Discussion
The genetic material (DNA) as well as plasma membranes get affected by the activity of chemical compounds and may pose threat to the proper functioning of the cells in organisms living in biosphere including aquatic biota (Livingstone, 1998, Arukwe et al., 2000; Eufemia and Epel, 2000; Roche et al., 2000; Schwaiger et al., 2000). In this study, genotoxic influence of PCP and 2,4-D has been assessed using mice Mus musculus as an experimental model.

The PCP and 2,4-D were further tested to study their effects on the chromosomes of somatic cells (bone marrow) in the treated mice. The present study has shown that not only a significant number of chromosomal aberrations could be induced by PCP and 2,4-D in bone marrow cells of Mus musculus but it has also proved that the frequency of CA has time and concentration dependent effects. The chromosome aberration test presented here may provide good indicator to assess the genotoxicity of environmental wastes as well as a viable laboratory alternative. The positive results reported here support the clear indication of the mutagenicity of both PCP and 2,4-D, which are of serious concern especially in case of PCP as it induces higher level of CA than the 2,4-D does.

The production of either chromatid or chromosome type of aberration by an agent depends on the nature of clastogen (chromosomal breaking agents) and the cell cycle stage and the target cell at the time of exposure. Majority of chromosome type effects are described from lesions induced in the G1 phase. A G2 exposure generally results in chromosome aberrations. Most chemical mutagens, however, induce chromatid type aberrations independent of cell cycle stage provided the cells are examined in the first mitosis (M1) after treatment. The greatest frequency of structural aberrations should be exposed in M1 cells (Brusick, 1987).

In the experiments carried out, chromatid breaks were more prevalent than chromosome breaks in metaphase plates observed. This clearly
establishes the fact that the damage affects the DNA strand in its late S phase or after the DNA has underwent replication (Bird et al., 1982). The concentration and duration response clearly reveals significant increase in chromosomal aberrations at 24hr of treatment and a decrease later on suggesting that the chemicals or its metabolites are active during this period. The decline after 24hr may be probably due to the reasons summarized (i) repairment of damage by various polymerases, (ii) removal of the cells or chromosomes with damaged genetic material and lastly (iii) by the inactivation of the chemicals or its metabolites.

The stickiness and pulverization level shows an increase starting from 12hr to 24hr followed by rapid decrease with time. The fact that oxidative metabolisms coincide with the resumption of cytochrome oxidase and formation of ATP enables repairment of chromosomal damage to proceed (George and Cramp, 1986). The PCP acts as an effective inhibitor of mitochondrial ATPase synthesis and acts at cellular level to uncouple oxidative phosphorylation in mitochondria.

For the CA assay be it in vitro or in vivo tests, PCP was labelled as clastogenic by Seiler (1991), although extensive testing in Chinese Hamster Oocytes (CHO) were ambiguous, consisting of a weakly positive response (Galloway et al., 1987).

Findings by Obe et al., (1982) suggested that in case of chromosomal damage, DNA is the primary target which arise as a consequence of either misrepair or mis-replication of DNA (Evans, 1977). The covalent binding of PCP to DNA was confirmed in an in vitro incubation with a metabolic activation system (Van Omen et al., 1986) amounting to about 1/5th of the observed protein binding. Rat microsomal preparations were able to convert PCP to tetrachlorohydroquinone and tetrachlorocatechol in changing ratios (1.2-2.5) depending upon enzyme induction procedure used (Van omen et al., 1986) and both the isomers were proposed to form semiquinone radicals in presence of oxygen and this mechanism produces DNA strand breaks. In
various *Salmonella* strains, (TA 97, TA 102, TA 104), tested by PCP with metabolic activation, were found to be highly susceptible to oxidative DNA damage. Tetrachlorohydroquinone also binds to calf thymus DNA in the absence of metabolic activation (Witte, 1985). The PCP was able to induce micronuclei in DNA strand breaks in zebra mussels and snails (Pavlica *et al.*, 2000, 2002). Various forms of structural chromosomal damage in *H. fossilis* was observed by Ali and Ahmad (1998). Recent reports on Allium root tip showed it to be clastogenic as it inhibited root growth and caused reduction in mitotic index with increased CA frequency (Ateeq *et al.*, 2002). It was also reported that micronucleus formation was due to aneugenic effects of PCP (Pavlica, 1999).

On the other hand 2,4-D was slightly mutagenic in *Drosophila* in recessive lethal tests (Seiler 1978) In the embryonic bovine kidney cells, multipolar spindles and polyploid mitotic states were observed suggesting an influence of 2,4-D on the spindle protein (Bongo and Basrur, 1973) In human cell cultures, dimethy amine salts of 2,4-D increased the frequency of CA in human embryonic fibroblast cultures and in human lymphocyte culture (Berin *et al.*, 1973). The *in vivo* tests also gave positive results with respect to chromosome breakage under the influence of 2,4-D (Pilinscaya 1974).

Garret *et al.*, (1986) evaluated the genotoxic levels of 2,4-D with a moderate response. The positive correlation between the cytogenic damage and spleen abnormality in 2,4-D exposure was evidently showing a dose dependent increase in the percentage of CA in spermatocytes and sperm head abnormalities (Amer and Aly, 2001). Reports of 2,4-D as a moderate genotoxicants also appeared simultaneously (Madrigal-Bujaider 2001). Some studies indicate an increase in malignant tumors in 2,4-D exposed people (Hardle et al, 1994), while Ateeq et al (2002) confirmed their clastogenic effects in plant system.
An issue of considerable controversy in cytogenetic evaluations is the significance of lack of gaps in chromatid and chromosomes. Some investigators feel that identification of gaps is extensively subjective and that they do not constitute true aberrations (Brogger, 1982). This group of investigators have suggested that gaps may be quantified and reported but should not be used in the evaluation of the study. Though gaps are defined as achromatic lesions or non-staining regions, faintly stained regions are frequently judged to be gaps, perhaps, connected between both distal parts, therefore, these gaps are not considered real discontinuities and hence are excluded from the structural aberrations in the present study.

Irrespective of the nature of the primary lesion, induced by chromosome breaking agents, the ultimate lesions responsible for the formation of aberrations seems to be DNA strand break (Natarajan and Obe, 1978). Following the treatment with chemical agents, most lesions, are reported in the form of adducts in which the agent is bound to the DNA with covalent bonds although some chemicals may break the DNA strand directly (Sharma, 1984).

The chromosome aberration test is especially relevant to assessing mutagenic hazard, in that it allows consideration of factors of in vivo metabolism, pharmacokinetics and DNA repair process, despite the fact these may vary among species and tissues. An in vivo test is also useful for further investigation of mutagenic effects detected by in vitro test. Chromosomal mutations and related events are the cause of many human genetic diseases and there is substantial evidence that chromosome mutation and related events causing alteration in oncogenes and tumor suppressor genes are involved in cancer in humans and experimental systems (OECD, 1997).

More reliable cytogenetic techniques have been developed to detect structural aberrations. A relatively recent development of fluorescent based staining method called fluorescent in situ hybridization (FISH) has heralded
a significant improvement in the ability of metaphase based cytogenetic analysis (Tucker and Preston, 1996) in the laser scanning cytometry (LSC). The DNA content of each chromosome, chromatin condensation level and size of individual chromosomes can be measured within few minutes (Ishidate Jr, 1998). With the incorporation of sophisticated and innovative methods like FISH and LSC, the detection frequency and accuracy of mutagenic potential is bound to increase.

The correlation between mutagenicity and carcinogenicity has been a major approach in changes in genetic toxicology. Although the critical importance of DNA in cancer induction is beyond any reasonable doubt, the actual relationship between mutagenic and carcinogenic properties of a chemical is more complex than previously conceived. A primary reason for this complexity is the multistep nature of cancer induction which implies both genetic and non-genetic events (Harris, 1991). Agents of various types may induce chromosomal breaks in cells, which become visible when the cell is arrested in division at metaphase. The agents have been termed clastogens (Ishidate & Sofuni, 1988) and may be having chemical, physical or biological nature. The chemicals may also be of various types, medicinal substances, food derivates or additives, pesticide drugs, organic solvents or metals. The majority of chemical agents which are carcinogenic in mammals interact with DNA and induce damage which, if unrepaired can result in chromosome rearrangements. Epidemiological studies indicate specific chromosomal aberrations in congenitally defective children. With regard to carcinogenicity, many tumors in mammals (including man) showing an altered karyotype and thus chromosomal changes have also been correlated with neoplasia (Radman et al. 1982). The chemical induction of mutations involve a series of events including some or all of the following process.

The process includes metabolic activation and / or detoxification, the formation of reactive electrophilic metabolites, the interaction of these metabolites with DNA, error-free and / or error-prone DNA repair and
altered cell selection. The fact that cancer is a multistep process and that all
tumors contain chromosomal alterations, and exhibit variable degrees of
chromosomal instability has been known for quite some time (Nowell,
1976). With the advent of first chromosomal banding technique, the
fluorescence in situ hybridization (FISH) and wide range of molecular
techniques for identifying genomic rearrangement, more detail of the
specific genetic alterations associated with specific steps in multistep
process of tumor formation have been described (Fearon and Vogelstein,
1990; Sandberg, 1993), and also the chromosomal changes associated with
particular tumor types have been comprehensively classified by Mitelmains

A key step in the formation of tumor is activation of oncogenes and / or inactivation of tumor suppressor genes, resulting in altered cell
proliferative capacity or alterations in other house keeping processes. These
genetic alteration can be produced by point mutation, deletion, translocation,
amplification or non-disjunction leading to aneuploidy. For example, a child
born with one mutant allele of the recessive gene for retinoblastoma, R b 1,
there is frequently a deletion at chromosome 13q 14, loosing only the one
copy of the wild type allele, in a single retinal blast in order for a tumor to be formed during development of the retina. This loss of wild type of allele can arise by non disjunction, loss by non-disjunction followed by chromosomal reduplication of the mutant chromosome mitotic recombination, deletion that includes Rb1 locus, gene inactivation or mutation of the Rb1 locus.

Thus, given that there is a strong association between chromosomal aberration and tumor formation, an appropriate assay for clastogenecity is a good predictor for carcinogenicity at least for directly acting mutagenic chemicals. It is further suggested that at least some indirectly acting mutagenic chemicals can produce chromosomal aberrations (Elia et al.,
1994).
The usefulness of clastogenicity assay for detecting mutagenic chemical is dependent on its design as most tumors are aneuploids, an exception being some haematopoietic tumors, although detailed analysis of these by G banding and most recently by spectral karyotyping (Veldman et al., 1997) shows that hidden chromosomal abnormalities, including aneuploidies, are present. The most difficult goal however, is to establish whether most chromosome numerical changes are a consequence of tumor development rather than cause. This should also be considered in the light of the fact that genomic instability is a hallmark of tumor development (Nowell, 1976). Thus, assays are designed to assess chromosome gains and losses which are not necessarily useful for identifying carcinogens. Nevertheless, a threshold response seems to be a reasonable additional factor for describing the shape of dose response curves for cancer at low levels of exposure when aneuploidy has been shown to be involved rather directly in tumor development (Elhajouji et al., 1997).

The mammalian in vivo micronucleus test is especially relevant to assessing mutagenic hazards, in that it allows consideration of factors of in vivo metabolism, pharmacokinetics and DNA repair processes. An in vivo assay is also useful for further investigations of mutagenic effects detected by an in vitro system. This test was initially developed in mouse bone marrow erythrocytes (Schmid, 1976) but it also has been conducted in rats (George et al., 1990), hamsters (Basler, 1986), and monkeys (Choy et al., 1993). The routine micronucleus test is conducted in mouse bone narrow erythrocytes (Mavournin et al., 1990). Micronuclei are small nuclei that arise from chromosomal fragments resulting from chromosomal breaks (double stranded DNA breaks), or detached chromosomes (microtubule malfunction in cell division). In the mouse micronucleus test the target cells are the bone narrow erythroblasts, while chemically induced micronuclei in the erythroblasts are retained in the erythrocytes after the extrusion of the main nuclei from the cells during maturation and can be scored in
polychromatic erythrocytes PCEs. An increase of micronuclei in PCEs indicate genotoxicity of the test agent.

The procedure of micronuclei test varies by the number of designs and the number of harvests (Tinwell, 1990). All common mouse strains can be used for this assay, toxicity is monitored by animal death or by bone marrow suppression, or by decrease of the ratio of PCEs to normochromatic erythrocytes (NCEs) or to total erythrocytes in the bone marrow. Micronuclei can also be detected in PCEs in mouse peripheral blood (MacGregor et al., 1980). The main advantages of peripheral blood assays are that the sampling and multiple sampling are easier from the same animal and also kinetic studies are possible. The peripheral blood micronucleus test, however can be performed only in mice. The ability of accumulating micronucleated erythrocytes also permit the scoring of micronuclei in NCEs obtained from routine blood smears in multiple dose studies. Retrospectic evaluations induced, of micronuclei in NCEs of peripheral blood have been performed in National Toxicology Progress (NTP) Cancer Biossays (Choy et al., 1985; Mac Greyor et al., 1990). Incorporation of the micronucleus test into chronic animal bioassays provide early information on the genotoxicity of the test agent in the same system for the carcinogenicity bioassay.

The occurrence of the cytogenetic damage in mice Mus musculus exposed to 2,4-D and PCP results into an enhanced frequency of micronuclei when observed in polychromatic erythrocyte, variation in the P/N in bone marrow cells. The genotoxic effects of these compounds had earlier been shown to induce abnormal aberration in Heteropneutis fossilis (Ahmad and Ali, 1996; Ali and Ahmad, 1998) and micronucleus generation in C. batrachus (Ahmad et al 2002; Ateeq et al 2002). Some work on PCP was carried on mutation system and in the intragenic recombination system of MP1 strain of Saccharomyces cerevisae is reported (Fahrig et al., 1978) while 2,4-D is found to induce sister chromatid exchanges in somatic and germ cells of mice (Madrigal-Bujaidar et al., 2001).
The mechanism of depressed adenosine triphosphate (ATP) has been suggested to be the cause of abnormal forms of erythrocytes in fish. A report on dichlorophenoxy herbicides has focussed on uncoupling of the oxidative phosphorylation of various compounds and disruptions of acetyl coenzyme A metabolism (Bradberry et al., 2000) with consequent fall resulting into the level of adenosine triphosphate and the cation pump of the cell. The cation pump of the cell using ATP as an energy source actually breaks down, allowing an influx of the sodium, chloride and calcium ions and water (Wendricks and Mayer, 1985). There has been various technical innovations in mammalian system aimed at improving the sensitivity and reproducibility of MN assay. The development of the chalasin block method is one such method. The technique enables MW to be stored in binucleated cells that have recently gone through cell division, since the addition of cytochalasin B, inhibits cytokinesis (Fenech and Morley, 1985). The hepatic assay has been developed in rodents (Tates et al., 1980).

It was suggested that a number of mechanisms could be involved in formation of MN (Heddle et al., 1983) including chromosomes breakages (clastogenesis) and spindle disruptions (aneuploidogenesis) Until few years ago, cytological evaluation of MN provided little or no information concerning the type of damage involved in their formation. There is considerable debate concerning the size of micronucleus as to whether it can be used to elicit mechanism of formation. The basic premise was that the larger MN were caused by spindle disruptions and would contain whole chromosome fragment, although some relationship between the MN size and the mechanism of origin might be true (Trucker and Preston, 1996). There are speculations about the size of micronucleus. Heddle (1973) and Schmid (1975), suggested their size varies between 1/5-1/20 of principal nucleus in rodents.

It is now known the MN formation depends upon the time needed for replication of DNA and performance of the nuclear division. In both human
and mice the cell cycle is well documented. Thus, so the importance of sampling times for micronucleus production have been highly emphasized with the objective of coinciding with maximum responses of the test system. For this atleast three samples are recommended (OECD, 1983) Before this Salamone et al. (1980) also proposed multiple sampling times keeping in mind that optimum sampling may also vary from chemical to chemical. Scholars like Vanparyas et al. (1992) and Al-Sabti and Metcalfe (1995) have also emphasized the same. The most frequently analyzed cells for MN are those of hematopoietic system, i.e. peripheral lymphocytes and erythrocytes. These cells are readily available from human and laboratory animals. In human erythrocytes, MN exists only in spleenectomised individuals, because spleen effectively removes micronucleated cells from the peripheral pool (Evans, 1988). Present results support the conclusion that the erythrocyte MN test in mice is indicative of short-term cytogenetic damage. The candidate compound PCP and 2,4-D have also been shown to induce both clastogenic and mitotic poisoning effects on plant system as well. Root tip cells of onion experienced inhibitory effects on mitosis as measured by mitotic index values along increased frequencies (Ateeq et al, 2002). Similar observations were also made with almost similar effects by Mohapatra et al. (1995).

Another additional criterion suggested by various workers was to assess cytotoxicity in abnormal cell morphology. Altered nuclear morphology and some abnormal variation in cell morphology are not new to PCP and 2,4-D. The present observation confirmed various forms of nuclear and loss of normal structure.

Current studies have shown that chromosome material can significantly be disrupted by PCP insults and PCE can affect more than one nucleus. Chromosomes may be found to be completely fragmented. These chemicals may directly damage the DNA as well as the mitotic spindles. In case of P. corneus, the nuclei of specific morphology and structure were
recognized as apoptotic cell induced by PCP. Apoptotic nucleus can be recognized by sticking chromatin marginalization forming dense crescent shaped aggregates by nuclear membrane (Pavlica et al., 2000). Adjacent cells may engulf the so called apoptotic bodies during the later phase (Corcoran et al., 1994).

Other studies on the basis of pure PCP showed that it can reduce the number of hemoglobin content of erythrocytes (Renner et al., 1987). In *heteropneustes fossilis* the volume of erythrocytes is reduced by 600 dot units in computer image analysis (CIA) due to drop in hemoglobin content (Ahmad et al., 2002). The volume ratio has been suggested to be an indicator of possible cytotoxic and genotoxic effect of the test compound (Reosenkranz and Klopman, 1993).

The PCP is metabolized to quinols i.e. tetrachlorhydroquinone (Cl₄H₂), which undergo auto-oxidation and / or enzyme-mediated oxidation to the corresponding semiquinone tetrachloro 1,2 benzosemiquinones (Cl₄-1,4-SQ) and quinones are tetrachloro-1,2, benzoquinone (Cl₄1,2-BQ) and tetrachloro 1,4-benzo-quinone (Cl₄ 1,4-BQ). Subsequent reduction of quinones to semiquinones intitiates redox cycling cascades and generates reactive oxygen species i.e. H₂O₂ which induce oxidative DNA damage. Additionally, PCP quinones and semiquinones are capable of reacting with genomic DNA to form direct DNA adducts.

Oxygen derived reactive species are capable of altering biological macromolecules like DNA, proteins, lipids and to certain extent carbohydrates. The primary site of oxidative damage is unequivocally considered to be DNA. The type of alteration can be single or double strand breaks, base alteration, damage to deoxyribose sugar, formation of DNA-protein crosslinks etc. Hydroxyl radical can predominantly cause strand breaks, while singlet oxygen mainly induce base alterations like and hydroxydeoxyadenine and hydroxydeoxyguanine and thymine glycol. The DNA alteration can affect the cell structure function and possibly leads to
mutagenesis and carcinogenesis. The consequences also can be structural or conformational changes by point mutations, rearrangements, deletions and insertions. The presence of 8 hydroxydeoxyguanine in DNA seems to be associated with mutagenicity and carcinogenicity due to its ability to interfere with DNA replication. The ROS can induce mutations in protooncogenes like C-Ha-ras-1 and in p 53 tumour-suppressor gene, and interfere with normal cell signaling resulting in the alteration of gene expression.

The liver is often the target organ for chemically induced tissue injury due to its role in biotransformation of xenobiotics and its unique position within the circulating system. Liver is primary site for metabolism of pesticides. It is, therefore obvious that a high level of LPO coupled with depletion of antioxidant enzymes is noted. Lipid peroxidation has been extensively used as marker of oxidative stress (Huggett et al., 1992) Present findings also show that LPO estimation could provide useful information about the concentration and dose dependent exposure. A significant increase was observed in LPO values in liver of pentachlorophenol and 2,4-D exposed group of mice at 24 and 48hr. All the three concentrations of both the chemicals showed peak LPO at 24hr time interval. Though somewhat reduced values were obtained at 48hr after treatment, still they were statistically significant. The slight reduction at 48hr of treatment might be due to various reasons, like elimination of chemical or its metabolite from the body or the inactivation of the chemical or its metabolites (Shyama et al., 1991). It can be suggested that PCP and 2,4-D or their metabolites can initiate LPO because of the formation of free radicals which induce oxidation processes in the cell. Estimation of lipid peroxidation (LPO) in particular has been found to have high predictive importance as revealed from a credible number of research papers describing its use as a biomarker (Lakner, 1998).
Lipid peroxidation could have both direct and indirect consequences. Selective permeability was also lost due to lipid peroxidation. Its products modified the physical characteristic of biological membranes. They also altered cell signaling or acted as toxic second messengers that amplify damage. Such by products include 4hydroxynonenal, malonaldehyde etc. These by products can bind DNA and induce mutagenicity and carcinogenicity. By products can also result in the induction of apoptosis.

The compound 2,4-D at higher concentration of 10-100 µM 2.2-220 mg/L increases bilayer width and cause deep structural perturbation of hydrophobic region of model membrane system (Suwalsky et al., 1996). The 2,4-D-induced inhibition of ion channels, with the potential to severely disrupt the regulation and maintenance of cellular functions. There are evidence for occurrence of lipid peroxidation by 2,4-D. (Palmeria et al., 1994). The 2,4-D at 1-10mm (220-2200 mg/L) has been shown to induce dose dependent hepatotoxicity in vitro. The depletion of hepatic protective agent such as glutathione, proteins and thiols has also been demonstrated in vitro (Palmeira et al. 1994, 1995).

The glutathione (GSH) is the major cytosolic low molecular weight sulfuysdryl compound that acts as a cellular reducing agent and provides protection against numerous toxic substances including pesticides (Stryer, 1988) by forming GS complexes throughout its thiolate sulfur atom. The GSH has profound importance for cellular homeostasis and for diverse cellular functions. GSH plays a role in such diverse biological processes as protein synthesis, enzyme catalysis, transmembrane transport, receptor action, intermediacy metabolism and cell maturation. The GSH depletion may be the ultimate factor determining vulnerability to oxidant attack.

In pentachlorophenol (PCP) and 2,4-dichlorophenoxyacetic acid (2,4-D) exposed animals maximum, glutathione depletion was recorded for maximum at 24hr of treatment with all concentration of PCP, whereas 2,4-D exposed animals recorded maximum glutathione depletion was seen at 48hr.
The GSH depletion has been suggested to represent an important contributory factor to liver injury and enhanced morbidity related to liver hypofunction. The redox phenomena are intrinsic to life processes, and GSH is a major pro-homeostatic modulator of inter cellular sulhydryl (–SH) groups in proteins (Ondarza et al., 1989, Crane et al., 1988, Hidago et al., 1990). Many important enzymes (such as adenylate cyclase, glucose-6-phosphate, pyruvate kinase, transmembrane Ca-ATPases and at least eight reactions participating in glucose metabolism) are regulatable by redox balance. It is evident that glutathione’s reducing power is used in conjugation with ascorbate and other antioxidants to protect the entire spectrum of biomolecules to help regulate their functions, and to facilitate the survival and optimal performance of a cell as living unit. An estimated 2-5 percent of electrons that pass through oxphos’ system are converted into superoxide and other oxygen radicals (Forman, et al., 1982). Superoxide, peroxide, hydroxyl radical, and other free radicals derived from oxygen are highly reactive and are, therefore, threatening to the integrity of essential biomolecules such as nucleic acids enzymes & other proteins, and the phospholipids responsible for membrane integrity.

It is known that the cellular environment influences the cell function and that cellular environment also regulates the response to genotoxic stress. These factors activate receptors to transmit signals in the cell. These signals may affect to response to DNA damage. The DNA strand breaks activate special signaling system. Consequently, the cells resist toxicity or it may actually mediate the toxic impact on DNA damage. In the present study, the cellular response in terms of DNA damage was analyzed by melting temperature (T_m), agarose gel electrophoresis and fluorescence spectroscopy.

The DNA denaturation, also called DNA melting, is the process by which double-stranded deoxyribonucleic acid unwinds and separates into single strands through the breaking of hydrogen bonding between the bases.
For multiple copies of DNA molecules, the T_m is defined as the temperature at which half of the DNA strands are in the double helical state and half are in the random-coil states (Lucia, 1998). The melting temperature depends on both the length of the molecule and the specific nucleotide sequence of that molecule.

The process of DNA denaturation can be used to analyze some aspects of DNA. Because cytosine-guanine base pairing is generally stronger than adenosine-thymine base pairing, the amount of cytosine and guanine in a genome called the "GC content" can be estimated by measuring the temperature at which the genomic DNA melts (Mandel and Marmur 1968). Higher temperatures are associated with high GC content. In vivo studies revealed genotoxic damage in hepatic cells of mice by PCP and 2,4-D. The rise in the concentration of these compounds was accompanied by increase in DNA damage and decrease in T_m.

As depicted by melting temperature analysis of hepatic DNA due to PCP, it was observed that maximum damage occurred at 24hr of treatment for all the three concentrations as early onset of T_m was observed as compared to the solvent. Lower concentration did show repair of damage at 48hr of treatment, but higher concentration did not show a noticeable amount of DNA repairs.

It is assumed that the early onset of T_m in case of treated hepatic DNA by PCP might be due to induction of apurinic / apyrimidinic sites in DNA by an oxygen radical mechanism, that involves cleavage of the deoxyribose, as well as by glycosylase cleavage of oxidized bases in DNA (Lin et al., 2001; Lin, et al., 2001).

DNA denaturation can also be used to detect sequence differences between two different DNA sequences. On a genomic scale, this method has been used by researchers to estimate the genetic distance between two species, a process known as DNA- hybridization (Sibley and Ahlquist,
1984). In context of single isolated regions of DNA, denaturing gradient gels and temperature gradient gels can be used to detect the presence of small mismatch between two sequences, a process known as temperature gradient gel electrophoresis (Mayers et al., 1987).

The process of DNA melting is also used in molecular biology techniques, notably in the polymerase chain reaction (PCR). DNA melting temperature can also be used as a proxy for equalizing the hybridization strengths of a set of molecules, e.g., the oligonucleotide probes of DNA micro-arrays.

The level of ROS generated causes different types of DNA damage; a basic site as well as single and double strand breaks both in vivo and in vitro systems (Dahlhaus and Appel, 1993).

The damaged hepatic DNA was subjected to alkaline gel electrophoresis. In case of PCP treated hepatic DNA, DNA molecules moved faster than the native hepatic DNA molecule, indicating fragmentation. As the concentration of PCP increased, it resulted in more damage, and the modified DNA moved more rapidly.

Furthermore, it is evident from the gel pattern that at 48hr the DNA damage is repaired for lower concentrations but at the higher concentration the damage is not corrected, because the repair enzymes are also damaged by the chemical or the damage is beyond repairable limits.

In case of 2,4-D, it did induce DNA damage in increasing order as concentration increased. Here, the damage was less as compared to the PCP and hence the repair of damage was more as compared to PCP.

Oxidative tissue damage leads to various toxic manifestations (Hincal et al., 1995). Due to high reactivity of ROS, most components of cellular structure are likely to be potential targets of oxidative damage. This may be reflected in DNA damage, lipid peroxidation (Kappus, 1987) protein
damage (Bring et al., 1996). Typical reaction during ROS-induced damage involves the peroxidation of unsaturated fatty acids (Kappus, 1987).

The generation of reactive oxygen species and oxidative DNA damage from PCP exposure results in carcinogenesis. There are species differences in PCP toxicity, with greater hepatotoxicity reported in mice than in rats. PCP induces hepatic cellular karyomegaly, cytomegaly and degeneration in mice, whereas only mild hepatotoxicity has been observed in exposed rats (Schwetz et al., 1978; Kimbrough et al., 1978). The PCP exposure induces a sustained increase in cell proliferation in the liver of B6C3F1 mice (Umemura et al., 1996). While the effects of PCP on cell proliferation have not been investigated in rats, the species difference in toxicity suggests a possible proliferation of covalent modification of DNA by endogenous and exogenous electrophiles and is generally considered to be important in carcinogenesis. In addition to ohdG, PCP induces other DNA lesions. Randerath et al., (1997) reported that wood preserving waste extracts, which induce PCP, increased bulky oxidative DNA lesions. The quinone and semiquione metabolites of PCP also are strongly electrophilic and capable of binding to macromolecules (Van omen et al., 1986; Witte et al., 1985). The covalent modification of DNA was analyzed by 32p-post labeling after enrichment of assay sensitivity by nuclease P1 digestion as described (Reddy et al., 1986).

In rats bearing Ehrlich tumors, injection of 2,4-D lead to a slight inhibition of tumor development as compared to controls. Concomitantly, the average survival time of mice bearing the same tumors got increased by about 25% by 2,4-D treatment (Walker et al., 1972).

These experiments show that the inhibition of RNA, DNA and protein synthesis seem to be due to some disturbing affect on the biosynthesis of the respective precursors. In the chicken cell cultures (Haag et al., 1975; Preiss et al., 1992) the cells under the influence of 2,4-D caused stimulation to progress from G1 to S phase. The S phase, however, was
prolonged because of inhibition of DNA synthesis. In these cells morphological alterations are also observed, which points to influences on the cell differentiation by 2,4-D.

Among other changes, a heterochromatinization of the nuclear material is noteworthy. This seems to indicate a more direct action of 2,4-D on the structure of interphase chromatin material, and such as influence would either account for inhibition of DNA synthesis. In mouse fibroblasts (L cells) dose-dependent inhibition of growth was observed under the influence of 2,4-D (Cohlberg et al., 1971). It has already been mentioned that chlorophenoxy herbicides (like 2,4-D) may cause uncoupling of oxidative phosphorylation and also of the phospholipid bilayer of mitochondrial membrane (Zychlinski et al. 1990; Palmeira, 1994). In earlier studies, it was found that uncoupling of oxidative phosphorylation can presensitize some cells for a Fas-death signal and provide information about the extensive pathways in induction of apoptosis (Lin-Singer et al., 1999; Krocher, 1998). The 2, 4-D has already been shown to induce hepatotoxic damage which are correlated to cell death (Arias, 1994; Palmeira et al., 1994). The results of these effects agree with the reported activity of the herbicide as a peroxisome proliferation in mice, rats and Chinese hamsters, (Linnainma, 1994; Blair et al., 1990). With regard to chemicals having such activity, a hypothesis has been developed suggesting that the induction of peroxisomal beta oxidation increases the hydrogen peroxide levels and cause oxidative stress in the cell (Blair et al., 1990). Under this process, it is known that free radicals as well as the depletion of the cellular GSH, renders the cell more rather susceptible to the lipid peroxidation and the oxidation of thiols, proteins and DNA (Palmeira et al. 1995). These actions may explain indirectly that the genetic material could well be affected by these chemicals (Madrigal-Bujaidar, 2001).

Further confirmation of DNA damage was supported by florescence studies. Ethidium bromide forms a strong and highly fluorescent complex
with native DNA by intercalation between base pairs, with up to one dye produced per five bases (Lepeeq and Paoletti, 1967). The fluorescence yield reduces to about 50% upon DNA denaturation in neutral solution and becomes very weak when intramolecular hydrogen bonds in single strands are further destabilized (Morgans and Pulley blank, 1974). The DNA-EthBr fluorescence, thus, provide a convenient probe to test (at least qualitatively) radiation or chemical induced alteration in DNA base pair region, a fluorometric method for rapid detection of DNA strand breaks (Birnboim and Jeevak, 1981). In the present study, the results obtained were further investigated by DNA-EthBr fluorescence. It was observed that in case of PCP and 2,4-D, the DNA-EthBr fluorescence is rather specifically inhibited after ·OH attack on DNA. As the concentration increased, the fluorescence got decreased. Maximum reduction was observed at higher concentration. Peak response was observed at 24 hr after treatment for each concentration. However, at 48 hr the reduction was lesser as compared to 24 hr profile. This was because the damage got repaired at lower concentration but for higher concentrations the damage was not repaired to a marked extent. It may be assumed that the DNA backbone gets damaged by PCP to a great extent. The free radicals generated by the PCP caused strand breaks while singlet oxygen induced base alterations like 8-hydroxydeoxyadenine, 8-hydrodeoxyguanine and thymine glycol.