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

NOTE FOR GUIDANCE ON CARCINOGENIC POTENTIAL

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Note:

This Note for Guidance (3BS7a), first adopted in 1983, has been updated to refer to the ICH-guidelines on carcinogenicity testing (S1A, CPMP/ICH/140/95; S1B, CPMP/ICH/299/95; S1C, CPMP/ICH/383/95 and S1CR, CPMP/ICH/366/96) and other relevant ICH guidelines.

1. INTRODUCTION

The objective of carcinogenicity studies is to identify a tumourigenic potential in animals as part of the assessment of the relevant risk in humans.

1.1 Objective of the guideline

The purpose of this guideline is to give guidance particularly on the practical features of the conduct of long-term carcinogenicity studies, but also on statistical design and the principles of reporting and analysis of the data.

This note for guidance concerns the application of chapter I (E) of part 2 of the Annex to Directive 75/318/EEC, with a view to the granting of a marketing authorisation for a new drug.

1.2 Scope of the guideline

This guideline concerns carcinogenicity studies of pharmaceuticals and should be read in conjunction with ICH-guidelines on carcinogenicity testing (S1A, CPMP/ICH/140/95; S1B, CPMP/ICH/299/95; S1C, CPMP/ICH/383/95; S1CR, CPMP/ICH/366/96), the ICH Note for Guidance on preclinical safety evaluation of biotechnology-derived pharmaceuticals (S6, CPMP/ICH/302/95) as well as the ICH Note for Guidance on the Assessment of Systemic Exposure in Toxicity Studies (S3A, CPMP/ICH/384/95).

This note for guidance is not applicable to short and medium term carcinogenicity studies mentioned in the ICH S1B document.

2. STUDY DESIGN

2.1 Animals

The choice of species should be appropriate, based on considerations outlined in ICH-guideline S1B.

Carcinogenicity studies should commence as soon as possible after weaning, i.e. as soon as the animals are accustomed to their diet and surroundings.

Animals should be specific pathogen free, in good general health initially and this should be maintained throughout the study. High standards of animal husbandry are essential.

Animals should be housed under standardised environmental factors, such as humidity and light/dark cycles, as well as diet, manner of feeding, and drinking water, which should be specified and documented.

2.2 Test substance

The characteristics of the test substance, including impurity profile and stability, should be clearly characterised prior to the study and documented in the final report.

2.3 Dosing and dose levels

Carcinogenicity studies should normally be conducted at three dose levels. These should be selected based on the considerations outlined in ICH-guidelines S1C and S1C-R.

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

2.4 Duration of studies

The currently recommended practice is to conduct life-span studies in the rat (usually 24 months). If another species is more appropriate the recommended duration is minimally 18 months in the mouse and 18 months in the hamster.

2.5 Number of animals per group

For routine tests in the species mentioned under 2.4 it is suggested that for each sex there should initially be at least 50 animals per treated group, and one control group of the same number for each sex dosed with the vehicle by the same route. If animals for interim sacrifice are included in the study plan, the number of animals scheduled for the interim sacrifices should increase the initial number. The initial number should be adjusted upwards if necessary to take account of the expected survival of the animals.

The survival characteristics of the chosen strain of animal should be considered when determining group size. Approximately 25 animals per sex per group is desired at the scheduled terminal necropsy for histopathological evaluation. However, this number may be smaller if most deaths occur late in the study (e.g. >90 weeks), and if adequate histopathological evaluation is possible on the majority of animals that die or are sacrificed moribund during the study, since tumour incidence is statistically evaluated on a survival weighted basis.

2.6 Historical control data

The concurrent control group should always be the primary reference with respect to treatment-related tumourigenicity.

If historical control data are used, they must be primarily derived from the same strain and testing facility. Data should have been obtained from several studies during the last 5 years prior to the study, taking into account genetic drift. Data from literature might be added if thought to be informative.

2.7 Allocation and randomisation of animals

The cages containing the animals in the treatment and control groups should be distributed within the animal house so as to eliminate bias due to the effects of any local environmental factor.

Animals should be allocated at random to the various experimental units (e.g. cages or cage levels) and the method used to achieve this randomisation should be clearly stated.

If large numbers of animals are used, and it is decided to stagger the start of the study it is desirable that all groups should be represented at each start in equal numbers. If the study is conducted using a staggered start, the times at which the various batches of animals enter the study must be stated.

2.8 Diet

Commercial diets are variable and steps should be taken to provide as uniform a diet as possible throughout the duration of the carcinogenicity study. Full specification (including important minerals) of the diet should be given.

Dietary restriction should be considered if a low survival rate is anticipated.

3. ROUTINE MONITORING

Body weights, food consumption, overt signs of toxicity, palpable masses and ophthalmoscopy should be routinely monitored.

Monitoring of biochemical and haematological parameters as well as urinalysis should be considered during the study and should be performed at study termination.

4. ADDITIONAL MONITORING

The primary purpose of a carcinogenicity study is to evaluate a drug's tumourigenic potential. However, studies should be designed to obtain the maximum amount of information from the animals used.

If a specific effect is expected on the basis of the properties of the compound then additional groups might be helpful for monitoring toxicological effects at an earlier time point supporting the mechanism of induction of tumours observed.

Information on systemic exposure to the parent drug (and metabolites) should be provided (cf. ICH-guideline S3A)

Analysis of blood samples of the control groups should be considered to check that exposure by contamination with the compound under study has not occurred.

Mechanistic studies are important for the interpretation of tumour findings in a carcinogenicity study. The need for an investigative study will be dictated by the particular properties of the compound and/or the specific results from the carcinogenicity testing. Suggestions of investigative mechanistic studies are given in ICH-guideline S1B.

5. TERMINAL INVESTIGATIONS

5.1 Necroscopy

A full necropsy should be made on all animals dying during the study or killed in extremis. Euthanasia in extremis is preferred to reduce the suffering of the animal and to prevent autolysis.

At the conclusion of the study all surviving animals should be sacrificed and a full necropsy conducted on each animal. Previously demonstrated toxic effects may indicate particular areas for investigation.

5.2 Histopathology

Listed tissues (see Appendix) from all animals in all groups killed during or at termination of the study should be examined microscopically. This list defines the maximum number of tissues that should be fixated and embedded at necropsy for histopathological examination in order to have tissues from all groups available if later during necropsy specific tissues show deviations. The list further specifies the minimum number of tissues, which should be examined microscopically.

In addition, any additional tissues in which macroscopic lesions are present, including tissue masses found at necropsy should also be examined microscopically.

Tissue processing should allow standard and special stains, in particular stains allowing immunohistochemical determination of tumour origin. Conditions of tissue trimming (number of sections, section size, presence of critical organ and tissue features, etc.) should be carefully considered. For tissue accountability, internal record standards should be applied and reported.

6. REPORTING ON CARCINOGENICITY STUDIES

6.1 General principles

Pre-neoplastic and neoplastic lesions should be described in conventional histopathological terms according to commonly used classifications (e.g. ILSI, STP, IARC, RENI and other recent texts on rodent pathology). Deviations from standard diagnoses should be explained in the report.

Ideally, one pathologist should be responsible for the histological evaluation. If several pathologists are involved, slides from all treatment groups must be distributed evenly among them. Peer-review of slides is required for all identified target organs and for at least 10% of all tumours. A complete review of 10% of the animals in each group should also be performed. If more than one pathologist is involved more extensive peer review is needed to assure consistency. The peer review should be documented in raw data and in the study report. Board certification or equivalent should qualify pathologists.

6.2 Presentation of the data

Findings should be presented for each treatment group and each control group separately, keeping the sexes separate, in terms of:

- number of animals examined and their individual gross and microscopic examination
- numbers of animals with tumours of each identified type in a specified tissue, distinguishing malignant from benign tumours, wherever possible
- time to each unscheduled death
- time of appearance of any mass (documented by clinical palpitation) and its progress, as well as its eventual histopathology

7. ANALYSIS OF THE DATA

The form of the analysis and the tests of statistical significance used should be appropriate to the type of data and to the basic experimental design. The statistical procedures used should be clearly stated.

The following types of responses can be addressed:

- occurrences of neoplastic lesions (and nonneoplastic lesions if they are related)
- number of animals at risk and examined
- the incidence of combined tumours of common histiogenic origin, if applicable
- the incidence of tumours judged to be malignant
- the sum of benign and malignant tumours in the same tissue when applicable
- the latent period to tumour appearance (using actuarial approaches)

The analysis should be directed towards:

- the assessment of the presence of any effect of the substance under study, as shown by the contrast between the response in the three treatment groups, separately and as a set, and the response in the control group,
- the assessment of whether any effect is dose-related, as shown by a trend in the responses in the low-, mid- and high-dose groups, This assessment is statistically independent of the assessment of treatment-related effect.

If historical control data are used to support the interpretation they should be included in the study report.

Any increase in tumour incidence should also be interpreted in the light of the historical incidence of that tumour. The occurrence of a rare tumour, even at statistically non-significant levels, may be viewed as being of biological significance. Similarly, a tumour causing an increase in premature deaths, although not showing an overall significant increase, may be considered to be of biological significance.

The influence of other factors, such as deaths of test animals because of other diseases, and premature killing of animals because of clinical detection of tumours, should be statistically analysed if applicable. The particular tests of statistical significance, which should be used in assessing the presence of an effect or a dose-relationship, are intentionally not specified in this note for guidance.

The test substance should be regarded as having the potential to increase the risk of neoplastic in the test species change if any of the above responses is materially increased (or the latent period is materially decreased). The compound may be regarded as possessing more powerful activity for the animal if several of the above responses are affected and if there is evidence of a dose-response as well as the presence of the effect. An increased incidence of tumours in treated as compared with control animals is of significance for the conclusion of the study whatever the mechanism postulated or defined for their development.

In view of risk assessment those increased incidences should be interpreted in light of the overall weight of evidence including pharmacological effects of the compound, genotoxicity findings, changes in husbandry, diet, background health status of the animals etc.

Other findings that should be discussed include:

- an increased incidence or reduced latency of malignant tumours
- an increased incidence of benign tumours
- the local induction of tumours at the site of injection
- the biological significance of tumour increases, e.g. an increased incidence of tumours rarely seen in controls and an increased incidence of tumours at very high systemic exposure only

Conclusions from the studies should be included in the study reports.

References:

- ICH Guideline S1A Note for Guidance on the need for Carcinogenicity Studies of Pharmaceuticals (CPMP/ICH/140/95)
- ICH Guideline S1B Note for Guidance on Carcinogenicity: Testing for Carcinogenicity of Pharmaceuticals (CPMP/ICH/299/95)
- ICH Guideline S1C Note for Guidance on Dose Selection for Carcinogenicity Studies of Pharmaceuticals (CPMP/ICH/383/95)
- Addendum S1C(R) Note for Guidance on Dose Selection for Carcinogenicity Studies of Pharmaceuticals: Addition of a Limited Dose and related Notes (CPMP/ICH/366/96)
- ICH Guideline S6 Note for Guidance on preclinical safety evaluation of biotechnology-derived pharmaceuticals (CPMP/ICH/302/95)
- ICH Guideline S3A Note for Guidance on the Assessment of Systemic Exposure in Toxicity Studies (CPMP/ICH/384/95).

Tissue list

List of Tissues to be Examined Histopathologically in Carcinogenicity Studies (for All Species, Where Applicable)

Adrenal Gland
Aorta
Bone, femur with articular cartilage (* bone marrow)
Brain inclusive cerebellum (3 levels)
Cecum
Colon
Duodenum
Epididymis
Esophagus
Eye ball with optic nerve (* harderian gland)
Gallbladder
* Glandula mandibularis/glandula parotis/glandula sublingualis
Heart
Ileum
Jejunum
Kidney
Larynx
Liver
Lung
Lymph node(s)
Mammary Gland (Females only). (* males)
* Nasal cavity with nasopharinx and paranasal sinus
Oesophagus
Ovary with oviduct
Pancreas
Parathyroid Gland
Peripheral Nerve
Pituitary
Preputial/clitoral gland
Prostate
* Rectum
Salivary Gland
Seminal Vesicle
Skeletal Muscle
Skin
Spinal Cord
Spleen
Stomach
Testis
Thymus
Thyroid Gland
Tongue
Trachea
Urinary Bladder
Uterus
Vagina
* Zymbal gland with external ear

Other organs or tissues with gross lesions

Tissue masses (tumors)

* These tissues should be fixated and embedded at necropsy for histopathological examination if needed