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TRENDS

Trends in human risk assessment of pharmaceuticals

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Pharmaceutical products used in human and veterinary medicine are a class of great importance in our modern society. The scientific community has recently recognized that after elimination from the body of humans and domestic animals, active ingredients are found in treated and untreated sewage effluents, surface water, groundwater, and drinking water [21]. Many therapeutic classes are commonly found, for example anti-inflammatory drugs, cholesterol-lowering drugs (e.g. statins), antidepressants, anticonvulsants, synthetic steroids (e.g. the contraceptive pill), antineoplastics, beta-blockers, bronchodilators, lipid regulators, hypnotics, antibiotics, antiseptics, X-ray contrast agents, and caffeine. Such contamination should be assessed in relation to possible environmental and human risks, because pharmaceutical products are biologically active, for example synthetic hormones which cause endocrine disruption in fish at very low levels of exposure (ng L^{-1}) [27]. Recent reviews describing detrimental effects of pharmaceutical compounds on the ecosystem are available, and also presented in this issue [3, 10, 13]. Advanced technologies using granular activated carbon, membrane technology, ozonation, and ultraviolet radiation have been used with relative success to remove pharmaceutical and environmental contaminants from water destined for human consumption. Several pharmaceutical products, for example anti-epileptics (carbamazepine, primidone), non-steroidal anti-inflammatory drugs (diclofenac, ibuprofen, ketoprofen, indomethacin), and lipid regulators (clofibric acid, gemfibrozil) are known to resist such treatment, because of their high solubility and/or poor degradability in water. Hence, concentrations of pharmaceutical products in drinking water have been shown to reach ng L^{-1} levels, and even μg L^{-2} levels for compounds such as diclofenac, the pharmaceutical compound present at the highest concentration in the environment [15]. Risk assessments for potential adverse effects in humans at these concentrations are scarce particularly for chronic exposure and sub-therapeutic concentrations. Such assessments should become a scientific priority for the "International Decade for Action: Water for Life" launched in 2005 and the Millennium Development Goals (MDGS), the objective of which is to double the amount of the world's population with sustainable access to safe drinking water and sanitation by 2015. The UNICEF and WHO have estimated that over a billion and 2.6 billion people are currently deprived of safe water supplies and adequate sanitation, respectively [19].

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Trends in the risk assessment of pharmaceutical products for human health are addressed here for non-cancer and cancer outcomes, including the threshold and safety factor approach, mixtures of pharmaceutical products, and use of emerging technology, for example toxicogenomics, quantitative structure–activity relationships, and probabilistic methods.

Human risk assessment of pharmaceutical products—trends and future refinements

The objective of human risk assessment is to protect susceptible individuals of the human population from potential chemical harm by derivation of safe levels of exposure "without appreciable health risk", for example the acceptable or tolerable daily intake (ADI and TDI) (WHO) or the reference dose (RfD) (US-EPA). For non-genotoxic carcinogens present in food and water contaminants, these have been set using thresholds (below which no toxicity is predicted), expressed in mg kg^{-1} diet or volume of water per day, to relate them to human oral exposure. The surrogate for the threshold, for example the lowest and/or no observed adverse effect level (LOEL, NOEL) or the benchmark dose (BMD) is determined from chronic or subchronic animal studies and then divided by an uncertainty factor of 100 (interspecies differences tenfold and human variability tenfold) to derive the ADI or RfD. For genotoxic carcinogens, dose–response relationships from experimental animal data are combined with low dose extrapolation to relate a human health risk to an estimated exposure or an estimated exposure to a human health risk ([9, 11].

Guideline values and Ambient Water Quality Criteria thresholds have also been developed by the US-EPA and WHO for pharmaceutical products, based on therapeutic effects and adverse effects, i.e. probable idiosyncratic and/or hypersensitivity reactions. In the WHO approach the guideline value is derived using daily water consumption, the fraction of the tolerable daily intake allocated to water consumption, and the body weight of the subjects [28]. Uncertainty factors (30) are applied to take into account human variability (tenfold) and extrapolation from LOEL to the NOEL derived from animal data (threefold).

A recent example of human health risk assessment for pharmaceuticals was performed by a cohort of pharmaceutical companies on twenty-six pharmaceutical products and their metabolites from fourteen pharmacological classes. For each compound, the risk assessments were conducted using safe levels (ADI), measured environmental concentrations (MECs) based on using US and worldwide environmental monitoring data for current levels of exposure from drinking water and fish ingestion in children and healthy adults, and predicted no effect and effect environmental concentrations (PECs and PNECs). Overall and for each compound, the ratio between the measured or predicted concentration to the PNEC for children was used as risk indicator i.e. increase in risk with a ratio 1. However, these ratios were between 10^{-5} and 10^{-1} indicating that these levels of exposure do not result in human health risk. The authors have, however, stressed the need to assess individually and thoroughly antibiotics with a non-human target, cytotoxic drugs with therapeutic doses at or above toxic doses, compounds with potential allergenic potential, and compounds with high bioaccumulation potential [26]. Such compound specific-assessment has been considered for the synthetic oestrogen 17-α-ethinylestradiol, the antibiotic phenoxymethylpenicillin, and the antineoplastic drug cyclophosphamide using Danish scenarios of worst-case emissions [4]. Another study was carried out using US-EPA methodology for non-cancer effects (clofibrate, indomethacin, and acetylsalicylic acid) and cancer effects (cyclophosphamide) using risk-specific doses for linear carcinogens (mg kg^{-1} day^{-1}), i.e. the dose associated with a cancer risk of 10^{-5}. The latter study took into account human exposure to contaminated water, intake via bioaccumulation in fish, the toxicological and pharmacological nature of the compound, exposure assessment, and environmental fate and transport of each pharmaceutical product [25]. Both studies showed that the concentration of each pharmaceutical product in drinking water was below safe limits and no appreciable risk to humans would be expected [4, 25]. Although these conclusions are based on scientific studies using sound toxicological, mechanistic, and exposure data, several issues require further assessment:

- These data are based on thirty pharmaceuticals but do not cover other classes, for example pharmaceuticals used in veterinary medicine.
- Some pharmaceutical products have been designed for specific subgroups of the human population. Therapeutic doses and toxic doses may vary widely between subgroups and these should also be assessed for the most susceptible subgroups, for example neonates [9].
- These human health risk assessments have been conducted for given scenarios of exposure in water sources yet levels of pharmaceuticals may fluctuate in different regions of the globe and/or bioaccumulate, depending on human activity and therapeutic use of pharmaceutical compounds. Hence, regular biomonitoring of concentrations of pharmaceutical products in water sources is also a major priority.
- Pharmaceuticals are always present as mixtures, an issue which still greatly troubles regulatory agencies

[1]; a major challenge to scientists is to evaluate whether these mixtures in surface and ground water could constitute a health risk to humans now or in the future.

– Finally, another important aspect is the scientific validity of the tenfold uncertainty factor allowing for human variability and used to derive safe levels of exposure for food and water contaminants for humans. Over the last ten years, regulators and scientists have subdivided the human variability factor (10) into toxicokinetics (TK, ($10^{0.5}$, 3.16), elimination of compounds and toxicodynamics (TD), and differences in the expression of toxicity ($10^{0.5}$, 3.16) [24]. Depending on the availability of chemical-specific data, such factors could be replaced with chemical specific adjustment factors (CSAFs) usually developed from a physiologically based TK-TD model (PB-TK-TD). The human body uses different pathways to eliminate compounds and a recent approach has derived pathway-related uncertainty factors to replace the TK uncertainty factor for each route and for different percentiles (95–99%) of human subgroups (genetic polymorphism, disease, age and ethnicity). Such an approach enables incorporation of in vivo human metabolism and TK data in risk assessment and provides flexible options between uncertainty factors and CSAFs. This approach has been based on meta-analyses of human studies describing the TK of pharmaceuticals metabolised primarily (>60% dose) by major human phase-I (mostly CYP) metabolism, phase-II hepatic metabolism, and renal excretion. The phase-I metabolic routes include the major human CYP isoforms (CYP1A2, CYP2E1, CYP3A4, CYP2C9, CYP2C19, CYP2D6), alcohol dehydrogenase (ADH), and major hydrolysis metabolic routes. Phase-II metabolism includes *N*-acetylation (NAT-2), glucuronidation, glycine and sulfate conjugation, and renal excretion [9]. Overall, the TK uncertainty factor would not cover neonates, the elderly for most elimination routes, and any human subgroup for compounds metabolised by polymorphic CYP (for example as CYP2C9, CYP2C19, CYP2D6, and NAT-2).

These conclusions raise questions about the safety of pharmaceuticals in water, as individuals vary in their susceptibility. This is of particular relevance to compounds metabolised by polymorphic CYP enzymes for which elimination differences are very wide with over 3–20-fold differences between extensive metabolisers (EMs) and poor metabolisers (PMs). Extra uncertainty factors would have a large impact on the setting of safe levels and it is important that future approaches take these quantitative differences into account. For polymorphic metabolism it can be assumed that the proximate toxicant is the parent compound and PMs would be the susceptible subgroup, i.e. a decrease in clearance would increase adverse effect risk. The reverse situation is also commonly seen, however, i.e. metabolic activation to a toxic species so that EMs would be the susceptible subgroup [8].

Metabolism and pharmacodynamic (PD) effects of some pharmaceutical products of environmental relevance are presented in Table 1; for a large number of these compounds pharmacodynamic effects are known to be affected by polymorphic metabolism.

Table 1 Metabolism and pharmacodynamics for several pharmaceutical products of environmental relevance

Therapeutic/ chemical class	Metabolic route	PD mode of action	Pharmaceutical product substrates
Methylxanthines	CYP1A2	Inhibition of phosphodiesterase	Caffeine, theophylline
Nicotine	CYP2A6	Nicotinic receptor agonist	Nicotine
Non-steroidal anti-inflammatory	CYP2C9, glucuronidation, renal excretion	Inhibition of cyclooxygenase	Ibuprofen, indomethacin, diclofenac
Antidiabetics	CYP2C9, renal excretion	Reducing glucose levels	Tolbutamide, glyburide
Anticoagulants	CYP2C9, CYP1A2	Inhibition of blood coagulation factors	Warfarin, acenocoumarol
Antimalarial drugs	CYP2C19, CYP3A4, renal excretion	Inhibition of plasmodium growth	Proguanil, chloroguanide
Antidepressants	CYP2D6, CYP2C19, CYP3A4, CYP1A2	Inhibition of serotonin reuptake	Fluoxetine, paroxetine, citalopram
Proton-pump inhibitors	CYP2C19, CYP3A4	Inhibition of H^+-ATPase pump	Omeprazole, lansoprazole
Beta-blockers	CYP2D6	Beta-adrenoceptor antagonism	Metoprolol, propranolol
Antimicrobials (tuberculosis)	*N*-acetylation	Inhibition of bacterial growth	Sulfamethazine, isoniazid
Antibiotics	Renal excretion	Inhibition of bacterial growth	Amoxicillin
Statins	CYP3A4, CYP2C9	Reducing cholesterol levels	Lovastatin, simvastatin, fluvastatin

Human risk assessment of pharmaceutical mixtures

Risk assessment of pharmaceutical mixtures should also be taken into account, e.g. characterization and modelling of interactions at the TK and TD level. In humans, metabolism-based interactions between potential CYP substrates can result in inhibition or induction of particular CYP enzymes with potential TD consequences. The magnitude of the TK effect will depend on: (i) the potency of the inhibitor/inducer (competitive, non-competitive, and inhibition constants), (ii) the compound's specific metabolism (metabolism by one or more CYP) and its consequence (toxication/detoxication), (iii) the TK differences between EMs and PMs, and, consequently, (iv) the presence of alternative routes of metabolism in the PM subgroup. The TD consequences of CYP-based interactions will depend on whether or not metabolism itself is critical to the expression of toxicity. There is a large amount of published data on drug–drug interactions; examples of potent CYP-based interactions (competitive inhibition and induction) with potential TD consequences are listed in Table 2.

These examples are based on therapeutic concentrations (mg day^{-1}) that would be higher than environmental exposure to pharmaceutical products (ng or μg day^{-1}). The effects of chronic exposure to low or sub-therapeutic doses of pharmaceutical products in the presence of potent competitive inhibitors (for example paroxetine for CYP2D6, protease inhibitors (ritonavir, indinavir) and antifungal drugs (ketoconazole, itraconazole) for CYP3A4) or inducers has not yet been assessed, however. A recent study has shown that current levels of exposure to organophosphate pesticides (<10 μmol L^{-1}) inhibit metabolism of the antidepressant imipramine by human recombinant enzymes and in liver microsomes [6]. This raises important issues for the risk assessment of mixtures and the potential for interaction between pharmaceutical products and other environmental contaminants. The human health relevance of these observations must be assessed more thoroughly with a wide range of pharmaceutical products, pesticides, and other environmental contaminants as potential inhibitors or inducers of drug-metabolising enzymes.

One of the objectives of the 6th framework European project NOMIRACLE (http://viso.jrc.it/nomiracle/) is to improve the scientific basis of uncertainty factors with particular reference to chemical mixtures. Recent analysis of TK interactions between probe substrates of polymorphic CYPs (CYP2C9, CYP2C19 and CYP2D6) and known inhibitors or inducers, has shown that the current TK uncertainty factor (3.16) would not cater for such interactions if the parent compound or its metabolite(s) were the proximate toxicant. Although these results are based on therapeutic concentrations rather than the low concentrations of pharmaceutical products in drinking water, genetic variability and potential interactions (including potency of the inhibitor or inducer) should be taken into account in human risk assessment. This may be of particular relevance to anti-cancer drugs and endocrine disrupters present in the environment as potential carcinogens, and their effect(s) may be modified during chemical interactions [12]. Such information can be obtained routinely in the laboratory by use of recombinant technology and toxicokinetic assays [14].

Future approaches: toxicogenomics, QSAR 282 and probabilistic approaches

The emergence of the field of systems biology, seeking to integrate different levels of biological information to understand how biological systems function, has introduced to toxicology a set of experimental methods and tools with potential application for refining and improving approaches

Table 2 CYP-based interactions and their toxicokinetic and toxicodynamic consequences

Compound(s)	Interacting compound	Mechanism of TK interaction	Toxicodynamic consequence
Inhibition			
Statins	Grapefruit juice	CYP3A4	Increased risk of adverse effects
Metoprolol	Paroxetine	CYP2D6	Increase in heart rate
S-Warfarin	Fluconazole	CYP2C9	Increase anticoagulation
Omeprazole	Ticoldipine	CYP2C19	Reduce ADP-induced platelet aggregation
Induction			
Propafenone	Rifampicin	CYP2D6, CYP1A2, glucuronidation	Loss of dysrhythmia control in heart
Valproate	Gingko biloba	CYP2C9, CYP2C19	Fatal seizures
Cyclosporin	St John's wort	CYP3A4	Risk of rejection of organ transplants
Omeprazole	Gingko biloba	CYP2C19	Reduce effect but needs further investigations
Oestradiol	St John's wort	CYP3A4	Increase bleeding time

to risk assessment. A recent workshop on toxicogenomic methods involving European and US experts, regulators, and principal validation bodies has addressed biological validation of such methods in the regulatory arena. Toxicogenomics and its cousin disciplines (proteomics, transcriptomics, metabolomics, and metabonomics) can help define mechanisms and identify biomarkers in combination with gene expression signatures to predict toxicity. Although such data are, currently, often derived from in vitro assays and have limited regulatory applications, they would still enable prescreening and complementary toxicological tests for single compounds and priority mixtures, i.e. microarrays indicating changes in gene expression after chemical exposure (potentially defining mechanisms of action, and potential alternatives to refine, reduce, and replace animal use). Other long-term goals can also be foreseen, for example the use of human or animal *in vitro* or *in vivo* data derived from omic technology to derive adverse (or no adverse) effect levels or to determine dose–response relationships for quantitative risk assessments [2].

Combining toxicogenomics data with other toxicological information, for example quantitative structure–activity relationships, can also provide very useful information for scientists assessing pharmaceutical products. The objective of quantitative structure–activity relationships (QSARs) is to correlate the structure of chemicals with their activity by use of statistical tools. These *in silico* approaches can be of great value for predicting the activities of chemicals that have not been tested, including pharmaceutical products [20]. QSAR models with good predictive power (>92%), using clinical trials and maximum recommended therapeutic doses (MRTD) for high and low-toxicity compounds, have been recently been developed for estimation of no observed effect levels (NOELs) of pharmaceutical products in humans. Such human data provide more specific estimates of toxic dose thresholds of chemicals in humans than do extrapolated animal data; e.g. there is poor correlation between MRTD in rodents and humans (R^2= 0.2005, n=326) [18]. QSAR is a very powerful tool for molecular modelling of enzyme–substrate interactions when investigating CYP metabolism, because quantitative information is available on the linear relationship between the strength of substrate binding and the hydrophobicity log P (where P is the octanol/water partition coefficient), on hydrogen bonding, and on aromatic π–π stacking [16]. QSAR models have also been developed for CYP3A4, which metabolises 50% of all known pharmaceuticals, and known competitive inhibitors, and there is good agreement between these models, molecular models of the enzyme itself, and known mechanisms of inhibition [17].

Science-based risk assessment has benefited from use of probabilistic risk assessment techniques which can replace "single value deterministic uncertainty factors" by distributions and quantify the uncertainty of the assessment explicitly. The most popular technique is Monte Carlo modelling in which the deterministic input values of the assessment (i.e. human uncertainty factors) are replaced by input distributions and, subsequently, an output distribution of risk is produced by repetitive drawings from the input distributions [23]. Such models have enabled good prediction of human variability in kinetics (and uncertainty factors) for compounds handled by multiple pathways of elimination with known in-vitro and in-vivo quantitative metabolic profiles in man [7, 8].

Monte Carlo simulation can also be combined with Bayesian statistics, enabling the combination of different sources of probabilistic information in one assessment. An example is the study by Roelofs et al. [22] in which an ecological NOEL is predicted on the basis of a combination of substance-specific toxicity data and non-substance-specific information from a toxicity database. These techniques can have disadvantages, however; e.g. different types of input variation are combined into one output distribution which is a mixture of the variance and the uncertainty of the output. One means of looking at both aspects is use of 2nd-order Monte Carlo simulation [5] so that uncertainty and variability are nested in two different simulations and can be combined in a final distribution. This type of analysis can produce quantitative results giving information about chemical risk, i.e. "10% probability (= uncertainty) that 5% of the population (interindividual variability) exceeds the safe concentration".

Conclusion and future work

Assessment of risk to humans of environmental exposure to pharmaceutical products (mostly drinking water and fish) can be regarded as a new field of risk assessment and risk analysis characterised by its inherent multidisciplinary nature. The link between human and ecological risk assessment is clear, because the effect of chemical species in the environment may have direct or indirect effects on human health. Advances in toxicology have helped scientists define pathways of metabolism (toxicokinetics) and mechanisms of toxicity (toxicodynamics) for single pharmaceutical products and, increasingly, for mixtures. New tools, for example the "omic" sciences, QSAR modelling, and probabilistic models have great potential to assist scientists in quantifying biological effects of pharmaceutical products and identifying toxicity mechanisms at the level of populations, individuals, cells, and molecular targets. These results can then be used to refine uncertainty factors used to set safe human levels or, ideally, derive chemical-specific adjustment factors.

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