Pyrethroids are the widely used insecticides due to their potential insecticidal activity in India and other countries to get protection against mosquitoes and other insects for various domestic and agricultural purposes\textsuperscript{1,2}. Over the half of world population have been using pyrethroid insecticides which may account for more than 25% of the insecticide market of the industrial countries in 90's and their demand/use is increasing now in these countries\textsuperscript{3,4} as prevalence of mosquitoes and other insects are more in many endemic parts of the world.

Initially, these pyrethroids were thought to be highly toxic to insects and less toxic to humans\textsuperscript{5}. Now pyrethroid induced neurotoxicity and other toxic effects ranging from whole body tremors to convulsions and death are well documented\textsuperscript{4,6-9}. Surprisingly, limited literature is available so far on the effects of pyrethroids on humans, and now slowly the facts related to their toxicity are coming into light. The fatality with pyrethroids in India has been reported to be 12.5 to 25%\textsuperscript{10}. Allethrin and prallethrin are among the most widely used pyrethroid insecticides. Allethrin poisoning can be much frequent due to its easy accessibility as mosquito repellent and/or insecticidal sprays etc\textsuperscript{11} and often reports of pyrethroid poisoning in India are evident\textsuperscript{12,13}. However, it is clear that no relevant data on chronic effects exist in open scientific literature related to pyrethroid toxicity in humans and animals\textsuperscript{10,14}. Since these pyrethroids are used routinely and/or regularly as mosquito repellents and/or through agricultural/gardening sprays exposing people continuously to the inhalation of these compounds for longer durations, their inevitable chronic use aroused a concern among public now, which formed the basis for the design of the present study.
Chemistry and metabolism/biotransformation of pyrethroids

Pyrethroid insecticides were first isolated from an extract obtained from flowers of a plant *Chrysanthemum cinerariaefolium* as pyrethrum and all esters extracted from the same exerted insecticidal action and are toxic to insects but relatively safe to mammals and birds\(^1\), \(^15\), \(^16\). The extracts contain pyrethrin I and pyrethric esters. Later pyrethroids, the synthetic analogs of pyrethrin are discovered and these pyrethroids are esters\(^17\), \(^18\). These pyrethroids are ester compounds formed from an acid and alcohol moieties. (Fig.1), which fall into two distinct categories viz. type I and type II based on their structure and toxic symptoms in experimental animals.

**Fig 1. Acid moiety and Alcohol moiety of pyrethroids**

![Chemical structure of pyrethroids](image)

Type I such as allethrin and prallethrin are devoid of \(\alpha\)-cyano group and the type II compounds contained \(\alpha\) - cyano constituent. Example: Cypermethrin, Deltamethrin.

Type I pyrethroid toxicity leads to a common T-syndrome and type II compounds cause CNC syndrome. Several pyrethroids have isomeric forms which show different
toxicities and insecticidal potencies. The cis-isomers are more toxic than trans isomers often.

Fig 2. Chemical structures of pyrethroids with and without an α-cyano groups.

Permethrin

Cypermethrin

deltamethrin

Fenvalerate
Chemical structures

Fig. 3 Chemical structures of pyrethroids used in the present study (Allethrin and prallethrin).

Allethrin — $C_{19}H_{26}O_3$

Prallethrin — $C_{19}H_{24}O_3$

The biological activity characteristics of pyrethroids responsible for their use for household insecticidal and for agricultural purposes are furnished below:

1. Quick knock-down effect against insects,
2. Low mammalian toxicity;
3. Efficacy against insects with organophosphorus and/or carbamate-resistant strains and
4. Easy decomposition in the environment;

Biological membranes are the primary targets for the action of pyrethroids. They produce membrane depolarization due to the prolonged opening of sodium channels ultimately cause block of nerve impulse transmission causing death of the animal (insects and other animals). Type II pyrethroids are generally more potent than type I pyrethroids. However type I pyrethroid are the most commonly used compounds due to their high insecticidal and low toxicity to mammals. All these compounds exert inhibitory action on nicotinic receptors, GABA gated chloride channels and Ca\(^{2+}\)-Mg\(^{2+}\) ATPases. Pyrethroids are lipophilic compounds and probably partition into the hydrophobic environment of lipids by binding to the hydrophobic domains of membrane proteins and perturb the bilayer and there by causing characteristic features.

D-allethrin and prallethrin (type -I pyrethroids) are the commonly used pyrethroid mosquito repellents and also as agricultural insecticidal sprays in India and all over the world. Now-a-days many useful compounds with varied insecticidal activities are derived from the structural modification of natural pyrethrin I and Fig 3 shows various synthetic pyrethroids invented by retaining chrysanthimic acid (pyrethrin) as the acid moiety and modifying the alcohol moiety. By modifying acid moiety and/or ester linkage also a variety of synthetic compounds are produced now. However, the information presented in the study is confined to type I pyrethroids viz., allethrin and prallethrin.
Inhalation is the main route of exposure for humans, as pyrethroid (Allethrin or prallethrin) based mosquito repellents, (mats, coils, liquid vaporizers)/insecticidal sprays are used for 8 to 12 hours/day by exposing all humans including newly born babies and infants. These inhaled pyrethroids enter the circulation exposing plasma, blood cells (RBC, WBC, platelets) and other tissues to the continued presence of pyrethroids or their derivatives. Lipophilic nature of these compounds and their interaction with some specific and non-specific components of membrane, and also the physico-chemical properties of biomembranes are responsible for the effects of pyrethroid toxicity.

And now it is clear that these compounds stimulate insect nerves to discharge repetitive impulses and to block nerve conduction and these actions were thought to be the major mechanisms (by their interaction with voltage gated sodium channels) responsible for insecticidal action. Besides, IP3 breakdown signaling also seems to be playing a role. Pyrethroids get degraded and metabolized rapidly in various living organisms. Many synthetic pyrethroids have an ester group which is an important moiety of the structure of the molecule, and in mammals in vivo the primary detoxification step is ester cleavage possibly by non specific carboxylases (carboxyl esterases), followed by hydroxylation reactions in cytochrome p450 system by several conjugation reactions. The low toxicity in mammals suggests that the metabolites are not significantly toxic. Only lipophilic unmetabolized pyrethroids are active in exerting their effects on neuronal and other membranes. However now there is enough evidence that pyrethroid compounds may not be safe as claimed previously. Since, the rate of elimination determines the toxicity of xenobiotics, the inhibition of
hydrolytic activities produces a significant increase in the toxicity\textsuperscript{40}. Indeed, it has been described that synergist compounds, through inhibition of CbES (Carboxyl esterases) enhance the toxicity of pyrethroids\textsuperscript{41}. Similarly, certain synergists, such as PBO (Pypronyl butaoxide) which are used as constituents of commercial pyrethroids and their products increase the toxicity by enhancing the potency of pyrethroid from 10-300 fold range by inhibition of oxidative degradation of pyrethroid in insects\textsuperscript{42,43}. The ambient temperature has a profound effect on insecticidal activity of pyrethroids and an effect that is augmented by lowering temperature due to the slow metabolism of pyrethroids in insects at low temperature\textsuperscript{44,47}.

Ramesh and Vijayalakshmi\textsuperscript{48} have reported maximal accumulated concentrations of the pyrethroids allethrin, transfluthrin, deltamethrin, esobiothrin and prallethrin in air circulation in the room when these pyrethroid based repellents were used. Leng \textit{et al.}\textsuperscript{49} performed blood and urine analysis for determining the levels of pyrethroids and reported the presence of low levels of these pyrethroids in normal subjects and also revealed by storage experiments which demonstrated that no significant loss of pyrethroids with in a year from frozen samples. Studies of Leng \textit{et al.}\textsuperscript{50} on extensive hydrolytic and oxidative degeneration of pyrethroids bioallethrin and related compounds showed a peak urinary excretion of Chrysanthemum dicarboxylic acid (CDCA) occurred within 24 hours of exposure. Pankaj and Prahlad\textsuperscript{10} reported that acute allethrin poisoning led to deaths where convulsions increase in duration and frequency and do not stop with in 2 to 3 weeks. Synthetic pyrethroid adsorption to sediment could render them (SP) unavailable to the degraders, and there by prolonging their persistence in aquatic system. Though the metabolism of allethrin
and prallethrin is not completely known based on earlier work it is understood that allethrin and prallethrin could have been metabolized by any of the following 5 transformation pathways.

1. Hydrolysis to allethrolone and a smaller extent CDCA.
2. Formation of 2,3 diol from allyl moiety
3. A hydroxylation of methylene position of allyl grouping
4. Hydroxylation at one of the geminal dimethyl groups
5. Oxidation at trans methyl group of isobutonyl moiety to carboxylic acid.

Along with parent compounds (allethrin & prallethrin), their degradatory products may accumulate in lipid environment and these degradatory products are more toxic than the parent compounds often. Important information concerning the effects of pyrethroids can be either acute and/or chronic.

**Short term or acute toxic effects**

Mode of administration, absorption and detoxification rates determine the toxicity in humans and experimental animals. Inhalation is the chief and also an effective route of exposure to these compounds in humans. The acute toxicity seems to be based on the allergenic properties of pyrethroids. Human exposure to pyrethroids causes contact dermatitis (from local erythroma to serve vasicular eruption), asthma like attacks, the anaphylactic reactions. The dermal absorption of pyrethrin is slow and usually prevents systemic poisoning. Although a significant reservoir of pyrethrin may remain bound to epidermis. Dermal absorption and poisoning can be avoided by decontamination of the skin.
Immunosuppressive effects of pyrethroids (type I and type II) are reported by earlier workers\textsuperscript{56-58}. Pyrethroids which are known to induce cutaneousparethesia, which is characterized by impairment of facial sensation and dizziness combined with a burning, itching or tingling sensation of the exposed skin. Studies of Diel \textit{et al.}\textsuperscript{59} demonstrated the immunotoxicological properties of the synthetic pyrethroid \textit{s-bioallethrin} suggesting bioallethrin induced histamine release from human basophiles and inhibition of lymphocyte proliferation and some other immunoallergotoxicological properties in human lymphocytes and basophiles. Liu \textit{et al.}\textsuperscript{60} have observed smoke and emissions containing formaldehyde polycyclic aromatic carbon aldehydes and ketones from one burning mosquito-coil, and was comparable to the pollutants released from 51 cigarettes.

Animals exposed to inhalation of pyrethroid tetramethrin exhibited changes in their physiological activities. Besides liver injury and other tissue pathological changes were noticed by many researchers\textsuperscript{35, 61}. On the contrary there are limited reports stating no acute toxic effects in experimental animals and humans when pyrethroids are administered in low doses\textsuperscript{10}. The possibility of chronic toxicity cannot be ruled out due to their routine and frequent use for longer durations with prolonged periods as mosquito repellents. However no relevant data on chronic effects of allethrin, prallethrin and other pyrethroids is available in literature now. Though some experiments were conducted on chronic toxicity using animals, those were also designed at scientists' discretion and the results obtained were also ambiguous.

The results of certain short term studies on experimental animals suggest that allethrins are weakly to moderately toxic when exposed to the pyrethroids in
concentrations. When used continuously for periods the toxic effects may be severe and were also not studied. Allethrin induces mild primary eye and skin irritation in rabbits. Allethrin induced neurotoxic responses are well documented and the symptoms of acute poisoning follow the typical pattern\textsuperscript{62}.

1. excitation
2. paralysis
3. convulsions
4. death

Allethrin and prallethrin produce neurotoxic responses by acting on brain and spinal levels and there by evoking allethrin induced tremor. Pyrethroid induced delay in closure of sodium channel resulting in a tail current that is characterized by a slow influx of sodium during the end of depolarization ultimately leading to excitation and followed by other symptoms.

**Chronic studies**

Surprisingly, no data on the effects of chronic use of type I pyrethroids such as allethrin and prallethrin are available on humans. As world population get exposed to these compounds for long time with routine and frequent use to get protection from mosquitoes and other insects as mosquito repellents and/or as agricultural/gardening sprays chronic effects of pyrethroids, chiefly of allethrin and prallethrin received much attention. Complications frequently observed after more than two years have been cerebro organic disorders (personality disorders), Visual disturbance, tinnitus, S-enbo motor polyneuropathy in the lower legs, and vegetative nervous disorder (peroxisomal, tachycardic increased heat sensitivity). Many of these patients exhibit pathological auto immune findings and develop auto immune diseases. Example: Autoimmune haemolysis in experimental animals.
Chronic use of prallethrin in diet at different concentration 120, 600 or 3000ppm in diet for one year induced significant changes in haemoglobin concentration, haematocrit value, MCH, MCV and alterations in blood biochemistry (blood cholesterol, phospholipids, albumin, creatinine, urea, bilirubin at different time intervals. 13, 26 and 52 weeks with a decrease in body weight gain, food consumption and water intake. Liu et al. showed biochemical and cellular changes in bronchoalveolar lavaged samples from rats after inhalation of smoke of allethrin based mosquito coils for 6 months. Such observations were noticed by many earlier researchers. Jeffrey and Jeffrey reported that pyrethroid class of insecticides can modulate the dopaminergic nervous system at low doses in persistent manner which may render nerves more vulnerable to toxicant injury.

**Pyrethroids Vs membranes**

It has been generally accepted that pyrethroid membrane interactions are chiefly responsible for the toxicity. Pyrethroid interactions induce changes in physico-chemical properties of biomembranes and membrane components (lipids, phospholipids, cholesterol and proteins-integral and peripheral). The fluidity of membrane has been shown to depend on cholesterol content and the orientation of phospholipid molecules and their composition of fatty acyl chains in the membrane. Pyrethroid insecticide affects the bilayer lipid order and lipid packing. The lipophilicity of pyrethroids facilitates their rapid access to tissues including nervous system and their incorporation into cell membranes of the nervous tissues.

ATP hydrolyzing enzymes are sensitive to the action of pyrethroids and possible disturbances in Na⁺-K⁺ ATPase activity and changes in the membrane fluidity have
been found to be mediating neurotoxicity\textsuperscript{64,65}. Besides a receptor mediated messenger pathway of an insecticide breakdown of IP\textsubscript{3} mechanism may also play role in the causation of toxicity. Wang \textit{et al.}\textsuperscript{66} reported that the pyrethroid induced neurotoxicity may involve at least, in part, in impairment of the physiological action of T\textsubscript{3} at its sub cellular targets. Hossain\textsuperscript{67} reported that allethrin had an interesting dual effect on Ach (Acetyl cholinesterase) release, increasing Ach efflux at lower dose (20mg/kg, ip) with a peak time of 60 min and decreasing the efflux after higher dose (60mg/kg) upto 3 hours after administration and suggested that pyrethriods modulate Ach release in the hippocampus of rat brain. Studies of Sinha \textit{et al.}\textsuperscript{33} revealed that mosquito repellents (allethrin 3.6\%, 8 h for day through inhalation) exert adverse effects on infants leading to CNS abnormalities, if a mechanism operates in humans similar to that in rat pups. Righi and Palermo\textsuperscript{68} reported some signs and symptoms of synthetic pyrethroid intoxication and stated that pyrethroid induced anxiety like symptoms with dose related effect. Wu \textit{et al.}\textsuperscript{69} reported that type I and type II pyrethroids inhibit the uptake of glutamate that might play vital role in neurotoxicity induced by pyrethroid insecticides. Surprisingly Tsuji \textit{et al.}\textsuperscript{33} reported that d-allethrin by inhalation did not induce effects in neonates. Imamura \textit{et al.}\textsuperscript{70} have reported that lactational exposure to pyrethroid might affect postnatal development of mammalian brain. Vais\textsuperscript{71} has shown that pyrethroid resistance mutations cause differences in pyrethroid sensitivity of replacing isoleucine and methionine of sodium channel. Vais\textsuperscript{72} has proved that a single amino acid change (the substitution of isolation) for methionine makes a rat neuronal sodium channel highly sensitive to pyrethroid insecticides. Go \textit{et al.}\textsuperscript{73} have demonstrated that pyrethroid compounds disrupt endocrine functions and also reported the estrogenic
potential of several synthetic pyrethroid compounds *in vitro*, which influenced several sub-cellular pathways. Sinha *et al.*\(^{33}\) have reported that pyrethroid based mosquito repellents induced dysfunction of blood brain barrier (BBB) permeability with the developing brain. Gupta *et al.*\(^{74}\) have reported that allethrin based mosquito repellent inhalation induced biochemical changes in BBB permeability and oxidative damage in selective organs in developing rats. Michael *et al.*\(^{75}\) has shown that calcium channels are another primary targets for allethrin and the sodium channels being the other target for pyrethroid poisoning suggesting some chronic effects by blockade of different types of Ca\(^{+}\) channels. Nasuti *et al.*\(^{76}\) has reported a pyrethroid-induced alteration in membrane fluidity and different changes induced by pyrethroids on lipidperoxidation, osmotic fragility on antioxidant enzymes. Irma *et al.*\(^{7}\) have supported the idea that membrane ATPases are the chief targets of neurotoxic effects of pyrethroids. Moyaquiles *et al.*\(^{19}\) reported that allethrin influences membrane lipid order in the bilayer and alters the properties of membranes. Cremer and Seville\(^{77}\) have reported changes in rate of general cerebral blood flow and glucose metabolism associated with pyrethroid toxicity in rats. Cremer *et al.*\(^{78}\) have made some relationship between the rat of glucose metabolism and the transport of the glucose between plasma, brain, cerebral blood flow and blood content in cismethrin administrated rats. They also compared the conscious control in rats with intense tremors induced with cismethrin. Dorman and Beasley\(^{8}\) showed that pyrethroid insecticides are toxic and produce a syndrome characterized by tremors prostration and altered startle flexes.
References


