Chapter-I

INTRODUCTION
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The first group of synthetic organic pesticides used on large scale was organochlorines. It was well established that the benefits of these compounds conferred on mankind during early years of use in fields of agriculture and public health management could not be ignored. However during 1950’s and 1960’s, reports of large quantities of residues of these long persistent pesticides were discovered. In addition, many pests have become resistant to these compounds. The high level of environmental awareness was dawn in the western countries. Once the environmental problems were unmasked, environmental laws were enacted, regulatory agencies (like EPA in USA and others elsewhere) were created and recalcitrant pesticides were banned in the western countries. Going the brink, the western world pulled itself back from disaster just in time.

The situation in the developing countries like India, however, has been vastly different. India did not wake up fully to the use of synthetic organic pesticides till 1971. When green revolution was ushered in, for better prospects, the increased use of pesticides and increased input of agricultural fertilizers, besides using better farm techniques were initiated. Compelling economic reasons also necessitated continuation of the use of some of the environmentally problematic compounds. Not only persistent compounds like DDT and HCH are continued to be used in India but also the total quantity of consumption of these compounds has steadily increased hand in hand with the spread of green revolution in our country. It is ironical that the year in which many of environmentally problematic compounds had been banned in the industrialized nations, i.e. 1971 also happens to be the year that heralded increased use of these compounds in our country.

In India, pesticide manufacture was started in 1952. The consumption of pesticides has increased from 150 metric tones to 80,000 metric tones, since 1953 to 1983. The problems that India has been facing can be gathered from the fact that the
population increased from 361 million in 1951 to over 860 million in 1991. The Indian pesticide industry with 82000 MT of production for 2005-06 is ranked second in Asia (behind China) and twelfth globally. In value terms, the size of the Indian pesticide industry was estimated at Rs 68 bn for 2006, including exports of Rs.28bn.

**Agrochemicals In 2009 (Metric Tones)**
- Agrochemicals & Pesticides: 19,687
- Fungicides: 2,946
- Herbicides: 4,975
- Insecticides: 8,152
- Other Agrochemicals & Pesticides: 1,451
- Plant Growth Regulators: 2,163

In the initial stages, food production did not keep pace with population growth. Since the dawn of “green revolution” in India, food production has gone up considerably so that from an initial grain yield of 59 metric tones from a cropland area of 9.73 lakh acres, the food production has gone up to 176 million metric tones from 12.85 lakh hectares in 1991. In other words, a mere 31.7% increase in arable land between 1951 and 1991 had to register a 245% increase in grain yield to take care of the needs of a 137% increase in the population during the same period. This by any count is a stupendous achievement and it could only be achieved by liberal use of pesticides. It is not without the attendant environmental problems that self sufficiency on the food front was achieved, but it is not as though Indians are not aware of the environmental hazards of the use of recalcitrant pesticides.

The undue persistence, high mammalian toxicity and developing resistance of the organo chlorine, organophosphate and carbamate insecticides led to a ban or restriction on their use in many developed and developing countries. Thus, attention was focused on less persistent, low mammalian toxicity compounds like pyrethrins.
According to Science April 29, 2010, over 47,677 species may soon disappear. 12% of birds, 21% of mammals, 30% of amphibians, 27% of coral reefs, 35% of conifers and cycads face extinction. Due to depredations approximately the extinction rate is 40,000 times higher than background rate [The Hindu dt: 27.05.2010 (Thursday)]

India is one of the worlds 12th mega biodiversity centres and the subcontinent is one of the sixth vavilovian centres of origin of species. Some 45,000 Plant species and over 89,000 species of animals have been documented. 2,500 fishes 150 amphibians 450 reptiles 1,200 birds 850 mammals and 68,000 insects are in the faunal diversity paradieses of valuable genes but are in changing towards the paradise lost. It is the human greed with habitat degradation causing the damage the biodiversity which is main natural resources for the foe.

The pyrethroids constitute another group of insecticides in addition to organo chlorine, organo phosphate, carbamate and other new generation compounds. Commercially available pyrethroids to date include allethrin, resmethrin, d-phenothrin, and tetramethrin (for insects of public health importance), and cypermethrin, deltamethrin, fenvalerate, and permethrin (mainly for agricultural insects). Other pyrethroids are also available including furamethrin, kadethrin, and tellolethrin (usually for household insects), fenpropathrin, telomethrin, cyhalothrin, lambda-cyhalothrin, tefluthrin, cyfuthrin, flucythrin, fluvalinate, and bifenate (for agricultural insects).

Chemically, synthetic pyrethroids are esters of specific acids (e.g., chrysanthemum acid, Halo-substituted chrysanthemum acid, 2-(4-chlorophenyl) -3-methyl butyric acid) and alcohols (e.g., allethrolone, 3-phenoxybenzyl alcohol). For certain pyrethroids, the asymmetric centre exist in the acid and/or alcohol moiety and the commercial products sometimes consists of a mixture of both optical (1R/1S or d/l) and geometric (cis/trans) isomers. However, most of the insecticidal activity of
such products may reside in only one or two isomers. Some of the products (e.g., d-phenothrin, deltamethrin) consist only of such active isomer(s).

Synthetic pyrethroids are neuropoisons acting on the axons in the peripheral and central nervous systems by interacting with sodium channels in mammals and or insects. A single dose produces toxic signs in mammals, such as tremors, hyper excitability, salivation, choreoathetosis and paralysis. The signs disappear fairly rapidly, and the animals recover, generally within a week. At near-lethal dose levels, synthetic pyrethroids cause transient changes in the nervous system, such as axonal swelling or breaks and myelin degeneration in sciatic nerves. They are not considered to cause delayed neurotoxicity of the kind induced by some organo phosphate compounds. Cyhalothrin and lambda-cyhalothrin are rapidly hydrolyzed under alkaline conditions but not in neutral or acidic media.

Pyrethroids constitute a new generation of highly active synthetic pesticides. They are derived from a group of insecticidal esters; the pyrethrins. These are extracted from the flower heads of certain Chrysanthemum species. Many plants contain toxic compounds some of which are selectively poisonous to insects; and have proved to be valuable insecticides. The early pyrethroids are rapidly degradable in the environment. After the replacement of photosensitive group in the compounds, a great range of stable insecticides has been developed. These are more effective against a broad spectrum of economically important pest than the most potent organo chlorine, organophosphate and carbamate pesticides. Further modification of the chemical structure of pyrethroids by the incorporation of a cyano group has led to the development of the most effective insecticides. The high insecticidal potency is combined with a low oral toxicity to mammal and the non-target species excluding aquatic organisms, if they are in limited quantities. These don’t accumulate in the ecosphere. These favorable toxicological and ecological properties instigate the wide spread application of pyrethroids for the control of disease vectors, ectoparasites and pests infecting important agricultural crops. Around 1980 photo stable pyrethroids had already gained 30% of the world market of insecticides (Naumann, 1981).
**Types of Pyrethroids:**
Pyrethroids are of two types.
1. Natural pyrethrins
2. Synthetic pyrethroids

1) **Natural pyrethrins**

They are also called ‘pyrethrum’ or ‘pyrethrum extract’. These were extracted from the flower heads of Chrysanthemum. The pyrethrums contain insecticidally active components. Pyrethrins I and pyrethrins II (Head, 1973; Elliot and Jones, 1978), these are widely used for domestic purposes for the protection of stored food, but for general pest control in agricultural, they are applied in limited scale only. Because of their highly degradability, they are applied in combination with metabolic synergists.

2) **Synthetic pyrethroids**

Pyrethroids intoxication to insects include hyper-excitability, tremors and convulsions, is later followed by paralysis and eventually to death. Yamamoto (1919) and Yamamoto and Sumi (1923) were the eminent pioneers of pyrethrum chemistry and recognized many features necessary for insecticidal activity. Knowledge about the natural pyrethrum and its efficient insecticidal action stimulated research for effective synthesis of pyrethroid insecticide (Metcalf, 1955; Elliot et al., 1978). The first synthetic pyrethroid to be synthesized was Allethrin (Schechter et al., 1949). Though it has strong insecticidal properties, it is photo-labile and less effective than the pyrethrins against many insects (Wouters and Van den Breken, 1978). The synthetic pyrethroid with potent activity like Resmethrin Cismethrin (Lhoste et al., 1971), (Elliott et al., 1973), Phenothrin (Fujimoto et al., 1973), Permethrin (Elliott et al., 1973), Deltamethrin and Decamethrin (Elliott et al., 1974), Cypermethrin (Elliott et al., 1975), Fenvalerate (Ohno et al., 1974) and Fenpropathrin (Matsuo et al., 1976) have been introduced subsequently. Today pyrethroids comprise one of the most powerful insecticidal groups widely used against insects (Elliott, 1977).
Lambda-cyhalothrin

Lambda-cyhalothrin is a synthetic pyrethroid, of the alpha-cyano group, with a core (-CCOOCHCN-), as in alpha-cypermethrin and deltamethrin. Lambda-cyhalothrin has low vapor pressure, is essentially insoluble in water, and has low volatility. It is available in WP formulation and is used at a dosage of 20-30 mg/m² giving a residual effect of 3-6 months.

Cyhalothrin was developed in 1977. It is principally used to combat a wide range of pests in public health and animal health, but is also employed in agriculture against pests of pome fruit. Lambda-cyhalothrin is mainly used as an agricultural pesticide on a wide range of crops and is being developed for public health.

Lambda cyhalothrin is a synthetic pyrethroid insecticide and acaricide used to control a wide range of pests in a variety of applications. Pests controlled include aphids, Colorado beetles and butterfly larvae (Kidd and James, 1991). Crops on which it may be applied include cotton, cereals, hops, ornamentals, potatoes, vegetables or others (Kidd and James, 1991). It may also be used for structural pest management or in public health applications to control insects such as cockroaches, mosquitoes, ticks and flies which may act as disease vectors (Kidd and James, 1991). Lambda cyhalothrin is available as an emulsifiable concentrate, wettable powder or ULV liquid (Meister, 1992, Kidd and James, 1991), and is commonly mixed with buprofezin, pirmicarb, dimethoate or tetramethrin (Kidd and James, 1991). It is compatible with most other insecticides and fungicides (Kidd and James, 1991).

Unless otherwise stated, data presented herein refer to the technical product.

Mode of action:

Lambda-cyhalothrin mode of action is the same as that of other alphacyano pyrethroids, primarily affecting the sodium channels in the nerve membrane and causing a long-lasting prolongation of the transient increase in sodium permeability of the membrane during excitation.
Fig: 1.1 Chemistry, structure, properties and other details of the test toxicant: Lamda Cyhalothrin

CAS registry : cyhalothrin: 68085-85-8
Number : lambda-cyhalothrin: 91465-08-6
Common : cyhalothrin: R114563, PP563
Synonyms : lambda-cyhalothrin: R119321,PP321
Trade names : cyhalothrin: Grenade Lambda-cyhalothrin: karate, Matador, Icon
Cyhalothrin was developed by ICI in 1977. It is acid moiety and one in the alcohol moiety, as well as Z and E forms. Thus, there are 16 possible isomeric forms (eight enantiomeric pairs). However, in practice cyhalothrin is produced only in the Z and cis-forms, reducing the number of isomers to four. These comprise two cis enantiomeric Pairs: Enantiomer pair A: (Z), (1R, 3R), R Alpha Cyano (z),

(1S, 3S) S-alpha-cyano;
Enantiomer pair B: (Z), (1R, 3R), S- alphacyano (z).
(1S, 2S) R=alpha-cyano.

Lambda-cyhalothrin is manufactured by crystallization of the more active pair of enantiomers from cyhalothrin. The less active pair of enantiomers is recycled. Pure lambda-cyhalothrin is a racemic mixture of the enantiomer pair B isomers. The enantiomer pair A is present in low concentration in the commercial product. Technical grade cyhalothrin contains more than 90% of the pesticide and is formulated in 5%, 10% and 20% emulsifiable concentrates. Technical grade lambda-cyhalothrin also contains more than 90% active ingredient. It is formulated as 2.5%, 5.0%, 8.3% and 12% emulsifiable concentrates and as a 0.8% ultra-low volume concentrate.

Physical and chemical properties
Table 1.1 Some physical and chemical properties of cyhalothrin and lambda-cyhalothrin.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Cyhalothrin</th>
<th>Lambda cyhalothrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical state</td>
<td>viscous liquid</td>
<td>solid</td>
</tr>
<tr>
<td>Color</td>
<td>yellow-brown</td>
<td>beige</td>
</tr>
<tr>
<td>Odor</td>
<td>mild</td>
<td>mild</td>
</tr>
<tr>
<td>Relative molecular mass</td>
<td>449.9</td>
<td>449.9</td>
</tr>
<tr>
<td>Melting point</td>
<td>below 10 °</td>
<td>49.2 °C</td>
</tr>
<tr>
<td>Decomposes</td>
<td>&gt; 275 °C</td>
<td>&gt; 275 °C</td>
</tr>
<tr>
<td>Water solubility</td>
<td>4 x 10⁻³ mg/litre</td>
<td>5 x 10⁻³ mg/litre</td>
</tr>
<tr>
<td>Solubility in organic solvents</td>
<td>soluble</td>
<td>soluble</td>
</tr>
<tr>
<td>n-octanol water-partition</td>
<td>6.9</td>
<td>7.0</td>
</tr>
<tr>
<td>coefficient (log P&lt;sub&gt;ow&lt;/sub&gt;) at 20 °C</td>
<td>1.25</td>
<td>1.33</td>
</tr>
<tr>
<td>Relative density</td>
<td>1 x 10⁻⁹ kPa</td>
<td>2 x 10⁻¹⁰ kPa</td>
</tr>
<tr>
<td>Vapour pressure at 20 °C</td>
<td>4 x 10⁻⁶ kPa</td>
<td>3 x 10⁻⁶ kPa</td>
</tr>
<tr>
<td>Vapour pressure at 80 °C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
 Persistence of Cyhalothrin in the Environment:

The environmental degradation of pyrethroid insecticides has been reviewed by Roberts (1981). These compounds are all esters and are rapidly degraded both by macroscopic and microscopic organisms. The rate of degradation in soil depends on many factors; compounds, type of soil, aerobic or anaerobic conditions, etc. hence, it is difficult to quote the average half lives and these can range from days to some months. Cypermethrin, fenpropathrin and fenvalerate have a half-life of approximately two to four weeks in mineral and organic soils. Deltamethrin is more persistent with a half-life of more than two months in soil (Chanpman et al., 1981). Permethrin one of the more widely used agricultural pyrethroid, with a half life of 1 to 3 weeks in various silt and clay soils and up to 15 weeks in organic soils (Belenger and Hamilton, 1979; Williams and Brown, 1979; Chapman et al., 1981).

No data are available on actual levels in the environment, but with the low current use pattern and low application rates, these are expected to be low. Under laboratory conditions of constant toxicant concentrations, cyhalothrin and lambda-cyhalothrin are highly toxic to fish and to aquatic invertebrates. The 96-h LC50 values for fish range between 0.2 and 1.3 µg/litres, whereas for aquatic invertebrates the 48 hrs LC50 values range between 0.008 and 0.4 µg/liter.

Animal studies have been conducted in various species to investigate the toxicokinetics of cyhalothrin and lambda-cyhalothrin. Oral cyhalothrin is readily absorbed, metabolized thoroughly, and eliminated as polar conjugates in the urine (IPCS,1990a). Studies with lambda-cyhalothrin have shown that it also is rapidly metabolized into less toxic water-soluble compounds and excreted in the urine and feces (EXTOXNET, 1996). In mammals, cyhalothrin is metabolized as a result of ester cleavage to cyclopropane carboxylic acid and 3-phenoxybenzoic acid, and eliminated as conjugates. Tissue levels decline after exposure stops and residues in the body are low (IPCS, 1990a).
Fig: The metabolic pathways that have been established for cyhalothrin in mammals.

![Diagram of metabolic pathways for cyhalothrin and lambda-cyhalothrin](image)

Proposed degradation pathways for cyhalothrin and lambda-cyhalothrin in man.

- Presumably formed via cyanohydrin and aldehyde intermediates

No boiling point data are available as both forms decompose on heating above 275°C. Cyhalothrin is highly stable to light and at temperatures below 220°C.

Lambda-cyhalothrin is stable in water at pH 5. At pH 7 and pH 9, there is racemization at the alpha-cyano carbon to yield a 1:1 mixture of enantiomer pairs A and B. At pH 9, the ester bond is fairly readily hydrolyzed (half-life, 7 days) (Collis & Leahey, 1984). Dilute aqueous solutions are subject to photolysis at a moderate rate (Hall & Leahey, 1983; Curl et al., 1984a).
**Effects on Aquatic Organisms:**

Lambda cyhalothrin is very highly toxic to many fish and aquatic invertebrate species. Reported LC50s in these species are as follows: bluegill sunfish, 0.21 ug/L; rainbow trout, 0.24ug/L; Daphnia magna, 0.36 ug/L; mysid shrimp, 4.9 ng/L; sheep head minnow, 0.807 ng/L. A median effect concentration, EC50 (i.e. the concentration at which the effect occurs in 50% of the test population), for the eastern oyster of 0.59 ng/L has been reported. Bioconcentration is possible in aquatic species, but bioaccumulation is not likely. Bioconcentration in channel catfish has been reported as minimal, with rapid depuration (elimination). A bioconcentration factor of 858 has been reported in fish, species unspecified, but concentration was confined to non-edible tissues and rapid depuration was observed.
Biology of The Selected Test Fish

*Labeo rohita* (Hamilton, 1822):

Body bilaterally symmetrical, moderately elongate, its dorsal profile more arched than the ventral profile; body with cycloid scales, head without scale; snout fairly depressed, projecting beyond mouth, without lateral lobe; eyes dorso lateral in position, not visible from outside of head; mouth small and inferior; lips thick and fringed with a distinct inner fold to each lip, lobate or entire; a pair of small maxillary barbells concealed in lateral groove; no teeth on jaws; pharyngeal teeth in three rows; upper jaw not extending to front edge of eye; simple (unbranched) dorsal fin rays three or four, branched dorsal fin rays 12 to 14; dorsal fin inserted midway between snout tip and base of caudal fin; pectoral and pelvic fins laterally inserted; pectoral fin devoid of an osseous spine; caudal fin deeply forked; lower lip usually joined to isthmus by a narrow or broad bridge; pre-dorsal scale 12-16; lateral line distinct, complete and running along median line of the caudal peduncle; lateral line scales 40 to 44; lateral transverse scale-rows six or six and a half between lateral line and pelvic fin base; snout not truncate, without any lateral lobe; color bluish on back, silvery on flanks and belly.

![Image of Labeo rohita](image.png)

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Chordata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subphylum</td>
<td>Vertebrata</td>
</tr>
<tr>
<td>Class</td>
<td>Osteichthyes</td>
</tr>
<tr>
<td>Subclass</td>
<td>Actinopterygii</td>
</tr>
<tr>
<td>Order</td>
<td>Cypriniformes</td>
</tr>
<tr>
<td>Family</td>
<td>Cyprinidae</td>
</tr>
<tr>
<td>Genus</td>
<td>Labeo</td>
</tr>
<tr>
<td>Species</td>
<td>rohita</td>
</tr>
</tbody>
</table>
Catla catla (Hamilton):

Body short and deep, somewhat laterally compressed, its depth more than head length; head very large, its depth exceeding half the head length; body with conspicuously large cycloid scales, head devoid of scales; snout bluntly rounded; eyes large and visible from underside of the head; mouth wide and upturned with prominent protruding lower jaw; upper lip absent, lower lip very thick; no barbells; lower jaw with a movable articulation at symphysis, without a prominent process; gill rakers long and fine; pharyngeal teeth in three row, 5.3.2/2.3.5 pattern; dorsal fin inserted slightly in advance of pelvic fins, with 14 to 16 branched rays, the simple rays non-osseous; anal fin short; pectoral fins long extending to pelvic fins; caudal fin forked; lateral line with 40 to 43 scales. Grayish on back and flanks, silvery-white below; fins dusky.

Phylum : Chordata
Subphylum : Vertebrata
Class : Osteichthyes
Subclass : Actinopterygii
Order : Cypriniformes
Family : Cyprinidae
Genus : Catla
Species : catla
Cirrhinus mrigala (Hamilton, 1822):

Body bilaterally symmetrical and streamlined, its depth about equal to length of head; body with cycloid scales, head without scales; snout blunt, often with pores; mouth broad, transverse; upper lip entire and not continuous with lower lip, lower lip most indistinct; single pair of short rostral barbels; pharyngeal teeth in three rows, 5.4.2/2.4.5 pattern; lower jaw with a small post-symphysial knob or tubercle; origin of dorsal fin nearer to end of snout than base of caudal; dorsal fin as high as body with 12 or 13 branched rays; last unbranched ray of dorsal fin non-osseous and non-serrated; pectoral fins shorter than head; caudal fin deeply forked; anal fin not extending to caudal fin; lateral line with 40-45 scales; lateral transverse scale rows 6-7/5½-6 between lateral line and pelvic fin base; usually dark grey above, silvery beneath; dorsal fin greyish; pectoral, pelvic and anal fins orange-tipped (especially during breeding season).

Phylum : Chordata
Subphylum : Vertebrata
Class : Osteichthyes
Subclass : Actinopterygii
Order : Cypriniformes
Family : Cyprinidae
Genus : Cirrhinus
Species : Cirrhinus mrigala
*Cyprinus carpio* (Linnaeus, 1758)

Body elongated and somewhat compressed. Lips thick. Two pairs of burbles at angle of mouth, shorter ones on the upper lip. Dorsal fin base long with 17-22 branched rays and a strong, toothed spine in front; dorsal fin outline concave anteriorly. Anal fin with 6-7 soft rays; posterior edge of 3rd dorsal and anal fin spines with sharp spinules. Lateral line with 32 to 38 scales. Pharyngeal teeth 5:5, teeth with flattened crowns. Colour variable, wild carp are brownish-green on the back and upper sides, shading to golden yellow ventrally. The fins are dusky, ventrally with a reddish tinge. Golden carp are bred for ornamental purposes.

![Illustration of a fish](image)

**Phylum** : Chordata  
**Subphylum** : Vertebrata  
**Class** : Osteichthyes  
**Subclass** : Actinopterygii  
**Order** : Cypriniformes  
**Family** : Cyprinidae  
**Genus** : *Cyprinus*  
**Species** : *Cyprinus carpio*
*Ctenopharyngodon idellus* (valenciennes)

The grass carp grows very rapidly, and young fish stocked in the spring at 20 centimetres (7.9 in) will reach over 45 centimetres (18 in) by fall, and adults often attain nearly 1.2 metres (3.9 ft) in length and over 18 kilograms (40 lb) in weight. According to one study, they live an average of 5-9 years with the oldest gaining 11 years. They eat up to 3 times their own body weight daily. They thrive in small lakes and backwaters that provide an abundant supply of fresh.

![Image of Ctenopharyngodon idellus](image)

**Phylum**: Chordata  
**Subphylum**: Vertebrata  
**Class**: Osteichthyes  
**Subclass**: Actinopterygii  
**Order**: Cypriniformes  
**Family**: Cyprinidae  
**Genus**: *Ctenopharyngodon*  
**Species**: *Ctenopharyngodon idellus*
The present study was under taken to study the toxic effects of cyhalothrin on the fresh water fish *Catla catla, Labeo rohita, Cirrhinus mrigala Ctenopharyngodon idella*, and *Cyprinus carpio*. With the following objectives

To evaluate the toxic levels of the toxicant cyhalothrin to the selected fresh water fish *Catla catla, Labeo rohita, Cirrhinus mrigala Ctenopharyngodon idella*, and *Cyprinus carpio*.

To study the impact of the test toxicant cyhalothrin on the respiratory metabolism of the test species of selected fresh water test fish, *Labeo rohita*.

To study the cyhalothrin induced biochemical changes, enzymatic changes, and nucleic acid changes in the test fish.

To study the histopathological changes in the vital organs viz Gills, liver and kidney of the reference test fish.

To identify and quantify the uptake of pesticide residues of repository of the organs such as liver, brain, gills, kidney and muscles of the test fish.

To identify the changes in the Nucleic acid and amino acid profiles of the test fish by using SDS-PAGE electrophoretic studies.