Chapter 3

Toxicokinetics

ANDREW G. RENWICK

3.1 INTRODUCTION

The sequence between exposure to a chemical and the generation of an adverse effect can be divided into two aspects (Figure 3.1); toxicokinetics or the delivery of the compound to its site of action and toxicodynamics or the response at the site of action. This subdivision is particularly useful in risk assessment (see later).

Toxicokinetics is the study of the movement of chemicals around the body. It includes absorption (transfer from the site of administration into the general circulation), distribution (*via* the general circulation into and out of the tissues), and elimination (from the general circulation by metabolism or excretion). The term toxicokinetics has useful connotations with respect to the high doses used in toxicity studies, but it may be misleading if interpreted as the 'movement of toxicants around the body' since, as all toxicologists agree, 'all things are toxic and it is only the dose which renders a compound toxic'. Toxicodynamics relates to the processes and changes that occur in the target tissue, such as metabolic bioactivation and covalent binding, and result in an adverse effect.

Useful toxicokinetic data may be derived using a radiolabelled dose of the chemical, *i.e.* in which a proton in the molecule is replaced by a tritium atom or a carbon or sulfur atom is replaced by the radioactive equivalent (^{14}C or ^{35}S). Such studies are invaluable in following the fate of the chemical skeleton as it is transferred from the site of administration into the blood, is distributed to the tissues, and is eliminated as carbon dioxide or more likely as metabolites in air, urine, or bile. The advantage of using the radiolabelled chemical is that measured radioactivity reflects both the chemical and its metabolites, and this allows quantitative balance studies to be performed, *e.g.* to determine how much of the dose is absorbed, which organs accumulate the compound, and the pathways of metabolism. However, such simple radioactive absorption, distribution, metabolism, and excretion (ADME) studies provide only a part of the total picture, because the lack of chemical specificity in the methods does not allow an assessment of how much of the chemical is absorbed intact and how much is distributed around the body as the parent chemical. A further advantage of radiolabelling studies is that radiochromatographic methods can be invaluable in the separation and identification of metabolites, which is an important aspect of the fate of the chemical in the body. Thus, initial ADME studies define the

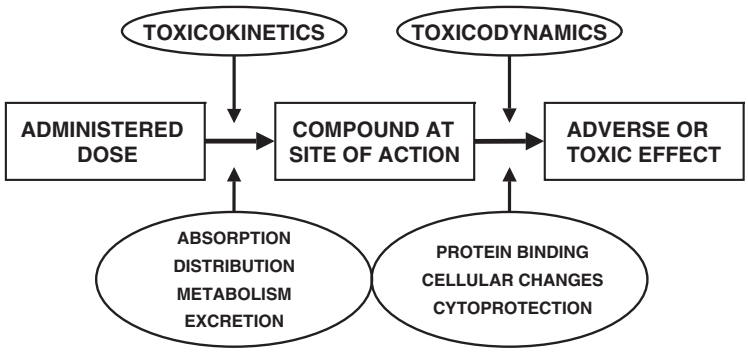

Figure 3.1 *The relationship between delivery of the administered dose to the target site and the generation of the adverse or toxic response*

overall fate of the chemical in the body and recognize the main chemical species (parent compound and/or metabolites) that are present in the circulation and in the urine and faeces following metabolism and excretion.

In recent years, it has been recognized that measurement of the circulating concentrations of the chemical and/or its metabolites can provide useful information on both the magnitude and the duration of exposure of targets for toxicity. The term toxicokinetics is sometimes restricted to studies based on measurements of blood or plasma concentrations, since these provide a vital link between the dosing of experimental animals and the amounts of the chemical in the general circulation (Figure 3.2). Such information can be of great value in the interpretation of species differences in toxic response, and in estimating the possible risk to humans of hazards identified in animal experiments. Toxicokinetic data are also useful in extrapolating across different routes of exposure or administration, as well as from single doses to chronic administration. Chemical-specific toxicokinetic measurements are essential if the results of *in vitro* toxicity tests are to be interpreted logically.

The ever increasing sensitivity of modern analytical techniques should allow the measurements of 'toxicokinetics' in humans receiving the compound at safe exposure levels. Thus, toxicokinetic differences between test animals and humans are open to direct measurement, and such data should increase confidence in the extrapolation process. In contrast, it is unethical intentionally to generate potentially adverse effects in humans and therefore data on inter-species differences in toxicodynamics are limited to observations following accidental poisonings, mild and reversible biomarkers of the potential adverse effect, and *in vitro* studies related to the mode of action of the chemical in animals.

The toxicokinetics of a chemical are determined by measuring the concentrations of the chemical in plasma (usually) or blood at various times following a single dose. The fundamental parameters that define the rates and extents of distribution and elimination are derived from data following an intravenous dose (Figure 3.2). The parameters relating to absorption from an extravascular site of administration, such as gut, lungs, *etc.*, are derived from comparisons of data following an extravascular dose with an intravenous dose. Additional useful information can be obtained from

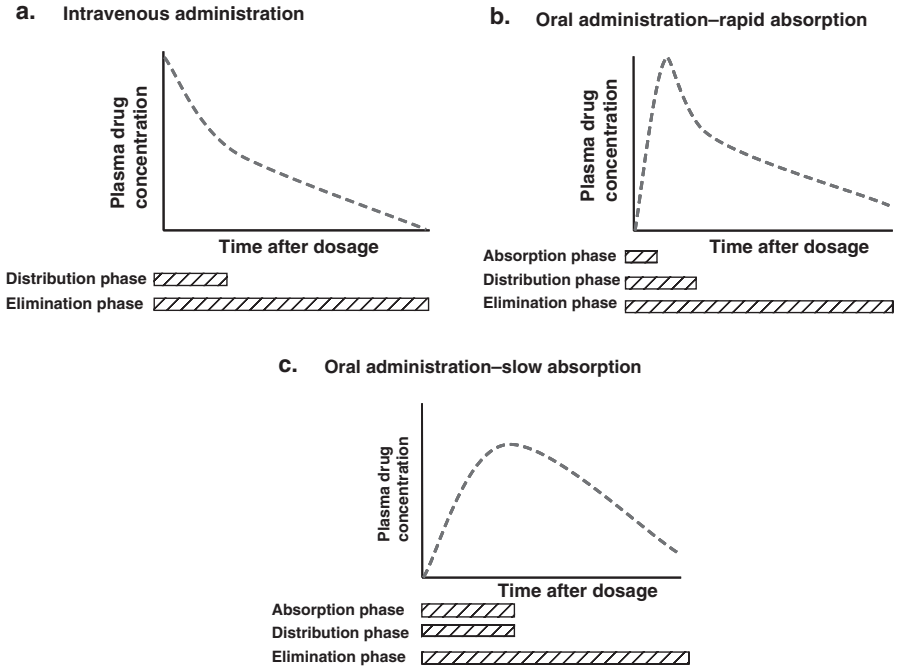

Figure 3.2 *The plasma concentration–time profiles of a chemical following intravenous and oral dosage*

measurements of the concentrations in plasma (or blood) over a period of 24 h in animals treated chronically with the chemical since the area under the plasma concentration–time curve often referred to as 'area under the curve' (AUC) is the best indication of exposure.

The interpretation of toxicokinetic data requires an understanding of both the biological basis of the processes of absorption, distribution, and elimination and the way that simple measurements of plasma or blood concentrations can be converted into useful quantitative kinetic parameters that describe these processes. The mathematics used to define and describe the movement of a chemical around the body can display various levels of sophistication and complexity. Compartmental analysis (Figure 3.3) allows the derivation of a mathematical equation which fits the data and allows the prediction of plasma concentrations at time points that were not measured directly and also outside the confines of the period of experimental observations. Physiologically based pharmacokinetic (PBPK) modelling (Figure 3.4) allows a greater interpretation of the data in biologically relevant terms but requires a sophisticated database to produce valid results. PBPK models (see below) can be used to bridge the gap between species, based on physiological differences and *in vitro* metabolic data, and extended to a biologically based dose–response model by the incorporation of *in vitro* response data.

This chapter will consider the biological basis of the processes of absorption, distribution, and elimination and describe the basic parameters, *e.g.* bioavailability, apparent volume of distribution, clearance, and half-life, which are most valuable

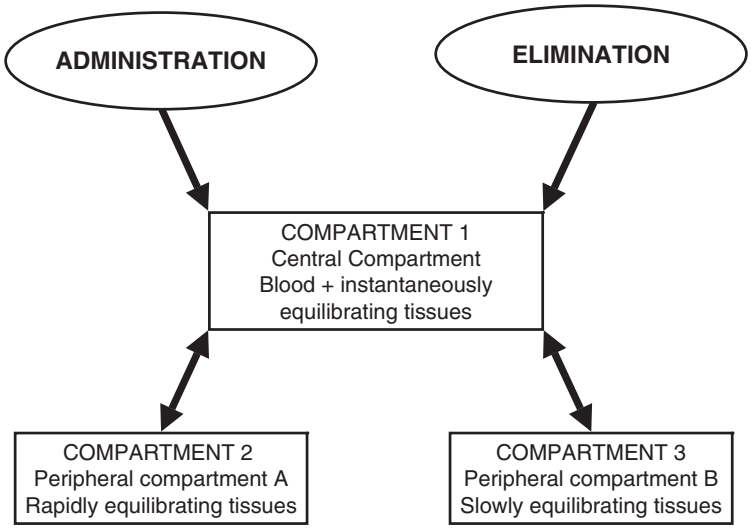

Figure 3.3 *Compartmental analysis. In the example shown, the body is considered to consist of two peripheral compartments that equilibrate with the central compartment. Strictly speaking the only property that links tissues that are part of the same "compartment" is the rate of transfer into and out of the tissue. The central compartment usually comprises blood and well-perfused tissues and equilibrates instantaneously. In the example shown, the compound is eliminated from the central compartment, for example by extraction by the liver or kidneys. The number of compartments necessary in the mathematical model fitted to the data depends on the number of exponential terms necessary to describe the plasma concentration–time curve. The mathematical model can be used to estimate the concentration in plasma or blood at any time after dosage*

because they are open to physiological interpretation. Absorption, distribution, and elimination can be considered in terms of the rate and the extent of the process.

3.2 ABSORPTION

The term absorption describes the process of the transfer of the parent chemical from the site of administration into the general circulation, and applies whenever the chemical is administered *via* an extravascular route (*i.e.* not by direct intravascular injection). The term 'absorption' is also used to describe the extent to which the radioactivity from a radiolabelled chemical is transferred from the site of administration into the excreta and/or expired air. However, many chemicals will be metabolized or transformed during their passage from the site of administration into the general circulation, so that little parent chemical may reach the general circulation, despite the fact that all of the radiolabel may leave the site of administration and be eliminated in the urine. This raises the possibility of confusion in discussing the 'extent of absorption' depending on whether the data refer to the parent chemical *per se*, or to radiolabel (which will include the chemical plus metabolites). This confusion is resolved by the proper use of the term bioavailability given below to describe the extent of absorption.

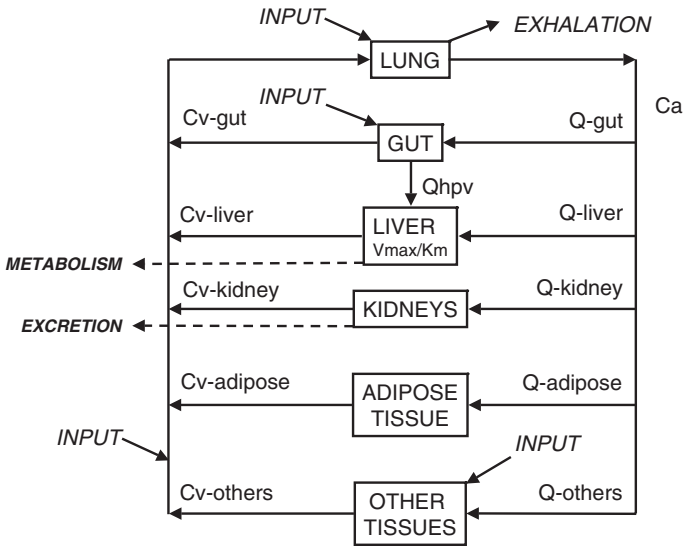

Figure 3.4 *Physiologically based pharmacokinetic model (PBPK). The PBPK model is derived from known rates of organ blood flow, the partition coefficient of the chemical between blood and the tissue, and the rates of the process of elimination, such as V_{max} and K_m for enzymes. PBPK modelling represents a powerful technique for estimating the dose delivered to specific tissues and can facilitate inter-species extrapolation by replacing animal blood flows and enzyme kinetic constants with human data. Removal across an organ equals (C_a–C_v) times the organ blood flow (Q)*

3.2.1 Rate of Absorption

The rate of absorption may be of toxicological importance because it is a major determinant of the peak plasma concentration and, therefore, the likelihood of acute toxic effects. Transfer of chemicals from the gut lumen, lungs, or skin into the general circulation involves movement across cell membranes, and simple passive diffusion of the unionized molecule down a concentration gradient is the most important mechanism. Lipid-soluble molecules tend to cross cell membranes easily and are absorbed more rapidly than water-soluble ones. The gut wall and lungs provide a large and permeable surface area and allow rapid absorption; in contrast the skin is relatively impermeable and even highly lipid-soluble chemicals can enter only slowly. The lipid solubility and rate of absorption depend on the extent of ionization of the chemical. Compounds are most absorbed from regions of the gastrointestinal tract at which they are least ionized. Weak bases are not absorbed from the stomach, but are absorbed from the duodenum which has a higher luminal pH, whereas weak acids are absorbed from the stomach. The rate of absorption can be affected by the vehicle in which the compound is given, because rapid absorption requires the establishment of a molecular solution of the chemical in the gut lumen. Extremely lipid-soluble compounds, such as dioxins, may be only partially absorbed, because they do not form a molecular solution in the aqueous phase of the intestinal contents. There are few membrane barriers to absorption following subcutaneous or intramuscular dosage, and the absorption rate may be limited by the water solubility of

the injected materials; slow absorption occurs with lipid-soluble compounds injected in an oily vehicle (which contrasts with the rapid absorption possible if such a dose is given *via* the gastrointestinal tract). Irrespective of the route of administration, the rate of absorption is determined from the early time points after dosing (Figure 3.5), and is usually described by an absorption rate constant or absorption half-life.

3.2.2 Extent of Absorption

The extent of absorption is important in determining the total body exposure or internal dose, and therefore is an important variable during chronic toxicity studies and/or chronic human exposure. The extent of absorption depends on the extent to which the chemical is transferred from the site of administration, such as the gut lumen, into the local tissue, and the extent to which it is metabolized or broken down by local tissues prior to reaching the general circulation. An additional variable affecting the extent of absorption is the rate of removal from the site of administration by other processes compared with the rate of absorption (see below).

Chemicals given *via* the gastrointestinal tract may be subject to a wide range of pH values and metabolizing enzymes in the gut lumen, gut wall, and liver before they reach the general circulation. The initial loss of chemical prior to it ever entering the blood is termed first-pass metabolism or pre-systemic metabolism; it may in some cases remove up to 100% of the administered dose so that none of the parent chemical reaches the general circulation. The intestinal lumen contains a range of hydrolytic enzymes involved in the digestion of nutrients. The gut wall can perform similar hydrolytic reactions and contains enzymes involved in oxidation, such as

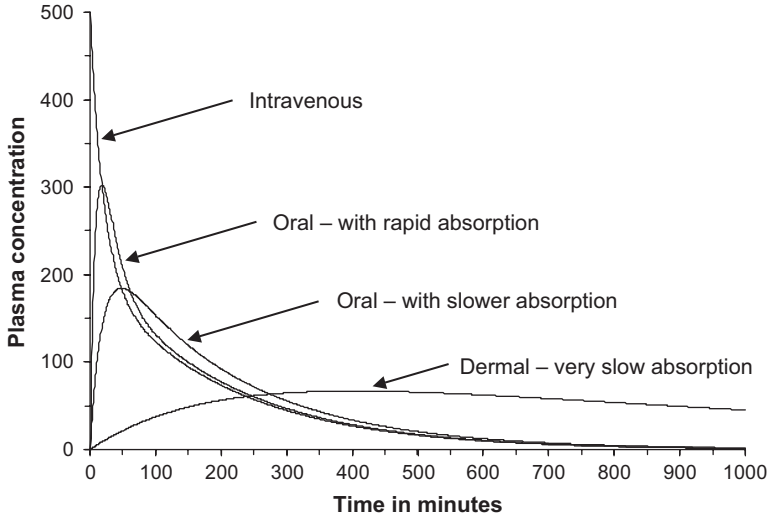

Figure 3.5 *The influence of the rate of absorption of a chemical on the plasma concentration–time curve. A relatively flat low profile is obtained when the rate of absorption is less than the rate of elimination, and this pattern is normally seen with transdermal absorption*

cytochrome P450 3A4, and conjugation of foreign chemicals. Enterocytes contain P-glycoprotein (PGP) which transports a range of absorbed complex foreign chemicals from the cytosol back into the gut lumen, which can increase the likelihood of first-pass metabolism in the gut lumen or gut wall, or incomplete absorption from the gut lumen. The portal circulation drains into the hepatic portal vein which carries compounds absorbed across the gut wall to the liver, which is the main site of foreign compound metabolism, and is responsible for most first-pass metabolism. The other main reason for incomplete absorption of the parent chemical occurs when the rate of absorption is so slow that the chemical is lost from the body before absorption is complete. Examples of this include incomplete absorption of very water-soluble chemicals from the gut and their loss in the faeces, or incomplete dermal absorption, before the chemical is removed from the skin by washing.

Irrespective of the reason that is responsible for the incomplete absorption of the chemical as the parent compound, it is essential that there is a parameter which defines the extent of transfer of the intact chemical from the site of administration into the general circulation. This parameter is the bioavailability, which is simply the fraction of the dose administered that reaches the general circulation as the parent compound. (The term bioavailability is perhaps the most misused of all kinetic parameters and is sometimes used incorrectly in a general sense as the amount available specifically to the site of toxicity.)

The fraction absorbed or bioavailability (F) is determined by comparison with intravenous (i.v.) dosing (where $F = 1$ by definition). The bioavailability can be determined from the area under the plasma concentration–time curve (AUC) of the parent compound (see Figure 3.6), or the percentage dose excreted in urine as the parent compound, *i.e.* for an oral dose:

$$F = \frac{\text{AUC oral}}{\text{AUC}} \times \frac{\text{dose i.v.}}{\text{dose oral}}$$

$$F = \frac{\%\text{ in urine as parent compound after oral dosing}}{\%\text{ in urine as parent compound after intravenous dosing}}$$

3.3 DISTRIBUTION

Distribution is the reversible transfer of the chemical between the general circulation and the tissues. Irreversible processes such as excretion, metabolism, or covalent binding are part of elimination and do not contribute to distribution parameters. The important distribution parameters relate to the rate and extent of distribution.

3.3.1 Rate of Distribution

The rate at which a chemical may enter or leave a tissue may be limited by two factors:

(i) the ability of the compound to cross cell membranes and
(ii) the blood flow to the tissues in which the chemical accumulates.

The rate of distribution of highly water-soluble compounds may be slow due to their slow transfer from plasma into body tissues such as liver and muscle; water-soluble

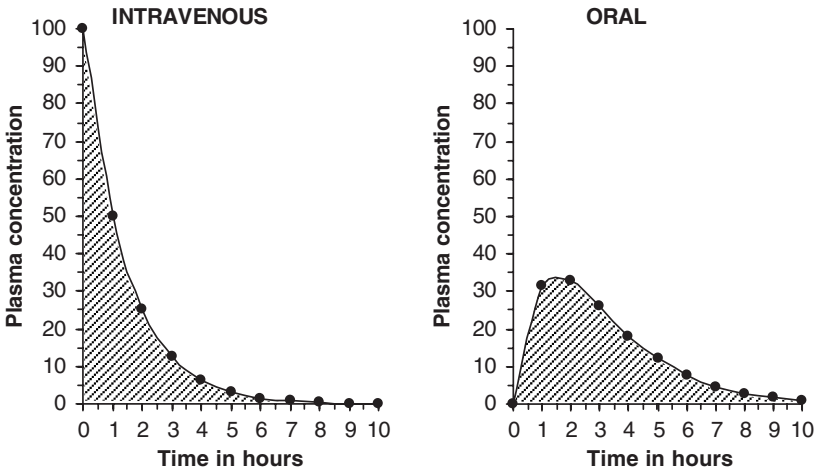

Figure 3.6 *The relationship between the area under the plasma concentration–time curve (AUC) and bioavailability. By definition, the bioavailability (fraction absorbed as the parent compound) is 1 for an intravenous dose. For other routes the bioavailability is given by the AUC for that route divided by the AUC after an intravenous dose (normalized to the same dose in mg kg^{-1})*

compounds do not accumulate in adipose tissue. In contrast, very lipid-soluble chemicals may rapidly cross cell membranes but the rate of distribution may be slow because they accumulate in adipose tissue, and their overall distribution rate may be limited by blood flow to adipose tissue.

Highly lipid-soluble chemicals may show two distribution phases: a rapid initial equilibration between blood and well perfused tissues, and a slower equilibration between blood and poorly perfused tissues (Figure 3.7). The rate of distribution is indicated by the distribution rate constant(s), which is(are) determined from the decrease in plasma concentrations in early time points after an intravenous dose. The rate constants refer to a mean rate of removal from the circulation and may not correlate with uptake into a specific tissue (for which the PBPK approach is more appropriate; see Figure 3.4). Once an equilibrium has been reached between the general circulation and a tissue, any process which lowers the blood (plasma) concentration will cause a parallel decrease in the tissue concentration (see Figure 3.8). Thus the elimination half-life measured from plasma or blood samples is also the elimination half-life from tissues.

3.3.2 Extent of Distribution

The extent of tissue distribution of a chemical depends on the relative affinity of the blood or plasma compared with the tissues. Highly water-soluble compounds that are unable to cross cell membranes readily (*e.g.* tubocurarine) are largely restricted to extracellular fluid (about 13 L per 70 kg body weight). Water-soluble compounds capable of crossing cell membranes (*e.g.* caffeine, ethanol) are largely present in total body water (about 41 L per 70 kg body weight). When one or more body tissues

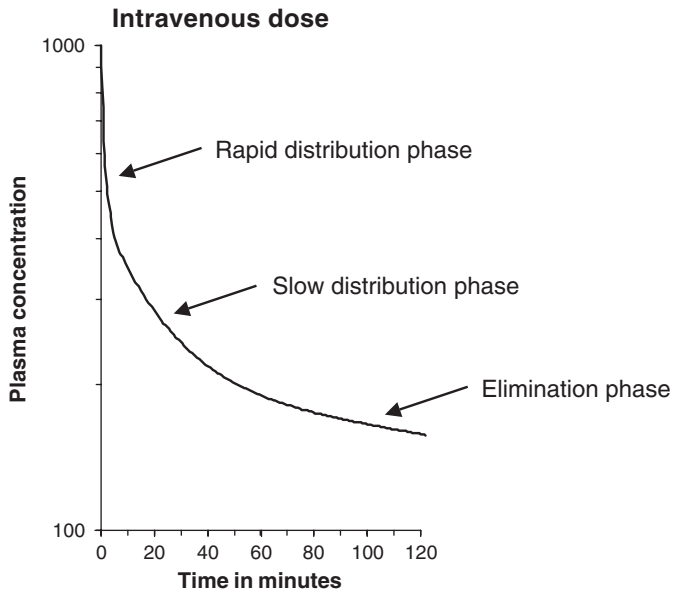

Figure 3.7 *The plasma concentration–time curve for a chemical that requires a three-compartment model (see Figure 3.3)*

has an affinity for the chemical, such as reversible tissue binding, then the blood (plasma) concentration will be lower than if the compound was evenly distributed through body water. Lipid-soluble compounds frequently show extensive uptake into tissues and may be present in the lipids of cell membranes, adipocytes, central nervous system (CNS), *etc.*; the partitioning between circulating lipoproteins and tissue constituents is complex and may result in extremely low plasma concentrations. A factor which may further complicate the plasma/tissue partitioning is that some chemicals bind reversibly to circulating proteins such as albumin (for acid molecules) and α_1-acid glycoprotein (for basic molecules).

The internal environment of the brain is controlled by the endothelial cells of the blood capillaries to the brain which have tight junctions between adjacent cells, fewer and smaller pores, little endocytosis, and the presence of transporters such as PGP which can extrude chemicals that diffuse across the blood brain barrier. In consequence, water-soluble molecules cannot 'leak' into the brain between endothelial cells (as could happen, for example, in muscle capillaries) and are excluded from the brain. The endothelial membranes have specific transporters for the uptake of essential water-soluble nutrients and some ions and also for the exclusion of organic acids. This so-called blood–brain barrier serves to exclude most water-soluble compounds, so that CNS toxicity may be limited. In contrast, lipid-soluble chemicals readily cross the blood–brain barrier and the CNS is a common site for toxicity (*e.g.* organic solvents). Similar permeability barriers are present in the choroid plexus, retina, and testes.

The extent and pattern of tissue distribution can be investigated by direct measurement of tissue concentrations in animals. Tissue concentrations cannot be

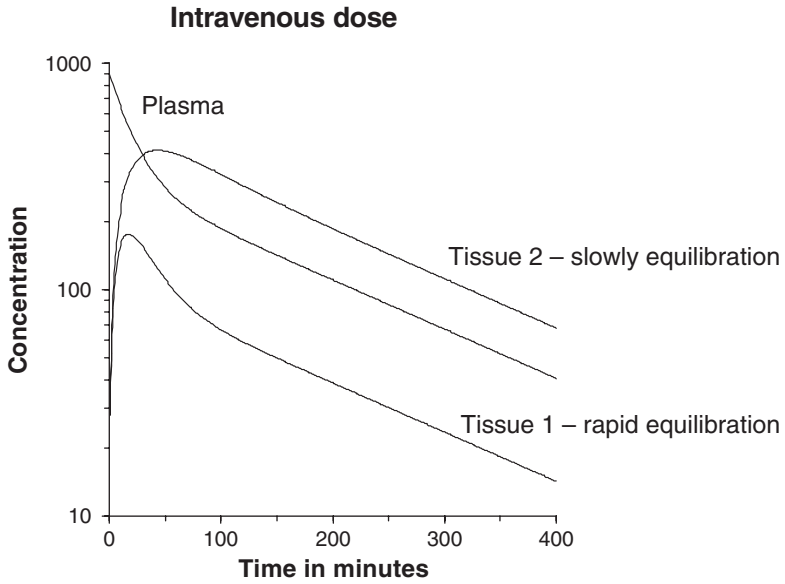

Figure 3.8 *Tissue distribution of a chemical after an intravenous bolus dose. Tissue 1 shows a greater rate of uptake and reaches equilibrium before tissue 2. Tissue 1 shows a lower affinity than tissue 2, so that the concentrations are lower. The concentrations measured in toxicokinetic studies are usually the total concentration (free + bound to proteins or present in cellular lipids) and tissue 2 may show greater tissue binding than tissue 1. The concentrations in all tissues decrease in parallel once all tissues have reached equilibrium with plasma*

measured in human studies and, therefore, the extent of distribution in humans has to be determined based solely on the concentrations remaining in plasma or blood after distribution is complete. The parameter used to reflect the extent of distribution is the apparent volume of distribution (V), which relates the total amount of the chemical in the body (Ab) to the circulating concentration (C) at any time after distribution is complete:

$$V = \frac{Ab}{C}$$

V may be regarded as the volume of plasma in which the body load appears to have been dissolved and simply represents a dilution factor. The volumes of distribution of tubocurarine and caffeine are about 13 and 41 L per 70 kg because of their restricted distribution (see above). However, when a chemical shows a more extensive reversible uptake into one or more tissues the plasma concentration will be lowered and the value of V will increase. For highly lipid-soluble chemicals, such as organochlorine pesticides, which accumulate in adipose tissue, the plasma concentration may be so low that the value of V may be many litres for each kilogram of body weight. This is not a real volume of plasma and therefore V is called the apparent volume of distribution. It is an important parameter because extensive reversible distribution into tissues, which will give a high value of V, is associated with a low

elimination rate and a long half-life (see below). It must be emphasized that the apparent volume of distribution simply reflects the extent to which the chemical has moved out of the site of measurement (the general circulation) into tissues, and it does not reflect uptake into any specific tissue(s).

Information on the uptake into specific tissues requires sampling of that specific tissue, although PBPK modelling can provide useful estimates of tissue concentrates based on *in vitro* partition coefficients and organ blood flows. Once equilibrium has been reached for a tissue, the tissue/plasma ratio will remain constant, so that as the chemical is eliminated from the plasma, the chemical will leave the tissue, maintaining the same ratio (Figure 3.8).

3.4 ELIMINATION

The parameter most commonly used to describe the rate of elimination of a chemical is the half-life (Figure 3.9). Most toxicokinetic processes are first-order reactions, *i.e.* the rate at which the process occurs is proportional to the amount of chemical present. High rates (expressed as mass/time) occur at high concentrations and the rate decreases as the concentration decreases; in consequence the decrease is an exponential curve. The usual way to analyse exponential changes is to use logarithmically transformed data which converts an exponential into a straight line. The slope of the line is the rate constant (k) for the process and the half-life for the

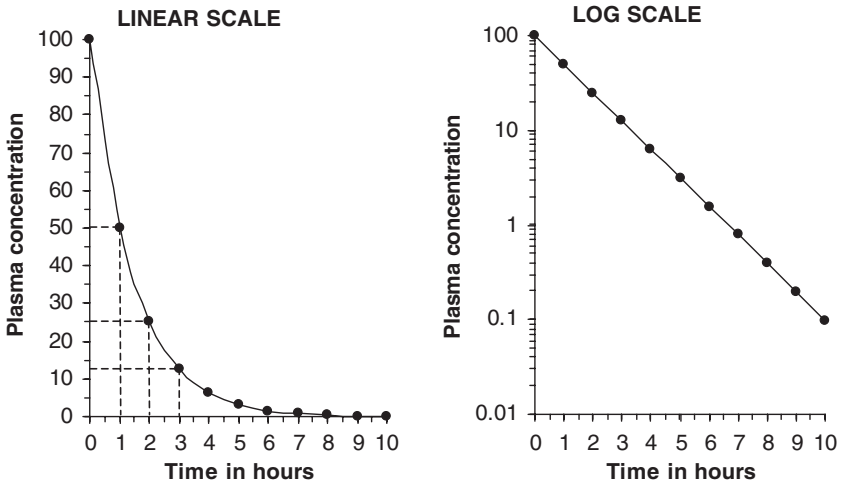

Figure 3.9 *The half-life of a chemical and its determination from plasma data. In the example in this figure the half-life is 1 h. Logarithmic conversion allows the concentration data to be fitted by linear regression analysis; the half-life is calculated as 0.693/slope. Plasma kinetic data are usually fitted by a non-linear least-squares method and there are various programmes available, such as Win-Nonlin*

process is calculated as 0.693/k. Rate constants and half-lives can be determined for absorption, distribution, and elimination processes.

There are two important biological variables that determine the rate at which a chemical can be eliminated from the body: (i) the functional capacity/ability of the organs of elimination to remove the chemical from the body (the clearance) and (ii) the extent of distribution of the chemical from the general circulation into tissues.

The clearance of a chemical is determined by the ability of the organs of elimination (*e.g.* the liver, kidney, or lungs) to extract the chemical from the plasma or blood and permanently remove it by metabolism or excretion. (Note that this is different from distribution in which the chemical is free to leave the tissue and re-enter the blood when the concentration in the general circulation decreases.)

The mechanisms of elimination depend on the chemical characteristics of the compound:

- volatile chemicals are exhaled,
- water-soluble chemicals are eliminated in the urine and/or bile and
- lipid-soluble chemicals are eliminated by metabolism to more water-soluble molecules, which are then eliminated in the urine and/or bile.

Foreign compound metabolism is an enormous subject and involves a wide range of enzyme systems. Foreign chemicals (xenobiotics) may be metabolized by the enzymes of normal intermediary metabolism, *e.g.* esterases will hydrolyse ester groups. Alternatively, chemicals may be metabolized by enzymes such as cytochrome P450, a primary function of which is xenobiotic metabolism. Species differences in metabolism can be a major source of differences in toxic response. The usual consequence of metabolism is the formation of an inactive excretory product so that species with low metabolizing ability will be likely to show greater toxicity. However, for many compounds, metabolism is a critical step in the generation of a toxic or reactive chemical entity (bioactivation), and for such compounds high rates of metabolism will be linked with greater toxicity. If a chemical undergoes metabolic activation then toxicokinetic studies should measure both the parent chemical and the active metabolite. If the metabolite is so reactive that it does not leave the tissue in which it is produced (*e.g.* alkylating metabolites of chemical carcinogens), then toxicokinetic studies should define the delivery of the parent chemical to the tissues, and the process of local activation should be regarded as part of tissue sensitivity (toxicodynamics) because it is not strictly speaking part of toxicokinetics, *i.e.* the movement of the chemical and/or metabolites around the body.

The best measure of the ability of the organs of elimination to remove the compound from the body is the clearance (CL):

$$CL = \frac{\text{rate of elimination}}{\text{plasma concentration}}$$

Because the rate of elimination is proportional to the concentration (see Figure 3.9), clearance is a constant for first-order processes and is independent of dose. It can be regarded as the volume of plasma (or blood) cleared of compound within a unit of time (*e.g.* mL min^{-1}).

Renal clearance depends on the extent of protein binding, tubular secretion and passive reabsorption in the renal tubule; it can be measured directly from the concentrations present in plasma and urine:

$$CL = \frac{\text{rate of elimination in urine}}{\text{plasma concentration}}$$

The total clearance or plasma clearance (which is the sum of all elimination processes, *i.e.* renal + metabolic, *etc.*) is possibly the most important toxicokinetic parameter. It is measured from the total amount of compound available for removal (*i.e.* an intravenous dose) and the total area under the plasma concentration–time curve (AUC) extrapolated to infinity.

$$CL = \frac{\text{Dose i.v.}}{\text{AUC i.v.}}$$

Plasma clearance reflects the overall ability of the body to remove permanently the chemical from the plasma. Plasma clearance is the parameter that is altered by factors such as enzyme induction, liver disease, kidney disease, inter-individual or inter-species differences in hepatic enzymes or in some cases organ blood flow. Once the chemical is in the general circulation, the same volume of plasma will be cleared of chemical per minute (*i.e.* the clearance value) applies irrespective of the route of delivery of chemical into the circulation. However, the bioavailability (F) will determine the proportion of the dose reaching the general circulation. Therefore, bioavailability has to be taken into account if clearance is calculated from data from a non-intravenous route (*e.g.* oral):

$$CL = \frac{\text{dose oral} \times F}{\text{AUC oral}}$$

Measurement of dose/AUC for an oral dose determines *CL/F*, which contains two potentially independent variables – the amount of chemical delivered to the blood from the site of administration and the clearance of chemical present in the blood.

The overall rate of elimination, as indicated by the terminal half-life ($t_{\frac{1}{2}}$), is dependent on two physiologically related and independent variables:

$$t_{\frac{1}{2}} = \frac{0.693V}{CL}$$

where CL is the ability to extract and remove irreversibly the compound from the general circulation, and V the extent to which the compound has left the general circulation in a reversible equilibrium with tissues.

Therefore, a chemical may have a long half-life because the organs of elimination have a low ability to remove it from plasma and/or because it is extensively distributed to body tissues and only a small proportion of the total body burden remains in the plasma and is available for elimination. Chemicals that are extremely lipid-soluble and are sequestered in adipose tissue are eliminated slowly. Lipid-soluble organochlorine compounds, which are not substrates for P450 oxidation, due to the blocking of possible sites of oxidation by chloro-substituents, are eliminated extremely slowly: for example, the half-life of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) is about 8 years in humans.

3.5 CHRONIC ADMINISTRATION

Most toxicity studies involve continuous, or chronic, administration of the chemical either *via* incorporation into the diet or by daily gavage doses. The kinetic concepts and parameters of a single dose (as discussed above) apply to chronic administration, but the exposure has to allow for the fact that not all of the previous dose(s) may have been eliminated when the subsequent dose is given. Therefore, there may be an increase in plasma concentration (and body load) until an equilibrium is reached in which the rate of elimination balances out the rate of input (Figure 3.10).

The description of clearance given above can be rewritten as

$$\text{rate of elimination } (\mu g\ d^{-1}) = CL\ (mL\ d^{-1}) \times \text{plasma concentration } (\mu g\ mL^{-1})$$

i.e. the rate of elimination (in mass per unit time) is proportional to plasma concentration. When doses of a chemical with a long half-life are given every day the low plasma concentrations from the first dose will give a low rate of elimination, such that not all of the chemical would be eliminated before the next dose is given. In consequence, the next dose will give higher concentrations (due to carryover from the first dose) and, therefore, the rate of elimination will be higher. In consequence, a greater proportion of the daily dose will be eliminated on the second day. The plasma concentrations and rates of elimination will continue to increase each day until the plasma concentrations are such that the daily dose is eliminated each day (Figure 3.10), *i.e.* an

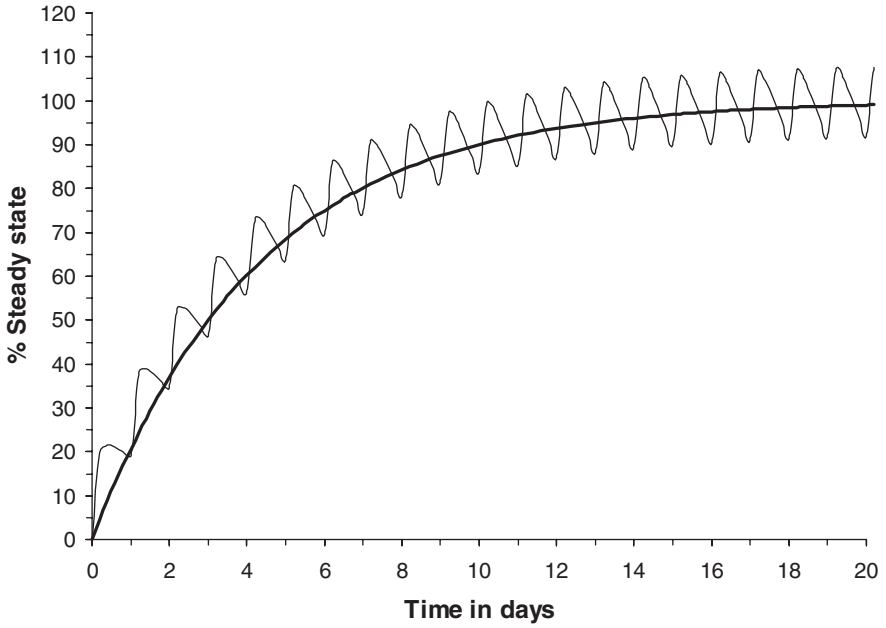

Figure 3.10 *The increase in plasma concentration following both continuous intravenous administration (thicker line) and once daily oral administration (thinner line). The chemical has a half-life of 3 days and it takes 4–5 half-lives, i.e. 12–15 days, to approach steady state (strictly speaking the true steady state is never achieved because it is an exponential increase)*

equilibrium or steady state is reached. At equilibrium the balance between input and output can be written as

$$\text{input} = \frac{\text{dose} \times F}{\text{dose interval}} = CLC_{ss} = \text{output}$$

C_{ss} is the average steady-state plasma concentration which can be calculated by

$$C_{ss} = \frac{\text{dose} \times F}{CL \times \text{dose interval}}$$

It is important to realize that clearance (CL) is the same value throughout the build-up to steady state. Once steady state has been reached,

$$CL = \frac{\text{dose} \times F}{\text{AUC for a dose interval}}$$

The equations above assume that CL is not altered by repeated exposure; the assumption is not correct if the chemical induces or inhibits its own elimination because clearance would be increased or decreased, respectively, after the period of chronic intake. The possibility that metabolism or excretion is saturated at the higher plasma concentrations during chronic intake is discussed below.

A further important toxicokinetic variable to be considered in the design and interpretation of chronic studies is the time taken to reach steady state. The extent of toxicity is usually proportional to the dose or the body load and the body load is given by the plasma concentration (at any time) multiplied by V. Because the plasma and therefore tissue concentrations increase during chronic intake until an equilibrium is reached (Figure 3.10), the amount in the body (Ab) will also increase to reach a steady state. The time taken to reach steady state is 4–5 times the elimination half-life and, therefore, the true duration of steady-state exposure in a toxicity study is the study duration minus 4–5 half-lives of the chemical. This is particularly important for chemicals that have a very long half-life; for example in rodents the steady-state body load of TCDD, which has a half-life in rats of about 1 month, will not be reached until after about 4–5 months of continuous treatment.

3.6 SATURATION KINETICS

All the parameters described above relate to first-order processes and therefore are independent of dose at low doses. However, at high doses and/or during chronic studies it is possible to overload or saturate compound–protein interactions. Under such circumstances any increase in the concentration of the compound cannot give a proportional (first-order) increase in the rate of the process. When a process is saturated the rate is at the maximum possible and is essentially independent of concentration.

In simple mathematical terms this means that the reaction changes from first to zero order. This is best described by Michaelis–Menten kinetics, *i.e.*

$$\text{rate} = \frac{V_{max} C}{K_m + C}$$

At low concentrations, C is less than the Michaelis constant K_m and, therefore, ($K_m + C$) approximates to K_m. At such low concentrations the rate equals (V_{max}/K_m) C and since V_{max} and K_m are constants the rate is proportional to the concentration (first-order).

At high saturating concentrations, C is greater than K_m and, therefore, ($K_m + C$) approximates to C. At such high concentration the rate equals ($V_{max}/C \times C$, *i.e.* V_{max}) and therefore is a fixed maximum rate (zero-order).

The consequences of this for a plasma concentration–time curve are shown in Figure 3.11. Michaelis–Menten kinetics can be included in PBPK models based on *in vitro* enzyme kinetic measurements. It is important to note that the terminal elimination half-life is always determined at low concentrations and therefore is a first-order parameter, which does not show saturation kinetics. The best parameter to reflect saturation kinetics is the *CL*, which is based on the total AUC and includes the slower zero-order elimination phase.

A classic example of this type of data is given in the studies of Dietz *et al.* (1982) on the solvent dioxane, in which saturation of metabolism resulted in a change of clearance in rats from 13.3 mL min^{-1} at 3 mg kg^{-1} to 1.0 mL min^{-1} at 1000 mg kg^{-1}. Renal tubular secretion can also be saturated, as demonstrated for cyclohexylamine in rats by Roberts and Renwick (1989), which resulted in a non-linear accumulation of the compound in the testes of rats (but not mice), which correlated with the dose–response for the testicular toxicity.

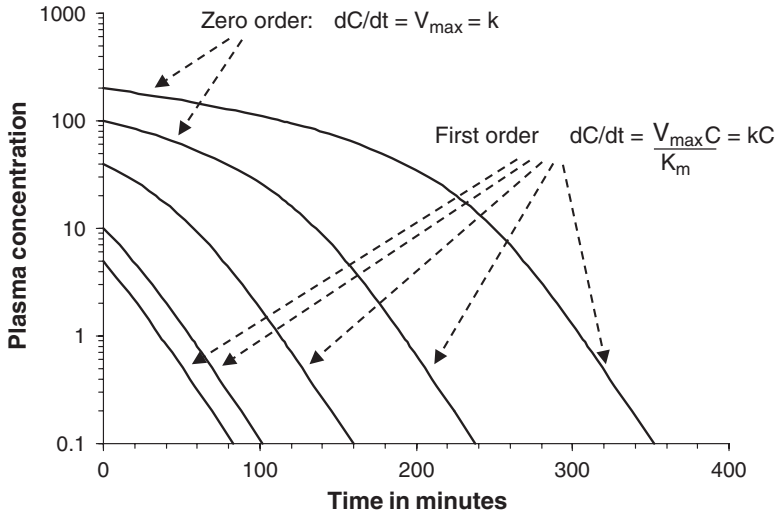

Figure 3.11 *The influence of saturation of elimination on the shape of the plasma concentration–time curve for a chemical. At low doses and low initial concentrations the decrease shows a simple exponential decrease (the example chosen represents the simplest case, i.e. a one-compartment model). At high doses high plasma levels cause saturation of the elimination process so that at very high initial concentrations the decrease is at V_{max} and is essentially independent of concentration. As the concentration deceases eventually the enzyme will no longer be saturated and the elimination will revert to a simple exponential decrease*

Important possible consequences of saturation of metabolism or excretion are that the chemical will accumulate to higher concentrations and that some normally minor alternative routes of elimination may become involved in the elimination of the chemical. Toxic effects seen at saturating doses may be of little or no relevance to lower non-saturating doses if the alternative route is a different pathway of metabolism which results in bioactivation of the chemical.

3.7 TOXICOKINETICS AND RISK ASSESSMENT

As described in the introduction, toxicokinetics is one of two aspects that link exposure to a chemical to the development of toxicity. Unlike toxicodynamics, kinetic processes can be studied ethically in humans, and this allows the potential for chemical-specific data on this aspect of inter-species differences and human variability to be taken into account is the establishment of safe human exposures, such as the acceptable daily intake (ADI). Traditionally, 10-fold uncertainty factors have been used to allow for possible species differences and human variability, and the no-observed-adverse-effect level of intake in an animal study (in mg kg^{-1} body weight) would be divided by 100 to calculate the ADI. Subdivision of each 10-fold factor into toxicokinetic and toxicodynamic aspects allows relevant chemical-specific data on toxicokinetics to replace the relevant default (see Figure 3.12). Replacement of one of the factors for kinetics or dynamics in Figure 3.12 requires an extensive database; this subdivision has been used in recent evaluations of the sweetener cyclamate, dioxins, and methylmercury.

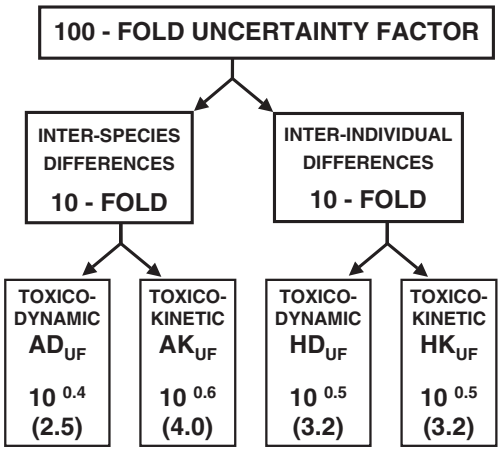

Figure 3.12 *Subdivision of the 10-fold uncertainty factors to allow for species differences and human variability in toxicokinetics or toxicodynamics (based on IPCS, 1994). The total composite factor would be the product of any chemical-specific values and the remaining default uncertainty factors that had not been replaced: for example, if the inter-species toxicokinetic UF were replaced by chemical-specific value of 6.0, then the total factor would be 2.5 × 6 × 3.2 × 3.2 = 150*

3.8 CONCLUSIONS

A common criticism of animal experiments is that they are 'not relevant' to humans. This is not true as a generalization, but there are instances where animal data are not relevant to human risk assessment owing to the nature of the target organ for toxicity or to the fate of the chemical in the body. Even when the target organ and pathways or metabolism are similar, inter-species differences in the fate of a chemical in the body complicate the interpretation of animal data in relation to human risk assessment. Information on the toxicokinetics of a chemical can provide an understanding of the extent of absorption and distribution and the pathways and rates of elimination. Such data provide a vital link between animal experiments and human safety.

BIBLIOGRAPHY

J.D. de Bethizy and J.R. Hayes, Metabolism: a determinant of toxicity, in *Principles and Methods of Toxicology*, 3rd edn, A.W. Hayes (ed), Raven Press, New York, 1994, pp. 59–100.

F.K. Dietz, W.T. Stott and J.C. Ramsey, Non-linear pharmacokinetics and their impact on toxicology: illustrated by dioxane, *Drug Metab. Rev.*, 1982, **13**, 963–981.

E.R. Garrett, Toxicokinetics, in *Toxic Substances and Human Risk*, R.G. Tardiff and J.V. Rodricks (eds), Plenum, London, 1987, pp.153–237.

J.D. Harvell and H.I. Maibach, Percutaneous absorption and inflammation in aged skin: a review, *J. Am. Acad. Dermatol.*, 1994, **31**, 1015–1021.

IPCS, *Assessing Human Health Risks of Chemicals: Derivation of Guidance Values for Health-based Exposure Limits*. Environmental Health Criteria, 170, World Health Organisation, International Programme on Chemical Safety, Geneva, 1994, 73 pp.

K. Krishnan and M.E. Andersen, Physiologically based pharmacokinetic modelling in toxicology, in *Principles and Methods of Toxicology*, 3rd edn, A.W. Hayes (ed), Raven Press, New York, 1994, pp.149–188.

J.H. Lin, Species similarities and differences in pharmacokinetics, *Drug Metab. Dispos.*, 1995, **23**, 1008–1021.

E.J. O'Flaherty, Differences in metabolism at different dose levels, in *Toxicological Risk Assessment*, Vol 1, D.B. Clayson, D. Krewski and I. Munro (eds), CRC Press, Boca Raton, FL, 1985, pp.53–91.

A.G. Renwick, Data-derived safety factors for the evaluation of food additives and environmental contaminants, *Food Addit. Contam.*, 1993, **10**, 275–305.

A.G. Renwick, Toxicokinetics, in *General and Applied Toxicology*, B. Ballantyne, T. Marrs and P. Turner (eds), 2 Vols, Macmillan, London, 1993, pp.121–151.

A.G. Renwick, Toxicokinetics – pharmacokinetics in toxicology, in *Principles and Methods of Toxicology* 3rd edn, A.W. Hayes (ed), Raven Press, New York, 1994, pp.101–147.

A. Roberts and A.G. Renwick, The pharmacokinetics and tissue concentrations of cyclohexylamine in rats and mice, *Toxicol. Appl. Pharmacol.*, 1989, **98**, 230–242.

J.A. Timbrell, Biotransformation of xenobiotics, in *General and Applied Toxicology*, B. Ballantyne, T. Marrs and P. Turner (eds), Macmillan, London, 1993, pp.89–119.

WHO, *Principles of Toxicokinetic Studies*, Environmental Health Criteria 57, World Health Organisation, Geneva, 1986.

J.R. Withey, Pharmacokinetic differences between species, in *Toxicological Risk Assessment*, Vol 1, D.B. Clayson, D. Krewski and I. Munro (eds), CRC Press, Boca Raton, FL, 1985, pp.41–52.

L. Zhang, C.M. Brett and K.M. Giacomini, Role of organic cation transporters in drug absorption and elimination, *Ann. Rev. Pharmacol. Toxicol.*, 1998, **38**, 431–460.