4.4 Genotoxicity studies

For negative results of in vivo genotoxicity studies, it may be appropriate to have demonstrated systemic exposure in the species used or to have characterized exposure in the indicator tissue.

4.5 Carcinogenicity (Oncogenicity) studies1

4.5.1 Sighting or dose-ranging studies

Appropriate monitoring or profiling of these studies should be undertaken in order to generate toxicokinetic data which may assist in the design of the main studies (see 4.5.2.). Particular attention should be paid to species and strains which have not been included in earlier toxicity studies and to the use of routes or methods of administration which are being used for the first time.

Particular attention should be paid to the establishment of appropriate toxicokinetic data when administration is to be in the diet (Note 13).

Toxicokinetic data may assist in the selection of dose levels in the light of information about clinical exposure and in the event that nonlinear kinetics (Note 3) may complicate the interpretation of the study.

In principle, the ideal study design would ensure that dose levels in oncogenicity studies generate a range of systemic exposure values that exceed the maximum therapeutic exposure for humans by varying multiples. However, it is recognized that this idealized selection of dose levels may be confounded by unavoidable species-specific problems. Thus, the emphasis of this guidance is on the need to estimate systemic exposure, to parent compound and/or metabolite(s) at appropriate dose levels and at various stages of an oncogenicity study, so that the findings of the study may be considered in the perspective of comparative exposure for the animal model and humans.

A highest dose based on knowledge of probable systemic exposure in the test species and in humans may be an acceptable end-point in testing for carcinogenic potential. Historically, a toxicity end-point¹ has been often used to select the top dose level.

4.5.2 The main studies

The treatment regimen, species, and strain selection should, as far as is feasible, be determined with regard to the available pharmacokinetic and toxicokinetic information. In practice, the vast majority of these studies is conducted in the rat and mouse.

As mentioned in the "Introduction" to this section, it is recommended that reassurance be sought from monitoring that the exposure in the main study is consistent with profiles of kinetics established in free-standing or specific dose-ranging studies. Such monitoring will be appropriate on a few occasions during the study, but it is not considered essential to continue beyond 6 months.

4.6 Reproductive toxicity studies²

4.6.1 Introduction

It is preferable to have some information on pharmacokinetics before initiating reproduction studies since this may suggest the need to adjust the choice of species, study design, and dosing schedules. At this time, the information need not be sophisticated or derived from pregnant or lactating animals. At the time of study evaluation, further information on pharmacokinetics in pregnant or lactating animals may be required depending on the results obtained.

The limitation of exposure in reproductive toxicity is usually governed by maternal toxicity. Thus, while toxicokinetic monitoring in reproductive toxicity studies may be valuable in some instances, especially with compounds with low toxicity, such data are not generally needed for all compounds.

Where adequate systemic exposure might be questioned because of absence of pharmacological response or toxic effects, toxicokinetic principles could usefully be applied to determine the exposures achieved by dosing at different stages of the reproductive process.

A satellite group of female animals may be used to collect the toxicokinetic data.

4.6.2 Fertility studies

The general principles for repeated dose toxicity studies apply (see 4.3). The need to monitor these studies will depend on the dosing regimen used and the information already available from earlier studies in the selected species.

4.6.3 Studies in pregnant and lactating animals

The treatment regimen during the exposure period should be selected on the basis of the toxicological findings and on pharmacokinetic and toxicokinetic principles.

Consideration should be given to the possibility that the kinetics will differ in pregnant and nonpregnant animals.

Toxicokinetics may involve exposure assessment of dams, embryos, fetuses, or newborn at specified days (Note 14). Secretion in milk may be assessed to define its role in the exposure of newborns. In some situations, additional studies may be necessary or appropriate in order to study embryo/fetal transfer and secretion in milk.

Consideration should be given to the interpretation of reproductive toxicity tests in species in which placental transfer of the substance cannot be demonstrated.

5. Supplementary Notes

Note 1: Definitions of expressions appearing in this "Note for Guidance:"

Analyte: The chemical entity assayed in biological samples.

Matrix: Blood, plasma, urine, serum, or other fluid or tissue selected for assay.

Concomitant toxicokinetics: Toxicokinetic measurements performed in the toxicity study, either in all animals or in representative subgroups or in satellite groups.

Exposure: Exposure is represented by pharmacokinetic parameters demonstrating the local and systemic burden on the test species with the test compound and/or its metabolites. The area under the matrix level concentration-time curve (AUC) and/or the

measurement of matrix concentrations at the expected peak-concentration time $C_{max},$ or at some other selected time $C_{(time)}$ are the most commonly used parameters. Other parameters might be more appropriate in particular cases.

Monitor: To take a small number of matrix samples (e.g., 1 to 3) during a dosing interval to estimate $C_{(time)}$ or C_{max} .

Profile: To take (e.g., 4 to 8) matrix samples during a dosing interval to make an estimate of C_{max} and/or $C_{(time)}$ and area under the matrix concentration-time curve (AUC).

Satellite: Groups of animals included in the design and groups: conduct of a toxicity study, treated and housed under conditions identical to those of the main study animals, but used primarily for toxicokinetics.

Support: In the context of a toxicity study—to ratify or confirm the design of a toxicity study with respect to pharmacokinetic and metabolic principles. This process may include two separate steps:

(a) Confirmation using toxicokinetic principles that the animals on a study were exposed to appropriate systemic levels of the administered compound (see 3.4) and/or its metabolite(s).

(b) Confirmation that the metabolic profile in the species used was acceptable; data to support this will normally be derived from metabolism studies in animals and in humans.

Validate: In the context of an analytical method—to establish the accuracy, precision, reproducibility, response function, and the specificity of the analytical method with reference to the biological matrix to be examined and the analyte to be quantified.

Note 2: Symbols and definitions according to "Manual of Symbols, Equations and Definitions in Pharmacokinetics," Committee for Pharmacokinetic Nomenclature of the American College of Clinical Pharmacology, Philadelphia, PA, May 1982:

C_{max}-Maximum (peak) concentration.

 $C_{(\text{time})}$ -Maximum concentration at a specified time after administration of a given dose.

 $t_{\text{max}}\mbox{-}\mbox{Time}$ to reach peak or maximum concentration following administration.

 $AUC_{(0-t)}$ -Area under concentration-time curve from zero to time t. It should be noted that $AUC_{(O-infinity)}$ is a special case of $AUC_{(0-t)}$.

Other measurements, for example, urinary excretion, may be more appropriate for some compounds. Other derived parameters, for example, bioavailability, half-life, fraction of unbound drug, and volume of distribution, may be of value in interpreting toxicokinetic data. Thus, the selection of parameters and time points has to be made on a case-by-case basis considering the general principles as outlined in Section 3.

Note 3: Increases in exposure may arise unexpectedly as a result of nonlinear kinetics due to saturation of a clearance process. Increasing exposure may also occur during the course of a study for those compounds which have a particularly long plasma halflife. Careful attention should also be paid to compounds which achieve high C_{max} values over comparatively short time periods within the dosing interval. Conversely, unexpectedly low exposure may occur during a study as a result of auto-induction of metabolizing enzymes.