CHAPTER 1  GENERAL INTRODUCTION

1. Occurrence and exposure

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1.1 Occurrence and exposure

Cesium is an alkali metal with certain unique properties which may lead to its extensive use in the field of alternate energy resources. It is the most electropositive of all stable atoms. The high atomic weight and low ionisation potential of this element suggest its suitability as a future fuel in ion propulsion engines. The low melting and boiling points of cesium metal, together with its high vapour pressure, may indicate a possible use in very high power gas-ion laser devices. Cesium is already widely used for different industrial processes (see page 41 under Review of this thesis).

The importance of the study of the effects of exposure to cesium (Cs) was realised following the Chernobyl accident, in which large amounts of radioactive Cs were released in the biosphere (see page 41 of Review). The radioactive forms of Cs, as Cs$^{134}$ and Cs$^{137}$, are produced during the fission of uranium and plutonium and form a major contamination problem following nuclear fallout, particularly in areas close to nuclear testing sites (see Wallace et al., 1971, 1981, 1982, 1983; Marckwordt and Lehr, 1971; Wallace and Romney, 1972a, b; Dahlman et al., 1975). When used in radiotherapy, as a substitute for radium, Cs has been suggested to pose radiation hazards (Vera, 1985).

Initial interest in the possible health hazards from the radioisotopes of Cs led to the publication of numerous articles dealing with mainly the determination of the whole body retention of the radionuclides and methods for their elimination (see Leggett, 1986 for review). Later, attention was diverted to the toxic effects of stable Cs as well, following the observation that the stable form can moderate to a certain extent, the uptake and retention of the radioactive forms in plants from the soil (Wallace et al., 1982). In addition, Cs enters easily into the plant and animal systems and ultimately the food chain. Further interest was generated by the realisation that its properties are closely related to the other alkali earth metals, namely sodium, potassium, lithium and rubidium, which are known to be biologically important elements.

Cs is transferred from soil, where it exists in a stable form in a K:Cs ratio of 1:5000, into pasture vegetation (see Wallace et al., 1982) and finally to the food chain (see Boikat et al., 1985). Cs isotopes, have been identified in high doses in wheat, rye and barley flour (see Bunzl and Kracke, 1987). The amounts are highest in cereals and fruits, followed successively by leafy vegetables and then underground tubers (see Jackson et al., 1987). The concentration of radioactive Cs was increased drastically in fruit bodies.
due to its easy penetration in soil-plant-animal-food chain, Cs has been identified in variable amounts in different organs in plant, animal and human systems (Ishikawa, 1987; Kasperek et al., 1979; Lin and Wen, 1988; Roomans et al., 1976; Sato and Kato, 1979; Seeger and Schweinshaut, 1981; Versieck et al., 1977). Since Cs can act as an analogue of K, it can be potentially harmful (Windholz, 1983).

1.2 Effects on living organisms

Cesium has been found to be toxic to lower organisms, like spiroplasmas (Chang, 1986), certain viruses (Nookt et al., 1982), bacteria (Demidkina et al., 1989), algae, yeast and Aspergillus (Kurita and Funabashi, 1984). The effects were, however, related to the species tolerance and the concentration of the metal used. In some cases, the presence of Cs increased the rate of growth, as in Chara fragilis (Strauss, 1980). In higher plants, Cs hampered chlorophyll synthesis and affected the protein metabolism in sprouting seeds (for details, see page 56 of the Review). Cs⁺ cation has been shown to be responsible for the adverse photosensitive growth behaviour of the hypocotyls of light-germinated seedlings of tomato (Kordan and Oritseja, 1984).

In lower animals, the activity of Cs was found to be related to the concentration to which the system had been exposed. Large concentrations of Cs in the ionic form affected the conduction of K ions; Cs interfered with the Na:K exchange mechanism in frog muscles (Beauge, 1973) and blocked the adenosine-5'-triphosphate-dependent K channels in sarcolemma vesicles in a voltage-dependent fashion (Quayle et al., 1988).

Considerable information is available on the toxic effects of Cs salts on mammals. The degree of toxicity differed according to the form of the compound. Most reports indicate that Cs compounds have a lower level of bioactivity and toxicity than rubidium. The effects depend on the concentration of the salt and the mode of administration (Walbum, 1929). Intravenous injection was twice as effective as oral administration. Direct harmful effects on rodents included poisoning and irritation of skin and mucous membrane (Bruk, 1969). The reports are variable, because in certain experiments, multiple intraperitoneal injections of CsCl to rats and mice daily for more than a month did not induce any significant difference from the controls.

Most workers have reported a general moderate toxicity of Cs, usually as CsCl, in mice and rats. The organs most affected appeared to be the liver, intestine, heart and kidney (Pinsky et al., 1981). The absorption of Cs from the gastrointestinal system is rapid and its movement is affected by the
presence of food (Moore, 1962). In laboratory rats, the total excretion of Cs was cumulative, between 50 to 20% of the dosage in 4 days (Gregus and Klassen, 1986). Administration of repeated and acute injections of CsCl to mice and subsequent study of brain tissue showed that the accumulation was considerably higher with repeated treatment (Messiha, 1976). In common with other alkali metals, Cs affects central nervous functions in mice and other mammals. A central depressant action in mice was reported by Bose and Pinsky (1984), amongst others. These authors suggested that Cs⁺ can be used for the treatment of motor side effects of antipsychotic agents.

In the human body, orally administered Cs¹³⁷ is rapidly and almost completely absorbed; and is taken up by the red cells from the plasma (Rosoff et al., 1963). The retention pattern of Cs in adult humans may vary widely with age, sex, state of health and may also show substantial variations for healthy persons of the same age and sex. The biological half-life of Cs has also been suggested to be an increasing function of age throughout life (McCraw, 1965). Cs follows the movement of K in the body and competes with K for transport across cell membranes, but substantial differences in the distribution and retention of Cs develop because of differences in transport rate across membranes (Leggett, 1986). The amount of Cs has been measured in patients suffering from nervous disorders. Persistent imbalance has been recorded of Cs, together with Na,K and Rb, in the brain of patients with Alzheimer's disease (Thompson et al., 1988).

Very little information is as yet available on the effects of Cs and its compounds on cell division, chromosomes and cellular components in higher organisms, which initiated the present work.

1.3 Screening for clastogenic effects of environmental agents

The awareness of genotoxic effects of environmental chemicals have led to the development of a number of tests. In screening for the cytogenetic effects, both in vivo and in vitro systems have been widely used. Chromosome damage has been scanned in a battery of biological systems. In general, it has been found to be a reliable index of the measure of genetic damage to man (Hsu, 1982; Sharma A., 1984; Sharma and Sharma, 1989; Mason et al., 1990).

Following a spate of publications on genotoxicity of environmental pollutants and of chemicals of daily use, certain guidelines were set up for minimal criteria of acceptability of the numerous short-term assays developed for genotoxicity (see Naismith, 1987). Mammalian test systems include mice and rats and a favoured tissue is the bone marrow, where the frequency of
chromosomal aberrations induced gives the potential clastogenic effects of the chemical, when compared with control animals (Preston et al., 1987). Amongst plants, in vivo effects of chemicals have been assessed extensively using *Allium*, either *cepa* or *sativum*. This test, first devised by Levan (1949) has been very useful for effects following prolonged chronic exposure. Of a number of other plants used in testing for genotoxicity, *vicia faba* and (Kihlman, 1975), maize (Plewa, 1985), are well known. Acute exposure can be screened after exposure of seeds of leguminous plants (Sharma, 1986).

Human leucocytes, from peripheral blood of healthy donors are cultured and are used for testing levels of genotoxicity. Over the last decades, these tests have been extensively employed in our laboratory to assess the genotoxicity of a wide variety of chemical agents, including metals, pesticides, insecticides, food additives, colourants and others. The first observation was that even traces of metals in water distilled in a copper retort and in tap water were able to induce spindle disturbances in *Allium* root-tip cells (Sharma A.K. and Sen, 1954). Spontaneous chromosome aberrations were recorded after exposure to a variety of chemicals (Sharma and Sharma, 1960).

With most of the chemical agents, cytotoxic effects could be induced in most of the higher organisms used as test systems, when exposed for sufficiently long periods to higher concentrations of the clastogen (Sharma and Sen, 1954; Sharma and Mukherjee, 1955; Sharma and Roy, 1955; Sharma and Bhattacharyya, 1956; Sharma and Sarkar, 1957; Sharma, 1959; Sharma and Chaudhuri, 1959; Sharma and Varma, 1959; Sharma and Bal, 1959; Sharma and Gupta, 1959a,b; Sharma and Sharma, 1960; Sharma and Datta, 1962; Sharma et al., 1963; Sharma and Ghosh, 1965; Sharma and Talukder, 1965; Sharma and Bhattacharyya, 1967; Sharma and Sarkar, 1967; Sharma and Ghosh, 1969; Chatterjee and Sharma, 1970; Banerjee and Sharma, 1971; Roy, 1973; Talukder, 1975; Sikka and Sharma, 1976; Singh and Sharma, 1980; Sahu et al., 1980; Banerjee et al., 1981a, 1984, 1986; Bandopadhyay and Sharma, 1982; Chatterjee et al., 1982; Giri et al., 1984, 1986a,b, 1988, 1990; Sharma A., 1984, 1985, 1986, 1989; Roy Talukder et al., 1985; Chatterjee et al., 1986; Mukherjee et al., 1988; Sharma and Sharma, 1989). The clastogenic effects of the metals were found to depend on the test system used, the dose and duration of exposure, the mode and vehicle of administration and the form of the chemical used in addition to the rates of absorption, distribution and retention in the tissues (Giri et al., 1978, 1979, 1980, 1981, 1984; Singh and Sharma (1980, 1981; Sanyal et al., 1980; De and Sharma, 1981; Das et al., 1983, 1983; Mukherjee et al., 1984, 1985, 1988a, b, c, d;

A major factor in the screening of cytotoxic effects was the presence of other metals in the system, since industrial exposure is usually to a complex mixture, rather than to a single metal. Experiments were carried out to test the effects of mixtures, as related to the life-style and genetic polymorphisms in exposed populations from the industrial belts of the Eastern India. Surveys of populations indicated a marked influence of diet and addiction on the levels of cytotoxicity (Sharma, 1989).

Interaction between different metals, as seen in laboratory animals in vivo, modified to a significant extent the clastogenic activity of individual metals. Such modifications were observed in counteracting the effects of Pb by Se (Chakraborty et al., 1987); Hg by Se (Das et al., 1985); Cd by Se (Mukherjee et al., 1988); Al by Ca (Roy et al., 1990) and Co by Ca (Palit et al., 1991) in our laboratory. These studies are of considerable importance in view of the occurrence of metals in combination in workplace environment (see Sharma and Talukder, 1987; Sharma, 1984,1985,1990a,b; Dhir et al., 1985c,1990a,b; Das et al., 1985; Nag et al., 1986,1987; Sen et al., 1986,1987,1991; Banerjee et al., 1987; Giri et al., 1988; Agarwal et al., 1989; Talukder, 1990; Ghosh B.B. et al., 1990; Roy et al., 1990,1991; Mukherjee et al., 1990,1991; Ghosh A. et al., 1991c).

A number of plant and vegetable constituents have been observed to reduce or suppress mutagenic, clastogenic and carcinogenic activities of different chemicals, including metals and pesticides when given as dietary supplements to laboratory animals (Morita et al., 1978; Lai et al., 1980; Giri and Banerjee, 1986c; Dhir, 1989,1991; Sharma A., 1990c). Chlorophyll and its component, chlorophyllin, are universally present in green plant parts and are known to be non-toxic and able to reduce the mutagenic activity of complex mixtures like cigarette smoke, tobacco chew, coal dust and fried shredded pork (Lai, 1979; Ong et al., 1986; Hayatsu et al., 1988). The action of chlorophyllin in reducing clastogenic effects in mice after exposure to nicotine and heavy metals has been observed in our laboratory (Sen et al., 1991; Ghosh A.K. et al., 1991) as well.
Parts of plants and crude plant products have also been recorded to decrease the toxic effects of known clastogens, mutagens and carcinogens (Sharma A., 1990a). The plant products include, amongst others, leaves of *Piper betel* L. (Sen et al., 1989) and fruits of *Phyllanthus emblica* L. The various plant products shown to have anti-cytotoxic effects are condiments (Amonkar et al., 1986); extracts (Ito et al., 1986); pigments (Arimoto et al., 1980a,b); flavonoids and polyphenolic acids (Huang et al., 1983; Wood et al., 1983; Steele et al., 1985; Alldrick et al., 1986). The fruits of *Phyllanthus emblica* L. of the family Euphorbiaceae contain a high amount of vitamin C. The extracts of these fruits and of related species have been extensively used in the Ayurvedic and Unani systems of India for the treatment of a wide variety of diseases (see Chopra et al., 1956). The aqueous extract has been shown to reduce the clastogenic effects of known clastogens in our laboratory. These include zinc chloride, ethyl parathion, metanil yellow (Giri et al., 1989), lead and aluminium salts (Dhir et al., 1990a,b), and nickel (Agarwal et al., 1989). These activities have been attributed principally to the vitamin C present in the extract. Ascorbic acid, as the principal component of vitamin C, shows antimutagenic and anticlastogenic effects (Parshad et al., 1978; Mirvish, 1981; Gebhardt et al., 1985). It is in addition a widely investigated inhibitor of the nitrosation reaction (Bartsch et al., 1988) and inhibits the formation of N-nitroso compounds (Licht et al., 1988). It has also been shown to antagonise the toxic activity of metallic salts in mammalian systems (Chakraborty et al., 1977). However, in all the experiments carried out in our group, the crude extract gave significantly greater protection against the clastogenic effect of the chemical tested, than an equivalent amount of vitamin C, indicating that the other components of the extract, like ellagic and gallic acids, though present in very small amounts, give a synergistic effect (Agarwal et al., 1989a, 1991; Dhir et al., 1990a,b; Roy et al., 1990, 1991; Ghosh et al., 1991d).

### 1.4 Programme followed to test cytotoxicity of cesium

In the present investigation, due to the paucity of the available information on Cs, a detailed programme has been worked out to assess the cytotoxic effects of cesium chloride - the most common salt of Cs - on a battery of test systems, both in vivo and in vitro. In the second part of the programme, the cytotoxic effects on cesium chloride have been modified by the administration of other metals and dietary components to laboratory animals in vivo, in order to find out, if possible, the scope of the use of such dietary supplements in counteracting the effect of exposure to Cs.
Part I : Direct effects - Experiments were planned to:

a) Study the effects of inorganic cesium salt, as chloride, on plant system in vivo following acute and chronic treatments and subsequent recovery in nutrient medium.

b) Study the effects of single exposure to CsCl in different concentrations on animal system in vivo.

c) Compare the cytotoxic effects of different concentrations of the metal, and periods of exposure in vivo.

d) Study the effects of CsCl in short term human lymphocyte in vitro as related to various associated factors such as age, sex and dose of the chemical.

The following endpoints were screened:

In plant system:

i. alterations in divisional frequency

ii. alterations in chromosomal structure and number and induction of spindle disturbance.

In animal experiments:

i. alterations in frequency of divisional cells

ii. frequency of aberrations in chromosomal structure and number.

In vitro experiments:

i. Changes in divisional frequency

ii. changes in chromosomal aberrations including structure and number.

For in vivo studies, both plant and animal systems were selected. Bulbs of Allium sativum and seeds of Trigonella foenum-graecum were used for assay. Laboratory bred swiss albino mice (Mus musculus L., 2n=40) maintained on standard diet were used for the animal experiments.

These protocols were followed so as to cover model systems of the higher organisms which have been exposed to acute dose of the chemical following different methods of administration. The observations were made on different experimental sets after 6, 12, 18 and 24 hours for short term tests and after 72 hours and up to 120 hours in longer tests so as to cover recovery of the cell during several cycles.

Different concentrations of the chemical were used in each case to find out the relationship of the concentration with the degree of clastogenicity, if any.
These studies were aimed to understand the action of Cs, in its most commonly available form, chloride, on chromosomes and cell division and the factors influencing the action. These data are needed to understand the enigmatic role of this trace element in the functioning of the life systems.

Part II: Modification of the direct effects in animal test systems in vivo through interaction with:

a) Other metals - calcium chloride, given by gavaging before, after and simultaneously with CsCl in different concentrations.

b) Plant product - chlorophyllin, in the same way as calcium chloride.

c) Crude plant extract - aqueous extract of fruits of *Phyllanthus emblica* L. (family Euphorbiaceae), given by gavaging for seven days daily to mice before exposure to CsCl.

d) Vitamin C (synthetic) in amount equivalent to that in the fruit extract, given daily for seven days before exposure to CsCl.

The endpoints screened were the same as those applied in testing for clastogenicity of CsCl in vivo in animal systems.