

September 2007

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34

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.



1 The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture
2 of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO)
3 and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the
4 scientific basis for assessment of the risk to human health and the environment from exposure to
5 chemicals, through international peer review processes, as a prerequisite for the promotion of
6 chemical safety, and to provide technical assistance in strengthening national capacities for the sound
7 management of chemicals.

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

17
18
19
20
21
22
23
24
25
26 © World Health Organization 2007
27

28 All rights reserved. Publications of the World Health Organization can be obtained from WHO
29 Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel: +41 22 791
30 2476; fax: +41 22 791 4857; email: bookorders@who.int). Requests for permission to reproduce or
31 translate WHO publications — whether for sale or for noncommercial distribution — should be
32 addressed to WHO Press, at the above address (fax: +41 22 791 4806; email: permissions@who.int).

33 The designations employed and the presentation of the material in this publication do not imply
34 the expression of any opinion whatsoever on the part of the World Health Organization concerning
35 the legal status of any country, territory, city or area or of its authorities, or concerning the
36 delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for
37 which there may not yet be full agreement.

38 The mention of specific companies or of certain manufacturers' products does not imply that they
39 are endorsed or recommended by the World Health Organization in preference to others of a similar
40 nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are
41 distinguished by initial capital letters.

42 All reasonable precautions have been taken by the World Health Organization to verify the
43 information contained in this publication. However, the published material is being distributed
44 without warranty of any kind, either express or implied. The responsibility for the interpretation and
45 use of the material lies with the reader. In no event shall the World Health Organization be liable for
46 damages arising from its use.

47
48 Technically and linguistically edited by Marla Sheffer, Ottawa, Canada

1	TABLE OF CONTENTS	
2		
3		
4	FOREWORD	1
5	ACKNOWLEDGEMENTS	2
6	LIST OF PARTICIPANTS IN THE WHO/IPCS INTERNATIONAL DRAFTING GROUP	
7	MEETING ON MUTAGENICITY TESTING FOR CHEMICAL RISK ASSESSMENT ..	3
8	LIST OF ACRONYMS AND ABBREVIATIONS	5
9		
10	1. INTRODUCTION	6
11		
12	2. STRATEGY FOR MUTAGENICITY TESTING	7
13	2.1 Development of a testing strategy for a particular compound	7
14	2.2 In vitro testing	7
15	2.2.1 In vitro tests	8
16	2.2.2 Evaluation of in vitro testing results	8
17	2.2.3 Follow-up to in vitro testing	8
18	2.3 In vivo testing	9
19	2.3.1 In vivo tests	9
20	2.3.2 Follow-up to in vivo testing	9
21	2.4 Strategy for germ cell testing	9
22		
23	3. REFERENCES	11
24		
25	GLOSSARY OF TERMS	16
26		
27		

1 FOREWORD

2
3 Harmonization Project Documents are a family of publications by the World Health
4 Organization (WHO) under the umbrella of the International Programme on Chemical Safety
5 (IPCS) (WHO/ILO/UNEP). Harmonization Project Documents complement the
6 Environmental Health Criteria (EHC) methodology (yellow cover) series of documents as
7 authoritative documents on methods for the risk assessment of chemicals.
8

9 The main impetus for the current coordinated international, regional and national efforts on
10 the assessment and management of hazardous chemicals arose from the 1992 United Nations
11 Conference on Environment and Development (UNCED). UNCED Agenda 21, Chapter 19,
12 provides the “blueprint” for the environmentally sound management of toxic chemicals. This
13 commitment by governments was reconfirmed at the 2002 World Summit on Sustainable
14 Development and in 2006 in the Strategic Approach to International Chemicals Management
15 (SAICM). The IPCS project on the Harmonization of Approaches to the Assessment of Risk
16 from Exposure to Chemicals (Harmonization Project) is conducted under Agenda 21, Chapter
17 19, and contributes to the implementation of SAICM. In particular, the project addresses the
18 SAICM objective on Risk Reduction and the SAICM Global Plan of Action activity to
19 “Develop and use new and harmonized methods for risk assessment”.

20
21 The IPCS Harmonization Project goal is *to improve chemical risk assessment globally,*
22 *through the pursuit of common principles and approaches and, hence, strengthen national*
23 *and international management practices that deliver better protection of human health and*
24 *the environment within the framework of sustainability.* The Harmonization Project aims to
25 harmonize global approaches to chemical risk assessment, including by developing
26 international guidance documents on specific issues. The guidance is intended for adoption
27 and use in countries and by international bodies in the performance of chemical risk
28 assessments. The guidance is developed by engaging experts worldwide. The project has
29 been implemented in a step-wise approach, first sharing information and increasing
30 understanding of methods and practices used by various countries, identifying areas where
31 convergence of different approaches would be beneficial, and then developing guidance that
32 enables implementation of harmonized approaches. The project uses a building block
33 approach, focusing at any one time on the aspects of risk assessment that are particularly
34 important for harmonization.
35

36 The project enables risk assessments (or components thereof) to be performed using
37 internationally accepted methods, and these assessments can then be shared to avoid
38 duplication and optimize use of valuable resources for risk management. It also promotes
39 sound science as a basis for risk management decisions, promotes transparency in risk
40 assessment, and reduces unnecessary testing of chemicals. Advances in scientific knowledge
41 can be translated into new harmonized methods.
42

43 This ongoing project is overseen by a geographically representative Harmonization Project
44 Steering Committee and a number of ad hoc Working Groups that manage the detailed work.
45 Finalization of documents includes a rigorous process of international peer review and public
46 comment.

1 ACKNOWLEDGEMENTS

2

3 The World Health Organization thanks the Fraunhofer Institute for Toxicology and
4 Experimental Medicine, Hanover, Germany, for its assistance in preparing for and convening
5 the expert meeting that developed this draft document.

6

1 LIST OF PARTICIPANTS IN THE WHO/IPCS
2 INTERNATIONAL DRAFTING GROUP MEETING ON
3 MUTAGENICITY TESTING FOR CHEMICAL RISK
4 ASSESSMENT

5
6 The international drafting group meeting was hosted by the Fraunhofer Institute for
7 Toxicology and Experimental Medicine, Hanover, Germany, on 11–12 April 2007. The
8 participants at this meeting are listed below.

9
10 **Dr Michael C. Cimino**

11 Biologist, Science Support Branch, Risk Assessment Division, Office of Pollution Prevention
12 and Toxics, United States Environmental Protection Agency, Washington, DC, USA

13
14 **Dr George R. Douglas**

15 Head, Mutagenesis Section, Healthy Environments and Consumer Safety Branch, Health
16 Canada, Ottawa, Canada

17
18 **Professor David A. Eastmond** (*Meeting Chair*)

19 Chair, Environmental Toxicology Graduate Program, University of California, Riverside,
20 CA, USA

21
22 **Professor Andrea Hartwig** (*Meeting Rapporteur*)

23 Institut für Lebensmitteltechnologie und Lebensmittelchemie, Technische Universität Berlin,
24 Berlin, Germany

25
26 **Dr Andreas Luch**

27 Head, Departments of Consumer Safety and Scientific Services, Federal Institute for Risk
28 Assessment, Berlin, Germany

29
30 **Professor David Phillips**

31 Institute of Cancer Research, Sutton, United Kingdom

32
33 **Dr Atsuya Takagi**

34 Cellular & Molecular Toxicology Division, National Institute of Health Sciences, Tokyo,
35 Japan

36
37 **Dr Raymond Tennant**

38 Head, Cancer Biology Group/Laboratory of Molecular Toxicology, National Institute of
39 Environmental Health Sciences, Research Triangle Park, NC, USA

1 *Observer*

2

3 **Professor David Kirkland¹**

4 Vice President, Scientific & Regulatory Consulting, Covance Laboratories Limited,
5 Harrogate, United Kingdom

6

7

8 *Secretariat*

9

10 **Dr Janet Kielhorn**

11 Department of Chemical Risk Assessment, Fraunhofer Institute for Toxicology and
12 Experimental Medicine, Hanover, Germany

13

14 **Ms Carolyn Vickers**

15 Harmonization Project Lead, International Programme on Chemical Safety, World Health
16 Organization, Geneva, Switzerland

17

18

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.

1 LIST OF ACRONYMS AND ABBREVIATIONS

2
3

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

4
5

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

1 aneuploidy (numerical chromosome aberrations), taking into account physicochemical
2 properties of substances under consideration.

3 4 *2.2.1 In vitro tests*

5
6 Screening should be based on a limited number of tests that are well validated and
7 informative. Genetic toxicity test batteries generally include the following:

- 8
9 1) **A test for gene mutation in bacteria (bacterial reverse mutation assay):** Organisation
10 for Economic Co-operation and Development (OECD) Guideline 471 recommends the
11 use of at least five strains of bacteria: (i) *Salmonella typhimurium* TA1535, (ii) *S.*
12 *typhimurium* TA1537 or TA97 or TA97a, (iii) *S. typhimurium* TA98, (iv) *S. typhimurium*
13 TA100, and (v) *Escherichia coli* WP2 or *E. coli* WP2uvrA or *S. typhimurium* TA102. The
14 choice of additional tests depends on the chemical structure and class of the agent (see
15 section 2.1). Table 1 describes the most commonly used bacterial mutagenicity tests.
16
17 2) **In vitro mammalian assays:** These assays should evaluate the potential of a chemical to
18 produce point mutations, clastogenicity and/or aneugenicity, by using either mammalian
19 cell lines or primary human cell cultures such as fibroblasts or lymphocytes (e.g. mouse
20 lymphoma TK assay or cytogenetic evaluation of chromosomal damage in mammalian
21 cells via in vitro micronucleus test) (see Table 2).
22

23 *2.2.2 Evaluation of in vitro testing results*

- 24
25 • Evaluation of results and classification into (i) positive results, (ii) negative results, and
26 (iii) inconsistent, conflicting, or equivocal results.
27
28 ○ *Positive:* Substance is positive at one or more end-points of mutagenicity.
29 ○ *Negative:* Substance is negative in all test systems under appropriate in vitro
30 conditions; the substance is not mutagenic (genotoxic) in vitro and is predicted not to
31 be mutagenic in vivo (for exceptions, see Tweats et al., 2007a, 2007b).
32 ○ *Inconsistent, conflicting, or equivocal* (i.e. borderline biological or statistical
33 significance): All other substances.
34

35 *2.2.3 Follow-up to in vitro testing*

- 36
37 • Positive results
38 ○ In vivo test; selection of an appropriate end-point; if necessary, further in vitro studies
39 to optimize in vivo testing (e.g. kinetochore staining as an addition in the
40 micronucleus assay of in vitro aneugens).
41
42 • Negative results
43 ○ Further in vivo testing is required only in the case of “high” or “moderate and
44 sustained” exposure, or for chemicals of high concern.
45

- 1 • Inconsistent, conflicting, or equivocal results
2 ○ Further in vitro testing to clarify positive or negative results; depending on whether
3 the situation is resolved by further in vitro testing, proceed according to “Positive” or
4 “Negative”.

5 6 7 2.3 In vivo testing

8 9 *2.3.1 In vivo tests*

- 10
11 • In vivo tests (see Table 3) should be chosen carefully to avoid an uninformative outcome.
12 Therefore, toxicokinetics, metabolism, chemical reactivity, and mode of action have to be
13 considered carefully.
14
15 • Typically, a bone marrow micronucleus or clastogenicity test is conducted. However, if
16 there are indications that point to a more appropriate assay, then this assay should be
17 conducted instead (e.g. mutagenicity study with transgenic animals; comet assay in
18 stomach/small intestine/colon if there is no uptake via gastrointestinal tract; comet assay
19 in the liver if there is metabolism to toxic species).

20 21 *2.3.2 Follow-up to in vivo testing*

- 22
23 • Positive results
24 ○ “In vivo somatic cell mutagen”. Testing for germ cell mutagenicity (see section 2.4)
25 may be required.
26
27 • Negative results
28 ○ Further in vivo testing is required only in the case of positive in vitro studies; again,
29 the second in vivo test is chosen on a case-by-case basis as stated above. If the test is
30 negative, it is concluded that there is no evidence for in vivo mutagenicity.
31
32 • Equivocal results
33 ○ Equivocal results may be due to low statistical power, which can be improved by
34 increasing the number of treated animals and/or scored cells.
35 ○ If the situation is unresolved, a second in vivo test is required, chosen on a case-by-
36 case basis (ordinarily on a different end-point or in a different tissue, depending on
37 toxicokinetics, metabolism, and mode of action); proceed according to “Positive” or
38 “Negative”.

39 40 41 2.4 Strategy for germ cell testing

42
43 When information on the risk to the offspring of exposed individuals is important, the
44 following germ cell testing strategy is recommended.

1 For substances that give positive results for mutagenic effects in somatic cells in vivo, their
2 potential to affect germ cells should be considered. If there is toxicokinetic or toxicodynamic
3 evidence that germ cells are actually exposed to the somatic mutagen, it is reasonable to
4 conclude that the substance may also pose a mutagenic hazard to germ cells and thus a risk to
5 future generations.

6
7 Where germ cell testing is required, judgement should be used to select the most appropriate
8 test strategy. There are a number of tests available, which fall into two classes:

- 9
10 1) tests in germ cells per se (“class 1”);
11 2) tests to detect effects in the offspring (or potential offspring) of exposed animals (“class
12 2”).

13
14 Three internationally recognized guidelines are available for such studies:

- 15
16 1) clastogenicity in rodent spermatogonial cells (class 1): OECD Guideline 483 (OECD,
17 1997f);
18 2) the dominant lethal test (class 2): OECD Guideline 478 (OECD, 1984);
19 3) the mouse heritable translocation assay (class 2): OECD Guideline 485 (OECD, 1986).

20
21 In order to minimize the use of animals in germ cell testing, it is advisable to start with tests
22 that detect effects in germ cells per se (class 1). These methods include (but are not limited
23 to) gene mutation tests in transgenic animals, gene mutations in the Expanded Simple
24 Tandem Repeat (ESTR) assay, chromosomal assays (including those using fluorescence in
25 situ hybridization [FISH]), comet assay, and DNA adduct analysis.

26
27 Following the use of such tests, if quantification of heritable effects is required (class 2), an
28 assay for ESTR mutations can be performed with the offspring of a low number of exposed
29 animals. Tests used historically to investigate transmitted effects (i.e. the heritable
30 translocation test and the specific locus test) can also be performed; however, they use large
31 numbers of animals.

32
33 Class 1 and class 2 germ cell assays are summarized in Table 4. The strategy used in germ
34 cell mutagenicity testing is outlined in Figure 2.

1 3. REFERENCES

- 2
- 3 Aardema MJ et al. (1998) Aneuploidy: A report of an ECETOC task force. *Mutation*
- 4 *Research*, 410: 3–79.
- 5
- 6 Adler ID (1986) Clastogenic potential in mouse spermatogonia of chemical mutagens related
- 7 to their cell-cycle specifications. In: Ramel C, Lambert B, Magnusson J, eds. *Genetic*
- 8 *toxicology of environmental chemicals, Part B: Genetic effects and applied mutagenesis*.
- 9 New York, Liss, pp. 477–484.
- 10
- 11 Ames B, McCann J, Yamasaki E (1975) Methods for detecting carcinogens and mutagens
- 12 with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutation Research*, 31: 347–
- 13 364.
- 14
- 15 Ashby J et al. (1996) IPCS harmonization of methods for the prediction and quantification of
- 16 human carcinogenic/mutagenic hazard, and for indicating the probable mechanism of action
- 17 of carcinogens. *Mutation Research*, 352: 153–157.
- 18
- 19 Brambilla G, Martelli A (2003) Failure of the standard battery of short-term tests in detecting
- 20 some rodent and human genotoxic carcinogens. *Toxicology*, 196: 1–19.
- 21
- 22 Burlinson B et al. (2007) Fourth international workgroup on genotoxicity testing: Results of
- 23 the in vivo comet assay workgroup. *Mutation Research*, 627: 31–35.
- 24
- 25 Cimino MC (2006) Comparative overview of current international strategies and guidelines
- 26 for genetic toxicology testing for regulatory purposes. *Environmental and Molecular*
- 27 *Mutagenesis*, 47: 362–390.
- 28
- 29 Dearfield KL, Moore MM (2005) Use of genetic toxicology information for risk assessment.
- 30 *Environmental and Molecular Mutagenesis*, 46: 236–245.
- 31
- 32 Dubrova YE et al. (1998) Stage specificity, dose response, and doubling dose for mouse
- 33 minisatellite germ-line mutation induced by acute radiation. *Proceedings of the National*
- 34 *Academy of Sciences of the United States of America*, 95: 6251–6255.
- 35
- 36 Ehling UH et al. (1978) Standard protocol for the dominant lethal test on male mice set up by
- 37 the work group “Dominant Lethal Mutations of the ad hoc Committee Chemogenetics”.
- 38 *Archives of Toxicology*, 39: 173–185.
- 39
- 40 Fenech M (2000) The in vitro micronucleus technique. *Mutation Research*, 455: 81–95.
- 41
- 42 Gomes-Pereira M, Monckton DG (2006) Chemical modifiers of unstable expanded simple
- 43 tandem repeats: What goes up, must come down. *Mutation Research*, 598: 15–34.
- 44
- 45 Haines GA et al. (2002) Germ cell and dose-dependent DNA damage measured by the comet
- 46 assay in murine spermatozoa after testicular X-irradiation. *Biology of Reproduction*, 67: 854–
- 47 861.

- 1
2 Hartmann A et al. (2003) Recommendations for conducting the in vivo alkaline Comet assay.
3 4th International Comet Assay Workshop. *Mutagenesis*, 18: 45–51.
4
5 Hayashi M et al. (2007) In vivo erythrocyte micronucleus assay. III. Validation and
6 regulatory acceptance of automated scoring and the use of rat peripheral blood reticulocytes,
7 with discussion of non-hematopoietic target cells and a single dose-level limit test. *Mutation*
8 *Research*, 627: 10–30.
9
10 Heddle JA et al. (2000) In vivo transgenic mutation assays. *Environmental and Molecular*
11 *Mutagenesis*, 35: 253–259.
12
13 Hill FS et al. (2003) A new FISH assay to simultaneously detect structural and numerical
14 chromosomal abnormalities in mouse sperm. *Molecular Reproduction and Development*, 66:
15 172–180.
16
17 Horak S, Polanska J, Widlak P (2003) Bulky DNA adducts in human sperm: Relationship
18 with fertility, semen quality, smoking, and environmental factors. *Mutation Research*, 537:
19 53–65.
20
21 ICH Steering Committee (1997) *Guidance for industry. S2B Genotoxicity: A standard battery*
22 *for genotoxicity testing of pharmaceuticals*. International Conference on Harmonisation of
23 Technical Requirements for Registration of Pharmaceuticals for Human Use, pp. 1–8
24 (<http://www.fda.gov/CDER/GUIDANCE/1856fnl.pdf>).
25
26 IPCS (2006) *Transgenic animal mutagenicity assays*. Geneva, World Health Organization,
27 International Programme on Chemical Safety (Environmental Health Criteria 233).
28
29 Josephy PD, Gruz P, Nohmi T (1997) Recent advances in the construction of bacterial
30 genotoxicity assays. *Mutation Research*, 386: 1–23.
31
32 Kirkland D et al. (2003) Summary of major conclusions. *Mutation Research*, 240: 123–125.
33
34 Kirkland D et al. (2005) Evaluation of the ability of a battery of three in vitro genotoxicity
35 tests to discriminate rodent carcinogens and non-carcinogens. I. Sensitivity, specificity and
36 relative predictivity. *Mutation Research*, 584: 1–256.
37
38 Kirkland D et al. (2006a) Evaluation of the ability of a battery of three in vitro genotoxicity
39 tests to discriminate rodent carcinogens and non-carcinogens. II. Further analysis of
40 mammalian cell results, relative predictivity and tumour profiles. *Mutation Research*, 608:
41 29–42.
42
43 Kirkland DJ et al. (2006b) Summary of major conclusions from the 4th IWGT, San
44 Francisco, 9–10 September, 2005. *Mutation Research*, 627: 5–9.
45

- 1 Kirsch-Volders M et al. (1997) The in vitro micronucleus test: a multi-endpoint assay to
2 detect simultaneously mitotic delay, apoptosis, chromosomal breakage, chromosome loss and
3 non-disjunction. *Mutation Research*, 392: 19–30.
4
- 5 Kirsch-Volders M et al. (2003) Report from the in vitro micronucleus assay working group.
6 *Mutation Research*, 540: 153–163.
7
- 8 Lambert IB et al. (2005) Detailed review of transgenic rodent mutation assays. *Mutation*
9 *Research*, 590: 1–280.
10
- 11 Léonard A, Adler ID (1984) Test for heritable translocations in male mammals. In: Kilbey BJ
12 et al., eds. *Handbook on mutagenicity test procedures*. Amsterdam, Elsevier, pp. 485–494.
13
- 14 Levin D et al. (1982) A new *Salmonella* tester strain (TA102) with A*T base pairs at the site
15 of mutation detects oxidative mutagens. *Proceedings of the National Academy of Sciences of*
16 *the United States of America*, 79: 7445–7449.
17
- 18 Lewis SE et al. (1986) Dominant visible and electrophoretically expressed mutations induced
19 in male mice exposed to ethylene oxide by inhalation. *Environmental Mutagenesis*, 8: 867–
20 872.
21
- 22 Lorge E et al. (2006) SFTG international collaborative study on in vitro micronucleus test. I.
23 General conditions and overall conclusions of the study. *Mutation Research*, 607: 13–36.
24
- 25 Lorge E et al. (2007) Genetic toxicity assessment employing best science for human safety
26 evaluation: Performances of the in vitro micronucleus test compared to the mouse lymphoma
27 assay and the in vitro chromosome aberration assay. *Toxicological Sciences*, 96: 214–217.
28
- 29 Madle S et al. (1994) Recommendations for the performance of UDS tests in vitro and in
30 vivo. *Mutation Research*, 312: 263–285.
31
- 32 Maron DM, Ames BN (1983) Revised methods for the *Salmonella* mutagenicity test.
33 *Mutation Research*, 113: 173–215.
34
- 35 Matthews EJ et al. (2006) An analysis of genetic toxicity, reproductive and developmental
36 toxicity, and carcinogenicity data: I. Identification of carcinogens using surrogate endpoints.
37 *Regulatory Toxicology and Pharmacology*, 44: 83–96.
38
- 39 Moore M et al. (2002) Mouse lymphoma thymidine kinase gene mutation assay: follow-up
40 International Workshop on Genotoxicity Test Procedures, New Orleans, Louisiana, April
41 2000. *Environmental and Molecular Mutagenesis*, 40: 292–299.
42
- 43 Moore M et al. (2003) Mouse lymphoma thymidine kinase gene mutation assay: International
44 Workshop on Genotoxicity Tests Workgroup report—Plymouth, UK 2002. *Mutation*
45 *Research*, 540: 127–140.
46

- 1 Moore MM et al. (2007) Mouse lymphoma thymidine kinase gene mutation assay: Meeting
2 of the international workshop on genotoxicity testing—San Francisco, 2005,
3 Recommendations for 24-h treatment. *Mutation Research*, 627: 36–40.
4
- 5 Müller L et al. (1999) ICH-harmonised guidances on genotoxicity testing of pharmaceuticals:
6 evolution, reasoning and impact. *Mutation Research*, 436: 195–225.
7
- 8 OECD (1984) *Genetic toxicology: Rodent dominant lethal test*. Paris, Organisation for
9 Economic Co-operation and Development (OECD Guideline 478).
10
- 11 OECD (1986) *Genetic toxicology: Mouse heritable translocation assay*. Paris, Organisation
12 for Economic Co-operation and Development (OECD Guideline 485).
13
- 14 OECD (1997a) *Bacterial reverse mutation test*. Paris, Organisation for Economic Co-
15 operation and Development (OECD Guideline 471).
16
- 17 OECD (1997b) *In vitro mammalian cell gene mutation test*. Paris, Organisation for Economic
18 Co-operation and Development (OECD Guideline 476).
19
- 20 OECD (1997c) *In vitro mammalian chromosome aberration test*. Paris, Organisation for
21 Economic Co-operation and Development (OECD Guideline 473).
22
- 23 OECD (1997d) *Mammalian erythrocyte micronucleus test*. Paris, Organisation for Economic
24 Co-operation and Development (OECD Guideline 474).
25
- 26 OECD (1997e) *Unscheduled DNA synthesis (UDS) test with mammalian liver cells in vivo*.
27 Paris, Organisation for Economic Co-operation and Development (OECD Guideline 486).
28
- 29 OECD (1997f) *Mammalian spermatogonial chromosome aberration test*. Paris, Organisation
30 for Economic Co-operation and Development (OECD Guideline 483).
31
- 32 OECD (1997g) *Mammalian bone marrow chromosome aberration test*. Paris, Organisation
33 for Economic Co-operation and Development (OECD Guideline 475).
34
- 35 OECD (2006) *OECD guideline for the testing of chemicals. Draft proposal for a new*
36 *Guideline 487. In vitro micronucleus test*. Paris, Organisation for Economic Co-operation and
37 Development, pp. 1–16 (Draft Guideline 487, 2nd version, 21 December 2006).
38
- 39 Parry JM, ed. (1996) Molecular cytogenetics. *Mutation Research*, 372: 151–294.
40
- 41 Phillips D et al. (2000) Methods of DNA adduct determination and their application to testing
42 compounds for genotoxicity. *Environmental and Molecular Mutagenesis*, 35: 222–233.
43
- 44 Russell LB et al. (1981) The mouse specific locus test with agents other than radiations:
45 interpretation of data and recommendations for future work. *Mutation Research*, 86: 329–
46 354.
47

- 1 Singer TM et al. (2006) Detection of induced male germline mutation: Correlations and
2 comparisons between traditional germline mutation assays, transgenic rodent assays and
3 expanded simple tandem repeat instability assays. *Mutation Research*, 598: 164–193.
4
- 5 Speit G, Hartmann A (2005) The comet assay: a sensitive genotoxicity test for the detection
6 of DNA damage. *Methods in Molecular Biology*, 291: 85–95.
7
- 8 Thybaud V et al. (2003) In vivo transgenic mutation assays. *Mutation Research*, 540: 141–
9 151.
10
- 11 Thybaud V et al. (2007) Strategy for genotoxicity testing: hazard identification and risk
12 assessment in relation to in vitro testing. *Mutation Research*, 627: 41–58.
13
- 14 Tice RR et al. (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic
15 toxicology testing. *Environmental and Molecular Mutagenesis*, 35: 206–221.
16
- 17 Tweats DJ et al. (2007a) Report of the IWGT working group on strategies and interpretation
18 of regulatory in vivo tests. I. Increases in micronucleated bone marrow cells in rodents that
19 do not indicate genotoxic hazards. *Mutation Research*, 627: 78–91.
20
- 21 Tweats DJ et al. (2007b) Report of the IWGT working group on strategy/interpretation for
22 regulatory in vivo tests. II. Identification of in vivo-only positive compounds in the bone
23 marrow micronucleus test. *Mutation Research*, 627: 92–105.
24
- 25 UKCOM (2000) *Guidance on a strategy for testing of chemicals for mutagenicity*. United
26 Kingdom Committee on Mutagenicity of Chemicals in Food, Consumer Products and the
27 Environment, pp. 1–36 (<http://www.dh.gov.uk/assetRoot/04/07/71/96/04077196.pdf>).
28
- 29 USFDA (2000) *Toxicological principles for the safety assessment of food ingredients*.
30 *Redbook 2000. IV.C.1. Short-term tests for genetic toxicity*. United States Food and Drug
31 Administration, Center for Food Safety and Applied Nutrition, Office of Food Additive
32 Safety, 5 pp. (<http://www.cfsan.fda.gov/~redbook/red-ivc1.html>).
33
- 34 Wyrobek AJ, Adler ID (1996) Detection of aneuploidy in human and rodent sperm using
35 FISH and applications of sperm assays of genetic damage in heritable risk evaluation.
36 *Mutation Research*, 352: 173–179.
37
- 38 Yauk CL (2004) Advances in the application of germline tandem repeat instability for in situ
39 monitoring. *Mutation Research*, 566: 169–182.
40

1 GLOSSARY OF TERMS

2 **Agent**

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

4 **Aneugen**

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

6 **Aneuploidy**

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

11 **Apoptosis**

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

15 **Bioavailability**

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

19 **Chromosomal aberration**

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

22 **Clastogenicity**

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

25 **Comet assay**

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

32 **Fluorescent in situ hybridization (FISH)**

33 A technique in which a chemically modified DNA (or RNA) probe is hybridized with target
34 DNA, usually present as a chromosome preparation on a microscopic slide. The chemical
35 modification can be visualized using a fluorescent microscope either directly when the
36 modification involves use of a fluorescent dye or indirectly with the use of a fluorescently
37 labelled affinity reagent (e.g. antibody or avidin). Depending upon the type of probe used,
38

1 this approach can be used to precisely map genes to a specific region of a chromosome in a
2 prepared karyotype, enumerate chromosomes, or detect chromosomal deletions,
3 translocations, or gene amplifications in cancer cells. [adapted from Sci-Tech Dictionary]
4

5 **Gene mutation**

6 A change of the DNA sequence within a gene of an organism, which may or may not lead to
7 functional alterations in cells. [adapted from American Heritage Dictionaries]
8

9 **Genotoxic carcinogen**

10 Carcinogen whose primary action involves DNA alterations. [adapted from IPCS, 2006]
11

12 **Genotoxin**

13 Used for agents able to alter the structure, information content, or segregation of DNA.
14 [adapted from IPCS, 2006]
15

16 **Hazard identification**

17 The identification of the type and nature of adverse effects that an agent has an inherent
18 capacity to cause in an organism, system, or (sub)population. Hazard identification is the first
19 stage in hazard assessment and the first of four steps in risk assessment. [IPCS, 2004]
20

21 **Heritable translocation test**

22 A test that detects heritable structural chromosome changes (i.e. translocations) in
23 mammalian germ cells as recovered in first-generation progeny. [European Community,
24 1988]
25

26 **Indicator test**

27 A test that provides evidence of interaction with DNA and is thus an indicator of mutagenic
28 potential (e.g. test systems for DNA adducts, DNA strand break assays such as the comet
29 assay, sister chromatid exchange, DNA repair tests, unscheduled DNA synthesis). [adapted
30 from Health Canada & Environment Canada, 1986]
31

32 **Kinetochores staining**

33 An immunochemical technique used to detect the presence of centromeric kinetochores
34 proteins in micronuclei and to identify the origin of micronuclei. In all but a few cases, the
35 presence of kinetochores in a micronucleus indicates that it was formed by loss of an entire
36 chromosome, whereas a micronucleus that lacks a kinetochores originated from an acentric
37 chromosome fragment. [adapted from Schuler et al., 1997]
38

39 **Micronucleus assay**

40 The in vitro micronucleus assay is a mutagenicity test system for the detection of chemicals
41 that induce the formation of small membrane-bound DNA fragments, i.e. micronuclei, in the
42 cytoplasm of interphase cells. These micronuclei may originate from acentric fragments
43 (chromosome fragments lacking a centromere) or whole chromosomes that are unable to
44 migrate with the rest of the chromosomes during the anaphase of cell division. [Parry, 1998]
45

1 **Mode of action (MOA)**

2 A biologically plausible sequence of key events leading to an observed effect supported by
3 robust experimental observations and mechanistic data. An MOA describes key cytological
4 and biochemical events—that is, those that are both measurable and necessary to the
5 observed effect—in a logical framework. [adapted from Boobis et al., 2006]

6
7 **Mutagen**

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

9
10 **Mutagenicity**

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

12
13 **Mutation**

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

18
19 **Numerical chromosome mutation**

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

23
24 **Polyploidy**

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

28
29 **Risk assessment**

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

36
37 **Risk characterization**

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

42
43 **Specific locus test**

44 A technique used to detect recessive induced mutations in diploid organisms; a strain that
45 carries several known recessive mutants in a homozygous condition is crossed with a non-
46 mutant strain that has been treated to induce mutations in its germ cells; induced recessive

1 mutations allelic with those of the test strain will be expressed in the progeny. [adapted from
2 Sci-Tech Dictionary]

3
4 **S phase**

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

7
8 **Structural chromosome mutation**

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

11
12 **Target**

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

18
19 **Toxicokinetics**

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

26
27 **Toxicodynamics**

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

30
31 **Transgenic**

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

35
36 **Transgenic animal**

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

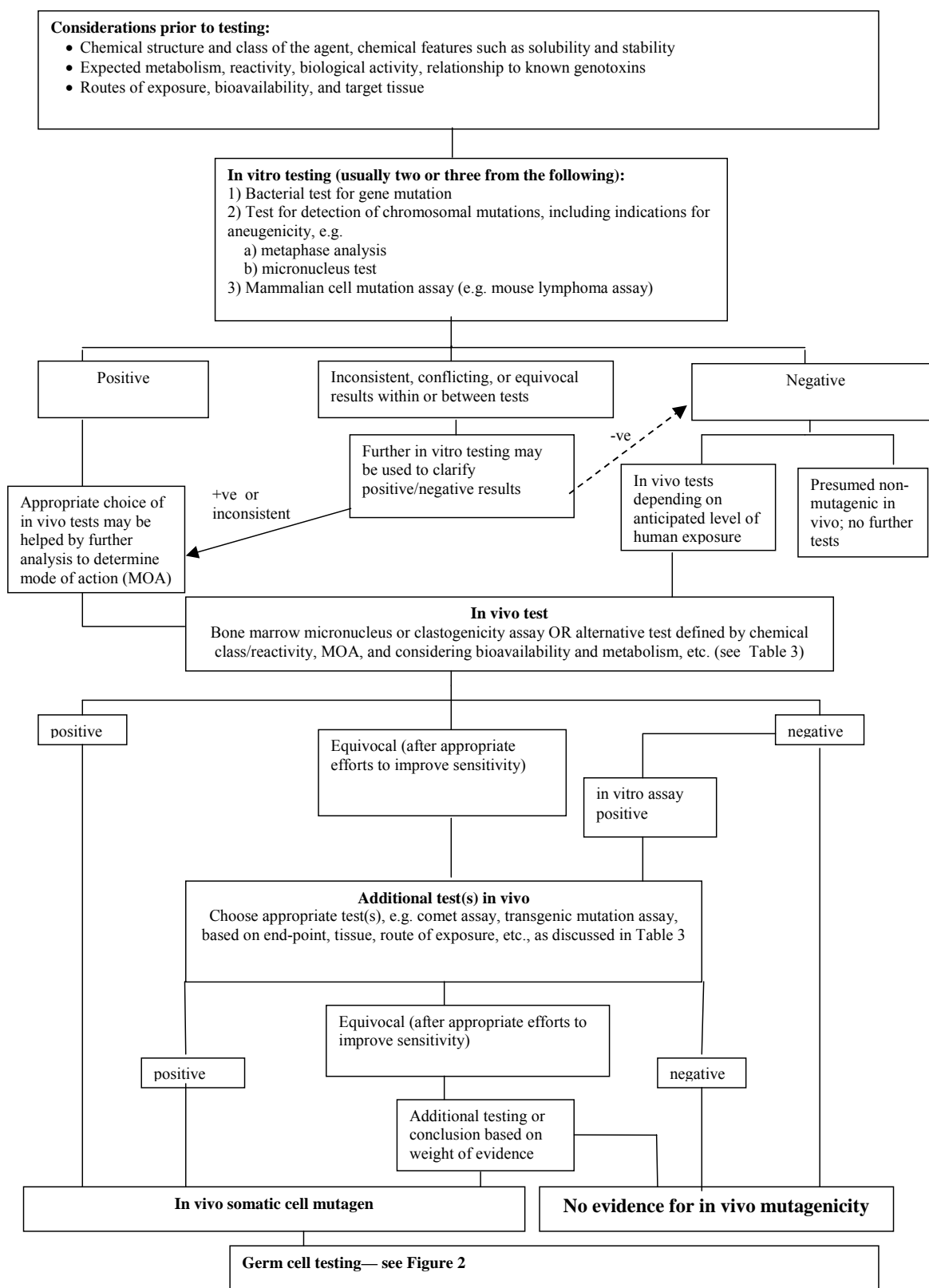
38
39
40 **Sources of glossary definitions:**

41
42 *The American Heritage Dictionary of the English Language*, 4th ed. (Updated in 2007)
43 Published by Houghton Mifflin Company.

44
45 *The American Heritage Stedman's Medical Dictionary* (2007) Published by Houghton
46 Mifflin Company (<http://www.answers.com/library/Medical%20Dictionary>). [Medical
47 Dictionary]

- 1
2 Boobis AR et al. (2006) IPCS framework for analyzing the relevance of a cancer mode of
3 action for humans. *Critical Reviews in Toxicology*, 36: 781–792.
4
5 Collins AR (2004) The comet assay for DNA damage and repair: Principles, applications and
6 limitations. *Molecular Biotechnology*, 26: 249–261.
7
8 European Community (1988) Mouse heritable translocation. *Official Journal of the European*
9 *Community*, L 133 ([http://ecb.jrc.it/documents/Testing-](http://ecb.jrc.it/documents/Testing-Methods/ANNEXV/B25web1988.pdf)
10 [Methods/ANNEXV/B25web1988.pdf](http://ecb.jrc.it/documents/Testing-Methods/ANNEXV/B25web1988.pdf)).
11
12 Health Canada, Environment Canada (1986) *Guidelines on the Use of Mutagenicity Tests in*
13 *the Toxicological Evaluation of Chemicals*. Ottawa, Ontario, Department of National Health
14 and Welfare and Department of Environment [reprinted in *Environmental and Molecular*
15 *Mutagenesis*, 11: 261–304, 1988].
16
17 IPCS (1994) *Assessing Human Health Risks of Chemicals: Derivation of Guidance Values*
18 *for Health-based Exposure Limits*. Geneva, World Health Organization, International
19 Programme on Chemical Safety (Environmental Health Criteria 170).
20
21 IPCS (2004) *IPCS Risk Assessment Terminology*. Geneva, World Health Organization,
22 International Programme on Chemical Safety (Harmonization Project Document No. 1).
23
24 IPCS (2006) *Transgenic Animal Mutagenicity Assays*. Geneva, World Health Organization,
25 International Programme on Chemical Safety (Environmental Health Criteria 233).
26
27 King RC, Stansfield WD (2002) *A Dictionary of Genetics*, 6th ed. New York, Oxford
28 University Press.
29
30 *McGraw-Hill Dictionary of Scientific and Technical Terms*, 6th ed. Published by The
31 McGraw-Hill Companies, Inc.
32 (<http://www.answers.com/library/Sci%252DTech+Dictionary>). [Sci-Tech Dictionary]
33
34 Parry JM (1998) *A Proposal for a New OECD Guideline for the In Vitro Micronucleus Test*.
35 Swansea, University of Wales (<http://www.swan.ac.uk/cget/ejgt/article1.htm>).
36
37 Schuler M, Rupa DS, Eastmond DA (1997) A critical evaluation of centromeric labelling to
38 distinguish micronuclei induced by chromosome loss and breakage in vitro. *Mutation*
39 *Research*, 392: 81–95.
40
41 WHO (2002) *Genomics and World Health. Report of the Advisory Committee on Health*
42 *Research*. Geneva, World Health Organization.
43

Strategy in mutagenicity testing



1
2 **Figure 1. Strategy in mutagenicity testing**

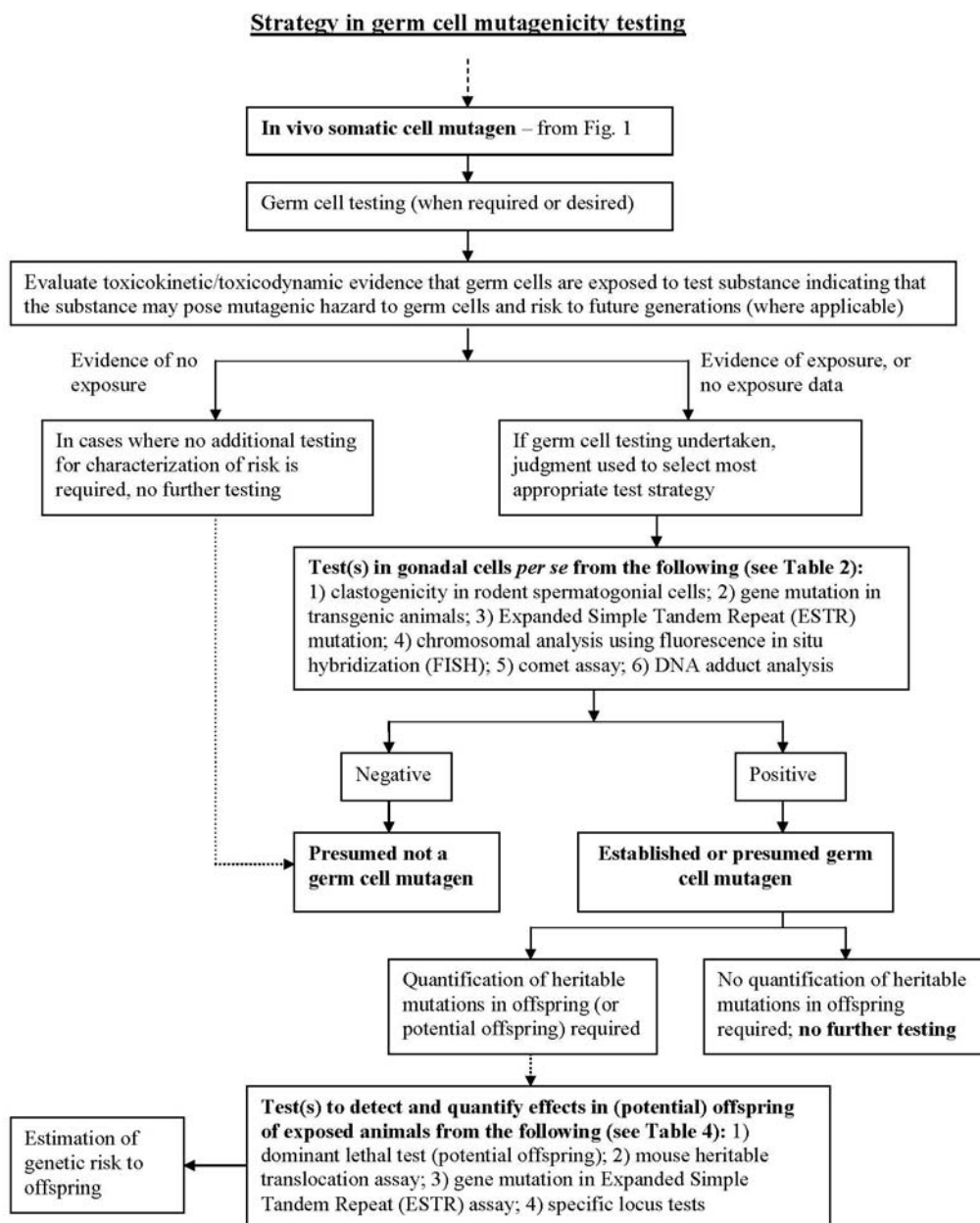


Figure 2. Strategy in germ cell mutagenicity testing

Note that this figure was prepared by two of the experts following the drafting group meeting.

1
2
3
4
5
6
7
8

Table 1. Common in vitro bacterial assays.^a

Assay	Strain	End-point	Comments	Published guidelines	References
<i>Salmonella typhimurium</i> reverse mutation assay	TA1535, TA1537 (or TA97 or TA97a), TA98, TA100	Detect G/C base pair and frame-shift mutations	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.	OECD Guideline 471 (replaces old OECD Guidelines 471 and 472)	Ames et al. (1975), Maron & Ames (1983), OECD (1997a)
<i>S. typhimurium</i>	TA102	Detects A/T base pair damage	Detects oxidizing mutagens and cross-linking agents.	OECD Guideline 471	Levin et al. (1982), OECD (1997a)
Other <i>S. typhimurium</i> mutants	TA98NR (deficient in nitroreductase) TA/981,8-DNP ₆ (deficient in NAT) YG1024 YG1029 (NAT overexpression)		For detection of mutagenicity of nitroaromatic compounds that are bioactivated by NR and NAT. More sensitive than conventional strains. Used for detecting mutagenicity of toxic pollutants in air, water, and food.		Josephy et al. (1997) (review)
<i>Escherichia coli</i> reverse mutation assay	WP2, WP2uvrA	Detect A/T base pair damage	Detect oxidizing mutagens and cross-linking agents.	OECD Guideline 471	OECD (1997a)

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

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

Table 2. Common in vitro mammalian assays.^a

Assay	Method/end-point	Main attributes	Comments	Published guidelines	References
Mouse lymphoma thymidine kinase (TK) gene mutation assay	L5178Y mouse lymphoma cell line; using a selective media, the mutant frequencies in treated and untreated cultures are compared with each other.	Detects not only point mutations but also various sizes of chromosome deletions.	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.	OECD Guideline 476; IWGT guidelines	OECD (1997b), Müller et al. (1999), Moore et al. (2002, 2003, 2007)
Metaphase analysis (in vitro mammalian chromosome aberration test)	A metaphase-arresting substance (e.g. colchicine) is applied; metaphase cells are analysed for the presence of structural chromosome aberrations.	Detects clastogenicity. Some information on aneugenicity can be obtained with extended culture times.	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).	OECD Guideline 473	Parry (1996), OECD (1997c), Aardema et al. (1998)
Micronucleus test	Detects micronuclei in the cytoplasm of cultured mammalian cells during interphase.	Detects both aneugenic and clastogenic chemicals. Cultured human peripheral blood lymphocytes or Syrian hamster embryo cells may be used.	Several developments in updating the protocol. Immunochemical 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).	Draft OECD Guideline 487	Kirsch-Volders et al. (1997, 2003), Fenech (2000), Lorge et al. (2006, 2007), OECD (2006)

FISH, fluorescence in situ hybridization; TK, thymidine kinase

^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.

Assay	End-point	Main attributes	Comments	Published guidelines	References
Micronucleus test in erythropoietic cells	Structural and numerical chromosome alterations	Long history, regulatory acceptance, high relevance of end-point.	Has potential for application to other tissues.	OECD Guideline 474	OECD (1997d), Hayashi et al. (2007) and references cited therein
Metaphase analysis in vivo	Structural and numerical chromosome aberrations.	Long history, regulatory acceptance, high relevance of end-point.	Has potential for application to other tissues.	OECD Guideline 475	OECD (1997g)
Transgenic animal models	Gene mutation	Can be applied to many tissues. Gene specific. No selective pressure on mutations. Relevant end-point.	Need to optimize protocols for different tissues. <i>lacI</i> , <i>lacZ</i> systems not sensitive to the detection of large deletions.	IWGT, IPCS guidance	Heddle et al. (2000), Thybaud et al. (2003), Lambert et al. (2005), IPCS (2006)
Chemically modified DNA	Covalent DNA adducts, oxidative lesions (e.g. 8-OH-dG)	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).	Indicator test detecting premutagenic lesions. Interpretation of results can be complicated.	IWGT guidance	Phillips et al. (2000)
DNA strand breakage assays (e.g. comet assay)	DNA strand breaks, alkali-labile lesions	Can be applied to many tissues. Incorporation of enzymes can improve specificity. Cell division not required.	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.	IWGT guidance	Tice et al. (2000), Hartmann et al. (2003), Speit & Hartmann (2005), Burlinson et al. (2007)
Liver UDS	Thymidine incorporation outside S phase	Long history of use; useful for some classes of chemicals.	Indicator test detecting repair activity. Uncertain acceptability and questionable sensitivity. Limited use in other tissues.	OECD Guideline 486	Madle et al. (1994), OECD (1997e)

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

1
2**Table 4. Germ cell assays.^a**

Assay	End-point	Main attributes^b	Comments	Published guidelines	References
Class 1: Tests in germ cells per se					
Transgenic animal models	Gene mutation	Gene specific. No selective pressure on mutations. Relevant end-point.	See Table 3	See Table 3	See Table 3
ESTR assay	Non-coding tandem repeat DNA mutation	Potentially relevant end-point. Detects heritable mutations at ambient exposure levels. Uses relatively few animals. Can be conducted in humans.	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.		Dubrova et al. (1998), Yauk (2004), Singer et al. (2006), Gomes-Pereira & Monckton (2006)
Mammalian spermatogonial chromosome aberration test	Structural chromosome aberrations	Relevant end-point.		OECD Guideline 483; EPA OPPTS 870.5380	Adler (1986)
FISH assay(s)	Structural chromosome aberrations; sperm aneuploidy	Relevant end-points.	See Table 3	See Table 3	Wyrobek & Adler (1996), Hill et al. (2003)
Comet assay	DNA strand breaks or alkali-labile sites	See Table 3	See Table 3	See Table 3	Haines et al. (2002)
Chemically modified DNA	DNA adducts	See Table 3	See Table 3	See Table 3	Horak et al. (2003)

<i>Assay</i>	<i>End-point</i>	<i>Main attributes^b</i>	<i>Comments</i>	<i>Published guidelines</i>	<i>References</i>
Class 2: Tests to detect effects in the offspring (or potential offspring)					
ESTR assay	As above for Class 1 tests	As above for Class 1 tests	As above for Class 1 tests		As above for Class 1 tests
Dominant lethal test	Reduction in viable embryos attributed to chromosome or gene mutations	Relevant end-point. Provides data for quantification of pregnancy loss.		OECD Guideline 478; EPA OPPTS 870.5450	Ehling et al. (1978)
Mouse visible specific locus test	Gene mutation	Provides data for quantification of inherited mutation frequency. Relevant end-point.	Uses large number of animals.	EPA OPPTS 870.5200	Russell et al. (1981)
Mouse biochemical specific locus test	Gene mutation	Provides data for quantification of inherited mutation frequency. Relevant end-point.	Uses large number of animals.	EPA OPPTS 870.5195	Lewis et al. (1986)
Mouse heritable translocation assay	Structural chromosome aberrations	Provides data for quantification of inherited mutation frequency. Relevant end-point.	Uses large number of animals.	OECD Guideline 485	Léonard & Adler (1984)

1 EPA OPPTS, United States Environmental Protection Agency Office of Prevention, Pesticides and Toxic Substances; ESTR, Expanded Simple

2 Tandem Repeat; FISH, fluorescence in situ hybridization

3 ^a This table was prepared by two of the experts following the drafting group meeting.

4 ^b “Relevant end-point” means relevant to the estimation of human heritable health risk.

5