This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization, the International Labour Organization, the United Nations Environment Programme, or the experts’ employers.

Harmonization Project DRAFT Document for Public and Peer Review

MUTAGENICITY TESTING FOR CHEMICAL RISK ASSESSMENT

This project was conducted within the IPCS project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals.

Published under the joint sponsorship of the World Health Organization, the International Labour Organization and the United Nations Environment Programme, and produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals.
The International Programme on Chemical Safety (IPCS), established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO) and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.
# TABLE OF CONTENTS

1. INTRODUCTION .................................................................................................................6

2. STRATEGY FOR MUTAGENICITY TESTING .................................................................7
   2.1 Development of a testing strategy for a particular compound ...............................7
   2.2 In vitro testing ..........................................................................................................7
      2.2.1 In vitro tests .......................................................................................................7
      2.2.2 Evaluation of in vitro testing results .................................................................8
      2.2.3 Follow-up to in vitro testing ..........................................................................8
   2.3 In vivo testing ...........................................................................................................9
      2.3.1 In vivo tests .......................................................................................................9
      2.3.2 Follow-up to in vivo testing ..........................................................................9
   2.4 Strategy for germ cell testing .................................................................................9

3. REFERENCES ....................................................................................................................11

GLOSSARY OF TERMS ........................................................................................................16
FOREWORD

Harmonization Project Documents are a family of publications by the World Health Organization (WHO) under the umbrella of the International Programme on Chemical Safety (IPCS) (WHO/ ILO/UNEP). Harmonization Project Documents complement the Environmental Health Criteria (EHC) methodology (yellow cover) series of documents as authoritative documents on methods for the risk assessment of chemicals.

The main impetus for the current coordinated international, regional and national efforts on the assessment and management of hazardous chemicals arose from the 1992 United Nations Conference on Environment and Development (UNCED). UNCED Agenda 21, Chapter 19, provides the “blueprint” for the environmentally sound management of toxic chemicals. This commitment by governments was reconfirmed at the 2002 World Summit on Sustainable Development and in 2006 in the Strategic Approach to International Chemicals Management (SAICM). The IPCS project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals (Harmonization Project) is conducted under Agenda 21, Chapter 19, and contributes to the implementation of SAICM. In particular, the project addresses the SAICM objective on Risk Reduction and the SAICM Global Plan of Action activity to “Develop and use new and harmonized methods for risk assessment”.

The IPCS Harmonization Project goal is to improve chemical risk assessment globally, through the pursuit of common principles and approaches and, hence, strengthen national and international management practices that deliver better protection of human health and the environment within the framework of sustainability. The Harmonization Project aims to harmonize global approaches to chemical risk assessment, including by developing international guidance documents on specific issues. The guidance is intended for adoption and use in countries and by international bodies in the performance of chemical risk assessments. The guidance is developed by engaging experts worldwide. The project has been implemented in a step-wise approach, first sharing information and increasing understanding of methods and practices used by various countries, identifying areas where convergence of different approaches would be beneficial, and then developing guidance that enables implementation of harmonized approaches. The project uses a building block approach, focusing at any one time on the aspects of risk assessment that are particularly important for harmonization.

The project enables risk assessments (or components thereof) to be performed using internationally accepted methods, and these assessments can then be shared to avoid duplication and optimize use of valuable resources for risk management. It also promotes sound science as a basis for risk management decisions, promotes transparency in risk assessment, and reduces unnecessary testing of chemicals. Advances in scientific knowledge can be translated into new harmonized methods.

This ongoing project is overseen by a geographically representative Harmonization Project Steering Committee and a number of ad hoc Working Groups that manage the detailed work. Finalization of documents includes a rigorous process of international peer review and public comment.
The World Health Organization thanks the Fraunhofer Institute for Toxicology and Experimental Medicine, Hanover, Germany, for its assistance in preparing for and convening the expert meeting that developed this draft document.
LIST OF PARTICIPANTS IN THE WHO/IPCS INTERNATIONAL DRAFTING GROUP MEETING ON MUTAGENICITY TESTING FOR CHEMICAL RISK ASSESSMENT

The international drafting group meeting was hosted by the Fraunhofer Institute for Toxicology and Experimental Medicine, Hanover, Germany, on 11–12 April 2007. The participants at this meeting are listed below.

Dr Michael C. Cimino
Biologist, Science Support Branch, Risk Assessment Division, Office of Pollution Prevention and Toxics, United States Environmental Protection Agency, Washington, DC, USA

Dr George R. Douglas
Head, Mutagenesis Section, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Canada

Professor David A. Eastmond (Meeting Chair)
Chair, Environmental Toxicology Graduate Program, University of California, Riverside, CA, USA

Professor Andrea Hartwig (Meeting Rapporteur)
Institut für Lebensmitteltechnologie und Lebensmittelchemie, Technische Universität Berlin, Berlin, Germany

Dr Andreas Luch
Head, Departments of Consumer Safety and Scientific Services, Federal Institute for Risk Assessment, Berlin, Germany

Professor David Phillips
Institute of Cancer Research, Sutton, United Kingdom

Dr Atsuya Takagi
Cellular & Molecular Toxicology Division, National Institute of Health Sciences, Tokyo, Japan

Dr Raymond Tennant
Head, Cancer Biology Group/Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA
Observer

Professor David Kirkland\(^1\)
Vice President, Scientific & Regulatory Consulting, Covance Laboratories Limited,
Harrogate, United Kingdom

Secretariat

Dr Janet Kielhorn
Department of Chemical Risk Assessment, Fraunhofer Institute for Toxicology and
Experimental Medicine, Hanover, Germany

Ms Carolyn Vickers
Harmonization Project Lead, International Programme on Chemical Safety, World Health
Organization, Geneva, Switzerland

\(^1\) Professor Kirkland was invited in his capacity as Steering Committee Chair for the 4th International Workshop on Genotoxicity Testing. He did not participate in the last session when the meeting finalized the agreed text.
### LIST OF ACRONYMS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OH-dG</td>
<td>8-hydroxy-2′-deoxyguanosine</td>
</tr>
<tr>
<td>AMS</td>
<td>accelerator mass spectrometry</td>
</tr>
<tr>
<td>DNA</td>
<td>2′-deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECD</td>
<td>electrochemical detection</td>
</tr>
<tr>
<td>EPA</td>
<td>United States Environmental Protection Agency</td>
</tr>
<tr>
<td>ESTR</td>
<td>Expanded Simple Tandem Repeat</td>
</tr>
<tr>
<td>FISH</td>
<td>fluorescence in situ hybridization</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use</td>
</tr>
<tr>
<td>IPCS</td>
<td>International Programme on Chemical Safety, World Health Organization</td>
</tr>
<tr>
<td>IWGT</td>
<td>International Workshop on Genotoxicity Testing</td>
</tr>
<tr>
<td>MOA</td>
<td>mode of action</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>NAT</td>
<td>N-acetyltransferase</td>
</tr>
<tr>
<td>NR</td>
<td>nitroreductase</td>
</tr>
<tr>
<td>OECD</td>
<td>Organisation for Economic Co-operation and Development</td>
</tr>
<tr>
<td>OPPTS</td>
<td>Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency</td>
</tr>
<tr>
<td>TK</td>
<td>thymidine kinase</td>
</tr>
<tr>
<td>UDS</td>
<td>unscheduled DNA synthesis</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

Since the publication of the International Programme on Chemical Safety (IPCS) Harmonized Scheme for Mutagenicity Testing (Ashby et al., 1996), there have been a number of publications addressing test strategies for mutagenicity, including the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) harmonized guidelines on genotoxicity testing of pharmaceuticals (ICH Steering Committee, 1997; Müller et al., 1999; UKCOM, 2000; USFDA, 2000; Dearfield & Moore, 2005) and reviews thereof (e.g. Cimino, 2006). In addition, analyses of test batteries and their correlation with carcinogenicity (Brambilla & Martelli, 2003; Kirkland et al., 2005, 2006a; Matthews et al., 2006) have indicated that an optimal solution to this issue has not yet been found. The 2005 International Workshop on Genotoxicity Testing (IWGT) meeting in San Francisco, USA, discussed many of these problems, and reports of this meeting (Kirkland et al., 2006a, 2006b) and companion papers (Lorge et al., 2006; Burlinson et al., 2007; Hayashi et al., 2007; Thybaud et al., 2007) have recently been published.

It is now clear from the results of international collaborative studies and the large databases that are currently available for the assays evaluated that no single assay can detect all genotoxic chemicals (Kirkland et al., 2003). This is not surprising, as a wide variety of possible genetic events can occur. For example, mutagens may act to cause base pair substitutions or by frameshifting, causing gene mutations but not chromosome mutations. Conversely, some chemicals produce chromosome mutations but show no evidence of inducing gene mutations. Therefore, safety assessments of chemicals with regard to genotoxicity are generally based on a combination of tests to assess effects on three major end-points of genetic damage associated with human disease: gene mutation (i.e. point mutations or deletions that affect single or blocks of genes), clastogenicity (i.e. structural chromosome changes), and aneuploidy (i.e. a numerical chromosome aberration) (Cimino, 2006).

The World Health Organization (WHO) therefore decided to update the IPCS Harmonized Scheme for Mutagenicity Testing (Ashby et al., 1996) as part of the IPCS Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals. The following draft for public and peer review was prepared by an International Drafting Group Meeting of experts held at the Fraunhofer Institute for Toxicology and Experimental Medicine in Hanover, Germany, on 11–12 April 2007.
2. STRATEGY FOR MUTAGENICITY TESTING

The approach presented in this document (see Figure 1) focuses on the identification of mutagens and genotoxic carcinogens. The term “mutation” as understood in this document comprises gene mutations as well as structural and numerical chromosome alterations. The group is aware of other mechanisms leading to carcinogenicity and other heritable diseases, but their identification requires additional types of mechanistic studies.

At various stages of the outlined testing strategy, a weight of evidence approach should be used. However, a clear positive result at a single mutagenicity end-point, even when multiple negative results in other end-points have been reported, is generally sufficient for the classification “positive”.

Most short-term tests in bacteria and mammalian cell cultures have been designed primarily for hazard identification and thus can represent only the starting point in the process of risk assessment. Whether or not the observed effects are relevant for human exposure depends on bioavailability, absorption, metabolism, half-lives, and other factors that require investigation in vivo.

Especially when choosing in vivo assays and when proceeding into germ cell mutagenicity studies (see section 2.4), expert judgement is required to select the appropriate test system(s) and to avoid uninformative and thus unnecessary animal experiments.

2.1 Development of a testing strategy for a particular compound

Before initiating mutagenicity testing on a particular compound, the following aspects should be considered:

- chemical structure and class of the agent (possible structure–activity relationships) and physicochemical properties, such as solubility and stability;
- expected routes of metabolism, chemical and biological reactivity/activity, and relationship to known genotoxic chemicals;
- routes of exposure, bioavailability, and target organ(s).

Critical evaluation of available data prior to testing usually provides important information for choosing the appropriate in vitro assay(s), but even more so for the selection of appropriate in vivo studies.

Distinction needs to be made between mutagenicity tests in the strict sense and indicator tests. As the latter may or may not indicate mutations, preference should be given to mutagenicity tests whenever possible.

2.2 In vitro testing

Usually two or three different tests in bacteria and mammalian cells are selected to cover the end-points of gene mutations, clastogenicity (structural chromosome aberrations), and
aneuploidy (numerical chromosome aberrations), taking into account physicochemical properties of substances under consideration.

2.2.1 In vitro tests

Screening should be based on a limited number of tests that are well validated and informative. Genetic toxicity test batteries generally include the following:

1) A test for gene mutation in bacteria (bacterial reverse mutation assay): Organisation for Economic Co-operation and Development (OECD) Guideline 471 recommends the use of at least five strains of bacteria: (i) Salmonella typhimurium TA1535, (ii) S. typhimurium TA1537 or TA97 or TA97a, (iii) S. typhimurium TA98, (iv) S. typhimurium TA100, and (v) Escherichia coli WP2 or E. coli WP2uvrA or S. typhimurium TA102. The choice of additional tests depends on the chemical structure and class of the agent (see section 2.1). Table 1 describes the most commonly used bacterial mutagenicity tests.

2) In vitro mammalian assays: These assays should evaluate the potential of a chemical to produce point mutations, clastogenicity and/or aneugenicity, by using either mammalian cell lines or primary human cell cultures such as fibroblasts or lymphocytes (e.g. mouse lymphoma TK assay or cytogenetic evaluation of chromosomal damage in mammalian cells via in vitro micronucleus test) (see Table 2).

2.2.2 Evaluation of in vitro testing results

- Evaluation of results and classification into (i) positive results, (ii) negative results, and (iii) inconsistent, conflicting, or equivocal results.
  - **Positive**: Substance is positive at one or more end-points of mutagenicity.
  - **Negative**: Substance is negative in all test systems under appropriate in vitro conditions; the substance is not mutagenic (genotoxic) in vitro and is predicted not to be mutagenic in vivo (for exceptions, see Tweats et al., 2007a, 2007b).
  - **Inconsistent, conflicting, or equivocal** (i.e. borderline biological or statistical significance): All other substances.

2.2.3 Follow-up to in vitro testing

- Positive results
  - **In vivo test**: selection of an appropriate end-point; if necessary, further in vitro studies to optimize in vivo testing (e.g. kinetochore staining as an addition in the micronucleus assay of in vitro aneugens).

- Negative results
  - **Further in vivo testing**: required only in the case of “high” or “moderate and sustained” exposure, or for chemicals of high concern.
• Inconsistent, conflicting, or equivocal results
  o Further in vitro testing to clarify positive or negative results; depending on whether
    the situation is resolved by further in vitro testing, proceed according to “Positive” or
    “Negative”.

2.3 In vivo testing

2.3.1 In vivo tests

• In vivo tests (see Table 3) should be chosen carefully to avoid an uninformative outcome.
  Therefore, toxicokinetics, metabolism, chemical reactivity, and mode of action have to be
  considered carefully.

• Typically, a bone marrow micronucleus or clastogenicity test is conducted. However, if
  there are indications that point to a more appropriate assay, then this assay should be
  conducted instead (e.g. mutagenicity study with transgenic animals; comet assay in
  stomach/small intestine/colon if there is no uptake via gastrointestinal tract; comet assay
  in the liver if there is metabolism to toxic species).

2.3.2 Follow-up to in vivo testing

• Positive results
  o “In vivo somatic cell mutagen”. Testing for germ cell mutagenicity (see section 2.4)
    may be required.

• Negative results
  o Further in vivo testing is required only in the case of positive in vitro studies; again,
    the second in vivo test is chosen on a case-by-case basis as stated above. If the test is
    negative, it is concluded that there is no evidence for in vivo mutagenicity.

• Equivocal results
  o Equivocal results may be due to low statistical power, which can be improved by
    increasing the number of treated animals and/or scored cells.
  o If the situation is unresolved, a second in vivo test is required, chosen on a case-by-
    case basis (ordinarily on a different end-point or in a different tissue, depending on
    toxicokinetics, metabolism, and mode of action); proceed according to “Positive” or
    “Negative”.

2.4 Strategy for germ cell testing

When information on the risk to the offspring of exposed individuals is important, the
following germ cell testing strategy is recommended.
For substances that give positive results for mutagenic effects in somatic cells in vivo, their potential to affect germ cells should be considered. If there is toxicokinetic or toxicodynamic evidence that germ cells are actually exposed to the somatic mutagen, it is reasonable to conclude that the substance may also pose a mutagenic hazard to germ cells and thus a risk to future generations.

Where germ cell testing is required, judgement should be used to select the most appropriate test strategy. There are a number of tests available, which fall into two classes:

1) tests in germ cells per se (“class 1”);
2) tests to detect effects in the offspring (or potential offspring) of exposed animals (“class 2”).

Three internationally recognized guidelines are available for such studies:

1) clastogenicity in rodent spermatogonial cells (class 1): OECD Guideline 483 (OECD, 1997f);
2) the dominant lethal test (class 2): OECD Guideline 478 (OECD, 1984);
3) the mouse heritable translocation assay (class 2): OECD Guideline 485 (OECD, 1986).

In order to minimize the use of animals in germ cell testing, it is advisable to start with tests that detect effects in germ cells per se (class 1). These methods include (but are not limited to) gene mutation tests in transgenic animals, gene mutations in the Expanded Simple Tandem Repeat (ESTR) assay, chromosomal assays (including those using fluorescence in situ hybridization [FISH]), comet assay, and DNA adduct analysis.

Following the use of such tests, if quantification of heritable effects is required (class 2), an assay for ESTR mutations can be performed with the offspring of a low number of exposed animals. Tests used historically to investigate transmitted effects (i.e. the heritable translocation test and the specific locus test) can also be performed; however, they use large numbers of animals.

Class 1 and class 2 germ cell assays are summarized in Table 4. The strategy used in germ cell mutagenicity testing is outlined in Figure 2.
3. REFERENCES


detect simultaneously mitotic delay, apoptosis, chromosomal breakage, chromosome loss and


Levin D et al. (1982) A new *Salmonella* tester strain (TA102) with A*T base pairs at the site
of mutation detects oxidative mutagens. *Proceedings of the National Academy of Sciences of
the United States of America*, 79: 7445–7449.

Lewis SE et al. (1986) Dominant visible and electrophoretically expressed mutations induced
in male mice exposed to ethylene oxide by inhalation. *Environmental Mutagenesis*, 8: 867–
872.

Lorge E et al. (2006) SFTG international collaborative study on in vitro micronucleus test. I.

Lorge E et al. (2007) Genetic toxicity assessment employing best science for human safety
evaluation: Performances of the in vitro micronucleus test compared to the mouse lymphoma

Madle S et al. (1994) Recommendations for the performance of UDS tests in vitro and in


Matthews EJ et al. (2006) An analysis of genetic toxicity, reproductive and developmental
toxicity, and carcinogenicity data: I. Identification of carcinogens using surrogate endpoints.

Moore M et al. (2002) Mouse lymphoma thymidine kinase gene mutation assay: follow-up
International Workshop on Genotoxicity Test Procedures, New Orleans, Louisiana, April

Research*, 540: 127–140.


GLOSSARY OF TERMS

Agent
A chemical, biological, or physical entity that contacts a target. [IPCS, 2004]

Aneugen
An agent that induces aneuploidy in cells or organisms. [adapted from IPCS, 2006]

Aneuploidy
A condition in which the chromosome number of a cell or individual differs from a multiple of the haploid component for that species. It is a type of numerical aberration that involves an individual chromosome or chromosomes but not entire set(s) of chromosomes. [adapted from King & Stansfield, 2002; IPCS, 2006]

Apoptosis
Programmed cell death characterized by a series of steps leading to a disintegration of cells into membrane-bound particles that are then eliminated by phagocytosis or by shedding. [modified from Sci-Tech Dictionary]

Bioavailability
The rate and extent to which an agent can be absorbed by an organism and is available for metabolism or interaction with biologically significant receptors. Bioavailability involves both release from a medium (if present) and absorption by an organism. [IPCS, 2004]

Chromosomal aberration
Modification of the normal chromosome complement due to deletion, duplication, or rearrangement of genetic material. [Sci-Tech Dictionary]

Clastogenicity
The capacity to give rise to structural chromosomal aberrations in populations of cells or organisms. [adapted from IPCS, 2006]

Comet assay
The comet assay (single-cell gel electrophoresis) is a simple method for measuring deoxyribonucleic acid (DNA) strand breaks in eukaryotic cells. Cells embedded in agarose on a microscope slide are lysed with detergent and high salt to form nucleoids containing supercoiled loops of DNA linked to the nuclear matrix. Electrophoresis at high pH results in structures resembling comets, observed by fluorescence microscopy; the intensity of the comet tail relative to the head reflects the number of DNA breaks. [Collins, 2004]

Fluorescent in situ hybridization (FISH)
A technique in which a chemically modified DNA (or RNA) probe is hybridized with target DNA, usually present as a chromosome preparation on a microscopic slide. The chemical modification can be visualized using a fluorescent microscope either directly when the modification involves use of a fluorescent dye or indirectly with the use of a fluorescently labelled affinity reagent (e.g. antibody or avidin). Depending upon the type of probe used,
this approach can be used to precisely map genes to a specific region of a chromosome in a prepared karyotype, enumerate chromosomes, or detect chromosomal deletions, translocations, or gene amplifications in cancer cells. [adapted from Sci-Tech Dictionary]

**Gene mutation**
A change of the DNA sequence within a gene of an organism, which may or may not lead to functional alterations in cells. [adapted from American Heritage Dictionaries]

**Genotoxic carcinogen**
Carcinogen whose primary action involves DNA alterations. [adapted from IPCS, 2006]

**Genotoxin**
Used for agents able to alter the structure, information content, or segregation of DNA. [adapted from IPCS, 2006]

**Hazard identification**
The identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system, or (sub)population. Hazard identification is the first stage in hazard assessment and the first of four steps in risk assessment. [IPCS, 2004]

**Heritable translocation test**
A test that detects heritable structural chromosome changes (i.e. translocations) in mammalian germ cells as recovered in first-generation progeny. [European Community, 1988]

**Indicator test**
A test that provides evidence of interaction with DNA and is thus an indicator of mutagenic potential (e.g. test systems for DNA adducts, DNA strand break assays such as the comet assay, sister chromatid exchange, DNA repair tests, unscheduled DNA synthesis). [adapted from Health Canada & Environment Canada, 1986]

**Kinetochore staining**
An immunochemical technique used to detect the presence of centromeric kinetochore proteins in micronuclei and to identify the origin of micronuclei. In all but a few cases, the presence of kinetochore in a micronucleus indicates that it was formed by loss of an entire chromosome, whereas a micronucleus that lacks a kinetochore originated from an acentric chromosome fragment. [adapted from Schuler et al., 1997]

**Micronucleus assay**
The in vitro micronucleus assay is a mutagenicity test system for the detection of chemicals that induce the formation of small membrane-bound DNA fragments, i.e. micronuclei, in the cytoplasm of interphase cells. These micronuclei may originate from acentric fragments (chromosome fragments lacking a centromere) or whole chromosomes that are unable to migrate with the rest of the chromosomes during the anaphase of cell division. [Parry, 1998]
Mode of action (MOA)
A biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. An MOA describes key cytological and biochemical events—that is, those that are both measurable and necessary to the observed effect—in a logical framework. [adapted from Boobis et al., 2006]

Mutagen
An agent capable of giving rise to mutations. [adapted from IPCS, 2006]

Mutagenicity
The capacity to give rise to mutations. [adapted from IPCS, 2006]

Mutation
A heritable alteration in the genetic composition of a cell. In most cases, these involve changes in DNA structure that either have no effect or cause harm. Occasionally a mutation can improve an organism’s chance of surviving and passing the beneficial change on to its descendants. [adapted from IPCS, 2006]

Numerical chromosome mutation
Modification of the normal chromosome complement due to deletion or duplication of whole chromosomes or sets of chromosomes (aneuploidy or polyploidy). [adapted from Sci-Tech Dictionary]

Polyploidy
Used for agents giving rise to numerical chromosomal aberrations in cells or organisms, involving entire set(s) of chromosomes and not only an individual chromosome or chromosomes. [adapted from IPCS, 2006]

Risk assessment
A process intended to calculate or estimate the risk to a given target organism, system, or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system. The risk assessment process includes four steps: hazard identification, hazard characterization, exposure assessment, and risk characterization. It is the first component in a risk analysis process. [IPCS, 2004]

Risk characterization
The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system, or (sub)population, under defined exposure conditions. Risk characterization is the fourth step in the risk assessment process. [IPCS, 2004]

Specific locus test
A technique used to detect recessive induced mutations in diploid organisms; a strain that carries several known recessive mutants in a homozygous condition is crossed with a non-mutant strain that has been treated to induce mutations in its germ cells; induced recessive
mutations allelic with those of the test strain will be expressed in the progeny. [adapted from Sci-Tech Dictionary]

**S phase**
The phase of the cell cycle during which DNA synthesis occurs. [adapted from Medical Dictionary]

**Structural chromosome mutation**
Modification of the normal chromosome complement due to rearrangement of genetic material (breaks, translocations, inversions). [adapted from Sci-Tech Dictionary]

**Target**
Any biological entity that receives an exposure or a dose (e.g. a human, a human population, or a human organ). [IPCS, 2004] In transgenic animal mutation systems, the target organ is the organ of a transgenic animal in which mutagenic effects (increased mutation frequency) were detected after exposure to the test substance. Target organs for carcinogenicity are those organs where tumours arise. [IPCS, 2006]

**Toxicokinetics**
The process of the uptake of potentially toxic substances by the body, the biotransformation they undergo, the distribution of the substances and their metabolites in the tissues, and the elimination of the substances and their metabolites from the body. Both the amounts and the concentrations of the substances and their metabolites are studied. The term has essentially the same meaning as pharmacokinetics, but the latter term should be restricted to the study of pharmaceutical substances. [IPCS, 1994]

**Toxicodynamics**
The process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects. [IPCS, 1994]

**Transgenic**
An experimentally produced organism in which DNA has been artificially introduced and incorporated into the organism’s germline, usually by injecting the foreign DNA into the nucleus of a fertilized embryo. [IPCS, 2006]

**Transgenic animal**
A fertile animal that carries an introduced gene(s) in its germ-line. [WHO, 2002]

**Sources of glossary definitions:**

*The American Heritage Dictionary of the English Language, 4th ed. (Updated in 2007)*
Published by Houghton Mifflin Company.


Strategy in mutagenicity testing

Considerations prior to testing:
- Chemical structure and class of the agent, chemical features such as solubility and stability
- Expected metabolism, reactivity, biological activity, relationship to known genotoxins
- Routes of exposure, bioavailability, and target tissue

In vitro testing (usually two or three from the following):
1) Bacterial test for gene mutation
2) Test for detection of chromosomal mutations, including indications for aneugenicity, e.g.
   a) metaphase analysis
   b) micronucleus test
3) Mammalian cell mutation assay (e.g. mouse lymphoma assay)

Inconsistent, conflicting, or equivocal results within or between tests

Positive

Further in vitro testing may be used to clarify positive/negative results

Negative

Appropriate choice of in vivo tests may be helped by further analysis to determine mode of action (MOA)

In vivo test
Bone marrow micronucleus or clastogenicity assay OR alternative test defined by chemical class/reactivity, MOA, and considering bioavailability and metabolism, etc. (see Table 3)

In vitro assay positive

Equivocal (after appropriate efforts to improve sensitivity)

Additional test(s) in vivo
Choose appropriate test(s), e.g. comet assay, transgenic mutation assay, based on end-point, tissue, route of exposure, etc., as discussed in Table 3

Positive

Additional testing or conclusion based on weight of evidence

Negative

Equivocal (after appropriate efforts to improve sensitivity)

In vivo somatic cell mutagen

No evidence for in vivo mutagenicity

Germ cell testing—see Figure 2

Figure 1. Strategy in mutagenicity testing

Considerations prior to testing:
- Chemical structure and class of the agent, chemical features such as solubility and stability
- Expected metabolism, reactivity, biological activity, relationship to known genotoxins
- Routes of exposure, bioavailability, and target tissue
**Figure 2. Strategy in germ cell mutagenicity testing**

Note that this figure was prepared by two of the experts following the drafting group meeting.
Table 1. Common in vitro bacterial assays.\(^a\)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Strain</th>
<th>End-point</th>
<th>Comments</th>
<th>Published guidelines</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella typhimurium reverse mutation assay</td>
<td>TA1535, TA1537 (or TA97 or TA97a), TA98, TA100</td>
<td>Detect G/C base pair and frame-shift mutations</td>
<td>Contain specific mutations in one of several genes involved in histidine biosynthesis that must be reverted to function normally. Testing with and without appropriate exogenous metabolic activation system. May not detect some oxidizing mutagens and cross-linking agents.</td>
<td>OECD Guideline 471 (replaces old OECD Guidelines 471 and 472)</td>
<td>Ames et al. (1975), Maron &amp; Ames (1983), OECD (1997a)</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>TA102</td>
<td>Detects A/T base pair damage</td>
<td>Detects oxidizing mutagens and cross-linking agents.</td>
<td>OECD Guideline 471</td>
<td>Levin et al. (1982), OECD (1997a)</td>
</tr>
<tr>
<td>Other S. typhimurium mutants</td>
<td>TA98NR (deficient in nitroreductase)</td>
<td></td>
<td>For detection of mutagenicity of nitroaromatic compounds that are bioactivated by NR and NAT.</td>
<td></td>
<td>Josephy et al. (1997) (review)</td>
</tr>
<tr>
<td></td>
<td>TA/981,8-DNP(_6) (deficient in NAT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>YG1024</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>YG1029 (NAT overexpression)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli reverse mutation assay</td>
<td>WP2, WP2uvrA</td>
<td>Detect A/T base pair damage</td>
<td>Detect oxidizing mutagens and cross-linking agents.</td>
<td>OECD Guideline 471</td>
<td>OECD (1997a)</td>
</tr>
</tbody>
</table>

\(^a\) This table was prepared by the Fraunhofer Institute for Toxicology and Experimental Medicine following the drafting group meeting.

3 NAT, N-acetyltransferase; NR, nitroreductase; OECD, Organisation for Economic Co-operation and Development

4
Table 2. Common in vitro mammalian assays.\(^a\)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Method/end-point</th>
<th>Main attributes</th>
<th>Comments</th>
<th>Published guidelines</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse lymphoma thymidine kinase (TK) gene mutation assay</td>
<td>L5178Y mouse lymphoma cell line; using a selective media, the mutant frequencies in treated and untreated cultures are compared with each other.</td>
<td>Detects not only point mutations but also various sizes of chromosome deletions.</td>
<td>Use of positive controls and colony sizing essential for quality control. Evaluation and interpretation changed over the years. Recent protocol updates recommendations. Can be used as alternative to metaphase analysis.</td>
<td>OECD Guideline 476; IWGT guidelines</td>
<td>OECD (1997b), Müller et al. (1999), Moore et al. (2002, 2003, 2007)</td>
</tr>
<tr>
<td>Metaphase analysis (in vitro mammalian chromosome aberration test)</td>
<td>A metaphase-arresting substance (e.g. colchicine) is applied; metaphase cells are analysed for the presence of structural chromosome aberrations.</td>
<td>Detects clastogenicity. Some information on aneugenicity can be obtained with extended culture times.</td>
<td>A variety of cell lines, strains, or primary cell cultures, including human cells, may be used (e.g. Chinese hamster fibroblasts, human or other mammalian peripheral blood lymphocytes) (OECD, 1997d). Chinese hamster cells are now considered by some to be insufficiently sensitive (UKCOM, 2000).</td>
<td>OECD Guideline 473</td>
<td>Parry (1996), OECD (1997c), Aardema et al. (1998)</td>
</tr>
<tr>
<td>Micronucleus test</td>
<td>Detects micronuclei in the cytoplasm of cultured mammalian cells during interphase.</td>
<td>Detects both aneugenic and clastogenic chemicals. Cultured human peripheral blood lymphocytes or Syrian hamster embryo cells may be used.</td>
<td>Several developments in updating the protocol. Immunohistochemical labelling of kinetochores or hybridization with general or chromosome-specific centromeric/telomeric probes gives information on the nature and mechanism of formation of micronuclei induced (whole chromosomes or fragments).</td>
<td>Draft OECD Guideline 487</td>
<td>Kirsch-Volders et al. (1997, 2003), Fenech (2000), Lorge et al. (2006, 2007), OECD (2006)</td>
</tr>
</tbody>
</table>

\(^a\) This table was prepared by the Fraunhofer Institute for Toxicology and Experimental Medicine following the drafting group meeting.
Table 3. Common in vivo genotoxicity assays.

<table>
<thead>
<tr>
<th>Assay</th>
<th>End-point</th>
<th>Main attributes</th>
<th>Comments</th>
<th>Published guidelines</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micronucleus test in erythropoietic cells</td>
<td>Structural and numerical chromosome alterations</td>
<td>Long history, regulatory acceptance, high relevance of end-point.</td>
<td>Has potential for application to other tissues.</td>
<td>OECD Guideline 474</td>
<td>OECD (1997d), Hayashi et al. (2007) and references cited therein</td>
</tr>
<tr>
<td>Metaphase analysis in vivo</td>
<td>Structural and numerical chromosome aberrations</td>
<td>Long history, regulatory acceptance, high relevance of end-point.</td>
<td>Has potential for application to other tissues.</td>
<td>OECD Guideline 475</td>
<td>OECD (1997g)</td>
</tr>
<tr>
<td>Transgenic animal models</td>
<td>Gene mutation</td>
<td>Can be applied to many tissues. Gene specific. No selective pressure on mutations. Relevant end-point.</td>
<td>Need to optimize protocols for different tissues. lacI, lacZ systems not sensitive to the detection of large deletions.</td>
<td>IWGT, IPCS guidance</td>
<td>Heddle et al. (2000), Thybaud et al. (2003), Lambert et al. (2005), IPCS (2006)</td>
</tr>
<tr>
<td>Chemically modified DNA</td>
<td>Covalent DNA adducts, oxidative lesions (e.g. 8-OH-dG)</td>
<td>Can be applied to many tissues. Can be highly sensitive ((^{32})P-post-labelling or AMS) or chemically specific (MS). Other methods include immunochemical techniques, fluorescence, ECD (for 8-OH-dG).</td>
<td>Indicator test detecting premutagenic lesions. Interpretation of results can be complicated.</td>
<td>IWGT guidance</td>
<td>Phillips et al. (2000)</td>
</tr>
<tr>
<td>DNA strand breakage assays (e.g. comet assay)</td>
<td>DNA strand breaks, alkali-labile lesions</td>
<td>Can be applied to many tissues. Incorporation of enzymes can improve specificity. Cell division not required.</td>
<td>Indicator tests. May be unable to detect mutagens that do not produce strand breaks or alkali-labile lesions, but may detect repair-induced breaks. Apoptosis/ necrosis need to be controlled.</td>
<td>IWGT guidance</td>
<td>Tice et al. (2000), Hartmann et al. (2003), Speit &amp; Hartmann (2005), Burlinson et al. (2007)</td>
</tr>
</tbody>
</table>

3 8-OH-dG, 8-hydroxy-2-deoxyguanosine; AMS, accelerator mass spectrometry; DNA, 2-deoxyribonucleic acid; ECD, electrochemical detection; IPCS, International Programme on Chemical Safety, World Health Organization; IWGT, International Workshop on Genotoxicity Testing; MS, mass spectrometry; OECD, Organisation for Economic Co-operation and Development; UDS, unscheduled DNA synthesis
Table 4. Germ cell assays.a

<table>
<thead>
<tr>
<th>Assay</th>
<th>End-point</th>
<th>Main attributesb</th>
<th>Comments</th>
<th>Published guidelines</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1: Tests in germ cells per se</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transgenic animal models</td>
<td>Gene mutation</td>
<td>Gene specific. No selective pressure on mutations. Relevant end-point.</td>
<td>See Table 3</td>
<td>See Table 3</td>
<td>See Table 3</td>
</tr>
<tr>
<td>ESTR assay</td>
<td>Non-coding tandem repeat DNA mutation</td>
<td>Potentially relevant end-point. Detects heritable mutations at ambient exposure levels. Uses relatively few animals. Can be conducted in humans.</td>
<td>Some tandem repeat mutations also occur in, or near, coding genes. Although there are parallels with mutations in coding genes, the human health outcomes require further study.</td>
<td></td>
<td>Dubrova et al. (1998), Yauk (2004), Singer et al. (2006), Gomes-Pereira &amp; Monckton (2006)</td>
</tr>
<tr>
<td>Mammalian spermatogonial chromosome aberration test</td>
<td>Structural chromosome aberrations</td>
<td>Relevant end-point.</td>
<td></td>
<td>OECD Guideline 483; EPA OPPTS 870.5380</td>
<td>Adler (1986)</td>
</tr>
<tr>
<td>FISH assay(s)</td>
<td>Structural chromosome aberrations; sperm aneuploidy</td>
<td>Relevant end-points.</td>
<td></td>
<td>See Table 3</td>
<td>See Table 3</td>
</tr>
<tr>
<td>Comet assay</td>
<td>DNA strand breaks or alkali-labile sites</td>
<td>See Table 3</td>
<td></td>
<td>See Table 3</td>
<td>See Table 3</td>
</tr>
<tr>
<td>Chemically modified DNA</td>
<td>DNA adducts</td>
<td>See Table 3</td>
<td></td>
<td>See Table 3</td>
<td>See Table 3</td>
</tr>
<tr>
<td>Assay</td>
<td>End-point</td>
<td>Main attributes&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Comments</td>
<td>Published guidelines</td>
<td>References</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>---------------------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td><strong>Class 2: Tests to detect effects in the offspring (or potential offspring)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESTR assay</td>
<td>As above for Class 1 tests</td>
<td>As above for Class 1 tests</td>
<td>As above for Class 1 tests</td>
<td>As above for Class 1 tests</td>
<td></td>
</tr>
<tr>
<td>Dominant lethal test</td>
<td>Reduction in viable embryos attributed to chromosome or gene mutations</td>
<td>Relevant end-point. Provides data for quantification of pregnancy loss.</td>
<td></td>
<td>OECD Guideline 478; EPA OPPTS 870.5450</td>
<td>Ehling et al. (1978)</td>
</tr>
<tr>
<td>Mouse visible specific locus test</td>
<td>Gene mutation</td>
<td>Provides data for quantification of inherited mutation frequency. Relevant end-point.</td>
<td></td>
<td>EPA OPPTS 870.5200</td>
<td>Russell et al. (1981)</td>
</tr>
<tr>
<td>Mouse biochemical specific locus test</td>
<td>Gene mutation</td>
<td>Provides data for quantification of inherited mutation frequency. Relevant end-point.</td>
<td></td>
<td>EPA OPPTS 870.5195</td>
<td>Lewis et al. (1986)</td>
</tr>
<tr>
<td>Mouse heritable translocation assay</td>
<td>Structural chromosome aberrations</td>
<td>Provides data for quantification of inherited mutation frequency. Relevant end-point.</td>
<td></td>
<td>OECD Guideline 485</td>
<td>Léonard &amp; Adler (1984)</td>
</tr>
</tbody>
</table>

<sup>a</sup> This table was prepared by two of the experts following the drafting group meeting.  
<sup>b</sup> “Relevant end-point” means relevant to the estimation of human heritable health risk.

---

1. EPA OPPTS, United States Environmental Protection Agency Office of Prevention, Pesticides and Toxic Substances; ESTR, Expanded Simple Tandem Repeat; FISH, fluorescence in situ hybridization

---

---