REVIEW OF LITERATURE
In modern times, particularly within this twentieth century, man-kind has played wonders. Rapid strides in almost all the fields of physical, chemical and biomedical sciences, only befitting to be labelled as "knowledge explosion" have been made. With the result that newer chemicals, newer disciplines, and newer technologies have come up with astonishing uses and promises of comfortable living and further expansion of frontiers of knowledge. It is on this account that modern man has become now so much dependent and thereby surrounded by a vast variety of natural and man-made chemicals that his exposure to the latter is now almost inescapable.

The development of modern technology has brought an astonishing proliferation in the production and consumption of chemicals. In a few cases, however, the benefits of chemical use, have been accompanied by unexpected adverse effects. Beginning with environmental chemicals in the broad sense, a recent count has shown that 4 million different
chemicals have been isolated from natural sources or synthesized in the laboratories. Of these, about 60,000 are used in daily life. A further break up reveals that about 1500 chemicals are active ingredients of pesticides, 4000 are used as drugs or drug intermediates, 5,500 are food additives and the remaining 47,000 are broadly classified as industrial and agricultural chemicals, fuels for power production and chemical consumer products. The number of chemicals put to diverse uses is increasing by about 4000 every year (Ames, 1979).

There are about 63,000 chemicals in common use in the United States only (Maugh, 1978). According to U.S. Council of Environmental Quality, a large number of the synthetic chemicals (of the order of one percent of them), are latently toxic. The Registry of Toxic Effects of Chemical Substances (RTECS) currently listed 911 chemicals that are teratogenic or produce reproductive defects (Idem, 1981). That list includes metals, pesticides, laboratory solvents, sterilants, drugs, anesthetic gases and other chemicals. The RTECS also listed 3,201 chemicals as mutagens and 3,128 chemicals as suspected carcinogens (Idem, 1981).

A successive chain of public scares about some chemicals in the environment in recent times has come as a rude shock to everybody's governments and administrative agencies responsible for public health and environmental
quality, industries manufacturing or using these chemicals and to the public at large. Hitherto unsuspected and unexpected hazards from chemicals that have permeated everyday life like, mercury, lead, asbestos, vinyl chloride, DDT and other organochlorides, nitrites used in food preservation and even chloride in drinking water have made sensational headlines.

It is no wonder, therefore, that many of these chemicals appear in the work environment, air, water, and soils as pollutants from the ways of production and consumption. Crampton and Charlesworth (1975) reported that natural food substances may contain a frightening number of potentially carcinogenic or teratogenic toxins including aflatoxins, nitrosamines, mycotoxins and trace metals. Drinking water could, at one time, be regarded as safe for human consumption if it was free from pathogenic organisms, but this assumption is now no more valid. The practice of dumping industrial effluents in the nearest rivers or lakes has created new and complex problems, and the lesson of methylmercury and Minamata disease must be remembered (Matsumoto et al., 1965). Furthermore, so far as the drugs are concerned, thalidomide has taught us the lesson we are unlikely to forget (Lenz, 1961 and McBride, 1961). As a result, obviously, many nations have set up machinery for controlling the manufacture and marketing of drugs.
We live in an age of increasing awareness and concern about occupational health and disease. Of particular interest to us are the special hazards uniquely faced by the increasing number of women involved in various industrial professions.

These days, women constitute a substantial proportion of the work population and their numbers are still increasing; in U.K. women now comprise 36% of the workforce, in the U.S.A. 40% and in Germany 35% (Sullivan and Barlow, 1979). The extent of employment during pregnancy is also considerable. In 1963, an American survey showed 31% of 3780 mothers who had legitimate births were employed outside the home at some stage of pregnancy and half of these were still working after the sixth month (Diddle, 1970). The proportion of women working during pregnancy is likely to have increased since then, and the range of chemicals to which they may have been exposed is large (Kullander et al., 1976). Compounding the problem of women of child bearing the exposed to drugs during pregnancy is the problem of occupational exposure to industrial chemicals. A recent publication estimates that in United States alone, there are approximately 1,25,000 women working in various laboratories who are at risk due to exposure to such potential teratogens as laboratory reagents, solvents, etc. The author inferred that the incidence of birth defects in the offsprings of these women could run two to three times higher than the expected rate of malformation (Raymond, 1980).
When a woman is pregnant, the health hazards to which she is exposed are a threat to her as well as to the unborn. Many toxic substances that enter her bloodstream may find their way to the fetus. Because the fetus is small and rapidly growing, it is often more sensitive and susceptible than adults to small doses of dangerous substances.

From the number of disasters which have already occurred throughout the world, it has been evident that developmental process in both animals and man may be severely affected by chemicals. The range of effects includes not only fetal death or malformation but also affects on the subsequent behaviour, intelligence, reproductive capacity and general health of offsprings which appear otherwise normal at birth. According to the National Foundation, U.S.A. out of about 3 million births in the United States, each year some 2,00,000 or about 7% of all live borns, will have birth defects. More than 5,60,000 lives a year are claimed through infant death, spontaneous abortion, still birth and miscarriage due to defective fetal development (Anon, 1975). In India, it has been estimated that 1,50,000 infants are born with malformation and defects every year. The social burden can be more fully appreciated when one considers that birth defects affect the daily lives of some 15 million persons in these two countries alone.

The agents including vast variety of chemicals causing these adverse effects on developmental processes both in man and animals, are termed as 'teratogens' or in other words
a teratogen (derived from Greek word "teras" meaning monster) is an agent that acts during pregnancy to produce physical or functional defects in the conceptus or offspring (Shepard, 1980). Environmental agents that cause birth defects have usually first been recognised by alert medical practitioners (Miller, 1978). The history of the teratologic effects of X-rays is typical. In the 1920's individual cases were reported of infants with small head circumference and mental retardation attributed to maternal exposure to radiotherapy early in pregnancy. In 1929 Goldstein and Murphy, Philadelphia Obstetricians, created a case series from the literature, and added a series of their own assembled by the simple procedure of a small survey inquiring about cases at other obstetric centres. After the relationship between exposure and teratogenic effects was observed by retrospective study in man, similar effects were induced experimentally in rats (Miller, 1978).

When the atomic explosions occurred in 1945 in Japan, a prospective study was made that defined the dose-response, gestational interval of susceptibility, and the range of effects (Miller and Mulvihill, 1976).

Experimental teratology in the modern sense can be said to have begun in 1940's when Warkany and associates (Warkany and Nelson, 1940; Warkany and Schraffenberger, 1947), for the first time, forcefully called attention to the fact that environmental factors could adversely affect the intrauterine development in mammals. It was widely assumed in biology, and
in medicine that the mammalian embryo developed within the virtually impervious shelter of the uterus and maternal body where it was protected from extrinsic factors. Even the revelation by Gregg (1941) and others that the rubella virus could indeed divert the course of normal development in the human embryo did not immediately alert biomedical scientists to the possibility that other extrinsic agents might also penetrate the protective mechanism of mother to damage the conceptus. The real implications of this possibility were realised only after McBride (1961) and Lenz (1961) related the appearance of an epidemic of limb-reduction malformations in new born infants to the taking, by the pregnant mothers, of a presumably harmless sedative-hypnotic drug, thalidomide.

Thus, despite its cost in terms of human suffering, the thalidomide catastrophe can also be regarded as having long range benefits for mankind. It called the attention to the fact that human and other mammalian embryos can be highly vulnerable to certain environmental agents even though these have negligible or non-toxic effects in post-natal individuals.

**Principles of prenatal toxicology**

Pharmacologists have only quite recently become interested in the action of drugs during pregnancy. The thalidomide accident in 1961 is generally accepted as the beginning of a new discipline, teratology. Wilson and coworkers (1973, 1977) have during the last 20 years
investigated and reported on the action of toxic substances during pregnancy. Wilson (1977) has derived 6 general principles on the mechanism of action of teratogens from animal studies.

**1st principle**

Susceptibility to teratogens depends upon the genotype of conceptus and the manner in which this interacts with environmental factors.

Differences in sensitivity of various animal species to the action of teratogens is due to their different genotypes. Environmental factors which are important for normal embryonic development in animal studies seem to be maternal diet and humidity and temperature of the animal facility.

**2nd principle**

Susceptibility to teratogenic agents varies with the developmental stage at the time of exposure.

As demonstrated in Fig. 1, the embryo is very insensitive to teratogens during the preimplantation period in the uterine mucosa. However, immediately following implantation during early organogenesis the embryo is extremely sensitive to embryotoxic effects. Also, that the sensitivity of the embryo decreases during later stages of organogenesis and is even lower at the end of the gestational period.
Fig. 1. A curve approximating the susceptibility of the embryo to teratogenesis from fertilization until after birth.
Functional Maturation

ENTIRE DEVELOPMENTAL SPAN

DEGREE OF SENSITIVITY

Fertilization

Implantation

Organogenesis

Histogenesis

Embryonic period

Fetal period

Functional Maturation

Birth
3rd principle

Teratogenic agents act in specific ways (mechanisms) on developing cells and tissues to initiate abnormal embryogenesis (pathogenesis).

4th principle

The final manifestations of abnormal mammalian development are summarised as follows:

i) normal development (reparation)
ii) intrauterine death (resorption)
iii) malformation
iv) growth retardation
v) functional disorder
vi) transplacental carcinogenesis.

Manifestations (i) and (vi) have not been originally postulated by Wilson, but were added according to Neubert (1977, 1978).

5th principle

The access of adverse environmental influences to developing tissues depends on the nature of the influences (agent). This principle just describes a few simple basic facts of mammalian embryology. The embryo is protected by mother from most of the physical influences except for X-rays and possibly microwaves. On the other hand, the chemical nature of a substance, its metabolism in maternal tissues
and pharmacokinetics are some of the important determining factors which only selectively permit certain metabolites of maternal origin in finally reaching the embryonic tissues.

6th principle

Manifestations of deviant development increase in degree as dosage increase from no effect to the totally lethal level.

This principle is of particular importance to the toxicologists since it postulates well defined dose response relationship in prenatal period following exposure to the toxic agents vis-a-vis their teratogenic influence on the conceptus. This principle has been diagramatically presented in Fig. 2.

Mechanisms of teratogenesis

As indicated in principle 3, the mechanisms are thought to occupy a pivotal position in the series of events taking place between the causative factor(s) in the environment and the conceptus and their ultimate expression as developmental abnormality and the final defect (Fig. 3). The accumulated literature on experimental embryology and teratology provides evidences and clues that there may be eight or ten such mechanisms which influence the embryonic development.
Fig. 2. Diagram of the toxic manifestations shown by the embryo and the maternal organism as dosage of a drug or chemical increases.
MECHANISMS → PATHOGENESIS → COMMON PATHWAYS → FINAL DEFECT

Initial types of changes in developing cells or tissues after teratogenic result:
1. Mutation (gene)
2. Chromosomal breaks, nondisjunction etc.
3. Mitotic interference
4. Altered nucleic acid integrity or function
5. Lack of normal precursors, substrates, etc.
6. Altered energy sources
7. Changed membrane characteristics
8. Osmolar imbalance
9. Enzyme inhibition

Fig. 3 — Diagram of the successive stages in the pathogenesis of a developmental defect, beginning initial types of changes in developing cells or tissues (the mechanism) and continuing to the final defect.
1. **Mutation**

   This is the most firmly established mechanism of teratogenesis and rather constitutes the basis of all heritable developmental defects. Mutation essentially consists of a change in the number of the nucleotides on the DNA molecule. It is estimated that some 20-30% of human developmental errors can be attributed solely or primarily to mutation in a prior germ line. Mutations are caused by ionizing radiation, a number of chemical mutagens and many carcinogens, which might lead to chromosomal breaks or crossovers (Freese, 1971).

2. **Chromosomal non-dysjunction and breaks**

   These give rise to microscopically visible excesses, deficiencies or rearrangements of chromosomes, chromatids, or parts thereof. They have been associated casually with only a small portion (3%) of human developmental defects. They differ from the point mutations at least in quantitative terms, and are not hereditary in usual sense, although translocations of chromatid parts may be transmitted to half of the offsprings.

3. **Mitotic interference**

   This mechanism very likely includes more than one type of primary effect because the mitotic process apparently can be interfered in a number of ways (Table 1). Many
<table>
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<tr>
<th>Apparent mechanisms</th>
<th>Some known causative agents</th>
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<tr>
<td>1. Slowing or arrest of DNA synthesis</td>
<td>Cytosine arabinoside, hydroxyurea, irradiation.</td>
</tr>
<tr>
<td>2. Failure of mitotic spindle by preventing microtubule formation</td>
<td>Colchicine, vincristine some anesthetics.</td>
</tr>
<tr>
<td>3. Improper formation or separation of chromatids ('stickiness', bridges, etc.)</td>
<td>Irradiation, radiomimetic chemicals.</td>
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cytotoxic agents are known to act by inhibiting synthesis of DNA, thereby slowing or arresting mitosis, since the process cannot progress beyond the S phase. The mitotic spindle can be prevented from forming, or be dissolved after formation, by several chemical agents (Malowists et al., 1968). Finally, even when DNA is synthesized and spindle is formed, chromosomes may not be able to separate owing to an apparent 'stickiness' or physical continuity known as 'bridges'.

4. **Altered nucleic acid integrity or function**

In addition to mutation, this is the mechanism by which many antibiotic and antineoplastic drugs are teratogenic. Biochemical changes that interfere with nucleic acid replication, transcription, natural base incorporation, or RNA translation (protein synthesis) without producing heritable changes in the DNA of germ cells, are included here.

5. **Lack of precursors and substrates needed for biosynthesis**

This is probably one of the better established mechanisms. The materials essential for biosynthesis and maintenance of growth and differentiation can be withheld from the sites where they are utilized in the growing embryo by four means as summarized in Table 2.

6. **Altered energy sources**

In view of the need for uninterrupted high levels
<table>
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<th>Cause of deprivation</th>
<th>Agents</th>
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<tr>
<td>1. Specific dietary deficiency</td>
<td>Of riboflavin, Vit. A, E, Folic acid, Zn, Mg, Mn.</td>
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<tr>
<td>2. Specific analogues or antagonists</td>
<td>For purines, pyrimidines, glutamine, etc.</td>
</tr>
<tr>
<td>3. Failure of absorption from maternal gut</td>
<td>Of copper due to excess presence of Zn or SO$_3$ or iodine in presence of Cu.</td>
</tr>
<tr>
<td>4. Failure of placental transport</td>
<td>Caused by azo dyes, tissue antisera, etc.</td>
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of energy by proliferating and rapidly growing tissues reduced and inadequate supply of energy can lead to developmental anomalies. Evidence is now accumulating to indicate that energy supply may be impaired by teratogens in 4 ways (Table 3).

7. Inhibition of enzymes

Enzymatic functions are essentially required in all aspects of differentiation and growth, hence they would almost certainly be interfered with factors adversely affecting these developmental processes. This subject has not been extensively studied, but in few instances, such as those listed in Table 4, agents shown to be teratogenic in mammals are either already known or have been extrapolated from studies conducted on specific enzymes in other growing systems (Runner, 1974). In addition to the enzymes listed in the table, it is now recognized that some mutagenic agents also act by inhibiting the enzymes which are responsible for repairing breaks induced in DNA strands (Freese, 1971).

8. Osmolar imbalance

This teratogenic mechanism is one of the few which can be traced stepwise through the pathogenesis of developmental anomalies.

Based on 'edema syndrome' as studied by Grabowski
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<tr>
<th>Affected pathways</th>
<th>Causes associated with teratogenesis</th>
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<tr>
<td>1. Inadequate glucose sources</td>
<td>Dietary deficiency induced hypoglycemia.</td>
</tr>
<tr>
<td>2. Interference with glycolysis</td>
<td>6-Aminonicotinamide, iodoacetate.</td>
</tr>
<tr>
<td>3. Interference with citric acid cycle</td>
<td>6-Aminonicotinamide, riboflavin deficiency.</td>
</tr>
<tr>
<td>4. Impairment of terminal electron transport system</td>
<td>Hypoxia, dinitrophenol, cyanide.</td>
</tr>
</tbody>
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Table 4

Inhibition of some specific enzymes thought to be involved in teratogenesis

<table>
<thead>
<tr>
<th>Enzymes inhibited</th>
<th>Teratogenic agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dihydrofolate reductase</td>
<td>Folic acid antagonists.</td>
</tr>
<tr>
<td>2. Thymidylate synthetase</td>
<td>5-Fluorouracil.</td>
</tr>
<tr>
<td>3. Ribonucleoside diphosphate reductase</td>
<td>Hydroxyurca.</td>
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<tr>
<td>5. DNA polymerase</td>
<td>Cytosine arabinoside.</td>
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(1964), in chick embryos subjected to hypoxia, it is possible
to delineate some of the successive events in teratogenesis
of the head, limb, and rump malformations (Fig. 4). Similar
pathogenetic events are thought to follow osmolar imbalances
resulting from such agents as trypan blue, hypertonic solu-
tions, and adrenocortical hormones. Malformations in the
tail and extremities of mice with hypertonic saline solutions,
have been attributed to tissue damage following edema, blis-
ters, and haemorrhages (Tanaka et al., 1968) providing a
mammalian example of the "edema syndrome".


Abnormal membrane permeability can lead to osmolar
imbalance as described above, but in other cases, the alte-
red membrane rather than the fluid compartments which it
separates would be the primary site of teratogenic action.
Solvents such as Dimethylsulfoxide (DMSO) have been noticed
to produce swellings and blisters associated with ionic
shifts between compartments in chick embryos and it was pos-
tulated that the solvent had altered the permeability of cell
and other membranes (Browne, 1968). Teratogenic doses of
vitamin A have been reported to cause ultrastructural damage
to cellular membranes in rodent embryos (Morriss, 1973).

TERATOGENICITY OF HEAVY METALS

The undesirable environmental effects of heavy metals
Fig. 4: The "edema syndrome" illustrates the sequence of events

Hypoxia (in chick embryo) → Hypoosmolarity in extra embryonic compartments → Inrush of fluid → Hypervolemia and increased blood pressure in embryo → Edema hematomas, blisters → Mechanical distortion and ischemia in tissues → Abnormal embryogenesis in eye, brain, limbs, rump, face, etc. → Final defects

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<thead>
<tr>
<th>Cause</th>
<th>Mechanism</th>
<th>Pathogenesis</th>
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and other toxic elements have been recognised for many years. These agents first affect the workers in industry, next that segment of general population which comes in contact with the industrial effluents and finally, the consumers exposed to these products, such as lead paints. At times, a more subtle and sinister effect occurs in the form of terata seen at birth in both the human and animal populations. Acute poisoning of humans and animals has been occasionally reported following industrial or agricultural accidents, but many of today's problems arise as a result of long-term exposure to heavy metals as environmental contaminants.

The event of contamination is many times related to the amount of concentration of a substance in question. The mere presence of a metal species does not constitute a threat. Indeed, the presence of certain amount of some metals is rather indispensable to the living cell. Iron and Copper are required as constituents in various respiratory pigments. Cobalt is required for the synthesis of vitamin $B_{12}$ and zinc as an ingredient for the enzyme carbonic anhydrase. Molybdenum is necessary for all the organisms which derive nitrogen from inorganic sources. Other metals such as antimony, arsenic, barium, beryllium, bismuth, cadmium, mercury, lead, silver, tellurium and thorium have no known nutritive value and are commonly considered as toxicants (Nilsson, 1969).

**Arsenic (inorganic)**

Large quantities of arsenic (As) compounds have been
introduced into the environment for over 100 years through processes like smelting and manufacturing of arsenic compounds in paints and as industrial chemicals.

Egg inoculation with inorganic As can result in stunting, micromelia, abdominal edema, and a high percentage of dose-related deaths (Franke et al., 1936; Ridgway and Karnofsky, 1952). Arsenic produces serious developmental malformations in golden hamster (mild encephalocoeles to complete exencephaly) when injected during critical stages of embryogenesis (Ferm and Carpenter, 1968).

Hamsters are most sensitive to the teratogenic effects of sodium arsenate at 20-25 mg/kg body weight (Ferm et al., 1971); rats are affected at 30 mg/kg body weight (Beaudoin, 1974) and mica at 45 mg/kg body weight (Hood and Bishop, 1972).

**Cadmium**

Cadmium (Cd) ranks 67th out of 97 most common elements in the earth's crust and occurs in the association with zinc in a ratio of 1:445. It is a general cytotoxic agent and a potent inhibitor of several enzyme systems.

Ribus and Schmidt (1973) found that Cd when applied to the intravitelline circulation of chick embryos, manifested teratogenic effects at the end of the trunk and hind extremities. Supravitelline application resulted in premature death of embryo.
Cd induces serious facial malformations in hamsters fetuses when injected on the eighth day of pregnancy (Perm and Carpenter, 1967). Mulvihill et al. (1970) concluded that Cd has a marked effect on the mesoderm of the head of the golden hamster, causing malformations including unilateral and bilateral cleft lip and palate. Cd is known to cross the placental barrier in the hamsters (Ferm et al., 1969).

Flick et al. (1971) concluded that the teratogenic effect of Cd in man is unresolved, although both acute and chronic intoxications have been reported.

**Copper (Cu)**

Absorption of copper is limited, which decreases its potency as a teratogen. However, laboratory experiments have demonstrated Cu teratogenicity in a few species. Copper salts injected intravenously into pregnant golden hamsters on the eighth day of gestation cause an increase in embryonic resorptions as well as developmental deformities. Radioactive copper citrate passes the placental barrier of hamsters, indicating that the metal may have a direct teratogenic effect on the developing embryo (Ferm and Hanlon, 1974).

**Mercury (Hg)**

The more severe expression of mercury (Hg) teratogenicity in laboratory animals are the result of intravenous injections of relatively large doses.
Methyl mercury (Me Hg) is the most common form of metal, as an environmental contaminant. Majority of reports of human intoxication are the result of methyl mercury either working its way up through the food chain, as in Minamata Bay, or being directly consumed due to mishandling of treated seed grain, as in Iraq (Francis and Theodore, 1979).

McLaughlin et al. (1963) found that the inoculation of chick embryo with 0.5 mg HgCl caused the death of all embryos. Lower doses (0.25 mg) reduced the hatchability by 80% (Kauhara, 1970). Organic Hg contaminated fertile eggs were less successful in hatching; 85% died as compared to 15% of controls. The hatching time was prolonged by 1-2 days and the body weight was less. No malformations were found, but a slightly disturbed gait was noted. Histopathological changes were occasionally observed in the neurons of the brain (Kojima, 1972).

A single high dose or 16 low doses of methyl mercury hydroxide (Me Hg OH) had an embryotoxic effect, i.e. dose related incidence of cleft palates and decreased behavioural emotionality and locomotor activity in mice (Okita et al., 1974).

Continuous low dose exposure to MeHgOH in pregnant rats increased fetal deaths, runting and edema. No gross behavioural changes were noted during the first three weeks post-partum in the pups (Mottet, 1974). Malformations of
the cerebellum have been induced following treatment of pregnant rats on day 9 to 11 of gestation with orally administered MeHgCl (Matsumoto et al., 1967).

The adverse effects of mercurial pollution have been reported from Minamata and Niigata, Japan, as Minamata disease. It affected both sexes, all age groups including the newborns (Muro and Goyer, 1969). Tejning (1961) reported the passage of MeHg across the placenta in women and its possible preferential concentration in the fetus. Twenty-three (6%) of 359 children born in the villages near Minamata Bay were affected with cerebral palsy-like disease (Nelson, 1971).

**Molybdenum (Mo)**

Molybdenosis has appeared in many parts of the world due to industrial molybdenum (Mo) contamination of nearby pastures. Ferm (1972), found that Mo produced no embryocidal or teratogenic effects up to 100 mg/kg. However, Schroeder (1973) demonstrated that 10 ppm in the drinking water of breeding mice caused a significant increase in young deaths in $F_1$ and $F_3$ generations and in dead litters in the $F_3$ generation.

Mills and Fell (1960) found severe demyelination of the CNS in newborn lambs from the sheep grazing at pastures near dams. It was subsequently revealed that their fodder was rich in Mo.
Selenium (Se)

Selenium (Se), as a teratogen and a toxicant, is an environmental hazard predominantly to grazing animals.

Workers as early as in 1936 injected Se into the air sac of hen eggs and found that the Se salts produced a large number of deaths and a high percentage of abnormalities (Franke et al., 1936).

Golden hamsters when given intravenously alone half the lethal dose (LD$_{50}$) did not develop teratogenic or embryo lethal effects (Homberg and Ferm, 1969).

Zinc (Zn)

Zinc deficiency has been found to be more detrimental to the health of embryo than its excess (Ferm and Hanlon, 1974). Excess zinc does not cause malformations in hamsters even though it has been shown to cross the placenta in significant amounts (Ferm and Carpenter, 1968). Similar findings have also been reported in guinea pigs, sheep and man (James et al., 1966).

Manganese (Mn)

Ferm (1972) reported that in preliminary experiments in pregnant golden hamsters has been shown to be embryocidal but not teratogenic. Interestingly, a deficiency of Mn produced an ataxic condition characterized by incoordination
and retracted head in the rats and guinea pigs (Hurley et al., 1958).

Nickel (Ni)

Ferm (1972) found that nickelous acetate was toxic to golden hamster embryos when 30 mg/kg body weight was injected into pregnant mothers on day 8 of gestation. A few general malformations were induced in some of the surviving embryos. Nickel (Ni) added to the drinking water of breeding rats produced a significant number of young deaths in $F_1$, $F_2$ and $F_3$ generations as well as runts in the $F_1$ pups (Barr, 1973).

MISCELLANEOUS ELEMENTS

1. Lithium (Li)

Although lithium is not a heavy metal and does not constitute a contaminant of the environment, its wide use in treating mental disorders in man and its possible teratogenic effects deserve some mention. It has been shown to be teratogenic in chick embryos, frogs, toads, mice, and rats (Loevy and Catchpole, 1973).

2. Rhodium (Ro)

Ridgway and Karnofsky (1952) found that injection of rhodium onto the chorioallantoic membrane of chick embryos produced stunting, feather inhibition, micromelia and mild edema.
3. **Tellurium** (Te)

Tellurium (Te) fed to the rats after being mixed in their diet has been shown to cause up to 100% hydrocephalus depending upon its level in the feed (Garro and Pentschew, 1964; Duckett, 1971). Placental transmission of radiotellurium in rats has been demonstrated in offspring of dams injected with the metal (Duckett and Ellem, 1971). The critical period of hydrocephalus induction is day 9-10 of gestation in the rat (Agnew, 1972). Absorbed Te reached the fetal brain within minutes and presumably caused maturation arrest of the telencephalic vesicles (Duckett et al., 1971).

A single injection of 50 mg Te on any one day did not produce hydrocephalus. No other abnormalities have been observed under the conditions studied (Agnew, 1972). James et al. (1966) fed potassium tellurate to four pregnant ewes for 45 to 151 days and was unable to produce any abnormalities in their offsprings.

4. **Thallium** (Th)

Thallium has been reported to induce achondroplasia, when injected in chick embryos on the chorioallantoic membrane on days 4 and 19 (Ridgway and Karnofsky, 1952). The cartilage appeared to be the primary site of action. These findings were later confirmed by Hall (1972) who showed that there was a defect in cartilage maturation and in the gross skeleton.
The growth of the long bones was specifically affected and smaller bones contained less organic material and more water. A similar finding has been observed in rats by Nogami and Terashima (1973).

5. Titanium (Ti)

Titanium in the drinking water of breeding rats caused a significant number of runts in the F₁, F₂ and F₃ generations (Schroeder, 1973). A significant increase in the number of young deaths also occurred in the F₂ generation. There was a marked reduction in the number of animals surviving to the F₃ generation.

TERATOGENIC EFFECTS OF LEAD

Lead has probably exerted its deleterious action on human development since antiquity, but scientific evidence for such effects has been forthcoming only at the end of the last century when it was recognised that women working in lead industries were frequently sterile, suffered from amenorrhoea and menstrual disorder (Bourret and Mehl, 1966) and had high rates of spontaneous abortions and fetal and neonatal loss (Paul, 1860; Oliver, 1911; Legge and Goedby, 1912). Earlier, in the twentieth century, the embryotoxic properties of lead were used to induce criminal abortion, a procedure that often gave rise to serious intoxications (Hall, 1905; Taussig, 1936; Pindborg, 1945). Abnormalities
surviving fetuses and infants such as retardation of intra-uterine and post-natal growth and neurological damage were often noted after accidental lead into intoxication during pregnancy. Wilson (1966) claimed the absorption of large amounts of lead in water during pregnancy which caused nystagmus and partial albinism, but this must be required with suspicion because no similar case has been reported in the literature. The observation of a higher rate of mental retardation in children from women living in certain districts of Glasgow where the water passes through leaden pipes has been reported (Beattie et al., 1975). For lead to act on human embryonic development, it must cross the placental barrier and has, therefore, been intensively studied in man as well as in lower mammals. Studies indicated that lead is rapidly transferred to the fetus (Hubermont et al., 1978; Ryu et al., 1978; Singh et al., 1978) at least from the third month of pregnancy (Barltop, 1969).

Placental permeability of lead has also been investigated in animals. Morris et al. (1938) were first to report the presence of lead in fetal rat tissue and more recently, it was reported that the placenta of mice and rats acted as a partial barrier to lead during the late pregnancy (McClain and Becker, 1970, 1972). Metllan et al. (1973) reported that significant amounts of lead crossed the hamster placenta during early pregnancy.
Studies on the teratogenic effects of lead on lower vertebrates and mammals and proper clinical and laboratory check up, along with detailed history taking of children suspected to be born or brought up in environment with higher lead contents may help in evaluating the hazard of lead to human development.

Studies on the teratogenic effects of lead in fish and amphibians reared in water containing various concentrations of lead showed retardation in the development of eggs and embryo (Dilling and Healey, 1926) and an increase in the fetal death rate (Ferm, 1974). Salamander tadpoles displayed, in addition, twisting of the tail (Ferm, 1974). The mechanisms of the teratogenic effects of lead on these species, however, have not been further investigated.

Nearly all the investigations done on mammals have shown that lead affects fertilization and embryonic development. The effects seen in mammals resemble those in lower vertebrates, i.e. retardation in fetal and post-natal growth and embryonic or fetal death. This was observed in sheep (James et al., 1966), dogs (Azar et al., 1972), guinea pigs (Woller, 1915), hamsters (Ferm and Carpenter, 1967), rats (Stowe and Goyer, 1971; Kimmel et al., 1976) and mice (Schroeder and Mitchener, 1971; Maisin, 1977). In addition to interfering with embryonic and fetal development, lead can also induce specific malformations in the CNS and the
skeleton, depending on the stage of pregnancy when the lead
was injected.

Teratogenic effects of lead on the skeleton were
observed in hamster (Ferm and Carpenter, 1967), rat (McClain
and Becker, 1975) and mouse (Jaequet and Gerber, 1979) when
the mothers were injected lead at day 8-9 of pregnancy.
Hamster fetuses displayed abnormal caudal and several vertele­
brate associated with tissue loss (Ferm and Perm, 1971).
When lead was injected together with cadmium, an enhancement
of the effect of lead was seen and sympodia developed, a
severe caudal malformation that was never observed in hamsters given lead alone (Ferm, 1969).

LYSOSOMES AND TERATOGENESIS

Although many chemical agents are known to produce
abnormalities in foetuses, the underlying mechanisms for
most of them, however, still remain unknown. Clearly, any
interference with the growth or differentiation of cells in
the foetus, or with materno-foctal relationship, might have
teratogenic effects. Lysosomal changes including autophagy
are associated with certain types of differentiation, notably
the regression of the Mullerian ducts (Scheib, 1965 a, 1965 b),
the resorption of the tadpole's tail (Weber, 1969) and bone
resorption (Vaes, 1969), etc. Selective and programmed cell
death and removal of the consequent debris by lysosomal
enzymes is an important factor in the remodelling of tissues
during morphogenesis (Saunders, 1966). Interference with lysosomal functions might, therefore, play a role in teratogenesis by affecting such functions.

During early development the conceptus depends upon the histiotrophic (or embryotrophic) nutrition, on the absorption of materials locally present in the endometrium. These include uterine secretions and products of endometrial hydrolysis which become locally available to the embryo following implantation in most species. The embryonic membranes involved in histotrophic nutrition vary from one animal form to another, but in all cases appear to be highly phagocytic and well endowed with lysosomal hydrolases (Lloyd and Beck, 1969). It seems likely that for the most part, ingested macromolecules are broken down in secondary lysosomes before transfer to the embryo. This process has been studied by Lloyd and Beck (1969) who injected horseradish peroxidase intravenously in pregnant rats and found it to be concentrated in secondary lysosomes of epithelial cells in the yolk-sac placenta. The concentration of peroxidase in the placenta falls steadily (as the enzyme is digested) and some of the intact enzyme is found in the embryo. Similar results have been obtained by Schultz (1966) using yeast invertase as a marker protein; again the highest concentration was found in the yolk-sac, and in this case also, small amounts could be detected in the embryo.

Histiotrophic nutrition is not the only source of material used as building blocks for the embryo, for example,
nucleotides are probably transferred directly (Glassi, 1967). Nevertheless, there is little doubt that this mode of nutrition is important for the embryo.

**TERATOGENIC EFFECTS OF POLYCYCLIC AROMATIC HYDROCARBON (PAH)**

A striking observation has emerged from enormous volume of data on experimental carcinogenesis. Many agents known as carcinogens in post-natal life have been found to be teratogenic to the foetus or embryo. Di Paolo and Kotin (1966) tested 26 chemical agents for both carcinogenic and teratogenic activities in animals. They found 20 (out of 26) as carcinogens and 19 (out of the latter 20) as teratogens.

Moreover, polycyclic aromatic hydrocarbons (PAH) known to be the most potent class of chemical carcinogens, have become synonymous with environmentally related hazardous products. The cytotoxic, mutagenic and carcinogenic actions of several members of this class of PAH, such as benzo(a)-pyrene (BP), have been well documented in mammals (Miller, 1978) and aquatic animals (Kocan et al., 1979). Longwell (1977) suggested that accumulation of PAH by maturing gonads would be particularly serious since these compounds could, deleteriously, affect maturation of gametes, physiological control of reproduction, or subsequent development of the resulting embryos. Ellen et al. (1981) reported that substantial decrease was seen in hatching success of egg from BP flat fish during development. The test eggs demonstrated treated/a significantly lower hatching success of (11.9%)
than control eggs (56.6%). Almost four times as many grossly visible abnormalities were noted in eggs from fish exposed to BP compared to eggs from control fish. Many of the abnormalities in the BP treated group consisted of malformations of tail region.

The surface application of small quantities (1-20 μl) of crude and fuel oils (rich in PAH) to mallard (Anas platyrhynchos) and chicken eggs caused significantly high incidence of embryotoxicity (Albers, 1978; Hoffmann, 1978; Szaro, 1979). Southern Louisiana crude applied to the egg surface on day 2 of incubation produced teratogenic effects in both chicken and mallard embryos (Hoffman, 1978, 1979 a). These investigators also showed a similar response for a mixture of PAH delivered in a vehicle of aliphatic hydrocarbons. Hoffman and Gay (1981) reported that addition of BP, chrysene, and 7,12-dimethyl benz(a)anthracene (DMBA) resulted in enhanced embryo toxicity when applied externally to mallard eggs in the vehicle of a synthetic petroleum hydrocarbon mixture. While evaluating the activity of aliphatic and aromatic fractions of crude and fuel oil on developing chick embryos, Ellenton (1982) showed that aromatic fraction had both teratogenic and embryotoxic activities causing ontogenic aberrations. These included gastroschisis, edema, beak abnormalities, haemorrhages and limb defects and he concluded that teratogenic activity in developing chick embryos was due to PAHs themselves. Moreover, the effects of 50 different major aliphatic hydrocarbons,
aromatic hydrocarbons and combinations of those similar in class composition to South Louisiana, crude oil, have been investigated in mallards. The studies have shown that the embryotoxicity is dependent upon aromatic hydrocarbon content and is enhanced by the presence of PAHs (Hoffman, 1979b).

**INTERACTION OF CHEMICALS**

Accessibility to a very large number of chemicals in our environment, both naturally occurring and man-made, many of which are toxic and the very fact that the list of the latter is ever expanding, judiciously warrants our immediate attention to the necessity to evaluate their interaction when an organism is simultaneously exposed to them. Interactions can occur in a variety of ways. The substances can interact with each other chemically, which usually results in a decrease response, produce altered rates of absorption, changes degrees of protein binding, and alters the rates of metabolism or excretion of one or both of the interacting toxicants. In addition to these known modes of interaction, the response of the organism to combinations of toxicants may be increased or decreased because of the toxicologic responses at the receptor sites.

Two chemicals given simultaneously will produce an effect that may be simply additive of other individual responses separately, or may be greater or less than that expected by addition of their individual responses. Study of these
interactions often leads to a better understanding of the mechanism of action of the chemicals involved. A number of terms have been used to describe pharmacologic and toxicologic interactions. An additive effect is the situation in which the combined effect of two chemicals is equal to the sum of the effect of each agent given alone (example: $2 + 3 = 5$). The effect, most commonly observed when two chemicals are given together, is an additive effect. For example, when two organophosphate insecticides are given together, the cholinesterase inhibition is usually additive.

A synergistic effect is the situation in which the combined effect of two chemicals is much greater than the sum of the effect of each agent given alone (example: $2 + 3 = 20$). For example, both carbon tetrachloride and ethanol are hepatotoxic agents, but together they produce much more liver injury than the mathematical sum of their individual effects on the liver would suggest.

Potentiation is the situation when one substance does not have a toxic effect on a certain organ or system, but when added to another chemical, it makes the latter much more toxic (example: $0 + 2 = 10$). Isopropanol, for example, is not hepatotoxic, but when added to carbon tetrachloride, the hepatotoxicity of the latter is much greater than when given alone.
Antagonism is the situation in which two chemicals, when given together, interfere with each other actions or one interferes with the action of the other chemical (example: \(4 + (-4) = 0, \ 4 + 0 = 1, \ 4 + 6 = 8\)). Antagonistic effects of chemicals are often very desirable effects in toxicology and are the basis of many antidotes. There are four major types of antagonism: functional antagonism, chemical antagonism, dispositional antagonism and receptor antagonism.

Functional antagonism is when two chemicals counterbalance each other by producing opposite effects on the same physiologic mechanism. Advantage is taken of this principle in that the blood pressure can markedly fall during severe barbiturate intoxication, and it can be effectively antagonized by intravenous administration of a vasopressor agent such as norepinephrine or metaraminol. Similarly, many chemicals when given at toxic dose levels, produce convulsions and the convulsions can often be controlled by giving anticonvulsants such as the short-acting barbiturates (example: amobarbital).

Chemical antagonism or inactivation is simply a reaction between two chemicals to produce a less toxic product. For example, dimercaprol (BAL) chelates with various metals such as arsenic, mercury and lead which decreases their toxicity. The use of anti-toxins to treat various toxins is an example of chemical antagonism. The use of the strongly basic low-molecular weight protein, protamine sulfate to form
a stable complex with heparin which abolishes its anti-coagulant activity, is another example.

Dispositional antagonism is the situation in which the disposition, i.e. the absorption, distribution, metabolism or excretion of the chemical, is altered so that lesser quantities of the toxic compound reach the target organ or that the duration of exposure at the target organ is less. Thus, the prevention of absorption of a toxicant by ipecac or charcoal and the increased excretion of a chemical by administering an osmotic diuretic or by altering the pH of the urine are examples of dispositional antagonism. If the parent compound is responsible for toxicity of the chemical (such as the organophosphate insecticide, paraxon) and its metabolites are less toxic than the parent compound, then increasing the compounds biotransformation by a microsomal enzyme inducer like phenobarbital will decrease its toxicity. However, if the chemical toxicity is largely due to a metabolic product (such as the organophosphate insecticide parathion) then inhibiting its biotransformation by an inhibitor of microsomal enzyme activity (SKF 525a or piperonyl butoxide) will decrease its toxicity.

Receptor antagonism is when two chemicals that bind to the same receptor produce less of an effect when given together than the addition of their separate effects (example: $4 + 6 = 8$) or when one chemical antagonizes the effect of the
second chemical (example: $0 + 4 = 1$). Receiver antagonists are often termed blockers. This concept is used to advantage in the clinical treatment of poisoning. The receiver antagonist naloxone (nalorphine: N-allyl normorphine) is used for treating the respiratory depressant effects of morphine and other morphine-like narcotics. The treatment of organophosphate insecticide poisoning with atropine is an example not of the antidote competing with the poison for the receptor but rather blocking the receptors responsible for the toxic effect. These organophosphates act as "antiacetyl cholinesterases" and exposure to them leads to excess accumulation of acetylcholine at the target site.

Zinc and selenium have been shown to protect the animals from the toxic effects of cadmium and mercury, respectively. Thus, the toxicity through metal pollution may be modified in the presence or absence of their metal ions.

Zinc and selenium salts did not protect rat testes from the toxic effects of cadmium when injected locally along with cadmium. However, when administered subcutaneously, these salts were found to protect testes from cadmium toxicity (Kar and Kamboj, 1965).

Administration of manganese to iron deficient animals leads to marked damage to the brain and some other organs which have been due to significant absorption and accumulation of manganese in the iron deficient animals (Shukla and
Chandra, 1976). Combined treatment of manganese and nickel significantly decreases the accumulation of later in liver, kidney, spleen and testes compared to the animals treated with nickel alone (Murthy and Chandra, 1976).

The toxic effects of manganese in combination with copper have been investigated on the brain of mice. Conclusion was drawn that combined exposure produced marked accumulation of copper in the brain and altered the levels of brain tryptophan and 5-hydroxytryptamine to a greater extent than with any of the single exposure (Chandra et al., 1980).

Simultaneous administration of molybdenum and copper in rats showed no traces of copper in tissues indicating that copper is ineffective in presence of molybdenum exhibiting physiological antagonism. Molybdenum may act on copper as a chelating agent which is ultimately excreted. Workers suggested intake of molybdenum with the diet for treatment of copper intoxication (Rana and Kumar, 1978).

Ribas and Schmidt (1973) reported that supravitelline application of cadmium on chick embryos resulted in malformations. The addition of Zn to the mixture prevented these malformations even though the dose of Cd was doubled.

Mercuric acetate given along with CdSO₄ intravenously on 8th day of gestation proved to be more detrimental to the embryos of golden hamsters than Hg with ZnSO₄ (Gale, 1973).
Lead has also been reported to protect the embryo from Cd induced facial defects (Ferm, 1969). Ferm (1971) reported that lead formed a complex inter-relationship with Cd which delayed the Cd effect in the presence of lead ions. The same inhibitory effect was seen with Zn, Se and As (Holmberg and Ferm, 1969).

In a study carried out with various doses of cadmium chloride injected into the chick embryos between 7th and 14th day of incubation, different types of malformations were seen to develop consisting of haemorrhagic atrophy of the distal feathers. Whereas, simultaneous injection of an equimolar amount of zinc sulfate prevented the feather malformation (Roberto et al., 1983).

Effect of lead toxicity coupled with zinc deficiency was investigated in the rats. It was seen that zinc deficiency exerted additive effects on body weights (Bushnell and Levin, 1983).

The response of embryos of C. virginiae to various combinations of copper, mercury and zinc was studied. Highly significant toxic synergism was observed with the copper-zinc mixture and 3 metal mixtures particularly at high concentrations (MacInnes, 1981).

In one study, Rystedt and Fislher (1983) reported that 853 hard metal workers were examined and patch tested with 20
substances from their environment, including nickel and cobalt. It was found that workers with simultaneous nickel and cobalt sensitivities had a more severe hand eczema than those with either cobalt or nickel sensitivity or only irritant dermatitis.

Effects of oral cadmium and polychlorobiphenyl (PCB) therapy on metabolism of drugs, ascorbic acid and cholesterol were studied by Suzuki (1980). The results suggested that effects of simultaneous ingestion of cadmium and PCB on rats were additive.

A synergistic enhancement of the neoplastic transformation frequency was found following combined treatment with organic carcinogens and some metabolites, e.g. nickel sulfate, cadmium acetate or potassium chromate. When the cells were exposed to BP both nickel and cadmium showed a promotion like effect. The data suggest that the metal salts are more potent as promoters than they are as initiators (Riredal and Sanner, 1981).

**SCOPE OF THE PRESENT STUDY**

As already reviewed, out of a very large number of chemicals found in global environment, consequent to human activities and otherwise also naturally present, some may behave as sources of environmental hazard, particularly to the
growing embryos of humans and other animals of socio-economic value. These deleterious effects can, however, be studied in depth, with an aim to abolish or minimise them through a series of studies conducted over suitable experimental animal models. Moreover, in actual experience, many times, the organism is simultaneously exposed to more than one such harmful chemical agents. Thus, the interaction of such toxic chemicals in the same animal in different ways may further complicate the situation. In view of the above facts, the present study was, therefore, planned to investigate the effects of some important chemicals, viz. salts of lead, zinc, calcium and nickel and a polyaromatic hydrocarbon acting as carcinogen and most commonly found in our environment - benzo-(a)pyrene (BP) individually and in combination after inoculating them into the yolk sac of developing chick embryos.

While taking into consideration the large variety and numbers of congenital malformations already witnessed in humans, the list of known human teratogens is comparatively very small. This fact itself demonstrates the importance and pressing need for such studies, which will be extremely helpful in establishing the identity of new teratogens and overall effect of simultaneous exposure to more than one toxic chemical agent over growing conceptus as evaluated in this study.