Chapter 1

INTRODUCTION
A key contributor to human health and disease is our environment. Exposure to many environmental stressors such as pesticides have detrimental effects on health and are considered to contribute substantially to most diseases of major public health significance (Olfa et al., 2011). As pesticides play an important role in increasing crop yield, agricultural productions and in public health, their use has become a necessary evil in the developing countries (Narra et al., 2012). These chemicals are continuously developed and used in developing country like India where about 80% of the population living in rural area depends upon agriculture as their food source.

Pesticides are products or any substance or group of substances manufactured and used to prevent, destroy, repel or mitigate any such type plant or animal that cause harm to human food supply, his health and comfort (Delaplane, 1996). Pesticide is a generic name which includes a variety of agents classified more specifically on the basis of their target species, mode of action, chemical structure and also on pattern of use. The major classes of pesticides include insecticides, fungicides, herbicides, larvicides, acaricides, miticides, rodenticides, molluscides, pheromones and plant growth regulators etc. (Ecobichon, 2001). The class insecticide which is used to kill harmful insect can be further subdivided into organophosphate, carbamate, organochlorine and pyrethroid insecticides on the basis of their chemical structure.

A large number of benefits have been derived from the use of pesticides in public health, forestry and domestic sphere and of course, in agriculture. Many other kinds of benefits which are often going unnoticed by general public may be attributed to the use of pesticides. From this point of view, pesticides can be considered as efficient tool of pest management being as economic and labor-saving with their great popularity in agricultural sectors (Damalas and Eleftherohorinos, 2011). Today, the worldwide annual consumption
of pesticides is about two million tons, of which 24% is consumed in United States alone followed by 45% in Europe, and 25% in rest of the world (Uggini et al., 2012).

The use of chemical pesticides has a long history. The first recorded use of insecticides was in 2500 B.C by Sumarians, who used sulphur compounds to control insects and mites. Later in 1200 B.C, Chinese farmers used various natural organic substances to protect their seed from insects, mice and birds, while inorganic mercury and arsenic compounds were used to control body lice (Pretty and Hine, 2005). It was only after World War II that chemical pesticides such as dichlorodiphenyltrichloroethane (DDT), dinitrocresol, 4-chloro-2- methoxyacetic acid (MCPA), 2,4-dichlorophenoxyacetic acid (2,4-D) and other agrochemicals were widely adopted as part of “Green Revolution”. A large proportion increase in productivity seen during the Green Revolution may only be due to the widespread use of these new pesticides. Since then, use of the chemical pesticides had become an integral part of agriculture. Public confidence in pesticide use was shaken only with the publication of book Silent Spring by Rachael Carson in 1962 in which she painted a grim picture of environmental consequences of careless pesticide use (Delaplane, 1996). In India, first report of pesticide poisoning was declared in year 1958 from the state Kerala, where over 100 people died after consuming parathion contaminated wheat flour (Karunakaran, 1958).

Pesticide production was started in India after establishment of a BHC plant near Calcutta in 1952. And after that, a steady growth has been reported in production of various technical grade pesticides from 5,000 metric tons in 1958 to 102,240 metric tones in 1998 (Aktar and Paramasivam, 2008). According to Mathur (1999), India was the second largest manufacturer of pesticides in Asia after China which ranks twelfth globally. And today about 500 pesticide formulations are used in India which average for 0.5 kg ha⁻¹ with an annual consumption of 164, 080 tons of active ingredients (Uggini et al., 2012).
According to a report published in Times of India, the latest compendium of “Environment Statistics India 2012” released by the ministry of statistics and programme implementation (MOSPI) indicates that environment situation of Rajasthan is appalling as the state has been ranked fifth for highest consumption of pesticides in the country after Andhra Pradesh, Punjab & Maharashtra (Sharma, 2013).

Nowadays, second and third generation derivatives of the early harmful pesticides are being developed which are more specific and selective toward their target species. But unfortunately, non-target species also get affected because they are physiologically and biochemically similar to target species. Therefore, the use of these modern derivatives of pesticides should not be treated as completely safe as they can cause accidental and/or intentional poisoning to wildlife, domestic stock, and humans (Ecobichon, 2001).

ORGANOCHLORINE INSECTICIDES

These are the large class of multipurpose chlorinated hydrocarbon chemicals. Organochlorine pesticides are no longer considered an important class of insecticides in North America and Europe, but these are still being used in developing and tropical countries because they are effective, inexpensive, essential chemicals in agriculture, forestry, structural protection, and public health (Ecobichon, 2001). The chlorinated hydrocarbons are stimulants of the nervous system with similar mode of action in both insects as well in the human. These chemicals affect the nerve fibers by disrupting the sodium/potassium balance surrounding them and ultimately result in a nerve that sends transmissions continuously rather than in response to stimuli.

A large number of chemical substances are grouped together under chlorinated hydrocarbon insecticides which are represented in Table 1.
Table1. Structural classification of organochlorine insecticides

<table>
<thead>
<tr>
<th>Classification of Organochlorine insecticides</th>
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<tr>
<td><strong>Dichlorodiphenylethanes</strong></td>
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<tr>
<td>DDT, DDD, Dicofol, Perthane, Methoxychlor, Methlochlor</td>
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<tr>
<td><strong>Cyclodienes</strong></td>
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<tr>
<td>Aldrin, Dieldrin, Heptachlor, Chlordane, Endosulfan</td>
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<tr>
<td><strong>Chlorinated Benzenes</strong></td>
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<tr>
<td>HCB, HCH, Lindane (α-BHC)</td>
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Modified from Ecobichon (2001)

All of these substances within each of these subgroups share in common the ability to modify nervous function but there are considerable differences, both qualitative and quantitative, in their symptomologies of intoxication and their effects on nervous system (Joy, 1982).

DDT type insecticides interact with the neuronal membrane by altering the membrane permeability for potassium and sodium and the calcium mediated processes. By inhibiting these functions, the repolarization of nerves is disturbed resulting in hyperexcitability.

Cyclodienes and cyclohexane compounds have a central nervous system stimulating mode of action. These compounds antagonize the neurotransmitter, gammaminobutyric acid (GABA), permitting only partial repolarization of the neuron and thus, uncoordinated nervous excitation.
If the properties such as low volatility, chemical stability, lipid solubility, slow rate of biotransformation and degradation had made organochlorine chemicals such effective insecticides then other properties such as persistence in environment, bioconcentration and biomagnification within various food chains, has bought their demise. These insecticides were also found to interfere with reproductive success of many wildlife species, particularly avian species such as grebes, pelicans, falcons and eagles (Carson, 1962; Ecobichon, 2001).

Many organochlorine pesticides are endocrine disrupting chemicals having subtle toxic effects on the body’s hormonal system which contributes to adverse health effects as these chemicals mimic the body’s natural hormones and disrupt their normal functions (Lemaire et al., 2004).

There are many ways by which people can be exposed to different types of organochlorine chemicals. Atmospheric and oceanic currents move these pesticides away from where they were used or manufactured, causing contamination of surface waters, ground water and/or soil (Bouman et al., 2002; Shomar et al., 2005). Consumption of contaminated animal products, mostly meat, dairy, fish and marine mammals have been reported to be linked with organochlorine pesticides exposure (Fitzgerald et al., 2001; Hagmar et al., 2001; Mwevura et al., 2002; Bradman et al., 2007). Fetuse and children may be exposed to pesticides in utero as well as through breast milk of exposed mother during their pregnancy (Jurewicz and Hanke, 2008). Organochlorine pesticides exposure has been linked to decreased psychomotor function and mental function, including memory, attention, and verbal skills in children (Jurewicz and Hanke, 2008). Lower performance in numerous neurobehavioral assessments has been reported in children who were born in agricultural areas where the pesticides were applied (Ribas-Fito et al., 2006).
There is clear evidence that exposure to organochlorine pesticides disrupts normal development depending on pesticide specificity, the level of exposure, duration of exposure and individual.

**Dicofol**

Dicofol [4-chloro-\textit{alpha}-(4-chlorophenyl)-\textit{alpha}-(trichloromethyl) benzene-methanol], an organochlorine pesticide is an acaricide which is very effective against red spider mite and has been approved for the use on agricultural crops such as apples, cotton and citrus cultivates, tomatoes, walnuts, mint, cucurbits, beans and peppers etc. and also non-residential lawns. It was first introduced in 1957 by US-based multinational company named as Rohm and Haas. Today, it is manufactured in countries like India, Spain and Israel and sold under a number of trade names such as Kelthane, Colonel, Decofol and Acarin etc.

Dicofol, a nerve poison is synthesized from technical DDT and also structurally similar to it. Earlier, dicofol products used to contain higher amount of DDT and chlorinated form of DDT as impurities which were ending up during their synthesis. Nowadays modern processes of manufacturing are used to produce technical grade of dicofol which contain only 0.1% of DDT. The World Health Organization (WHO) classifies dicofol as "slightly hazardous" (Level III) pesticide (Extoxnet, 1996).

Animal exposure studies of dicofol, DDT and their analogs, have demonstrated neurological, hepatic, and renal toxicities. Deposition of the compound in blood, liver, kidney, heart, and the central nervous system has also been reported, with highest concentrations in adipose tissue (Lessenger and Riley, 1991). Between 1982 and 1992, 38 incidents of dicofol toxicity alone were reported. The number of incidents of illnesses ranged from 0.11 to 0.21 per 1,000 of dicofol applications (Extoxnet, 1996).
In mammals including human, dicofol is believed to be related to inhibition of certain enzymes (ATPases) of the central nervous system and causes hyper stimulation of axonal transmission of nerve impulse. High dose of dicofol exposure may result in nausea, weakness and vomiting, dizziness, skin irritation or rash and eye conjunctivitis. In severe cases of poisoning, it may affect certain organs such as liver, kidney and the central nervous system of animals which ultimately result in coma, convulsions, or even death due to their respiratory failure. Neuropsychological and psychological problems such as headaches, irritability, insomnia, and a poorly described “neurasthenic” or “asthenoaustonomic” syndrome characterized by difficulty in thinking has been associated with chlorinated hydrocarbon- dicofol (Grasso et al., 1984). Studies of the dicofol metabolism in various experimental animals have shown that the chemical is rapidly absorbed and get accumulated in steroid producing organs such as adrenal gland, testes and ovary of rats and mice. And, there it has antispermogenic and antiandrogenic activity (Jadaramkunti and Kaliwal, 2001; 2002; El-Kashoury et al., 2009; 2010). Some primary effects such as increase in liver weight and enzyme induction can reappear long after dicofol exposure due to intense activity and starvation which mobilize the chemical stored in fatty tissue of exposed animals. Reports are also available regarding certain toxic effect of dicofol which includes altered adrenocorticoid metabolism and hormonal changes accompanied by the histological changes in the rats. It is harmful to aquatic animals such as fish, invertebrate and algae. In various species of birds, it is known to be responsible for causing eggshell thinning and reduced offspring survival (Extoxnet, 1996).

Dicofol has a half-life of 60 days as it is moderately persistent in the soil. It is susceptible to chemical breakdown and degradation by UV light only in condition where pH level is above 7. Therefore, in slity loam soil and anaerobic soil conditions, its half-life decreases to 30 and 15.9 days, respectively. Practically, it is insoluble in water and get
adsorb to soil particles very strongly. And because of its immobile nature, it does not infiltrate in groundwater but can enter surface water if soil erosion occurs (Extoxnet, 1996).

**SYNTHETIC PYRETHROID**

A large number of pyrethrin derivatives called as pyrethroids with insecticidal properties came into the market in early 1970s for agricultural purposes (Fishel, 2005). Today, at least 2 dozen pyrethroids are used extensively for controlling insect and mite pests along with treatment for ectoparasitic diseases and these chemicals make up one of the most popular classes of pesticides. The properties such as low mammalian toxicity, quick knockdown effect on insects and improved stability in outdoor environments with limited soil persistence has increased their market value worldwide in agriculture sector. Their commercial formulations are available in the form of emulsifiable concentrates, aerosols, wettable powders, dips and granules. For broad spectrum use, these chemical may be mixed with other pesticides for controlling insect pest (Fishel, 2005).

Pyrethrins were originally derived naturally from pyrethrum or chrysanthemum flowers (*Chrysanthemum cinerariifolium* and *Chrysanthemum coccineum*) which have insecticidal activity. These flowers which are mainly found in Kenya contain six insecticidal esters or toxins: pyrethrin I and II, jasmolin I and II, and cinerin I and II. In a natural environment, these compounds are chemically unstable and broke down rapidly on exposure to air, sunlight and under ultraviolet radiation. Therefore, many pyrethroids with increased photostability have been synthesized which may be divided into two large categories: type I pyrethroids, which do not have a cyano moiety at α position; and type II pyrethroids, which do have a cyano group at α-benzyllic position (Anon, 2003).
Type I and type II pyrethroids exert their toxicity by affecting both peripheral and central nervous systems of organisms. These chemicals act by modifying a small fraction of the voltage-gated sodium channels of nerve cells which stimulate them to generate repetitive discharges until the level of hyperexcitability (Davies et al., 2007). This modification results in prolonged opening of individual sodium channels from the tens or hundreds of milliseconds by type I pyrethroids and last for several seconds or longer by type II pyrethroid compounds (Anon, 2003; Davies et al., 2007). Type I pyrethroids produce the T (tremor) syndrome characterized by restlessness, un-coordination, prostration, paralysis, sparring, aggressive behaviour and enhanced startle response in both insects as well as in mammals. Type II compounds produce CS (choreoathetosis/salivation) syndrome resulting in intense hyperactivity, un-coordination and convulsions in cockroaches, while burrowing behavior, coarse tremors, chronic seizures, sinuous writhing (choreoathetosis), and profuse salivation without lacrimation has been observed in rats (Ecobichon, 2001; Anon, 2003; Davies et al., 2007).

Pyrethroids are known to be least toxic to mammals than insects as these chemicals are quickly deactivated by their metabolic processes (Fishel, 2005). The major reasons underlying behind selective toxicity of pyrethroids in insects are due to sodium- channels sensitivity which are 100-1000 times more sensitive in insects than that of mammals. Also,
the activity of pyrethroids depends on temperature and cause greater effect on sodium channels at a lower body temperature (about 27°C) of insects than at higher temperature of the mammals (about 37°C). However, Fishel (2005) reported that these chemicals can cause liver damage, asthmatic condition and a skin rash or inflammation in mammals if exposed with these sensitizing agents. No data is available on carcinogenic and teratogenic effects of synthetic pyrethroids.

Pyrethroids are reported to be highly toxic to aquatic animals such as fish and tadpoles as these chemicals affect their sensitive skin touch receptors and balance organs (Fishel, 2005). They are moderately toxic to birds. Birds can also be directly affected through their food supply contaminated with these pyrethroids. Waterfowl and small insectivorous birds are the most susceptible. Because pyrethroids application are toxic to all insects, they may affect beneficial insects specially predator insects disrupting the predator- prey relationship (Mueller- Beilschmidt, 1990).

**Deltamethrin**

Deltamethrin, [(S)-a-cyano-3-phenoxybenzyl-(1R)-cis-3-(2, 2-dibromovinyl)-2, 2-dimethylcyclopropane carbo-xylate] belongs to type II synthetic pyrethroid which was synthesized in 1974. It is used as an active ingredient in the number of commercially available insecticide formulations which are applied on a variety of agricultural crops such as cotton, coffee, corn, hops, maize, artichokes cereals and fruits for controlling their insect pests like mealy bugs, apple and pear suckers, various caterpillars, plum fruit moths, aphids and whiteflies. Chemically, it is the [1R, cis; alpha S]-isomer of 8 stereo isomeric esters of the dibromo analogue of chrysanthemic acid, 2, 2-dimethyl-3-(2, 2-dibromovinyl) cyclopropanecarboxylic acid (Br₂CA) with alpha-cyano-3-phenoxybenzyl alcohol.
As a pyrethroid, deltamethrin acts on insect’s nervous system, paralyzes it and ultimately leads to death (Extoxnet, 1995). It is absorbed by the insect pests through both dermal and oral means. The susceptibility of these insects to toxic effects of deltamethrin depends on their own physiological structure as well as on the surrounding environmental conditions. Deltamethrin is considered as most powerful and toxic among the pyrethroids (Extoxnet, 1995). A variety of acute health conditions such as allergic reactions, writhing syndromes, ataxia, convulsions and salivation, diarrhea, dermatitis, tremors and vomiting due to deltamethrin exposure has been monitored in farmers and workers of agricultural sectors. The chemical has also been reported to carry several ecological risks particularly by causing algal blooms in water bodies which in turn harms a range of aquatic life and decrease the oxygen level of water. It also reduces bee populations and their associated pollination service. Deltamethrin is reported to have a fast metabolism in living organisms and a low level of residues in the environment; these may vary depending on the environmental conditions. Though this chemical is broken down via UV and sun lights, it is quite tolerant to storage and can preserve its activity for 6 months at 40ºC and pose risks to mammals and ecosystem as whole (Ozkan and Ustuner, 2012). Deltamethrin has been listed as “moderately hazardous” by both United States Environmental Protection Agency (USEPA) and the World Health Organization (WHO). But under laboratory conditions, it is reported to be extremely toxic to a variety of aquatic organisms such as fishes, amphibians, mollusks, crustaceans and planktons.

Children and unborn babies are particularly more susceptible to pesticide poisoning in developing nations which is of great concern. Developing embryos can be exposed to pesticides in mother’s blood as it crosses the placenta during the “critical periods “of their embryogenesis. Pesticides affecting cellular proliferation, differentiation, or apoptosis can produce many embryotoxic and teratogenic effects which may result in permanent
congenital malformations, functional abnormalities, or even death of embryo (Gilbert, 2006).

**CHICK EMBRYO: AN EXPERIMENTAL MODEL**

For obvious reasons no studies of teratogenicity or developmental toxicity are conducted during embryogenesis of humans. Therefore, a wide variety of test animals have been employed in detecting important toxic and teratogenic properties of these pesticides and, for estimating risk to human and environmental health. Chick embryo; a non mammalian model have made significant contributions in many toxicological studies because of many advantages such as known embryonic development and lack of placenta, which may reveal the extent of maternal protective factors, the large size of the embryo and *in vivo* development makes it accessible to surgical and biochemical manipulation and easy to observe embryos at different stages and follow them through developmental changes, each embryo develops in self –contained environment, easily accessible because of short 21- days of gestation period, cost efficient and possibility of experimenting on large scale for statistically valid results. Nevertheless, tests on chicken embryo will never replace tests performed with mammals (Pageze *et al.*, 1996), but they would easily satisfy the first requirement for a preliminary teratological screen because developing morphogenetic system (MGSs) of this quicker *in vitro* system interact directly with chemical substances (Jelinek, 1982; Kotwani, 1998).

In many avian developmental toxicological studies, eggs are exposed to toxicants either by injection or by immersion technique. From the point of view of environmental contamination, the injection method does not reflect field exposure because this method is used only for evaluating the risk of embryo toxicity and teratogenic potency of toxicants, while immersion method intends to mimic the exposure associated with agricultural
practices, more closely related to normal mammalian exposure and allows us to assess the potential hazard posed by these chemicals (Varga et al., 2002).

**OBJECTIVES**

As far as teratological effects of the deltamethrin and dicofol on chick development are concerned, there is practically no information available in literature regarding their teratogenicity. Therefore, the present teratological study has been planned taking the commercial formulations of above mentioned insecticides on developing chick embryo with the following objectives-

1. To observe the congenital anomalies (morphological and skeletal), if any, in the developing chick embryo exposed with commercial formulations of insecticide dicofol and deltamethrin.

2. To assess the effect of these insecticides on certain biochemical parameters of chick embryo.

3. To determine the effect on activity of brain acetylcholinesterase enzyme of chick embryo.

4. To know the hepatotoxic (pathological and biochemical) changes in the liver of developing chick embryo exposed to above mentioned insecticides.

The findings of this study will definitely help in detecting the adverse effects of deltamethrin and dicofol which could support the idea of the generalized harmfulness of these insecticides in environment. This knowledge could later be used in making a judicious or harmonious use of these popular insecticides.