INTRODUCTION
It is generally accepted that a high proportion of human cancers is attributable to environmental agents, mainly environmental chemicals. The distribution of potential carcinogens in the environment is essentially ubiquitous. The human diet contains a variety of naturally occurring mutagens and carcinogens (Ames, 1983). The predominance of certain foods in some countries has been related to the incidence of certain types of cancers in their populations. Therefore dietary mutagens have attracted considerable interest in the past decade and a number of studies on dietary practices in relation to cancer have been undertaken. These studies suggest that a greater intake of fibre rich cereals, vegetables, fruits and a lower consumption of fat rich products and alcohol would be advisable (Doll and Peto, 1981; Peto and Schneiderman, 1981). Although quite a large number of dietary components have been evaluated in microbial and animal test systems, there is still a lack of definitive evidence about their carcinogenicity and mechanism of action. A majority of chemical carcinogens are known to form covalent adducts with DNA and there is a large body of evidence implicating DNA as a critical target in chemically induced cancer (Miller, 1978; O'Connor, 1981). In order to understand carcinogenesis at the molecular level, it is essential to determine the conformational changes in the target macromolecules and relate these findings to possible aberrations in the functioning of modified macromolecules. Of late, there has also been an increasing interest in oxygen radicals and
lipid peroxidation as a source of damage to DNA and therefore as promoters of cancer (Harman, 1981; Gensler and Bernstein, 1981; Totter, 1980; TappeI, 1980). In addition, mammalian systems have evolved many defence mechanisms as protection against mutagens and carcinogens. The most important of such mechanisms may be those against oxygen radicals and lipid peroxidation.

Mutagens and carcinogens in dietary plant material;
It is obvious that food is a very complex substance to which humans are exposed. Most people perceive food substances of natural origin as free of risk. Such acceptance is largely based on faith because our objective knowledge on this topic is relatively poor. A large number of chemicals are synthesized by plants, presumably as a defence against a variety of invasive organisms, such as bacteria, fungi and insects (Kapadia, 1982; Clark, 1982; Pamukcu et al., 1980; Stich et al., 1981a). The number of these toxic chemicals is extremely large and new plant chemicals are being continuously discovered (Jadhav et al., 1981; Griesebach and Ebel, 1978). It has been known for many years that plants contain carcinogens and a number of edible plants have shown experimental carcinogenic activity for several species and various tissues. Wide use of recently discovered short term tests for detecting mutagens (Ames, 1979; Stich and San, 1981) 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,
Safrole and estragole are related compounds, which occur in certain spices and essential oils and are weak hepatocarcinogens (Fenaroli, 1971; Guenther and Althausen, 1949). Recent studies have implicated 1'-hydroxysafrole and 1'-hydroxyestrargole, respectively as proximate carcinogenic metabolites of safrole and estragole (Drinkwater et al., 1976; Borchert et al., 1973). Eugenol and anethole are structurally related to safrole and estragole and are widely used as flavouring agents or as food additives. Black pepper contains small amounts of safrole and large amounts of closely related compound piperine (Concon et al., 1979). Extracts of black pepper cause tumours in mice at a number of sites at a dose equivalent to 4 mg of dried pepper per day given for 3 months.

Ivie et al. (1981) have reported that linear furocoumarins (psoralens), which are widespread in plants of the Umbelliferae family, are potent light activated carcinogens and mutagens. Three of the most common phototoxic furocoumarins are psoralen, xanthotoxin and bergapten. In addition to Umbelliferae, psoralen also occurs in plants from several other families (Ivie, 1978). Psoralen are potent photosensitizers and highly mutagenic in the presence of activating long wavelength UV light. They readily intercalate into duplex DNA where they form light
induced mono- or diadducts with pyrimidine bases. Psoralen, in the presence of light, is also effective in producing oxygen radicals (Ya et al., 1982).

Pyrrolizidine alkaloids are naturally occurring carcinogens and have been found in some fifty species of the families Compositae, Boraginaceae and Leguminosae (Schoental, 1982), which are used as foods or herbal remedies. Several of these alkaloids are hepatotoxic and certain hepatotoxic pyrrolizidine alkaloids are also carcinogenic (Hirono et al., 1977; Schoental, 1976). Testing of pure pyrrolizidine alkaloids for carcinogenicity has been extensive for reasons of a limited supply of these chemicals. However, a number of these alkaloids have been reported to be mutagenic (Clark, 1960) in Drosophila and Aspergillus system (Alderson and Clark, 1966). Recently, Mori et al. (1985) have used a hepatocyte primary culture-DNA repair test to screen seventeen pyrrolizidine alkaloids for their DNA damaging property. This test is highly responsive to carcinogenic pyrrolizidine alkaloids (Williams et al., 1980). Among the results obtained by these authors is the indication of a species difference in liver bioactivation of these alkaloids. This implies that there may be species difference in the carcinogenic potential of pyrrolizidine alkaloids.

Edible mushrooms contain various hydrazine derivatives in relatively large amounts. Most hydrazines that have been tested have been found to be carcinogenic and
mutagenic. The most common commercial mushroom, Agaricus bisporus contains about 300 mg of agaritine, the \( \xi \)-glutamyl derivative of the mutagen 4-hydroxymethylphenylhydrazine, per 100 g of mushrooms as well as smaller amounts of the closely related carcinogen N-acetyl-4-hydroxymethylphenylhydrazine (Toth et al., 1982). Some agaritine is metabolized by the mushroom to a diazonium derivative, which is a potent carcinogen and is also present in the mushroom in smaller amounts. Many hydrazine carcinogens may act by producing oxygen radicals (Hochstein and Jain, 1981).

A number of 1, 2-dicarbonyl compounds e.g., maltol, kojic acid, ethylmaltole, diacetyl and glyoxal have been found to be mutagenic in the Salmonella/Microsome assay. Several compounds in this class are of toxicological interest because they occur in various foods. For example, maltol is a product of carbohydrate dehydration and is present in coffee, soyabean and baked cereals such as bread. Kojic acid is a metabolite of many microorganisms including several fungi used in food production, while diacetyl is an aroma component of butter, beer, coffee, etc. (Fishbein, 1983).

A number of furans, such as 2-methylfuran, 2, 5-dimethylfuran, furfural, 5-methylfurfural and 2-furyl methylketone are found in numerous food products including meat, milk products, various nuts, tea and coffee (Maga, 1979). Stich et al. (1981b) have reported that these furans
induced relatively high frequencies of chromatid breaks and chromatid exchanges when they were exposed to cultured Chinese hamster ovary (CHO) cells in the absence of a liver microsomal preparation. The clastogenic doses of many of the furans were relatively high (100-3900 ppm), whereas the concentration in food products was relatively low. However, Stich et al. (1981b) cautioned that the furans are not the only genotoxic chemicals in the complex mixture of heated, roasted or boiled food products and even if the furans do not pose a serious health hazard by themselves due to their small amounts in most food items, they do contribute significantly to the total genotoxicity of many consumable foods and beverages.

In addition to pyrrolizidine alkaloids, certain glycoalkaloids found in potato, such as solanine and chaconine, have been reported to be highly toxic as they are strong inhibitors of cholinesterase (Jadhav et al., 1981). Pyrrolizidine alkaloids and other glycoalkaloids can reach levels which can be lethal to humans in potatoes that are diseased or exposed to light (Katsui et al., 1982).

Cyclopropenoid fatty acids, present in cotton seed and other oils, have been reported to be carcinogenic and mitogenic having various toxic effects in farm animals. Among these, sterculic acid and malvalic acid are widespread in the human diet. They are also potentiaters of carcinogenicity of aflatoxins (Hendricks et al., 1980). Human exposure to these fatty acids results from the
consumption of products of animals fed on cotton seed. Another major toxin in cotton seed is gossypol, which accounts for about 1% of its dry weight. Gossypol causes male sterility through formation of abnormal sperm and is carcinogenic as well (Xue, 1980). It is a potent initiator and also promoter of carcinogenesis in mouse skin (Haroz and Thomassan, 1980). Gossypol has been tested in China as a possible male contraceptive as it is inexpensive and causes sterility during use. Its mode of action as a spermicide is presumably through the production of oxygen radicals.

A number of quinones and their phenolic precursors are found in the human diet and have been shown to be mutagens (Stich et al., 1981b; Brown, 1980; Levin et al., 1982). Quinones are quite toxic as they can act as electrophiles or accept a single electron to yield the semiquinone radicals which can react directly with DNA or generate superoxide radicals (Morimoto et al., 1983; Kappus and Sies, 1981). Many dietary phenols can autoxidize to quinones generating hydrogen peroxide at the same time. The amounts of these phenols in human diet are appreciable, for example, catechol which is mainly derived from metabolism of plant substances and is a potent promoter of carcinogenesis and an inducer of DNA damage (Carmella et al., 1962).

In addition, there are many other dietary compounds which have been shown to be mutagenic and carcinogenic in various test systems. Allylthiocyanate, a major flavour ingredient of mustard oil, is one of the
main toxins of mustard seeds and has been shown to be a carcinogen in rats (Dunnick et al., 1982). Phorbol esters, present in plants of Euphorbiacea family, are potent promoters of carcinogenesis and cause nasopharyngeal and oesophageal cancers (Hecker, 1981). A variety of carcinogens and mutagens are present in mold contaminated food grains, nuts and fruits. Some of these, such as various aflatoxins, are among the most potent carcinogens and mutagens known (Hirono, 1981; Tazima, 1982). Nitrosoamines and other nitroso compounds formed from nitrate and nitrites in food have been directly related to the incidence of stomach and oesophageal cancer. Nitrates are present in large amounts in spinach, radish, lettuce and beans (Magee, 1982). Although alcohol is not a constituent of a normal human diet, in view of its widespread use, it would be relevant to mention its toxic role. Alcohol has long been associated with the cancer of mouth, pharynx and liver (Tuyns et al., 1982). Alcohol metabolism generates acetaldehyde, which is a mutagen and possibly a carcinogen (Stich and Rosin, 1983; Campbell and Fantel, 1983). It also generates radicals that produce lipid hydroperoxides and other mutagens and carcinogens (Winston and Cederbaum, 1982; Videla et al., 1982).

**Dietary fat - a possible source of carcinogens:**

Fat accounts for approximately 40% of the calories in the human diet. There is epidemiological evidence relating high fat intake with colon and breast cancer. Animal studies have
indicated that high dietary fat is a promoter and a presumptive carcinogen (Kinlen, 1983; Fink and Kritchevsky, 1981; Welsch and Aylsworth, 1983). Two plausible mechanisms, involving oxidative processes, have been considered to account for the relationship between high fat intake and the occurrence of cancer and heart diseases. According to the first mechanism, rancidity of fat yields a variety of mutagens and carcinogens, such as fatty acid hydroperoxides, cholesterol hydroperoxides, fatty acid epoxides and aldehydes (Simic and Karel, 1980; Bischoff, 1969; Petrakis et al., 1981; Imai et al., 1980; Ferrali et al., 1980). Alkoxy and hydroperoxy radicals are also formed (Pryor, 1976-1982). Therefore the colon and digestive tract are exposed to a variety of fat derived carcinogens. The second possible mechanism involves hydrogen peroxide, which is generated by the oxidation of dietary fatty acids by peroxisomes. Each oxidative removal of two carbon unit generates one molecule of hydrogen peroxide, a known mutagen and carcinogen (Reddy et al., 1982; Plain, 1955). Some hydrogen peroxide may escape the catalase in the peroxisomes and thus contribute to the supply of oxygen radicals (Speit et al., 1982; Jones et al., 1981). Oxygen radicals in turn can damage DNA and can start the rancidity chain reaction, which leads to the production of the mutagens and carcinogens mentioned above (Pryor, 1976-1982).

**Mutagens and carcinogens produced in cooking:**

The burnt and browned materials from heating protein during
cooking is reported to be highly mutagenic (Nagao et al., 1978; Sugimura and Nagao, 1979; Pariza et al., 1983). Pyrolysis of protein produces strong frameshift mutagens that require metabolic activation by rat liver S9 fraction (Nagao et al., 1977). Pyrolysates of amino acids also show various mutagenic activities (Matsumoto et al., 1977). Among the various amino acids, the pyrolysate of tryptophan has been found to be most mutagenic followed by those of serine, glutamic acid, ornithine and lysine.

Pyrolysates of various sugars, such as glucose, arabinose, fructose and sorbitol, are all mutagenic in S. typhimurium system without metabolic activation. Pyrolysate of glucose was found to contain acetaldehyde and glyoxal which are mutagenic to S. typhimurium (Nagao et al., 1978). Caramel, which is sugar derived and widely used as a food colouring and flavouring agent is also mutagenic in Salmonella test systems but had no carcinogenic effect when fed to rats as 6% of the diet for two years (Evans et al., 1977). Coffee contains a considerable amount of burnt material including the mutagenic pyrolysis product methylglyoxal (Sugimura and Sato, 1983). One cup of coffee also contains about 250 mg of the natural mutagen chlorogenic acid (Stich et al., 1981a) and about 100 mg of caffeine which can cause birth defects at high levels in several experimental species (Fabro, 1982). There is inconclusive evidence to suggest that heavy coffee drinking is associated with cancer of the ovary, bladder, pancreas
and the large bowel (Trichopoulos et al., 1981). Rancidity reaction of cooking oils and animal fat is accelerated during cooking, thus increasing intake of mutagens and carcinogens (Simic and Karel, 1980).

Food additives:
Sodium nitrite is extensively used as a preservative in meat, fish, and cheese. A possible formation of nitrosamines from amines, present in or derived from the diet, occurs by reaction with nitrous acid at acidic pH. In humans, gastric juice attains a pH of nearly 1.0. Such high concentration of hydrogen ions gives rise to the nitrosyl cation $\text{NO}^+$, which is a highly reactive nitrosylating agent. Nitrous acid itself is a known mutagen for various bacterial and fungal cells. Its mutagenicity is presumably related to the deamination of adenine and cytosine (Fishbein et al., 1970).
Sodium bisulphite is used as a bacterial inhibitor in a variety of beverages and as a preservative in canned fruits and vegetables. The bisulphite anion reacts, rather specifically, with uracil and cytosine, within single-stranded regions of DNA and RNA. It is also mutagenic to bacteria and bacteriophages (Singer, 1983). EDTA and its alkali salts are widely used as sequestrants in various foods. They are useful as antioxidants due to their property of forming poorly dissociable chelate complexes with trace quantity of metal ions such as copper and iron in fats and oils. EDTA has been shown to induce chromosome aberrations and breakage in various plant species.
Saccharin was synthesized in the last century and since then it has been widely used as an artificial sweetener. Reports on the mutagenicity and carcinogenicity of saccharin are conflicting and there is some suggestion that these activities are thought to be due to impurities present in saccharin preparations (Kramers, 1975). The possibility of an in vivo conversion of saccharin into a mutagenic metabolite has also been suggested (Batzinger et al., 1977). Another artificial sweetener, which was widely used but is now banned in USA and many other countries, is cyclamate. Cyclamate induces chromosome breakage in cells of several plants and animal species. It is converted in vivo into cyclohexylamine, which is also an inducer of chromosome breaks (Fishbein et al., 1970).

Oxygen radicals and cancer:
One of the theories of etiology of cancer which is being widely accepted, holds that the major cause is damage to DNA by oxygen radicals and lipid peroxidation (Ames, 1983; Totter, 1980). Several enzymes produce superoxide anion (O$_2^-$) during the oxidation of their substrates, for example, xanthine oxidase and peroxidase (Buettner et al., 1978; Duran et al., 1977). Numerous substances such as reduced flavins and ascorbic acid upon autoxidation produce superoxide anion. This radical further accepts an electron from a reducing agent, such as thiols to yield peroxide (H$_2$O$_2$). There is in vitro evidence that H$_2$O$_2$ may then react with certain chelates of copper and iron to yield the highly
reactive hydroxyl free radical (OH) (Wolff et al., 1986). That the superoxide anion actually appears in metabolism is confirmed by the ubiquitous occurrence of superoxide dismutase. Indeed, certain white blood cells generate superoxide deliberately by means of a specialized membrane bound NADPH oxidase and this participates in the killing of microorganisms and tumour cells (Wolff et al., 1986).

It has been suggested that certain promoters of carcinogenesis act by generation of oxygen radicals, this being a common property of these substances. Fat and hydrogen peroxide are among the most potent promoters (Welsch and Ayisworth, 1983). Other well known cancer promoters are lead, calcium, phorbol esters, asbestos and various quinones. Inflammatory reactions lead to the production of oxygen radicals by phagocytes and this is the basis of promotion by asbestos (Hatch et al., 1980). Many carcinogens which do not require the action of promoters and are by themselves able to induce carcinogenesis (complete carcinogens), also produce oxygen radicals (Demopoulos et al., 1980). These include nitroso compounds, hydrazines, quinones and polycyclic hydrocarbons. Much of the toxic effect of ionizing radiation damage to DNA is also due to the formation of oxygen radicals (Totter, 1980). The mechanism of action of promoters involves the expression of recessive genes and an increase in gene copy number through chromosome breaks and creation of hemizygosity (Kinsella, 1982; Varshavsky, 1981). Promoters also cause modification
of prostaglandins which are intimately involved in cell division, differentiation and tumour growth (Fischer et al., 1982). Most data on radical damage to biological macromolecules concern with the effects of radiation on nucleic acid because of the possible genetic effects. However, in view of the catalytic role of enzymes, damage to proteins is also considered important. It has been suggested that primary oxygen radicals, produced in cells and their secondary lipid radical intermediates, modify and fragment proteins. The products are often more susceptible to enzymatic hydrolysis leading to accelerated proteolysis inside and outside the cells (Wolff et al., 1986).

Anticarcinogens:
The protective defence mechanisms against mutagens and carcinogens include the shedding of surface layer of the skin, cornea and alimentary canal. If oxygen radicals play a major role in DNA damage, defence against these agents is obviously of great importance (Totter, 1980). The major source of endogenous oxygen radicals are hydrogen peroxide and superoxide which are generated as side products of metabolism (Pryor, 1976-1982). In addition, oxygen radicals also arise from phagocytosis after viral and bacterial infection or an inflammatory reaction (Tauber, 1982). The exogenous oxygen radical load is contributed by a variety of environmental agents (Pryor, 1976-1982). The enzymes that protect cells from oxidative damage are superoxide dismutase, glutathione peroxidase (Pryor, 1976-1982). D.T.
diaphorase (Lind et al., 1982) and glutathione transferases (Warholm et al., 1981). In addition to these enzymes, some small molecules in the human diet act as antioxidative agents and presumably have an anticarcinogenic effect. Some of these compounds are discussed below.

Tocopherol (vitamin E) is an important trap of oxygen radicals in membranes (Pryor, 1976-1982) and has been shown to decrease the carcinogenic effect of quinones, adriamycin and daunomycin which are toxic because of free radical generation (Ames, 1983). Protective effect of tocopherols against radiation induced DNA damage and dimethylhydrazine induced carcinogenesis have also been observed (Beckman, et al., 1982). β-carotene is a potent antioxidant present in the diet and is important in protecting lipid membranes against oxidation. Singlet oxygen is a highly reactive form of oxygen, which is mutagenic and is mainly generated by pigment mediated transfer of energy of light to oxygen. Carotenoids are free radical traps and are remarkably efficient as quenchers of singlet oxygen (Packer et al., 1981). β-carotene and similar polypropene are also the main defence in plants against singlet oxygen generated as a byproduct of the interaction of light and chlorophyll (Krinsky and Deneke, 1982). Carotenoids have been shown to be anticarcinogens in rats and mice and may also have a similar effect in humans (Mathews-Roth, 1982; Peto et al., 1981). Glutathione is present in food and is one of the major antioxidants and is antimutagenic in cells.
Glutathione transferases are a major defence against oxidative and alkylating carcinogens (Warholm et al., 1981). Dietary glutathione is an effective anticarcinogen against aflatoxins (Novi, 1981). The cellular concentration of glutathione is influenced by dietary sulphur amino acids (Tateishi et al., 1981). Selenium, which is present in the active site of glutathione peroxidase, is another important dietary anticarcinogen. Glutathione peroxidase is essential for destroying lipid hydroperoxides and endogenous hydrogen peroxide and therefore helps to prevent oxygen radical induced lipid peroxidation (Flohe, 1982). Several heavy metal toxins, such as Cd$^{2+}$ (a known carcinogen) and Hg$^{2+}$ decrease glutathione peroxidase activity by interacting with selenium (Flohe, 1982). Some other dietary antioxidants include ascorbic acid and uric acid. The former has been shown to be anticarcinogenic in rodents treated with UV light and benzo (a) pyrene (Hartman, 1982). Uric acid is present in high concentrations in the blood of humans and is a strong antioxidant (Ames et al., 1981). A low uric acid level has been considered a risk factor in cigarette caused lung cancer; however, too high levels may cause gout.

In addition, edible plants contain a variety of substances such as phenols that have been reported to inhibit or enhance carcinogenesis and mutagenesis in experimental animals (Ames, 1983). The inhibitory action of such compounds may be due to the induction of cytochrome P-450 and other metabolic enzymes (Boyd et al., 1982). The
optimum levels of dietary antioxidants have not been determined; however, there might be considerable variation among individuals. On the other hand, high doses of such compounds may lead to deleterious side effects. The differences in cancer rates of various populations are generally considered to be due to environmental and lifestyle factors such as smoking, dietary carcinogens and promoters. However, these differences may also be due, in good part, to insufficient amounts of anticarcinogens and other protective factors in the diet (Maugh, 1979).

In the past two decades, there has been much emphasis on the induction of cancer by occupational and industrial pollution factors. There is growing recognition, however, that these may account for only a small fraction of human cancer. It is becoming increasingly clear from epidemiological and laboratory data that diet is an important factor in the etiology of certain human cancers. It has been suggested by Doll and Peto (1981) that in the United States diet accounts for 35% of cancer deaths. According to these authors, there are five possible ways whereby diet may affect the incidence of cancer; (i) ingestion of powerful direct acting carcinogens or their precursors (ii) affecting the formation of carcinogens in the body; (iii) affecting transport, activation or deactivation of carcinogens; (iv) affecting "promotion" of cells that are already initiated and (v) overnutrition. Normal individual consumption of potentially mutagenic
substances per day from foods and beverages is estimated to be between 1 to 2\(^\text{nd}\) gm. In addition, the endogenous conditions favour the formation of still more mutagens \textit{in vivo} in humans (Oshshima and Bartsch, 1981).
SCOPE OF THE WORK PRESENTED

Mutagenicity of methylglyoxal:

Methylglyoxal (MG), also known as pyruvaldehyde or acetylformaldehyde, is a ketoaldehyde and may arise in the cell both enzymatically (Cooper and Anderson, 1970; Elliot, 1960; Sato et al., 1980) and nonenzymatically (Riddle and Lorenz, 1968) from free trioses. Besides, it has also been reported to be present in various foods, such as roasted coffee, beans, tea, whisky and soy sauce (Sugimura and Sato, 1983). Whether the enzymatic MG formation actually occurs in mammals has been controversial for many years (Meyer, 1953; Bonsignore et al., 1976; Salem, 1975; Van Eys et al., 1962; Riddle and Lorenz, 1968). The isolation of MG synthase from the enterobacteriaceae (Cooper, 1974; Yuan and Gracy, 1977) and its presence in rat liver cells (Sato et al., 1980) confirmed that MG can be formed enzymatically from triose phosphates. Riddle and Lorenz (1968) observed that MG formation from both dihydroxyacetone phosphate (DHAP) and DL-glyceraldehyde is accelerated by polyvalent cations at physiological pH values.

Szent Gyorgyi (1967, 1977) proposed that MG interacts with the highly reactive sulfhydryl groups that may participate in the regulation of cell division in tissues and that this MG-SH complex can arrest cell division in rapidly dividing cells. MG and other similar aldehyde compounds exert significant effects on certain cancers by reducing the ascites fluid formation, prolonging the
survival time of animals bearing those tumours and decreasing the mitotic index of normal and tumour cells (Jerzykowski et al., 1970; Fenselau and Long, 1976; Dianzani et al., 1978, 1980). The uncontrolled proliferation of tumour cells is supposed to be due to destruction of MG (Szent Gyorgyi, 1977) by two enzymes, namely glyoxalase and \( \alpha \)-ketoaldehyde dehydrogenase, which catalyze its oxidation to lactate (Racker, 1951) and pyruvate (Monder, 1967), respectively.

In millimolar amounts, MG exerts several damaging effects on various biochemical parameters (Dianzani, 1979) and has been found to inhibit in vitro the growth of a variety of mammalian cell lines (Gregg, 1968; Klamerth, 1968; Scaife, 1969). It has been shown to be mutagenic in Salmonella typhimurium TA100 (Kasai et al., 1982; Fujita et al., 1985) and to induce DNA repair in the pyloric mucosa of rats by gastric incubation (Furihata et al., 1985).

Recently, in a screening performed to elucidate the DNA damaging activities of a series of biotic and xenobiotic aldehydes, Brambilla et al. (1984) found that exposure of cultured mammalian cells to non-toxic concentrations of MG resulted in the formation of macromolecular cross-links, mainly of the DNA protein type (Brambilla et al., 1985). Sister-chromatid exchanges in Chinese hamster ovary cells (Faggin et al., 1985) and mutagenicity in cultured Chinese hamster lung cells, assessed by using diphtheria toxin resistance as a selective marker have also been reported.
(Nakasato et al., 1984). MG causes a dose-dependent increase in the frequency of HGPRT deficient mutants in V79 cells (Cajelli et al., 1987). Efforts have also been made to investigate the effect of MG on the microtubular system in order to understand the mechanisms of its antiproliferative activity (Gabriel et al., 1985). Studies in our laboratory have shown that the reaction of MG with DNA leads to the formation of strand breaks and interstrand crosslinks as a function of MG concentration and also its time of reaction with DNA (Rahman et al., 1990a).

Fugita et al. (1985) have recently reported that MG considerably enhances the mutagenicity of H2O2 which is otherwise weakly mutagenic in Salmonella typhimurium TA100. In view of the above observation it was of obvious interest to examine if MG generate oxygen free radicals in solution, which are responsible for its increased mutagenicity. Using an absorbance method, the present work demonstrates that MG undergoes photo-oxidation leading to the generation of superoxide dependent OH radical formation through the Haber-Weiss reaction.

Mutagenicity of quercetin:
Of late, there has been increasing interest in naturally occurring compounds, ingested as part of the normal diet, which are potentially mutagenic/carcinogenic (Ames, 1983). One such class of compounds is the flavonoids which occur in large amounts in a wide range of food plants including many
fruits, vegetables, tea and the skin of tubers and roots (Herman, 1976). There are many flavonoids in plants and the mutagenicity of more than seventy naturally occurring flavonoids has been tested (Nagao et al., 1978). Of these, quercetin was the strongest mutagen, followed by kaempferol, rhamnetin, galangin, isorhamnetin and fisiptin (Brown, 1980; Nagao et al., 1981). It has been estimated that the average daily intake of flavonoids in the American diet is about 1 gm and thus there is clearly a potential hazard. All these compounds except quercetin required metabolic activation by rat liver enzymes when tested in microbial systems (Nagao et al., 1978). The mutagenicity of quercetin was further enhanced by rat liver enzymes. This suggests that quercetin may interact directly with cellular DNA. Besides the microbial system, flavonoids especially quercetin and kaempferol have also been tested for mutagenicity in higher systems, such as rat, hamsters and Drosophila. However, in these systems there have been conflicting reports on the mutagenicity and carcinogenicity of quercetin (Hirono et al., 1981; Pamukcu et al., 1980; Watson, 1982). Whereas studies of Ambrose et al. (1952) reported quercetin to be non-carcinogenic to rats fed 1% quercetin for 410 days, more recent definitive studies of Pamukcu et al. (1980) demonstrated that quercetin was carcinogenic for the intestinal and bladder epithelium of the rat when fed as a basic grain diet of 0.1% quercetin (of purity > 99%) for 58 weeks. Although the mechanism of carcinogenicity of quercetin is not known, it has shown significant effects on
DNA synthesis, lactate production and cyclic adenosine 3',5'-monophosphate level in neoplastic cells (Podhajcer et al., 1980).

Quercetin in common with other flavonoids is a candidate substance for the development of antiviral agents (Vanden et al., 1986; Van Hoof et al., 1984) and is a promising compound for the inhibition of tumor invasion (Bracke et al., 1987). The mechanism by which quercetin exhibits its antitumour activity is not understood. Since it is a frame-shift mutagen in S. typhimurium (Ames, 1972) it has been argued that it might be an intercalating agent (Bjeldanes, 1977). However, there are no chemical data to support this view. The genotoxicity of quercetin correlates with the ability of the substance to cause DNA strand scission in the presence of Cu(II) and molecular oxygen (Rahman et al., 1989). Previous studies have suggested that flavonoids function as scavengers of reactive species of oxygen such as singlet oxygen (Takahama et al., 1984), superoxide anion (Takahama, 1983), and H2O2 (Takahama, 1984). From the studies on the effect of metal ions, antioxidants and pH on the mutagenicity of quercetin in S. typhimurium, Hatcher and Bryan (1985) concluded that this reaction is antimutagenic. Contrary to the above observations the strand scission reaction of quercetin is associated with the formation of a ternary complex of DNA, quercetin and Cu(II) (Rahman et al., 1990b), followed by a transient reduction of Cu(II) to Cu(I) and the generation of
active oxygen species (Rahman et al., 1989). These conflicting reports led me to investigate if quercetin is capable of generating oxygen free radicals in solution.

Using fluorescence and absorbance techniques it is reported here that quercetin upon photo-oxidation or metal-catalysed oxidation leads to the generation of $O_2^-$ dependent OH radical production through the Haber-Weiss reaction (Haber and Weiss, 1934; Beauchamp and Fridovich, 1970).

Structural requirement for mutagenicity of quercetin and related flavonoids:

There are conflicting reports on the structural features essential for the mutagenicity of flavonoids. Macgregor and Jurd (1978) using S. typhimurium reversion assay found that it is the ability of the free OH groups at positions 3' and 4' of the B-ring to oxidize to a quinoid intermediate and of the proton of the 3-OH group to tautomerize or rearrange to keto compounds which is responsible for the mutagenicity of flavonols. Hatcher and Bryan (1985), on the other hand, using the same strain TA98 obtained opposite results. The discrepancy in these results led Rahman et al. (1989) to compare the DNA breakage activity of different flavonoids which are structurally related with quercetin. Their findings suggest that the OH groups in the position 3' and 4' of the B-ring in quercetin are required for the reaction and that O- substitution in rutin and absence of 5-
OH in fisetin result in the diminution of the reaction. The structures of quercetin and other flavonoids used are summarized in Fig. 1. Since none of these flavonoids is similar to quercetin with respect to number and positions of OH groups it does not establish whether the diminution of the reaction is due to the lesser number of OH groups or due to their positions other than 3' and 4'. In order to precisely determine the involvement of 3' and 4' OH groups in the reaction, the DNA breakage activity of quercetin should be compared with a related flavonoid which differs from quercetin only in having OH groups at positions different from those of quercetin in the B-ring. One such flavonoid is morin (Fig. 1) which has OH groups in the B-ring at 2' and 4' positions. Preliminary experiments indicated that morin does not cleave DNA as efficiently as quercetin. In order to account for the low DNA breakage activity of morin and to define in a more precise manner the structural features of flavonoids responsible for their binding and cleavage of DNA, it seemed logical to carry out parallel studies on morin.
Fig. 1. Structure of naturally occurring flavonoids showing numbering of ring atoms.

In morin R₁, R₃, R₄ and R₅ are OH whereas in quercetin R₁, R₂, R₄ and R₅ are OH. Rutin is a glycoside of quercetin in which R₅ is the disaccharide, rutinose (α-1-L-rhamnosido-6-D-glucose). R₁, R₂ and R₅ are OH in fisetin; R₄ and R₅ are OH in galangin; R₁ and R₄ are OH in apigenin. In all the flavonoids, unspecified R groups are H.
Backbone structure