B. 36. TOXICOKINETICS

1. METHOD

1.1. Introduction

See General Introduction Part B.

1.2. Definitions

See General Introduction Part B.

1.3. Reference substances

None.

1.4. Principle of the test method

The test substance is administered by an appropriate route. Depending on the purpose of the study, the substance may be administered in single or repeated doses over defined periods to one or several groups of experimental animals. Subsequently, depending on the type of study, the substance and/or metabolites are determined in body fluids, tissues and/or excreta.

Studies may be done with 'unlabelled' or 'labelled' forms of the test substance. Where a label is used it should be positioned in the substance in such a way to provide the most information about the fate of the compound.

1.5. Quality criteria

None.

1.6. Description of the test method

Preparations

Healthy young adult animals are acclimatized to the laboratory conditions for at least five days prior to the test. Before the test, animals are randomized and assigned to the treatment groups. In special situations, very young, pregnant or pre-treated animals may be used.

Test conditions

Experimental animals

Toxicokinetic studies may be carried out in one or more appropriate animal species and should take account of the species used or intended to be used in other toxicological studies on the same test substance. Where rodents are used in a test the weight variation should not exceed ± 20% of the mean weight.

Number and sex

For absorption and excretion studies, there should be four animals in each dose group initially. Sex preference is not mandatory, but under some circumstances both sexes may need to be studied. If there are sex differences in response, then four animals of each sex should be tested. In the case of studies with non-rodents fewer animals may be used.

When tissue distribution is being studied, the initial group size should take into account both the number ofanimals to be sacrificed at each time point and the number of time points to be examined.

When metabolism is being studied, the group size is related to the needs of the study.

For multiple-dose and multiple-time-point studies, the group size should take into account the number of time points and planned sacrifice(s), but may not be smaller than two animals. The group size should be sufficient to provide an acceptable characterization of uptake, plateau and depletion (as appropriate) of the test substance and/or metabolites.

Dose levels

In the case of single-dose administration, at least two dose levels should be used. There should be a low dose at which no toxic effects are observed and a high dose at which there might be changes in toxicokinetic parameters or at which toxic effects occur.

In the case of repeated-dose administration the low dose is usually sufficient, but under certain circumstances a high dose may also be necessary.

Route of administration

Toxicokinetic studies should be performed using the same route and, where appropriate, the same vehicle as that used or intended to be used in the other toxicity studies. The test substance is usually administered orally by gavage or in the diet, applied to the skin, or administered by inhalation for defined periods to groups of experimental animals. Intravenous administration of the test substance may be useful in determining relative absorption by other routes. In addition, useful information may be provided on the pattern of distribution soon after the intravenous administration of a substance.

The possibility of interference of the vehicle with the rest substance should be taken into consideration. Attention should be given to differences in absorption between the administration of the test substance by gavage and in the diet and the need for an accurate determination of dose particularly when the test substance is given in the diet.

Observation period

All the animals should be observed daily and signs of toxicity and other relevant clinical features recorded, including time of onset, degree and duration.

Procedure

After weighing test animals, the test substance is administered by an appropriate route. If considered relevant, anirnals may be fasted before the test substance is administered.

Absorption

The rate and extent of absorption of the administered substance can be evaluated using various methods, with and without reference groups ([1]), for example by:

-determination of the amount of test substance and/or metabolites in excreta, such as urine, bile, faeces, exhaled air and that remaining in the carcase,

-comparison of the biological response (e.g. acute toxicity studies) between test and control and/or reference groups,

-comparison of the amount of renally excreted substance and/or metabolite in test and reference groups,

-determination of the area under the plasma-level/time curve of the lest substance and/or metabolites and comparison with data from a reference group.

([1]) In this method a reference group is one in which the test substance is administered by another route that ensures complete bioavailability of the dose.

Distribution

Two apptoaches are available at present, one or both of which may be used for analysis of distribution patterns:

-useful qualitative information is obtained using whole body autoradiographic techniques,

-quantitative information is obtained by sacrificing animals at different times after exposure and determining the concentration and amount of the test substance and/or metabolites in tissues and organs.

Excretion

In excretion studies, urine, faeces and expired air and, in certain circumstances, bile are collected. The amount of test substance and/or metabolites in these excreta should be measured several times after exposure, either until about 95% of the administered dose has been excreted or for seven days, whichever comes first.

In special cases, the excretion of the test substance in the milk of lactating test animals may need to be considered.

Metabolism

To determine the extent and pattern of metabolism, biological samples should be analysed by suitable techniques. Structures of metabolites should be elucidated and appropriate metabolic pathways proposed where there is a need to answer questions arising from previous toxicological studies. It may be helpful to perform studies *in vitro* to obtain information on metabolic pathways.

Further information on the relationship of metabolism to toxicity may be obtained from biochemical studies, such as the determination of effects on metabolizing enzyme systems, depletion of endogenous non-protein sulphydryl compounds and binding of the substance with macromolecules.

2. DATA

According to the type of study performed, data should be summarized in tabular form supported by graphical presentation whenever appropriate. For each test group, mean and statistical variations of measurements in relation to time, dosage, tissues and organs should be shown when appropriate. The extent of absorption and the amount and rates of excretion should be determined by appropriate methods. When metabolism studies are performed, the structure of identified metabolites should be given and possible metabolic pathways presented.

3. REPORTING

3.1. Test report

According to the type of study performed, the test report shall, if possible, contain the following information:

-species, strain, source, environmental conditions, diet,

-characterization of labelled materials, when used,

-dosage levels and intervals used,

-route(s) of administration and any vehicles used;

-toxic and other effects observed,

-methods for determination of test substance and/or metabolites in biological samples, including expired air,

-tabulation of measurements by sex, dose, regimen, time, tissues and organs,

-presentation of the extent of absorption and excretion with time,

-methods for the characterization and identification of metabolites in biological samples,

-methods for biochemical measurements related to metabolism,

-proposed pathways for metabolism,

-discussion of the results,

-interpretation of the results.

3.2. Evaluation and interpretation

See General Introduction Part B.

4. REFERENCES

See General Introduction Part B.