COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

GUIDANCE ON THE SIGNIFICANCE OF CHEMICAL-INDUCED MUTATION FOR HUMAN HEALTH

Executive Summary

The Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) is an independent body that advises Governmental departments and agencies on the potential mutagenicity of natural and man-made chemicals.

The focus of this document is on the role of mutagenic chemicals in the cause and development of cancer and inherited genetic disease.

a) Carcinogenesis: There is good evidence from laboratory studies in animals and epidemiological studies in humans to conclude that mutagens are often carcinogenic
b) Germ cell mutagenesis: There is good evidence from laboratory studies in animals that chemical mutagens cause inherited genetic disease and malformations in offspring. There is only limited evidence from epidemiological studies for such effects in humans.

An important concept is that for those chemicals which cause disease through mutagenicity, it is not possible to identify a threshold dose below which the effect does not occur, and that even low exposures can be associated with a small increase in risk.

Introduction

1. The Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) is an independent body that advises Governmental departments and agencies on the potential genetic toxicity of natural and man-made chemicals. The Committee has the following terms of reference as detailed on the COM website page

a) To assess and advise on the mutagenic risk to man of chemical substances
b) To advise on important general principles or new scientific discoveries in connection with mutagenic risks, to co-ordinate with other bodies concerned with the assessment of mutagenic risks and to present recommendations for mutagenicity testing.

2. The COM remit involves providing advice on a wide range of chemicals including chemicals in food (including those that are naturally part of the food, those that occur as contaminants, or pesticides or veterinary medicines which may be present as residues in food), chemicals that are used in manufacturing processes or in household goods, and those that may become environmental pollutants. The COM is also concerned with providing generic advice on testing methods and strategies and has recently published a Guidance document on a strategy for the testing and evaluation of chemical mutagens
http://www.iacom.org.uk/guidstate/documents/COMGuidanceFINAL2.pdf

Basic Concepts and General Principles

Genotoxicity and mutagenicity

3. Genotoxicity is the process by which chemicals interact with or damage DNA and/or the cellular apparatus which regulates the accuracy and efficiency of the DNA replication and repair processes. Mutation is defined as permanent change in the amount or structure of the genetic material of an organism.

4. Point mutations are small changes in individual genes. Mutations resulting from chromosome aberrations can be either a structural rearrangement of the chromosomes or a change in the number of chromosomes in a cell (aneuploidy)

5. A mutation results in a change to the genetic material - a genotypic change. However not all genotypic changes lead to observable physical or biochemical changes. A change to the cell's genotype that manifests as a functional change (such as a change in cell biochemistry), is a phenotypic change.

6. Mutations can arise from DNA damage caused by radiation (ionizing and non—ionizing), exogenous chemicals (those that do not occur naturally in the body) or endogenous chemicals, such as reactive oxygen species, and can also arise spontaneously during normal cellular function such as during cell division.

7. Chemical-induced mutagenicity occurs by a variety of mechanisms. For example, a chemical or its metabolites may bind to DNA and this bound chemical (DNA adduct) may cause an error in the replication of the DNA resulting in a mutation.

8. The detection and measurement of chemical-induced DNA adducts can be used as an effective biomarker of exposure to certain mutagenic chemicals.[1,2] However, the formation of an adduct does not automatically result in a mutation because there are a variety of repair mechanisms that can restore the DNA strand to its correct structure (see DNA repair). Therefore detection of adducts demonstrates genotoxicity but not necessarily mutagenicity.

9. Further details and discussion of the basic principles which underpin the processes of genotoxicity and mutagenicity can be found in a number of published reference text books.[3-5]

Mutagenesis - the role of DNA repair

10. DNA is intrinsically fragile and it has been estimated that 1,000 to 1,000,000 DNA damaging events, due to environmental factors and normal metabolic processes, occur in each cell per day [6]. To ensure a continued maintenance of DNA (genomic) integrity, complex DNA repair pathways exist which respond to a variety of different types of DNA damage.

11. Accuracy of the DNA repair processes is important for cell function and survival, and the capacity of cells to repair lesions induced by exogenous chemicals will in part determine the potency of a mutagen.

Mutagenesis - the role of apoptosis

12. Apoptosis, also termed programmed cell death, is a process by which cells are removed from an organism in a controlled, regulated manner. Cells with DNA damage can be removed by this process. Apoptosis is an integral aspect of the maintenance of cell and tissue growth and integrity, and is regulated by complex signal transduction pathways.

Understanding Hazard and Risk

13. Classical definitions of hazard and risk state that: a hazard is ‘the potential to cause harm’ and risk is ‘the likelihood of the harm occurring in a

given circumstance'. Chemical risk assessment comprises hazard identification, hazard characterization, exposure assessment and risk characterization. If there is no exposure then there is no risk. More details are provided on the Health Protection Agency website :
http://www.hpa.org.uk/ProductsServices/ChemicalsPoisons/ChemicalRiskAssessment/RiskAssessment
and in the recent COM Guidance
http://www.iacom.org.uk/guidstate/documents/COMGuidanceFINAL2.pdf

14. An important concept when considering chemical-induced mutagenicity, is that it is generally not possible to identify a threshold dose, and therefore there is a default assumption that where mutagenicity leads to disease, even low exposures can be associated with a small increase in risk.

Biomarkers of Exposure and/or of Effect

15. Many of the mutagenic endpoints examined in genotoxicity testing are also used to evaluate DNA damage in humans following exposure to potentially genotoxic substances in the workplace, the environment or following treatment with cytostatic medicines.[7] These investigations are principally conducted using collected blood samples.

16. Biomarkers that are considered adequate for measuring exposure and/or effect include chromosome aberrations and micronuclei, DNA strand breaks (as measured in the Comet assay) and DNA adducts. Investigations using biomarkers such as DNA adducts are often designed with the aim of establishing whether there are associations between exposure, DNA damage and disease (cancer in particular).[1,8,9] Such links have been demonstrated but, as yet, the findings do not firmly establish that biomarkers can be used as prognostic or diagnostic tools for human disease. However, the field is developing rapidly, with wider use of proteomic techniques and it is likely that the role of these methods in understanding the effect of genotoxic chemicals on human health will increase as a consequence.
http://www.iacom.org.uk/statements/COM04S5.htm.

Significance of Mutations for Human Health

17. As described in sections 3-14, a genotoxic chemical may cause a variety of different DNA lesions by a number of different modes of action and this damage may be repaired by DNA repair pathways. If the damage is not repaired, or is not repaired correctly, and the lesion is copied during the next round of cell division, a mutant cell may arise. Most chemically induced mutations can be lethal to a cell, or the cell is stimulated to undergo programmed cell death (apoptosis) and is removed. A mutation in the germ cells of sexually reproducing organisms may be transmitted to the offspring (germ cell mutagenesis) whereas a mutation that occurs in a somatic cell will be transferred only to daughter cells in the organism itself (possibly leading to cancer).

18. This document focusses on the role of mutagenic chemicals in cancer and inherited genetic disease. Genetic changes are implicated in many disease processes. Studies of identical (genetically identical) compared to non-identical (genetically different) twins have shown that the chance of some disorders (such as cleft lip/palate, spina bifida, hypertension, schizophrenia and Alzheimer's disease) occurring in identical twins is much higher than in non-identical twins [10]. It is thought that mutations in a large number of genes each act in a small but significant manner to predispose towards disease.

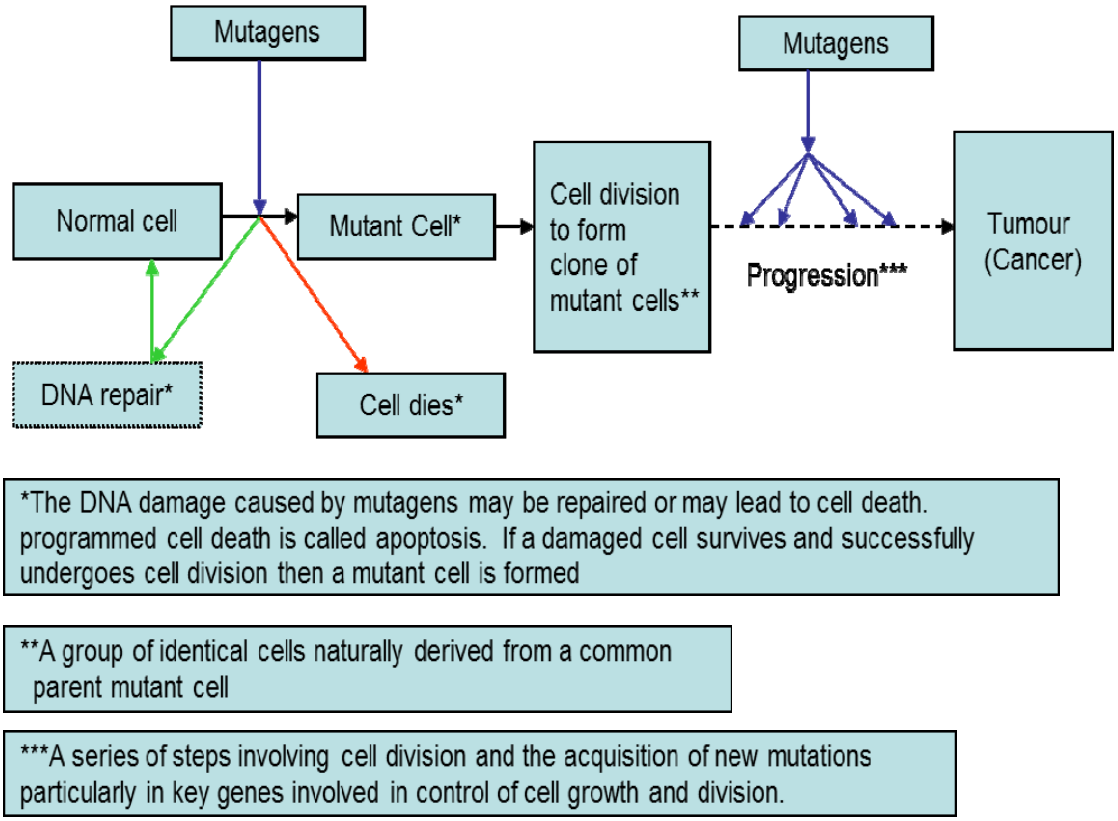
19. As cancer is a complex, multi-stage process there is often a significant lag-period (time interval) between mutation and the occurrence of disease. Therefore it may take several decades for enough mutations to accumulate for a tumour to develop and to be detected and recognised as cancer in an individual.[11] The complexity of genetically induced disease processes and the long periods of time between mutation and onset of disease make it very difficult to investigate the role of chemical-induced mutation in human disease.

20. There have been major advances in human genetics and in the identification of genetic variability since the previous COM guidance through the work on the human genome project and the availability of affordable whole genome scanning [12]. The identification of genetic variations, such as single nucleotide polymorphisms (SNPs), where a DNA sequence exists in two forms, and copy number variants (CNV), large number of multiple copies of sections of DNA have shown that alterations (e.g. insertions or deletions) and rearrangements in the genome are more common than previously thought. However, the cause of these SNP and CNV changes and the role, if any, of chemical exposure is not yet fully understood.

Carcinogenesis

21. DNA damage resulting in mutation may lead to malignant transformation (cancer). Cancer is a tumour that grows progressively, invades local tissues and may spread to distant sites. Carcinogenesis, the origin, causation and development of tumours, - is considered to be a multi-stage process. A simplified flow diagram of the role of mutagens in carcinogenesis is given below.

Figure 1: Role of mutagens in carcinogenesis



A good example of the process is colon cancer where it is known that multiple mutations are associated with the final development of a tumour[13].

Specific mutations in cancer - the human cancer / tumour genome

22. Considerable endeavour is directed towards unravelling the genetic alterations in human tumours and the patterns of somatic mutations in the human cancer genome.[10-11] Currently this information is most widely used to elucidate the dynamics of tumour growth with a view to developing therapies. However there is a possibility that the evaluation of altered genomic fingerprints (patterns of mutations) will provide insight into the causes and development of specific tumours and the role that exogenous chemicals play in their formation.

23. The affected genes are often elements of pathways implicated in the maintenance of the cell cycle or that affect the cell's ability to undergo apoptosis, which is a mechanism used to remove a damaged cell from the body. The p53 oncogene, a tumour suppressor gene, is the most extensively researched gene with regard to cancer and is implicated in a majority of cancer types[14,15] An alteration in the p53 gene is found in 70% of colon cancers, 30-50% of breast cancers and 50% of lung cancers. p53 genes with normal function act to induce cell cycle arrest to allow either repair and

survival of the cell or programmed cell death (apoptosis) to discard a damaged cell.

Association between mutagenic chemicals and cancer

24. The ability of a chemical to induce mutations can be rigorously examined in *in vitro* systems and in animal models, and from these data it is possible to predict chemicals that may cause cancer in humans. However, in man, there is very little data enabling these predictions to be confirmed, as it is often difficult to quantify human exposures to specific chemicals, and there is a long time period between mutation(s) and the occurrence of disease such as cancer. Where possible, epidemiological studies underpin these assessments, In these studies, human populations with known exposures (e.g. an an occupational exposure) are compared with relatively unexposed groups. Epidemiological studies need to account for confounding factors which may also contribute to cancer (such as smoking, age, dietary or other lifestyle factors) and to employ appropriate statistical methods.[16]

25. The International Agency for Research on Cancer (IARC), which is affiliated to the World Health Organisation, assesses dietary, environmental and occupational factors that may contribute to cancer risk in humans. If they are available, epidemiological data underpin these assessments, but mutagenicity data are also of significant value in determining whether the results in experimental animals are relevant to humans. Whilst there are some examples of chemicals classified as carcinogenic to humans (Group 1) which are not mutagenic, the most common mode of carcinogenic action of chemicals in Group 1 is mutagenicity.

Some examples of mutagenic carcinogens

Aflatoxin-B1

26. Aflatoxin B1 is a naturally occurring mycotoxin produced by species of the fungus *Aspergillus*. Human exposure occurs following the ingestion of crops contaminated with the fungus, particularly peanuts, maize and oilseeds. Aflatoxin B1 is metabolised to a reactive intermediate in the liver where it binds to hepatocyte DNA. Chronic low-level exposure results in an increase in liver cancer, especially in geographical regions with a high incidence of hepatitis B infection. It is one of the most potent mutagens known, generating tumours at very low doses in animals (µg/kg/day quantities).[17,18]

Chromium VI

27. Notable human exposure to hexavalent chromium (Cr-VI) occurs occupationally in industrial settings, but as it is a naturally occurring chemical species, there is also documented low-level exposure from environmental sources including air. IARC considers that there is sufficient evidence to classify hexavalent chromium compounds as carcinogenic in humans. The epidemiological evidence for this is an observed increased risk of respiratory

cancers in workers occupationally exposed to Cr-VI compounds. The exposures in these circumstances were high and there is no evidence of this increased risk from low, environmental exposures.[19] However, Cr-VI compounds are mutagenic and so it is not possible to assume that there is a level at which there is no cancer risk. [20]

Cyclophosphamide

28. Cyclophosphamide is an anti-neoplastic pharmaceutical drug used for the treatment of a range of cancers including lymphomas, leukaemia and neuroblastomas. It is an established carcinogen in life-time rodent studies. Cyclophosphamide is genotoxic, and, generates DNA adducts in the target tissues of treated animals with a frequency that is dose-related.[21] There is also epidemiological evidence that it induces tumours in patients treated with cyclophosphamide, particularly bladder tumours.[22]

1,3 –butadiene

29. 1,3-butadiene is used principally in the production of synthetic rubbers and polymers, and workers in the industries concerned can be exposed to the compound. It is carcinogenic in rats and mice and a comprehensive review of epidemiological data gathered in occupational settings revealed increases in cancers such as leukaemia and lymphomas. 1,3-butadiene has been classified by IARC as 'carcinogenic to humans [23]. However molecular epidemiology studies in humans examining biomarkers such as adduct formation or chromosome damage yield inconclusive results [24]. Studies generally show that 1,3-butadiene is genotoxic and mutations of a number of key oncogenes (*p*53 and *ras*) have been identified in tumours from mice treated with butadiene.

4-aminobiphenyl (4-AB)

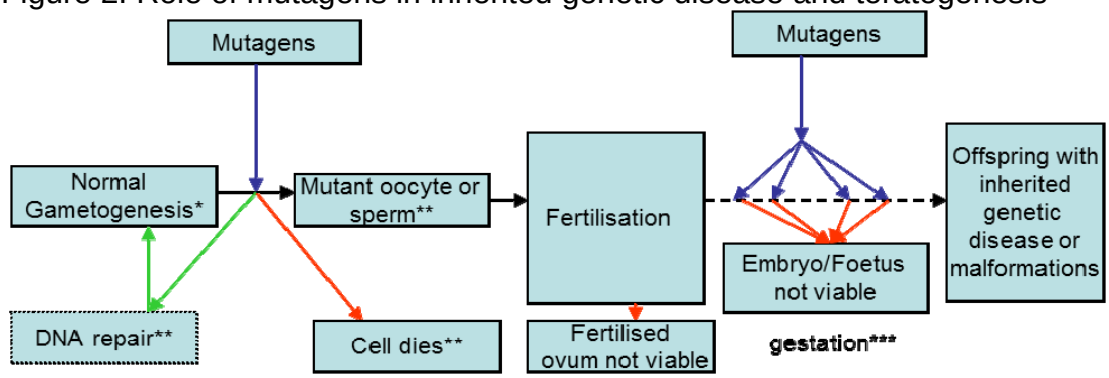
30. 4-AB is another known human carcinogen which induced a high incidence of bladder tumours in exposed workers. In mice, it causes an increase in the occurrence of bladder and liver tumours and it is clearly mutagenic, giving positive findings in all the standard genotoxicity assays, *in vitro* and *in vivo*. 4-AB and a number of other structurally similar chemicals (aromatic amines) are also constituents of cigarette smoke. DNA adducts derived from 4-AB metabolites have been detected in human bladder tumours and protein adducts are found in the blood of smokers.[25]

Germ Cell Mutagenesis

31 Cell division which leads to the production of germ cells is termed meiosis which differs from mitosis (see glossary). Mammalian oocytes begin meiosis in the foetal ovary, but only complete it when fertilized in the adult reproductive tract. Spermatogenesis, the process by which sperm is produced, takes approximately 11 weeks in the human male and occurs continuously from puberty onwards.

32 If a mutation arises in a germ cell (i.e. an oocyte or sperm cell) it is theoretically possible that this mutation will be transferred to the off-spring (heritable germ cell mutagenicity) and can result in genetic diseases such as Downs Syndrome. The potential role of mutagens in infertility, inherited genetic disease and teratogenic effects is shown in Figure 2 below. Heritable genetic damage has been demonstrated for a number of well known mutagenic chemicals in animal models (e.g. antineoplastic drugs, acrylamide, ethylene oxide)[26] and this results in embryonic lethality or genetic disease in off-spring. In human studies, it has been shown that the sperm of smokers have elevated levels of DNA damage and DNA adducts from tobacco-derived carcinogens, and there is evidence that these DNA adducts are transmitted to embryos.[27] A possible link between parental smoking and the incidence of cancer in children has been widely investigated, but the evidence for a causal association is inconclusive.

Figure 2: Role of mutagens in inherited genetic disease and teratogenesis



* The process whereby germ cells (i.e. oocytes (eggs) and sperm) are formed

** The DNA damage caused by mutagens may be repaired or may lead to cell death. programmed cell death is called apoptosis. If a damaged cell survives and successfully undergoes cell division then a mutant oocyte or sperm is formed

***The period of development in the uterus from conception until birth; pregnancy.

33. Heritable mutations in animals have been induced by some mutagenic chemicals (e.g. ethylnitrosourea[28]) and by chemical mixtures, such as tobacco smoke in male mice [29]. Germ cells are particularly susceptible to aneuploidy during meiosis and there are notable examples of chemically induced aneuploidy in animal models (e.g acrylamide in mouse sperm,[30] and cyclophosphamide;[31]). For most chromosomes, aneuploid fetuses are not viable. However, exceptions exist, and in man aneuploidy gives rise to a number of well characterized syndromes; for example, an additional chromosome 21 leads to Downs syndrome, and a missing X chromosome to Turners syndrome.[32] Links have been made with risk factors, maternal age in particular, but there is no convincing evidence for a causal association of these conditions with maternal chemical exposures from occupational,

medicinal or environmental sources, including cigarette smoking. There is some evidence that mothers' exposure to trichlorfon, an insecticide, was associated with an increase in babies born with Downs' syndrome[33]

34. Overall, whilst it is acknowledged that exposure to chemicals has the potential to adversely affect human disease outcomes, there is no convincing evidence for chemically-induced heritable germ cell mutations in human populations despite a large number of studies examining the effects of mutagenic carcinogens in germ cells in animals.[34] It is probable that emerging molecular epidemiological methods and techniques will allow improved study designs, providing new insights into this possible effect of mutagenesis.[35]

Teratogenicity

35. Teratogenicity is the ability of a chemical to cause foetal anomalies following *in utero* exposure to a chemical (see Figure 2). Although many of the well established human chemical teratogens do not act via a mutagenic mechanism (e.g thalidomide, diethylstilbestrol), there is evidence that a number of *in vivo* mutagens produce birth defects if administered to pregnant women. These include the anti-cancer drugs busulfan, 6-mercaptopurine and daunorubicin.[36] The effects reported are largely congenital abnormalities such as cleft palate, hypospadia and limb defects. Although these chemicals are known mutagens, the precise modes of action underlying their teratogenesis are largely uninvestigated. Understanding the causal associations between chemical exposures and teratogenic effects is complicated by the knowledge that exposures at different gestational times may produce a different spectrum of effects, and by the normal variation in the incidence of congenital effects. There is no convincing evidence of an increase in the induction of diseases in off-spring through parental exposure to mutagens nor of chemically induced germ cell mutations that have been passed on to successive generations.

Summary

36. The COM has a remit to provide advice on, and evaluate information relating to chemical-induced mutations.

37. Mutations have the potential to result in the development of cancer and, if they occur in the germ cells, may also affect future generations. There is a clear causal association between exposure to some mutagens and increased incidence of cancer in humans. The impact of germ cell mutations on human health is less well understood, and currently there are no clear causal associations. There is evidence, however, for heritable genetic effects of mutagenic chemicals in animal models.

July 2012

GLOSSARY

Aneuploidy: The occurrence of an abnormal number of chromosomes in a cell, such that the total number of chromosomes within the cell is not an exact multiple of the normal (haploid) number. Chromosomes may be lost (monosomy) or gained (trisomy) during cell division. An extra or missing chromosome is a common cause of genetic disorders (birth defects or spontaneous abortions). Some cancer cells also have abnormal numbers of chromosomes. (Chemical induction of aneuploidy is aneugenicity, cf **aneugenic** chemcals)

Altered genomic fingerprint: An alteration in the sequence of DNA base pairs, the form of which may point to the mutagen responsible. For example, tobacco smoke has been shown to cause specific changes to the P53 gene.

Apoptosis: A form of active cell death resulting in break-up of a cell into membrane-bound fragments (apoptotic bodies). These are usually rapidly removed *in vivo* through engulfment by phagocytic cells. Apoptosis can occur normally during development, but can also be triggered by toxic stimuli.

Biomarker: An observable change (not necessarily pathological) in an organism, related to a specific exposure or effect. The term is used in a broad sense to include almost any measurement reflecting an interaction between a biological system and an environmental agent, which may be chemical, physical or biological. For example, responses to a mutagenic chemical can include the formation of micronuclei in polychromatic erythrocytes. Biomarkers of disease are specific changes in biochemistry which can be used to infer the presence or prognosis of disease. (see also Exposure biomarker)

Carcinogenesis (Cancer): The process by which tumours are caused or develop (q.v.). The term applies to benign as well as malignant neoplasms

Cancer: a synonym for a malignant neoplasm – that is, a tumour that grows progressively, invades local tissues and can spread to distant sites (see also tumour).

Cell cycle (cell cycle arrest): The cell cycle is a series of events involving the growth, replication, and division of a eukaryotic cell. **Cell cycle arrest**: A regulatory process that halts progression through the cell cycle during one of the normal phases (G1, S, G2, M).

Chemical Risk Assessment: In the context of genotoxicity, an assessment of the probability that exposure to a chemical will cause cancer. The assessment is specific to the chemical and exposure conditions under consideration.

Chromosomal aberrations: Collective term for particular types of chromosome damage induced after exposure to exogenous chemical or physical agents which damage the DNA. (see clastogen).

Chromosome: The DNA and associated proteins in the nucleus of cells that carries the genes and functions in the transmission of hereditary information. In simple organisms, such as bacteria and many viruses, the chromosome consists of a single circular molecule of DNA containing the entire genetic material of the cell. In eukaryotic cells, the chromosomes are thread-like structures, composed mainly of DNA and protein, which are present within the nuclei of cells. They occur in pairs, the numbers varying from one to more than 100 pairs per nucleus in different species. Normal somatic cells in humans have 23 pairs of chromosomes, each consisting of linear sequences of DNA which are known as genes.

Clastogen: An agent that produces chromosome breaks and other structural aberrations in chromosomes such as translocations. Clastogens may be viruses or physical agents as well as chemicals. Clastogenic events play an important part in the development of some tumours (**clastogenicity**)

Clone of mutant cells: A group of identical cells naturally derived from a common parent mutant cell

Comet assay: A genotoxicological technique for measuring DNA damage in an individual cell using single-cell gel electrophoresis. Cell DNA fragments assume a "comet with tail" formation on electrophoresis and are detected with an image analysis system. Alkaline assay conditions facilitate sensitive detection of single-strand damage.

Copy number variants: Alterations in the DNA of a genome that results in the cell having an abnormal number of copies of one or more sections of the DNA. CNVs correspond to relatively large regions of the genome that have been deleted (fewer than the normal number) or duplicated (more than the normal number) on certain chromosomes.

Cytostatic medicines: Drugs which work by inhibiting or suppressing cell growth and division. e.g most medicines used in cancer chemotherapy

Diagnostic tools: Sources of information (e.g. medical history and physical examination of the patient), which help to distinguish the nature of a disease or illness.

DNA: DNA stands for deoxyribonucleicacid and is a nucleic acid that carries the genetic information in the cell and is capable of self-replication and synthesis of RNA. DNA consists of two long chains of nucleotides twisted into a double helix and joined by hydrogen bonds between the complementary pairs of bases adenine/ thymine and cytosine/guanine. The sequence of nucleotides determines individual hereditary characteristics.

DNA adducts: Covalent adducts between chemical mutagens and DNA. Such couplings activate DNA repair processes and, unless repaired prior to DNA replication, may lead to mutations such as nucleotide substitutions, deletions, and chromosome rearrangements.

DNA repair: Processes that correct potentially damaging mutations in DNA, including those induced by chemical mutagens. (*See* mutagen.) Through the action of enzymes, individual DNA bases may be replaced, or part of a strand of DNA may be replaced, using its opposite, paired strand as a template.

DNA repair pathways: The sequence of steps in the repair of DNA. Each step is governed by an enzyme. Some examples are outlined below.
The **Base Excision Repair (BER) pathway**. The damaged base is removed by an enzyme called DNA glycosylase.. Several types of DNA glycosylases exist, each one specifically excising a different type of damaged base. In order to fill the gap (replace the missing nucleotide), an enzyme specialised in synthesizing DNA, a DNA polymerase, will insert the correct nucleotide into the gap and link it to the paired normal nucleotide. The enzyme DNA ligase joins the strands by creating a phosphodiester bond between them,

DNA damage that involves particularly "bulky" molecules or chemical bonds between bases, or that significantly distorts the double-stranded structure of DNA, is subject to repair by the **nucleotide excision repair (NER) pathway**. The NER pathway is complex. The key action is to detect DNA damage that induces distortion of the DNA structure.

The **DNA mismatch repair (MMR) pathway** has evolved to correct errors made by DNA polymerase during DNA replication.

Embryonic (and fetal) lethality: Embryotoxicity that causes death of the embryo. Toxicity that causes death of the foetus.

Epidemiological studies: Studies designed to investigate associations , distribution, and control of disease (such as cancer) in human populations

Exposure biomarker: an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent (chemical) and some target molecule or cell that is measured in a compartment within an organism (e.g. blood or urine)

Gene: The functional unit of inheritance: a specific sequence of nucleotides along the DNA molecule, forming part of a chromosome

Gene mutation: A mutation resulting from a change in a single base pair in the DNA molecule (also called point mutation)

Genotoxicity: Genotoxicity refers to interaction with, or damage to, DNA and/or other cellular components which regulate the fidelity of the genome. It is a broad term that, as well as mutation (see paragraph 7) includes damage to DNA such as the production of DNA adducts, by the chemical itself or its metabolites. Cells have the capacity to protect themselves from such potentially lethal or mutagenic genotoxic effects by many repair processes and therefore many genotoxic events do not become evident as mutations. However, the capacity to damage the genome (genotoxicity) is an indicator of

potential mutagenicity. Thus, some methods that measure genotoxicity maynot provide direct evidence of heritable mutation.

Genotypic change: The genotype is the genetic makeup, as distinguished from the physical appearance, of an organism or a group of organisms. The combination of alleles located on homologous chromosomes that determines a specific characteristic or trait. Mutation results in a change to the genotype.

Germ cell mutagenesis: Germ cells are cells that give rise to the gametes of an organism that reproduces sexually. The cells undergo mitotic and meiotic cell division in the gonads followed by cellular differentiation into mature gametes, either oocytes or sperm. Germ cell mutagenesis involves mutation of the germ cells during mitotic and/or meiotic cell division.

Haematopoietic and lymphatic cancers: cancers that affect the blood, bone marrow and lymph nodes. Chromosomal translocations are a common cause of these diseases.

Hazard: Any source of potential damage, harm or adverse health effects on something or someone under certain conditions, for example at work or at home. Basically, a hazard can cause harm or adverse effects (to individuals as health effects or to organizations as property or equipment losses). Chemicals can have intrinsic hazardous properties (e.g. potential to be mutagenic or induce cancer).

Heritable Genetic Damage (inherited genetic disease): Germ cell mutagenesis that leads to the inheritance of a mutation from one generation to the next generation.

Heterozygous: having two different forms of a gene that controls a particular characteristic, one inherited from each parent, and therefore able to pass on either form

I**n vivo:** taking place within a living organism

Malformations: The inheritance of an abnormal or anomalous formation of tissues and organs often referred to as a deformity .

Meoisis: The process of cell division in sexually reproducing organisms that reduces the number of chromosomes in reproductive cells from diploid to haploid leading to the production of gametes in animals and spores in plants. During the first meiotic division there is homologue pairing, efficient intergenic recombination between homologues during pairing, and the suppression of sister chromatid separation. S phase is absent at the start of the second meiotic division. Thus the outcome of meiosis should be four genetically unique haploid cells.

Mitosis: The process in cell division by which the nucleus divides, typically consisting of four stages, prophase, metaphase, anaphase, and telophase, and normally resulting in two new nuclei, each of which contains a complete

copy of the parental chromosomes. The outcome of mitosis should be two genetically identical diploid cells.

Molecular epidemiology: A study that combines the tools of standard epidemiology–e.g. case-control studies, questionnaires and monitoring of exposure to external factors with the tools of molecular biology–eg, mutations of the p53 gene in smokers.

Micronuclei: Whole or broken chromosomes that fail to segregate normally during cell division and may be lost from the main nuclei **----** Centromere positive micronuclei contain DNA and/or protein material derived from the centromere. The presence of centromere positive micronuclei following exposure to chemicals *in vitro* or *in vivo* can be used to evaluate the aneugenic potential of chemicals.

Mutation: A permanent change in the amount or structure of the genetic material in an organism or cell, which can result in a change in phenotypic characteristics. The alteration may involve a single gene, a block of genes, or a whole chromosome. Mutations involving single genes may be a consequence of effects on single DNA bases (point mutations) or of large changes, including deletions, within the gene. Changes involving whole chromosomes may be numerical or structural. A mutation in the germ cells of sexually reproducing organisms may be transmitted to the offspring, whereas a mutation that occurs in somatic cells may be transferred only to descendent daughter cells.

Mutagenic endpoints: These comprise three levels of genetic change, namely **gene mutation, clastogenicity** and **aneuploidy**

Mutagenic risk: The probability that an agent, such as a chemical, ultraviolet light, or a radioactive element, will induce or increase the frequency of mutation in an organism.

Mycotoxin: a toxic substance produced by fungi, some of which may affect food.

Oncogene: A gene that played a normal role in the cell as a **proto-oncogene** and that has been altered by mutation and now may contribute to the growth of a tumour.

Oxidative: relating to or characterised by oxidation.

P53 oncogene: The p53 gene can act to stop cells with damaged DNA from replication. The mutated form of the p53 gene has lost its capacity to halt DNA replication. Thus cells with damaged and /or mutated DNA are able to grow and divide when the p53 function is lost. These cells can form the basis of a carcinogenic response.

Phenotypic Change: A change in the observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences

Point mutation (see gene mutation)

Prognostic tools: Signs, symptoms, clinical measurements which give an indication of the future course of a disease.

Proteomic: The analysis of the expression, localizations, functions, and interactions of proteomes**.** The proteome is a term which refers to all the proteins expressed by a genome

Proto-oncogene: A normal gene which, when altered by mutation, becomes an oncogene that can contribute to cancer. Proto-oncogenes may have many different functions in the cell. Some proto-oncogenes provide signals that lead to cell division. Other proto-oncogenes regulate programmed cell death (apoptosis) (see p53 oncogene).

ras oncogene: The Ras protein family are a class of protein called small GTPase, and have important roles in cell signalling The ras gene is the most common oncogene in human cancer - mutations that permanently activate *ras* are found in 20-25% of all human tumours and up to 90% in certain types of cancer (e.g. pancreatic cancer).

Risk: Probability that a harmful event (death, injury or loss) arising from exposure to a chemical or physical agent may occur under specific conditions.

Signal Induction pathway: The molecular pathways that signal (i.e turn on or off) biochemical pathways or biological functions (e.g biochemical pathways leading to nerve conduction)

Single nucleotide polymorphism: A DNA sequence variation occurring when a single nucleotide –A, T, C or G - — in the genomoediffers between members of a single biological species chromosomes in an individual.

Single Strand Breaks: A break in double-stranded DNA in which only one of the two strands has been cleaved; the two strands have not separated from each other.

Teratogenicity: The capability of a chemical to produce fetal malformations (**teratogenesis**). Chemicals which cause teratogenicity are **teratogens**

Threshold Dose: Dose or exposure concentration below which an effect (e.g. response in a genotoxicity assay) is not expected.

Tumour: Tumour (Synonym - neoplasm): A mass of abnormal, disorganised cells, arising from pre-existing tissue, which are characterised by excessive and uncoordinated proliferation and by abnormal differentiation. **Benign** tumours show a close morphological resemblance to their tissue of

origin; grow in a slow expansile fashion, and form circumscribed and (usually) encapsulated masses. They may stop growing and they may regress. Benign tumours do not infiltrate through local tissues and they do not metastasise (spread). They are rarely fatal. **Malignant tumours** (synonym - cancer) resemble their parent tissues less closely and are composed of increasingly abnormal cells in terms of their form and function. Well differentiated examples still retain recognisable features of their tissue of origin but these characteristics are progressively lost in moderately and poorly differentiated malignancies: undifferentiated or anaplastic tumours are composed of cells which resemble no known normal tissue. Most malignant tumours grow rapidly, spread progressively through adjacent tissues and metastasise to distant sites. Tumours are conventionally classified according to the anatomical site of the primary tumour and its microscopical appearance, rather than by cause. Some common examples of nomenclature are as follows:

- Tumours arising from epithelia, e.g. benign - adenomas, papillomas; malignant - adenocarcinomas, papillary carcinomas.
- Tumours arising from connective tissues such as fat, cartilage or bone: benign - lipomas, chondromas, osteomas; malignant - fibrosarcomas, liposarcomas, chondrosarcomas, osteosarcomas.
- Tumours arising from lymphoid tissues are malignant and are called lymphomas; they are often multifocal. Malignant proliferations of bone marrow cells are called leukaemias.

Benign tumours may evolve into the corresponding malignant tumours; examples are the adenoma to carcinoma sequence in the large bowel in humans, and the papilloma to carcinoma sequence in mouse skin.

REFERENCES

1. Phillips, D.H. (1996) DNA adducts in human tissues: biomarkers of exposure to carcinogens in tobacco smoke. Environ. Health Persepct. **104 (suppl 3)** 453-458
2 Farmer, P.B. (2004) Exposure biomarkers for the study of toxicological impact on carcinogenic processes. IARC SCi Pub **157** 71-90
3. Lynch, A.M. (2009) Mutagenesis In: General and Applied Toxicology (3rd Edition). Ballantyne, B., Marrs, T. and Syversen, T. (eds). *John Wiley and Sons Ltd., Chichester, UK.* pp 1601-1681.
4. Turpenny, P., Ellard, S. (2005) in Emery's Elements of Medical Genetics, 12th ed Elsevier Churchill Livingstone Edinburgh
5. Preston, R.J., Hoffman, G.R (2008) Genetic Toxicology. In: Casarett and Doull's Toxicology, the basic science of poisons. Klaasen, C.D (ed), 7th Ed, McGraw-Hill Medical Publishing
6. Lodish H, Berk A, Matsudaira P, Kaiser CA, Krieger M, Scott MP, Zipursky SL, Darnell J. (2004). Molecular Biology of the Cell, p963. WH Freeman: New York, NY. 5th ed.
7. Bonassi, S., Ugolini, D., Kirsch-Volders, M., et al (2005) Human population studies with cytogenetic biomarkers: review of the literature and future prospectives. *Environ Mol Mutagen*, **45**, 258-270
8. Farmer, P.B., Brown, K., Tompkins, E., et al (2005) DNA adducts: mass spectrometry methods and future prospects. Toxicol. Appl. Pharmacol. **207** 293-301
9. Boffetta, P. (2010) Biomarkers in cancer epidemiology : an integrative approach. Carcinogenesis. **31(1)** 121-126
10. Greenman, C., Stephens, P., Smith, R. et al (2007) Patterns of somatic mutation in human cancer genomes. Nature **446** 153-158
11. Stratton M.R., Campbell, P.J., Futreal P.A. (2010) The cancer genome. Nature **458 (7239)** 719
12. Lander, E.S. (2011) Initial impact of the sequencing of the human genome. Nature **470** 187-197
13. Moolgavkar S.H, Luebeck EG. (1992) Multistage carcinogenesis: population-based model for colon cancer. J Natl Cancer Inst. **84(8)** : 610-8.
14. Nigro,J.M., S. J. Baker, A. C. Preisinger, et al (1989) Mutations in the *p53* gene occur in diverse human tumour types. Nature **342**, 705–708
15. Levine, L., Oren, M., (2009) The first 30 years of p53: Growing ever more complex. Nat. Rev. Cancer 9, 749–758 (2009).
16. Bailey L, Vardulaki K, Langham J, and Chandramohan Dl (2005) Introduction to Epidemiology (Understanding Public Health). Open University Press. pp 176 ISBN-10 / ASIN: 0335218334 ISBN-13
17. IARC (2002). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, volume 82. Some traditional Herbal medicines, Some Mycotoxins, Naphthalene and Styrene. IARC, Lyon France.
18. Smela , M.E., Currier, S.S., Bailey, E.A., Essigmann, J.M. (2001) The chemistry and biology of aflatoxin B1 : from mutational spectrometry to carcinogenesis. Carcinogenesis **22(4)** 535-545
19. De Flora, S. (2000) Threshold mechanisms and site specificity in chromium (VI) carcinogenesis. Carcinogenesis **21(4)** 533-541.
20. McCarroll, N., Keshava, N., Chen, J. et al (2010) An evaluation of the mode of action framework for mutagenic carcinogens case study II Chromium (VI). Environ. Mol. Mut. **51** 89-11.
21 Mc Carroll, N., Keshava, N., Cimino, M., et al (2008) An evaluation of the mode of action framework for mutagenic carcinogens case study: cyclophosphamide. Environ. Mol. Mut. **49** 117-131.

22. IARC (2011) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, volume 100A. A review of Human Carcinogens. Pharmaceuticals. IARC, Lyon France.
23. IARC (2008) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, volume 97. 1,3-Butadiene, Ethylene Oxide and Vinyl Halides (Vinyl Fluoride, Vinyl Chloride, and Vinyl Bromide. IARC, Lyon France.
24. Kirman, C.R., Albertini, R.A., Gargas, M.L. (2010) 1,3-butadiene: III. Assessing carcinogenic modes of action. Crit.Rev. Tox. **40(S1)** 74-92
25. Vineis, P. (1992) Epidemiological models of carcinogenesis: the example of bladder cancer. Cancer Epi. Biomarkers. Prev. **1** 149-153
26. Shelby, M.D., Bishop, J.B., Mason, J.M., Tindall, K.R. (1993) Fertility, reproduction and genetic disease : studies on the mutagenic effects of environmental agents on mammalian germ cells. Environ. Health Perspec. **100** 283-291.
27. Chang ,J., (2009) Parental smoking and childhood leukaemia . Meth Mol. Biol./Cancer Epi. **472** 103-137
28. Lewis SE, Barnett LB and Shelby MD (1992) ENU Mutagenesis in mouse electrophoretic specific-locus test 2. Mutational studies of mature oocytes. Mutat Res, 296, 129-33.
29. Marchetti F, Rowan-Carroll A, Williams A, Polyzos A, Berndt-Weis ML, Yauk CL. (2011) Sidestream tobacco smoke is a male germ cell mutagen. Proc Natl Acad Sci U S A. **108(31)**: 12811-4
30. Pacchierotti, F., Tiveron, C., D'Archivio, M., et al (1994) Acrylamide-induced chromosomal damage in male mouse germ cells detected by cytogenetic analysis of one-cell zygotes. Mutat. Res. **309** 273-284
31. Adler, I-D., Schmid, T.E., Baumgartner, A. (2002) Induction of aneuploidy in male mouse germ cells detected by the sperm-FISH assay: a review of the present data base. Mutat. Res. **504** 173-182
32. Hassold, T., Hall, H., Hunt, P. (2007) The origin of human aneuploidy :where we have been, where are we going. Hum. Mol. Genetics **16** R203-208
33. Czeizel A.E, Elek C, Gundy S, Métneki J, Nemes E, Reis A, Sperling K, Tímár L, Tusnády G, Virágh Z. (1993) Environmental trichlorfon and cluster of congenital abnormalities, The Lancet, **341** 539-542.
34. Singer TM and Yauk CL (2010) Germ cell mutagens: Risk assessment challenges in the 21st century. Env Mol Mutagen, 51, 919-928.
35. Verhofstad, N., Linschooten, J.O., van Benthem., J. et al (2008) New methods for assessing germ line mutations in humans and genetic risks in their offspring. Mutagenesis **23** 241-247
36. Bishop, J.B., Will, K.L, Sloane, R.A (1997) Genetic toxicities of human teratogens. Mut. Res. **396** 9-43.