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*Evaluation of Medicines for Human Use*

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**COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS  
(CPMP)**

**NOTE FOR GUIDANCE ON REPEATED DOSE TOXICITY**

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This Note for guidance (3BS2a), first adopted in October 1983, has been revised to update the guidance on immunotoxicity provided in section 6. It has also been updated to refer to the *Note for guidance on Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals* (ICH M3, CPMP/ICH/286/95) and other relevant ICH guidelines.

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# REPEATED DOSE TOXICITY

## 1. INTRODUCTION

### 1.1 Objectives

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 marketing authorisation.

This note for guidance should be read together with the general requirements set out in Council Directive 75/318/EEC, as amended. This document should also be read in conjunction with other CPMP Notes for Guidance, especially, CPMP/ICH/300/95 *Note for guidance on Duration of chronic toxicity testing in animals (Rodent and non-rodent toxicity testing)*, CPMP/ICH/384/95 *Note for guidance on Toxicokinetics: A Guidance for assessing systemic exposure in toxicology studies*, CPMP/ICH/286/95 *Note for guidance on Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals*.

### 1.2 Scope

This Note for guidance concerns the conduct of repeated dose toxicity studies of compounds intended for human use. For certain types of compounds, 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*).

## 2. 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.

## 3. GENERAL RECOMMENDATIONS ON SUBSTANCE QUALITY

### 3.1 Substance quality

Each batch used in the repeated dose toxicity studies should be identified. The physico-chemical 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 the same pattern of impurities as the product intended for marketing, when 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*).

### **3.2 Excipients**

The toxicology and pharmacokinetics of an excipient used for the first time in the pharmaceutical field shall be investigated (cf Council Directive 75/318/EEC, as amended). 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.

## **4. GENERAL RECOMMENDATIONS CONCERNING THE EXPERIMENTAL ANIMAL**

### **4.1 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.

### **4.2 Sexes**

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

### **4.3 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 to 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

### **4.4 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 (cf Council Directive 75/318/EEC, as amended). 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.

## 4.5 Animal husbandry

A high standard of animal husbandry is required (cf *The Council Directive on animal welfare 86/609/EEC and Council Decision on the European Convention on the protection of vertebrate animals, 1999/575/EC*). 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.

## 5. 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.

When toxicity studies of three months duration or longer are necessary, it is recommended that a repeated dose toxicity study of two or four weeks duration is carried out in such a manner that it can serve as a dose-finding study for the longer term investigation.

### 5.1 Duration of administration

The duration of repeated dose toxicity studies depends on the duration of the proposed therapeutic use in humans (see CPMP/ICH/286/95: *Note for guidance on Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals* and CPMP/ICH/300/95: *Duration of chronic toxicity testing in animals (Rodent and non-rodent toxicity testing)*).

### 5.2 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 (see *Note for guidance on Non-clinical local tolerance testing of medicinal products* Vol. III: Eudra/S/90/024).

### 5.3 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. In some cases more frequent administration in animals than anticipated in clinical use may be appropriate.

### 5.4 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.

## **6. OBSERVATIONS**

### **6.1 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.

### **6.2 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 (*see Note 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.

### **6.3 Toxicokinetics**

Information on systemic exposure of animals during repeated dose toxicity studies are essential for the interpretation of study results, to the design of subsequent studies and to the human safety assessment. For detailed guidance see CPMP/ICH/384/95 (*Note for guidance on Toxicokinetics: A Guidance for assessing systemic exposure in toxicology studies*).

## 6.4 Guidance on immunotoxicity

All new medicinal products should be screened for immunotoxic potential in at least one repeated dose toxicity study. The duration of the experiment(s) should be 28 days. If this is not feasible the immunotoxic potential should be screened in 14 days or 3 months studies. The interpretation of the initial immunotoxicity screen should be based on an integrative analysis of changes in lymphoid tissues and immune cell populations as well as other types of toxicity and the health status of the test animals.

If the initial screening phase suggests direct immunotoxicity, follow-on studies in animals may be warranted on a case-by-case basis to further study the altered immune response.

The design of extended animal studies will depend on the nature of the immunological changes observed in the initial screening phase. It should include comprehensive *in vivo* or *ex vivo* assays of immune function. For more detailed information see Appendix B.

## 6.5 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 CPMP/ICH/136/95 *Note for guidance on Reproductive toxicity: Toxicity on male fertility*.

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

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.

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 (for detailed information see Annex B).

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 known.

## 7. 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.

*Note 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).*

## **APPENDIX A**

### **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 (when relevant including Peyer's Patches)
- 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

## APPENDIX B

### GUIDANCE ON IMMUNOTOXICITY

Immunotoxicity concerns direct or indirect adverse effects on the immune system resulting from therapeutic exposure. It encompasses altered immunologic events including immune dysregulation (suppression or enhancement), allergy, and autoimmunity. Tiered testing strategies have been developed to assess this direct type of immunotoxicity (i.e. suppression or enhancement). Signs of direct immunotoxicity in either exploratory or extended animal studies may trigger the incorporation of immunotoxicological endpoints into the safety monitoring in clinical trials. This tiered approach is recommended for conventional medicinal products, but in general does not apply to either biotechnology-derived medicinal products (cf. *Note for guidance on Preclinical safety evaluation of biotechnology-derived pharmaceuticals*, CPMP/ICH/302/95) or vaccines (cf. *Note for guidance on Preclinical pharmacological and toxicological testing of vaccines*, CPMP/465/95).

#### INITIAL SCREENING PHASE

The initial screening phase consists predominantly of non-functional parameters. Addition of functional parameters will increase the predictive value of the screening phase and is therefore encouraged. The initial screening phase can be incorporated within at least one standard repeated dose toxicity study. Immunotoxicity should be incorporated in a 28 days study, if this is not feasible 14 days or 3 months studies are acceptable. Rats or mice are the species of choice, unless another species are better justified.

The initial immunotoxicity screen consists of the following parameters: haematology (i.e. differential cell counting) lymphoid organ weights (i.e. thymus, spleen, draining and distant lymph nodes), microscopy of lymphoid tissues (i.e. thymus, spleen, draining and distant lymph nodes, Peyer's patches), bone marrow cellularity, distribution of lymphocyte subsets and NK-cell activity. If the latter two are unavailable the initial screening phase should be completed with the primary antibody response to a T-cell dependent antigen (e.g. sheep red blood cells).

Changes in the above mentioned parameters may be a trigger for additional testing.

Interpretation of the initial immunotoxicity screen should be based on an integrative view of changes in lymphoid tissues and immune cell populations as well as taking into account other types of toxicity and the health status of the test animal. For instance, involution of the thymus in the presence of overt systemic toxicity may be too easily explained as a secondary (stress-related) response to deterioration of the general health status. It may instead reflect a direct immunotoxic insult.

#### EXTENDED STUDIES

The primary aims of extended animal studies are to define the immunotoxicity and the dose-response relationship in order to facilitate risk assessment. Additionally they may indicate the target cell population(s) involved. The extended studies consist of functional assays, however, if data on lymphocyte subset distribution and NK cell activity are not available at this time, they should be considered as part of the extended studies. The design of extended animal studies will depend on the nature of the immunological changes observed in the initial screening phase. A scientifically motivated choice should be made from the following test parameters:

- delayed-type hypersensitivity (DTH)
- mitogen- or antigen-stimulated lymphocyte proliferative responses
- macrophage function

- primary antibody response to T-cell dependent antigen
- *In vivo* models of host resistance, these may be employed to detect increased susceptibility to infectious agents (bacteria, parasites or viruses) or tumours. Such models are particularly important for risk assessment, as they may be tools to elucidate the actual consequences of disturbed immune function.