

---

## Preface I

Evaluation of potential mutagenic activity is a critical step in the assessment of the safety of both new and preexisting chemical types. Such assessments are critical in the development of new pharmaceuticals and consumer products. The UK Environmental Mutagen Society has identified an unsatisfied demand for education in the discipline of genetic toxicology, which provides the academic basis for the science behind mutagenicity testing.

To provide for education in genetic toxicology, the UKEMS is sponsoring postgraduate education and the production of both electronic and printed materials.

This book covers three basic areas: the scientific basis of the discipline, the methodologies of the main test assays, and the application of the methods.

The text is aimed primarily at workers in the safety departments of the industries working with both natural and synthetic chemicals. Such workers need to undertake continual updating in assay methods and their application. Changes in regulations for the assessment of chemical safety in areas such as the EU are resulting in substantial increases in the demand for mutagenicity testing. We aim to provide support to both laboratory workers in providing quality information on the appropriate application of techniques and to study directors in their assay selection and protocol design. The text will provide information for both individuals undertaking personal study programs and for those undertaking formal qualifications in genetic toxicology.

Jim Parry was a leading light and inspiration in this field and my lifelong collaborator. The project was devised, driven, and produced due to his unbounded enthusiasm, and he would have relished its completion. This book is dedicated to his memory.

*Elizabeth M. Parry*



---

## Preface II

Mutation is a broad term covering a whole range of changes to the informational molecule, DNA (made up of the four nucleotides: the purines, adenine and guanine, and the pyrimidines, thymine and cytosine) packaged into chromosomes, of an organism from point/gene changes to modifications of the number and/or structure of chromosomes.

Point/gene mutations are changes to the sequence of nucleotides and may involve the substitution of individual bases (classified as base substitution mutations). When one purine nucleotide is replaced by another purine or a pyrimidine by a pyrimidine, it is called a transition mutation. When a purine is replaced by pyrimidine or vice versa, the mutation is called a transversion.

Since the genetic code is degenerate, not all base substitutions will result in coding changes leading to changes at the protein level. When mutations result in amino acid changes, they are classified as missense, and when they lead to a codon that terminates protein production, they are classified as nonsense mutations.

Mutations involving the loss or gain of DNA may range from a single base pair change, called a frameshift mutation, to many bases (often megabases), called deletion or duplications, or to whole chromosome changes called aneuploidy. All these types of change are produced spontaneously during the life cycle of living organisms and may also be produced by exposure to some chemicals which interact with the DNA (adduct formation) and to ionizing radiation and nonionizing radiation such as ultra violet light. Such chemical and physical agents are classified as mutagens and/or genotoxins.

Not all the DNA in a cell carries coded information for protein synthesis. Some of it is noncoding and is important for chromosome structure.

Although all cells of an organism contain the same DNA, somatic cells in different organs and tissues of the adult body become specialized to perform defined functions so that only some parts of the genome are expressed. A common feature of mutations in cancer causing genes, such as those controlling cell division and proliferation, is that this results in genes being expressed in the wrong tissue at the wrong time. The effect of a mutation will depend upon the position of the mutation within the DNA and the location and activity of the particular gene in which the mutation has been induced.

Mutations in the many genes that have been implicated in the multistage events leading to cancer can be produced by a variety of mechanisms and interactions and modifications of the genetic material, as is illustrated by the molecular changes in the DNA that occur in the progression of colorectal cancer as identified by Vogelstein and colleagues [1]. Chemicals that induce mutations in cancer causing genes are classified as genotoxic carcinogens and the potential of mutagen test systems to detect such compounds has been a major stimulus to the development and application of the science of environmental mutagenesis and genetic toxicology.

There are a number of mechanisms by which chemicals can interact with DNA and lead to the induction of mutations. We have summarized some of these mechanisms below:

1. Direct interaction with the components of the DNA as illustrated by the reaction of alkylating agents such as methyl methanesulfonate with the components of the DNA helix [2].
2. The activation of a compound by cellular metabolism to produce compounds which are now capable of reacting with the DNA. An example of the production of active metabolites is the metabolism of benzo(a)pyrene by the arylhydrocarbon hydroxylases into the DNA reactive diol epoxide [3]. The metabolic activation of potential mutagens is a property of intact animals. However, such metabolically activated compounds may be detected using in vitro test systems by the incorporation into the test protocols of metabolic activation preparations, most frequently based upon the inclusion of microsomes prepared from rat liver (generally called S9 mix). A standard feature of in vitro protocols for the screening of chemicals for potential mutagenic and genotoxic activity is that chemicals are tested for activity in both the presence and absence of S9 mix.
3. The test compound may react with cellular components which may lead to the production of secondary active molecules which are themselves capable of reacting with the DNA. An example of such a mechanism is the production of reactive oxygen species (ROS). In the case of ROS, their level of production can be reduced by the cellular antioxidant mechanisms [4, 5].

Not all of the agent-induced changes in DNA lead to mutations, as all living cells have been shown to possess repair mechanisms which are capable of removing the damaged and modified DNA and reconstituting the original DNA structure (for review *see* ref. 5).

The potency of a compound can be modified by metabolic interactions prior to DNA reaction, which can prevent and/or increase the formation of mutagenic DNA changes, for example, the reduction of the levels of the benzopyrene diol epoxide produced by phase II conjugation reactions in the intact animal [6].

DNA modifications can be processed by the mammalian cell to produce mutations or cellular repair systems can “correct” the compound related modifications before they are processed to produce mutagenic changes (reviewed by Friedberg et al. [5]).

The effects of mutations upon an individual animal will depend upon the site of the mutation within the DNA and the location of the mutated cell within the body. Some mutations will have little or no effect upon protein production, whereas others may produce major changes. Mutations in somatic cells will depend for their effects upon whether or not the mutated cell is expressing that particular gene and if the cells are dividing. Thus, mutations in somatic cells that change normal growth controls are important in the development of cancer. If mutations occur in germ cells, the changes involved may be passed on to the next generation.

Mutagenic chemicals can potentially induce genetic changes in somatic cells in those genes (the oncogenes) whose modifications may be involved in cancer formation and in germ cells where gene modifications may lead to various types of birth defects. Because of the potential health hazard represented by exposure to mutagenic chemicals, it is important that all chemicals for which there is possible human exposure be screened for mutagenic activity. If mutagenic hazard is detected, then the risks of exposure can be assessed and the use of the chemical controlled and when appropriate eliminated from the market and the environment.

Over the past 20 years, more than 300 methods have been developed which were proposed for use as test systems suitable for use in the detection of mutagenic activity and assessment of risks. There have been a number of international collaborative research projects which have evaluated the various methods and determined their reliability, sensitivity, and cost-effectiveness. The aims of these collaborative projects have been to:

1. Provide in vitro methods capable of detecting most if not all of those chemicals with mutagenic potential at early stages of product development without the use of animal experiments.
2. Provide methods to determine whether the mutagenic activity detected by the in vitro methods is reproduced in experimental animals and thus potentially in humans.

Regulatory bodies for all chemical types and products at both the national and international level have produced guidance documents which recommend and/or require mutagenicity testing involving the use of specific types of assays. Although there are some minor differences between the requirements of the various regulators, there is now considerable international agreement on the use and application of the recommended methods. Basically, all international regulations require compounds to be initially evaluated using in vitro assays which measure their ability to induce DNA damage and chromosome damage, the induction of the repair of DNA and the induction of point/gene and chromosome mutations.

In this book, we have provided protocols for those methods which have been extensively validated and in most cases have received approval (or are currently undergoing the stages leading to approval) for usage by International regulatory bodies such as the Organization for Economic Cooperation and Development (OECD).

This collection of mutagenicity testing protocols has been organized on the basis of the testing strategy recommended by the UK Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) published in 2000.

- Stage 1. Initially those organizing testing programs should consider factors which may be relevant to the potential mutagenic activity of a compound such as possible metabolism, physiochemical properties, purity, and the nature of contaminants. Structure/activity models and software can be applied at this early stage.
- Stage 2. The application of in vitro tests methods measuring the induction gene and chromosome mutation. In this volume, we have outlined protocols for:

1. The measurement of gene mutations in bacterial cultures.
2. The measurement of gene mutation in the Thymidine kinase gene of mouse lymphoma cells.
3. The measurement of gene mutations in the HPRT gene of cultured mammalian cells.
4. The measurement of the induction of chromosome aberrations in cultured mammalian cells.
5. The classification and analysis of chromosome aberrations.
6. The measurement of the induction of micronuclei in cultured mammalian cells.

We have also reviewed methodologies which enable the identification of modifications of DNA (adducts) or the induction of the repair of DNA damage.

7. The measurement DNA adducts by the use of P32 postlabeling.
8. The measurement of damage to the chromatin material of the cell which when placed on an electrophoretic gel results in the production of "flares" of DNA from the cell, the

so-called Comets. In this volume, we describe the application of the Comet assay to assess chromatin damage in both cultured cells and in a range of tissues in intact animals. An additional protocol which can be used to identify those compounds which cross-link DNA molecules is also described.

9. The measurement of the activity of cell damage and repair related genes in the Green Screen assay is described here. This assay is yet to be extensively validated but is showing considerable potential for application in high-throughput programs where there is a need to screen substantial numbers of chemicals.
10. Although not a part of the standard mutagenicity testing packages, the measurement of the effects of test compounds upon gene activity is proving to be a valuable methodology when identifying the mechanisms of action of potential mutagens. In this volume, we outline a protocol for the use of real time reverse-transcription chain reaction for gene expression analysis. This methodology can be particularly informative when used to determine the effects of mutations induced at specific genes.

If a compound induces genetic damage and/or genetic changes *in vitro*, the next question to be asked is whether this activity is reproduced *in vivo* in intact animals and potentially in humans. *In vivo* assays can be divided into those undertaken in somatic cells, such as rodent bone marrow and peripheral blood, and those in germ cells. Although chromosome aberrations and the induction of sister chromatid exchange can be measured in blood, in this volume we have focussed upon the measurement of the induction of micronuclei in rodents. The micronucleus assay has the advantage of being able to detect and quantify the ability of a compound to induce both structural and numerical chromosome mutations.

In those situations where actual or suspected exposure to mutagenic chemicals has occurred in a human population, it may be necessary to monitor the population for genetic damage and to estimate the hazards and risks of mutagen exposure. In this volume, we review biomonitoring methods which can be applied to human populations. The methods described here are based upon chromosome endpoints, i.e., the quantification of sister-chromosome exchange, chromosome aberrations, and micronuclei.

The *in vivo* methods described thus far have been based upon the detection and quantification of chromosome mutations. The Comet assay can also be used to measure the induction of chromatid damage *in vivo*.

The *in vivo* genetic toxicology assays thus far described are utilized to detect and assess the potential of compounds to induce mutations in somatic tissues. When a compound produces positive results in somatic cells, then it can be considered to be a potential carcinogen and a possible germ cell mutagen.

We have described in this volume a range of cytogenetic methods which can be used to detect and assess the induction of structural and numerical chromosome mutations in germ cells:

- (a) Metaphase analysis of mitotically dividing spermatozoa
- (b) Metaphase analysis of meiotically dividing primary and secondary spermatocytes
- (c) The spermatid micronucleus assay
- (d) Sperm FISH assay
- (e) Analysis of metaphase II oocytes
- (f) Dominant lethal assay

Gene mutations can be monitored in human blood using modifications of the HPRT assay described in the in vitro section. However, considerable progress has been made in the development of genetically engineered rodents carrying genes that can be exposed to potential mutagens in vivo and analyzed in vitro. The basic principles and applications of rodent transgenic mutation models are described in this volume. An important feature of transgenic animals is that they can be used to analyze the induction of mutations in both somatic tissues and in germ cells.

## References

1. Feron E R and Vogelstein B (1996) A genetic model for colorectal tumour genesis. *Cell* **62**: 759–767.
2. Singer B (1975) The chemical effects of nucleic acid alkylation and their relationship to mutagenesis and carcinogenesis. In *Progress in Nucleic Acid Research and Molecular Biology*. **15**: 218–184.
3. Levin W A Y, Luz H, Ryan D, Wood A W, Kpitulnik J, West S, Huang M T, Conney A H, Thakker D R, Holder G, Hogi G and Jerina D M. (1977). Properties of the liver microsomal monooxygenase and epoxide hydrolase: factors influencing the metabolism and mutagenicity of benzo(a)pyrene. P659–682. In H.H. Hiatt and J.A. Watson (Ed) *Origins of Human Cancer*. Cold Spring Harbour Laboratory. N.Y.
4. Riley PA (1994) Free Radical Biology: Oxidative Stress and the effects of ionising radiations. *International J. Radiation Biology* **65**: 27–33.
5. Freidberg E C, Walker G C, Siede W, Wood R D, Shultz R D and Ellenberger T (2006) *DNA Repair and Mutagenesis* 2nd Edition ASM Press Washington DC
6. Sies H and Ketterer B eds (1988) *Glutathione Conjugation: Mechanisms and Biological Significance*. Academic Press, London.



Genetic Toxicology

Principles and Methods

Parry, J.M.; Parry, E.M. (Eds.)

2012, XVI, 433 p. 114 illus., 46 illus. in color., Hardcover

ISBN: 978-1-61779-420-9

A product of Humana Press