- 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 for 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.

B. DESCRIPTION OF THE TEST PROCEDURE

- Preparations

Healthy young adult animals are acclimatised to the laboratory conditions for at least five days prior to the test. Before the test, animals are randomised and assigned to treatment groups. In special situations, other animals, such as the very young or pregnant, may be used.

- Experimental animals

Selection of species

Toxicokinetic studies may be carried out in one or more appropriate animal species, taking due 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 per cent 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 sexual dimorphism exists, then four animals of each sex should be studied. 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 the number of animals at each time point and the need for different times of sacrifice. 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 characterisation of uptake, plateau and depletion (as appropriate).

Housing and feeding conditions

The temperature of the experimental animal room should be 22°C (± 3°C) and the relative humidity 30 to 70 per cent. Animals are caged individually in metabolism cages. Where lighting is artificial, the sequence should be 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. For non-rodents other appropriate housing and feeding conditions may be used. Whichever diet is used, appropriate information on its composition should be available and the possibility of interfering constituents should be considered.

- Test conditions

Test substance

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

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 preferably be performed using the same route and, where appropriate, the same vehicle as that 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 when determining the amount of absorption and the pattern of distribution soon after the administration of a substance.

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

Timing of the study

Toxicokinetic studies are used as an aid in the interpretation of toxicity data. Consequently, the type of study undertaken and the timing of the study depend on the need for appropriate data.

- Performance of the test

After weighing the test animals, the test substance is administered by an appropriate route.

Absorption

The rate and extent of absorption of the administered substance can be determined by various methods, with and without reference groups*, such as:

* A reference group is one in which the test substance may be administered via another route that ensures complete availability of the dose.

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

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

- comparison of the amount of dose excreted renally in test and reference groups;

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

Distribution

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

- useful quantitative 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 at several times after exposure, until about 95 per cent 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 determined.

Metabolism

For determining the extent and pattern of metabolism, biological samples should be analysed by suitable techniques. Structures of metabolites should be elucidated and the metabolic pathways proposed in relation to the need to answer questions arising from previous

toxicological studies. It may be helpful to perform studies *in vitro* to obtain information about the pathways of metabolism. To obtain further information relating metabolism to toxicity, biochemical studies, such as the determination of effects on metabolising enzyme systems, depletion of endogenous non-protein sulfhydryl compounds and binding of the substance with macromolecules, may be performed.

3. DATA AND REPORTING

- Treatment of results

Where possible, data should be summarised 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 of 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 metabolic pathways proposed.

- Evaluation of results

Absorption

The determination of the absorption of the test substance may aid in the evaluation of acute toxicity studies and in the planning and evaluation of repeated-dose toxicity studies. The complete absence of absorption and acute toxic effects of a test substance (e.g. polymeric material) may suggest that further repeated-dose studies are not needed.

Distribution

The determination of the distribution pattern of the test substance in tissues and organs may aid in the evaluation of repeated-dose studies. A study of the distribution of the test substance in pregnant animals makes it possible to assess the amount of placental transfer at critical periods of organogenesis in relation to maternal exposure. Data from distribution studies may indicate accumulation of the test substance in the body or in selected tissues and organs of the body.

Excretion

Knowledge of the excretory patterns of the test substance may be useful in the evaluation of repeated-dose toxicity studies. The assessment of the amounts and rates of excretion will indicate whether retention of the test substance and/or metabolites can occur. Retention of the test substance and/or metabolites may be associated with changes in toxic response.

Metabolism

The determination of the pattern and the rates of metabolism of the test substance may aid in the interpretation of long-term toxicity studies. Such studies may detect changes in metabolic parameters across a dose range which may be reflected in disproportionate changes in toxicological response. Dose selection for long-term studies may be based on such information about dose-dependent metabolism.

The observed changes in toxicological response under repeated-dose conditions may be related to the induction of metabolising enzymes.

Radioactivity bound to macromolecules after administration of radiolabelled substances may result from normal intermediary metabolism, or it can indicate the formation of reactive intermediates.

Knowledge of the depletion of endogenous sulfhydryl compounds may be useful in evaluating toxic effects related to the formation of reactive metabolites and for dose selection in repeated-dose studies.

- Test report

According to the type of study performed, the test report should include the following information:

- species/strain and number of animals used;
- characterisation of labelled and reference materials, when used;
- dosage levels and intervals used;
- route(s) of administration and any vehicles used;
- diet used;

- 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 the extent of excretion with time;
- methods for the characterisation and identification of metabolites in biological samples;
- methods for biochemical measurements related to metabolism;
- proposed pathways for metabolism; and
- discussion and interpretation of the results.

• Interpretation of results

Information on the absorption of a test substance provides an estimate of the amount and rate by which the substance enters the body of the test animal by oral, dermal, inhalation or other routes of administration.

Information on the distribution of a test substance provides an estimate of the pattern by which the absorbed substance and/or its metabolites circulate and partition with various tissues and organs in the body.

Information on the excretion of a test substance provides an estimate of the amount and the rates by which the administered substance and/or its metabolites are eliminated from the body.

A study of the metabolism of a test substance provides information on the way in which the administered substance is structurally changed in the body by either enzymatic or non-enzymatic reactions.

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