REVIEW OF LITERATURE
1. HISTORY

The date of the first use of pyrethrum as an insecticides is unknown. Probably it was used by Caucasian tribemen at an early date. Its source however, was kept secret until an Armenian named Jumtikoff learnt that the powder used as an insecticide was obtained from the flower heads of certain species of *Pyrethrum*. In 1928, using this knowledge, Jumtikoff's son began to manufacture the powder on a large scale (Lodeman, 1896). Use of pyrethrum flowers for insecticidal purpose originated in Persia (Gnadinger, 1936, 1945) and was introduced into Europe early in the nineteenth century.

Initially pyrethrums (sometimes called Trieste flowers) were used in the powder form to control only household insects, while being non-toxic to warm-blooded animals. At about 1916, kerosene extracts of pyrethrum flowers were employed extensively as a spray, particularly against house-flies and mosquitoes.

*Sumicidin* is a pyrethroidal type of the new generation of insecticides discovered by Sumitomo Chemical Co. Ltd., Japan in 1972. It was at first introduced by the pesticide research experts with the Sumitomo's Code No. S.5602 and later was given the common name *fenvalerate*. It is a synthetic pyrethroid type in its activity. It belongs to the fourth generation of insec-
ticides, following the three earlier dominant groups of insecticides namely, chlorinated hydrocarbons, organo-phosphate and carbamates. The pyrethroids are used extensively for household purposes due to (i) their extremely low toxicity to man and animals, (ii) absence of residual problem in the environment and (iii) high performance against household pests. However, the cost of pyrethroids in outdoor application as compared to the conventional insecticides is much higher.

The later pyrethroids have certain entirely new characteristics such as (a) high biological performance over a wide range of pesticidal spectrum, (b) extremely low dosage per unit area, made possible by its high efficacy, (c) quick killing and knock-down effects, (d) necessary duration of effect (good photo-stability), (e) outstanding biological control over insect pests, resistant to chlorinated hydrocarbons, organophosphates and carbamates, (f) the low toxicity to men and animals common among pyrethroids (Technical Information Sheet: Sumitomo Chemical Co. Ltd., Osaka, Japan 1972).

2. **ACTIVE PRINCIPLES OF PYRETHROIDS**

The active principles of a typical extract of pyrethrum are (i) pyrethrin I 35%, (ii) pyrethrin II 32%, (iii) cinerin I 10%, (iv) cinerin II 14%, (v) jasmolin I 5% and (vi) jasmolin II 4%. The basic structure of active ester is:
Pyrethrin I is the most important natural constituent lethal to insect while pyrethrin II provides much of the rapid knockdown effect. The jasmolins are lower in activity than the four main insecticidal constituents.
3. **ISOLATION AND IDENTIFICATION**

Partition of petroleum ether extract from dried flower heads between the hydrocarbon and nitromethane solutions through a short column of activated charcoal gives an active concentrate of 90% purity (Barthel and Haller 1945). Molecular distillation is then employed to purify and decolorize the concentrate (Elliot et al. 1959). The insecticidal activity of pyrethrum is attributed to the action of six constituents given earlier. Pyrethrin I and cinerin I are fairly easily separated from pyrethrin II and cinerin II by column chromatography on alumina or silica gel. Good separation may also be obtained by paper and thin-layer chromatography (TLC). The pyrethrins and cinerins form crystalline 2,4-dinitrophenylhydrazones and are further identified by degradation, hydrogenolysis, ozonolysis, ultra-violet (UV), infra-red (IR), nuclear magnetic resonance (NMR), spectroscopy and optical rotation (Crombie and Elliott 1961). The jasmolines are identified by UV, IR and NMR spectroscopy. Donegan et al. (1971) developed a rapid assay method for pyrethrin content in the field by decoupling with proton resonances.

With the orthophosphoric acid method for the pyrethrum assay Naomichi et al. (1972) observed that only pyrethrins I and II alone showed colour with phosphoric acid reagent. The different methods commonly used to determine the pyrethrins and its related compounds are 'Sel' procedure mercury reduction and sulphur
colorimetry (Balbaa et al. 1972). A colour specific reagent (a mixture of phosphoric, tannic and acetic acids and acetone) was used for the thin layer chromatographic identification and quantitation of pyrethrins and piperonyl butoxide in pesticide formulations (Olive 1973). Gas chromatographic techniques were applied by Kawano and Kawai (1974) to study the insecticidally active esters to pyrethrum. $^{13}$C NMR data for naturally occurring insecticidal pyrethrin esters (found in Chrysanthemum cinerariaefolium), synthetic allethrin and the chrysanthemic acid and rethrolone molecules from which the esters are constituted, were measured by Crombie et al. (1975).

4. PROPERTIES THAT INFLUENCE BIOLOGICAL ACTIVITY OF PYRETHROIDS

The biological activities of pyrethroids are closely related with their chemical structure (Elliott 1974, 1977; Elliott et al. 1974a, Elliott and Janes 1978). Effect on insects, mammals and other groups, such as fish, depend on the optical and geometrical configurations of their acidic and alcoholic components.

In the natural pyrethrin, all the esters are present in their most active optical and geometrical configurations, but some commercial pyrethroids are a mixture of two (bioallethrin, the forte mixture); four (tetramethrin, resmethrin, phenothrin, permethrin, fenvalerate) or eight (allethrin, cypermethrin) isomers. In other cases (S-bioallethrin, bioresmethrin and
decamethrin), the most active isomers have produced by using advanced techniques of synthesis and resolution. It is clearly beneficial to use such individual isomers, if economically feasible, to minimise the risk of environmental contamination.

The possibility of interaction between isomers in racemic mixtures of pyrethroids that enhances or diminishes their activities has not been widely investigated.

Wickham (1976) showed that 5-benzyl-3 furylmethyl and (S)-allethronyl (1R-trans)-chrysanthemates were more active only when present as a pure isomer. An enhancement of activity in an (IR, trans) isomer by a small amount of (IR, cis) isomer is claimed by Sumitomo Chemical Co. (1976).

5. GENERAL PROPERTIES

Pyrethroids are traditionally photolabile compounds, without persistent residues. Thus there is less danger to predators and other beneficial insects and no risk of long-term environmental contamination. The natural pyrethrins and synthetic compounds such as, bioresmethrin have at least two centres of instability to light. Much of this loss of insecticidal activity in the chrysanthemate is restored in the dichloro-vinyl ester. The latter is relatively more effective, especially against mustard beetles, than would have been predicted from comparable results with alternative acidic and alcoholic components.
Field and laboratory tests show that pyrethroids have a promising action against a wide range of pests. It is, therefore, important now to consider the implications of widespread use of more stable pyrethroids in place of other insecticides such as organophosphorus and organochlorines.

All the pyrethroids are lipophilic compounds, at most insoluble in water. In this respect, they resemble the chlorinated hydrocarbon insecticides and differ from most organophosphorus and carbamates (Briggs et al. 1974). Although their precise mode of action in insects is not established, yet they are recognised as nerve poison (Narahashi 1976). They do not interact with acetyl cholinesterases as do organophosphorus and carbamates. Physical properties probably determine their rapid action in insects (Briggs et al. 1974) and also minimize translaminar action in leaves and systemic movement in plants. Therefore, pyrethroids are effective as contact insecticides and to a lesser extent, as stomach poisons. Their efficacy in practice is enhanced by resistance to rain leaching (Roscoe 1977). This property is due to their lipophilic nature. Most pyrethroids are relatively high-boiling viscous liquids with low vapour pressures (Nishizawa 1971). Only a few are sufficiently volatile (Maciver 1963). For the same reason even the more stable synthetic pyrethroids are unlikely to be dispersed through the environment.
Little has been reported so far on the behaviour of pyrethroids in soil. However, their physical properties indicate that mobility in soil will be small. Vapour diffusion in the soil-air space is unlikely to be significant because their vapour pressures are low. Their highly lipophilic characters give a octanol-water coefficient ($P$) greater than $10^5$ (Briggs et al. 1974). Breese (1977) suggested that pyrethroids would be strongly absorbed in most soils and thus not leached readily from the point of application or deposition. Therefore, ground water will receive insecticides only by direct application or by the erosion of treated soil and not by diffusion or leaching.

6. POLARITY AND PHYSICAL CHARACTERISTICS

Polarities of various groups of insecticides were estimated by their octanol-water partition coefficients (Briggs et al. 1974b). The pyrethroids are much more lipophilic than the majority of organophosphorus compounds and carbamates. Of the 300 pyrethroids studied, the nine most potent insecticides fall within a very narrow range of polarities close to that of the organochlorine compounds. For both organochlorine compounds and pyrethroids, lipophilicity is probably an important factor determining their ability to penetrate to and act intercellularly in the nervous systems of insects (Burt and Goodchild 1974).

With the less stable pyrethroids used at present this
lipophilicity is not a disadvantage from the environmental standpoint, because the compounds are rapidly metabolised in mammalian systems (Elliott et al. 1972, Casida et al. 1975/6, Ueda et al. 1973) and are excreted as polar metabolites. The products of photo-decomposition are also relatively harmless. Therefore, neither the pyrethroids nor the metabolites or photo-decomposition products are stored in mammalian body fat and thus do not pass into food chains, or contaminate the environment in other ways. Although some of the latest pyrethroids are more stable in air and light, they too, have low mammalian toxicities (Elliott et al. 1973a, b; Barnes and Verschoyle 1974). Preliminary studies indicate that they are also very rapidly metabolized and excreted from mammalian system (Elliott et al. 1974b) and their photo-decomposition products, when formed, are innocuous.

Knowledge of the relative polarities of pyrethroids also helps to interpret the influence of structure on the rapid knock-down action of these compounds. Knock-down compounds are generally more polar than those with good killing action (Briggs et al. 1974a). The difference in speed of action appears to be related to the greater speed of penetration to the central nervous system (Burt and Goodchild 1974), although insecticidal properties are modified by changes in molecular structure which influence polarity.
7. PREPARATION OF SYNTHETIC PYRETHROID

A significant step in study of synthetic pyrethroids was the synthesis of chrysanthemuric acid by Campbell and Harper (1945). They adopted the method of Staudinges and Ruzicka (1924). It is essentially the one now used commercially (Sanders and Taft 1954), except for the preparation of the diene hydrocarbon starting material (see scheme below):

Campbell and Harper (1945) Sanders and Taft (1954) Inoue et al. (1951)

\[
\begin{align*}
2\text{CH}_2 &= \text{C(\text{CH}_3)CH}_2\text{Cl} \\
\text{Mg} &\quad \text{KOH} \\
\text{CH}_2 &= \text{C(\text{CH}_3)CH}_2\text{CH}_2\text{C(\text{CH}_3)=CH}_2 \\
250-275^\circ\text{C} &\quad \text{Alumina and Chromic oxide} \\
\text{N}_2 &\quad \text{H}_2 \\
\text{N}_2\text{CH}_2\text{COOC}_2\text{H}_5 &\quad 100-125^\circ\text{C} \\
\text{Ethyl chrysanthemumate}
\end{align*}
\]
Harper and Reed (1951a) and Harper et al. (1951b) later improved the methods, particularly by the use of tetrahydrofuran as a solvent for the reaction between magnesium and methyl chloride.

Inoue et al. (1951a, b), Katsuda et al. (1951, 1958) and Barkal (1961) studied the synthesis of the so-called 'synthetic pyrethrins', now known to be synthetic pyrethroids. Instead of acetylene-acetone synthesis used in the USA for the preparation of 2,5-dimethyl 2,4-hexamethylene, they examined ethyl levulinate, diethyl succinate and methallyl chloride as a starting material, finally adopting the methallyl chloride route used by Campbell and Harper (1945).

At Rothamstead experimental station in 1972 an exceptionally valuable combination of properties was found in the esters of 3-phenoxybenzyl alcohol with cis and trans dichlorovinyl analogues of chrysanthemic acid, having chlorine atoms in place of methyl groups in the isobutenyl side chain. These esters were active against a number of insect species and were very much more stable in oxygen and light than the other pyrethroids and exerted a prolonged residual action. This opened up for the first time the possibility of using synthetic pyrethroids for control of pests and plants and animals in the field. As the speed of knock-down of insects is less than that of the natural pyrethrins, these synthetic forms exert both contact and stomach action.
In 1974, the Japanese workers obtained highly active esters (e.g., S-5439) when various isopropylarylactic acids replaced chrysanthemic acid. Further increase of insecticidal potency was generally obtained by substituting a cyano group into 3-phenoxybenzyl esters (e.g., Fenvalerate). In the chrysanthemic series, esters derived from 3-cyano-3-phenoxybenzyl alcohol were two to three times more active than those from 3-phenoxybenzyl alcohol.

8. APPLICATION AND USE OF PYRETHROIDS

The various uses of pyrethroid insecticides (Elliott et al. 1978), require different optimum combinations of properties. Two contrasting situations may be distinguished. In enclosed spaces such as houses, hostels, bakeries, flour mills, food processing plants and miscellaneous industrial premises, where timber, fabric, greenhouse and veterinary pests and some human disease vectors must be controlled, low mammalian toxicity is particularly important, but photostability is less so. For outdoor applications, as in general plant protection and silviculture, and for controlling many veterinary pest and disease vectors, residual activity is essential, but because direct exposure is improbable, very low mammalian toxicity, although desirable, is less crucial. The effect of the toxicant on parasites, predators and wild-life in general must also be considered in these applications.
The natural pyrethrins and some synthetic pyrethroids have been used for many years in the first type of environment because of their low mammalian toxicity, rapid action, outstanding insecticidal efficiency and degradation to innocuous residues. Their cost and instability, however, have excluded them from many other applications, especially outdoors, where the newer compounds, more active and adequately stable, can compete with the established insecticides in cost effectiveness.

As broad-spectrum insecticides, the more stable pyrethrroids tend to be toxic to beneficial insects, including bees and predators, although approximate methods of formulation and application may diminish their adverse effects.

Synthetic pyrethrroids developed recently are equally toxic to bees. The more recent, stable compounds (permethrin, decamethrin) are relatively less active by contact than resmethrin and bioresmethrin. The effect of the new compounds on the predators of the major pests of monocultures (cotton, tobacco) in practice cannot yet be assayed. In one instance, decamethrin was the least harmful of a group of insecticides against a parasitic species (Plapp and Vinson 1977).

9. **TOXICITY ON DIFFERENT ORGANISMS**

Mode of action

Pyrethrins paralyze insects very rapidly (Matsui and
Yamamoto 1971). Even in small dosages, knock-down occurs almost instantaneously but the effect is usually temporary. The dosage required to kill insects is usually much higher than that needed to paralyze them. Both paralytic and killing effects can be potentiated several fold by adding certain compounds which by themselves have little or no toxicity. Pyrethrins show almost no significant toxicity to mammals when applied dermally or orally. For example, the acute oral toxicity (LD$_{50}$) in mice in mg/kg is 300–650 for allethrin and 320–720 for pyrethrins. In rats the corresponding figures are 680 and 200. These characteristics of pyrethrins make them valuable for household use, as livestock sprays, for stored grains, and for vegetables and fruits.

However, the toxic mechanism of pyrethrins is not yet fully elucidated (Kearns 1956, Dahm 1957, Brown 1963). Pyrethrins definitely act on the nervous system. Many histopathological changes are observed, but they are probably the result rather than the cause of the lethal paralysis. The mechanism of action of pyrethrins on the nerve fibre at the cellular levels was studied by Narashashi (1962a,b, 1965) and O'Brien (1967). Pyrethrins and allethrin first stimulated the nerve cells and nerve fibres, then paralyzed them. This is probably due to the accumulation of certain depolarizing substances around the nerve fibres. The increased negative after potential, initiates repetitive discharges which in turn cause the hyperactivity and convulsions of the poisoned insect. In higher concentrations,
Pyrethrins and allethrin block nerve conduction which in turn causes paralysis. The nerve membrane is the major site of excitation. Upon stimulation sodium and potassium conductance increases, which accounts for the excitation or production of action potentials.

Allethrin inhibits conductance increases, thereby causing the blockage of nerve conduction (Fig. 1). The sizes of Na⁺ and K⁺ demonstrate the sodium and potassium concentration gradients across nerve membrane, and the arrows demonstrate the path of the ions. Both sodium and potassium conductances in the active state of the nerve are blocked by allethrin (from Narahashi 1965).

Biochemical changes associated with the action of pyrethroids are not known. Insect cholinesterase is not inhibited \textit{in vivo}, and the \textit{in vitro} inhibition of cytochrome oxidase seems to be an artifact. A neuroactive toxin is released from the nerves of the pyrethrin-poisoned cockroach (Blum and Kearns 1956). This toxin is produced by the hyperactivity of the poisoned nerve, and is also responsible for further stimulation and eventual paralysis of nerve.
Tissue-bound conjugates

Casida and Ruzo (1980) suggested that, in contrast to the organophosphorus and methylcarbamate insecticides, the pyrethroids do not form covalent derivatives at the neuroreceptor site, so that their insecticidal activity is not dependent on their reactivity. Bound conjugates are the exception rather than the rule with pyrethroids. Extensive fragmentation occurs in soil and some plants but generally not in mammals or insects. Some attention has been given to reactive metabolites that combine with tissue components. Cyanide from the cyano-pyrethroids is converted to thiocyanate which is localized in hair, skin and stomach probably as the result of forming disulphide linkages with protein. A metabolite of the resmethrin alcohol moiety, possibly 5 benzyl-3-furaldehyde, reacts with microsomal components. Pyrethroids with phenoxy substituent may bind via phenolic metabolites and the corresponding quinones.

In soil

Kaufman and Jordan (1977) showed that persistence of permethrin in soil is limited because it is extensively degraded by soil micro-organisms in four weeks. Ester cleavage is followed by oxidation, eventually to carbon dioxide. The stability of decamethrin in dried African soil depends on the relative humidity. In Ugandan soil, esters without an α,β-cyano group were relatively more stable (Barlow et al. 1977). The most
important conclusions from these and other studies are that pyrethroids are fully degraded in various soil types in 2-4 weeks, in contrast to organochlorine insecticides. At recommended rates, permethrin is probably metabolised sufficiently rapidly, microflora and fauna.

In lower organisms

Pyrethroid (EMC 33297) was found to be compatible with the bacterium Bacillus thuringiensis var. kurstati (Habib and Garcia 1981). Pyrethroid at 1.44 ppm at pH 6.5 and 2.89 ppm at 8.6 pH, stimulated bacterial germination.

Propoxus and d-resmethrin, both at 1:500, were sprayed in a hen house at 7 days intervals, to compare their control of intermediate host of the leucocytozoon, Culicoides. The carbamate propoxus was markedly more efficient in controlling Culicoides than d-resmethrin and the controlling power lasted for 7 days (Yoshioka et al. 1981).

Insects: Although the metabolic fate of many pyrethroids in mammals had been examined in detail, both qualitatively and quantitatively, the number of detailed investigation in insects was limited, because experimental difficulties are greater and such information is not required to establish the safety of an insecticide (Yamamoto et al. 1969). The ability to metabolize pyrethroids varies greatly with compound and insect species,
but the extent to which this affected insecticidal potency, was not yet precisely defined. The situation in resistant strains is further complicated by interaction between the various factors of resistance (Sawicki 1975). However in house flies, the enhanced activity of natural pyrethrin and some synthetic esters when synergized showed that metabolism limits the toxicity of these compounds (Casida 1970). Fundamental investigations by Burt et al. (1976) on the action of synergists such as, sesamex with later pyrethroids, however have shown much lower levels of synergism, even at constant high synergist doses, so that extensive use of the known synergists with them, seems unlikely.

In plants: The natural pyrethrins and earlier synthetic pyrethroids had few uses on plants and knowledge of the rate and mode of degradation of the compounds was unnecessary. However, because the newer synthetic compounds will be used much more extensively on a wide range of outdoor crops, more detailed knowledge of their behaviour on and in plants is essential. Much can be inferred from their physical characteristics and behaviour in other metabolizing systems since it is established that in general, plants, micro-organisms, insects and mammals metabolize foreign compounds by similar pathways, although relative rates can vary greatly (Casida and Lykken 1969).

The lipophilic character of the pyrethroids indicates that they will be absorbed readily onto the waxy layer of plant
surface and penetrate to the aqueous inner phases predominantly as more polar metabolites. After application of $^{14}$C-labelled (IR, trans) and (IR, cis) - permethrin to beans, Ohkawa et al. (1977) recovered 99% of the radiocarbon that had not evaporated from the leaf 14 days after application and less than 1% from other parts. Neither cis nor trans permethrin moved detectably from the position at which they were applied, and half-lives remained at 9 and 7 days, respectively, on bean and cotton leaves. Gaughan et al. (1977a) detected 6-13% of trans - cis isomerization in 21 days. The metabolites included hydroxysteres and their glycosides, hydrolysis products and their glycosides, and 3-phenoxybenzoic acid. Trans - permethrin was degraded more rapidly than cis - permethrin partly because the trans compounds were hydrolyzed faster.

Hargreaves and Cooper (1979) observed that a compound formulation of pyrethroid insecticide fenvalerate caused leaf chlorosis when sprayed on seedlings of tomato cultivars. Phytotoxic symptoms were noted with 25 ppm fenvalerate. Compound formulations of the other pyrethroid insecticides cypermethrin, decamethrin and permethrin at their expected usage rates for tomatoes, were not phytotoxic.

Ohkawa et al. (1980) observed that fenvalerate (I) labelled at CN with $^{14}$C and its S-acid isomer (II) $^{14}$C-labelled at CN or at the $\alpha$-position at benzyl, applied in Phaseolus vulgaris
leaves at 10\(\mu\)g leaf in MeOH solution, disappeared from the leaves with half-lives of approx. 14 days, very little \(^{14}\)C was translocated into other parts of the plant. 2-(3-phenoxyphenyl)-3-(4 chlorophenyl)-4 methyld pentanenitrile III, IV, V, 2-(4-chlorophenyl) isovaleric acid IV and 3-phenoxybenzoic acid (VII) and conjugates of VI, VII, 3-phenoxybenzyl and \(\alpha\)-cyano-3-phenoxybenzyl alcohol, and 3-phenoxymandelic, 3-(2-hydroxyphenoxy)-3-(4-hydroxyphenoxy) benzoic acids were detected on and in leaves treated with I and II. III was a photo-product and IV and V appeared to be produced partly by photo-chemical and/or physico-chemical reactions. Roots and shoots grown for 30 days on Kodaira light clay soil treated with II-\(^{14}\)C at 1 ppm contained \(^{14}\)C equivalent to 140-200 and 14-23 ppb respectively of II. Those grown on similarly treated Katano sandy loam soil contained a little more \(^{14}\)C. The \(^{14}\)C in pods and seeds corresponded to 3-8 ppb when given as I. No intact I was found in shoots.

Amer and Aboul-ela (1985) observed that cypermethrin induced a statistically significant percentage of abnormal mitoses in root meristems of Vicia faba.

Fish: Pyrethroids have long been reputed to be toxic to fish. The nature and extent of fish toxicity are relatively unimportant in relation to the traditional uses of the less stable compounds that are unlikely to be used near open water with the prospect that agricultural crops and forests will be treated. More infor-
mation about the action of each compound on a range of organisms is needed to provide informed guidance about possible hazards.

The lipophilia of pyrethroids indicates that they are absorbed strongly by the gills of fish even from very low concentrations in water. Quélenec (1972) observed that resmethrin was toxic to fish. Pillmore (1973) collected information on the toxicity of the natural pyrethrins, with and without piperonyl butoxide, on a range of aquatic organisms.

Marking (1974) suggested that pyrethroid SBP-1382 (S-benzyl-3 furyl ester of chrysanthemate) was highly toxic to fish and could be used as a piscicide under conditions in which other toxicants were ineffective. The 96 hours LD50 ranged from 1-5 μg/l of pyrethrin for coho salmon (Oncorhynchus kisutch), chinook salmon (O. tshawytscha), rainbow trout (Salmo gairdneri), lake trout (Salvelinus namaycush), brook trout (S. fontinalis), carp (Cyprinus carpio), white sucker (Catostomus comersoni), green sunfish (Lepomis cyanellus), blue gill (L. macrochirus) and yellow perch (Perca flavescens). Toxicity increased as temperature decreased but was not influenced by changes in pH or water hardness. Toobey et al. (1975) observed that pyrethroids were more toxic to killfish than organophosphates and carbamates. Skea et al. (1975) stated that the toxicity of two synthetic pyrethrins, SBP 1382 EC (24.3% active ingredient) and formula 7067 (5% active ingredient) was determined for fingerling brown trout (Salmo
Medium tolerance limit for SBP 1382 EC was approximately 1.2 μg/l at 24 hours and at 24 and 48 hours TLM for formula 7067 were 4.9 and 3.5 μg/l, respectively.

In killfish (Oryzias latipes) relative toxicity of optical and geometrical isomers of phenothrin, permethrin, resmethrin and tetramethrin varied with species, and in any one species the toxicity generally paralleled insecticidal activities (Miyamoto 1976). As with insects, esters of IR-cyclopropane acids (e.g., IR, trans and IR, cis permethrin and resmethrin) were much more toxic to killfish than the (IS)-forms. Therefore, no greater hazard to fish is likely from the more active compounds used at proportionately lower rates of application than from the less active analogues.

Miura and Takahashi (1976) showed that fenvalerate was relatively more toxic on 2-day old mosquito fish (Gambusia affinis). Mauck et al. (1976) compared the toxicity of the natural pyrethrins and five synthetic pyrethroids on cold and warm water species of fish. Cypermethrin (100 ha⁻¹) applied to a pond did not affect amphibia or fish, including fish fry (Breese 1977).

Roscoe (1977) suggested that rapid absorption onto river banks, pond sediments and organic matter in general, greatly diminished concentrations in the water, so that under practical conditions, at application rates up to 70g ha⁻¹ (a level unlikely to
be attained in normal practice), permethrin shows no direct action on fish and there was little significant effect up to three times that rate on worms, leaches, snails, bivalves, tadpoles, newts and aquatic flora.

**Sumicidin** gives a high order of acute toxicity on both fresh water and marine animals. However, it is absorbed strongly on the surfaces and this property may effect the toxicity of the material on fish in practical field situations. When sumicidin was applied to soil which was then added to aquarium water, its LD₅₀ value was increased by a factor of approximately X40 (Technical Information, Sumitomo Chemical, Osaka, 1978).

**Glickman et al.** *(1981)* suggested that little ester hydrolysis of cis- or trans-permethrin occurs in vivo in rainbow trout, the major metabolite being the glucuronide conjugate of 4'-OH permethrin. This is significantly different from the metabolite profiles reported in rats, cows and hens where the great majority of permethrin metabolites were products of ester hydrolysis.

**Birds:** From all the evidences published so far toxicity of the natural and synthetic pyrethroids to birds is low. Gnadinger *(1945)* has shown large doses of oleoresin of pyrethrum to chicken without any ill effect. Pillmore *(1973)* stated that natural and synthetic pyrethroids were less toxic to birds, for example, the acute mean lethal dose of permethrin to Japanese quail...
exceeds 13,500 mg kg\textsuperscript{-1} (plant protection division - ICI 1976) and decamethrin to wild ducks exceeds 4000 mg kg\textsuperscript{-1} (Roussel uclaf/procida\textsuperscript{technical data sheet 1977}).

Eight day feeding studies in mallards and bobwhite quails were done by Sumitomo Chemical Co (1978). Fourteen day old mallard ducklings and bobwhite quails (50/dose group) were fed 0, 464; 1,000; 2,150; 4,640 and 10,000 ppm sumicidin for 14 days. Except for reduced food intake and some deaths at 1,000 ppm and higher feeding levels, no signs of intoxication were seen in the mallards and the dietary LD\textsubscript{50} value was calculated to 5,502 ppm (95\% confidence limits 3,194\textendash 9,478 ppm). In bobwhite quail at 10,000 ppm groups signs of intoxication were found and there were some deaths. The dietary LD\textsubscript{50} value for bobwhite quail was > 10,000 ppm. For one generation reproduction studies, the birds were fed 0, 0.2 and 2.0 ppm sumicidin for 21 weeks. Eggs from each species were incubated and the young were maintained on the control diet for 14 days after hatching. No reproductive effects attributable to sumicidin were seen.

**Mammals : Rats and Mice**

Carpenter \textit{et al.}(1950) made a detailed study of the toxicity of allethrin and pyrethrins using white rats, guinea pigs, rabbits, dogs and white mice as test animals. Two separate inhalation studies on aerosols with 1\% by weight of allethrin or of pyrethrins, 9\% of peanut oil and 90\% of freon - 12 were carried out at concentrations in excess of 50g of total formulation per
100 cft of space. In the first study, laboratory produced allethrin and comparative aerosols caused no detectable injurious effects on rats exposed twice daily for 30 minutes up to a total of 85 such periods within 67 calendar days. In a second similar study a sample representing commercially produced allethrin caused no injury to rats or dogs receiving 40 exposures, each of 30 minutes duration within 27 calendar days.

Single 30 minutes exposures of rats to 15 concentrations of aerosols of commercial allethrin and pyrethrins in the order of 350 times of level used for the repeated exposures caused no visible damage, nor did they depress weight gain during a subsequent 14 day observation period. A fog of commercially allethrin produced by a vaponefrin nebulizer was lethal to one of 10 rats in a two hour exposure at a concentration of 19 mg/l. The LD50 of undiluted commercial allethrin for rabbits by the percutaneous route was 11.2 ml/kg. Dilution in deodorized kerosene markedly increased toxicity by skin penetration.

Starr et al. (1950) conducted toxicity tests with technical allethrin on white rats. White rats exhibited no harmful effects when fed 0.2% in diet for 24 weeks. When a 1% aerosol mist was inhaled 30 minutes per day, 6 days a week for 22 weeks, microscopic examination of the organ showed no abnormalities. Ambrose and Robbins (1951) administered allethrin gastrically and subcutaneously to rats and also to the skin of guinea pigs and
three humans. They concluded that allethrin was no more toxic than pyrethrins. The little toxic reaction observed may have been due to impurities.

Oral administration of pyrethrum to male rats at 200 mg/kg for 23 days resulted in liver enlargement with decreases in hepatic DNA concentration, while total lipid concentrations were increased. Protein and hepatic water concentrations were not different from control. A significant decrease in hexa-barbital induced hypnosis without concomitant changes in barbital suggested a pyrethrum caused alteration in hepatic drug metabolism (Springfield et al. 1973).

In the 1000 ppm group no clinical signs of intoxication were seen but there was some reduction in body weight gain with reduced food intake. Blood urea concentrations were increased and plasma alkaline phosphatase activity decreased. No pathological changes were seen. Special preparations of sciatic nerves from rats of the 1000 and 2000 ppm groups showed no neuropathological changes. At 500 ppm (equivalent to approximately 25 mg/kg day) the only differences between the control and experimental rats were increases in liver weight (female only) and kidney weights (both sexes). Plasma alkaline phosphatase activity was decreased but not in a dose-related manner. In the absence of any pathological changes these apparent effects are considered to be without any toxicological significance.
In another experiment, sumicidin was administered orally to mice of ddy strain for three consecutive months. At the higher dosages (1000 and 3000 ppm) the animals exhibited hypersensitivity during the first month of feeding. Body weight gain with higher two dosage levels (males only at 3000 ppm) was significantly low. Hematological, blood biochemical, gross and histopathological examinations revealed some adverse effects, namely multiple focal necrotic foci with infiltration of oval cells in liver with abnormalities of some parameters in liver functional testing.

At a dietary concentration of 300 ppm, no toxic effects were seen. Toxicity of sumicidin was observed after subacute inhalation at the aerial concentrations of 0, 2, 7 and 20 mg/m³ in mice and rats. With the highest concentration, both mice and rats exhibited daily transient symptoms of intoxication such as, hypersensitivity during the experimental period. No lethality was observed in any group and there was no significant difference between groups exposed to sumicidin and solvent or non-treatment groups in body weight gain. Hematologic and biochemical parameters at the termination of the experiment were quite normal. No pathological effects were seen. The aerial concentration of sumicidin having no effect in rats and mice under the conditions of this study is therefore 7 mg/m³.

Poisoning of rats with 5-benzyl-3-furylmethyl (IR)-cis-chrysanthemate (White et al. 1976) is associated with critical
brain concentrations of these insecticides. There are large differences in the toxicity of decamethrin to mice depending on the route of administration and the carrier vehicle.

Irrespective of these factors, a critical concentration of decamethrin in brain is seen, which correlated with the onset of tremors or the time of death. Further direct intracerebral administration of decamethrin at approximately this brain level gives a similar poisoning effect. The brain appears to be a primary target in decamethrin poisoning of mice and possibly also on a more general basis in pyrethroid poisoning of mammals (Ruzo et al. 1979).

Wouter and Vander Bercken (1978) accepted that pyrethroids produce effects by a prolongation of the transient increase in the sodium permeability of the nerve during the excitatory phase of the action potential. This effect is directly responsible for the repetitive nerve activity. These poisoning syndromes are apparently not due to primary interference with single inhibitory central nervous system transmitters (Cremer et al. 1980, Smith 1980). Yet there is little evidence to suggest a selective action on any of the other central neurotransmitters since a number of centrally acting pharmacological antagonists produce little modification of poisoning syndromes (Staatz et al. 1980).
The toxicity of 36 pyrethroids given intravenously to rats was examined (Verschoyle and Aldridge 1980). These cause either T-syndrome, consisting of aggressive sparring, sensitivity to external stimuli, finally progressing to gross whole body tremor, and cs-syndrome, consisting of pawing and burrowing behaviour, salivation, coarse tremor, progressing to sinuous writhing (choreathetosis) and chronic seizures. The poisoning effect may be due to action of the increased sodium conductivity produced by the pyrethroid at different durations. The motor and other behavioural changes observed during poisoning in animals may also be explained by a differential susceptibility of certain neurons.

Bradbury et al. (1981) stated that pyrethroid produces the classic syndromes of toxicity progressing to death. Animals injected with cismethrin died within 16 minutes of injection and those with deltamethrin died within 35 minutes.

Mouse intracerebral toxicity was studied with 29 pyrethroids. Alpha-cyano-3 phonylobenzyl esters produced choreathetosis, convulsion and salivation. High stereospecificity lacking the alpha-cyano group induced tremors and convulsions (Lawrence and Casida 1982). Intracerebral investigation established that profuse salivation was not unique for pyrethroids with the alpha-cyano group. Most rapid action in the brain was of those with the highest knock-down activity for insects. The cyanophenoxybenzyl
esters, in comparison with the non-cyanopyrethroids, had a high intracerebral toxicity relative to their synergized intraperitoneal toxicity.

The cardiovascular action of two pyrethroids, deltamethrin and cismethrin were assessed by Forshaw and Bradbury (1983) using the pithed rat, the isolated working heart and perfused mesentery preparations. Deltamethrin but not cismethrin, increased mean arterial and differential pressures in the pithed rat, and the aortic heart. Reserpine pretreatment reduced, but did not abolish the response to deltamethrin, and did not reduce the increase in aortic output. In the profused mesentery, deltamethrin did not modify the action at exogenous noradrenaline (norepinephrine) but increased the response to 10 Hz stimulation. The cardiovascular effects of deltamethrin are evidently due to both increased catecholamine release in peripheral vascular beds, and to a direct positive inotropic effect on the heart.

A heterogeneous particulate fraction of mouse brain homogenate bound NRDC 157, a potent pyrethroid insecticide was studied by Soderlund et al. (1983). Stereospecific binding was a minor component of total binding (2.8%). The remainder of observed binding was predominantly nonspecific and unsaturable, stereospecific binding was half saturated at $4 \times 10^{-3}$ and fully saturated at $>1 \times 10^{-7} M$. The stereospecific binding capacity of this preparation was 200-250 pmol NRDC 157/g equivalent brain tissue (2.3-2.8 pmol/mg protein). This binding site may have represented the
neural receptor involved in stereospecific toxic action of pyrethroid. To better characterize the behavioural toxicity of pyrethroid insecticides, comparisons were made of the effects of cismethrin and deltamethrin on motor activity and the acoustic startle response in male Evans rats. Deltamethrin (0, 2, 4, 6 and 8 mg/kg) was administered orally in 0.2 ml/kg corn oil. Cismethrin and deltamethrin produced similar dosage-dependent decrease in motor activity. The time course of onset and recovery of this decreased activity was rapid (1–4 hours) (Crofton and Lawrence 1984).

**Rabbits:**

Carpenter *et al.* (1950) stated that undiluted commercial allethrin and dilutions in deodorized kerosene were harmless to rabbit eyes, but caused moderate erythema of the clipped skin of the rabbit belly when applied in singly or repeatedly. Cloth impregnated with allethrin at the rate 4g per square foot caused marked erythema of the hair-free trunk of rabbits when worn for three days. Subsequently these reactions subsided even though the impregnated bands were applied twice each week for 3 weeks. No systemic injury, as judged by weight changes, resulted and all skin reaction had subsided in this interval.

Extracts of pyrethrum flowers, obtained by treatment with various aqueous and organic solvents, were tested for immediate and delayed hypersensitivity reactions in guinea pigs previously
sensitized to pyrethrum by a single subcutaneous injection of the ground flowers suspended in Freund's complete adjuvant. Strong reactions were elicited by various aqueous extracts of the flowers. An extract of the spent flower with methylene chloride showed irritant effects in unsensitized guinea pigs (Rickett et al. 1972).

Five patients with allergic contact dermatitis from chrysanthemum oil of turpentine and its sensitizing compounds gave no patch test responses (Schulz et al. 1975). No relationship was seen between contact allergy due to chrysanthemum and to turpentine oil. The pyrethrins play no role in chrysanthemum allergy.

A series of experiments conducted by the manufacturers, Sumitomo Chemical Co., Japan (1978), suggested that undiluted technical sumicidin gave a low order of acute percutaneous toxicity. The LD₅₀ value in the rabbit is between 1000 and 32,000 mg/kg. No death occurred at 1000 mg/kg but signs of intoxication, which included convulsion and ataxia, were observed at this dosage and in one out of six rabbits given 560 mg/kg. In another experiment, rats (12 of each sex per dose group with 5 each sex controls) were fed 0, 125, 500, 1000 and 2000 ppm of sumicidin for three months. After one week all rats in the last group showed hypersensitivity to sound and touch, tremors and ataxia with splayed hind limbs. One male and three females from this group survived. No compound-related pathological effects were seen
although most of the animals which died showed contraction of the muscle coat of the bladder wall.

Undiluted technical sumicidin was mildly irritant to occluded rabbit skin and moderately irritant to rabbit's eyes. However, the undiluted emulsifiable concentrate showed moderate to severe irritation potential to occluded rabbit skin and was severely irritant to rabbit's eyes. But undiluted ULV formulation was moderately irritant to rabbit skin and moderately to severely irritant to rabbit eyes. A skin sensitization test with technical sumicidin in guinea pigs was negative (Technical Information Sheet, Pesticide Division, Sumitomo Chemical 1978).

Some tests carried out by Dangogo and Jeanpaul (1983) in the field have shown irritant effect of all insecticides. The irritability was highest in OMS 570 (enclosulfan). It was lowest in OMS 2000 (Sumicidin), OMS 1998 (Decamethrin), OMS 2002 (Cypermethrin) and a mixture of OMS 1998 and OMS 570.

Dogs:
Yamamoto (1923) found that pyrethrin had no effect on dogs when introduced into the stomach. Zeigler (1923) also reported that guinea pigs showed no toxic symptoms from subcutaneous injections or from applications of powdered pyrethrum to the mouth and nostril. Dogs were not affected by feeding or by subcutaneous injections, but intravenous injection produced convulsions, sometimes resulting in death. Chevalier (1930) found that intraperitoneal injections of 10 to 15 mg of pyrethrins per kg of body weight caused nervous disturbances and 6 to 8 mg of pyrethrin per kg in dog caused death.
Effect on Man:

Some workers are affected with dermatitis when handling pyrethrum or its extracts (Mc Cord et al., 1921). Garratt and Bigger (1923) reported asthma due to the use of pyrethrum in the patient's bed. Cases of pyrethrum allergy has also been reported (Ramirez, 1930). Allergic reactions have also been observed among those picking the fresh flowers. Feinberg (1934a) stated that pyrethrum apparently contains three toxic principles: (a) the active insecticidal esters, the pyrethrins; (b) the substance that causes the dermatitis; (c) the specific allergen, which may give rise to symptoms of allergy, particularly in respiratory tract.

Feinberg (1934b), from a series of 225 patients sensitive to ragweed pollen, obtained cutaneous reactions with extract of pyrethrum in 46 percent of the cases. He attributed it to a group sensitiveness occurring only in ragweed sensitive individuals. Since the allergenic substances of ragweed pollen and pyrethrum were closely related, he warned that ragweed sensitive people may be susceptible to allergic attacks at any time of the year as the result of exposure to pyrethrum products.

Schwartz (1934) found 20 cases of dermatitis in a factory manufacturing pyrethrum-oil sprays. Patch tests on the most susceptible individual, with all the ingredients used in the plant, showed that he was markedly hypersensitive to pyrethrum flowers. The perfume and oil used gave negative or slight reactions. The belief that pyrethrum is toxic to cold blooded animals and non-toxic to warm blooded ones seems to be well founded. Sweitzer and Tedder (1935) reported only four cases of allergic sensiti-
vity to pyrethrum in 618 patients treated.

Audiffren (1934) found chrysantheum monocarboxylic acid in the urine of a patient to whom pyrethrum had been administered as a vermifuge. Shimkin and Anderson (1936) had also investigated the action of pyrethrins on mammals. On the other hand, Cnadinger (1945) had handled pure pyrethrin in considerable quantities without ill effect and had taken orally doses of about 50 mg with no effect except the numbing of the tongue and lips.

Mitchell et al. (1972) observed that in a patient with allergic dermatitis from pyrethrum, pyrethrosis produced strong and pyrethrin II weak, positive patch test responses. Some other compounds derived from pyrethrum, viz., chrysanthemumic acid, its ethyl ester and its naturally occurring insecticidal esters pyrethrin I, onerin I and II, jasmolin I and II; the synthetic ester allethrin; chrysanthemin taraxaterol (pyrethrrol) and tiglic acid produced negative patch test responses. In this patient, pyrethrosis, a sesquiterpene lactone which does not have insecticidal properties, was the principal allergenic fraction of pyrethrum derived from species of Chrysanthemum. Of the 8 esters of chrysanthemumic acid tested pyrethrin II was also allergenic in this case.

The pyrethroids received further attention when their use was rapidly becoming widespread. Under normal spraying conditions, these compounds have a more than adequate margin of
safety with regard to toxicity to man, but when directly applied they are known to be potent neurotoxic agents in mammals (Verschoyle and Barnes 1972, Barnes and Verschoyle 1974). With the increase in use and availability of these compounds, the possibility of people who handle some of the more toxic pyrethroids being intentionally or accidentally exposed to toxic amount, becomes more likely. As yet, however, there is no compound available that is capable of protecting mammals against pyrethroid-induced toxicity. Cismethrin produced a fine tremor rapidly after injection while deltamethrin caused a rapid onset of profuse salivation in rat with a more gradual development of whole body tremor and writhing seizures (Verschoyle and Barnes 1972).

Apparently, in mammals decamethrin is approximately twice as toxic as cismethrin and its mechanism of toxicity is different from the resmethrin-type pyrethroid. The neurotoxicity of pyrethroid, on both mammals and insects, depends on their stereochemical configuration suggesting that a stereo-specific binding possibly within the spinal cord is responsible for pyrethroid toxicity (Gray et al. 1980).

10. METABOLIC PATHWAYS

Fenvalerate is the member of the $\alpha$-cyano-3 phenoxy-benzyl group. The fate of the $\alpha$-cyano-3-phenoxybenzyl moiety
is similar to that of the 3-phenoxybenzyl moiety in that hydroxylation occurs at the 2'-4' positions. 3-phenoxybenzoic acid and its 2' and 4' hydroxy derivatives are formed and then conjugated (Casida and Ruzo 1980). There is an exception, however, because hydroxylation occurs at the 5-position in the α-cyano-3-phenoxybenzyl esters rather than the 6-position noted with the 3-phenoxybenzyl esters. In soils, the cyano group is hydrated to the amide which is then hydrolysed to the carboxylic acid. The 3-hydroxybenzyl ester is also formed in soils. The 2-hydroxynitrile liberated on ester hydrolysis is mostly cleaved to the aldehyde and hydrogen cyanide, but on cotton a portion undergoes glycoside formation. The aldehyde is reduced to alcohol, which is partially conjugated, or is oxidised to acid. Cypermethrin, deltamethrin and 3-phenoxybenzoic acid give N-(3-phenoxybenzyl) taurine in mice but not in rats. Cyanide is converted by rhodanese to thiocyanate or it is conjugated with cysteine to give the 2-iminothiazolidine-4-carboxylic acid, excreted by rats but not by mice (see figure II).
FIG. METABOLIC PATHWAY OF \( \alpha - \text{CYANO-3-PHENOXYBENZYL} \) MOIETY. (CASIDA AND RUZO 1980)
Metabolism: An outstanding characteristic of pyrethroids, which distinguishes them from other lipophilic insecticides, such as the chlorinated hydrocarbons, is susceptibility to attack metabolic systems. All known active pyrethroids are esters, and one route by which they are inactivated is ester cleavage, of varying importance, according to the degree of steric hindrance by groups around the linkage. Most pyrethroid structures also have numerous sites susceptible to attack by mixed function oxidase systems. This susceptibility is independent of other properties, e.g. photostability, so that compounds have been discovered that persist in abiotic environments, yet are rapidly decomposed in metabolizing systems (Elliott et al. 1978).

In Mammals

The favourable toxicological properties of pyrethroids are related to their ability to penetrate rapidly to and interact with, the site of action in insects, whereas after external or oral administration to mammals they are eliminated, intact or modified by metabolism before reaching sensitive regions. That sites in mammals are susceptible to the action of pyrethroids, is indicated by the higher intravenous toxicity of some compounds, which is much greater than oral administration (Verschoyle and Barnes 1972). Elliott et al. (1972b) studied the structure of three metabolites: from pyrethrins I and II. Pyrethin I probably was first oxidized at the transmethyl group of iso-
butenyl side chains of the acid, to a carboxyl group. The methoxy-carbonyl group of the acid side chain of pyrethrin II was rapidly hydrolyzed to the free acid. The methyl group was less easily attacked than the methoxy-carbonyl group, which explained the high toxicity of pyrethrin I to rats and the large proportion recovered, that was unchanged. The carboxylic acid derived from both pyrethrin I and II was a common intermediate for the next stages in the metabolism. This occurred at the terminal double bond in the alcoholic side chain. The products were 2 diols and conjugate of I group of the isobutenyl side chain. This was the position in pyrethroids most susceptible to metabolic attack. Unlike the terminal double bond in the alcoholic side chain of the pyrethrins, there was no dominantly reactive centre in allethrin, so several other creations were favoured. To a small extent, the central ester link in allethrin, but not in the pyrethrins, was cleared because small quantities of the chrysanthemum dicarboxylic acid allethrolone was isolated. The cyclopropane ester link was apparently hydrolyzed after methyl group was oxidized. The hydroxylated products of the pyrethrin probably were less toxic than the parent compounds to mammals. Mammalian systems quickly and extensively degraded pyrethroids, which may explain their low mammalian toxicity.

Elliott et al. (1972b) again suggested that the oral administration at radio-labelled pyrethrin I, pyrethrin II and allethrin to rats produced several urinary metabolites, identified
by chromatographic and spectroscopic analysis. Each isolated metabolite contains a trans-2-carboxyprop-1-enyl side chain resulting from oxidation of the chrysanthemate isobutenyl group of hydrolysis of the pyrethrate methoxycarbonyl group. Also, the cis-2, 4'-pentadienyl side chain of pyrethrin I and pyrethrin II is modified to give a cis-4', 5''-dihydroxypent-2' enyl group, 4' conjugate of this diol, or a trans-2', 5''-dihydroxypent-3-enyl group. Allethrin is oxidised not only at the chrysanthemate isobutenyl moiety but also at the allyl group to the 1'-hydroxyprop-2' enyl and 2', 3'-dihydroxypropyl derivatives, or a methyl on the cyclopropyl moiety to a hydroxymethyl derivative. Allethrin gives some chrysanthemum dicarboxylic acid and allethrolone. Rapid detoxification in mammals by these metabolic pathways is probably an important factor in the selective toxicity of pyrethroids (White et al. 1976).

The mammalian toxicities of pyrethroids are generally lower than those of the other classes of insecticides (Elliott 1977) and they are unlikely to prevent any of the compounds being used in the applications for which they are best suited by physical properties and insecticidal activity. Even the very potent insecticide, decamethrin is less toxic by the intravenous route (Barnes and Verschoyle 1974, Roussel uclaf/Procida 1977) than the natural constituent pyrethrin II. Detailed investigations of mammalian metabolism and toxicology of an increasing number of synthetic pyrethroids (Miyamoto et al. 1971, Elliott et al. 1972, A, technical data sheet).
Casida et al. 1975/6, Elliott et al. 1976, Miyamoto 1976, Gaughan et al. 1977b) are providing a rational basis for interpreting relative potencies to mammals and the information needed to establish their safety for domestic and agricultural application.

Decamethrin, a highly potent synthetic pyrethroid insecticide, is metabolized by rat (Elliott et al. 1974b, Elliott 1977, Ruzo et al. 1978) and mouse liver microsomal enzymes (Shono and Casida 1978, Shono et al. 1979) by pathways that included hydroxylation of methyl group trans to the carboxyl group, hydroxylation at the 2', 4' and 5 positions of the alcohol moiety and hydrolysis of parent compound or its hydroxy derivatives.

Ruzo et al. (1978) observed that in rats the phenolic metabolites are excreted as glucoronides and sulphate and the carboxylic acid metabolites as glucuronides and glycine conjugates. The cyano fragment is converted via HCN to SCN, which is temporarily localized in the stomach and skin prior to excretion and small amounts of 2-imino thiazolidine-4-carboxylic acid, which are excreted more rapidly. Analogous reactions (Crawford and Hutson 1977, Casida et al. 1979) are established in rats for compounds with the unresolved alcohol moiety (i.e., $\alpha$-$\text{RS}$) esterified with $\alpha$-(4-chlorophenyl) isovaleric acid or 2,2,3,3-tetramethyl cyclopropane carboxylic acid.

Ruzo et al. (1977, 1978) suggested that decamethrin metabolism in mice involves four sites of oxidative attack, hydro-
lysis and a variety of conjugation process. Mice excreted less unmetabolized decamethrin than rats, suggesting a more efficient absorption and/or metabolism. Whereas rats hydroxylate decamethrin predominately at the 4' position, mice produce considerable amount of t-2' and 5-hydroxy derivatives.

Decamethrin hydrolysis yields phenoxybenzylcyanohydrin (Shono et al., 1979) which readily degrades to Piperonylbutoaldehyde (PB ald) and HCN (Ruzo et al., 1979). The alcohol moiety metabolites found from piperonylbutoxide (PB) generally followed the same pathways in mice as in rats (Ruzo et al., 1978) with some important exceptions. Only mice extensive conjugate PB acid with taurine (Hutson and Casida, 1978) and excreted glucuronide of 4'-OH-PB ald and 5-OH-PB acid. Mouse but not rat excreta contain PB ald and PB ald and its glucuronide. The aldehyde appears to be less easily oxidized in mice than in rats, and a portion is reduced to the benzyl alcohol. Rats but not mice convert HCN to iminothiazolidine carboxylic acid in addition to SCN.

Ruzo et al. (1979) suggested that the decamethrin is detoxified in mice by both oxidative and hydrolytic processes. Thus pretreatment with either PB and S,S,S,tributylphosphorotrithioate (DEF) increases decamethrin toxicity, reduces its rate of in vivo metabolism, and of product excretion and elevates decamethrin levels in fat and brain. Decamethrin-hydrolyzing esterases are generally sensitive to organophosphate inhibitions. This factor contributes to the
rapid detoxification of decamethrin. The relevant esterases are present in many tissues and the oxidases in at least liver microsomes.

Casida and Ruzo (1980) found that the parent pyrethroid is the neuroactive agent and its metabolism is a detoxification process relative to the poisoning of insects and mammals. Possible exceptions for acute or chronic toxicity are cyanide released from cyano-pyrethroid (Casida et al., 1979, Ohkawa et al., 1977) and (IR)-trans chrysanthemic acid from low toxicity chryanthemates (Ueda et al., 1974). Nakayama et al. (1978) reported that the (s)-acid, (r)-alcohol isomer of fenvalerate is biologically recemised to the (S)-alcohol compound (esfenvalerate), a product of greatly increased insecticidal activity. Shono et al. (1979) and Ruzo et al. (1978) stated that however, with deltamethrin this epimerisation is a slow process which is probably not enzymically mediated.

11. ENZYME SYSTEMS

(a) Esterases

Abernathy et al. (1973) found that the ester group of primary alcohol chrysanthemates is cleaved by mouse hepatic microsomal esterases, more rapidly for the (+) transthan for the (+) cis isomers. Substrate specificity and inhibition studies in vivo established that these pyrethroid hydrolyzing esterases probably contribute to the low mammalian toxicity of biores-
methrin and other (+) trans chrysanthemate insecticide chemicals derived from primary alcohol. Springfield et al. (1973) observed that after oral administration of pyrethrum to male rats, the activity of hepatic microsomal enzymes responsible for EPN \(-\text{O-ethyl-0(4-nitrophenyl)-phenylphosphonothioate}\) detoxification, P-nitroanisole demethylation and hexobarbital oxidation were increased over control. No significant increase were noted in the methylation of aminopurine or oxidation of L-tryptophan. Increase in liver weight, the detoxification of EPN and demethylation of P-nitroanisole were dose related.

Jao and Casida (1974) and Abernathy (1973) found the assay of some insect and mammalian esterases to be facilitated by preparation of 'acetone powder' to remove lipids or interfering materials. Attention should be given to possible changes in substrate specificity during enzyme preparation. Esterases from rat plasma (White et al. 1976) and five insect species (Jao and Casida 1974) vary in their trans/cis substrate specificity.

Much is known about the substrate specificity, purification and properties (Suzuki and Miyamoto 1978) of pyrethroid-hydrolysing esterases from liver microsomes. The organophosphate-sensitivity esterase, that hydrolyses the \(3\)-(2,2 dihalo-vinyl) 2, 2-dimethylcyclopropanecarboxylates, at low substrate levels, are present in the blood, kidney, liver, lung and stomach of rats, mice and rabbits. Optimal conditions remain to
be developed for assay of most of these esterases (Casida and Ruzo 1980).

(b) Oxidases:

The preferred site of pyrethroid hydroxylation varies with the source of the insect or mammalian microsomal oxidase (Soderlund and Casida 1977, Shono and Casida 1978 and Shono et al. 1979). The oxidation rate also depends on the history of previous exposure to inducing agent (Crawford and Hutson 1977).

Metabolic pathways in the enzyme systems were examined for trans and cis permethrin and cis-cypermethrin and for decamethrin (Shono et al. 1979). The preferred sites of hydroxylation, based on all identified metabolites in the oxidase and esterase-plus-oxidase system are generalized relative to the acid moiety. Mouse and rat enzymes preferentially hydroxylate the cismethyl group of the trans pyrethroids and the transmethyl group of the cis-pyrethroids. The opposite relationship in the preferred methyl group is observed with housefly microsomes. Looper enzymes hydroxylate only the transmethyl group of cis-permethrin. The preferred site of hydroxylation in the alcohol moiety is the 4' position with all substrates, but in second site preference varies with the pyrethroid. The cyano group appears to influence the substrate orientation of the oxidase site or the reactive of various positions in the benzyl moiety. Alternatively, the cyanopyrethroids may be acted on
in part by an oxidase not involved with the noncyano compounds.

Soderlund and Casida (1977) studied the effect of structural modification on biodegradability or enzymatic metabolism with 44 pyrethroids using mouse liver microsomal esterase and oxidase preparations. The esterases were found to be the most important in metabolizing primary alcohol esters of cyclopropanecarboxylic acids with trans side chains such as 150 butyl or dihalovinyl substituents of cyclopropene c-3. The mixed function oxidase system dominated the metabolism of alcohol cis-substituted cyclopropanecarboxylates. An α-cyano group is the rate of both enzymatic hydrolysis and oxidation. Pretreatment of mice with an appropriate esterase or oxidase inhibitor usually showed increased susceptibility to pyrethroid intoxication.

(c) Cytochrome-c-reductase

Synthetic pyrethroids have an ability to alter microsomal cytochrome P-450 and NADPH cytochrome-c-reductase in rats. Permethrin (80:20 cis-trans) 50 mg/kg per day orally, increased cytochrome P-450 after 4, 8 or 12 days of administration and NADPH cytochrome-c-reductase after 8 or 12 days. A mixture containing less cis form(40:60) cis-trans did not alter either cytochrome P-450 or NADPH cytochrome-c-reductase after 4 days of administration but increased both after 8 or 12 days. L-cyano
anologs of permethrin (40:60 cis-trans and 97:3 cis-trans) did not induce either cytochrome P-450 or NADPH cytochrome-c-reductase (Carlson and Gerald 1980).

12. ISOMERS, IMPURITIES AND PHOTOPRODUCTS OF PYRETHROIDS

In addition to the major insecticidal components, each pyrethroid contains from trace level to major amounts of isomeric materials and other impurities. Even isomerically pure pyrethroids are photodecomposed under environmental conditions to yield many photoproducts of widely varied chemical types. Among the pesticides, the pyrethroids yield an unusually large number of metabolites and photoproducts, for instance, 79 metabolites from the permethrin isomers occur in various species and systems. The variety of biodegradable substituents in pyrethroids and their impurities and photoproducts help to insure their environmental degradation (Casida and Ruzo 1980).

13. UNIDENTIFIED METABOLITES OF PYRETHROIDS

Investigations by Casida and Ruzo (1980) focus on maximum accountability by identifying as many metabolites as possible using various combinations of isolation, spectroscopy, derivative formation, degradation and synthesis. Low percentages of identified products are evident in the following cases: allethrin for which the studies emphasized identification but
not quantitation (Elliott et al. 1972b), acid-labelled chrysanthemates (upto 34% identified and no studies on most compounds); alcohol-labelled benzylfurymethyl esters (upto 32% identified) where much of the difficulties arises from unstable metabolites; and cis as compared with trans isomers where the greater stability of the cis isomers gives more parent compound, but their greater degree of oxidation results in lower amounts of identified metabolites.

14. MUTAGENICITY STUDIES

Little work has been done on mutagenicity of pyrethroids so far. Rec assay by Bacillus subtilis H45, mutagenic assay by Salmonella typhimurium TA 1535, TA 1538, TA 100, TA 98 with and without rat liver microsomal enzyme preparation, as well as host-mediated assay and dominant lethal assay in mice and a chromosome study in Chinese hamsters, all indicated no mutagenic potential of sumicidin (Sumitomo Chemical Co., 1978). Deltamethrin was not found to be mutagenic in assays with Salmonella typhimurium strains, Escherichia coli WP2 or Saccharomyces cerevisiae D3, with or without rat liver S9 metabolic activation (Kavlock et al. 1979). Similar result was obtained by Miyamoto (1976) with permethrin, cismethrin, bioresmethrin and resmethrin. These were neither mutagenic nor DNA-damaging in various strains of Salmonella typhimurium, Bacillus subtilis or E. coli using
the spot test nor in the host mediated assay in mice with Salmonella typhimurium G46 strain.

Cypermethrin is negative in several mutagenicity studies. For example, it did not induce mutation in the mouse host-mediated assay (Brooks 1976) or in E. coli or S. typhimurium TA 1538 in vitro either in the presence or absence of the microsomal oxidation system (Brooks 1976) nor did cypermethrin (at a dose level up to 1 mg/plate) increase the number of revertant colonies of S. typhimurium (TA 1535, TA 1537, TA 1538, TA 98 and TA 100) in the presence or absence of mouse liver subcellular activation preparation obtained from 6 strains of PCB-treated mice (Suzuki 1977).

Permethrin and resmethrin showed no genetic activity in any of six in vitro short-term tests including assays with bacteria, yeast and human fibroblasts (Simmon et al. 1977). Fenvalerate was not found to be carcinogenic in B6C3F1 mice (Parker et al. 1983). In the absence of an exogenous metabolic activation system, bioresmethrin, resmethrin and cismethrin, at concentrations higher than 5 \( \mu g/ml \) were highly toxic to V79 Chinese hamster cells, permethrin and cypermethrin were weakly toxic and fenvalerate and deltamethrin were not toxic at concentrations ranging from 5 to 4 \( \mu g/ml \).

None of these compounds induced significant delayed
toxicity. No cytotoxicity was observed with any compound when assays were carried out in the presence of rat hepatocytes. Thus, rat hepatocytes protected V79 cells from the toxicity induced by some of the pyrethroids. The protective effect may be attributable to metabolic detoxification of the pyrethroids within rat hepatocytes and/or their lower accessibility to V79 cells, since the density of the rat hepatocytes was 4-fold higher than that of V79 cells. Permethrin, cypermethrin, deltamethrin, fenvalerate and bioresmethrin were not found to be mutagenic for either genetic locus (OUA' and TH') in V79 cells when tested in the presence or absence of rat hepatocytes. In the presence of rat hepatocytes, neither resmethrin nor cis-methrin, induced mutagenic effects in V79 cells. In the absence of rat hepatocytes, however, cismethrin at concentrations of 10 and 11.3 μg/ml produced TG1 but not OUA mutants, although this effect could be reproduced in only 2 out of 4 experiments. In this experiments (in accordance with the observations with cismethrin) resmethrin caused a slight increase in the number of TG1 mutants, but no dose response curve was obtained. The weak mutagenic effects of cismethrin and resmethrin are consistent with other findings, since resmethrin is a mixture (1:1) of cismethrin and bioresmethrin. In view of the absence of dose-response curves and of the poor reproducibility of genetic effects, unequivocal evidence that cismethrin and
resmethrin are mutagenic could not be obtained. However, the negative results obtained in the battery of tests cannot be taken as proof that these pyrethroids are devoid of carcinogenic activity (Pluijmen et al. 1984).

Cypermethrin showed a mutagenic potential in the study by Amer and Aboul-ela (1985). In dermal treatment cypermethrin (120 and 360 mg/kg body weight) was applied as a solution in 0.1 ml DMSO on a preshaved part of the back of the mice (about 2 cm). Applications were performed twice weekly for 2 weeks. This multiple treatment (total 4) caused toxicity. Signs of acute toxicity were observed and then disappeared rapidly after treatment of mice with 360 mg/kg body weight. At this dose mortality was rapid and reached 20%. Double and multiple treatments induced a significant increase in the frequency of polychromatic erythrocytes with micronuclei.

Oral administration of 300 and 900 ppm cypermethrin for 14 consecutive days increased the percentage over that of the control. The increase in the frequency of PEs was statistically significant but not dose-related. 900 ppm of the insecticide induced a statistically significant percentage of PEs with micronuclei after treatments for 7 and 14 days. This increased frequency of PEs may be a result of accumulation of pesticide within the body due to the slow elimination of cypermethrin from the tissue of mice.
A significant increase in the percentage of PEs after intraperitoneal treatment was observed, 1 and 2 days after injection with cypermethrin. The percentage of PEs returned to normal 7 days after cessation of the treatment, indicating complete recovery. Repeated treatment with cypermethrin at 60 mg/kg body weight induced a significant increase in the frequency of PEs over that of the control, after the third injection. This increase returned to normal 1 (one) week after the treatment. Toxic signs of poisoning were rapid and disappeared in the survivors after a few days. Mortality was rapid (within 30 minutes) and reached 20% and 60% after injection with 60 and 180 mg/kg body weight respectively.