

HANDBOOK

NON-CLINICAL
SAFETY
TESTING



UNICEF/UNDP/World Bank/WHO
Special Programme for Research and Training in Tropical Diseases (TDR)



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This Handbook on Non-clinical Safety Testing is designed to serve as an aid for scientists who wish to undertake non-clinical safety testing for regulatory purposes during product development. It has been developed as part of a significant and wide-ranging technology transfer and capacity building programme in the area of pre-clinical product development for disease endemic countries.

The Non-clinical Safety Testing Handbook was produced by a Scientific Working Group (SWG) on pre-clinical issues, convened by the UNICEF/UNDP/World Bank/WHO Special Programme for Research & Training in Tropical Diseases (TDR) and consisting of independent scientific specialists from around the world. The Handbook is broadly based on current safety testing guidelines including those of the Organisation for Economic Cooperation and Development (OECD) and the International Conference on Harmonisation (ICH).

The Handbook will provide scientists and laboratories in disease endemic countries with the necessary technical aid for planning and implementing non-clinical safety testing programmes. The Handbook attempts to highlight the differences between synthetic chemical drug, vaccine and traditional herbal (botanical) medicine development programmes.

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FOREWORD

One of the most important milestones in product development is the decision to enter into clinical trials with a candidate product. This important decision is based, in part, on data produced during non-clinical safety testing of the candidate during the preclinical phases of development. The quality and reproducibility of safety data are hereby key components of their utility for supporting the assumption of safety in humans. Therefore, when the non-clinical programme in TDR was started four years ago, Good Laboratory Practice (GLP) was viewed as an important starting point. The GLP activities have progressed quite successfully, and documents have been developed for use in training and upgrading of laboratories to GLP status.^{1, 2, 3, 4} However, GLP is a managerial tool concerned with the way non-clinical studies are planned, performed, monitored, recorded, reported and archived, and it is not concerned with the question about why the study is being conducted (i.e. is it justified? will it achieve the results?). The scientific issues of why the study is being conducted are as equally important as the technical, quality issues if a successful and internationally acceptable non-clinical data package for submission of a product for registration and marketing permits is to be achieved. It was therefore considered important to equip our target countries, the disease endemic countries (DECs), with a sound blueprint for developing non-clinical testing programmes that takes into consideration both the scientific and the technical, quality aspects of the studies. Especially important is a system for decision-making and planning of non-clinical activities.

The need for this technical guidance was expressed during the first Scientific Working Group (SWG) meeting on GLP issues, held at WHO, Geneva, in 1999, when participants from the DECs indicated an immediate need for a glossary for valid models

¹ *Quality standards in basic biomedical research. Draft handbook.* Geneva, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, 2001 (document no. TDR/PRD/QSBR/01.1).

² *Good Laboratory Practice (GLP). Handbook.* Geneva, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, 2001 (document no. TDR/PRD/GLP/01.2. TDR).

³ *Good Laboratory Practice (GLP). Training manual for the trainer.* Geneva, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, 2001 (document no. TDR/PRD/GLP/01.1A. TDR).

⁴ *Good Laboratory Practice (GLP). Training manual for the trainee.* Geneva, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, 2001 (document no. TDR/PRD/GLP/01.1B).

and a listing of appropriate tests. During the various TDR-sponsored GLP training workshops to introduce the concepts of GLP to scientists in DECs (1999-2002), scientists also expressed the need for guidance on how to plan a non-clinical safety testing programme. This demonstrated that, although the technical knowledge is available in these countries, there is an apparent need for guidance on appropriate tests that are necessary to support registration of a product. While guidance on technical requirements for non-clinical development does exist in many organizations (e.g. US Food and Drug Administration [FDA] Redbook; European Union, Committee on Proprietary Medicinal Products [CPMP] Notice to Applicants; International Conference on Harmonisation of Technical Requirements [ICH] Common Technical Document and specific Safety Testing Guidelines; Organisation for Economic Co-operation and Development [OECD] Guidelines for Testing of Chemicals, Part 4), such guidance might not be easily accessible to scientists in DECs. Furthermore, while it is true that product development should be evaluated on a case-by-case basis, a general list of tests would be useful for DECs and would provide the required glossary.

The possibility of developing a glossary for non-clinical testing was first discussed at the third meeting of the SWG on GLP issues, Geneva, November 2001, when it was recommended that a small working group be established to draft a document on “General guidance on non-clinical testing strategies” which would focus on toxicology and safety issues. The document would lay emphasis on specific aspects of programme development and testing of chemical drugs, vaccines, and traditional, herbal medicines, make reference to existing documents, and include relevant website links. It was proposed that the working group include representatives from ICH, FDA, the European Medicines Evaluation Agency (EMA) and members from DECs.

To follow up this recommendation, a preparatory regional meeting was held in Johannesburg, South Africa, 27-28 June 2002. It was attended by representatives from South Africa, Nigeria, Zimbabwe, Kenya, Cameroon, Madagascar, Tanzania, the World Health Organization Regional Office for Africa (WHO-AFRO), and Switzerland. The meeting was viewed as a fact-finding mission whereby knowledge gaps would be identified in order to enable development of a document clearly addressing the real needs of DECs in planning and conducting non-clinical safety tests. The objectives of the meeting were: 1 to present the possibilities that already exist in the African region for developing drugs and performing testing activities, and 2 to delineate the needs for guidance in developing non-clinical, safety testing strategies for a full non-clinical safety package that would also satisfy the registration requirements of industrialized

countries. The outcome of that meeting was endorsement of the need for such a document, and consensus on a possible outline of a draft document.

The recommendations of this meeting were that:

- 1) The outline presented to the participants should be elaborated as a draft document and sent to the attendees for comment, then later discussed and possibly finalized in a working group meeting in Geneva later in the year.
- 2) Since there is a need for training in toxicology testing methodology in DECAs, WHO/TDR should look at possibilities for cooperation with international toxicology organizations, e.g. the International Union of Toxicology (IUTOX) and the Drug Information Association (DIA), with the aim of promoting educational toxicology activities, e.g. training workshops.
- 3) WHO/TDR should provide support to institutions that conduct non-clinical product development activities in DECAs.
- 4) Support by national governments to institutions active in non-clinical product development should be encouraged.

The draft document on non-clinical safety testing was then prepared and tabled for discussion at the meeting of the working group in Geneva, 28-29 November 2002.

The objectives of this meeting in November 2002 were to review the draft document and:

- 1) Judge the contents from the perspective of DECAs worldwide.
- 2) Discuss, improve and correct it, completing and enhancing the information content through the expertise and experience of the members of the Working Group.
- 3) Finalize the document.

During the two-day meeting, reviews were presented on the approaches taken, both in DECAs and industrialized countries (Europe and USA), to the safety testing of drugs, vaccines and traditional medicines, and the draft non-clinical safety testing document was reviewed. Some issues were identified as important and in need of clarification. The following key points were discussed and agreed as indicated.

GENERAL

- It was considered that a single document which referred to non-clinical safety testing approaches for drugs, vaccines and traditional medicines would be most suitable for the target audience.
- The title “Handbook on non-clinical safety testing” was proposed.

- The audience for the handbook was identified as research workers needing guidance in the identification of a safety testing strategy. It would not be directed at regulatory agencies although it was recognized that it may also provide support for regulators.
- The handbook would include an explanation of the purpose of each test and how it may be used, with cross references to published test guidelines. Where possible, flow charts/decision trees would be used to emphasize the flexibility required by such a process. The handbook should also identify the circumstances where certain tests would not be considered suitable.
- The Handbook would make it clear that the tests conducted should follow a standard of science and quality that would be acceptable to international regulatory agencies.
- It should be recognized that any strategy proposed could eventually be discussed with relevant regulatory agencies before starting.
- The Handbook should be generated in a form that allows periodic updating.
- The Handbook would provide a general strategy to be followed for chemical drugs, identifying specific differences in the approaches to be adopted for other materials such as vaccines and traditional, herbal medicines.
- Several levels of training were recognized, ranging from helping researchers to understand the document and the concepts expressed therein, and providing more detailed training on specific toxicological issues, to training individuals as toxicologists (study directors) or toxicological specialists.

VACCINES

- A need was recognized to distinguish between this guideline and draft guidelines for non-clinical evaluation of vaccines that are also being developed by WHO. It was agreed that the two were directed at different audiences. However the two documents should complement one another and appropriate cross-referencing should be included.
- The section on vaccines should identify specific issues where a required safety testing programme differs from that for drugs.
- It was considered that it would not be useful, at this stage, to recommend use of “alternative” in vitro tests, as these are not yet fully validated.
- Some advice should be included regarding the correct species to use for testing, i.e. clarification of the term “most relevant species” that should be used for safety testing.
- The document should provide a set of common tests to which additional studies may be added depending on existing knowledge of the material.

TRADITIONAL MEDICINES

- The section would be limited to herbal (botanical) medicinal products.
- The section should identify specific issues where a required safety testing programme differs from that for chemical drugs.

At the end of two days, it was decided that:

- 1) The title of the document should be “Handbook on non-clinical safety testing”.
- 2) Taking into consideration the target audience, the handbook should be a single document containing the non-clinical safety testing approaches for chemical drugs, vaccines and traditional, herbal medicines.

WHO/TDR was invited to provide help in establishing training workshops to assist researchers in using the handbook and improving their understanding of safety testing programmes in the non-clinical development of medicinal products for human use.

This handbook discusses the problems and issues of preparing a development programme for toxicological studies in non-clinical safety testing. Since there is no “one approach that fits all”, the document focuses on general issues, but also identifies the differences between the various classes of pharmaceuticals (chemical drugs, vaccines, and traditional, herbal medicines), as well as between different compounds, different patient populations and different galenical forms. The specific process of developing the programme and the basic considerations are outlined, with reference to the fact that the final decision is usually made on a case-by-case basis depending on the individual circumstances cited above.

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1. INTRODUCTION

1.1 GENERAL REMARKS

A number of diseases place heavy burdens in terms of morbidity and mortality, and economic losses, onto the populations and health systems of disease endemic countries (DECs). Fighting these diseases (malaria, tuberculosis, filariasis, trypanosomiasis, etc.) is thus of great importance for DECs; however, only a limited number of affordable drugs are available for their treatment. Furthermore, the available drugs are mainly “old” and of partially limited efficacy, while problems of resistance have emerged and are of major concern. Development of new drugs being rather slow, due to the limited interest of multinational pharmaceutical companies in developing drugs for diseases which offer only a small chance of sufficient returns, this situation has created a need for DECs to engage in developing new treatment options from existing sources and knowledge.

WHO has been active for some time in this field and has developed some guidance documents on traditional and herbal medicines that are intended to facilitate the development of such pharmaceuticals. Generally speaking, their purpose, however, has been more in the direction of clinical research and testing in order to obtain better efficacy data. Non-clinical development and safety testing, although mentioned in the documents, has not been treated in the extensive way needed to provide real guidance on the design of a comprehensive non-clinical testing package and on the components that are really necessary for an acceptable submission dossier.

While many laboratories in DECs are involved in pharmaceutical research and discovery activities on preparations based on local or regional experience and knowledge (e.g. traditional medicine), such projects are rarely developed to the point where the preparation, even if considered promising from a therapeutic point of view, undergoes formal non-clinical testing with a view to creating the full-fledged submission dossier necessary for obtaining marketing permits either in the country of origin or abroad. The situation is worsened by the fact that, in these countries, there is often not only insufficient technical capacity and experience but also poor accessibility to relevant information on current methodologies and regulatory requirements. On the other hand, since there is a growing interest in herbal medicines on a global scale, the development of pharmaceuticals based on the experiences and expertise of traditional medicine has the potential to reach a

wider market, which in turn could create economic benefits for countries developing such pharmaceuticals which go beyond the immediate health benefits to their own populations. Exploring this potential, however, requires the ability to design and produce submission dossiers which meet the formal and scientific requirements of drug regulatory authorities worldwide. In this sense, developing a guidance document which contains information on non-clinical testing methods and presents basic considerations for the design of non-clinical testing strategies in different situations was seen as a major means to provide incentives for the development and production of safe, effective, accessible, affordable and easy-to-use medicines in DECAs. The purpose of the present draft guidance document is therefore to fill this gap, and to provide the necessary guidance for developing a scientifically well reasoned and justified safety testing package, as well as to provide information on the available guidelines for safety testing of pharmaceuticals.

1.2 SCOPE

This document will provide general guidance for the non-clinical safety testing of pharmaceuticals (synthetic drugs, vaccines, herbal medicines, etc.). It will focus on aspects of toxicological investigations, and is directed towards the investigator or sponsor responsible for designing a safety testing programme.

1.3 OBJECTIVES

The intention of this document is to:

- guide researchers and laboratories in the design of non-clinical safety testing programmes
- help in the design, conduct, reporting and assessment of non-clinical studies
- enable users to undertake research and development at internationally acceptable regulatory and scientific standards
- provide a framework for guidance on regulatory aspects of non-clinical product development, as well as on non-regulatory applications of non-clinical safety testing.

It is not the intention, however, to provide specific and detailed guidelines on the design of individual study types, or detailed information that can be obtained elsewhere (e.g. in various appendices).

2. CONSIDERATIONS IN THE NON-CLINICAL DEVELOPMENT OF PHARMACEUTICALS

2.1 REGULATORY ASPECTS OF PHARMACEUTICAL DEVELOPMENT AND SUBMISSIONS

For regulatory purposes, and derived from their scientific objectives, non-clinical studies are divided into three parts: investigations of the pharmacodynamic actions of a compound (primary and secondary pharmacodynamics and safety pharmacology); investigations of the compound's behaviour and fate in the organism (pharmacokinetics; absorption, distribution, metabolism, excretion [ADME]); and investigations to ensure that the substance does not pose any potential untoward safety risk (toxicology) when it may finally be administered to humans. This document mainly covers the toxicological types of investigation; the other two parts of a non-clinical study programme and their regulatory requirements are only briefly described.

The strategy for developing a pharmacodynamic research programme is results driven in the sense that, after initial screens which identify promising leads, there are well defined ways of investigating pharmacodynamic efficacy and mode of action in vitro and in vivo, in animal disease models (if available). Regulatory requirements for these studies are less stringent than for other non-clinical or clinical studies since they are regarded more as "hypothesis generating" than as definitive "proofs" for therapeutic efficacy; pharmacodynamic activities and their utility in treating the targeted affliction will ultimately be proven only by results generated from clinical studies in patients.

A pharmacokinetic investigational programme may be a relatively straightforward exercise. Plasma toxicokinetic data have to be generated for the mode of application foreseen for the clinical situation, as well as for intravenous administration, in order to allow the absolute bioavailability to be determined. ADME studies will have to address these four areas in a rather standard way, although certain special studies may be considered appropriate depending on the properties of the pharmaceutical (e.g. investigations in bile-cannulated animals may be necessary if there are indications of secretion into bile or enterohepatic re-circulation).

The purpose of toxicology studies is, ultimately, non-clinical safety evaluation through characterization of the toxic effects with respect to type of toxicity, target

organs, dose dependence, relationship to exposure, and potential reversibility. This information is important for estimating an initial safe starting dose for human trials and for identifying the parameters that need to be clinically monitored for potential adverse effects. Results from the completed package of toxicology studies will also be used to determine a compound's potential for adverse, toxic effects, and to assess its risk-benefit ratio. Thus, toxicology studies cover a wide range of investigations, and a lesser or greater number of different types of study in different animal species.

In regulatory terms (e.g. see the table of contents of the ICH common technical document, [1]), safety studies encompass:

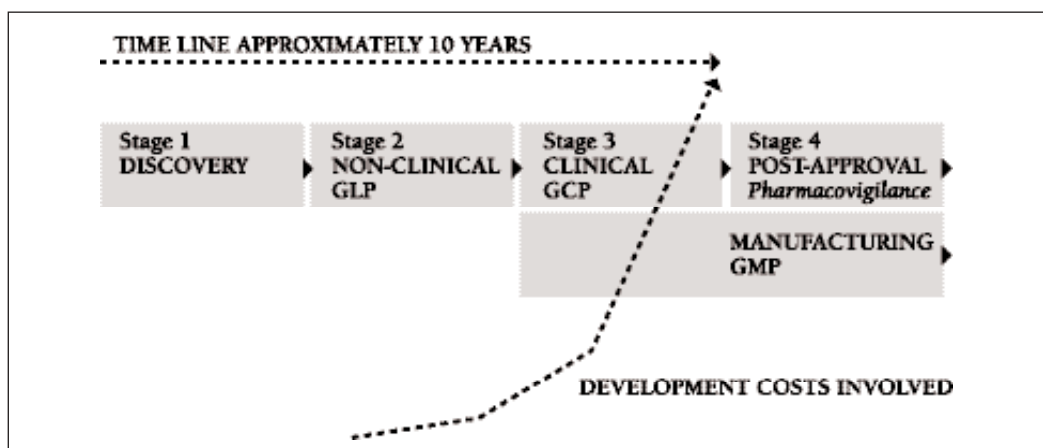
- Safety pharmacology
- Acute (single dose) toxicity
- Subchronic/chronic (repeated dose) toxicity
- Genotoxicity
- Carcinogenicity
- Reproductive toxicity
- Special toxicity (immunotoxicity, local tolerance, environmental risk assessment, etc.).

Depending on the type of pharmaceutical and the knowledge already available, all or some of these areas will have to be assessed by conducting the corresponding studies. It is important to design a scientifically justified programme of investigations addressing all areas of identified or assumed safety concerns, without unnecessarily inflating the number and kinds of studies conducted. Such an approach, i.e. developing a toxicity testing programme that provides all the necessary safety information with the least possible amount of testing, makes economic sense. Toxicity studies are undoubtedly expensive in financial and resource terms. While part of the cost is related to the number of animals used and to the extent of the clinical-chemical, haematological, macroscopic (necropsy) and microscopic (histopathology) investigations required, what adds unnecessarily to the development costs are studies that can be considered not relevant to the safety assessment of the specific pharmaceutical under investigation.

A number of criteria may be envisaged that will help in deciding the scientific and regulatory acceptability of a non-clinical safety testing programme, as well as the selection and design of the testing procedures. These criteria will be different for different types of pharmaceuticals as well as for different galenical forms. In general, new active substances (NAS), whether of synthetic chemical, biotechnological or herbal origin, will need more extensive non-clinical investigation of their toxicological properties

and non-clinical safety than preparations for which there is already a certain amount of (toxicological or clinical) experience, as may e.g. be the case for herbal preparations derived from traditional usage. In selecting a testing strategy, two main considerations have therefore to be taken into account: the extent of existing knowledge about safety aspects of the pharmaceutical under investigation, and the extent of the toxicological information necessary for a reliable safety assessment.

In general, the non-clinical testing programme should be designed so as to allow the “right” decisions to be made as early as possible, in order to prevent unnecessary expenditures on developing a compound that could be destined to failure. Thus it is generally advisable to start a toxicological investigation with those tests and assays that generate important information in a short time and with relatively low amounts of compound, efforts and resources. Such assay systems are generally those used for the detection of genotoxic effects, single dose toxicity tests, and the “core battery” safety pharmacology assays. From there on, taking into account the results obtained in, and insights gained from, the early experiments, further development of the non-clinical database may proceed in a scientifically justified way. The following graph demonstrates why it is advisable to proceed in this manner, since it can plainly be seen that, as development progresses, the costs of the programme rise not linearly but exponentially.

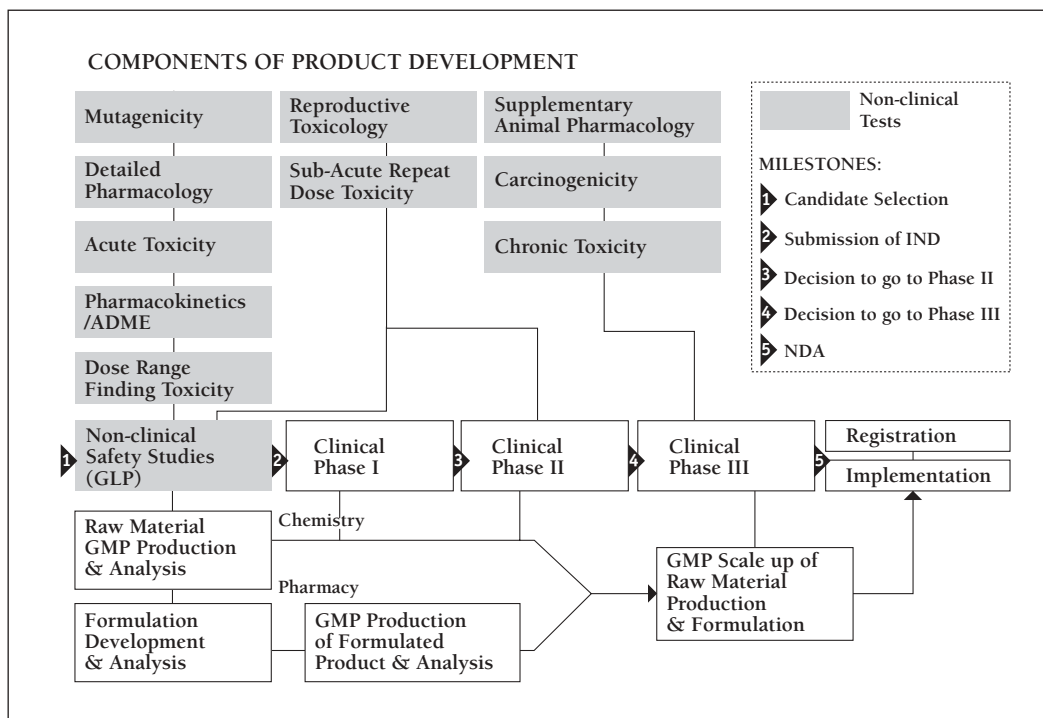


The development of a pharmaceutical is not a completely continuous process. While, in theory, the various phases of pharmaceutical development – research and early pharmacology investigations, non-clinical testing, clinical trials, post-registration marketing (see graphic representation below) – can be described as separate entities, sequentially following each other, they are by no means purely successive activities but

will be conducted in a partially parallel approach. At various time points the pharmaceutical development process may be stopped, either temporarily because of open questions or unresolved concerns, or finally because of insufficient efficacy or relevant, unacceptable toxicity. Therefore, the optimal design of the safety testing programme will also be based on a step-wise approach that uses all available information at each of these steps.

This is addressed, too, in the ICH Guideline M3(M) *Maintenance of the ICH guideline on non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals* [2], concerned with the timing of non-clinical studies in relation to clinical trials:

“The development of a pharmaceutical is a stepwise process involving an evaluation of both the animal and human safety information.....This information is important for the estimation of an initial safe starting dose for the human trials and the identification of parameters for clinical monitoring for potential adverse effects. [Therefore,] the non-clinical safety studies, although limited at the beginning of clinical development, should be adequate to characterise potential toxic effects under the conditions of the supported clinical trial.”



Thus, for example, phase I clinical trials will be started as soon as permissible, before all non-clinical investigations are completed. In the same way, some early toxicity investigations will be performed before the whole research programme into the pharmacodynamic properties and mechanism of action is completed. In consequence, there are certain “milestones” in the development process, decision points, where the further proceedings have to be determined and decided upon. Criteria have therefore to be applied at these various checkpoints to determine whether or not to continue with the development, and under what conditions to do so. The major milestones may be characterized as follows:

- The earliest checkpoint will certainly be connected with the primary pharmacodynamic data obtained in the research phase of the project. For synthetic chemicals, it is usual to test a number of substances that are derived from a “lead compound” with the objective of deciding which show the most promising properties. When single preparations, e.g. herbal preparations derived from traditional experience, are tested, the results of primary pharmacodynamic studies will demonstrate whether the preparation can indeed exhibit the desired therapeutic activity at biologically relevant concentrations or doses.
- A second checkpoint will be reached when the results of the early toxicology studies are available. If an assessment of these results should demonstrate the presence of unacceptable toxic hazards, the project may be terminated, or the compound replaced by another one from the investigated series of substances. This possibility is most probable when data on genotoxic activity give rise to major concerns about potential mutagenicity and carcinogenicity in humans.
- At later stages in the development, further concerns may emerge from data obtained in non-clinical studies if and when specific organ toxicities are demonstrated, e.g. neurotoxicity, that could negatively impact on the usefulness of the pharmaceutical, especially if these effects should prove to be irreversible. Also the detection of reproductive toxicity, specifically of clear-cut teratogenicity, could influence the decision on whether or not to continue the development.
- As the development moves further along the path to submission and marketing, the more prominent do clinical data and concerns become as decision criteria. Lack of

adequate efficacy, or the emergence of intolerable side-effects (intolerable in terms of either frequency or seriousness), may at least curtail, if not terminate, the development and marketing potential of an investigational drug.

At any one of these checkpoints, even if some concerns would seem to call for terminating the project, it might be advisable to contemplate conducting further investigative studies. Already at the earliest stages of the development the question of risk-benefit relationship may have to be asked, as a compound or preparation showing great potential for utility (due to efficacy, ease of application, or broad availability and inexpensiveness) should not be terminated without good reason. Thus, apart from performing the conventional and mandatory assays and tests, it may prove useful to remain alert to the scientific need for additional investigations, the results of which could justify the continuation of a project even in the face of some reason for concern.

At the beginning of clinical development therefore, the data from non-clinical safety studies, although limited at that stage, should nevertheless be adequate to characterize any potential toxic effects that might occur under the conditions of the planned clinical trials. Assays of longer duration and more complex design, e.g. long-term chronic toxicity and carcinogenicity studies, if needed, are most likely to be conducted late in the development process when the therapeutic value of the compound or preparation has already been ascertained in (limited) clinical studies.

In many cases it will be advisable to contact the regulatory authority of the country where it is intended to submit the product for registration and marketing, in order to obtain pre-submission advice about the planned non-clinical (and clinical) testing programme. Such advice is provided by a number of regulatory authorities worldwide (e.g. US FDA, European EMEA, other national authorities); in other cases, however, regulatory authorities may not be prepared to advise a sponsor about the acceptability of a proposed testing programme. If, because of the nature of the pharmaceutical under investigation, a restricted testing programme is contemplated, early contact with regulatory authorities is advisable; another possibility is to seek the advice of a consultant who is familiar with the general opinions held by specific regulatory authorities with respect to such issues.

Very important considerations in this context are not only questions about selection of the most appropriate non-clinical models and assay procedures, but the availability of necessary resources and the possibility of conducting the assays under conditions of Good Laboratory Practice (GLP) will also have to be considered. While the availability

and timely allocation of resources to various projects will influence development speed and success, the conduct of non-clinical testing under GLP conditions will ultimately have an impact on acceptability of the data for regulatory purposes. Therefore it is very important that even the earliest toxicological investigations are conducted in conformity with GLP requirements [3, 4, 5]. By failing to observe the GLP principles in these early studies, the possibility exists that the experiments will need to be repeated under GLP conditions, thus delaying the project and adding unnecessarily to its costs.

Finally, there is another aspect to the question of resources, their availability and allocation. Multinational pharmaceutical companies provide the example: often they conduct only part of the non-clinical safety studies in their own laboratories – if their own resources are not sufficient to conduct a study on time, or if they need special expertise for specific experiments, they contract the studies out. In an analogous way, for laboratories situated in DECs, the possibilities of national and international networking should be seriously considered. Cooperation or work-sharing agreements could provide opportunities to support the development of promising pharmaceuticals, especially where resources, including technical as well as professional experience and expertise, are scarce. Although it may be difficult to persuade multinational pharmaceutical companies to take part in a project that does not promise huge financial gains, there are other ways to gain access to the technology or expertise required in specific cases. One of the most important ways is through the personal networks of international friends and colleagues – if not able to provide the necessary technical help themselves, they might know other persons in the professional environment who can. Another possibility is provided by international (professional) organizations or funds, which can help identify and locate potential partners, or help to finance the out-sourcing of safety studies.

2.2 METHODOLOGICAL ASPECTS OF SAFETY TESTING

While the design of a safety testing programme is governed by both the necessity to obtain specific toxicological information and the reliance on pre-existing toxicity data, i.e. by the necessity to fill the gaps in knowledge of the toxicological properties of the compound or preparation, the choice and design of the single assay systems to be used also follow general guiding principles.

These principles concern, for example, the choice of test system (animal species in the case of in vivo assays, or cell or bacterial strains in the case of in vitro tests), test

size (numbers of wells, plates or test tubes, or animal numbers), selection and number of concentrations or doses to be tested, statistics to be employed.

2.2.1 Selection of test system

Although there are obvious similarities in function between humans and experimental laboratory animal species, no single species can be considered identical to humans in terms of morphology, physiology or biochemistry. Even in the case of non-human primates, straightforward extrapolations from animal tests to humans are not possible. Because no single species can provide a completely human-like model, it is generally agreed that at least two different species, one rodent and one non-rodent, should be included in the conventional toxicity testing programmes – it is assumed that the use of two, instead of one, species will provide a broader basis for the extrapolation to humans of any toxic effects observed in the animal models. On the other hand, knowledge of the similarities and dissimilarities in pharmacokinetics (ADME: absorption, distribution, metabolism, excretion) between humans and the animal species used will help to establish the meaningfulness of animal study results for humans, and such data should be available at least for the final assessment of preclinical data in order to support the risk evaluation.

Accepting the fact that no ideal animal analogue is available for laboratory testing, the choice of test species can be influenced by other considerations of a logistic nature, such as ease of breeding or purchasing, animal husbandry, speed of growth/development and handling under the experimental conditions, thus recognizing that a number of factors might dictate the number or choice of species for study. These considerations have led to the extensive use of rats and dogs in toxicology investigations, and indeed the major amount of safety testing is performed on these two species. Thus, a laboratory wishing to undertake regulatory toxicology studies will have to give priority consideration to using these two species, if possible (and if not scientifically justified otherwise); in practice, however, it may be better for the single laboratory to use an animal strain or species with which it is familiar, and for which it has already assembled experience and extensive baseline data on the parameters to be determined in a toxicity study (e.g. haematology, clinical chemistry, histopathology), than to use a species which it is not used to handling.

A special problem may not only be the availability, but also the cost of certain animals or animal strains. This may, under financial strain, induce laboratories to rely on local sources for animals, whose origin, health status, etc., might be in doubt. Regulatory

expectations are an important consideration here. While e.g. in the case of non-human primates, the use of wild-caught animals would most probably be admissible to many regulatory authorities, the use of e.g. mongrel dogs instead of pure- and purpose-bred Beagle dogs will certainly not be acceptable and should therefore be most urgently discouraged, since adherence to the above recommendations of known origin and consistency in certain parameters will not be possible. As stated above, it is not only necessary to be able to conduct an experiment well, but in order to reach a scientifically valid interpretation of the information obtained, extensive knowledge about the baseline properties of the assay system is an absolute requirement.

2.2.2 Conditions for test systems

In all cases the animals should be healthy, of known origin, reasonably consistent in terms of age and body weight, and suitably acclimatized to the experimental environment before the study commences. In general, the internationally available and accepted guidelines do not specify specific age or weight ranges for test animals, but instead there may be general recommendations, for example, on the use of young adult animals.

Newly arrived animals should be acclimatized to the conditions of the test facility for a certain time, generally not less than seven days. During this time the necessary health checks may be performed, weight ranges determined, animals marked individually, and preparations made for randomization into the study. In certain circumstances, e.g. when it is necessary to immobilize animals for some time in order to perform certain measurements (e.g. for taking electrocardiograms from conscious dogs), training in these procedures and in the necessary handling will ensure that the data obtained will not be jeopardized by undue stress and accompanying physiological reactions.

Stringent control of environmental conditions (room temperature and humidity, cage size, living environment of the animals within the cages, etc.) and proper animal care techniques are mandatory for meaningful results. Diet should meet all the nutritional requirements of the species used in the tests. It is highly desirable to know the effect of the dietary regimen on metabolism and on animal longevity as well as on the development of toxicity. Since e.g. mycotoxins or polychlorinated biphenyls will influence the metabolic capacity of the liver, such dietary constituents which are known to influence toxicity should not be present in interfering concentrations.

2.2.3 *Size of the experiment*

One critical issue is the size of the experiment, especially when conducting in vivo tests, where the number of animals to be used has to be set. For in vitro assays, such as those used in genotoxicity experiments, the size of the assay (number of plates in a bacterial mutagenicity test, or number of metaphases to be scored in a chromosomal aberration test with mammalian cells) is described in a clear-cut way in the respective methodological guidelines. When it comes to using live animals, in many cases a decision has to be made between an upper limit, e.g. the number of animals to be used for detecting a defined extent or magnitude of change from baseline in a certain parameter (e.g. for detecting an increase of 10% in the level of liver enzymes with a certain statistical power), and a lower limit that may be dictated by animal protection considerations or by economic reasons. Certainly, with the objective of an efficient approach to testing in mind, there is no point in having more groups or more animals per group than are strictly necessary to attain the purpose of the study. Some guidance, however, can be derived from the internationally used “conventional” number of animals, which depends also on study type. Thus, for single dose, acute toxicity tests, the requirement for groups and number of animals in groups is related to the reliable determination of acute toxic effects in rodents, for which purpose ten animals per group may constitute a sufficient number. The same number per sex and group can also be considered sufficient for most cases of subchronic and chronic toxicity testing. Due to the specific endpoints that are to be monitored, and due to their incidence in untreated control animals, the number per sex and group has to be increased in tests for reproductive toxicity, where 20 to 24 pregnant animals are conventionally used. In tests for carcinogenicity, where additionally the long duration of the experiment makes the surviving fraction a statistical issue, generally 50 to 60 animals per sex and group are used. For non-rodents, e.g. dogs or non-human primates, a number of four is conventionally accepted as sufficient also in chronic toxicity studies.

2.2.4 *Selection of doses and concentrations*

Depending on the type of test, there are different requirements for setting the concentration or dose levels. The information available in international guidelines may range from relatively firm requirements to rather vague recommendations.

For instance, in vitro bacterial mutagenicity tests need to be performed up to a limit concentration of 5 mg/plate, unless bacterial toxicity or solubility problems preclude the use of concentrations in this range. Concentrations to be used in other in vitro genotoxicity tests will also be limited by cytotoxicity; for mammalian cells in culture, survival of around 50% may constitute such a limit, which has, however, to be converted to actual concentration values through the preliminary conduct of a cytotoxicity study.

Other considerations may apply to the setting of doses for studies with mammals in vivo. For acute, single dose testing, international guidelines provide a limit dose of 2000 mg/kg. Doses for subchronic and chronic, repeated dose studies will have to be set according to different considerations; in general, these call for a high dose which produces some evidence of toxicity, a low dose which does not influence the well-being of the animal, and a mid dose that is at the borderline of toxicity. Deriving, from the results of shorter-term studies (in the first instance from single dose toxicity), the respective dose levels for the longer-term investigations so as to meet these requirements, may be regarded as much an art as a science. For carcinogenicity studies, still other considerations apply. Here, the historical convention has been to use, as the high dose, the “maximum tolerated dose” (MTD), defined as the dose that produces a body weight loss of 10% in comparison with untreated controls. Since, for relatively non-toxic compounds, this limit may be hard to achieve unless exaggerated amounts of test item are administered, other definitions for the high dose in carcinogenicity tests have been put forward. These are specified in the ICH Guidelines *Dose selection for carcinogenicity studies of pharmaceuticals (guideline S1C)*, and *Addendum to S1C: Addition of a limit dose and related notes (guideline S1C[R])* [6, 7]; they include, among other possibilities, the use of doses that will yield an exposure (measured as the area under the plasma concentration curve [AUC]) of at least 25 times the expected (or determined) human exposure under therapeutic conditions.

In essence, however, the setting of concentrations and doses to be tested calls again for a scientific judgement of all the available information, as well as for a justification of the actual values chosen.

2.2.5 Monitoring of exposure and reversibility of effects

The requirement to monitor exposure and perform toxicokinetic measurements [8], and to provide information on reversibility of effects, will lead to an increase in the numbers of animals needed in a study. On the one hand, reversibility of effects will be

determined in recovery groups, comprising the controls and the high-dose group. On the other hand, satellite groups for toxicokinetic determinations will be needed for rodents, but not for dogs and non-human primates since these are of a size which allows for some within-study blood-lettings without any undue consequences to the health of the animal and the quality of the study.

2.2.6 Test item characterization

Another requirement which is mandatory for the conduct of a GLP compliant study is exact (analytically supported) knowledge about the nature of the test item, the pharmaceutical and safety properties of which are to be determined [3, 5]. The identity of the test item should be known and defined; its physical and chemical properties will provide important information for the design of the studies as well as for handling and storage of the test item. For herbal preparations this will be confined, in the first instance, to an exact botanical identification of the plant material, while for chemically defined compounds it is important to characterize them and identify impurities that are known or likely to be present.

2.2.7 Good Laboratory Practice

Since compliance with the Principles of GLP [3, 4, 5] is a mandatory requirement for the conduct of non-clinical safety studies that are submitted to a regulatory authority for purposes of supporting clinical trials (investigational new drug [IND]) or obtaining a marketing permit (new drug application [NDA]), laboratories performing safety pharmacology and toxicology studies should work under the conditions of GLP. The “proof” of GLP compliance involves two different levels. On the one hand, the study director must sign a claim of GLP compliance for each study, and the quality assurance (QA) statement must list all QA activities and confirm that the study report reflects the raw data. On the other hand, the test facility itself should be part of a national compliance monitoring programme and be listed as a compliant facility. If this latter prerequisite cannot be complied with because of lack of a national compliance monitoring programme, it is probable that the drug registration agency to which the study is submitted will call for a study audit to be conducted by GLP inspectors of its own compliance monitoring

authority. If the results of this audit show no major deviations from GLP principles, the study will be accepted as GLP compliant, and this status will then also be accepted by other drug registration authorities to which the study may subsequently be submitted.

2.3 LEGAL ASPECTS IN PHARMACEUTICAL DEVELOPMENT

If development of a promising pharmaceutical candidate compound or preparation for ultimate regulatory acceptance and licensing for marketing is contemplated, the legal aspects of property rights and patentability should be considered and addressed well in advance of submitting the documentation for regulatory assessment.

An inter-regional workshop (Bangkok, Thailand, December 2000) organized by WHO addressed this question with respect to traditional medicines. The report of this workshop [9] may be used to obtain more information about these aspects.

The complexity of the questions, problems and issues involved calls for expert help, but these aspects of pharmaceutical development exceed the scope of this document.

3. NON-CLINICAL SAFETY TESTING STRATEGY

3.1 GENERAL ISSUES

Toxicology studies are conducted to determine the degree of toxicity of a chemical substance, to establish the relationship between dose and adverse effects, and to provide information on target organs and target functions, allowing for a meaningful assessment of the data obtained, and a scientifically supported extrapolation of the effects to the human situation. For emerging areas of concern for human safety, additional studies or investigations may become necessary, while in areas where there is no concern, or where the absence of concern is suggested by other information, the examination of potential effects may be reduced or even left out completely. In essence, most guidelines provide introductory statements, stressing the need for flexibility in the conduct of the single study as well as in the design of a complete toxicity testing programme. The objectives of the non-clinical safety studies are thus:

- To identify hazards.
- To evaluate risks.
- To provide the basis for risk management.

Risk evaluation of identified hazards in animals includes determination of their degree of severity and potential reversibility or irreversibility. This means e.g. to determine the type and degree of toxicity of a chemical substance, to establish the relationship between dose/exposure and adverse effects, to provide information on target organs and target functions, and to provide, if possible, information on the mode of toxic action. Finally, the characterized risks need to be extrapolated to clinical situations and patients. This will be achieved by conducting the relevant toxicity tests in a way that allows for a meaningful assessment of the data obtained and for a scientifically supported extrapolation of the effects in the human situation. For identified areas of concern, the conduct of additional studies or investigations may become necessary, while in areas of no concern, or where the absence of concern is suggested or proven by other information, the examination of potential effects may be reduced or even left out completely.

The European “Notice to applicants” [10] phrases the purpose of the non-clinical part of the submission as follows:

“The toxicological and pharmacological tests must show:

- a) the potential toxicity of the product and any dangerous or undesirable toxic effects that may occur under the proposed conditions of use in human beings; these should be evaluated in relation to the pathological condition concerned;*
- b) the pharmacological properties of the product, in both qualitative and quantitative relationship to the proposed use in human beings.”*

Non-clinical safety testing is highly regulated by regional, national and international guidelines. While the details of requirements may differ from region to region, and while the International Conference on Harmonisation (ICH) is trying to harmonize these requirements, in essence, most guidelines uniformly provide useful information about designing, conducting and reporting safety studies, and they generally contain introductory statements, stressing the need for flexibility in conducting the individual studies as well as in designing a complete non-clinical safety toxicity testing programme.

The introduction to the OECD guidelines for the testing of chemicals [11], for example, draws attention to the fact that for a sound determination, in both economic and scientific terms, of the whole array of toxicological properties and hazards of a substance, there cannot be a rigid framework of required tests that have to be conducted according to fixed rules:

“There must be provision for the exercise of toxicological skill and judgement during the course of the study, even where this forms part of a prescribed set of test requirements, and so guidelines or similar defined procedures should allow for this; obviously, the rationale for changes in procedure must be explained and supported scientifically. The emphasis on a flexible approach should not be construed as a recommendation for a lack of order. It should be seen as creating a situation in which the examination of the toxicity of a chemical substance is conducted as a scientific exercise rather than as a set of stereotyped tests to be conducted in a routine.”

In order to profit from the flexibility that is evoked in these guidelines, and that makes sense economically, scientifically and ethically, it is necessary to plan the safety testing strategy in a well considered way. The first step is to collect pre-existing knowledge about any safety-related properties of the preparation or compound under consideration for pharmaceutical development. This information gathering exercise ultimately leads to the identification of relevant gaps in the knowledge of its safety aspects. Drug toxicity

data are sometimes evaluated on a comparative basis; the results obtained with the new compound are compared to the known animal and human toxicity profile of other, marketed preparations to better predict its risks for patients.

3.2 THE GENERAL DESIGN OF NON-CLINICAL SAFETY (TOXICITY) TESTING PROGRAMMES FOR PHARMACEUTICALS IN INTERNATIONAL GUIDELINES

Most pharmaceutical products are still derived from synthetic chemicals, so guidance on layout and design of safety testing programmes that can be gleaned from international guidelines is geared to dealing with the properties of these synthetic chemical compounds. Because these are newly developed compounds, it is assumed that, at the beginning of testing, nothing is known about their potential toxicity and therefore a complete investigation into all aspects of this potential is necessary.

Thus, the ICH guideline M3 [2] states:

“The non-clinical safety study recommendations for the marketing approval of a pharmaceutical usually include single and repeated dose toxicity studies, reproduction toxicity studies, genotoxicity studies, local tolerance studies and, for drugs that have special cause for concern or are intended for a long duration of use, an assessment of carcinogenic potential. Other non-clinical studies include pharmacology studies for safety assessment (safety pharmacology) and pharmacokinetic (ADME) studies.”

This general statement, however, is then qualified by stressing the importance of scientific judgement in the design of a non-clinical safety testing programme:

“This guideline applies to the situations usually encountered during the conventional development of pharmaceuticals and should be viewed as providing general guidance for drug development. Animal safety studies and human clinical trials should be planned and designed to represent an approach that is scientifically and ethically appropriate for the pharmaceutical under development.

[For certain classes of pharmaceuticals]... the existing paradigms for safety evaluation may not always be appropriate or relevant. The safety testing program evaluation in such cases should be considered on a case by case basis ... to optimise and expedite drug development. In these cases, particular studies may be abbreviated, deferred or omitted.”

In section 3.1 of the present handbook it is stated that, for the purposes of safety testing, i.e. of obtaining data and information on the inherent hazards of a compound or preparation, it is first and foremost necessary to identify important gaps in the knowledge about the toxic properties of the pharmaceutical. To derive, from these identified gaps, the nature of the necessary tests that need to be performed, it is essential to be aware of the possibilities and limitations of the different test systems and models with respect to the knowledge obtainable from them.

Of special importance are those non-clinical safety tests which provide information that is hard or impossible to obtain from testing in humans. To these belong the reproductive toxicity, genotoxicity and carcinogenicity tests. They deal with toxic properties which are irreversible, not immediately apparent, and the detection of which – due to their relative rarity on the one hand and their spontaneous occurrence on the other – can be hard to detect in human epidemiological studies. Therefore, these are areas of major concern which can only be dealt with through thoughtful conduct of the respective non-clinical studies.

Below, the salient features of the various types of non-clinical safety studies are summarized again.

3.2.1 Single dose acute toxicity studies

Single dose acute toxicity studies deal with the potential adverse effects of single doses. These investigations should provide information about possible dose levels for first applications to humans, and give indications as to the possible effects to be expected with (accidental or intentional) over-dosing. For pharmaceuticals, “pseudo-exact” lethal doses (e.g. LD50) are no longer derived, as has been the practice for decades [12]. A single acute toxicity test will, however, in most instances not be considered sufficient evidence for the single-dose safety of a new pharmaceutical substance, and a “subacute” study of relatively short duration, but with repeated administration of the test substance, is considered a necessary prerequisite to support the first clinical trials in humans.

3.2.2 Repeated dose toxicity studies

The primary goal of repeated dose toxicity is to characterize the toxicological profile of the test substance following repeated administration. This will provide detailed

information on toxic effects, identification of potential target organs, effects on physiological functions, haematology, clinical chemistry, pathology and histopathology; it may furthermore provide an indication of no observed effect levels (NOEL). Also information on the persistence or reversibility of effects will be obtained from recovery groups.

3.2.3 *Reproductive toxicity studies*

The term “reproductive toxicity” covers the whole cycle of reproduction, fertility, embryo- or foetotoxicity and teratogenicity, as well as the toxic consequences in offspring with regard to survival, physical and mental development, and reproductive capacity. The primary goal of reproductive toxicity studies is therefore to characterize the toxicological profile of the pharmaceutical with respect to effects on:

- Fertility and early embryonic development.
- Embryo-foetal development.
- Pre- and postnatal development, including maternal function.

Fertility can be affected in males and females, and effects can range from slightly decreased reproductive capability to complete sterility. Embryo- and foetotoxicity will influence the capability of a conceptus to survive to term, while teratogenicity represents adverse effects on the developing embryo and foetus that will have irreversible consequences on the physical integrity of the offspring.

3.2.4 *Genotoxicity studies*

The aim of genotoxicity studies is to detect compounds which induce genetic damage directly or indirectly. Since no single test is capable of detecting the different relevant end points simultaneously, the usual approach is to carry out a battery of tests which provide information on gene mutations, structural chromosome aberrations and numerical chromosome aberrations (polyploidy, aneuploidy). Genotoxicity tests may also be used to provide tentative evidence for potential to induce carcinogenic effects.

3.2.5 Carcinogenicity studies

The aim of carcinogenicity studies is to identify the tumorigenic potential of a test substance in animals and to assess the relevant risk to humans. In assessing the need to conduct carcinogenicity studies, the duration of patient treatment, therapeutic indication, intended patient population, systemic exposure and cause for concern from other investigations (especially from the genotoxicity test battery) have to be considered.

3.2.6 Concluding remarks

In summary, a whole array of questions is answered through studying the toxicological properties of a pharmaceutical preparation. Some, or many – depending on the nature of the preparation – of these answers may already be known, and it is the task of the expert to ultimately summarize knowledge about the hazardous properties of the pharmaceutical under scrutiny for admission to the market, provide an expert statement about the expected risks to the patient, and present the reasons for and justify the extent of the non-clinical testing programme chosen. The ICH guideline on the Common Technical Document [1] phrases this requirement very succinctly when it states that the Non-clinical Overview, an integral component of a submission dossier, should not only “*present an integrated and critical assessment of the pharmacological, pharmacokinetic, and toxicological evaluation of the pharmaceutical*”, but also that “*the non-clinical testing strategy should be discussed and justified.*”

3.3 GENERAL GUIDANCE TO A FULL NON-CLINICAL SAFETY TESTING PROGRAMME

The actual testing strategy chosen, of course, depends very much on the nature of the pharmaceutical preparation, i.e. on the extent of safety knowledge (derived either from existing non-clinical testing information, or from information about safe use in humans) which is utilizable for defining risk-benefit. Such “prior knowledge” will certainly vary depending on the pharmaceutical preparation’s origin and nature so, in order to provide more concrete guidance to the development of non-clinical testing strategies for an array of potential pharmaceutical preparations with regard to the need for safety

testing and choice of most relevant assay type, it is important to distinguish between the various categories of pharmaceutical preparations. The timing aspects of non-clinical safety studies, in relation to clinical development, also have to be observed. In the ICH Guideline M3, the type and duration of non-clinical safety studies is therefore correlated to the clinical development programme [2].

Usually it is recommended that the following non-clinical safety studies are conducted before the first administration of the new compound to humans: single dose and repeat dose studies of two weeks minimum duration; if necessary, local tolerance studies; two *in vitro* genotoxicity studies; the core battery of safety pharmacology studies; a first characterization of the kinetic profile.

During clinical phases 2 and 3, and in preparation for an NDA, the results of long-term repeated dose studies, the full genotoxicity battery, assessment of carcinogenic potential, and evaluation of reproductive toxicity should be provided.

3.3.1 *Before phase I*

Primary and secondary pharmacodynamic studies will have characterized the effects of the investigational product. General evaluation of the pharmacological effects on physiological functions in bioassays can also provide information on the modes and sites of action of the compound. When significant and/or unique toxicity is noticed, additional explanations of mechanisms of toxicity could be provided by further *in vivo* and *in vitro* experiments.

Single dose toxicity should be evaluated in two rodent species by two routes, one of which should mimic the clinical application while the other should provide maximal exposure (and therefore intravenous application is most often chosen). In most instances, a single acute toxicity test will not be considered sufficient evidence for the single-dose safety of a new pharmaceutical substance, and a “subacute” study of relatively short duration, but with repeated administration of the test substance, is a necessary prerequisite to support the first clinical trials in humans.

The Safety Pharmacology Core Battery [13] comprises the assessment of the effects on vital functions such as the central nervous (CNS), respiratory, and cardiovascular (CVS) systems. These studies may be conducted separately or along with specially designed toxicity studies.

3.3.2 *Safety information to support phase I*

A repeated dose toxicity study in two species (one rodent and one non-rodent mammal) for the anticipated duration of the clinical trial (e.g. toxicity studies of two weeks duration for clinical trials of up to 14 single dose daily treatments) could support phase I clinical studies [2]. In some instances, a single dose toxicity study as previously mentioned can suffice. An initial pharmacokinetic analysis should also be performed, as well as two genotoxicity studies [14] such as a bacterial mutation test (i.e. Ames test) and an in vitro mammalian test for chromosomal damage (e.g. mouse lymphoma test).

The design of repeated dose studies should be justified and be based on the physico-chemical nature of the test substance, on pharmacodynamic, pharmacokinetic and toxicological results (if available), and on the intended clinical use. In general, repeated dose toxicity studies should be performed in two mammalian species, of which one should be a non-rodent. One species can be acceptable if it is unequivocally demonstrated that other species are not relevant to the human safety assessment. In general, a vehicle control and three dose groups are recommended in repeated dose toxicity studies. The dose regimen, frequency, route of administration and duration should be based on intended clinical use. In terms of maximum study duration, six months for rodents and nine months for non-rodents are internationally acceptable. In general, the duration of the clinical study should not exceed the duration of the non-clinical repeated dose studies. Animals should be observed for morbidity and mortality, changes in clinical chemistry, haematology and urinalysis, and for organ weight and gross pathologic changes at necropsy; organs should be investigated histopathologically. Any adverse effects in animals should be investigated further. Concerns may arise if specific adverse effects are noticed which would require clarification by additional and appropriately designed studies on a case-by-case basis (e.g. tests for immunotoxicity in test animals).

3.3.3 *Safety information to support phase II*

Repeated dose toxicity studies of appropriate duration in two mammalian species (one rodent e.g. rat, and one non-rodent e.g. dog) could support phase IIa trials (clinical studies of up to two weeks duration for therapeutic exploratory trials). The duration of repeated dose non-clinical studies depends on the anticipated length of the clinical trials (see *ICH Guideline M3* [2]).

The full standard battery of tests for genotoxicity, according to current recommendations (see *ICH Guidelines S2A and S2B* [14, 15]), should be available. The following tests are recommended:

- A test for gene mutation in bacteria.
- An in vitro test in mammalian cells with cytogenetic evaluation of chromosomal damage or an in vitro mouse lymphoma tk+/- assay.
- An in vivo test for chromosomal damage using rodent haematopoietic cells.

Reproductive toxicity studies (see *ICH guidelines S5A and S5B* [16, 17]) should be conducted within the timeframe provided by *ICH guideline M3*. All available information, e.g. pharmacological, kinetic, and toxicological data for the test substance, should be considered in deciding the most appropriate strategy and choice of study design. A three-study design in rats (or other rodents) covering fertility, embryotoxicity/ foetotoxicity/teratogenicity, and peri/postnatal developmental toxicity is adequate in most instances. A combination of different reproductive toxicity endpoints in one single study could also be valid, provided that all stages of the reproductive process can be evaluated.

To investigate potential effects on reproductive organs, histopathology results from repeated dose toxicity studies of at least two weeks duration may, in general, provide sufficient information. If specific studies for fertility and early embryonic development to implantation are performed, the following considerations apply: for **females**, the study should detect potential effects on the oestrus cycle, tubal transport, implantation, and development of preimplantation stages of the embryo. For **males**, the study should detect potential functional effects that may not be detected by histological examination of the male reproductive organs. The pre-mating exposure time for **males** should be nine to ten weeks (i.e. covering at least one full spermatogenic cycle), and for **females**, two weeks. Provided no effects on spermatogenesis are found in a repeated dose toxicity study, a pre-mating exposure of four weeks in **males** can be sufficient. When exposure of females terminates at implantation, the animals should be sacrificed between days 13 and 15 of pregnancy. Males can be sacrificed if the result of mating is known; males can be mated with untreated females, or exposure can be continued beyond mating to detect potential toxicity to the male reproductive system. A mating ratio of 1:1 is advisable. Potential effects should be assessed on:

- Maturation of gametes.
- Mating behaviour.
- Fertility.

- Preimplantation stages of the embryo.
- Implantation.

These studies should be performed in at least one mammalian species, preferably rats.

The aim of studies looking at effects on pre-and postnatal development, including maternal function, is to detect potential adverse effects on the pregnant/lactating female and on development of the conceptus. Exposure time of the female should be from implantation to the end of lactation. The offspring are exposed *in utero* and via breastfeeding until weaning. Studies should be performed in at least one mammalian species, preferably rats.

The aim of studies looking at effects on embryo-foetal development is to detect potential adverse effects on the pregnant female and on development of the embryo and foetus. Exposure time of the female should cover the whole period of organogenesis and thus be from implantation to closure of the hard palate in the foetus. The following potential adverse effects should be assessed:

- Enhanced toxicity relative to that in non-pregnant females.
- Altered foetal growth and embryo-foetal mortality.
- Pre- and post-implantation loss.
- Structural changes (skeletal and soft tissue variations and malformations).

Studies should be performed in two mammalian species, one rodent and one non-rodent. Conventionally, rats and rabbits are used; special justification would be needed for use of other species.

3.3.4 *Safety information to support phase III*

Studies with repeated dosing of longer duration (i.e. to support the intended duration of the clinical trials) are necessary. This could also include carcinogenicity studies, as appropriate: The ICH Guideline S1A [18] provides guidance on whether or not carcinogenicity studies need to be conducted. Carcinogenicity studies are needed under one or more of the following circumstances:

- Long-term therapy with duration of at least six months through continuous or intermittent use.
- Pharmaceutical from a pharmacological/chemical class with a proven carcinogenic potential.

- Structure-activity relationship suggesting a carcinogenic risk.
- Positive or equivocal genotoxicity.
- Preneoplastic lesions observed in repeated dose toxicity studies.
- Long-term tissue retention of parent compound or metabolite(s).

Carcinogenicity studies may not be needed in the case of:

- Short-term exposure or infrequent use for short duration in humans.
- Short life-expectancy in the respective patient population.
- Unequivocal genotoxic activity of the substance (where the carcinogenic potential can be assumed and thus may not need to be tested).
- Poor systemic exposure, e.g. from topical use.
- Different chemical forms, especially salts or esters, of the same therapeutically active moiety for which prior carcinogenicity studies are available, provided there is no significant change in pharmacodynamics, pharmacokinetics, or toxicity.

To speed up the availability of pharmaceuticals for certain life-threatening, serious or debilitating diseases, carcinogenicity studies that may be needed can be conducted post-approval.

Historically, regulatory requirements provided for the conduct of long-term (life-time) carcinogenicity studies in two rodent species, usually the rat and the mouse (or, for special cases, the hamster), with a duration of 24 months in the rat and at least 18 months in the mouse and hamster. This design is still considered acceptable. The basic scheme more recently provided by *ICH guideline S1B* [19] comprises one long-term study, usually in rats, and one other test model to be chosen from various possibilities, such as:

- Several transgenic mouse assays.
- The neonatal rodent tumorigenicity model.
- The initiation-promotion model (this latter model today is only used for assessing hepatotoxins/hepatocarcinogens).

3.3.5 Safety information to support product label

For obtaining a marketing permit, information to support appropriate labelling has to be available. This may include mutagenicity, carcinogenicity, impairment of fertility, teratogenicity, and peri/post-natal developmental effects. Other studies may also generate safety data to include in the product label. Data regarding overdosing, morbidity, and toxicity in animals, that could be of potential significance in humans but which effects were not observed in clinical trials, may also be included.

3.3.6 Additional non-clinical safety information that may be required

If the pharmaceutical is to be used for paediatric indications, it may be necessary to develop safety data for this population through conducting studies in juvenile animals. For drugs to be used in pregnant women, it may also be necessary to perform special toxicity studies. As stated above, any newly identified animal or human toxicity may need further investigation on a case-by-case basis.

3.4 SPECIFIC ASPECTS OF DIFFERENT GROUPS OF PHARMACEUTICALS

This handbook deals equally with the regulatory requirements for safety testing in the fields of synthetic or semi-synthetic chemical pharmaceuticals and for herbal medicines and vaccines. Definitions for the different classes of pharmaceuticals are given in the glossary (section 5).

As there will be major differences in the “knowledge database” for these different classes of pharmaceuticals, there will be special aspects and specific requirements for individual cases, although the basic requirements for safety assessment are the same for all. Thus, while in principle a non-clinical safety assessment will follow the steps outlined above and provide information on the various safety aspects of the pharmaceutical under investigation, deviations from this general testing programme are possible and will, in view of resources and other considerations, even be advisable.

These special aspects may lead to specifically designed non-clinical safety testing programmes, and they will be dealt with in a concise form in the next section, where as

much information as possible is extracted from official publications – official, regulatory guidelines, as well as draft guidelines which, however, mirror the current thinking of regulatory authorities – to provide a general and pragmatic approach to non-clinical safety testing, the design of the respective testing programmes and the kind of strategies that should be followed when developing a potentially useful pharmaceutical preparation through the non-clinical stages.

3.4.1 *Synthetic chemical compounds*

As mentioned above, non-clinical testing programmes for “conventional drug substances” have been delineated in a number of guidelines, and are customarily designed to cover broadly all aspects of potential toxicity of the compound under investigation since, for new active substances, knowledge about possible toxic effects will, for all practical purposes, be considered nil. In these instances, existing guidelines such as *ICH guideline M3* [2] spell out in more or less detail what kind of documentation regulatory authorities expect to be submitted for scrutiny and assessment when the compound is intended to be applied for the first time to humans (IND) or when it is ready to be marketed (NDA). Such applications must address the whole field of safety testing, and the studies will be expected to cover more or less everything from acute toxicity to carcinogenicity following the outline given in the previous section of this handbook and in, e.g., the table of contents of the ICH Common Technical Document [1].

Even in these instances, however, there are possible exemptions from the complete array of studies. Thus, compounds that are to be applied for short-term treatments only (i.e. for less than six months) and which do not show cause for concern through positive genotoxicity results are exempt from carcinogenicity testing. Logically, there is no need to perform embryo/foetotoxicity testing for pharmaceuticals which target patient populations where child bearing is no longer an issue (e.g. post-menopausal women).

Another area where requirements have to be adapted to actual necessity are the various possibilities for galenical formulations. The general assumption is that a pharmaceutical will be administered in a form that will lead to significant systemic exposure (as most pharmaceuticals are targeted to oral administration). There are, however, exemptions to this rule: for instance, a pharmaceutical which is only to be applied topically will have to satisfy specific requirements as to the detection of potential local reactions, but – with appropriate scientific justification for lack of potential for sys-

temic exposure – will not have to undergo the full toxicity testing procedures that are intended to provide information about systemic toxicity. In the European “Notice to Applicants” [10], this is made very clear, as these guidelines declare that: “Where a medicinal product is intended for topical use, systemic absorption must be investigated, due account also being taken of the possible use of the product on broken skin and absorption through other relevant surfaces. Only if it is proved that systemic absorption under these conditions is negligible may repeated dose systemic toxicity tests, foetal toxicity tests and studies of reproductive function be omitted.

If, however, systemic absorption is demonstrated during therapeutic experimentation, toxicity tests shall be carried out on animals, including where necessary, foetal toxicity tests.

In all cases, tests of local tolerance after repeated application shall be carried out with particular care and include histological examinations; the possibility of sensitisation shall be investigated and any carcinogenic potential investigated in the cases referred to in paragraph II E of this Part [of the Notice to Applicants].”

Thus, while the requirements are relatively well defined, it is nevertheless useful and good practice to design the whole programme very carefully, to assess at every step the results and their consequences, in order to determine the best, shortest, easiest and most economical way to arrive at a full understanding of the potential toxicity of the compound under investigation.

3.4.2 Traditional and herbal medicines

Traditional medicine products are natural drug products that have a documented, historical human use and may also be available in dietary supplement markets in some countries. They include botanicals (plant materials, algae, macroscopic fungi), minerals, materials of animal origin, combinations of the above, and fermented products, all of which may not easily be chemically characterized. This drug category should not include highly purified or chemically modified substances derived from natural products because purified products can easily be fully characterized.

Because of the extensive, though mainly uncontrolled, use in humans, traditional and herbal medicines may be supported by sufficient information for the initial, limited, clinical study without prior conduct of standard non-clinical testing. Sufficient non-clinical and clinical data could furthermore obviate the need for some types of data that are mentioned in this document. In this situation, an investigator could gather and

submit available animal toxicity data concerning the traditional product and the individual traditional medicine ingredients in the product to justify the proposed clinical trial. A database search should be conducted to identify information relevant to the safety and efficacy of the final formulation of the intended commercial traditional medicinal drug product, the individual traditional medicinal ingredients, and the known chemical constituents of the traditional medicinal ingredients, when feasible. Emphasis is placed on the flexibility that sufficient non-clinical and clinical data allow in the development of such traditional and herbal drugs (*Directive 2001/83 EC, article 10A*) [20]. Many important aspects of research into non-clinical and especially clinical development of traditional and herbal medicines have already been covered in various guidelines, especially in the WHO document *General guidelines for methodologies on research and evaluation of traditional medicines* [21] and the *ICH guideline M3* [2]. The reader is referred to these documents (as well as other applicable regulatory guidance) for questions relating to research and early development and to clinical trials in healthy volunteers and patients.

The aspect of non-clinical safety testing has, however, not been addressed in more than a cursory way in these documents. This is probably just an expression of the general uncertainty over how to deal with these products in the context of non-clinical safety testing. However, through a logical process starting from the different categories as previously described, and from the identified gaps in non-clinical information needed to support a safety assessment, a scientifically justified programme can be designed and the necessary methods identified.

There are indeed some recent efforts to guide the “herbal drug developer” in these matters, in sharing the thoughts of the regulators with respect to what, in their opinion, should be considered an acceptable non-clinical testing strategy and submission dossier. **In Appendix F, the draft “Guidance to Industry” of the US FDA is provided in a condensed version that highlights the non-clinical issues as they are presented for the different possibilities of herbal submissions** [22].

In short, the more information that is available from human use, the less testing needs to be performed in non-clinical assays. The caveats to this approach have already been cited, and they are also reiterated in the ICH, FDA and EC guidelines mentioned above. While human experience with the traditional use of plant-derived pharmaceuticals may be well documented, it may also be anecdotal only. The former case would call for less rigorous non-clinical testing than the latter, and might be limited to some genotoxicity testing and, possibly, some interaction studies with metabolizing enzymes. On the

other hand, a new indication for a well documented traditional drug, that would introduce a longer duration of treatment than in the traditional use, may necessitate re-evaluation of the long-term safety. This could mean having to perform conventional chronic toxicity studies in two mammalian species as for any new synthetic chemical.

Requirements for non-clinical safety testing of herbal preparations therefore span a wide array of possibilities, and depend on the gaps identified and the needs perceived.

- Following the purely intellectual exercise of collecting and assessing clinical and published information on a traditional medicine, the data may be considered sufficient (and sufficiently trustworthy) to cover the safety requirements of the intended use of this preparation. If traditional herbal medicines are to be developed and employed in the traditional form and for the traditional indications, they will mostly fall into this category.
- In many cases, the botanical information may indicate the presence of certain suspicious or even dangerous natural compounds (e.g. certain alkaloids or flavonoids), so that some further non-clinical studies may be considered useful for the safety assessment. Especially genotoxicity studies may be warranted under such circumstances, but also investigations into the inducing or inhibitory properties towards metabolizing enzymes (e.g. liver cytochrome P450 isoenzymes) may be warranted. Depending on the “safety information gaps” identified, these safety concerns will arise mainly in those instances where new information, especially from analytical data about natural constituents of traditional herbs, becomes available during the scientific development of such preparations.
- If the herbal preparation contains plants, or plant parts, or extracts therefrom, which have not been used traditionally, or which have been used rather rarely, and for which there is thus no extensive evidence of human safety, further non-clinical safety studies should be conducted. Consideration should again be given to those aspects that could be seen as missing out important safety information. Even though an acute single dose toxicity study may provide certain evidence of safety, more emphasis will have to be placed on repeated dose toxicity and especially on embryotoxicity/teratogenicity and genotoxicity investigations.

The following strategies may be regarded as a blueprint for consideration by drug developers with sufficient flexibility based on scientific judgement and national regu-

lations. However, it is recommended that the appropriate regulatory authorities be contacted regarding drug development plans prior to the initiation of any studies.

PRELIMINARY CONSIDERATIONS

The non-clinical safety testing strategies considered for traditional and herbal medicines apply to all herbal extractives from water or any traditionally used solvent. The strategies to be developed must take into account the basic preliminary concerns that exist or arise before commencement of non-clinical and clinical trials (i.e. proper identification of materials etc.), safety issues that correlate with clinical phases I, II and III, and other important general considerations that will ensure an adequate scientific and ethically justifiable dossier.

EVIDENCE OF TRADITIONAL USE AND LITERATURE DATA

The first step in a literature search is to consult a reference text on plant toxicology [23, 24] and a pharmacopoeia or similar monographs [25]. Secondly, databases such as EMBASE, NAPRALERT or *Biological Abstracts* may be consulted.

A retrospective survey of cases treated with a particular medicine by traditional healers, and the healers' observations of possible side-effects or contraindications to the medicine, will provide some vital information that can be used to guide non-clinical safety studies. Most crucial of all is to record the methods of preparation and the doses used. Most useful medicinal plants, like most useful pharmaceuticals, may be toxic if prepared incorrectly or given in an incorrect dose because they contain pharmacologically active compounds [26]. A well conducted literature search therefore may help to identify any potential toxicity of herbal products.

Furthermore, most common medicinal plants have been the subject of more or less extensive scientific research. There is a large body of literature on the chemical constituents of such plants, and their pharmacological activities, and substantial ethnobotanical research results already exist [27] to support safety studies. This literature should be consulted before embarking on any study that is aimed at answering the following types of question:

- Are there any reports of human toxicity associated with ingestion of the plant and, if so, which part of the plant, in what preparation, and at what dose?
- Have any laboratory studies of toxicity been carried out on the relevant preparation of the plant? If so, what did the results show?
- What pharmacologically active compounds does this plant species (or genus) contain? In which parts of the plant are they found?
- What are their principal pharmacological effects, and at what doses?

IDENTIFICATION AND STANDARDIZATION OF MATERIALS

In the absence of a reliable chemical basis for identifying the herbal materials, proper botanical identification of the plant species and/or sub-species becomes imperative. Identification should be based on the currently accepted Latin binominal system, with the synonyms listed. For safety studies, materials with verifiable botanical identification and characterization are required.

Besides pharmacognostic methods (macroscopy, microscopy), phytochemical evaluation and fingerprinting may also provide independent verification of identity and certification for quality. Such information may also be valuable during monitoring and evaluation of human studies.

Pharmaceutical standardization of the drug product should aim to establish additional parameters for monitoring and evaluation of safety during clinical trials. This may include qualitative and quantitative description, dosage forms, route of administration, names of ingredients, etc.

ADDITIONAL NON-CLINICAL SAFETY TESTING

Once important gaps in knowledge about the toxic properties of a plant-derived pharmaceutical have been identified, any non-clinical investigations needed can be outlined. As already stated, human experience with such drugs may obviate the need to conduct acute and chronic toxicity tests. However, reproductive toxicity, genotoxicity and carcinogenicity may not be well characterized since these areas of major concern can only be dealt with through careful conduct of non-clinical studies. Since these potential toxic properties are of special importance, and the respective tests provide information which is hard or impossible to obtain from testing in humans, the conduct of the respective assays should certainly be contemplated in any such non-clinical safety testing programme.

3.4.3 Toxicology safety testing for vaccines

INTRODUCTION

Vaccines are medicinal products and as such must undergo non-clinical toxicity safety testing (see, e.g., the European pharmaceutical legislation [28]); they pose very special problems since their use as well as their mode of action differs fundamentally from those of other medicinal products. It is necessary to identify the critical safety issues

and not to embark on a fixed programme of conventional toxicity assays [29, 30, 31]. Vaccines are intended to induce specific immunity against infectious pathogens or their toxins or antigens with the primary aim of preventing infectious diseases. They may contain:

- Inactivated organisms that retain adequate immunogenicity.
- Living organisms, either naturally avirulent or attenuated.
- Antigenic fractions extracted from the organisms or produced by recombinant DNA technology.

In this document, the use of vaccines for curative purposes is not considered. At the moment, only limited data and a few guidelines are available, so there are few established rules which can be used to test the non-clinical toxicity of vaccines [32, 33, 34]. However, this situation is rapidly evolving.

SAFETY CONCERNS

Potential safety concerns include general systemic toxicity, paradoxical enhancement of the intended disease, local tolerance, pyrogenicity, allergy, autoimmunity and reproduction toxicity. As adequately standardized and validated animal models are not available to address all safety issues, and as causes of concern may vary depending on the type or intended use of the vaccine, a case-by-case approach is recommended. It is logical to use a route of non-clinical administration as close as possible to the clinical route, wherever applicable.

SYSTEMIC TOXICITY

Two types of systemic toxicity studies must be performed. Generally it is recommended that only one species is used for these studies; however, two species are required for vaccines produced by recombinant DNA technology.

Single dose toxicity. The mouse or rat can be used and there is no established rule as to which of the two species is best. Immunogenicity and protection studies – the primary pharmacodynamic studies for vaccines – are commonly conducted in mice, whereas the rat is the most frequently used species for single dose toxicity studies of medicinal products. Several doses must be used to determine a safety margin between the immunogenic (effective) dose and the dose associated with systemic toxicity, if any.

Repeated dose toxicity. Because most vaccines are normally administered several times in the life of the same person and often within a relatively short period of time, at least one repeated dose toxicity study is recommended. There is no established rule regarding the most adequate species with the exception that the candidate vaccine should be immunogenic and protective in the selected species. The rat and the monkey are the most commonly used species. At least two doses are used: the low dose is the immunogenic dose in the selected species and the high dose is traditionally the human dose. Important issues are the duration of the study and the schedule of administration. A short-term, e.g. 14-28 days, repeated dose toxicity study is deemed acceptable for most vaccines. Even though no established rule is available, one may consider intermittent administration every other day or twice a week in order to mimic the clinical situation. Pharmacokinetic studies are not recommended; however, measurement of the antibody response is valuable for confirming adequate vaccine exposure. Endpoints to be assessed in such a repeated dose toxicity study are those normally assessed for any medicinal product. Special attention must be paid on a case-by-case basis to particular safety aspects, such as deposits of immune complexes on the basement membrane of renal glomeruli, inadvertent immune responses, safety pharmacology endpoints (CNS), or paradoxical enhancement of the targeted disease.

LOCAL TOLERANCE

A specific local tolerance study is not deemed necessary in most instances. Careful macroscopic and histological examination of the injection sites can be performed in the course of the repeated dose toxicity study.

PYROGENICITY

Pyrogenic reactions can be seen with some vaccines. Guidelines are available to test for pyrogenicity, and they are applicable to vaccines.

ALLERGY

Hypersensitivity reactions, although very rare, are the most common severe adverse effects of vaccines. No validated model is available to predict this risk reliably. Systemic anaphylaxis models in the guinea pig are debated, but have been shown to produce results comparable to the clinical experience [35]. However, they are not applicable when the final formulation contains substances of human origin or humanized antigens. The risk of serum sickness is extremely low and can be considered by careful histology

and immunohistochemistry examination of the glomeruli, or in experimental serum sickness models, e.g. in the rabbit [36].

AUTOIMMUNITY

Autoimmune diseases associated with vaccines are an unsolved question. The use of animal models of autoimmunity can be considered, but research efforts are needed to propose reliably predictive models. Two different types of animal models may be used, either genetically autoimmunity-prone animals, such as NZB/NZW F1 (lupus) or NOD (type I diabetes) mice, or models of experimental diseases, such as experimental allergic encephalomyelitis (multiple sclerosis) or collagen-induced arthritis (rheumatoid arthritis). In addition, autoimmunity can be induced by molecular mimicry, and this possibility should be considered on a case-by-case basis.

REPRODUCTION TOXICITY

In most cases, reproduction toxicity studies are not required as vaccines are usually administered to children. These studies may become necessary when vaccination is intended for use in women of child-bearing age and still more so in pregnant women. Specific designs should be used as conventional reproduction toxicity studies are not applicable [37].

OTHER ISSUES OF CONCERN

Genotoxicity studies are normally not needed, except when impurities of unknown origin are present. Carcinogenicity studies are normally not needed. The safety of adjuvants, preservatives and excipients must be considered, especially when novel derivatives are included in the final formulation.

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5. GLOSSARY OF TERMS

- “Traditional medicine” is a term that encompasses more than just the local or regional usage and application of (indigenous, herbal) drugs or “folk medicines”; it relates to the whole cultural environment of therapies delivered to the patient. In addition to the use of herbs and herbal or other preparations, important factors in traditional healing practices may include therapeutic measures of a physical, mental and/or spiritual nature.
- “Traditional herbal medicines” means those herbs and herbal preparations which have been used traditionally for a long time, sometimes for generations, in the treatment of illness. Extensive knowledge may therefore be assumed to exist about their safe application to patients.
- “New preparations from traditional herbal medicines” refers to more recent developments in the technology of preparing pharmaceutical products from plants and their parts, such as solvent extraction (instead of aqueous extracts), fractionation, concentration, or new combinations of different extracts.
- “New herbal medicines” covers the use of herbs that have not been used traditionally but for which some pharmacological activity has been discovered, e.g. by a research programme looking for pharmaceutically useful plants.
- “Natural compounds” denominates the pure active ingredients isolated from medicinal plants, such as artemisinin, quinine, or salicylic acid. Their safety aspects may be more or less well known, but when it comes to more recent discoveries of active ingredients that may have been isolated from natural sources, these may have to be treated like any new synthetic chemical, i.e. as a “new active substance” (NAS), and be assayed for safety in the whole toxicity testing battery. An example of such an NAS is taxol.
- “Semi-synthetic compounds” means those substances which are derived from natural products, but which have undergone some chemical modification; examples are the

various artemisinin derivatives (artemether, arteether, artesunate). In these cases, although some safety information may be deduced from the information available on the parent, natural compound, it will again be necessary to complement the available safety information with new non-clinical tests.

- “Synthetic (chemical) compounds” means all substances produced de novo by artificial processes, whether manufactured by a purely chemical synthesis (“small” molecules) or by a biological synthesis through any kind of fermentation (some antibiotics, or biotechnology-derived pharmaceuticals, e.g. recombinant proteins). Vaccines are exempt from this “working” definition and are dealt with separately in this document.
- “Vaccines” are pharmaceuticals intended to protect against transmissible diseases; vaccination can be active, through application of an antigen which elicits an immunogenic reaction and confers immunity to the vaccinated person, or passive, through application of a pre-formed antibody to combat an existing condition.
- “Safety pharmacology studies” are those studies that investigate the potential undesirable pharmacodynamic effects of a substance on physiological functions in relation to exposure in the therapeutic range and above.
- “Primary pharmacodynamic studies” are studies on the mode of action and/or effects of a substance in relation to its desired therapeutic target.
- “Secondary pharmacodynamic studies” are studies on the mode of action and/or effects of a substance not related to its desired therapeutic target (these have sometimes been referred to as part of general pharmacology studies).
- “Good Laboratory Practice (GLP)” is a quality system concerned with the organizational process and conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported.
- “Acute, single dose toxicity studies” are studies to investigate the immediate and delayed toxic effects, including mortality, of a single, high dose of a substance on a mammalian organism. Clinical symptoms, effects on body and organ weights and

gross findings at necropsy are the main end-points. Histopathology is only rarely performed in such studies.

- “Chronic toxicity studies” are conducted with repeated daily application of a substance, for a duration of between 14 days and 6 months in rodents and 9 months in non-rodents, to determine the effects of a test substance following prolonged and repeated exposure; also, effects which require a long latent period, or are cumulative, should become manifest. These studies should generate, by means of observing clinical symptoms, body and organ weight changes, changes in haematological, serum biochemical, urinary and other parameters, data on which to identify the majority of chronic effects, determine dose-response relationships, and define target organs for toxicity by determining histopathological changes related to exposure to the test substance. Ideally, the design and conduct of chronic toxicity studies should allow for the detection of general toxicity including neurological, physiological, biochemical effects and exposure-related, morphological effects. They also should enable the definition of a “no observed effect level” (NOEL) or a “no observed toxic effect level” (NOTEL).
- “Mutagenicity studies” investigate the effects of substances on the genetic material (DNA) for the potential to induce heritable mutations in genes (gene mutations).
- “Genotoxicity” is a more general term that encompasses all changes in the genetic material (structural and numerical chromosomal changes, DNA damage and repair, changes in arrangement of genetic material, and including gene mutations) that may lead to changes in the expression of the genetic make-up of a cell.
- “Reproductive toxicity studies” investigate the effects of treatment on the reproductive process (gamete development, sexual behaviour and fertility, conception, embryonic and foetal development, lactation, and physical and behavioural development, including reproductive capacity, of the offspring).
- “Developmental toxicity” is a term that covers any detrimental effect of exposure on a developing organism during the embryonic stages of development. The lesions can be irreversible or reversible. Embryo-lethal lesions are incompatible with survival of the conceptus and result in resorption, spontaneous abortion, or stillbirth.

- “Teratogenicity” is a specific term for severe, irreversible structural or functional abnormalities that are compatible with survival of the foetus, but may or may not be compatible with survival of the newborn.
- “Carcinogenicity studies” are intended to provide evidence for the induction of neoplastic changes in tissues and organs, leading to cancer, in mammalian organisms through long-term (i.e. for the major part of the life-span) exposure to chemical substances.

6. INFORMATION ON WHERE TO FIND OFFICIAL REQUIREMENTS, AND INFORMATION RESOURCES IN TOXICOLOGY

Important journals

American Industrial Hygiene Association Journal
Environment and Health Perspectives
Environmental Science and Technology
Environmental and Molecular Mutagenesis
Fundamental and Applied Toxicology
International Journal of Toxicology
Journal of Occupational and Environmental Medicine
Journal of Applied Toxicology
Journal of Toxicology and Environmental Health
Pesticide and Toxic Chemicals News
Regulatory Toxicology and Pharmacology
Toxicology and Applied Pharmacology
Toxicology and Industrial Health
Toxicology Letters
Toxicology

Abstracts and indices

Most data contained in abstracts are available through the US National Library of Medicine (NLM):

Biological Abstracts (1926 to date). Covers animal toxicology of toxic substances.
Index Medicus (1879 to date). Covers worldwide medical literature.
Industrial Hygiene Digest (1980 to date). Industrial medicine, chemical and physical hazards, environment and safety, accident prevention.

Pollution Abstracts (1970 to date). Air, water, noise and land pollution. Sewage management, pesticide and chemical contaminants.

Online data sources

General search technique - guidance on how to search the Internet is available:

Ralph B Stuart. *Safety and health on the Internet*. Government Institutes Inc. December 1996 (ISBN: 0865875235).

A good starting point to gain an idea of the physico-chemical nature of a material is one of the various material safety data sheet (MSDS) databases. After this, one may access the online chemical identification file (CHEMID) to locate any data within the NLM system and to check if the chemical is on any federal or state regulatory list.

National Library of Medicine

The Medical Literature Analysis and Retrieval System (MEDLARS) is the information retrieval system for NLM. A user identification code is needed to search all the files, except for PubMed and Internet Grateful Med (IGM).

Over 40 online databases and over 18 million citations are covered. The important databases are listed below.

TOXLINE (Toxicology Information Online)

Contains 18 secondary subfiles. The ones commonly needed are:

DART	Developmental and Reproductive Toxicology
EMIC	Environmental Mutagen Information Center
EPIDEM	Epidemiology Information System
IPA	International Pharmaceutical Abstracts
PESTAB	Pesticide Abstracts
PPBIB	Poisonous Plants Bibliography

TOXBIB	Toxicity Bibliography
BIOSIS	Toxicology Abstracts of Environmental Health
CRISP	Toxicology Research Projects

CHEMID (Online Chemical Identification File)

Available through MEDLARS for a fee. All 335 000 substances cited in NLM databases are identified. It also contains a SUPERLIST providing regulatory information maintained by federal and state regulatory agencies on the chemical in question.

MEDLINE (Medical Information Online)

Covers the articles listed in Index Medicus. Available free via Pubmed and Internet Grateful Med (IGM).

TOXNET (Toxicology Data Network)

Contains 11 files:

HSDB	Hazardous Substances Database
TRI	Toxic Chemical Release Inventory
IRIS	Integrated Risk Information System
RTECS	Registry of Toxic Effects of Chemical Substances
CCRIS	Chemical Carcinogen Research Information System
GENE-TOX	Genetic Toxicology
DART	Developmental and Reproductive Toxicology
EDICBACK	Environmental Teratology Information Center Backfile
EMIC	Environmental Mutagen Information Center
EMICBACK	Environmental Mutagen Information Center Backfile
TRIFACTS	Toxic Chemical Release Inventory Facts

Government websites

For a complete directory of online US government information resources, see official Federal Government website at: <http://lcweb.loc.gov/global/ncp/ncp.html>.

Twenty-five of the total sites listed are useful for toxicologists.

REGULATORY INFORMATION SOURCES

1. International Conference on Harmonisation (ICH):
<http://www.ich.org/>
2. Chemical Abstracts Registry File – STN or ETN Easy:
<http://www.cas.org/>
3. US Food and Drug Administration homepage:
<http://www.fda.gov/>
4. Chemicals on Reporting Rules:
<http://www.epa.gov/docs/oppyintr/CORR/index.html>
5. DOE Technical Standards homepage:
<http://apollo.osti.gov/html/tech/stds/techstds.htm>
6. EPA Federal Register environmental documents:
<http://www.epa.gov/fedrgstr/>
7. European regulations: H&H Scientific Consultants Ltd.
<http://dSPACE.dial.pipex.com/hhsc>
8. EXTOWNET – The EXTension ToXicology NETwork – Pesticide Toxicology Information:
<http://acc.orst.edu/info/exonet/>

9. Federal Register subscription information:
<http://www.epa.gov/fedrgstr/subscribe.htm>
10. HAZMAT:
<http://www.text-trieve.com/dotrspa/>
11. NFPA codes and standards. Server page:
<http://www.nfpa.org/>
12. Organisation for Economic Cooperation and Development
<http://www.oecd.org/>
13. OSHA standards and related documents:
<http://www.osha-slc.gov/>
14. Proposition 65 – list of chemicals:
<http://www.cdpr.ca.gov/docs/regulate/prop65/prop65.htm>
15. The APPA Regulatory Reporter:
<http://appa1.appa.org/pubpol/regrep.htm>
16. The Prop 65 page:
<http://www.calprop65.com/>

APPENDIX A

ICH COMMON TECHNICAL DOCUMENT –
SAFETY (CONDENSED VERSION)⁵

NON-CLINICAL SUMMARIES (MODULE 2)
AND ORGANIZATION OF MODULE 4

1. SUMMARIES IN MODULE 2 OF THE COMMON TECHNICAL DOCUMENT

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1.1 GENERAL PRINCIPLES

This guideline provides recommendations for the harmonisation of the Non-clinical Overview, Non-clinical Written Summary, and Non-clinical Tabulated Summaries. The primary purpose of the Non-clinical Written and Tabulated Summaries should be to provide a comprehensive factual synopsis of the non-clinical data. The interpretation of the data, the clinical relevance of the findings, cross-linking with the quality aspects of the pharmaceutical, and the implications of the non-clinical findings for the safe use of the pharmaceutical (i.e., as applicable to labelling) should be addressed in the Overview.

1.2 NON-CLINICAL OVERVIEW

The Non-clinical Overview should provide an integrated overall analysis of the information in the Common Technical Document. In general, the Non-clinical Overview should not exceed about 30 pages.

⁵ The complete text of this, and other ICH Guidelines, can be downloaded from the ICH website at: <http://www.ich.org/>

1.2.1 *General Aspects*

The Non-clinical Overview should present an integrated and critical assessment of the pharmacologic, pharmacokinetic, and toxicological evaluation of the pharmaceutical. Where relevant guidelines on the conduct of studies exist, these should be taken into consideration, and any deviation from these guidelines should be discussed and justified. The non-clinical testing strategy should be discussed and justified. There should be comment on the GLP status of the studies submitted. Any association between non-clinical findings and the quality characteristics of the human pharmaceutical, the results of clinical trials, or effects seen with related products should be indicated, as appropriate.

Except for biotechnology-derived products, an assessment of the impurities and degradants present in the drug substance and product should be included along with what is known of their potential pharmacologic and toxicological effects. This assessment should form part of the justification for proposed impurity limits in the drug substance and product, and be appropriately cross-referenced to the quality documentation. The implications of any differences in the chirality, chemical form, and impurity profile between the compound used in the non-clinical studies and the product to be marketed should be discussed. For biotechnology-derived products, comparability of material used in non-clinical studies, clinical studies, and proposed for marketing should be assessed. If a drug product includes a novel excipient, an assessment of the information regarding its safety should be provided.

Relevant scientific literature and the properties of related products should be taken into account. If detailed references to published scientific literature are to be used in place of studies conducted by the applicant, this should be supported by an appropriate justification that reviews the design of the studies and any deviations from available guidelines. In addition, the availability of information on the quality of batches of drug substance used in these referenced studies should be discussed.

The Non-clinical Overview should contain appropriate reference citations to the Tabulated Summaries, in the following format: (Table X.X, Study/Report Number).

1.2.2 Content and Structural Format

The Non-clinical Overview should be presented in the following sequence:

- Overview of the non-clinical testing strategy
- Pharmacology
- Pharmacokinetics
- Toxicology
- Integrated overview and conclusions
- List of literature citations.

Studies conducted to establish the pharmacodynamic effects, the mode of action, and potential side effects should be evaluated and consideration should be given to the significance of any issues that arise.

The assessment of the pharmacokinetic, toxicokinetic, and metabolism data should address the relevance of the analytical methods used, the pharmacokinetic models, and the derived parameters. It might be appropriate to cross-refer to more detailed consideration of certain issues within the pharmacology or toxicology studies (e.g. impact of the disease states, changes in physiology, anti-product antibodies, cross-species consideration of toxicokinetic data). Inconsistencies in the data should be discussed. Inter-species comparisons of metabolism and systemic exposure comparisons in animals and humans (AUC, C_{max}, and other appropriate parameters) should be discussed and the limitations and utility of the non-clinical studies for prediction of potential adverse effects in humans highlighted.

The onset, severity, and duration of the toxic effects, their dose-dependency and degree of reversibility (or irreversibility), and species- or gender-related differences should be evaluated and important features discussed, particularly with regard to:

- Pharmacodynamics
- Toxic signs
- Causes of death
- Pathologic findings
- Genotoxic activity – the chemical structure of the compound, its mode of action, and its relationship to known genotoxic compounds
- Carcinogenic potential in the context of the chemical structure of the compound, its relationship to known carcinogens, its genotoxic potential, and the exposure data

- The carcinogenic risk to humans - if epidemiologic data are available, they should be taken into account
- Fertility, embryofetal development, pre-and post-natal toxicity
- Studies in juvenile animals
- The consequences of use before and during pregnancy, during lactation, and during paediatric development
- Local tolerance
- Other toxicity studies/ studies to clarify special problems.

The evaluation of toxicology studies should be arranged in a logical order so that all relevant data elucidating a certain effect / phenomenon are brought together. Extrapolation of the data from animals to humans should be considered in relation to:

- Animal species used
- Numbers of animals used
- Routes of administration employed
- Dosages used
- Duration of treatment or of the study
- Systemic exposures in the toxicology species at no observed adverse effect levels and at toxic doses, in relation to the exposures in humans at the maximum recommended human dose. Tables or figures summarising this information are recommended
- The effect of the drug substance observed in non-clinical studies in relation to that expected or observed in humans.

If alternatives to whole-animal experiments are employed, their scientific validity should be discussed.

The Integrated Overview and Conclusions should clearly define the characteristics of the human pharmaceutical as demonstrated by the non-clinical studies and arrive at logical, well-argued conclusions supporting the safety of the product for the intended clinical use.

Taking the pharmacology, pharmacokinetics, and toxicology results into account, the implications of the non-clinical findings for the safe human use of the pharmaceutical should be discussed (i.e., as applicable to labelling).

2. THE NON-CLINICAL WRITTEN AND TABULATED SUMMARIES

2.1 NON-CLINICAL WRITTEN SUMMARIES

2.1.1 *Introduction*

This guideline is intended to assist authors in the preparation of non-clinical pharmacology, pharmacokinetics, and toxicology written summaries in an acceptable format. This guideline is not intended to indicate what studies are required. It merely indicates an appropriate format for the non-clinical data that have been acquired. The sequence and content of the Non-clinical Written Summary sections are described below.

It should be emphasised that no guideline can cover all eventualities, and common sense and a clear focus on the needs of the regulatory authority assessor are the best guides to constructing an acceptable document. Therefore, applicants can modify the format if needed to provide the best possible presentation of the information, in order to facilitate the understanding and evaluation of the results.

Whenever appropriate, age- and gender-related effects should be discussed. Relevant findings with stereoisomers and/or metabolites should be included, as appropriate. Consistent use of units throughout the Summaries will facilitate their review. A table for converting units might also be useful.

In the Discussion and Conclusion sections, information should be integrated across studies and across species, and exposure in the test animals should be related to exposure in humans given the maximum intended doses.

2.1.2 *General presentation issues*

ORDER OF PRESENTATION OF INFORMATION WITHIN SECTIONS:

When available, in vitro studies should precede in vivo studies. Where multiple studies of the same type need to be summarised within the Pharmacokinetics and Toxicology sections, studies should be ordered by species, by route, and then by duration (shortest duration first).

Species should be ordered as follows:

1. Mouse
2. Rat
3. Hamster
4. Other rodent
5. Rabbit
6. Dog
7. Non-human primate
8. Other non-rodent mammal
9. Non-mammals.

Routes of administration should be ordered as follows:

1. The intended route for human use
2. Oral
3. Intravenous
4. Intramuscular
5. Intraperitoneal
6. Subcutaneous
7. Inhalation
8. Topical
9. Other.

USE OF TABLES AND FIGURES

Although the Non-clinical Written Summaries are envisaged to be composed mainly of text, some information contained within them might be more effectively and/or concisely communicated through the use of appropriate tables or figures.

Examples of formats that might be included in the Written Summaries are shown in Appendix A. To allow authors flexibility in defining the optimal structure for the Written Summaries, tables and figures should preferably be included within the text. Alternatively, they could be grouped together at the end of each of the Non-clinical Written Summaries. Throughout the text, reference citations to the Tabulated Summaries should be included, in the following format: (Table X.X, Study/Report Number).

LENGTH OF NON-CLINICAL WRITTEN SUMMARIES

Although there is no formal limit to the length of the Non-clinical Written Summaries, it is recommended that the total length of the three Non-clinical Written Summaries in general not exceed 100-150 pages.

SEQUENCE OF WRITTEN SUMMARIES AND TABULATED SUMMARIES

The following order is recommended:

1. Introduction
2. Written Summary of Pharmacology
3. Tabulated Summary of Pharmacology
4. Written Summary of Pharmacokinetics
5. Tabulated Summary of Pharmacokinetics
6. Written Summary of Toxicology
7. Tabulated Summary of Toxicology.

2.2 CONTENT OF NON-CLINICAL WRITTEN SUMMARY

2.2.1 *Introduction*

The aim of this section should be to introduce the reviewer to the pharmaceutical and to its proposed clinical use. The following key elements should be covered:

1. Brief information concerning the pharmaceutical's structure (preferably, a structure diagram should be provided) and pharmacologic properties.
2. Information concerning the pharmaceutical's proposed clinical indication, dose, and duration of use.

2.2.2 *The pharmacology written summary*

Within the Pharmacology Written Summary, the data should be presented in the following sequence:

- Brief Summary
- Primary Pharmacodynamics
- Secondary Pharmacodynamics
- Safety Pharmacology
- Pharmacodynamic Drug Interactions
- Discussion and Conclusions
- Tables and Figures (either here or included in text).

BRIEF SUMMARY

The principal findings from the pharmacology studies should be briefly summarized in approximately 2 to 3 pages. This section should begin with a brief description of the content of the pharmacologic data package, pointing out any notable aspects such as the inclusion/exclusion of particular data (e.g., lack of an animal model).

PRIMARY PHARMACODYNAMICS

Studies on primary pharmacodynamics* should be summarised and evaluated. Where possible, it would be helpful to relate the pharmacology of the drug to available data (in terms of selectivity, safety, potency, etc.) on other drugs in the class.

SECONDARY PHARMACODYNAMICS

Studies on secondary pharmacodynamics* should be summarised by organ system, where appropriate, and evaluated in this section.

SAFETY PHARMACOLOGY

Safety pharmacology studies* should be summarised and evaluated in this section. In some cases, secondary pharmacodynamic studies can contribute to the safety evaluation when they predict or assess potential adverse effect(s) in humans. In such cases, these secondary pharmacodynamic studies should be considered along with safety pharmacology studies.

* See: ICH Guideline S7A: Safety pharmacology studies for human pharmaceuticals, note 2, p. 8, for definitions

PHARMACODYNAMIC DRUG INTERACTIONS

If they have been performed, pharmacodynamic drug interaction studies should be briefly summarised in this section.

DISCUSSION AND CONCLUSIONS

This section provides an opportunity to discuss the pharmacologic evaluation and to consider the significance of any issues that arise.

TABLES AND FIGURES

Text tables and figures can be included at appropriate points throughout the summary within the text. Alternatively, tables and figures can be included at the end of the summary.

THE PHARMACOLOGY TABULATED SUMMARY (see Appendix B).

2.2.3 The pharmacokinetics written summary

The sequence of the Pharmacokinetics Written Summary should be as follows:

- Brief Summary
- Methods of Analysis
- Absorption
- Distribution
- Metabolism
- Excretion
- Pharmacokinetic Drug Interactions
- Other Pharmacokinetic Studies
- Discussion and Conclusions
- Tables and Figures (either here or included in text).

BRIEF SUMMARY

The principal findings from the pharmacokinetics studies should be briefly summarized in approximately 2 to 3 pages. This section should begin with a description of the scope of the pharmacokinetic evaluation, emphasising, for example, whether the species and strains examined were those used in the pharmacology and toxicology evaluations, and whether the formulations used were similar or identical.

METHODS OF ANALYSIS

This section should contain a brief summary of the methods of analysis for biological samples, including the detection and quantification limits of an analytical procedure. If possible, validation data for the analytical method and stability of biological samples should be discussed in this section. The potential impact of different methods of analysis on the interpretation of the results should be discussed in the following relevant sections.

ABSORPTION

The following data should be summarised in this section:

- Absorption (extent and rate of absorption, in vivo and in situ studies)
- Kinetic parameters, bioequivalence and/or bioavailability (serum/plasma/blood PK studies).

DISTRIBUTION

The following data should be summarised in this section:

- Tissue distribution studies
- Protein binding and distribution in blood cells
- Placental transfer studies.

METABOLISM (INTER-SPECIES COMPARISON)

The following data should be summarised in this section:

- Chemical structures and quantities of metabolites in biological samples
- Possible metabolic pathways
- Pre-systemic metabolism (GI/hepatic first-pass effects)
- In vitro metabolism including P450 studies
- Enzyme induction and inhibition.

EXCRETION

The following data should be summarised in this section:

- Routes and extent of excretion
- Excretion in milk.

PHARMACOKINETIC DRUG INTERACTIONS

If they have been performed, non-clinical pharmacokinetic drug-interaction studies (in vitro and/or in vivo) should be briefly summarised in this section.

OTHER PHARMACOKINETIC STUDIES

If studies have been performed in non-clinical models of disease (e.g., renally impaired animals), they should be summarised in this section.

DISCUSSION AND CONCLUSIONS

This section provides an opportunity to discuss the pharmacokinetic evaluation and to consider the significance of any issues that arise.

TABLES AND FIGURES

Text tables and figures can be included at appropriate points throughout the summary within the text. Alternatively, there is the option of including tables and figures at the end of the summary.

THE PHARMACOKINETIC TABULATED SUMMARY (see Appendix B)

2.2.4 The toxicology written summary

The sequence of the Toxicology Written Summary should be as follows:

- Brief Summary
- Single-Dose Toxicity
- Repeat-Dose Toxicity
- Genotoxicity
- Carcinogenicity
- Reproductive and Developmental Toxicity
- Studies in Juvenile Animals
- Local Tolerance
- Other Toxicity Studies
- Discussion and Conclusions
- Tables and Figures (either here or included in text).

BRIEF SUMMARY

The principal findings from the toxicology studies should be briefly summarized in a few pages (generally not more than 6). In this section, the extent of the toxicological evaluation can be indicated by the use of a table listing the principal toxicological studies (results should not be presented in this table), for example:

TOXICOLOGY PROGRAMME

Study type and duration	Route of administration	Species	Compound administered*
Single-dose toxicity	po and iv	Rat and mouse	Parent drug
Single-dose toxicity	po and iv	Rat and mouse	Metabolite X
Repeat-dose toxicity			
1 month	po	Rat and dog	Parent drug
6 months	po	Rat	Parent drug
9 months	po	Dog	Parent drug
etc.			

* This column required only if metabolite(s) are investigated.

The scope of the toxicological evaluation should be described in relation to the proposed clinical use. A comment on the GLP status of the studies should be included.

SINGLE-DOSE TOXICITY

The single-dose data should be very briefly summarised, in order by species, by route. In some instances, it may be helpful to provide the data in the form of a table.

REPEAT-DOSE TOXICITY (including supportive toxicokinetics evaluation)

Studies should be summarised in order by species, by route, and by duration, giving brief details of the methodology and highlighting important findings (e.g., nature and severity of target organ toxicity, dose (exposure)/response relationships, no observed adverse effect levels, etc.). Non-pivotal studies can be summarized in less detail (pivotal studies are the definitive GLP studies specified by ICH Guideline M3).

GENOTOXICITY

Studies should be briefly summarised in the following order:

- In vitro non-mammalian cell system
- In vitro mammalian cell system
- In vivo mammalian system (including supportive toxicokinetics evaluation) other systems.

CARCINOGENICITY (including supportive toxicokinetics evaluations)

A brief rationale should explain why the studies were chosen and the basis for high-dose selection. Individual studies should be summarised in the following order:

- Long-term studies (in order by species; including range-finding studies that cannot appropriately be included under repeat -dose toxicity or pharmacokinetics).
- Short- or medium-term studies (including range-finding studies that cannot appropriately be included under repeat-dose toxicity or pharmacokinetics).
- Other studies.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY**(including range-finding studies and supportive toxicokinetics evaluations)**

Studies should be summarised in the following order, giving brief details of the methodology and highlighting important findings:

- Fertility and early embryonic development.
- Embryo-fatal development.
- Prenatal and postnatal development, including maternal function.
- Studies in which the offspring (juvenile animals) are dosed and/or further evaluated, if such studies have been conducted.

If modified study designs are used, the sub-headings should be modified accordingly.

LOCAL TOLERANCE

If local tolerance studies have been performed, they should be summarised in order by species, by route, and by duration, giving brief details of the methodology and highlighting important findings.

OTHER TOXICITY STUDIES (if available)

If other studies have been performed, they should be summarised. When appropriate, the rationale for conducting the studies should be provided.

- Antigenicity
- Immunotoxicity
- Mechanistic studies (if not reported elsewhere)
- Dependence
- Studies on metabolites
- Studies on impurities
- Other studies.

DISCUSSION AND CONCLUSIONS

This section should provide an opportunity to discuss the toxicological evaluation and the significance of any issues that arise. Tables or figures summarizing this information are recommended.

TABLES AND FIGURES

Text tables and figures can be included at appropriate points throughout the summary within the text. Alternatively, tables and figures can be included at the end of the summary.

THE TOXICOLOGY TABULATED SUMMARY (see Appendix B)

2.3 NON-CLINICAL TABULATED SUMMARIES

It is recommended that summary tables for the non-clinical information in the Common Technical Document be provided in the format outlined in this Guideline. Applicants can modify the format if needed to provide the best possible presentation of the information and to facilitate the understanding and evaluation of the results.

This Guideline is not intended to indicate what studies are requested, but solely to advise how to tabulate study results if a study is performed. Applicants might need to add some items to or delete some items from the cited format where appropriate. One tabular format can contain results from several studies. Alternatively, it may be appropriate to cite the data resulting from one study in several tabular formats.

3. THE ORGANISATION OF MODULE 4: NON-CLINICAL STUDY REPORTS

This guideline presents an agreed format for the organisation of the non-clinical reports in the Common Technical Document for applications that will be submitted to Regulatory Authorities. This guideline is not intended to indicate what studies are required. It merely indicates an appropriate format for the non-clinical data that have been acquired. The appropriate location for individual -animal data is in the study report or as an appendix to the study report.

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3.1 TABLE OF CONTENTS

A Table of Contents should be provided that lists all of the non-clinical study reports and gives the location of each study report in the Common Technical Document.

3.2 STUDY REPORTS

The study reports should be presented in the following order:

4.1 Pharmacology

- 4.1.1 Primary Pharmacodynamics
- 4.1.2 Secondary Pharmacodynamics
- 4.1.3 Safety Pharmacology
- 4.1.4 Pharmacodynamic Drug Interactions

4.2 Pharmacokinetics

- 4.2.1 Analytical Methods and Validation Reports (if separate reports are available)
- 4.2.2 Absorption
- 4.2.3 Distribution
- 4.2.4 Metabolism
- 4.2.5 Excretion

- 4.2.6 Pharmacokinetic Drug Interactions (non-clinical)
- 4.2.7 Other Pharmacokinetic Studies
- 4.3 Toxicology
 - 4.3.1 Single-Dose Toxicity (in order by species, by route)
 - 4.3.2 Repeat-Dose Toxicity (in order by species, by route, by duration; including supportive toxicokinetics evaluations)
 - 4.3.3 Genotoxicity
 - 4.3.3.1 In vitro
 - 4.3.3.2 In vivo (including supportive toxicokinetics evaluations)
 - 4.3.4 Carcinogenicity (including supportive toxicokinetics evaluations)
 - 4.3.4.1 Long-term studies (in order by species; including range-finding studies that cannot appropriately be included under repeat-dose toxicity or pharmacokinetics).
 - 4.3.4.2 Short- or medium-term studies (including range-finding studies that cannot appropriately be included under repeat-dose toxicity or pharmacokinetics)
 - 4.3.4.3 Other studies
 - 4.3.5 Reproductive and Developmental Toxicity (including range-finding studies and supportive toxicokinetics evaluations) (If modified study designs are used, the following sub-headings should be modified accordingly.)
 - 4.3.5.1 Fertility and early embryonic development
 - 4.3.5.2 Embryo-fatal development
 - 4.3.5.3 Prenatal and postnatal development, including maternal function
 - 4.3.5.4 Studies in which the offspring (juvenile animals) are dosed and/or further evaluated
 - 4.3.6 Local Tolerance
 - 4.3.7 Other Toxicity Studies (if available)
 - 4.3.7.1 Antigenicity
 - 4.3.7.2 Immunotoxicity
 - 4.3.7.3 Mechanistic studies (if not included elsewhere)
 - 4.3.7.4 Dependence
 - 4.3.7.5 Metabolites
 - 4.3.7.6 Impurities
 - 4.3.7.7 Other
- 4.4 Key Literature References.

APPENDIX B

ICH GUIDELINE M3 – (Condensed version) ⁶

MAINTENANCE OF THE ICH GUIDELINE ON NON-CLINICAL SAFETY STUDIES FOR THE CONDUCT OF HUMAN CLINICAL TRIALS FOR PHARMACEUTICALS

1. INTRODUCTION

1.1 OBJECTIVES OF THE GUIDELINE

The purpose of this document is to recommend international standards for and promote harmonisation of the non-clinical safety studies needed to support human clinical trials of a given scope and duration.

Harmonisation of the guidance for non-clinical safety studies will help to define the current recommendations and reduce the likelihood that substantial differences will exist between regions. This guidance should facilitate the timely conduct of clinical trials and reduce the unnecessary use of animals and other resources. This should promote safe and ethical development and availability of new pharmaceuticals.

1.2 BACKGROUND

The recommendations for the extent of non-clinical safety studies to support the various stages of clinical development differ among the regions of Europe, USA and Japan. This raises the important question of whether there is scientific justification for these

⁶ The complete text of this, and of other ICH Guidelines, can be downloaded from the ICH website at: <http://www.ich.org/>

differences and whether it would be possible to develop a mutually acceptable guidance.

The present guideline represents the consensus that exists regarding the scope and duration of non-clinical safety studies to support the conduct of human clinical trials for pharmaceuticals.

1.3 SCOPE OF THE GUIDELINE

The non-clinical safety study recommendations for the marketing approval of a pharmaceutical usually include single and repeated dose toxicity studies, reproduction toxicity studies, genotoxicity studies, local tolerance studies and for drugs that have special cause for concern or are intended for a long duration of use, an assessment of carcinogenic potential. Other non-clinical studies include pharmacology studies for safety assessment (safety pharmacology) and pharmacokinetic (ADME) studies. These types of studies and their relation to the conduct of human clinical trials are presented in this guideline.

This guideline applies to the situations usually encountered during the conventional development of pharmaceuticals and should be viewed as providing general guidance for drug development. Animal safety studies and human clinical trials should be planned and designed to represent an approach that is scientifically and ethically appropriate for the pharmaceutical under development.

There have been marked changes in the kinds of therapeutic agents being developed (e.g., biotechnology derived products), and the existing paradigms for safety evaluation may not always be appropriate or relevant. The safety evaluation in such cases should be considered on a case by case basis (1). Similarly, pharmaceuticals under development for indications in life threatening or serious diseases without current effective therapy may also warrant a case by case approach to both the toxicological evaluation and clinical development to optimise and expedite drug development. In these cases, particular studies may be abbreviated, deferred or omitted.

1.4 GENERAL PRINCIPLES

The development of a pharmaceutical is a stepwise process involving an evaluation of both the animal and human safety information. The goals of the non-clinical safety evaluation includes a characterisation of toxic effects with respect to target organs, dose dependence, relationship to exposure, and potential reversibility. This information is important for the estimation of an initial safe starting dose for the human trials and the identification of parameters for clinical monitoring for potential adverse effects. The non-clinical safety studies, although limited at the beginning of clinical development, should be adequate to characterise potential toxic effects under the conditions of the supported clinical trial.

Human clinical trials are conducted to demonstrate the efficacy and safety of a pharmaceutical, starting with a relatively low exposure in a small number of subjects. This is followed by clinical trials in which exposure usually increases by dose, duration and/or size of the exposed patient population. Clinical trials are extended based on the demonstration of adequate safety in the previous clinical trial(s) as well as additional non-clinical safety information that is available as the clinical trials proceed. Serious adverse clinical or non-clinical findings may influence the continuation of clinical trials and/or suggest the need for additional non-clinical studies and a re-evaluation of previous clinical adverse events to resolve the issue.

Clinical trials are conducted in phases for which different terminology has been utilised in the various regions. This document uses the terminology as defined in the ICH guideline “General Considerations for the Clinical Trials” (2). Clinical trials may be grouped by their purpose and objectives. The first human exposure studies are generally single dose studies, followed by dose escalation and short term repeated dose studies to evaluate pharmacokinetic parameters and tolerance (Phase I studies--Human Pharmacology studies). These studies are often conducted in healthy volunteers but may also include patients. The next phase of trials consists of exploratory efficacy and safety studies in patients (Phase II studies-- Therapeutic Exploratory studies). This is followed by confirmatory clinical trials for efficacy and safety in patient populations (Phase III studies—Therapeutic Confirmatory studies).

2. SAFETY PHARMACOLOGY

Safety pharmacology includes the assessment of effects on vital functions, such as cardiovascular, central nervous and respiratory systems, and these should be evaluated prior to human exposure. These evaluations may be conducted as additions to toxicity studies or as separate studies.

3. TOXICOKINETIC AND PHARMACOKINETIC STUDIES

Exposure data in animals should be evaluated prior to human clinical trials (3). Further information on absorption, distribution, metabolism and excretion in animals should be made available to compare human and animal metabolic pathways. Appropriate information should usually be available by the time the Phase I (Human Pharmacology) studies have been completed.

4. SINGLE DOSE TOXICITY STUDIES

The single dose (acute) toxicity for a pharmaceutical should be evaluated in two mammalian species prior to the first human exposure (Note 1). A dose escalation study is considered an acceptable alternative to the single dose design.

5. REPEATED DOSE TOXICITY STUDIES

The recommended duration of the repeated dose toxicity studies is usually related to the duration, therapeutic indication and scale of the proposed clinical trial. In principle, the duration of the animal toxicity studies conducted in two mammalian species (one non-rodent) should be equal to or exceed the duration of the human clinical trials up to the maximum recommended duration of the repeated dose toxicity studies (Tables 1 and 2).

In certain circumstances, where significant therapeutic gain has been shown, trials may be extended beyond the duration of supportive repeated dose toxicity studies on a case by case basis.

5.1 PHASE I AND II STUDIES

A repeated dose toxicity study in two species (one non-rodent) for a minimum duration of 2 weeks (Table 1) would support Phase I (Human Pharmacology) and Phase II (Therapeutic Exploratory) studies up to 2 weeks in duration. Beyond this, 1, 3 or 6 months toxicity studies would support these types of human clinical trials for up to 1, 3 or 6 months, respectively. Six month rodent and chronic non-rodent studies (11) would support clinical trials of longer duration than 6 months.

TABLE 1

Duration of Repeated Dose Toxicity Studies to Support Phase I and II Trials in EU and Phase I, II and III Trials in the US and Japan*

Duration of Clinical Trials	Minimum Duration of Repeated Dose Toxicity Studies	
	Rodents	Non-rodents
Single Dose	2 Weeks**	2 Weeks
Up to 2 Weeks	2 Weeks**	2 Weeks
Up to 1 Month	1 Month	1 Month
Up to 3 Months	3 Months	3 Months
Up to 6 Months	6 Months	6 Months***
> 6 Months	6 Months	Chronic***

* In Japan, if there are no Phase II clinical trials of equivalent duration to the planned Phase III trials, conduct of longer duration toxicity studies is recommended as given in Table 2.

** In the US, as an alternative to 2 week studies, single dose toxicity studies with extended examinations can support single-dose human trials (4).

*** See (11). Data from 6 months of administration in non-rodents should be available before the initiation of clinical trials longer than 3 months. Alternatively, if applicable, data from a 9 month non-rodent study should be available before the treatment duration exceeds that which is supported by the available toxicity studies.

5.2 PHASE III STUDIES

For the Phase III (Therapeutic Confirmatory) studies, the recommendations for the US and Japan are the same as those in Table 1. In EU, a one month toxicity study in two species (one non-rodent) would support clinical trials of up to 2 weeks duration (Table 2). Three month toxicity studies would support clinical trials for up to 1 month duration, while 6 month toxicity studies in rodents and 3 month studies in non-rodents would support clinical trials of a duration up to 3 months. For longer term clinical trials, a 6 month study in rodents and a chronic study in non-rodents are recommended.

TABLE 2
Duration of Repeated Dose Toxicity Studies to Support Phase III Trials in the EU and Marketing in all Regions*

Duration of Clinical Trials	Minimum Duration of Repeated Dose Toxicity Studies	
	Rodents	Non-rodents
Up to 2 Weeks	1 Month	1 Month
Up to 1 Month	3 Months	3 Months
Up to 3 Months	6 Months	3 Months
> 3 Months	6 Months	Chronic**

* The above Table also reflects the Marketing recommendations in the 3 Regions that a Chronic non-rodent study is recommended for clinical use > 1 month

** See (11)

6. LOCAL TOLERANCE STUDIES

Local tolerance should be studied in animals using routes relevant to the proposed clinical administration. The evaluation of local tolerance should be performed prior to human exposure. The assessment of local tolerance may be part of other toxicity studies.

7. GENOTOXICITY STUDIES

Prior to first human exposure, in vitro tests for the evaluation of mutations and chromosomal damage are generally needed. If an equivocal or positive finding occurs, additional testing should be performed (5).

The standard battery of tests for genotoxicity (6) should be completed prior to the initiation of Phase II studies.

8. CARCINOGENICITY STUDIES

Completed carcinogenicity studies are not usually needed in advance of the conduct of clinical trials unless there is cause for concern. Conditions relevant for carcinogenicity testing are discussed in the ICH document (7).

For pharmaceuticals developed to treat certain serious diseases, carcinogenicity testing, if needed, may be concluded post-approval.

9. REPRODUCTION TOXICITY STUDIES

Reproduction toxicity studies (8,9) should be conducted as is appropriate for the population that is to be exposed.

9.1 MEN

Men may be included in Phase I and II trials prior to the conduct of the male fertility study since an evaluation of the male reproductive organs is performed in the repeated dose toxicity studies (Note 2). A male fertility study should be completed prior to the initiation of Phase III trials (8,9).

9.2 WOMEN NOT OF CHILDBEARING POTENTIAL

Women not of childbearing potential (i.e., permanently sterilised, postmenopausal) may be included in clinical trials without reproduction toxicity studies provided the relevant repeated dose toxicity studies (which include an evaluation of the female reproductive organs) have been conducted.

9.3 WOMEN OF CHILDBEARING POTENTIAL

For women of childbearing potential there is a high level of concern for the unintentional exposure of an embryo/foetus before information is available concerning the potential benefits versus potential risks. There are currently regional differences in the timing of reproduction toxicity studies to support the inclusion of women of childbearing potential in clinical trials.

In Japan, assessment of female fertility and embryo-fatal development should be completed prior to the inclusion of women of childbearing potential using birth control in any type of clinical trial. In the EU, assessment of embryo-fatal development should be completed prior to Phase I trials in women of childbearing potential and female fertility studies prior to Phase III trials.

In the US women of childbearing potential may be included in early, carefully monitored studies without reproduction toxicity studies provided appropriate precautions are taken to minimise risk. These precautions include pregnancy testing (for example, based on the b-subunit of HCG), use of a highly effective method of birth control (Note 3) and entry after a confirmed menstrual period. Continued testing and monitoring

during the trial should be sufficient to ensure compliance with the measures not to become pregnant during the period of drug exposure (which may exceed the length of study). To support this approach, informed consent should include any known pertinent information related to reproductive toxicity, such as a general assessment of potential toxicity of pharmaceuticals with related structures or pharmacological effects. If no relevant information is available, the informed consent should clearly note the potential for risk.

In the US, assessment of female fertility and embryo-fatal development should be completed before women of childbearing potential using birth control are enrolled in Phase III trials.

In the 3 Regions, the pre- and post-natal development study should be submitted for marketing approval or earlier if there is cause of concern. For all regions, all female reproduction toxicity studies (8) and the standard battery of genotoxicity tests (6) should be completed prior to the inclusion, in any clinical trial, of women of childbearing potential not using highly effective birth control (Note 3) or whose pregnancy status is unknown.

9.4 PREGNANT WOMEN

Prior to the inclusion of pregnant women in clinical trials, all the reproduction toxicity studies (8,9) and the standard battery of genotoxicity tests (6) should be conducted. In addition, safety data from previous human exposure are generally needed.

10. SUPPLEMENTARY STUDIES

Additional non-clinical studies may be needed if previous non-clinical or clinical findings with the product or related products have indicated special safety concerns.

11. CLINICAL TRIALS IN PEDIATRIC POPULATIONS

When paediatric patients are included in clinical trials, safety data from previous adult human exposure would usually represent the most relevant information and should generally be available before paediatric clinical trials. The necessity for adult human data would be determined on a case by case basis. In addition to appropriate repeated dose toxicity studies all reproduction toxicity studies (8) and the standard battery of genotoxicity tests (6) should be available prior to the initiation of trials in paediatric populations. Juvenile animal studies should be considered on an individual basis when previous animal data and human safety data are insufficient.

The need for carcinogenicity testing should be addressed prior to long term exposure in paediatric clinical trials considering the length of treatment or cause for concern (7).

12. CONTINUING EFFORTS TO IMPROVE HARMONISATION

It is recognised that significant advances in harmonisation of the timing of non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals have already been achieved and are detailed in this guideline. However, differences remain in a few areas. These include toxicity studies to support first entry into man and the recommendations for reproduction toxicity studies for women of childbearing potential. Regulators and industry will continue to consider these differences and work towards further improving the drug development process.

13. ENDNOTES

NOTE 1

For the conduct of single dose toxicity studies, refer to the ICH-1 recommendations (10) and the regional guidelines.

NOTE 2

In Japan, unlike the EU and US, the male fertility study has usually been conducted prior to the inclusion of men in clinical trials. Since an assessment of male fertility by careful histopathological examination in the rodent 2 week repeated dose toxicity study has been found to be more sensitive in detecting toxic effects on male reproductive organs than fertility studies (9, 12), it is now recommended prior to the first clinical trial in Japan.

NOTE 3

A highly effective method of birth control is defined as those which result in a low failure rate (i.e. less than 1% per year) when used consistently and correctly such as implants, injectables, combined oral contraceptives, some IUDs, sexual abstinence or vasectomised partner. For subjects using a hormonal contraceptive method, information regarding the product under evaluation and its potential effect on the contraceptive should be addressed.

14. REFERENCES

1. ICH Topic S6 Document “Safety Studies for Biotechnological Products”.
2. ICH Topic E8 Document “General Considerations for Clinical Trials”.
3. ICH Harmonised Tripartite Guideline (S3A) “Note for Guidance on Toxicokinetics - The Assessment of Systemic Exposure in Toxicity Studies”.
4. United States DHHS Federal Register notice August 26, 1996 (61 FR 43934).
5. ICH Harmonised Tripartite Guideline (S2A) “Guidance on Specific Aspects of Regulatory Genotoxicity Tests”.
6. ICH Topic S2B document “Standard Battery of Genotoxicity Tests”.
7. ICH Harmonised Tripartite Guideline (S1A) “Guideline on the Need for Carcinogenicity Studies for Pharmaceuticals”.
8. ICH Harmonised Tripartite Guideline (S5A) “Detection of Toxicity to Reproduction for Medicinal Products”.
9. ICH Harmonised Tripartite Guideline (S5B) “Toxicity to Male Fertility”.
10. P.F. Arcy and D.W.G. Harron, “Proceeding of The First International Conference on Harmonisation”, Brussels 1991, Queen’s Univ. of Belfast, pp 183-184 (1992).
11. ICH Topic S4 Document “Duration of Chronic Toxicity Testing in Animals (Rodent and Non Rodent Toxicity Testing)”.
12. Sakai T, Takahashi M, Mitsumori K, Yasuhara K, Kawashima K, Mayahara H, Ohno Y; Collaborative work to evaluate toxicity on male reproductive organs by 2-week repeated dose toxicity studies in rats. Overview of the studies. *J. Toxicol. Sci.* 25, 1-21 (2000).

APPENDIX C

EU-CPMP – THE RULES GOVERNING MEDICINAL PRODUCTS IN THE EUROPEAN UNION (Condensed version) ⁷

- Volume 1 Pharmaceutical legislation (Medicinal products for human use)
- Volume 2 A Notice to applicants, Procedures for marketing authorisation (Medicinal products for human use)
- Volume 2 B Presentation and content of the dossier, Common Technical Document (Medicinal products for human use)
- Volume 2 C Regulatory guidelines
- Volume 3 Guidelines (Medicinal products for human use)
- Volume 4 Good manufacturing practices (Medicinal products for human and veterinary use)
- Volume 9 Pharmacovigilance (Medicinal products for human and veterinary use).

This Notice to Applicants (NTA) has been prepared by the European Commission, in consultation with the competent authorities of the Member States and the European Agency for the Evaluation of Medicinal Products. This Notice has no legal force and does not necessarily represent the final views of the Commission. In case of doubt, therefore, reference should be made to the appropriate Community Directives and Regulations.

The Notice to Applicants is prepared by the Commission in accordance with article 6 of Regulation (EEC) No. 2309/93 and the Annex of Directive 2001/83/EC as amended by Directive 2003/63/EC. It is important when reading this text to appreciate that the legal requirements of the Directives and the Regulations must be met and that this Notice presents the harmonised views of the Member States and the European Agency for the Evaluation of Medicinal Products on how those requirements may be met.

⁷ *The complete European legislation on pharmaceuticals may be obtained from the website:*
<http://pharmacos.eudra.org/>

The Notice to Applicants (Volume 2 in the series The Rules Governing Medicinal Products in the European Union) is presented in three parts:

- Volume 2A is dealing with procedures for marketing authorisation
- Volume 2B is dealing with the presentation and content of the application dossier
- Volume 2C contains the regulatory guidelines.

Volume 2A introduces the legislative basis for marketing authorisations in the European Union, covers the operational procedures for applications for a marketing authorisation using Community procedures (centralised, mutual recognition and referrals) as well as national procedures. In addition, information on the procedures that apply for variations to a marketing authorisation are set out.

Volume 2B is concerned with the presentation and content of the application dossier. It provides guidance for the compilation of dossiers for applications for marketing authorisation, and is applicable for the centralised procedure and national procedures, including mutual recognition. The latest update takes account of the international agreements on the structure and format of the Common Technical Document.

Volume 2C describes the various regulatory requirements to be observed in applications for new, and for renewal of existing, marketing authorisations, as well as those for the Summary of Product Characteristics and packaging and labelling requirements.

APPENDIX D

OECD GUIDELINES FOR THE TESTING OF CHEMICALS (TABLE OF CONTENTS OF PART 4: HEALTH EFFECTS) ⁸

- 402 Acute Dermal Toxicity
 - 403 Acute Inhalation Toxicity
 - 404 Acute Dermal Irritation/Corrosion
 - 405 Acute Eye Irritation/Corrosion
 - 406 Skin Sensitisation
 - 407 Repeated Dose 28-day Oral Toxicity Study in Rodents
 - 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents
 - 409 Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents
 - 410 Repeated Dose Dermal Toxicity: 21/28-day Study
 - 411 Subchronic Dermal Toxicity: 90-day Study
 - 412 Repeated Dose Inhalation Toxicity: 28-day or 14-day Study
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 - 414 Prenatal Developmental Toxicity Study
 - 415 One-Generation Reproduction Toxicity Study
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 - 417 Toxicokinetics
 - 420 Acute Oral Toxicity - Fixed Dose Method
 - 421 Reproduction/Developmental Toxicity Screening Test
 - 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test
 - 423 Acute Oral toxicity - Acute Toxic Class Method
 - 424 Neurotoxicity Study in Rodents
 - 425 Acute Oral Toxicity: Up-and-Down Procedure
- *Acute Oral Toxicity (OECD Test Guideline 425) Statistical Programme (AOT 425 StatPgm). Version: 1.0, 2001. Not yet available, but coming soon.

⁸ The complete set of OECD Testing guidelines for chemicals may be obtained from the OECD bookshop at: 2, rue André-Pascal, F-75775 Paris Cedex 16, France; also at: <http://www.oecd.org/bookshop>.

- 451 Carcinogenicity Studies
- 452 Chronic Toxicity Studies
- 453 Combined Chronic Toxicity/Carcinogenicity Studies
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- 475 Mammalian Bone Marrow Chromosomal Aberration Test
- 476 *In vitro* Mammalian Cell Gene Mutation Test
- 477 Genetic Toxicology: Sex-Linked Recessive Lethal Test in *Drosophila melanogaster*
- 478 Genetic Toxicology: Rodent Dominant Lethal Test
- 479 Genetic Toxicology: *In vitro* Sister Chromatid Exchange Assay in Mammalian Cells
- 480 Genetic Toxicology: *Saccharomyces cerevisiae*, Gene Mutation Assay
- 481 Genetic Toxicology: *Saccharomyces cerevisiae*, Mitotic Recombination Assay
- 482 Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells *in vitro*
- 483 Mammalian Spermatogonial Chromosome Aberration Test
- 484 Genetic Toxicology: Mouse Spot Test
- 485 Genetic Toxicology: Mouse Heritable Translocation Assay
- 486 Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo*

DRAFT GUIDELINES

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- 404 Acute Dermal Irritation/Corrosion
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- 426 Developmental Neurotoxicity Study
- 429 Skin Sensitisation: Local Lymph Node Assay
- 430 *In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test (TER)
- 431 *In Vitro* Skin Corrosion: Human Skin Model Test
- 432 *In Vitro* 3T3 NRU Phototoxicity Test
- *** Somatic Mutation and Recombination Tests (SMART) in *Drosophila melanogaster* (Draft New Guideline, May 1994)
- *** Acute Dermal Photoirritation Screening Test (Draft New Guideline, February 1995)
- *** Acute Dermal Photoirritation Dose-Response Test (Draft New Guideline, February 1995)
- *** *In Vitro* Syrian Hamster Embryo (SHE) Cell Transformation Assay (Draft New Guideline, March 1996)
- *** Acute Dermal Irritation Study in Human Volunteers (Draft New Guideline, April 1997)

*** these numbers will be assigned when the guidelines are finalized.

APPENDIX E

UNITED STATES FOOD AND DRUG ADMINISTRATION (US-FDA) TOXICOLOGICAL PRINCIPLES FOR THE SAFETY OF FOOD INGREDIENTS (REDBOOK 2000) ⁹

INTRODUCTION AND TABLE OF CONTENTS

INTRODUCTION

This guidance represents the Agency's current thinking on the information needed for the Safety Assessment of Food Ingredients. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. Alternative approaches to the guidance documents may be used if such approaches satisfy the requirement of the applicable statute, regulations, or both.

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⁹ This publication is available at: <http://www.cfsan.fda.gov/~redbook/red-toca.html>; new information will be added as it becomes available.

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VIII. Glossary: Acronyms and Definitions

APPENDIX F

US-FDA DRAFT GUIDANCE FOR INDUSTRY “BOTANICAL DRUG PRODUCTS” (Condensed version) ¹⁰

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES FOOD
AND DRUG ADMINISTRATION CENTER FOR DRUG EVALUATION AND
RESEARCH (CDER) AUGUST 2000

BOTANICAL DRUG PRODUCTS

101

I. Introduction

This guidance... provides guidance to sponsors on submitting investigational new drug applications (INDs) for botanical drug products, including those botanical products (or botanicals) currently lawfully marketed as foods and dietary supplements in the United States.

This guidance also discusses several areas in which, because of the unique nature of botanicals, FDA finds it appropriate to apply regulatory policies that differ from those applied to synthetic, semisynthetic, or otherwise highly purified or chemically modified drugs (including antibiotics). (This latter group of drug substances is referred to in this guidance as synthetic or highly purified drugs.) In particular, the guidance states that applicants may submit reduced documentation of preclinical safety and of chemistry, manufacturing, and controls (CMC) to support an IND for initial clinical studies of botanicals that have been legally marketed in the United States as dietary supplements or cosmetics without any known safety concerns.

II. Background

For the purposes of this document, the term botanicals includes plant materials, algae, macroscopic fungi, and combinations thereof. It does not include fermentation products

¹⁰ The full document can be downloaded from: <http://www.fda.gov/cder/guidance/1221dft.htm>

such as products fermented with yeast, bacteria, and other microscopic organisms, even if previously approved for drug use or accepted for food use in the United States, nor does it include highly purified or chemically modified substances derived from botanical sources, such as paclitaxel, because these substances can readily be fully characterized. This guidance addresses only those botanical products that are regulated by CDER.

III. General Regulatory Approaches

A. MARKETING UNDER OTC MONOGRAPH VERSUS APPROVED NDA

A botanical drug product may be marketed in the United States under (1) an OTC monograph or (2) an approved NDA or ANDA. A botanical product that has been marketed in the United States for a material time and to a material extent for a specific OTC drug indication may be eligible for inclusion in an OTC monograph codified in 21 CFR Parts 331-358. The manufacturer would need to submit a petition to amend the monograph to add the botanical substance as a new active ingredient in accordance with 21 CFR 10.30.

Under current regulations, if there is no marketing history in the United States for a botanical drug product if available evidence of safety and effectiveness does not warrant inclusion of the product in an OTC monograph, or if the proposed indication would not be appropriate for non-prescription use, the manufacturer must submit an NDA to obtain FDA approval to market the product for the proposed use (sections 201(p) and 505 of the FD&C Act). An NDA for a botanical drug could seek approval for either prescription or OTC use, depending on the indication and characteristics of the product and whether it is safe for use outside of the supervision of a practitioner licensed by law to administer it. If existing information on the safety and efficacy of a botanical drug product is insufficient to support an NDA, new clinical studies will be needed to demonstrate safety and effectiveness.

When a final OTC drug monograph is published for a specific use of a botanical drug, any person can market a product containing the same substance and for the same use, provided the labelling and other active ingredients (if present) are in accord with all relevant monographs and other applicable regulations. In contrast, when a product is approved under an NDA, the approval is specific to the drug product that is the subject of the application (the applicant's drug product), and the applicant may be eligible

for marketing exclusivity for either 5 years (if it is a new chemical entity) or 3 years from the time of approval, even in the absence of patent protection. During the period of exclusivity, FDA will not approve, or in some cases even review, certain competitor products unless the second sponsor conducts all studies necessary to demonstrate the safety and effectiveness of its product. Therefore, if a person who wishes to market a botanical drug product that is not included in an existing OTC monograph desires marketing exclusivity for the product, they should seek approval of an NDA rather than petition the agency to amend a monograph. Appendix A contains a schematic showing different regulatory approaches that can be taken for marketing botanical drug products in the United States, including OTC monograph and NDA procedures.

B. CMC INFORMATION FOR BOTANICAL DRUG PRODUCTS

Botanical drug products have certain unique characteristics that should be taken into account in the application of FDA regulations and guidance. Botanical drugs are derived from vegetable matter and are usually prepared as complex mixtures. Their chemical constituents are not always well defined. In many cases, even the active constituent in a botanical drug is not identified, nor is its biological activity well characterized. Therefore, the CMC documentation that should be provided for botanical drugs will be different from that for synthetic or highly purified drugs, whose active constituents can be more readily chemically identified and quantified. For example, active constituents in a botanical drug might not need to be identified during the IND stage or in an NDA submission if this is shown to be infeasible. In such circumstances, FDA will rely instead on a combination of other tests (e.g., spectroscopic or chromatographic fingerprints, chemical assay of characteristic markers, and biological assay), controls (e.g., strict quality controls of the botanical raw materials and adequate in-process controls), and process validation (especially for the drug substance) to ensure the identity, purity, quality, strength, potency, and consistency of the botanical drug.

C. CMC AND TOXICOLOGY INFORMATION TO SUPPORT INITIAL STUDIES

Many botanical products are legally available in the United States as dietary supplements. Given the wide availability of such products outside of clinical trials, it is important to assess the effectiveness of such products. The preclinical pharmacology and toxicology information that should be provided for legally available botanical products with no known safety issues during initial clinical trials may be markedly reduced (in most cases, additional toxicology and CMC data will not be required) compared to that

expected for synthetic or highly purified new drugs that are not legally marketed and for which there is no prior human experience (see 21 CFR 312.22(b)).

D. APPLICABILITY OF COMBINATION DRUG REGULATIONS

Botanical drug products that are derived from a single part of a plant (e.g., leaves, stems, roots, seeds), or from an alga or macroscopic fungus (e.g., a mushroom), are not considered to be fixed-combination drugs within the meaning of 21 CFR 300.50 and 330.10(a)(4)(iv).

Consequently, they would not have to meet the requirements for combination drugs, principally the need to demonstrate that each component or active ingredient makes a contribution to claimed effects. Botanical drugs composed of multiple parts of a single plant species, or of parts from different plant species, currently are subject to the combination drug requirements. However, FDA intends to propose revisions to its regulations to allow for the exemption of such botanical drugs from application of the combination drug requirements under certain circumstances.

IV. Marketing A Botanical Drug Under An OTC Monograph

.... [not included]

V. Marketing A Botanical Drug Under An NDA

Any botanical drug product that is not generally recognized as safe and effective for its therapeutic claims is considered a new drug under section 201(p) of the FD&C Act. Section 505(a) of the Act requires any person wishing to market a botanical drug product that is a new drug to obtain FDA approval of an NDA or ANDA for that product. According to section 505(d) of the Act and 21 CFR 314.50, an NDA must contain substantial evidence of effectiveness derived from adequate and well-controlled clinical studies, evidence of safety, and adequate CMC information. The format of an NDA submission and the requirements for its various sections are set forth in 21 CFR Part 314 and discussed in several CDER guidance documents.

VI. INDs For Botanical Drugs

If available information is insufficient to support an NDA for a botanical drug, the sponsor will need to develop further data. If the sponsor wishes to conduct clinical trials in the United States to support an NDA, it will have to submit an IND under section 505(i) of the FD&C Act and 21 CFR Part 312. An IND is also required when a botanical product is studied for a drug use (see 21 U.S.C. 321(g)), even if such study is intended solely for research purposes. Under 21 CFR 312.22, an IND must contain sufficient information to demonstrate that the drug product is safe for testing in humans and that the clinical protocol is properly designed for its intended objectives.

A. IND INFORMATION FOR DIFFERENT CATEGORIES OF BOTANICALS

Under 21 CFR 312.22(b), the amount of information that must be submitted in an IND for a particular drug product depends on, among other things, the novelty of the drug, the extent to which it has been studied previously, the drug product's known or suspected risks, and the developmental phase of the drug. Sections VII and VIII of this guidance describe the information that a sponsor should provide in an IND for initial (i.e., phase 1 and phase 2) clinical studies of a botanical drug. As noted above, for botanicals legally marketed under the DSHEA, there will often be very little new CMC or toxicological data needed to initiate such trials, as long as there are no known safety issues associated with the product and it is used at approximately the same doses as those currently or traditionally used or recommended. When properly conducted, these early investigations, including controlled effectiveness trials in phase 2, should allow a determination of whether there is a clinical effect worth pursuing and will provide a more systematic evaluation of safety than previously available. Should a botanical drug product show promise of effectiveness in such early trials, the potential for wider use for particular purposes will create a need for greater assurance of product quality and consistency and for expanded (i.e., phase 3) clinical studies of safety and effectiveness (21 CFR 312.22(b)). IND information appropriate for expanded clinical studies of botanical drugs is discussed in section IX.

The IND sponsor of a botanical product that has been previously marketed but not in the United States should provide certain additional information to assist FDA in determining the safety of the product for use in initial clinical studies (section VII). Such additional information is appropriate under 21 CFR 312.22(b) because these products

are not already marketed in the United States, and evidence of safety is needed before patients should be exposed to them.

This guidance also addresses the type of information that should be provided in INDs for initial studies on botanical products that have not been lawfully marketed anywhere or have known safety issues (section VIII). In contrast to botanical products that have been marketed in some form without any known safety issues, considerably less information may be available on the safety of a new botanical product that has not been marketed anywhere as a food or dietary supplement and has not been tested as a drug in humans. Consequently, it is appropriate that, under 312.22(b), sponsors of INDs for initial trials of botanical products that have not previously been lawfully marketed anywhere, or for which there are known safety issues, should provide certain additional information to FDA.

The information to be provided in an IND for a botanical drug product is illustrated schematically in Appendix B and discussed in this section and sections VII-IX below. CDER reviews INDs and NDAs based on the clinical indication being sought for labelling. FDA encourages sponsors of INDs for initial studies of botanical drugs to seek input from CDER review divisions to ensure that the appropriate information is submitted and that the clinical protocols are well designed.

Many guidance documents specific to certain indications or dosage forms are also available from the respective review divisions. FDA will place an IND on clinical hold (i.e., an order issued by the Agency to delay a proposed clinical study) if it finds that the IND does not contain sufficient information required under 21 CFR 312.23 to assess the risk to subjects of the proposed studies (21 CFR 312.42(b)(1)). However, the lack of any specific item of information listed in ' 312.23 for a phase 1 study will not necessarily be grounds for a clinical hold. Possible grounds for a clinical hold are set forth in 21 CFR 312.42(b) and discussed in CDER=s guidance for industry on Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-Derived Products (November 1995).

B. BASIC FORMAT FOR INDs

The format and general requirements for IND submissions are stated in 21 CFR 312.23 and discussed in several CDER guidance documents, including the phase 1 guidance

referenced above. These requirements are summarized below, with guidance on the type of information that sponsors of botanical drug products should provide under particular provisions:

1. *Cover Sheet*

2. *Table of Contents*

3. *Introductory Statement and General Investigational Plan*

4. *Investigator's Brochure*

5. *Protocol*

... [not included]

6. *Chemistry, Manufacturing, and Controls.*

... [not included]

7. *Pharmacological and Toxicological Information*

The content and format for pharmacological and toxicological information to be provided in an IND are stated in 21 CFR 312.23(a)(8). Preclinical pharmacology and toxicology studies are useful in guiding early clinical studies and in predicting the potential toxicity of a new drug. Traditional herbal medicines or currently marketed botanical products, because of their extensive though uncontrolled use in humans, may require less pre-clinical information to support initial clinical trials than would be expected for synthetic or highly purified drugs. When early clinical studies are to be conducted with a botanical product that is not currently lawfully marketed in the United States, but is prepared, processed, and used according to methodologies for which there is prior human experience, sufficient information may be available to support such studies without standard pre-clinical testing. After initial clinical studies, further pharmacology and toxicology studies of a botanical drug would generally be needed prior to later phases of clinical development and prior to approval for marketing. Sections VII.C, VIII.C, and IX.C provide details on the pharmacological and toxicological information that should be provided in clinical trials on botanical drugs.

VII. INDs For Phase 1 And Phase 2 Clinical Studies Of Lawfully Marketed Botanical Products

This section provides more detailed guidance on the submission of certain types of information for INDs for initial clinical studies on botanical products that have been lawfully marketed and that do not raise safety issues (for drugs with known safety concerns,

see section VIII). This section also notes where additional information should be provided when an IND is for a botanical product that has been marketed in one or more foreign countries but not the United States.

A. DESCRIPTION OF PRODUCT AND DOCUMENTATION OF HUMAN USE

... [not included]

B. CHEMISTRY, MANUFACTURING, AND CONTROLS (' 312.23(a)(7))

... [not included]

C. PHARMACOLOGY/TOXICOLOGY INFORMATION (' 312.23(a)(8))

... [not included]

1. All marketed botanical products

To support initial clinical trials (phase 1 and phase 2) of a botanical drug product, previous human experience and available animal toxicity data concerning the clinical formulation and the individual botanical ingredients within the formulation should be provided to support the proposed use. As noted in section VI.A, initial studies for U.S.-marketed products may generally be conducted without further pharmacological/toxicological testing. Nevertheless, available information should be provided. A database search should be conducted, when feasible, to identify information relevant to the safety and effectiveness of (1) the final formulation of the intended commercial botanical drug product, (2) the individual botanical ingredients, and (3) the known chemical constituents of the botanical ingredients. An integrated summary of available data from medical and toxicological databases (e.g., Medline, Toxline, TOMES, RTEC) should be submitted for review. Using the information gathered from this literature, the sponsor should address, as appropriate for the proposed study, the following issues concerning the botanical drug product: (1) general toxicity; (2) target organs or systems of toxicity; (3) teratogenic, carcinogenic, or mutagenic potential of any botanical ingredient in the product; (4) relationship of dosage and duration to toxic responses; and (5) pharmacological activity.

2. Foreign-marketed botanical products

For the reasons discussed in section VI, for a botanical product with which there is some foreign marketing experience, but which is not marketed in the United States, in

addition to information listed above, the sponsor should provide data that support safe human use and should include the annual sales volume, an estimate of the size of the exposure population, and available data on the rate of adverse effects. The nature of preclinical pharmacology/toxicology information needed before a sponsor conducts an initial clinical study will be determined on a case-by-case basis depending on the indications, dose proposed, and available supporting safe human experience.

D. BIOAVAILABILITY

... [not included]

E. CLINICAL CONSIDERATIONS

... [not included]

VIII. INDs For Phase 1 And Phase 2 Clinical Studies Of Nonmarketed Botanical Products

This section discusses the type of information that should be provided in INDs for initial trials of botanicals that have not previously been lawfully marketed in the United States or elsewhere or that have known safety issues.

A. DESCRIPTION OF PRODUCT AND DOCUMENTATION OF HUMAN USE

... [not included]

B. CHEMISTRY, MANUFACTURING, AND CONTROLS (' 312.23(a)(7))

... [not included]

C. PRECLINICAL SAFETY ASSESSMENT

... [not included]

1. Traditional Preparations

Preclinical pharmacology and toxicology studies are particularly important in establishing the safety of a new botanical drug for which there is no current marketing experience. The information is used for assessing the botanical drug=s risk-to-benefit ratio, guiding early clinical studies, and predicting potential toxicity. Because of their extensive

though uncontrolled use in humans, there may be sufficient information on traditional herbal medicines to support initial clinical studies without standard preclinical testing. Therefore, such products may require different preclinical safety information under 21 CFR 312.23(a)(8) than that expected for synthetic or highly purified drugs for which there is little experience.

A traditional herbal preparation, which may have evolved over time, generally has the following characteristics: (1) It meets official compendia or other published standards in terms of the botanical identity and plant part used for each botanical raw material; (2) in the case of a multi-herb substance, it is composed of the same formulation as a historical formula, with the amount of each botanical ingredient falling within the range of traditional usage; (3) it is prepared by the same processing methodology as traditionally used; and (4) it is used in the traditional manner in terms of therapeutic indication, route and schedule of administration, and quantities or doses.

For initial clinical studies on a botanical drug product that is not currently lawfully marketed in the United States or elsewhere but is prepared, processed, and used according to methodologies for which there is prior human experience, sufficient information might be available to support the studies without standard preclinical testing. In general, the considerations listed under section VII.C are applicable. When the initial clinical study for such a drug shows promising results and further clinical development of the drug is intended, pharmacology and toxicology studies carried out prior to the later phases of the clinical trials may be needed to support a risk-benefit assessment and to identify potential toxicities not readily detected in clinical studies (see section IX.C below).

2. Others

For a botanical product that is not prepared according to a traditional methodology, the extent of variation from the traditional formulation, preparation, or processing should be described in full detail. The nature of preclinical pharmacology/toxicology information needed before conducting an initial clinical study (in addition to that described under section VII.C) will be determined on a case-by-case basis, depending on the indications, extent of safe human experience, and safety concerns about the new formulation, preparation, or processing methodology used.

D. BIOAVAILABILITY

... [not included]

E. CLINICAL CONSIDERATIONS

... [not included]

IX. INDs For Phase 3 Clinical Studies Of All Botanical Products

When conducting expanded (i.e., phase 3) clinical studies on a botanical drug product, an IND sponsor is expected to provide more detailed information on CMC and preclinical safety than when conducting a phase 1 or phase 2 study (21 CFR 312.22(b), 312.23(a)(7)(i), 312.23(8)). The better definition of the product will ensure an ability to apply data from trials to a well-controlled, reproducible substance. The additional toxicology data is needed to support wider use. This additional information should be provided regardless of whether the product is currently lawfully marketed in the United States or elsewhere as a dietary supplement. For phase 3 clinical studies of a botanical product, the following information should be provided in accordance with 312.23:

A. DESCRIPTION OF PRODUCT AND DOCUMENTATION OF HUMAN EXPERIENCE

... [not included]

B. CHEMISTRY, MANUFACTURING, AND CONTROLS (' 312.23(a)(7))

... [not included]

C. PRECLINICAL SAFETY ASSESSMENT (including pre-NDA)

To support safety for expanded clinical studies or to support marketing approval of a botanical drug product, toxicity data from standard toxicology studies in animals may be needed. A botanical product submitted for approval for marketing as a drug will be treated like any other new drug under development. Previous human experience may be insufficient to demonstrate the safety of a botanical product, especially when it is indicated for chronic therapy. Systematic toxicological evaluations could be needed to supplement available knowledge on the general toxicity, teratogenicity, mutagenicity, and carcinogenicity of the final botanical product. Depending on the indication (e.g., target patient population, disease to be treated), route of administration, and duration

of recommended drug exposure, the timing of these animal studies in relation to concurrent clinical trials and other requirements for preclinical animal studies can vary. The following are points to consider in preparing a preclinical pharmacology/toxicology development plan for a botanical drug product that is intended to be used in large-scale human trials or to support an NDA. If questions arise during any stage of the clinical development of a botanical drug, sponsors are encouraged to consult the appropriate review division in CDER.

1. Repeat-dose General Toxicity Studies

The primary objective of long-term, repeat-dose toxicity studies in animals is to identify the target organs and/or systems for toxicity and the threshold doses for producing toxic effects. The studies provide information valuable for designing long-term clinical studies at safe doses with appropriate monitoring for predicted adverse reactions. Existing literature on the animal toxicity of a botanical drug product is often limited to single-dose (acute) toxicity studies. These studies may be inadequate to support the conclusion that a botanical drug product is non-toxic for multiple administrations because they were not designed to monitor the usual parameters of toxicity (e.g., clinical pathology and histopathology) or take into consideration the effect of more frequent dosing. To support expanded clinical trials, repeat-dose toxicity of a drug product should usually be evaluated in two mammalian species (one of which is a non-rodent) by employing sufficiently high doses to produce a toxic effect or by using a maximum feasible dose. If possible, the drug should be tested using the same formulation and route of administration as proposed for clinical use. Animal studies should be of a duration at least equal to that of the clinical trial (usually a minimum of two weeks). General animal toxicity studies need not exceed 6 months of testing in a rodent species and 9 months testing in a non-rodent species. For additional information on the timing of animal toxicity studies in relation to clinical trials, see the International Conference on Harmonisation (ICH) guidance *M3 Non-clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals* (November 1997).

2. Non-clinical Pharmacokinetic/Toxicokinetic Studies

In the development of a new drug that is a single molecular entity, pharmacokinetic studies are often carried out to demonstrate systemic exposure and to relate exposure levels to toxicities in both animals and humans. Because botanical products usually consist of more than one chemical constituent, standard pharmacokinetic measurements

to substantiate the systemic exposure of a botanical drug product in animals may be technically infeasible. However, monitoring major or representative chemical constituents in a botanical drug product can provide valuable information regarding systemic exposure. Depending on the complexity of the botanical drug product to be studied, pharmacokinetics could be helpful in the design and interpretation of toxicity studies. For additional information on toxicokinetic evaluations, see the ICH guidances *S3A Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies* (March 1995), and *S3B Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies* (March 1995).

3. Reproductive Toxicology

Reproductive toxicology studies, such as those on fertility/reproductive performance, teratology, and prenatal/perinatal development in animals, provide information on the potential of a botanical drug product to produce toxicity during the different stages of reproductive and developmental processes. In the absence of documentation on reproductive toxicity in humans or animals, these tests should be conducted prior to expanded clinical trials. For detailed information regarding reproductive toxicology sponsors should refer to the ICH guidances *S5A Detection of Toxicity to Reproduction for Medicinal Products* (September 1994), and *S5B Detection of Toxicity to Reproduction for Medicinal Products: Addendum on Toxicity to Male Fertility* (April 1996).

4. Genotoxicity Studies

Information on the potential of a botanical drug product to produce genetic toxicity should be obtained as early as possible, preferably before the initiation of human clinical trials. A complete assessment of genetic toxicity may be needed prior to expanded clinical trials. A standard battery of tests is defined in the ICH guidances *S2A Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals* (April 1996), and *S2B Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals* (November 1997). If the tests chosen indicate that a drug is devoid of genetic toxicity, additional studies may not be needed. If one or more test results are positive, the sponsor may need to carry out additional genotoxicity tests in consultation with the appropriate CDER review division.

5. Carcinogenicity Studies

Carcinogenicity studies may be needed to support marketing approval of a botanical drug, depending on the duration of therapy or any specific cause for concern. The toxicity

profile of the botanical drug product and the indication and duration of the intended use may influence the need for carcinogenicity studies and their timing relative to clinical development (see ICH guidance *S1A The Need for Long-Term Rodent Carcinogenicity Studies of Pharmaceuticals* (March 1996)). Draft protocols for carcinogenicity studies should be submitted to the appropriate review division and the CDER Carcinogenicity Assessment Committee for review and concurrence prior to the initiation of such studies to ensure the acceptability of dose selection and study design. Study types should be in accordance with the ICH guidance *S1B Testing for Carcinogenicity of Pharmaceuticals* (February 1998). Doses used should be chosen according to the principles outlined in the ICH guidances *S1C Dose Selection for Carcinogenicity Studies of Pharmaceuticals* (March 1995), and *S1C(R) Dose Selection for Carcinogenicity Studies of Pharmaceuticals: Addendum on a Limit Dose and Related Notes* (December 1997).

6. Special Pharmacology/Toxicology Studies

A general evaluation of pharmacological activity on organs and/or systems is often performed during new drug development. This evaluation can be accomplished using established in vitro and in vivo assays of broad specificity that screen for the modes and sites of action of the botanical drug. When significant and unique toxicities to certain organs and/or systems are evident, the sponsor should provide further explanation of the mechanism of toxic actions, if necessary by performing additional in vitro or in vivo studies.

7. Regulatory Considerations

Preclinical toxicity studies conducted as part of botanical drug development and intended to support safety must be in accordance with regulations governing good laboratory practices under 21 CFR Part 58. To the extent possible, a botanical drug substance tested in animals should be prepared and processed in the same manner, and the botanical drug product should have the same formulation, as the product intended for human use. Both the drug substance and the drug product should be made with batch-to-batch consistency. If changes occur in the drug substance or product during clinical development, bridging toxicity studies might be needed.

D. BIOAVAILABILITY AND DRUG-DRUG INTERACTIONS

... [not included]

E. CLINICAL CONSIDERATIONS

... [not included]

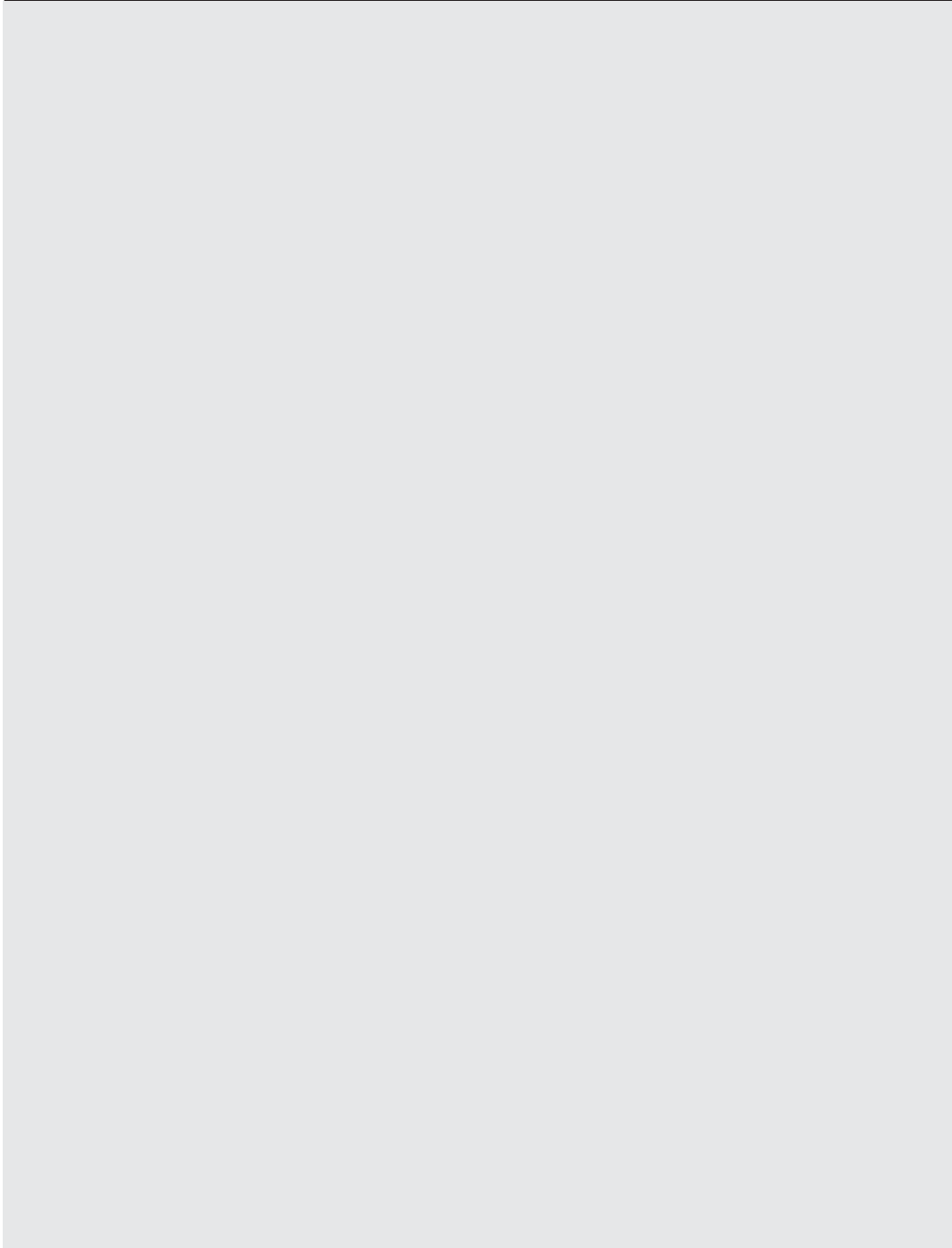
APPENDIX G

EU-CPMP NOTE FOR GUIDANCE ON VACCINES

See text of guidance at: <http://www.emea.eu.int/pdfs/human/swp/046595en.pdf>
(read only format, not downloadable).

PERSONAL NOTES

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