INTRODUCTION
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It is well recognised that an unparalleled expansion has occurred in the chemical industry which is witnessed by the development of enormous new organic chemicals and their wider application. These varieties of agents along with their derivatives induce plethora of changes in the hereditary material directly or indirectly, generally termed as mutation. It does not mean either that we are swimming in the sea of carcinogens or mutagens or being bombarded ceaselessly by their uncontrolled number, but we must recognise that many factors from our environment are potential causes of cancer or contribute to the mutagenic risk. These include a broad spectrum of chemicals both naturally as well as synthetic, of both simple or complex nature. Most of the chemicals have already exhibited carcinogenic and mutagenic properties in animal models and in varieties of short term tests (IARC 1982; Wynder 1982).

One of the most striking properties of the neoplastic state is its heritable nature. This is also true of the pre-neoplastic state.

The heritable nature of both the preneoplastic and the neoplastic state has geared to the speculations that the pathogenesis of cancer involves permanent alterations in gene expression, accompanied perhaps by permanent alterations in gene structure. These concepts are now clumped under the general theory of carcinogenesis called the somatic mutation theory (Florey 1962).
It has been known that cancer can arise as a result of exposure to a variety of agents, and studies on the mechanism of the induction process have revealed that pure chemicals themselves are able to produce cancer (Miller 1978). Damage to DNA by environmental mutagen may be the main cause of death and disability in advanced societies (Cairns 1975). Since, a large proportion of human cancer may arise from chemical causes, cancer prevention will depend to a large extent upon the recognition and possible elimination of environmental and endogenous exposure to carcinogenic/mutagenic agents (Doll and Peto 1981). Rapid and accurate in vitro tests such as Salmonella and various mammalian cytogenetic test assays play a crucial role in the identification of environmental mutagens and minimizing human exposures (McCann and Ames 1976).

**Mechanisms of action of the Carcinogenic/Mutagenic Agents:**

A high proportion of human cancers are attributed to environmental agents, brought about by the xenobiotics. Epidemiological evidences strongly suggest that there could be major "environmental" factors behind the real occurrence of cancer. Such factors, rightly speaking, are a combination of all aspects of our life styles.

It is assumed that DNA damaging agents are carcinogenic because they induce mutations. These damage lead to heritable changes in the methylation of cytosine in DNA. Considerable evidence exists to show that gene
expression in mammalian cells is in part controlled by methylation of specific DNA sequences (Robin 1987).

Most chemical carcinogens and alkylating or acylating agents (electrophilic reagents) produce such compounds following metabolism in the body. This means that they have electron deficient centre and will combine with electron rich centre within the cell. Reaction with DNA is the most important process in the initiation of mutagenesis and carcinogenesis, although reaction with certain amino acids in proteins will also occur (Farmer 1982). Alkylating agents are able to react at many sites in DNA, in particular at the nitrogen ring and the exocyclic oxygen atoms of the DNA bases and at the oxygen atoms of the phosphate internucleotide linkages (Margison and O'Connor 1979; Singer and Kusmierek 1982). Substitution at the N-7 position of guanine in DNA is usually the major reaction product with alkylating carcinogens and mutagens, although many other products such as 3-alkyladenine, 3-alkylguanine, O\textsuperscript{6}-alkylguanine, alkylthymidine, 3-alkylcytosine and exocyclic N\textsuperscript{2}-alkylguanine are also formed (Farmer 1982).

Since virtually every chemical known to cause cancer in human also causes cancer in animals (IARC Monographs, 1972-1975; Tomatis et al. 1970; Epstein 1974), the simplest assumption is that any chemical which is a carcinogen in an animal test is likely to be a human carcinogen, though, there are many uncertainties in determining the risk to humans from animal data (Epstein 1974; Mantal and
as mutagens) of only 54% and a specificity (percentage of non-carcinogens identified as non-mutagen) of only 70%. Even worse, Tennant et al. (1987) found that the Salmonella assay would only identify about 45% of carcinogens. These results are at variance with the observations made a decade ago when sensitivities and specificities of 90% or more were claimed for the Ames test (McCann et al. 1975a; McCann and Ames 1976). This looked disastrous for carcinogenisity screening, as for expensive animals, the carcinogenicity studies were the only way to identify carcinogens. But the new work of Ashby and Tennant (1986) (the same authors who forced re-evaluation of the Ames test) lifts this gloom.

Nestman (1986) stated that a mutagen is a mutagen, not necessarily a carcinogen. For instance Caramel, a sugar derivative which is widely used as a good colouring and flavouring agent, is mutagenic in Salmonella test system, but has no carcinogenic effect at all when fed to rats as 6% of the diets for as far as 2 years (Evans et al. 1977). Despite the conclusive evidence of the role of mutagenesis in carcinogenesis, certain chemicals are known to be nonmutagenic carcinogens (Carl et al. 1988).

From the discovery of Brookes and Lanley (1964) of correlation between carcinogen and mutagenic potency of alkylating agents, on one hand, to their ability to bind to DNA on the other, considerable proof has accumulated to show that this signifies a general biological principle. Miller and Miller (1977) concluded that most carcinogens and
Schneiderman 1973; Ames et al. 1987). In general, chemicals carcinogenic in one species are carcinogenic in other species (Tomatis et al. 1970) although the carcinogenic potency of a particular chemical can vary considerably depending upon the animal species in which it is tested and the manner in which the chemical is administered (IARC Monograph 1974; Weisburger 1973). Chemicals of very similar structure can also differ greatly in carcinogenic potency (McCann et al. 1975a).

Correlation of Mutagenesis and Carcinogenesis:

Ames test has been widely used to investigate the mutagenic potential because of the fact that many carcinogens are also mutagenic although a high degree of variation has been seen with regard to the sensitivity of Ames test ranging from as much as about 45% to more than 90% (McCann et al. 1975b; Levin et al. 1984; Zeiger et al. 1987). It was also suggested that a high level of sensitivity is very often a characteristic of the composition of the materials used for testing than that of the test system itself (McCann et al. 1975b). It is also noteworthy that as time has elapsed since the first Ames test was conducted by McCann et al. (1975a) to find the mutagenic potential of various carcinogens. There appeared a continuous decrease in the degree of sensitivity about the Ames testing. Zeiger et al. (1987) reported that the Ames test has a sensitivity (percentage of carcinogens identified
mutagens are electrophilic reagents or they, in a majority of cases, give rise to electrophilic reagents through metabolism (bioactivation), or through various chemical changes.

\[
\begin{array}{ccc}
(A) & \text{Precarcinogen} & (R_iX) \\
\text{Metabolism} & \text{Carcinogen/Mutagen or} & \text{electrophilic metabolite} \\
\text{(in certain cases of)} & \text{electrophilic metabolite} & \\
\text{chemical activations)} & \\
\end{array}
\]

A consequence of random reaction pattern is that if only certain sites in DNA are critical in carcinogenesis or mutagenesis, the appearance of an electrophile in the DNA space will lead to chemical changes at critical sites (DNA). This suggests that any electrophile appearing in the DNA space of a cell is potentially mutagenic or carcinogenic.

Genotoxic effects are induced at varying doses or levels, apparently and at the present state of the art, without any no effect threshold (Ehrenberg et al. 1983). Therefore, with low levels of potentially electrophilic chemicals in human environments, the hazards of raised incidence of cancer and heritable damages possibly of embryonal nature, will be predominating over those of other kinds of toxicity, and hence may be characterised by a dose threshold below which the risk is zero.

**DNA Damage**:

Damage to DNA is likely to be a major cause of cancer and other diseases (Haitt et al. 1974; Ames 1970). There is an evidence which supports that carcinogens and
radiations are likely to initiate majority of human cancers and other genetic defects by way of damage to DNA (McCann et al. 1975a). Defined precisely, DNA damage is an alteration that constitute a stumbling block for the replication machinery and hence hampers the replication of DNA, endangering the survival of the cell (Devoret, 1979).

The correlation between radiation-induced damage and cancer has long been apparent to the radiation biologists but through molecular evidence, it has been found that DNA damage is a direct cause of cancer. The evidence comes from patients suffering from Xeroderma pigmentosum (Davoret, 1979). With certain eukaryotic cells, the consequences of DNA damage can also be assessed by cytogenetic analysis. The human disorders ataxia telangiectasia, Bloom's syndrome and Fanconis anemia are genetic diseases characterised by an increased susceptibility to cancer (Walker et al. 1985).

**DNA Repair**

DNA is the primary carrier of genetic information and the structural integrity of DNA is a prerequisite for gene expression (Modak, 1972). It is a known fact that primary structure of DNA is dynamic and subject to a constant change. All ionizing and UV-radiations as well as multitude of other chemical agents upset the genetic and metabolic machinery of the living system. This would, perhaps render our planet barren, were it not subjected to the constant cellular monitoring and repair. Moreover, the contemporary
global environment has also posed a continuous threat to the hereditary material. The living systems have, therefore, evolved repair processes to maintain structural and functional fidelity of DNA against a large range of insults (Friedberg 1985b). The molecular mechanism involved in repair of damaged portions of DNA and their restriction into functionally intact informational units is fundamental to the maintenance of the functional integrity (Modak, op cit.). Defective DNA repair could cause an accumulation of lesions or mutations which might either be lethal or lead to an altered phenotype, or cause neoplastic transformation. Repair at the cellular and macromolecular level is multiple in its form and varies as a function of species, tissues and stage of the cell cycle (Hart et al. 1979).

A great deal of research has been directed towards gaining new insights into the mechanistic regulation of repair machinery in *Escherichia coli*. The genetic studies have led to identification of a large number of genes participating in their repair of damaged DNA (Walker 1985).

The earliest suggestion on recovery of bacterial after exposure to ultraviolet (UV) light was made by Hollander and Curtis in 1935. Setlow and Carrier (1964) and Boyce and Howard-Flanders (1963) independently demonstrated that the UV induced thymine dimers in bacterial DNA were not excised in a UV sensitive strain but were excised in the wild-type strain. This suggested that excision of thymine dimers from bacterial DNA may be important for cell survival.
and that it is genetically controlled. The following repair systems have been shown to be existing in bacteria.

Photoreactivation:

The simplest class of repair pathway is photoreactivation that directly rectifies the cyclobutane type pyrimidine dimers in UV-irradiated DNA without the formation of new phosphodiester bonds (Walker et al. 1985). It was the first system to be observed in vitro (Rupert et al. 1958). Photoreactivation is a universal phenomenon because it is known to occur in E. coli, yeasts and possibly in higher animals and plants (Schild et al. 1984).

Excision Repair:

An important mechanism for cell survival after UV-irradiation depends upon the release or excision of enzymes, and the subsequent reconstruction of the twin helix by repair enzymes that make use of the intact opposite strand as template. Excision repair appears to be a significant source of DNA repair virtually in all organisms (Walker et al. 1985). Mechanism of repair of DNA by excision has been studied extensively using mutants of E. coli sensitive to ultraviolet radiations (Howard-Flanders et al. 1966). This repair system has at least four steps viz. incision, excision, gap filling and scaling (Hanwalt et al. 1979; Walker 1985).
Post-Replication Recombination Repair:

The DNA lesions, especially the UV induced pyrimidine dimers, that are neither split photoenzymatically nor removed from DNA by excision repair, block the continuous progress of the DNA replication fork. However, they do not prevent the initiation of DNA synthesis at a point beyond the dimer (Rupp and Howard-Flander 1968). Gaps are produced in the daughter strand opposite the lesions and as a result of this the continuity of daughter strand is interrupted by gaps (Benlow et al. 1974). This is called post-replication repair. This was first demonstrated in *E. coli* by Rupp and Howard-Flanders (op cit.).

Inducible Error-prone "SOS" Repair:

The term 'SOS' (International Distress Signal) implies to an error prone repair, induced under enormously stressed condition of growth as a last resort of the survival of the cells. It was described by Defais et al. (1971) and was amplified by Radman (1974, 1975). 'SOS' repair is a highly integrated and sophisticated regulatory network that require de novo protein synthesis for expression (Koval 1986). It is an inducible repair process and is believed to be responsible for common mutagenic pathways (Radman 1974; Wilkin 1976).

Mutagenesis resulting from 'SOS' processing of damaged DNA template is targeted and is not due to the induction of some random mutator activity (Miller 1983).
Mutagenecity and Carcinogenicity Testing Systems:

It has become increasingly apparent that the traditional methods for identifying carcinogens by using long-term studies in rodents are unable to meet demands for a quick, sure and inexpensive identification of environmental carcinogens. This has brought about an intensive search for appropriate test system and over the last few decades a series of short-term tests have been published (Dyrby and Ingvardsen 1983).

Long term tests, however, are expensive and time consuming; they have given considerable evidence in estimating the potency of a carcinogen (Farmer 1982). But there is a problem of predicting whether humans will respond to the carcinogen in the same way as the animals (Farmer 1982). There are several instances in which only one species like mouse was found to respond to the test and the rat was ineffective. Then, how can we extrapolate the risk from rodents to humans, a very dissimilar long-lived species. Opposite was also found in which a chemical was found to be carcinogenic by epidemiological studies but was non-carcinogenic in rodents (Ames et al. 1987).

Short term tests (STTs) for genotoxic chemicals were originally developed to study mechanisms of chemically induced DNA damage and to assess the potential genetic hazard of chemicals to humans. In the following, better known short-term tests are listed (table 1). The role of
Table 1: Methods for the assessment of mutagenicity and carcinogenicity (From annexures V and VIII of Council Directives 79/831/EEC).

<table>
<thead>
<tr>
<th>Test for gene mutations:</th>
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<tr>
<td><em>Salmonella typhimurium</em> reverse mutation assay</td>
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<tr>
<td><em>E. coli</em> reverse mutation assay</td>
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<tr>
<td>Gene mutation, <em>Saccharomyces cerevisiae</em></td>
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<tr>
<td><em>In vitro</em> mammalian cell gene mutation</td>
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<td>Sex-linked recessive lethal test in <em>Drosophila melanogaster</em></td>
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<td>Mouse spot test</td>
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<th>Tests for chromosome mutations:</th>
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<tr>
<td><em>Mitotic aneuploidy, Saccharomyces cerevisiae</em></td>
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<tr>
<td><em>In vitro</em> mammalian cytogenetic test</td>
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<tr>
<td><em>In vivo</em> mammalian bone marrow cytogenetic test</td>
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<tr>
<td>Micronucleus test</td>
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<tr>
<td>Mammalian germ cell cytogenetics</td>
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<tr>
<td>Mouse heritable translocation</td>
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<td>Rodent dominant lethal tests</td>
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<th>Indicator tests for DNA effects:</th>
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<tr>
<td><em>Mitotic recombination, Saccharomyces cerevisiae</em></td>
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<tr>
<td>DNA damage and repair, unscheduled DNA synthesis</td>
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<tr>
<td>Sister chromatid exchange <em>in vitro</em></td>
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<th>Other indicator tests for carcinogenic potential:</th>
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<tr>
<td><em>In vitro</em> mammalian cell transformation test</td>
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these tests has increased, however, because of accumulating evidence in support of the somatic mutation theory of carcinogens (Strauss 1981; Crawford 1985; Ames et al. 1987) and because of reports that many rodent carcinogens in vitro are genotoxic in short term tests (Ames 1979). The in vitro short-term test have the advantage that they can be conducted relatively quickly and inexpensively compared to long term carcinogenicity assays with rodents and do not involve testing in animals. Early studies of concordance between results from in vitro short-term test and rodent carcinogenicity tests were highly encouraging (Durston et al. 1973; Sugimura et al. 1976).

Wide use of short-term tests (STTs) for detecting mutagen (Ames, 1979) and a number of animal cancer tests on plant substances have contributed to the identification of many natural mutagens and carcinogens in the human diet (Kapadia 1982). There is a range of applications in which STTs have been used successfully from the identification of mutagenic fractions in complex mixtures such as cooked meat (Hatch et al. 1984; Sugimura 1985) or air pollutants to the early identification of genotoxicity in the development of new chemical products (Tassignon 1985).

**Ames Testing System:**

Among the various bacterial manifestations of DNA damage, Ames chose mutagenesis as the basis of his pioneering work to develop a test for potential carcinogen
(Devoret 1979). The \textit{Salmonella} mutagenicity test (Ames \textit{et al.} 1975) along with other short-term assays (Hollstein \textit{et al.} 1979), is being extensively used to survey a variety of substances in our environment for mutagenic activities. The test measures back mutation in several specially constructed mutants of \textit{Salmonella}.

The test has been adopted for use in detecting chemicals which are potential human carcinogen or mutagen by adding homogenates of rat liver enzymes (\textit{S}_{g} \textit{mix}) directly to the petriplates as an approximation of mammalian metabolism into the \textit{in vitro} test (Ames \textit{et al.} 1973b). Several histidine requiring mutants in the standard set of \textit{Salmonella} tester strains have GC base pairs at the critical site for reversion (Isono and Yorno, 1974).

\textbf{Cytogenetic studies for the assessment of mutagenicity}:

Chromosome damage indicates a biological effect on the genome, the implications of which for carcinogenesis and mutagenesis are still unknown. It is generally believed that increased rates of chromosomal aberration in the organism may indicate an increased cancer risk for the individual (Forni 1984).

\textbf{Heritable Cytogenetic Damage}:

Human cytogenetics began in the mid 1950s with the development of human tissue culture technique (Hsu 1952; Tjio and Levan 1956; and Moorhead \textit{et al.} 1960). The improvement of this technique which analyses the numerical
chromosome aberration (Lejuene et al. 1963) in human beings, initiated a new branch of human genetics called clinical cytogenetics. The work done on chromosome analysis in adults, newborns and abortuses, permits an estimate of the load of chromosome mutations in man.

The mutations are often called "spontaneous" mutations. It was supposed that part of these new mutations are induced by exogenous agents, including drugs and chemicals (Luers 1955a; Rohrborn 1965). However, for a long time, the geneticist have underestimated the role of chemically induced germ cell mutations. It was due to the knowledge of epidemiological evidence that genetic disease were the result of exposure to chemical mutagens. The direct evidence of induced germ cell mutations, the selection of various stages of prenatal development and the outcome of chemically induced mutations were experimentally examined using 2,3,5-triethyleneimonobenzoquinone-1,4 (Trenimon) (Basler et al. 1976) and mitomycin C (Basler 1980). About 43% zygotes showed induced chromosome aberration.

Short-term Cytogenetic Tests:

It has become feasible by use of cytogenetic methods to detect mutagenic potentiality of chemicals at biologically significant levels and in so doing to consider the complexity of human exposure patterns with various interacting factors and modifying responses.
Some of the recommended methods for the in vitro cytogenetic tests are structural chromosome aberrations, sister chromatid exchanges (SCEs), Micronuclei, cell kinetics etc.

Induced chromosome aberrations in human lymphocytes were well-developed by the mid-1970s (Evans and O'Riorcan 1978). Recommended methodology, classification and scoring of structural aberrations and statistical methods have been described (Evans 1982, Forni 1979). Chromosome aberrations consist of breakage and rearrangements of chromosome visualized in the metaphase plate. Chromosome aberrations are most sensitive to agents that can directly break DNA duplex. However, since most chemical mutagens which cause chromatid type damage, are 'S' phase dependent, the highest yield of aberration is likely to be seen in the first division of the metaphases (Table 2).

Sister chromatid exchanges (SCEs) involve breakage of double stranded DNA in both chromatids followed by an exchange of whole DNA duplexes. The SCEs, like chromosome aberrations, induced by S-dependent agents, are formed by unpaired lesions that are present when the cell passes through S-phase and the chromosome replicates. This is the most sensitive method to detect the mutagenic property of the chemical and to determine which of the metabolites of a premutagenic and precarcinogenic agent might be the most likely ones to interact with DNA and cause its effect (Perry and Evans 1975; Latt et al. 1981).
Table 2: Some typical properties of ionizing radiation and mutagenic chemicals in the induction of chromosome damage in peripheral lymphocytes (PL).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ionizing radiation</th>
<th>Mutagenic chemicals</th>
</tr>
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<tbody>
<tr>
<td>Typical lesions</td>
<td>DNA double-stranded breaks</td>
<td>Base, alkylation, DNA cross links, intercalation.</td>
</tr>
<tr>
<td>Dependency of DNA synthesis (S)</td>
<td>S-independant</td>
<td>S-dependant (Except few radiomimetic chemicals).</td>
</tr>
<tr>
<td>Types of chromosome aberrations induced</td>
<td>G0, Gl: Chromosome type. S: Chromatid chromosome type. G2,: Chromatid type</td>
<td>Mostly chromatid type.</td>
</tr>
<tr>
<td>Induction of SCEs</td>
<td>Ineffective as SCE inducer</td>
<td>Effective SCE inducer</td>
</tr>
<tr>
<td>Relationship to repair in PL.</td>
<td>Lesions repaired of misrepaired immediately. Aberrations produced remain permanent.</td>
<td>Misrepaired can be expressed in vitro</td>
</tr>
</tbody>
</table>

Micronuclei arise from chromosome fragments or lagging whole chromosomes that are not incorporated into daughter nuclei at the time of cell division. The test assay detects both clastogens and agents that affect the spindle apparatus. Cells in first interphase are scored for the presence of micronuclei (Hedde et al. 1978; Fenech and Morley 1985).

An additional check on the potential of the mutagen to produce an effect on the cells can be made by analyzing the proportion of mitotic cells in their first, second or subsequent metaphase for each treatment group and calculating a proliferation index (PI).
Steroids: Uses and Applications:

Naturally occurring as well as synthetic steroids have since long been used in various industries. Several steroids have been reported to possess anti-inflammatory (Boltralik 1988), anesthetic (Evelyne et al. 1988), anabolic (Fennessey et al. 1988), angiostatic (Larrian 1989), antiangiogenic (Judah et al. 1989), cardioactive (Karel and Thomas 1987) and antidiabetic (Ryoji et al. 1988) activities.

Certain steroids have also been reported to control energy metabolism throughout pregnancy (Baird et al. 1985), steroid alkaloid formulations have been used for skin disorder treatment (Chain et al. 1984). Also, there are some steroidal compounds which increase resistance against drugs and toxic agents (Kourounakis 1986).

Sex hormonal steroids are used in human medicine for a large variety of conditions, apart from their uses as oral contraceptive agents viz. in the treatment of dysmenorrhea, endometriosis and dysfunctional urine bleedings and climacterics. Their use in the prevention of postmenopausal osteoporosis, is also there. Progestine have been used in the management of threatened abortion and to prevent premature labour.

Metabolism of the Steroid Hormone:

Steroid hormones appear to enter all cells by passive or facilitated diffusion, but they are retained only in their target organs due to the presence of specific cytoplasmic
receptors.

The natural steroids are rapidly metabolised and eliminated. Oestradiol-17β and progesterone have half lives of 20 minutes, and testosterones are of 11 minutes (Sandberg and Slaunwhite 1958). Some conversions occur peripherally, but the major site of metabolism is in the liver. Elimination of glucuronide/or sulphate conjugates of metabolites occurs via the urine or the bile. The main urinary metabolites of Oestradiol 17β and Oestrone are the conjugates of Oestriol, 2-hydroxy-oesterone, oesterone, 16α-hydroxyoestrone and oestradiol. The main urinary metabolite of progrestron is the fully reduced compound, pregnanediol (Klopper and Michie 1956).

Synthetic steroids may be metabolized in human by the same mechanisms as are the natural compounds. The main metabolites of norethynodrel, ethynodiol diacetate and norgestrel are the 3- and 3 alcohols (Cook et al. 1973). The products are eliminated as the glucuronides and sulphates (Layne 1963).

**Steroid Hormones and Carcinogenesis**

There is a high risk of development of cancer with the use of steroids. Several steroids have been reported to possess tumourogenic and carcinogenic activi^t̂es (Kay 1981). Several steroids have been reported to be mutagenic and carcinogenic on the basis of short term tests (Dunkel et al. 1985). Yet various steroids have been shown to play an
active role in human carcinogenesis (Lipsett 1986). Especially the steroidal hormones and their derivatives have been demonstrated to be mutagenic and carcinogenic in the bacterial as well as in the animal testing systems (Kay 1981; Metzler 1984).

Estrogenic hormones have been shown to possess the tumourogenic activity and are assumed to serve as regulators of tumourous growth. These hormones probably maintain the neoplastic state of the cells in a variety of well characterised experimental animal tumour systems (Katzenellenbogen, 1986). Growth prompting effect on hepatocarcinoma has also been demonstrated to be mediated by Oestrogen receptor in the mated rats (Kohigashi et al. 1986). Tissue steroids have been found to play a role in regulating aromatase activities in breast and endometrial cancer (James et al. 1986). Contraceptive steroids have been assessed for toxicological and carcinogenic hazards (Heywood 1986). Moreover, the stereochemical complementarity of DNA and reproductive steroid hormones have been found to correlate with biological activity (Lawrence et al. 1986).

The following points of possible mechanism is speculative for neoplastic growth:

1. Steroid hormone may increase the binding of chemical carcinogens to cellular constituents — Metabolic activation system.

2. It may activate oncogenic virus production.
3. It may be immunosuppressive and could thus influence tumour occurrence and growth.

4. Exposure to hormones may result into preneoplastic condition which provide an environment for the survival of cells with abnormal growth potentials.

5. It may influence the rate of progression of preneoplastic cells to neoplastic cells.

6. It may preferentially estimate proliferation of abnormal cell populations.

7. It may stimulate the DNA synthesis and mitosis essential for fixation of the transformed state.

8. Hormones, by stimulating the proliferation of normal cells with a definite number of cell divisions, may exhaust the normal cell population and thus eliminate their inhibitory influence over the proliferation of abnormal cells (Nandi 1978).

Steroid Hormones and Embryotoxicity and Teratogenicity:

Many steroidal sex hormones cause antifertility, embryotoxicity and foetotoxicity in several species, and such effects are usually dose-related. Some oestrogens also produce teratogenic effects and impaired fertility in exposed offspring.

In humans, birth defects have been observed in foetuses after maternal ingestion of various drugs. However, only in a limited number of cases, is it possible to ascribe a particular defect to a specific drug. Masculinization of
external genitalia in female foetuses have been observed after the exposure of progesterone. Advancement of skeletal maturation has been noted (Breibart et al. 1983). It has been reported that more congenital abnormalities occur in infants born to women who became pregnant while actually taking oral contraceptives (Nora and Nora 1978). Significant embryolethality was observed after the administration of steroid (Ethinyld Estradiol) in rats (Joshi et al. 1983).

Steroid Hormones and Mutagenicity/Genotoxicity:

Hormones play an important role in the growth, metabolism and control of many vital body functions. Hormone diffuses through the cell membrane and after conjugating with specific receptor proteins in the cytoplasm, passes through the nuclear pore to form steroid DNA complexes that influence the gene (Rubin 1982; Makin 1984).

Estrogens were identified as mitotic inhibitors by Rao and Engelberg (1967) who suggested that the structural specificity is required for mitotic inhibition. Lyette et al. (1970) observed significant increase of hyperploid cells in cultured human synovial cells treated with high concentration of estradiol. Estradiol binds to isolated DNA (Blackburn et al. 1974) and induces fragmentation in isolated DNA (Yamafuji et al. 1971). The hormone is negative in Amest test and does not induce point mutations in mammalian cells in vitro (Lang and Redmann 1979; Drevon et al. 1981). In human, embryonal fibroblasts and kidney epithelial cells, estradiol was
reported to induce structural chromosomal aberrations (Serova and Kekis 1974).

Some of the commonly used oral contraceptive (OCs) which are steroids have been found to be mutagenic in plant chromosomes (Hakeem and Amer 1965; Kabarity and Mazrooei 1984). The species Ovral 50 was found to be genotoxic in mammals such as mice and dogs (Williams et al. 1968). Badr and Badr (1974) demonstrated a high incidence of dominant lethal mutations in female mice. The OCs were not mutagenic in Drosophila melanogaster (Paradi 1981). Chromosomal abnormalities were found in spontaneous abortions in women who became pregnant after stopping the intake of oral contraceptive pills. Carr (1967) and Goh (1967) reported an increased number of chromosomal breaks and rearrangements in lymphocyte cultures of woman taking OCs. This report was confirmed by McQuarrie et al. 1970; Littlefield, 1971. An increased frequency of chromosomal aberration and micronucleus was reported using a combination of drugs containing oestrogen ethinyl estradiol and the progestin norethisterone acetate (Shayma et al. 1991).

Murthy and Prema (1983) observed a significantly increased frequency of SCE in OC users. Simultaneous addition of estradiol, progesterone, and human chronic gonadotrophins increased the SCE frequency in human lymphocyte culture, but their separate administration did not induce SCEs (Sharma and Das 1986).
The mutagenicity of hydrocortisone both with and without metabolic activation (S9 mix) and the inhibition of the bacterial lawn formation could not produce significant result (Maron and Ames 1983). Therefore, glucocorticoid appears to be a highly potent steroidal drug capable of directly attacking the genetic material despite the negative findings with Ames test (Rali et al. 1990).
Definition of the problem and objectives of the present work

These days man is being exposed to a great number of substances which were not encountered by his ancestors, and to which he has not been specifically adapted. Among these substances are food additives, drugs, narcotics, antibiotics, cosmetics, air and water pollutants and the contraceptives. Of these the chemicals/drugs may be far more dangerous to the population in terms of genetic defects than radiation and, as a result, it is important to determine their mutation frequency in mammalian cells.

Sex hormones and the corticosteroid hormones have potential application in pharmacology (Loeb et al. 1973; Kochar 1987). These steroids possess antitubercular, antitumour, antiinflammatory activities and antineoplastic activities (Tait and Burstein, 1961; Killie, 1971). Corticosteroid is reported to be antiinflammatory and immuno-suppressive, antibacterial, antimicrobial and antiviral (Haynes, 1974). Glucocorticoids have important effects on the nervous system. Large doses of glucocorticoids stimulates excessive production of acid and pepsin in the stomach and may cause peptic ulcer.

Both the synthetic steroids have also been shown to possess diversified types of biological activities including their contraceptive, antitumour, antineoplastic, cytotoxic and antibacterial characters (Briggs and Christie, 1976).
The cytogenetic studies conducted on the possible chromosome damaging effect of steroidal hormones so far are inconclusive and controversial. A large number of steroids have been studied for their teratogenic (Joshi et al. 1983), carcinogenic (Rosenfield et al. 1983), mutagenic and clastogenic (Wheeler et al. 1986) potentialities. Although numerous literature have been produced on the genotoxicity of sex steroids, very little attention has been paid on the other steroids like corticosteroids being extensively used.

Keeping in view the practical applications of these compounds, genotoxicity study through a battery of in vitro assays have been attempted. Two groups of steroidal compounds have been chosen viz - (A) Sex steroids, and (B) Corticosteroids. The genotoxic effects of these steroids have been studied to know their harmful effects on humans.

These steroids had been used with a view to study the following objectives:

1. To study the in vitro effects of the steroids through human lymphocyte culturing system.
2. To study the genotoxic effects of the steroids on human chromosomes.
3. The evaluation of the chromosome damage have been paralleled with the presence of metabolic activation (S$_9$ mix).
4. To study the genotoxic effects of the steroid on the frequency of sister chromatid exchange (SCEs).
5. The frequency of SCEs has also been compared in the presence of $S_9$ mix.

6. The effects on cell growth kinetics of the steroid was ascertained in the presence and absence of $S_9$ mix (metabolic activation).