COUNCIL RECOMMENDATION

of 26 October 1983

concerning tests relating to the placing on the market of proprietary medicinal products

(83/571/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community, and in particular Article 235 thereof,

Having regard to the proposal from the Commission,

Having regard to the opinion of the European Parliament (1),

Having regard to the opinion of the Economic and Social Committee (2),

Whereas Council Directive 65/65/EEC of 26 January 1965 (3) led to the approximation of provisions laid down by law, regulation or administrative action relating to proprietary medicinal products in the Member States; whereas Directives 75/318/EEC (4) and 75/319/EEC of 20 May 1975 (5) continued this approximation and developed the principles set out in Directive 65/65/EEC;

Whereas, in particular, Council Directive 75/318/EEC lays down a general framework for the testing of proprietary medicinal products, lists the various types of tests to be conducted and defines certain principles to be followed in the examination of applications for authorizations to place a proprietary medicinal product on the market;

Whereas experience has shown that the conduct and content of these tests should be defined more precisely so as to make possible an identical implementation of the Community Directives when such tests are carried out and when applications are examined by national authorities;

Whereas Notes for guidance are therefore necessary in order to prevent differences of interpretation in the implementation of the standards and protocols referred to in Directive 75/318/EEC; whereas this will help to promote the free movement of proprietary medicinal products;

Whereas tests designed to evaluate the quality, safety and efficacy of proprietary medicinal products must be constantly adapted to take account of the latest scientific and technical knowledge without, however, giving rise either to a waste of resources or to the more than necessary use of laboratory animals;

Whereas it is therefore highly desirable that the Notes for guidance be periodically revised to take account of the state of the art and that new Notes for guidance be drawn up as and when required, in agreement with the national authorities;

Whereas such progress in harmonization, essential at Community level, will also promote international recognition of tests on medicinal products conducted in accordance with these Notes and will therefore tend to render unnecessary the repetition of tests on products intended for export to third countries;

Whereas the Pharmaceutical Committee and the Committee for Proprietary Medicinal Products have been consulted on the measures contained in this recommendation,

HEREBY RECOMMENDS THE MEMBER STATES TO:

1. Ensure that, in the conduct of tests and in the presentation of results, applicants for authorization to place proprietary medicinal products on the market comply with the principles and adhere to the methodology set out in the Notes for guidance annexed hereto.

2. Examine and evaluate, in accordance with the Notes for guidance, all applications for marketing authorization.

Done at Luxembourg, 26 October 1983.

For the Council

The President

G. MORAITIS

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(1) OJ No C 287, 9. 11. 1981, p. 127
(2) OJ No C 189, 30. 7. 1981, p. 39.
(3) OJ No 22, 9. 2. 1965, p. 369/65.
(4) OJ No L 147, 9. 6. 1975, p. 1.
(5) OJ No L 147, 9. 6. 1975, p. 13.

ANNEX I

REPEATED DOSE TOXICITY

Note for guidance concerning the application of chapter I (B) (2) of part 2 of the Annex to Directive 75/318/EEC, with a view to the granting of a marketing authorization for a new drug

1. INTRODUCTION

The purpose of these studies is to obtain information on the toxicity of a product when repeated exposure to this medicinal product is anticipated, in order that an assessment may be made of the risk resulting from therapeutic administration of the product, taking into account the products of biotransformation.

The duration of these studies will be determined by the proposed use in man or by the intended duration of human exposure. The following periods of administration are suggested as a guidance to correlate the duration of the repeated dose toxicity studies with the proposed duration of human exposure to the drug:

Proposed duration of human treatment	*Suggested duration of repeated dose toxicity studies*
One or several doses within one day	two weeks
Repeated doses for up to seven days	four weeks
Repeated doses for up to 30 days	three months
Repeated doses beyond 30 days	six months

When human exposure is likely to be longer term, for example when frequent discontinuous administration results in a total period of exposure of one month or more in a period of one year, or when retention in the body of a single dose of the drug is prolonged, then the duration of the repeated dose toxicity studies will be six months.

When it is necessary to conduct toxicity studies of three or six months duration, a subacute toxicity study of two or four weeks duration may be designed and carried out in such a manner that it acts as a range-finding study for the longer term investigation (see 2.5).

The reason is that administration of too high a dose would leave too few animals alive at the end of the study lasting three months or more; administration of too low a dose would prevent the development of toxic changes.

1.1. General specifications with regard to the repeated dose toxicity study

In the Annex to Directive 75/318/EEC, part 2 concerning toxicological and pharmacological tests states that these tests should show *inter alia* the toxicity thresholds:

'Repeated dose toxicity tests are intended to reveal any physiological and/or pathological changes induced by repeated administration of the active substance or combination of active substances under examination, and to determine how these changes are related to dosage.

Generally it is desirable that two tests be performed: one short-term, lasting two to four weeks, the other long-term. The duration of the latter shall depend on the conditions of clinical use. Its purpose shall be to determine by experiment the non-toxic dose range of the product'.

As these thresholds can only be determined when they have been crossed the repeated dose toxicity study must be conceived in such a way as to demonstrate signs of toxicity. This implies that:

1.1.1. In the selection of species for long-term studies prior to marketing, it is desirable that, with regard to the metabolism and the pharmacokinetics of the drug, the species should be as closely similar to man as is possible within the usual spectrum of laboratory animals used for repeated dose studies. If there are significant differences in metabolism these should be taken into account in evaluating the results.

1.1.2. It is desirable that the pharmacological target organs and the pharmacological effects of the product in the species used should be the same as those involved in the therapeutic effect envisaged for man where this is known and is practical.

1.1.3. The dosage, the route and the frequency of administration should be planned so as to promote a loading of the animal organism with the product and its metabolites sufficient to demonstrate the target organ(s) in terms of harmful secondary effects. In designing the protocol, account will be taken of the pharmacokinetics of the drug.

By continuous administration of a drug in sufficiently high doses the following principal stages may be reached:

(a) the load of the drug in the organism builds up until a steady state is reached;

(b) adaptation of the organism to the load with regard to the pharmacological target organs or the biotransformation enzymes or the excretory mechanisms;

(c) in some cases a second metabolic pathway may be activated due to an overloading of the primary detoxication pathway; as a result a new toxic metabolite may be formed;

(d) a phase of manifestation of target organ toxicity manifested by failure of physiological functions and ultimately by pathological changes.

Administration of the product in the repeated dose toxicity studies should be sufficient with regard to dosage and duration to reach this final stage (d) so that the type of toxicity produced by an excessive dose and the multiple of the therapeutic dose producing toxicity may be assessed. Not all drugs can be practically administered in dosages that produce target organ toxicity. Under such circumstances, evidence should be produced that the highest possible dose has been given and that the drug has been systemically absorbed.

2. SPECIFICATIONS WITH REGARD TO THE DRUG AND ITS ADMINISTRATION

2.1. Drug quality

The active substance should present the same pattern of impurities as the product to be marketed, when possible. Should drug substance in the final dosage form be shown to have impurities significantly different either in quantity or quality from those in the test batch then further steps should be taken to ascertain their possible toxicity. When the drug is given orally its physical characteristics such as particle size may be important; therefore the physical characteristics and stability of the material used in the repeated dose toxicity studies should be stated in each case. Whenever more than one batch of active substance is used in repeated dose toxicity studies this must be stated and each batch identified. The batch or batches used in the repeated dose toxicity studies should not be of a higher degree of purity than those intended for the final product. When the drug is given in the diet or the drinking water it should be established that it is stable in that medium.

When a new excipient is used for the first time, it should be tested in accordance with the same criteria as a new active substance.

2.2. Duration of administration

The duration of these studies should be related to the duration of the proposed therapeutic use in man (see 1).

2.3. **Route of administration**

Whenever this is technically feasible, the product should be administered by the route intended for use in man and it is desirable that the pharmacological effect is demonstrable by this route. When this cannot be shown, the use of other routes should be considered. (Administration by inhalation is discussed in the Appendix.) The quantity of drug absorbed from the proposed site of administration should be known from pharmacokinetic studies. When the product is administered in the food or the drinking water, the applicant must give assurance that a reasonable and known amount of the drug ingested is absorbed. Dosing by incorporation of the test substance in the diet or drinking water requires regular adjustment of the amount of drug in the diet or drinking water to compensate for growth and changing consumption.

In addition to systemic toxicity the possibility of local toxicity at the site of application should be given due attention, for example in the case of application to the skin, intravaginal application, intravenous, intramuscular, rectal, subcutaneous, intra-articular, intrathecal, conjunctival, intranasal and aural application, or when the drug is given by inhalation.

2.4. **Frequency of administration**

The steady state of the loading of the organism with some products is reached only when they are administered for seven days a week. Usually the administration of a drug to animals should be conducted on this basis. If this is not possible the reason should be given. When the rate of elimination is slow, less frequent administration may be acceptable. A rapid rate of elimination or gastric intolerance may make it necessary to administer the products more than once a day.

2.5. **Dose levels**

The treatment should include:

(a) a high dose, selected for the purpose of causing target organ toxicity whenever possible or, failing this, other non-specific toxicity, or until limited by volume of dose;

For a toxicity study of three or six months duration this dose should be derived from a subacute toxicity test of two or four weeks duration, designed and carried out as a dose range-finding study;

(b) a low dose, sufficient to produce a pharmacodynamic effect or the desired therapeutic effect, or to produce blood levels comparable to those expected to produce these effects in man;

(c) an intermediate dose, such as the geometric mean between the high dose and the low one;

(d) The study should include appropriate control group(s); in special cases a positive control group may be necessary.

However, the above considerations do not apply when the pharmacodynamic effect by itself will cause toxicity; hypoglycaemia by antidiabetic agents serves as an example.

The investigator should indicate the rationale on which the dose levels were selected.

2.6. **Pro-drugs**

When the product administered is a pro-drug, its conversion to an active drug should be demonstrated in the species under study.

3. SPECIFICATIONS PERTAINING TO THE EXPERIMENTAL ANIMAL

3.1. Animal species: choice and characterizations

As far as possible, the species should be chosen on the basis of their similarity to man with regard to the pharmacokinetics including the biotransformation of the product (see 1.1.1).

The pharmacodynamic effect of the drug should, if possible, be demonstrated in at least one of the species, so as to provide information about the margin between therapeutic and toxic effects.

The investigator should justify the choice of the species and strain. Use of SPF animals usually increases the value of the study.

3.2. Sexes

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

3.3. Size of treatment groups

When the size of the treatment groups is being considered, attention should be given to the following:

(a) the size of the treatment groups should be such that all toxicologically important effects due to treatment will be revealed;

(b) 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;

(c) 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;

(d) background knowledge concerning the ranges of variables to be studied in the species and strains used is also relevant to consideration of group size.

However, the size of control and treatment groups will always be limited for practical and financial reasons and for humane considerations.

3.4. Number of species

The purpose of the repeated dose toxicity studies is to provide an animal model for the repeated administration of the product to man. The value of the model for extrapolation to man depends to a large extent on the qualitative similarity between the animal model and man; this is usually unknown. To reduce the risk of error in extrapolation, due to effects or lack of effects, which are peculiar to one species, at least two species should be used, one being a non-rodent. The choice of species should be justified (see 1.1.1).

4. ANIMAL HUSBANDRY

A high level of animal husbandry is required and the environmental conditions should be controlled and the diet should be of known constant composition throughout. Measures taken to obtain these conditions should be recorded in the report.

5. OBSERVATIONS

5.1. Pre-treatment and control values

Control data from the colony are necessary for small mammals for all morphological, biochemical and physiological variables. In the case of larger animals, pre-treatment values should be obtained from the animals used in the study.

5.2. **Monitoring during the study**

5.2.1. *General monitoring*

General monitoring should be carried out during the study and should include food intake, body weight, haematology, clinical chemistry, urinanalysis, ophthalmology, ECG and general behaviour. The selection of techniques used and the choice of other tests should be appropriate to the current state of knowledge and to the animal species being used. In rodents, if ECG and ophthalmological or other specialized examinations are required, it is acceptable that these are conducted in a limited number of animals at each dose level.

5.2.2. *Frequency of monitoring*

The frequency of monitoring in addition to pre-treatment studies and final monitoring should be adapted to the manifestations of toxicity and also to the pharmacokinetics of the drug. The tests performed (including the collection of blood samples) during the monitoring should also be performed on the controls, and should not affect the experimental animals in a way which would influence the final interpretation of the toxicity test results.

5.2.3. *Food intake*

When products are administered in the food, particular attention should be given to the effect of the product on food consumption. Allowance should be made for consequent effects on drug intake.

5.3. **Terminal monitoring**

Terminal observations should be as complete as possible. Autopsy must be conducted on all animals. Histopathology should be performed on all organs and tissues of the high dose and the control groups listed in the table. In rodents the examination of the lower dosed groups may be restricted to those organs and tissues showing pathological changes at autopsy. In other species where small numbers of animals are used, histopathology on those tissues listed should be conducted in all animals at all doses.

If organs are not examined microscopically, wax blocks or slides should be prepared and conserved for five years from the date of marketing for examination if required. Peculiarities in the distribution of the drug may necessitate further histopathological studies.

6. IMMUNO-INTERFERENCE

The expansive growth of immunology and the recognition of its importance has made it necessary to pay attention to interference with the immunologic system by drugs even when this does not belong to their intended activity. Such an interference may cause undesired side effects (interference with infection; carcinoma). Therefore it is particularly important to examine the spleen, the thymus and some lymph nodes macroscopically and microscopically at the termination of the toxicity study. These should indicate any effect on the immune system and thus the need for further tests.

Since our present knowledge in this field is rapidly increasing, any test used to investigate the immunological effects of a drug should rely on the state of the scientific knowledge at that moment.

7. CONCLUSIONS

Conclusions should be drawn from these studies by the investigator.

Appendix A

List of tissues to be studied histologically in a repeated dose toxicity study

— Gross lesions

— Tissue masses or tumors (including regional lymph nodes)

— Blood smears (in case of anaemia, enlarged thymus, lymphadenopathy)

— Lymph nodes

— Mammary glands

— Salivary glands

— Sternebrae, femur or vertebrae (including bone marrow)

— Pituitary

— Thymus

— Trachea

— Lungs

— Heart

— Thyroid

— Oesophagus

— Stomach

— Small intestine (Swiss roll method)

— Colon

— Liver

— Gall-bladder

— Pancreas

— Spleen

— Kidneys

— Adrenals

— Bladder

— Prostate

— Testes

— Ovaries

— Uterus

— Brain (coronal sections at three levels)

— Eyes

— Spinal cord

Appendix B

Conduct of toxicity studies by inhalation

1. INTRODUCTION

Medicinal products intended for administration to humans by inhalation may either be aerosols containing the pharmacologically active substance in solid or liquid state or they may be vapors or gases. The latter mentioned products are used as inhalation anaesthetics while aerosols in general contain a drug material in the form of particles delivered in a propellant which in principle ought to be biologically inactive.

Toxicological studies conducted by inhalation are necessary where:

(a) pharmacokinetics after administration by inhalation may differ qualitatively or quantitatively from the pattern after other routes of administration; or

(b) drug and propellant may interact in the body; or

(c) the inhaled product may have a local effect in the airways, either a short-term effect (effect on ciliary function or other signs of local irritation) or a long term effect (emphysema, bronchitis, malignancy).

Aerosols are used for the administration of drugs either (i) to obtain a local effect in the respiratory system, or (ii) for the purpose of obtaining systemic effects by using the lining of the airways for absorption of the active compound or (iii) for circumventing the alteration of drugs in the gastro-intestinal tract.

In some circumstances toxicological studies on the drug may have been performed using other routes of administration, therefore extensive toxicological investigation by other routes of administration may already have been performed when inhalational studies are planned. In other cases, for example, locally acting compounds, such as mucolytic agents, toxicological investigation by other routes of administration may be non-existent or of little relevance. Planning of the toxicology studies by inhalation should take into consideration any already existing toxicological or pharmacological knowledge of the substance.

2. PHYSICO-CHEMICAL PROPERTIES

The information of the active substance's physico-chemical properties should be provided in the same way as for any other toxicological study. Additional information should be provided on the characteristics of the aerosol, which should include the distribution of the particle or droplet size of the active substance and the physico-chemical specification of the substance or substances used as the propellant.

The propellant system used in these studies should be that proposed for the final product. If a novel propellant system is used, this should itself be investigated to the same standard as a new active substance.

3. DOSING SCHEDULES

3.1. **Administration**

The method of administration depends on the nature of the substance and the intended use in man. In acute studies it may be reasonable to administer the substance directly into the airways via a nasotracheal tube or through a tracheotomy. In this way the quantity administered can be determined directly.

In the case of long-term exposure studies it will usually be necessary to use either 'head only' or 'nose only' exposure chambers or masks for inhalation. If whole body exposure is used,

deposition of the drug on the skin, in the pelt, in the upper airways and the amount swallowed should be taken into account in determining the dose of substance administered.

It should be demonstrated that the method of administration ensures that the substance reaches the desired site.

3.2. **Dose levels**

In these studies, normally three dose levels and one or more control groups as appropriate should be used in both single dose and repeated dose studies. Different levels of drug exposure can be achieved by alteration of concentration of substance inhaled or by alteration of the duration of exposure. In the selection of dose levels the same principles should apply, as far as is possible to those used for toxicity studies by other routes.

The reasons for selecting particular dose levels should be given.

3.3. **Duration of study**

The duration of the study should be related, at least to some extent, to the proposed human exposure.

4. PHARMACOKINETICS AND METABOLISM

The metabolic pattern of the drug administered by inhalation may differ from the pattern observed following other routes of administration. The investigator should determine whether there are any pharmacokinetic or metabolic differences of relevance for the interpretation of the toxicological studies conducted by the inhalation route.

When biotransformation occurs in the lung itself, the possibility of enzyme induction of this process should be taken into consideration.

5. ANIMALS

The experimental animals used in these studies should be free of pulmonary infection and have a low incidence of other pulmonary pathology.

The number of experimental animals to be used in each group should be adequate for statistical analysis and will be determined by the duration of the experiment and by the number of observations, measurements and interim sacrifices to be made during the exposure periods. At least one rodent and one non-rodent species should be used for all repeated exposure studies.

6. OBSERVATIONS

Interim monitoring and terminal studies should be conducted as indicated for other toxicity studies. Particular attention should be paid to any local effects. If the drug is to be administered repeatedly, special studies of ciliary function and on the microflora may be necessary.

Blood-level monitoring of drug and propellant or other methods of assessing absorption of drug and propellant should be performed at intervals during repeated dose studies.

Terminal examination

At the conclusion of the study all animals should be subjected to autopsy and examination of tissues should be conducted as with other toxicological studies.

In studies conducted by the inhalational 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.

7. PRESENTATION OF RESULTS AND CONCLUSIONS

These should be drawn up in the same manner as in other toxicity studies and the investigator should draw appropriate conclusions from the study.

ANNEX II

REPRODUCTION STUDIES

Note for guidance concerning the application of chapter I (C) and (D) of part 2 of the Annex to Directive 75/318/EEC, with a view to the granting of a marketing authorization for a new drug

GENERAL STATEMENT

The study of drug effects on reproduction should be conducted on all new drugs in such a manner as would reveal the presence of any effect on mating behaviour and of any effect which might result in fetal loss, fetal abnormality and damage to the offspring in later life, e.g.:

(i) changes in fertility or in the production of abnormal young due to damage to the male and/or female gametes;
(ii) interference with preimplantation and implantation stages in the development of the conceptus;
(iii) toxic effects on the embryo;
(iv) toxic effects on the fetus;
(v) changes in maternal physiology producing secondary effects on embryo or fetus;
(vi) effects on uterine or placental growth or development;
(vii) interference with parturition;
(viii) effects on postnatal development and suckling of the progeny, and on maternal lactation;
(ix) late effects on the progeny.

SPECIFIC GUIDELINES

In the interpretation of the following notes for guidance it must be appreciated that they are not rigid requirements and may not be universally applicable. Interpretation should therefore be flexible and related to the proposed use of the drug; justification for choice of studies must be given.

1. SELECTION OF SPECIES

Embryotoxicity studies should normally be conducted on two mammalian species one of which should be other than a rodent. Fertility and perinatal studies should be conducted in at least one species. Where metabolism of a drug in a particular species is known to be similar to that in man, it is desirable to include this species. It is desirable that one of the species is the same as in the long term toxicity studies. Studies in a third species may be helpful if conflicting results are obtained in the initital two species. The species and strains used in the studies should be specified.

2. DOSAGE

Dosing should normally be conducted at three dose levels. The high dose usually should be such that evidence of some maternal toxicity is produced, for example decrease in body weight gain. The low dose should be sufficient to produce a pharmacodynamic effect similar to the desired therapeutic effect, or to produce blood levels comparable to those required to produce the effect (this does not apply if the pharmacodynamic effect by itself will cause toxicity). The intermediate dose should be the geometric mean of the high and low doses.

Dosing should be conducted by the proposed route or routes of clinical administration.

Dosing schedules for investigation of drug effects on reproduction should normally include:

(a) embryotoxicity studies; dosing throughout the period of embryogenesis (organogenesis) in two species one of which should be other than a rodent;

(b) a fertility study which should be conducted in at least one species; dosing should commence in male and female animals at a sufficient time before the proposed mating so that any effects of the drug on gametogenesis could be revealed. Dosed animals may be mated with dosed partners but in the event of positive findings of a reproductive defect then the study should be repeated using dosed animals mated with undosed partners. After mating, the dosed females should continue to be dosed throughout pregnancy.

Half the females should be killed during gestation, preferably some days before the expected date of parturition, and the fetuses removed by Caesarean Section and examined. The remainder of the females should be allowed to litter normally and rear their progeny.

(c) Perinatal studies which should be conducted in at least one species; dosing should cover the period of gestation from the end of organogenesis parturition and should extend throughout the period of lactation up to weaning.

3. NUMBERS OF ANIMALS

An adequate number of animals should be used at each dose level to enable valid assessments to be made. With the exception of primates, the following minimum numbers per dose level are suggested:

(a) embryotoxicity studies: 20 pregnant females in rodents, 12 pregnant females in non-rodents;

(b) fertility studies: 24 females and 24 males;

(c) perinatal studies: 12 pregnant females.

Where a third species is used it is suggested that adequate numbers of dosed animals should be used together with controls to allow a clear conclusion to be drawn from the study.

4. HOUSING AND DIET

Full details of the housing and caging conditions of the animals must be given. The full specification of the diet (including additives) must be provided.

5. PHARMACOKINETICS

In the conduct of reproduction studies, account should be taken of the pharmacokinetics of the drug in the pregnant animal. The level of exposure of the fetus to the drug should have been determined as far as this is technically possible.

6. EVALUATIONS

(a) Examinations should be made of the fetuses from the animals dosed during the period of embryogenesis. Animals should be killed and the fetuses removed by Caesarean Section. In these animals the numbers of corpora lutea, implantation sites (visible and those determined by special techniques e.g. by the Salewsky method), resorptions, the weight and sex of their individual fetuses should be recorded. The individual fetuses should be examined for external abnormalities and adequate examiniation of the skeleton or viscera or both made on all fetuses. Where obvious abnormalities are found further appropriate examination should be conducted. Special attention should be paid to abnormally high numbers of resorptions as this might indicate the need for further studies for teratogenic effects in the early stages of pregnancy.

(b) In the fertility study, females killed during the period of gestation should have their fetuses delivered by Caesarean Section and the following information should be recorded: number of corpora lutea, implantation sites, resorptions, weight and sex of individual fetuses. All individual fetuses should be examined for either skeletal and/or visceral abnormalities.

From animals dosed during the fertility study and allowed to litter normally and rear their progeny to the stage of weaning, a large enough number of progeny to allow for the subsequent investigations should be allowed to live and reach maturity. Late effects of the drug on the progeny in terms of auditory, visual and behavioural impairment should be assessed. Reproductive function should be determined in the progeny by allowing at least one male and one female from each litter of dosed animals to breed and produce one litter (brother/sister mating is not envisaged).

(c) The females dosed through the pre- and post-natal period should be allowed to litter spontaneously and the progeny examined at weaning. All animals killed at the end of lactation should be subjected to a thorough autopsy examination. Under certain circumstances some of the progeny may be allowed to live and reach maturity so that their reproductive capacity could be assessed, and other late effects of the drug on the progeny in terms of behavioural, visual and auditory impairment determined.

7. CONCLUSIONS

The investigator is required to draw overall conclusions from the results of these studies indicating either:

(a) there is no evidence of adverse effects of the drug on reproductive function; or

(b) there is evidence of adverse effects on reproductive function(s) to be specified; or

(c) the data are inadequate to draw conclusions.

If particular studies have been omitted the investigator should justify the omission.

Appendix

Duration of gametogenesis

In rodent studies, dosing of the male should be for a minimum of 60 days and the female for a minimum of 14 days prior to mating. Animals should be about 40 days of age at the commencement of dosing. In the present stage of knowledge these times would be regarded as acceptable.

ANNEX III

CARCINOGENIC POTENTIAL

Note for guidance concerning 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 authorization for a new drug

The strongest evidence that a compound is a carcinogenic hazard for man is epidemiological although most known human carcinogens are found to be carcinogenic for experimental animals. There is no evidence that all substances which are carcinogenic for animals are also carcinogenic for man, but it is difficult to declare any compound as being non-carcinogenic for man when it has been shown to be carcinogenic in animal studies.

Extrapolation to man is a difficult, sometimes arbitrary, procedure, and the ideal would be to analyze the mechanisms involved in increasing the incidence of the experimental tumours and to determine whether such mechanisms involving specific biochemical pathways and the formation of the proximate carcinogen would be applicable to man. The criteria on which extrapolation is based may vary with the agent under consideration, its projected use, dosage and mode of administration on the one hand, and the species, sites, incidence of tumours and required test dosage, on the other.

The likelihood of carcinogenic risk in man is increased if there is a high yield of malignant tumours involving a specific tissue when the test animal is given the test substance by the route to be used in man and at a dosage equal to or lower than that which induces minimal toxicity. However, since there is no good evidence of a threshold level, an increase in yield of benign tumours, or malignant tumours at higher dosage, or reduction of latency should be interpreted as constituting a possible risk for man. In such circumstances, the agent is generally regarded as less potent and the risk may more easily be reconciled with benefits associated with the therapeutic use of the compound.

1. REQUIREMENTS FOR CARCINOGENICITY STUDIES

Carcinogenicity studies will usually be required in the following circumstances:

(a) when the medicine is likely to be administered regularly over a substantial period of life (continuously during a minimum period of six months, or frequently in an intermittent manner so that the total exposure is similar); or

(b) where a substance has a chemical structure that suggests a carcinogenic potential; or

(c) where a substance causes concern due to:

  (i) some specific aspects of its biological action (e.g. a therapeutic class of which several members have produced positive carcinogenic results);

  (ii) its pattern of toxicity or long-term retention (of drug or metabolites) detected in previous studies;

  (iii) the findings in mutagenicity tests and/or short-term carcinogenicity tests.

Carcinogenicity testing may not be regarded as necessary where the substance in question will be used only in patients with a life expectancy shorter than that in which a chemical might reveal any carcinogenic hazard in man. If circumstances alter and a drug of the latter variety were used in less serious conditions then carcinogenicity testing would become necessary. Insoluble substances which are not absorbed may not require formal carcinogenicity studies.

2. SPECIES AND STRAIN SELECTION

Where carcinogenicity studies are required, they should normally be conducted on two species. The metabolic handling of the drug should be known in the species used and should preferably show similarities to the metabolism in man. Cognizance should be taken of known species and strain responses with similar chemicals. Species and strains with a high incidence of spontaneous tumour formation should normally be avoided. Those undertaking the study should select species and strains known to be sensitive to one or more carcinogens. Positive controls will not be required routinely but the spontaneous tumour incidence of the strains used should be recorded.

3. DOSAGE

(a) *Route and frequency of dosing*

Where possible, dosing should be conducted by the proposed clinical route of administration. Where relevant, evidence of absorption should be provided. The frequency of dosing normally will be daily.

(b) *Dose levels*

Carcinogenicity testing should normally be conducted at three dose levels. The top dose should produce a minimum toxic effect, for example a 10 % weight loss or failure of growth, or minimal target organ toxicity. Target organ toxicity will be demonstrated by failure of physiological functions and ultimately by pathological changes. The lowest dose should be of the order of two to three times the maximum human therapeutic dose or the dose that produces a pharmacological effect in animals. The middle dose should be the geometric mean of the high and low dose.

Exceptions to these principles may occur, for example where the toxic dose of the drug is a high multiple of the therapeutic dose; in these circumstances it is acceptable if the top dose is set at approximately 100 times the human therapeutic dose, where technically feasible.

4. PRACTICAL FEATURES

Animals should be in good general health initially and this should be maintained throughout the study. High standards of animal husbandry are essential. Special precautions are necessary when inhalational or volatile carcinogens are being tested.

The pharmaceutical quality of the batch(es) used should be clearly characterized.

(a) *Age of animals at commencement of study*

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

(b) *Duration of studies*

The currently recommended practice is to conduct studies for 24 months in the rat and 18 months in the mouse and hamster. Where the survival rate is high there may be advantages in extending studies for 30 months in the rat and 24 months in the mouse, or for the lifespan of the animals; i.e. to 20 % survival in the controls.

(c) *Number of animals per group*

For routine tests with mice, rats and hamsters it is suggested that for each sex there should be 50 animals per treated group, and two control groups of 50 for each sex dosed with the vehicle by the same route (in most cases the drug will be administered in the diet).

(d) *Composition of 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 of the diet should be given.

5. ADDITIONAL MONITORING

Carcinogenicity studies should be designed to obtain the maximum amount of information from the animals used but any investigations undertaken to elicit additional toxicological data should not prejudice the prime purpose of evaluating a drug's carcinogenic potential. Information on absorption, distribution and metabolism of the drug and on whether the drug accumulated or was an enzyme inducer should have been determined during other toxicity studies.

6. STATISTICAL DESIGN OF STUDY

An appropriate experimental design should be selected and in particular:

(i) 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;

(ii) animals should be allocated at random to the various experimental units (e.g. cages) and the method used to achieve this randomization should be clearly stated;

(iii) if for practical reasons of handling such large numbers of animals 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.

7. TERMINAL INVESTIGATIONS

7.1. **Autopsy**

A full autopsy should be made on all animals dying during the study or killed because of their poor condition.

At the conclusion of the study all surviving animals should be sacrificed and a full autopsy conducted on each animal. Previously demonstrated toxic effects may indicate particular topics for investigation. Haematological and biochemical investigations may be helpful in the interpretation of any lesions found.

7.2. **Histopathology**

7.2.1. *Carcinogenicity screening*

(i) Microscopical examination should be carried out on all listed tissues and organs from all high-dose animals and from all controls; and

(ii) tissues from any animal in any group in which macroscopical lesions of any kind are found at autopsy.

If the results from (i) indicate that tumours occur in one or more organs or tissues, then:

(iii) those tissues and organs should be examined in the mid- and low-dose groups even when macroscopically normal.

All listed tissues should be microscopically examined from all animals dying or killed during the course of the study.

7.2.2. *Toxicity screening*

Previously demonstrated toxic effects may indicate particular aspects for investigation.

Haematological and biochemical investigations may be helpful in the interpretation of any lesion found. Particular attention should be paid to the site of application if the drug is administered other than by the oral route.

8. PRINCIPLES OF REPORTING ON CARCINOGENICITY STUDIES

8.1. Definitions

A neoplasm (tumour) is regarded as a population of abnormal cells with uncontrolled and usually increased proliferative activity and other less well-defined morphological and functional features.

A malignant neoplasm is one which invades surrounding tissues or metastasises. In general terms, the tumour is considered to be benign or malignant on the basis of its histopathological appearances and the correlation by the pathologist of such charges with biological behaviour known from previous experience to occur in tumours displaying comparable characteristics.

Tumours should be described in conventional histopathological terms according to well-defined classifications (e.g. WHO).

8.2. Presentation of the data

In the first instance the findings should be summarized for each treatment group and each control group separately, keeping the sexes separate, in terms of:

1. number of animals examined and their individual gross and microscopic examination;

2. numbers (and percentages) of animals with tumours of each identified type in a specified tissue, distinguishing malignant from benign tumours, wherever possible;

3. a frequency distribution of the total number of tumours found in an animal with one or more tumours of the same or different types; and another of the total number of malignant tumours found in the animal. If the tumours cannot be enumerated some grading of multiplicity of tumours should be used instead;

4. time to each interim death;

5. time of appearance of any mass (starting from clinical palpation) and its progress, as well as its eventual histopathology.

9. 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 responses should be assessed in the following ways:

(i) the total incidence of tumour bearing animals;

(ii) the total incidence of tumours;

(iii) the incidence of tumours involving a specific tissue;

(iv) the incidence of tumours judged to be malignant;

(v) the latent period to tumour appearance (using actuarial approaches).

The analysis should be directed towards:

(a) 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, as a set, and the response in the two control groups, as a set;

(b) 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 that in (a).

Professional statistical advice should be available in order to assess the influence of other factors, such as death of test animals because of other diseases, and premature killing of animals because of clinical detection of tumours. 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. The data in one experiment may require a different approach from the data in another.

The test substance should be regarded as having the potential to increase the risk of neoplastic 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. A raised incidence of tumours in treated as compared with control animals is of significance whatever the mechanism postulated or defined for the development of such tumours, but any particular circumstances should be identified or remarked. Examples include known pathways which may be peculiar to one species (griseofulvin and porphyrin metabolism in the mouse), severe stimulation of endocrine glands (especially in the dog), or the development of physical features peculiar only to the test species (vesical calculi in the rat).

Different circumstances may result in:

(i) an increased incidence or reduced latency of malignant tumours;

(ii) an increased incidence of benign tumours;

(iii) local induction of tumours at the site of injection.

10. USE OF SHORT-TERM CARCINOGENICITY STUDIES

Evaluation of new compounds in a mutagenicity screen is desirable. However, available techniques involving short-term testing of chemicals for mutagenicity/carcinogenicity are not capable of replacing formal carcinogenicity testing in animals as a means of evaluating a drug's carcinogenic potential. Short-term studies giving positive results will always indicate the need for formal carcinogenicity studies if the drug is to be developed further; those giving negative results do not preclude the need for formal studies when these are advisable for the reasons given in paragraph 1.

11. CONCLUSIONS

Conclusions from these studies should be drawn by the investigator.

Appendix

List of tissues to be studied histologically in a carcinogenicity study

— Gross lesions

— Tissue masses or tumors (including regional lymph nodes)

— Blood smears (in case of anaemia, enlarged thymus, lymphadenopathy)

— Lymph nodes

— Mammary glands

— Salivary glands

— Sternebrae, femur or vertebrae (including bone marrow)

— Pituitary

— Thymus

— Trachea

— Lungs

— Heart

— Thyroid

— Oesophagus

— Stomach

— Small intestine (Swiss roll method)

— Colon

— Liver

— Gall-bladder

— Pancreas

— Spleen

— Kidneys

— Adrenals

— Bladder

— Prostate

— Testes

— Ovaries

— Uterus

— Brain (coronal sections at three levels)

— Eyes

— Spinal cord

ANNEX IV

PHARMACOKINETICS AND METABOLIC STUDIES IN THE SAFETY EVALUATION OF NEW DRUGS IN ANIMALS

Note for guidance concerning the application of chapter I (F) and (G) of part 2 of the Annex to Directive 75/318/EEC, with a view to the granting of a marketing authorization for a new drug

1. INTRODUCTION

These notes are concerned with the time course of the absorption, distribution and excretion of new drugs and with their metabolism in relation to their safety. For many steps in the evaluation of a drug such data are essential, for example:

(a) to assess the levels of the drug and of its metabolites and their kinetics in blood, body fluids and organs;

(b) to obtain information on the relationship between target organ toxicity and the blood, body fluids and organ concentrations of the drug;

(c) to assess the possibility of enzyme induction and of cumulation of the drug with repeated administration;

(d) to choose where possible the animal species to be used in toxicological studies on the basis of their similarity to man in handling the drug, and to determine the relevance of these toxicity studies to man.

2. DRUG SPECIFICATION

Specification of the physical and chemical properties of the drug substance must be given and the stability of the preparation should be provided.

When a labelled drug is used the position of the label in the molecule and the specific activity of the material must be stated. Consideration should be given when selecting the position of the label to its likely metabolic fate.

3. METHODS

Data on the levels of drug and metabolites in blood, body fluids, organs and in the excreta can be obtained by physical, chemical or biological methods. The investigator should justify the details of the methods used, their validity and reproducibility, including the specificity, precision and accuracy. (The study of the time course of its pharmacodynamic effects may provide useful additional information.)

When using labelled drugs attention must be given to the fact that the measured label in body fluids may not correspond to that of the unmodified drug, but may include labelled metabolites and conjugates. Attention should be given to the possibility of isotope exchange with endogenous compounds.

4. SPECIES

The animal species in these studies usually should be those normally used in the laboratory for pharmacological and toxicological investigations. The reasons for selection of any other species should be given.

A preliminary study of kinetics and metabolism of the drug in a few human subjects could provide useful information in choosing the animal species to be used in repeated dose toxicity studies.

5. DRUG ADMINISTRATION

Doses and routes of drug administration should be related, when possible, to the proposed clinical use of the drug. One of the routes selected should ensure the absorption of the drug if this is relevant to human usage.

6. PRESENTATION OF RESULTS

Information should be presented on the following items:

(i) absorption (fractional absorption, kinetics);

(ii) distribution in the principal organs and tissues and the time course in body fluids;

(iii) blood, plasma or serum half-life;

(iv) plasma protein binding;

(v) characterization of the pattern of metabolites in excreta, and where practicable, identification of major metabolites;

(vi) route and time course of excretion of drug and metabolites;

(vii) if biliary excretion is a major route of elimination, then the possibility of enterohepatic recycling should be investigated;

(viii) a quantitative account of the fate of the administered dose should be attempted;

(ix) possibilities of enzyme induction should be investigated. If enzyme induction is found its relevance in the context of the proposed use of the drug should be examined.

7. CONCLUSIONS

Appropriate conclusions should be drawn from these studies in the context of the objectives indicated in paragraph 1.

ANNEX V

FIXED-COMBINATION PRODUCTS

Note for guidance concerning the application of chapter II (C) (2) of part 3 of the Annex to Directive 75/318/EEC, with a view to the granting of a marketing authorization for a new drug

1. JUSTIFICATION

Applicants will be required to justify the particular combination of active ingredients proposed. Fixed combination products will only be considered acceptable if the proposed combination is based on valid therapeutic principles.

For any individual fixed combination it will be necessary to assess the potential advantages in the clinical situation against possible disadvantages, in order to determine whether the product meets the requirements of the standards and protocols with respect to efficacy and safety.

Potential advantages of fixed combinations include:

1. an improvement in the therapeutic:toxic ratio, e.g. as a result of the potentiation of the therapeutic effect;

2. simplification of therapy, resulting in improved patient compliance.

Disadvantages of fixed combinations include:

1. the fact that even a combination which meets the need of the average patient is unlikely to be ideally formulated for the needs of the individual;

2. accumulation of adverse reactions.

Combinations, in principle, may not be considered rational if the half-life and/or the duration of action of the components differ significantly, but this may not necessarily apply where it can be shown that the combination is clinically valid despite differences in this respect, e.g. if one component is intended to enhance absorption of the other or where the components are intended to exert their effects successively.

The inclusion of an ingredient to counteract an adverse effect of one of the other components may be considered justified, but only if the adverse reaction is a commonly occurring one.

The inclusion of a component intended to produce unpleasant side-effects as a means of preventing abuse is undesirable.

Substances having a critical dosage range or a narrow therapeutic index are unlikely to be suitable for inclusion in fixed combinations.

2. INDICATIONS

The indications claimed for a fixed-combination product should be such that the presence of each component is justified for each indication. The product should be formulated so that the dose and proportion of each component present is appropriate to all the recommended uses.

Clearly, an 'indication' must be a well-recognized disease state, dysfunctional state, syndrome or pathological entity. The individual components of a fixed combination may be intended to relieve simultaneously different symptoms of such a disease state, but it will not be proper to regard each individual symptom as an indication for the fixed combination, since it may also occur in other diseases and for treating this symptom alone the other constituents may be irrelevant.

3. SAFETY AND EFFICACY

It is permissible to distinguish between the extent of the studies required in the case of those fixed combinations which correspond closely to combinations which are already in widespread use provided these are thoroughly and reliably documented, and those studies required in the case of those combinations which are essentially new (either because the drugs involved are not usually combined, because the quantitative composition is unusual, or because one of the components is entirely new).

Safety studies in animals with fixed combinations should, as a general rule, have been performed with the active ingredients in the proportions present in the product. Such studies will not be required where all the components have been extensively and safely used in humans in identical or very similar combinations for a long period and the safety of such combinations is well-documented.

Both the efficacy and the safety of a fixed-combination product should have been investigated in man. For well-recognized combinations well-founded bibliographical data will be acceptable in some cases. It will be necessary to test a new combination clinically against one or more of the components in order to define the role played by each in the total.

4. INTERACTIONS

The possibility of interactions between the components should always be considered. Where a pharmaceutical, pharmacokinetic or pharmacodynamic interaction appears possible, the applicant should submit data either to establish that such interaction does not occur, or that it is clearly recognized and defined.

5. ADVERSE EFFECTS

Where there are grounds to expect that a fixed-combination product may be substantially more harmful or give rise to much more frequent adverse effects than any individual components given alone, the applicant should provide evidence that this does not occur in therapeutic use, or that the advantages of the combination, e.g. increased efficacy, outweigh such disadvantages.

6. DOSAGE

The combination product must be safe and effective throughout the whole of the recommended dosage range.

7. COMBINATION PACKS

The principles applicable to fixed-combination products will also be applied in the assessment of preparations consisting of different medicinal products in combination packs where the products are intended for simultaneous or sequential administration.

8. CHEMICAL COMBINATIONS AND COMPLEXES

Substances of this type which dissociate prior to absorption into two or more active components may be regarded for the application of the above provisions as fixed combinations of these substances rather than as chemical entities.