

PRE-CLINICAL BIOLOGICAL SAFETY TESTING ON MEDICINAL PRODUCTS DERIVED FROM BIOTECHNOLOGY

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Additional Notes	This note for guidance concerns the application of Part 3 of the Annex to Directive 75/318/EEC as amended with a view to the granting of a marketing authorisation for a medicinal product derived from biotechnology. It classifies products derived from biotechnology into groups and provides a summary of general methodologies pertaining to pre-clinical safety testing and minimum testing requirements for each group.

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PRE-CLINICAL BIOLOGICAL SAFETY TESTING ON MEDICINAL PRODUCTS DERIVED FROM BIOTECHNOLOGY

1. INTRODUCTION

A wide range of proteins, peptides and other substances for use as biological medicinal products can now be produced by novel biotechnological processes, including recombinant DNA (rDNA) methods and large scale cell culture. These products include naturally occurring human proteins and peptides such as Hormones (e.g. insulin, human growth hormone, erythropoietin), Blood Products (e.g. factor VIII, tissue plasminogen activator), Cytokines (e.g. interferons, interleukins, colony stimulating factors, cytotoxins) as well as Monoclonal Antibodies of murine or human type. Viral and bacterial Antigens, used for new vaccines, may also be prepared. It is anticipated that many new monoclonal antibody- and cytokine-products will be rapidly developed and that sustained progress will occur in the development of blood products and vaccines. Furthermore, availability of techniques for the single- and multi-site mutagenesis of protein-coding DNA and for the complete chemical synthesis of genes provides prospects of modifying the amino acid sequences of known proteins and peptides at will. This will lead to the generation of molecular variants of naturally occurring human proteins for clinical assessment.

The large scale availability of biotechnology-derived products now makes feasible their evaluation in the diagnosis, prevention and treatment of disease. However, there are potential safety concerns that arise from the novel processes used in their manufacture and from the complex structural and biological characteristics of the products. Such products therefore require thorough testing by relevant laboratory methods which allow adequate assessment of safety before clinical investigation. This is particularly important where repeated, or large or non-physiological doses of a particular product may be required for a given therapeutic effect and when knowledge of its biological effects in man remains incomplete.

The general aim of pre-clinical safety testing is to ascertain whether new products have the potential to cause unexpected and undesirable effects. Existing pre-clinical notes for guidance for the safety testing of new chemical medicinal products recommend that studies be conducted on each new substance to investigate pharmacokinetics, pharmacodynamics, acute and chronic toxicity, reproductive effects and carcinogenicity in suitable experimental systems. These notes for guidance will provide the basic framework for recommendations on testing specific biotechnology-derived products, but future amendments and alterations to these are likely to be necessary to take account of the particular nature of the biotechnology-derived product considered. It is expected that only certain aspects of the studies recommended for chemical substances will be applicable to biotechnology-derived products before marketing authorisation and that expert assessment will usually be based on extended pharmacological investigations.

The present document is intended to cover products derived from new biotechnological methods including rDNA techniques, hybridoma technology etc., and does not apply to vaccines containing live organisms. However, certain aspects of this document may also be relevant to chemically synthesised peptides and some non-peptide products (e.g. polysaccharides).

Products derived from biotechnology may be highly pharmacologically active, or be active on the immune or other physiological systems. Based on such classification and for ease of reference in

this document, biotechnology-derived products are subdivided into four broad product groups* depending on whether they are Hormones or Cytokines or Other Regulatory Factors, Blood Products, Monoclonal Antibodies or Vaccines – see Table 1. In addition each group of products may be divided, on biochemical grounds, into three categories (Table 1):

- I polypeptides and proteins shown to be identical to naturally occurring human polypeptides and proteins.
- II polypeptides and proteins closely related to human polypeptides and proteins, but containing known differences in amino acid sequences and/or post-translational modification(s) that may affect biological activity or immunogenicity or both. This category also includes proteins whose structure may be identical to the natural product but where this cannot yet be verified.
- III polypeptides and proteins distantly related or unrelated to human polypeptides and proteins (e.g. murine monoclonal antibodies and viral/bacterial antigens).

The above categories provide a basis for determining the strategy of safety testing requirements. In addition, other factors, including method of production, and the quality with respect to the precise physico-chemical nature of the impurities and any excipients (e.g. human serum albumin) present in the pharmaceutical formulation may all have a bearing on testing requirements.

Biotechnology-derived products are likely to pose particular problems in the area of pre-clinical safety testing, especially in relation to toxicity testing in animals *in vivo*. Their safety evaluation will therefore have to take into account many factors: for example, certain proteins (e.g. human interferons) are highly species-specific and are thus much more pharmacologically active in man than in any other animal species. In addition, the amino acid sequences of human proteins will often be significantly different to their natural counterparts in other species. Thus, these proteins frequently produce immunological responses in foreign hosts which may ultimately modify their pharmacological (biological) effects and which may result in toxicity due to immune complex formation. Such toxicity would, of course, have little bearing on the safety of the product in man, the intended (natural) host. Standard toxicological procedures applying to the pre-clinical safety testing of chemical substances may therefore be inappropriate because they will not give a useful measure of the toxic potential of some protein products in man. In such situations *in vitro* biological tests, e.g. to ascertain specific activity, species specificity and immunochemical characteristics may aid the choice of test species.

As for all toxicological investigations, other factors to be considered include:

- general and specific pharmacological activity,
- mechanism of action,
- intended dosage and route of administration,
- clinical status of the recipient,
- prophylactic/therapeutic/diagnostic indication,
- and results from preliminary clinical investigations.

* It is recognised that certain products may not fit easily into the stated categories. Further developments are known to be taking place which are expected to lead to entirely novel products, e.g. adjuvants, plant toxins, inhibitors and carrier molecules.

In this note for guidance a summary of general methodologies pertaining to pre-clinical safety testing precedes separate guidance notes on the minimum testing requirements for each product group (Table 1) which are outlined under the following headings*:

- 3.1 Hormones and cytokines and other regulatory factors
- 3.2 Blood products
- 3.3 Monoclonal antibodies
- 3.4 Vaccines

Some in vitro studies included herein are also covered by the following notes for guidance:

- Production and quality control of monoclonal antibodies of murine origin;
- Production and quality control of medicinal products derived by recombinant DNA technology.

It is emphasised that this document is not intended to provide rigid requirements of universal applicability; a degree of flexibility in the application of the recommendations made herein relating to the proposed use of any particular product is implicit and case by case evaluation will be essential.

2. SAFETY TESTS FOR BIOTECHNOLOGY-DERIVED PRODUCTS

As discussed in the introductory section there are a number of factors that may limit the usefulness of the well established toxicological investigations for chemical active principles when applied to biotechnology-derived products. For this reason it is likely that a more flexible approach is necessary for biotechnology-derived products. A wide range of investigative techniques (e.g. pharmacological, biochemical, immunochemical, toxicological and histopathological) should be used where appropriate in the assessment of the product's effect, over an appropriate range of doses, during both acute and chronic exposure.

Tests recommended in this note for guidance should be carried out on the final product or material consistent with the final product with an impurity profile which has been established by analytical tests. As far as possible, information should be obtained on whether any observed adverse effects are due to the intended therapeutic/diagnostic substance or to the impurities contained in the product.

The possibility that there may be differences in the contaminants according to production methods is predominantly an issue that requires definition by quality control/assurance procedures rather than the application of new toxicity tests (see also section 2.5.5).

Although purities of 95% can now be achieved for some biological products (including biotechnology products) they remain in general more variable batch to batch than the equivalent chemically defined products, because of the nature of biological production. In these circumstances, in-process control of production and/or GMP procedures assume much greater importance and pre-clinical toxicity testing much less importance than would be the case for defined chemical entities.

* It is recognised that certain products may not fit easily into the stated categories. Further developments are known to be taking place which are expected to lead to entirely novel products, e.g. adjuvants, plant toxins, inhibitors and carrier molecules.

2.1 Selection of animal species

Appropriate mammals should be selected on the basis of a number of factors. These should include the findings of pharmacokinetic and pharmacodynamic studies with respect to immunoreactivity and species-specific pharmacological effects. Pre-existing knowledge, where available, on the suitability of animal species in relation to specific product groups should be taken into account. Where it is not possible to predict the value of particular animal species for safety testing, initial investigations may be considered in any of several rodent and non-rodent species. Where studies are expected to exceed a duration of four weeks, test species known to be low responders with regard to antibody production against the test substance should be considered.

2.2 Selection of doses

The rationale for the doses selected, route and frequency of administration in each study should be provided. This should take into account the therapeutic and/or maximum doses proposed to be used in man and the intended clinical use of the product. A series of increasing doses should be used to investigate dose-target organ toxicity. However, it may not always be possible to use doses that produce toxic effects. In some cases there may be large differences in pharmacological responsiveness between man and the test species which should be taken into consideration. Doses should be expressed where possible as mg or units per body weight or surface area.

2.3 Pharmacokinetic studies

The aim should be to determine the pattern and time course of absorption, distribution, metabolism and elimination of the active product and its metabolites in animals and to relate these data to man.

It is recognised, however, that it is not always possible to conduct such wide ranging studies, for example, it is not current practice in the field of 'conventional' vaccines to conduct ADME (Adsorption, Distribution, Metabolism, Elimination) studies on antigens, which are administered in very small amounts, and which may have a very short half-life.

An assessment of the pharmacokinetics of the biotechnology-derived product should be carried out as early as possible during the development of the product (within the limitations of analytical methods). This should include measurement of the concentration of active principle with time and the time-course of the pharmacological effect. Such information will supply useful guidance on species selection for pharmacology and toxicology tests and on the determination of the duration of repeated dose studies.

2.4 Pharmacodynamic studies

Methods of pharmacodynamic testing (pharmacodynamic effects referring to envisaged indications; routine pharmacodynamics; drug interactions) will vary with the type of preparation under investigation. The aim should be to provide evidence of a pattern of primary effects and mode of actions within the pharmacological major physiological systems using a variety of experimental techniques.

Pharmacodynamic studies are required to establish the therapeutic rationale and should address dose-response relationships. Such studies should investigate the effects and functional interactions of a product at physiological doses, where known, and at higher doses. The latter

should aim to reveal any unexpected effects at the target organ and at other organs/tissues. The pharmacodynamic effects of the test product should be compared with those of the natural substance where possible.

2.5 Toxicological studies

For all toxicological tests the reason for the selection of species, dose, route of administration, duration of treatment/experiment and the number of animals used should be justified, having regard to the data available on the pharmacokinetics, metabolism, pharmacodynamics and immunoreactivity of the product, and to the potential differences between the animal species and man (Notice to Applicants, Volume II of this series). Non-physiological doses or routes of administration of the product may affect the distribution, the biological effects and toxicological profile of the product.

2.5.1 Single dose toxicity

A note for guidance on the qualitative and quantitative study of toxic phenomena and their occurrence related to time after a single administration of the substance, or combination of substances to be tested, has been published (Note for guidance on *Single Dose Toxicity*).

The effects of a single high dose of the product on the major physiological systems should be investigated using a wide range of techniques. The dose selected for this purpose and for

other toxicity studies should be appropriate to the intended dosage and duration of dosage in man.

2.5.2 Repeated dose toxicity

A note for guidance on the qualitative and quantitative study of toxic phenomena after repeated administration pertaining to reason, purpose, design, duration and evaluation of such studies, has been published (Note for guidance on *Repeated Dose Toxicity*). Such repeated dose toxicity tests should be conducted where the results from pharmacodynamic and/or single dose toxicological studies are considered inadequate in assessing the safety of the product under investigation. The recommended duration of repeated dose studies already published may require modification because of immunological incompatibility of the product with the animal species used. It may also be necessary to vary the dosing schedule and to consider the use of low responding species with respect to immunoreactivity.

2.5.3 Local tolerance

Assessment of potential toxicity following local application should be performed as for a chemically synthesised compound.

2.5.4 Reproduction toxicity

A note for guidance on the requirements and experimental design of studies investigating potential embryotoxicity, peri- and post-natal toxicity, and fertility and general reproductive performance has been published (Note for guidance on *Detection of Toxicity to Reproduction for Medicinal Products*). These guidelines are not rigid requirements, but should be interpreted in relation to the proposed use of the product.

Reproduction toxicology testing may not be necessary in the following situations:

- where satisfactory evidence of the degree of safety of an identical product has been obtained from previous studies;
- where there is appropriate labelling (patient and/or product information) on the use of the product in women of child bearing potential, pregnancy or lactation;
- where testing is inappropriate because of the intended clinical use of the product.

Where reproduction toxicity studies or segments thereof are justifiably required, dosing regimens may have to be adjusted to minimise expected/potential immunological responses. Where appropriate, regular treatment periods may be shortened or sub-divided.

2.5.5 Mutagenic and oncogenic/carcinogenic potential

The description of testing of medicinal products for their mutagenic potential has been published (Note for guidance on *Testing of Medicinal Products for their Mutagenic Potential*). Requirements for carcinogenicity studies have also been published (Note for guidance on *Carcinogenic Potential*). In most cases it is likely that studies will be inappropriate or not feasible for the assessment of the mutagenic and carcinogenic potential of biotechnology-derived products.

The requirements for the application of short-term tests for detecting mutagenic and carcinogenic potential will depend largely on the availability and sensitivity of analytical methods for the assessment of the identity, quality and purity of the product.

Appropriate testing for oncogenic/carcinogenic potential should be based on the type of product, its possible contaminants and its actions. Long-term studies in animals to detect an oncogenic/carcinogenic potential would, however, be only applicable where a product causes concern as a result of:

- some specific aspects of its biological action (e.g. induction of hyperplastic or neoplastic cellular responses) if observed in long term studies,
- its pattern of toxicity or long-term retention detected in previous studies.

Where oncogenicity/carcinogenicity testing is indicated studies should be undertaken in one or more species (preferably using species in which biological effects have been demonstrated in response to the product) and conducted with high doses. Where practical the proposed therapeutic route of administration should be used. Treatment should be for as long as is compatible with the immunological response of the animal. If the duration of treatment is short, observation should be continued for a biologically relevant time period after the termination of treatment.

2.5.6 Immunological aspects of toxicology

This is a difficult area for investigation as the observed effects of biotechnology-derived products on the immune system of chosen test species may have little relevance to the safety of such products in man, for example, when the product induces antibody formation and subsequent immune-complex formation. Also, many new products, e.g. cytokines, have profound effects on the immune system and these can be an essential part of their therapeutic action(s). Therefore, similar effects in test species should be carefully monitored in relation to intended actions on the immune system. No fixed battery of tests is recommended. In some cases, the following investigations may be appropriate to monitor whether a product:

- a) is immunogenic in the test species, and thus induces antibody formation. The pharmacokinetics of potential antibody formation (including neutralising antibodies) should be determined if possible;
- b) forms complexes with host immunoglobulins or immunofunctional molecules, e.g. complement;
- c) interacts with cells of the immune system causing their dysfunction;
- d) causes the release of pharmacologically active molecules which themselves affect the functions of the immune system.

2.5.7 Homologous animal systems

Therapeutic models in animal species may be useful to aid clinical investigations. For this purpose animal homologous systems, i.e. the preparation of the product in a form specific for the animal test species, may have to be created in exceptional circumstances. Since clinical studies may have been initiated, close co-operation between pre-clinical and clinical investigators will provide guidance for tests in experimental animals to address specific clinical problems.

2.6 Conclusions

At the present time no battery of safety tests can be described which would be applicable to all types of product groups and all biochemical groups available today. The usefulness of performing various combinations of tests should be ascertained by discussions amongst pharmacologists, toxicologists and clinicians, both from the pharmaceutical company (including those making the product) and the competent authorities. The outcome of these discussions would be expected to be reflected in the Expert Reports submitted for each product, which would explain the case by case approach employed.

In all cases toxicological investigations would be preceded by studies addressing the biotechnological problems of production, quality control, contaminants and impurities.

Although the animal testing programme outlined above can provide a core of knowledge about a particular product, this may only be of limited value in predicting problems in clinical practice. This has major implications regarding the exclusion of experimental studies in the investigation of a specific biotechnology product, and on the clinicians' assessment of the relevance of experimental data to the therapeutic purposes of the compound in patients.

3. DESCRIPTION OF SAFETY TESTING BY PRODUCT GROUP

3.1 Hormones, cytokines and other regulatory factors

Hormones, cytokines and other regulatory factors are here defined as secreted, non-antibody, soluble mediators of cellular function or behaviour and include:

- products of the endocrine system (e.g. insulin, growth hormone),
- growth factors (e.g. erythropoietin, epidermal growth factor),
- alpha and beta interferons (i.e. anti-viral cytokines),
- lymphokines (i.e. mediators secreted by regulatory lymphocytes, e.g. interleukin 2, gamma interferon),
- monokines (i.e. factors or mediators secreted by monocytes and macrophages, e.g. interleukin 1),
- cytotoxins (i.e. factors or mediators known to cause cytotoxicity in vitro but which probably also have complex physiological effects in vivo, e.g. tumour necrosis factor),
- other regulatory factors (e.g. atrial natriuretic factor, streptokinase, bombesin).

Hormones and cytokines may be highly species specific for expression of biological activity, e.g. gamma interferon. Hormones are used clinically as replacement therapies, e.g. where a hormone is absent or present in insufficient amount. Cytokines and other regulatory factors are used as biological response modifiers.

3.1.1 For biochemical group I: where it is intended to produce by biotechnological techniques a biologically active protein (hormone, cytokine or other regulatory factor) which is already prepared by a non-biological technological process, and has an established place in medicine, pre-clinical studies should demonstrate that conventionally and biotechnologically produced proteins are equivalent.

For biologically active proteins that are not already established medicinal products, whether they are used in physiological or pharmacological dosage, safety testing in addition to pharmacodynamics may be applicable. Pharmacokinetic studies (see section 2.3) and acute toxicological studies (see section 2.5.1) may be required and (sub)-chronic toxicological and local tolerance studies (see sections 2.5.2 and 2.5.3) may additionally become necessary. In certain circumstances, embryotoxicity/teratogenicity and fertility studies might be necessary, and mutagenicity and tumourigenicity testing might occasionally be needed.

3.1.2 For biochemical group II: testing described under sections 2.3, 2.4, 2.5.1 and 2.5.2 will be applicable in most cases. Requirements for local tolerance studies (see section 2.5.3) will depend on the proposed route of administration. In addition reproduction toxicity studies may be necessary (see section 2.5.4).

Immunological aspects of toxicology (see section 2.5.6) may need to be assessed, especially for immunomodulating substances. The necessity for investigations on mutagenicity/tumourigenicity (see section 2.5.5) will depend on the product and its uses: in most cases they are unlikely to be appropriate.

3.1.3 For biochemical group III and biologically active substances not presently in use in man testing described under sections 2.3, 2.4, 2.5.1, 2.5.2 (to reflect anticipated clinical use as far as possible), should be applicable. Requirements for local tolerance studies (see section 2.5.3) will depend on the proposed route of administration. In addition reproductive toxicity studies (see section 2.5.4) may be necessary.

For immunological aspects of toxicology see section 3.1.2.

3.2 Blood products

Blood products are here defined as major components of blood including albumins, complement components and enzymes regulating the blood clotting and fibrinolytic processes, e.g. factor VIII, factor IX, tissue plasminogen activator, but excluding hormones and cytokines.

Comparison of the rDNA-derived blood products with their naturally derived counterparts is highly desirable.

3.2.1 For biochemical group I in addition to pharmacodynamic studies (see section 2.4) testing according to sections 2.3 and 2.5.1 may be necessary. More extensive safety testing is unlikely to be required.

3.2.2 For biochemical group II studies described under section 2.5.2 will often additionally be necessary. More extensive safety testing is unlikely to be required.

3.3 Monoclonal antibodies

It is expected that the majority of products included in this group will be monoclonal antibodies produced conventionally from murine or human hybridomas or EBV-transformed B cell lines. However, it is also expected that in future some monoclonal antibodies will be produced by rDNA techniques. The latter will include monospecific antibodies of known amino acid sequence, and various modified, hybrid or 'designer' monoclonal antibodies of mono- or bi-specific types.

Methods of pharmacodynamic screening will vary with the type of preparation under investigation; the aim should be to provide evidence of a pattern of pharmacological activity within the major physiological systems using a variety of experimental techniques. Particular attention should be given to binding of human complement and undesirable cross reactivity with a variety of human tissues. A suggested list of tissues to be used for immunohistochemical or cytochemical investigations of cross reactivity of monoclonal antibodies is given in Annex II of the note for guidance on *Production and Quality Control of Monoclonal Antibodies*. If the antibody is conjugated to a toxin or pharmacologically active substance, the structure, stability and pharmacokinetic pattern of the conjugate should be established.

Selective toxicological tests outlined in sections 2.4, 2.5.1 and 2.5.2 may be required for these products depending on the proposed frequency of application for diagnostic or therapeutic uses. The choice of doses should reflect the effects aimed at clinically.

The necessity for additional studies, e.g. reproduction toxicity (section 2.5.4), mutagenic/tumourigenic potential (section 2.5.5), immunological aspects (section 2.5.6) and local effects (section 2.5.3) relate to the intended clinical dose/effect and should be decided for each product on a case by case basis.

3.4 Vaccines

These include bacterial and viral immunogens (e.g. hepatitis B surface antigen made in yeast or continuous mammalian cell lines) for vaccination against infectious agents and antigens designed for other purposes (e.g. anti-fertility vaccines). This section does not apply to vaccines containing live organisms. Monoclonal antibodies may also be used as immunogens, i.e. anti-idiotypic vaccines, and should be considered to belong to both this section and section 3.3.

The duration of safety testing will depend on the proposed vaccination schedule. However, testing may be inapplicable for certain vaccines against infectious agents.

The pharmacokinetic studies (see section 2.3) should include assessment of retention of the product at the site of injection and its further distribution. Local tolerance testing is essential (see section 2.5.3).

Reproduction toxicity studies (see section 2.5.4) are not normally required. They may become necessary if the vaccine is intended for use in women of child bearing age or during pregnancy. Mutagenicity and tumourigenicity studies (see section 2.5.5) will usually not be required for the final product.

In addition to immunological aspects of toxicity testing (see section 2.5.6) the effects of any adjuvants and undue cross reactivity of induced antibodies with intrinsic human tissue antigens should be assessed.

ANNEX

TABLE 1

Provisional Categorisation of Biotechnology Products for Pre-Clinical Safety Testing

This table is provided to establish some general principles.

BIOCHEMICAL GROUP	PRODUCT GROUP			
	Hormones & Cytokines and Other Regulatory Factors	Blood Products	Monoclonal Antibodies	Vaccines (Antigens)
I Identical Polypeptides & Proteins	+	+	+ ^a	- ^c
II Closely Related but Different Polypeptides & Proteins	+	+	+ ^a	+ ^c
III Distantly Related or Unrelated Polypeptides & Proteins	+ ^d	-	+ ^b	+

+/- Products in this class are currently available (+), are not available (-).

- I Polypeptides and proteins shown to be identical to naturally occurring human polypeptides and proteins.
- II Polypeptides and proteins closely related to human polypeptides and proteins, but containing known differences in amino acid sequences and/or post-translational modification(s) that may affect biological activity or immunogenicity or both. This category also includes proteins whose structure may be identical to the natural product but where this cannot yet be verified.
- III Polypeptides and proteins distantly related or unrelated to human polypeptides and proteins (e.g. murine monoclonal antibodies and viral/bacterial antigens).
- a) Human monoclonal antibodies.
- b) Murine monoclonal antibodies.
- c) In some instances, e.g. anti-fertility vaccines, products may be identical to or closely related to human polypeptides and proteins.
- d) Polypeptides and proteins having regulatory activity, but having little homology with known human polypeptides and proteins.