Lecture -2-

Environmental Biotechnology

1-General Bioassay in pollution Monitoring

1-1 Genotoxicity test

At the early testing stages, the genotoxicity assays for predicting potential heritable germ cell damage are the same as used for predicting carcinogenicity because the endpoints measured in genotoxicity tests are common precursors for both of these adverse health outcomes. There is a need to evaluate diverse types of biological alterations in order to thoroughly assess the genotoxic/mutagenic potential of a substance; this requires the use of a battery of tests. Germ cell mutagens/genotoxins are substances that cause heritable (passed on to progeny) changes in the genetic material in germ cells, namely spermatocytes or oocytes. The term *mutagen* refers to a substance that induces transmissible changes in DNA structure involving a single gene or a group of genes. Genotoxins are a broader category of substances that induce changes to the structure or number of genes via chemical interaction with DNA and/or non-DNA targets

In vivo tests for assessing potential heritable genotoxicity include:

1. Heritable germ cell mutagenicity tests that include a component that measures damage passed onto progeny are: the mouse heritable translocation test, the mouse specific locus test, and the rodent dominant lethal test.

2. Assays for measuring genotoxicity induction in germ cells that are used to predict chemicals that might induce heritable damage include the mammalian spermatogonial chromosome aberration test, the spermatid micronucleus assay, the mammalian oocyte chromosome

aberration/aneuploidy test, and unscheduled DNA synthesis test in testicular cells.

3. Assays for measuring genotoxicity induction in somatic cells that are used to predict whether a chemical has the potential to induce germ cell genotoxicity (these are also used in predicting potential carcinogenicity) include the mammalian erythrocyte micronucleus test , the mammalian bone marrow chromosome aberration test, the liver unscheduled DNA synthesis (UDS) , and the mouse spot test which measures genotoxicity in fetal somatic cells.

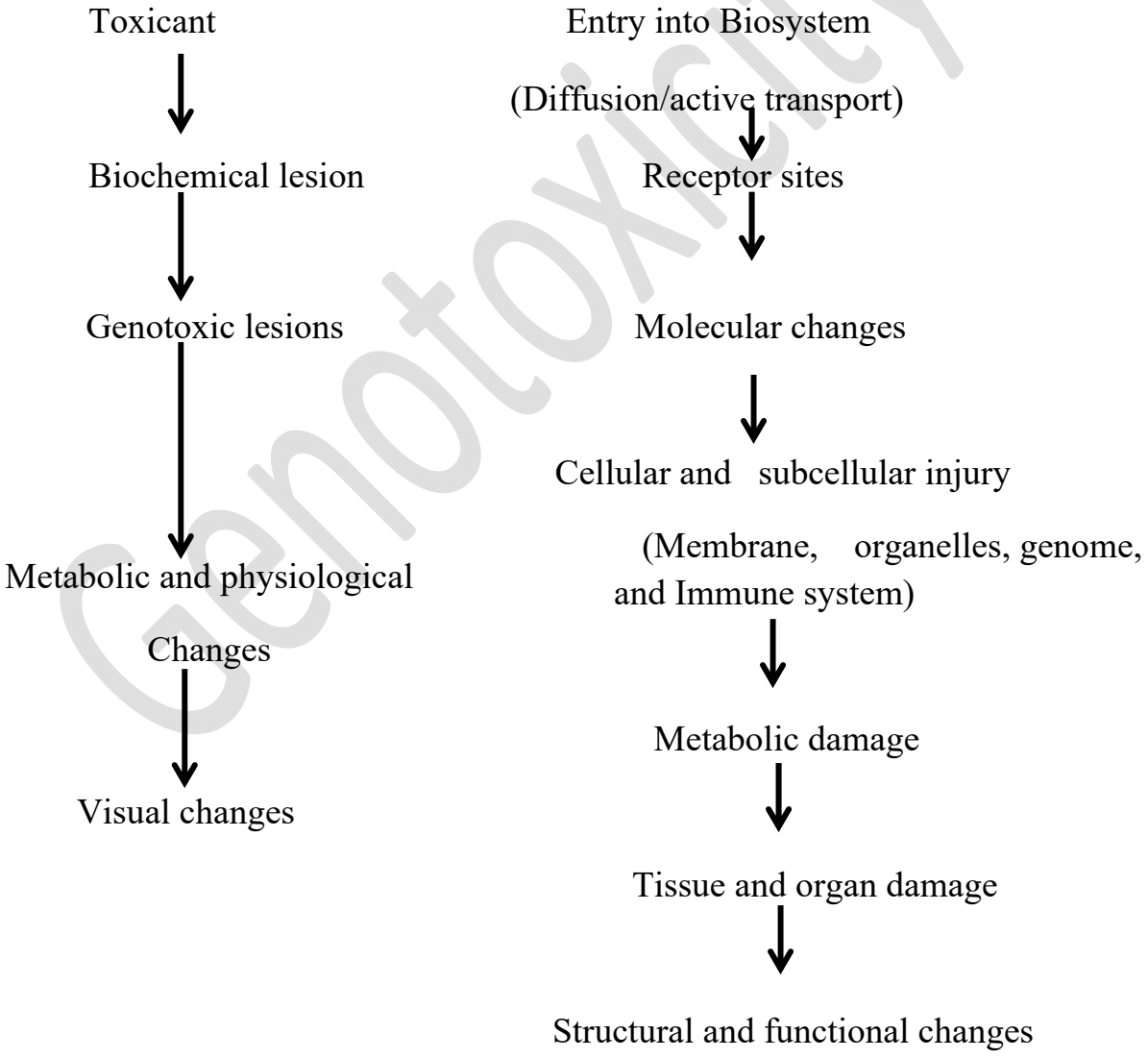


Figure 1: Toxic effect and Bioassay levels

The standard test organism should meet certain criteria which are as follow:

1- It should be sensitive to pollutants.
2- Able to readily absorb/adsorb the toxicants.
3- Available throughout the year
4- Directly measurable and predicative
5- Providing inexpensive or cost effective measure.

1-2 Test system for assessment of Genetic damage.

1-Ames test

The Ames test is a biological assay to assess the mutagenic potential of chemical compounds. A positive test indicates that the chemical is mutagenic and therefore may act as a carcinogen, since cancer is often linked to mutation. However, a number of false-positives and false-negatives are known. The test serves as a quick and convenient assay to estimate the carcinogenic potential of a compound since standard carcinogen assays on rodents are time-consuming (taking two to three years to complete) and expensive.

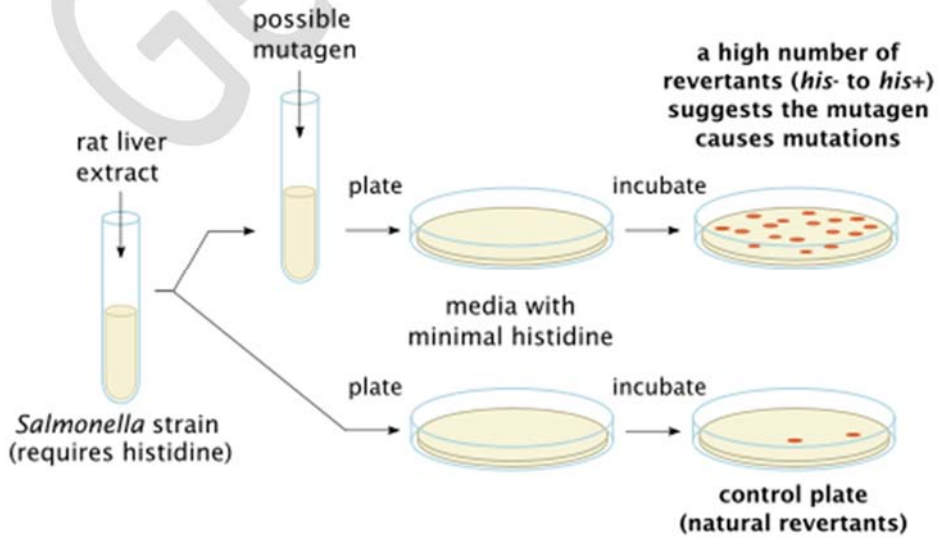


Figure 2: Ames test

2- Micronucleus test : is a test used in toxicological screening for potential genotoxic compounds. The assay is now recognized as one of the most successful and reliable assays for genotoxic carcinogens, i.e., carcinogens that act by causing genetic damage and is the OECD guideline for the testing of chemicals. There are two major versions of this test, one in vivo and the other in vitro.

The in vivo test normally uses mouse bone marrow or mouse peripheral blood. When a bone marrow erythroblast develops into a polychromatic erythrocyte, the main nucleus is extruded; anymicronucleus that has been formed may remain behind in the otherwise anucleated cytoplasm. Visualisation of micronuclei is facilitated in these cells because they lack a main nucleus. An increase in the frequency of micronucleated polychromatic erythrocytes in treated animals is an indication of induced chromosome damage.

3-Chromosome damage:

Chromosome damage arises when a single chromosome is broken before duplication in S phase. Chromatid damage results from damage to one arm of the duplicated chromosome, with no damage to the other.

Chromosome damage types according to induction:

1- broken ends may rejoin to their original positions and no obvious chromosomal defects will be identifiable

2-A fragment of the chromosome may be lost if several double strand breaks occur within a single chromosome

3-Broken ends may attach to incorrect broken ends, leading to the formation of lethal abnormalitie

Lethal Chromosomal Damage

Three lethal effects seen following radiation include:

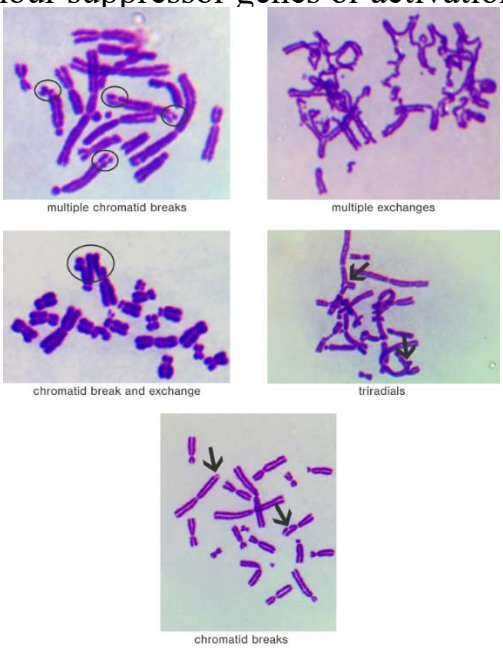
1-A dicentric chromosome is formed when part of an arm of two separate chromosomes are separated. The two chromosomes then attach to each other rather than their separated arms. The new chromosomes contains two centromeres, making it impossible for the chromosome to divide normally at mitosis

2-An anaphase bridge is formed when a duplicated chromosome loses both ends of a paired arm. The arms then unite, and when the cell tries to divide at mitosis it is unable to separate the fused arms.

3-A ring is formed when both ends are lost from the same chromosome. The chromosome then attaches its new ends together, leading to formation of a ring.

Other chromosomal abnormalities

It is possible for chromosomes to reattach in a non-lethal fashion. For example, the ends of two chromosomes may become swapped. This may cause no effect, or may lead to abnormal gene expression if there is an alignment of promoter regions with incorrect genes. These translocations are seen in many malignancies, either causing deactivation of tumour suppressor genes or activation of oncogenes.

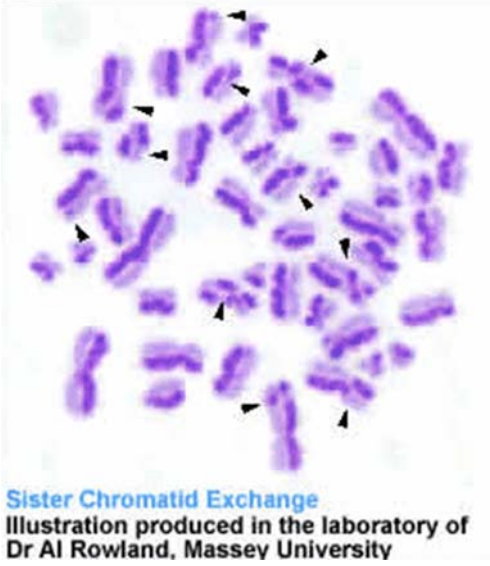


4- Sister chromatid exchange (SCE):

Sister chromatid exchange (SCE) is the exchange of genetic material between two identical sister chromatids.

It was first discovered by using the Giemsa staining method on one chromatid belonging to the sister chromatid complex before anaphase in mitosis. The staining revealed that few segments were passed to the sister chromatid which were not dyed. The Giemsa staining was able to stain due to the presence of bromodeoxyuridine analogous base which was introduced to the desired chromatid.

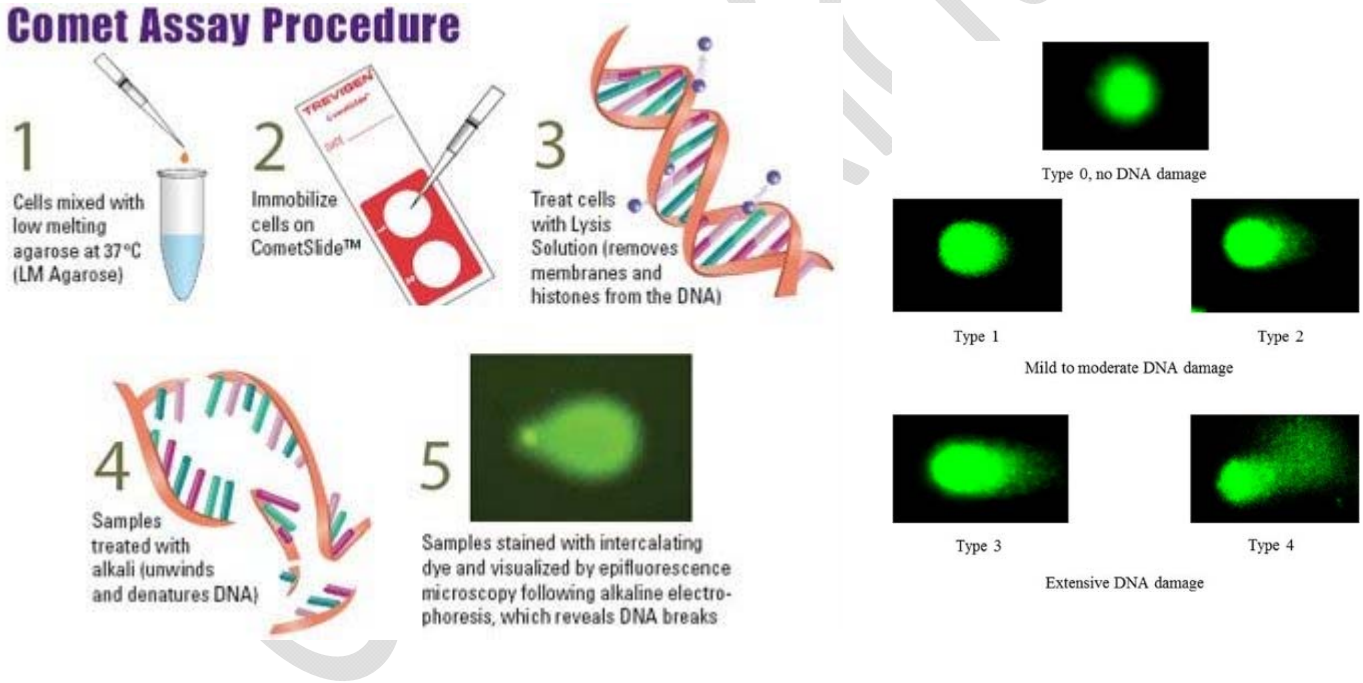
The reason for the (SCE) is not known but it is required and used as a mutagenic testing of many products. Four to five sister chromatid exchange per chromosome pair, per mitosis is in the normal distribution, 14-100 exchanges is not normal and presents a danger to the organism. SCE is elevated in pathologies including Bloom syndrome, in which distributions reach 100-160 per chromosome pair per mitosis. Frequent Sister Chromatid Exchange may also be related to formation of tumors.



Sister Chromatid Exchange
Illustration produced in the laboratory of
Dr Al Rowland, Massey University

5-Comet Assay

The Single Cell Gel Electrophoresis assay (also known as comet assay) is an uncomplicated and sensitive technique for the detection of DNA damage at the level of the individual eukaryotic cell. It was first developed by Östling & Johansson in 1984 and later modified by Singh et al. in 1988. It has since increased in popularity as a standard technique for evaluation of DNA damage/repair, biomonitoring and genotoxicity testing. It involves the encapsulation of cells in a low-melting-point agarose suspension, lysis of the cells in neutral or alkaline (pH>13) conditions, and electrophoresis of the suspended lysed cells.



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