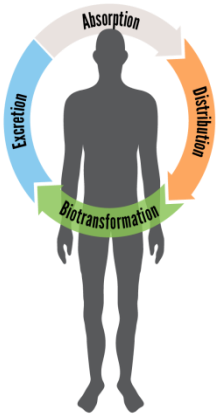
www.toxgurukul.org.in

ToxGurukul Foundation



TOXICOLOGY
NEWSLETTERS

"Toxicokinetics (TK) is a term used to describe the analysis of analyte concentration data collected during pre-clinical or nonclinical safety/toxicology studies conducted in animals. The primary goal of TK is to correlate findings of toxicity with a corresponding level of drug exposure using the primary endpoints of maximum concentration (C_{max}) and the area under the curve (AUC)."

June, 2019

Toxicokinetics in Animal Toxicology Studies

K.S. Rao M.V.Sc. Ph.D., DABT ✉ and Amol A Raje, M.V.Sc. ✉
[1]EurofinsAdvinus Limited

1. Preamble

Toxicokinetics (TK) is a composite term for the characterization of the exposure of a drug or chemical to test animal at doses which elicit a toxic response. Typically, toxicokinetic data are obtained by analysis of blood or plasma samples collected sequentially from test animals during the course of exposure of drug or chemical at different dosages. Thea toxicity of many drugs and chemicals has been well characterized for more than a century by noting their effects on the behavior and survival of test animals and by observing changes in organ function and morphology. *The additive value of toxicokinetic data derives from the fact that knowledge of the amount of drug and the manner in which it exists in the test animal during a given interval permits a more meaningful correlation between dosage and the observed toxic effects.* This additional refinement also enables us to evaluate the quantitative and qualitative significance of the toxic effects observed and their potential relationship to the concentration of drug or chemical in the blood.

Toxicology studies require toxicokinetics to check whether systemic exposure reflects the administered dose. There is a broad consensus among toxicologists in the field of drug metabolism that knowledge of toxicokinetics adds a valuable perspective to the interpretation of observations made in toxicology studies. Indeed, in many instances, toxicokinetic data contribute to the elucidation of mechanisms underlying the toxic effects produced by drugs or chemicals. *In particular, it is important to know whether the absence of toxicity at a given dose is due to the innocuousness of the compound or to its poor bioavailability.*

Editor
Alex Thomas
+91-974-545-0045
alexthomas.a@gmail.com

2. Regulatory Guidance and Significance of Toxicokinetics

The International Conference on Harmonization (ICH) has issued a global definition of toxicokinetics, describing it as *'the generation of pharmacokinetic data, either as an integral component in the conduct of non-clinical toxicity studies or in specially designed supportive studies, to assess systemic exposure'.* Overall, it is safe to say that pharmacokinetics is used as an in-depth characterization of the properties of a drug, while toxicokinetics is used in the safety assessment of a drug, usually at doses and systemic exposure of drug or chemical associated with undesired toxic effects.

Several regulatory guidelines recommend toxicokinetic measurement during toxicology studies.

- *First, there is a need to describe the systemic exposure achieved in animals, and its relationship to dose level and the time-course of the toxicity study.*
- *Second, exposure data in animals should be evaluated before human clinical trials.*
- *Third, information on the systemic exposure of animals during repeated-dose toxicity studies is essential for the interpretation of study results, to the design of subsequent studies and to the human safety assessment.*

Exposure in the animal species needs to be related to administered dose and any observed toxicity. In addition, species, sex, and inter-animal variability also need to be compared. The resulting data are used both to set plasma limits for clinical exposure (based on potential toxicity) and to calculate safety margins. Many drugs show species difference; for example, clearance of nicardipine in the plasma of rats is high compared with other species, including humans. Proxicromil causes liver toxicity (with elevated plasma levels) in the dog but not in the rat or monkey owing to their inability to metabolize the drug through hepatobiliary saturation. Gender also affects the kinetics of drug metabolism. For example, drugs, such as pentobarbital, morphine, and methadone, male rats have a much higher liver metabolism than females, resulting in lower plasma levels. *A recent survey by the Japanese Pharmaceutical Manufacturers Association compared the results from 102 repeat-dose toxicity studies (ranging from one to 12 months) in mouse, rat, dog, and monkey. Sex differences were observed in 41 out of 92 of the studies, primarily consisting of higher exposure in female rats. Exposure, as observed from plasma levels, increased in 35 out of 96 studies and decreased in 14 out of 96 studies.*

3. Typical Study Design for Assessment of Toxicokinetics

Ina typical repeat dose toxicity study, TK is an integral part of the study. TK animals are a satellite (subset) group or animals which are distinct from the accompanying main toxicology animals in the repeat dose toxicity study protocol.

Animals The same strain and species as in the toxicology studies should be used in the TK studies. Young healthy adults (10-12 weeks of age, matched for body weight) should be used. Sufficient animals should be included in the TK study design to guarantee a minimum of three data samples per time point of analysis.

Chemical Radiolabeled chemical is not required although it can be used if available

Route of exposure The same route in the toxicology study or the most common route of human exposure should be used.

Dose levels The same doses used or anticipated in the toxicity studies should be used. A minimum of three doses is recommended. Three dose levels may be sufficient to demonstrate dose proportionality; however, additional doses may be needed to more accurately estimate where nonlinear kinetics occur.

Samples For an IV administration, samples should be collected at multiple (7 to 8) time points. For example, if the plasma concentration of a chemical after a specified dose reaches the limit of detection of the analytical method at approximately 24hr, samples might be collected at 5, 15, 30, and 60 min and 2, 4, 8, and 24 hr after dosing. Rats can be sampled from the tail vein, implanted jugular cannula, or by puncture of retro-orbital plexus. For non-iv routes of exposure, blood samples (plasma or serum) should be collected at multiple time points (8-10) sufficient to characterize the chemical concentration in plasma or other tissues versus time profiles. Sampling interval and times will depend on the rate of absorption and clearance of the chemical.

Following is a typical study design of a repeat dose toxicity study with the inclusion of satellite TK rats:

Repeat Dose Toxicity and Toxicokinetic(TK) Study

Dose group	Dose levels (mg/kg/day)	Number of Animals		
		Main study group	Recovery group	Toxicokinetic Satellite group*
Vehicle control	0	10M/10F	5M/5F	3M/3F
Low	TBD**	10M/10F	-	9M/9F
Mid	TBD**	10M/10F	-	9M/9F
High	TBD**	10M/10F	5M/5F	9M/9F

Toxicokinetic animals will have for blood sample analysis and will not be evaluated with the main study animals. TBD - To be determined*

Toxicokinetics

8 sampling time points (sparse sampling on rotation) on Day 1 and last treatment day from 3 animals/ sex/ group for each time point Parameters: C_{max}, T_{max}, $T_{1/2}$ $AUC_{0\text{-}24h}$ and $AUC_{0\_infinity}$

Blood samples for bioanalysis (BA) towards toxicokinetic (TK) assessment drawn on **Day 1 of treatment**

Treatment Group	No. of Rats/ group/sex	Total no. of Rats per group	No. of rats bleeding per time point (M + F combined)								
			Sample Collection Times (Hours)								
			T 1	T2	T 3	T4	T5	T6	T7	T8	Total samples
Control	3	6	6			6		-	-	-	12
Low Dose	9	18	-	6	6	6	6	6	6	6	42
Middle Dose	9	18	-	6	6	6	6	6	6	6	42
High Dose	9	18	-	6	6	6	6	6	6	6	42
Total samples		-	6	18	18	24	18	18	18	18	138

Blood samples for bioanalysis (BA) towards toxicokinetic (TK) assessment
Last Day of Treatment:

Treatment Group	No.of Rats/ group/sex	Total no. of Rats per group	No. of bleeding per time point (M + F combined)								
			Sample Collection Times (Hours)								
			T1	T2	T3	T4	T5	T6	T7	T8	Total samples
Control	3	6	6	-	-	6	-	-	-	-	12
Low Dose	9	18	6	6	6	6	6	6	6	6	48
Middle Dose	9	18	6	6	6	6	6	6	6	6	48
High Dose	9	18	6	6	6	6	6	6	6	6	48
Total Samples	-	-	24	18	18	24	18	18	18	18	156

TK in Non-Rodent (dogs, monkeys, etc): In non-rodent studies, generally, main toxicology animals are used for TK blood collection. All other features remain the same with the exception of the number of animals which will be in the range of 3 to 5 per group per sex.

TK Parameters: The following parameters are usually measured:

a. *Maximum plasma concentration (C_{max})*
b. *Area Under Curve (AUC) of plasma concentration versus time*
c. *Time to reach maximum plasma concentration (T_{max})*

d. Time to 50% plasma level ($t_{1/2}$; often, this parameter is not measured in routine toxicity studies owing to limited datasets).

4. Bioanalysis of Plasma Samples For Analytes

a. **Analytical Method for the Analyte in Plasma:** If the study is a GLP, it is mandatory to develop and validate the method of analysis under GLP conditions, unless in rare cases where it is not possible to do so, then one can claim an exemption to the GLP (which should be rare).

b. **Analyte(s):** Typically, the parent compound is monitored. However, if one expects a metabolite (toxic or non-toxic) formation, it is mandatory to analyze for the metabolite(s) in addition to the parent compound. In this way, you have covered for human clinical trials, if humans do show the same metabolite(s).

c. **Analyses** Blood/plasma samples should be assayed for the administered compound. *For some chemicals (e.g., esters that are rapidly hydrolyzed), a major metabolite must be measured instead of the parent chemical*. The minimal design almost completely ignores the issue of metabolism and identification of the toxic agent (if presumed different than the parent chemical). This design is purposely modest in scale. In most cases, parameters obtained from the disposition or toxicokinetic studies provide some indication of the processes involved. *In general, a chemical that is readily absorbed in the rat, it is assumed that it may also readily absorbed in humans, though the rate of absorption may not be identical.* Additional prior knowledge about a chemical could further change this proposed design. *For instance, the study of a chemical known to undergo significant enterohepatic recirculation may call for the use of bile duct-cannulated animals*

d. **The analytical methods** to be used in toxicokinetic studies should be specific for the entity to be measured and of adequate accuracy and precision. The limit of quantification should be adequate for the measurement of the range of concentrations anticipated to occur in the generation of the toxicokinetic data.

e. **The toxicokinetic analysis** *identifies the level of test item/article exposure which elicits an adverse event in animals.* Most short and long-term toxicity studies include ‘main study animals’ which are used to determine potential adverse effects, plus ‘satellite animals’ for toxicokinetics. *Direct biological comparison of exposure and adverse events in the same animal is limited by the volume of blood required for analysis – typically around 200µl per time point*.

f. For small molecules, bioanalytical methods exist that allow drugs to be measured in blood samples of less than 50µl per time point. This provides the opportunity to take micro samples of blood from the main study group without the need for satellite animals, giving scientific as well as 3Rs benefits. *Removing the need for specific groups of rodents for the sole purpose of toxicokinetic represents the single biggest opportunity to reduce the use of animals in regulatory toxicology studies – providing up to a 55% reduction for some studies.*

g. **Dried Blood Spot Method**: There is also growing interest in the use of dried blood spot technology combined with high-performance liquid chromatography-mass spectrometry (HPLC–MS). *This technology permits high-quality TK information to be obtained using significantly smaller volumes of blood that are traditionally required, avoiding or reducing the need for satellite animals in the case of rats and mice, respectively.*

5. Toxicokinetic Data Analysis

*Toxicokinetic data is analyzed by either non-compartmental or compartmental model analysi*s. Although these compartments usually have no physiological or anatomical relevance to the body. It is to assume that the rate of transfer between compartments and the rate of drug elimination from compartments follow first-order or linear kinetics.

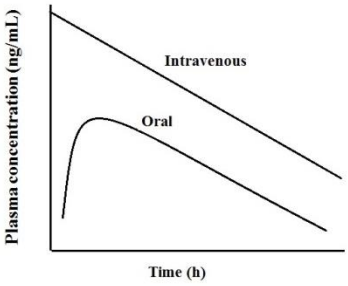
Compartment models are of two types- one compartment and two compartment/multi-compartment models.

The one compartment model is the simplest model which considers the body as a single, kinetically homogenous unit.

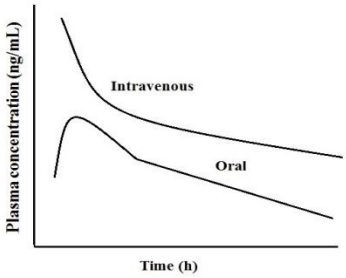
This model is useful for pharmacokinetic/toxicokinetic analysis of test item/ article which distributes (equilibrates) instantaneously throughout the body. In this model, plasma/serum is anatomical reference compartment. It is assumed that the rate of change of test item/article concentration in plasma/serum quantitatively reflects the change of concentration of test item/article throughout the body. This means if we observe 50% decrease in test item/article concentration over a certain period of time in plasma/serum, we assume test item/article concentration in kidney, liver, cerebrospinal fluid and other body fluids and tissues also decreases by 50% during this time. Kinetic homogeneity does not necessarily mean that test item/article concentration in all tissues of the central compartment at any given time is same. However, it assumes that any change that occurs in plasma levels of test item/article quantitatively reflects a change that occurs in central compartment tissue levels.

The two-compartment model applies to the test item/article which does not instantaneously distribute (equilibrate) between the blood and those other body fluids or tissues which it eventually reaches. This means the distribution of test item/article in blood and other soft tissues (highly perfused) occurs at a different rate than those into other deep tissues (poorly perfused /peripheral). The body fluids or tissues which are in equilibrium with the circulatory system comprise the central compartment. Those body fluids and tissues in which test item/article distributes (equilibrates) slowly comprise the peripheral compartment. In two-compartment model, an initial rapid decline indicates the distributive phase and later phase is post distributive phase (elimination phase).

One compartment model (IV and PO)



Two compartment model (IV and PO)



The decision on selection of compartment model:

Selecting the correct compartment model is essential to understand the true pharmacokinetic or toxicokinetic properties of test item/article. There are many methods reported in the literature for selecting the compartment model, but most commonly used methods are as follows-

1. Visual inspection of plasma concentration-time profile on semi-log plot:

Plot concentration-time data in semi-log plot and calculate the terminal slope. Draw a straight line through terminal points of the plot. This terminal line is then back-extrapolated to the ordinate. If no concentration point lies above the line, then it means the first compartment model is applicable. If initial concentration lies above the line, it may follow two-compartment model.

2. AUC method:

Calculate the AUC of points, which are above the back-extrapolated terminal line. If this calculated AUC is less than 5% of total AUC, then it follows the first compartment model and if it more than 5%, then it may follow two-compartment model.

Sometimes, the data points are scattered or insufficient due to blood withdrawal limitation, in such scenario, the selection of right model is difficult. Selection of right model is utmost important if the wrong model is selected, obtained PK/TK parameters might not reflect true PK/TK profile which in turn will not serve the purpose of the PK/TK study.

Hence, to avoid this, non-compartmental analysis is widely used during pre-clinical pharmacokinetic/toxicokinetic data analysis. Non-compartmental analysis (NCA) does not require the assumption of specific compartmental model. The NCA uses the trapezoidal rule for measurement of area-under plasma concentration curve (AUC). Additionally, the selection of the model depends on the specific objective of the study protocol. If the primary requirement of the study is to determine the rate and extent of exposure (AUC)following administration of a test item/article and associated pharmacokinetic parameters, such as clearance, elimination half-life, T_{max}, C_{max}, etc., then NCA is generally the preferred methodology to use, as it requires few assumptions compared to model-based approaches. In general, toxicokinetic

data is analyzed by using NCA model.

Phoenix WinNonlin a software developed by Certara Inc. USA is widely used to calculate pharmacokinetic/toxicokinetic data in the pharmaceutical industry.

While analyzing the toxicokinetic data using Phoenix WinNonlin, make sure you have clearly defined the following points-

- Enter the plasma concentrations data in Phoenix WinNonlin worksheet with respect to its animal ID, treatment group ID, gender, day, time point, concentration and analyte (in case of multiple analytes eg. Parent and metabolites) as per the study plan.
- Ensure the unit of plasma concentration and time is correctly captured in the worksheet as per bioanalytical data.
- Make sure that plasma concentrations below quantification limit are appropriately entered as "BLQ/BQL/Zero" as per the study plan.
- Consider using the nominal time of blood sample collection if there is not too much deviation in sample collection. If there is a deviation in blood sample collection, then use the actual time of blood collection.
- Select an appropriate model type of matrix to be analyzed (eg. Plasma, urine, drug effect, etc.)
- Make sure that right dosing option is selected (extravascular, IV bolus, IV infusion) and define the dose normalization pattern i.e. none, kg, g, mg or m2) and fix the unit of dose (eg. mg, g, µmoles, International units (IU), etc).
- Select the appropriate calculation method for NCA as defined in study plan i.e. linear log trapezoidal, linear trapezoidal -linear interpolation, linear up-log down or linear trapezoidal-linear interpolation.
- Select the right blood sampling design used i.e. sparse sampling or serial. If it is sparse sampling, then click on the option of sparse sampling with uniform weighing.
- Make sure to enter the dosing time and dosing interval details in dosing tab.
- Select the "best fit" option for slope selection or select the terminal time points (minimum three-time points) in elimination phase (by excluding the Cmax time point and regression coefficient r2> 0.80 for calculation of elimination rate constant (Kel)). If r2 is less than 0.80 then do not report the elimination half-life, AUCinf, clearance, and volume of distribution as these parameters are dependent on elimination rate constant.

6. Factors to Consider in Measuring Toxicokinetics

Following major factors need to be considered when measuring toxicokinetic and in interpreting the results:

a. Unbound drug versus bound drug. Unbound drug in plasma is the most relevant indirect measure of tissue concentration. It has been reported that similar exposure of a drug was noted at a low toxic level in rat, dog and monkey compared to humans, using the area under the curve (AUC) measurements of the total drug (i.e. with no safety margin). However,

unbound drug exposure was >20-fold the safety margin.

b. Exposure-based on active entity and not based on salt (assume dissociation to the active form occurs in the blood).
c. Racemate versus enantiomer analyte. A method for chiral conversion might be needed early in development.
d. Non-linear dose kinetics; for example, increased exposure owing to saturation of a clearance process or a long plasma half-life, or decreased exposure owing to autoinduction of metabolizing enzymes.
e. Parent (always) versus metabolite(s) (rarer) analysis.
f. Pro-drugs, where the metabolite is the active moiety (e.g. cyclosporin, enalapril, levodopa, and cyclophosphamide).
g. The drug is metabolized to pharmacological or toxicological metabolites that contribute to the overall response.
h. When extensive metabolism occurs, and the measurement of a major metabolite is the only measure for estimating exposure.
i. Human metabolites not found in animal studies.

7. Setting Doses in Toxicity Studies Based on Toxicokinetics

Historically, high doses have been included in toxicology studies to maximize the potential for identifying adverse effects in target organs to support hazard identification, and the concept of the maximum tolerated dose (MTD) became well established. *For pharmaceuticals, a limit dose of 1000 mg/kg body weight (BW)/day has been proposed as a top dose in the absence of a demonstrable MTD.*

It is important to avoid 'false negative' results through the selection of doses that are too low and to ensure an adequate margin between test dose(s) and likely human exposure. *However, it is also important to avoid 'false positives' from the use of excessively high doses that may have little or no relevance to human safety*. The objective of repeat dose studies should be more than just hazard identification; there is also a need to ensure they will be informative for risk assessment.

The use of excessively high doses can result in saturation of TK processes, resulting in systemic exposure to either parent compound or metabolites that no longer increases with increasing dose or a systemic exposure that increases massively with an only small incremental increase in dose. Dose-dependent transitions in mechanisms of toxicity and/or alteration of TK processes at such high doses may lead to other adverse effects that have no relevance to the much lower levels to which humans are likely to be exposed. *Excessively high doses and associated non-linear TK may generate data of questionable value and relevance for human health risk assessment.*

Following aspects of TK data need to be considered:

a. *If saturation of TK processes occurs and the dose–AUC relationship deviates from linearity above a certain dose, the dose level corresponding to the inflection point can be regarded as the Kinetically Derived Maximum (KMD) dose*, and selection of this as the maximum dose should be considered.

The selection of a high dose in repeat dose studies at the inflection point of nonlinear TK behavior, i.e. a 'kinetically derived maximum dose' (KMD), has been suggested as preferable to selection of a MTD or even to a dose causing toxicity, provided there is an adequate margin between test dose and predicted human exposures and the TK processes in the test species are relevant to humans. This concept is also acknowledged in the OECD test guideline for the extended1-generation reproductive toxicity study (OECD, 2011)as well as the draft OECD guidance document on the design and conduct of chronic toxicity and carcinogenicity studies (OECD, 2010). *Indeed, the draft guidance on chronic toxicity and carcinogenicity studies notes that 'although top dose selection based on the identification of inflection points in TK nonlinearity may result in study designs that fail to identify traditional target organ or body weight effects,* it must be appreciated metabolic saturation, in fact, represents an equivalent indicator of biological stress.

In addition to supporting selection of high doses, TK information can be useful in demonstrating that the doses selected produce a range of systemic exposure levels to the test compound and/or its metabolites. It can also confirm that the low dose results in adequate systemic exposure.

Consideration of TK parameters during dose selection can thus help to ensure that the doses tested are of most relevance for assessing risks in humans, refine animal use by avoiding testing at inappropriately high doses, and reduce further unnecessary animal use.

8. Toxicokinetics in Early Toxicology Studies

Toxicology studies conducted before the first introduction of a new drug into humans (Phase I studies) are designed to reveal its full toxic potential in animals.

Studies of relatively short duration (dosing up to 7 to10 days) using small groups of animals, exposed to a wide range of dosages, are conducted initially to determine the broad nature and extent of the toxic response. These initial observations guide dosage selection in subsequent studies, usually of 1-month duration, which is completed prior to initiating Phase I studies. Toxicokinetic data may influence the range of dosages employed in these studies but, generally, do not place a limit on the highest dosage. Selection of the highest dosage in these studies is usually based on combinations of clinical, functional, or pathological endpoints, or limitations of the formulation. *The concentration of the drug, and occasionally its principal and/or active metabolite(s), in blood or plasma of test animals, may be determined in range-finding studies and usually on the first and last days of the1-month study.*

These data provide useful information about the rate of absorption, the peak plasma concentration achieved C_{max}, the time taken (T_{max})after dosing to reach C_{max}, the half-life during the elimination phase, the area under the concentration-time curve (AUC), and the potential for changes in pharmacokinetics of the parent drug or its metabolites in the test animal upon repeated dosing (e.g.,accumulation, auto-induction). A less than dose proportional increase in plasma exposure between the oral dosage given and the resultant plasma concentration would suggest that systemic exposure may be limited by absorption. Alternatively, a more than proportional increase may suggest

saturation of elimination or first pass metabolism. *Toxicokinetic data may reveal a temporal relationship between plasma concentration and toxic response.* Although, toxicokinetic data make only a minor contribution to dosage selection in toxicology studies in these early stages of drug development, but they add another dimension to the interpretation of the toxic response manifested by the test animal, aid in the design of chronic toxicology studies, and in establishing safe starting dosages for Phase I studies in human subjects.

9. Application of Toxicokinetic Data to Dosages Selection In Chronic Studies

Chronic toxicity studies of 6- or 12-month duration in rodent and nonrodent species and lifetime carcinogenicity studies in 2 rodent species are conducted to support studies in Phase II/III of clinical development. At this stage of drug development, exposure data obtained from acute and subchronic studies in animals, and pharmacokinetic data from Phase I and Phase II studies in humans, should guide dosage selection as well as the design of chronic toxicity studies in animals.

Toxicokinetic data can provide a useful guide to dosage selection provided consideration is given to the entire spectrum of drug-induced toxicity and an attempt is made to relate each toxic manifestation to the most appropriate and concurrent measure of exposure. It is not logical to use dosages that saturate clearance mechanisms, thus causing progressive damage to these organs of excretion. Toxicity from the saturation of excretory processes is compounded in chronic studies by the fact that the clearance capacity of excretory organs may be further diminished as a result of spontaneous disease in aging animals. Thus, the amount of the dose to which the body is exposed over time and the amount of the dose subject to metabolic processing are the key determinants of toxicity in chronic toxicity studies. Steady-state toxicokinetics should be used to assist dosage selection. The extent of systemic exposure is dependent on a variety of host factors, the physicochemical nature of the drug (lipophilicity, hydrophilicity) as well as the frequency and route of administration. The extent of binding to plasma protein may be an important factor because it is the free fraction of the drug which gains access to critical cellular and subcellular sites to evoke toxicity. The fraction of the oral dose absorbed, and the rate of absorption are determinants of the plasma concentration achieved as well as the total exposure to the drug and drug-related products over a given interval of time.

10. Interpretation of TK Data

Interpretation of toxicological studies often hinges on the actual exposure of the animals to the test item/article. The amount of the test item/article administered to an animal is not necessarily the same as the amount reaching the intended site of action. Knowledge of the systemic exposure of an animal to a test item/article gives us an indication of the amount of the test item/article that is responsible for the actions. *The exposure information allows an assessment of the linear correlation with pharmacodynamic measurements. For example, if the heart rate was the biological/toxicological endpoint of interest, then increase in the area under the plasma concentration-time curve (AUC) could be compared to increase in the heart rate to determine a correlation between the*

pharmacodynamic and pharmacokinetic measurements. If there is a good correlation, then the area under the plasma concentration-time curve can be used to predict heart rate changes. If there is not a good correlation, this would suggest that the test item/article may be sequestered in tissues and, thus, have biological activity beyond the exposure measured in the blood. Alternatively, it could indicate that the dose range used was not wide enough to cause a range of responses in the heart rate.

As toxicokinetic studies are often conducted at multiple dose levels in both the genders, TK data interpretation in terms of test item/article dose-exposure relationship (dose proportionality), effect of gender on exposure, accumulation index and metabolite to parent ratio (if applicable) would add more information about the TK profile of test item/article. Evaluating the data in this way would help toxicologist to understand, dose proportionality of test item/article. *Whether the test item/article is showing linear/nonlinear kinetics. Is the kinetics same or different in both the genders? Is there a correlation between plasma exposure and observed adverse clinical signs/biochemical/hematological/histopathological changes?* Many times, due to different plasma exposures observed in males and females, dose of test item/article has to reduce to ensure study completion and there will not be any loss of data due to untimely mortality observed during the study.

Following a single oral gavage administration on Day 1, the T_{max} of test item/article was observed at 0.5 h in both the genders across the tested dose levels suggesting a rapid rate of absorption. The T_{last} was measured between 8.0 h and 24 h in both genders across the tested dose levels on study Day 1. The C_{max} values of test item/article in males were 399, 1530 and 2810 ng/mL at 15, 45 and 75 mg/kg/day dose levels, respectively. The corresponding C_{max} values in females were 182, 1380 and 1340 ng/mL, respectively, on Day 1. The AUC_{last} values of test item/article in males were 955, 5640 and 11900 h.ng/mL at 15, 45 and 75 mg/kg/day dose levels, respectively. The corresponding AUC_{last} values in females were 585, 2730 and 5450 h.ng/mL, respectively, on Day 1.

An example of the typical TK data and its interpretation is shown below.

Day	Gender	Dose (mg/kg/day)	Tmax (h)	Cmax (ng/mL)	AUClast (h.ng/mL)	Tlast (h)	C_{last} (ng/mL)
1	Male	15	0.5	399	955	8.0	43.2
		45	0.5	1530	5640	24.0	6.31
		75	0.5	2810	11900	24.0	35.3
	Female	15	0.5	182	585	8.0	23.6
		45	0.5	1380	2730	8.0	38.1
		75	0.5	1340	5450	24.0	12.9
28	Male	15	0.5	313	869	8.0	54.7
		45	0.5	1190	3290	8.0	133
		75	0.5	1590	7480	24.0	14.8
	Female	15	0.5	177	482	8.0	27.3
		45	0.5	1620	2920	8.0	86.4
		75	0.5	1240	4380	24.0	12.1

On study Day 1, a 3.0- fold increase in dose from 15 to 45 mg/kg/day, led to a 3.83- and 7.58- fold increase in peak plasma concentration (C_{max}) in male and female, respectively. Further 1.67- fold increase in dose from 45 to 75

mg/kg/day, led to a 1.84- and 0.97-fold increase in peak plasma concentration (C_{max}) in males and females respectively. These results suggest that as the dose increases, the C_{max} values also increased with more than dose proportional manner from 15 to 75 mg/kg/day. On study Day 1, a 3.0- fold increase in dose from 15 to 45 mg/kg/day, led to a 5.91- and 4.67- fold increase exposure (AUC_{last}) in male and female, respectively. Further 1.67- fold increase in dose from 45 to 75 mg/kg/day, led to a 2.11- and 2.00- fold increase exposure (AUC_{last}) in males and females, respectively. These results suggest that as the dose increased, the AUC_{last} values also increased with more than dose proportional manner from 15 to 75 mg/kg/day in both the genders. The male to female exposure (AUC_{last}) ratios were 1.63, 2.07 and 2.18 at 15, 45 and 75 mg/kg/day, respectively. The Corresponding C_{max} ratios were 2.19, 1.11 and 2.10, respectively. The result suggested that in general, males showed moderately higher plasma exposure than females on study Day 1.

Following repeated once-daily oral gavage administration for 28 days, the T_{max} of test item/article was observed at 0.5 h in both the genders across the tested dose levels, suggesting a rapid rate of absorption. The T_{last} was measured between 8.0 to 24 h in both genders in both the genders across the tested dose levels on study Day 28. The C_{max} values of test item/article in males were 313, 1190 and 1590 ng/mL at 15, 45 and 75 mg/kg/day dose levels, respectively. The corresponding C_{max} values in females were 177, 1620 and 1240 ng/mL, respectively, on Day 28. The AUC_{last} values of test item/article in males were 869, 3290 and 7480 h.ng/mL at 15, 45 and 75 mg/kg/day dose levels, respectively. The corresponding AUC_{last} values in females were 482, 2920 and 4380 h.ng/mL, respectively, on Day 28.

On study Day 28, a 3.0- fold increase in dose from 15 to 45 mg/kg/day, led to a 3.79- and 6.06- fold increase exposure (AUC_{last}) in male and female, respectively. Further 1.67- fold increase in dose from 45 to 75 mg/kg/day, led to a 2.27- and 1.50- fold increase in exposure (AUC_{last}) in males and females, respectively. These results suggest that as the dose increased, the AUC_{last} values also increased with more than dose proportional manner from 15 to 75 mg/kg/day in both the genders. The male to female exposure (AUC_{last}) ratios were 1.80, 1.13 and 1.71 at 15, 45 and 75 mg/kg/day, respectively. The Corresponding C_{max} ratios were 1.77, 0.73 and 1.28, respectively. The result suggested that in general, males showed marginally higher plasma exposures than females on study Day 28, except at 45 mg/kg/day dose level where females showed comparable plasma exposures with that of males.

On repeated once daily oral gavage administration for 28 consecutive days, the Day 28 to Day 1 exposure (AUC_{last}) ratios in males were 0.91, 0.58 and 0.63 at 15, 45 and 75 mg/kg/day dose levels, respectively. The corresponding exposure ratios in females were 0.82, 1.07 and 0.80, respectively. These results suggest that in general, there is no accumulation upon repeated oral administration at tested dose levels in both the genders.

11. Clinical Relevance of TK Data from Animal Safety Studies

Integration of TK into a testing program can play an important role in the design of toxicity studies, helping to ensure the use of doses, routes and regimens

relevant to humans, avoid the generation of irrelevant findings at very high doses and reduce animal welfare concerns

Advances in analytical technologies and sampling strategies mean that basic TK data across a wide range of dose levels cannot be derived from toxicology study animals, without substantially modifying existing tests or costs, providing an opportunity for TK to become an integral part of a testing program. Clinical pathology evaluation and TK has become routine in most repeat dose toxicology studies.

Toxicokinetic data from either NOEL or NOAEL can be used to give guidance to the clinical investigator by providing suitable safe starting and upper doses in the initial single-dose Phase I study. For further clinical studies using multiple dosing, toxicokinetic data from toxicity studies provide information on possible increase or decrease of the drug in plasma. Cases where human plasma levels in a Phase I study are higher than in the animal study NOEL or NOAEL values need to consider the effects of different metabolism and plasma protein binding. This might result in the use of a different species in the toxicity study and/or a change of formulation to enable a reassessment of safety margins.

Effects occurring at doses exceeding the KMD are thus also not of relevance for hazard classification, which as with risk assessment has the goal of protecting human health.

12. Use of Toxicokinetic Data in Reducing the Default Factors in Risk Assessment

The greatest utility of preclinical toxicokinetic data has been in the interspecies comparison of product toxicity. It is now widely accepted that toxic effects can be better extrapolated from animals to humans when these comparisons are based on toxicokinetic and disposition (absorption, distribution, metabolism, and excretion) data in preclinical species and humans. *In this context, the safety margin that is based on the ratio of animal AUC at NOAEL to human AUC at an efficacious dose is the key predictor of human toxicity risk. It is generally accepted that when the AUC ratio to support safety margin is large, the expected risk of toxicity in humans is low*. Although model-independent or compartment-based plasma/blood toxicokinetic has served well as a practical means of assessing systemic exposure, they provide no information on the time course of exposure of target organs to drug or metabolites. *Overall, toxicokinetic have greatly enhanced our understanding of interspecies differences in toxicity and significance of safety margins.*

Traditionally, a default factor of 10 has been used to account for interspecies variation. *The International Program on Chemical Safety (IPCS) project has proposed that this factor be subdivided into a subfactor to address the toxicokinetic aspects and a second subfactor for the toxicodynamic aspects.* The subfactors are considered default values, which can be replaced with data-derived values when appropriate data are available. To address the toxicokinetic aspects, the active species, the relevant internal exposure, and the adequate metrics must be considered. *Based on compound-specific data the default factor can be brought down from the traditional 10 to 3 - 5, depending upon the quality and robustness of the TK data.* Proper application of pharmacokinetic and

pharmacodynamic data reduces the uncertainties when establishing limits for specific compounds and provides better assurance that established limits are adequately protective. *It also provides scientific support for more cost-effective exposure control strategies in establishing Occupational Exposure Limits (OELs).*

13. Conclusion

In the development process of human pharmaceutical therapeutics, toxicology studies play an important role in establishing the safety profiles of compounds. A battery of *in vitro* and *in vivo* studies is conducted for this purpose. Animals commonly used in toxicology studies include rats, mice, dogs, and monkeys. Interpretation of toxicological studies often hinges on the actual exposure of the animals to the compound. *The amount of the compound administered to an animal is not necessarily the same as the amount reaching the intended site of action.*

Toxicokinetic evaluation is both a regulatory and scientific requirement in the drug development process. Toxicokinetic is the generation of kinetic data to assess systemic exposure, either as an integral component of preclinical toxicity studies or in specially designed supportive studies. These data help to understand the relationship between observed toxicity and administered dose. They also play a role in the clinical setting, assisting in the setting of plasma limits for early human exposure and in the calculation of safety margins.

Toxicokinetic (TK) information can substantially enhance the value of the data generated from toxicity testing and is an integral part of pharmaceutical safety assessment. It is less widely used in the chemical, agrochemical and consumer products industries, but recognition of its value is growing, as reflected by increased reference to the use of TK information in new and draft OECD test guidelines.

The measurement of peak and total exposure in these studies helps to determine the relationship between the toxicological effects and the exposure. Compared to the actual dose administered in the toxicology studies, the data on exposure is more relevant for comparing effects in animals and man. This is because the pharmacokinetics of a drug varies extensively between the species. This information is valuable in interpreting toxic effects, or lack thereof, and may assist in the extrapolation of animal toxicity data to humans.

Robust toxicokinetic evaluation is an essential part of drug development. Assessment should comprise effective analytical methods (performed to GLP), adequate sampling (including controls where relevant), sufficient results (through the collection procedure), evaluation of metabolites, and measurements of human metabolite(s) in an animal species (where appropriate). Effective interpretation of toxicokinetic data in regulatory dossiers should also occur.

The TK data relates the exposure achieved in toxicity studies to toxicological findings and contributes to the assessment of the relevance of these findings to human safety. *It provides information on linear/non-linear pharmacokinetics, accumulation, and whether the effects are related to C_{max} (peak concentration) or total exposure (AUC).* Toxicokinetic data helps to determine the appropriate species, study design, and treatment regimen in subsequent non-clinical toxicity

studies. Toxicokinetic information also helps in evaluating the impact of a proposed change in the clinical route of administration. *An increased understanding of the relationships between external exposure, target organ dosimetry, and adverse effects should provide greater confidence in making low-dose extrapolations of human risk.*

Interpreting preclinical data from the range of studies performed during drug development requires a good understanding of the observed toxic response(s) versus drug exposure. This understanding is crucial for setting safe dose levels for clinical use of a potential new drug. *Both species and sex-differences exist in animal toxicity studies for toxicokinetic measurements, which obviously have relevance to the clinical evaluation*. A lack of dose proportionality also has relevance to the clinical use of a drug and can be related to saturated mechanisms or auto-induction. These mechanisms include intestinal-absorption saturation (e.g.with verapamil, cimetidine, and salbutamol), enzyme-metabolizing system saturation (e.g. with salicylates, theophylline, paroxetine, phenytoin, and acyclovir) and tubular-absorption saturation (e.g. with L-carnitine).

Another important factor is the effect of higher plasma values observed later in a toxicity study, possibly caused by accumulation from a long half-life, reduced clearance, metabolizing-enzyme inhibition or enterohepatic re-circulation. Clearance can be affected either by capacity-limited elimination or impairment of hepatic function (e.g. with proxicromil). Drugs such as methadone have a long half-life and are subject to accumulation. Several examples of enzyme-inhibiting drugs can be found in the literature, including cimetidine, ciprofloxacin, and ketoconazole. Enterohepatic cycling might prolong the action of some benzodiazepines and components of the contraceptive pill. In the dog, the ulcerogenic effects of indomethacin have been related to increased cycling compared with other species. *Lower plasma values observed later in a toxicity study could be related to enzyme induction or first-pass metabolism.* A range of enzyme-inducing agents are known to exist and include carbamazepine, phenobarbitone, and phenytoin. The first pass of a drug (e.g. morphine) through the liver can reduce systemic bioavailability and can be considered a form of pre-systemic metabolism.

Questions

1. *Define Toxicokinetics?*
2. *Toxicology studies require toxicokinetics to check whether systemic exposure reflects _____*
3. *The global definition of toxicokinetics, issued by ICH?*
4. *Differentiate Pharmacokinetics and toxicokinetics?*
5. *What are the parameters analyzed in a TK study?*
6. *Explain one compartment and two compartment/multi-compartment models?*
7. *The _____ model considers body as a single, kinetically homogenous unit*
8. *Phoenix _____, a software developed by Certara Inc. USA is widely used to calculate pharmacokinetic/toxicokinetic data in the pharmaceutical industry.*

9. *Define Kinetically Derived Maximum (KMD) dose?*
10. *Write on Clinical Relevance of TK Data from Animal Safety Studies?*
11. *Explain the use of Toxicokinetic Data in Reducing the Default Factors in Risk Assessment?*
12. *What are the factors to be Considered in Measuring Toxicokinetics?*
13. *Write on the decision on selection of compartment model?*

---------End of the Document----------

ToxGurukul Foundation

ToxGurukul Foundation is a registered non-profit organization for professionals in the field of toxicology who are in search of a platform to learn and share the vast knowledge in this area. This syndicate belongs to independent professionals from different backgrounds of toxicology who share their knowledge to un-puzzle the Rubik's cube that each face in their daily work routine.

Website: www.toxgurukul.org.in
Email: toxgurukul.india@gmail.com

Important Links – ToxGurukul Foundation

Follow us

Join us

Subscribe

Library

Feedback

ToxGurukul Foundation (A Non - Profit Organization)
Regd. Office: Fl. No.10, New Ajanta Avenue, Building-4, Wing- A1,
S. No. 135/136 Part, Kothrud, Pune-411038
Corporate Identity Number: U80904PN2019NPL182886
Emai Id: toxgurukul.india@gmail.com