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2

What is toxicology and how does toxicity occur?

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Toxicology has matured since it was defined as the 'science of poisons'. Modern toxicology is no longer anthropocentric but takes on different views at various biological systems, including ecosystems. Each will interact specifically when exposed to defined chemical agents, including drugs. Adverse effects during drug therapy or after (accidental) poisoning are the result of some negative interactions between the agent and the exposed biological system. Toxicity is no longer a specific property of drugs and chemicals but an operative term to describe the adverse outcome of a specific drugs–host interaction.

Newer developments in toxicology have focused on the host. Toxicogenetics continues to provide answers to variations of host response to xenobiotics, including drugs. Clinically relevant genetic polymorphisms and gene defects have been detected, and their number is rapidly growing. The key to understanding is in the host proteins that interact with the drug and mediate the cellular response. Hence, the proteom, i.e. the complete set of proteins of a cell, an individual or a species, determines how an exposed biological system may interact with the manifold of different xenobiotics. Structure–activity studies try to find out useful predictive parameters for risk and toxicity assessment.

Key words: toxicodynamics; toxicokinetics; toxicogenetics; drug–host interactions.

THE SCOPE OF TOXICOLOGY

In the latest edition of a highly respected textbook, Curtis D. Klassen[1] provided a simple answer to the title question when he wrote that 'toxicology is the science of adverse effects of chemicals on living organisms'. This traditional view of toxicology will be compared with an enhanced concept that has become increasingly popular in recent years.

Like many scientific disciplines, modern toxicology has experienced a division into specialized fields, depending on which aspects of the problem in question are being emphasized. At the interface of medicine and life and earth sciences, toxicologists have always been stimulated by case reports of poisoning and toxic actions, but it has become increasingly popular to work on mechanisms, models and scenarios in order to predict and prevent rather than to merely explain afterwards.

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Figure 1 is an illustration of the many faces of toxicology. Depending on how a toxicological issue is approached, the traditional descriptive toxicologist is now in good company with the mechanistic, the molecular (biochemical or genetic), the regulatory, the forensic, the clinical, the industrial and the environmental toxicologist. This picture leaves out theoretical or comparative approaches to toxicology. Also, there is no mention of epidemiological, industrial or food toxicologists. It ignores sub-specialities of environmental toxicology, such as aquatic toxicology, and it does not account for facets of clinical toxicology such as behavioural toxicology, inhalational toxicology, immunotoxicology or neurotoxicology. All of these specializations are reflected by the growing number of publications which, at present, comprise more than 60 active periodicals with the TOX string in their title.

This chapter provides some insight into current toxicological research. We will walk along the border between knowledge and ignorance and will deliberately omit some important aspects—most noteworthy, biomonitoring and carcinogenesis. Similarly, most toxicological aspects of environmental toxicology will be skipped—for example, tobacco, food toxins and air pollution.

SOME DEFINITIONS

Toxicity is no longer a specific biomedical property of a given chemical but an operative descriptor of the adverse outcome of a specific drug–host interaction. When we say that the toxicity of cyanide is extreme, we often imply that *relatively* little cyanide is sufficient to cause a life-threatening intoxication in man, but in another context we may focus on the large-scale destruction of biomass in a river.

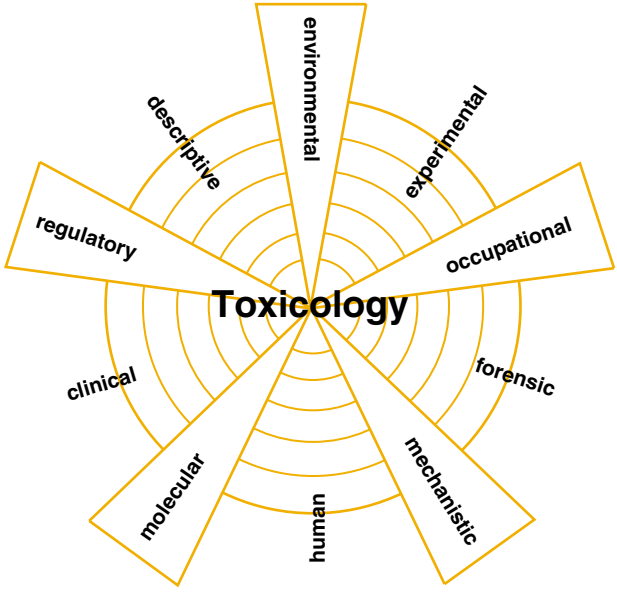

Figure 1. The scope of toxicology—depicted as a 'toxicology butt'. The traditional 'subspecialities' of toxicology form the background of the butt. More recent developments (with a strong inheritance from life and earth sciences and epidemiology)—by no means exhaustive—are represented as darts.

Sometimes the word *toxicity* is combined with the target organ to specify which tissues are most likely adversely affected by the poison (Table 1). It is also common to differentiate *acute* and *chronic* toxicity when emphasizing that a toxic effect had occurred after single exposure to a given chemical (acute exposure) or after equal doses were given repeatedly over a certain period of time (chronic exposure; minimum 3 months, depending on the species), respectively. Finally, *immediate* and *delayed* toxicity are terms which characterize the onset of harm and symptoms after exposure.

The three terms *toxicity*, *toxicant* and *toxin* require careful distinction, as do the terms *danger*, *hazard* and *risk* (Table 2).

In this context, pharmaceuticals are beneficial xenobiotics (unless, for example, an overdose exceeds therapeutic needs), and toxicants or toxins are harmful xenobiotics expected not to exert beneficial effects to the exposed biological system. However, many authors simply appear to designate any toxic agent a toxicant or use toxin synonymously.

Instead of focusing on dose–response relationships in an individual, toxicologists sometimes describe the observations of some toxic effects in a population, a quantal relationship. Due to biological variation, a specified endpoint will be present in some individuals but absent in others, even though all have received the same mg/kg dose of a drug. To estimate the impact of toxicity, *risk assessment* strategies are being devised. Risk assessment involves the calculation of the individual hazards of a given toxicant (expressed as a probability figure) times the frequency of exposure to the toxicant (sometimes other relationships have been found). Even the most toxic chemical poses little risk when the frequency of exposure is low.

With the above vocabulary in mind, we will review some widely used definitions of toxicology. I borrow the formulations from the script of Deon Canyon[2], with some modifications.

1. The traditional definition has seen toxicology as the 'science of poisons' focusing on the chemical agent, rather ignoring the exposed human being. It suggests that toxic effects are determined by the toxic agent.
2. A newer definition resembles the one of Klaassen (cited earlier) and describes toxicology as 'the study of the adverse effects of chemical or physical agents on living organisms'. It appreciates that a given toxicant may provoke different effects in various species. For example, it is well known that 1 mg/kg of TCDD (2,3,7,8-tetrachlorodibenzodioxin) given to guinea pigs is sufficient to kill half of the exposed

Table 1. Examples of target-specific toxicity. While toxic phenomena always reflect an adverse interaction between a poison and a (cellular) host, it may still be useful from a clinical or descriptive point of view to focus on specific targets within the wide spectrum of interactions.

Term	Example
Neurotoxicity	Organophosphates inhibit acetylcholine esterase
Cardiotoxicity	Doxorubicin induces free radical formation and mitochondrial damage in cardiac myocytes
Myelotoxicity	Mercaptopurine depresses bone marrow
Immunotoxicity	Low-dose TCDD suppresses delayed-type hypersensitivity reaction to tuberculin in guinea pigs
Cytotoxicity	Etoposide induces apoptosis in HL60 cells
Genotoxicity	Non-lethal cisplatin concentrations induce a positive micronucleus test

Table 2. Some useful definitions in toxicology and risk assessment. While the first four terms are tightly linked to the science of toxicology, the latter three may play a role in other situations of danger, rather unrelated to toxicology, for example, car driving.

Term	Definition (example)
Poison	Any toxic compound without beneficial effects
Toxicant	Any toxic substance that is made by, or a by-product of, human activities (carbon tetrachloride)
Toxin	Natural toxic substances produced by living organisms (diphteria toxin; snake, spider, fish venoms)
Xenobiotic	Any foreign substance taken into the body (any administered drug, personal care product)
Danger	Any possible adverse outcome or situation (car crash or injury or potential toxic exposure)
Hazard	A probability number of frequency expression assigned to a specific danger (number of car crashes in a town, per day, with 100 000 cars moving around for 1 hour, or the number of victims after a defined toxic exposure)
Risk	An expression that combines a given hazard with the exposure frequency (risk for car crash increases proportionally to the number/duration of drives, risk is zero when the car is in the garage; similarly, an extremely toxic substance causes little harm when no exposure occurs)

animals, but $>$1000 mg/kg are required to achieve the same effect with hamsters. This definition also takes into account that physical phenomena (light, heat, radiation) may contribute to toxicity. Drugs such as tetracyclines may pose skin problems, even in subtherapeutic doses, when the affected body is exposed to intense sunlight (*phototoxicity*).

3. Toxicologists have become increasingly aware that an *interaction* between the toxic agent and the exposed organism, both qualitatively, is the key to understanding. For example, diphtheria toxin produces overt mucosal necrosis in humans, guinea pigs and sheep, but not in mice or rats. In rats, the membrane receptor, an HBEGF-like growth factor precursor, simply does not interact with the toxin. Alternatively, consider the metabolic activation of acetaminophen or cyclophosphamide. Both can be presented to isolated cells in huge concentrations without causing any harm. Only if the cells are competent to metabolize the compounds is toxicity observed. Thus, the exposed organism plays a crucial role for the quality and intensity of effects (beneficial or detrimental). A modern definition therefore extends the concept of toxicity to the mutual interaction of toxicants and exposed biological systems, organisms and ecosystems alike. It also includes risk assessment. Toxicology could then be defined as the study and assessment of adverse or harmful interactions between xenobiotics and biological systems. It is this interactive view of toxicology that has spawned the most fruitful advances of our understanding of modern toxicology.

TOXICANT–HOST INTERACTIONS

Drug–host and toxicant–host interactions obey the same pharmacological principles. However, the terms *toxicodynamics* and *toxicokinetics* are often used when we deal

with overdose and/or adverse effects. Toxicodynamics and toxicokinetics traditionally indicate a different approach to toxicity: the former zooms in on the drug, the latter on the host.

Toxicodynamics

In order to study various toxic phenomena, it has been useful to look at a certain level of detail. As there is no sharp boundary between these levels, appropriateness is sometimes a matter of 'taste', sometimes limited by the lack of knowledge. The following paragraphs discuss some examples of current toxicology research, including adverse drug reactions.

Organ- and tissue-level interactions

Toxicologists have been interested in drug-induced dysfunction of the central nervous system (CNS) such as seizures, loss of consciousness, and eventually coma in predisposed patients, even at therapeutic dosages.[3] Examples of such drugs are antidepressants, lithium, H1 antihistamines, non-steroidal anti-inflammatory drugs (NSAIDs), antimicrobial agents, antineoplastic drugs and many more. Seizures may be induced directly (via excitatory stimulation, as with pentetrazol or cocaine) or indirectly (through a variety of epileptogenic mechanisms, e.g. hypoxia, oxygen utilization blockade, hyperpyrexia, electrolyte imbalance, hypoglycaemia). While some mechanisms of triggering—such as electrolyte shifts and the intracellular accumulation of glutamate and other excitotoxins—have now been elucidated, nobody knows precisely why one brain would synchronize after drug administration but another one would not.

Hepatotoxicity is another organ-wide example and has been noted with many drugs, for example, nitrofurantoin, phenylbutazone, carbontetrachloride and rarely sulphonamides, but apparently the underlying mechanisms are heterogeneous and poorly understood.[4] Toxic hepatitis can be indistinguishable from viral hepatitis, with hepatocellular necrosis, inflammation and cholestasis. In other instances, isolated cholestasis with or without inflammation may be present, as occurs after exposure to oral steroids (contraceptives, anabolics) or gold, azathioprine, captopril and cyclosporine, respectively. Non-caseating granulomatous hepatitis has been diagnosed after allopurinol, diazepam, isoniazide, phenytoin and sulphonamides.

Macrovascular or microvascular steatosis has been observed in patients on glucocorticoids and methotrexate or on tetracyclines and valproate, respectively. Cholangitis and cholangiolitis after chlorpromazine exposure closely resemble primary cirrhosis. However, there is no clue, yet, as to how liver cells influence the ultimate histological picture. Several mechanisms are being discussed among which cytokines, oxidative stress and immunological reactions have received the widest acceptance. Interleukin-10 (IL-10) has been shown to play an important role in hepatotoxic failure. Mice that lack the IL-10 gene are significantly more susceptible to hepatotoxic drug reactions.[5]

SIADH (syndrome of inappropriate secretion of anti-diuretic hormone) has been observed in up to 1% of patients on drugs such as omeprazole, antidepressants (tricyclic, selective serotonin re-uptake inhibitors), antipsychotics and monoamine oxidase (MAO) inhibitors. These drugs probably induce hormonal actions at the pituitary gland.[6] The consequence is water retention, hyponatraemia, high osmolar urine production and sometimes increased intraocular pressure. However, other

drugs such as carbamazepine, angiotensin converting enzyme (ACE) inhibitors and dopaminergic agents appear to act at both the pituitary and kidney levels. The underlying mechanism is unknown.

Toxic lung oedema fits neatly between organ-level and tissue-level interactions. It is a special type of acute lung injury that has long puzzled toxicologists, when observed, for example, after exposure to toxic gases, for example, chlorine.[7] Although the whole respiratory tract may be affected, the focus of research has shifted to the alveolar/capillary interface where irreversible damage occurs when the primary lesion is exacerbated by the subsequent inappropriate repair processes. Cytokines and chemokines and pro-coagulant stimuli trigger the proliferation of fibroblasts, eventually replacing the thin alveolar linings with fibrotic deposits, thus leading to an acute respiratory distress syndrome.[8] A similar clinical picture is observed after paraquat poisoning.[9] Paraquat is a bispyridylium herbicide that has been shown to generate reactive oxygen species from its radical interaction with molecular oxygen. While paraquat is a potent killer of type II pnemocytes, for unknown reasons its chemical sister diquat is not.[10]

Rhabdomyolysis is a massive destruction of muscle fibres. It has been associated with hereditary defects, such as McArdle's disease (myophosphorylase deficiency) or carnitine palmitoyltransferase deficiency (CPD), but is also seen after exposure to toxins from snakes, quails, etc. and after drug administration.[11] ε-Aminocaproic acid and statins such as cerivastatin are potent triggers of the disorder[12,13], which appears to start as a massive calcium release and a breakdown of the sarcolemma. Alcohol-induced rhabdomyolysis has been associated with ATP depletion due to intracellular hypophosphataemia[14], while cerivastatin-induced rhabdomyolysis may be initiated by massive apoptosis of myocytes.

Cell-live interactions

Apoptosis is a major endpoint of cytotoxicity.[15] It designates the programmed death of a cell, and is clearly distinguished from necrosis (1) by an *ordered* sequence of events and (2) by massive consumption of metabolic energy (ATP). Two mechanisms have been identified[16]: apoptosis triggered by *internal* and by *external* signals (Figure 2). To proceed to completion, apoptosis has to pass several checkpoints that are currently under intense research.[17]

Endocrine disrupters represent a heterogeneous class of chemicals that have in common the ability to stimulate the cellular oestrogen receptor.[18] While they are generally several orders of magnitude less potent than natural oestrogens, their almost ubiquitous presence in the environment has raised concerns about impaired fertility (reduced sperm counts, cryptorchidism), increased cancer incidence in the male, and abnormal maturation processes, like in puberty. Compounds such as polychlorinated biphenyls and nonylphenol stimulate oestrogenic receptors but they also interfere with steroid synthesis and biotransformation. A list of candidates is shown in Table 3.

Organelle-level interactions

Mitochondrial dysfunction has long been known to be caused by a variety of chemicals [e.g. 2,4-dinitrophenol (DNP), cyanide *m*-chloro phenyl hydrazone (CCCP)] that are effective uncouplers of mitochondrial ATP production and deplete cellular energy stores. Only recently has interest shifted to certain anti-HIV drugs that cause lactic acidosis by interfering with mitochondrial DNA synthesis.[19]

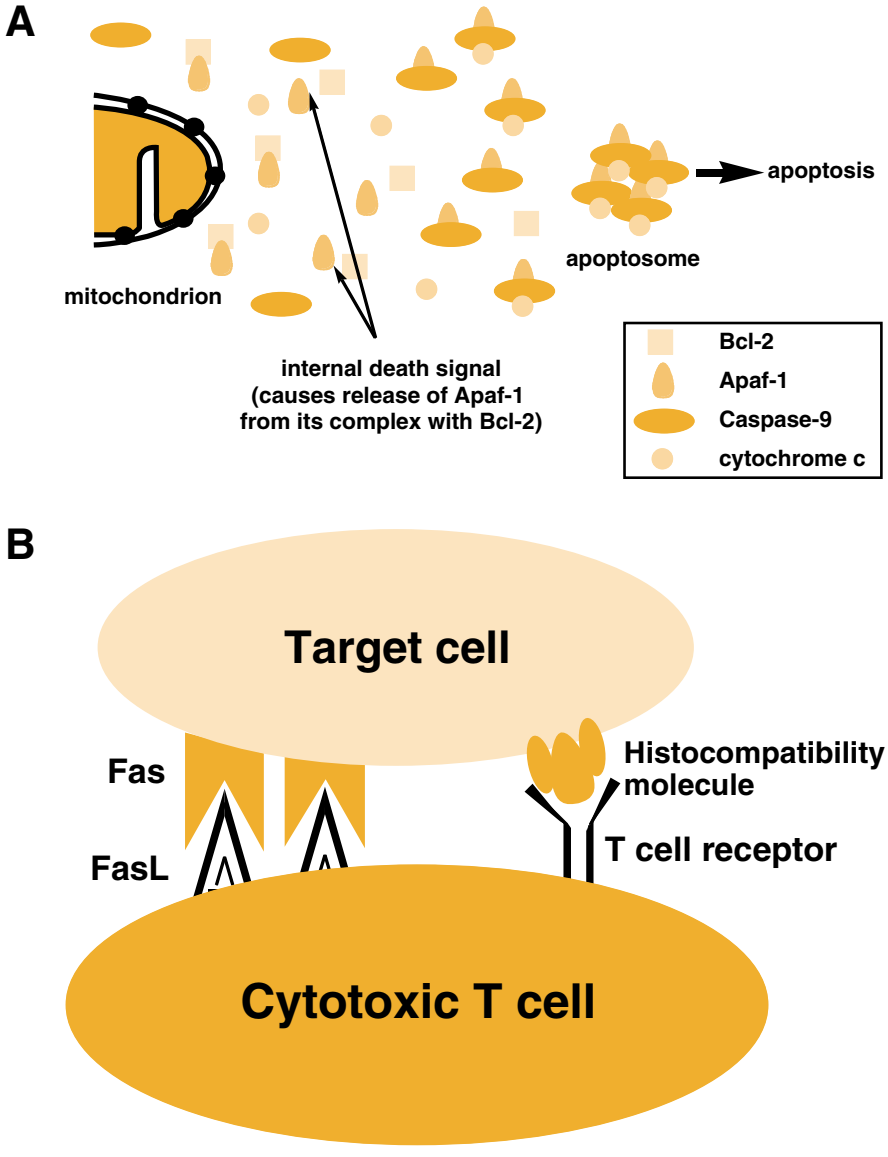

Figure 2. Intracellular (A) and extracellular (B) signalling in apoptosis. (A) In the internal sequence, Apaf-1 protein (normally bound as a protien–protein complex to Bcl-2 in the outer mitochondrial membrane) is released upon cellular damage (e.g. changes in redox or ATP status) Subsequently, an *apoptosome* is formed in the cytosol as a ternary complex of caspase-9, Apaf-1 and cytochrome *c*. Apoptosomes bind ATP, and this activated complex activates other hydrolytic enzymes, thus inducing massive proteolysis and breakdown of cellular organelles, including the nucleus and DNA. (B) External signalling requires the interaction of cytotoxic T cells with the apoptotic cell. The death signal is triggered by antineoplastic drugs, for example, taxanes, vinca alkaloids, anthracyclines, or radiation. Appearance of the *Fas* or TNF receptor at the surface of the damaged cell causes cytotoxic T cells to bind via their *Fas ligand* molecule. When the T cell detects the presenting major histocompatibility complex (MHC) molecule on the surface of the target cell, it recruits even more Fas ligand. The Fas/Fas ligand complex formation exposes its 'death' domain in the cytosol of the target cell, activates capase-8 and induces a cascade of proteolytic and endonucleatic activities.

Table 3. Chemicals associated with endocrine effects in animals and humans (*) or in vitro (+) (from US EPA Illinois, 1999).

Known	Probable	Suspect
Atrazine	Alachlor	Aldicarb
Chlordanes	Aldrin	Butyl benzyl phthalate
Chlordecone (*)	Amitrole (aminotriazole)	Tert-Butylhydroxyanisole (+)
DDD	Benomyl	*p*-sec-Butylphenol (+)
DDE	Bisphenol a (+)	*p*-tert-Butylphenol (+)
DDT	Cadmium (*)	Carbaryl
1,2-dibromo-3-chloropropane (*)	2,4-D	Cypermethrin
Dicofol	Di(2-ethylhexyl)phthalate	2,4-dichlorophenol (+)
Dieldrin	Endrin	Dicyclohexyl phthalate
Diethylstilbestrol (*)	Heptachlor	Di(2-ethylhexyl)adipate (+)
Dioxins (2,3,7,8-)	Hepatchlor epoxide	Di-*n*-butyl phthalate (+)
Endosulphans	Hexachlorobenzene	Di-*n*-hexyl phthalate
Furans (2,3,7,8-)	*p*-hexachlorocyclohexane	Di-*n*-pentyl phthalate
Lindane	Lead (*)	Di-*n*-propyl phthalate
Methoxychlor	Mancozeb	Esfenvalerate
p-Nonlyphenol	Maneb	Fenvalerate
PCBS	Mercury (*)	Malathion
Toxaphene	Methyl parathion	Methomyl
Tributyl tin	Metiram	Metribuzin
	Mirex	Nitrofen
	p-Octylphenol	Octachlorostyrene
	Parathion	PAHS
	Pentachlorophenol	*p*-iso-Pentylphenol (+)
	Polybrominated biphenyls	*p*-tert-Pentylphenol (+)
	Styrene (+)	Permethrin
	2,4,5-T	Ziram
	Trifluralin	
	Vinclozolin	
	Zineb	

Peroxisome proliferation is another example of organelle-related interaction.[20] First discovered as elusive microbodies in liver and kidneys, peroxisomes were regarded as (per)oxidative centres involved in the oxidation of D-amino acids, uric acid and L-α-hydroxyacids (e.g. lactate). When it was shown that peroxisomal defects were involved in inherited disorders—such as Schilder's and Zellweger's disease—it subsequently became evident that they were essential for the oxidation of (long-chain) fatty acids and for the biosynthesis of ether-lipid glycerolipids. In the late 1980s, toxicologists learnt that a variety of drugs (fibrates) and consumer chemicals (plasticizers such as dibutylphthalate) were effective inducers of peroxisome proliferation—and that their non-genotoxic carcinogenicity in rodent liver may be related to the activation of peroxisomes and the ensuing increase in hydrogen peroxide production. It became evident that peroxisome proliferation was the result of the binding of these xenobiotics to a cytosolic protein that would, in turn, form a complex with another cytosolic receptor protein which directly travels to the nucleus. Gene recombination experiments and base sequence analysis then revealed that this isolated receptor (PPAR = peroxisome proliferation activated receptor) was a member of the steroid receptor superfamily. While not directly carcinogenic (in humans), *peroxisome*

proliferation activators were shown to have a stimulating effect on tumour growth and on atherosclerosis.

Nuclear interactions

Transcription factors are nuclear proteins that act as promoters or suppressors of gene expression. These nuclear factors (nF) have attracted toxicologists' interest because it has been found that many 'old' drugs such as salicylates, tamoxifen and immunosuppressants (tacrolimus, cyclosporin) exert some of their effects by interfering with certain types of transcription factor (e.g. nF-κB).[21,22] Furthermore, it was shown that they might play a role in a special kind of chronic toxicity, namely, addiction: a *c*AMP-*r*esponsive element *b*inding (CREB) protein has recently been shown to act as an nF in vitro and in vivo by mediating morphine and cocaine-related changes in CREB protein expression and phosphorylation, which is associated with alterations of animal behaviour in reward experiments.[23,24]

Toxicogenetics: dynamic aspects

Increasing evidence indicates that genetic predisposition plays a critical role in toxicodynamics. *Alcoholic liver damage* has been linked to a polymorphism in the promotor region of the IL-10 gene. IL-10 is an anti-inflammatory cytokine that, when secreted by monocytes, exerts an inhibitory effect on macrophages. Monocytes of patients with alcoholic cirrhosis failed to secrete normal amounts of IL-10 in response to an LPS stimulus.[25]

Malignant hyperthermia is a rare genetic disorder that may be triggered by administering a neuromuscular blocking agent (e.g. suxamethonium) and certain volatile anaesthetics.[26] It is a life-threatening condition due to uncontrolled release of intracellular calcium from the sarcoplasmic reticulum and subsequent hyperthermia, acidosis and cardiac tachyarrhythmia. Mutations in several skeletal muscle ion channel genes, such as the ryanodine receptor, the voltage-gated dihydropyridine receptor and some others, have been associated with malignant hyperthermia.[27] This example neatly demonstrates that an individual anaesthetic agent may be only the trigger—but not the 'cause'—of the disorder and the observed toxic effects.

After the complete deciphering of the human genome, microarray techniques are now being developed that tackle a vast amount of multidimensional analyses on a few silicon microchips.[28,29] In this way, genetic defects and polymorphisms may eventually be detected *before* therapy has done harm.

Toxicokinetics

Toxic effects may sometimes push the affected biological system to a completely new state that becomes irreversible after a certain point-of-no-return has been reached, for example, death. Often irreversible damage remains when the triggering agent has long left the system. Fortunately, most adverse or harmful effects start no sooner than the administration of the toxicant, but usually subside more or less slowly after the last dose due to its continuing elimination from the system. Most elimination processes are active contributions of the host system in response to a variety of metabolic challenges. What the host does to a toxicant is the objective of toxicokinetics.

A typical time course of drug concentration in plasma after a single (oral) dose is shown in Figure 3. At some time, t_{max}, the maximum concentration, C_{max} is reached.

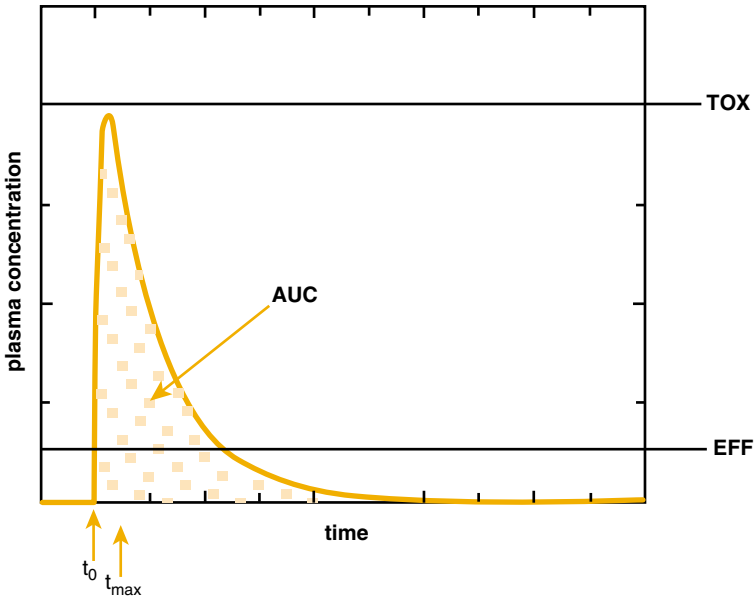

Figure 3. Typical time course of the plasma concentration after oral administration of an absorbable drug. Absorption starts at t_0 and by t_{max} the plasma concentration will reach a maximum (C_{max}) and then declines due to elimination processes. A threshold labelled EFF represents the minimum concentration required for efficacy (e.g. with an antibiotic) and the line labelled TOX indicates a hypothetical threshold above which unacceptable toxicity will accrue (especially with repeated doses). The AUC is the (shaded) area-under-the-curve that is proportional to the amount of absorbed drug.

The *area-under-the-curve* (AUC) of concentration versus time is directly proportional to the amount of drug or toxicant that has entered the body. The three parameters AUC, C_{max} and t_{max} are important characteristics of drug therapy. An increase in the AUC above therapeutic levels often heralds increased toxicity, while a decrease in the AUC below therapeutic levels results in suboptimal therapeutic efficacy.

We will briefly review the so-called *LADME* scheme that has been successfully applied to pharmaco- and toxicokinetics and which deals essentially with the influence that an exposed body exerts on the invading xenobiotic. The acronym stands for *L*iberation, *A*bsorption, *D*istribution, *M*etabolism (biotransformation) and *E*xcretion. The term *elimination* has been reserved to sum up metabolism *and* excretion.

Liberation

Many poisons come to the exposed body in a matrix. Research in environmental toxicology has taught us to differentiate *nominal* from *actual* (=inner) exposure:[30] a person may be exposed to a nominal burden of dust particles in a closed room (calculated by multiplying the particle concentration of a single breath with the number of breaths), but the *inner exposure* may be different because the particles may also be absorbed through exposed skin areas (thus increasing the actual particle burden) and a fraction of particles might be exhaled again (thus decreasing the actual particle burden). A proof of inner exposure is the detection of the agent or its

metabolites in body fluids and/or tissues. Even the subcutaneous administration of a drug quite often requires liberation of the active principle from the vehicle before absorption can occur.

Absorption

For many toxicants to reach their targets, absorption from the gastrointestinal tract, respiratory tract or skin is mandatory. Absorption across epithelial barriers may occur via the paracellular or transcellular pathway. Epithelia, such as the intestinal mucosa, have been shown to act as physical and metabolic barriers. Conversely, breakdown of the barrier may occasionally result in increased toxicity.

The *paracellular pathway* is controlled by several specialized cellular structures, the junctional belt.[31] Most epithelial (endothelial) cells form intercellular junctions, gap junctions, desmosomes and tight junctions, for electrochemical coupling, mechanical adhesion, and paracellular sealing, respectively. The tight junction (zonula occludens, ZO) is a membrane area where two or more cells get into close contact so that their lipid bilayers fuse by means of integral membrane proteins that assemble spontaneously after close contact to form anastomosing TJ strands. The tightness of the ZO can be assessed by measuring the transepithelial electrical resistance. Gastrointestinal mucosa or the alveolar linings have rather low resistance (100–600 ohms cm^2), whereas the kidney and bladder epithelia are highly resistant (10 000–50 000 ohms cm^2). Lipophilic solutes and dissolved gas molecules can pass the ZO, but their diffusion rates correlate inversely with their water solubility. The driving force for the paracellular transport is a concentration gradient; it is not dependent on metabolic energy. The ZO—once formed—is maintained with almost no input of metabolic energy, but any repair (due to loss of constituting cells, for example) requires ATP. The functional integrity depends on (1) the complex integrity of the TJ strands and (2) the availability of calcium. Hardly surprisingly, any chemical that interferes with protein synthesis or lipid–protein interactions (e.g. puromycin, proteases, saponins) or chelates calcium (e.g. EDTA) will sooner or later 'open' the paracellular pathway and abolish the transepithelial electrical resistance.

The *transcellular pathway* requires uptake of the toxicant into the epithelial cell (mucosa, alveolar epithelium, endothelium). Several uptake mechanisms have been identified[31], for example, the exploitation of membrane pores, carrier proteins, endocytosis, some of which hardly discriminate their payload, while others show a high degree of specificity. Small lipophilic molecules may enter the cells without any specialized mechanism, driven only by concentration gradients—for example, ethanol or diethyl ether.

Endocytosis is an energy-dependent process. For small solutes (< 150 nm in diameter), pinocytosis is an efficient way to enter a cell. Pinocytotic droplets are engulfed by invagination of the cell membrane at specialized areas, called pits, and pinch off to become *coated vesicles*, coated with the protein clathrin. After shedding their clathrin coats in the cytosol, these vesicles undergo several transformations to become early and late endosomes, lysosomes, until their contents have been assimilated by the cell. It has been calculated that macrophages continually ingest 25% of their fluid volumes each hour, while fibroblasts endocytose at a somewhat lower rate. The cell membrane undergoes a rapid turnover. This 'waste' of resources is balanced by the effective recycling of endocytotic vesicles, called the endocytotic–exocytotic cycle. Larger molecules can be effectively internalized by specialized receptors that assemble at the membrane pit where a vesicle begins to form.

This *receptor-mediated endocytosis* is the most important pathway by which lipoproteins get into the cells, but many toxins (e.g. diphtheria toxin) and viruses (HIV) exploit this pathway successfully, too.

Other toxicants require carrier molecules in the cell membrane that facilitate their transport along a concentration gradient or—at the expense of ATP—they provide an active 'uphill' transport. *Facilitated diffusion* or *active transport* of toxicants is another example of how even ionized or polar solutes that cannot diffuse across a lipid bilayer may enter the cytosol. The past few years have also provided evidence that transmembrane transporters not only import xenobiotics into cells but also effectively extrude a variety of xenobiotics out of affected cells (see later under 'transporters').

Distribution

When a toxicant has reached the systemic circulation, it will be rapidly distributed via the blood until equilibrated with all accessible tissues. Whereas the time to travel with the blood is short (the average circulation time is 60 seconds), it takes much longer to equilibrate with remote tissues, especially at those regions that are effectively shielded by a blood–organ barrier (e.g. brain, testis).

$$D_L = V_d \cdot c \qquad \text{(Eqn 1)}$$

In pharmacokinetics, the *volume of distribution* V_d is defined as the proportionality factor between the amount of drug D_L in the body (which equals the loading dose, when a drug is administered for the first time) and the drug concentration in plasma c (Eqn 1). The major physiological spaces of distribution are (1) the intravascular blood plasma space (approximately 0.043 l/kg), (2) the extracellular space (approximately 0.16 l/kg), and (3) the whole-body water (approximately 0.57 l/kg). However, calculations of volumes of distributions on the basis of plasma concentration measurements rarely match these physiological spaces. Therefore, the term 'apparent volume of distribution' has been introduced. Table 4 lists some typical examples. A possible explanation for the observed discrepancies is that a considerable part of the administered dose is bound to tissues (e.g. adipose tissue). This 'escape' from blood decreases the plasma concentration and, conversely, increases the apparent volume of distribution. Equation 1 is a useful expression to estimate the amount of drug or toxicant in the body that causes an observed plasma concentration when elimination fails or may be ignored.

Elimination

As soon as a drug enters the body, mechanisms that tend to decrease its amount become effective. Two different ways to get rid of a xenobiotic have been identified: *metabolism* (=biotransformation) and *excretion*. Metabolites may be toxicologically active or inactive. They can be metabolized further, but eventually all derivatives of the parent compound will be removed from the body by excretion (see below).

$$D_M = Cl_{tot} \cdot c \qquad \text{(Eqn 2)}$$

Table 4. Pharmacokinetic data of drugs that are being used in anaesthesiology and intensive care units, sorted by apparent volumes of distribution. Note the large standard deviations of many examples.

Compound	Apparent volume of distribution (in l/kg ± SD)	Clearance (in ml/min/kg ± SD)	Route
Amiodarone	66 ± 44	1.9 ± 0.4	H
Fluoxetine	35 ± 21	9.6 ± 6.9	H
Imipramine	18 ± 2	13 ± 1.7	H
Fentanyl	4 ± 0.4	13 ± 2	H
Morphine	3.3 ± 1.9	24 ± 10	H
Codeine	2.6 ± 0.3	11 ± 2	H
Ondansetron	1.9 ± 0.05	5.9 ± 2.6	H
Sufentanil	1.7 ± 0.6	12.7±	H
Propofol	1.7 ± 0.7	27 ± 5	H
Diazepam	1.1 ± 0.3	0.38 ± 0.06	H
Lidocaine	1.1 ± 0.4	9.2 ± 2.4	H
Midazolam	1.1 ± 0.6	6.6 ± 1.8	H
Alfentanil	0.8 ± 0.3	6.7±2.4	H
Ethosuximide	0.71 ± 0.16	0.19 ± 0.04	H
Thiopental	0.71±0.11	3.9± 1.2	H
Lithium	0.66 ± 0.16	0.35 ± 0.11	R
Ethanol	0.54 ± 0.05	V_{max} = 2.07 ± 0.67 mg/kg/min, K_m = 0.082 mg/ml[a]	H
Thenphylline	0.5 ± 0.16	0.65 ± 0.20	H
Remifentanil	0.3 – 0.4	40 – 60	H
Pancuronium	0.26 ± 0.07	1.8 ± 0.4	R
Dobutamine	0.2 ± 0.08	59 ± 22	H
Furosemide	0.13 ± 0.06	1.66 ± 0.58	R
Heparin	0.058 ± 0.011	$(0.65 + 0.008\ D)^{-1}$ ± 0.1[b]	H

H, hepatic clearance; R, renal clearance. Data from Thummel KE and Shen DD[41] (in Hardman JD et al. (eds) *Goodman & Gilman's The Pharmaceutical Basis of Therapeutics*, 10th edn. New York: McGraw-Hill, 2001; 1917–2023, with permission from the publisher.

[a]Ethanol is metabolized by a saturable (Michaelis–Menton) process involving ADH. The V_{max} value represents the fastest possible.

[b]Heparin clearance is dose-dependent (D in IU/kg).

For a quantitative estimate of how much drug is 'lost' over time, the clearance concept has been useful. The total clearance Cl_{tot} is a proportionality factor between the amount of drug D_M eliminated from the body in a given time interval (which equals the maintenance dose, when a drug is given repeatedly) and the drug concentration in plasma c (Eqn 2). The total clearance is the sum of various contributions, for example, hepatic clearance, renal clearance, etc. (Table 4). Mostly, it will be sufficient to consider hepatic and renal clearance. When a drug is eliminated exclusively via the kidneys, toxicity from accumulation is likely to occur in renal failure. On the other hand, a significant loss of functional hepatic parenchyma, for example, with cirrhosis, may predispose to toxicity from drugs that are predominantly eliminated by biotransformation.

Sometimes the half-life time of a drug is falsely used to estimate elimination from the body. While the half-life is indeed related to the clearance it is also affected by the volume of distribution (Eqn 3)! The half-life $t_{1/2}$ is directly proportional to the apparent

volume of distribution, but inversely proportional to the clearance; the natural logarithm of 2 may be taken as 0.69.

$$t_{1/2} = \ln 2 \cdot \frac{V_d}{Cl_{tot}} = 0.69 \cdot \frac{V_d}{Cl_{tot}} \qquad \text{(Eqn 3)}$$

Metabolism

Many drugs can leave the body unaltered, no matter whether they are being exhaled, like anaesthetic gases, or excreted by the kidneys, like many antibiotics. However, equally important is the elimination by *biotransformation*, i.e. the metabolic degradation of xenobiotics. This happens predominantly in the liver, but lungs, intestine and kidney—or even plasma—may contribute in certain cases. Two phases, functionalization and conjugation, are distinguished. Metabolic functionalization usually renders a lipophilic molecule more hydrophilic and suitable for conjugation, while the conjugation reactions add polar moieties, thereby increasing water solubility, molecular weight and particle size so that the conjugate may partition into the bile. Both steps usually alter not only the pharmacological activity but also the toxicity of a xenobiotic. But there are known examples where biotransformation actually increases toxicity. The insecticide parathion is rapidly converted to the 1000 times more potent paraoxon. The larger the fraction of a drug that becomes metabolically inactivated upon arrival at the intestinal mucosa, or at the liver, the smaller the amount of active substance that becomes available systemically. This phenomenon, called *first-pass effect*, is the limiting factor that virtually prevents oral therapy with nitroglycerin and some other drugs due to the heavy pre-systemic extraction and inactivation.

Functionalization

Despite a great variety of possible substrates and reactions, the predominant reaction type is mono-oxygenation, i.e. the transfer of activated oxygen. The most important catalyst family is the cytochrome P450 (*CYP*) system.

Cytochrome P450 enzymes

All known CYP enzymes are haemoproteins that exhibit a characteristic absorption band around 450 nm when exposed to carbon monoxide (CO) under reducing conditions. Their molecular weight ranges from 45 to 55 kDa, and each carries a haem moiety with an essential iron (Fe) atom. They are typically located in the membranes of the endoplasmatic reticulum, mostly in the liver, but also occur in enterocytes and other cells. They require phospholipids, molecular oxygen, NADPH and NADPH–CYP-reductase activity. Oxygen activation occurs via electron transfer (Figure 4). All CYP enzymes transfer activated oxygen, but differ in the specificity. Substrate specificity comes from the side chains of substrates and the pockets of the enzymes, but there is considerable overlap so that many drugs can be processed by different isoenzymes. Phylogenetic analysis suggests a common ancestor gene some 3 billion years ago. More than 1000 different isoenzymes have been characterized to date[32], about 50 being active in human beings. They are grouped in three families with several subfamilies (A–F), encoded on six chromosomes (Figure 5). Twelve isoenzymes are important for the biotransformation; other CYP enzymes are required for

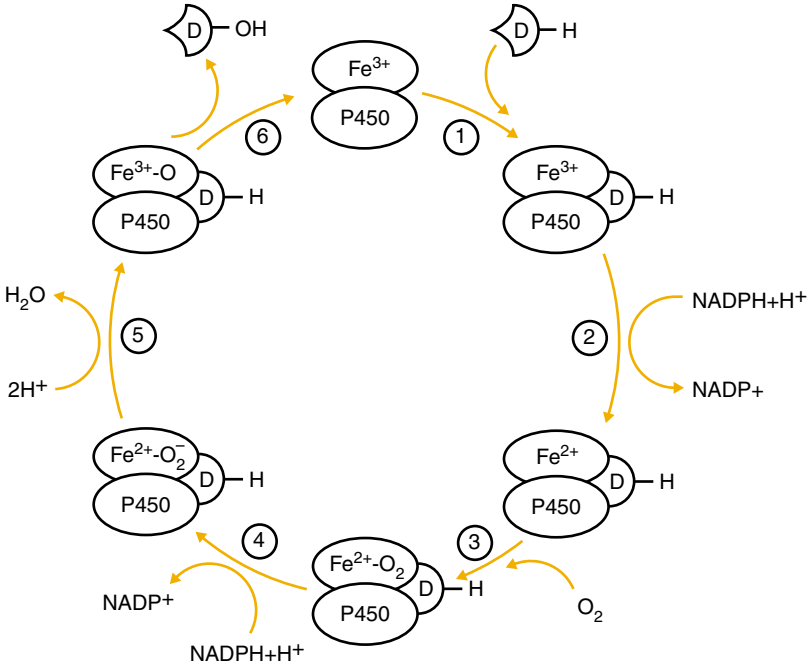

Figure 4. Activation of substrates by CYP enzyme activity. The complete P450 reaction sequence comprises at least six discernible steps for a drug (or xenobiotic) D to become functionalized. (1) Substrate D enters the cycle and becomes bound to a P450 Fe-containing catalytic site. (2) An electron is transferred in a 1st reduction step from NADPH, thus reducing Fe^{3+} to Fe^{2+}. (3) Molecular oxygen binds to the D–Fe^{2+} complex. (4) A 2nd electron is transferred from NADPH to reduce the oxygen. (5) The charged oxygen molecule is cleaved, and one atom picks up two H^+ ions from the surrounding milieu to form water, leaving the other O attached to the P450 site. (6) The Fe-attached O atom is transferred to the substrate, forming a hydroxylated form of the substrate. The hydroxylated substrate (D–OH) then leaves the P450 site.

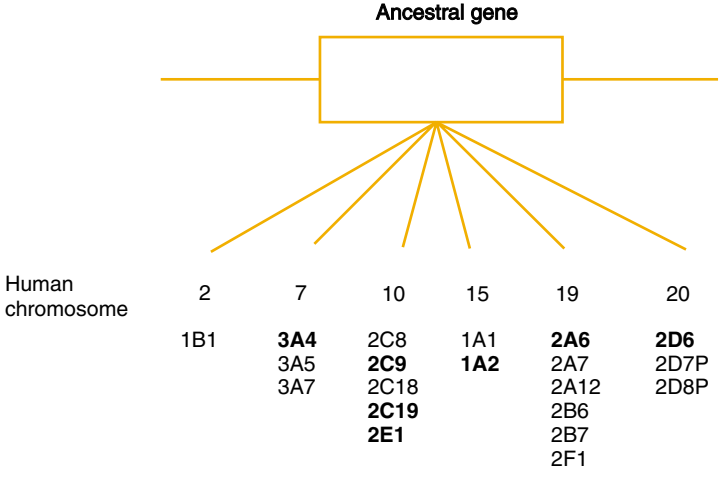

Figure 5. Location of human CYP genes on chromosomes and their putative origination from a common ancestor gene. (Most important genes in **bold**.)

the biosynthesis of prostaglandins, steroid hormones, cholesterol, bile salts and vitamin D3. Some 30% of the total CYP content is CYP3A4, 20% belongs to the CYP2C subfamily, 10–12% to the CYP1A2 and 3–6% to the CYP2E1 isoform. Table 5 lists the isoenzymes that are most important in humans and gives some of their typical substrates. Being a substrate for a certain CYP isoenzyme means a significant affinity for its catalytic centre. Some substrates decrease or abolish CYP activity (*inhibitors*), some induce the biosynthesis of new copies of the enzyme—especially after prolonged exposure—thereby increasing catalytic activity (*inducers*). There is no easy way to predict whether a substance will simply bind, inhibit or stimulate a certain CYP activity (Table 5). CYP interaction can markedly alter the AUC of xenobiotics: inhibition of liver enzymes slows down elimination (increased AUC!), while CYP induction may accelerate elimination (decreased AUC!). Recent interest concerns food and spice constituents because some food (grapefruit juice) and herbs (St John's wort) have dramatically altered the AUC of concomitantly given drugs while inhibiting intestinal CYP activities (thus increasing drug bioavailability).

CYP-catalysed oxidation reactions are driven by the presence of oxygen. When oxygen tension is low, as may happen in the centrolobular part of the liver, some of the mixed-function oxygenases may rather reduce their substrates. A notable example

Table 5. Some human CYP isoenzymes with relevant drug substrates (examples taken from the 'Cytochrome P450 Drug Interaction Table', http://medicine.iupui.edu/flockhart/).

Isoform	Substrates	Inhibitors	Inductors	Comments
CYP1A2	Imipramine, clomipramine, clozapine, caffeine, propranolol, theophylline	Fluvoxamine, cimetidine	Smoking	Activates some procarcinogenes
CYP2C9	NSAID, fluvastatin, phenytoin, tolbutamide, warfarin	Fluconazol, isoniazid, ticlopidine	Rifampin	
CYP2C19	Amitriptyline, citalopram, diazepam, imipramine, moclobemide, omeprazol	Fluvoxamine, fluoxetine, ketoconazol, moclobemide, cimetidine	Carbamazepine	Genetic polymorphism
CYP2D6	Tricyclic antidepressants, metoprolol, timolol, propranolol, codein, tramadol	Amiodarone, fluoxetine, paroxetine, quinidine, sertraline	Rifampin	Genetic polymorphism
CYP3A4	Calcium channel blockers, statins, macrolides, carbamazepine, quinidine, ciclosporin, tamoxiphen	HIV protease inhibitors, intraconazole, ketoconasole, erythromycin, cimetidine, grapefruit juice	Carbamazepine, rifampin, St John's wort	High activities in gut mucosa (fist-pass effect!), shares many substrates with P-glycoprotein

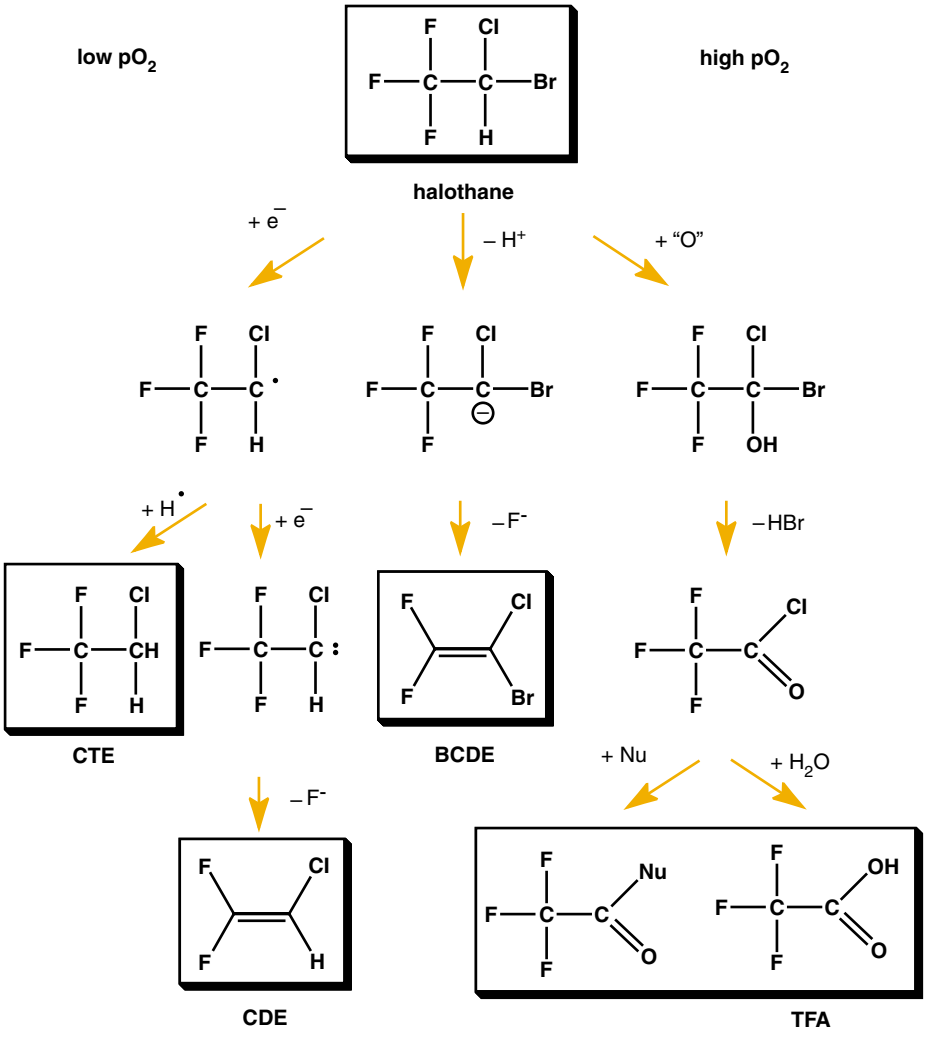

Figure 6. CYP-mediated biotransformation of halothane in liver tissue. Three different pathways have been identified depending on oxygen tension: the oxidative pathways leads to trifluoroacetic acid (TFA) or a reaction product with nucleophiles (Nu, e.g. lysine or serine residues). The reductive pathway yields bromochlorodifluoroethene (BCDE)—which is conjugated with glutathione and cleaved to yield the corresponding mercapturic acid (not shown) or chlorotrifluoroethene (CTE) and chlorodifluoroethene (CDE), both of which have been detected in exhaled air. Modified after Gut J et al[44] (1993, *Pharmacology and Therapeutics* 58: 133–155) with permission.

is the complex metabolization of halothane by CYP2B isoforms (Figure 6), which has been proposed as the biochemical cause of halothane hepatitis.

Functionalizing non-cytochrome P450 enzymes

Some phase I enzymes are not CYP-related. They are oxidoreductases (which may oxidize alcohols and aldehydes) and hydrolases (which cleave ester bonds and open

epoxide rings). Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), xanthin oxidase, flavin-containing monoxygenases (FMO) are examples of the non-CYP class. Hydrolytic enzyme activities have been detected not only in liver tissues but also in plasma—for example, butyrylcholine esterase which catalyses ester cleavage in acetylsalicylic acid, procain and other esters and amides. Epoxide hydrolase converts epoxides into diols.

Conjugation

Phase II reactions (conjugation) promote the excretion of metabolites with suitable functional groups (–OH, –NHR, –SH). There are several families of conjugating enzymes. All increase the molecular weight of their substrates, thus making biliary elimination a possible alternative route. Some conjugations will enhance water solubility, while others decrease polarity, but may in turn provide suitable substrates for a second round of functionalization.

Most important is *glucuronidation*, i.e. the conjugation with glucuronic acid, a highly polar and water-soluble acid that increases the molecular weight of the conjugate by 193 Da. Glucuronic acid is derived from glucose-6-phosphate attached to uridine diphosphate (UDP) and requires oxidative activation of UDPG to yield UDP-glucuronate (UDPGA). The transfer enzyme *UDPGA-transferase* (UGT) is ubiquitous, but highest activities are again found in the liver. Seventeen human UGTs have been characterized so far, with UGT1A1-10 belonging to the UGT1 and seven others to the UGT2A or UGT2B subfamily. UGT2B7 is responsible for the glucuronidation of morphine, a notable exception to the 'rule of detoxification': morphine-6-glucuronide is 1000 times (!) more potent than morphine at the μ-receptor.

Sulphotransferases (SULT) catalyse the esterification of –OH groups with sulphate, yielding highly polar and water-soluble sulphuric acid esters. It is an important reaction with steroids, phenols and catechols. The co-factor is 3′-phosphoadenosine-5′ phosphosulphate (PAPS). Reduced glutathione (GSH), the most important redox buffer in animal cells, is linked to xenobiotics by *glutathione-S-transferases* (GST). Six human families of GSTs have been characterized, designated α, μ, κ, π, ζ and θ. GST activity detoxifies many antineoplastic drugs, but conjugation of 1,2-dibromoethane actually increases reactivity towards nucleophilic sites. The nucleophilic –SH group of GSH substitutes –OH groups to yield thioethers or it adds to epoxides to yield a thioether with a vicinal hydroxy group. Glutathione conjugates may undergo further reactions involving cleavage of the glutamate moiety and finally removal of the glycin residue, leaving the corresponding mercapturic acid for excretion.

N-Acetyltransferases (NAT) are important for the biotransformation of aromatic amines and sulphonamides. Two isoforms (NAT1 and NAT2) are known in humans. The chemotherapeutic drug isoniazid (INH) is completely stripped of its anti-tuberculosis activity after acetylation by NAT2.

The transfer of methyl groups by *methyl transferases* such as catechol-*O*-methyl-transferase (COMT) or thiopurine-*S*-methyl-transferase (TPMT) is another important conjugation reaction. While the transfer of methyl groups, which requires *S*-adenosylmethionine (SAM) as cofactor, appears to abolish the activity of some xenobiotics with phenolic, S- or N- functional groups, it does not enhance water solubility.

Toxicogenetics: kinetic aspects

Table 6 gives some examples of important genetic variants of phase I and II enzymes along with their frequency in the Western European population. For example, CYP2D6 has at least 70 single nucleotide polymorphisms and other variants, some with reduced catalytic activity, while others are non-functional. Thus, affected populations may be divided into poor (*PM*), intermediate (*IM*), extensive (*EM*) and ultra-rapid metabolizers (*UM*). About 5–10% of white Caucasians are PM-type and thus at greater risk of adverse reactions when a CYP2D6-affine drug is given (Table 6). Physicians will be surprised that usual doses of drugs have little effect in UM patients (e.g. timolol), but sometimes an exaggerated response is noticed when the metabolite is pharmacologically active (e.g. formation of morphine from codeine). Adding an inhibitor of CYP2D6 to the regimen may convert a UM to a PM, while the opposite (adding an inductor to a PM) is usually less effective. The former type of *phenocopying* has been exploited to reduce drug burden: fixed combinations of inhibitors plus effectors have been purposefully designed for 'pharmacokinetic maximization' (sometimes called *PK enhancers*). For example, a small dose of the HIV protease inhibitor ritonavir with a fat meal added to another protease inhibitor, lopinavir, will almost double the lopinavir AUC.

Another example to illustrate the role of toxicogenetics relates to TPMT: treating homozygotes (0.3–1% of the population) of inactive TPMT with standard doses of azathioprin will almost inevitably cause severe toxicity with pancytopenia. The desired therapeutic effect would be achieved with 10–15% of the usual dose.

Excretion

Renal excretion means filtration of plasma through the pores of the renal tubules, which are about 8 nm in diameter, so that any compound with a molecular weight below 7000 Da can pass freely, but those of $>70\,000$ Da are retained. Thus, renal excretion occurs (1) by passive filtration in the glomerula only (Cl ≈ GFR = 120 ml/min/1.73 m^2); H (2) by subsequent re-absorption along the nephron (Cl < GFR); or (3) by additional tubular secretion (Cl > GFR).

Table 6. Frequencies of selected genetic polymorphisms that affect drug metabolism.

Enzyme	Frequency of low-activity polymorphisms in Western Europe
CYP2A6	1–2%
CYP2D6	5–10%
CYP2C9	Approx. 2%
ADH2 (alcohol dehydrogenase 2)	2–5%
ALDH2 (aldehyde dehydrogenase 2)	Rare in Caucasians, but $\leqslant$50% of Asians
FMO 3 (flavine mono-oxygenase 3)	Unknown (bromhidrosis, fish-odour syndrome)
DPD (dihydropyrimidin dehydrogenase)	0.1%
PCHE (butyrylcholinesterase)	0.05%
Paroxonase	5–10%
UGT1A1	5–7%
GST (Glutathion-*S*-transferase)	GST T1 $\approx$ 38%; GST M1 $\approx$ 30–60%
NAT 2 (*N*-Acetyltransferase)	50%
TPMT (thiopurine-*S*-methyl-transferase)	0.3%

Adapted from Eichelbaum M and Schwab M[42] (in Forth W et al. (eds) *Allgemeine und spezielle Pharmakologie und Toxikologie*, 8th edn. München: Urban & Fischer, 2001; 39–52, with permission from the publisher.

Table 7. Factors that affect creatinine plasma concentrations.

Factor	Effect on plasma creatinine	Comment
Kidney disease	Increase	Decreased GFR (increase is blunted by increased tubular secretion of creatinine and by reduced creatinine generation)
Reduced muscle mass, immobilization	Decrease	Reduced creatinine generation (common in children, women, and older and malnourished patients)
Ingestion of cooked meat	Increase	Transient increase in creatinine generation (increase may be blunted by transient increase in GFR)
Malnutrition	Decrease	Reduced creatinine generation (caused by reduced muscle mass and reduced meat intake)
Use of cimetidine, trimethoprim	Increase	Inhibition of tubular creatinine secretion
Use of flucytosine, some cephalosporins	Increase	Positive interference with iminohydrolase and picric acid assays for creatinine, respectively
Ketoacidosis	Increase	Positive interference with picric acid assay for creatinine

Adapted from Manjunath G et al.[43] (2001, *Postgraduate Medicine* 110: (55–62) with permission.

It has been common to use the endogenous creatinine clearance (Cl_{Cr}) or, even worse, the creatinine plasma concentration, as a surrogate parameter for renal function, although recent reports[33–35] have warned against relying on creatinine plasma data because of many pitfalls (Table 7).

Tubular secretion is one example of *cellular* export mechanisms.[36] Export proteins that have been associated with multiple drug resistance (MDR) in antineoplastic chemotherapy include P-glycoprotein and various ATP-binding cassette (ABC)-containing transporters also play a major role in (pre-systemic) drug elimination. They have been found in the kidneys, but also in brain, intestine and liver. Typical substrates are basic and lipophilic drugs with planar conformation and a molecular weight >400 Da, but other molecules, such as steroids, digoxin, leukotrienes and oxidized glutathione (GSSG), are effectively exported too. Cellular export of drugs may provide an explanation for therapeutic failure. On the other hand, inhibition of cellular drug export may significantly increase adverse reactions.[37]

MODELLING AND QSAR IN TOXICOLOGY

A recent trend in predictive toxicology is the prediction of potential toxicity of xenobiotics using computational methods.[38] An ever-growing number of chemicals used for various industrial, cosmetic and medicinal purposes may eventually be released into the environment and thus adversely affect human and environmental health. To arrive at a reasonable estimate of their toxic potential, means for 'calculating' their toxicity from structural properties are highly desirable.[39] It is beyond the scope of this overview to present the details, but some basic concepts may be outlined here.

Research objects primarily include three different areas: (a) hierarchical quantitative structure–activity relationships (QSARs) (b) quantitative molecular similarity analysis (QMSA), and (c) prediction of modes of action (MOA) of chemicals from structure.

In QSAR, one uses primarily computed parameters, for example, topostructural, topochemical, geometrical and quantum chemical indices, in a hierarchical manner. In this approach, more complex parameters are used for model building, if necessary. If computed parameters are not sufficient to develop good models, simple experimental properties may be used for QSAR development. In QMSA, computed structural indices (based on angles, bond lengths, and atom or charge distances) are used to define intermolecular similarity and intermolecular distance in structure spaces. The computed similarity scores are then used to select analogues. Properties of selected analogues are then used to estimate properties of query chemicals based on the K-nearest neighbour method.[40] Such estimated properties can be used when good QSARs are not available for a given chemical. Generally, it is better to use a QSAR model for a specific class of compound (with specific MOA) than to use any arbitrary QSAR equation blindly. This involves a two-tier approach: (a) first predict the MOA of the chemical from structure, and (b) then predict the potential toxicity of the chemical using the appropriate class-specific model.

These techniques are increasingly being used to select new or even hypothetical compounds from a bunch of similar structures and/or to predict properties of hypothetical compounds in pharmacology and toxicology.

Research agenda

- advances in toxicogenetics using microarray techniques will help in profiling and screening for abnormal metabolic patterns in patients and in the general population
- proteomics has gained momentum as a powerful approach to relevant susceptibilities or resistances. This speciality is still in its infancy and it is too early to draw important conclusions, but further research is warranted
- computational databases have to be advanced in order to better link physicochemical properties to biomedical effects. The conflict between the public interest to learn as early as possible about the profile of a new drug and the manufacturers' interest to extend patent protection and secrecy as far as possible is a political issue

SUMMARY

Toxicology is no longer *only* the science of poisons, high doses and forensic enigma. It has embraced chemical, physical, life and earth sciences, and medicine, in order to obtain answers to molecular as well as global problems. The crisis that is currently felt by activists and observers in the field alike may herald another alternation in generations.

Toxicodynamics, toxicokinetics, toxicogenetics and various modelling techniques, applied to improve risk assessment and to increase predictive power, benefit from joint efforts, with input from various disciplines. The issue is no longer to understand and explain why a certain chemical or drug exerts adverse effects but to learn how a specific biological system, including man, can and will react to exposure. Increasing

efforts are currently being devoted to make reasonable assumptions and predictions about scenarios and interactions, including xenobiotics that have yet to be discovered or invented.

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