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DRAFT

GUIDELINE ON REPEATED DOSE TOXICITY

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GUIDELINE ON REPEATED DOSE TOXICITY

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EXECUTIVE SUMMARY

The purpose of testing toxicity after repeated dosing is to contribute to the development of safe medicinal products that need repeated administration to patients. General principles are provided on substance quality and excipients. The criteria discussed takes into account the choice of animal species, the size of groups and animal husbandry. Dose regimen, duration and route of administration should be selected based on the intended clinical use. Guidance is given on the parameters to be monitored during the in-life phase, and which special studies may be needed in case of a special activity of a certain medicinal product. A list of recommended tissues to be studied histopathologically is attached.

1. INTRODUCTION

The primary goal of repeated dose toxicity studies is to characterise the toxicological profile of the test compound following repeated administration. This includes identification of potential target organs of toxicity and exposure/response relationships, and may include the potential reversibility of toxic effects. This information should be part of the safety assessment to support the conduct of human clinical trials and the approval of a marketing authorisation.

2. SCOPE

This Guideline concerns the conduct of repeated dose toxicity studies of active substances intended for human use. For certain types of substances, such as biotechnology-derived compounds, vaccines and anticancer medicinal products, specific guidance is available (see CPMP/ICH/302/95 *Note for guidance on Safety studies for biotechnological products*, CPMP/SWP/465/95 *Note for guidance on Pre-clinical pharmacological and toxicological testing of vaccines*, CPMP/SWP/997/96 *Note for guidance on the Pre-clinical evaluation of anticancer medicinal products).* The Guideline may be considered also in the case of herbal medicinal products

3. LEGAL BASIS

This guideline should be read in conjunction with Directive 2001/83/EC as amended, and all ICH and CHMP guidelines as applicable. The guideline is also applicable for Clinical Trial Applications in line with Directive 2001/20/EC as amended.

With respect to animal husbandry, the Council Directive on animal welfare 86/609/EEC and Council Decision on the European Convention on the protection of vertebrae animals, 1999/575/EC) should be also be taken into account.

4. GENERAL PRINCIPLES

Repeated dose toxicity studies should be carried out in conformity with the provisions relating to good laboratory practice laid down by Council Directives 87/18/EEC and 88/320/EEC.

The design of the study, including selection of test species, dose levels, route and frequency of administration, should be based on available pharmacodynamic, pharmacokinetic and toxicological information as well as the intended clinical use. The investigator should justify the selected study design.

5. GENERAL RECOMMENDATIONS ON SUBSTANCE QUALITY

5.1. Substance quality

Each batch used in the repeated dose toxicity studies should be identified. The physicochemical characteristics should be presented and certified for each batch and the stability of the material stated.

Furthermore, the stability of the substance in the tested dose formulation should be known. The substance used in the repeated dose toxicity studies should present a similar pattern of impurities as the product intended for use in human (clinical trials and marketing), as much as possible. Should the medicinal product intended for marketing have impurities significantly different from those in the test batches, either in terms of quality or quantity, these may need further qualification (see Notes for guidance on impurities: CPMP/ICH/142/95 *Note for guidance on Impurities in new drug substances*, CPMP/ICH/282/95 *Note for guidance on Impurities in new drug products*).

5.2. Excipients

The toxicology and pharmacokinetics of an excipient used for the first time in the pharmaceutical field shall be investigated. In principle, the same pivotal studies as for a new active substance should be performed.

In certain cases, studies with the active substance together with the excipient(s) used in the final product may be needed.

6. GENERAL RECOMMENDATIONS CONCERNING THE EXPERIMENTAL ANIMAL

6.1. Animal species

Within the usual spectrum of laboratory animals used for toxicity testing, the species should be chosen based on their similarity to humans with regard to pharmacokinetic profile including biotransformation. Exposure to the main human metabolite(s) should be ensured. If this can not be achieved in toxicity studies with the parent compound, specific studies with the metabolite(s) should be considered. When the product administered is a pro-drug, its conversion to the active substance should be demonstrated in the species under study.

Whenever possible, the selected species should be responsive to the primary pharmacodynamic effect of the substance.

In certain cases e.g. when the pharmacodynamic effect by itself will cause toxicity, studies in disease models may be warranted.

6.2. Sexes

Normally, equal numbers of male and female animals should be used.

6.3. Size of treatment groups

The size of the treatment group should be sufficient to allow meaningful scientific interpretation of the data generated. However, ethical considerations as well as practical aspects are also of importance. The following should be considered:

- Background knowledge concerning the ranges of variables to be studied in the species and strains used is also relevant for consideration of group size.
- In case of interim sacrifice, the size of the treatment groups should be large enough to permit the sacrifice of animals at intervals before the end of the study without interfering with the final statistical analysis.
- In case of a recovery period, the size of the treatment groups should be large enough to allow some animals to be retained at the completion of the period of dosing so that the reversibility of toxic changes at the end of the treatment may be evaluated

6.4. Number of species

In general, repeated dose toxicity studies shall be carried out in two species of mammals, one of which must be a non-rodent. The use of one species is acceptable if it has been unequivocally demonstrated that other available species are irrelevant as models for human safety assessment.

6.5. Animal husbandry

A high standard of animal husbandry is required. The environmental conditions should be controlled. The diet and water should be of known quality and composition throughout the study period. These conditions should be recorded in the report.

7. GENERAL RECOMMENDATIONS CONCERNING DOSE AND ADMINISTRATION

The dose regimen and route of administration should be chosen based on the intended clinical use with the aim to obtain sufficient exposure of the animals to the substance and its metabolites. In designing the study, all available information on pharmacodynamics, pharmacokinetics and toxicity of the medicinal product should be considered.

7.1. Duration of administration

The duration of repeated dose toxicity studies depends on the duration of the proposed therapeutic use in humans. When toxicity studies of three months duration or longer are needed, it is recommended that a repeated dose toxicity study of two or four weeks duration is carried out in such a way that it can serve as a dose-finding study for the longer term investigation.

7.2. Route of administration

In general, the medicinal product should be administered by the same route as that intended for humans. Other routes of administration may be selected, if justified on the basis of pharmacological, pharmacokinetic/toxicokinetic and/or toxicological information.

In addition to systemic toxicity, effects at the site of administration, and if different, the intended clinical site of administration should be evaluated.

7.3. Frequency of administration

The frequency of administration should be determined on a case-by-case basis taking account of the intended clinical dosing regimen and the toxicological/pharmacokinetic/pharmacodynamic profile of the test compound. Generally, once a day administration is adequate. In some cases more frequent administration in animals than anticipated in clinical use may be appropriate.

7.4. Dose levels

In general, the treatment should include:

- appropriate control group(s); in special cases a positive control group may be necessary
- a low dose, sufficient to produce a pharmacodynamic effect or the desired therapeutic effect, or result in systemic exposure comparable with that expected at the intended clinical use
- a high dose, selected to enable identification of target organ toxicity or other non-specific toxicity, or until limited by volume of dose
- an intermediate dose, such as the geometric mean between the high and the low dose.

Ideally, at the high dose level, the systemic exposure to the drug and/or principal metabolites should be a significant multiple of the anticipated clinical systemic exposure.

Dosing by incorporation of the test substance in the diet or drinking water will require regular adjustment of the amount of substance in the diet or drinking water to compensate for growth and changes in consumption.

Dose levels may need to be adjusted, if unexpected toxic responses or lack of responses occurs during the study.

When the medicinal product is administered via inhalation, the respirable dose should be determined.

Special care should be taken to eliminate contamination of the control group with the compound under study.

8. OBSERVATIONS

8.1. Pre-treatment and control values

For both rodents and non-rodents, historical control data should be available for the morphological, biochemical and physiological variables studied. In the case of non-rodents, pre-treatment values should be obtained from the animals used in the study.

8.2. Monitoring during the study

During the study, food intake, general behaviour, body weight, haematological parameters, clinical chemistry, urinalysis and ophthalmology should be monitored. Electrocardiographic recordings should be obtained in non-rodent species. Within each of the above-mentioned areas, relevant parameters should be selected to enable an identification of the toxicity profile. The parameters should be determined at relevant time points, taking the pharmacodynamic/pharmacokinetic profiles into account. In addition to final observations, these parameters should be monitored with a frequency that allows an assessment of changes over time. The selection of methodologies should be according to the current state-of-the-art [1]. In species where small numbers of animals are used, examinations should be conducted in all animals at all doses. In rodents, specialised examinations may be performed in a subset of animals at each dose level.

The examinations performed during the study should also be performed in the controls. The testing/sampling should not be performed in a way, which could influence the outcome and reliability of the study.

Animals that die or are sacrificed during the study should be autopsied and if feasible, subjected to microscopic examination.

8.3. Toxicokinetics

Information on systemic exposure of animals during repeated dose toxicity studies is essential for the interpretation of study results, for the design of subsequent studies and for the human safety assessment. For detailed guidance see Note for Guidance on Toxicokinetics: A Guidance for assessing systemic exposure in toxicology studies (CPMP/ICH/384/95). Analysis of blood samples of the control groups should be considered to check whether exposure due to contamination with the compound under study has occurred or not (see Guideline on the Evaluation of Control Samples in Nonclinical Safety Studies: Checking for contamination with the test substance; CPMP/SWP/1094/04).

[1] With respect to clinical pathology (i.e. haematology, clinical chemistry, urinalysis), the specific parameters to be monitored will depend on animal species and study design. Recommendations regarding core tests and standard sampling intervals can be found in the literature (e.g. Weingand et al, Fundam. Appl. Toxicol. 1996; 29:198-201).

8.4. Terminal monitoring

Terminal observations should be as complete as possible. Autopsy must be conducted on all animals. In non-rodent species where small numbers of animals are used, histopathology on the organs and tissues listed (Appendix A) should be conducted in all animals at all dose levels. In rodents, histopathology should be performed on all organs and tissues in Appendix A from the high dose and the control groups. Examination of the lower dosed groups may be restricted to those organs and tissues showing gross pathological changes at autopsy.

Furthermore, if histopathological changes are identified in the high dose group, lower dose groups should be examined to clarify the exposure/response relationship.

For specific guidance on the evaluation of the male genital tract, reference is made to the Note for Guidance on the Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility (CPMP/ICH/386/95; ICH S5).

Further histopathological examination may be necessary depending on the medicinal product tested.

In the case of CNS active substances, systematic histopathological examinations should be extended to the target cells or the CNS regions that are affected directly during treatment because of the receptor binding profile of the substance or other substance related pharmacodynamic effects (in addition to the structures listed in appendix A). If there are findings suggesting a specific neurotoxicity then further investigations should be conducted to identify and assess the damage and its functional consequences.

In studies conducted by the inhalation route, the lungs should be weighed in all animals and histopathological examination conducted on tissues taken from all exposed levels of the respiratory tract and from associated lymphoid tissue.

If needed, bone marrow cellularity, lymphocyte subsets and NK-cell activity or the primary antibody response to a T-cell dependent antigen should be monitored in at least one rodent study taking into consideration the signals from pharmacodynamics, toxicity studies and intended patient population, in accordance with the Guideline on Immunotoxicity of Human Pharmaceuticals (CPMP/ICH/SWP/167235/2004; ICH S8).

All tissues (see Appendix A) from all animals in the study should be conserved and wax blocks should be prepared. This material should be archived, and the site for archiving should be documented.

9. DATA ANALYSES, PRESENTATION OF RESULTS AND CONCLUSIONS

The study report should in an adequate and reliable way reflect all the raw data and information gathered during the course of the study. The study results should be analysed according to the state of the art, including relevant statistical analyses. Results should be presented in a clear and concise manner. Group summary values should be presented in a form that reflects the distribution of the variable. Individual values of all recorded parameters should be appended to the study report. Finally, a conclusion based on the study results should be drawn. Although statistics are important for the analysis of the data, interpretation of the results and conclusions drawn should be based on biological significance and plausibility.

10. REFERENCES

- Note for Guidance on Toxicokinetics: A Guidance for Assessing Systemic Exposure in Toxicology Studies (CPMP/ICH/384/95; ICH S3A).

- Guideline on the Evaluation of Control Samples in Nonclinical Safety Studies: Checking for Contamination with the Test Substance (CPMP/SWP/1094/04).

- Note for Guidance on Non-clinical Local Tolerance Testing of Medicinal Products (CPMP/SWP/2145/00).

- Note for Guidance on Non-clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals (CPMP/ICH/286/95; ICH M3).

- Note for Guidance on Duration of Chronic Toxicity Testing in Animals (Rodent and Non-rodent Toxicity Testing)) (CPMP/ICH/300/9; ICH S4B).

- Note for Guidance on the Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility (CPMP/ICH/386/95; ICH S5).

- Note for Guidance on Immunotoxicity Studies for Human Pharmaceuticals (CHMP/ICH/SWP/167235/2004; ICH S8)

APPENDIX

LIST OF TISSUES TO BE STUDIED HISTOLOGICALLY IN A REPEATED DOSE TOXICITY STUDY

Application site (when relevant)
Gross lesions
Tissue masses of tumours
Blood smears
Lymph nodes (mesenteric and any peripheral)
Mammary glands
Salivary glands (mandibular, parotid, sublingual)
Skeletal muscle
Sternebrae, femur or vertebrae (including bone marrow)
Pituitary gland
Thymus
Trachea
Lungs with bronchi and bronchioles
Heart
Aorta
Thyroid / Parathyroid glands
Oesophagus
Stomach
Small intestines
Large intestines (including Peyers Patches when relevant)
Liver
Gall-bladder (when relevant)
Pancreas
Spleen
Kidneys and ureters
Adrenal glands
Urinary bladder
Prostate
Testes
Epididymides
Seminal vesicles (rodents)
Ovaries
Uterus with uterine cervix and oviducts
Vagina
Brain (coronal sections at three levels to include cerebrum, cerebellum and brain stem)
Peripheral nerves
Eyes and optic nerves
Spinal cord

Skin and subcutaneous tissue
Joint with bone
Larynx
Tongue