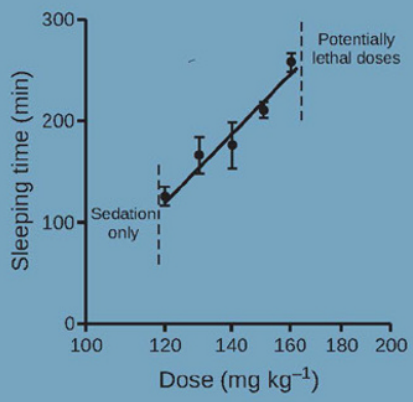
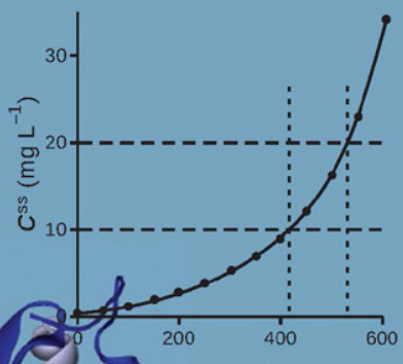
Second Edition

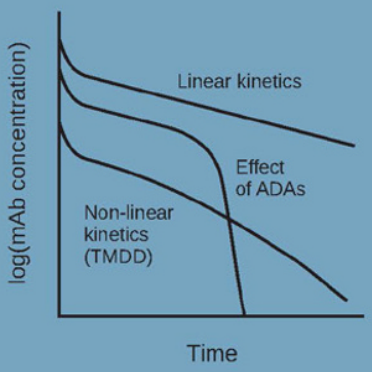
Drug Disposition and Pharmacokinetics

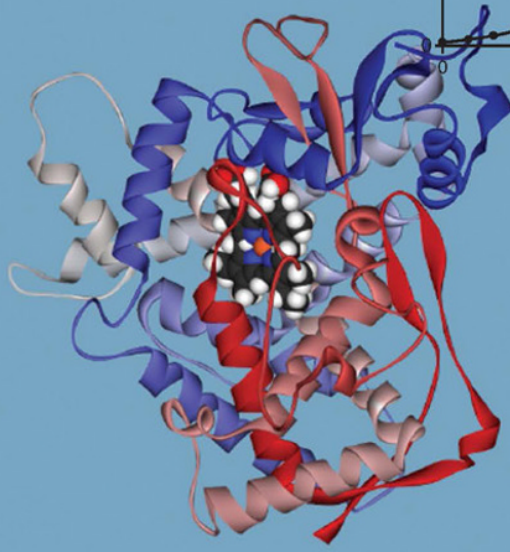
PRINCIPLES AND APPLICATIONS FOR MEDICINE, TOXICOLOGY AND BIOTECHNOLOGY

Stephen H. Curry
Robin Whelpton









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Principles and Applications for Medicine, Toxicology and Biotechnology

Second Edition

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Preface

The origins of this book can be traced back nearly half a century. The title '*Drug Disposition and Pharmacokinetics*' was devised by SHC to embrace the broad collection of processes involved with the fate of drugs in the body, and their mathematical assessment. With the subtitle '*With a consideration of pharmacological and clinical relationships*' the title was first used for a slim volume of the subject published by Blackwell in 1974, at a time when many of the drugs then in use had been introduced with little or no available scientific information concerning how long they stayed in the body after dosing, and why. The original version of the book went through three editions, plus a Japanese translation and a reprint for the Indian market, between its introduction and approximately the year 2000, before re-appearing under our joint authorship as *Drug Disposition and Pharmacokinetics: from Principles to Applications* in 2011 and, as what we consider to be a student edition, *Introduction to Drug Disposition and Pharmacokinetics* in 2017.

The title has nearly spanned the fifty years of our collaboration, starting when SHC was a Senior Lecturer and RW was a Postgraduate Student at The London Hospital Medical College. The present edition reflects the phenomenal growth in the relevant body of knowledge during this time, and the problems involved in the objective use of scientific literature highlighted by Landhuis[1], and also the broadening of the potential readership that is occurring. Many of the fundamental concepts, including several of the illustrations from that first edition remain, although some of the material has been rearranged to save space and present a more logical order. For example, rather than having its own chapter, consideration of protein binding now features largely in a newly titled Chapter 6, along with the more advanced features of clearance, and extrapolation from animals to human beings, which was the topic of a single chapter in the previous edition. In Chapter 1 we have restored discussion of bioanalysis and emphasized the fundamental growth and decay curves that are all-pervasive in the time course of drug action, something that has been a feature of the book since its inception. The chapters on drug administration, distribution and elimination of the previous edition have been combined into one, as have the chapters on elementary pharmacokinetics and more advanced models, partly to save space, but also to blur the edges between compartmental and non-compartmental approaches. The chapter on metabolite kinetics and elimination (Chapter 4) has been enlarged, and the chapters on quantitative pharmacological relationships and pharmacokinetic–pharmacodynamic modelling have been combined and brought forward to Chapter 5, which we felt was a more logical place. Thus, the first six chapters now form what could be considered as the *principles* of the subject, knowledge of which will help the appreciation of the examples of the *applications* of the topic in the remaining chapters, hopefully aided by the numerous cross-references that we have included. The growth in the clinical use of monoclonal antibodies means that these now merit a chapter of their own (Chapter 8) and new chapters on veterinary pharmacokinetics (Chapter 9) and stereochemical aspects of drug metabolism and pharmacokinetics (Chapter 15), have been introduced. The consideration of special populations, including the importance of pharmacogenetics, sex, age and disease, can be found in enlarged Chapters 10–14. As previously, considerations of drug interactions and toxicity, and the roles of therapeutic drug monitoring, are placed towards the end of the book (Chapters 16 and 17) because they illustrate all the aspects of drug disposition and pharmacokinetics that are described in the chapters that precede them.

[1] Landhuis E. Scientific literature: Information overload. Nature. 2016 Jul 21; 535: 457-8.

The final chapter is a somewhat indulgent account of a brief history of the subject, the current role of artificial intelligence and tongue-in-cheek predictions for the future.

Those wishing more background into the clinical use of the drug examples that we have chosen may find a general text such as Goodman and Gilman's *The Pharmacological Basis of Therapeutics*[2] useful in this context. We have noted the status of this now serial publication, which could be cited in every chapter, but referring to it, not in relation to specific references, but by the general citation that is here in the Preface.

In relation to history, readers may find the third edition of the original Drug Disposition and Pharmacokinetics (DDP3) interesting, for contemporaneous coverage of areas of interest that have faded in topicality as others have arisen to compete for space in books, and as the readership has evolved. It is the author's intention to make DDP3 available free to the scientific community during the year following the publication of this edition, and to that end, a submission has been made to the Open Library (openlibrary.org), which has its headquarters in San Francico, USA, for inclusion in its digitized collection online lending programme, in perpetuity.

This edition is presented in full colour, thanks to the support of John Wiley & Sons, following our adoption of colour in *Introduction to Drug Disposition and Pharmacokinetics* – something that was very much appreciated by reviewers of that volume who recognized that appropriate use of colour aids understanding and clarification of information: "*extremely effective use of color in both the text and graphs are immeasurable guides and assets for the reader*"[3]. The web site that accompanies our student edition is a further useful resource[4]. All the illustrations in that volume are presented as Powerpoint slides that can be viewed and freely used for non-commercial purposes.

We would like to express our gratitude to the students, both undergraduate and postgraduate. whom we have taught over the years, some participating in projects that generated some of the data that we have included, and others who by their astute questions required us to provide focused and comprehensible replies. Then there are the many colleagues and fellow scientists who have shaped our careers, some mentioned by name in the final chapter, and some who have made their data available for inclusion – they are acknowledged in the text, as appropriate. Thanks go to Ms J Cossham of John Wiley & Sons for her guidance and encouragement. Lastly, but most importantly, we thank our wives, Susan and Elizabeth, for their immeasurable patience.

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[2] "The Blue Bible": Goodman and Gilman's *The Pharmacological Basis of Therapeutics,* New York and London, Macmillan, thirteen editions 1941 – 2017, with various editors and contributors.

[3] Budny JA. Book review: Introduction to Drug Disposition and Pharmacokinetics. *Int J Toxicol*. 2018; 38: 76-7.

[4] https://bcs.wiley.com/he-bcs/Books?action=index&itemId=111926104X&bcsId=10602

1

Setting the Scene: Concepts, Nature of Drugs, and Quality of Results

1.1 Introduction

This book is about the time course of drug action and how that relates to concentrations of the chemicals concerned in body fluids, principally plasma. Obviously relevant are drug properties such as solubility and pK_a, properties induced by the pharmaceutical formulation, the physiological and biochemical influences such as metabolism and excretion, and the interaction of drugs and disease, because drugs both treat disease in animals and humans, and are affected differently by diseased and healthy organs. Drugs in this context include xenobiotics old and new, molecules small and large, and both natural and synthetic examples and importantly refers to the so-called 'biologicals' – products of the modern pharmaceutical industry with its focus on peptides, synthetic proteins, and oligonucleotides. It also includes certain molecules of a similar nature that are of importance because of their toxicity, while having little or no therapeutic significance.

1.2 Concepts and terminology: disposition and pharmacokinetics

Drug disposition is a collective term used to describe the processes of drug absorption, distribution, metabolism, and excretion, often given the acronym ADME, which is extended in some literature to LADME, recognizing L for liberation of a drug from its dosage form, a process that can be crucial to the successful development and use of many drugs. The importance of toxicological studies in the development of new drugs is reflected by the extension of ADME to ADMET. Promising drug candidates have been withdrawn because of unfavourable toxicological data – a process referred to as 'attrition'. A further acronym that will be encountered frequently is DMPK, drug metabolism and pharmacokinetics (PK), an area of study that clearly overlaps with ADME.

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PK is somewhat more conceptual in nature, being the study of the rates of the drug disposition processes. By subjecting the observed changes, for example, in plasma concentrations as a function of time to mathematical equations (models), pharmacokinetic parameters such as elimination half-life ($t_{½}$), volume of distribution (V), and plasma clearance (CL) can be derived. Pharmacodynamics (PD) is the study of how the drug interacts with the body to cause an effect, and increasingly models are being developed that integrate quantitatively pharmacokinetics and dynamics (PK–PD) as discussed briefly later and in subsequent chapters, particularly Chapter 5.

Drug disposition can be considered to be a 'natural' science, whilst the *mathematical* modelling employed in PK is a 'formal' science (Patel, 2013). When natural science requires measurement of a phenomenon, there will be inevitable errors of observation leading to uncertainty about the result. Furthermore, when biological systems are studied, there will be variability as a result of differences between the samples, be they *in vitro* or *in vivo* – often referred to as 'biological variation'. The variation is likely to be less in studies with laboratory animals, such as an inbred colony of rats, than in human beings with their wide diversity of genetic differences and exposure to various agents that might influence the result. Being a formal science, mathematics is expected to provide a definitive answer; however, pharmacokinetic models not only use inputs from the natural sciences but also often make assumptions, approximations, and may be only applicable under clearly defined conditions. Thus, the results of mathematical modelling of pharmacokinetic data will also have attendant degrees of uncertainty. This chapter examines some of the issues that measurement and modelling raise as these need to be controlled and any errors minimized to ensure the most reliable outcomes.

1.3 Pharmacokinetic parameters

Pharmacokinetic parameters are the variables that are used for modelling, although this may be little more than a description of the time course of a substance in the body. At a first level of approximation, there are only a few key ones that need consideration: half-life and its attendant rate constant to which it is inversely proportional (Equation 3.9), systemic clearance, apparent volume of distribution, and the fraction of an extravascular dose that reaches the systemic circulation (F, often referred to as bioavailability). These parameters are the tools of the trade and like any other tools can be misused or used inappropriately. It is of interest that these tools have moved between disciplines over time, with half-life having been used with radioactivity before it found its place in DMPK, and more recently, bioavailability has become commonly used in relation to plant feeding and pesticides, such that these terms are now commonplace in conversational language.

1.3.1 Half-life

Half-life can be defined as the time that it takes for a defined concentration of drug to fall to half that value, analogous to the use of the term in describing the time course of decay of a radioactive substance. Radioactive decay is random, and the rate of decay is directly proportional to the number of unstable nuclei. This is exponential decay, a first-order rate process, and the rate of decay can be calculated from knowledge of the decay (rate) constant, λ (Equation 3.6). The half-life of a first-order reaction is constant and can be derived from any arbitrarily chosen concentration on the decay curve (Section 3.3). Because the elimination of most drugs is exponential when they are used at therapeutic doses, it makes perfect sense to describe their elimination kinetics in terms of half-lives.

A really useful feature of half-life is that, for many people, it is much easier to visualize what is happening in terms of the changes in drug concentrations, or amount of drug in the body, as a function of time. The simplest of models is that which looks solely at the elimination of drug after administration. However, declining concentrations from an instant high concentration cannot realistically occur, because the drug has to be put there in the first place, and even after intravenous bolus doses a finite time is required for the concentration to become homogeneous throughout the plasma. The practical way to reduce this potential error is to measure plasma concentrations as a function of time and to extrapolate to zero time to obtain the intercept on the

concentration axis – this gives another very important variable, C_0, which is referred to as the initial concentration even though it is impossible to measure it directly. The half-life can then be obtained from the time it takes for the concentration to fall to $C_0/2$. This does not address potential problems of inhomogeneity of plasma, which can be an issue, particularly in behavioural studies (Section 5.4.4).

By definition, 50% of an administered intravenous bolus dose will be eliminated in the duration of one half-life, a further 25% in the next half-life, and so on, until <1% remains after seven half-lives have elapsed. As discussed later, half-life is a useful predictor of when the plasma concentrations of a drug reach their maximum during therapy with multiple doses.

A pedant may say that drugs do not 'have' half-lives, as this is not an inherent property of a drug, but arises as a result of several factors, including the chemical nature of the drug and the environment that it is in. However, under similar conditions, some drugs have longer half-lives than others, so that a drug may be chosen for clinical use or further development as drug, on the basis of its elimination half-life relative to those of other potential candidates.

1.3.1.1 Non-linear kinetics

There is nothing mystical about the fact that drugs exhibit first-order kinetics at therapeutic doses. It is reasonable to assume that the processes of elimination are not saturated by large amounts of drug, and that any enzymes and/or transporters that are involved are in such excess, relative to their substrates, that that their concentrations are not rate-limiting. Under these circumstances, the rate of elimination is determined purely by the amount/concentration of drug or xenobiotic. Even with newly developed drugs, those exhibiting first-order kinetics will have been chosen over any candidates that do not. However, it must be stressed that exponential decay is unique to first-order reactions (often referred to as linear kinetics, every other kind being non-linear) and so the concept of half-lives is of limited value with drugs such as phenytoin and ethanol that are not eliminated according to first-order kinetics at the doses that are usually administered. Furthermore, drugs that show linear kinetics at therapeutic doses often do not at higher doses when, for example, drug-metabolizing enzymes may become saturated with drug, such as in the case of drug overdose or when the drug metabolizing capacity is reduced because of disease or age.

It is important to ensure that the data show linear kinetics if first-order equations are to be used for modelling. It may not be sufficient to claim that a plot of ln(concentration) versus time can be fitted to a straight line. Preferably, it should be possible to produce a series of straight lines, all with the same slope, for different doses of drug, indicating that the half-life is independent of dose. If the lines have different slopes, for example, as in Figure 1.4 (Section 1.6.3.1), then the kinetics are non-linear, and application of linear equations will lead to erroneous conclusions. One example would be the use of area under the plasma concentration–time curve to assess ethanol absorption (Section 1.3.3).

In the clinic, it could be dangerous to use published values of half-lives when assessing and treating patients with suspected overdoses, if the half-life is markedly prolonged as a result of saturation of the elimination pathways, i.e. non-linear elimination.

1.3.2 Clearance and apparent volume of distribution

Despite the obvious simplicity of describing kinetics in terms of half-life values, they do not give an indication *per se* as to why a value is what it is or if it changes, the reasons for that change. While it is true that enzyme induction (Chapter 17) will increase the rate of metabolism of drugs that are substrates for the induced enzyme(s), the rates of metabolism and excretion are not the only determinants of the half-life. The extent to which a drug is distributed in body tissues is the other, as will be explained later. The parameter to assess the ability of the body to eliminate a drug is systemic (plasma) clearance, which is an extension of the concept of renal clearance that is used to assess the functioning of the kidneys. The renal clearance of a substance is the volume of plasma flowing through the kidneys from which the substance is removed per unit time. It is usually

calculated in units of mL min^{-1} and may be normalized to body surface area. The *rate* at which the substance is excreted via the kidneys must be the renal clearance multiplied by the concentration of drug in the plasma entering the kidney. Realizing that the plasma clearance is the sum of all the individual organ clearances (Section 3.2.2), even though it may not be possible to measure each one, provides a method of quantifying the ability of the body, as a whole, to eliminate a drug. The rate of elimination is the plasma concentration, multiplied by *CL* so that the half-life will be shorter for drugs with higher values of clearance.

The distribution of the drug within the body is a second factor that determines the half-life – the more a drug is distributed in body tissues (assessed as apparent volume of distribution, *V*), the longer the half-life. The plasma concentration for a widely distributed drug will be less than for one with a smaller value of *V*. Thus, less drug will be available to the enter organs of metabolism and excretion, the liver and the kidneys. Mathematically, the rate of elimination will be the plasma concentration, $C \times CL$. The lower the value of *C*, the lower the rate of excretion and, as a consequence, the longer the half-life. Thus, as shown in Chapter 3, the half-life is directly proportional to *V* and inversely proportional to *CL*. This does *not* mean that *V* is proportional to half-life because half-life is the dependent variable and can only change as a result of changes in *CL*, *V*, or both.

The answer to the response, 'but surely half-lives have been used to investigate enzyme induction', is yes, but under carefully controlled conditions. It was usual to use a 'test' or 'probe' drug, such as phenazone (antipyrine), that is distributed in total body water, with little or no tissue or plasma protein binding so that *V* can be expected to be constant. Therefore, any reduction in half-life must be as a result of an increase in clearance, and because over 95% of a dose of phenazone was metabolized, renal clearance was considered to be negligible. It was under these conditions that the half-life was taken to be inversely proportional to *hepatic* clearance. This assumes the enzymes for which phenazone is a substrate are induced by the drug being investigated. The alternative is to determine the clearance of the drug directly when assumptions about a second test compound such as phenazone are not required.

The original edition in the series of books of which this current book is a member had the title 'Drug Disposition and Pharmacokinetics: with a Consideration of Pharmacological and Clinical Relationships' and was published in 1974, which was before the appreciation of 'clearance-based' PK became widespread (Curry, 1974). This is not to imply that the use of half-lives has been consigned to history. Far from it, half-lives, or at least the associated rate constants, have to be determined, not only to be able to model the concentration–time data but also to estimate *CL*. So, clearance-based PK is not a newer branch of the subject, but merely an extension. Being able to tease out if the change in some phenomenon is a result of changes in *V* or *CL* is important in understanding pharmacokinetic changes in disease, age, enzyme induction, etc., as discussed throughout the book, but particularly in the later chapters.

1.3.3 Area under the plasma concentration–time curve

The area under the plasma, or blood, concentration–time curve (*AUC*) is an indicator of the amount of drug that has entered and passed through the systemic circulation and is used to assess the *exposure* of a subject to that particular drug. As a pharmacokinetic tool, it is used, amongst other uses, to assess the fraction, *F*, of an extravascular dose of drug that reaches the systemic circulation. However, *AUC* is not only directly proportional to the amount of drug absorbed but also inversely proportional to clearance and to the apparent volume of distribution, both of which reduce the plasma concentration and, hence, *AUC*. Therefore, it would be a misconception to assume that a reduction in *AUC* after an oral dose is unequivocal proof that the absorption of a drug has been affected by a particular treatment, for example, unless it is possible to demonstrate that *CL* or *V* has not been altered. It is for these reasons that bioavailability studies (Section 3.8) are carefully controlled, using cross-over studies in the same individuals in the hope that there is not a significant change in *CL* or *V* between the two occasions.

A further potential pitfall is to use *AUC* to quantify bioavailability when the elimination kinetics are nonlinear. In the case of ethanol, which displays zero-order kinetics, the *AUC* is reduced by delayed absorption (Figure 3.13), so that estimates of *F* are erroneously underestimated if *AUC* is used.

1.3.4 Apparent oral clearance

It is not always possible to administer an intravenous dose of drug, which is often the case with clinical studies, those involving children, the elderly, and the ill, for example. Such studies may not have deriving pharmacokinetic parameters as the primary objective; however, attempts to measure clearance using the same approaches as those used with intravenous doses provide a parameter known as 'apparent oral clearance' or more commonly 'oral clearance', CL_{oral}. Unfortunately, by the time original work has been cited in other publications, it is not always clear whether what was measured was CL or CL_{oral}. The dilemma arises because to calculate CL, one has to know the amount of drug that was absorbed, which requires knowing F and that can only be achieved from an intravenous study. Therefore, when comparing values of oral clearance, it is paramount that one is aware that any change might be as a result of a change in oral bioavailability. To avoid any ambiguity, we have chosen to use CL/F to indicate oral clearance, and by analogy, V/F, when a 'value' of apparent volume of distribution has been derived without an intravenous study.

1.4 Time–concentration–effect relationships

When a drug is first ingested, there will be none in the body, but the concentration in the plasma will rise as it is absorbed into the circulation. A simple way to depict this is with one of the pharmacokinetic models described in Chapter 3, as shown in Figure 1.1. The green line represents the model-predicted concentrations following, for example, oral administration of a tablet containing the drug. There is a finite lag at the beginning of the growth phase, resulting from multiple potential factors, most obviously delayed release of drug from its pharmaceutical dosage form, and delay in the medicament reaching its site of absorption (the location where it first transfers into the blood), for example, the intestine after swallowing. This model applies to intramuscular and subcutaneous as well as, most obviously, to oral doses. According to the model, drug is eliminated from the body by metabolism and excretion so that the plasma concentration continues to increase only to the point at which the rate of absorption equals the rate of elimination when the concentration is maximal, after which the concentration declines. Thus, at early times, the overriding feature is absorption, but at later times, it is mainly elimination. Note that the peak concentration does not denote the time that absorption ceases and elimination

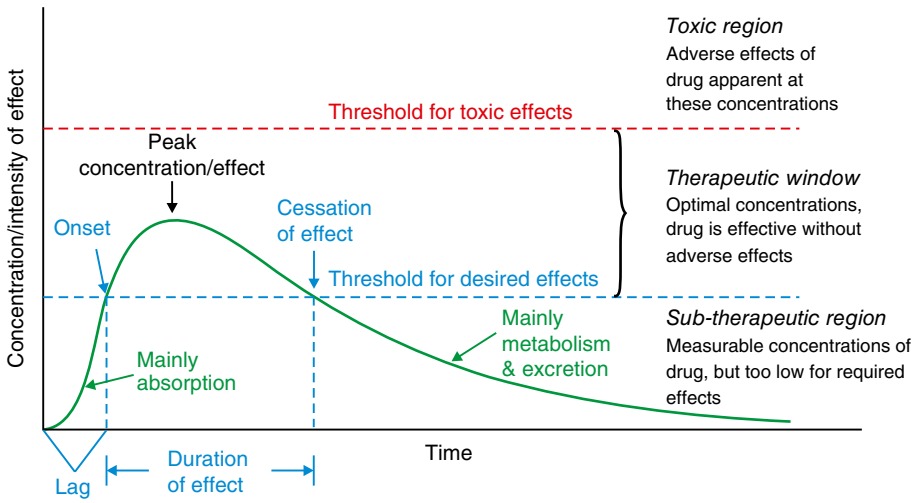

Figure 1.1 *Model curve showing a theoretical relationship between drug effect and drug concentration for a hypothetical 'ideal' drug following a single dose given orally, intramuscularly, or subcutaneously, as a function of time.*

begins – in fact the model to generate the curve assumes that absorption and elimination continue throughout (Equation 3.65).

Also represented by the curve of Figure 1.1 is the time course of a hypothetical drug whose pharmacological activity is directly proportional to the plasma concentration and whose intensity of activity peaks when the plasma concentration peaks. We know from studying concentration–effect relationships *in vitro* that there is a threshold concentration below which there are no drug effects. The same is true *in vivo* and the plasma concentration has to reach a threshold value before effects are discernible. Concentrations below the threshold concentration are in the subtherapeutic region. Above this value, the action is apparent but when the elimination has reduced the concentration to below the threshold concentration then the effect ceases. As a consequence, the duration may be considerably less than the time over which plasma concentrations are measurable. However, the duration is to some extent dependent on the threshold value. From inspection of the figure, it is clear that if the threshold were lower, the onset would be earlier and the duration longer. The same effect on the time course can be achieved by increasing the dose of drug. However, there is a limit to the size of the dose that can be administered because eventually unacceptable effects will become apparent. This introduces the concept of a threshold concentration for toxic effects, with a region between the two threshold concentrations where the therapeutic effects are optimal. This region may be given several names, *therapeutic window* being one. The concept is important in designing dosage regimens and in therapeutic monitoring (Chapter 17) when the values may define a therapeutic or reference range. Occasionally, concentrations are true target ranges, as with lithium (Section 17.3.9). Unsurprisingly, drugs with narrow therapeutic windows – digoxin, warfarin, gentamicin, and ciclosporin, for example – are more difficult to prescribe safely, but despite this, are used widely. They have been studied extensively, and feature large as examples in this book. The way to maintain the concentration of a drug in its therapeutic window is to give it as smaller doses at regular intervals (Figure 1.2). This figure contains several important features: (i) it illustrates the use of a relatively simple pharmacokinetic model (Equation 3.82) (the parameters for which can be obtained from a single dose study as depicted in Figure 1.1) to predict the plasma concentration as a function of dose and dose interval, (ii) it shows the peak and trough concentrations associated with each dose rising to maximum levels, (iii) those levels are only reached after an infinite number of doses, that is, at infinite time, and (iv) the desired effect may not occur until after several doses have been administered – particularly, for a drug with a narrow therapeutic window when the size of the individual doses must be limited to avoid toxicity.

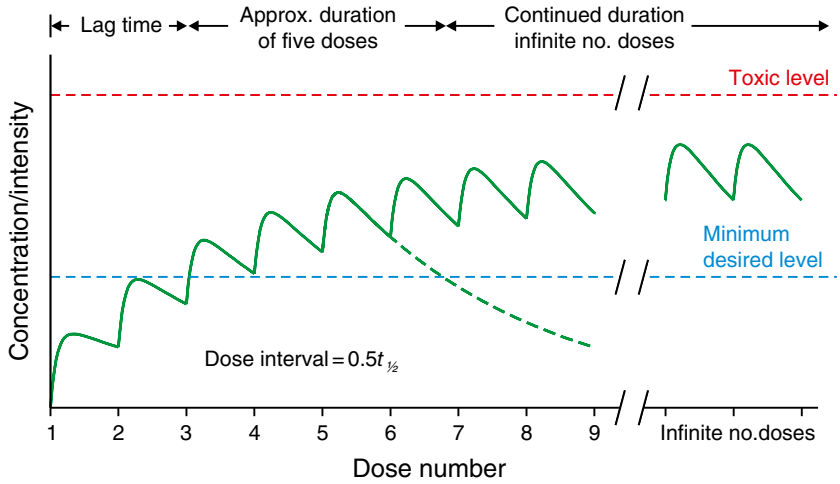

Figure 1.2 *Model curves showing a theoretical relationship between drug effect and drug concentration during a series of eight doses given with a dose interval of 0.5$t_{½}$, for an 'ideal' drug. The broken line shows the predicted time course had the fifth dose been the last one. Also shown are the concentrations after an infinite number of doses.*

The manner in which the concentrations plateau to what is called steady-state is a feature of drugs that are eliminated according to first-order kinetics. Just as the elimination half-life can be used to predict how long it will be for a defined proportion of a dose of drug to be eliminated, as discussed earlier, it can also be used to predict how long it takes to reach, say, 95% of the steady-state.

It would indeed be an 'ideal' drug that had *exactly* the same time course of concentration and effect; it would certainly make PK–PD modelling easy. However, for both pharmacokinetic and pharmacodynamic reasons such 'ideal' relationships do not exist; however, there are a few examples where the relationship is almost 'ideal' (Figure 5.11(a)). The reader is probably aware that concentration–effect relationships (particularly when investigated *in vitro*) may be modelled to derive the maximum effect that can be achieved, E_{max} and EC_{50}, the concentration at 50% of the maximum effect (Figure 5.9).

Figures 1.1 and 1.2 reflect common sense intuitive conceptual thinking from before the majority of scientists were applying mathematical equations to the patterns of plasma concentrations that characterize this subject. They reflect no particular controlling influences or mathematical models; we used models to generate the curves because that was convenient, but they would have had the same utility had they been roughly sketched by hand. They provide concepts over which it is possible to lay more sophisticated models that are especially useful in making predictions. They also address the critical requirement that DMPK studies are seen to be related to drug effects in some systematic way. They were in the backs of the minds of the scientific community when the first edition of Drug Disposition and Pharmacokinetics was published (Curry, 1974), although they first appeared in a mature form in the second edition (Curry, 1977) and subsequent editions (Curry, 1980; Curry & Whelpton, 2011). They record conceptual thinking that facilitated more rigorous studies, especially providing useful first approximations, and proving to be invaluable teaching aids since they were first introduced in print. They were not particularly original – they encapsulated established thinking – even that of lay people in regard to aspirin tablets and headache and drinking of alcohol. Variations of them have facilitated forward thinking about loading doses in multiple dose regimens, especially with antibiotics, intravenous dosing versions of Figure 1.2, administration by other routes such as via the lungs with volatile anaesthetics, administration directly to the site of action, such as with topical skin treatments, activity of drug metabolites, changes in the underlying processes such as when interacting drugs intervene, pharmaceutical formulation as a therapeutic strategy for long durations of action, interspecies differences, disease processes affecting elimination, etc., all of which are discussed in the ensuing chapters.

1.5 Properties of drugs and xenobiotics

There is little point in having a long debate into what is, and is not, a drug. Our definition is that it is a substance that is taken, or administered, to provide an effect (usually a desirable one) that is manifested as physiological, biochemical, or behavioural changes. This catch-all definition includes substances that are used for therapy, whether prescribed or bought from the pharmacy, over the counter (OTC) medications, and substances that are misused. The latter includes drugs intended for legitimate use, e.g. morphine, and substances that have been synthesized for 'recreational' purposes, such as ecstasy. So called 'designer' drugs are those that have been synthesized with the aim of circumventing legislation that makes other drugs in the same class illegal. Some of these arise from the pharmaceutical industry, being compounds that were synthesized but never marketed, or were marketed in some countries but not others. The designer benzodiazepines are a widely known example.

Drugs in current use are, for the most part, fine chemicals that are produced by medicinal chemists in the pharmaceutical industry, others are naturally occurring substances, some of which have been used for millennia, but are now purified and standardized prior to use. The complex structures of the drugs in this group mean that it is not usually commercially viable to produce them *de novo* by total synthesis. One exception is chloramphenicol (Figure 2.15), but because it contains two chiral centres (Chapter 15) chemical synthesis gives four compounds, the unwanted isomers having to be removed before use. Thus, the majority of drugs can be classed as xenobiotics (i.e. foreign to the body). However, there is a small group of pharmacologically interesting endogenous substances, for example, hormones and neurotransmitters such as hydrocortisone, insulin,

thyroxine, and epinephrine. Insulin can be genetically engineered using a laboratory strain of *Escherichia coli* bacteria to produce human insulin, rather than the previously used porcine insulin. Similarly, desmopressin is used therapeutically rather than the naturally occurring vasopressin. A further rapidly expanding group is the 'biologicals'. These are defined as substances produced from living organisms or contain components of living organisms, being derived from human, animal, or microorganisms by the use of biotechnology. The class includes vaccines, blood, blood components, cells, allergens, genes, tissues, and recombinant proteins some of which are considered in Chapter 7. Also included are the monoclonal antibodies, which because of their rapid development and widespread indications for use merit a chapter of their own (Chapter 8).

Agrochemicals, household products, and the like are considered to be xenobiotics rather than drugs. They can include almost anything that is foreign that might be introduced, either deliberately or by accident, to the body. Some may also be drugs, for example, when the insecticide malathion was applied to treat head lice. Investigational studies, which may be referred to as toxicokinetics, follow the same principles and approaches as those described for PK.

A diverse range of substances encompass what are classed as drugs. These include atoms (helium) and ions from inorganic compounds such as lithium carbonate, ferrous sulfate, magnesium sulfate, and sodium bicarbonate, but most are organic molecules of varying degrees of complexity, from simple ones (diethyl ether, ethanol) to complex proteins, oligonucleotides, vaccines, and even cells. Structures in the molecule may be recognizable as being necessary for therapeutic activity, the pharmacophore, or as being associated with toxicity, the toxicophore, or structural alert, as it is often called (Chapter 16). From a knowledge of the physicochemical properties (molecular size, pK_a, lipophilicity, etc.) and the functional groups (esters, amides, aliphatic sidechains, aromatic rings, heterocycles, etc.), it is usually possible to predict some of the DMPK properties. Furthermore, the knowledge of the functional groups in a drug and/or its metabolites is a requirement for developing suitable quantitative assays, for without them there can be no PK. It falls to the analyst to develop assays that will accurately measure the compounds of interest with the required sensitivity. Aromatic groups impart ultraviolet absorption and planar molecules are often fluorescent, characteristics that might be exploited, usually in conjunction with some form of chromatography to increase the selectivity of the assay. Flame ionization detection (FID) can be employed for most organic molecules provided that they are amenable to gas chromatography. FID lacks the sensitivity of some detectors. Electron capture detection (ECD) can be very sensitive for electron-deficient compounds. Lovelock developed the ^{63}Ni-ECD primarily to measure halogenated insecticides. Phenols and quinols are easily oxidized (sometimes annoyingly so) and may be candidates for electrochemical detection (ED). ECD and ED are potentially very sensitive to some compounds and so impart a degree of selectivity but are difficult to use and maintain over a period of time and therefore not the methods of choice for large pharmacokinetic studies or routine drug screening. Despite the high cost and need for trained operators, mass spectroscopic (MS) techniques are becoming widely used. Tandem MS (MS/MS), where selected ions from one MS are passed to a second MS, can be highly selective and, as a consequence, sensitive. Various modes of ionization and mass analysers are available so that small molecules (up to ~800 Da) can be assayed using, for example, electrospray ionization (ESI) interfaced to liquid chromatography, while large peptides and proteins can be separated using time of flight MS. Negative ion MS of electron-deficient molecules is very sensitive and has the advantage that it does not have the safety and legislative issues associated with the use of ^{63}Ni-ECD. The combination of short liquid chromatography columns with MS/MS provides selectivity, sensitivity, and high throughput and is used widely in pharmaceutical and forensic laboratories. Immunoassays may be suitable for peptides, proteins, and monoclonal antibodies, for which enzyme-multiplied immunoassay (EMIT) is commonly used.

1.6 Quality of the data

Clearly, there is a mathematical aspect of DMPK, and at first sight the equations can appear to be formidable, especially for those interested mainly in descriptive science. However, the underlying mathematics is not particularly challenging – the use of calculus, for example, barely goes beyond simple differentiation and integration.

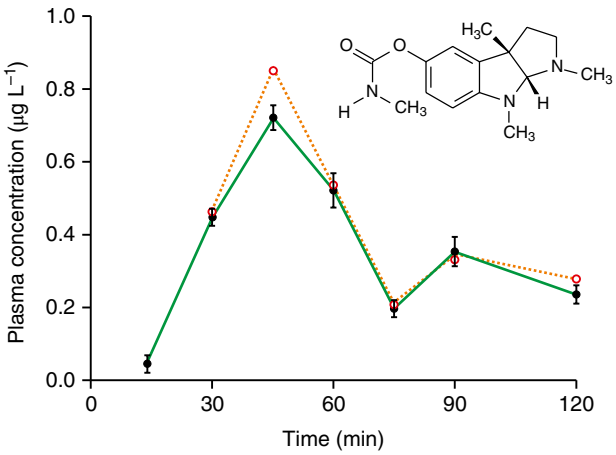

Figure 1.3 *Physostigmine plasma concentrations in a male subject given a 4 mg tablet of physostigmine salicylate. Each point is the mean of four determinations (±SD) carried out on separate days. From Hurst and Whelpton (1989) – with permission of John Wiley & Sons. The circles and broken orange line are data from a second laboratory courtesy of Ralph Brodie, FRSC.*

Also, computers are essential in 'number-crunching', and in modelling of 'what if' questions, although the concept of artificial intelligence (AI) is increasingly invoked for the purpose of extracting increasing quantities of interpretative numbers from large volumes of data. AI receives special attention in Chapter 18, alongside further information on the history of the subject. However, PK is essentially a practical subject. Concentration–time data such as those in Figure 1.3 have to be collected and the quality of the results of a pharmacokinetic investigation can be no better than the quality of those data. Thus, quality assurance (QA) is paramount, and due consideration should be given to not only to the quality of the analytical results but also the sample collection, including the accuracy of the timing of the collection, storage, and sample handling. The properties of the chemicals govern their assay methods just as much as they govern their DMPK properties. The way in which data are obtained governs every aspect of the conclusions to be drawn, and the remainder of this chapter is devoted to an appreciation of this key area of skills that are so closely associated with the core subject matter of the book.

1.6.1 Quantification of analyte concentrations

There was an almost complete dearth of pharmacokinetic information on new drugs until about 1960 onward. One of the reasons for this was the lack of suitable chemical analyses. Whatever method is chosen it should be validated to demonstrate that it is fit for purpose and uses appropriate standards and quality control (QC) samples. Such validation is normally required by the regulatory authorities when pharmacokinetic data are presented. Journals often require similar evidence of method validation before accepting pharmacokinetic data for publication.

1.6.1.1 Specificity

In the majority of cases, it is necessary to show that it is the compound under investigation that is being assayed and not interfering substances. Interferences can be from endogenous compounds in the samples or impurities in the reagents. Metabolites can pose particular problems, and it should be demonstrated that metabolites are not being quantified as parent drug. The presence of other drugs and their metabolites is yet another issue. In some instances, it may be possible to exclude the presence of other drugs in the study design, but this may not

be the case with clinical samples. Separation of drug and interfering substances may be possible during sample preparation, by chromatographic resolution and/or judicial choice of *m*/*z* ratios in MS detection. Because interferences can arise from so many sources, continued vigilance is required.

Under some circumstances, therapeutic monitoring, for example, quantification of drug and metabolite(s) combined, may be acceptable, or even required (Section 17.2.2). Usually, the clinician will understand the significance of the numbers produced.

1.6.1.2 Accuracy

The measured value of the concentration must be close to the actual value. This requires the preparation of standardized samples with 'known' concentrations that are assayed and the results compared with the notional concentrations. The required level of accuracy will normally be predetermined arbitrarily depending on the requirements of the study and inaccuracies of 10% or less may be acceptable. High-quality reference materials, which should be dried, if necessary, before use are required for the preparation of suitable standard solutions. Accurate weighing and good quality volumetric procedures should be the norm. If stock solutions are stored, then these should be checked for decomposition. The concentration range of the standard solutions should encompass the concentrations determined in the samples. Any samples with concentrations higher than the highest standard should be diluted appropriately and re-assayed.

1.6.1.3 Precision

Precision is usually accessed from repeated measurements of sample concentrations and expressed as the relative standard deviation (RSD) of the mean concentration. Acceptable values are usually chosen arbitrarily, for example, 10%, or less, for the majority of concentrations but up to 20% at the lowest quantifiable concentration. Precision values may be determined from replicate samples run in a single assay: within-batch, once known as within-day, or from several separate assay runs when it is referred to as the between-batch (between-day) RSD. The concentration range in a study may cover several orders of magnitude, and it is usual to develop assays with a limited range of RSD values. Understanding this is important when fitting time–concentration data, because if the RSD is constant throughout the range of concentrations, then it is appropriate to weight the concentration data by $1/C^2$.

1.6.1.4 Sensitivity

Sensitivity is important because for each halving of the lower limit of quantification (LLoQ), the decline in concentrations can be followed for a further half-life. The LLoQ is determined from the arbitrarily defined RSD and accuracy that have been set for the assay. Modern analytical methods with high sensitivities allow for small sample sizes, which may be advantageous in small animal, paediatric, and microdosing studies. Smaller sample sizes often mean that more samples may be taken and there is likely to be sufficient sample for replicate analyses, should this be necessary. This allows for statistical analysis of 'real' samples and can be invaluable in separating biological variation from analytical variation. Figure 1.3 shows replicate analyses of physostigmine conducted on four separate days. Inspection of the error bars that represent the between-batch standard deviations clearly show that the apparently low concentration at 75 minutes is not as a result of gross analytical error. This pattern of concentrations was obtained by a second laboratory using a different analytical technique (Brodie *et al*., 1987), confirming that the issue was not an error in the quantification of the plasma sample taken at 75 minutes, but a valid assessment of the plasma concentration in that sample. What was assumed to be a low value could have been as a result of some unknown biological phenomenon. The analyte was unstable in plasma (Figure 1.4) so inappropriate sample handling and storage prior to delivery to the respective laboratories might be the explanation. This exemplifies the need to reduce the analytical error to a minimum if subtle biological differences are to be discovered.

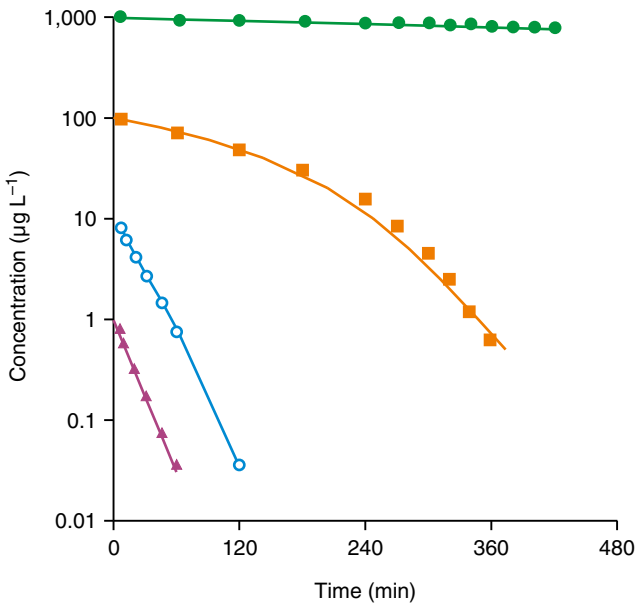

Figure 1.4 *Disappearance of physostigmine from plasma samples at room temperature (21.4 °C) as a function of time. Initial concentrations were 1,000, 100, 10, and 1 µg L^{-1}. Solid orange line calculated using: initial concentration = 100 µg L^{-1}, V_{max} = 0.55 µg L^{-1} min^{-1}, K_m = 20 µg L^{-1}. From Hurst and Whelpton (1989) – with permission of John Wiley & Sons.*

1.6.1.5 Recovery

Plasma, serum, urine, and blood are usually considered to be homogenous and when standard solutions are prepared with these materials it is usually assumed that the analyte is distributed homogeneously. Analysis of these 'spiked' materials enables an estimate of the recovery (i.e. how much is 'found' compared to the amount added to the sample). High recoveries may impart a degree of confidence – values >98% cannot vary by very much – but low recoveries need not be a problem provided that it can be shown that the variation is small and the result repeatable.

An issue that has not, and probably never will be fully resolved, is the assessment of recovery from tissues. The question remains: 'How does one introduce a standard amount of drug to a tissue so that it is distributed in exactly the same way as it is distributed when the drug was administered *in vivo*?' The best that can usually be done is to add standards to tissue homogenates or the homogenizing solution. Additionally, tissues can be exhaustively extracted until no more drug and metabolites can be recovered; for example, ^{14}C-labelled temoporfin was extracted from rat faeces with acetone–water (4+1) until no further radioactivity could be extracted. The amount of radioactivity recovered was compared with the amount of $^{14}CO_2$ released when samples of the faeces were combusted in oxygen. The recovery using acetone–water was shown to be 101 ± 4% (Whelpton *et al.*, 2002).

1.6.2 Timing of samples

Errors associated with the times when samples are taken should be small, provided that the instructions are followed. Study protocols may use sample tubes pre-labelled with collection times, possibly using bar codes so that they can be read by machine; however, procedures should be in place for whoever is collecting the sample to record the actual times if they differ from those of the protocol. Errors in timing will be more important when the drug has a short elimination half-life.

1.6.2.1 Number and spacing of samples

There is no *a priori* way of knowing the optimum times at which samples should be collected or the total number of samples that will be required. These have to be determined empirically. However, most drugs show biphasic decay, with steep declining phases in plasma concentrations initially, followed by a shallower decay in the terminal phase (Chapter 3). Therefore, samples need to be more closely spaced at early times. Fitting data to a biphasic decay (two-compartment) model following an oral dose of drug requires at least five variables, so a study design with only six samples will have only one degree of freedom, which is extremely unlikely to give a reliable statistical result. Even with more samples these must be spaced to define the rising phase, the peak, and the two phases of decline. Thus, it is considered better to have more time points to ensure better statistical fit and derivation of parameters than to assay *all* samples in duplicate, say.

1.6.2.2 Use of replicate samples

An early radioimmunoassay kit for plasma digoxin required samples to be assayed in triplicate to obtain the required precision and accuracy. However, with modern methods replicate assays are rarely required. A proportion of unknown samples may be assayed in duplicate for QC purposes with the majority of the samples being only assayed once – 'singletons'. Under these circumstances, when fitting the data only singletons should be used; for those time points where duplicates were used for QC purposes, only one of the results should be used; the one to be used having been decided in advance.

1.6.3 Quality control and method validation

Having developed or chosen an established method for analysing samples, it is necessary to demonstrate that it is appropriate and fit-for purpose. This should be done initially and then throughout its application by the use of QC samples and charts. At least one pharmacological journal requires assay details, such as precision and accuracy, quoted for the data *being presented* even if these values have been published for previous studies.

1.6.3.1 Samples and sample integrity

It is important to indicate whether plasma, serum, or blood has been sampled. The concentration values may vary depending on the distribution of the analyte between these fractions and the haematocrit. Serum is usually used for lithium assays to prevent potential contamination by lithium heparin that might have been used as the anticoagulant for preparation of plasma. Because it is normally necessary to store samples prior to analysis, method validation should include an investigation into the stability of the analyte(s) on storage. Refrigerator and freezer temperatures should be monitored to ensure that they do not exceed stipulated values. For forensic samples, in particular, records of when samples are removed and by whom should be kept to ensure that there can be no tampering with what could be evidence.

When investigating analyte stability, the concentrations tested should be in the relevant range (i.e. as expected in samples collected *in vivo*) as failure to do so may result in erroneous conclusions. Hartvig *et al.* (1986) determined the apparent half-life of physostigmine in plasma at room temperature as ~3 h. However, the initial concentration chosen (100 $\mu g\,L^{-1}$) was 2 orders of magnitude above that expected in patient samples. The loss is chiefly a result of hydrolysis by plasma esterases (Hurst & Whelpton, 1989) and at lower concentrations the *in vitro* half-life is ~15 min (Figure 1.4).

Sometimes it may be necessary to add stabilizers and these should be chosen carefully. In the case of physostigmine, blood samples were collected into tubes containing neostigmine or pyridostigmine, which were shown to prevent *in vitro* degradation of the analyte but not to interfere with the assay. The use of antioxidants may inadvertently reduce metabolites such as amine oxides and sulfoxides. Agents such as ascorbic acid and even ferrous sulfate may also demethylate drugs producing qualitative as well as quantitative errors (Curry & Evans, 1976).

1.6.3.2 Use of incurred samples

For years, some analysts have understood the value of reanalysis of 'real' samples, 'incurred' samples as they have become known, because these can differ significantly in their composition when compared with calibration and QC samples that have been used to validate the analytical method. Incurred sample reanalysis (ISR) now forms part of regulatory guidelines as a means of establishing assay reproducibility (Viswanathan, 2011). ISR can be very informative, particularly if metabolites, either known or unknown, revert to parent drug or interfere with the analysis. For a more recent commentary, see Kall *et al*. (2018).

Summary

This introductory chapter has examined the basic concepts that are required to perform satisfactory investigations into drug disposition, while at the same time addressing many commonly held misconceptions. The nature of the drug or xenobiotic is crucial to developing satisfactory analyses has been considered.

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2

Drug Disposition and Fate

2.1 Introduction

For a drug to be successful, it must have the required pharmacodynamic properties, *and* it must reach its site of action in sufficient quantities and remain there long enough to have the desired clinical effect(s). Drugs do not have an itinerary directing them to their receptors, and they move about the body by what has been referred to as random walk – even 'targeted' drugs have to do this. Drugs cross those biological membranes that they can but not those barriers that are impermeable to them and so will enter some tissues but not others. The nature of the membranes and the physicochemical properties of the drug will dictate where a particular drug may go. Initially, movement will be from the site of administration to the tissues, the drug being carried in the bloodstream or lymphatic flow. For the majority of drugs, the driving force is the concentration gradient on either side of a membrane, and as the concentration in blood falls (because of distribution and elimination), the gradient is reversed and the net flow will then be from tissues to blood.

Ideally, a drug would only penetrate tissues containing its receptors, 'on-target' tissues, but not others, the so-called 'off-target' tissues, thereby giving a degree of selectivity of action and hopefully less incidence of unwanted effects and toxicity. Thus, understanding the issues around the administration, distribution metabolism, excretion, and toxicity (ADMET) is crucial to developing a new chemical entity (NCE) into a successful drug. Much time and expense are spent on investigating the ADMET of candidate molecules. Many of these fail during development (attrition) because of unsuitable ADMET.

Drug Disposition and Pharmacokinetics: Principles and Applications for Medicine, Toxicology and Biotechnology, Second Edition. Stephen H. Curry and Robin Whelpton.

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