2. SCIENTIFIC BACKGROUND
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2.1 RADIATION PHYSICS AND X-RAYS: Ionizing Radiations (X-rays, Gamma rays, Neutrons and Alpha particles) while passing through matter, eject electrons from the atoms through which they travel, leaving positively charged ions. The distribution of the primary events, (ionisation and excitation along the track of an ionizing particle) will vary according to the type of radiation. The pattern of energy deposition will determine the effectiveness of a particular radiation in inducing the biological end-point. The term used to quantify this energy deposition is LINEAR ENERGY TRANSFER (LET), which is defined as:

\[ \text{LET} = \frac{dE}{dl} \]

where \( dE \) is the average energy locally imparted to the medium by a charged particle in traversing a distance of \( dl \) (ICRU, 1970; IAEA, 1986). LET is expressed as KeV/\( \mu \)m. Ionizing radiation can be divided into sparsely ionizing (Low LET) and densely ionizing radiation (High LET) depending upon the energy deposited per micron unit track. Low LET radiation varies between 1-99 KeV/\( \mu \)m and the High LET radiation varies from 100-200 KeV/\( \mu \)m. The effectiveness of different types of ionizing radiation in the induction of a particular biological end point,
is commonly represented by the term Relative Biological Effectiveness (RBE) and is defined as follows:

\[
RBE = \frac{\text{dose of } 250 \text{ KVp X-rays producing effect } E}{\text{dose of test radiation producing effect } E}
\]

RBE can also be considered in terms of effect per unit dose.

\[
RBE = \frac{\text{effect produced by } D \text{ rads of test radiation}}{\text{effect produced by } D \text{ rads of } 250 \text{ KVp x-rays}}
\]

i.e. ratio of the effect produced by a particular test radiation to the effect produced by the same dose of reference radiation (IAEA, 1986).

The absorbed dose is defined as \( D = \frac{dE}{dm} \), where \( D \) is the dose, \( dE \) is the mean energy imparted by ionizing radiation in a volume element, and \( dm \) is the mass in that volume element. The unit of absorbed dose, according to the International System of Units (SI) is Gray (Gy): and 1 Gy = 1 Joule/kg. In the old terminology, the unit for absorbed dose was the rad, and 1 rad = 1/100 Gy (ICRU 1971).

X-rays are low LET radiation, and for 250 KVp (kilovolt peak) the LET is 2 keV/µm. RBE increases with increase in LET, up to the 100 keV/µm, beyond which the RBE
is reduced. This is the optimum level for production of RBE. With the further rise in LET, excess energy will be deposited in the target, resulting in 'over-kill' - wastage of energy (IAEA, 1986). For low LET radiations the induction of chromosomal aberrations fits into a Poisson distribution because the DNA lesions are distributed randomly, due to the large number of tracks. However, for high LET radiation, the ionizing tracks will be non-randomly distributed among the cells, with energy deposited in more discrete pockets (Lea, 1947). For X-rays the dose response curve will fit the linear quadratic equation:

\[ Y = A + \alpha D + \beta D^2 \]

where A is the background aberrations, Y is the yield of dicentrics, D is the dose, alpha is the linear coefficient, and beta is the dose squared coefficient. This means that dicentrics will be produced when there is a combination of one and two track events. One track events (alpha component), will be more frequent at lower doses and two track events (beta component), will be more frequent at higher doses. But for high LET radiations the dose response curve will be linear (Lloyd et al., 1975).

2.2 DNA DAMAGE AND REPAIR: Mutagens cause a variety of primary lesions in the DNA, such as single strand breaks.
(SSB), double strand breaks (DSB), different types of base damages (BD) such as DNA-DNA cross links, DNA-Protein cross links, phosphotriester formation, radical formation, intercalation, formation of bulky adducts, thymine dimers, apurine and apyrimidine sites (Obe et al., 1982; Collins, 1987).

X-rays mainly cause SSB, DSB and BD. Double strand breaks and base damages are the two lesions mainly responsible for formation of chromosomal aberrations (Van Zeeland, 1984). Natarajan and Obe, (1978, 1984); Natarajan et al. (1980, 1986) and Natarajan and Zwanenberg (1982) gave evidence that DSB is the most important lesion for the formation of chromosomal aberrations. They demonstrated this, by treating the X-irradiated cells with restriction enzyme, Neurospora endonuclease (NE), which converts the SSB into DSB. They found a concomitant increase in chromosomal aberrations with the increase in DSB, and concluded that, DSB is the most important lesion for induction of chromosomal aberrations. However, Preston (1980 and 1982) postulated that BD is the most important lesion in production of chromosomal aberrations. He demonstrated that, when the lymphocytes were X-irradiated and treated with Cytosine Arabinoside (Ara-C), it produced time-dependent
increase in chromosomal aberrations. Since DSB are repaired faster than DB, he interpreted the time dependent increase in aberrations as due to inhibition of 'slow-repair' processes in general and in particular excision of radiation-induced base damages. Thus, he concluded, that incomplete repair of BD leads to the formation of chromosomal aberrations.

For every 10 to 20 SSB, one DSB was introduced by X-rays (Vander Schans et.al., 1982). It was still not clear which of the lesions were responsible for induction of chromosomal aberrations. Natarajan et.al., (1984) investigated the relationship between induced cell killing, chromosomal aberrations, sister chromatid exchanges (SCEs) and point-mutations in chinese hamster Ovary cell lines. While there was a good correlation between SCEs, chromosomal aberrations and cell killings, induction of point-mutations did not correlate with any of these end-points. It was, therefore, difficult to establish a 1:1 relationship between the biological effects and the DNA lesions produced (Natarajan et. al, 1984).

There are three major pathways of DNA repair:
A. Photoreactivation: This repair is specific for pyrimidine dimers (Rupert et.al, 1958). In humans, the
enzyme which catalyses this repair system is photolyase (Sutherland, 1974). This repair system is well understood in prokaryotes and a few eukaryotes especially in mammals (Cook and Regan, 1988).

B. Excision Repair: This repair system has been well studied, and the enzymes involved in this repair process can react on different types of DNA damage (Setlow and Carrier, 1964; Van Zeeland, 1984). Excision repair was demonstrated for the first time, while studying the repair of UV-induced pyrimidine dimers in E.coli (Setlow and Carrier, 1964). Cleaver (1978) first demonstrated the defective UV-induced excision-repair in cells derived from patients with the disease xeroderma pigmentosum. This repair pathway involves various enzymes such as, the endonucleases, exonucleases, polymerases and ligases in a sequential order (Wilkins and Hart, 1974; Cleaver, 1977, 1978). Lesions repaired by this pathway range from physically small lesions like BD and SSB to larger lesions like pyrimidine dimers, DNA-DNA, DNA-protein cross links and adducts (Van Zeeland, 1984). The repair of lesions by the action of endo-and exo-nucleases show greater complexity due to the complex structure of chromatin (Wilkin and Hart, 1974; Cleaver 1977, 1978). The steps in excision-repair
involving polymerases and ligases are simpler. The differing rates in excision repair may be due to many reasons, such as the degree of accessibility to the lesions by the enzymes, etc. (Cleaver, 1977).

C. Post-Replicative Repair: This is a recovery process rather than a repair process. It allows the cell to replicate its DNA, despite lesions (Van Zeeland, 1984). The replicating DNA leaves a gap in the lesion, and this is later filled by a de novo replication of single strand recombination between homologous daughter molecules (Lehman, 1972).

2.3 DNA-REPAIR-INHIBITORS: Taylor and Haut (1962) demonstrated that 5-fluorodeoxyuridine (FudR), a thymidine-analogue, when treated after X-irradiation, enhances the yield of chromosomal aberrations. They postulated that, the chromosomal aberrations were enhanced because, X-ray induced lesions were not allowed to repair by FudR, which resulted in the increase in chromosomal aberrations.

DNA-repair-inhibitors have been classified as:

(a) Inhibitors of Deoxynucleotide synthesis: Egs: 5-Fluorodeoxyuridine, Hydroxyurea and 2'-Deoxyadinosine. These inhibitors act at a late stage of the synthesis of
the immediate precursors of DNA. This effects the rate of DNA synthesis (Kihlman and Natarajan, 1983).

(b) Inhibitors of DNA polymerases: Egs; Cytosine Arabinoside and Aphidicolin. These inhibitors inhibit the action of the enzymes alpha-, beta-, and gamma-DNA polymerases. The specificity depends on the cell type and the status of the cell during treatment (Kihlman and Natarajan, 1983).

(c) Inhibitors of ADP-Ribosyl Transferase: Egs: 3-Aminobenzamide. This enzyme is responsible for maintaining the integrity of the chromatid during replication and repair of the DNA. Inhibiting this enzyme action will also delay the ligation step in the excision repair (Shall, 1983).

DNA-repair-inhibitors inhibit the repair processes in the DNA, leading to the enhancement of mutagen induced chromosomal aberrations. The DNA-repair-inhibitors also inhibit certain metabolic processes in the cell, which also can alter the rate of DNA-repair. This makes it difficult to assess at which stage the DNA-repair processes, are inhibited (Kihlman and Natarajan, 1983).

2.4 CHROMOSOMAL ABERRATIONS: Induced-chromosomal aberrations at present serve as the most reliable and
important biological indicators of accidental or professional radiation exposures (Heddle, 1969). Sax (1938) first reported the induction of chromosomal aberration by X-rays in Tradescantia microspores, and postulated the mechanism of formation of chromosomal aberration after X-irradiation and proposed the 'Breakage-First' hypothesis (Sax 1939, 1940 and 1941).

The important discovery of stimulated peripheral blood lymphocytes culture in vitro (Moorhead et. al., 1960) paved the way to study the effects of radiation on chromosomes on human materials (Bender and Gooch, 1962). This was followed by many reports on the various types and number of chromosomal aberrations induced by different LET radiations in peripheral blood lymphocytes (Dolphin 1978; Lloyd et. al., 1973 and 1975; Bauchinger et. al., 1975; Vulpus et.al., 1976).

Ionizing radiation produces chromosomal aberrations at all stages of the cell cycle. Irradiation of cells at G\(_2\) and G\(_1\) will produce an exclusively Chromosome-type of aberration; irradiation at S-phase will produce both Chromatid and Chromosome-types of aberration and irradiation at G\(_2\) will produce only the chromatid-types of aberration. It is this unique induction of chromosomal aberration which makes this the best assay
for assessing ionization radiation exposures (Sax, 1941; Lea and Catcheside, 1942; Evans, 1962). Peripheral blood lymphocytes are non-dividing (Trepal, 1978). Several years after in vivo exposure to ionizing radiation, the lymphocytes when stimulated to grow in vitro they will reveal a previous history of exposure in the form of chromosomal aberrations (Evans and Lloyd, 1978). About forty years after exposure to the atomic bomb irradiations at Hiroshima and Nagasaki (Japan), chromosomal aberrations continue to persist in peripheral blood lymphocytes of the survivors (Iseki, 1966; Boom et al, 1966; Awa et al, 1983 and 1984 and Aikyma et al, 1983).

After the discovery of the Fluorescent Plus Giemsa (FPG) staining technique (Perry and Wolff, 1974), identification of exclusively first cell cycle metaphases was possible. This is important because ionizing radiation causes mitotic delay and cell death. Further, it induces unstable chromosomal aberrations like dicentrics, rings and acentric fragments. These aberrations will be eliminated in the subsequent cell cycles due to their failure to separate out at anaphase during cell division (Crossen and Morgan, 1977; Scott and Lloyd, 1979). After X-irradiation, when scoring was
restricted exclusively to the first cell cycle, the yield of chromosomal aberration was higher than in conventional staining method. This was due to loss of aberrations in subsequent cell cycles, mitotic delay and cell death (Purrot et al., 1980; Wagner et al., 1983). Purrot et al., (1980) reported that X-ray and gamma rays induce mitotic delay at the rate of about one hour per 100 rads exposure.

Induction of dicentrics is the most important aberration produced by ionizing radiation, and is more frequently used in biological dosimetry (Lloyd et al., 1975). In order to produce a dicentric aberration, DNA damage must be induced in such a way that, two unreplicated chromosomes are involved, such that, the damaged chromosomes can undergo exchange, either as a result of the two misreplication of DNA strand, break, or as a result of misreplication during the excision repair of the base damage. Hence lesions on the chromosomes should be within the target distance (zone of interaction), which is less than 1 μm. With X-rays there is a low probability that two forms of ionization from a single track will occur within the unit track length. Dicentrics will be produced in single and two tract events, while the former is more frequent at lower
doses, the latter is more frequent at higher doses. The
dose response curve fits well into a linear quadratic
equation. For high LET radiations the dose response
curve will be linear (Lea and Catcheside, 1942;
Catcheside et al., 1946).

There are 3 main theories concerning the formation of
structural chromosomal aberration. They are as follows:

A. Breakage-first hypothesis: This theory was postulated
by Sax (1939-41). He postulated that the primary lesion
is a break caused in the DNA by ionizing radiation. The
breaks may be restituted to the original configuration,
or they may interact and rejoin, to form an exchange
aberration, or may remain open to form a terminal
deletion.

B. The Exchange Hypothesis: This theory was postulated
by Ravell (1954, 1959), and was based on the results of
the experiments with radiation-induced chromatid
aberrations in Vicia faba. It is postulated that, the
primary lesion is not a break but a local instability in
the chromosome structure. It can either be directly
repaired, or subsequently lead to an exchange
initiation; or it may interact with another exchange to
form the actual aberration. So, chromatid deletion results from incomplete exchange.

C. The Molecular Theory: This theory was postulated by Chadwick and Leenhouts (1978). It is accepted that the eukaryotic chromosomes are unineme i.e. each chromatid has a single DNA double helix backbone extending continuously from one end, through the centromere to the other end of the chromatid (Du Praw, 1970; Kavenoff and Zimm, 1973 and Kavenoff et al., 1974). Based on this unineme concept, Chadwick and Leenhouts (1978) had postulated that all the radiation-induced chromosomal aberrations are double strand breaks (DSBs) visualized as chromatid-arm breaks at the first posttreated metaphases. The terminal deletions are formed as a result of unrepairsed DNA-DSBs in the chromatid arm. The exchange-type of aberrations are formed due to some form of incorrect rejoicing of strand breaks (Chadwick and Leenhouts, 1978).

2.5 SISTER CHROMATID EXCHANGES: Sister Chromatid Exchanges (SCEs) refer to reciprocal inter-change of DNA between chromatids (Taylor et al., 1957 and Taylor 1958), and can be easily visualized by Fluorescent Plus Giemsa (FPG) staining technique (Perry and Wolff, 1974). The possibility of reciprocal inter-change of chromatids in
somatic cells was first hinted at by Mc Clintock (1938). The first direct observation was made by Taylor (1958). The principle of this procedure is to allow the cells to divide in the presence of tritiated-thymidine, which gets incorporated into the DNA of the chromosomes after the first cell cycle. On replication for the second time, one chromatid is unfillerly substituted, and the other, bifillerly substituted. This can be detected using autoradiographic techniques (Taylor, 1958). Using this technique the chromatid exchanges between the sister chromatids could be identified and quantified. Also, metaphases that had divided once, twice, thrice or more, could also be identified depending upon the incorporation of tritiated-thymidine (Taylor et. al., 1958). This discovery correlated with the discovery that DNA replicates in a semi-conservative manner (Meselson and Stahl, 1958).

The autoradiography technique had some technical problems like clarity of the chromosomes etc. Instead of Tritiated Thymidine, Bromodeoxyuridine (BrdU), a thymine analogue, was used for sister chromatid differentiation by Zakhorov and Egoline, (1972). This procedure involves BrdU incorporation and staining of the slides with Giemsa after heat treatment. Soon, another procedure was
introduced in which, BrdU substituted chromosomes were stained with a fluorescent dye Bisbenzamide (133258) staining, followed by fluorescent microscopy (Latt, 1973). The best resolution of sister chromatid differentiation was obtained after the discovery of the FPG staining technique (Perry and Wolff, 1974). Various explanations were offered for the principle of sister chromatid differentiation (SCD) staining. In the BrdU incorporated strand, protein synthesis is altered, and as a result chromosomal condensation and spiralization is delayed (Zakhorok and Egolina, 1972). The proteins on the BrdU substituted strand are bound more tightly than those on the unsubstituted strand. Due to this, differential staining is observed after heat and Giemsa staining (David et al., 1974). Latt (1973) postulated that the BrdU-incorporated-strand quenches the Hoechst dye much faster, and so the BrdU substituted strand appears lighter. Differentiation is not believed to be due to quenching of the Hoechst dye, but is also attributed to the difference in protein-binding to the BrdU substituted strand (Perry and Wolff, 1974).

SCEs are good indicators of DNA damage induced by chemicals (Carrano et al., 1978); for detecting chromosomal damage (Perry and Evans, 1975); for
identification of chromosomal instability diseases (Chaganti et al., 1974; Wolff et al., 1977; Latt et al., 1975; Galloway and Evans, 1975) and for the detection of cells exposed to carcinogens and mutagens (Perry and Evans, 1975; Carrano et al., 1978; Soloman and Bobrow, 1975; Latt et al., 1975; Takihisa and Wolff, 1977). The first report on radiation inducing SCEs was from Martin and Prescott (1964) and Wolff (1964). In general ionizing radiations are poor inducers of SCEs, except when cells were irradiated at S-phase (Wolff, 1977). Gundy et al., (1984) reported a slight increase in SCEs after X-irradiation below 50 rads. Tofilon and Meyn (1987) reported that when cells were X-irradiated under hypoxic conditions, there was an increase in SCEs.

The number of lesions induced by various chemicals is large and most of them are not identified and quantitated (Natarajan et al., 1984). Studies on the type and the amount of lesions, needed to increase the SCEs have been carried out. About 29,000 pyrimidine dimers or about 16,000 ethylations have to be induced in the DNA to increase the number of SCEs per cell by 1 (Reynolds et al., 1979; Natarajan et al., 1984). SCEs are good indicators for DNA damage induced by UV radiation as well as chemicals, which induce chromosomal
aberrations in an S-dependent manner, but not for ionizing radiation, which induce chromosomal aberrations in an S-independent manner (Review - Wolff, 1977).

SCEs result from different lesions and processes from those that cause chromosomal aberrations. The chromosomal aberrations are associated with cell death whereas SCEs are the representatives of events compatible with cell survival such as mutagenesis (Wolff et al., 1977). Majority of SCEs are genetically neutral because there is almost always a reciprocal exchanges without genetic loss (Wolff, 1977). The actual biological significance of SCEs is not clear (Taylor, 1958; Latt, 1979 and Wolff, 1977).

2.6. MICRONUCLEUS TEST: Radiation induces a variety of chromosomal aberrations viz., dicentrics, rings, acentric fragments, breaks and gaps. The lack of centromere in the aberrations formed (like acentric fragments) prevents them from proper alignment in the spindle fibres, and proper distribution to daughter cells during cell division. These fragments may fuse with the nucleus, or may remain in the cytoplasm and form a micronucleus (Resnick and Martin, 1976; Vender and Schans, 1981). It is observed that about 60% of the induced acentrics are converted into micronuclei (Evans
et. al., 1959). In the first report on induction of micronucleus in human peripheral blood lymphocytes following X-irradiation and dose response curve was reported by Countryman and Haddle (1976). They confirmed that the dose-response-curve fitted into a linear quadratic equation.

The Micronucleus test is ideal for exposures ranging from 50 to 300 rads. It is not a very accurate test for assessing chronic exposure in a human population receiving doses up to 25 rads, because the frequency of induction is too low. Exposure beyond 300 rads also shows no proportionate increase in frequency of micronucleus. This is due to radiation-induced distortion in cells and proliferation, which in turn reduces the kinetics of micronucleus formation (Karepinsky and Haddle, 1983).

The use of BrdU incorporation, and the FPG staining technique has permitted the identification of first post-irradiated cells exclusive, (Pincu et.al,1984). The latest technique (Cytokinetic Block) allows the nucleus to divide without intervening cytokinesis. This is prevented by a chemical, Cytocalasin-B. Only binucleate cells are scored for micronucleus, because they have already grown once in culture (Fenech and Moerly, 1985).
This technique will take care of the mitotic delay and cell cycle progression, and there is a good correlation between the observation of induced-micronuclei and the acentric fragment (Ramalho et al., 1988).

2.7 CELL CYCLE KINETICS AND MITOTIC INDEX: It is possible to detect the cells which have divided once ($M_1$), twice ($M_2$) and thrice or more ($M_3^+$) by FPG staining technique and is necessary to score chromosomal aberrations from first post-treated metaphases (Perry and Wolff, 1974; Scott et al., 1978). Many investigators reported that radiation-induced cell cycle/mitotic delay, is dose dependent. For X-rays the delay is in the order of one hour/100 rads (Purrot et al., 1960). The proportion of metaphases at I, II and third and subsequent cell cycles provide information on the rate of proliferation. The mitotic index is useful in judging the size of the proliferating population. Generally, at 72 hour culture, the proportion of first-cycle metaphases increases, and the proportion of third or subsequent metaphases decreases with an increase of the X-ray dose (Carossen and Heddle, 1983). The second cycle metaphases is in a state of equilibrium between the two. Both these increase of first cycle metaphases and the reduction of second cycle
metaphases are dose dependent. The mitotic index increases with the increase in dosage. While studying the cell cycle kinetics and mitotic index the following must be taken into account: culture medium, serum concentration, antibiotic concentration and the storage of blood before inoculating cultures. This is necessary, since these parameters influence to a great extent the variation in the cell proliferation (Crossen and Morgan, 1977; Lloyd et al., 1977; Munro, 1970).

2.8 RADIO-SENSITIVE DISEASES: It has been known that there are many, relatively small groups of patients in the human population with the following:

(a) increased frequency of chromosomal aberrations and/or SCEs;
(b) hypersensitivity to a few mutagens and
(c) predisposition to cancer.

These patients are collectively said to be suffering from 'cancer prone syndrome' or 'chromosomal instability diseases'. Incidentally, the increased sensitivity to mutagens in these diseases is attributed to inefficiency in the repair of damage sustained by the DNA. However, in no disorder is the precise nature of the cause and its relationship to cancer well established. For the mutation to occur, leading to cancer, there may be
several pathways including, genetic defects and interactions with endogeneous and exogenous environmental agents. Even in the general population there is a fairly wide variation in the response to ionizing radiation as detected by induced chromosomal aberrations (Natarajan et.al., 1982).

Patients with several 'cancer prone diseases' are known to be hypersensitive to X-rays as estimated by induced chromosomal aberrations:


7. Familial polyposis: Parshad et.al.,(1983)
Fanooni's anaemia: Higurashi and Conen (1973 and Biglow (1979)
Retinoblastoma: Morten et al., (1981)

Except in the cases of ataxia telangiectasia and Down syndrome, there are contradicting reports on the hypersensitivity occurring in other diseases. Evans et al (1978) and Kuhn (1980) could not detect hypersensitivity in xeroderma pigmentosum. Evans et al., (1978) and Tice et al., (1978) could not detect hypersensitivity in Bloom syndrome. Bender et al., (1988) did not find hypersensitivity in blood lymphocytes of 'cancer family syndromes' susceptible to colon cancer and epithelial cancers which are inherited in an autosomal dominant pattern.

Radiosensitive diseases estimated by cell viability (dye-exclusion) technique are:


Radiosensitive diseases detected by 'clonogenic survival assay' are established cell lines of:
1. Ataxia telangiectasia;
2. Progeria
3. Two forms of retinoblastoma and partial trisomy of chromosome 13 (Weichselbaum et al., 1980)

Radiosensitivity have been detected by 'colony forming ability' in established cell lines of Usher's syndrome and Huntington's disease: Nova et al., (1987)

2.9 PERIPHERAL BLOOD LYMPHOCYTES: Human peripheral blood lymphocytes are at a presynthetic phase i.e. G0 phase of cell cycle, and less than 0.2% replicate their DNA (Bond et al., 1958; Trepal, 1976). There are three types of lymphocytes in the proportions mentioned below:

(a) upto 85% small lymphocytes consisting of T- and B-lymphocytes.
(b) upto 5% medium sized lymphocytes and
(c) upto 15% large lymphoid cells (Ling and Kay, 1975; Trepal, 1976).

Nowell et al., (1980) first demonstrated that leucocytes could be stimulated by phytohemagglutinin (a protein extracted from a plant Phaseolus vulgaris) to undergo
mitosis in vitro (Ling and Kay, 1975). Carstairs (1962) showed that small lymphocytes are the target cells for mitotic activity of PHA, and that predominately stimulates T-lymphocytes (Janossy and Greaves, 1972; Link and Kay, 1975). The sensitivity to different physical and chemical agents depends upon the proportion of T- and B-lymphocytes in the blood (Natarajan and Obe, 1982), and this proportion is also age-dependent (Augeher et. al, 1974; Steel et. al, 1975). Gamma rays and X-rays have revealed a higher sensitivity of B-lymphocytes, as compared to T-lymphocytes (Santos Mello et. al, 1974; Prosser, 1976; Kwan and Norman, 1977). Spontaneous SCE rate in T-lymphocytes is higher than in B-lymphocytes. A small amount of BrdU is sufficient for SCE differentiation in T-lymphocytes as compared to B-lymphocytes (Santesson et. al, 1979).

Peripheral blood lymphocytes are usually chosen for the test system which monitors the in vivo exposure to various mutagens. This is because about 80% of the lymphocytes belong to the re-distribution pool ie. they leave the blood stream and enter various organs like the kidney, liver, thymus etc (Trepal, 1975 and 1976). Mutations occurring therefore, in the peripheral blood may be distributed to various organs, and mutations
occurring elsewhere in the body may consequently manifest in the peripheral blood lymphocytes. Secondly, they are long lived and nondividing. The lesions caused at any time will be fixed and can be seen several years after exposure. The lesions can be detected as induced-chromosomal aberrations when lymphocytes are stimulated. Hence the peripheral blood lymphocyte test is the best system for both in vivo as well as in vitro studies (Natarajan and Obe, 1982).