CHAPTER 9

Toxicokinetics

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9.1 *Introduction*

In the pharmaceutical industry, the term 'toxicokinetics' is generally used to describe the pharmacokinetics (PK) performed at the dose levels used in the toxicological risk assessment of drugs. The aim of the toxicokinetic evaluation is to define the relationship between systemic exposure to test compound and the administered dose, and to provide information on potential dose- and time-dependencies in the kinetics. Toxicokinetic studies can also aid in determining the effect of age on the PK in animals, provide clearer delineation when there are sex-related differences, determine whether there are any changes in kinetics in pregnancy (during reproductive toxicology studies) and also provide greater detail on interspecies comparisons. However, the overall aim in conducting toxicokinetics during safety studies is to extrapolate the risk assessment from the toxicity test species to humans.

Toxicokinetics is usually monitored as part of the toxicity studies, but is sometimes carried out as a separate study and is always derived from multiple doses. This chapter describes the process for conducting toxicokinetic studies and the application of the data obtained.

9.2 *Study design*

Toxicokinetic study design is well described in the ICH guidelines, note for guidance on toxicokinetics, ICH Harmonised Tripartite Guideline (1994). Multiple dose toxicokinetic studies are usually run concomitantly during the toxicity studies, and may be conducted with serial bleeds or as part of a destructive design, and in individual animals or by using a composite design. Table 9.1 shows an example of a composite design for a small animal toxicokinetic study.

The study should be designed to define the exposure of the drug during a dosing interval, and blood should be collected over a sufficient period of time to estimate the *AUC*. Single time points are not considered suitable for evaluating the toxicokinetics of a compound. There is no requirement to obtain blood samples for 'proof of absorption'. It is not necessary to perform a toxicokinetic assessment if data can be reliably extrapolated from another study.

Relatively large volumes of blood may be demanded from toxicity test animals for toxicokinetic analysis, haematology and clinical chemistry; therefore, it is often necessary to sample blood from a separate ('satellite') group housed under the same conditions (e.g. rats and mice). The blood volume in larger species like dogs is less restrictive, and rather than a satellite group, the test animals themselves are usually used for toxicokinetic bleeds. The collection of blood samples for the derivation of toxicokinetic parameters is dependent on the number of animals and the dosing regimen. For example, a preliminary MRD study (maximum repeatable dose study) may use incremental dosages in one group of animals, and due to haematological or regulatory limitations on blood sampling, it may not always be possible to obtain PK parameters at all dose levels to assess dose proportionality. Monitoring authorities generally accept that maximum blood withdrawal should not exceed 15 per cent of blood volume within a four-week period. For rats and dogs, this represents approximately 1.1–1.3 mL/100 g bodyweight. This has been tested in-house, resulting in an agreement that the haematology parameters may be more seriously affected if removal of 15 per cent blood volume is exceeded.

TABLE 9.1 *Example of a composite design for a small animal toxicokinetic study*

	Animal numbers (n=6)					
	001	**002**	**003**	**004**	**005**	**006**
Bleed times (hours) for each animal ($n = 3$ bleeds/animal)	1 24 12	2 1 24	4 2 1	8 4 2	12 8 4	24 12 8

Resulting in *AUC* of 6 time points with $n = 3$ samples per time point.

The following sampling scheme is generally recommended for toxicokinetic evaluation:

1 *After intravenous administration*:
Five to six points for the *AUC* with at least two to three time points during the elimination phase.

The sampling may be limited to three time points in subsequent studies in total if the profile proves to be monophasic and dose proportional.

2 *After oral administration*:
Five to six points for the *AUC* with at least:

- one time point during the absorption phase
- one time point at the expected T_{max}
- and two to three time points during the elimination phase.

Pre-dose samples may also be required if the period of drug circulation is longer than the dosing interval (i.e. if drug was still in the blood from the previous day's dose). Alternatively, if a 24-hour time point is selected, the concentration at this time point may be extrapolated as a pre-dose value.

3 *After intravenous infusion*:

- at least one time point during the infusion
- one time point at the end of the infusion
- at least two to three time points during the elimination phase.

The sampling may be limited to one point at steady state during a constant rate infusion.

In formal studies conducted with multiple oral doses, it is often possible to collect concentration–time profiles over a number of time points during the dosing interval, at various occasions throughout the study. These studies should be sampled on the first and last (or close to last) days of the study. In longer-term studies, the kinetics should be monitored with full *AUC* profiles at intervals during the study. For example, blood sampling is strongly recommended during long-term (3–6 months) toxicity studies and over the complete time course of a carcinogenicity study to monitor any changes in the pharmacokinetic as a result of ageing. The toxicokinetics from various studies may be combined to provide a representative picture of the relationships between the kinetics and time/age (i.e. it is not necessary to sample on the same occasions in different studies). During continuous infusion, a number of important pharmacokinetic parameters may be derived from only very few time points. Blood sampling can be minimised by means of a sparse data design resulting in reduced animal numbers with associated ethical and cost benefits.

In general, there is no requirement to evaluate systemic drug levels in animals not receiving test compound; however, this may change in future as more and more often low levels are detected in controls, and the results must be explained. It is important though to bleed control animals to maintain consistency between groups. In these cases, samples from control animals may be stored without analysis and subsequently analysed if the results of the study dictate, or as a standard procedure, only a representative few may be analysed. Exceptions may include dietary and inhalation studies where the likelihood of cross-contamination is increased, in which case all samples should be analysed. Blood sampling is also recommended to monitor the recovery phase of toxicology studies particularly if the elimination half-life of the test compound is relatively long.

Any study done directly in support of a formal safety study must be conducted in compliance with good laboratory practice (GLP). Dosing and bleeding records should be collected and referred to by the kineticist during the toxicokinetic parameter derivation, just in case the doses or bleed times deviated from the nominal protocolled values. Adequate records should be maintained to account for all blood samples taken during a study and their storage conditions. The bioanalytical method is important in underlining the integrity of the toxicokinetic evaluation. The method used to measure test compound should be validated in accordance with the FDA position paper of Shah *et al*. (1992) and a validation report should be available. The validation method should include analyte stability, specificity, precision, accuracy and sensitivity. A calibration line with appropriate limits of quantification should also be defined.

Statistical analysis of toxicokinetic data may overcome uncertainty associated with dose- or time-dependencies in the systemic exposure, or may provide information to optimise study design. A statistical analysis may be appropriate to assess dose proportionality, time-dependence, sex differences or estimate the reliability of *AUC*s obtained from a minimal sampling approach. The kinetics of drugs may become non-linear (supra- or sub-proportional) at higher dose levels. A common method of assessing dose proportionality is to dose-adjust *AUC*s and test for a constant ratio between dose levels. In some studies non-linearity may not be evident because of variability. In this case a non-linear power model may be effective.

The proportionality relationship may be written as a power function:

$$AUC = a \cdot dose^{b}$$

where, b is the proportionality constant and a the intercept. Linearisation of this relationship gives:

$$\log AUC = \log a + \log \ dose \cdot b$$

The relationship is dose proportional when $b = 1$. Confidence intervals around b can be produced to estimate plausible ranges of true values. This type of model can, therefore, provide a quantitative measure of the deviation from dose proportionality.

Depending on the metabolism of the test compound, it may also be necessary to determine the exposure and kinetics of the metabolites. Optimally, the metabolites in the pre-clinical species should be relevant for humans (i.e. the same metabolites or products of further metabolism). If there were a different metabolite or range of metabolites in humans than in the animals used in the toxicity study, the animal species may not be exposed to the appropriate compound, or desired levels of material, and the risk assessment of the metabolite not adequately evaluated. To support exposure to metabolites, sometimes it is necessary to quantify the levels of the metabolites and perform metabolite toxicokinetics, which ideally should be done in the same assay. Metabolite toxicokinetics is recommended when the test substance is a prodrug, the metabolite(s) are known to be pharmacologically active or toxic, or the toxicity of metabolite is unknown but metabolite levels are high. The toxicokinetics of the metabolite should also be assessed if the test substance undergoes rapid or extensive metabolism, in which case the metabolite may act as a surrogate for exposure to the parent compound. Metabolite toxicokinetics is also useful to help describe the kinetics of the parent drug, particularly if dose- or time-dependent kinetics is seen. However, conducting metabolite kinetics must be carefully assessed on a 'needs basis' prior to such work being conducted.

An approach which is sometimes used in toxicokinetics is that of 'population pharmacokinetics'. This approach takes into account unexplainable inter- and intra-subject effects (random effects) and measured concomitant effects (fixed effects), and such data may be subjected to mixed effect modelling. These methods employ sparse sampling from large study populations, and thus may be suitable for determining the toxicokinetics from oncogenicity studies.

9.3 *PK parameters for toxicokinetic evaluation*

PK parameters have been previously defined in the pharmacokinetics chapter of this book. The most important parameters in toxicokinetic evaluation are those which describe exposure (AUC and C_{max}) and those which may help to assess dose- and time-dependent kinetics ($t_{1/2}$ and CL). Figure 9.1 shows an example of multiple dose concentration–time profile.

As toxicokinetics is concerned with multiple doses (compared to the single dose PK parameters previously described) additional parameters may be described as follows.

9.3.1 CMAXSS, TMAXSS

These are the maximum plasma concentrations at steady state for a multiple dosing profile (C_{maxss}) or a continuous infusion (C_{ss}), or the time at which the steady state

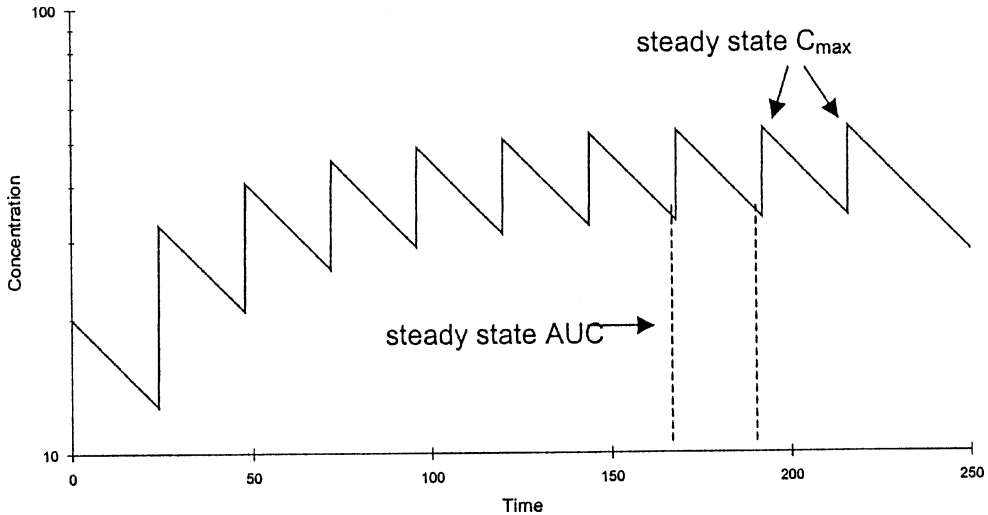

FIGURE 9.1 *Example of a multiple dose concentration–time profile.*

maximum plasma concentration was measured (T_{maxss}). These parameters are obtained directly from the concentration–time data.

9.3.2 AUC_τ

The AUC for the dosing interval (AUC_τ) after multiple dosing should be calculated using a trapezoidal method (see the pharmacokinetics chapter for more details). The log-linear trapezoidal method is recommended as time points are generally widely spaced in these types of studies, and using the linear trapezoidal rule in these cases (where concentrations have been sampled at intervals greater than the elimination half-life) can overestimate the AUC. Dosing intervals in toxicokinetic studies are usually 24 hours (a dose every day), but may be 12 hours if twice daily dosing is employed. Sometimes the intervals are irregular (e.g. 6 and 18 hours). However, it is common for the AUC for a 24-hour period to be reported, irrespective of the dosing interval.

For a continuous infusion, AUC_τ may be estimated from a single sample at steady state:

$$C_{ss} = \frac{AUC_\tau}{\tau}$$

It is important to note that AUC for the dosing interval (AUC_τ) = AUC at steady state (AUC_{ss}) = AUC extrapolated to infinity for a single dose (AUC_∞). This will aid in the interpretation of whether there are any time-dependent changes in the kinetics.

9.3.3 *R* (ACCUMULATION)

The administration of a drug on a multiple dose regimen will generally result in its accumulation. This type of accumulation is expected and is a function of the half-life of the drug and the dosing interval (τ). Generally when τ is equal to or greater than the half-life of the drug, the extent of accumulation is relatively modest (≤ 2). If τ is much less than the half-life, the extent of accumulation could be substantial. The accumulation factor (R) may be calculated (9.1) or predicted (9.2) as follows:

$$R = \frac{AUC_\tau}{AUC_t} \tag{9.1}$$

$$R = \frac{1}{1 - e^{-\lambda_z \tau}} \tag{9.2}$$

where AUC_τ is the AUC over a dosing interval at steady state, AUC_t the AUC up to time $t = \tau$ after a single dose, and λ_z the terminal elimination phase rate constant. The use of equation 9.2 assumes that each dose is administered in the post-distributive phase of the preceding dose or that the PK is monophasic.

The above equations are useful for predicting what concentrations could be achieved following multiple dosing for different dosing intervals; however, in the toxicology studies, accumulation is generally perceived as a negative factor and is often confused with increased concentrations or exposure resulting from changes in the PK of the drug following multiple dosing. These changes in PK can result in a greater than expected degree of accumulation. Changes in the PK of the drug upon multiple dosing are most easily evaluated by comparing the AUC_∞ following a single dose (e.g. on day 1 of the study) to the AUC over a dosing interval (AUC_τ) at steady state. If the PK of the drug does not change with time, then these two AUCs should not be different. It is this concept/comparison which should be emphasised in the analysis of the multiple dose studies and not the '*R*' values.

9.3.4 TIME TO STEADY STATE AND 'EFFECTIVE HALF-LIFE'

After dosing, drug is distributed and eliminated at the same time. When a drug distributes rapidly relative to its elimination, the body acts as one compartment, and it takes 3.3 half-lives to reach 90 per cent steady state. In a more slowly equilibrating pool, the body acts as two compartments, and the elimination half-life of the terminal phase determines the time to steady state. If a lot of drug is eliminated before distribution equilibrium is achieved, the elimination may appear biphasic, but the body acts as a single compartment, and it is the half-life of the initial phase that determines the time to steady state.

A basis for deciding which is the relevant half-life to estimate time to steady state rests on area considerations. The areas associated with the two phases of a biexponential curve are $C1/\lambda_1$ and $C2/\lambda_2$.

The area associated with the terminal portion of the curve is defined as:

$$f_2 = \frac{C2/\lambda_2}{C2/\lambda_1 + C2/\lambda_2}$$

where $f_1 + f_2 = 1$. If $f_2 > f_1$ (which is the normal case), then elimination half-life $= 0.693/\lambda_2$. If $f_1 > f_2$ (>than 0.8), then the terminal half-life is not appropriate to describe time to steady state.

The half-life that controls the time to steady state is the one that is involved with the predominant phase involved in the majority of the area. As a reasonable approximation:

$$\text{'Effective' half-life} = f_1 \frac{0.693}{\lambda_1} + f_2 \frac{0.693}{\lambda_2}$$

When sample time points are limited, as in toxicokinetics studies, the pre-clinical data generally show a monophasic elimination. However, from single dose definitive PK studies, the concentration–time profiles are more rigorously monitored, and often times reveal a biphasic elimination (where the terminal phase of elimination is slower than the initial phase of distribution and elimination). Consideration should be made of the extent that the terminal phase contributes to the total AUC. If not predominant, an 'effective half-life' should be estimated based on the elimination rates in the relative fractions of the AUC, and these used for any allometric scaling, rather than the terminal half-life.

9.4 *Reporting*

Toxicokinetic parameters should be clearly defined in regulatory submissions. The data should take the presentation of tabulated exposure data reflecting the relationship between the exposures (C_{max} and AUC) in animals compared to man (a clinical safety matrix). The AUC should be clearly defined (e.g. AUC_∞ or AUC_τ or AUC_t). It is recommended that the toxicokinetics be discussed in the pre-clinical PK section of submission documents and also in the toxicology section. The exposure ratios should be discussed directly in relation to the findings of the toxicity studies in the toxicology section of the document. The method of calculation of the exposure ratios should also be defined (see below). The multiple dose kinetics should be discussed, including any implications these may have on the toxicity findings. Cross referencing of the toxicokinetic data between the PK section and the toxicology section should occur throughout to guide the reviewer to the relevant information.

9.5 *Application of toxicokinetic data*

The primary use of toxicokinetic data is as a measure of systemic exposure. The safety margin is an assessment of safety in man relative to the toxicological species based on extent of systemic exposure. It is generally expressed as:

$$\frac{AUC \text{ at NOTEL or NOAEL for animals}}{AUC \text{ at therapeutic dose level for man}}$$

where NOTEL is the no-toxic-effect-dose-level and NOAEL is the no-adverse-effect-dose-level (generally related to pharmacological effects). C_{max} may be used instead of *AUC*, depending on which parameter is considered to be more relevant to the toxicological findings.

Regulatory authorities expect to see this ratio to provide comfort in the assessment of risk (i.e. the animals have been exposed to substantially more drug than in humans), and this data should be constructed into a clinical safety matrix (e.g. a table) in the submission document. There are no common recommended ratios which this comfort factor can be based on. Basically, exposure ratios must be 'suitable' for the proposed clinical trial or for the marketing application. The ratios depend on the therapeutic indication (e.g. treatment of chickenpox in children would likely require a much larger exposure ratio than a curative agent) and dosing regimen (e.g. short-term versus chronic administration). In general, the bigger the exposure ratio the better. Therefore, if exposure at a no-effect-level can be maximised, for example by increasing the mid-dose level, this should be done.

One exception where a ratio has been defined is the ICH proposed 25-fold exposure ratio (on a mg/m^2 basis) as a method for selection of the high dose in rodent carcinogenicity studies (ICH Harmonised Tripartite Guideline, 1994 and 1997). Other acceptable criteria for the selection of the high dose may be dose-limiting PD effects, maximum tolerated dose, saturation of absorption or the maximum feasible dose based on formulation. In the ICH guidelines, the justification for the 25-fold exposure ratio includes the statement 'Those pharmaceuticals tested using a 25 fold or greater *AUC* ratio for the high dose will have exposure ratios greater than 75 per cent of pharmaceuticals tested previously in carcinogenicity studies performed at the MTD'. This suggests that in 75 per cent of cases, the dose level required to reach a 25-fold AUC ratio will exceed the MTD (maximum tolerated dose), a position which is totally unacceptable. Therefore, the MTD is likely to remain the major criteria for high dose selection in carcinogenicity studies.

The exposure ratio is also applicable to extrapolating animal data to humans to aid in the design of the first human volunteer trials. Suitable doses for trial in humans are often selected by extrapolation of the pre-clinical PK/PD data to man, and then the top dose levels restricted by the exposure ratio of the NOTEL in animals compared to the predicted exposures in man.

Toxicokinetic data plays an important role in determining optimal dose levels. Apart from its direct utility in limiting dose due to supra- or sub-proportional kinetics, toxicokinetic data may also be used to refine the mid-dose, for example, when a higher exposure is desired at a no-toxic-effect level.

Toxicokinetics may also be useful in the species selection process. Typically the rat and dog are chosen for pre-clinical toxicity testing. Using sensitive mass spectrometry techniques, it is possible to compare the metabolite profiles in the species of choice, and this may be done at discovery or early development stage. Furthermore, toxicokinetics may supersede single dose studies in some species (e.g. mouse, rabbit) for the provision of pre-clinical PK data and often provide additional information to ADME studies.

Toxicokinetic data may also be used in allometric scaling to predict PK parameters in humans. This is a method of interpolation and extrapolation based on the anatomical, physiological and biochemical similarities in mammals, and has been described in more detail in the pharmacokinetics chapter of this book. The application of an equation ($y = aW^b$) may permit the extrapolation of animal data to human data and thus predict PK parameters and, together with PK/PD data, therapeutic dose levels in man. However, this author has found that scaling with toxicokinetic data may be misleading due to the limited number of time points from this type of data. The use of single dose PK data for allometric scaling has proven more successful.

9.6 *Toxicokinetic–toxicodynamic relationships*

To achieve maximal application of the toxicokinetic data, it is recommended that the relationships between toxicokinetics and toxicodynamics (TK/TD) are investigated whenever possible. Toxicodynamic parameters include organ and body weight changes, histochemistry and pathology scores. TK–TD relationship is often difficult to assess in general toxicity studies due to limited measurements, and may require a dedicated time-course study.

The TK–TD relationship may aid in the selection of candidate compounds, aid in the selection of the clinical starting dose, characterise active or toxic metabolites, elucidate time-dependent toxicokinetic alterations (e.g. induction, inhibition) and help in the assessment and utilisation of safety margins.

The following considerations should help to achieve useful TK–TD relationships:

1 Selection of the most appropriate toxicokinetic parameter AUC, C_{max}, systemic clearance for intravenous administration, cumulative AUC over complete study period.
2 Protein binding and measurement of unbound drug.

3 Tissue concentration measurements (PET scan, microdialysis).
4 Pooling of plasma concentration data across studies and different species.

Notwithstanding the difficulties associated with establishing a TK/TD model, it is recommended that systemic exposure to test substance is linked in some way to toxicity. In this context, consideration should be given to constructing an exposure–toxicity matrix in the submission, based on the most appropriate indices of systemic exposure (C_{max}, AUC), similar to, or in conjunction with, the clinical safety matrix described previously. The exposure–toxicity relationship should be evaluated across species and any differences assessed in terms of protein binding.

Importantly, toxicokinetic data helps explain unanticipated toxicity. For example, decreases in clearance due to saturation of a metabolic pathway may cause non-linear increases in the exposures and concurrent toxicity. Increases in clearance due to enzyme induction may cause decreases in exposure to the test compound and increases in exposure to a metabolite. In these cases, it may be necessary to quantify the metabolite to provide an exposure ratio to aid in the risk assessment to man. Some real examples follow.

9.7 *Dose- and time-dependencies*

Dose- or time-dependencies in the kinetics of drugs indicate that the kinetics is not linear (Figure 9.2). The term 'non-linear' kinetics most often relates to a dose- or time-dependency which causes a supra-proportional increase in exposure relative to the dose increment and are often related to saturable pathways of elimination. Dose-dependencies which result in sub-proportional increases in the exposure relative to the dose increment are often related to saturable absorption. Time-dependencies resulting in decreases in exposure with time are often related to enzyme induction.

L-N^G-monomethylarginine (LNMMA) is eliminated primarily by metabolism and subsequent putative amino acid catabolism (Schwartz *et al.*, 1997). These pathways appear to become saturated, and the exposures to LNMMA increase supra-proportionally with the dose increment in rats and dogs. This results in the kinetics becoming non-linear even at low dose levels. With these types of kinetics, there is also a tendency for the exposures to increase with time due to the saturation of the elimination pathway. Upon multiple dosing, the clearance of LNMMA eventually reaches a new steady state, which may be related to a secondary pathway of elimination (possibly renal excretion). This exemplifies a dose-dependency in the toxicokinetics of LNMMA that results in an increase in exposure with time (Table 9.2).

An example of time-dependent kinetics with opposite connotations is from a 5-lipoxegenase inhibitor programme conducted some years back, prior to the consistent subscription to toxicokinetic assessment. The metabolism of some of these compounds was induced by approximately 50 per cent during multiple dosing for as little as 5–7 days. The candidate series suffered from an inherent potential for renal

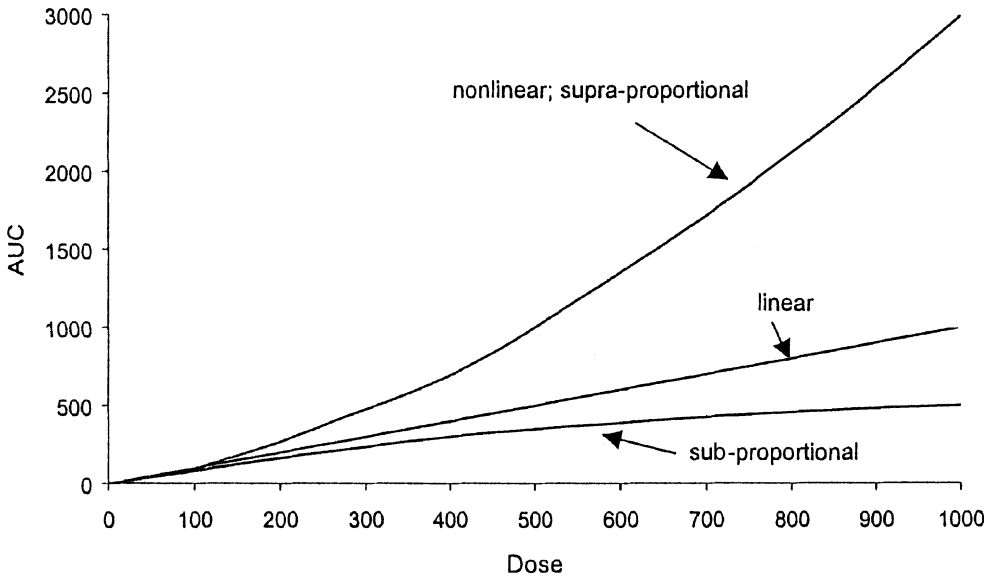

FIGURE 9.2 *Illustration of exposure relationship with dose.*

toxicity, and the successful candidates were selected based on their safety profile. When toxicokinetic data was finally generated, it was clear that the compounds which appeared to produce the best safety profile (minimal or no renal toxicity) were the greatest auto-inducers and thus the compounds which produced the lowest exposures. Indeed, if exposures were increased by incremental dosing following induction, renal toxicity was evident (Figure 9.3). The series was not developed.

Another slightly different example relates to an antiviral compound which was terminated in late phase development. There was renal toxicity associated with this compound also, but it did not manifest itself until the longer-term oncogenicity studies in rats and mice. The exposures to this compound were dose related and remained constant throughout the dosing period. The exposure to the metabolite was sub-proportional with the dose increment (exposures to the metabolite were equal at all doses irrespective of the exposure to the parent compound) during studies of up to approximately three months duration. During longer-term studies, the exposures to

TABLE 9.2 *The toxicokinetics of LNMMA following constant rate infusion; illustrating non-linear kinetics and achievement of steady state clearance*

	0.5 mg/kg/h	1 mg/kg/h	5 mg/kg/h	15 mg/kg/h
C_{ss} (μM)	12	190	630	1190
AUC_{ss} (μM h)	280	4710	15200	28600
CL (mL/h/kg)	230	56	42	67

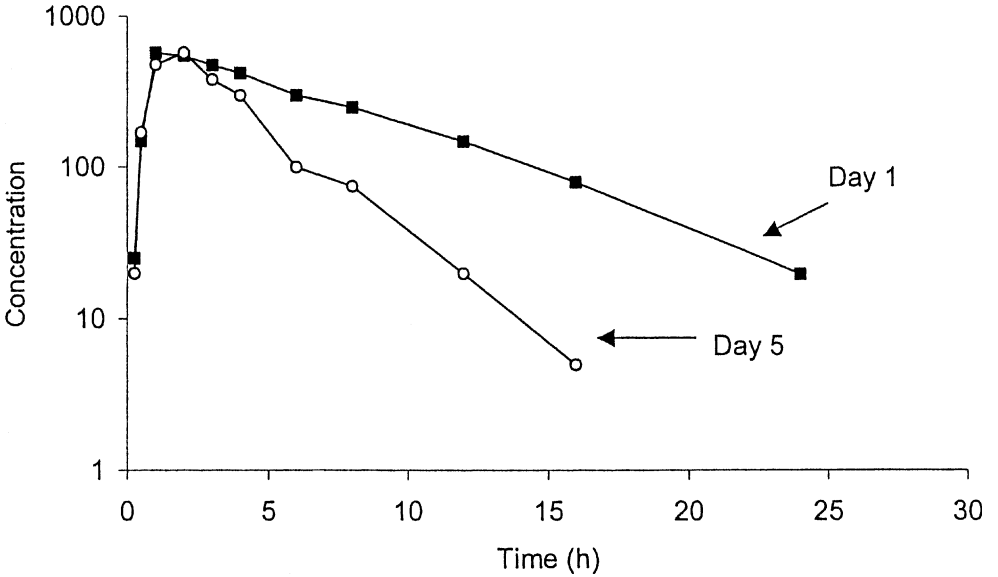

FIGURE 9.3 *Plasma concentration profile of 5-lipoxegenase inhibitor showing enzyme induction in rats.*

the metabolite increased substantially, in a dose-related fashion, without any concomitant decreases in exposure to the parent compound. It was postulated that this was possibly due to an age-related phenomenon (e.g. increased extravascular formation of the metabolite combined with a decrease in clearance of the metabolite). In any case, without the metabolite kinetics, the association between the renal toxicity and the metabolite may never have been drawn (Figures 9.4 and 9.5).

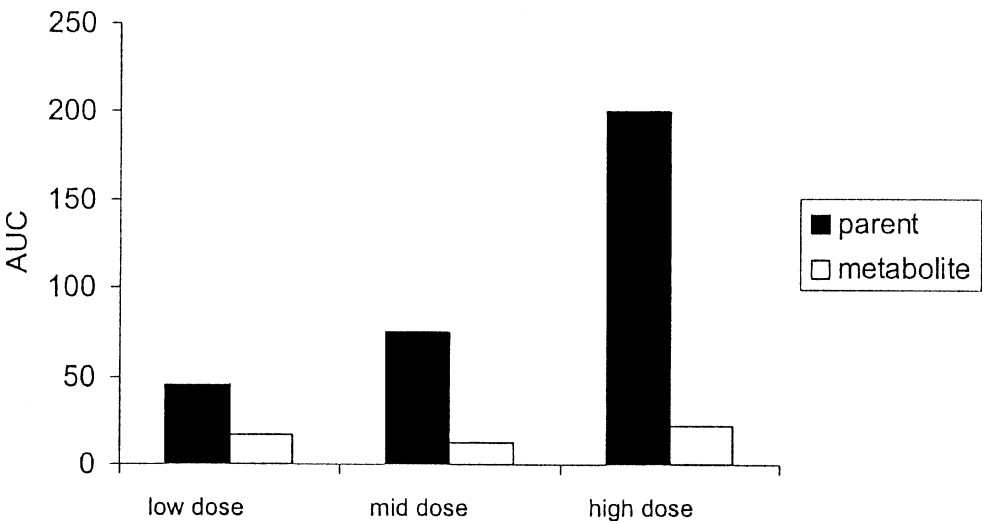

FIGURE 9.4 *Exposure versus dose relationship of an antiviral compound during a 3-month toxicity study in rats; metabolite shows sub-proportional exposure.*

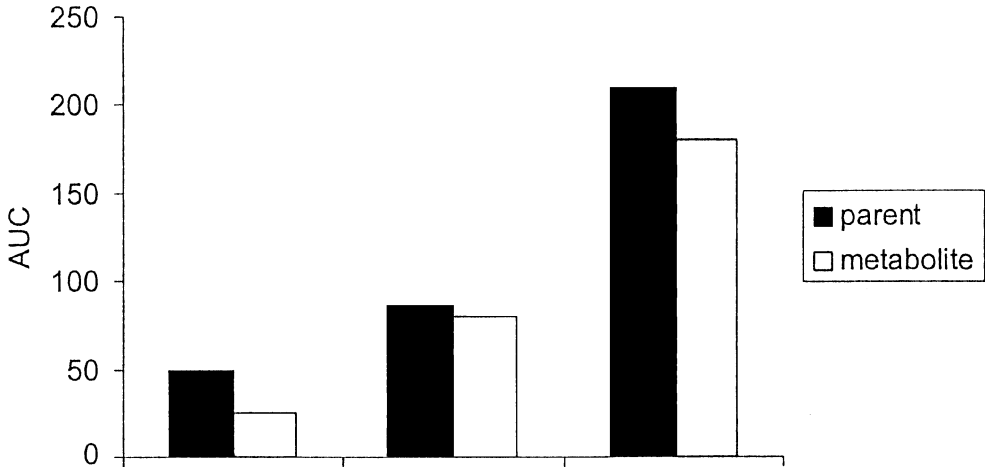

FIGURE 9.5 *Exposure versus dose relationship of an antiviral compound during a 12-month toxicity study in rats; metabolite shows dose-related increase in exposure.*

The reader may find other examples of toxicokinetic applications in the published literature. However, it is often the case that data is not published if there is failure of a developmental project due to toxicity (most often for nothing more than a lack of resource). To truly learn more about how TK and TK–TD may be applicable to the drug development process, scientists should be encouraged to publish more of their successes and failures.

9.8 *References*

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