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Advisory Document of the Working Group on Good Laboratory Practice

The Application of the Principles of GLP to in vitro Studies

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Series on Principles of Good Laboratory Practice

and Compliance Monitoring

No. 14

Advisory Document of the Working Group on GLP

The Application of the Principles of GLP to *in vitro* Studies

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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Also published in the Series on Principles of Good Laboratory Practice and Compliance Monitoring

No. 1, *OECD Principles of Good Laboratory Practice* (as revised in 1997)

No. 2, *Revised Guides for Compliance Monitoring Procedures for Good Laboratory Practice* (1995)

No. 3, *Revised Guidance for the Conduct of Laboratory Inspections and Study Audits* (1995)

No. 4, *Quality Assurance and GLP* (as revised in 1999)

No. 5, *Compliance of Laboratory Suppliers with GLP Principles* (as revised in 1999)

No. 6, *The Application of the GLP Principles to Field Studies* (as revised in 1999)

No. 7, *The Application of the GLP Principles to Short-term Studies* (as revised in 1999)

No. 8, *The Role and Responsibilities of the Study Director in GLP Studies* (as revised in 1999)

No. 9, *Guidance for the Preparation of GLP Inspection Reports* (1995)

No. 10, *The Application of the Principles of GLP to Computerised Systems* (1995)

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No. 12, *Requesting and Carrying Out Inspections and Study Audits in Another Country* (2000)

No. 13, *The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies* (2002)

About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 30 industrialised countries in North America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in nine different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring**; **Pesticides and Biocides**; **Risk Management**; **Harmonisation of Regulatory Oversight in Biotechnology**; **Safety of Novel Foods and Feeds**; **Chemical Accidents; Pollutant Release and Transfer Registers; and Emission Scenario Documents.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (http://www.oecd.org/ehs/).

This publication was produced within the framework of the Inter-Organisation Programme for the Sound Management of Chemicals (IOMC).

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The participating organisations are FAO, ILO, OECD, UNEP, UNIDO, UNITAR and WHO. The World Bank and UNDP are observers. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

Foreword

As efforts to decrease the use of animals in safety testing are intensifying, *in vitro* methods are gaining a more prominent role as alternatives or supplements to *in vivo* safety testing. Anticipated developments in the fields of toxicogenomics, toxicoproteomics, toxicometabonomics and in various high through-put screening techniques are expected to enhance the importance of *in vitro* methodologies for safety testing, beyond their traditional use as test systems in the area of genetic toxicity testing. The OECD Working Group on Good Laboratory Practice considered it therefore worthwhile to develop further guidance specifically of relevance to the application and interpretation of the OECD Principles of GLP[1] to *in vitro* studies.

The Working Group established a Task Force under the leadership of Switzerland, which met in Bern on 12 to 13 February 2004. The Task Force comprised members of the Working Group or experts in *in vitro* testing nominated by them representing Belgium, France, Germany, Japan, the Netherlands, Switzerland, the United States and the European Commission.

The draft Advisory Document developed by the Task Force was examined by the Working Group at its 18th Meeting in May 2004, where it was amended and endorsed. The Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology at its 37th Joint Meeting in turn endorsed the document and recommended that it be declassified under the authority of the Secretary-General.

[1] See No. 1 of the Series on Good Laboratory Practice and Compliance Monitoring

Advisory Document of the Working Group on GLP

THE APPLICATION OF THE PRINCIPLES OF GLP TO *IN VITRO* STUDIES

Introduction

Studies involving *in vitro* test systems have long been used to obtain data on the safety of chemicals with respect to human health and the environment. National legislation usually requires that these studies be conducted in accordance with Good Laboratory Practice (GLP) requirements.[2] Traditionally *in vitro* methods have been mainly used in the area of genetic toxicity testing, where the hazard assessment is based to a large extent on data derived from studies using *in vitro* test systems. As efforts to decrease the use of animals in safety testing are intensifying, *in vitro* methods are gaining a more prominent role as alternatives or supplements to *in vivo* safety testing. Furthermore, developments in the area of toxicogenomics, toxicoproteomics, toxicometabonomics and various (e.g., micro-array) high through-put screening techniques will also enhance the importance of *in vitro* methodologies for safety testing.

The requirement that safety studies be planned, conducted, recorded, reported and archived in accordance with the *OECD Principles of Good Laboratory Practice* (hereafter the GLP Principles) does not differ for different study types. Therefore, the GLP Principles and the associated Consensus Documents[3] describe requirements for and provide general guidance on the conduct of all nonclinical health and environmental safety studies, including *in vitro* studies. In order to facilitate the application and interpretation of the GLP Principles in relation to the specific *in vitro* testing situation, further clarification and guidance was considered useful.

Purpose of this Document

The purpose of this document is to facilitate the proper application and interpretation of the GLP Principles for the organisation and management of *in vitro* studies, and to provide guidance for the appropriate application of the GLP Principles to *in vitro* studies, both for test facilities (management, QA, study director and personnel), and for national GLP compliance monitoring authorities.

This Advisory Document intends to provide such additional interpretation of the Principles and guidance for their application to *in vitro* studies carried out for regulatory purposes. It is organised in such a way as to provide easy reference to the GLP Principles by following the sequence of the different parts of these GLP Principles.

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[2] Revised OECD Principles of Good Laboratory Practice [C(97)186 (Final)]

[3] See OECD series on Good Laboratory Practice and Compliance Monitoring

Scope

This document is specific to the application of the Principles of GLP to *in vitro* studies conducted in the framework of non-clinical safety testing of test items contained in pharmaceutical products, pesticide products, cosmetic products, veterinary drugs as well as food additives, feed additives, and industrial chemicals. These test items are frequently synthetic chemicals, but may be of natural or biological origin and, in some circumstances, may be living organisms. The purpose of testing these test items is to obtain data on their properties and/or their safety with respect to human health and/or the environment.

Unless specifically exempted by national legislation, the Principles of Good Laboratory Practice apply to all non-clinical health and environmental safety studies required by regulations for the purpose of registering or licensing pharmaceuticals, pesticides, food and feed additives, cosmetic products, veterinary drug products and similar products, and for the regulation of industrial chemicals.

Definitions

a) In vitro Studies

In vitro studies are studies which do not use multicellular whole organisms, but rather microorganisms or material isolated from whole organisms, or simulations thereof as test systems.

Many *in vitro* studies will qualify as short-term studies under the definition provided by the GLP Principles. For these studies, the *OECD Consensus Document on The Application of the GLP Principles to Short-Term Studies* should be consulted and used as appropriate, in order to allow for the application of the provisions facilitating the work of Study Director and QA.

b) Reference Item

Test guidelines for *in vitro* studies mandate in many cases the use of appropriate positive, negative and/or vehicle control items which may not serve, however, as the GLP definition of "reference items" implies, to grade the response of the test system to the test item, but rather to control the proper performance of the test system. Since the purpose of these positive, negative and/or vehicle control items may be considered as analogous to the purpose of a reference item, the definition of the latter may be regarded as covering the terms "positive, negative, and/or vehicle control items" as well. The extent to which they should be analytically characterized may, however, be different from the requirements of reference items.

Responsibilities

a) Test Facility Management

Most of the responsibilities of test facility management are of a general nature and are equally applicable to *in vivo* and *in vitro* studies, such as the requirements that test facility management has to ensure the availability of qualified personnel, and of appropriate facilities and equipment for the timely and proper conduct of the study. However, test facility management should be aware that *in vitro* testing may influence the execution of some of their responsibilities For example, test facility management must ensure that personnel clearly understand the functions they are to perform. For *in vitro* studies this may entail

ensuring that specific training is provided in aseptic procedures and in the handling of biohazardous materials. *In vitro* testing may also necessitate the availability of specialized areas and the implementation of procedures to avoid contamination of test systems. Another example is provided by the requirement that test facility management should ensure that test facility supplies meet requirements appropriate to their use in a study. Certain *in vitro* studies may necessitate the use of proprietary materials or test kits. Although the *OECD Consensus Document on Compliance of Laboratory Suppliers with GLP Principles* states that materials to be used in a GLP compliant study should be produced and tested for suitability using an adequate quality system, thus placing the primary responsibility for their suitability on the manufacturer or supplier, it is the responsibility of the test facility management to confirm that these conditions are adequately fulfilled through assessment of the suppliers practices, procedures and policies.

b) Study Director

The general responsibilities of the Study Director are independent of the type of study and the responsibilities listed in the Principles apply to *in vitro* studies as well. The study director continues to be the single point of study control and has the responsibility for the overall conduct and reporting of the study.

In *in vitro* studies the Study Director should pay particular attention to documenting the justification and characterization of the test system, an activity which may be more difficult to accomplish for *in vitro* studies. See the section on Test Systems, below, regarding the documentation required to justify and characterize the test system. In *in vivo* studies these activities have been rather straightforward. For example, the use of a particular species may be justified by documenting the characteristics of that species that make it an appropriate model for assessing the effect of interest. Characterization of a particular animal may be accomplished by simply documenting the animal species, strain, substrain, source of supply, number, body weight range, sex, and age.

These required activities may be more difficult to accomplish for *in vitro* studies:

Justification of the test system may require that the Study Director document that the test method has been validated or is structurally, functionally, and/or mechanistically similar to a validated reference test method. Prior to the use of new test methods that are structurally, functionally and/or mechanistically similar to a validated reference test method, the Study Director should therefore provide documented evidence that the new test method has comparable performance when evaluated with appropriate reference items.

Characteristics of *in vitro* systems may also be difficult to document. Although the Study Director may be able, with the assistance of the supplier, to document some characteristics of the test system (e.g., cell line, age/passage, origin) he/she should also characterize the test system by documenting that the test system provides the required performance when evaluated with appropriate reference items, including positive, negative, and untreated and/or vehicle controls, where necessary. A special case may be seen in the use of proprietary materials or test kits in the conduct of *in vitro* studies. While the performance of such materials or test kits should be assured by the supplier, producer or patent holder, and while the test facility management is responsible for ensuring that the supplier meets the quality criteria as mentioned above, e.g., by reviewing vendor practices, procedures and policies, it is the responsibility of the Study Director to ensure that the performance of these materials or kits indeed meets the requirements of the study, and to ensure that test kits have been adequately validated and are suitable for their intended purpose. Since the quality and reliability of study results will be influenced directly by the quality and performance of these materials or test kits, it is especially important that the completeness and acceptability of the quality control documentation provided by the supplier should be thoroughly examined and critically evaluated by the Study Director. At a minimum, the Study Director should be able to judge the appropriateness of the quality system used by the manufacturer, and have available all documentation needed to assess the fitness for use of the test system, e.g., results of performance studies.

c) Study Personnel

Personnel should meticulously observe, where applicable, the requirements for aseptic conditions and follow the respective procedures in the conduct of *in vitro* studies to avoid pathogen contamination of the test system. Similarly, personnel should employ adequate practices (see "Sources for Further Information", ref 1) to avoid cross-contamination between test systems and to ensure the integrity of the study. Study personnel should be aware of, and strictly adhere to, the requirements to isolate test systems and studies involving biohazardous materials. Appropriate precautions to minimize risks originating from the use of hazardous chemicals should be applied during *in vitro* studies as well.

Quality Assurance

In general, Quality Assurance (QA) activities will not be greatly different between *in vitro* and *in vivo* studies. *In vitro* studies may qualify in certain cases for treatment under the conditions of short-term studies; in these cases, the *OECD Consensus Document on The Application of the GLP Principles to Short-Term Studies* will be applicable. Thus, such studies may be inspected, if applicable and permitted by national regulations, by QA on a process-based inspection programme. Since the GLP Principles require QA to inspect especially the critical phases of a study, it is important that, in the case of *in vitro* studies, QA is well aware of what constitutes critical phases (and critical aspects) of such studies. Corresponding guidance for QA inspections should be developed in co-operation with Study Directors, Principal Investigators and study personnel in the relevant areas. Since the QA programme should, wherever indicated, explicitly cover specific aspects of *in vitro* testing, education and training of QA personnel should also be explicitly directed towards the ability to recognise potential problems in specific areas of *in vitro* testing.

Specific areas to be inspected may include, but not be limited to, the procedures and measures for:

- monitoring of batches of components of cell and tissue culture media that are critical to the performance of the test system (e.g. foetal calf serum, etc.) and other materials with respect to their influence on test system performance;
- assessing and ensuring functional and/or morphological status (and integrity) of cells, tissues and other indicator materials;
- monitoring for potential contamination by foreign cells, mycoplasma and other pathogens, or other adventitious agents, as appropriate;
- cleaning and decontamination of facilities and equipment and minimizing sources of contamination of test items and test systems;
- ensuring that specialised equipment is properly used and maintained;
- ensuring proper cryopreservation and reconstitution of cells and tissues;
- ensuring proper conditions for retrieval of materials from frozen storage;
- ensuring sterility of materials and supplies used for cell and tissue cultures;
- maintaining adequate separation between different studies and test systems.

Facilities

a) General

The GLP Principles mandate that test facilities should be suitable to meet the requirements of the studies performed therein, and that an adequate degree of separation should be maintained between different activities to ensure the proper and undisturbed conduct of each study. Due to the fact that *in vitro* studies generally occupy only limited workspace and do not normally require dedicated facilities that exclude the performance of other studies, measures should be taken to ensure the appropriate separation of *in vitro* studies co-existing in the same physical environment.

b) Test System Facilities

The GLP Principles require that a sufficient number of rooms or areas should be available to ensure the isolation of test systems, and that such areas should be suitable to ensure that the probability of contamination of test systems is minimized. The term "areas", however, is not specifically defined and its interpretation is therefore adaptable to various *in vitro* situations. The important aspect here is that the integrity of each test system and study should not be jeopardised by the possibility of potential contamination or cross-contamination or mix-up.

In this way it may be possible to incubate cells or tissues belonging to different studies within the same incubator, provided that an adequate degree of separation exists (e.g., appropriate identifiers, labelling or separate placement to distinguish between studies, etc.), and that no test item is sufficiently volatile so as to contaminate other studies that are co-incubated.

Separation of critical study phases may be possible not only on a spatial, but also on a temporal basis. Manipulation of cell and tissue cultures, e.g., subcultivation procedures, addition of test item, etc., is normally performed in vertical laminar flow cabinets to assure sterility and to protect the test system as well as study personnel and the environment. Under these circumstances, adequate separation to prevent cross-contamination between different studies will be achieved by sequential manipulation of the test systems used in the individual studies, with careful cleaning and decontamination/sterilization of the working surfaces of the cabinet and of related laboratory equipment performed between the different activities, as necessary.

Another important aspect is the availability of devoted rooms or areas with special equipment for the long-term storage of test systems. The equipment, including storage containers, should provide adequate conditions for maintenance of long-term integrity of test systems.

c) Facilities for Handling Test and Reference Items

While the requirements of the GLP Principles for handling test and reference items apply equally to *in vitro* tests as far as the prevention of cross-contamination by test and reference items is concerned, another aspect needs to be taken into account: Since sterility is an important consideration in *in vitro* studies it should be ensured that rooms or areas used for preparation and mixing of test and reference items with vehicles be equipped so as to allow working under aseptic conditions, and thus protecting the test system and the study by minimizing the probability of their contamination by test and reference item preparations.

Apparatus, Material, and Reagents

While the commonly observed, routine requirements for apparatus used in a GLP compliant environment apply equally to apparatus used for *in vitro* studies, there are some specific points and issues of particular importance. As an example, it may be of importance for the integrity and reliability of some *in vitro* studies to ensure that the proper conditions of certain equipment, like microbalances, micropipettes, laminar air flow cabinets or incubators are regularly maintained, and monitored and calibrated where applicable. For specific equipment, critical parameters should be identified requiring continuous monitoring or the setting of limit values together with installation of alarms.

The requirements in the GLP Principles for reagents with respect to labelling and expiry dates apply equally to those used for *in vitro* studies.

Test Systems

In vitro test systems are mainly biological systems, although some of the more recent developments in alternatives to conventional *in vivo* testing (e.g., gene arrays for toxicogenomics) may also exhibit some attributes of physical-chemical test systems, and still others, e.g., toxicometabonomics, may mainly rely on analytical methodology. Test kits, including proprietary test kits, should also be considered as test systems.

a) Conditions for Test Systems

As for any other biological test systems, adequate conditions should be defined, maintained and monitored to ensure the quality and integrity of the test system. during storage and within the study itself. This includes the documented definition, maintenance and monitoring of the viability and responsiveness of the test system, including recording of cell passage number and population doubling times. Records should also be kept for environmental conditions (e.g., liquid nitrogen level in a liquid nitrogen cryostorage system, temperature, humidity and CO_2 concentration in incubators, etc.) as well as for any manipulation of the test system required for the maintenance of its quality and integrity (e.g., treatment with antibiotics or antifungals, subcultivation, selective cultivation for reducing the frequency of spontaneous events). Since maintenance of the proper environmental conditions during the storage of test systems may influence data quality to a greater degree than for other biological systems, these records may be of special importance in the maintenance of data quality and reliability.

b) Newly Received Test Systems

Documentation obtained from the supplier of in vitro test systems (e.g., origin, age/passage number, cell doubling time and other relevant characteristics that help identify the test system) should be reviewed and retained in the study records. Predefined criteria should be used to assess the viability, suitability (e.g. functional and/or morphological status of cells and tissues, testing for known or suspected microbial or viral contaminants) and responsiveness of the test system. Results of such evaluations should be documented and retained in the study records. If no such assessment is possible, as, e.g., with primary cell cultures or "reconstituted organs", a mechanism should exist between the supplier and the user to ascertain and document the suitability of the test system. Monitoring and recording performance against negative and positive control items may constitute sufficient proof for the responsiveness of a given test system. Any problems with the test system that may affect the quality, validity and reliability of the study should

be documented and discussed in the final report. Problems with vendor-supplied test systems should be brought to the attention of the vendor and corrective measures sought.

c) Test System Records

The GLP Principles require that records be maintained of source, date of arrival and arrival condition of test systems; for cells and tissues these records should include not only the immediate source (e.g., commercial supplier), but also the original source from where the cells or tissues have been derived (e.g., primary cells or tissues with donor characteristics; established cell lines from recognized sources, etc.). Other information to be maintained should include, but not be limited to, the method by which cells or tissues were originally obtained (e.g., derived from tissue explants, biopsies of normal or cancer tissues, gene transfer by plasmid transfection or virus transduction, etc.), chronology of custody, passage number of cell lines, culture conditions and subcultivation intervals, freezing/thawing conditions, etc. For transgenic test systems, it is necessary, in addition, to ascertain the nature of the transgene and to monitor maintenance of expression with appropriate controls.

Special attention should be paid to the proper labelling of test systems during storage and use, which includes measures to ensure the durability of labelling. Especially where the size of containers and the conditions of storage (e.g., cryovials in liquid nitrogen, multiple test systems stored in one container) may be critical factors for labelling, measures should be in place to ensure the correct identification of test systems at all times.

The requirements in the OECD Principles of GLP for test items and reagents with respect to labelling and expiry dates apply equally to test kits used as *in vitro* test systems. Test kits, whether used as test systems or in any other way, e.g., for analytical purposes, should have an expiry date. Extending this expiry date can be only acceptable on the basis of documented evaluation (or analysis). For test kits used as test systems, such documented evaluation may, e.g., consist of the historical record of observed responses obtained with the respective batch of the test kit to positive, negative and/or vehicle control items, and proof that, even after the expiry date, the response did not deviate from the historical control values. A documented decision of the Study Director as to the extension of the expiry date should provide evidence for this evaluation process.

In order to avoid possible confusion, the nomenclature for the test systems should be clearly defined, and test system labels as well as all records obtained from individual studies should bear the formally accepted designation of the test system.

Test and Reference Items (including Negative and Positive Control Items)

In general, there are no specific requirements for receipt, handling, sampling, storage and characterisation for test and reference items that are used in studies utilising *in vitro* test systems besides those listed in the GLP Principles. Aseptic conditions may, however, be required in their handling to avoid microbial contamination of test systems.

For negative, vehicle and positive control items, it may or may not be necessary to determine concentration and homogeneity, since it may be sufficient to provide evidence for the correct, expected response of the test system to them.

The expiry date of such control items may also be extended by documented evaluation or analysis. Such evaluation may consist of documented evidence that the response of the respective test systems to these positive, negative and/or vehicle control items does not deviate from the historical control values recorded in the test facility, which should furthermore be comparable to published reference values.

Standard Operating Procedures (SOPs)

In addition to the examples cited in the GLP Principles (see section 7.4.1 – 7.4.5) there are activities and processes specific to *in vitro* testing that should be described in Standard Operating Procedures. Such SOPs should therefore be additionally available for, but not be limited to, the following illustrative examples for test facility activities related to *in vitro* testing.

a) Facilities

Environmental monitoring with respect to pathogens in the air and on surfaces, cleaning and disinfection, actions to take in the case of infection or contamination in the test facility or area.

b) Apparatus

Use, maintenance, performance monitoring, cleaning, and decontamination of cell and tissue culture equipment and instruments, such as laminar-flow cabinets and incubators; monitoring of liquid nitrogen levels in storage containers; calibration and monitoring of temperature, humidity and CO_2-levels in incubators.

c) Materials, Reagents and Solutions

Evaluation of suitability, extension of expiry dates, assessment and maintenance of sterility, screening for common pathogen contaminants; description of procedures for choice and use of vehicles; verification procedures for compatibility of vehicles with the test system.

d) Test Systems

Conditions for storage and procedures for freezing and thawing of cells and tissues, testing for common pathogens; visual inspection for contaminations; verification procedures (e.g., use of acceptance criteria) for ensuring properties and responsiveness on arrival and during use, whether immediately after arrival or following storage; morphological evaluation, control of phenotype or karyotype stability, control of transgene stability; mode of culture initiation, culture conditions with subcultivation intervals; handling of biohazardous materials and test systems, procedures for disposal of test systems.

e) Performance of the Study

Aseptic techniques, acceptance criteria for study validity, criteria for assay repetitions.

f) Quality Assurance

Definition of critical phases, inspection frequencies.

Performance of the Study and Reporting of Study Results

The GLP requirements for the performance of *in vitro* studies are identical to those provided for the more conventional safety studies. In many cases, the *OECD Consensus Document on The Application of the GLP Principles to Short-Term Studies* may be consulted in combination with the OECD GLP Principles in order that *in vitro* studies may be conducted in a GLP compliant way.

There are a number of issues specific to *in vitro* testing that should be addressed in the study plan as well as in the final study report. These issues, however, are mainly of a scientific, technical nature, such as the (scientific) requirement that any internal controls (appropriate positive, negative, and untreated and/or vehicle controls), carried out in order to control bias and to evaluate the performance of the test system, should be conducted concurrently with the test item in all *in vitro* studies. More specific guidance as to what topics should be addressed in the study plan and the final report will be found in the respective OECD test guidelines or other appropriate references.

Storage and Retention of Records and Materials

The general retention requirements of the GLP Principles apply to *in vitro* studies as well. Additionally, it should be considered to retain samples of long-term preservable test systems, especially test systems of limited availability (e.g., special subclones of cell lines, transgenic cells, etc.), in order to enable confirmation of test system identity, and/or for study reconstructability.

Retention of samples of test item should be considered also for such *in vitro* studies which can be categorised as short-term studies, especially in cases where *in vitro* studies constitute the bulk of safety studies.

Records of historical positive, negative, and untreated and/or vehicle control results used to establish the acceptable response range of the test system should also be retained.

Glossary of Terms

Within the context of this document the following definitions are used:

Aseptic conditions: Conditions provided for, and existing in, the working environment under which the potential for microbial and/or viral contamination is minimized.

Cell lines: Cells that have undergone a genetic change to immortalization and that, in consequence, are able to multiply for extended periods *in vitro*, and can be expanded and cryopreserved as cell bank deposits. A continuous cell line is generally more homogeneous, more stable, and thus more reproducible than a heterogeneous population of primary cells.

Control, negative: Separate part of a test system treated with an item for which it is known that the test system should not respond; the negative control provides evidence that the test system is not responsive under the actual conditions of the assay.

Control, positive: Separate part of the test system treated with an item the response to which is known for the test system; the positive control provides evidence that the test system is responsive under the actual conditions of the assay.

Control, untreated: Separate untreated part of a test system that is kept under the original culture conditions; the untreated control provides baseline data of the test system under the conditions of the assay.

Control, vehicle: Separate part of a test system to which the vehicle for the test item is added; the vehicle control provides evidence for a lack of influence of the chosen vehicle on the test system under the actual conditions of the assay.

Critical phases: Individual, defined procedures or activities within a study, on the correct execution of which the study quality, validity and reliability is critically dependent.

Cross-contamination: Contamination of a test item by another test item or of a test system by another test item or by another test system that is introduced inadvertently. and taints the test item or impairs the test system.

Cryopreservation: Storage of cells and tissues by keeping them frozen under conditions where their viability is preserved.

Cryovial: Special vial used for cryopreservation. A cryovial has to satisfy special conditions such as tightness of closure even at extremely low temperatures and extreme temperature changes encountered during freezing and thawing.

Ex vivo: Cells, tissues, or organs removed for further analysis from intact animals.

Gene transfection: The introduction of foreign, supplemental DNA (single or multiple genes) into a host cell.

High through-put screening: The use of miniaturized, robotics-based technology to screen large compound libraries against an isolated target gene, protein, cell, tissue, etc. to select compounds on the basis of specific activities for further development.

Micro-arrays: Sets of miniaturized chemical reaction areas arranged in an orderly fashion and spotted onto a solid matrix such as a microscope slide. A DNA microarray provides a medium for matching known and unknown DNA samples based on base-pairing rules and allows for the automation of the process of identifying unknown DNA samples for use in probing a biological sample to determine gene expression, marker pattern or nucleotide sequence of DNA/RNA.

Primary cells: Cells that are freshly isolated from animal or plant sources. Freshly isolated primary cells may rapidly dedifferentiate in culture, and they often have a limited lifespan. Primary cultures isolated from animals or humans may represent heterogeneous populations with respect, for example, to differences in cell types and states of differentiation depending on purification techniques used. Each isolate will be unique and impossible to reproduce exactly. Primary cell cultures commonly require complex nutrient media, supplemented with serum and other components. Consequently, primary cell culture systems are extremely difficult to standardise.

Proprietary material: Material protected by (patent, copyright, or trademark) laws from illicit use.

Test kit: Ready-to-use compilation of all components necessary for the performance of an assay, test or study.

Tissues: Multicellular aggregates of differentiated cells with specific function as constituents of organisms.

Toxicogenomics: The study of how genomes respond to environmental stressors or toxicants. The goal of toxicogenomics is to find correlations between toxic responses to toxicants and changes in the genetic profiles of the objects exposed to such toxicants. Toxicogenomics combines the emerging technologies of genomics and bioinformatics to identify and characterize mechanisms of action of known and suspected toxicants. Currently, the premier toxicogenomic tools are the DNA microarray and the DNA chip, which are used for the simultaneous monitoring of expression levels of hundreds to thousands of genes.

Toxicometabonomics: The quantitative measurement of the time-related multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification by the systematic exploration of biofluid composition using NMR/pattern recognition technology in order to associate target organ toxicity with NMR spectral patterns and identify novel surrogate markers of toxicity.

Toxicoproteomics: The study of how the global protein expression in a cell or tissue responds to environmental stressors or toxicants. The goal of toxicoproteomics is to find correlations between toxic responses to toxicants and changes in the complete complements of proteins profiles of the objects exposed to such toxicants.

Transgenic cells: Cells transfected with one or more foreign gene(s) which consequently express characteristics and functions that are normally not present, or at low expression levels only, in the parental cell.

Sources for Further Information on In Vitro Testing

Webpages of:

1. Good Cell Culture Practices

   http://ecvam.jrc.it/publication/index5007.html

2. MIAME Guideline

   http://www.mged.org/Workgroups/MIAME/miame.html

3. ECVAM

   http://ecvam.jrc.it/index.htm

4. ICCVAM

   http://iccvam.niehs.nih.gov/