CHAPTER 1

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INTRODUCTION

Pesticides in minute quantities are commonly cited as significant contaminants because of their general environmental ubiquity and ease of assimilation in the food. The environmental and toxicological impacts of pesticides are highly dependent not only on the parent compound, but also on their metabolites **(Kulkarni and Mitra, 1990)**. As per the report of Food Monitoring Institute, USA, 75% of today's consumers consider pesticides as serious hazards. The US Environmental Protection Agency (EPA) ranked pesticides in food as one of the nation's most serious health and environmental problems **(Singh and Dhaliwal, 1993)**.

The bulk of pesticides residues enter the human body through dietary products and also through agriculture products like cereals and vegetables. The chemical pesticides like most other invention of modern science constitute a double-edged weapon. The contribution of pesticides to the increased agriculture products and public health has been substantial, but the hazards associated with their injudicious use present a serious problem. In agriculture, it has become a common practice to use combination of pesticides in order to combat insecticide resistance associated with the use of a single class of pesticide. One of the combinations that have become most popular in agriculture is a combination of pyrethroids and organophosphates.

Organophosphates and synthetic pyrethroids are widely used in agriculture to control crop pests and livestock ectoparasites **(Nolan *et al*, 1979)**. Cypermethrin together with profenofos is an examples of wide spectrum pesticide used in a variety of agriculture and non-agriculture application.

The pyrethroids constitute a major class of highly active synthetic insecticides derived from the natural pyrethrins. Since 1970's when the first photostable

pyrethroids were discovered **(Elliot *et al*, 1978)**, numerous new pyrethroids have been synthesized, some of which seem only remotely related to the parent compounds **(Casida, 1980)**. The synthetic pyrethroids are the most extensively used insecticides against a large variety of pests of household **(Blackman and Hudson, 1977)**, veterinary **(Gupta and Bhaumik, 1988)**, agriculture and public health importance **(Hirano, 1989)** due to their excellent insecticidal properties **(Elliot *et al.*, 1978)** rapid decomposition in soil **(Agnihotrudu, 1988)** and low mammalian toxicity **(Bradbury and Coats, 1989)** owing to rapid metabolization and elimination from the body **(Gupta *et al.*, 1988)**. It has been often said, "no pesticide is perfect, but the pyrethroids come close."

Cypermethrin is a synthetic pyrethroid, which was initially synthesized in 1974 and was first marketed in 1977 as a highly active synthetic pyrethroid insecticide, effective against a wide range of pests in agriculture, public health, and animal husbandry. Its structure is based on pyrethrum, a natural botanical from chrysanthemum flowers, but it has a higher biological activity and is more stable than its natural analogue. It has a low oral toxicity to mammals, and in general, its insect (topical) to mammals (oral) toxicity ratio is much higher than that of the other major classes of insecticides **(Elliot *et al.*, 1978)**. The favorable properties have promoted the wide spread application of cypermethrin in virtually all sectors of insect control. Although severe acute human poisoning with pyrethroids seems unlikely, recent experience in the People's Republic of China shows that these insecticides should certainly not be considered harmless **(Henk *et al.*, 1990)**.

Synthetic pyrethroids control a wide range of insects in a wide range of crops. Unfortunately, this broad-spectrum nature can adversely affect many non-target beneficials whereby disrupting, in particular, integrated pest management (IPM) programs.

In 1988, pyrethroids amounted to 40% of the sales for insecticides for cotton treatment in the world. Cypermethrin is one of the most important insecticides for cereals and vegetables in the UK. There has been a dramatic increase in the use of cypermethrin for crops in the UK: from approximately 216,000ha in 1988 to

1,582,000ha sprayed in 1992, falling back to 863,000ha in 1994. It is also used for impregnation of mosquito bed nets to prevent malaria, and also used extensively for indoor pests (**Bhalla *et al*, 1994**).

World Health Organization (WHO) has classified cypermethrin as 'moderately hazardous' (Class-II). It interacts with the sodium channels in nerve cells making them open for up to seconds, compared to the normal period of a few milliseconds, after a signal has been transmitted. Cypermethrin also interferes with other receptors in the nervous system.

The effect is that of long-lasting trains of repetitive impulses in sense organs. Symptoms of poisoning include abnormal facial sensations, dizziness, headache, nausea, anorexia and fatigue, vomiting and increased stomach secretion. Cypermethrin is also a skin and eye irritant. Normally, symptoms should disappear after some days but severely exposed patients additionally may suffer from muscular twitching, coma and convulsive attacks.

In such cases, symptoms may persist for some weeks. Most cases of pyrethroid poisoning have been reported in China (nearly 600 between 1983 and 1988, of which 45 involved cypermethrin). They occur among farmers, mostly after misuse. Recently, poisonings have as well been reported after indoor use of pyrethroids in Germany among pest controllers and private users.

Chronic symptoms after exposure to pyrethroids have now been reported (**WHO, 1990**). Symptoms include brain and locomotor disorders, polyneuropathy and immune-suppression, and resemble the multiple chemical sensitivity syndromes (MCS).

Reproductive toxicity

When administered to pregnant and nursing rats, cypermethrin may lead to a functional delay in the brain maturation of the pups. The toxicity to young rats is higher since the pathway for degrading cypermethrin is not readily developed in young rats. Synthetic pyrethroid (phenothrin) induced gynecomastia was reported amongst Haitian refugees (Casida, 1980). Experimental evidence suggests the

pyrethroid molecule may bind to sex hormone binding globulin (SHBG) *in vitro*. Chronic exposure to pyrethroids may result in disturbances in the androgen action. Pyrethrins and in particular bioallethrin interact strongly with SHBG at concentrations of 40mg/kg **(Abd El-Raheeml *et al.*, 1987)**.

Environmental effects

The pyrethroids are widely used because of their general low toxicity to birds and mammals. However, they are highly toxic to aquatic organisms and fish as well as to bees - with the same mode of action in each organism. The LC_{50} values for small fish and other aquatic organisms typically lie below 1µg/l, and the LD_{50} value for bees is 0.03 - 0.12µg/kg. For use with conventional hydraulic sprayers, buffer zones of 16-24m are needed to reduce mortality of butterflies in the surroundings.

Although the direct acute toxicity towards birds is rare, they are affected via the food chain. Other beneficial organisms that can be affected by cypermethrin include ground dwelling beetles, spiders and centipedes, and predatory mites. Populations are reduced to 20% in some experiments, but recover after some weeks.

Despite earlier findings, the microbial population of soil is affected by cypermethrin: the ammonification and nitrification in treated soils is enhanced, a sign of the environmental impact of cypermethrin **(Bhunya and Pati, 1988).**

Once applied, cypermethrin is bound strongly by soil components and is therefore not likely to enter ground water. Cypermethrin is not persistent in soil and quickly degrades to less toxic products (with a half life of 2 to 4 weeks). In contrast, cypermethrin persists in treated wood for up to seven months.

Residues

After indoor use, cypermethrin residues may be found in dust and carpets with a concentration up to 4 mg/kg. The concentration in the air after an indoor treatment increases rapidly, but can then stay relatively constant for months at values for which pyrethroids can cause adverse health effects (3-8 $\mu g/m^3$).

New studies show that the health effects of cypermethrin and pyrethroids in general may be more severe than previous toxicological evaluations suggest. Further toxicological studies on neutotoxictiy, sub chronic toxicity and developmental toxicity are required.

Profenofos

Currently large number of organophosphorus (OP) compounds are being extensively used for insect and pest control. Profenofos is an organo-thiophosphate insecticide that is mainly applied to control cotton bollworm and tobacco budworm. Also profenofos is being extensively used to protect vegetables, fruits, rice and other food crops from insects such as fruit borer, leafhopper, aphid, yellow stem borer etc.

Salim *et al.*, (1988) studied the effect of organophosphorus pesticides on semen characteristics in rabbit and demonstrated deleterious effect on sperm formation together with the decline in testosterone secretion by the pesticides treatment. **Kimbrough and Gaines (1968)** reported embryotoxicity and increased resorptions in pregnant rats given a single high dose of organophosphorus pesticides on 11^{th} day of gestation. However, these effects were associated with maternal toxicity. Similarly, trichlorofon produced defects in offsprings and cholinergic symptoms at high dose level when administered during 6-15 day of gestation **(Staples and Goulding, 1979)**.

Profenofos is a broad spectrum organophosphate insecticide and acaricide. Its mode of action is by inhibiting acetlycholinesterase.

Absorption, Distribution and Excretion

Ifflaender *et al.*, (1974) dosed four male and three female rats a single dose of ring labeled-^{14}C profenofos at 4.8 mg/kg b. wt. Within 6 days essentially the entire radioactive dose was eliminated in the urine (81.8 % in males and 96.4% in females) and faeces (15.7% in males and 2.5 % in females). Most of the urinary and faecal excretions occurred within the first 24 hours of dosing. When the animals were sacrificed 6 days after dosing, residual radioactivity was found only in the liver (0.013ppm in males and 0.023ppm in females) and kidney (0.007ppm in males and 0.008ppm in females), while radioactivity in other tissues (fat, muscle, testis, ovary, brain) was below the limit of detection.

Oakes *et al.*, (1986) observed notable amount of radioactivity only in kidney of Leghorn hens given oral dose of ring-labeled -^{14}C profenofos for 14 consecutive days at dose rate equivalent to 5 mg/kg in the feed, whereas, negligible levels were found in liver, blood, muscle, skin, and fat.

Biotransformation

Ifflaender *et al.*, (1974) studied the metabolism of ring labeled -^{14}C Profenofos over 24 hour period in the urine of RAI rats given a single oral dose of approximately 4.8 mg/kg b.wt. Analysis of urine via TLC in rats of both sexes indicated complete degradation of Profenofos, as no unchanged parent compound was present.

Effect on Enzymes and other Biochemical Parameters

Wing *et al.*, (1983) observed that profenofos is stereo specifically converted to a more potent inhibitor of acetylcholinesterase by mouse liver microsomal mixed-function oxidase system.

Acute Toxicity

Profenofos has a moderate order of acute toxicity following oral and dermal administration. **Kobel and Gfeller (1983)** reported oral LD_{50} values of 358-502 mg/kg in rats. It has been classified as moderately hazardous by World Health Organization **(WHO, 1990)**. The adverse signs that were observed were generally similar for each route of compound administration. These included non cholinergic symptoms such as dyspnoea, exophthalmos, ruffled fur and crooked body posture, and cholinergic symptoms such as sedation, salivation, discharge from eyes and nose, tremors, and tonic clonic convulsions. The symptoms were reversible in surviving animals.

Justification of Experimental Design

The literature survey of pesticides revealed that they are harmful to man and environment **(FAO and WHO working groups, 2000; Kartikeyan *et al.*, 2001)**, but the data available are only of the technical grade products or their formulations **(Richardson and Chambers, 2002)**. The combination of two pesticides may have additive, synergistic, potentiative or antagonistic effect **(Ballantyne *et al.*, 1995)**. Considerable body of literature has been found on toxicity related studies of

organophosphorous and synthetic pyrethroid insecticides independently but very limited information is available on the toxic manifestations of combination of these classes of insecticides. Particularly, there is a paucity of data regarding neurotoxicity and postnatal development aspects in rats. Hence, the present investigation has been taken up to determine the toxic effects of combination insecticide (Profenofos 40% + Cypermethrin 4% EC) on nervous system, general health, reproduction and post natal development in rats when exposed over a period of time.

Oral route is the most common route of exposure encountered in toxicological literature. It is a route by which a large number of substances gain entry into the animal's body. Many pharmaceutical preparations are also designed to administer orally. Environmental contaminants, which enter drinking water supplies, will be inadvertently ingested.

It is virtually impossible to avoid ingestion of traces of chemical residues used on field crops, in animal husbandry and food processing or food packaging.

It should be remembered that one of the precepts of toxicology is that, whenever technically feasible, test article should be studied by the oral route of administration. This is appropriate to the problem under investigation. Particularly in the field of risk assessment, where route-to-route extrapolations add to uncertainty already inherent in the imprecise approximations and assumptions used to obtain the risk estimates. Hence, the oral route of exposure is preferred. An attempt has been made to explore the possibilities of adverse effects caused by insecticide combination cypermethrin and profenofos on rat.

In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. It provides information on health hazards likely to arise from a short-term exposure by the oral route. Data from an acute study may serve as a basis for classification and labeling. It is an initial step in establishing a dosage regimen in sub chronic and other long term studies and may provide initial information on the mode of toxic action of a substance. Acute toxicity studies will thus identify highly toxic chemicals and provide information on the possible hazards, which could occur where humans are, exposed **(OECD, 1996).**

In the present investigation, an acute oral LD_{50} study was conducted in order to determine LD_{50} value and also to establish possible toxicity by single dose.

Followed by acute oral toxicity study, an acute (single dose) neurotoxicity screening battery tests were conducted to evaluate the neurotoxic propensity of the test article. The test substance was administered to several groups of experimental animals, one dose being used per group. The animals were observed under carefully standardized conditions with sufficient frequency to ensure the detection and quantification of behavioral and/or neurologic abnormalities, if present. Various functions that could be affected by neurotoxicants were assessed during each observation period. In addition, serum cholinesterase (ChE) was estimated after 24 hours of dosing. Measurements of motor activity of individual animals are made in an automated device. The animals were perfused and tissue samples from the nervous system were prepared for microscopic examination.

After obtaining initial information regarding neurotoxic potentiality of the test article following single oral dose, a repeated dose 90-Days oral neurotoxicity study of combination insecticide in rats was performed. Animals of 5-6 week old were treated at 3 graded dose concentrations for a period of 90 consecutive days. During this period, animals were observed for clinical/neurological signs, body weight and food consumptions were monitored weekly and at the end of this period, animals were subjected to functional observational battery tests, motor activity and complete clinical pathology estimations were performed to obtain information regarding effect of the test article on general health of animals.

Following 90 days of treatment, same males and females were allowed to cohabitate at 1:2 ratios. Treatment was continued through mating, gestation, lactation and weaning of pups (F1). The present study was designed to evaluate the:

- Reproductive toxicity induced by the combination insecticide
- Effect on organ weight
- Effect on pathological changes on reproductive and other organs
- Effect on reproductive parameters such as:

- Male fertility index
- Female fertility index
- Parturition index
- Gestation index
- Live birth index
- Pups survival index
- Litter weight and litter size