Concomitant toxicokinetics may be performed either in all or a representative proportion of the animals used in the main study or in special satellite groups (Notes 1 and 5). Normally, samples for the generation of toxicokinetic data may be collected from main study animals, where large animals are involved, but satellite groups may be required for the smaller (rodent) species.

The number of animals to be used should be the minimum consistent with generating adequate toxicokinetic data. Where both male and female animals are utilized in the main study it is normal to estimate exposure in animals of both sexes unless some justification can be made for not so doing.

Toxicokinetic data are not necessarily required from studies of different duration if the dosing regimen is essentially unchanged (see also 4.3).

## 3.6 Complicating factors in exposure interpretation

Although estimating exposure as described above may aid in the interpretation of toxicity studies and in the comparison with human exposure, a few caveats should be noted.

Species differences in protein binding, tissue uptake, receptor properties, and metabolic profile should be considered. For example, it may be more appropriate for highly protein bound compounds to have exposure expressed as the free (unbound) concentrations. In addition, the pharmacological activity of metabolites, the toxicology of metabolites, and antigenicity of biotechnology products may be complicating factors. Furthermore, it should be noted that even at relatively low plasma concentrations, high levels of the administered compound and/or metabolite(s) may occur in specific organs or tissues.

### 3.7 Route of administration

The toxicokinetic strategy to be adopted for the use of alternative routes of administration, for example, by inhalation, topical, or parenteral delivery, should be based on the pharmacokinetic properties of the substance administered by the intended route.

It sometimes happens that a proposal is made to adopt a new clinical route of administration for a pharmaceutical product; for example, a product initially developed as an oral formulation may subsequently be developed for intravenous administration. In this context, it will be necessary to ascertain whether changing the clinical route will significantly reduce the safety margin.

This process may include a comparison of the systemic exposure to the compound and/or its relevant metabolite(s) (AUC and C<sub>max</sub>) in humans generated by the existing and proposed routes of administration. If the new route results in increased AUC and or C<sub>max</sub>, or a change in metabolic profile, the continuing assurance of safety from animal toxicology and kinetics should be reconsidered. If exposure is not substantially greater, or different, by the proposed new route compared to that for the existing route(s) then additional nonclinical toxicity studies may focus on local toxicity.

#### 3.8 Determination of metabolites

A primary objective of toxicokinetics is to describe the systemic exposure to the administered compound achieved in the toxicology species. However, there may be circumstances when measurement of metabolite concentrations in plasma or other body fluids is especially important in the conduct of toxicokinetics (Note 9).

- When the administered compound acts as a "pro-drug" and the delivered metabolite is acknowledged to be the primary active entity.
- When the compound is metabolized to one or more pharmacologically or toxicologically active metabolites which could make a significant contribution to tissue/organ responses.
- When the administered compound is very extensively metabolized and the measurement of plasma or tissue concentrations of a major metabolite is the only practical means of estimating exposure following administration of the compound in toxicity studies (Note 10).

#### 3.9 Statistical evaluation of data

The data should allow a representative assessment of the exposure. However, because large intra- and inter-individual variation of kinetic parameters may occur and small numbers of animals are involved in generating toxicokinetic data, a high level of precision in terms of statistics is not normally needed. Consideration should be given to the calculation of mean or median values and estimates of variability, but, in some cases, the data of individual animals may be more important than a refined statistical analysis of group data.

If data transformation (e.g., logarithmic) is performed, a rationale should be provided.

#### 3.10 Analytical methods

Integration of pharmacokinetics into toxicity testing implies early development of analytical methods for which the choice of analytes and matrices should be continually reviewed as information is gathered on metabolism and species differences.

The analytical methods to be used in toxicokinetic studies should be specific for the entity to be measured and of an 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.

The choice of analyte and the matrix to be assayed (biological fluids or tissue) should be stated and possible interference by endogenous components in each type of sample (from each species) should be investigated. Plasma, serum, or whole blood are normally the matrices of choice for toxicokinetic studies.

If the drug substance is a racemate or some other mixture of enantiomers, additional justification should be made for the choice of the analyte (racemate or enantiomer(s)).

The analyte and matrix assayed in nonclinical studies should ideally be the same as in clinical studies. If different assay methods are used in non-clinical and clinical studies they should all be suitably validated.

#### 3.11 Reporting

A comprehensive account of the toxicokinetic data generated, together with an evaluation of the results and of the implications for the interpretation of the toxicology findings, should be given.

An outline of the analytical method should be reported or referenced. In addition, a rationale for the choice of the matrix analysed and the analyte measured (see 3.8 and 3.10) should be given.

The positioning of the report within the application will depend upon whether the data are specific to any one toxicity study or is supportive of all toxicity testing.

# 4. Toxicokinetics in the Various Areas of Toxicity Testing—Specific Aspects

#### 4.1 Introduction

Based on the principles of toxicokinetics outlined above, the following specific considerations refer to individual areas of toxicity testing. The frequency of exposure monitoring or profiling may be extended or reduced where necessary.

It may be appropriate to take samples from some individual animals only, where this may help in the interpretation of the toxicology findings for these animals.

#### 4.2 Single dose toxicity studies

These studies are often performed in a very early phase of development before a bioanalytical method has been developed and toxicokinetic monitoring of these studies is therefore not normally possible. Plasma samples may be taken in such studies and stored for later analysis, if necessary; appropriate stability data for the analyte in the matrix sampled would then be required.

Alternatively, additional toxicokinetic studies may be carried out after completion of a single dose toxicity study in order to respond to specific questions which may arise from the study.

Results from single dose kinetic studies may help in the choice of formulation and in the prediction of rate and duration of exposure during a dosing interval. This may assist in the selection of appropriate dose levels for use in later studies.

#### 4.3 Repeated dose toxicity studies

The treatment regimen (Note 11) and species should be selected whenever possible with regard to pharmacodynamic and pharmacokinetic principles. This may not be achievable for the very first studies, at a time when neither animal nor human pharmacokinetic data are normally available.

Toxicokinetics should be incorporated appropriately into the design of the studies. It may consist of exposure profiling or monitoring (Note I) at appropriate dose levels at the start and towards the end of the treatment period of the first repeat dose study (Note 12). The procedure adopted for later studies will depend on the results from the first study and on any changes in the proposed treatment regimen. Monitoring or profiling may be extended, reduced, or modified for specific compounds where problems have arisen in the interpretation of earlier toxicity studies.