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ENVIRONMENTAL MUTAGENESIS AND GENETIC HAZARDS OF AGRICULTURAL CHEMICALS

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Animals including man and plants are exposed to a variety of chemicals in the environment, especially in recent years. Since industrial development and needs of the modern society are interlinked, pollution and ecological changes resultant of industrial advancement have their own mutagenic effects on plant and animal life. A broad spectrum of chemicals both naturally occurring and applied for different purposes of both simple and complex structure, occur around us and are present in the air we breathe, the water we drink and the food we eat. Since environmental mutagens pose a potential genetic hazard for man, both for the present and future generations, efforts must be made to detect them in our environment and eliminate or restrict their use. In other words, it will be essential to evaluate all chemicals applied to the environment for their mutagenicity.

There are manifold problems in evaluating the mutagenicity of chemicals. It is also necessary to know the types of genetic changes induced by a chemical and how persistant they are in biological systems.

The genetic changes induced by chemicals which have significance in human health, can broadly be grouped into the following categories.

1. *Point mutations:* These could be base-pair substitutions and frame shift mutations. The number of abnormalities in man, associated with monogenic inheritance has increased to over 1000 in the past fifteen years with an additional 1000 suggested, for which proof is incomplete.

2. *Reciprocal translocation:* Next is reciprocal translocations which involve breakage and exchange of segments between two non-homologous chromosomes and are transmitted in a regular manner through mitosis. Such translocations are transmitted as dominants, may be maintained in the population for many generations, and as heterozygotes produce unbalanced chromosome sets at meiosis. Viable mosaic aneuploids may arise following loss of a small translocation element at mitosis-earlier the loss, more severe the abnormality. Karyotype survey of 31,000 new born children has shown that almost 0.2% of them are translocation carriers.

3. *Non disjunction:* The third one is non-disjunction. Aneuploid individuals (monosomics and trisomics) arise due to meitic non-disjunction and non-disjunctional mosaics arise when homologues fail to separate at mitotic division. Fortunately, in man most of the monosomic and trisomic conditions lead to dominant lethals (abortion, still births, etc.) and most of them go undetected.

4. *Chromosome losses:* The fourth is the chromosome losses which may occur when a broken piece of a chromosome does not get incorporated in the daughter cells. This is detected as monosome. Almost all conditions of monosomy are uterine lethal except Turnors' syndrome (XO) which has a high frequency of survival. Also the monosomic mosaics are quite high and these are associated with mild to severe congenetial malformation Hence, even those environmental chemicals which produce chromosome breakage without any rearrangements (eg. phenols and caffeine) could constitute potential risk for future generations.

Test systems for detecting environmental mutagens

Man and other organisms are exposed continuously to the chemicals in the environment, which occur at very low concentrations. It is therefore necessary to assess properly the small mutational effects of the active ingredients of these chemicals at low concentrations. It is also necessary to detect simultaneously many types of changes which may ultimately lead to genetic hazards. Various systems are employed according to their ability and sensitivity to detect different kinds of genetic hazards. There are problems of extrapolating the genetic hazards of a chemical to human beings, once it has been shown to be mutagenic in other sub mammalian systems. Mammals and man, due to their unique mechanisms of metabolic conversion and detoxification, alter a chemical after it enters the body. Thus

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a mutagenic chemical may be converted into a non-mutagenic one or *vice-versa*, inside the body. Various test systems and their suitability for detecting different forms of mutagenic action are briefly described in the following paragraphs.

Tests to detect direct acting compounds

These employ micro-organisms and mammalian *in vitro* cell culture systems and are very useful for rapid screening of large nu.nber of chemicals, Micro-organisms used are *Neurospora*, various strains of yeast and bacteria. Tester strains of *Salmonella typhimurium* have been extensively used to detect frame-shift mutations and base-pair substitutions.

In vitro microsomal enzyme activation: To overcome the problem that some chemicals show mutagenic effects only after microsoma enzyme metabolism, bacterial indicator organisms have been coupled with liver homogenate from mammals. Alfatoxins and polycyclic hydrocarbons are some such compounds which can be detected by this system. This however, cannot test the metabolities produced inside a mammal, by routes other than liver microsomal enzymes.

Screening for mutagenic compounds produced within the Animals

Host-mediated assay: In this test the animal (a mammal) during and after treatment with a potential chemical mutagen is injected with an indicator organism in which mutation frequency can be measured. After sometime the indicator organism is withdrawn and tested for mutations induced. Blood, urine and other body fluids from animals treated with a chemical, can be tested for its mutagenic activity on indicator organisms. Mutagenicity tests using *Drosophila* offer greater advantage because one can simultaneously detect a wide spectrum of genetic changes ranging from chromosome loss to non-disjunction. There are large number of tester strains available in *Drosophila* and it is possible to run host-mediated assays by feeding *Drosophila* on plasma from mice treated with different chemicals. Moreover, microsomal enzyme activation has also been demonstrated in *Drosophila*. Hence, mutagenicity tests on environmental chemicals using *Drosophila* can give relevant and useful information.

Chemicals with mutagenic activity

Eventhough only relatively limited studies have been conducted to test the wide array of chemicals, sufficient information is available to indicate that at least some of the well known and widely used chemicals are mutagenic. Captan, a well known fungicide, is known to cause about 41% increase in chromatid break at a concentration of 10 ppm, congenital mal-formation in chicken embryo and an increase in mitotic gene conversion. Due to its hazard it was suggested by the EPA that the use of this chemical should be banned/ restricted. Among other fungicides commonly used, Benlate (Benomyl) has been tested. While no detectable increase in sex-linked recessive lethals in *Drosophila* was observed, mild chromosome breaking effects of Benlate in cultured human lymphocytes have been reported. Some of the mercurial fungicides were found to increase the frequency of sex linked recessive lethals in *Drosophila*.

Dichlorvos (DDVP) is an insecticide which has been shown to be mutagenic in different *in virto* experiments as well as lower organisms Both positive and negative mutagenicity of DDVP has been reported in *Drosophila*. This compound has also been known to increase sister chromatid exchanges in *in virto* tests. In mice and other mammalian systems, no significant increase in mutation has been induced by DDVP. It appears that DDVP which is a strong mutagen in the lower organisms is converted into non-mutagenic forms within the mammalian systems. Several other organo-phosphate pesticides which have been tested are found to be mutagenic in different systems However, malathion and metasystox have been found to be non-mutagenic in different test systems ranging from bacteria to mammals. However, malathion induced significant decrease in the content of RNA and DNA and also reduced survival of cultured human lymphocytes Tests with methylparathion have given varying results and from the available reports so far this pesticide cannot be considered free from genetic hazards. 2, 4-D and Diquat showed genetic activity. Another herbicide 2, 4, 5-T also induced chromosomal disturbances in *Drosophila*.

Sodium bisulfite, a commonly used food preservative, is known to cause ceamination of cytosine and is found to be mutagenic in *E. coli*. Widespread use of this compound in the animal systems and its continued use is not considered free from genetic hazards. According to Doll and Peto (1981) there are five possible ways or means whereby diet may affect the incidence of cancer (Table 1). More than 20% commercially available tranquilizer based on phenothiazines, have been studied for their mutagenic effects. Chlorpromazine is known to cause genetic damage. Therefore these products are believed to be posing a potential mutagenic hazard. Many products such as 'Flaggyl' which are based on related compounds have been banned in the USA and other countries.

The artificial sweetner saccharine has also been suspected to be mutagenic in action. Tests on rats have suggested an increase in the incidence of lymphosarcoma as well as blood cancer. An increase in the incidence of chromosomal aberrations in onion root tip has been shown after treatment with saccharine.

The available data on this artificial sweetner indicate that this chemical is not free from genetic hazards. There are quite a lot of industrial chemicals comprising of halogenated hydrocarbons and alkylating agents and dietary factors which are chemical carcinogens and mutagens causing mutations in many organisms.

Thus, chemicals found in the environment must be assessed for their mutagenic effects, because they can cause gene mutations and chromosome damage. Observations made on non-mammalian system provide positive indications for thorough tests in mammalian systems which are very essential. Man's g[illegible] constitute his most p[illegible]us heritage, that a deterioration in gene quality can result in a corresponding decrease in the quality of life. Steady progress in the control of infectious diseases, lengthening human life span and improved procedure for identifying genetic disorders have revealed an important residue of genetic disease in human populations. We must, therefore, detect chemicals which are mutagenic in our environment, assess their risk-benefit ratio and eliminate *them from our environment*, or at least minimise their use when they are absolutely essential.

Further Reading:

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TABLE 1. WAYS OR MEANS WHEREBY DIET MAY AFFECT THE INCIDENCE OF CANCER

Possible Ways or Means	Example
1. Ingestion of powerful, direct acting carcinogens or their precursors.	a. Carcinogens in natural foodstuffs (Plant products) b. Carcinogens products in stored food by micro-organisms (bacterial and fungal) c. Carcinogens products in stored food by micro-organisms.
2. Affecting the formation of carcinogens in the body	a. Providing substrates for the formation of carcinogens in the body. (e. g. nitrites, nitrates, secondary amines) b. Altering intake of excretion of cholesterol and bile acids (and hence the production of carcinogenic metabolites in the bowel) c. Altering the bacterial flora of the bowel (and hence the capacity to form carcinogenic metabolites)
3. Affecting transport, activation or deactivation of carcinogens	a. Altering concentration in, or duration of contact with feces (fiber) b. Altering transport of carcinogens to stem cells (alcohol?) c. Induction or inhibition of enzymes (which affect carcinogen metabolism or catabolism) d. Deactivation or prevention of short-lived intracellular species (eg. use of selenium vitamin E, trapping free radicals, use of beta-carotene or otherwise quenching singlet oxygen; use of other antioxidants)
4. Affecting "promotion" of cells (that are already inactivated)	a. Vitamin A deficiency (clinical or subclinical) b. Retinol binding protein. c. Otherwise affecting stem cell differentiation (carotenoids ? determinants of lipid "profile")
5. Overnutrition	a. Age of menarche b. Adipose-tissue-derived estrogens c. Other effects.