholds for genotoxic carcinogens and it is likely to be taken case-by-case, based on reliable data on the Mode of Action (Pratt et al., 2009).

4. Non-genotoxic carcinogens

In contrast, non-genotoxic carcinogens and their metabolites are assumed to act via an epigenetic mode of action without covalent binding to DNA, however, such effects in target cells can either indirectly lead to neoplasms or facilitate their development from cryptogenically transformed cells. Scientists and risk assessors assume a threshold level of exposure below which no significant effects are induced implying that homeostatic mechanisms can counteract biological perturbations produced by low levels of intake, and that structural or functional changes leading to adverse effects, that may include cancer, would be observed only at higher intakes (Dorne et al., 2009). Health-based guidance values are then derived by applying an uncertainty factor of a 100-fold, to allow for interspecies differences (10) and human variability (10) to surrogates for the threshold such as the non-observed-adverse-effect-level (NOAEL) or the BMD or BMDL from laboratory animal species used in risk assessment (mouse, rat, rabbit, dog).

Such level of exposure is defined as “without appreciable health risk” when consumed everyday or weekly for a lifetime such as the ‘Acceptable and Tolerable Daily Intakes (ADI/TDI)’ or provisional tolerable weekly intake (PTWI) in Europe and the ‘Reference dose’ in the United States. For thresholded chemicals with acute toxic effects, the acute reference dose approach (ARID) has been defined as by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) as “an estimate of the amount a substance in food and/or drinking water, normally expressed on a body weight basis, that can be ingested in a period of 24 h or less without appreciable health risk to the consumer on the basis of all known facts at the time of the evaluation” (JMPR, 2002). Recent risk assessment for which ARID have been derived for humans based on the consumption of marine biotoxins (EFSA, 2009a; Dorne et al., 2009).

5. Uncertainty factors: default assumptions, pathway-related uncertainty factors and Monte Carlo models

5.1. Default assumptions

The scientific basis of the 100-fold uncertainty factor used for thresholded toxicants has been criticised because it was not defined clearly when it was first introduced to the scientific community by Lehman and Fitzugh (1958). A number of analyses have been performed to investigate its scientific basis and Renwick (1993) suggested that the interspecies and the human uncertainty factors of 10-fold should be subdivided to allow for the toxicokinetic aspect (TK) relating the external dose and the internal dose; absorption of the chemical from the site of administration, its distribution, metabolism and excretion and the toxicodynamic aspect (TD) dependent upon the concentration of the proximate toxicant (parent compound, metabolite or both) in the target organ(s) and the sensitivity of the target organ(s) itself (Dorne et al., 2001a). Renwick (1993) proposed to subdivide the uncertainty factors for the TK and TD aspects of 4 and 2.5 were first proposed for the interspecies dif-
ferences and even values of 3.16 (100.5) for the human variability aspect from the analysis of a small database describing interspecies differences, expressed as the ratio between the animal species and humans for TK processes and parameters (e.g. liver weight, liver blood flow, renal blood flow, absorption, elimination) as well as for TD sensitivity to a chemical (e.g. sedation, pain relief) (Dorne, 2007). The subdivision was subsequently adopted by the International Programme on Chemical Safety (IPCS) workshop on the derivation of guidance values (WHO, 2005). The main aim of such subdivision was to allow chemical-specific TK or mechanistic data to contribute quantitatively to the magnitude of the uncertainty factor (a data-derived factor renamed recently chemical-specific adjustment factor) (Dorne et al., 2005a,b; WHO, 2005). Renwick and Lazarus assessed human variability using therapeutic drugs that were subject to a range of metabolic and elimination pathways and showed cases for which the current factors for TK and TD would not cover human variability: polymorphic pathways of metabolism, differences between preterm infants and adults. As illustrated in Fig. 1 and based on the extent of knowledge of the chemical under assessment, the authors proposed flexible options to replace default TK and TD uncertainty factors for interspecies differences and human variability with:

1. A chemical-specific adjustment factor (CSAF) or a physiologically based toxicokinetic model when compound-specific data are available as recommended recently by the WHO (WHO, 2005). This approach has been recently explored for the risk assessment of cadmium in food for which a PB-PK model was developed from human data together and a human BMD/BMDL was derived from a meta-analysis of the published studies relating urinary cadmium and biomarkers of renal effects (β-2 microglobulin). In this case, the provisional tolerable weekly intake (PTWI) for cadmium was derived without the need to extrapolate from animals to humans and the use of the 100-fold uncertainty factor was replaced by the PB-PK model together with the use of a CSAF for cadmium variability in toxicodynamics (EFSA, 2009b; EFSA Report, 2009).

2. Pathway-related uncertainty factors when the pathway of metabolism is known but compound-specific TK data are not available. For the toxicodynamic aspects, quantitative estimates of class-related mechanisms of toxicity could be used to derive process specific uncertainty factors but human variability data for contaminants are rarely available and previous analysis have used pharmacodynamic data from the therapeutic drug database (Renwick and Lazarus, 1998; Dorne et al., 2007a).

5.2. Pathway-related uncertainty factors

The derivation of pathway-related uncertainty factors required the analysis of “pathway-related variability” for fourteen of the major human routes of xenobiotic elimination (phase I, phase II metabolism and renal excretion) in subgroups of the human populations using the therapeutic drug database. Values for “pathway-related variability” were derived, as a overall coefficients of variation for a each metabolic pathway, subgroups of the population and markers reflecting chronic and acute exposure from meta-analyses of lognormal kinetic studies for probe substrates eliminated by a single metabolic/renal route (>60% of the dose) (Dorne et al., 2001a,b, 2002a,b; Renwick et al., 2001). Pathway-related uncertainty factors were then derived to cover given percentiles of the human population (95, 97.5, 99th percentiles). For interspecies differences, TK data for test species used in chemical risk assessment (rat, dog, mouse and rabbit) were compared to human data and pathway-related uncertainty factors were derived for CYP1A2 (Walton et al., 2001b), glucuronidation (Walton et al., 2001c) and renal excretion (Walton et al., 2004), for other pathway of eliminations (Walton et al., 2001a; Dorne and Renwick, 2005a,b).

Metabolic pathways were described as monomorphic phase I (CYP1A2, CYP2A6, CYP2E1, CYP3A4, ADH and hydrolysis), phase II (glucuronidation, glycine and sulphate conjugation) and renal excretion for which no clinically relevant polymorphism and no significant differences in internal dose have been demonstrated in humans (Dorne et al., 2001a,b,c,d, 2002a,b; Dorne, 2004a,b). In contrast, phase I (CYP2C9, CYP2C19 and CYP2D6) and phase II (NAT-2) polymorphic pathways have been characterized with regard to pharmacokinetics with in vivo kinetic data in phenotyped individuals (i.e. extensive and poor metabolizers (EM and PM) for CYP2C9, CYP2C19 and CYP2D6 and fast and slow acetylators for NAT-2). Differences in internal dose between each subgroup and healthy adults for kinetic parameter reflecting chronic and acute exposure (AUC/clearance and Cmax, respectively) were calculated based on the geometric mean ratio and the variability ratio between general healthy adults and each subgroup. Such ratios were expressed to reflect differences in internal dose so that a value >1 indicated a higher internal dose and greater variability in the subgroup. Pathway-related uncertainty factors, that would cover 95th, 97.5th and 99th centiles of each subpopulation (here only 99th centile values are discussed), were derived using pathway-related variability (and the difference in internal dose compared with healthy adults for subgroups) (Dorne et al., 2001a,b,c,d, 2002a,b; Dorne and Renwick, 2004a,b). A brief discussion of the differences in pathway-specific variability and uncertainty factors for subgroups of the population follows. However for a full quantitative account of these differences, the reader is referred to recent reviews (Dorne, 2004a,b, 2007; Dorne et al., 2005a,b; Dorne and Renwick, 2005a,b).

Data for healthy adults showed overall that human variability in toxicokinetics was predictably higher for polymorphic pathways in comparison with monomorphic pathways. For monomorphic pathways, pathway-related uncertainty factors (99th centiles) were all below the kinetic default uncertainty factor (3.16) with a range comprised between 1.6 and 2.2 for all pathways and up to 2.8 for CYP3A4 metabolism. For polymorphic pathways, uncertainty factors for phase I (CYP2C9, CYP2C19, CYP2D6) and phase II (N-acetyltransferase, NAT 2) exceeded the kinetic default factor with values up to 4.7 in non-phenotyped CYP2D6 healthy adults and 5.2, 26 and 52 in slow acetylators (NAT-2) and CYP2D6, CYP2C19 PM respectively (Dorne et al., 2002a,b, 2003b, 2005a,b; Dorne and Renwick, 2005a,b). This analysis assumed that the parent compound was the proximate toxicant that faster elimination is protective. However, bioactivation can frequently lead to the production of a toxic metabolite as exemplified by the organophos-
phorothioate chloropyrifos (activated partially by CYP2D6 and CYP2C19) and this case in the EM subgroup would be potentially be more susceptible to toxicity. Another important aspect that has been considered is the effect of the quantitative involvement of the polymorphic route (CYP2D6 and CYP2C19) in EMs and the differences in elimination (clearances) between EMs and PMs has also been investigated for major and minor substrates of each CYP isofrom (10–100% metabolism) and exponential relationships ($R^2 > 0.80$) have been shown to relate the two variables so that interphenotypic differences would be covered by the kinetic uncertainty factor for compounds metabolized to a minor extent (30% of an oral dose) (Dorne et al., 2002a,b, 2003b). This relationship is illustrated in Fig. 2 for the CYP2D6 pathway.

The limited database related to TK differences according to ethnicity and age showed that a number of metabolic routes are affected by these factors. Regarding inter-ethnic differences, both south Asian and African healthy adults had lower CYP3A4 activity; a reverse situation has been shown for CYP2C19 with 18% poor metabolizers in Asian subgroups compared with 3% in Caucasian subgroups (Dorne and Renwick, 2005a,b).

Age differences in TK particularly for the elderly and neonates constitute another source of variability. This variability applies to most metabolic routes as a result of lower hepatic metabolism and renal excretion due to slower and immature metabolism in the elderly and the neonate, respectively. This general principle has been shown for CYP1A2, CYP3A4, CYP2D6, CYP2C19, renal excretion for the elderly and CYP1A2, CYP3A4, glucuronidation, glycine conjugation and renal excretion for neonates: CYP1A2, CYP3A4 and glucuronidation, glycine conjugation and renal excretion in neonates. Pharmacokinetic data for polymorphic pathways in neonates were available in only 2 subjects for CYP2D6 and were associated with a 19- and 33-fold lower clearance (Ito et al., 1998; Dorne et al., 2004a,b). Overall, it is probable that neonates would be the most susceptible subgroup when exposed to compounds handled by CYP2D6 and CYP2C19 metabolism but the database was too small to provide even a quantitative approximation. In contrast, for most elimination routes, hepatic metabolism and renal excretion was shown to be faster in children than adults with the exception of CYP2D6 and CYP2C19 metabolism for which data were limited.

An important question for risk assessors when dealing with inter-individual variability in toxicokinetics is the choice of a cut-off value in the distribution that can be considered as sufficiently protective for specific subgroups of the population and consequently whether the required uncertainty factor should cover a percentile of a subgroup or of the combined total population. As an illustration, when considering polymorphic pathways, the 99th PM subgroup representing a 5% frequency of the total population would represent the 99.95th percentile of the total population. For neonates and children, although they may only represent a small percentage of the total population at a fixed point in time, every individual has been part of these subgroups and their potential susceptibility to chemicals can be of public health concern (Dorne and Renwick, 2005a,b). In terms of ethnic differences, it has been discussed above that frequencies of polymorphism differ between different ethnic groups of the human population, i.e. CYP2D6 PMs in Caucasian versus Asian populations (8% versus 2%), CYP2C19 pathways (2.5% versus 15%) (Wedlund, 2000). Given these differences, uncertainty factors could be developed to cover a particular percentile of the total population or subgroup of concern depending on the end point and percentage of the population to be covered (Dorne et al., 2005a,b).

5.3. Monte Carlo models

Pathway-related variability has also been applied to validate Monte Carlo models predicting variability in toxicokinetics and uncertainty factors for compounds metabolised by a range of monomorphic and polymorphic pathways in non-phenotyped healthy adults and phenotyped healthy adults (Dorne and Renwick, 2003; Dorne et al., 2006). Toxicokinetic variability for each compound was predicted using Latin hypercube sampling, a variant of the Monte Carlo sampling, by simulating quantitative metabolism data (as the fraction of a dose handled by each metabolic route, with the sum of all fractions set equal a 100%) and pathway-related lognormal variability. Seven compounds covering a wide range of monomorphic (antipyrine and paracetamol) and polymorphic pathways (codeine, diazepam, imipramine, proguanil and propranolol) were selected to validate the model. For all compounds, uncertainty factors were calculated from the predicted inter-individual variability and compared with the uncertainty factors derived from variability derived from meta-analyses of published kinetic studies. The results of the Latin hypercube (Monte Carlo) models are illustrated in Fig. 3 and show that uncertainty factors can be predicted with accuracy for compounds handled by multiple pathways (Dorne and Renwick, 2003). Such models can potentially be of use to risk assessors to predict uncertainty factors when in vivo metabolism data is available for a particular compound and have the major advantage over the inappropriate use of default uncertainty factors (Dorne and Renwick, 2003; Dorne, 2007; Dorne et al., 2009).

6. NOMIRACLE, toxicokinetic interactions and uncertainty factors in ecological and human risk assessment

Human and ecological risk assessment (HRA and ERA) of chemical mixtures is difficult to perform because of the complexity of the potential toxicokinetic interactions between the
components of the mixture and the difficulty to characterize the potential consequence of these interactions on the toxicodynamics of the mixture in the target species. Under the 6th EU framework program, the integrated project NOMIRACLE (NOvel Methods for the risk Assessment of Cumulative stressors in Europe) (http://nomiracle.jrc.ec.europa.eu/default.aspx; Science of the total environment-special issue 2010) aimed to develop new methods to support current and environmental and public health risk assessment strategies with regard to combined exposure to complex mixtures. Two aspects are relevant to this review: (1) toxicokinetic interactions and uncertainty factors (2) harmonisation of uncertainty factors in ecological and human risk assessment.

6.1. Toxicokinetic interactions and uncertainty factors

Toxicokinetic interactions of statistical significance for substrates of polymorphic CYP2D6, CYP2C9, CYP2C19 enzymes have been analysed to address whether the default kinetic uncertainty factor (3.16) would cater for mixture effects in humans for inhibitors (competitive and non-competitive) and inducers. The only available in vivo database quantifying human variability in toxicokinetic interactions was the therapeutic drug database with doses (mg) larger than trace contaminant or pesticide levels (µg). Studies for which statistically significant toxicokinetic interactions were analysed and changes in internal dose for markers of chronic/acute exposure were quantified to derive pathway-specific uncertainty factors (95th, 97.5th and 99th percentiles) (Dorne et al., 2001a,b, 2002a,b) taking into account interactions according to the biochemical mechanism involved (inhibition and induction). Overall, inhibition or induction would increase/decrease exposure to chemicals in EMs and PMs for induction and the default uncertainty factor for toxicokinetics (3.16) would not cater for such interactions particularly for potent inhibitors/inducers (Dorne and Papadopoulos, 2008). This human database, although based on therapeutic doses higher than current contaminant levels in food and may require a low dose extrapolation step, represents a useful tool to quantify the magnitude of toxicokinetic interactions in humans. Moreover, the major advantage of this database when considering polymorphic pathways such as CYP2C9, CYP2C19 and CYP2D6 is the actual use of human data because the metabolic route in test species (rat, mice, dog, rabbit) diverges from that in humans (Dorne et al., 2007a,b). Many contaminants are known to be substrates for such CYP enzymes as well as inhibitors or inducers at relatively low doses in mammals depending on their potency and current exposure levels to organophosphates (chloropyrifos, diazinon) (<5–10 µM) have been shown to inhibit imipramine metabolism mediated by multiple CYPs in recombinant enzymes and liver microsomes (Di et al., 2005). Recently, the in vivo toxicodynamic consequence of lindane on CYP-mediated steroid hormone metabolism in male mice following in utero exposure. Results showed changes of male reproductive endpoints (testis weight, spermatid number) together with dramatic reduction of CYP-mediated testosterone metabolism. The authors concluded that lindane-induced toxicity in males is linked to an impairment of steroid hormone homeostasis, due to the modulatin of CYP-mediated testosterone catabolism, this mechanism diverges from the receptor-mediated mechanism previously reported in females (Di et al., 2009).

Identifying the potentially susceptible subgroup when taking into account mixture effect (inhibition/induction) requires an understanding of the consequence of metabolism (bioactivation/detoxification) so that for polymorphic pathways, EM or PMs could be the susceptible subgroup, i.e. EMs would be susceptible to toxicity if the compound was activated to a toxic species and PMs would be at risk if the parent compound was the toxicant (Dorne et al., 2002a,b, 2003a; Dorne and Papadopoulos, 2006, 2008).

For genotoxic carcinogens, such as ethyl carbamate, bioactivated by CYP2E1 to genotoxic metabolites including vinyl carbamate, the presence of CYP2E1 inhibitors such as isothiocyanates and diallyl sulfone from garlic would inhibit such bioactivation. Such inhibition has been shown to have consequences on the toxicodynamics in mice for which the frequency of carcinomas was lower than that in controls. Additionally, the frequencies of adenomas of the lung and of the Harderian gland in CYP2E1 knockout mice (CYP2E1−/−) were significantly lower reduced compared to the wild type (CYP2E1+/+) (Ghanayem and Hoffler, 2007; EFSA, 2007).

Further research is needed to characterize and quantify the potential magnitude of interactions between contaminants on individual CYP at their level of exposure relevant to the human diet. Relevant information can be obtained routinely in the laboratory using recombinant technology and toxicokinetic assays (Hodgson and Rose, 2008; Dorne and Papadopoulos, 2008).

6.2. Harmonisation of uncertainty factors in ecological and human risk assessment

Integrated risk assessment has been defined as “a science-based approach that combines the process of risk estimation for humans, biota and natural resources in one assessment” (WHO, 2001). Harmonising the use of uncertainty factors used in both human and ecological risk assessment to derive safe exposure levels for humans and ecosystems may improve the quality and efficiency for both disciplines. Two quantitative dimensions constitute the basis for such harmonisation: the toxicological dimension estimating the difference between measured and predicted endpoints for both toxicokinetics and toxicodynamics, and the precautionary dimension quantifying the uncertainty involved in predicting such differences between the endpoints (Ragas and Dorne, 2005; Dorne et al., 2006). The derivation of uncertainty factors based on variability data from mechanistic information (e.g. metabolism, toxicokinetics, mode of action, mechanism of toxicity), as described here within the human context for variability in toxicokinetics can be applied to ERA if such information is available. For vertebrates, a number of enzymes are highly conserved i.e. CYP2E1, CYP1A, CYP3A; and such toxicokinetic variability is available for some substances (pharmaceuticals, pollutants, etc.) and species (fish, dogs, seals, etc.) (Dorne et al., 2007a, 2009; Wolkers et al., 2009). The fact that basic mechanistic and toxicokinetic data are not available for all ecological species is one of the major challenges to such harmonisation.

7. Conclusions

Chemical risk assessment is moving towards more quantitative approaches and such an evolution has been stimulated by researchers, international public health agencies as well as.
international Organization guidelines. The reasons beyond this move towards “science-based” quantitative approaches are complex and are influenced by public perception of chemical risk but an important component is the historical result of the bio-informatics era, i.e. the evolution and interface between biological/biomedical sciences (molecular biology, pharmacology, toxicology, epidemiology), mathematics/statistics and computer sciences. The application of systems biology with the emergence of the OMICS (e.g. genomics, proteomics, metabolomics, metabonomics) provides global views on gene, protein or metabolite profile changes to understand the mode of action of specific chemicals and opportunities to develop new biomarkers. Together with systems biology, the implementation of new methods such as quantitative structure-activity relationship (QSAR) and biologically based or physiologically based models to quantify variability and uncertainty have been central to these developments (Dorne and Renwick, 2005a,b; Dorne et al., 2009). In practice, several levels of evidence from molecular to population level can be combined to perform risk assessments and derive safe levels of exposure for individual or groups of chemicals. One of the consequences for risk assessment being that scientists rely on default assumptions only in the total absence of data.

This review has illustrated the potential use of human variability data in metabolism and toxicokinetics, as “pathway-related uncertainty factors and Monte Carlo models, to replace default uncertainty factors for non-genotoxic carcinogens. Pathway-related uncertainty factors were derived for 14 major human routes of xenobiotic elimination (phase I, phase II metabolism and renal excretion) using meta-analyses of pharmacokinetic studies for probe drugs eliminated by a single metabolic/renal route (>60% of the dose) and to cover given percentiles of the human population.

Overall, four main scenarios in humans have been identified for which the current default factor for toxicokinetic (3.16) does not cater for human variability namely:

1. Genetic polymorphism in any subgroups of the population including healthy adults (phase I (CYP2C9, CYP2C19, CYP2D6) and phase II (N-acetyltransferase, NAT 2)) (Dorne et al., 2002a,b, 2003a; Dorne, 2007).
2. Inter-ethnic differences for which a number of metabolic routes showed lower activities in south Asian and African populations compared to Caucasians (CYP3A4, CYP2D6 (Africans), CYP2C19 and NAT-2 (Asians)) (Dorne, 2004a,b).
3. Age differences: Most metabolic routes are affected by age because of lower hepatic metabolism and renal excretion due to slower metabolism in the elderly (CYP3A4, CYP2D6, CYP2C19, renal excretion) and immature metabolism in the neonates (CYP1A2, CYP3A4, glucuronidation, glycine conjugation and renal excretion) (Dorne et al., 2005a,b; Renwick et al., 2000). Very limited data was available quantifying the effect of age on polymorphic pathways but it can be it is probable that neonates would be the most susceptible subgroup when exposed to compounds handled by CYP2D6 and CYP2C19 metabolism. In children, hepatic metabolism and renal excretion was shown to be faster compared with adults with the exception of the limited database for polymorphic metabolism (CYP2D6 and CYP2C19) (Dorne et al., 2007a,b; Renwick et al., 2000).
4. Toxicokinetic/metabolic interactions between substrates of polymorphic CYPs (CYP2D6, CYP2C9, CYP2C19) and inhibitors/inducers. For interspecies differences, the incorporation of TK data for interspecies differences is more limited because of qualitative and quantitative differences in enzymatic expression profiles and pathway-related uncertainty factors can be generated for metabolic routes with evolutionary conservation. Such pathway-related uncertainty factors have been derived for CYP1A2 and glucuronidation which are highly conserved in mammals (Walton et al., 2001a,b); other potential pathways include CYP2E1 and CYP3A (Dorne et al., 2007a).

The use of pathway-related uncertainty factors as intermediate option can be replaced ideally by chemical-specific adjustment factors when sufficient data and physiologically based models as recommended recently by the WHO (WHO, 2005). The possible use of physiologically based pharmacokinetic (PBPK) models in risk assessments in Europe, Canada, and the United States has been explored during two International Workshops for the Development of good modelling practice in Greece on April 27–29, 2007 and their application in risk assessment in Germany on July 6–8, 2009 (Loizou et al., 2008; WHO, 2009).

From a methodological point of view, the pathway-related uncertainty factors and Monte Carlo models were developed using meta-analysis of continuous (lognormal) toxicokinetic data in humans and such methods generally represents a very useful tool to improve evidence-based risk assessment. Full Bayesian inference has recently been proposed to improve the meta-analysis method previously published and applied to the polymorphic CYP2D6 pathway to derive CYP2D6-related uncertainty factors for subgroups of the population (healthy adults, extensive and poor metabolisers) (Dorne et al., 2002a,b; Amzal and Dorne, 2008; Dorne and Amzal, 2008). The method is based the incorporation of population variability and uncertainty and includes hierarchical modeling taking into account covariates, combined with compartmental models to account for inter-individual, inter-compound and inter-study variability and sensitivity analyses to assess the impact of model assumptions on the analysis of variability.

Beyond the analysis of variability in toxicokinetics, availability of epidemiological data relating dose and response in humans would potentially allow to apply these models to the derivation of human BMDL/BMDL for a particular chemical. Such an approach has been recently explored to the derivation of a BMDL in humans for cadmium in food using a meta-analysis of clinical studies reporting the relationship between urinary cadmium and the excretion of β-2 microglobulin as a biomarker for renal effects (EFSA, 2009a,b). Meta-analysis has also been applied to ecological risk assessment using abundance data for a number of naturally occurring terrestrial invertebrates (Carabidae, Heteroptera, Staphylinidae, Lepidoptera and grouped chick-food insects) to investigate the impact of pesticide restriction in arable crop edges for 12 broad types of pesticide manipulation in crop edges. Overall, this recent meta-analysis confirms that restriction of pesticide inputs in crop edges benefits arthropod populations at the edges of arable fields but highlights data gaps on the ecological consequences of excluding insecticides and fungicides from crop edges, and the clear need to improve the clarity of reporting in agro-ecology studies (Frampton and Dorne, 2007).

As illustrated in this review, a key issue in chemical risk assessment is the integration of quantitative descriptors regarding variability and uncertainty in the toxicokinetics and toxicodynamics of single toxicants and chemical mixtures. Such quantitative approaches will prove useful to risk assessors to provide science-based risk assessment that are more transparent.

Acknowledgements

In loving memory of my mother Annie GATEL (1940–2000) and Professor George Gordon Gibson (1949–2008)

We are grateful to the Health and safety Executive, the department of Health, Health Canada and the European Commission under the 6th framework project NO MIRACLE (project number 003956) for funding this research. The views reflected in this review are the author’s only and do not reflect the views of the University
of Southampton, any of the sponsors or the European Food Safety Authority.

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