INTRODUCTION

Throughout most of history, the world's population has been much smaller than it is now. Before the invention of agriculture, for example, the human population was estimated to be around 15 million. The introduction of agriculture and the gradual movement of humanity into settled communities saw the global population increase gradually to around 300 million by AD 0. To give an idea of scale, the Roman Empire, which many regard as one of the strongest empires the world has ever seen, probably contained only around 50 million people at its height; that's less than the number of people in Gujarat today (The current US Census Bureau world population, 2013).

The world population growth (timeline): Year 1: 200 million, Year 1000: 275 million, Year 1500: 450 million, Year 1650: 500 million, Year 1750: 700 million, Year 1804: 1 billion, Year 1850: 1.2 billion, Year 1900: 1.6 billion, Year 1927: 2 billion, Year 1950: 2.55 billion, Year 1955: 2.8 billion, Year 1960: 3 billion, Year 1970: 3.7 billion, Year 1985: 4.85 billion, Year 1999: 6 billion, Year 2011: 7 billion, Year 2025: 8 billion (The current US Census Bureau world population, 2013).

World population is expected to grow by over a third, or 2.3 billion people, between 2009 and 2050. This is a much slower rate of growth than the one seen in the past four decades during which it grew by 3.3 billion people, or more than 90 percent. Nearly all of this growth is forecast to take place in the developing countries. Among the latter group, sub-Saharan Africa's population would grow the fastest (+114 percent) and East and Southeast Asia's the slowest (+13 percent). Urbanization is foreseen to continue at an accelerating pace with urban areas to account for 70 percent of world population in 2050 (up from 49 percent at present) and rural population, after peaking sometime in the last decade, actually declining (High Level Expert Forum - How to Feed the World in 2050, 2009).

Taking in to consideration the above mentioned upward trend in human population size in the years to come it is but logical to presume that more than anything else, adequate food and environment security would remain as the two key issues that confront mankind in the third millennium. The future strategies should therefore be carefully planned to sustain food production with least disruption to the fragile agro ecosystem (Dhaliwal *et al.*, 1999).

While the first recorded use of chemicals to control pests date back to 2500 BC, it is really only in the last 50 years that chemical control has been widely used (Morley *et al.*, 1996). Many of the earliest pesticides were either inorganic products or derived from plants (i.e. burning sulphur to control insects and mites). Other early insecticides included hellebore to control body lice, nicotine to control aphids, and pyrethrin to control a wide variety of insects. Some heavy metals also became popular - lead arsenate was first used in 1892 as an orchard spray - while about the same time it was accidentally discovered that a mixture of lime and copper sulphate (Bordeaux mixture) controlled downy mildew, a serious fungal disease of grapes. It is still one of the most widely used fungicides (Morley *et al.*, 1996).

Pesticides are an undeniable part of modern life, used to protect everything from flower gardens to agricultural crops and specific pests. Pesticides have contributed significantly to improving quality of life and safeguarding the environment. Although often taken for granted, without these important products, food production would decline, many fruits and vegetables would be in short supply and prices would rise. Some 20 to 40 percent of the world's potential crop production is already lost annually because of the effects of weeds, pests and diseases (according to the FAO reports, 2005). These crop losses would have doubled if existing pesticides were abandoned and the food prices also would have increased significantly. Even after harvest, crops are susceptible to attack by a diverse pest population. Insects, rodents and fungi can harm grains. In addition to increasing crop yields, crop protection products used in stored food grains can also prolong the shelf-life of products by preventing huge post-harvest losses to insect pests. On the other hand, although pesticides are now commonplace, concerns still exist about their safety and proper use. Pesticides can be used safely and effectively. But if proper care is not taken, pesticides can harm the environment by contaminating soil, surface and ground water, and ultimately challenging the very existence of life. Moreover, modern man is constantly exposed to a wide array of toxic chemicals primarily due to changes in life style. The food we eat, the water we drink, the air we breathe, and the environments we live in are all contaminated with toxic chemicals. Humans are exposed to such chemicals while still in the womb of the mother (Lederman, 1996). Therefore, human life would be threatened not only directly by pesticides in environment, but indirectly by a contaminated food chain. It is now well understood that the conventional chemical pesticides have environmental inconvenience and disadvantages for human health (Lederman, 1996). Hence, the problems along with the

benefits of pesticides in improvement of quality of agricultural products and food production and storage are discussed herein.

The term pesticide includes substances intended for use as a plant growth regulator or an agent used for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport (FAO, 2005). A pesticide may be a chemical substance, biological agent (such as a virus or bacterium), antimicrobial, disinfectant or device used against any pest. We use pesticides to cover a wide range of chemicals used to control insect pests, plant diseases, weeds, rats or other unwanted organisms. Currently, more than 800 pesticide active ingredients in a wide range of commercial products are registered for use in agriculture to meet food supply demands (FAO, 2005; Stoytcheva, 2011).

Pesticides can be classified by target organism, chemical structure and physical state (Council on Scientific Affairs, American Medical Association, 1997). Pesticides can also be classed as inorganic, synthetic, or biologicals that include microbial pesticides and biochemical pesticides. Plant-derived pesticides or botanicals include pyrethroids, rotenoids, nicotinoids, and a fourth group that includes strychnine and scilliroside (Kamrin, 1997). In addition, pesticides can be classified based upon their biological mechanism function or application method.

Basically, agricultural pesticides are divided into five categories, depending on the target pest, *viz.*, insecticides, herbicides, fungicides, rodenticides and fumigants.

All pesticides are toxic to some plant or laboratory animal species. At higher doses, they can also be toxic to farm animals, pets, and humans. In general, prominent insecticide families include organochlorines, organophosphates, and carbamates. Acute toxicity of insecticides for mammals ranges from low to high. Herbicides, which are used to control weeds, are reported to have low acute toxicity for mammals and fungicides are characterized as moderately toxic (Shokrzadeh *et al.*, 2009).

However, pesticides are the most essential agricultural inputs for protecting crop plants and increasing agricultural production. In India, 241pesticides and 41 combination products are registered as on date for use in agriculture. Out of these 62 insecticides, 40 fungicides and 7 plant growth promoters have been registered for use only in vegetable crops (Kodandaram *et al.*, 2013).

ADVANTAGES OF USING PESTICIDES

Uninhibited supply of fresh food products is vital for a healthy population. Numerous scientific studies demonstrate the health benefits of regularly eating a variety of fresh fruit and vegetables. Moreover, consumers today are increasingly aware of these benefits. To ensure this demand at an affordable price, agricultural productivity should be increased. The best way to achieve this task is through enhanced yet judicious use of crop protection products (Anon, 1990).

Therefore, the primary aim of crop protection industry is to enable farmers to grow an abundant supply of food in a safe manner and prevent costs from increasing. Furthermore, continual advancements in agricultural technologies and practices are also playing a positive role in improving food production. In fact, a population now nearly twice as large has more food available per capita than four decades ago. Tools such as insecticides and fungicides reduce crop losses both before and after harvest, hence increasing crop yields (Saravi and Shokrzadeh, 2011).

The major benefits of pesticides and their role in food production are highlighted in the following section.

PESTICIDES IN THE MODERN WORLD - RISKS AND BENEFITS

Increase in food quality and quantity: Crop protection technologies allow producers to increase crop yields and efficiency of food production processes. It is reported that up to 40 percent of the world's potential crop production is already lost annually because of the effects of weeds, pests and diseases (Saravi and Shokrzadeh, 2011). These crop losses would be doubled if existing pesticide uses were abandoned. In addition, pesticides allow consumers to consume high-quality products that are free of insect blemishes and insect contamination. Moreover, crop protection chemicals that reduce or eliminate insect damage allow the consumers to buy high-quality food products.

Decrease in price of food: Since the use of pesticides and crop protection technologies improves crop yields, it might also impact the cost of food. Without crop protection chemicals, food production would decline, many fruits and vegetables would be in short

supply and prices would rise. Therefore, one of the large benefits of pesticides is to help keep food prices in check for the consumers.

Human health protection: Pesticides are the most effective substances to eliminate insects that transmit human diseases such as malaria and dengue fever. Human health is also supported by preventing fungi- borne carcinogens, like aflatoxins, which is known to induce hepatic and other cancers.

Environmental protection: Other positive aspects of crop protection chemicals, in responsible and safe use, include household pest control, control of vegetation in industry and infrastructure, and recreation and protection of areas against environmental pests like noxious weeds, feral animals, etc., which cause land degradation (Saravi and Shokrzadeh, 2011).

Disadvantages of using pesticides

It is well known that food is the basic necessity of life and food contaminated with toxic pesticides might result in severe health effects. Hence, it is pertinent to explore strategies that address this situation of food safety especially for a country like India where pesticide contamination is reported to be widespread due to indiscriminate usage and is hugely populated (Saravi and Shokrzadeh, 2011).

The four major groups of pesticides such as the organochlorine, organophosphate, carbamate, and pyrethroid are of particular concern because of their toxicity and persistence in the environment (Ahmed *et al.*, 2000; Smith and Gangolli, 2002). Even though many of the pesticides from the above classes are banned, several of them are still used on a large scale in developing countries and continue to pose severe health and environmental problems (Ahmed *et al.*, 2000). Pesticide use raises a series of environmental concerns including human and animal health hazards. It has been reported that over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water and soil (Miller, 2004). Pesticides are one of the causes of water pollution and some pesticides are persistent organic pollutants, contributing to soil contamination. Since these chemicals are non-biodegradable, they get accumulated in the environment and ultimately into the human food chain. Despite regulatory measures, these compounds continue to be detected in measurable amounts in the ecosystem including marine life (Smith and Gangolli, 2002).

In addition, there are reports that pesticide use reduces biodiversity, hampers nitrogen fixation (Rockets, 2007) and adversely affect the population size of pollinators (Haefeker, 2000; Zeissloff, 2001; Wells, 2007).

PRODUCTION AND USAGE OF PESTICIDES IN INDIA

Pesticide manufacturing in India started in the year 1952 with the establishment of a plant for the production of BHC near Kolkata. Currently India is the second largest manufacturer of pesticides in Asia after China and ranks twelfth globally (Mathur, 1999). There has been a steady growth in the production of technical grade pesticides in India, from 5,000 metric tons in 1958 to 102,240 metric tons in 1998 (Mathur, 1999). In 1996-97, the demand for pesticides in terms of value was estimated to be around Rs. 22 billion (USD 0.5 billion), which was equivalent to 2% of the total world market then.

One of the recent reports indicates that in the past few years, Indian agriculture has done remarkably well in terms of output growth. The Five year plan (2007-12) witnessed an average annual growth of 3.6% in the gross domestic product (GDP) from agriculture and allied sector. The growth target for agriculture in the 12th five year plan is estimated to be 4%. Indian agriculture is benefitting hugely from rising external demand and the sector's aggressive participation in the global economy. The report further predicts that the market for agrochemicals will grow at the rate of 7.5% to reach USD 1.95 billion by financial year 2014 and USD 3.5 billion by financial year 2015 (Figure i). The expected growth is supported by the fact that India's agrochemical consumption is one of the lowest in the world with per hector consumption at just 0.58 kg compared to USA (4.5 kg/ha) and Japan (11 kg/ha). This suboptimal and inadequate usage of agrochemicals incurs 20% to 30% crop losses ever year (Insecticide (India) Limited, 2012-13).

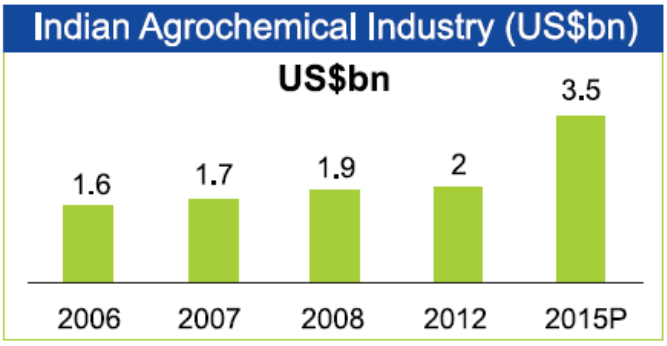


Figure i. The market share of Indian agrochemical industry over the years (Source: FICCI and Indian Brand Equity Foundation)

Curiously, the pattern of pesticide usage in India is markedly different from that of the global pesticide usage pattern. As can be seen in Figure ii, in India 76% of the pesticides used are insecticides, as against 44% globally (Mathur, 1999). Further it is clear from the figure ii that the use of herbicides and fungicides is less in India on a global comparison. It has been further reported that the main use of pesticides in India is for cotton crops (45%), followed by paddy and wheat (Mathur, 1999). Moreover, though there are several newer classes of pesticides which are in use world over, in India we still continue to use largely the old generation insecticides. Most notable among these is the organophosphate (OP) group of insecticides, which though contributed greatly to pest control and agricultural output, the adverse health effect associated with casual intake of OP compound is yet to be ascertained.

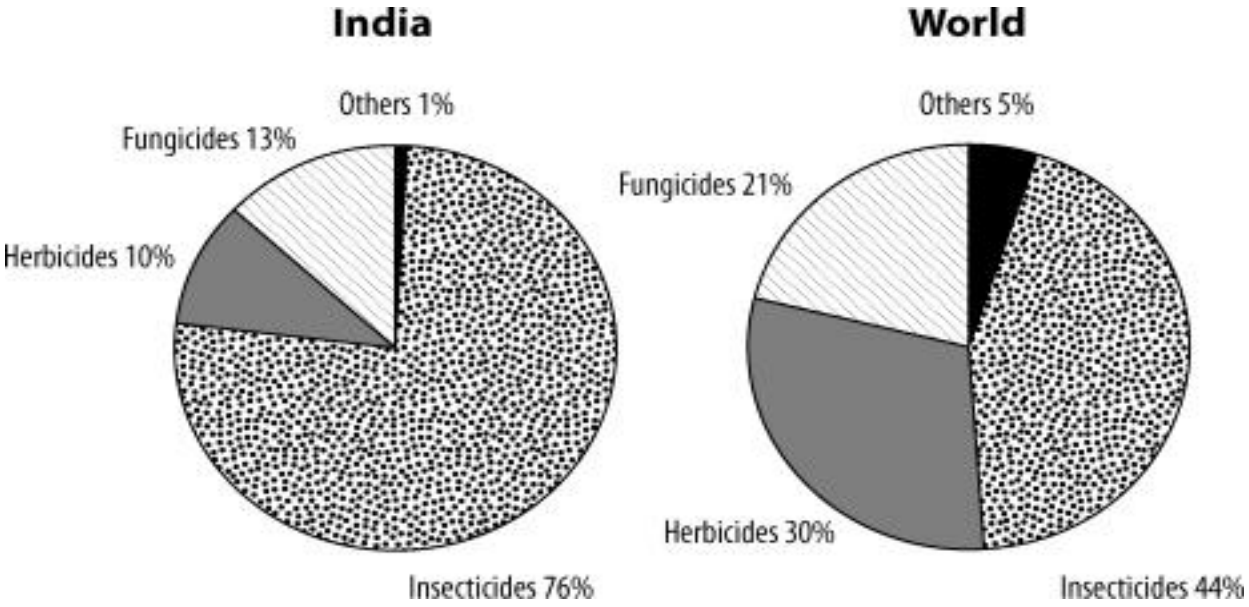


Figure ii: Consumption pattern of pesticides in India and in the rest of the world (adopted from Mathur, 1999)

ORGANOPHOSPHATES

Organophosphates are classified as neurotoxic and they adversely influence the cholinergic neurons of animals, especially insects. They are esters of phosphoric or phosphorothioic or related acids and are akin to the chemical warfare nerve agents, which are often phosphonofluoridates (Timbrell, 1995). Many pesticidal organophosphates are phosphorothioates and those containing P = S groups tend to be of lower mammalian toxicity than their corresponding phosphates and phosphonates. The reason for this is that the phosphorothioates moiety is metabolised to a less toxic oxon containing compound in a mammalian system (WHO, 1986). Since the P = S form is intrinsically more stable, many

insecticides are manufactured in this form, which can be converted to the biologically active form in the tissues of insects. Sulphur atoms from the P = S form are removed oxidatively and replaced by oxygen P = O (Timbrell, 1995).

The term organophosphates are used often to describe virtually any organic phosphorus (V) containing compound, especially when dealing with neurotoxic compounds. Many of the so called organophosphates contain C-P bonds. For example, sarin is O-isopropyl methylphosphonofluoridate, which is formally derived from phosphorous acid ($HP(O)(OH)_2$), not phosphoric acid ($P(O)(OH)_3$). Yet another group of organophosphates includes derivatives of phosphinic acid (Timbrell, 1995).

At a mechanistic level, OP pesticides irreversibly phosphorylate the active centre and inactivate the enzyme acetylcholinesterase, which is essential to maintain the normal function of cholinergic neurons in insects, humans, and many other animals (Timbrell, 1995). However, the affinity to phosphorylate the enzyme varies with the chemical nature of the OP. For instance, parathion, one of the first OPs commercialized, is many times more potent than malathion.

Moreover, it has been reported that organophosphates degrade rapidly in nature by hydrolysis on exposure to sunlight, air, and soil, although small amounts can be detected in food and drinking water (Timbrell, 1995). Their ability to degrade made them an attractive alternative to the persistent organochloride pesticides, such as DDT, aldrin, and dieldrin. Although organophosphates degrade faster than the organochlorides, they have greater acute toxicity, posing risks to people who may be exposed to large amounts (Timbrell, 1995).

ORGANOPHOSPHATES: DEVELOPMENT THROUGH YEARS

The famous two in the field of developing chemical agents that hamper nervous system were Jean Louis Lssaigne (early 19th century) and Philippe de Clermont (1854). Several years later German chemist Willy Lange and his graduate student, Gerde von Krueger, first described the cholinergic nervous system effects of organophosphates and reported choking sensation and a dimming of vision after exposure (Lange and Krueger, 1932). This observation inspired German chemist Gerhard Schrader at company IG Farben in the 1930s to explore the possibility of these compounds as potential insecticides. British scientists experimented with a cholinergic organophosphate of their own, called diisopropylfluorophosphate (Stenersen, 2004).

During the late 1940s, American companies gained access to some information from Schrader's laboratory, and began synthesizing organophosphate pesticides in large quantities. Parathion was among the first marketed OP, which is followed by malathion and azinphosmethyl. The acceptance of these insecticides increased after many of the organochlorine insecticides like DDT and dieldrin were banned by 1970s (Stenersen, 2004).

Since the initial molecules were found to be severely toxic to the non-targeted organisms including humans, relatively safer OP compounds like Acephate were developed and marketed subsequently. Acephate is an organophosphate insecticide of moderate persistence with residual systemic activity of about 10–15 days at the recommended use rate (CDC, 2009). It is used primarily for control of scale insects, including resistant species, in vegetables (e.g. potatoes, carrots and tomatoes) and in horticulture. It also controls leaf miners, caterpillars and thrips in the previously stated crops as well as in lawns and forestry (CDC, 2009).

Acephate affects the nervous system, causing over-activity in the nerves, muscles, or brain. In addition, acephate is absorbed into plants, and insects that feed on treated plants may contract acephate (Nigg and Knaak, 2000). Acephate can kill target insects when they touch it or eat it. When insects eat acephate, their body metabolically activates it to methamidophos, which is more toxic than the parent compound and proves lethal to insects. However, acephate is less toxic in mammals because mammalian system do not turn it into methamidophos very well (Nigg and Knaak, 2000; Harrington, 2002).

Nevertheless, applicators and formulators may be exposed through dermal and inhalational (e.g. dust or aerosol) routes. Lower levels of exposure can happen if people work with acephate-treated soils or plants. General population exposure to acephate may also occur from consuming foods that have been treated with acephate and by direct contact with treated surfaces. Estimated dietary and water intakes have not exceeded recommended limits (Gunderson, 1995), but potential exposures from residential use have posed concern, hence residential indoor and lawn registrations have been discontinued in US (USEPA, 2006).

However, the acephate is used unrestricted in India in diverse commercial formulations. The formulation insecticide Acephate used for control of pests on selected agricultural crops and in specified non-crop areas. Registered formulations of acephate include wetable powder,

soluble powder, soluble extruded pellets, granules, water soluble bags, and liquid. A wide range of application methods are approved for acephate, ranging from hose-end sprayers and belly grinders, to high pressure sprays and aerial application by aircraft, for agricultural use. Application rates vary considerably between food and non-food uses and for specific crops, structural uses, and pests (Patterson, 2004).

For the current study a commercial formulation of acephate (acephate 97% DF) manufactured by UPL Ltd. under the trade name *Lancer 97 DF* was selected. The active ingredient in acephate 97% DF is acephate (O,S-Dimethyl acetylphosphoramidothioate), a water-soluble insecticide readily absorbed by plant roots and foliage to give systemic control of feeding insects. Insect pests are generally controlled more effectively by acephate 97% DF through ingestion than by contact.

PHYSICAL / CHEMICAL PROPERTIES OF ACEPHATE 97% DF

Active Ingredient: By Weight.

Acephate : 97.0%

Other Ingredients : 3.0%

Total : 100.0%

The International Union of Pure and Applied Chemistry (IUPAC) chemical Name: O,S-Dimethyl acetylphosphoramidothioate

Chemical Abstracts Service (CAS) registry number: 30560-19-1.

Acephate has a strong odor similar to mercaptan, which smells like sulfur.

Vapor pressure (USEPA, 2006): 1.7×10^{-6} mmHg at 24 °C

Octanol-Water Partition Coefficient (Kow) (Downing, 2000): 0.13 at 25 °C

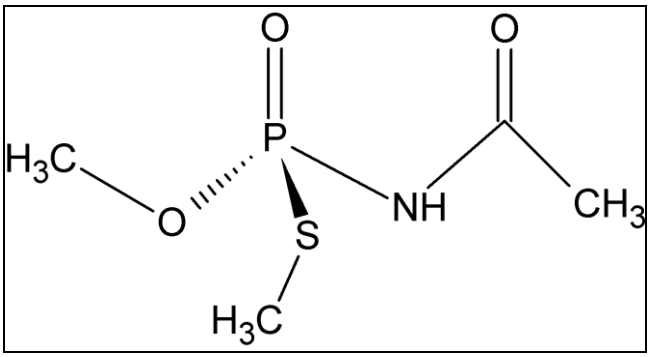
Henry's constant (Yen *et al.*, 2000; Davy *et al.*, 2007):

3.1×10^{-7} atm·m^3/mol; 5.1×10^{-13} atm mole/m^3

Molecular weight (USEPA, 2006): 183.16 g/mol

Solubility (water) (USEPA, 2006; Yen *et al.*, 2000): 79 - 83.5 g/100 mL

STRUCTURE:



MODE OF ACTION:

In Target Organisms

Acephate is a systemic insecticide used to control sucking and biting insects by direct contact or ingestion (Thomson, 1989; Tomlin, 2006). Studies of Acephate revealed that acephate is soluble in polar solvents and solubility increased with temperature (Geen *et al.*, 1981; Yen *et al.,* 2000; Wang *et al.,* 2010) hence the lifetime, persistence and leaching of acephate increases with a polar solvent. It binds to and inhibits the enzyme acetylcholinesterase (AChE) in nervous system. As a result, the neurotransmitter acetylcholine accumulates and repeatedly activates cholinergic receptors (Reigart and Roberts, 1999; Klaassen, 2001). However, as mentioned earlier, acephate as a parent compound is a weak inhibitor of AChE (Chuckwudebe *et al.*, 1984). But its metabolic product methamidophos is a more potent AChE inhibitor than acephate (Reigart and Roberts, 1999; Kumar and Upadhay, 2013). Insects metabolize acephate into methamidophos by hydrolysis, whereas mammals metabolize acephate more readily into des-O-methylacephate, accounting for acephate's relatively high selectivity against insects (Mahajna *et al.*, 1997; Roberts and Hutson, 1999; Farag *et al.*, 2000;).

Methamidophos inhibits acetylcholinesterase through phosphorylation (Farag *et al.,* 2000). Acetylcholine is the prominent insect stimulatory neurotransmitter for motor, sensory, and intermediate neurons (Chapman, 1998) and is broken down by acetylcholinesterase (Smith and Treherne, 1965). Organophosphates cause acetylcholine levels to increase and over-excite target nerves, muscles or tissues (Reigart and Roberts, 1999; Yen *et al.*, 2000).

In Non-target Organisms

Mammals are less sensitive to acephate than insects (Chuckwudebe *et al.*, 1984; Roberts and Hutson, 1999). The selectivity or potency of acephate is linked to mammalian metabolism (Mahajna *et al.*, 1997). Mammalian metabolism of acephate into the cholinesterase inhibitor methamidophos is minor, compared to the main pathway of its conversion into des-O-methylacephate, also known as S-methylacetyl phosphoramidothiolate (SMPT), which is not a cholinesterase inhibitor (Chuckwudebe *et al.*, 1984; Roberts and Hutson, 1999).

In one of the earlier studies, authors have suggested that acephate blocks methamidophos from binding to acetylcholine directly or by an allosteric site in mammals (Spassova *et al.*, 2000). In another study, methamidophos inhibited erythrocyte and brain acetylcholinesterase a thousand times more effectively than acephate (WHO, 2005). In an experiment using mice, the investigators reported that methamidophos is ten times stronger in inhibiting cholinesterase than acephate (Chuckwudebe *et al.*, 1984). Evidence of carcinogenicity is limited to liver tumors that occurred in rats fed high doses of acephate over several months, and USEPA considers acephate to be a possible human carcinogen (USEPA, 2012).

Vyas and coworkers, while performing an ecotoxicology study, observed inhibition of cholinesterase in the brain of white throated sparrow challenged with acephate (Vyas *et al.*, 1996). In another experiment, where hens were given acephate by gavage, there was inhibition of AChE and, to a much lesser extent, neuropathy target esterase (Wilson *et al.*, 1990).

It has been reported that in humans, acephate is quickly absorbed through the skin, lung and gastrointestinal tract and is subsequently broken down in the liver; breakdown products are readily excreted via respiration and urine (Chuckwudebe *et al.* 1984). Studies have also shown that acephate intoxication might induce renal tubular cell injury due to heightened lipid peroxidation in the wake of compromised local antioxidant defence (Poovala *et al.*, 1998; Datta *et. al*., 2010).

RATIONALE OF THE CURRENT STUDY

Though there are large bodies of evidence to show that acephate is by and large safe for the non-target organisms because of the safe metabolic by-product that is getting generated in intoxicated animals, there are also ample evidences available of acephate poisoning in these (Chuckwudebe *et al.*, 1984; Nigg and Knaak, 2000; Harrington, 2002; CDC, 2009; USEPA, 2012; Kumar and Upadhay, 2013). Moreover, a scan through literature has revealed that whatever systematic *in vivo* studies had been attempted previously using acephate as test substance had all chosen oral route for application. Therefore, the current study was designed wherein it was thought pertinent to study the toxic characteristics of the formulation insecticide Acephate 97% DF in rat/mouse via inhalation route, since that is the route through which the applicators or formulators might accidently/passively contract acephate.

As a prelude, the median lethal concentration of Acephate 97% DF was studied in rats (Chapter 1) since it provides the basic information about the signs of toxicity and also acts as baseline information for further long term toxicological evaluation. Subsequently, in order to evaluate the systemic toxicity of the formulated insecticide, a 14day repeated-dose study was conducted in rats wherein haematological, biochemical and histopathological parameters were evaluated at the end of the experimental schedule. Moreover, to understand the test compound's effect on the central and peripheral nervous system, blood cholinesterase activity too was evaluated after repeated exposure to acephate formulation (Chapter 2). Lastly to check the immunosuppression potential, if any, of the selected test substance the mouse local lymph node assay (LLNA) was attempted (Chapter 3).