CHAPTER - 1

INTRODUCTION
Rapidly growing industrialization in the present day world poses a serious threat to the environment as it is getting deteriorated due to a variety of pollutants being let off into the biosphere in bulk quantities. These pollutants have the potential to interfere directly with certain biological, biochemical and physiological processes (Venugopal and Lucky, 1978).

The potential toxicity of many trace metals including Aluminium (Al), Cadmium (Cd), Mercury (Hg), Lead (Pb), Copper (Cu), Zinc (Zn) and Arsenic (As) is well established. Among these trace metals Hg, Cd and Pb constitute the greatest risk to the well being of human population (Chang et al., 1980 and Kumar et al., 1991).

Lead is an ubiquitous element with numerous industrial and domestic applications (Chisolm and Barltrap, 1979). Recently, a serious concern has been expressed over the adverse effects of lead on human health (Posne et al., 1978 and Landrigan, 1990) due to its wide spread use (Grandjean, 1978 a and 1978 b; Chaner et al., 1986; Svoboda, 1981; Schwartz et al., 1990 and Refowitz, 1984).

Adverse health effects from occupational lead exposure have long been recognized, especially when lead is heated, or sprayed. Canned food may also be contaminated with lead from soldering which is used to seal the canes. Drinking water, generally has low lead concentrations but occasionally may have elevated levels due to leaching of lead from pipes especially if the water has low pH. Atmospheric lead from leaded gasoline and unchecked industrial emissions can pollute crops and reservoirs. Other sources of lead include bone meal, fertilizers made from sewage sludge, fruits treated with lead arsenate insecticides, ceramic pots, newsprint including the burning newspapers and flakes of old lead paint (Underwood, 1977).

The daily lead intake in an adult ranges from 0.1-0.6 mg (Kehoe, 1963). There are two major routes of lead absorption viz. gastrointestinal tract and respiratory tract (Berman, 1966).

Lead has been postulated to be retained in three body compartments i.e., blood, soft tissues and calcified tissues. In the blood, the first
compartment, about 95% of Pb is bound to erythrocytes and has a biological half life ($T_b$) of 25-30 days (Robinowitz et al., 1975). In soft tissues, the second compartment, lead has a $T_b$ of a few months, and in the brain it may be somewhat longer (Grandjean, 1978c and Robinowitz, 1975). In the calcified tissues, the third compartment, where more than 90% of body lead is accumulated, it has $T_b$ of 30 to 40 years (Barry and Mossman, 1970 and Schroedar and Tipton, 1968).

The effect of lead pollution on biological systems has been extensively reported (Klein, 1974; Baloh, 1974; Stofen, 1974; Scoppa et al, 1973 and Singh et al., 1992). It has been established that lead toxicity results in the alterations of various biochemical parameters which include hepatic marker enzymes, brain enzymes, neurotransmitters, haematological parameters and thyroid functions (Flora and Tondon, 1987; Louis et al., 1978; Silbergeld and Lamon, 1980; Sterling et al., 1982; Ragunathan and Sundaresan, 1983; Vyskosil et al., 1990; Tandon and Flora, 1989 and Refowitz, 1984). Further, lead has also been shown to interact with Fe,Cd, Cu, Zn, K, Rb and Br in body tissues (Finelli et al., 1975; Ragan, 1977; Kumar et al., 1991 and Singh et al., 1993).

Despite, the large accumulation of lead in liver, where it is metabolized, an area of lead toxicity that has received little attention is its influence on the various hepatic functions in a time and dose dependent manner. The effects of lead toxicity on various functional enzymes viz., hepatic transaminases, alkaline phosphatase and mixed function oxidase, as a function of time and lead body burden, have been undertaken in the present study. Moreover, we could also develop an expertise for studying the biological half-life of Iodine-131 Rose Bengal in order to assess the in vivo liver functions of rats in toxic conditions.

Silbergeld et al (1988) demonstrated that lead stored in bone (calcified tissues) is released into systemic circulation when its capacity to accumulate any more lead reaches the peak levels. It is this mobile lead which exerts its adverse effects on various soft tissues. It is therefore evident that there is a
need for more comprehensive study on various haematological parameters as these studies are also indicative of lead toxicity in different organ systems. Lead had been reported to cause anemia (Schwartz et al., 1990) and lead induced anemia was assigned to cause impairment of heme biosynthesis and increased rate of red cell destruction (Goyer and Rhyne, 1973). Further, lead has been reported to block the entry of iron from non-haemoglobin protein and also into haemoglobin (Santra et al., 1986). Lead has also been reported to cause the inhibition of δALAD (delta-aminolaevulinic acid dehydratase) activity, an enzyme responsible for incorporation of iron into heme moiety (Paglieuca et al., 1990). In addition to anaemia, reticulocytosis and basophilic stippling occurs due to lead poisoning (White and Selhi, 1975). Thus, keeping in view, the lacunae in the literature, the various parameters viz: trace elemental distribution, serum transaminases, serum alkaline phosphatase, leukocyte counts (total as well as differential) and serum thyroid hormones have been included to assess the dose and time dependent responses of lead in rat blood.

Another focus of concern lies in the neurotoxicity of lead, for it is the property of all neurotoxins to cause effects on the nervous system. Lead has been described as an inhibitory agent towards Na⁺/K⁺ ATPase, acetylcholine esterase, GABA transaminase and tetrahydrobiopterin synthetase (Silbergeld & Hruska, 1980). The biopterins are enzyme co-factors, which play an important role in the metabolism of the amino acid phenylalanine which itself is a part of the biosynthesis of the neurotransmitters, dopamine and nor-adrenaline and the hormone adrenaline (Ramsey et al., 1980) and have been assigned to be affected by lead supplementation.

The levels of various amino acids, including those which are important for synthesis of putative neurotransmitters, have been shown to be affected by lead toxicity (Patel et al., 1974a and Gerber et al., 1978). Patel et al (1974b) described inorganic lead to disturb glucose metabolism. Many of the neurological manifestations of heavy metal toxicity are certainly a
consequence of the disruption of normal actions of the essential divalent cations by heavy metal ions (Copper et al., 1984).

However, there is a paucity of information regarding the dose dependent and time related effects of lead on various brain functions and also on the levels of various elements in brain. Therefore, the present study was undertaken to study the distribution of different essential and non essential elements in brain. Other investigations with lead include the effects on the uptake of radio labelled nutrients viz: glucose, alanine and leucine, in brain as a function of dose and time. Alterations in the activities of acetylcholine esterase, succinate dehydrogenase and Na\(^+\)/K\(^+\) ATPase in brain following lead supplementation has also been investigated. Lead has been explored for its neurological manifestations as regards to catecholaminergic levels in rat brain synaptosomes.

Another area of lead toxicity that remains to be explored more is its effect on various thyroid functions. So, the present study includes the effects of lead on the in vivo biokinetics of I-131 in rat thyroid and estimations of serum thyroxine (T4) and Tri-iodothyronine (T3) following lead administration.

Lithium is found in nature not as a pure metal, but as salts especially silicates. Its concentration in the earth’s crust is 30 ppm. The lithium content of water and vegetables varies from region to region and is related to the hardness of water. The presence of lithium has been verified in many plant and animal tissues and the daily intake is estimated to be about 2 mg (WHO, 1973).

Lithium is probably not an essential element, but it has a role in psychiatry both as an antimaniac drug and in the prophylaxis against recurrent attacks of manic depressive mood swings. Lithium is ubiquitous among the non-essential elements as one of the few cations that are tolerated in concentrations up to 1 mmol/kg body wt. (Gabay et al., 1985).

Lithium ions are apparently effective in the treatment of affective disorders (Baastrup et al., 1970; Hanna et al., 1972 and Schou et al., 1970).
Many studies have apparently considered interactions between lithium and sodium or potassium ions (Goodwin and Bunny, 1969 and Baer et al, 1970). Birch (1970) however, emphasized the 'diagonal relationship', in the periodic table, between lithium and magnesium and calcium ions, reflected in terms of polarizing powers and ionic radii. Recently, Schrauzer (1990) indicated lithium interactions in human scalp hair with vitamin B₁₂ and some trace elements.


Lithium, in general, has not been considered hepatotoxic (Shou, 1986 and Jefferson, 1987) and questions have been raised as to whether it is totally benign with regard to its effects on this organ. Direct effects of lithium on the liver are scantily studied. A few investigations that have been done yielded conflicting results (Viegut, 1990). Animal investigations have shown variable results due to differences in experimental species and lithium dosages. For example, some studies show no change in liver weight with lithium administration (Messiha and Dunn, 1987 and Canolty, 1987), while others indicated a decrease in liver weight (Ibrahim et al, 1987). However, there is a paucity of information on the effects of short and long term supplementation with therapeutic doses of lithium on various haematological parameters (Hb, TLC, DLC) and trace element profile, hepatic parameters and 1-131 Rose Bengal clearance and Mixed function oxidase system.

Further, lithium treatment has been reported to alter thyroid functions with respect to uptake and biological half-life of 1-131 and circulating thyroid hormones (Dhawan et al., 1985; Maarjerg et al., 1987 and Dhawan et al., 1988). However, not much attention has been paid on the interaction of lithium with other metals in thyroid. Several investigations have shown that lithium influences the metabolism of glucose (Vendsburg and Vilstrap, 1976; Shah and Pishdad, 1980 and Dhawan et al., 1992). Ghoshdastidar and Poddar
(1990) indicated that LiCl produced alterations in catecholamines. There is still lack of information regarding the effects of lithium on in vitro incorporation of radiolabelled nutrients as a function of time and also on the status of other elements in brain.

A few studies have revealed combined effects of lead and lithium on body systems. Batuman et al (1989), reported that lead increases sodium-lithium counter transport in normal human RBC's in vitro and are consistent with the inhibitory effect of lead on red cell ATPase (Hernberg et al., 1967). A report by Mailman et al (1978) documents that massive oral doses of lead administered postnatally lead to subsequent enhancement of lithium induced polydipsia.

Since lithium is widely used as a mode of therapy for manic depressive patients and it is likely that few patients undergoing lithium therapy may well be subjected to a exposure from diet or pollutants including lead. So it leads to an added interest in carrying out studies to explore the effects of lead and lithium and also in combination on the functions of thyroid, brain and serum and also on hematological parameters. Moreover, there is enough scope to investigate experimentally the combined effects of lead and lithium on the status of various elements and other parameters involved in the function of blood, thyroid and liver and serum of rat.

Interactions among the trace elements are prevailing and biologically so influential that nutritional and toxicological studies carried out with single elements might project inconclusive picture unless the levels of interacting elements in dietary samples and body tissues are known simultaneously. Energy dispersive X-ray fluorescence (EDXRF) a multielement analysis technique meets these requirements adequately. With the advancement in experimental facilities, the values of different parameters involved in EDXRF techniques, such as photoelectric cross-sections, mass absorption coefficients and incident radiation intensities could be computed with better accuracy.
Therefore, the present study aims at investigating the effects of short and long term treatment with lead and lithium treatment alone, and in combination, in experimental rats to understand:

i) **Interactions of lead and lithium on various essential and non-essential elements in rat blood, liver and brain tissues after 1, 2, 3 and 4 months of treatment.**

ii) **Effects of lead and lithium on various haematological parameters viz., haemoglobin levels, total leukocyte counts, differential leukocyte counts and also on serum proteins, transaminases and alkaline phosphatase as a function of time.**

iii) **Influence of lead and lithium on thyroid functions viz., biokinetics of I-131 in rat thyroid, serum thyroxine and tri-iodothyronine levels as a function of time.**

iv) **Assessment of various liver functions following lead and lithium supplementation at time intervals of 1, 2, 3 and 4 months i.e. biological half-life of I-131 Rose Bengal in rat liver, hepatic enzymes viz., transaminases, alkaline phosphatase, cytochrome b5 and P450, glutathione, glutathione S-transferase and glucose-6-phosphatase.**

v) **Role of parameters related to brain functions of rats exposed to lead and lithium at time intervals of 1, 2, 3 and 4 months; In vitro uptake of nutrients viz., glucose, alanine and leucine in brain slices; status of epinephrine and nor-epinephrine in synaptosomes; brain enzymes viz., succinate dehydrogenase, Na⁺/K⁺ ATPase and acetylcholine esterase.**