Guidelines for preclinical and clinical testing of new medicinal products

Part 1—Laboratory investigations
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Part 1 – Laboratory investigations

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Foreword

These guidelines, which are published as separate pre-clinical (Part 1) and clinical (Part 2) sections, have been prepared for the Scientific and Technical Council by its Research and Development and Medical Committees. They represent the consensus views of the Committees’ experts on good practice in the pre-clinical and clinical testing of new medicinal products for human use. As the title indicates, these documents are intended to provide guidance only and do not attempt to establish requirements for testing. The problems encountered in drug research demand a flexible approach and, depending on the precise nature of a new medicinal compound, it is appropriate to undertake evaluation by different methods or to different standards.

On behalf of Council I thank all the Committee members and other co-opted experts who have participated in the lengthy task of preparing these guidelines. I commend both documents to all member companies in anticipation that they will make a positive and helpful contribution to their experimental programmes.

P T Main
Chairman of the Scientific and Technical Council
General introduction

Early in 1973 the Scientific and Technical Council of the ABPI accepted a proposal from its Research and Development Committee that an effort be made to define good current practice for testing new medicinal products in human beings. This meant bringing up to date the ABPI First Report of the Expert Committee on Drug Toxicity which was published in 1964 and amended in 1968, and extending it to include a greater consideration of pharmaceutical and medical problems. Like that report, this also is mainly a guide to good current practice for the member companies of the ABPI. The detailed development programme for any new medicine depends on its nature and intended use. The definition of the programme and of the safety measures needed to protect all the individuals involved is the responsibility of the scientists and physicians of the company concerned.

The administration of any medicine to human beings inevitably carries some risk of side effects that cannot be foreseen because human beings differ from one another, and from the animals used for pharmacological and toxicological studies in their responses to drugs. That component of the risk associated with the drug substance itself is potentially greater in the early stages of the testing of new medicines in man and so there must always be a good reason for such experiments. Here it is assumed that each test compound or preparation has already been shown to possess properties which might reasonably lead to the development of an improved medicine for a significant human ailment.

The subjects who receive test substances must be safeguarded by adequate prior experimentation in animals and by close medical surveillance. Part 1 of this report, prepared under the aegis of the Research and Development Committee, deals with the type of pharmacological, toxicological and biochemical information that should be known about any medicament before it may reasonably be given to man. Part 2 was prepared by the Medical Committee and is concerned with the other essentially medical safeguards for the subjects involved in human pharmacological and clinical trials.

The guidelines relate to any substance or combination of substances intended to be used in man for the diagnosis, prevention, or treatment of disease, for the mitigation of symptoms of disease, or for the alteration of physiological function. Vaccines and serological preparations are excluded.

The bioavailability studies required in the development of new drugs have been considered by a Working Party set up by the
Research and Development Committee and are reported in a separate document.

Some important general considerations relating to the preclinical examination of new drugs are mentioned and each of the major types of study required are discussed. Brief guidelines intended to directly assist experimenters in setting up protocols for commonly used toxicological tests are provided as Appendices.

1 General considerations

1.1 Requirements for animal studies

Adequate studies in animals are an essential part of the rational basis for the use of new substances or preparations in man. The nature of the toxicological evidence required depends on the biological properties of the test substance, the nature of the disease to be treated, the probable dosage schedule in man and the proposed duration of treatment.

Expert supervision of the animals used in these studies is essential in order to identify unexpected effects and to ensure proper investigation of their possible significance in any subsequent human studies.

1.2 Quality of test substances

The substance or preparation used in animal tests preparatory to human studies must be a defined entity and, unless there is good reason for using less pure material, of the quality intended for use in human beings. It should be characterised by adequate provisional specifications supported by analytical methods capable of detecting instability of the test substance, or of any pharmaceutical formulation containing it.

1.3 Pharmaceutical preparations that contain more than one medicament

A mixture that contains a new test substance or any component that has not been the subject of adequate toxicity tests should be treated as a new test substance.

A mixture that consists of two or more medicaments of known acceptable toxicity in animals and/or man should be subjected to a systematic pharmacological examination and to acute toxicity tests as described in this report. If there is clear-cut potentiation of the pharmacological activity or toxicity with the mixture as compared
with its individual components, the mixture should be subjected to chronic toxicity tests and reproduction studies before the clinical trial and marketing of preparations containing it. Mixtures with unexpected properties should be treated as new test substances.

If no potentiation of activity is found in acute tests the mixture should be subjected to appropriate maternal and foetal toxicity tests. Satisfactory results in all these tests, and in the pharmacological and acute toxicity tests, constitute adequate evidence for properly supervised human pharmacological studies and clinical trials of unlimited duration, and for marketing the preparation if the clinical trial results are satisfactory.

1.4 Classification of studies

The following classification has been adopted for the studies discussed in this report:

Pharmacodynamic activity.

Acute toxicity tests which are concerned with the effects of single doses of the test substance, or occasionally with the effects of a few doses given during a 24-hour period.

Chronic toxicity tests in which the test substance is administered every day for varying periods of time.

Reproduction toxicity tests.

Carcinogenicity tests.

Pharmacokinetics and drug metabolism.

Chemistry and pharmacy.

2 Pharmacodynamic activity

2.1 General

Before a test substance is given to man its pharmacological effects on the major body systems should be investigated in a number of mammalian species. In each species the doses which cause effects likely to limit drug treatment, the lethal dose and, if practicable, the cause of death should be determined. The preferred species are the mouse, rat, guinea-pig, rabbit, cat, dog and certain primates, because much is known about their responses to different kinds of drugs.

Pharmacological tests should be done to investigate possible effects of the test substance on locomotion and other aspects of gross behaviour, and on the cardiovascular, respiratory and nervous systems. The methods referred to in this report have been found to
be satisfactory but other equally valid techniques might be used. Other potentially harmful effects of the test substance on other body functions should be considered when subsequent chronic toxicity tests are defined.

Many pharmacological effects, especially those involving neuromuscular function and the central nervous system, may be detected by giving graded doses of the test substance to mice. A range of doses should be given by various routes of administration including those likely to be used in man. The mice should be observed frequently during the first few hours after administration of the drug. All drug effects should be recorded together with the doses which caused them. A suitable test system has been described by Irwin¹.

2.2 Cardiovascular studies

The effects of the test substance on the cardiovascular system should be determined in at least two mammalian species. The dog and cat, appropriately anaesthetised, are generally suitable for this purpose. The substance is usually given intravenously in graded doses and its effects on the cardiac output, the rate and force of contraction of the heart, the ECG and the blood pressure are recorded. If a significant effect is found, the site and mechanism of action should be investigated in further experiments. Possible interference with the actions of physiologically important substances, such as acetylcholine, adrenaline, noradrenaline and tyramine, should be examined.

Further cardiovascular studies should be done in conscious animals by the route(s) of administration proposed for man, because cardiovascular responses in conscious animals often differ from those in anaesthetised animals. The dog is usually suitable for this purpose.

2.3 Respiratory studies

The effect of the test substance on the rate and depth of respiration should be investigated in the anaesthetised animals used for cardiovascular studies. The effect of the compound on respiratory smooth muscle should also be measured. The guinea-pig is suitable for this purpose, because the responses of its airways to pharmacologically active substances are usually similar to those in man. Methods that use anaesthetised guinea-pigs have been described by Dixon and Brodie², Konzett and Rössler³ and James⁴.

More detailed studies should be done on substances found to have significant activity.
2.4 Interaction studies

Simple experiments should be done to see if the test substance interferes with the actions of other medicines which, because of their specific effects or because of their common use, are likely to be taken concurrently with the test substance. Conscious mice and rats and anaesthetised cats and dogs are suitable for most investigations of this kind. Any synergism and antagonism of drug effects should be investigated and any necessary warning issued to clinical investigators.

An attempt should be also made to identify an antidote for the test substance for possible use in the management of overdosage, particularly if the therapeutic margin of the compound is small.

3 Acute toxicity tests

3.1 Qualitative tests

Small numbers of animals are used to establish the pharmacological effects of increasing doses of the test substance. Their purpose is to determine limiting pharmacological side-effects of the test substance. The cause of death should be established if practicable. The results obtained in these tests give a guide to maximum tolerated doses in subsequent chronic toxicity tests. Tests of this kind should be done for each compound in a number of mammalian species including at least two rodents and two non-rodents. The mouse and the rat are suitable rodents; the rabbit, cat, dog and monkey are suitable non-rodents.

3.2 Quantitative tests

These are used mainly for comparative purposes and require relatively large numbers of small animals such as mice and rats. For example, the pharmacologically effective dose of the test substance may be compared with that which causes some other effect which would limit drug treatment, or the efficacy and/or toxicity of the substance may be compared with that of related medicines.

It is usual to determine ED$_{50}$ or LD$_{50}$ values, which are the dosages that are effective or lethal in 50 per cent of animals.

These values are not absolute but vary with the strain, sex, weight and state of nutrition of the animals and with experimental conditions, such as the environmental temperature, the manner of housing, the time of day and the season of the year. For valid comparisons it is essential for each substance to be tested at the same
time and using the same experimental conditions. LD$_{50}$ and any appropriate ED$_{50}$ values should be determined for each test substance in at least two species of small animals; mice and rats are suitable. The drug is usually given by the oral and intravenous routes of administration and by any other routes likely to be used in man.

4 Chronic toxicity tests: general requirements

4.1 Purpose

The main purpose of chronic toxicity tests is to establish whether or not the test substance is adequately tolerated after administration to animals for a prolonged period in doses or concentrations at least equivalent to those intended for use in man. The effects of prolonged excessive dosage with the substance are a useful guide to possible adverse reactions in human beings. The size and frequency of the doses and the duration of the tolerated dosage schedule are major determinants of permissible tests in man. When toxic effects are produced it is highly relevant to know whether or not they are reversible.

4.2 Kinds of tests

There are two types of chronic toxicity tests:

i  Systemic tests in which the test substance is administered by mouth or by a parenteral route.

ii  Tests in which the test substance is applied topically to a body surface, external or internal.

Each test has to be specially designed to take account of the biological properties of the test substance and the nature and duration of the proposed human studies.

Topically applied substances that are absorbed into the body should also be subjected to appropriate systemic tests.

4.3 Animals

The animals must be healthy and of a defined stock without known metabolic peculiarities. Young animals are best for most tests since drug effects on growth rate and the developing gonads are sensitive measures of toxicity.
The animals used for toxicity tests should preferably be sensitive to the drug effect expected in man and also deal with the test substance in a similar way to man. For example, if the expected therapeutic effect depends on absorption and general distribution of the substance in man, the drug should be so absorbed and distributed in the animals. It is also desirable, but not always possible, to select species in which the major metabolites, if any, of the test substance are the same as in man.

Each test substance should be examined in at least two mammalian species, one of which is not a rodent or the rabbit. Mice and rats are suitable rodents; dogs, monkeys and pigs are suitable non-rodents.

### 4.4 Dose levels

Three dose levels are usually necessary but two may suffice for some drugs. The low daily dose should be about two to five times the estimated human daily intake. The high dosage regimen should be toxic, for preference cause the deaths of some of the animals and as a minimum cause a significant retardation of growth. When a substance is non-toxic the high dose need not exceed 200 times the low dose. The geometric mean of the high and low dose levels is usually a suitable middle dose. Each test must include a control group that is given an appropriate control preparation instead of the test substance but which is otherwise identically treated.

### 4.5 The ear

Regular tests to determine possible effects of the test substance on cochlear and vestibular function are an essential part of the chronic toxicity testing of any new substance. The functional integrity of the cochlea can be assessed by the pinnaal twitch response to an unexpected noise; the sound of a Galton whistle is a suitable stimulus. Vestibular function is assessed by clinical observation of dosed animals; changes in posture, gait and positional nystagmus are important.

Compounds which show effects in these tests and known ototoxic classes of drug, such as aminoglycoside antibiotics and certain diuretics, should be examined in greater detail.

### 4.6 The eye

Clinical examination of the eye is an essential part of the chronic toxicity testing of any test substance. The examinations should include ophthalmoscopy and, if necessary, slit lamp examination of the lens and appropriate staining of the cornea.
In the animals commonly used in chronic toxicity tests spontaneous eye lesions are not uncommon. Detailed examination of the eye should therefore be carried out before dosing starts and at regular intervals during the dosing period. The sizes and positions of any lesions should be recorded on charts; serial observations of this kind are necessary for assessing the significance of particular lesions.

The preparation of eyes for histological examination requires great care because artefacts are readily produced during their removal, fixation, processing and sectioning.

4.7 Biopharmaceutical and pharmacokinetic considerations

The formulation of a drug can greatly influence its bioavailability and consequently the pharmacological and toxicological effects produced. It is recognised also that comparative bioavailabilities may vary in different mammalian species.

The pharmaceutical formulations used in toxicity tests of orally administered drugs intended to be generally distributed in the body should be designed to achieve maximum absorption of the drug whether given by individual dosing or mixed in the diet. They need not be identical with those used subsequently for clinical trials or for marketed products but information on comparative availability of the active drug in these formulations will be required in due course. Water-soluble drugs should be given in aqueous solution or in readily dispersible tablets or capsules. Sparingly soluble drugs should be sufficiently comminuted to ensure as far as practicable that the rate of dissolution in the alimentary tract is not the limiting factor for absorption. They should be administered in aqueous suspension or in tablets or capsules formulated to ensure rapid dispersion of their drug content.

It is desirable for the degree of absorption of the drug, its half-life in the body and the blood levels achieved after varying doses to be determined in the proposed experimental species, using simple formulations to enable rational daily dosage schedules to be constructed. If specific analytical methods are not available for the drug or its relevant metabolites, an adequate answer is usually obtained by administering a suitably radio-labelled preparation, and then at various times later determining the levels of radioactivity in urine, faeces, blood and other appropriate tissues. Care is needed in the choice of the position of the radioactive label for the results to be meaningful in relation to the fate of the drug itself.

More elaborate pharmaceutical formulations may reduce the rate of solution or diffusion of medicaments. They should be specially tested before use in toxicity tests to make sure that the medicaments are adequately absorbed.
Toxicity tests on substances to be administered by a parenteral route or locally applied should, when practicable, be carried out using the pharmaceutical formulations intended for clinical use in man; more concentrated preparations may be necessary for the high-dose groups.

4.8 Photosensitisation

A specific animal test for photosensitisation is usually done only if the test substance is chemically similar to compounds known to sensitise human skin to sunlight.

Animal tests have not reliably identified drugs found to be photosensitising in man but the photosensitising actions of chlorpromazine and demeclocycline, for example, have been demonstrated in guinea-pigs.

Test substances may be administered by a systemic route to albino guinea-pigs or, if appropriate, applied locally to their skin (preferably the formulation intended for use in man is applied to the shaved skin daily for seven to 14 days). Areas of skin are then exposed to natural or simulated sunlight and the degree of any inflammatory response is assessed and compared with appropriate control reactions in untreated animals and/or untreated skin. These tests should be supplemented by skin patch tests in a few healthy volunteers before more extensive tests are done in man, if there is reason to suppose that the test substance may cause photosensitisation in man.

5 Chronic toxicity tests for systemic preparations

Brief guidelines for chronic toxicity tests in rats and non-rodents for drugs that are systemically active are presented in Appendices A and B.

5.1 Dosing

The dosing schedule should be such that the animals are exposed to the drug for a prolonged period each day for the duration of the test. For most drugs this is achieved by once daily dosing for seven days a week. More frequent dosing may be necessary for unusually short-acting drugs if they are not specially formulated to prolong their action. Tests on long-acting drugs that accumulate in the body are best controlled by monitoring blood levels of the drugs or their metabolites.
5.2 The duration of chronic toxicity tests

5.2.1 Animal tests prior to clinical trial

The duration of dosing in animals required to support pharmacological and clinical testing of a new medicine in man is largely determined by the intended duration of dosing in man. The recommended durations of chronic toxicity tests given in Table 1 refer to test substances whose observed toxic effects in animals are consequences of their known pharmacological or biochemical actions and are of a kind that are reversible after administration of the test substance is discontinued.

Tests of the recommended durations are considered to provide an adequate safeguard against the known toxic effects of drugs, except the induction of tumours and adverse effects on reproduction, which are considered separately.

Table 1 Recommended duration of chronic toxicity tests in animals prior to testing in human pharmacological or clinical studies

<table>
<thead>
<tr>
<th>Duration of dosing in human pharmacological or clinical studies</th>
<th>Duration of chronic toxicity tests in animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to three doses within a three-day period</td>
<td>14 days</td>
</tr>
<tr>
<td>Continuous medication with repeated doses for: a period up to seven days</td>
<td>28 days</td>
</tr>
<tr>
<td></td>
<td>a period up to 30 days</td>
</tr>
<tr>
<td></td>
<td>90 days</td>
</tr>
<tr>
<td>Longer periods of continuous medication</td>
<td>180 days</td>
</tr>
</tbody>
</table>

5.2.2 Animal tests prior to marketing

New medicines that are to be taken by man for a period of four weeks or more should be subjected to long-term toxicity tests in animals before they are marketed. Tests of at least six months' duration should be done in the dog and rat, or in other relevant species.

The necessary duration of the chronic toxicity studies required for a new medicine taken by man for shorter periods should be assessed according to the nature of the human disease, the mode of action of the drug and its persistence in the body.
5.3 Irreversible toxic effects

Test substances that cause irreversible toxic effects in animals should not be administered to human beings unless the potential benefit to man outweighs any risk involved. The decision to test such a compound in man depends *inter alia* on the nature of the disease to be treated, on the ratio of the toxic dose or tissue concentration to the expected therapeutic dose or concentration, and on establishing appropriate laboratory and clinical tests to safeguard the human beings involved. The observation of disproportionately high toxicity in one animal species should not deter examination in man if it can be shown that the effect is truly species specific, perhaps because of peculiar metabolism of the drug in that species. Each test substance requires individual assessment and the decision to proceed to man must be approved by the responsible expert toxicologist and the physicians responsible for the work in man (see Part 2).

6 Chronic toxicity tests for locally applied preparations

6.1 Skin preparations

6.1.1 Test for absorption of medicament

The nature of the toxicity tests required for a substance which is to be applied locally to the skin depends on whether it is absorbed from the site of application. This may be measured by applying the chosen formulation made with suitably radio-labelled drug to appropriate body surfaces in animals in a way that as far as possible prevents its ingestion and absorption by irrelevant routes. The degree of absorption is assessed from the amount of radioactivity in the blood and/or other tissues, and from the amounts in the urine and faeces of the test animals at various times after applying the test formulation.

The test formulation should be applied to areas of intact and abraded skin that are suitably covered to prevent its loss or excessive spread. The effect of a covering that prevents local loss of moisture should be investigated because such occlusive conditions facilitate the passage of many substances through the skin and occur naturally, for example, in skin folds.

Young pigs may be chosen for these tests because their skin is similar in many ways to that of man. Rabbits, rats and guinea-pigs are also suitable if the test areas are carefully shaved.
If the test substance is not significantly absorbed through the skin only tests for local toxicity need be done. If the test substance is significantly absorbed supplementary tests must be carried out to exclude unacceptable systemic toxic effects.

6.1.2 Tests for local toxicity

The purpose of these tests is to determine whether the preparation is toxic to skin as shown, for example, by inflammation, necrosis or delayed healing.

Test preparations containing varying concentrations of the drug should be applied daily under covering dressings, as described in the previous section, to defined areas of intact and abraded or scarified skin for periods up to 20 to 30 days. Longer tests are unnecessary, because the half-life of skin cells is short, but prolonged tests for possible carcinogenic activity may still be required. The test sites should be examined daily and subjected to detailed histological examination at the end of the test period. The choice of animals is the same as in Section 6.1.1. The use of young pigs facilitates direct comparison of a large number of preparations.

6.1.3 Sensitisation of skin

Many substances are known to induce hypersensitivity reactions after their repeated application to human skin. An indication of the skin sensitising potential of a test substance intended for local application may be obtained by injecting it intradermally, with or without Freund’s adjuvant, into shaved guinea-pigs. After 14–28 days the animals are challenged by injecting the drug intradermally at another site. Weak allergens may be detected by giving several sensitising doses during a period of about 14 days, or by giving a single dose together with adjuvant. Suitable methods have been described by Landsteiner and Jacobs and Magnusson and Kligman.

Allergenicity in a skin preparation may be detected by applying it daily for 14 days to intact or abraded skin, preferably under occlusive conditions, followed by similar challenge applications at other skin sites after a further seven to 14 days.

Although the antigenic potential of a test substance may be assessed by immunisation of guinea-pigs, the test does not give precise information about the ability of substances to act as contact allergens. These are more reliably identified by predictive patch tests such as are used to determine the skin sensitising potential of topical antibiotics and cosmetics. The Draize technique is most commonly used. It is advisable to carry out appropriate patch tests on the
skins of about 200 volunteers (100 men and 100 women of a wide age range) before treating patients with a preparation containing a new test substance. A more sensitive test has been described by Kligman. In this only 50 volunteers are used and sensitivity is enhanced by the use of an irritating concentration of sodium lauryl sulphate (5 per cent) either before or at the same time as application of the test substance.

6.1.4 Supplementary systemic toxicity tests

Substances that are absorbed through the skin should be subjected to appropriate systemic toxicity tests (Section 5). Most test substances can be administered in solution or as fine suspensions by subcutaneous injection but very insoluble substances that are not effectively absorbed from subcutaneous sites may have to be given intramuscularly as ultra-fine suspensions, or if need be, intravenously.

6.1.5 Skin preparations for use in infants

If it is likely that a skin preparation would be used in infants the possibility should be considered of its active ingredient being more effectively absorbed than through the skin of adults. Tests may be done using neonatal rats. The preparation may be applied to their skin, or the neonates may be immersed in solutions of the active ingredient. Examinations should be made for local and systemic effects. Tests for absorption of the active ingredient may also be appropriate.

6.2 Vaginal preparations

Tests for local toxicity should be done by intravaginal application; the dog and rat are usually suitable species.

Preparations that are absorbed should be subjected to supplementary systemic tests.

6.3 Intranasal and inhalation preparations

Most medicines administered by inhalation or by instillation into the lungs or the nose in man are significantly absorbed not only from the respiratory tract but also from the alimentary tract because much of each dose is swallowed. Accordingly, it is important for the animal toxicity tests to take account of possible systemic effects as well as local effects on the respiratory tract.

The test formulation for the low-dose group of animals should be the same as that intended for use in man, although a more concentrated form may be needed for the middle and high dose groups.
For preparations intended to act within the lungs, the effectiveness of the delivery system should be checked by determining the proportion of the drug deposited in different parts of the airways in a few test animals. This is most easily achieved by administering the pharmaceutical preparation made with radio-labelled drug. The animals are killed immediately after receiving the drug and the amounts of radioactivity present in different parts of their respiratory tracts determined. The half-life of the drug in the lungs may be determined in a similar way using animals killed at varying times after dosing.

The design of toxicity tests for inhaled drugs is governed by the general rules for systemically active substances. Special attention should obviously be paid to macroscopic and microscopic examinations of the respiratory tracts of the animals.

Methods for administering drugs from pressurised inhalers into the lungs of rats and dogs have been described by Poynter and Spurling⁹, Smith and Spurling¹⁰ and Burley, Clarke, Cuthbert, Paterson and Shelley¹¹. Mice and monkeys may also be used. Similar systems may be devised to administer solutions or dry powders.

It is not easy to administer a large amount of a drug by inhalation into the lungs of animals, so it is usually necessary to supplement inhaled doses in toxicity tests by concurrent administration of the test substance by mouth, if it is well absorbed, or by a rational parenteral route. An alternative is to carry out separate inhalation and systemic toxicity tests.

6.4 Preparations intended to have local effects on the alimentary tract

Whether the test substance is to be taken by mouth to exert a local therapeutic effect in the alimentary tract, or by the rectal route to act locally or systemically, it is usually advisable to assume its general distribution in the body and to carry out the full toxicity examination appropriate to a systemically active drug.

Test formulations for rectal administration in animals should be the same as those for use in man. If need be the rectal dose may be supplemented by concurrent administration of an appropriate preparation by mouth or by a relevant parenteral route. Alternatively, separate tests for local and systemic toxicity may be done.

6.5 Ophthalmic preparations

The nature and duration of toxicity tests needed for topical eye preparations depend on whether the active ingredient(s) is signifi-
cantly absorbed into the general circulation and/or into the eyeball. The degree of absorption and distribution of the drug may be determined by methods similar to those described for skin preparations.

Topical eye preparations containing medicaments that are not absorbed into the body of the eyeball, or that penetrate the eyeball but are not detectable in the general circulation, need be tested only for local toxicity. Preparations whose ingredients are absorbed into the body should also be subjected to chronic toxicity tests in which they, or their active ingredients, are administered by a relevant systemic route. The required durations of the systemic tests are the same as those defined in Table 1 for systemic preparations.

Topical eye preparations must be sterile and contain appropriate preservatives if they are packed in multidose containers. As a rule they should be isotonic with tear secretion. The formulation used in local toxicity tests should be that intended for use in human beings.

Toxicity tests on topical eye preparations should be done in two species; the guinea-pig, rabbit and dog are suitable.

7 Effects on reproduction

7.1 General considerations

The purpose of reproduction toxicity tests is to investigate possible adverse effects of test substances on reproductive processes in animals when they are given in doses significantly greater than those intended for man, or in doses that give significantly higher blood and/or other tissue concentrations than those achieved in man.

Test substances may adversely affect reproductive processes in many ways and the use of at least two of the following kinds of test is likely to be required for most new drugs:

a Maternal and foetal toxicity tests
b Three-generation reproduction tests
c Perinatal studies.

Reproduction toxicity tests are subject to the same general principles as chronic toxicity tests. The route of administration of the test substance in animals should be the same as that used in human beings, unless it is impossible thereby to achieve a high enough drug challenge to the parents and foetuses. In such an event the human route of administration should be supplemented or replaced by a route that ensures an adequate drug challenge. For example, sub-
cutaneous injection may be used to replace or supplement local application of the test substance to the skin or respiratory tract.

7.2 Maternal and foetal toxicity tests

The purpose of these tests is to detect possible adverse consequences to the mothers or their foetuses of administering the test substance during pregnancy.

The essential requirement of any reproduction and foetal toxicity test is that the foetus is exposed to an adequate concentration of the test substance during organogenesis in the species used. In the procedure described in Appendices c and d dosing is restricted to these periods. If, however, the metabolic fate of the drug does not greatly change during its continuous administration, the drug may be given daily for the whole duration of the pregnancy to monitor other possible adverse effects on reproduction. When the compound under study is an enzyme-inducer or has a cumulative toxic action on certain metabolic processes, the drug effects produced when dosing is commenced during organogenesis may differ from those produced when dosing is started sooner.

Three dose levels are required. They are selected as described in the section on chronic toxicity tests, except that the high dose should not be grossly toxic, because pregnancy is unlikely to be normal in mothers that are seriously stressed.

Each test substance should be examined in at least two mammalian species, one of which is not a rodent. The rat and rabbit are usually suitable; mice and some species of monkeys are also used.

Each test should include a control group which receives a placebo preparation and the same handling as the test groups. An untreated control group is desirable if the control preparation is not bland, or if the incidence of spontaneous malformations in the animal strain is not known. In the guidelines for the rat study (Appendix d) the intention is not only to reveal effects of the test substance that arise during organogenesis but also adverse effects on the neonates due to failure of lactation or the possible presence in the maternal milk of the test substance or a metabolite.

As a rule appropriate foetal toxicity tests should be carried out and acceptable results obtained before a test substance is given to humans. An exception is the administration of the test substance to male subjects, or to women incapable of becoming pregnant, if the test arrangements ensure that drug administration is restricted to these groups.

Drugs that cause foetal malformation when given in doses that show no maternal toxicity should not be given to humans, unless
significant therapeutic benefit is expected in a serious ailment that is inadequately controlled by existing medicaments, or unless there are valid scientific reasons for supposing that such malformations will not occur in humans.

7.3 Three-generation reproduction tests

The main purpose of these studies is to reveal adverse effects on reproduction that would escape detection in maternal and foetal toxicity studies of the kind described above. In particular they are intended to reveal adverse effects on the formation of gametes and on fertilisation, and to detect gross genetic mutations which may lead to foetal death, foetal abnormalities or inadequate development or abnormal reproductive capacity in the F1 generation. They are also intended to reveal adverse effects of the drug that occur during the pregnancy or during lactation.

Three dose levels of test substance should be used; they are determined in the same way as in the maternal and foetal toxicity tests. The test need be done in only one mammalian species, either the mouse or the rat being suitable. Guidelines for a three-generation reproduction test in the rat are given in Appendix E.

The likelihood of the adverse effects that are detected by the three-generation tests, other than those revealed by maternal and foetal toxicity tests, is small with nearly all substances likely to be used as medicines in man. Any risk to human beings should be adequately contained if the test is done concurrently with clinical trials and is completed before preparations containing the test substance are marketed.

7.4 Perinatal studies

Special tests in which dosing is started in late pregnancy and continued throughout lactation up to weaning may be required. In such tests dosing should cover the period of gestation not covered by the maternal and foetal toxicity tests referred to in paragraph 7.2. Such a test should be done in addition to or as an alternative to a three-generation reproduction test of the kind described in paragraph 7.3 whenever a drug might be used in late pregnancy or during lactation. For detecting adverse effects in these situations, the use of a perinatal test rather than a three-generation reproduction test may be advantageous, as for example when the drug stimulates its own metabolism or when the dosage which can be administered in the three-generation tests is unduly restricted by the drug interfering with some other stage of the reproductive cycle.

In perinatal studies it is usually appropriate to test the drug at three dose levels, using appropriate controls, in groups of at least 12 rats.
Dosing would usually be started on day 17 of pregnancy and continued until 21 days post partum. Observations should be made of weight and behaviour of the does and their offspring up to the time of weaning when the progeny are autopsied. Parts of Appendix E are relevant to these studies, i.e. 1, 2, 3, 4, 6, 8, 9 (d to f) and 12.

8 Carcinogenicity tests

8.1 Objective

The purpose of carcinogenicity tests is to determine whether chronic administration of the test substance causes tumours in animals and might therefore be expected to cause tumours in man.

8.2 Applicability

The need for a carcinogenicity study should be considered for all new medicines and especially those that are likely to be given to human beings continuously for long periods, or given in frequent shorter courses of treatment over a prolonged time. Carcinogenicity testing may not be regarded as necessary where the substance in question will only be used in patients with a life expectancy shorter than that in which a chemical might reveal any carcinogenic hazard in man. The test is usually started when the probable usefulness of the new medicine in man has been established. At the latest, it should be done concurrently with confirmatory clinical trials and may extend into the early period of more general use of preparations containing the new substance, provided that more than half of the dosing period has been satisfactorily completed before marketing is started.

The following classes of substances are exceptions and, if the risk is deemed high enough, carcinogenicity tests on them should have been satisfactorily completed before human pharmacological or clinical studies are started:

- Substances that are chemically similar to known carcinogens
- Substances found in chronic toxicity tests to be cytotoxic as shown, for example, by their effects on bone marrow, gonads, lymphoid tissues and/or gut epithelium.

8.3 Choice of species

Mice, rats and hamsters are the species most commonly used for carcinogenicity tests because of their small size, ready availability and relatively short life spans, and because the knowledge that has accumulated concerning spontaneous diseases and tumours in particular strains of these species helps greatly in the interpretation of results.
If the metabolic fate of the drug in man is very different from that in mice, rats or hamsters, other species may have to be considered. Similarly, it is sometimes necessary to use other species because they provide better models for the physiological and biochemical processes with which the drug interacts in man. For example, monkeys are more appropriate for hormonal contraceptive preparations and dogs and hamsters are more suitable for testing aromatic amines or other compounds that might cause bladder carcinomas.

Studies should be made in one species. The mouse and rat are usually suitable for drugs given orally or by inhalation. The mouse is preferable for substances given subcutaneously or locally applied. The strains of animals used in carcinogenicity tests should be healthy, should have a consistently low incidence of spontaneous tumours and should, if practicable, be free from tumours caused by viruses.

8.4 Design of test

Since interpretation of the results of carcinogenicity tests may ultimately depend on comparison of the differences in the observed incidences of tumours in the test and control groups, enough animals must be used to permit a statistically sound assessment of the experiment. Analysis of carcinogenicity tests must take into account the incidence, nature and behaviour of the many types of tumours that occur spontaneously in animals. Factors of particular importance in considering the results of such tests include whether or not in dosed animals there was premature appearance of neoplasms and/or an occurrence of tumours at sites rarely involved in untreated animals. It is also important to know whether the neoplasms found were more malignant than usual in the strain employed and what was the relationship between dose and response. The known pharmacodynamic actions of the compound examined should also be considered, particularly if it affected endocrine glands directly or by an action on their target tissues.

Critical appreciation of these factors, which include adequate knowledge of the spontaneous incidence of tumours in the animals used, requires careful planning of carcinogenicity experiments. Correct randomisation of the animals into groups taking account of the litter of origin, ages and weights of the animals is necessary for the experiment to be valid. A statistician should therefore be involved in the design as well as the interpretation of the results.

Three test groups and a similarly handled control group are usually required. Each test group should contain equal numbers of male and female animals. If mice or rats are used each test group may consist
of 50 males and 50 females and the control group of 100 males and 100 females.

The high dose of the test compound should not unduly shorten the life span of the animals and should produce at most a minimal toxic effect, such as slight retardation of growth. This dose may be a modest multiple of that effective in man or an experimental animal, or may be such as to produce a multiple of the therapeutic concentration of the drug in blood and/or another tissue. The low dose should be no more than twice either the estimated therapeutic dose or that producing a therapeutic concentration in man. Dosing with the test substance should be started as soon as possible after the animals are weaned and should be continued for a substantial part of the life span of the animals, or until the results of the test are obvious from the appearance of tumours.

Guidelines for systemic carcinogenicity tests in the mouse and rat are given in Appendix F.

9  Pharmacokinetics and drug metabolism

9.1  General considerations

Experiments should be carried out, using male and female animals of the same strains as those used in the toxicity tests, to establish the following characteristics of the test substance after its administration by routes relevant to those likely to be used in man:

Degree and rate of absorption
Distribution and persistence in important organs and tissues of the body
Metabolic fate
Rate and routes of excretion of the drug and any major metabolite.

The use of radio-labelled test substances is usually a great help in experiments of this kind, but the results must be interpreted with caution, at least until the metabolism of the drug is understood. A method of radio-labelling should be chosen in which the radioactive atoms are not easily disengaged from the parent molecule by known metabolic routes except when a particular metabolic pathway is being studied.

9.2  Absorption, distribution, metabolism and excretion

The rate and degree of absorption of the test substance are most easily assessed by comparing the levels of radioactivity in the blood
(or plasma), faeces and urine of the test animals at various times after drug administration by the chosen route with the results obtained after the same dose of the substance has been injected intravenously or, if this is not practicable, intraperitoneally. The effect of increasing doses of the test substance on the levels achieved and on its persistence in blood or plasma should be investigated as a guide to dosage in animal toxicity tests and subsequent studies in man.

Experiments of this kind should also be done in animals that have been given the test substance for a prolonged period, to ensure that the fate of the drug does not change greatly as a consequence, perhaps, of the induction of metabolising enzymes.

Similar studies should also be done to determine whether the pharmacokinetic properties of the drug change during pregnancy.

The distribution of the test substance in major organs and tissues should be determined in the species used in the toxicity tests because high local concentrations of a test substance may be associated with toxicity or indicate sites of its excretion and/or metabolism. An adequate quantitative estimate of the distribution of the substance is often obtained by removing selected tissues from animals killed at various times after administration of the labelled substance and then determining the levels of radioactivity in weighed samples by appropriate methods. The following should be examined: adrenal glands, bile, blood (and/or plasma), brain, fat, gonads, heart, liver, skeletal muscle, lungs, kidney, spleen, thyroid. A substance which concentrates in any tissue and has a half-life within that tissue of more than 48 hours should be subjected to special investigations to establish the possible implications of its persistence.

Similar methods using radio-labelled material may be used to determine the concentration and persistence of the test substance in the foetuses or amniotic fluid in pregnant animals in an extension of reproduction studies. The substance should be administered by the same route(s) as in those tests.

A useful semi-quantitative estimate of the distribution of the radio-labelled test substance in small animals may be obtained by whole body radio-autography. This method can have the advantage of revealing unusually high local concentrations of the substance, thus indicating possible sites of metabolism, transportation or excretion.

Methods should be developed for separating the test substance and its major metabolites from urine and faeces. The substance and these metabolites should be sufficiently characterised by chromatographic and/or mass spectrometric methods or other suitable means
to permit their identification in subsequent human studies; it is not essential to establish the chemical structures of the metabolites. An effort should be made to develop quantitative chemical, spectrophotometric and/or other methods for determining the test substance and its major metabolites in biological fluids in order to avoid unnecessary administration of radioactive materials to human beings.

9.3 Protein binding

The degree of binding of the test substance to proteins in human and animal plasma should be measured in order to detect species variation in the amount of free substance in plasma samples that contain similar total amounts of drug. The proposed dosage schedules for man should take account of any such differences.

It is important to determine which fraction of the plasma proteins binds the test substance, since this binding may displace natural substances or concurrently administered medicaments from the protein, and so alter their activities or toxicities; conversely, other medicaments may displace the test substance from its binding sites. An appropriate warning of possible drug interactions should be issued to the physicians in charge of subsequent studies of the test substance in human beings.

9.4 Studies in man

The fate of the test substance should be determined in human beings after graded doses have been administered by the intended therapeutic route. The concentrations and amounts of the substance and/or its metabolites in blood (and/or plasma), urine and faeces should be determined at varying times after administration. The main objectives of these tests are to establish a rational dosage regimen for subsequent clinical studies and to see if the metabolites of the test substance in man are the same as those in the animals used in the toxicity studies. Additional animal studies should be carried out before clinical studies are undertaken if a high proportion of the compound is converted to a different metabolite that might be expected to be toxic to human beings. Most stable polar metabolites would not be expected to be toxic to man, since they are usually excreted more rapidly and are less toxic than parent substances.

It is desirable to carry out similar studies in patients, both early in treatment and after prolonged treatment, in order to detect changes in the fate of the drug and therefore possibly a change in its efficacy or toxicity.
10 Chemistry and pharmacy

The chemical and pharmaceutical development programme for a new chemical entity should be planned with regard to the nature of the drug, the scale of the proposed clinical investigation and the intended duration of treatment in man. The type of information required at each stage is related to the nature and duration of the proposed use. Prior to the first administration to man, a tentative physico-chemical and analytical specification should be prepared for batches of active constituent to be used in toxicological investigations and for subsequent clinical trials. The profiles should include such parameters as description of physical properties and evidence of structure and potency. The material will also have been characterised by the method of synthesis and an impurity profile established by suitable chromatographic techniques. If the proposed dose of the drug is small and the percentage of impurities is low (often a higher percentage of impurities must be accepted in natural products than in synthetic drugs) it is not necessary fully to identify impurities, provided that the batches used for toxicity studies were of closely similar quality to those to be used clinically.

Stability studies should be undertaken on the active constituent and on any formulation intended for clinical use, but the use of simple formulations such as aqueous solutions and capsules is recommended for first trials. Only short-term stability studies will be required at the stages of animal toxicity and preliminary clinical tests. Their duration will usually be related to the time interval between manufacture and completion of the study. The expiry date of the material supplied for testing should be known.

The information about chemistry and pharmacy that should be available prior to the first administration of a new chemical entity to man has been listed in Appendix G.

As trials progress and become multicentre and possibly more prolonged, more extensive studies will be required. Sufficient batches of the active constituent should then have been prepared for a specification to be defined both for the active constituent prepared by the proposed method of manufacture and for the developed formulation. Stability studies should then be initiated on several batches both of active ingredient and the chosen dosage form.
References

1. Irwin, S (1964) from Animal and Clinical Pharmacologic Techniques in Drug Evaluation, Chap. 4, p36. Eds Nodinc, J E and Siegler, P E.


Appendix A

Experimental guidelines for chronic toxicity studies in rats

1 Animals
The age, strain and source of the animals and their diet should be specified. Young animals are preferable for long-term studies.

2 Route of administration
The route will usually be that intended for man.

The parenteral route is used in addition for some substances intended for local application (see text). Administration by gavage is preferable to administration in the diet unless the test is to continue for longer than six months (see also Appendix 1, 2).

3 Frequency of administration
Usually this should be once every day (see text).

4 Period of dosing
This will depend upon the intended clinical use of the drug (see text).

5 Dose levels
a Usually three are necessary but two may suffice in some instances.

b The highest dose should produce toxic effects and preferably kill some of the animals.

c The lowest dose should normally be two to five times the predicted therapeutic dose in man (calculated on a weight for weight basis), or two to five times the minimum dose producing the effect in the test species which is relevant to the expected therapeutic effect.

d Dosages should be spaced logarithmically.

e A control group, run concurrently, should receive the vehicle used for the drug.

6 Numbers of animals
Treated and control groups should be of equal size and consideration should be given to using 25 animals of each sex to allow the possibility of:
a Histological examination of 10 animals of each sex at the end of dosing at least in the control and highest dose groups.

b Histological examination of six animals of each sex one month after the end of dosing to determine whether or not any changes produced by the drug are reversible.

c The killing of nine animals for studies of blood, urine and tissue concentrations at intervals during and at the end of the experiment if a suitable analytical method is available.

7 General observations

a Before and daily during dosing:
   body weight
   overt behaviour
   average food intake

b Ophthalmoscopic examinations after dilatation of the pupil should be made before and at the end of the experiment and, in tests lasting several months, also at intermediate times.

8 Blood biochemistry

Examination is made of the blood of all animals at least in the high dose and control group at the termination of dosing; if deviations are found the blood of all groups should be examined. An examination of base line values prior to dosing is advantageous when there is insufficient background information concerning the animals. In experiments of long duration examination should also be made of blood taken from selected rats after treatment for various periods.

Determinations should be made of a relevant selection from:
   total protein, albumin, globulin and electrophoretic pattern
   urea, creatinine
   sodium, potassium, calcium, inorganic phosphate
   glucose
   transaminases: SGOT (AST) and SGPT (ALT)
   alkaline phosphatase, CPK
   other enzymes if appropriate
   total bile pigment.

9 Haematology

In parallel with the biochemical studies, determinations are usually made of:
haemoglobin and methaemoglobin concentration
packed cell volume
platelet count
total and differential leucocyte counts including examination of stained film
total red cell and reticulocyte counts

(Examination of differential leucocyte and reticulocyte counts is normally limited to the high dosage and control groups and extended to the other groups only if an abnormality is found in blood films, haemoglobin level or packed cell volume.)

10 Urine
Specimens should be taken from the bladder at autopsy for:
microscopic examination
specific gravity
detection by test paper of protein, pH, glucose, specific gravity and blood content.

11 Autopsy
a When performing autopsies, it is important to look for signs of local toxic effects at sites of parenteral administration or in the alimentary tract if the animals are dosed orally. The animals are weighed before killing. A gross examination is made and the findings recorded.

b The following organs are removed and weighed:
adrenals    pituitary
brain       prostate
gonads      seminal vesicles
heart       spleen
kidneys     thymus
liver       thyroid
lungs       uterus

c Microscopical examination should be made of the tissues listed below from all animals treated at the highest dose level and from the undosed controls. If any abnormalities are detected, the tissues of animals at the next lower dose level should be examined:
adrenal    aorta    bone marrow – section from
brain      colon    femur or rib and smear
<table>
<thead>
<tr>
<th>Organ</th>
<th>Organ</th>
<th>Organ</th>
</tr>
</thead>
<tbody>
<tr>
<td>eye</td>
<td>gonads</td>
<td>heart</td>
</tr>
<tr>
<td>kidney</td>
<td>liver</td>
<td>lung</td>
</tr>
<tr>
<td>lymph nodes</td>
<td>mammary gland</td>
<td>pancreas</td>
</tr>
<tr>
<td>pituitary</td>
<td>prostate</td>
<td>salivary gland</td>
</tr>
<tr>
<td>sciatic nerve</td>
<td>skeletal muscle</td>
<td>skin</td>
</tr>
<tr>
<td>small intestine</td>
<td>spinal cord</td>
<td>spleen</td>
</tr>
<tr>
<td>stomach</td>
<td>thymus</td>
<td>thyroid</td>
</tr>
<tr>
<td>tongue</td>
<td>urinary bladder</td>
<td>uterus</td>
</tr>
</tbody>
</table>

Other tissues as indicated as appropriate by the clinical or post mortem observations.

**Appendix B**

**Experimental guidelines for chronic toxicity studies in dogs or other suitable non-rodents**

The recommendations differ from those for the rat only in the following respects:

1. *Route of administration*

Usually that intended for man. When the drug is to be given orally to dogs it is recommended that capsules or tablets are used. The same procedures or gavage may be used for monkeys.

2. *Numbers of animals*

Each group should consist of at least six animals, three of each sex. Additional animals are needed if a recovery study is to be incorporated.

3. *General observations*

- behaviour (daily)
- food intake (daily)
- body weight (weekly)

**ECG:** before dosing and at regular intervals during the study.

Temperature, respiratory rate, pulse and hearing may also be assessed during the study.

4. *Blood biochemistry*

Samples should preferably be examined twice before drug administration and at suitable intervals during the study. The tests
applied depend upon the species, the selection being taken from those listed for the rat, with the addition of cholesterol.

5 *Haematology*

As for rat but in addition:

- one stage prothrombin time
- activated partial thromboplastin time.

6 *Urine*

Similar examinations are made as in the rat but when dogs are used specimens of urine can also be taken by catheterisation during the test.

7 *Autopsy*

As for the rat and also tissue examination of gall bladder and parathyroid glands.

**Appendix C**

**Experimental guidelines for maternal and foetal toxicity studies in rabbits**

1 *Objective*

The purpose of such studies is to look for adverse effects of the drug on the mother and foetuses when it is given to pregnant animals especially during the period of organogenesis.

2 *Animals*

The age, strain and source of the animals and their diet should be specified. It is desirable to use young uniparous rabbits of a thalidomide-sensitive strain.

3 *Group sizes*

Twelve is a suitable number.

4 *Mating procedure*

Either artificial insemination or natural mating may be used.

5 *Route of drug administration*

The route will usually be that intended for man. Administration by gavage is preferable to administration in the diet.
6 **Frequency of administration**

Usually this should be once every day.

7 **Period of dosing**

Dosing may be given from day 6 to day 18, the day of insemination being counted as day 0, but for drugs that may influence nidation it is preferable to start dosing on day 8.

8 **Dose levels**

a Usually three are necessary.

b The highest dose should produce some evidence of maternal toxicity, e.g. a reduction of weight gain.

c The lowest dose on a body weight basis should preferably be two to five times the amount expected to have a therapeutic effect.

d Dosages should be spaced logarithmically.

e If the reproductive characteristics of the strain of animals are not well known it is advisable to run two control groups, one untreated to serve as an environmental control and the other receiving the vehicle for the drug and, if appropriate, the excipients.

9 **Observations during pregnancy**

a Body weight – weekly or more frequently if necessary (excessive stress during handling can lead to abortion).

b Overt behaviour – daily.

c Abortions.

d Deaths – all animals dying during the study should be autopsied.

10 **Examination at termination of pregnancy**

a On day 28, 12 dams in each group are killed and counts are made of:

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>corpora lutea</td>
<td>live foetuses</td>
</tr>
<tr>
<td>implantations</td>
<td>dead foetuses</td>
</tr>
<tr>
<td>resorptions</td>
<td>other maternal and foetal abnormalities</td>
</tr>
</tbody>
</table>

b Each foetus is assigned an order number in the uterus, weighed, sexed and examined for external abnormalities.

c The foetuses from each mother are chosen at random for examination by:
open dissection
Wilson's section technique
Staples and Schnell's alizarin red method or X-ray for skeletal structure.

Appendix D

 Experimental guidelines for maternal and foetal toxicity studies in rats

1 Objective

The purpose of such studies is as defined for the similar studies using rabbits.

The experimental procedure recommended differs from that for the rabbit in the following respects:

2 Animals

Two- four month old nulliparous animals are suitable.

3 Mating procedures

It is usual to examine vaginal smears daily in order to select only those females with regular oestrous cycles. Two or three such animals are then caged with one fertile male. A female showing sperm in a subsequent vaginal smear is assumed to be pregnant (day 0 of pregnancy).

4 Period of dosing

Must include days 6 to 15 (inclusive) of pregnancy.

5 Numbers of animals

This should be sufficient to provide 20 pregnant animals in each group (12 for examination at term, eight to rear their young).

6 Observations during pregnancy

As for rabbits but recording body weight daily.

7 Examination at termination of pregnancy (day 20)

a Twelve dams in each group are killed and examined in the manner described for rabbits.

b Eight dams in each group are allowed to come to term. All
surviving young are weighed and sexed on the day of birth and examined for external abnormalities. A record is made of litter size and numbers of live, dead and malformed offspring.

The litters are usually standardised to eight by culling and/or redistribution.

8 Observations during lactation

a The dams and their offspring are weighed and examined at suitable intervals, e.g. on days 4, 12 and 21 post partum.

b At 21 days all pups are autopsied.

Appendix E

Experimental guidelines for three-generation reproduction studies in rats

1 Objectives

The main purpose of these studies is to reveal adverse effects on reproduction that might escape detection in maternal and foetal toxicity studies.

2 Animals

The age, strain and source of the animals and their diet should be specified. Previous knowledge of their breeding performance is of particular value. At the start of the test males should be approximately six weeks and females approximately four months old. The oestrous cycles of females are more regular when the two sexes are kept in adjacent cages.

3 Route of administration

Usually as intended for man.

4 Frequency of administration

Usually this should be once every day or continuous if the drug is added to the diet.

5 Period of dosing

The parental generation only is dosed.

a Males: from at least 60 days before mating until the animals are no longer required for mating purposes.
b Females: from at least 14 days before mating and throughout matings, pregnancy and lactation until 21 days post partum.

6 Dose levels
a Usually three are necessary but two may suffice in some instances.
b The dosages chosen for the females will usually be based on experience gained from maternal and foetal toxicity studies. It is usual to mate them with males receiving the same dose.
c Dosages should be spaced logarithmically.
d Control animals are run concurrently. It may be necessary to have an environmental control group (untreated) and a vehicle or excipient control group each containing the same number of animals as the drug treated groups. The environmental control group may be omitted if the vehicle is known to be bland and the breeding history of the strain is well known.

7 Number of animals
There are usually 12 males and 24 females in each treatment group and twice the number of controls (see 6d above).

8 Mating procedure
When mating is required it is usual to cage each male with two or three females.

9 Observations on the parent (F₀) and first filial (F₁) generation
a During the pre-mating period
   body weight – weekly
   overt behaviour – daily
   vaginal smears – daily until mating has been established by the presence of sperm. The occasion of finding sperm is designated day 0 of pregnancy.
b During pregnancy
   females should be weighed at intervals, e.g. on days 0, 6, 12 and 18.
c Autopsy
   On scientific grounds the preferred day for autopsy is day 20. Twelve females in each group are killed and their foetuses are examined as described for maternal and foetal toxicity studies (Appendix c). The remaining 12 females in each group are allowed to continue to term and deliver their young.
d Immediately following parturition:
    all dams are weighed
    all offspring are examined and a record is made of their
    weight and sex, and of the numbers alive, dead and/or
    abnormal.

The length of gestation for each dam is calculated and recorded for
each group.

Litters are usually standardised to eight by culling and/or redistribu-
tion.

e During lactation:
    dams are weighed weekly at a regular time from the day of
    parturition and their behaviour is scrutinised periodically
    the F₁ generation is weighed and their behaviour is scrutinised
    periodically
    simple tests for auditory and visual functions are applied to
    the F₁ generation.

f At 21 days post partum (weaning):
    the numbers and weights of all surviving young are deter-
    mined
    survivors are examined for external abnormalities.

All young except those needed for the studies described in the
following section are autopsied.

10 Studies of the reproductive capacity of the F₁ generation

One male and one female from every litter of each experimental
group are randomly selected and caged separately from 21 days post
partum. At 42–56 days post partum these F₁ progeny are paired to
provide F₂ foetuses for examination. Mating should be of animals
within the group – sib-mating is not recommended.

Pregnant F₁ females are killed between day 18 and day 21 of
pregnancy and examined internally for evidence of foetal and
maternal toxicity as described above for maternal and foetal toxicity
studies.

11 Further studies of the parent generation (F₀)

If the drug affects reproductive ability or if other abnormalities are
observed after mating, the same animals should be kept without
 treatment for one month and then mated again to assess whether
reproductive function recovers. The males may need to be kept
without treatment for a longer period if the early stages of spermato-
genesis are affected.
Deaths during these studies

All animals dying during these studies should be autopsied and the cause of death revealed if practicable.

Appendix F

Experimental guidelines for carcinogenicity studies in rats and mice

1 Animals

Newly weaned mice or rats from stocks that have been well studied are required. The strain selected should be healthy and not have a high incidence of spontaneous tumours. Particularly high standards of animal husbandry are required to minimise losses during these lifetime studies. Details concerning the animals and their diet should be specified.

2 Route of administration

The active ingredient is usually added to the diet. Its uniformity of distribution and stability in the diet should be checked. (Food intake is measured to ensure that an appropriate quantity of the active ingredient has been ingested.)

3 Period of exposure

The drug-containing diet is administered continuously to mice for 18 months or to rats for 24 months or until the result of the test is obvious from the incidence of tumours.

4 Dose levels

a Usually three are necessary but two may suffice in some instances.

b The high dose should produce minor toxic effects, e.g. cause a 10 per cent reduction in weight gain. It should not unduly shorten the life span of the animals.

c The low dose would normally be about twice the estimated therapeutic dose in man (calculated on a weight for weight basis), or about twice the minimum dose producing the effect in the test species which is relevant to the expected therapeutic effect.

d Dosages should be spaced logarithmically.

e A control group should be run concurrently. When formulated drugs are being tested it is appropriate to add the excipients to the diet of all or half the controls.
f In order to determine doses, and particularly the low dose, knowledge is required of the pharmacologically effective dose and/or the absorption of the drug by the method of dosing used in the carcinogenicity study, and use of a pilot test for this purpose is recommended.

5 Numbers of animals

Treatment groups – 50 males, 50 females.
Control group – 100 males, 100 females.
Additional animals may be required for pharmacokinetic investigations during the study.

6 General observations

a Before and daily during dosing
   food intake
   overt behaviour

b Before and weekly during dosing
   body weight
   clinical examination to reveal the incidence and appearance of tumours

c Periodically (e.g. three-monthly)
   inspection of blood films.

Sick animals should be isolated to prevent cannibalism.

7 Autopsy

a Full autopsy is required of:
   all animals that die or have to be killed during the test because of illness
   all animals that survive until termination of the experiment; they are weighed before being killed.

b A carcinogenicity study requires comparison of the incidence of tumours in treated and control groups. Thus it is advisable for the top dosage group to be examined first to demonstrate the susceptible organs.

Careful naked eye examination and dissection of all organs is made to detect the presence of tumours and the findings recorded. Attention should be focused on abnormalities and only those organs deemed to be affected need be weighed.
Following autopsy the suspected tissues are examined microscopically. The routine microscopy of all tissues is regarded as unnecessary, but as microscopic or diffuse neoplasms or metastases in the haemopoietic and reticulo-endothelial systems and in endocrine glands may not be easily visible to the naked eye, histological examination of the following tissues is recommended:

- adrenals
- blood film
- bone marrow
- liver
- lymph node
- pituitary
- spleen
- thymus
- lung
- thyroid

Appendix G

Chemical and pharmaceutical information required prior to the first administration of a new chemical entity to man

1. Names
Chemical name, laboratory code and official names if appropriate.

2. Description
Physical form, structural formula, molecular formula and molecular weight.

3. Method of synthesis
The final route of manufacture may not have been established at this stage and it is therefore recommended that a flow sheet diagram is prepared of the synthetic route by which the batch for use in the studies was prepared. Each stage of the process should be briefly outlined, together with full details of the final purification. Starting materials should be specified and it should be indicated what impurities are likely to be present in the final product.

4. Physico-chemical and analytical profile
A profile should be established of the batches proposed for use in toxicological and clinical investigations. Such profiles should include information on the following as appropriate:

- evidence of structure, results of elemental analysis, i.r., n.m.r. and u.v. spectra
- solubility, pKa, pH, $\varepsilon_{\text{ICM}}$, isomeric potential (optical rotation),
melting point, solvation and particle size analysis, the last being especially important for drugs of a low solubility because of its influence on bioavailability potency and/or purity chromatographic impurity profile together with comment on possible or known impurities other analytical profiles such as heavy metals, ash, water and solvent content and, when needed, the results of biological tests such as toxicity, safety, sterility or pyrogenicity.

A description of the methods used to establish the above profile should be presented.

5 Stability

Information on one batch of active constituent relevant to the proposed trial in man.

6 Formulation

The formulation of any presentation for use in the clinical investigation should be available, together with the preformulation studies (including those to establish compatibility with excipients) and short-term stability studies related in duration to the time interval between manufacture and completion of the proposed clinical study.