PREFACE

Over the last 50-60 years many types of man-made chemicals have been manufactured and many of them have become widespread environmental contaminants (Simonich and Hitesl, 1995; Loganathan and Kannan, 1994; Bajerregaard, 1995). There is now growing concern that some of these man-made chemicals (including pesticides, industrial chemicals, plastics, detergents, paints and cosmetics) are affecting the health of human and wildlife populations (Loganathan and Kannan, 1994; Fry, 1995; Dich et al., 1997; Longnecker et al., 1997). These substances can affect human health by upsetting the balance of the endocrine system, and they are known as hormone-disrupting chemicals or endocrine disrupters. As a result of mankind’s use of vast quantities of such chemicals, humans and wildlife are continually exposed to endocrine disrupters (O’Shea et al., 1980).

Environmental contaminants by pesticides has been documented in biotic and abiotic components. These persistent organic pollutants are lipid soluble, non-biodegradable and endocrine disrupters. The effect of various industrial chemicals and other environmental pollutants in altering human endocrine systems has drawn public attention. Man has utilized a wide variety of pesticides to combat the crop pests and vectors of human diseases. Pesticides have been largely responsible for increased yields and improved quantity of cereals, roof crops, frits and vegetables by making them free of insects.

Pesticides are such commodities the quantities used by farmers are governed by concepts of productivity and increasing expectations promoted by clever advertising. The British report identified four possible outcomes of long-term exposure to pesticides in man-carcinogenicity, mutagenicity, teratogenicity, allergies and effects on the immune and nervous system. Pesticides have been continuously produced with pressure to do so being compounded by commercialism and encouraged by promises of greater effectiveness and increased productivity. The killing of primary pests with pesticides has paved the way for secondary pests to come to the fore where previously there were 10 primary pest insect – defined as causing greater than one million dollars of crop damage per year – there are now 300 of the 25 most serious pests, 24 were previously secondary pests and 72 percent of these are now pesticide resistant (Ausuble, 1994; Walter and Crinnion, 2000). Hundreds of active ingredients and tens of thousands of formulations are used to control agricultural pests and disease carrying vectors (Meister, 1919). 1.5 million tones of

i
pesticides are manufactured every year, producing a business worth US$30 billion (Wood McKenzie, 2001). The widespread adoption of pesticides during the 1950s was associated with increased crop yields, opening up of new agricultural land and reduction in incidence of vector-borne diseases. However, increasing pest resistance has resulted in lower yields and a resurgence of vector-borne diseases such as malaria. At the same time, the many health and environmental costs of intensive pesticide deaths recorded in hospital surveys are the result of self-poisoning. (Eddlestone, 2000). The Global Burden of Disease Study (Murry and Lopez, 1996) estimated that 7,98,000 people died from deliberate self-harm in 1990, over 75% of whom were from developing countries (Murry and Lopez, 1996). More recent WHO estimates show that over 5,00,000 people died from self-harm in Southeast Asia and the Western Pacific during 2000 alone (WHO, the World Health Report, 2001). In extrapolation from every limited data, WHO estimates that three million pesticide poisoning cases occur worldwide every year with 2,20,000 deaths, most of which are international (WHO in collaboration with UNEP World Health Organization, 1990).

India is one of the largest user of agricultural insecticides in recent times (Allen et al., 1984). Pesticides are by nature toxic to all living things and they not only pollute the environment but also cause harm to humans as well as beneficial organism (Link et al., 1984). In 1997, WHO estimated that number of deaths occurred globally due to pesticides was about 20,640 a year. More recently the Economic and Social Commission of Asia and the Pacific (ESCAP) suggested that pesticide poisoning incidents might amount to two million a year of which 40,000 could be fatalities (Fod, 1985). In Sri Lanka 47.3 percent of the 30,490 hospitals admissions for poisoning 1986 were due to pesticides, 33.7 per cent being by cholinesterase inhibitors alone (Forget, 1991). Therefore, all the newly synthesized pesticides need to be screened well for their adverse effects to non-target organisms before their commercialization in large scale, safety evaluation of pesticides on the basis of target enzyme interaction has been given a push in the recent time for their on site evaluated and species elective toxicity. The low doses of certain synthetical chemicals in the environment can mimic hormones and disrupt natural growth and development in animals and humans (Colbron, et al., 1996). The most widely used pesticides are found in the form of insecticide organochlorines, organophosphates and carbamates.

The introduction of synthetic insecticides – organophosphate (OP) insecticides in 1960s, carbamates in 1970s and pyrethroids in 1980s and the introduction of herbicides and fungicides
in 1970s-1980s contributed greatly in pest control and agricultural output. The rampant use of these chemicals, under the adage, “if little is good, a lot more will be better” has played havoc with human and other life forms. In India the first report of poisoning due to pesticides was from Kerala in 1958, where over 100 people died after consuming wheat flour contaminated with parathion (Karunakaran, 1958). Certain environmental chemicals including pesticides termed as endocrine disrupters are known to elicit their adverse effects by mimicking or antagonizing natural hormones in the body and it has been postulated that their long-term, low-dose exposure are increasingly linked to human health effects such as immuno suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Crisp et al., 1998; Hurley et al., 1998; Brouwer et al., 1999). If the credits of pesticides include enhanced economic potential in terms of increased production of food and fibre, cod amelioration of vector borne diseases, then their debts have resulted in serious health implications to man and his environment. There is now overwhelming evidence that some of these pesticides do pose potential risk to humans and other life forms and unwanted side effect to the environment (Forget, 1993; Iqbedioh, 1991; Jayaratnam, 1985). No segment of the population is completely protected against exposure to pesticides and the potentially serious health effects, through a disproportionate burden is shouldered by the people of developing countries and by high risk groups in each country (WHO, 1990). The world wide deaths and chronic illnesses due to organophosphate pesticide poisoning number about 1 million per year (Environ News Forum, 1999). The Poison Information Centre in NIOH Ahmedabad reported that OP compounds were responsible for the maximum number of poisoning (73%) among all agricultural pesticides (Dewan et al., 1998). Patients of acute OP poisoning (N=190), muscarinic manifestations such as vomiting (96%) nausea (82%), miosis (64%), excessive salivation (61%) and blurred vision (54%) and CNS manifestation such as giddiness (44%), headache (84%), disturbances in consciousness (44%) were the major presenting symptoms (Agarwal, 1993). Organophosphate insecticides exert their toxicity by inhibition of Acetylcholinesterase (AChE), the enzyme responsible for degradation of the cholinergic neurotransmitter acetyl choline (Ach), neurotransmitter, or nerve-signaling chemical that acts as a signaling chemical both in the brain and elsewhere in the body; The inhibition of cholinesterase results in the accumulation of the cholinergic synapses leading to hyperstimulation of the cholinergic system. Studies on the biocidal effects of OPs are of immense important in the field of toxicology. Environmental pollution from OPs is an important issue that attracts widespread public concern. Owing to the extensive use of organophosphate pesticides in agriculture, there is a high risk of human
exposure to these chemicals. OPs are more toxic and have been responsible for more human deaths than other pesticides. Potential exposures of mammals to organophosphate pesticides and their metabolites are recognized (Frank et al., 1991). Studies have shown altered androgen metabolism (Krause, 1979), the pathological changes in testes and adrenals (Chapin et al., 1979; Usha Rani et al., 1980; Lander and Ronne, 1995; Amena et al., 2007; Pina-Guzman et al., 2006; Narayana et al., 2006; Prashanti et al., 2006). Other mechanisms have been proposed for the toxicity of OPs in animals and humans. Particularly oxidative stress (Akhgari et al., 2003; Ranjbar et al., 2002; Ranjbar et al., 2005; Abdollahi et al., 2004). The risk of acute exposure these compounds is a constant threat and they are responsible for numerous cases of poisoning annually in non-target wildlife and for acute mammalian toxicity and neurotoxicity (Lohan et al., 1986; Fautz and Miftenburger, 1994). Sub-lethal doses of these pesticides lead to alterations in reproductive performance in birds and mammals (Pope, 1999; Samkuli et al., 1987; Ray et al., 1987, 1991; Chapin et al., 1990; Maitra and Sarkar, 1995, 1996). In animals, several physiological and behavioural dysfunctions persist after exposure to high doses of organophosphates (Hall and Clark, 1982; Rattner et al., 1987). Exposure to vertebrate to organophosphate (OP) and carbamates results in the inhibition in the activity of cholinesterase (ChE) enzymes which are fundamental for the normal functioning of the cholinergic system. Sublethal depression of acetylcholinesterase (AChE) activity in the brain has been related to several physiological effects in laboratory rodents, such as changes in nerve conductance (Wison and Cohen, 1953; Greigh-Smith, 1991), respiratory reflexes (Metz, 1958; Nay and Nandi, 1991), and learning capabilities (Banks and Russell, 1967; Reddy et al., 1991). Behavioural consequences of exposure to anti-cholinesterase have also been documented. Many studies on laboratory rodents have indicated that both central and peripheral factors play a role in causing a general behavioural depression in the early phases of intoxication, with specific and/or non-specific alterations of a wide variety of parameters (Bignami et al., 1975). Inhibition of cholinesterase (ChEs) has been used widely as a biomarker to indicate the poisoning of non-target vertebrates with OPs (Greig-Smith, 1991), but there have been few attempts to examine the relationship between the severity of ChE inhibition and alteration in behaviour (Peakall, 1985; Hart, 1993). However, pesticide mediated changes in behaviour may have a significant impact on subsequent survival and indeed it has been suggested that behavioural alterations might provide a tool for predicting the ecological impact of OPs (Grue et al., 1983).
Indeed, interest in the potential adverse reproductive effects of these compounds has heightened in recent years as a result of studies which show that several organophosphorous compounds including dimethoate, methyl parathion, malathion, dichlorovos, chloropyrifos, phosphamidon and dimethyl phosphate, monocrotophos etc. impair fertility suppress libido, deteriorate semen quality, vaginal cyclicity, follicles of ovary, testicular degeneration and biochemical contents in rodents following repeated exposure (Krause and Honola, 1974; Krause, 1977; Haas et al., 1983; Dunnick et al., 1984; Sortur and Kaliwal, 1999; Jadaramkunti and Kaliwal, 1999, 2000; Mahadevaswamy and Kaliwal, 2002; Radhika and Kaliwal, 2002; Asmathbanu and Kaliwal, 1997; Akbarsha and Sivaswamy, 1997; Bhatnagar and Soni, 1990; Shreelakshmi and Kaliwal, 2007; Narayana et al., 2006; Prashanti et al., 2006; Amina et al., 2007). And a number of experimental works revealed that organophorous pesticides effect on male and female reproductive system (Budreau and Sing, 1973; Sciffer, 1975; Gallo and Lawryk, 1991).

It is very much clear from the above literature that pesticides cause deleterious effects, on nervous, endocrine, excretory, circulatory, digestive and reproductive systems. Biochemical indices are sensitive index to the changes due to pesticide toxicity and can constitute important diagnostic tool in toxicological studies (Dabrowska and Wlasow, 1986). The biochemical responses of non target organisms to pesticides can be used to predict early warning of pesticide toxicity. In non-target animals and these responses are quite effective for rapid detection (Pant et al., 1987; Mandal and Lahiri, 1989). Membrane bound enzymes are markers of tissue damage whereas cholinesterase and ATPases are primary targets of organophosphorus compounds.

It is therefore, that the present study was undertaken to elucidate sub-acute oral toxicity of phosphamidon in male and female albino mice with emphasis on biochemical contents, target enzyme interaction, reproduction, steriodogenesis, vital organs, behaviour and histopathology. Thesis of the present study was divided into four chapters.

I. Phosphamidon induced toxicity on estrous cycle, ovarian follicular kinetics and biochemical contents of the ovary and uterus in albino mice.

II. Phosphamidon intoxication on testis, accessory reproductive organs, biochemical contents and histopathology of testis.
III. Effect of phosphamidon exposure on liver and kidney biochemical contents and histopathological changes in albino mice.

IV. Neurobehavioural assessments, brain histology and biochemical changes after exposure to phosphamidon in albino mice.

Each chapter has a separate introduction, materials and methods, observations, discussion and summary with pertinent literatures of earlier works. The relevant literature is cited under references, to avoid disruption in the flow of text. Tables, graphs and figures are placed at the end of each chapters.