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Pyrethroids

From Chrysanthemum to Modern Industrial Insecticide

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Pyrethroids

From Chrysanthemum to Modern Industrial Insecticide

Volume Editors: Noritada Matsuo · Tatsuya Mori

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Aims and Scope

The series *Topics in Current Chemistry* presents critical reviews of the present and future trends in modern chemical research. The scope includes all areas of chemical science, including the interfaces with related disciplines such as biology, medicine, and materials science.

The objective of each thematic volume is to give the non-specialist reader, whether at the university or in industry, a comprehensive overview of an area where new insights of interest to a larger scientific audience are emerging.

Thus each review within the volume critically surveys one aspect of that topic and places it within the context of the volume as a whole. The most significant developments of the last 5–10 years are presented, using selected examples to illustrate the principles discussed. A description of the laboratory procedures involved is often useful to the reader. The coverage is not exhaustive in data, but rather conceptual, concentrating on the methodological thinking that will allow the non-specialist reader to understand the information presented.

Discussion of possible future research directions in the area is welcome.

Review articles for the individual volumes are invited by the volume editors.

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Preface

Pyrethrum has been used as an insecticide for around 150 years, and there has been no other insecticide which has so successfully contributed to the control of sanitary pests. Numerous analogs have been developed by chemists worldwide since the elucidation of the chemical structure of pyrethrins, which are the insecticidal ingredients of pyrethrum. As a result, their application has expanded extensively to various fields. To date, many eminent books have been published by scientists in this field and have contributed to advancing pyrethroid science.

Pyrethroids refer to the general name for pyrethrins, insecticidal ingredients of pyrethrum, and their synthetic analogs. They exhibit quick action on insects in a small amount. At the same time, they show selective toxicity to insects over mammals. These features of pyrethroids are therefore ideal for use as household insecticides. Since both humans and insects are organisms with a nervous system, compounds with high insecticidal potency may be highly toxic also to humans, as seen in many organophosphorous compounds and carbamates. In the previous century, the absolute configuration of 6 insecticidal ingredients consisting of natural pyrethrins were elucidated and, with the advancement from natural pyrethrins to synthetic pyrethroids, their applications have developed from household insecticides for indoor use against sanitary pests to outdoor use in agriculture, forestry, construction and livestock. The development of photostable pyrethroids has led to their infinite use in various fields throughout the world.

While many drugs and agricultural chemicals have been developed from natural products with biological activities, no other compounds have been studied for a longer time and in more countries than pyrethroids. Synthetic pyrethroids have advanced markedly by modifying the chemical structure of pyrethrins and now even compounds with structures far from natural pyrethrins are called pyrethroids. This is probably the result of pursuing higher insecticidal activities, although they belong to pyrethroids in terms of electrophysiological activities. Notably,

[*] Please see the section entitled "Further Reading" for details about these books.

household insecticides should be discriminated from photostable pyrethroids for outdoor use from development stages. For household insecticides, safety for humans and pets is extremely important, and residues of photostable synthetic pyrethroids and impurities, degraded products and secondary synthetic products contained in the compounds in rooms and their influence on the environment are to be evaluated strictly. In this century, the most awaited development is that of highly safe pyrethroids which are produced based on the original natural pyrethrins with excellent insecticidal activity, safety and less resistance. However, for pyrethrum, it takes about 2 years from seeding to flowering and therefore, investigations of the mechanism of biosynthesis to improve production efficiency and advancements in this field are also expected.

Although "pyrethroids" have been developed without a concrete definition, it is quite difficult to define this group of compounds based on their chemical structures. As such, I would like to propose the following definition:

"Pyrethroids" are a collective term for compounds that are obtained by modifying the structure of natural insecticidal ingredients, pyrethrins, contained in pyrethrum while maintaining safety, to improve efficacy and provide different characteristics from pyrethrins that show high selective toxicity comparable to pyrethrins.

Since 1995 some new types of pyrethroids with high insecticidal potency have been developed for practical use. For this reason we decided to publish a volume written by experts in various fields to review the development of new pyrethroids and offer future perspectives. This volume includes chapters on the progress and the future of pyrethroids, the biosynthesis of natural pyrethrins, newly developed polyfluorobenzyl-type pyrethroids with potent insecticidal activity, the mode of action, mammal toxicology, biotransformation and enzymatic reactions, environmental behavior, and ecotoxicology of pyrethroids. We hope that this book will contribute greatly to the further development of pyrethroids.

October 2011 Dr. Yoshio Katsuda

Further Reading

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Progress and Future of Pyrethroids

Yoshio Katsuda

Abstract After the chemical structure of "natural pyrethrins," the insecticidal ingredient of pyrethrum flowers, was elucidated, useful synthetic pyrethroids provided with various characteristics have been developed by organic chemists throughout the world, leading to the advancement of pyrethroid chemistry. Even in pyrethroids with high selective toxicity, a chemical design placing too much importance on efficacy improvements may invite loss of the safety margin. It is strongly hoped that the development of household pyrethroids and their preparations for use in living environments around humans and pets will be achieved in the future by retaining the characteristics of natural pyrethrins.

Keywords Cross resistance · Natural pyrethrins · Safety · Synthetic pyrethroid

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1 Introduction

Dr. Leslie Crombie, an honor professor, was awarded an international prize in the field of agricultural pesticides at the American Chemical Society, held on August 24, 1998, in Boston. At the memorial symposium, Katsuda [1] presented a lecture.

As novel pyrethroids developed in the 10 years since that time are described in detail by the respective authors, I would like to omit them and instead review the past development of pyrethroid chemistry and comment on the future of pyrethroids.

The development of pyrethroids over the last century can be divided into two categories: (1) ingredients of household insecticides for use in and around the home, emphasizing safety, and (2) photostable ingredients for outdoor use as agricultural chemicals and for larvicides of sanitary pests. Chemically stable pyrethroids, which were initially developed for outdoor use, are sometimes applied indoors. In such cases, it is absolutely essential to resolve problems, including persistent residues of such compounds indoors, and environmental issues.

In this chapter, emphasis is placed on pyrethroids for household use.

While dried flowers of pyrethrum have been used in mosquito coils since around 1890, they have been almost entirely replaced by allethrin which resembles cinerin I, an ingredient of pyrethrins since around 1955. Quick knockdown agents of phthalthrin together with highly lethal resmethrin have become dominant in aerosol formulations since around 1970. In addition, the use of permethrin, characterized by its long residual effect, was started in insecticides for cockroach control around 1977. Different from pyrethrins, the practical application of photostable pyrethroids raised resistance problems in mosquitoes, flies, and cockroaches, and stronger pyrethroids were developed as a consequence to deal with them. It is a reality that novel pyrethroids with high insecticidal potency, even at low concentration, show the development of cross-resistance as a matter of course, necessitating an increase in their usage concentrations. Residues of insecticides indoors and effects on humans and pets are important problems which cannot be ignored.

As described in the section on “Cross-resistance” in this chapter, it was found that some insect species showed extremely low cross-resistance to three ingredients, pyrethrins as well as d-allethrin and prallethrin, although they developed resistance to photostable synthetic pyrethroids. The latter two compounds of d-allethrin and prallethrin have quite similar chemical structures and the same configuration as cinerin I (an ingredient of pyrethrins). It is considered preferable to develop pyrethroids retaining the characteristics of natural pyrethrins and household insecticides containing them in the perspectives of safety and low cross-resistance.

When developing novel pyrethroids, particularly for household insecticides for indoor use, attention should be paid not to place too much importance on insecticidal potency and ease of use while giving sufficient consideration to the indoor persistence of chemicals and safety.

Research on pyrethroid chemistry will be overviewed in the following four sections.

2 Cultivation and Utilization of Pyrethrum

Pyrethrum, originally a wild plant, is native to the Dalmatian region of the former Yugoslavia and Persia.

It is classified taxonomically into the following three species [2]:

1. *Chrysanthemum cinerariaefolium* (*Tanacetum cinerariaefolium*)
2. *Chrysanthemum roseum* (*Tanacetum coccineum*)
3. *Chrysanthemum Marshalli Ascherson*

Chrysanthemum cinerariaefolium (1) is a species of white flower and contains more insecticidal ingredients than other species. This pyrethrum species originated from Dalmatia and has been used for cultivation. On the other hand, the origin of *Roseum* (2) is Persia and the Caucasus. It has beautiful red flowers but its pyrethrin content is extremely low compared to (1). Known as red-flowered pyrethrum, it is used merely as an ornamental plant. *Marshalli* (3) originated from Persia and contains pyrethrins in negligible amounts; therefore, the pyrethrum referred to in this text is from *C. cinerariaefolium* (1).

As mentioned above, the origin of pyrethrum is the Dalmatian region of the former Yugoslavia on the Mediterranean coast of the Adriatic Sea, east of Italy. It is said that pyrethrum was discovered in 1694. While inhabitants of the pyrethrum-growing region seem to have already known about the properties of this plant and to have utilized it in powder form for insecticide applications, its insecticidal activity was verified in around 1840.

According to the record of Gnadinger, pyrethrum powder, known as "insect powder," was imported from Europe to America in around 1855 and the demand for pyrethrum increased from 600,000 lbs in 1885 to 3,000,000 lbs in 1919. Pyrethrum cultivation in the USA was achieved in California with slight success in 1859, but the business was destroyed in the 1920s, although its content of pyrethrins was 1% or higher, being superior to that of Dalmatian products. McLaughin Gormley King Company, established in 1908, imported dried flowers, extracted them with petroleum in 1919, and started the manufacture of oil-based preparations. Spraying of oil-based preparations was established in the USA due to its higher efficacy and easier use than powders.

Meanwhile, pyrethrum was introduced into Japan for the first time in 1885. Pyrethrum flowers of German origin were planted in the Medical Herb Garden in Meguro, Tokyo. According to another record, pyrethrum flowers from an American source were grown in the test farm of the Agricultural College in Komaba, Tokyo. For industrial purposes, Eiichiro Ueyama, the founder of Dainihon Jochugiku Co., Ltd., obtained seeds of pyrethrum from H.E. Amoore, an American druggist, in 1886. After starting its cultivation in Wakayama prefecture for the first time, he

promoted its plantation in the coastal regions of the Inland Sea and popularized it for overseas exportation of the plant in 1898.

As in the Dalmatian region, pyrethrum was initially utilized as a powder in Japan. In 1890, a mosquito stick of about 30 cm length was devised which had a burning time of about 1 h. Subsequently, the cultivation and processing of pyrethrum in Japan advanced gradually. In 1938, Japanese pyrethrum reached peak production of 13,000 tons per annum in terms of dried flowers, occupying nearly 70% of the world's production at that time. Pyrethrum was mainly cultivated in the coastal regions of the Inland Sea and Hokkaido. Meanwhile, the mosquito stick was improved and developed into a coil type with a burning time prolonged to 7–8 h, enough to cover human sleeping time.

Pyrethrum became the main source of household insecticides in sprays in the USA (1919) and mosquito coils (1895) as well as oil-based preparations (1924) in Japan. Thereafter, the insecticidal ingredients shifted from pyrethrins to various synthetic pyrethroids, but mosquito coils have been used worldwide for more than 110 years without changing in shape.

The different types of insecticide formulations used in the USA and Japan are considered to be attributed to the differences in climate and house construction style. That is to say, mosquito coils are suitable to prevent mosquitoes from entering a house from outside in Japan where the weather is hot and humid in summer and the houses are of an open style. These conditions are similar in subtropical and tropical zones, including south-east Asia.

After World War II, the production of pyrethrum in Japan fell markedly and declined to only 1,000 tons in terms of dried flowers in 1965. At present, pyrethrum is not cultivated in Japan and the main producers are Kenya, Tanzania, Tasmania, and China, with worldwide production in 2010 amounting to around 10,000 tons of dried flowers. Dried flowers are extracted and purified at pyrethrum-extracting factories on the spot, producing 25–50% pyrethrin extracts. While pyrethrum extracts have been replaced with various synthetic pyrethroids, they are still used in houses, food factories, gardens, and organic farms, all of which emphasize the importance of safety. Katsuda [1] reported that natural pyrethrins showed a low development of resistance by flies and mosquitoes compared with many synthetic pyrethroids, against which a high development of cross-resistance was observed.

It has been said that pyrethrins are contained in the flowers of pyrethrum but not in the leaves and, therefore, dried flowers and extracts of dried flowers have been traded.

Regarding the analysis of pyrethrins around 1950, precise analytical instruments such as those used in the present day were not available. At that time, Katsuda et al. [3, 4] determined the amount of pyrethrins contained in the flowers by Seils' method and polarography. They reported that the content reached a peak at the time of full bloom followed by a gradual decrease, with the substance contained in the ovaries of flowers (seeds). Moreover, it was also reported that the pH of a juice of fresh pyrethrum flowers was strongly acidic from the bud stage to immediately post-full bloom and that the biosynthesis of pyrethrins in the plant was interestingly performed using the acidic region from the viewpoint of the stability of

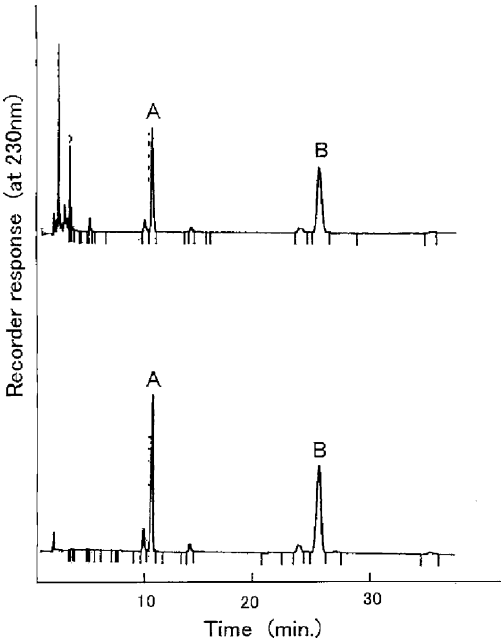


Fig. 1 HPLC chromatogram of pyrethrum leaves and authentic pyrethrins. (**a**) Extract from pyrethrum leaves. (**b**) Standard solution of pyrethrin I and pyrethrin II. *A*: pyrethrin II, *B*: pyrethrin I

pyrethrins. They questioned the biosynthesis of pyrethrins in the ovaries in such a short time and then analyzed the leaves, assuming that pyrethrins are biosynthesized by the function of enzymes in the leaves and then transported to the ovaries; however, the presence of the substance was not detected. Subsequently, spurred by the development of analytical instruments for minute amounts, Katsuda et al. [5] investigated the analysis of pyrethrum leaves from around 2000 again, and identified pyrethrins in young leaves of pyrethrum 2 months after seeding by HPLC, as shown in Fig. 1.

Determination of the contents of pyrethrin I and pyrethrin II was then made, for about 2 years (Fig. 2). Having detected pyrethrin I throughout the whole growing process of pyrethrum leaves, they reported that the pyrethrin I content, which had a close relationship with flowering, reached a peak of 0.27–0.40 wt% during flowering and was slightly lower than that in dried flowers.

Pyrethrin II was also detected in young leaves 2 months after seeding, similarly to pyrethrin I, but the content remained at about 0.05 wt% without seasonal change for 2 years. The insecticidal potency of pyrethrins obtained from pyrethrum leaves was confirmed with *Musca domestica*.

While it is conceivable that a part of pyrethrin I is biosynthesized in pyrethrum leaves and moves to flowers sequentially, biosynthesis of pyrethrin II is quite an interesting theme.

Katsuda et al. also confirmed the presence of six ingredients – pyrethrin I and II, cinerin I and II, and jasmolin I and II – in the young leaves and flowers of *C. roseum* (unpublished).

Meanwhile, this ingredient of pyrethrins has been re-evaluated as a safe raw material for insecticides, reflecting the recent trend of reverting to natural products.

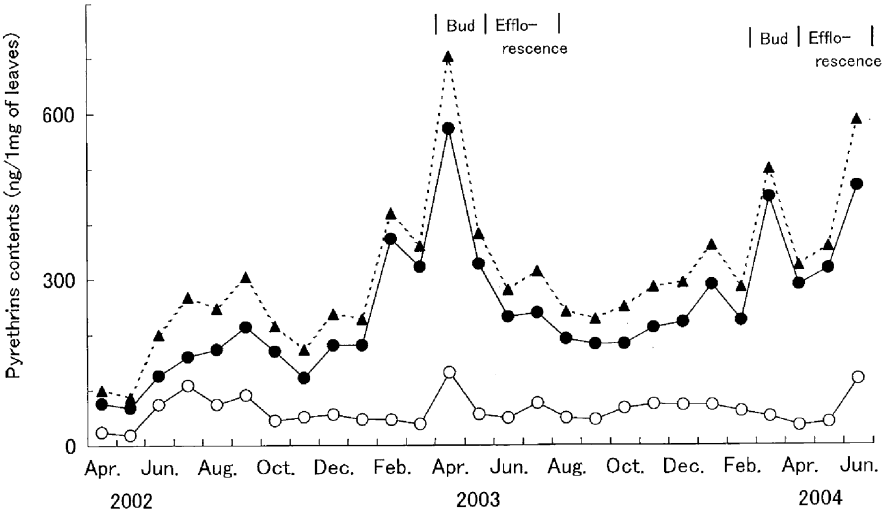


Fig. 2 Seasonal changes in pyrethrins contents in pyrethrum leaves. *Filled circles*: pyrethrin I, *open circles*: pyrethrin II, *filled triangles*: pyrethrin I + pyrethrin II. There were significant differences between changes in pyrethrin I contents and those in pyrethrin II contents (F test, $P < 0.05$)

Since it takes about 2 years from seeding to flowering of pyrethrum, it is important to elucidate the mechanism of the biosynthesis of pyrethrins in the plant to improve production efficiency.

3 Determination of the Structure of Natural Pyrethrin

Fujitani [6] separated the insecticidally active syrupy ester from pyrethrum flowers in 1909 and named the ester "pyrethron." Yamamoto [7, 8] subjected the hydrolysis product of this pyrethron to ozone oxidation, and isolated *trans*-caronic acid and aldehyde (**1** and **2**, respectively, Fig. 3). Although Yamamoto did not determine the structure of this acid, he presumed it to be "pyrethron acid" (Fig. 3). Eventually, the presence of a cyclopropane ring in the molecule of natural pyrethrins became clear for the first time in 1923.

In 1924, Staudinger and Ruzicka [9] proposed the structures of pyrethrin I and II (**3** and **4**, Fig. 4) constituting natural pyrethrins. Although there were some errors in the light of our present knowledge, their studies received widespread admiration as truly great achievements at that time. In 1945, LaForge and Barthel [10] reported that four homologs, pyrethrin I and II and cinerin I and II (**5**–**8**, Fig. 5) were contained in natural pyrethrins. The presence of jasmolin I and II (**9** and **10**, Fig. 5) was confirmed by Gordin et al. [11] in 1966, determining planar chemical structures of six ester components, as shown in Fig. 5.

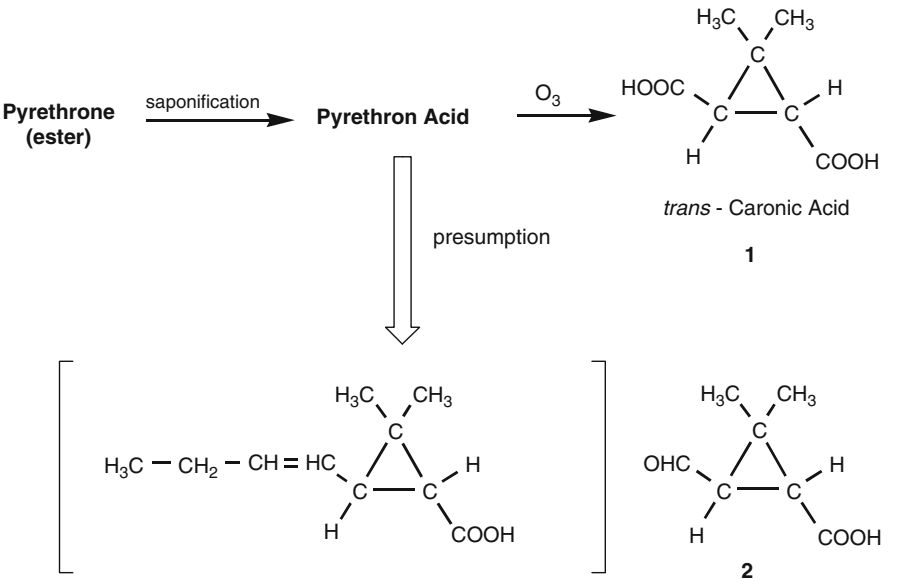


Fig. 3 Isolation of *trans*-caronic acid

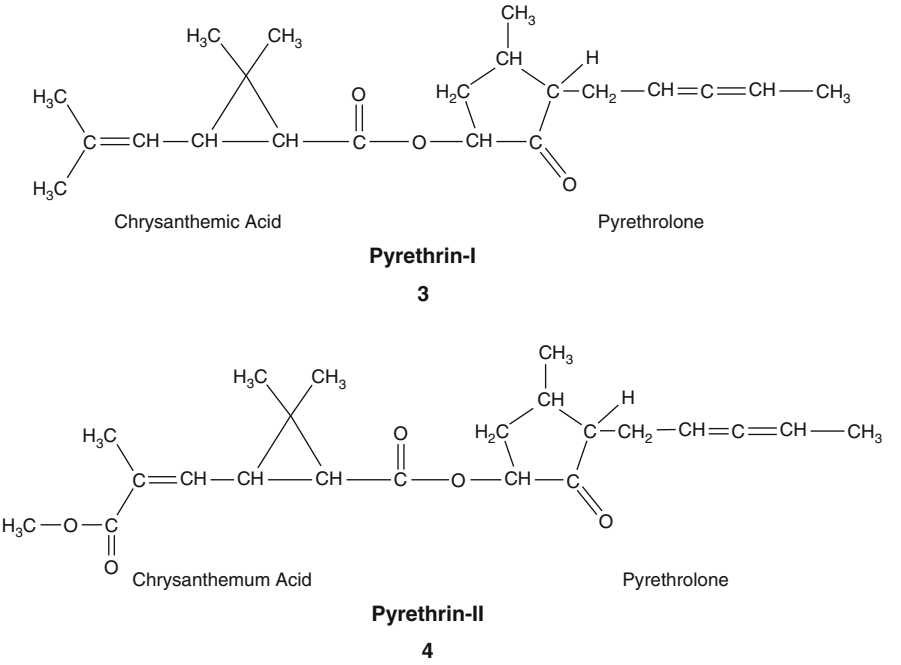
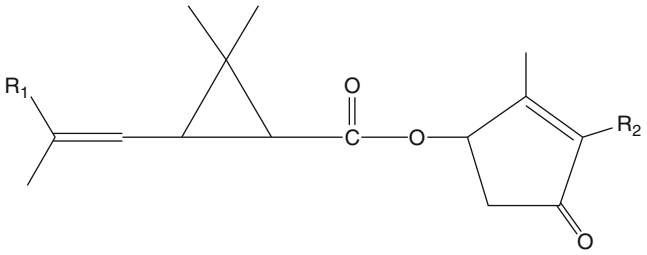


Fig. 4 Proposed chemical structures of pyrethrin I and pyrethrin II



	Compound	Acid (R_1)	Alcohol (R_2)
5	Pyrethrin I	$-CH_3$	$-CH_2\text{-}CH{=}CH{-}CH{=}CH_2$
6	Pyrethrin II	$-COOCH_3$	$-CH_2\text{-}CH{=}CH{-}CH{=}CH_2$
7	Cinerin I	$-CH_3$	$-CH_2\text{-}CH{=}CH{-}CH_3$
8	Cinerin II	$-COOCH_3$	$-CH_2\text{-}CH{=}CH{-}CH_3$
9	Jasmolin I	$-CH_3$	$-CH_2\text{-}CH{=}CH{-}CH_2\text{-}CH_3$
10	Jasmolin II	$-COOCH_3$	$-CH_2\text{-}CH{=}CH{-}CH_2\text{-}CH_3$

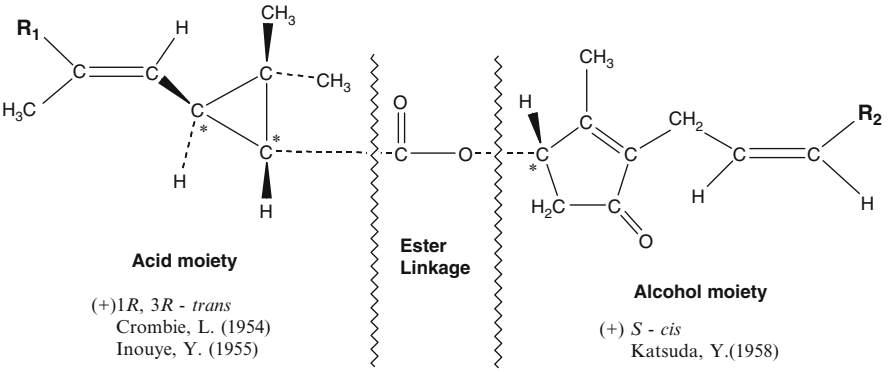
Fig. 5 Correction of the chemical structure of natural pyrethrins [12]

For the absolute configuration of the acid moieties, that of chrysanthemic acid was elucidated by Crombie et al. [13] in 1954 and that of chrysanthemum acid was determined by Inoue et al. [14] in 1955, respectively. The absolute configuration of the alcohol moiety was found by Katsuda et al. [15] in 1958. The complete elucidation of the absolute configuration of natural pyrethrins (Fig. 6) has led to the development of new useful synthetic products based on this model.

4 Development of Synthetic Pyrethroids

4.1 Modification of the Alcohol Moiety: Household Insecticides

Figure 7 shows the course of development of various synthetic pyrethroids developed by retaining chrysanthemic acid as the acid moiety and modifying the alcohol moiety. Numerous useful compounds with favorable characteristics have been derived from the structural modification of natural cinerin I (**7**). These underlined compounds have been put into practical use as active ingredients, mainly for household insecticides.



	Compound	R_1	R_2	%
5	Pyrethrin I	$-CH_3$	$-CH=CH_2$	38
6	Pyrethrin II	$-COOCH_3$	$-CH=CH_2$	35
7	Cinerin I	$-CH_3$	$-CH_3$	7.3
8	Cinerin II	$-COOCH_3$	$-CH_3$	11.7
9	Jasmolin I	$-CH_3$	$-CH_2CH_3$	4.0
10	Jasmolin II	$-COOCH_3$	$-CH_2CH_3$	4.0

Fig. 6 Absolute configuration of natural pyrethrins

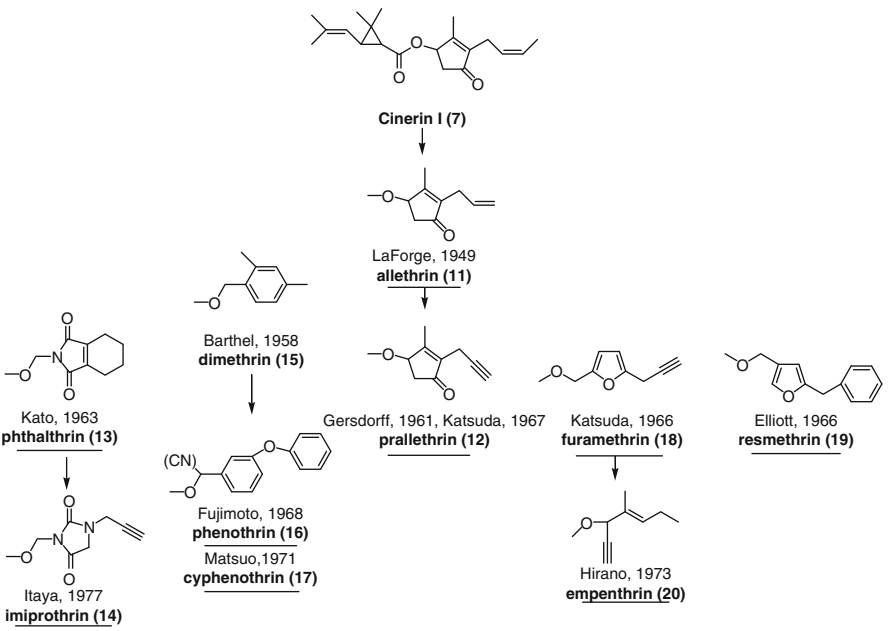


Fig. 7 Modification of the alcohol moiety (pyrethroids underlined have been commercially used)

4.1.1 Cyclopentenolone Ester

Allethrin (**11**), developed by LaForge et al. [16] in 1949, is a compound that lacks the terminal CH_3 in the side chain of the cyclopentenolone ring in cinerin I and it possesses eight isomers. Of them, d,d-*trans*-allethrin, with the same absolute configuration as cinerin I, exhibits the most potent insecticidal activity and is widely used in mosquito coils. Gersdorff et al. [17] reported in 1961 that the insecticidal activity of a compound (12) whose allyl group in the side chain of allethrin (**11**) was replaced with a propargyl group was only 60% of that of allethrin. On the other hand, it was reported by Katsuda [18] at the Second International Congress of Pesticide Chemistry (1971) that the racemic form of this compound (**12**) exhibited 1.2 times higher insecticidal activity than allethrin by the topical application method. The efficacy of mosquito coils containing the compound (**12**) was reportedly about three times as high as that of allethrin mosquito coils [19]. Then Matsuo et al. [20, 21] of Sumitomo Chemical Group accomplished the industrial synthesis of prallethrin, which has the same configuration as both chrysanthemic acid and alcohol moiety as cinerin I and d,d-*trans*-allethrin. These pyrethroids of d,d-*trans*-allethrin and prallethrin (ETOC®) possess (1*R*)-*trans*-chrysanthemic acid in common with cinerin I, one ingredient of natural pyrethrins, and their alcohol moieties all having the S-configuration differ only in the terminal of the side chain. Namely, d,d-*trans*-allethrin and prallethrin consist of only three elements, carbon, hydrogen, and oxygen, similarly to natural pyrethrins, their absolute configurations are basically the same, and they are pyrethroids with the structure most resembling that of natural pyrethrins. In terms of the LD_{50} values determined by topical application, prallethrin is more insecticidally potent than dl,d-*trans*-allethrin, being four times more effective against *M. domestica* and more than ten times against *Culex pipiens pallens*, respectively [22].

Since then, many photostable pyrethroids have been developed as agrochemicals, yet their repeated use has resulted in resistance by some insects in a short time. It has been recently reported that natural pyrethrins as well as allethrin and prallethrin showed markedly slow development of resistance, posing quite an interesting issue (described in the section “Cross-resistance”).

4.1.2 Imidomethyl Ester

Phthalthrin (**13**), developed by Kato et al. [23], shows outstandingly rapid knockdown efficacy, especially against *M. domestica*, and has been used as an active ingredient in aerosol formulations. From studies on fungicides with a hydantoin structure, Itaya et al. [24] developed imiprothrin (**14**), which is a knockdown agent in aerosol formulations for direct spraying against cockroaches. For controlling cockroaches, whose habits are usually nocturnal and latent behind objects, too much emphasis on rapid knockdown efficacy is unfavorable and the use of imiprothrin in excessive amount should be restricted from a safety viewpoint by including warnings on products.

4.1.3 Benzyl Ester

In 1958, Barthel et al. [25] reported dimethrin (**15**), which was the first substituted benzyl alcohol ester of chrysanthemic acid. This compound was not put into practical use due to its low insecticidal activities. Phenothrin (**16**), one of the *m*-phenoxybenzyl alcohol esters developed by Fujimoto et al. [26], was found to have superior chemical stability as well as safety, and has been the sole pyrethroid used as a lice control agent for humans. Further improvement was made by Matsuo et al. [27] who introduced a cyano function at the α position of the benzyl part of phenothrin, leading to α-cyano-*m*-phenoxybenzyl alcohol esters (**17**). Thereafter, this alcohol moiety has been used as a component for a number of photostable pyrethroids for agricultural purposes; however, the development of cross-resistance can be seen in some pests.

4.1.4 Furylmethyl Ester

Focusing on furan ring compounds, Katsuda [28] developed furamethrin (**18**) in 1966, which was suitable as an active ingredient of electric vaporizing insecticides due to its extremely low toxicity to mammals and its high volatility. Almost simultaneously, resmethrin (**19**) was reported by Elliott et al. [29] in 1967 as possessing a powerful lethal effect, and has been used in aerosol formulations.

4.1.5 Straight Chain Alkenyl Ester

Developed by Hirano et al. [30], empenthrin (**20**), the most volatile among the existing pyrethroids, has been in broad practical use as a moth-proofing agent. It is noted that a hint for empenthrin was taken from α-ethynyl furamethrin and acyclic alcohol ester obtained in the course of studies on the synthesis of furamethrin.

4.2 Modification of the Acid Moiety: Agricultural and Hygienic Insecticides

Pyrethroids for agricultural use were developed in the 1970s in Japan, USA, and Europe after research on photostable synthetic pyrethroids. Those compounds were composed of an acid moiety obtained by various modifications and a chemically stable alcohol component, such as benzyl group and *m*-phenoxybenzylalcohol. According to recent statistics, pyrethroids accounted for approximately 20% in value of agricultural insecticides used annually all over the world in 2009.

Insects have acquired resistance to organochlorine compounds, such as DDT and BHC, developed as agricultural and hygienic insecticides after World War II. This insect resistance was also acquired to subsequent organophosphorus compounds and carbamate insecticides. Photostable pyrethroids have been developed for outdoor use because pyrethroids were found to be effective against these resistant pests. As a matter of course, these pyrethroids are also effective against sanitary pests; however, problems associated with safety and chemical residues indoors must be resolved.

4.2.1 Cyclopropanecarboxylic Acid Esters

Figure 8a shows the development of synthetic pyrethroids with a cyclopropane ring in the acid moiety. Dihalovinyl chrysanthemic acid with halogens in place of the methyl group in the isobutenyl side chain of the parent chrysanthemic acid was first reported by Farkas et al. [31] in 1958. Later, Elliott et al. [32] prepared a series of acid esters combined with *m*-phenoxybenzylalcohol or α-cyano derivatives, such as permethrin (**21**), cypermethrin (**22**), and deltamethrin (**23**), followed by the development of cyfluthrin (**24**) [33], tralomethrin (**25**) [34], and so on. With marked improvement in photostability, these pyrethroids have a strong demand in the fields of agricultural and hygienic insecticides. Flumethrin (**26**) [35] works markedly well on ticks that are parasitic in cattle and is in wide use in Australia and New Zealand. In addition, cyhalothrin (**27**) [36] and bifenthrin (**28**) [37], in which a halogen atom of dihalovinyl chrysanthemic acid was substituted by a trifluoromethyl group, have been used as agricultural chemicals for orchard trees and vegetables and as termiticides. Tetramethyl cyclopropanecarboxylic acid ester (fenpropathrin (**29**)), developed by Matsuo et al. [38], is a compound developed on the basis of Matsui's terallethrin as a key compound and has been put into practical use as an acaricidal pyrethroid.

Transfluthrin (**30**) [39] is a compound obtained by esterification of dichlorovinyl chrysanthemic acid with 2,3,5,6-tetrafluolobenzylalcohol. With very high insecticidal potency against mosquitoes and flies, it is used as a household insecticide; however, as the promotion activity of the compound is known, its use should be restricted to preparations in which the issues of safety for humans and pets have been resolved.

Using norchrysanthemic acid, which lacks a methyl group in the side chain of chrysanthemic acid, metofluthrin (**31**) [40] was produced by esterification with 2,3,5,6-tetrafluoro-4-methoxymethylbenzylalcohol. Its vapor pressure at 25°C is 1.8 mPa (see Table 10), and its volatilization is not marked at room temperature. Nevertheless, as its basic insecticidal potency is particularly high against mosquitoes, a variety of formulations have been developed by adding a volatilization-assisting function, such as blowing and centrifugal force.

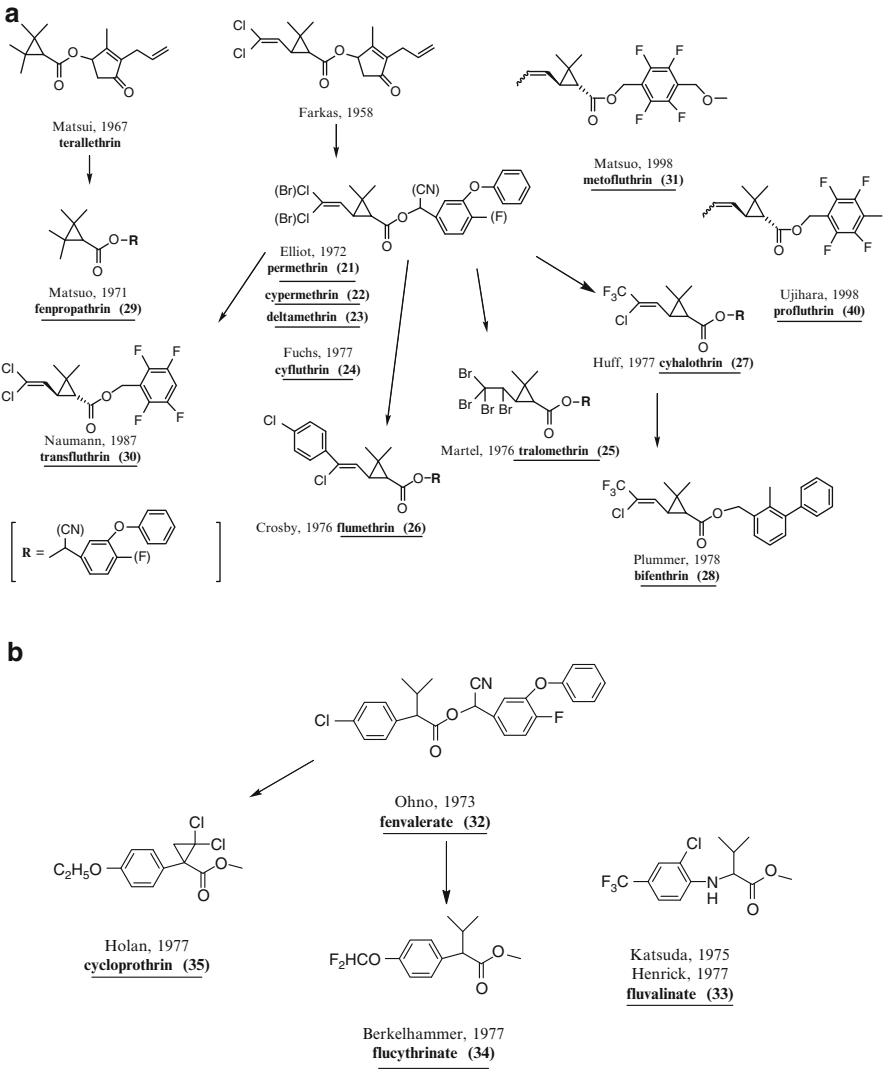


Fig. 8 Modification of the acid moiety. (**a**) Cyclopropanecarboxylic acid esters. (**b**) Non-cyclopropanecarboxylic acid esters

4.2.2 Non-cyclopropanecarboxylic Acid Esters

Figure 8b shows pyrethroid esters composed of an acid moiety without a cyclopropane ring and a phenoxybenzyl alcohol group. While a cyclopropane ring had long been considered an indispensable acid component constituting a pyrethroid skeleton, Ohno et al. [41] in 1974 developed fenvalerate (**32**), α-isopropylphenyl acetate derivative, with no cyclopropane ring in its acid moiety. This compound exhibits

insecticidal characteristics resembling conventional pyrethroids while having excellent photostability and antioxidative properties, and has been used especially for the control of cotton pests. Fluvalinate (**33**) [42, 43] and flucythrinate (**34**) [44] have been put into practical use for orchard trees and vegetables, and cycloprothrin (**35**) [45] for paddy fields, respectively.

4.3 Modification of the Alcohol, Acid, and Ester Linkage (Pyrethroid-Like Compounds): Agricultural Insecticides and Termiticides

Figure 9 shows compounds in which the bonding between an acid moiety and an alcohol moiety has been modified to a linkage other than an ester. Etofenprox (**36**) [46], silafluofen (**37**) [47], and flufenprox, for example, were developed in the 1980s. These compounds are completely different in structure from the prototype natural pyrethrins, and do not fall into the classical pyrethroid type of insecticides. Silafluofen, which Katsuda et al. developed, features a silicon atom in the molecule. There is no doubt, however, that the idea of this compound emerged in the course of pyrethroid development. It is truly interesting that silafluofen was independently patent applied almost at the same time in Japan (1984), Germany (1985), and the USA (1986). Silafluofen differs from classical pyrethroids in that it is a stomach

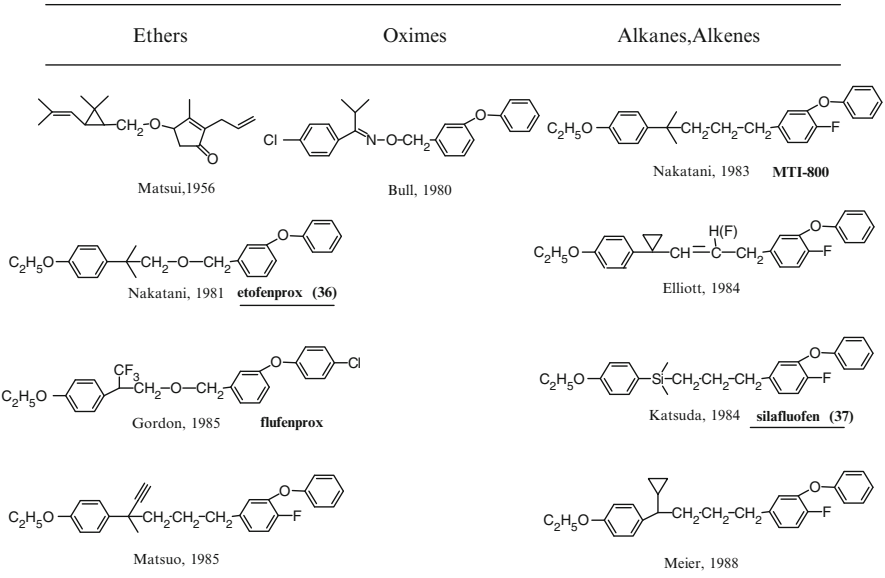


Fig. 9 Modification of the alcohol, acid moieties and linkage group (pyrethroid-like compounds)

poison as well as a contact poison and is stable for a long time in alkaline soils; therefore, it has taken root as a termiticide in Japan.

Moreover, while the use of pyrethroids has been greatly restricted in and around paddy fields and fishponds due to their high toxicity to fish, silafluofen, and etofenprox (see Table 2) show low fish toxicity and are commonly used as agricultural chemicals for paddy fields in Japan.

5 Problems with Pyrethroids

Natural pyrethrins, derived from pyrethrum, contain six insecticidal components. Due to their excellent insecticidal potency against insects in small amounts and their high safety for mammals, they are the only natural insecticidal components used for more than 100 years to date throughout the world as a household insecticide.

The main application fields of pyrethrins are limited to indoor use because of their instability to heat, light, and oxygen. Since the absolute configuration of the six insecticidal components of pyrethrins were elucidated in 1958, various researches on structural modifications have been carried out actively in many countries for more than half a century, leading to the development of a variety of photostable pyrethroids. As a result, they have been widely put into outdoor use for agriculture, forestry, animal health, termite control, and so on.

Table 1 shows the ratios of LD_{50} values of various insecticidal components for mammals and insects, i.e., indexes for selective toxicity.

For example, the average LD_{50} value of 15 carbamates for rats is 45 mg/kg, whereas for 27 carbamates for insects it is 2.8 mg/kg. Accordingly, the LD_{50} value for mammals and insects, an index of selective toxicity, is 16. The corresponding value of organophosphorus compounds is 33, and that of organochlorine compounds is 91. In contrast, the value of pyrethroids is 4,500, indicating much lower toxicity to mammals in spite of their excellent insecticidal activity.

Table 1 Selective toxicity of insecticides [48]

Insecticide	LD_{50} (mg/kg)		Ratio of selectivity	Mode of action
	Mammal (rat)[a]	Insect[a]		
Carbamate	45 (15)	2.8 (27)	16	Inhibition of acetylcholinesterase
Organophosphorus compound	67 (83)	2.0 (50)	33	Inhibition of acetylcholinesterase
Organochlorine compound	230 (21)	2.6 (26)	91	Action on the nervous system
Pyrethroid	2,000 (11)	0.45 (35)	4,500	Action on the nervous system

[a]Number of insecticides tested in parentheses

Table 2 Fish toxicity of pyrethroids for agricultural uses in Japan

Criteria for fish toxicity			Pyrethroid	LC_{50} value	
Class	LC_{50} value			Carp (ppm/48 h)	Daphnid (ppm/3 h)
	Carp	Daphnid			
A	>10 ppm	>0.5 ppm	Silafluofen	>100	7.66
B	>10 ppm	<0.5 ppm			
	0.5–10 ppm		Etofenprox	5	40
			Cycloprothrin	8	>10
C	<0.5 ppm		Fenvalerate	0.00075	0.3
			Permethrin	0.043	>10
			Cyfluthrin	0.012	0.94
			Tralomethrin	0.008	0.22
			Fluvalinate	0.00048	0.298

5.1 Fish Toxicity

Because of high fish toxicity, the use of pyrethroids is prohibited or greatly restricted in and around water systems in Japan; for example, in rooms with a water tank containing pet fish, or in and around paddy fields and fishponds.

In Japan, the fish toxicity of agricultural chemicals is classified into ranks A (>10 ppm), B (0.5–10 ppm), and C (<0.5 ppm) based on the LC_{50} values against carp, as shown in Table 2. Pyrethroids are all classified into rank C, showing high fish toxicity, except for cycloprothrin (**35**) and etofenprox (**36**), which fall into rank B; therefore, the use of pyrethroids is limited in and around paddy fields, fishponds, and other water systems. In contrast, silafluofen (**37**), with low fish toxicity, is the only compound classified into rank A among pyrethroids and has been used from 1995 as an insecticide in paddy fields in Japan. Although the reason for silafluofen's low fish toxicity is unknown, studies on this mechanism would be an interesting theme for the elucidation of fish toxicity.

5.2 Cross-Resistance

Resistance to insecticides has drawn global attention since the Korean War in 1950 when the mass use of organic synthetic insecticides, such as DDT and BHC, against agricultural pests and sanitary pests became common. Organophosphorus compounds and carbamates were used thereafter, but invited problems of safety concerns and insect resistance. Synthetic pyrethroids were watched with keen interest as alternatives and have become used widely not only for sanitary pests but also agricultural pests. The development of resistance to synthetic pyrethroids is also not a rare phenomenon and has spread all over the world.

The development of drug resistance is a phenomenon in which a resistance gene potentially present in a pest at low frequency is selected by exposure to a drug and

then its gene frequency is increased. Thus, the development of resistance is an adaptation phenomenon of living organisms.

While the mechanism of resistance to various synthetic pyrethroids in flies has been elucidated in terms of physiology, biochemistry, and genetics, it seems that the resistance mechanism is mostly common to mosquitoes.

The issue of pyrethroid resistance in houseflies and mosquitoes and the countermeasures are described below.

5.2.1 Houseflies

It is said that the action site of pyrethroids in flies is on the neuroaxonal excitatory membrane, similarly to that of DDT. Moreover, DDT-resistant *M. domestica* is known to show high cross-resistance to synthetic pyrethroids and the *kdr* gene is involved in the onset of the resistance. It has also been shown that such resistant flies exhibit high cross-resistance to many synthetic pyrethroids developed to date.

As shown in Fig. 6, the chemical structure of natural pyrethrins consists of six chemical components: pyrethrin I and II, cinerin I and II, and jasmolin I and II.

Allethrin, the first synthetic pyrethroid, is a compound which is the closest in structure to cinerin I. Pyrethroids developed subsequently are mostly esters of chrysanthemic acid, and cinerin II analogs, i.e., esters of chrysanthemum acid have not been industrialized.

Although pyrethroids consist of natural pyrethrins and many photostable synthetic pyrethroids, they must be discriminated when discussing cross-resistance.

By topical application, Katsuda et al. determined LD_{50} values of natural pyrethrins and several pyrethroids against the pyrethroid-susceptible CSMA strain and the pyrethroid-resistant Hiroyama strain of *M. domestica*. The resistant strain was originally collected in 1996 from a hog farm in Hiroyama, Niigata Prefecture. Their resistance ratios, R/S, are shown in Table 3.

Table 3 Pyrethroid resistance against *Musca domestica* (tested in 1997)

Compound	LD_{50} (μg/female)[a]		
	CSMA(S)[b]	Hiroyama (R)[c]	R/S ratio
Permethrin	0.028	5.700	204
Phenothrin	0.047	13.289	283
Natural pyrethrins[d]	0.784	5.384	7
Allethrin (racemic)[e]	0.417	40.355	97
Allethrin (bioallethrin)[f]	0.210	14.368	68
Allethrin (d,d-*trans*-allethrin)[g]	0.081	2.721	33

[a]Topical application method, 0.5 μL injection
[b]Susceptible strain of *M. domestica*
[c]Resistant strain of *M. domestica*
[d]Alcohol moiety, d form, and acid moiety; d-*trans* form
[e]Alcohol moiety, dl form, and acid moiety; dl-*cis,trans* form
[f]Alcohol moiety, dl form, and acid moiety; d-*trans* form
[g]Alcohol moiety, d form, and acid moiety; d-*trans* form

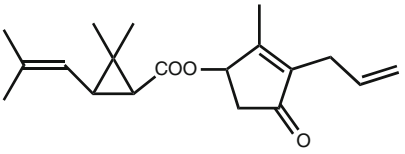
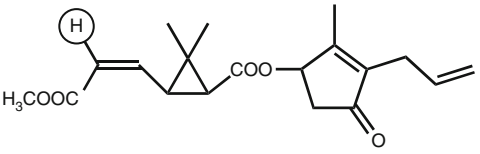
The R/S ratios for permethrin and phenothrin in Table 3 are 204 and 283, respectively. The Hiroyama strain showed high cross-resistance to permethrin and phenothrin, whereas the R/S ratio for natural pyrethrins was only 7. Moreover, the R/S ratio for racemic allethrin was 97, indicating the slow development of resistance compared with permethrin and phenothrin. These results agree well with the findings of Sawicki et al. [49], who reported that "pyrethroids with the cyclopentenolone ring showed only a slight resistance" at the International Congress of Pesticide Chemistry in Ottawa in 1986.

In the series of allethrin homologs, the R/S ratios of racemic allethrin, dl,d-*trans*-allethrin (bioallethrin) and d,d-*trans*-allethrin were 97, 68, and 33, respectively. The closer the steric configuration of allethrin homologs comes to that of natural pyrethrins, the smaller the R/S ratio; however, the R/S ratio of d,d-*trans*-allethrin with the same steric configuration as natural pyrethrins is still greater than that of natural pyrethrins.

Except for the side chain structure of the alcohol moiety, there is a great difference between natural pyrethrins and d,d-*trans*-allethrin in that the former consists of the mixture of pyrethrins I and II (**5**–**10**, Fig. 5), whereas the latter does not contain pyrethrin II homologs.

Thus, Katsuda [1] synthesized an allethrolone ester of chrysanthemum acid analog (allethrin II′, **38**, Table 4) and examined LD_{50} values of allethrin (**11**, Table 4), allethrin II′ (**38**, Table 4), and a mixture of allethrin and allethrin II′ against the pyrethroid-susceptible CSMA strain and the resistant Hiroyama strain of *M. domestica*. The test results, shown in Table 4, can be summarized as follows:

Table 4 Chrysanthemic acid ester (allethrin) and modified chrysanthemum acid ester (allethrin II′)

Compound	LD_{50} (μg/female)[a]		
	CSMA(S)	Hiroyama (R)	R/S ratio
 Allethrin **11**			
 Allethrin II' **38**			
Allethrin (bioallethrin)	0.210	14.368	68
Allethrin II′	0.077	3.368	44
Allethrin/allethrin II′ (1/1)	0.098 (0.144)[b]	3.870 (8.876)[b]	39

[a]Topical application method, 0.5 μL injection
[b]Calculated based on values of allethrin and allethrin II′

Table 5 Pyrethroid resistance against *Musca domestica* (tested in 2010)

Compound	LD_{50} (μg/female)[a]		
	CSMA(S)[b]	Obihiro (R)[c]	R/S ratio
Permethrin	0.0392	11.0255	281
Phenothrin	0.0416	12.5285	301
Natural pyrethrins	0.5554	6.3280	11.4
d,d-*trans*-Allethrin	0.1459	1.8968	13.0
Prallethrin (**12**)	0.1336	1.3904	10.4

[a]Topical application method, 0.5 μL injection
[b]Susceptible strain of *M. domestica*
[c]Resistant strain of *M. domestica*

1. Allethrin II′ was more effective than allethrin against both susceptible (S) and resistant (R) strains of *M. domestica*.
2. The mixture of allethrin and allethrin II′ showed a marked synergistic effect against both S and R strains of *M. domestica*.
3. R/S ratios decreased in the order of allethrin, allethrin II′, and their mixture.

Similarly to Table 3, LD_{50} values of several pyrethroids against the pyrethroid-susceptible CSMA strain and the pyrethroid-resistant Obihiro strain of *M. domestica*, originally collected in 2008 from a meadow in Obihiro district, Hokkaido, were determined by the topical application method. Their R/S are shown in Table 5.

Tests for Tables 3 and 5 were carried out in 1997 and 2010, respectively.

It is noteworthy that the Obihiro strain of *M. domestica* showed markedly high cross-resistance to photostable pyrethroids such as permethrin and phenothrin having a benzyl group in the alcohol moiety, with their R/S ratios being 281 and 301, respectively.

On the other hand, R/S ratios of natural pyrethrins as well as d,d-*trans*-allethrin and prallethrin (**12**) with the same steric configuration as natural pyrethrins were only as low as about 10, suggesting slow development of resistance.

5.2.2 Mosquitoes

Until recently, the resistance of mosquitoes to pyrethroids has not been taken as a serious issue. In Japan, *C. p. pallens* and *Aedes albopictus* (Skuse) are the main species living around houses. Although mosquito coils have utilized natural pyrethrins as insecticidal ingredients for about 50 years and then allethrin for about 50 years, there has been no report on resistance development. The reason for this is considered to be the short active time of 4–5 months per year for *C. p. pallens*. Yasutomi et al. [50] reported in 1989 the presence of pyrethroid-resistant *Culex tritaeniorhynchus* in Okinawa, but Japanese encephalitis transmitted by *C. tritaeniorhynchus* decreased markedly after 1992 and disappeared.

Katsuda et al. [51–53] performed joint research with Somjai, Supatra, Narumon et al. in the Department of Medical Entomology, Faculty of Tropical Medicine,

Mahidol University, Thailand, from 2006 to 2008 on the themes of allethrin susceptibility and the control of *Aedes aegypti* transmitting dengue fever. The incidence areas of dengue fever have expanded to over 100 tropical and subtropical countries, and the death rate from dengue hemorrhagic fever is especially high among infants. Unlike nocturnally active anopheles mosquitoes, *A. aegypti* is active in blood-sucking in the daytime. Moreover, an effective vaccine against dengue fever remains to be developed and there is no treatment available; therefore, it is essential to protect humans from mosquito bites and mosquito coils are considered to be the best method.

Using the topical application method, Katsuda et al. [52] examined the allethrin susceptibility of *A. aegypti* including two different standard SS and BS strains and two other field colonies. The SS strain larvae of *A. aegypti* were collected in 1977 in the Pom Prap Sattru Phai district of Bangkok, and the BS strain has been under successive breeding from larvae collected in 2005 in the Thung Kru district of Bangkok. The latter two field colonies were collected in 2007 in district A (Thung Kru) and district B (Thra Pha) of Bangkok suburbs, where dengue fever was prevalent. According to the data by the topical application in (Table 6), *A. aegypti* developed seven to ten times more resistance to dl,d-*T*80-allethrin over a period of about 30 years since 1977 when the SS strain was collected in the suburbs of Bangkok.

Moreover, mosquito coils containing allethrin as an active ingredient were tested for their efficacy against the four colonies of *A. aegypti* in a practical room of 25 m^3.

Table 7 shows that the dl,d-*T*80-allethrin 0.5% mosquito coil was effective on the susceptible SS strain of *A. aegypti* in the 25-m^3 semi-field test, but showed low efficacy against the BS strain and colonies collected in districts A and B, their KT_{50} values being uncalculated.

The blood-sucking suppressing effect of various mosquito coils was also investigated in a field test by volunteers in district A located in the suburbs of Bangkok. As a result, high repellent effect of 80% compared with the control was

Table 6 LD_{50} values of dl,d-T80-allethrin against various strains of *Aedes aegypti* by the topical application method

Strain	Collected year	LD_{50} (μg/insect)	Relative ratio against SS
SS	1977	0.0092	1.0
BS	2005	0.0637	6.9
A	2007	0.0702	7.6
B	2007	0.0925	10.1

Table 7 KT_{50} values of dl,d-T80-allethrin 0.5% mosquito coil against various strains of *Aedes aegypti* in 25-m^3 room semi-field tests (2 h burning, 2 h exposure)

Strain	KT_{50} (min)
SS	12.9
BS	170
A	ND[a]
B	ND[a]

[a]Not determined

Ae. Aegypti colony	Province and year of collection
SS	Pom Prap Sattru Phai, BKK (1977)
BS	Thung Kru, BKK (2005)
A	Thung Kru, BKK (2007)
B	Tha Phra, BKK (2007)
C	Uttaradit (2008)
D	Chiang Rai (2008)
E	Surat Thani (2008)
F	Kanchanaburi(2008)
G	Nakhon Sawan (2008)
H	Chanthaburi(2008)
I	Chonburi (2008)
J	Songkla (2008)
K	Phangnga (2008)

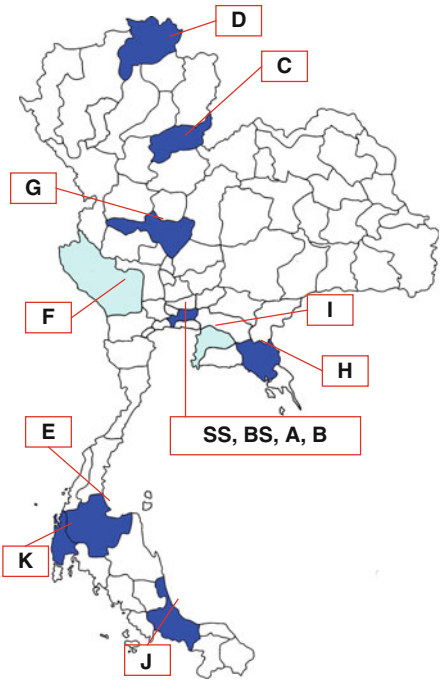


Fig. 10 *Aedes aegypti* colonies collected from various provinces

observed for mosquito coils containing 0.1–0.2% prallethrin, which has the same steric configuration as cinerin I with high insecticidal potency.

Katsuda et al. [53] collected *A. aegypti* larvae from 11 districts (Fig. 10) in Thailand where dengue fever was prevalent. After rearing in the laboratory, the colonies were tested for KT_{50} values using dl,d-*T*80-allethrin 0.5% mosquito coils in a 25-m^3 semi-field test in addition to KD_{50} and LD_{50} values to dl,d-*T*80-allethrin by the topical application method.

As shown in Table 8, mosquito colonies with KT_{50} values below 60 min in the 25-m^3 semi-field test were classified as susceptible (Group I), those with KT_{50} of 60–120 min as less susceptible (Group II), and those with KT_{50} of over 120 min as low susceptible (Group III).

It was found that two colonies were susceptible to allethrin, similar to the SS strain, six colonies had low susceptibility similar to the allethrin-resistant BS strain, and the remaining three colonies had susceptibility to allethrin between the SS and BS strains. The allethrin mosquito coils, even at higher concentrations, were ineffective on the six decreased susceptibility colonies, similar to the BS strain.

Table 8 Allethrin susceptibilities of *Aedes aegypti* colonies in 25-m^3 room semi-field tests by using dl,d-T80-allethrin 0.5% mosquito coil

Sensitivity group	Mosquito group	KT_{50} (min)		Ratio
		Range (min)	Mean value (min)	
	SS		13	1
	BS		>120	13
I	F	<60	13	1
	I		35	3
	J		76	6
II	H	60–120	96	7
	D		99	8
	E			
	G			
III	A	>120	KT_{50} not calculated	≫10
	K			
	B			
	C			

Table 9 Pyrethroid susceptibilities of *Aedes aegypti* colonies in 25-m^3 room semi-field tests by using mosquito coils with each pyrethroid

Sensitivity group	Mosquito group	KT_{50} (minutes)			
		dl,d-T80-Allethrin 0.5%	d,d-T-Prallethrin		Nat. Pyrethrins
			0.1% + S*	0.15% + S*	0.7–0.8%
	SS	13	12	–	
	BS	>120	120	100	
I	F	13	11	–	
II	D	99	87	–	
III	E	>120	60	44	–
	G	>120	81	–	65
	K	>120		92	82

*S: a synergist, N-(2-ethylhexyl)bicyclo[2,2,1]hept-5-ene-2,3-dicarboxyimide (**39**)

Mainly against the Group III mosquitoes, Katsuda et al. investigated the efficacy of mosquito coils containing various pyrethroids. In the 25-m^3 semi-field test shown in Table 9, mosquito coils with d,d-*T*-prallethrin at concentrations of 0.1–0.15% plus a synergist, *N*-(2-ethylhexyl)bicyclo[2,2,1]-hept-5-ene-2,3-dicarboxyimide (**39**, Fig. 11), were effective even for the allethrin-resistant *A. aegypti*.

A. aegypti colonies were found to have developed cross-resistance to even polyfluoro benzylalcohol ester pyrethroids with potent insecticidal activity. Mosquito coils of these compounds were effective against allethrin-susceptible *A. aegypti* colonies at ultra-low concentration, but needed several times higher concentrations for *A. aegypti* colonies in Group III in Table 8 (unpublished).

In the 25-m^3 semi-field test (Table 9), KT_{50} values of mosquito coils containing natural pyrethrins at about 0.7% were determined for *A. aegypti* colonies of G and K

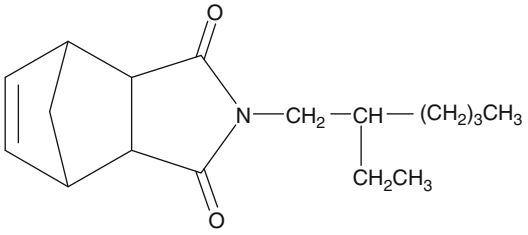


Fig. 11 Chemical structure of *N*-(2-ethylhexyl)bicyclo [2,2,1]hept-5-ene-2,3-dicarboxyimide (**39**)

in Group III. As a result, the KT_{50} values were 65 min for colony G and 82 minutes for colony K, respectively, whereas those for dl,d-*T*80 allethrin coils were 120 min or more, indicating the effectiveness of natural pyrethrin coils. Incidentally, mosquito coils around the 1950s consisted of dried pyrethrum flowers at about 60–70% and adhesives at about 30–40%. As the content of pyrethrins in dried flowers was 1.0–1.2%, that in one coil corresponded to about 0.7%. The results are related to those obtained in the test shown in Table 3 where the development of cross-resistance was examined using pyrethroid-resistant *M. domestica* strains as well as a susceptible strain. In the test, the resistance ratios obtained for permethrin and phenothrin were 204 and 283, respectively, whereas that for natural pyrethrins was only 7. We therefore still have much to learn from natural pyrethrins.

For the control of dengue fever-transmitting mosquitoes, there are measures for dealing with sources of larval emergence and adult mosquitoes. After World War II, the WHO took the lead and focused on residual spraying with DDT, organophosphorus compounds, and pyrethroids, as well as measures for sources of larval emergence; however, such measures did not achieve sufficient effects, inviting the development of chemical resistance and, now, the importance of imago control by individuals has been re-evaluated.

Thanispong et al. [54] (2008) tested *A. aegypti* colonies collected from nine areas in Thailand by exposure to insecticide-treated papers and reported strong development of resistance to DDT and permethrin. According to a report by Kawada et al. [55], the considerable development of resistance to photostable synthetic pyrethroids was observed with the involvement of a *kdr* mechanism in *A. aegypti* colonies in the central and southern areas of Vietnam. Kasai et al. [56] reported that *A. aegypti* colonies collected in Singapore had 35-fold resistance to permethrin by topical application and that the selection through 3 generations with permethrin increased the resistance ratio to 200 times or more.

5.2.3 Countermeasures and Future Perspectives

Against the above background, the development and perspective of cross-resistance to synthetic pyrethroids should be discussed as follows.

First, considering safety and resistance problems, agricultural pyrethroids used outdoors in large quantities should be discriminated from pyrethroids used as household insecticides in and around houses from the development stage of preparations.

According to the US national population census, it is said that the world population will increase by about 30% from about 6.9 billion (2010) to 9.2 billion in 2050. Since these figures include starving populations, a more than 30% increase in food supply will be needed. Agricultural chemicals are indispensable because farming areas are limited and, therefore, it is unavoidable that the development of novel agricultural chemicals including novel photostable pyrethroids is and will be repeatedly followed by the development of resistance in insects.

Some organochlorine, organophosphorus, and carbamate insecticides used after World War II (since 1945) were found to have various problems of adverse effects on mammals and environmental behavior and influences. The use of many industrial chemicals has been prohibited because those contained as impurities in minute quantities produced critical toxic substances by transformation and repeated chemical reactions in their environment.

The distribution of agricultural products is global now at a speed not so different from domestic transportation by the use of airplanes. For example, agricultural products cannot be imported if they contain any residues of agricultural chemicals at a concentration higher than the standard value set in the importing country. The World Trade Organisation (WTO) was started in 1995 as an international organization to discuss international trading rules, and 153 countries have joined. Items to be checked before practical use of a new agricultural chemical have been standardized tentatively, although there are some differences between countries.

Through various challenges, vast expense and time have become necessary for the risk assessment of agricultural chemicals, including efficacy and safety, environmental toxicology, and toxic effects due to impurities contained in agricultural chemicals and their behavior. These circumstances have led to the global integration of agricultural chemical manufacturers.

Second, meanwhile, no international guidelines have been provided for the manufacture, marketing, and distribution of household insecticides on such a level as those of agricultural chemicals, and not even manufacturing registration is required in some countries. Of course, the minimal required toxicity studies are conducted with synthetic pyrethroids for household insecticides to examine absorption, distribution, metabolism, and genotoxicity in animals.

However, we are concerned about the behavior, safety, and environmental problems of photostable synthetic pyrethroids remaining in and around houses, considering the present situation of their increased indoor use. In particular, compounds with strong insecticidal potency need long-term safety and residue studies for the health of infants and pets.

Cross-resistance to pyrethroids for outdoor use has developed markedly in *M. domestica*, mosquitoes, cockroaches, and so on; however, it has also been found that natural pyrethrins as well as d-allethrin and prallethrin (ETOC®), which have very similar chemical structures and the same configuration as natural pyrethrins, show an extremely low degree of cross-resistance development by these highly-resistant sanitary pests compared to photostable pyrethroids. Many novel synthetic pyrethroids recently developed as household insecticides have tended to pursue efficacy improvements in terms of rapid knock-down effects, residual efficacy or volatility.

As these compounds seem to lose the selective toxicity characterized by natural pyrethrins, we should learn from natural pyrethrins to develop safer pyrethroids.

Measures taken to control sources of larval emergence of sanitary pests are limited, and excessive treatments frequently induce the development of resistance in disease-transmitting insects. On the other hand, control measures by individuals are becoming a trend. For example, patients with malaria have decreased by the popularization of Olyset® mosquito nets, which were developed by Sumitomo Chemical Co., Ltd. to deal with nocturnally-active blood-sucking anopheles.

On the other hand, *A. aegypti*, a mosquito vector of dengue fever, is blood-sucking in the daytime, and its larvae emerge from small puddles; therefore, it is impossible to deal with the source as this measure requires the spraying of insecticides in innumerable puddles. At present, when a dengue fever vaccine is not available, it is preferable to prevent blood sucking using mosquito coils containing a safer pyrethroid.

5.3 Pyrethroids and Household Insecticides

5.3.1 Development Policy of Pyrethroids

Sanitary insects coming into houses are largely divided into two types – flying insects and crawling insects. The use of pyrethrum powders as an insecticide for crawling insects was started around 1855 in the USA and in 1886 in Japan. Mosquito coils were developed in 1890 in Japan and oil formulations containing pyrethrum extract were available in 1919 in the USA.

Natural pyrethrins are a neurotoxin and repel, knock down, and kill by contact with insects at a low concentration. On the other hand, they have ideal features for household insecticides because of their quite low dermal and oral toxicities to warm-blooded animals. Neither plants other than pyrethrum nor synthetic insecticides have been reported to have such properties. Numerous synthetic pyrethroids have been developed by chemists since the complicated chemical structure of natural pyrethrins was elucidated in the middle of the twentieth century. Allethrin was the first synthetic pyrethroid put into practical use.

While natural pyrethrins is a collective term for six components of similar structure, allethrin (**11**), the first synthetic pyrethroid, has a structure resembling that of cinerin I, which is one of the six components. Having been used for more than 50 years as an insecticidal ingredient of mosquito coils in Japan, allethrin has excellent efficacy without any resistance problems; however, studies by Katsuda et al. revealed that *A. aegypti* in south-east Asia, including Thailand, has acquired no little resistance to allethrin. They also reported that prallethrin (ETOC®) (**12**), with a chemical structure resembling those of cinerin-I and allethrin along with the same absolute configuration, was quite effective in dealing with the problem. Prallethrin is very attractive together with natural pyrethrins since its efficacy against mosquitoes is reported to be about four times that of allethrin [57] and it

is safe and capable of dealing with allethrin-resistant mosquitoes for a while. Although preparations at low concentrations have been developed using polyfluorobenzyl-type synthetic pyrethroids with dozens of times higher potency to mosquitoes than allethrin this century, it is important to examine the effects of these pyrethroids on cross-resistance.

In the example of *M. domestica* shown in Table 3, the development of resistance to permethrin (**21**) and phenothrin (**16**) was 204 and 283 times, respectively, but the resistance to natural pyrethrins was only 7 times and that to d-allethrin was very low, 33 times. Moreover, the development of resistance to natural pyrethrins and prallethrin (ETOC®) was found to be low in *A. aegypti* as described previously.

Again, we would like to encourage the sound development of pyrethroids for household insecticides based on natural pyrethrins, taking safety and resistance into consideration.

5.3.2 Pyrethroids and Forms of Household Insecticides

Since pyrethroids show contact toxicity, the first requirement for insecticides is contact between pyrethroids and insects (flying, crawling). To achieve this, a physical property of pyrethroids, i.e., vapor pressure, is an important indicator. High vapor pressure is usually associated with excellent volatilization; however, as the vapor pressures of individual pyrethroids are diverse due to different measurement methods and conditions (for example, temperature), it is difficult to capture the entire picture.

To deal with flying insects, the volatilization of pyrethroids is the first requirement and volatilization energy is needed for this property. In the case of crawling insects, persistent and residual effects of pyrethroids are required after residual application to floors and walls.

Table 10 shows the data on the vapor pressure values of some pyrethroids measured under the same conditions and their relative ratios against allethrin as

Table 10 Vapor pressure of synthetic pyrethroids (25°C)

	Compound	mmHg ($\times 10^{-5}$)	mPa (conversion)	Relative value	Vaporizing condition
1	Empenthrin	18	24	31	At room temperature
2	Profluthrin	7.7	10	13	
3	Transfluthrin	2.56	3.4	4.3	Wind, centrifugal force
4	Furamethrin	2.45	3.3	4.2	
5	Metofluthrin	1.35	1.8	2.3	
6	Allethrin	0.59	0.78	1.0	Heating
7	Prallethrin	0.48	0.64	0.8	
8	Phenothrin	0.016	0.021	0.03	
9	Permethrin	0.0055	0.0073	0.01	

Source: Data from Sumitomo Chemical Co., Ltd.
Reference: Vapor pressure of other chemicals at 25°C
Glycerin 10 mPa
Dichlorvos 2,100 mPa

1. Pyrethroids generally have low vapor pressures. Although empenthrin (**20**) volatilizes at room temperature without external energy and is 31 times more volatile than allethrin, its vapor pressure is only 1/88 that of dichlorvos, a volatile organophosphorus compound. As profluthrin (**40**) is 13 times more volatile than allethrin and volatilizes gradually for 6 months to 1 year at room temperature, it is suitable as an insecticide for the protection of clothes. The vapor pressure of profluthrin is 10 mPa/25°C, almost the same as that of glycerin.

For transfluthrin (**30**) and metofluthrin (**31**), volatilization at room temperature is hardly expected, requiring wind, centrifugal force, and other effects. Nevertheless, with a slight move of air, these compounds possibly have the effect of preventing insects from coming into a house to some extent because they are effective in minimal amounts against mosquitoes, with dozens of times higher potency than allethrin.

A recently commercialized U.L.V. (ultra-low volume) -type aerosol sprays a fixed volume of ultra-fine particles into the room and retains effectiveness for 12 h with one spray. The mechanism of the effectiveness was considered to be the contact of pyrethroid particles with mosquitoes in the air at the initial stage followed by re-volatilization into the air of pyrethroids attached to walls and floors; however, according to experiments by the present author et al., it was confirmed that mosquitoes are knocked down by contact with pyrethroid particles in the air during the first 1 h and then the lethal effect on mosquitoes is achieved by their contact with pyrethroids adhering to walls.

On the other hand, heating is needed for volatilization of allethrin, prallethrin, phenothrin, permethrin, and others. In the case of aerosol formulations, the energy from a liquefied gas or compressed gas encourages volatilization.

Thus, the form of insecticides should be selected in consideration of chemical and physical properties as well as the safety of pyrethroids, and their excessive use should be restricted in rooms.

6 Concluding Remarks

Since around 1855, when pyrethrum flowers imported into the USA from Europe were used as an insecticide in powder form, the use of dried pyrethrum flowers has continued for about 150 years, with annual demand amounting to about 10,000 tons even now. Soon after the chemical structure of "natural pyrethrins," the insecticidal ingredient of pyrethrum flowers, was elucidated, commercial production of the first synthetic pyrethroid of allethrin was started by Sumitomo Chemical Co., Ltd. in 1953. Successively useful synthetic pyrethroids with various characteristics have been developed by organic chemists throughout the world, leading to the advancement of pyrethroid chemistry. Generally, ingredients with high insecticidal effects are also highly toxic to humans. Even in pyrethroids with high selective toxicity, a chemical design placing too much importance on efficacy improvements may invite loss of the safety margin. It is strongly hoped that the development of

household pyrethroids and their preparations for use in living environments around humans and pets will be achieved in the future by retaining the characteristics of natural pyrethrins.

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Recent Advances of Pyrethroids for Household Use

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Abstract Development of pyrethroids for household use and recent advances in the syntheses of (1*R*)-*trans*-chrysanthemic acid, the acid moiety of most of the household pyrethroids, are reviewed. As another important acid moiety, we discovered norchrysanthemic acid to have a significant vapor action at room temperature when esterified with fluorobenzyl alcohols. In particular, 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (1*R*)-*trans*-norchrysanthemate (metofluthrin) exhibits the highest potency in mosquito coil formulations as well as the vapor action at room temperature against various mosquitoes. Structure-activity relationships of norchrysanthemic acid esters and synthetic studies of norchrysanthemic acid are discussed.

Keywords (1*R*)-*trans*-Chrysanthemic acid · Metofluthrin · Mosquito · Norchrysanthemic acid · Pyrethroids

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1 Introduction

The study of structural modification of natural pyrethrins has lasted for more than 60 years. Especially, the invention of allethrin, 2-methyl-4-oxo-3-allylcyclopent-2-enyl chrysanthemate, prompted chemists to make structural modifications of the pyrethroid alcohol and acid moieties. As a result, a number of pyrethroids with diversified characteristics have been invented not only for the control of household insect pests but also for agricultural use. With regard to household use pyrethroids commercialized, their acid moiety was mostly racemic (1*RS*)-*cis,trans*-chrysanthemic acid at the beginning. These household use pyrethroids have gradually been afforded in enatiomerically pure forms, (1*R*)-*trans*-chrysanthemic acid esters or (1*R*)-*cis,trans*-chrysanthemic acid esters by so-called "racemic switch" since the 1980s. Classical optical resolution of (1*RS*)-*cis,trans*-chrysanthemic acid with optically active amines is one of the practical methods toward the access to optically active (1*R*)-*trans*-chrysanthemic acid and (1*R)-cis,trans*-chrysanthemic acid. Various efficient synthetic processes have been reported by Sumitomo Chemical including enzymatic resolution and asymmetric synthesis.

Further exploratory work in our laboratory to find new pyrethroids with a higher vapor action and high effectiveness against mosquitoes resulted in the discovery of (1*R*)-*trans*-norchrysanthemic acid or (1*R*)-*trans*-2,2-dimethyl-3-(1-propenyl)-cyclopropanecarboxylic acid as another important acid moiety for household pyrethroids. Here we describe the structure-activity relationships of fluorobenzyl esters of (1*R*)-*trans*-norchrysanthemic acid regarding vapor activity against mosquitoes and the development of synthetic methods of norchrysanthemic acid.

2 Development of Pyrethroids for Household Use

Natural pyrethrins have long been used as most favored household insecticides. Pyrethrum flowers are still cultivated in certain areas including Africa, Australia, and China. On the other hand, commercial use of pyrethroids is one of the most remarkable success stories in insecticide development originated from natural products as a lead. It should be noted that in 1924 Staudinger and Ruzicka reported [1] several pyrethrin analogs including piperonyl chrysanthemate (**3**) as shown in Fig. 1.

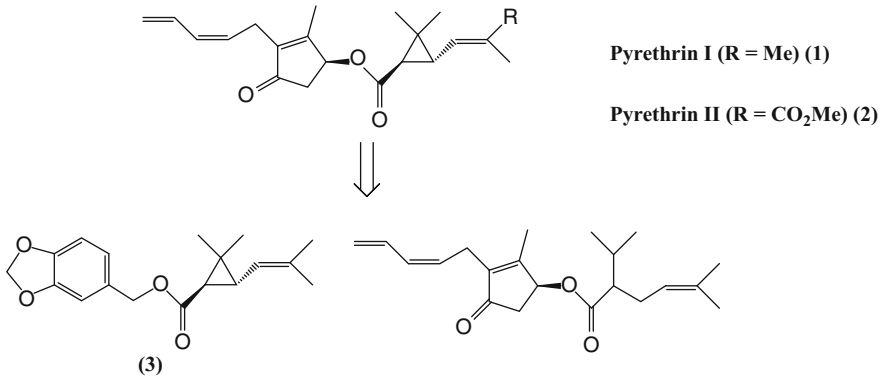


Fig. 1 Structural modifications by Staudinger and Ruzicka

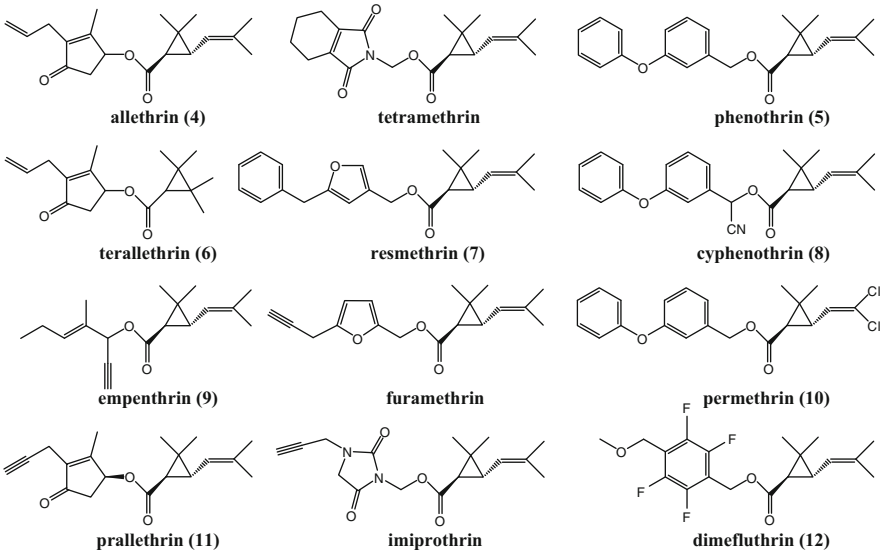


Fig. 2 Structures of pyrethroids commercialized by Sumitomo Chemical

Some of them were only slightly insecticidal. But their foresight is notable regarding natural pyrethrins as lead compounds without knowledge of the real structures of alcohol moieties at that time.

Through extensive studies during the past 60 years, natural pyrethrins proved to possess ample possibilities for structural modifications. Namely, just after elucidation of the structures of pyrethrin I (**1**) and pyrethrin II (**2**) of natural pyrethrins in 1947 [2], extensive efforts began to modify mainly the alcohol moieties. In Fig. 2 commercialized household use pyrethroids from Sumitomo Chemical Co.Ltd are listed.

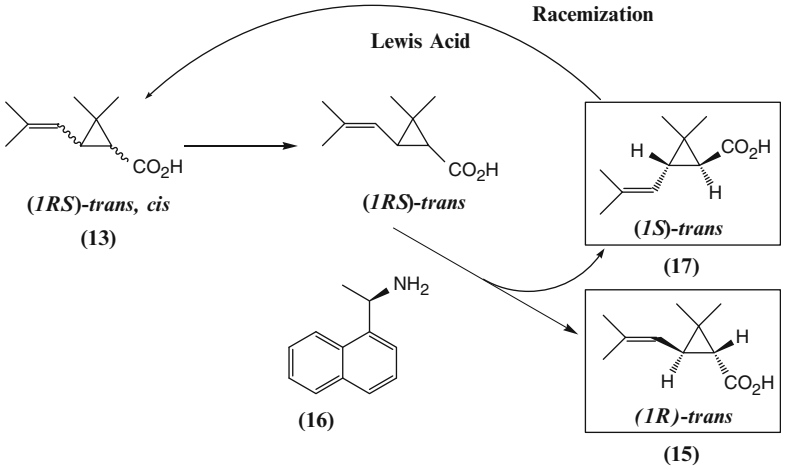
Simplification of the diene moiety of pyrethrin I resulted in finding allethrin (**4**) by Schechter [3], which was the first pyrethroid commercialized for household use. Matsui et al. made an extensive effort on the process of allethrin, which resulted in the launch of the first pyrethroid in Japan in 1954. As the propynyl analog of pyrethrin I, prallethrin (**11**) was commercialized by Sumitomo Chemical in 1988 in the most insecticidally active form [4]. Allethrin and prallethrin have been used widely for control of mosquitoes.

The most important breakthrough in terms of modifications of the alcohol moiety of pyrethrins was the aforementioned piperonyl ester (**3**) followed by the inventions of resmethrin (**7**) by Elliott in 1965 [5] and phenothrin (**5**) by Itaya in 1968 [6]. Further exploratory work in Sumitomo Chemical on modification of 3-phenoxybenzyl alcohol resulted in the invention of cyphenothrin (**8**) by Matsuo et al. in 1971 [7]. Resmethrin, phenothrin, and cyphenothrin have strong lethal activity against various insect pests. Phenothrin and cyphenothrin are used as components of aerosol insecticides. Phenothrin has also been used to kill head lice in humans as an active ingredient in shampoos. At the beginning the acid moiety of resmethrin, phenothrin, and cyphenothrin were racemic (1*RS*)-*cis*,*trans*-chrysanthemic acid (**13**). Latterly this acid has been switched to optically active forms, namely (1*R*)-*cis*,*trans*-chrysanthemic acid (**14**) or (1*R*)-*trans*-chrysanthemic acid (**15**) in order to enhance insecticidal activity. Notable is the fact that most pyrethroids for household use contain natural (1*R*)-*trans*-chrysanthemic acid (**15**) as the acid moiety as shown in Fig. 2, except terallethrin (**6**) and permethrin (**10**). Further exploratory work by Mori et al. [8] on modification of the alcohol moiety has recently resulted in the discovery of dimefluthrin (**12**), or 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (1*R*)-*trans*-chrysanthemate, which exhibits excellent potency against mosquitoes in mosquito coil formulations.

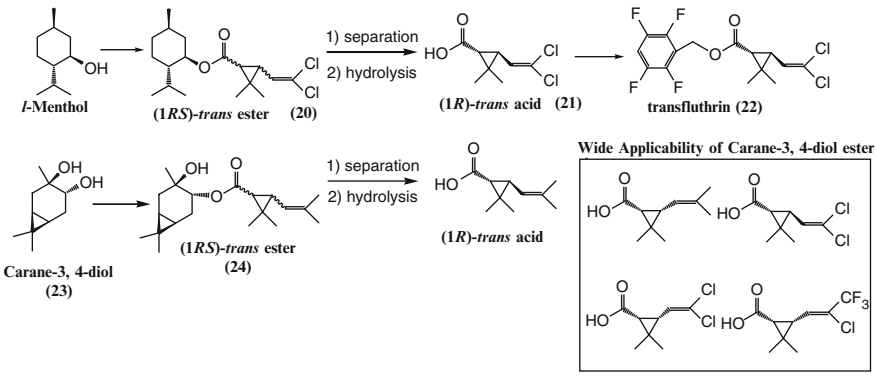
3 Recent Advances of the Syntheses of (1*R*)-*trans*-chrysanthemic Acid

(1*R*)-*trans*–Chrysanthemic acid (**15**) can be obtained by various methods. Classical optical resolution of the racemic *trans*-acid with (−)-naphthylethyl amine (**16**) is one of the efficient methods, although the theoretical yield is at most 50%. In this case, insecticidally unimportant (1*S*)-*trans*-chrysanthemic acid (**17**) is racemized back in order to make the whole process efficient. Classical resolution is practical but the efficiency is not always satisfactory for producing (1*R*)-*trans*-chrysanthemic acid (Scheme 1).

Recently novel methods were reported to make (1*R*)-*trans*-chrysanthemic acid including optical resolutions with the (+)-3-caranediol or 1,1′-binaphthol monoethylether, enzymatic resolution with *Arthrobacter globiformis* and the asymmetric synthesis with a new Cu catalyst. These methods are reviewed in this section.



Scheme 1 Optical resolution of (1*RS*)-*trans*,*cis*-chrysanthemic acid with (−)-1-naphthylethylamine



Scheme 2 Optical resolution with *l*-menthol and carane-3,4-diol

3.1 Optical Resolution with Carane-3,4-Diol

l-Menthol ester (**20**) with (1*RS*)-*trans*-2,2-dimethyl-3-(2,2-dichloroethenyl) cyclopropanecarboxylic acid (**19**) has been utilized to produce (1*R*)-*trans*-2, 2-dimethyl-3-(2,2-dichloroethenyl) cyclopropanecarboxylic acid (**21**), an acid moiety of transfluthrin (**22**) [9]. Matsuo et al. surveyed various optically active secondary alcohols for their potential in the optical resolution of (1*RS*)-*trans*-chrysanthemic acid [10] (Scheme 2).

As a result, the ester (**24**) of (1*S*,3*R*,4*R*,6*R*)-carane-3,4-diol (**23**) with (1*RS*)-*trans*-chrysanthemic acid could easily be separated by silica gel column chromatography to give two ester diastereomers. The Rf value of the ester of

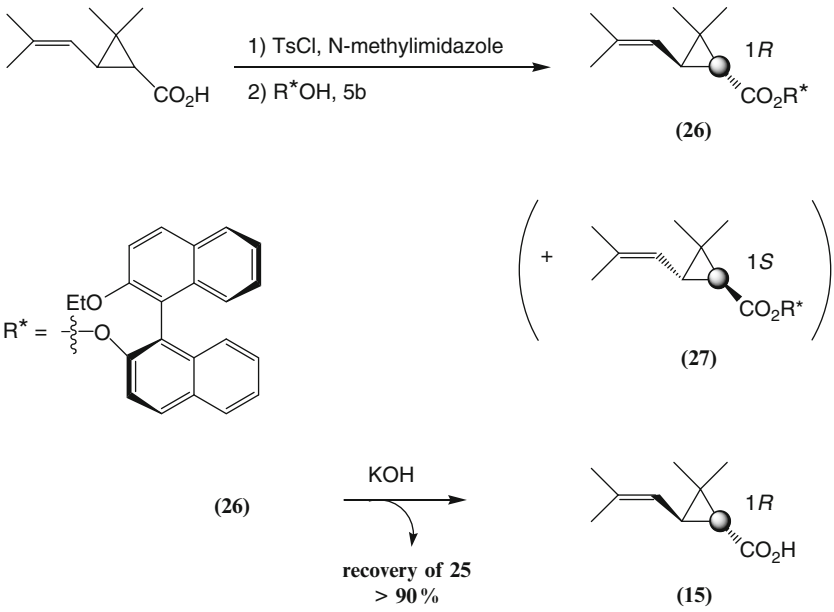
(1*S*,3*R*,4*R*,6*R*)-carane-3,4-diol with (1*R*)-*trans*-chrysanthemic acid is 0.62, and the Rf. value of the corresponding (1*S*)-*trans*-chrysanthemic acid ester is 0.65 (toluene: ethyl acetate = 3:1). However, the *l*-menthol ester of (1*RS*)-*trans*-chrysanthemic acid could not easily be separated by silica gel column chromatography to obtain (1*R*)-*trans*-chrysanthemic acid *l*-menthol ester.

Optical resolution methods with carane-3,4-diol are noteworthy for wide generality. Esters of various cyclopropane carboxylic acids with (1*S*,3*R*,4*R*,6*R*)-carane-3,4-diol were prepared and all (1*R*)-isomers could easily be obtained by a simple silica gel column chromatography.

3.2 Optical Resolution with 1,1′-Binaphthol Monoethyl Ether

Chiral 1,1′-binaphthol derivatives are well established as readily available chiral catalysts and auxiliaries for the production of various useful optically active compounds. Tanabe et al. investigated [11] a crystalline-liquid resolution of (1*R*)-*trans*-chrysanthemic acid utilizing 1,1′-binaphthyl monoethyl ether (**25**) (Scheme 3).

Thus, (1*RS*)-*trans*-chrysanthemic acid was condensed with 1,1′-binaphthol derivative using the TsCl-*N*-methylimidazole reagent to give the corresponding two sets of diastereomers (**26**) and (**27**). From the solution, only the (1*R*)-*trans*-chrysanthemic acid ester (**26**) crystallized from the diastereomer mixtures. The ester was readily



Scheme 3 Optical resolution with 1,1′-binaphthyl monoethyl ether

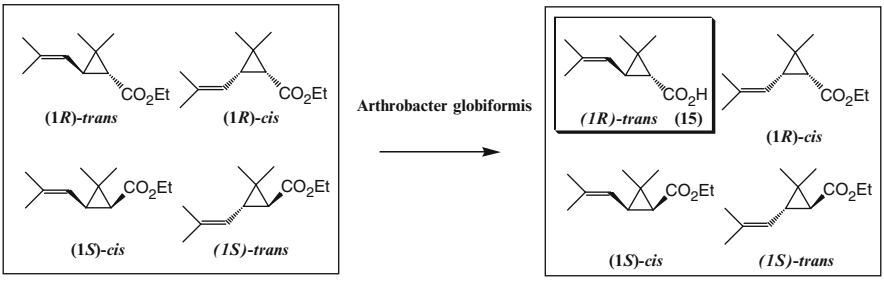
hydrolyzed under conventional conditions (KOH/THF-H_2O) to give the desired (1*R*)-*trans*-chrysanthemic acid (**15**) without any epimerization of the (1*R*)-position.

3.3 *Enzymatic Resolution of (1*R*)-*trans*-Chrysanthemic Acid with Bacterium*

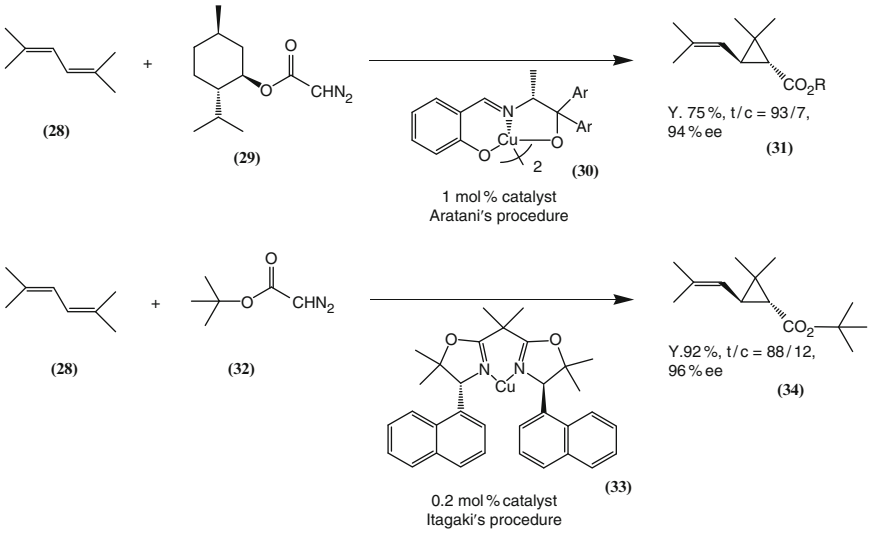
Nishizawa et al. found [12] an efficient esterase in *A. globiformis* (SC-6-98-28), which stereoselectively hydrolyzed only ethyl (1*R*)-*trans*-chrysanthemate among four stereoisomers of chrysanthemates to give the pure (1*R*)-*trans*-chrysanthemic acid (**15**). The gene coding was cloned from *A. globiformis* and overexpressed in *Escherichia coli*. Thus, the cellular content of enzyme reached 33% of the total soluble protein in the recombinant *E. coli*. cells. The hydrolysis activity of the recombinant *E. coli* cells for ethyl chrysanthemate were 2,500-fold higher than that of *A. globiformis* cells. The optimum pH and temperature were 9.5 and 50 °C, respectively, and more than 98% conversion and 100% stereoselectivity of (1*R*)-*trans*-chrysanthemic acid were accomplished (Scheme 4).

3.4 *Recent Advance of Asymmetric Synthesis of (1*R*)-*trans*-Chrysanthemic Acid with a New Chiral Copper Complex*

Asymmetric synthesis of 2,5-dimethyl-2,4-hexadiene (**28**) and *l*-menthyl diazoacetate (**29**) with chiral copper complexes (**30**) was successfully conducted by Aratani et al. [13] to afford the (1*R*)-chrysanthemic acid *l*-menthyl ester (**31**) in high optical and chemical yield. Since this finding, a lot of chiral copper complexes have been reported and applied to the asymmetric synthesis of (1*R*)-chrysanthemate. However, these copper complexes required more than 1 mol% of the catalyst and the *cis*/*trans* ratio still remains unsatisfactory. Moreover, *l*-menthyl ester was crucial for the high enantioselectivity. Given an industrial production of



Scheme 4 Synthesis of (1*R*)-*trans*-chrysanthemic acid with the bacterium



Scheme 5 Asymmetric synthesis of (1*R*)-*trans*-chrysanthemic acid

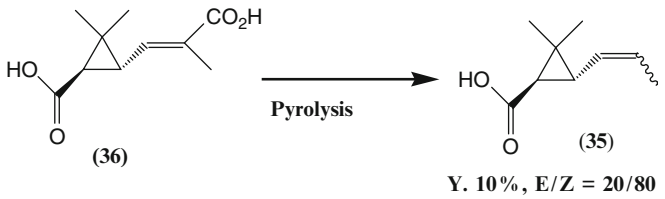
(1*R*)-*trans*-chrysanthemic acid by asymmetric synthesis, a much lower amount of the copper complex and a higher *trans*-isomer ratio of more than 80% should be attained. In addition, asymmetric synthesis using *l*-menthyl diazoacetate required extra steps. The use of a lower alkyl diazoacetate would be desirable for large scale production of (1*R*)-*trans*-chrysanthemic acid. Focusing on these points, Itagaki et al. screened various bisoxazoline type copper catalysts (Scheme 5).

As a result, 1-naphthylderivative (**33**) worked efficiently as a new copper complex combined with Ph_3CPF_6 as the catalysts to provide highly stereoselectivly *cis*/*trans* (88/12)-chrysanthemic *tert*-butyl ester (**34**) in 96% ee [14]. The ester (**34**) was easily hydrolyzed in acidic conditions to give (1*R*)-*cis*/*trans*-chrysanthemic acid. The required amount of the copper complex was only 0.2 mol%.

4 Invention of Metofluthrin [15]

Norchrysanthemic acid (**35**) was first synthesized by Staudinger in 1924 [16] as the pyrolytic decomposition product of chrysanthemun dicarboxylic acid (**36**) (Scheme 6). In the 1970s, Ohno and Elliott independently reported [17] insecticidal norchrysanthemic acid esters and these norchrysanthemic acid esters showed comparable insecticidal activity to the corresponding chrysanthemates (Fig. 3).

However, further studies were discontinued at that time because they could not find any advantage in developing these norchrysanthemic acid esters due to the increased difficulty in the synthesis of norchrysanthemic acid compared to chrysanthemic acid.



Scheme 6 The first synthesis of norchysanthemic acid by Staudinger and re-examination by Crombie

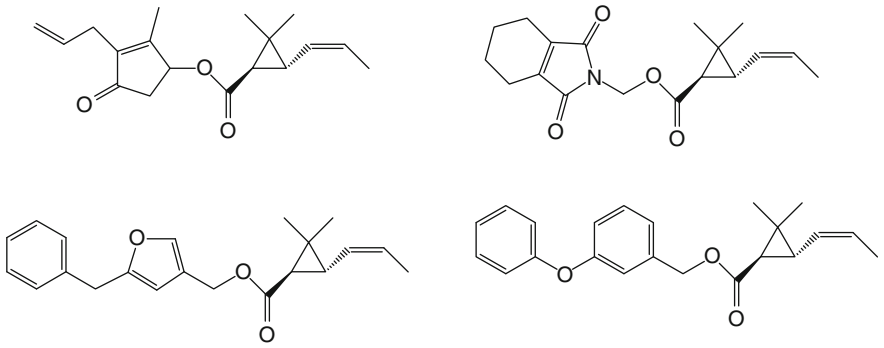


Fig. 3 Insecticidal norchrysanthemate by Ohno and Elliott

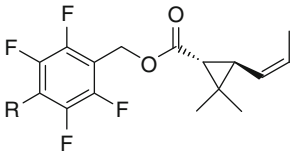
Recently much attention has been directed at the development of devices to control mosquitoes by using products in ambient temperature devices because of their increased safety and ease of use, especially during outdoor activities. This development has resulted in a variety of fan powered mosquito vaporizers and associated formulations which are now being marketed. These devices have limitations in performance that are imposed by the insecticidal activity of the active ingredient used. In order to overcome some of these limitations, we undertook extensive research to find new pyrethroids with higher vapor action which were highly active against mosquitoes.

We directed our attention to norchrysanthemic acid esters because they had a lower molecular mass (−14) and showed comparable insecticidal activity with corresponding chrysanthemates. We synthesized various insecticidal norchrysanthemic acid esters and tested their vapor activity against mosquitoes.

As a result of the screening, we found 2,3,5,6-tetrafluorobenzyl norchrysanthemate (**38**) had faster knockdown activity than the chrysanthemate (**37**) against mosquitoes as shown in Table 1. Then we synthesized the derivatives with substituents at the 4-position on the phenyl ring of the compound (**38**).

All analogs have much higher activity against mosquitoes than empenthrin and compound (**38**) by the standard topical application method as shown in Table 1. The relative toxicity reaches the maximum between two and three carbon atoms at the 4-position (**41** ~ **43**). Unsaturation (**43**) or incorporation of an oxygen atom

Table 1 Insecticidal activities of metofuthrin and its analogus against *Curex pipiens pallens* by the standard topical application method



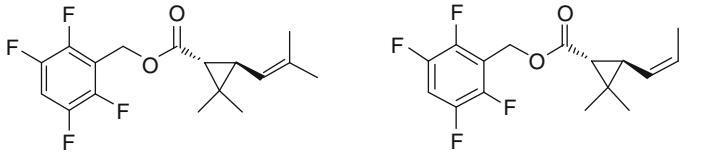
Compound	R	Relative toxicity
38	H	30
39	F	100
40	Me	200
41	Et	490
42	Pr	250
43	Allyl	500
44	OMe	360
45a	CH_2OMe	2,500
Empenthrin(**9**)		10
d-Allethrin(**4**)		100

Table 2 Efficiency of metofluthrin in a non-heating vapor formulation with a fan and mosquito coil formulations aganinst various mosquito species

Formulation	Species	Conc. (%)	KT_{50} (min)	
			Metofluthrin	*d*-Allethrin
Non-heating	*C. pipiens pallens*		27	>60
Mosquito coil	*C. quinquefasciatus*	0.013	49	
		0.02	35	
		0.04	22	
		0.2		54
Mosquito coil	*C. quinquefasciatus*	0.005	42	
		0.2		58

(**44**) also show substantial activity, inter alia, (1*R*)-*trans*-(*Z*)-2,3,5,6-tetrafluoro-4-methoxymethylbenzyl norchrysanthemate (**45a**) exhibits the highest potency being approximately 40 times as potent as *d*-allethrin in mosquito coil formulations when tested against southern house mosquitoes (*Culex quinquefasciatus*). Based on this structure-activity relationship, we have decided to develop the compound (**45b**), (1*R*)-*trans*-(*EZ*)-isomer (*E:Z* ratio is about 1:8), which is named metofluthrin (Fig. 5). Results as shown in Table 2 clearly demonstrate that metofluthrin exhibits significant levels of vapor action against mosquitoes at room temperature. Metofluthrin also exhibits high levels of knockdown activity in coil formulations when tested against mosquito species as shown in Table 2. In particular, metofluthrin exhibits similar activity to *d*-allethrin against the southern house mosquitoes (*Culex quinquefasciatus*) at only 1/40 of the active ingredient level.

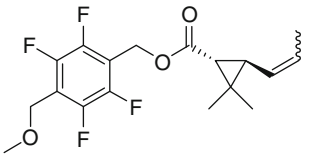
Metofluthrin has high knockdown activity against mosquitoes and has an excellent mammalian safety profile. Metofluthrin is suitable for use not only in various



chrysanthemate (37) **norchrysanthemate (38)**

KT_{50} = 79 [min] KT_{50} = 55 [min]

Fig. 4 Insecticidal activities of 2,3,5,6-tetrafluorobenzyl chrysanthemate and norchrysanthemate



Metofluthrin (45b) (E:Z = about 1 : 8)

Fig. 5 Structure of metofluthrin

existing emanating devices like mosquito coils but also in the more novel new devices such as fan vaporizers and treated paper strips.

4.1 Materials and Methods

Topical application for evaluating insecticidal efficacy against common house mosquitoes (*Culex pipiens pallens*) complied with the method described by Yamaguchi et al. [18].

4.1.1 Vapor Action Activity Evaluation in Non-Heating Formulation at Room Temperature Against Common House Mosquitoes (*C. pipiens pallens*)

A test compound (100 mg) was dissolved in 20 mL of acetone and impregnated onto a sheet of filter paper (20 cm × 50 cm) and the acetone was removed by air-drying. In the center of a 28-m^3 test chamber (4.3 m × 2.65 m × 2.45 m), the filter paper was hung from the ceiling with the upper end of the filter paper 1.7 m above the floor. Four nylon-net cages (cylindrical, 30 cm in diameter and 20 cm in height) each containing 20 female common house mosquitoes (*C. pipiens pallens*) were hung from the ceiling with the base of each cage 60 cm from the floor. One cage was placed in each corner of the room, 60 cm horizontally from the filter. The number of knocked down mosquitoes was counted at the designated intervals for

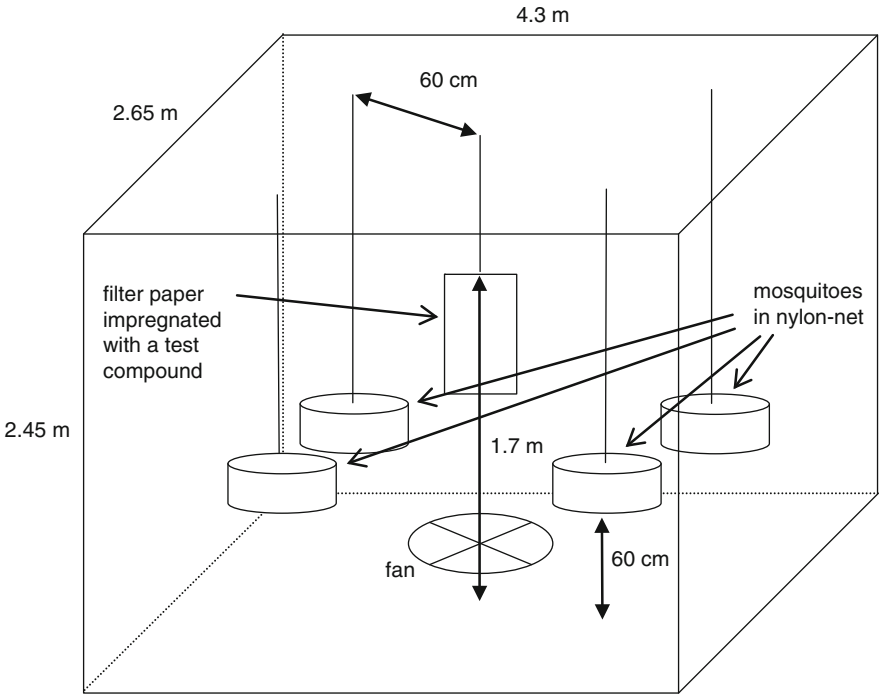


Fig. 6 Method of evaluating vapor activity of formulations at ambient temperature

60 min. In order to circulate air in the chamber, a fan was set under the treated filter paper and a board was placed between the fan and the paper to prevent the fan from directly blowing the filter paper (Fig. 6).

4.1.2 Biological Efficacy Evaluations in Mosquito Coil Formulations

The preparation of test mosquito coils complied with the method described by Yamaguchi et al. The test coil was fitted on a coil holder and placed at the center of the chamber (4.3 m × 2.65 m × 2.45 m). The coil was ignited and then 100 adult female mosquitoes were released into the chamber. The number of knocked down mosquitoes was counted at the designated intervals for 75 min.

5 Synthetic Studies of Norchrysanthemic Acid

The acid moiety of metofluthrin is (1*R*)-norchrysanthemic acid. In this section we describe the development of synthetic studies of *Z*-rich norchrysanthemic acid. Aforementioned norchrysanthemic acid was first synthesized by Staudinger in 1924 as the pyrolytic decomposition product of chrysanthemun dicarboxylic

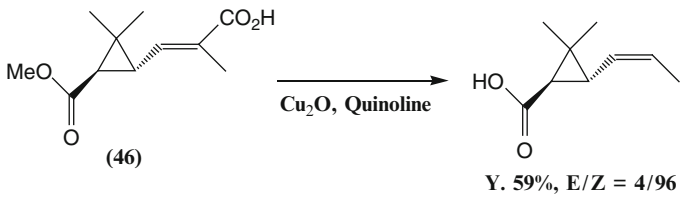
acid. However, the *E*/*Z* ratio of obtained norchrysanthemic acid was not mentioned. Crombie et al. re-examined [19] this pyrolytic reaction of chrysanthemic acid and found the yield of norchrysanthemic acid was only 10% and the *E*/*Z* ratio was 20/80.

5.1 *Synthesis of* (Z)-(1R)-trans-*Norchrysanthemic Acid by the Wittig Reaction*

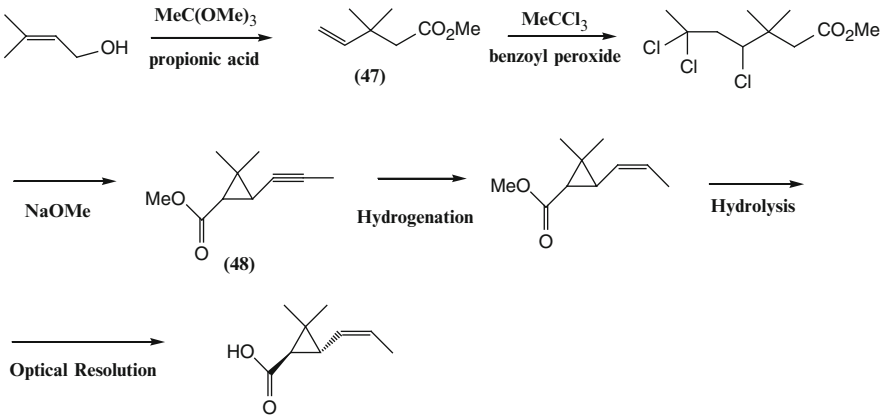
As mentioned, Ohno and Elliott reported the insecticidal (*Z*)-norchrysanthemate as shown in Fig. 3. They both used the Wittig reaction of (1*R*)-*trans*-caronaldehyde ester to obtain (*Z*)-(1*R*)-*trans*-norchrysanthemates. Although the Wittig reaction gives (*Z*)-(1*R*)-*trans*-norchrysanthemates predominantly, it was not practical due to the Wittig reaction conditions requiring very low reaction temperature, a strong base, and the phosphonium salt (much waste atom economy).

5.2 *Synthesis of* (Z)-(1R)-trans-*Norchrysanthemic Acid by Pyrolytic Reaction of Chrysanthemum Dicaroboxylic Acid Monomethyl Ester with a New Catalyst*

Hagiya et al. recently reported [20] the new decarboxylation catalyst of chrysanthemum dicarboxylic acid monomethyl ester (**46**) to give ethyl (*Z*)-norchrysanthemate (*E*/*Z* = 4/96) in 59% yield. This method efficiently gives (*Z*)-(1*R*)-*trans*-norchrysanthemic acid, but the yield is still moderate and the starting chrysanthemum dicarboxylic acid monomethyl ester is not a commercial product (Scheme 7).



Scheme 7 Synthesis of *Z*-norchrysanthemic acid by decarboxylation reaction with Cu_2O and quinoline



Scheme 8 Synthesis of *Z*-norchrysanthemic acid via the Claisen rearrangement

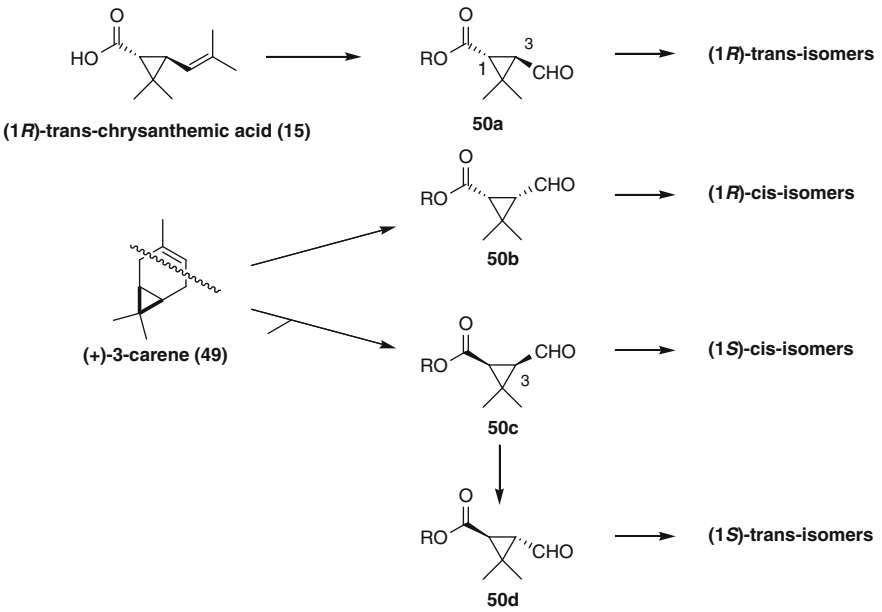
5.3 *Synthesis of (Z)-(1R)-trans-Norchrysanthemic Acid by the Claisen Rearrangement*

Mori et al. reported [21–23] a promising approach to synthesize ethyl (*Z*)-(1*RS*)-*trans*-norchrysanthemate utilizing a partial hydrogenation of ethyl 2,2-dimethyl-3-(1-propynyl)cyclopropanecarboxylate. This was synthesized starting from ethyl 3, 3-dimethyl-4-pentenoate via the Claisen rearrangement followed by radical addition of $MeCCl_3$ and the ring closure with NaOEt. (*Z*)-(1*RS*)-*trans*-Norchrysanthemate was hydrolyzed under basic conditions to give the acid. This was resolved using (−)-1-naphthylethylamine to give (*Z*)-(1*R*)-*trans*-norchrysanthemic acid (Scheme 8).

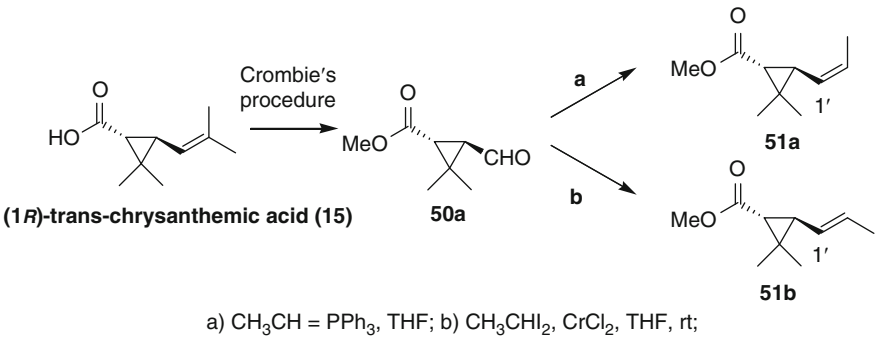
5.4 *Syntheses of All Stereoisomers of Norchrysanthemic Acid*

There are two asymmetric carbon atoms and *E, Z*-isomers in norchrysanthemic acid. So, there exist eight stereoisomers. In order to elucidate the structure activity relationship of metofluthrin stereoisomers, we investigated the synthetic pathways of all stereoisomers of norchrysanthemic acid.

We planned to introduce a 1-propenyl side chain at the C-3 position by the Wittig reaction with caronaldehyde acids or the corresponding esters (**50a–d**) on the last step. (1*R*)-*trans*-Caronaldehyde methyl ester (**50a**, R = Me) can be prepared by ozonolysis of methyl (1*R*)-*trans*-chrysanthemate (**15**). For *cis*-isomers, (+)-3-carene (**49**) was transformed to (1*R*)-*cis*-caronaldehyde acid (**50b**, R = H) and (1*S*)-*cis*-caronaldehyde acid (**50c**, R = H) according to Dev's and Ho's procedures. (1*S*)-*trans*-Isomer (**50d**, R = Me) could be prepared by epimerization at the C-3 carbon atom of (**50c**, R = Me) (Scheme 9).



Scheme 9 Synthesis of (1*R*)-*trans*-(*Z*)- and (*E*)-isomers

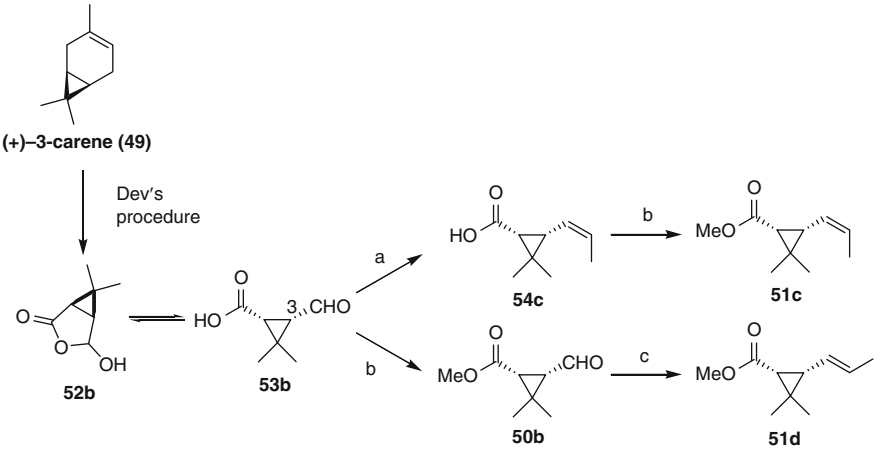


Scheme 10 Synthesis of (1*R*)-*trans*-(*Z*)- and (*E*)-isomers

Syntheses of (1*R*)-*trans*-isomers were reported by Crombie [24] and Elliott [25] starting from (1*R*)-*trans*-chrysanthemic acid by means of the Wittig reaction. Their method were convenient to obtain (*Z*)-isomer (Scheme 10, step a) but not appropriate for the synthesis of (*E*)-isomer because of the (*Z*)-selective nature of the Wittig reaction in the case of nonstabilized ylides. It was very difficult to separate the pure (*E*)-isomer out of the (*E*)- and (*Z*)-mixture. This problem was overcome by use of the Takai's method (Scheme 10, step b) [26]. The (*E*)-selectivity of the double bond was fairly high (*E*:*Z* = 89:11) (Scheme 10).

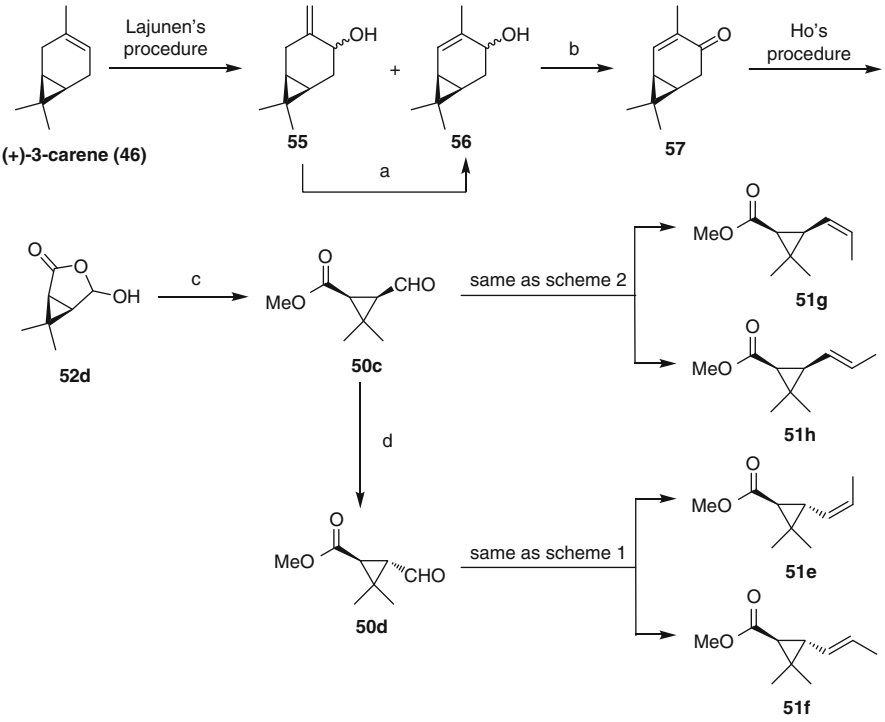
(1*R*)-*cis*-isomers were synthesized as shown in Scheme 11. (1*R*)-*cis*-Caronaldehyde acid hemiacetal (**52b**), which is the equivalent of (1*R*)-*cis*-caronaldehyde acid **53b**, could be prepared from (+)-3-carene (**49**) according to the Dev's procedure [27]. The Wittig reaction of hemiacetal (**52b**) under ice-cooling gave not only desired (1*R*)-*cis*-(*Z*)-norchrysanthemic acid (**54c**) but also undesired (1*R*)-*trans*-isomer (*cis*:*trans* = 80:20). Fortunately, this epimerization was prevented by performing the reaction at lower temperature. Thus, the Wittig reaction of hemiacetal (**52b**) with $CH_3CH = PPh_3$ at −40 °C gave (1*R*)-*cis*-(*Z*)-norchrysanthemic acid (**54c**) free from the *trans*-isomer. The acid (**54c**) was converted to the corresponding methyl ester (**51c**) using the Mitsunobu reaction. (1*R*)-*cis*-(*E*)-Isomer was prepared using the same procedure as the (1*R*)-*trans*-(*E*)-isomer via Takai's method. However, the reaction resulted in the lower stereoselectivity (*E*:*Z* = 72:28) because of the more steric hindrance of the (*E*)-isomer with the ester group (Scheme 11).

(1*S*)-isomers were synthesized as shown in Scheme 12. According to the Lajunen's procedure, (+)-3-carene was converted to a mixture of allyl alcohol derivatives (**55**) and (**56**) (9:1) in totally 60% yield. The mixture was treated under basic conditions to obtain the pure alcohol (**56**) via the isomerization of the double bond. Subsequently the alcohol (**56**) was oxidized with PCC to give 2-caren-4-one (**57**). This was converted to (1*S*)-*cis*-caronaldehydic acid hemiacetal (**52d**), which is an antipode of (**52b**), according to Ho's procedure [28]. The (1*S*)-hemiacetal was transformed to the (1*S*)-*cis*-(*Z*)- and (1*S*)-*cis*-(*E*)-isomers according to the same procedures as (1*R*)-*cis*-isomers. For *trans*-isomers, the methyl ester (**50c**) was epimerized at the C-3 position to give the (1*S*)-*trans*-isomer by sodium methoxide. (1*S*)-*trans*-(*Z*)- and (1*S*)-*trans*-(*E*)-norchrysanthemic acid methyl esters were obtained in the same manner as described in Scheme 10.



a) $CH_3CH_2PPh_3$·Br, *t*-BuOK, THF, -40 °C to 0 °C; b) MeOH, *i*-PrOCO-N = N-CO_2*i*-Pr, Ph_3P, THF; c) CH_3CHI_2, $CrCl_2$, THF, rt;

Scheme 11 Synthesis of (1*R*)-*cis*-(*Z*)- and (*E*)-isomers



a) *t*-BuOK, DMSO, 100 °C; b) PCC, CH_2Cl_2; c) MeOH, *i*-PrOCO-N = N-CO_2*i*-Pr, Ph_3P, THF; d) MeONa, MeOH.

Scheme 12 Synthesis of (1*S*)-*cis*-(*Z*)-, (1*S*)-*cis*-(*E*)-, (1*S*)-*trans*-(*Z*)-, and (1*S*)-*trans*-(*E*)-isomers

In conclusion, all of the eight stereoisomers of norchrysanthemic acid methyl esters were synthesized in stereoselective manner starting from (1*R*)-*trans*-chrysanthemic acid or (+)-3-carene. All stereoisomers of metofluthrin were synthesized in our laboratory. Their structure-activity relationship will be published elsewhere.

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Advances in the Mode of Action of Pyrethroids

J. Marshall Clark and Steven B. Symington

Abstract The ability to clone, express, and electrophysiologically measure currents carried by voltage-gated ion channels has allowed a detailed assessment of the action of pyrethroids on various target proteins.

Recently, the heterologous expression of various rat brain voltage-gated sodium channel isoforms in *Xenopus laevis* oocytes has determined a wide range of sensitivities to the pyrethroids, with some channels virtually insensitive and others highly sensitive. Furthermore, some isoforms show selective sensitivity to certain pyrethroids and this selectivity can be altered in a state-dependent manner. Additionally, some rat brain isoforms are apparently more sensitive to pyrethroids than the corresponding human isoform. These finding may have significant relevance in judging the merit and value of assessing the risk of pyrethroid exposures to humans using toxicological studies done in rat.

Other target sites for certain pyrethroids include the voltage-gated calcium and chloride channels. Of particular interest is the increased effect of Type II pyrethroids on certain phosphoforms of the N-type $Ca_v2.2$ calcium channel following post-translational modification and its relationship to enhanced neurotransmitter release seen in vivo.

Lastly, parallel neurobehavioral and mechanistic studies on three target sites suggest that a fundamental difference exists between the action of Types I and II pyrethroids, both on a functional and molecular level. These differences should be considered in any future risk evaluation of the pyrethroids.

Keywords CS-syndrome · Neurotransmitter release · Pyrethroids · T-syndrome · Voltage-gated calcium channels · Voltage-gated sodium channels

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Contents

1 Introduction

In our previous review [1], we summarized the mechanisms of pyrethroid neurotoxicity in mammals from the perspective of the "common mechanism" statute of the Food Quality Protection Act of 1996. In addition to discussing the structure and insecticidal properties, mammalian toxicity, toxicokinetics and metabolism, and physiological and neurochemical indices of intoxication of pyrethroids, we identified four sites of toxic action that pyrethroids act on in vitro: (1) voltage-gated sodium channels, (2) voltage-gated calcium channels, (3) voltage- and ligand-gated chloride channels, and (4) the γ−aminobutyric acid (GABA) receptor-chloride ionophore complex ($GABA_A$ receptor). Of the potential target sites implicated in the action of pyrethroids, only the voltage-gated sodium, calcium, and chloride channels are altered by relatively low concentrations of pyrethroids, elicit stereospecific actions, and have been implicated in the acute neurotoxicological response using function assays.

Biochemical evidence indicates that the Type II pyrethroids (α-cyano-containing pyrethroids) bind and block GABA receptors expressed in the mammalian brain [1]. Blocking GABA receptors would inhibit Cl^- flux and remove inhibitory neuronal input leading to an indirect neuroexcitatory effect, similar to the action of the convulsant, picrotoxin. Such an action is therefore consistent with the functional neuroexcitatory symptoms of the pyrethroids elicited in mammals. The action of pyrethroids on GABA receptors, however, is only semi-stereospecific for the neurotoxic Type II pyrethroids and block is only apparent at concentrations of pyrethroids that exceed those necessary to disrupt the function of voltage-gated sodium channels. Due to the incomplete stereospecificity and relatively low potency of pyrethroids at GABA receptors, it is unlikely that the GABA receptor is a significant target site, resulting in the choreoathetosis with salivation (CS) syndrome produced by Type II pyrethroids in vivo. Because of this conclusion, our current review will not include further discussion of the GABA receptor as a major target site for pyrethroid action.

There is strong agreement, however, that voltage-gated sodium channels are principal sites of pyrethroid action in mammals and may be the sole site of action in insects [1]. All pyrethroids have been shown to perturb the function of at least one type of voltage-gated sodium channel. Generally, most Type I pyrethroids (no α-cyano group) induce burst discharges (repetitive action potentials in nerve axons) in response to a single electrical depolarization, which do not substantially affect the resting membrane potential, and produce short lived sodium tail currents under voltage-clamp conditions. These events are widely believed to cause the tremor (T) syndrome of pyrethroid intoxication in mammals. Conversely, most Type II pyrethroids produce long-lived sodium tail currents under voltage-clamp conditions, which cause extensive depolarization of the resting membrane potential and result in relatively rapid, use-dependent, nerve conduction block. Most Type II pyrethroids produce the CS-syndrome of intoxication or some intermediate response, which is a mix of the T- and CS-syndromes, such as that elicited by fenpropathrin. The actual events that cause the CS-syndrome and how conduction block is involved in this process, nevertheless, are not currently well understood.

It is of interest that insects usually have only a single gene that expresses the α-subunit (the pore-forming subunit that is putatively considered the binding site of pyrethroids) of voltage-gated sodium channels, whereas mammals generally have nine such genes [1]. Recently, it has been determined that different channel isoforms, formed with these different α-subunits, vary widely in their sensitivity to pyrethroids when heterologously expressed and this aspect will be a major focus of the current review.

It was also concluded in our previous review that voltage-gated sodium channels may not be the only targets involved in the neurotoxicity of pyrethroids in mammals but that other voltage- and ligand-gated ion channels are of interest and may contribute to the neurotoxic action of at least some pyrethroids. Specifically, the action on voltage-gated calcium and chloride channels have been suggested to be involved in the CS-syndrome produced by most Type II pyrethroids.

Of high interest is the role of certain voltage-gated calcium channels (N-, P/Q-, R- and T-types), which are directly involved in the release of neurotransmitter from presynaptic nerve terminals following depolarization. Additionally, certain voltage- and ligand-gated chloride channels, which function as rectifying channels allowing Cl^- flux to balance depolarizing and hyperpolarizing conditions thereby buffering hyperexcitability in the nerve, have also been implicated. Early acute toxicity studies in rat indicated that the in vivo action of Type II pyrethroids that cause the CS-syndrome was different than that of T-syndrome pyrethroids. Deltamethrin, a Type II pyrethroid producing the CS-syndrome in mammals, decreased the acetylcholine (the neurotransmitter released by cholinergic presynaptic nerve terminals) content of the cerebellum by some 52%. DDT, a well-established voltage-gated sodium channel agonist that causes an intoxication syndrome indistinguishable from the T-syndrome elicited by most of the Type I pyrethroids in mammals, and cismethrin, a Type I pyrethroid that causes the T-syndrome in mammals, caused no significant reduction [2]. These highly relevant in vivo experiments clearly delineated a physiological response (enhanced neurotransmitter release) that was different

between the Type I pyrethroid cismethrin, which produces the classic T-syndrome, and the Type II pyrethroid, deltamethrin, which elicits the classic CS-syndrome. Because deltamethrin causes rapid and extensive resting membrane depolarization and conduction block of action potentials in the axon (an action at voltage-gated sodium channels that is not consistent with enhanced neurotransmitter release), other mechanisms, in addition to actions at voltage-gated sodium channels, may be involved with the neurotoxic action of CS-syndrome pyrethroids, including voltage-gated calcium and chloride channels.

Release of neurotransmitter from presynaptic nerve terminals following action potential depolarization occurs rapidly, is highly regulated [3], and is absolutely dependent upon Ca^{2+} entry via voltage-gated calcium channels (N-, P/Q-, R- and T-type), which are co-localized in active release zones along with synaptic vesicles and the SNARE protein apparatus [4–7]. This arrangement delivers small amounts of concentrated Ca^{2+} (~3 mM) briefly to the active release zone, which allows synaptic vesicles to fuse with the synaptolemma releasing neurotransmitter via exocytosis [7]. Any perturbation of this highly regulated process can have varied and devastating consequences for any organism with a nervous system. In view of the toxicological importance of this process, this present review will summarize the action of pyrethroids on voltage-gated calcium channels and voltage- and ligand-gated chloride channels that modulate this process.

To accomplish the above in an efficient manner, we have chosen to summarize the literature on the mode of action of pyrethroids in mammals published since our last review in 2002. During the time since our last review, there have been a number of excellent reviews that have dealt, in part, with this subject matter and we will use this information to guide our summarization and review new information published since 2009 [8–11].

2 Development and Neurotoxicity of Pyrethroid Insecticides

2.1 Chemistry and Structure-Activity Relationships

Pyrethroids are synthetic analogs that are based structurally on the six naturally occurring esters found in the apolar solvent extract from the flower heads of the Chrysanthemum plant [12]. Each of the six naturally-occurring esters is called a pyrethrin and together they comprise the pyrethrum extract. Pyrethrum itself is clearly the most successful botanical insecticide with high insecticidal potency but low mammalian toxicity. Unfortunately, these natural esters are susceptible to hydrolysis and photodegradation, which hampered its commercialization as an agricultural insecticide. Using a repetitive process of structural alteration followed by biological assessment, the first "synthetic pyrethroid," allethrin, was commercially introduced in 1949 [12]. Over the next 30 years, a variety of pyrethroids were developed, all of them mimicking the chemistry of and filling a three-dimensional space occupied by the pyrethrins [1] (Fig. 1).

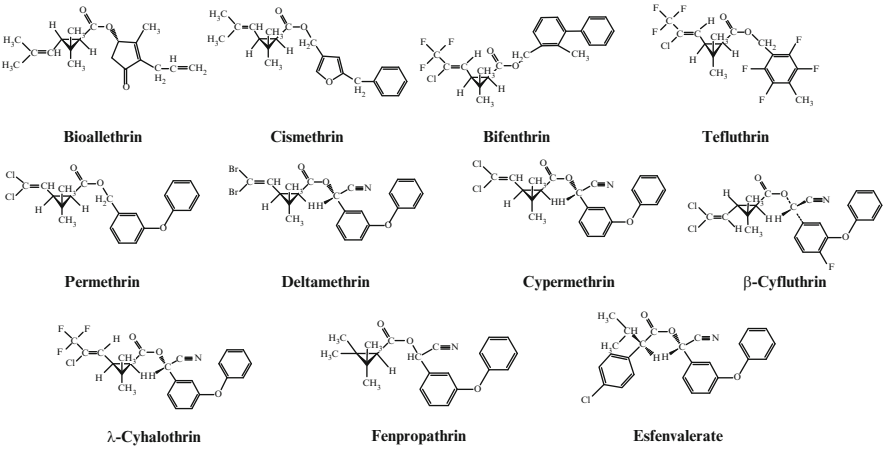

Fig. 1 Structures of selected Type I and Type II pyrethroids

Three major discoveries resulted in greatly increasing the environmental stability of the pyrethroids without compromising their insecticidal potency or mammalian safety [11]. The first alteration was to replace the photolabile dimethylvinyl group in the acid moiety of the natural ester by exchanging the methyl groups with halogens to obtain a much more photostable dichlorovinyl group. The second alteration was to replace the natural alcohols of the chrysanthemates with a 3-phenoxybenzyl alcohol. Coupling these two alterations gave rise to the development of permethrin, the first substantially field-stable pyrethroid in 1973. The introduction of a nitrile group onto the methylene carbon of the 3-phenoxylbenzyl alcohol was the third major alteration giving rise to the development of the α-cyano-containing pyrethroids (currently referred to as the Type II pyrethroids), such as deltamethrin (1974) and cypermethrin (1975). It was also found that an α-isopropylphenylacetic acid replacement of the 2,2-dimethylcyclopropanecarboxylic acid found in the pyrethrins resulted in pyrethroids that contained no cyclopropane rings, such as fenvalerate, and that replacement of the central ester moiety by ether linkages was possible, giving rise to non-ester pyrethroids, such as etofenprox.

Although structurally-diverse as evidenced above, the insecticidal pyrethroids still conform to a unique, operationally-defined, structure-activity relationship based on the physical characteristics and three-dimensional shape of the entire molecule conforming to those originally evidenced in the natural pyrethrins [13]. From this relationship, it becomes apparent that there is no single molecular aspect or reactive moiety that serves as a true toxophore for the pyrethroids and that their actions at target sites are dependent upon the entire stereospecific structure of these insecticides [1].

There are two sites where stereospecificity must be maintained in order to achieve optimal insecticidal action. As seen in the acid moieties of the natural pyrethrin esters, all pyrethroids that possess a cyclopropane ring must have the 1*R*

configuration at the C-1 carbon of that ring to be insecticidal. Enantiomeric 1*S* compounds are not insecticidal although they remain physically identical with their 1*R* enantiomers [13]. Even pyrethroids without a cyclopropane ring in their acid moiety must conform to this stereospecificity. As evidenced in fenvalerate, the 2*S* configuration, which mimics the 1*R* configuration of cyclopropane-containing pyrethroids, is insecticidal whereas the corresponding 1*R* configuration is non-insecticidal. Although less stringent, a stereoisomerism within the alcohol moiety also exists when chiral centers are present. As evidenced by deltamethrin, the α-cyano group within the 3-phenoxybenzl alcohol can be in the α*R* or the α*S* configuration, with only the α*S* configuration being highly insecticidal [14].

Generally, the structure-activity relationships initially determined in insects as discussed above have also been found to be obeyed in mammals (see 77.3.2, [10]).

2.2 Acute Toxicity and Syndromes of Intoxication

Pyrethroids have been generally determined to be relatively safe following their exposure to humans and other mammals. They are poorly absorbed dermally and when administered orally in aqueous suspensions due to their high lipophilicity. Oral toxicity can be enhanced by co-administration in vegetable oils [1]. They are readily metabolized and detoxified by hydrolytic cleavage of the central ester bond and by oxidative metabolism usually within the alcohol moiety, primarily producing hydroxylated metabolites. When orally dosed in vegetable oils, most pyrethroids are moderately toxic to rats (LD_{50} values 50–500 mg/kg, EPA Toxicity Category II) with a few exceptions. Tefluthrin is classified in EPA Toxicity Category I and both permethrin and resmethrin are classified in Category III [10].

Two distinct syndromes of acute toxicity of pyrethroids have been identified in rats following oral and intravenous administration. Verschoyle and Barnes [15] first described a common syndrome of intoxication following dosing, both orally and intravenously, for the natural pyrethrins bioallethrin and resmethrin (both Type I pyrethroids), which included hypersensitivity and aggression, followed by stimulus-induced bouts of general tremor, convulsive twitching, coma, and death. In 1994 following the discovery of deltamethrin (a Type II pyrethroid), these researchers reported a syndrome of intoxication, following either oral or intravenous dosing, that was noticeably different that previously reported, which included salivation without lacrimation, followed by jerking leg motions and progressive writhing convulsions (choreoathetosis) [16]. In 1980, Verschoyle and Aldridge [17] published a pivotal study, which described the signs of acute intoxication of 36 pyrethroids following intravenous dosing. Fifteen of the 18 esters of various primary alcohols examined (Type I pyrethroids) elicited signs of intoxication that were indistinguishable from those first described for the pyrethrins, bioallethrin and resmethrin. The other three esters were not toxic at the doses tested. This syndrome was designated as the “tremor” or T-syndrome. Twelve of the 17 esters, which had α-cyano-3-phenoxybenzyl alcohols (Type II pyrethroids), elicited signs of

intoxication similar to those initially described for deltamethrin, and was designated as the "choreoathetosis with salivation" or CS-syndrome. One α-cyano-3-phenoxybenzyl ester and one α-ethynyl-3-phenoxybenzyl ester produced intermediate syndromes of intoxication. The separation of pyrethroid intoxication into these two principle syndromes was confirmed by Lawrence and Casida [18] following the intracerebral injection of 29 pyrethroids into mice.

Although most Type I pyrethroids produce the T-syndrome and most Type II pyrethroids produce the CS-syndrome, there are exceptions to this classification. Fenpropathrin, a Type II pyrethroid, and permethrin, a Type I pyrethroid, produce mixed intoxication syndromes depending on the study and animal examined. Lastly, the signs of intoxication may not be independent of the routes of administration [1]. As reported recently, the results of a functional observational battery study of 12 pyrethroids in rats following acute oral exposure did not correlate well with the signs of intoxication following intravenous dosing [19].

3 Neurophysiological Effects of Pyrethroids

3.1 Modulation of Voltage-Gated Sodium Channels

3.1.1 Voltage- and Patch-Clamp Electrophysiology Studies

There have been many studies over the last 40 years that provide substantial evidence that voltage-gated sodium channels in the CNS are major sites of action for the pyrethroids in mammals [1, 20–25]. This literature has been recently critically reviewed by Soderlund [10] from the viewpoint of the neurotoxic action of pyrethroids in mammals and we will rely heavily on this review in our summary below.

The finding that pyrethroids generally elicit one of two distinct effects on nerve, depending on pyrethroid structure, was first demonstrated using intracellular electrophysiological recordings of action potentials by Lund and Narahashi [26]. Type I pyrethroids resulted in transient depolarizing after potentials that over time cause long trains of action potentials (repetitive discharges) to be produced following a single stimulus to the nerve. Under these conditions, there was little or no persistent alteration in the resting membrane potential. Most Type II pyrethroids produce a use-dependent conduction block of the action potential due to an extensive and rapid rise (depolarization) in resting membrane potential. Under these conditions, repetitive discharges are generally not seen. There are some pyrethroids (e.g., permethrin), however, that cause transient repetitive discharge followed by resting membrane depolarization and conduction block.

Using both voltage- and patch-clamp electrophysiological protocols, pyrethroids have been shown to affect the kinetic properties of voltage-gated sodium channels in a way that is consistent with their neuroexcitability [22]. With voltage-clamp,

pyrethroids slow activation, inactivation, and deactivation kinetics, leading to decreased transient peak current, enhanced late current, and prolonged tail current, respectively. The last two effects allow excess sodium ion to enter the nerve, resulting in nerve depolarization and hyperexcitability. A major difference between Types I and II pyrethroids is that Type II pyrethroids result in tail currents that are greatly prolonged in duration whereas the Type I pyrethroids result in tail currents that decay relatively rapidly. When individual channels are examined using patch-clamp techniques, results are comparable to their effects on macroscopic current as discussed above. For example, the Type I pyrethroid, tetramethrin, increases the mean opening time of single channels by ~10-fold, whereas deltamethrin, a Type II pyrethroid, can increase the opening time by ~200-fold.

A most interesting and significant finding is the recent elucidation of the differential sensitivity of voltage-gated sodium channel isoforms to pyrethroids. Because the nerve tissue preparations initially used to study the action of pyrethroids expressed multiple channel isoforms (there are nine pore-forming, sodium channel α-subunit genes, *$Na_v1.1$–1.9*, and four sodium channel β subunit genes, *β1-4,* in mammals, that can combine following translation to form a variety of heteromultimeric channels), there was no way to correlate the action of a pyrethroid to a specific channel isoform [27–29]. Nevertheless, early work in this area showed that allethrin, tetramethrin, and deltamethrin were all more efficacious on tetrodotoxin (TTX)-resistant channels than on TTX-sensitive channels [30–32], which indicated that a differential sensitivity of isoforms to the pyrethroids was likely.

This problem of overlapping expression of sodium channel isoforms has recently begun to be addressed using cloned channels heterologously expressed individually in *Xenopus laevis* oocytes with voltage-clamp techniques and the picture that is emerging is that there are substantial differences in the sensitivity of mammalian sodium channel isoforms to the pyrethroids.

Using this approach, Smith and Soderlund [33] initially reported that rat $Na_v1.2$, a sodium channel that is highly expressed in the CNS, was only slightly modified by deltamethrin and other pyrethroids. In comparison, rat $Na_v1.8$, a sodium channel that is TTX-resistant and expressed only in the PNS, was highly sensitive to pyrethroid modification [34]. Similarly, the rat $Na_v1.3$ channel, which is preferentially expressed in the embryonic and neonatal stages, is also highly sensitive to modification by Type II pyrethroids [35, 36]. Recently, Tan and Soderlund demonstrated that rat $Na_v1.6$, the most abundant sodium channel expressed in adult brain, was likewise highly sensitive to pyrethroid modification [37] but also showed a divergent action to Types I and II pyrethroids [38]. Bioallethrin, a Type I pyrethroid, only transiently modified the rapidly decaying tail current but weakly modified the channel under resting conditions, and modification of the channel was not increased following repetitive activation by high-frequency trains of depolarizing pulses. Deltamethrin, a Type II pyrethroid, however, resulted in tail currents that were ~ninefold more persistent than those caused by bioallethrin, again only weakly modified the resting channel but resulted in fourfold more channel modification when repeatedly activated. Tefluthrin, a highly toxic Type I pyrethroid, was intermediate in its effect on rat $Na_v1.6$. Using concentration-effect

data, the potency of tefluthrin, however, was greater than deltamethrin as a use-dependent modifier of this channel. Comparative experiments showed that the rat $Na_v1.6$ isoform was ~15-fold more sensitive to the action of tefluthrin and deltamethrin than the rat $Na_v1.2$ isoform. Given its high expression level in the CNS and high affinity, particularly under use-dependent conditions, rat $Na_v1.6$ isoform appears to be an important target site and may be implicated in the neurotoxic effects produced by pyrethroids.

Although only limited data are available, the single channel analysis using patch-clamp techniques of rat $Na_v1.2$ channels expressed in *Xenopus* oocytes is consistent with the results discussed above [39]. In this work, the histograms of the channel open times in the absence of deltamethrin were best fitted by single exponentials, which were fast and decreased with depolarization. Upon treatment with deltamethrin, opening times were best fitted by two exponentials, a fast one and a slow one. Only the population of long duration openings with the slow exponential increased when treated with increasing concentrations of deltamethrin.

An important ramification of the differential sensitivity of certain sodium channel isoforms to pyrethroids is that some channel isoforms are also developmentally regulated. A particularly relevant example of this process has been described [35] where the effects of deltamethrin and other Type II pyrethroids were determined on the sodium currents from *Xenopus* oocytes injected with different combinations of rat alpha $Na_v1.2$ or $Na_v1.3$ and beta(1) or beta(3) subunits. The $Na_v1.3$/beta(3) channels, which are expressed in the embryo and neonate, were found to be more sensitive to modification by deltamethrin and other Type II pyrethroids when compared to the $Na_v1.2$/beta(1) channels, which are highly expressed in adults. These toxicodynamic results are consistent with the in vivo finding that juvenile rats may be more sensitive to the toxic action of deltamethrin than adults and may augment the documented toxicokinetics differences between juvenile and adult rats.

Because sodium channel α-subunit genes from rat and human are highly conserved (>95% identical on an amino acid basis [28]), it has been assumed that toxicological studies of the pyrethroids in rat would be similar to those in humans. This level of similarity, however, still allows some 50–100 amino acids to be different between sodium channels from these two species and recent information indicates that there may be significant differences in the sensitivity of specific isoforms to pyrethroid modifications in rats vs humans [36]. Following heterologous expression in *Xenopus* oocytes and under voltage-clamp conditions, exposure of rat and human $Na_v1.3$ channels to tefluthrin resulted in channels that activated, inactivated, and deactivated more slowly than untreated channels. Modification by tefluthrin, however, was fourfold greater when applied to rat $Na_v1.3$ compared to human $Na_v1.3$. Additionally, human $Na_v1.3$ was also less sensitive to modification by tefluthrin when compared to rat $Na_v1.2$, a sodium channel, that has been determined to be relatively insensitive to pyrethroids. These findings may have significant relevance in judging the merit and value of assessing the risk of pyrethroid exposure to humans using toxicological studies done in rat.

Recent results that examined the effect of pyrethroids on voltage-gated sodium channels expressed in human embryonic kidney (HEK293) cells grown in

continuous culture are also consistent with the above findings based primarily on heterologously expressed channels. Using HEK293 cells, He and Soderlund [40] identified an endogenous TTX-sensitive current, principally due to the expression of the $Na_v1.7$ isoform, which is modified by pyrethroids. Tefluthrin prolonged the inactivation of the transient currents and induced slowly decaying tail currents, modifications that are consistent with the effect of pyrethroids on many voltage-gated sodium channels [41].

3.1.2 The "Pyrethroid Receptor" on Sodium Channels and Implications of the "State-Dependent" Actions of Pyrethroids

Numerous biochemical [42, 43] and radioligand binding studies [44–47] have determined the existence of a pyrethroid binding site on the α-subunit, which is allosterically coupled to the α-scorpion toxin, brevetoxin and veratridine/batrachotoxin binding sites. Insight as to the location of the receptor within the α-subunit has been inferred using the occurrence and location of point mutations within insect α-subunit genes that give rise to amino acid substitutions that are associated and in some cases functionally related to pyrethroid resistance and sodium channel insensitivity, respectively [48]. From analyses of these mutations [49–51] and recent modeling of voltage-gated sodium and potassium channels [52, 53], a pyrethroid receptor has been postulated that consists of amino acid residues in the intracellular linkers between transmembrane helices S4 and S5 or within the transmembrane helices S5 and S6 of the homology domain II, with additional interactions being supplied by the S6 helices and associated regions of homology domains I and III. Using this information, a structural model of the pyrethroid receptor of the house fly Vssc1 sodium channel has been recently developed based on the crystal structure of homologous voltage-gated potassium channels in the open configuration [54]. From computer-generated docking experiments using DDT and pyrethroids, four amino acids, M918, L925, T929, and L932 (all found within the linker between S4 and S5 and the S5 helices of homology domain II), were identified as important in the binding of these ligands. As recently pointed out by Soderlund [10], this model pertains only to insect sodium channels and has little relevance in understanding the differential sensitivity of mammalian sodium channel isoforms to pyrethroids as discussed above as all four amino acids identified are conserved across all nine mammalian sodium channel isoforms.

A main consideration in modeling the pyrethroid receptor on insect sodium channels in the open state was the determination that channel modification by cypermethrin and deltamethrin of cloned insect sodium channels expressed in *Xenopus* oocytes occurred only following repeated depolarizations [33, 55]. This use-dependency of some pyrethroids was the basis for the widely held opinion that these pyrethroids bind preferentially to open sodium channels and that state-dependent modification of sodium channels by pyrethroids was an important consideration for any receptor modeling.

There are several recent papers from the Soderlund research group that have examined the impact of use-dependency on the modification of rat sodium channel isoforms expressed in *Xenopus* oocytes [34, 36, 38]. The salient findings were that: (1) all 11 pyrethroids tested produced resting or closed-state modification of $Na_v1.8$ channels, with deltamethrin and three additional Type II pyrethroids also producing use-dependent modifications; (2) *S*-bioallethrin produced no enhancement of resting modification of $Na_v1.6$ upon repeated depolarizations; (3) modification of $Na_v1.2$ and $Na_v1.6$ by deltamethrin only occurred following repeated depolarizations; and (4) tefluthrin resulted in resting modification that were increased two- to fourfold following repeated depolarizations. Together, these findings substantiate that the relative importance of resting and use-dependent modification varies with pyrethroid structure. Implicit in this finding is that channel activation, leading to the open state, results in a pyrethroid receptor that has binding characteristics that differ from the pyrethroid receptor of channels in the resting state. Therefore, the existing pyrethroid receptor model, which is based on the "open state" of the insect sodium channel, will likely have to be modified to accommodate pyrethroids that bind channel isoforms in the resting or close-state, and channels that have activated but have not yet opened. Information gained from such state-dependent models may produce a better molecular-based understanding of the differential sensitivity of certain sodium channel isoforms to pyrethroids [10].

3.2 Modulation of Voltage-Gated Calcium Channels

3.2.1 Voltage- and Patch-Clamp Electrophysiological Studies

Evaluation of pyrethroid effects on expressed voltage-gated calcium channels indicates that these channels are modified at concentrations similar to voltage-gated sodium channels and suggests that pyrethroid modulation of these targets may play a role in acute neurotoxicity. Pyrethroids as a class modify the gating kinetics of voltage-gated calcium channels expressed in heterologous expression systems in dissimilar manners. Deltamethrin, a Type II pyrethroid, reduced the peak current of a rat N-type voltage-gated calcium channel ($Ca_v2.2$) expressed in *Xenopus* oocytes in a concentration-dependent and stereospecific manner with an estimated IC_{50} of ~2 nM [56]. Deltamethrin also increased the rate of activation and prolonged the inactivation rate of this channel. In similar electrophysiological experiments using differentiated PC12 cells, the Type I pyrethroid allethrin modified a variety of voltage-gated calcium channel properties independent of voltage-gated sodium channels. Neal et al. [57] report that allethrin inhibited the peak and tail currents of N-type voltage-gated calcium channel expressed in these differentiated PC12 cells with an estimated IC_{50} of ~10 μM [57, 58]. In other similar experiments, allethrin was also found to block P/Q-type voltage-gated calcium channels expressed in non-neuronal HEK cells with an estimated

IC_{50} of 7 μM [59]. These authors also found that allethrin elicited a hyperpolarizing shift in voltage-dependent inactivation and accelerated the inactivation kinetics under steady-state depolarization. Collectively, these results suggest that Type II pyrethroids are more potent antagonists of N- and P/Q-type voltage-gated calcium channels than Type I pyrethroids. However, differences in electrophysiological systems used in these experimental systems could also partly explain the differences in the IC_{50} values reported.

Electrophysiological approaches have also been used to investigate the effect of pyrethroids on other types of voltage-gated calcium channels and have shown that they are modified at concentrations similar to voltage-gated sodium channels. Allethrin was found to block L-type ($Ca_v1.2$) voltage-gated calcium channels expressed in HEK cells in a use-dependent manner with an estimated IC_{50} of ~7 μM and caused a hyperpolarizing shift in voltage-dependent inactivation [59]. Conversely, Neal et al. [57, 58] report that allethrin increased peak and tail currents of $Ca_v1.2$ expressed in PC12 cells in a concentration-dependent manner with an EC_{50} in the pM range. In addition, allethrin also significantly altered the voltage-dependence of activation of $Ca_v1.2$ [58].

Pyrethroid modification of low voltage-activated T-type calcium channels (Ca_v3) has likewise been investigated in a variety of experimental systems. The original electrophysiological analysis of the Type I pyrethroid, tetramethrin, in mouse neuroblastoma cells (N1E-115) reported that barium currents via T-type calcium channels were blocked [60]. Hilldebrand et al. [59] confirmed this report using $Ca_v3.1$ expressed in HEK cells. These authors showed that $Ca_v3.1$ was blocked by allethrin in a concentration-dependent manner with an IC_{50} of ~7 μM. The effect of fenvalerate on a T-type voltage-gated calcium channels in mouse spermatocytes has been characterized using a whole-cell patch-clamp technique. Fenvalerate significantly inhibited this calcium channel current in a concentration-dependent manner with an estimated IC_{50} of ~0.25 μM and also resulted in a 15 mV hyperpolarization shift of the voltage-dependent activation potential [61]. More recently, a suite of pyrethroids were examined on an expressed human T-type voltage-gated calcium channel ($Ca_v3.2$) in *Xenopus* oocytes. The results showed that Types I and II pyrethroids do not modify $Ca_v3.2$ in a consistent manner. Pyrethroids that possess an α-cyano moiety were more potent and efficacious inhibitors of peak current than those that lack this moiety [62].

In summary, pyrethroids appear to modify expressed voltage-gated calcium channels in substantially different ways depending on the experimental system utilized and compound examined. The collective results presented above suggest that pyrethroid action on voltage-gated calcium channels is both analog- and isoform-specific. Furthermore, the collective regulation of all the proteins and co-factors localized to the specific channel may alter the manner by which pyrethroids modify voltage-gated calcium channels. In support of this, Xiao et al. [61] report that fenvalerate inhibition of T-type voltage-gated calcium channel current in mouse spermatogenic cells was partially reversed when treated with calmodulin and was independent of changes in current density and suggest that the phosphorylation state of voltage-gated calcium channels may play a role in the

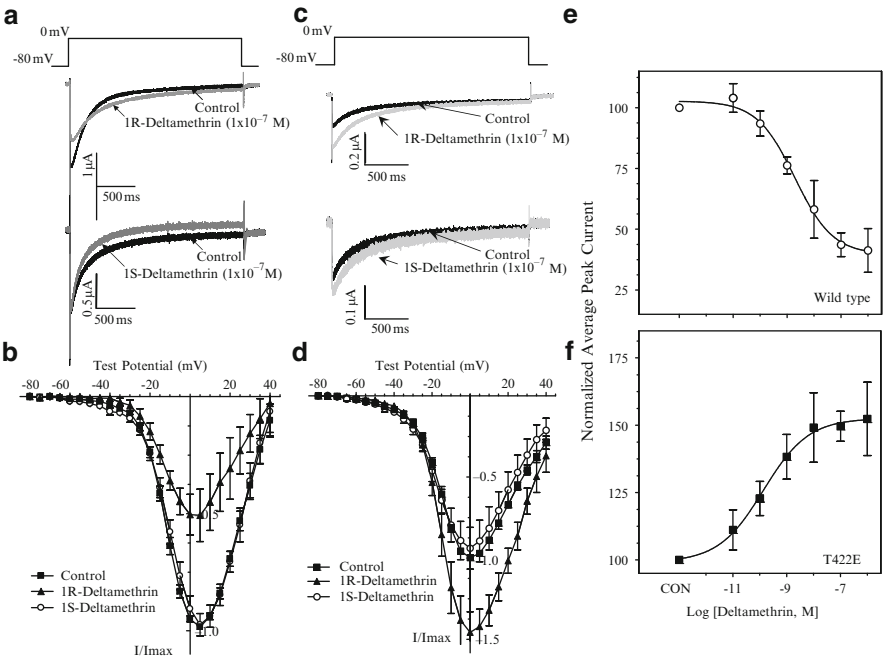

Fig. 2 Deltamethrin effects on $Ca_v2.2$ are altered by phosphorylation. **(a)** Representative current recordings illustrating the effects of 1R- and 1S-deltamethrin on $Ca_v2.2$ under steady-state depolarization. Currents were evoked by step depolarizations to 0 mV from a holding potential of −80 mV for 2.5 sec. **(b)** The current-voltage relationship of wild type $Ca_v2.2$ α_1-subunit co-expressed with the β_3-subunit in *Xenopus* oocytes in the presence and absence of 1R- and 1S-deltamethrin or nontreated control. Currents were evoked with 5 mV step depolarizations from a holding potential of −80 mV to +40 mV ($n > 5$). **(c)** Representative current recordings illustrating the effects of 1R- and 1S-deltamethrin on T422E $Ca_v2.2$ under steady-state depolarization. Currents were evoked by step depolarizations to 0 mV from a holding potential of −80 mV for 2.5 sec. **(d)** The current-voltage relationship of T422E $Ca_v2.2$ α_1-subunit mutant co-expressed with the b-subunit in *Xenopus* oocytes in the presence and absence of 1R- and 1S-deltamethrin or nontreated control. Currents were evoked with 5 mV step depolarizations from a holding potential of −80 mV to +40 mV ($n > 5$). Concentration-dependent response relationship for 1R-deltamethrin on the relative Ba^{2+} peak current of wild type (e) and T422E (f) $Ca_v2.2$ α_1-subunit co-expressed with the α_3-subunit in *Xenopus* oocytes. All recordings were made using 5 mM Ba^{2+} as the charge carrier. Data calculated as percent of nontreated control (normalized to 100) and normalized peak current values averaged from multiple current recordings ± SEM ($n > 5$)

pyrethroid modulation of these channels. Recently, voltage-clamp electrophysiological studies using wild type and mutant $Ca_v2.2$ and its β_3 subunit co-expressed in *Xenopus* oocytes have been carried out to investigate the role of phosphorylation in the mechanism of action of pyrethroids on voltage-gated calcium channels (Fig. 2). Deltamethrin treatment of wild type $Ca_v2.2$ reduced peak current in a stereospecific and concentration-dependent manner and slowed the rate of inactivation [56, 63]. However, PMA-activated phosphorylation of wild type $Ca_v2.2$ followed by deltamethrin treatment significantly increased peak current and slowed deactivation

of the phosphorylated channel [64]. Site-directed mutagenesis of threonine 422 to glutamic acid (T422E), a critical phosphorylation site in the regulation of $Ca_v2.2$ [65, 66], confirmed the experiments conducted with PMA. In these mutation studies, deltamethrin significantly enhanced peak current via the T422E mutant channel (1.5-fold) compared to the non-treated control and the increase was significantly greater than for either the wild type (T422) or T422A (permanently unphosphorylated mutant) channels. The effect of deltamethrin on T422E $Ca_v2.2$ was stereospecific and concentration-dependent with an EC_{50} that was approximately 100-fold more potent compared to the non-phosphorylated channel [67].

3.2.2 In Situ Functional Studies

Early in situ findings established that deltamethrin markedly stimulates the spontaneous release of [^{3}H]GABA from mammalian synaptosomes superfused with saline buffer [68]. The stimulatory effect is pronounced and mediated completely via sodium channels since the response is blocked by the voltage-sensitive sodium channel antagonist, tetrodotoxin (TTX). Fenvalerate, another CS-syndrome pyrethroid, evoked spontaneous [^{3}H]dopamine release from rabbit striatal slices that was also blocked by TTX [69]. The effect on spontaneous release appeared to be regional, however, since no release of neurotransmitters from hippocampal brain slices was observed. In a comprehensive analysis of 25 pyrethroids, it was found that most of the pyrethroids examined also increased the sodium-dependent release of neurotransmitters from rat brain synaptosomes, but release was only partially abolished by TTX [70]. Since neurotransmitter release evoked by CS-syndrome pyrethroids was not completely and reproducibly blocked by TTX or by substituting choline for sodium, additional actions at target sites other than voltage-sensitive sodium channels were suggested.

Neurotransmitter release induced by potassium-dependent depolarization is a physiologically relevant way to investigate pyrethroid effects on calcium-dependent neurotransmitter release since this process is independent of voltage-sensitive sodium channels [71]. Furthermore, potassium-stimulated calcium influx and subsequent neurotransmitter release by synaptosomes is blocked by a variety of voltage-sensitive calcium channel antagonists but not by TTX [4, 71, 72].

CS-syndrome pyrethroids enhanced calcium-dependent norepinephrine release from potassium-depolarized synaptosomes isolated from rat brain while T-syndrome pyrethroids were much less potent and efficacious in evoking release [73]. In the presence of TTX, deltamethrin still enhanced release. However, the specific calcium channel blocker, D595, inhibited the deltamethrin-stimulated release. In more recent studies, actions of cismethrin and deltamethrin were evaluated using rat brain synaptosomes [74]. Both pyrethroids stimulated calcium influx but only deltamethrin enhanced calcium-dependent glutamate release following potassium depolarization. The action of deltamethrin was stereospecific, concentration-dependent, and blocked by ω-conotoxin GVIA (a specific N-type voltage-gated calcium channel blocker), while cismethrin-stimulated calcium influx was blocked by TTX. These findings

delineate a separate action for deltamethrin and cismethrin at presynaptic nerve terminals and implicate voltage-gated calcium channels as a target sites for Type II pyrethroids.

In an extension to the studies mentioned above, the actions of 11 commercial pyrethroids on calcium influx and glutamate release were assessed using a high-throughput approach with rat brain synaptosomes [75, 76]. Concentration-dependent response curves for each commercial pyrethroid were determined and the data used in a cluster analysis. Previously characterized Type II pyrethroids that induce the CS-syndrome symptoms (cypermethrin, deltamethrin, and esfenvalerate) increased calcium influx and glutamate release, and clustered with two other α-cyano pyrethroids (β-cyfluthrin and λ-cyhalothrin) that shared these same actions. Previously characterized Type I pyrethroids (bioallethrin, cismethrin, and fenpropathrin) did not share these actions and clustered with two other non-cyano pyrethroids (tefluthrin and bifenthrin) that likewise did not elicit these actions.

Collectively, the in situ biochemical evidence suggests that voltage-gated calcium channels are modified by pyrethroids; however, the mechanism by which Types I and II pyrethroids accomplish this may be different. Specifically, Type II pyrethroids are more potent enhancers of calcium influx and glutamate release under depolarizing conditions than Type I pyrethroids and may contribute, in part, to different symptoms elicited by these classes of pyrethroids in vivo.

3.2.3 Mammalian Cell Cultures

Modern advances in high-throughput electrophysiological techniques with mammalian cell cultures are likely to elucidate the relative contribution of different ion channels to pyrethroid neurotoxicity. In mouse hippocampal neurons, repeated applications of nanomolar concentrations of deltamethrin increased potassium stimulated GABA and glutamate release [77]. These authors also found that deltamethrin differentially affected the survival of neuronal subtypes and concluded that deltamethrin disrupts neuronal organization and function in networks. In contrast, Meyer et al. [78] used microelectrode arrays (MEAs) to examine the effects of deltamethrin and permethrin on neuronal activity in hippocampal neuronal cultures. MEAs are a high-throughput electrophysiological approach that measures the spontaneous activity of neuronal cell cultures from multiple sites in the network simultaneously and can provide a robust measure of network activity and connectivity [79]. Both deltamethrin and permethrin decreased spontaneous excitatory post-synaptic currents (sEPSCs) and spike rate in the presence of GABA receptor antagonists. The pyrethroid response was mimicked by veratridine and unaffected by voltage-gated calcium channel blockers [78]. In the absence of GABA receptor antagonists, deltamethrin and permethrin increased spontaneous spike rates. Similar results were also observed in mouse primary cultures obtained from the frontal cortex and spinal cord. Both deltamethrin and permethrin reduced spike and burst rates in a concentration-dependent manner [80]. In mouse neocortical neurons, the effects

of 11 different pyrethroids were examined on spontaneous calcium influx using the calcium indicator dye fluo-3. Nine pyrethroids (tefluthrin, deltamethrin, λ-cyhalothrin, β-cyfluthrin, esfenvalerate, *S*-bioallethrin, fenpropathrin, cypermethrin, and bifenthrin) produced concentration-dependent increases in intracellular calcium concentration, while permethrin and resmethrin were without effect. Calcium influx under resting conditions was blocked by TTX, suggesting that these pyrethroids stimulated calcium influx subsequent to their actions on voltage-sensitive sodium channels under resting conditions [81].

Taken together, the limited experiments conducted using neuronal cell cultures illustrate a distinct difference in the way that pyrethroids modify ion conductance and subsequent neurotransmitter release under resting and depolarized conditions. Continued efforts utilizing recent new tools like automated patch-clamp systems and MEAs to assess the effects of pyrethroids on the kinetics and voltage-dependent gating of ion channels in primary cultures or transfected cells is likely to provide new insight into the neurotoxicity of pyrethroids [79, 82].

3.3 Modulation of Voltage-Gated Chloride Channels

3.3.1 Electrophysiological Studies

Unlike voltage-gated sodium and calcium channels, the pharmacology of voltage-gated chloride channels is not well characterized in that specific high-affinity ligands are not yet available [83]. Additionally, those ligands that do bind voltage-gated chloride channels (e.g., avermectins, barbiturates, *tert*-butylbicyclo-phosphorotrithioate) are non-specific as they also bind other channels, such as the GABA-chloride channel [10]. Nevertheless, two broad structural classes of voltage-gated chloride channels have been determined by molecular sequencing. The cystic fibrosis transmembrane conductance regulator (CFTR)-type channel is an example of one class [83, 84]. The other class is the multi-gene CLC family of voltage-gated chloride channels that function in the regulation of cell volume, rectification and stabilization of resting membrane potentials and transepithelial membrane transport [85]. It appears that it is this second type of voltage-gated chloride channels (CLC) that may function as alternative target sites for some pyrethroids.

Initial in vitro electrophysiology work established that deltamethrin increased skeletal muscle membrane resistance but cismethrin was without an effect, suggesting that the CS syndrome-producing Type II pyrethroid, deltamethrin, may block chloride ion permeability selectively [86]. Additional experiments confirmed this finding [87] and showed that either reduced concentrations of extracellular chloride ion or addition of ivermectin, which activates voltage-gated chloride channels, antagonized the effect of deltamethrin on increasing muscle membrane resistance [88, 89]. Using patch-clamp techniques on excised membranes from N1E-115 neuroblastoma cells, single voltage-gated chloride channels (maxi channels) were blocked by deltamethrin and cypermethrin but not by cismethrin,

again indicating that blockage of these channels by Type II pyrethroids may be involved in the production of the CS-syndrome [90, 91]. This study was expanded to examine the action of 14 pyrethroids on these channels, including both Type I and Type II pyrethroids [92], but failed to confirm their originals findings. Only five of the 14 pyrethroids blocked these maxi channels: *S*-bioallethrin, β-cyfluthrin, cypermethrin, deltamethrin, and fenpropathrin. *S*-Bioallethrin is a Type I pyrethroid that causes the T-syndrome, β-cyfluthrin, cypermethrin, and deltamethrin are Type II pyrethroids that cause the CS-syndrome, and fenpropathrin is a Type II pyrethroid that causes a mixture of T- and CS-syndromes of intoxication.

The above subset of five pyrethroids that do block maxi voltage-gated chloride channels, however, may play a role in the more-than-additive effect that certain pyrethroids produced on neurotransmitter (glutamate) release from isolated presynaptic nerve terminals when applied in binary mixtures with deltamethrin always as one of the two constituents [93]. In this study, only a subset of pyrethroids (*S*-bioallethrin, cismethrin, cypermethrin, and fenpropathrin) in binary mixtures with deltamethrin caused a more-than-additive effect on glutamate release. None of these binary mixtures resulted in increased calcium ion influx, indicating that the more-than-additive effect on neurotransmitter release is a calcium-independent event likely not mediated by voltage-gated calcium channels (e.g., N-type $Ca_v2.2$). Thus, except for β-cyfluthrin and cismethrin that were not tested in both systems, the subset of pyrethroids that produce the more-than-additive response on glutamate release when in binary mixture with deltamethrin were the same as those that blocked maxi voltage-gated chloride channels. These results are consistent with the possibility that the more-than-additive response on glutamate release is the result of binary mixtures where one pyrethroid acts as an agonist on voltage-gated calcium channels, such as $Ca_v2.2$, and the other pyrethroid has an antagonistic action at voltage-gated chloride channels, such as maxi CLC channels. Further investigation on the modulation of enhanced glutamate release due to selective pyrethroids in binary mixtures by a battery of well-defined chloride channel activators and blockers would begin to test the above hypothesis.

3.4 Ex Vivo Neurotoxicology

As previously mentioned, early in vivo acute toxicity studies indicated that the action of Type II pyrethroids on the nervous system was different from that of the Type I pyrethroids. Deltamethrin decreased the acetylcholine content of the cerebellum, whereas DDT, a well-established voltage-sensitive sodium channel agonist, and cismethrin, caused no significant reduction [2].

The effect of allethrin (a Type I pyrethroid), cyhalothrin, and deltamethrin (Type II pyrethroids) on neurotransmitter release from the hippocampus (acetylcholine, glutamate, and GABA release) and striatum (dopamine release) has recently been investigated using ex vivo microdialysis in freely moving rats exhibiting the symptoms of pyrethroid poisoning [94–97]. Deltamethrin increased the release

of the excitatory neurotransmitters acetylcholine, dopamine, and glutamate in a dose-dependent manner from each of the brain regions. Conversely, deltamethrin decreased the concentration of GABA released from the hippocampus in a dose-dependent manner. These physiological responses are consistent with the convulsive nature of pyrethroids. Infusion of 1 μM TTX into the hippocampus and striatum only partially prevented neurotransmitter release, whereas infusion of 10 μM nimodipine, a Ca_v1 channel antagonist, prevented neurotransmitter release. Unlike deltamethrin, cyhalothrin inhibited the release of acetylcholine, dopamine, and glutamate, and stimulated GABA in a dose-dependent manner. TTX completely blocked cyhalothrin-induced changes in neurotransmitter release while nimodipine had no effect. In both brain regions, allethrin-dosed rats exhibited two different effects on acetylcholine release. At low concentrations, allethrin increased acetylcholine release threefold compared to controls; however, at the highest doses, allethrin inhibited acetylcholine release. Similar effects were also observed in the striatum where low concentrations resulted in an increase in dopamine release and high concentrations resulted in inhibition. Like cyhalothrin, infusion of 10 μM TTX blocked allethrin-stimulated changes in neurotransmitter release from the different brain regions. Collectively, these ex vivo approaches suggest that other sites of action, in addition to TTX-sensitive voltage-gated sodium channels, may be involved with the neurotoxic action of pyrethroids in the central nervous system. Furthermore, these experiments delineate a physiological response (enhanced neurotransmitter release) that is different between the two types of pyrethroids.

4 Regulatory Neurotoxicology

4.1 Implications of the Food Quality Protection Act: Common Mechanism of Toxicity and Cumulative Risk Assessment

The Food Quality Protection Act (FQPA) of 1996 mandated that the US EPA carry out risk assessments that consider the cumulative effects of exposure to pesticides having a common mechanism of toxicity, as well as consider exposure to each pesticide by various routes of exposure (e.g., dermal, dietary, inhalation) and sources (e.g., residues in food and water) in an aggregate manner [19]. To accomplish this, there needs to be sufficient evidence supporting a common adverse effect that is associated with a common mechanism of action in specific target tissues. To date, the required criteria necessary to establish a common mechanism of toxicity with a specific toxic effect for the pyrethroids are not available [1, 8, 98].

The establishment of a common mechanism of mammalian toxicity for the pyrethroids is not a straight forward process, as it was for the organophosphorus and carbamate insecticides, due to the occurrence of multiple potential target sites and the varied action of pyrethroids at these sites as reviewed above. In view of

this complexity and lack of comparable data, parallel neurotoxicity [19] and mechanistic studies [98, 99] were undertaken under conditions suited to assist with the determination of whether the pyrethroids as a group act by a common mechanism of toxicity in mammals.

Neurotoxicity and mechanistic data were obtained for up to six Type I pyrethroids (bifenthrin, *S*-bioallethrin [or allethrin], permethrin, pyrethrins, resmethrin [or cismethrin], and tefluthrin) and six Type II pyrethroids (β-cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, and λ-cyhalothrin). Data obtained from the following four studies were subjected to a combination of principal components analysis, factor analysis and multidimensional scaling: (1) an in vivo neurotoxicity study based on a functional observational battery (FOB) evaluation in young adult male rats following acute oral exposure [19]; (2) an in vitro electrophysiology study based on the modification of channel gating characteristics using rat $Na_v1.8$ sodium channel isoform heterologously expressed in *Xenopus* oocytes evaluated with two-electrode voltage clamp techniques [34]; (3) an in situ biochemical study based on membrane depolarization, calcium ion influx, and neurotransmitter release from isolated adult rat brain presynaptic nerve terminals [56, 72]; and (4) an in vitro electrophysiology study based on open channel probability of voltage-gated chloride channels expressed in N1E-115 mouse neuroblastoma cells using patch-clamp techniques [92].

Using a principal components analysis of the FOB data, there were four patterns of variation (factors) that were responsible for between-groups differences that resulted from pyrethroid treatments: Factor 1 (CS_b) = abnormal posture, neuromuscular weakness, and writhing; Factor 2 (CS_a) = lacrimation and non-reactivity to sensory stimulation; Factor 3 (T_a) = difficulty in handling the animal and exaggerated response to sensory stimuli; and Factor 4 (T_b) = head-flicking, jerking movements, and prominent eye bulging. Using this approach and other related published information, two common mechanism groups were supported by this acute neurobehavioral data: Group 1 (bifenthrin, permethrin, pyrethrins, resmethrin, *S*-bioallethrin, and tefluthrin) and Group 2 (β-cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, and λ-cyhalothrin) [19].

Using the above FOB data and a principal components-based factor analysis, a single CS composite factor was calculated from the maximum of either Factor 1 or 2 above and was found to be based on treated rats eliciting excessive salivation, impaired mobility, and a lower body temperature. A single T composite factor was likewise characterized and found to be based on treated rats eliciting elevated temperature, myoclonus, and tremors [99]. The CS or T composite factor scores were overlaid onto multidimensional scaling maps (MDS) based on the dissimilarities scores determined from the mechanistic data generated on $Na_v1.8$, on calcium influx and neurotransmitter release, and on the open probability of voltage-gated chloride channels. Visual clustering of the principal components/factor and dissimilarity analyses gave similar groupings as for the FOB study and provided evidence for separate mechanisms of toxicity for the Types I and II pyrethroids. Only esfenvalerate and fenpropathrin resulted in mixed-type responses, indicating that they should be included in both mechanistic groupings.

Because similar pyrethroid groupings were obtained using neurobehavioral and mechanistic differences on three target sites, these findings suggest that a fundamental difference exists between the actions of Types I and II pyrethroids, both on a functional and molecular level [99].

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Pyrethrin Biosynthesis and Its Regulation in *Chrysanthemum cinerariaefolium*

Kazuhiko Matsuda

Abstract Pyrethrins are a natural insecticide biosynthesized by the plant pyrethrum [*Chrysanthemum cinerariaefolium* (Current species name: *Tanacetum cinerariifolium*)] of the family Asteraceae. Although pyrethrins have been used to control household pests for the past century, little is known about the mechanism of biosynthesis, contrasting with intensive research on their synthetic analogs, pyrethroids. The author studied pyrethrin biosynthesis in young seedlings of *C. cinerariaefolium*. The results of experiments using ^{13}C-labeled glucose as the biosynthesis precursor indicated that the acid and alcohol moieties are biosynthesized via the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) and oxylipin pathways, respectively. Further study on the effects of wound-induced signals in leaves showed that biosynthesis is enhanced in response to both volatile and nonvolatile signals.

Keywords Biosynthesis · *Chrysanthemum cinerariaefolium* · Natural pyrethrins · *Tanacetum cinerariifolium*

Contents

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1 Introduction

As far back as medieval times, pyrethrum *C. cinerariaefolium* was known to accumulate pyrethrins as the active components in flowers (precisely, in the achenes) that exhibit fast-acting toxicity to many insect pest species. This plant species originated in Persia and Middle Eastern Europe. Pyrethrins contain six components resulting from the esterification of two types of acid moieties [chrysanthemic acid (**1**) and pyrethric acid (**2**)] with three types of alcohol moieties [pyrethrolone (**3**), jasmololone (**4**), and cinerolone (**5**)] (Fig. 1). Little was known about the pyrethrin structures until two Swiss chemists, Staudinger and Ruzicka, uncovered the structures of a major component pyrethrin I (**6**) [1–10]. This brilliant work was achieved without the benefit of modern analytical techniques such as Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS), although the structures later underwent revision by the LaForge group [11]. Pyrethrin II (**9**) as well as minor components cinerins (**8**, **11**) [12] and jasmolins (**7**, **10**) [13] were discovered thereafter [14]. Pyrethrins possess three asymmetric carbons, resulting

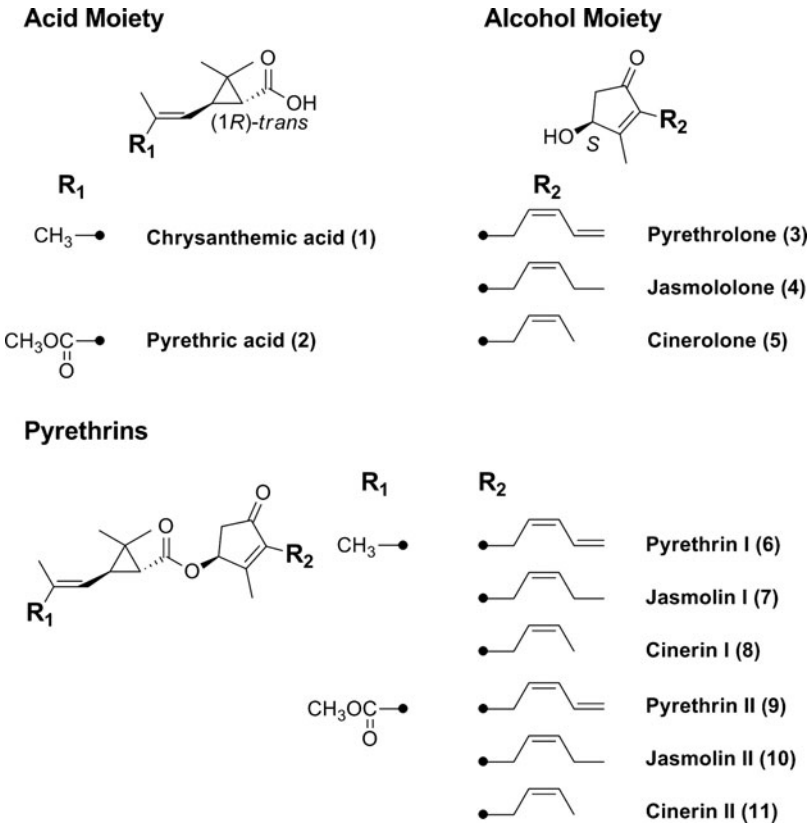

Fig. 1 Natural pyrethrins and their acid and alcohol moieties

in eight possible stereoisomers. Crombie et al. in the UK and Inouye et al. and Katsuda et al. in Japan respectively determined the absolute configuration of the cyclopropane carbons in the acid moiety to be (1*R*)-*trans* [15, 16], and that of the hydroxylated carbon in the alcohol moiety to be *S* [17] (Fig. 1). Eventually, NMR [16, 18] and X-ray crystallography [19, 20] confirmed the geometry and absolute configuration of pyrethrins (Fig. 1).

Natural pyrethrins are readily degraded by sunlight and oxygen, limiting their use to the control of household pests. To circumvent this property, many synthetic analogs, "pyrethroids," with higher stability and potency, have been developed, taking over the market from pyrethrins. In this respect, pyrethrins may appear outdated, yet they still show promise as little research has been conducted on pyrethrin biosynthesis and its regulation in plants.

Accordingly, the author studied the mechanisms of pyrethrin biosynthesis in *C. cinerariaefolium*. Young seedlings were employed since pyrethrins are synthesized in the seedlings as well as in the flowers, and this material is available throughout all seasons. Here, the author describes the biochemical aspects of pyrethrins in terms of biosynthetic pathways and factors influencing biosynthesis.

2 Elucidating the Biosynthetic Pathways

Chrysanthemic acid (**1**) consists of ten carbons, suggesting that it is a monoterpene. The cyclopropane ring of the acid moiety is a feature of pyrethrins. Rivera et al. isolated chrysanthemyl pyrophosphate synthase (CPPase or alternatively referred to as chrysanthemyl diphosphate synthase) underlying the formation of chrysanthemyl pyrophosphate (**16**) containing a cyclopropane ring from two molecules of dimethylallyl pyrophosphate (**15**) (DMAPP) and the gene thereof [21]. They found that the reaction involves the c1′-2-3 cyclopropanation of DMAPPs in a non-head-to-tail manner.

The CPPase substrate DMAPP (**15**) is formed from isopentenyl pyrophosphate (IPP) (**14**) via the IPP isomerase reaction. It had been assumed that IPP was generated only via mevalonic acid (**12**) (Fig. 2), but Rohmer discovered another route, 2-*C*-methyl-D-erythritol 4-phosphate (**13**) (MEP) pathway (Fig. 2) [22, 23]. A key step in the MEP pathway is the reaction catalyzed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS), which combines hydroxyethyl thiamine pyrophosphate (hydroxyethyl TPP) generated from pyruvic acid (**17**) and TPP with glyceraldehyde 3-phosphate (**18**) to yield 1-deoxy-D-xylulose 5-phosphate (**19**) containing five carbons. The mevalonate pathway operates in the cytosol of plants and animals, whereas the MEP pathway is present in the plastid of plants or in eubacteria [24–27].

Previously, the ^{14}C-labeled mevalonic acid (**12**) was shown to be incorporated into the acid moiety [28, 29]. However, monoterpenes are generally biosynthesized via the MEP but not the mevalonate pathway. Hence, the author employed [1-^{13}C] D-glucose (Fig. 2) as the precursor to examine whether the acid moiety is produced

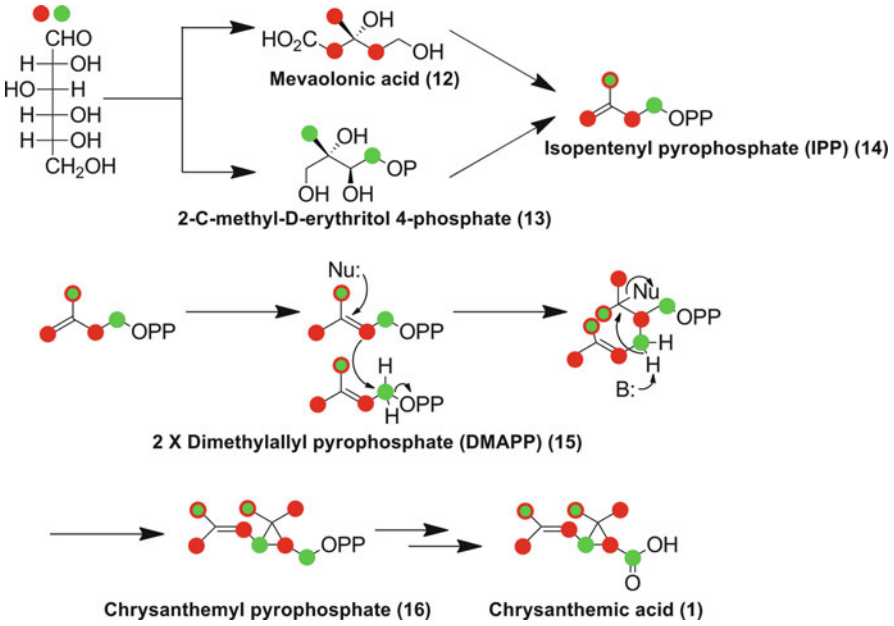

Fig. 2 Two possible biosynthetic pathways to pyrethrolone. The [1-^{13}C]D-glucose-derived ^{13}C labels that occur in the mevalonic acid and 2-*C*-methyl-D-erythritol 4-phosphate (13) pathways are colored in red and green, respectively. The phosphate moiety is indicated as "P"

by the mevalonate or MEP pathway, and to determine if the alcohol moiety is biosynthesized by the oxylipin pathway. The labeled glucose was supplied to the *C. cinerariaefolium* seedlings and pyrethrin I (**6**) was isolated from the seedlings to measure its ^{13}C-NMR spectrum. Figure 2 illustrates the acid moiety carbons predicted to be labeled when biosynthesized by the two pathways. The carbonyl carbon of chrysanthemic acid (**6**) should be labeled if the MEP pathway is employed, but not if the mevalonate pathway is used. The ^{13}C-NMR spectrum showed significant ^{13}C incorporation into the carbonyl carbon, indicating that the MEP pathway was predominantly used to synthesize the acid moiety, at least in part, in leaves (Fig. 3) [30].

The alcohol moiety is produced in a different manner from that of the acid moiety. The alcohol moiety resembles the plant hormone jasmonic acid (JA) (**26**) generated from linolenoyl moiety of lipids via (13*S*)-hydroperoxy-linolenic acid (**22**), (12,13*S*)-epoxylinolenic acid (**23**), and 12-oxo-*cis*-10,15-phytodienoic acid (**24**) by the oxylipin or octadecanoid pathway (Fig. 3) [31]. In fact, ^{13}C was incorporated at pyrethrolone (**1**) carbon positions that agreed with those predicted to be labeled when the alcohol moiety is produced via the pathway (Fig. 3) [30]. Figure 3 illustrates that *cis*-jasmone (**25**) is hydroxylated to yield jasmololone (**4**), which is then dehydrogenated to yield pyrethrolone (**5**). However, it has not yet been determined if this is actually the case.

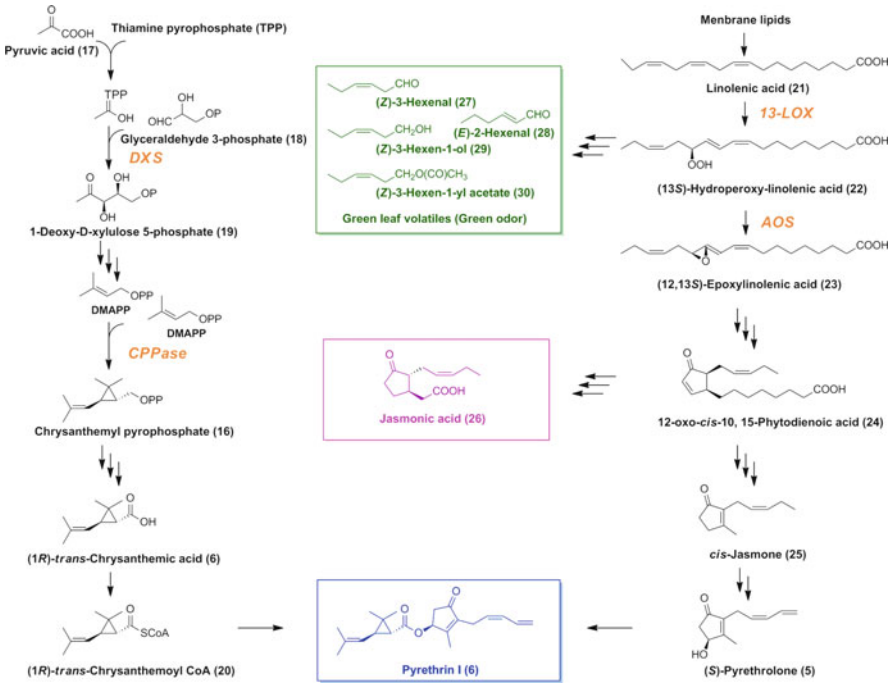

Fig. 3 Biosynthetic pathways to pyrethrins. The identified enzymes involved in biosynthesis are shown in orange. Note that green leaf volatiles and the plant hormone jasmonic acid share the oxylipin pathway. The phosphate moiety is indicated as "P"

The ester linkage is another feature of pyrethrins. This bond is established at the final step in the biosynthetic scheme. It is conceivably formed by sequential action of two enzymes, acyl-CoA ligase and acyltransferase. The former catalyzes the formation of chrysanthemoyl/pyrethroyl CoA (**20**) from the acid moiety and CoA with ATP hydrolysis to AMP. The resultant acyl-CoA is then transferred to the alcohol moiety to yield pyrethrins by acyltransferase. In the acyltransferase reaction, products coming from the two different biosynthetic pathways must arrive at the same location with synchronized timing. Considering the hydrophobicity of pyrethrins, the acyltransferase is perhaps located in a hydrophobic environment, namely the membranes. Identifying these two enzyme genes is essential for understanding the mechanism of the ester formation in terms of regulation of gene expression, substrate specificity, chemistry of the enzyme reaction, localization of the enzymes, etc.

Similar in importance to hunting for biosynthetic genes is clarifying whether other plant species are able to produce biosynthetic intermediates themselves using high-sensitivity LC-MS. If so, how close are such compounds located to pyrethrins in the pathway? It could be that pyrethrin production is too small to be noticed in other plant species. Even if this is not the case, it should be noted that some other plant species produce chrysanthemyl pyrophosphate (**16**) and *cis*-jasmone (**25**).

Such metabolomic information is useful for understanding the overall picture of biosynthesis and developing transgenic plants that overproduce pyrethrins.

3 Regulating Biosynthesis by Volatile and Nonvolatile Signals

In nature, pyrethrins are not produced by *C. cinerariaefolium* for the purpose of insect control, as is required by humans, but are instead produced for self-defense against herbivores. In this context, it is conceivable that pyrethrin biosynthesis is inducible by herbivore attack or mechanical wounding. To test this hypothesis, pyrethrin I (**6**) and II (**9**) in leaves were quantified in intact and wounded pyrethrum seedlings. It was found that pyrethrins in the intact seedling leaves increased in response to injury [32]. One possible mechanism accounting for this observation is that nonvolatile systemic molecules mediate the wound signals to intact leaves, resulting in enhanced production of pyrethrins. Yet mediators are not limited to nonvolatile molecules. It has been demonstrated that herbivory or wound-induced volatile organic compounds (VOCs) mediate not only plant–plant [33] but also within-plant communications [34]. To examine how VOC-mediated within-plant communication contributes to the increase in pyrethrins, the intact leaves of pyrethrum seedlings were wrapped and other leaves were mechanically wounded. Then the pyrethrins in the intact leaves of the wounded seedlings were quantified. In this case, pyrethrin I (**6**) did not increase in response to wounding, suggesting that the VOCs emitted by the wounded leaves promoted pyrethrin biosynthesis in intact leaves. In contrast, pyrethrin II (**9**) increased irrespective of wrapping, suggesting that nonvolatile molecules play a more important role than the wound-induced VOCs in producing the compound [32].

To clarify how volatile signals regulate pyrethrin biosynthesis, the wound-induced VOCs were quantified as well as identified by GC-MS. Also, the effects of VOCs on biosynthesis in intact plants placed in the vicinity of wounded seedlings were investigated in terms of expression of certain biosynthetic genes as well as changes in pyrethrin content in the intact leaves [35]. Upon injury, four green leaf volatiles [(*Z*)-3-hexenal (**27**), (*E*)-2-hexenal (**28**), (*Z*)-3-hexen-1-ol (**29**), and (*Z*)-3-hexen-1-yl acetate (**30**)] and one sesquiterpene [(*E*)-*β*-farnesene (**31**)] were significantly emitted by the seedlings with the amount changing dynamically with time (Fig. 4). Interestingly, green leaf volatiles are generated from 13-hydroperoxy-linolenic acid (**22**), which is also used as the precursor to biosynthesize pyrethrins via the oxylipin pathway. Hence, it is not surprising that green leaf volatiles contribute to the regulation of pyrethrin biosynthesis.

When the intact seedlings were placed in the vicinity of wounded seedlings, pyrethrin content in the leaves of the intact seedlings increased significantly. Also, exposing the intact seedlings to a mixture of (*Z*)-3-hexenal (**27**), (*E*)-2-hexenal (**28**), (*Z*)-3-hexen-1-ol (**29**), (*Z*)-3-hexen-1-yl acetate (**30**), and (*E*)-β-farnesene (**31**) at the same concentrations at which they were emitted in response to wounding resulted in enhanced pyrethrins. Thus, the effects of each component as well as

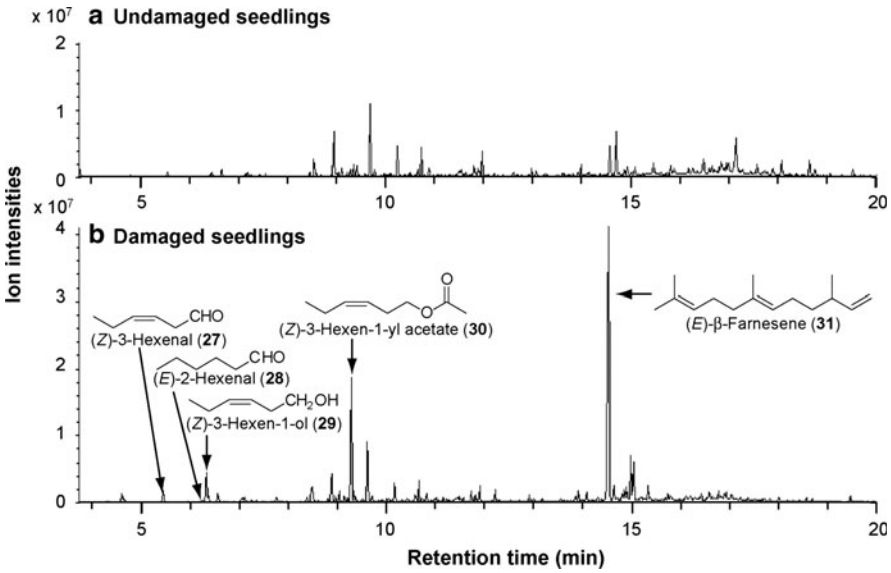

Fig. 4 VOCs emitted by young seedlings of *Tanacetum cinerariaefolium* in response to mechanical wounding. VOCs from undamaged and wounded seedlings were recorded by GS-MS. Reproduced from [35] with permission of Oxford University Press

the VOC mixture were tested as a gas on the intact seedlings to investigate the induction of gene expression of four biosynthetic enzymes [1-deoxy-D-xylulose 5-phosphate synthase (DXS), chrysanthemyl diphosphate synthase (CPPase), 13-lipoxygenase (13-LOX), and allene oxide synthase (AOS)] (Fig. 3). DXS and 13-LOX are located upstream of CPPase and AOS, respectively. Consistent with this sequence, *DXS* and *13-LOX* gene expression reached the maximum more quickly compared to *CPPase* and *AOS* gene expression.

The relationship between gene expression and concentration of wound-induced VOC mixture was examined for the four biosynthetic genes to show that the optimum expression excluding that for *13-LOX* is achieved at the concentration at which the mixture was emitted; both increasing and decreasing the concentration reduced the gene expression to the control level.

To evaluate the efficacy of biosynthesis control by the VOCs, they were individually tested at the concentration determined by GC-MS for the action on biosynthetic gene expression. Contrasting with the significant gene-expression promoting action of the VOC mixture, each VOC was ineffective in promoting gene expression when tested alone. Furthermore, eliminating just one component from the five-component mixture resulted in lower gene expression, demonstrating that the wound-induced VOCs act as a specific blend to enhance pyrethrin biosynthesis.

4 Summary

The author studied pyrethrins in terms of biosynthetic pathways and molecules influencing biosynthesis. Pyrethrins may partly substitute for synthetic pyrethroids in the control of sanitary pests because they are less persistent (or more environmentally benign), safer for mammals, and effective on pyrethroid-resistant species. In-depth studies on biosynthesis will lead to some general principles in biology. Notably, pyrethrin biosynthesis involves wound-induced signaling and related lipid metabolism, which are also faced by other organisms. It will be important in the future to clarify such general principles using modern technologies.

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Mammal Toxicology of Synthetic Pyrethroids

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Abstract Pyrethroids show moderate acute oral toxicity in rodents, and their typical toxicological signs are tremors (T syndrome) for Type I (generally non-cyano pyrethroids) and choreoathetosis with salivation (CS syndrome) for Type II (generally α-cyano pyrethroids). However, some pyrethroids show mixed clinical signs. Mainly Type II pyrethroids cause paresthesia, which is characterized by transient burning/tingling/itching sensation of the exposed skin. Also, it has been suggested that some pyrethroids cause developmental neurotoxicity, but available evidence has been judged to be insufficient. While some pyrethroids have been shown to cause tumors in rodent models, the tumor induction does not appear to reflect a common carcinogenic endpoint for this particular subset of compounds. Analysis of carcinogenic mode of action in some cases provides evidence not relevant in humans. Pyrethroids produce no common teratogenic effects in a particular species based on similarity in structure or mode of insecticidal action.

Keywords Carcinogencity · Developmental toxicity · Neurotoxicity · Pyrethroid

Contents

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1 Neurotoxicity of Pyrethroids

1.1 Acute Toxicity and Clinical Signs

Pyrethroids are a class of synthetic insecticides designed and optimized based on the structure of the pyrethrins found in natural pyrethrum extracted from chrysanthemum flowers [1, 2]. Pyrethroids are widely used to control insect pests in agriculture and public health because of their relative safety for humans and high insecticidal potency [3].

The acute oral toxicity in rats is summarized in Table 1. The LD_{50} values of most compounds following administration in vegetable oil are more than 50 mg/kg, and thus they are considered to be moderately toxic and equivalent to GHS category 3 or more. Although the acute inhalation toxicity is also considered to be moderate, the acute dermal toxicity is generally very low [4]. The acute toxicity of compounds with a cyano group is generally stronger than that of those without in results using vegetable oil as vehicle (Table 1).

Pyrethroids can possess one to three chiral centers, resulting in two to eight isomers. Stereospecificity of acute toxicity of pyrethroids are known as well as insecticidal action [17]. The toxicity depends on the stereochemical configuration at cyclopropane C-1 or the homologous position in compounds lacking the cyclopropanecarboxylate moiety. Only esters of 1*R*-cyclopropanecarboxylate and isosteric 2*S* isomers of non-cyclopropane acids are toxic. The absolute configuration at cyclopropane C-3 of cyclopropanecarboxylate esters of primary alcohols also strongly influences toxicity. The compounds having the 1*R*, *cis* configuration are toxic. In addition, the presence of an α-cyano substituent in *S* configuration in 3-phenoxybenzyl alcohol moiety enhances acute toxicity.

Historically, two distinct toxic syndromes have been described. Verschoyle and Aldridge classified poisoning syndrome by pyrethroids into two types, T (tremor) and CS (choreoathetosis with salivation), from the results of the acute intravenous toxicity of 36 pyrethroids in rats [3]. T syndrome, which was mainly observed for the compounds with non-cyano groups, such as permethrin and resmethrin, consists of aggressive sparring, sensitivity to external stimuli, fine progressing to gross whole body tremor, and prostration. Meanwhile, CS syndrome, which was mainly observed for compounds with an α-cyano group, such as deltamethrin, consists of pawing and burrowing behavior, salivation, and coarse tremor, progressing to

Table 1 Acute oral toxicity, structure class, and type of clinical sign in rats

Compound	Cyano group in structure	Acute oral toxicity LD_{50} (mg/kg)		Types of clinical signs	Comparative FOB study with low dose	
		Male	Female		LOAEL (mg/kg)	Type of clinical sign
Cypermethrin	+	297[a]	372[a]	CS[k,l]	65[m]	CS
Cyfluthrin	+	155*[a]	160*[a]	–	12.5[m]	CS
λ-Cyhalothrin	+	79[a]	56[a]	–	10[m]	CS
Deltamethrin	+	95[a]	87[a]	CS[k,l]	12.5[m]	CS
Esfenvalerate	+	90[b]	90[b]	CS[k,l]	15[m]	Mixed
Cyphenothrin	+	318[c]	419[c]	CS/T[k], CS[l]	60[n]	Mixed
Fenpropathrin	+	71[a]	67[a]	T[k], CS/T[l]	15[m]	Mixed
D-Allethrin	–	2,150**[d]	900**[d]	–	200[n]	T
S-Bioallethrin	–	370[a]	320[a]	T[l]	150[m]	T
Bifenthrin	–	70[a]	54[a]	–	40[m]	T
Imiprothrin	–	1,800**[e]	900**[e]	–	900[n]	T
Metofluthrin	–	>2,000**[f]	2,000**[f]	–	57[n]	T
Permethrin	–	430[g]	470[g]	T[k,l]	200[m]	T
D-Phenothrin	–	>10,000**[h]	>10,000**[h]	T[l]	>5,000[n]	ND
Prallethrin	–	640[i]	460[i]	–	150[n]	T
Pyrethrin	–	710[a]	320[a]	T[l]	400[m]	T
Resmethrin	–	1,695[a]	1,640[a]	T[k,l]	350[m]	T
Tefluthrin	–	22[a]	35[a]	–	10[m]	T
Tetramethrin	–	>5,000[j]	>5,000[j]	T[l]	>5,000[n]	ND

Corn oil was used as vehicle. *Acetone + peanut oil; **No vehicle; ND: not determined
[a]Soderlund et al. [4], [b]FAO/WHO [5], [c]EPA [6], [d]EPA [7], [e]EPA [8], [f]Matsuo et al. [9], [g]IPCS [10], [h]IPCS [11], [i]WHO [12], [j]IPCS [13], [k]Verschoyle and Aldridge [3], [l]Lawrence and Casida [14], [m]Weiner et al. [15], [n]Tsuji [16]

sinuous writhing (choreoathetosis) and clonic seizure. In addition, there were exceptions that presented intermediate symptoms. Lawrence and Casida confirmed the classification by administration of 29 pyrethroids to mice via intracerebral injection [14].

An alternative nomenclature (Type I and Type II) has been proposed for subgroups of pyrethroids based not only on the syndromes of intoxication produced in mammals but also on their chemical structures, their signs of poisoning in insects, and their actions on insect nerve preparations [2, 14, 18]. The Type I/II nomenclature has been used in parallel with the T/CS nomenclature, so that Type I and Type II pyrethroids are generally considered to induce T- or CS syndrome, respectively [4]. However, the relationship between the two syndromes and types are neither necessarily confirmed in all pyrethroids nor absolute from the recent available data.

Pyrethroid-induced neurobehavioral effects including clinical signs are known to be highly influenced by methodological changes in route, vehicle, dosing volume, species, and strain [19, 20]. The influence of the dose volume on the neurobehavioral effects after single oral administration was evaluated with

bifenthrin [21]. Effects of bifenthrin on motor activity and clinical signs were twofold more potent at 1 mL/kg than at 5 mL/kg. Increasing dose volume may delay the onset of toxicity and decreases the potency of bifenthrin. The reasons for the volume dependence of toxicity of bifenthrin are not currently known. The influence of each of four vehicles, namely corn oil, glycerol formal, emulphor, and methylcellulose, on the motor activity decrease induced by deltamethrin was examined [19]. The effect of deltamethrin with corn oil as vehicle was the most effective, and the difference in the potency was more than 200 times of that with methylcellulose.

Under the Food Quality Protection Act (FQPA), the U.S. EPA evaluates the potential for people to be exposed to more than one pesticide at a time from a group of chemicals with an identified common mechanism of toxicity. As part of the examinations, to clarify whether some or all of the pyrethroids share a common mechanism of toxicity, a comparative FOB (functional observational battery) studies with 12 pyrethroids were carried out under standardized conditions [15]. The FOB was evaluated at peak effect time following oral administration of non-lethal doses of pyrethroids to rats using corn oil as vehicle. Four principal components were observed in the FOB data [22]. Two of these components described behaviors associated with CS syndrome (lower body temperature, excessive salivation, impaired mobility) and the others described behaviors associated with the T syndrome (elevated body temperature, tremor myoclonus). From the analysis, pyrethroids can be divided into two main groups (Type I: T syndrome and Type II: CS syndrome) and a third group (Mixed Type) that did not induce a clear typical response. Five other pyrethroids were also classified by an FOB study conducted in the same manner [16]. The results of these classifications are shown in Table 1. The FOB results for all non-cyano pyrethroids were classified as T syndrome, and the results of four α-cyano pyrethroids were classified as CS syndrome; however, three of the α-cyano pyrethroids, esfenvalerate, cyphenothrin, and fenpropathrin, were classified as Mixed Type.

Although cumulative effects of cyano and non-cyano pyrethroids on motor activity were reported [23], it is difficult to demonstrate the common mechanism using such a non-specific endpoint, as motor activity is an apical measure of the disruption of nervous system function [24].

Pyrethroids have low oral toxicity to mammals, and in general their insect (topical) to mammal (oral) toxicity ratio is much higher than that of the other major classes of insecticides [25]. As the reason, at least the following mechanisms are conceivable: (1) negative temperature dependence – differences in body temperature between insects and mammals makes the insect nerves much more sensitive, (2) metabolic rate – insects metabolize the insecticide more slowly than mammals, and the metabolizing enzyme systems are different, and (3) differences in body size – insects will have less chance to metabolize the insecticides before reaching the target site [26].

Pyrethroid poisonings in humans have been reported, but a life-threatening risk has rarely occurred except in cases where concentrated formulations were swallowed. He et al. reviewed 573 cases of acute pyrethroid poisoning, including

229 occupational cases due to inappropriate handling and 344 accidental cases mostly due to ingestion, in China from 1983 to 1988 [27]. Most of the cases were related to α-cyano pyrethroids. About half of the occupational patients developed burning, itching, or tingling sensation of the face, and in some serious cases, systemic symptoms followed. When swallowed, the initial symptoms were epigastric pain, nausea, and vomiting. The systemic symptoms included dizziness, headache, nausea, anorexia, and fatigue, and in more serious cases, coarse muscular fasciculations in large muscles of extremities were also observed. However, with adequate therapeutic treatment, the prognosis of acute pyrethroid poisoning is generally good [27].

1.2 Neurobehavioral Effects

Various neurobehavioral effects induced by pyrethroids were reported. Motor activity represents a broad class of behaviors involving coordinated participation of sensory, motor, and integrative processes [28]. Motor activity measurements are required in guidelines for neurotoxicity studies (EPA, OECD) to help to detect neurotoxic effects, although changes in motor activity do not always show the neurotoxic effects. Most pyrethroids tested showed dose-dependent decrease in motor activity [29, 30]. Wolansky et al. compared the relative potencies of 11 pyrethroids under the same conditions, as motor activity data can be altered by a number of experimental factors [29]. Pyrethroids were orally administered to Long-Evans rats using corn oil as vehicle at 1 mL/kg of dose volume, and locomotor activity was measured in a figure-eight maze. The ED_{30} values of the pyrethroids are shown in Table 2. α-Cyano compounds are approximately 10 times more potent than non-cyano compounds in terms of decreasing motor activity. Pyrethroids display up to approximately 240-fold difference in relative potencies for motor activity.

Table 2 ED_{30} for acute effects of pyrethroids on motor activity in rats

Pyrethroid	Cyano group in structure	ED_{30} (mg/kg)
Esfenvalerate	+	1.2
λ-Cyhalothrin	+	1.32
β-Cyfluthrin	+	2.21
Deltamethrin	+	2.51
Tefluthrin	+	2.26
Bifenthrin	+	3.21
Fenpropathrin	+	7.70
Cypermethrin	+	10.70
Permethrin	–	42.66
S-Bioallethrin	–	90.48
Resmethrin	–	292.80

Data from Wolansky et al. [29]

Table 3 Effects on pyrethroids on acoustic startle response

Pyrethroid	Cyano group in structure	Dose range (mg/kg)	Effect on startle response	
			Amplitude	Latency
Cypermethrin[a]	+	37–150	Decrease	Increase
Cypermethrin[b]	+	60–120	Increase	ND
cis-Cypermethrin[c]	+	0.5–2	No effect	No effect
Deltamethrin[d]	+	2–6	Decrease	Increase
Deltamethrin[b]	+	2–6	Decrease	ND
Cyfluthrin[a]	+	25–75	Decrease	Increase
Flucythrinate[a]	+	3–15	No effect	Increase
Fluvalinate[a]	+	25–150	No effect	Increase
Fenvalerate[a]	+	10–40	Increase	No effect
Cismethrin[d]	–	6–18	Increase	No effect
Permethrin[a]	–	60–120	Increase	No effect
Permethrin[b]	–	30–90	Increase	ND
RU11679[a]	–	15–30	Increase	No effect
NAK 1901[b,c]	–	1–4	Increase	No effect

Revised from Wolansky and Harrill [20]
ND not determined
Data from [a]Crofton and Reiter [30], [b]Hijzen and Slangen [32], [c]Hijzen et al. [33], [d]Crofton and Reiter [31]

The effects of pyrethroids on acoustic startle response (ASR) were examined to detect the effects on sensorimotor function. Pyrethroids show various effects on ASR (Table 3). Crofton and Reiter reported that non-cyano pyrethroids showed no effect on the latency, while they increased the amplitude. α-Cyano pyrethroids showed increase or no change on the latency, while various effects on the amplitude were observed [30, 31]. Fenvalerate showed effects similar to non-cyano pyrethroids. In studies by Hijzen et al. [32, 33], the results of permethrin and deltamethrin on the amplitude were consistent with the findings by Crofton and Reiter, but cypermethrin induced contradictory effects. NAK 1901 showed similar effects to other non-cyano pyrethroids. The reason for the inconsistency of effects of pyrethroids on startle response remains unsolved.

Scheduled and controlled operant responses as learning and memory tests were examined with several pyrethroids, and the data showed that pyrethroids produced dose-related decreases of operant response rates [34–36]. However, interpretation of these data may be difficult due to their dependence upon the integrated performance of several neurobiological systems (e.g., motor coordination, sensory response, and crossmodal association) [21].

1.3 Peripheral Sensory Effects

It is known that sensory stimulations in skin, eye, or nose are induced in workers who handle pyrethroids. Itching and burning sensations, paresthesia, blisters, nasal hypersecretion, sneezing, coughing, dyspnoea, and irritation were reported as

symptoms [37]. Skin paresthesia is one of the specific symptoms of pyrethroids. The characteristics of skin paresthesia are as follows: (1) cyano pyrethroids have more potent effect than non-cyano pyrethroids; (2) transient burning/itching sensation of skin is observed; (3) the effect is limited to the exposed area; (4) the effect arises from 0.5–1 h to several hours after exposure; (5) light, heat, and wind are aggravating factors; (6) the reaction is not inflammatory (no erythema, no edema); (7) functional or histopathological changes are not observed; (8) protective clothing is effective; and (9) vitamin E, mineral oil, cream containing vitamins A and D, and benzocaine are effective preventive agents [38].

The phenomena were confirmed in experiments using human volunteers and experimental animals. In humans, fenvalerate, cypermethrin, and permethrin induced paresthesia by application on earlobe [39, 40], and the paresthesia was inhibited by Vitamin E and mineral oil. In experimental animals, two types of models were developed. Pyrethroids were applied to the flank of guinea pig or the back of rabbit, and reaction was quantified by counting frequency of licking, rubbing, biting, or scratching the exposed area [38, 41]. The results for pyrethroids in rabbit model are shown in Fig. 1. Consistent with observations in humans, cyano-containing pyrethroids lead to development of more severe symptoms of skin paresthesia. On the other hand, non-cyano compounds also possess such an activity, although quite weak, which has been occasionally reported for certain human populations. Vitamin E or benzocaine inhibited the paresthesia in both models [38]. No detailed molecular mechanism has been proposed, although repetitive firing of peripheral nerves in the skin has been postulated. Recently, several channels, including Transient Receptor Potential (TRP) channels, which sense environmental stimulation, have been revealed [42]. There is a possibility that pyrethroids may affect such channels. As the paresthesia is not serious, and effects

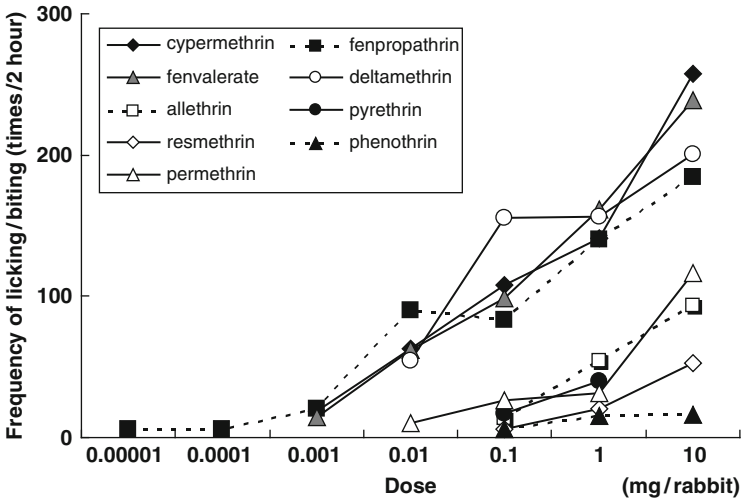

Fig. 1 Skin paresthesia by pyrethroids in rabbits (modified from Miyamoto et al. [38])

are local and do not progress into systemic neurotoxicity, the paresthesia is also considered as a kind of detector of the exposure to pyrethroids.

1.4 Developmental Neurotoxicity

The developing brain is vulnerable and prone to impairment by environmental insult. Social concerns regarding the effects of environmental factors on children's health, especially on their nervous systems, are increasing. In consideration of this issue, regulatory agencies, the U.S. Environmental Protection Agency (EPA, 1991) and the Organization for Economic Co-operation and Development (OECD, 2007) added the developmental neurotoxicity (DNT) test to the toxicological guideline studies to evaluate adverse effects on morphology and neurobehavioral functions of the developing nervous system before and after birth. For pyrethroids, this area has recently drawn much attention. Although publicly available literature suggests potential neurodevelopmental effects of some pyrethroids, available evidence has been judged to be insufficient [43].

1.4.1 Age-Related Differences in Sensitivity to Pyrethroids

Age-related sensitivity to pyrethroids in experimental animals has been reported. Cantalamessa reported that the acute oral LD_{50} of cypermethrin in 8-, 16-, 21-day-old, and adult rats were 14.9, 27.1, 49.3, and 250 mg/kg, respectively [44]. Sheets et al. reported that the acute oral LD_{50} of cypermethrin in 11-, 21-, and 72-day-old rats were 18, 73, and 439 mg/kg, respectively [45]. That is, in cypermethrin, the acute lethality is 17–24 times greater in neonates compared to adult rats. Similarly, deltamethrin, the α-cyano pyrethroid, showed 16-fold greater toxicity in neonatal rats [45]. Meanwhile, these age-related differences in acute lethality were not observed [45] or limited [44] with permethrin and/or cismethrin. The issue of age-related sensitivity to pyrethroids needs careful consideration, because administration of high dose levels is needed to compare the LD_{50}. Such dosing regimens likely exceed the limited capacity of the non-adult animals to metabolize a large bolus dose, as in vitro metabolism of deltamethrin by plasma carboxyesterases, hepatic carboxyesterases, and hepatic microsomes were 6, 35, and 7 times higher in adult rats than in postnatal day 10 rats, respectively [46]. Actually, effects on acoustic startle response were comparable in 21- and 72-day-old rats when the same low dose level of deltamethrin was administered [47]. Concentration of deltamethrin in brain was equivalent when each LD_{50} dose was administered in weanling rats and adults [47]. These results indicate that age-related differences in toxicity of pyrethroids are due to immature metabolite activity. However, it is still unclear whether this age-related difference would be relevant for human risk assessment at lower doses.

1.4.2 Developmental Neurotoxicity

Eriksson and co-workers have reported that neonatal exposure to low doses of the pyrethroids bioallethrin and deltamethrin by oral administration from postnatal days 10 to 16 induced an increase in muscarinic cholinergic receptor (MAChR) density in cerebral cortex at the age of 17 days. That resulted in a decrease in the cortical MAChR density, an increase in motor activity, and a lack of habituation at the adult age of 4 months in mice [48–50].

To confirm their results and check for methodological problems, some studies have been carried out. As there was a probability that hypothermic conditions during temporary removal from dam may have affected the results, Pauluhn and Schmuck administered *S*-bioallethrin and deltamethrin to neonatal mice from postnatal day 10 to 16 under a hypo-, normo-, or hyperthermic environment, and measured the MAChR density at the age of 17 days [51]. Increase in MAChR in Cortex at PND 17 in animals treated with *S*-bioallethrin was observed. Meanwhile, no changes were observed in animals treated with deltamethrin. In addition, an enormous influence of environmental temperature on the density of MAChR receptors in the crude synaptosomal fraction of the cerebral cortex was ascertained. Tsuji et al. exposed mouse dams with their litters to D-allethrin by inhalation for 6 h from postnatal day 10 to 16. The inhalation administration method is the most relevant route of exposure for humans, including babies and infants, after indoor use of D-allethrin. The neonatal exposure to D-allethrin by inhalation did not induce effects either on the brain MAChR density or motor activity at 17 days and 4 months of age, or on performance in the learning/memory test at 11 months of age [52]. Other unpublished studies with D-allethrin, *S*-bioallethrin, or deltamethrin were examined to confirm the results of Eriksson et al. and showed inconsistent results [53]. The reasons for discrepancy among these findings are unknown.

The effects of developmental exposure of pyrethroids on the dopaminergic system, which is considered to be related to behavior, were examined in several studies, but inconsistent results were obtained. Administration of deltamethrin between gestation day 6 and 15 induced increase of DOPAC (dopamine metabolite) levels in adult rats [54]. Exposure of fenvalerate on gestation day 18 and during postnatal days 2–5 produced no effect on monoamine levels on postnatal day 21 [55]. Gestational and lactational exposure to fenvalerate decreased and increased ^{3}H-spiroperidol binding in striatum after development, respectively, whereas only lactational exposure of cypermethrin induced increase in ^{3}H-spiroperidol binding [56].

Shafer et al. (2005) [53] reviewed 22 studies for DNT of pyrethroids including the above studies and summarized them. They insisted that additional, well-designed and well-executed DNT studies are needed because most of the studies suffered from inadequate design, problematic statistical analyses, use of formulated products, and/or inadequate control.

DNT studies on six pyrethroids (bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, fenpropathrin, and deltamethrin) were carried out in accordance with

U.S. EPA or OECD guidelines and submitted to the authorities [53]. The European Food Safety Authority (EFSA) released contents and evaluation results of a DNT study with deltamethrin [57]. Before the main DNT study, a preliminary study was conducted to verify exposure levels of rat offspring during lactation and to determine tolerance doses for the main study. Based on the deltamethrin levels in brain, pups in lactation period were confirmed to be exposed through dam's milk by dietary administration. In the main DNT study, deltamethrin was administered in diet from gestation day 6 to lactation day 21. FOB, motor activity, auditory startle response, learning and memory, morphological changes in neural tissues, brain weight, body weight, and developmental physical parameters were evaluated. Reduced body weight, body weight gain, and delayed balanopreputial separation, which were not adverse effects indicating DNT, were observed in offspring at the dose at which maternal effects were detected. The EFSA panel concluded that the available data did not indicate that deltamethrin is a developmental neurotoxic agent.

The EPA evaluated submitted DNT studies on six pyrethroids examined under their guidelines [58]. Generally, the toxic effects to offspring observed in DNTs were decreases in pup weight, pup weight gain, and/or brain weight, indicating that weight changes were a more sensitive indicator of toxicity in the pups than neurological effects. These available studies did not show effects on behaviors or parameters including learning, memory, auditory startle, or brain morphometries, although some exceptions were observed. In some studies, tremors were observed in both pups and dams, but tremors in pups were not seen at the LOAEL in any DNT studies. The adverse effects noted in the DNT studies were also noted in other guideline studies (reproduction study, acute neurotoxicity, subchronic neurotoxicity studies, etc., at similar or lower doses compared to the DNT studies. Thus, as the DNT studies did not provide sensitive endpoints for risk assessment, the EPA concluded that there would be little added value in conducting DNT studies for additional pyrethroids.

2 Carcinogenicity of Pyrethroids

2.1 Rodent Carcinogenicity Data of Selected Pyrethroids

Since pyrethroids, including pyrethrins, have been used for many years as insecticides for household, agricultural, and other applications, it is important to evaluate their carcinogenicity in humans. The Agency for Toxic Substances and Disease Registry (ATSDR) [59] provided an excellent review entitled "Toxicological Profile for Pyrethrins and Pyrethroids." According to this review, no reports were located regarding cancer in humans or animals following inhalation or dermal exposure to pyrethrins or pyrethroids. However, in the case of oral exposure to these chemicals, while no reports were located regarding cancer in humans, pyrethrins

and some pyrethroids have been shown to cause tumors in rodent models. Although the published papers regarding these findings are limited, there are several documents showing conclusions of evaluation of carcinogenic potential of these chemicals available in open documents on the Internet. A summary of these findings is shown in Table 4.

These findings show that most of the pyrethroids are not carcinogenic. However, some pyrethroids and pyrethrins increased the production of tumors in rodents: tumors of various types were produced by allethrin (kidney), bifenthrin (urinary bladder, liver, lung, leukemia), cypermethrin (lung), fenvalerate (testis), metofluthrin (liver), permethrin (liver and lung), pyrethrins (liver and thyroid), resmethrin (liver), tefluthrin (uterus and liver), tetramethrin (liver and testis), and transfluthrin (liver and urinary bladder). Of these, the liver was the most common target tissue. This may be because the liver is the major site of metabolic activation of chemicals, and furthermore, the liver is the first organ exposed to the chemical following absorption from the gastrointestinal tract if administered orally. These results are not specific to pyrethroids [108], and therefore these data indicate that tumor induction does not appear to reflect a common carcinogenic endpoint for this particular subset of compounds. Instead, tumorigenic responses appear to be specific to the compound and test organism employed.

2.2 *An Evaluation of the Human Relevance of the Selected Pyrethroids/Pyrethrins-Induced Tumors in Rodents Based on Mode of Action*

In the 1950s and 1960s, several chemicals were identified as rodent carcinogens that were also known to be carcinogenic in humans. The 2-year rodent bioassay was developed to provide a standardized screening procedure for evaluating chemicals with the assumption that these were predictive of human carcinogenic risk. In utilizing animals as a bioassay screening model, two fundamental assumptions are made: (1) the results observed in the animal model are relevant to humans (species extrapolation) and (2) the dose administered to the animals is relevant to the exposure levels in humans (dose extrapolation) [109]. For many chemicals, particularly DNA reactive carcinogens, these assumptions are reasonable. However, as has been clear for many chemicals, one or both of these assumptions may not be appropriate [109, 110].

In recent years, a Mode of Action (MOA) framework has been developed through the International Life Sciences Institute Risk Science Institute (ILSI/RSI) [111, 112] and the International Programme on Chemical Safety (IPCS) [113, 114], including an evaluation of the human relevance of the animal MOA data. Of pyrethroids showing positive findings of carcinogenic bioassays in rodents, those for metofluthrin, together with pyrethrins, are good examples of carcinogenic MOA analysis and evaluation of the human relevance of the animal MOA data.

Table 4 Carcinogenicity summary for selected pyrethrins/pyrethroids

Chemical (type)	Conclusion of evaluation of carcinogenicity
Allethrins (I)	• Suggestive evidence of carcinogenicity based on *kidney tumor* in rat (esbiothrin), but not sufficient to assess human carcinogenic potential [7]. • No carcinogenicity [60–62].
Bifenthrin (I)	• Because evidence of carcinogenicity in mice (*leiomyosarcoma in the urinary bladder, lymphoblastic lymphosarcoma and leukemia, and bronchiolar-alveolar adenocarcinoma and adenoma*) was obtained from a single study, it is considered that there is "limited evidence of carcinogenicity effects," which deserves a classification Category 2-H350 according to CLP criteria [63]. • Category C (possible human carcinogen) was evidenced by a dose-related increase in the incidence of *leiomyosarcomas in the urinary bladder*, a significant dose-related trend for combined *hepatocellular adenomas and carcinomas* in males, and a significantly higher incidence of combined *lung adenomas and carcinomas* in females. For the purpose of risk characterization, the RfD approach should be used for quantification of human cancer risk. The chronic exposure analysis revealed <100% RfD, and it is assumed that the chronic dietary endpoint is protective for cancer dietary exposure [64]. • R40 (Carc. Cat. 3) was proposed. The tumors occurred in mice exposed to bifenthrin on multiple sites (*urinary bladder, leukemia*), and therefore without robust mechanistic data the carcinogenic potential of bifenthrin could not be excluded. It was noted the tumors did not impact on the risk assessment [65]. • Unlikely to pose a carcinogenic hazard to humans [66].
Bioresmethrin (I)	• No increase in tumor incidence was found in rat or mouse [67].
Cyfluthrin (II)	• Not likely to be carcinogenic to humans [68]. • Unlikely to pose a carcinogenic risk to humans [69]. • No potential for carcinogenicity [70]. • Cyfluthrin treatment was not associated with increased tumorigenesis in either rats or mice [71].
Cyhalothrin (II)	• Lambda-cyhalothrin and an isomer gamma-cyhalothrin: not likely to be carcinogenic to humans [72]. • No oncogenic effects were observed in rats or mice [73].
Cypermethrin (II)	• Category C (possible human carcinogen) based on *lung tumor* in mouse. No quantification required [74]. • Zeta-cypermethrin is not carcinogenic under experimental conditions (carcinogenicity was tested with cypermethrin) [75]. • Unlikely to pose a carcinogenic risk to humans [76] • No evidence of carcinogenicity in rat or mouse [77] • No evidence for the carcinogenic potential [78].
Cyphenothrin (II)	• Not likely to be carcinogenic [6] • No evidence of carcinogenicity in rat or mouse [79]
Deltamethrin (II)	• No evidence of carcinogenicity in rat or mouse [80]. • Not likely to be a human carcinogen [81]. • Unlikely to be a carcinogenic hazard to humans [82]. • Group 3, not classifiable as to its carcinogenicity to humans [83] • No compound-related tumors were observed [84].

(continued)

Table 4 (continued)

Chemical (type)	Conclusion of evaluation of carcinogenicity
Esfenvalerate/ fenvalerate (II)	• Fenvalerate/esfenvalerate is currently classified as a Group E chemical: no evidence of carcinogenicity in rats or mice. The existing data base consisting mainly of a rat study with fenvalerate and a mouse study with esfenvalerate did not indicate increased incidence of neoplasia [85]. • Esfenvalerate: The weight of evidence led the meeting to conclude that esfenvalerate is not carcinogenic in rodents [5]. • Fenvalerate: Group 3, not classifiable as to its carcinogenicity to humans (inadequate evidence for the carcinogenicity of fenvalerate in experimental animals) [86] • Fenvalerate: Not carcinogenic to mice or rats [87].
Etofenprox (pyrethroid ether)	• Not likely to be carcinogenic to humans at doses that do not alter rat thyroid hormone homeostasis based on the following considerations: (1) treatment-related thyroid follicular cell tumors were seen in both male and female rats at 4,900 ppm, which was considered to be adequate, and not excessive, to assess carcinogenicity; (2) no treatment-related tumors were seen in male or female mice when tested at a dose that was considered adequate to assess carcinogenicity; (3) there is no mutagenicity concern for etofenprox based on in vivo and in vitro assays; (4) the non-neoplastic toxicological evidence (i.e., thyroid growth and thyroid hormonal changes) indicates that etofenprox disrupts the thyroid pituitary hormonal status; and (5) rats are substantially more sensitive than humans to the development of thyroid follicular cell tumors in response to thyroid hormone imbalance. The overall weight of the evidence was considered sufficient to indicate that etofenprox induced thyroid follicular tumors through an antithyroid mode of action. The quantification of carcinogenic potential was not applicable [88]. • No carcinogenic potential for human risk assessment. The etiology of the formation of the thyroid adenomas observed in rats was elucidated in a mechanistic study and considered not relevant for human risk assessment. Marginally increased renal cortical tumors found only in male mice at high doses were also not considered relevant to humans [89]. • There was an increased incidence of thyroid follicular adenomas among the 4,900 ppm animals, which was increased with statistical significance in females only. The absence of genotoxicity of etofenprox in combination with the observed activation of the thyroid gland, which might be related to the effects on the liver (the latter probably leading to increased breakdown of thyroid hormones), is a strong indication for a non-genotoxic mechanism of induction of the thyroid tumors [90].
Fenpropathrin (I/II)	• Not likely to be carcinogenic to humans [91]. • No evidence of carcinogenicity in mouse or rat [92].
Metofluthrin (I)	• The committee determined that the new data were sufficient to support a mitogenic mode of action for the development of liver tumors in rats exposed to metofluthrin in the carcinogenicity study. The report summarized mode of action study data that characterized effects such as increased P450 enzyme levels, increased smooth endoplasmic reticulum, hepatocellular hypertrophy, hepatocellular proliferation, and inhibition of intracellular communication, which were described as steps leading to tumor development via a nongenotoxic mechanism (i.e., mitogenicity). Some of these studies used sodium phenobarbital as a positive control,

(continued)

Table 4 (continued)

Chemical (type)	Conclusion of evaluation of carcinogenicity
	because this compound induces tumors in rodents via a mitogenic mode of action. The additional data on mode of action reviewed by the CARC in their second analysis addressed their previous uncertainties, and the committee revised their classification for metofluthrin to "not likely to be carcinogenic to humans at doses that do not induce a mitogenic response." The CARC further indicated that quantification of cancer risk is not required [93].
Permethrin (I)	• Likely to be carcinogenic to humans based on two reproducible benign tumor types (*lung and liver*) in mouse, equivocal evidence of carcinogenicity in Long-Evans rats, and supporting structural activity relationships (SAR) information. For the purpose of risk characterization, a low dose extrapolation model (Q1*) was used [94]. • Not carcinogenic to mouse or rat [95] • Group 3, not classifiable as to its carcinogenicity to humans (inadequate evidence for the carcinogenicity of permethrin in experimental animals) [96]. • Although there was a difference between the control and treated groups in terms of lung adenomas in mouse studies, these differences were not significant when compared with historical control values. The oncogenicity potential, as evaluated by the FIFRA Scientific Advisory Panel, was considered to be very weak. No tumors related to the ingestion of permethrin were observed in a rat study [10].
D-Phenothrin (I)	• Not likely to be carcinogenic to humans. Rat liver tumors occurred only at excessively toxic doses (limit dose) and mouse hepatocellular adenomas, which are common, did not achieve statistical significance ($p < 0.01$). Additionally, acceptable mutagenicity studies were negative for mutagenic potential [97] • No tumorigenicity was observed [11].
Prallethrin (I)	• There was no evidence of a carcinogenic response [98]. • No evidence of carcinogenicity [12]
Pyrethrins (I)	• Pyrethrins induce the formation of liver and thyroid tumors by mechanisms that appear to be similar to those of other non-genotoxic, mitogenic substances, e.g., phenobarbital, which produce tumors in rodents, and these tumors are not predictive of hazard in humans at relevant exposures [99]
Resmethrin (I)	• Likely to be carcinogenic to humans based on increased incidences of benign and malignant *liver tumors* in female rats and male mice. A low-dose extrapolation approach was applied to the experimental animal data in order to estimate human cancer risk [100]. • No oncogenic effects were seen [101].
Tau-fluvalinate (II)	• Not likely to be a human carcinogen [102].
Tefluthrin (I)	• In female rats, there was evidence of carcinogenicity in the form of *uterine adenocarcinomas*. Benign *liver tumors* were observed in female mice. Tefluthrin did not cause cancer in male mice or rats. There was no evidence that tefluthrin was genotoxic. Cancer risks are not of concern in humans [103]. • No evidence of carcinogenicity was demonstrated in studies conducted with mice or rats [104].

(continued)

Table 4 (continued)

Chemical (type)	Conclusion of evaluation of carcinogenicity
Tetramethrin (I)	• Suggestive evidence of carcinogenic potential (Group C, possible human carcinogen) based on evidence of *benign testicular tumors* in rats and increased *hepatocellular carcinomas* in male mice at 1,134 mg/kg/day. The Agency determined that no chronic cancer risk assessment was necessary based on the fact that this type of tumor (interstitial cell adenomas of the testes) is benign and does not progress to a malignant tumor in rats; the tumors occurred at a later stage of the study; the exposure started *in utero*; and the treatment did not cause reduction in latency [105]. • *Testicular interstitial cell tumors* occur spontaneously in aged rats, and the incidence can vary greatly in control groups. This tumor is believed to be hormonally mediated. There was no evidence of malignancy in three rat studies and no evidence of this type of tumor in mice. It can be concluded that the tumorigenic effect, if real, is most unlikely to be relevant to human exposure [13].
Tralomethrin (II)	• Not likely to be carcinogenic to humans [106].
Transfluthrin (I)	• Transfluthrin induced a low frequency of *urinary bladder adenomas/carcinomas* in rats at high doses – the NOEL for non-cancer endpoints was 20 ppm, for cancer, 200 ppm, and the urinary tumors were observed at a level of 2,000 ppm in diet. It also induced adenomas in female mice at a high dose level. Transfluthrin had no initiating activity, but was a weak promoter of carcinogenicity. Transfluthrin was consistently negative in in vitro and in vivo mutagenicity studies; it is concluded that the tumors induced at high dose in rats and female mice are probably not produced by a genotoxic mechanism [107].

***Case Study 1: An evaluation of the human relevance of the synthetic pyrethroid metofluthrin-induced liver tumors in rats based on mode of action* [115–117]**

Metofluthrin (CAS-No.240494-70-6; 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (EZ)-(1*RS*,3*RS*;1*RS*,3*SR*)-2,2-dimethyl-3-(prop-1-enyl)cyclopropanecarboxylate) is a new Type I pyrethroid insecticide for use in pest control. A basic genotoxicity assessment (including bacterial mutagenicity test, chromosomal aberration test in Chinese hamster lung cells, and micronucleus test in mice) demonstrated that metofluthrin is not genotoxic. The carcinogenicity of metofluthrin has been studied in male and female rats and mice in standard bioassays under the guidelines of Good Laboratory Practice. Male and female HanBrl:WIST rats were fed 0 (control), 20, 200, 900, or 1,800 ppm metofluthrin in their diet for two years (average chemical intakes: 0.84, 8.24, 38.08, and 77.79 mg/kg/day for males, 1.03, 10.12, 47.40, and 96.13 mg/kg/day for females, respectively). The incidence of hepatocellular adenomas and/or carcinomas was significantly increased in male rats given 900 or 1,800 ppm metofluthrin and in female rats given 1,800 ppm metofluthrin. The combined incidences of hepatocellular adenomas and carcinomas of 0, 20, 200, 900, and 1,800 ppm groups were, respectively, 2, 2, 6, 16, and 24% for males and 2, 6, 2, 10, and 24% for females. Historical background incidences for combined liver tumors in the laboratory conducting this bioassay were 0–6% for males and 1–12%

for females. It was concluded that metofluthrin has a weak carcinogenic potential in rats based on the liver tumors. In contrast, metofluthrin was not carcinogenic in the liver or any other tissues in male or female CD-1 mice when administered at dietary levels of 100, 1,000, and 1,750/2,500 ppm (average chemical intakes: 11.8, 115.7, and 208.7 mg/kg/day for males, and 15.4, 154.7, and 276.7 mg/kg/day for females, respectively).

To elucidate the MOA for liver tumor formation, a series of studies examining the effects of metofluthrin on hepatic microsomal cytochrome P450 (CYP) content, hepatocellular proliferation, hepatic gap junctional intercellular communication (GJIC), oxidative stress, and apoptosis was conducted after 1 or 2 weeks of treatment [115]. The global gene expression profile indicated that most genes with up-regulated expression with metofluthrin were metabolic enzymes that were also up-regulated with phenobarbital. Metofluthrin induced CYP2B and increased liver weights associated with centrilobular hepatocyte hypertrophy (with increased smooth endoplasmic reticulum), and induction of increased hepatocellular DNA replication. CYP2B1 mRNA induction by metofluthrin was not observed in CAR knockdown rat hepatocytes using the RNA interference technique, demonstrating that metofluthrin induces CYP2B1 through CAR activation. Metofluthrin also suppressed hepatic GJIC and induced oxidative stress and increased antioxidant enzymes, but showed no alteration in apoptosis. The above parameters related to the key events in metofluthrin-induced liver tumors were observed at or below tumorigenic dose levels. All of these effects were reversible upon cessation of treatment. Metofluthrin did not cause cytotoxicity or peroxisome proliferation. Thus, it is highly likely that the MOA for metofluthrin-induced liver tumors in rats is through cytochrome P450 induction and increased hepatocyte proliferation, similar to that seen for phenobarbital [115]. Other possible MOAs, including mutagenicity, cytotoxicity, hepatic peroxisome proliferation, porphyria, and hormonal perturbation, were excluded [117].

The postulated rodent MOA was tested against the Bradford Hill criteria based on the 2006 IPCS framework [113] and was found to satisfy the conditions of dose and temporal concordance, biological plausibility, coherence, strength, consistency, and specificity that fits with a well-established MOA for hepatocellular tumor [117]. In humans, there is currently no direct evidence for the effects of metofluthrin. It is unlikely that such data will become available, but data derived from in vitro studies on human hepatocytes can be contributory and of value. Such data have been obtained using an in vitro comparison of human hepatocytes to rat hepatocytes treated with metofluthrin or phenobarbital. Metofluthrin slightly induced CYP2B enzyme in rat and human hepatocytes as did phenobarbital, but phenobarbital appeared to have a more marked effect [116]. The CYP2B induction with metofluthrin in rats and humans is similar to that observed with pyrethrins [118]. Although CAR is expressed in humans, and phenobarbital induces CYP enzymes in human liver, phenobarbital can apparently act through other receptors as well, such as pregnane X receptor (PXR) [119]. In addition, human CAR has been suggested as being activated by phenobarbital, leading to not only activation of the CYP2B enzyme but also the induction of non-P450 genes, such as UGT1A1

[120]. The levels of exposure to phenobarbital in humans from therapeutic uses are comparable to serum levels that produce tumorigenic effects achieved in animals. Based on these analyses, the first key event, induction of CYP isoforms (CYP3A as well as CYP2B), occurs in humans at the levels of exposure that humans attain [121]. Based on the in vitro results and the rat in vivo studies at different exposure levels, induction of CYP isoforms in human hepatocytes is considerably lower for metofluthrin than phenobarbital at identical concentrations [115, 116].

A more critical step is the effect on hepatocellular proliferation. It has been thought that there is an increase in liver size in humans after prolonged treatment with phenobarbital, and that this increased liver size appears to be due to hepatocellular hypertrophy [110]. Thus, there appears to be the same proliferation of smooth endoplasmic reticulum in human liver in response to phenobarbital that is seen in the rat and the mouse. However, the in vitro studies with human hepatocytes suggest that the hepatocytes are refractory to the increased proliferative and antiapoptotic effects of phenobarbital that occur in rodents [122, 123]. In our study, dose-dependent increase of DNA synthesis was detected in rat hepatocytes [116], which is consistent with previous findings [122, 123], while various concentrations of phenobarbital had no effect on DNA synthesis associated with cell proliferation in humans [116]. Thus, there is significant evidence that this key event in phenobarbital-induced liver tumors in rodents, increased cell proliferation, does not occur in the human liver. Most importantly, there is also substantial epidemiological evidence for the non-carcinogenicity of phenobarbital in humans [124–127]. Again, the exposure levels in humans are similar to those in rodents, and administration to humans occurs over a period of many years, frequently beginning in childhood and continuing for essentially the lifetime of the individual. Such studies have demonstrated that in human subjects receiving phenobarbital for many years at doses producing plasma concentrations similar to those that are carcinogenic in rodents there is no evidence of increased liver tumor risk. As for phenobarbital, human hepatocytes were also refractory to the increased hepatocellular proliferation by metofluthrin that occurs in rodents [116]. Similar to phenobarbital, metofluthrin is not expected to produce an increase in hepatocellular proliferation, and therefore would not result in an increase in liver tumors. Based on this analysis, the answer to the question regarding plausibility in humans is likely to be "no" for metofluthrin. Based on the evidence, including a comparison with the results with another chemical, phenobarbital, acting by a similar MOA, it is reasonable to conclude that metofluthrin will not have any hepatocarcinogenic activity in humans [117].

***Case Study 2: An evaluation of the human relevance of the liver and thyroid tumors in rats induced by pyrethrins based on mode of action* [118, 128–130]**

Pyrethrum has been used for many years as an insecticide for household, agricultural, and other applications. Pyrethrins exhibit a low order of toxicity in mammals and are rapidly metabolized. However, the chronic administration of pyrethrins to rats has been shown to result in liver and thyroid gland tumor formation [128, 129]. The carcinogenicity of pyrethrins has been examined in both the mouse and rat.

Male and female Sprague-Dawley CD rats were fed 0 (control), 100, 1,000, and 3,000 ppm pyrethrins in the diet for 2 years. Average daily chemical intakes were 0, 4.37, 42.9, and 130 mg/kg/day for males, and 0, 5.39, 55.5, and 173 mg/kg/day for females, respectively. While 1,000 ppm pyrethrins had no effect on tumor incidence in both sexes, a small increase in the incidence of hepatocellular adenoma was observed in female rats given 3,000 ppm pyrethrins. Treatment with pyrethrins did not affect the incidence of hepatocellular carcinoma in female rats and had no significant effect on the incidence of hepatocellular adenoma or carcinoma in male rats. Furthermore, the combined incidence of thyroid follicular cell adenomas and/or carcinomas was significantly increased in male rats given 1,000 and 3,000 ppm pyrethrins and in female rats given 3,000 ppm pyrethrins. A basic genotoxicity assessment (including gene mutation, chromosomal aberrations, and other genotoxic effects) demonstrated that pyrethrins were not genotoxic [129]. In a mouse 18-month study, pyrethrins were not carcinogenic to male or female CD-1 mice when administered at dietary levels of 0 (control), 100, 2,500, and 5,000 ppm (average daily chemical intakes: 0, 13.8, 346, and 686 mg/kg/day for males, and 0, 16.6, 413, and 834 mg/kg/day for females, respectively).

To understand the MOA for liver tumor formation, the hepatic effects of pyrethrins have been investigated [130]. Male Sprague-Dawley CD rats were fed diets containing 0 (control) and 8,000 ppm pyrethrins and female rats diets containing 0, 100, 3,000, and 8,000 ppm pyrethrins for periods of 7, 14, and 42 days, and 42 days followed by 42 days of reversal. Rats were also fed diets containing 1,200–1,558 ppm sodium phenobarbital for 7 and 14 days as a positive control. The treatment of male rats with 8,000 ppm pyrethrins, female rats with 3,000 and 8,000 ppm pyrethrins, and both sexes with phenobarbital resulted in increased liver weights, which were associated with hepatocyte hypertrophy. Hepatocyte replicative DNA synthesis was also increased by treatment with pyrethrins and phenobarbital. The treatment of male and female rats with pyrethrins and phenobarbital produced significant increases in hepatic CYP content and a marked induction of CYP2B-dependent 7-pentoxyresorufin *O*-dependentylase and testosterone 16β-hydroxylase activities. Significant increases were also observed in CYP3A-dependent testosterone 6β-hydroxylase activity. The hepatic effects of pyrethrins were dose-dependent in female rats, with 100 ppm being a no-effect level, and on cessation of treatment were reversible in both sexes. This study demonstrates that pyrethrins are mitogenic CYP2B form inducers in rat liver. The MOA for pyrethrins-induced rat liver tumor formation appears to be similar to that of phenobarbital and some other non-genotoxic CYP2B inducers of hepatic xenobiotic metabolism [130]. Other possible MOAs including mutagenicity, cytotoxicity, hepatic peroxisome proliferation, porphyria, and hormonal perturbation were excluded [129].

To understand the MOA by which the thyroid tumors are produced, the effect of pyrethrins on rat thyroid gland, thyroid hormone levels, and hepatic thyroxine UDP-glucuronosyltransferase activity was also investigated [128]. The treatment of male rats with 8,000 ppm pyrethrins, female rats with 3,000 and 8,000 ppm pyrethrins, and both sexes with phenobarbital resulted in increased thyroid gland

weights, which were associated with follicular cell hypertrophy. Thyroid follicular cell replicative DNA synthesis was increased by treatment with pyrethrins and phenobarbital for 7 and/or 14 days. Treatment with pyrethrins and phenobarbital increased hepatic microsomal thyroxine UDP-glucuronosyltransferase activity and serum thyroid-stimulating hormone (TSH) levels, but reduced serum levels of thyroxine (T4) and/or triiodothyronine (T3). The effects of pyrethrins in female rats were dose-dependent, with 100 ppm being a no-effect level, and on cessation of treatment were essentially reversible in both sexes. The concordance between the effects of pyrethrins and phenobarbital suggests that the MOA for rat thyroid gland tumors induced by pyrethrins is similar to that of some other non-genotoxic inducers of hepatic xenobiotic metabolism. The MOA for pyrethrins-induced thyroid gland tumor formation appears to involve hormonal dysfunction due to hepatic enzyme induction: enhanced thyroxine glucuronidation, due to the stimulation of microsomal thyroxine UDP-glucuronosyltransferase activity, leads to decreased serum levels of T4/T3, which results in a compensatory increase in serum TSH levels, leading to increased thyroid gland weight due to thyroid follicular cell hypertrophy and hyperplasia; the chronic stimulation of the rodent thyroid gland by TSH is known to result in thyroid follicular cell hyperplasia and subsequently in the formation of thyroid follicular cell adenomas and carcinomas [128].

The postulated rodent MOA was evaluated based on the 2006 IPCS framework [113] and was found to satisfy the conditions of dose and temporal concordance, biological plausibility, coherence, strength, consistency, and specificity that fits with a well-established MOA for hepatocellular tumor and thyroid follicular-cell tumor. Regarding hepatocellular tumor, the proposed MOA is considered not to be plausible in humans, because pyrethrins, like phenobarbital, do not induce cell proliferation in human hepatocytes [129]. Moreover, as discussed in Case Study 1, epidemiological studies with phenobarbital demonstrate that such compounds do not increase the risk of liver tumors in humans. It is concluded that pyrethrins do not pose a hepatocarcinogenic hazard for humans [129]. As for thyroid follicular cell tumor, although the postulated MOA could theoretically operate in humans, there are well-known marked quantitative differences in the inherent susceptibility for neoplasia to thyroid hormone imbalance in rats [131]. Therefore, the findings from MOA analysis allow for the conclusion that pyrethrins do not pose a thyroid carcinogenic hazard to humans [128].

3 Developmental and Reproductive Toxicities

3.1 Developmental Toxicity

Pyrethroids produce no common teratogenic effects in a particular species based on similarity in structure or mode of insecticidal action. In most of the animal studies with pyrethroids, no increased incidences of morphological abnormalities were

observed. There have been no epidemiological investigations on the outdoor use of pyrethroids. A human study of a permethrin cream for head lice did not demonstrate an increased risk for birth defects [132].

Treatment with fenvalerate to pregnant mice at 200 mg/kg was associated with micromelia [133], although no treatment-related abnormalities were observed in rats [134]. Deltamethrin caused early embryonic deaths and growth retardation at high doses in rats, but did not increase the incidence of malformations in rats or mice [135, 136]. Experimental animal studies and limited published human data show permethrin is not expected to increase the risk of birth defects. Placental effect levels of exposure in pregnant animals produced increased resorptions but not congenital anomalies in rats and mice [137, 138]. A human study of first trimester exposure to permethrin and later exposure in pregnancy did not show a significant increase in congenital anomalies or other adverse outcomes [139].

Treatment of rats with cypermethrin up to 8 mg/kg/day produced no malformations [140]. Maternally toxic dose level of bifenthrin did not produce adverse effects on embryonic development in rats [141]. A rabbit teratology study with 30 or 90 mg/kg/day tetramethrin during fetal organogenesis demonstrated no adverse effects on skeletal or external development [137].

3.2 Reproductive Toxicity

Some reports suggested that pyrethroids might affect reproductive organs or sperm. Different modes of action mediating calcium, nitric oxide, or mitochondrial membrane are proposed for different chemicals investigated. Some studies have shown inhibitory or stimulating action of pyrethroids to sex hormones. In recent years, a number of research efforts to identify endocrine disruptors among international communities have been underway. It is expected that such efforts would show whether pyrethroids exhibit endocrine-disrupting action in mammals.

Prenatal and postnatal exposures to fenvalerate reduced prostate and seminal vesicle weights and plasma testosterone levels in male rats [55]. A chronic study showed no adverse effects on reproductive tissues at a high dose level of 1,000 ppm [142]. In vivo and in vitro studies with rats and mice suggested that fenvalerate may affect male and female reproduction, possibly due to calcium transport alteration [143–146]. One paper reported that fenvalerate affected human sperm count and sperm motility of male workers who were exposed to fenvalerate in a pesticide factory [147].

A subacute study in rats treated intraperitoneally with deltamethrin showed testicular degeneration and an inhibition of spermatogenesis, which seemed to be mediated by an elevation of nitric oxide levels [148]. Subcutaneous dosing also produced adverse testicular effects and reduced spermatogenesis [149].

Increased resorptions were observed in female mice mated to males treated with cypermethrin 40 or 80 mg/kg/day [150]. Reduced fertility and decreased body weight were noted in male rats at a level of 8,571 ppm cypermethrin in drinking

water [151]. Another study did not show adverse effects in male rats [152]. Treatment of male and female mice with cypermethrin 10 mg/kg/day prior to mating produced a decrease in fertility, litter size, pup viability, and pup weight, and effects on neonatal and juvenile behavior in the offspring [153].

Permethrin may behave like estrogen in females but antiandrogen in males when administered to immature female and male rats at 10–800 mg/kg [154]. Treatment of rats with permethrin at 10 mg/kg/day during gestation produced fetal death and feminization of male fetuses [155]. Reductions in sperm count and motility and in serum testosterone were noted in a 6-week repeated dose study in mice at 35 mg/kg/day. Permethrin may cause mitochondrial membrane impairment in Leydig cells, resulting in inhibition of testosterone biosynthesis [156].

There were no effects on the fertility or the incidence of anomalies or growth in offspring in a one-generation reproduction study in rats with an aerosolized repellent containing 3.6% allethrin for 8 h/day [157].

In recent studies, tetramethrin exhibited anti-estrogenic activity in some assays [158], but not in others [159].

There are conflicting findings on the possible estrogenic effects of phenothrin. One group reported that phenothrin had estrogen-like effects on the postnatal development of male and female rats [160]; in contrast, another group reported that there was no indication of estrogenic or (anti-)androgenic effects of D-phenothrin in uterotrophic and Hershberger assays [161].

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Biotransformation and Enzymatic Reactions of Synthetic Pyrethroids in Mammals

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Abstract Synthetic pyrethroids, a major insecticide group, are used worldwide for controlling indoor and agricultural pests. Extensive mammalian metabolism studies have been carried out since the late 1960s, and major metabolic reactions have been found to be oxidation of the acid or alcohol moiety, ester cleavage, and conjugation reactions. In addition, various conjugation reactions occur in mammals, forming hydrophilic and lipophilic conjugates. Pyrethroids are generally rapidly metabolized in mammals and completely excreted from the body in a short period. Human and laboratory animals share similar metabolic reactions for pyrethroids. Oxidation reactions in humans are mediated by several CYP isoforms. On the other hand, ester bonds of pyrethroids are hydrolyzed mainly by carboxylesterase(s).

Keywords Carboxylesterase · CYP · Ester hydrolysis · Metabolism · Oxidation · Pyrethroid

Contents

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1 Introduction

Natural pyrethrins, insecticidal ingredients occurring in the flowers of *Tanacetum cinerariaefolium* (also known as *Chrysanthemum cinerariaefolium* or *Pyrethrum cinerariaefolium*), have been modified for higher stability in the environment as well as better biological performance for more than 40 years, and consequently more than 30 synthetic pyrethroids have been marketed and used worldwide for controlling indoor and agricultural pest insects [1, 2].

Substantial metabolism studies of pyrethroids in mammals were launched starting from the late 1960s, and many in vivo and in vitro studies have been carried out so far. From the historical viewpoint, mammalian metabolism studies of pyrethroids can be roughly divided into three periods (first period, late 1960s–mid-1970s; second period, mid-1970s–2000; third period, 2000–present). During the first period, mammalian metabolism of the first-generation pyrethroids was investigated only in rodents. In the second period, many in vivo and in vitro metabolism studies of the first- and second-generation pyrethroids were carried out, and metabolic fates were extensively examined in several mammalian species, including humans, mostly using radiolabeled preparations. In addition, metabolic fates of pyrethroids were investigated in livestock animals, including lactating goats and laying hens, for regulatory purposes. Furthermore, metabolic fates of geometrical and chiral isomers of some pyrethroids were studied for elucidation of geometrical or chiral isomer-specific biological effects and for biological interactions among the isomers. Since 2000 (third period), genetically expressed CYP isoforms or carboxylesterases of animals or humans have become commercially available along with progress of molecular biology. Furthermore, human hepatic microsomes or frozen hepatic cells have been put on the market. Therefore, it has become possible to determine enzymes responsible for metabolic reactions and to make clear species differences between humans and laboratory animals in terms of enzyme levels. In addition, species differences in metabolism between human beings and laboratory animals can be quantitatively and qualitatively investigated to some extent, and now rather precise risk assessment of pyrethroids can be made [1, 2].

So far, metabolic studies of about 30 synthetic pyrethroids, including their chiral and geometrical isomers, have been carried out in mammals [1]. However, detailed metabolism data have not necessarily all been published in scientific journals. In some cases, the reports of joint World Health Organization–Food and Agricultural Organization (WHO/FAO) expert meetings on pesticide residues and the

International Program on Chemical Safety (IPCS), Environmental Health Criteria (WHO), were referred to [1, 2].

This chapter presents an overview of mammalian metabolic fates of pyrethroids, enzymes responsible for biotransformation, human metabolism and species, isomers, and age differences of pyrethroids.

2 Absorption, Elimination, Distribution

Regarding absorption, in general oral absorption rates are rather high in rats and mice, and dermal penetration rates are low. It should be noted that humans show much lower skin penetration rates than rats, indicating that rat data lead to overestimation of dermal penetration of pyrethroids [2]. The Cmax is commonly observed up to several hours after oral administration at low doses but it is delayed at higher oral doses. However, oral absorption rates may depend on the vehicles used for dosing. After systemic absorption, pyrethroids and their metabolites do not show accumulation in any specific tissues or organs [1, 2]. The second-generation pyrethroids appear to show somewhat higher tissue residue levels in fats as compared with other tissues, due to high lipophilicity. The acid and alcohol moieties of pyrethroids are rapidly and completely excreted into urine and feces within several days after oral administration. However, the carbon derived from the CN group of pyrethroids, α-cyano-3-phenoxybenzyl alcohol derivatives, shows incomplete excretion and longer bioretention in skin and stomach [3–6]. The CN group from these pyrethroids is mainly metabolized to thiocyanate after cleavage of the ester bond, and this slow and incomplete excretion of thiocyanate generated is likely due to distribution to the extracellular fluid and partial binding with serum albumin, as is the case with endogenous thiocyanate [3, 4].

3 Metabolic Reactions

Review of metabolic pathways of about 30 pyrethroids revealed that the major metabolic reactions are commonly oxidation, cleavage of ester bond, and conjugation, in all cases [1]. These metabolic reactions proceed in animals in two steps. As a first step, so-called phase I reactions occur, which are oxidation and cleavage of ester bond. In the second step, conjugation is a phase II reaction that generates hydrophilic and lipophilic forms. Hydrophilic conjugates are often found as glucuronides, sulfates, or amino acid conjugates, and these are readily excreted into urine due to high hydrophilicity. In less frequent cases, lipophilic conjugates are found and generally show longer bioretention than the hydrophilic conjugates. Though data in the public domain are limited, metabolites of pyrethroids normally show less acute oral toxicity than the corresponding parent compounds and thus rapid metabolism leads to low mammalian acute toxicity [7].

Oxidation reactions occur on several sites of the acid and alcohol moieties, depending on the chemical structures. For example, the *trans* methyl of the isobutenyl group in chrysanthemates is preferentially oxidized over the *cis* methyl group in rats, and the 4'-position of the phenoxy ring is oxidized to a larger extent as compared with other positions [8] (Fig. 1).

Ester hydrolysis occurs to a larger extent with the *trans* and primary alcohol derivatives as compared with the corresponding *cis* and secondary alcohol derivatives, respectively (Fig. 2). The chirality (*1 S* or *1R*) at the acid moiety of phenothrin [9], tetramethrin [10], and permethrin [11] does not significantly affect ester hydrolysis.

Hydrophilic conjugates, such as glucuronides, sulfates, and amino acid conjugates, are found in mammalian metabolism of pyrethroids [2]. 3-Phenoxybenzoic acid (3-PBacid), a common metabolite from pyrethroids having 3-phenoxybenzyl alcohol or α-cyano-3-phenoxybenzyl alcohol in the alcohol moiety, shows remarkably diversified amino acid conjugates: a glycine conjugate is the major form in sheep, cat, and gerbil, a taurine conjugate in mice, and a glycylvaline dipeptide conjugate in the mallard duck [12]. In addition, thiocyanate and sulfonic acid conjugates have been reported to be found in pyrethroid metabolism. Thiocyanate is formed by conversion of the CN ion released from ester hydrolysis of pyrethroids with the α-cyano-3-phenoxybenzyl alcohol derivative [3–6]. Sulfonic acid conjugates have a sulfonic acid group incorporated into the double bond of the 3,4,5,6-tetrahydrophthalimide moiety of tetramethrin, and are reported to be non-enzymatically formed in the intestinal tract [13]. A mercapturic acid conjugate is documented to be involved in the metabolism of prallethrin [14] (Fig. 3).

In addition, three types of lipophilic conjugates have been found in pyrethroid metabolism studies (Fig. 4). They are cholesterol ester (fenvalerate) [15], glyceride (3-PBacid, a common metabolite of several pyrethroids) [16], and bile acid conjugates (fluvalinate) [17]. It is noteworthy that one isomer out of the four chiral isomers of fenvalerate yields a cholesterol ester conjugate from its acid moiety [15]. This chiral-specific formation of the cholesterol ester has been demonstrated to be mediated by transesterification reactions of carboxylesterase(s) in microsomes, not by any of the three known biosynthetic pathways of endogenous cholesterol esters

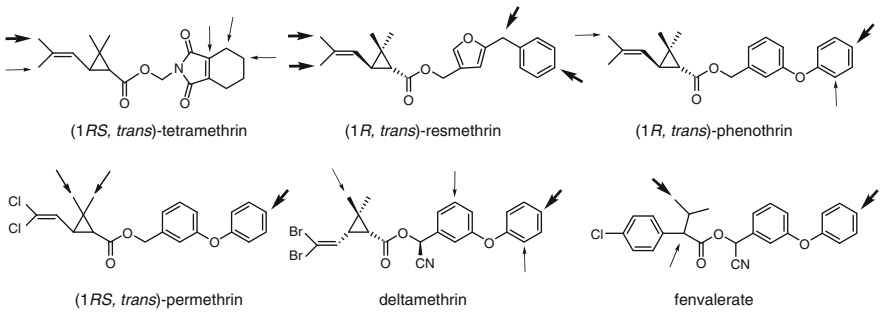

Fig. 1 Oxidation reactions

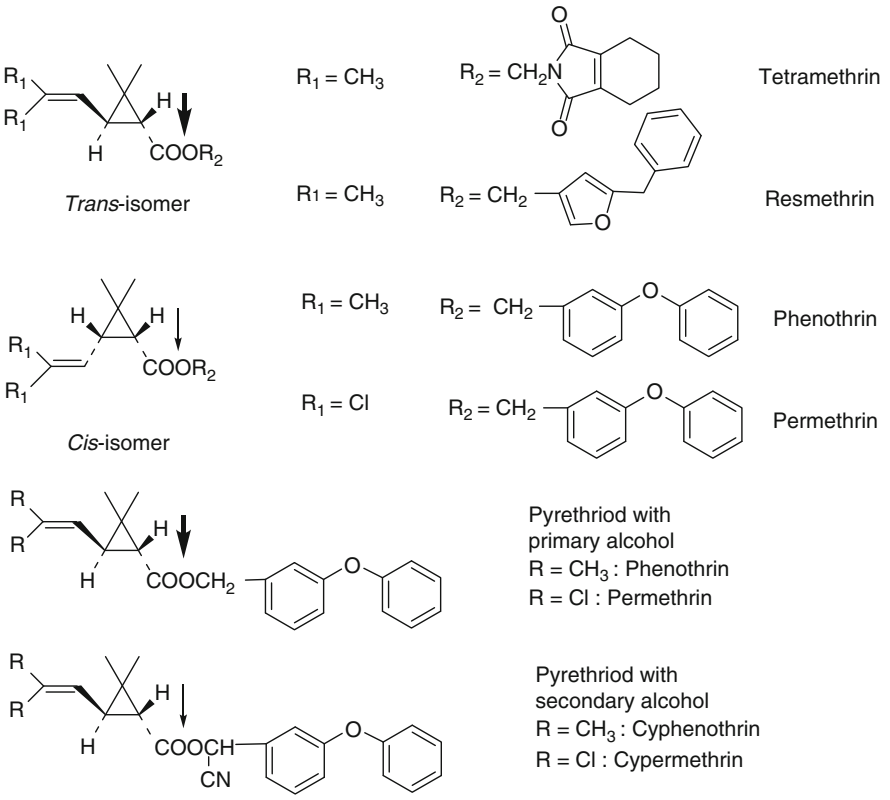

Fig. 2 Ester hydrolysis

(acyl-CoA:cholesterol *O*-acyltransferase (ACAT), lecithin:cholesterol *O*-acyltransferase (LCAT), or cholesterol esterase) [18]. This conjugate was demonstrated to be a causative agent for granulomatous changes that are observed in rats and mice when fenvalerate is administered for a long time [19]. This is the first example of a lipophilic conjugate showing toxicity.

4 Enzymes Involved in Pyrethroid Metabolism

Extensive metabolism studies carried out mainly in rats and mice show that pyrethroids are metabolized by oxidation and ester cleavage, which are mediated by CYP isoforms and carboxylesterases, respectively. CYP isozymes and carboxylesterases responsible for the metabolism are reviewed below.

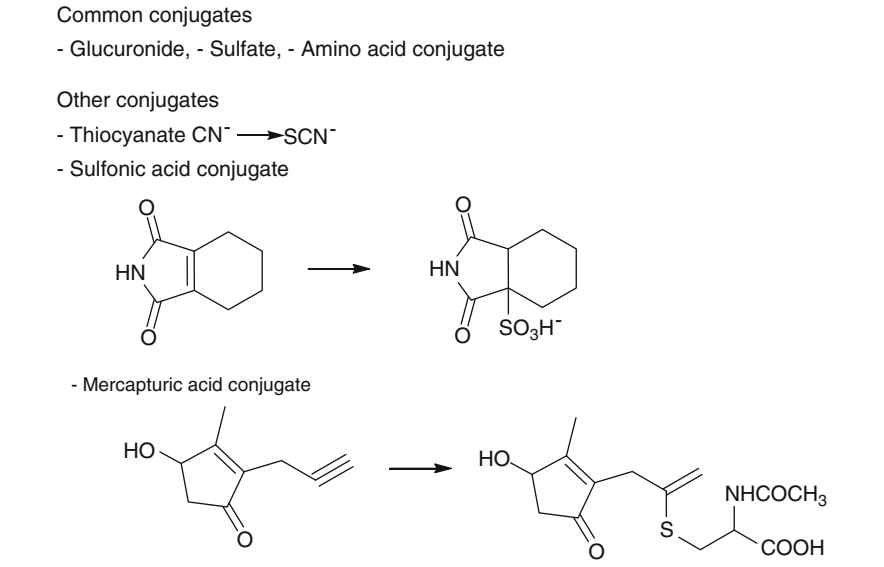

Fig. 3 Hydrophilic conjugates

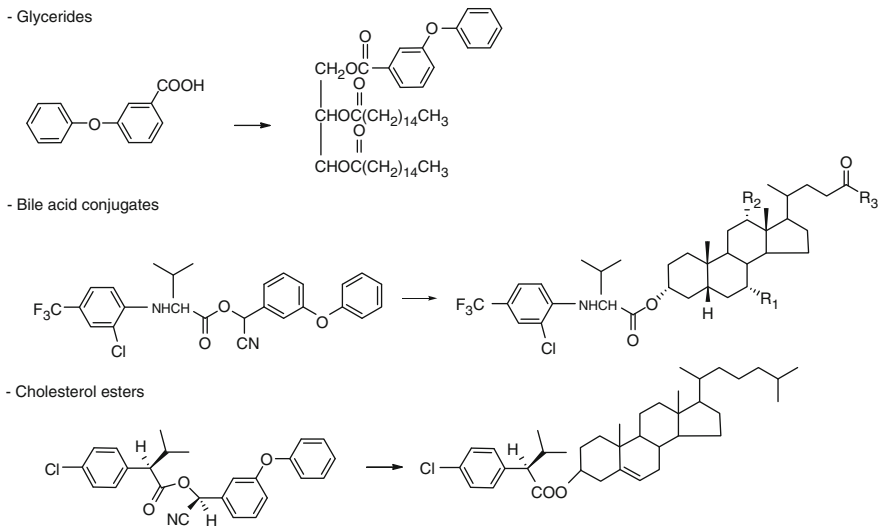

Fig. 4 Lipophilic conjugates

4.1 Cytochrome P450s

Metabolism of bifenthrin, *S*-bioallethrin, bioresmethrin, β-cyfluthrin, cypermethrin, *cis*-permethrin, and *trans*-permethrin were examined in rat and human hepatic

microsomes [20]. The intrinsic hepatic clearance (CL_{int}) of the pyrethroids was 5- to 15-fold greater in rat relative to human microsomes except for *trans*-permethrin, which was approximately 45% greater in human microsomes. The metabolism of bifenthrin, *S*-bioallethrin, and *cis*-permethrin in rat and human hepatic microsomes was solely the result of oxidative processes. The metabolism of bioresmethrin and cypermethrin in human hepatic microsomes was solely the result of hydrolytic processes. Bioresmethrin and cypermethrin in rat hepatic microsomes and *β*-cyfluthrin and *trans*-permethrin in microsomes from both species were metabolized by both oxidative and hydrolytic pathways. Rat cytochrome P450s (CYPs) that showed activity toward several pyrethroids included CYP1A1, CYP1A2, CYP2C6, CYP2C11, CYP3A1, and CYP3A2. Human CYPs that showed activity toward multiple pyrethroids were CYP2C8, CYP2C9, CYP2C19, and CYP3A4 (Table 1).

Godin et al. [21] reported differences in the metabolism of deltamethrin and esfenvalerate by rat and human liver microsomes with respect to the biotransformation pathway (oxidation vs hydrolysis) responsible for their clearance. Rat CYP1A1, CYP2C6, CYP2C11, and CYP3A2 and human CYP2C8, CYP2C19, and CYP3A5 were capable of metabolizing either pyrethroid. Human CYP2C9 metabolized esfenvalerate but not deltamethrin. Rat and human CYPs that metabolize esfenvalerate and deltamethrin do so with similar kinetics.

Nakamura et al. [22] reported the in vitro metabolism of permethrin and its hydrolysis products in rats. *cis*- and *trans*-permethrin were mainly hydrolyzed both by liver and small-intestinal microsomes of rats. *trans*-Permethrin was much more effectively hydrolyzed than the corresponding *cis*-isomer. Three metabolites, 3-phenoxybenzyl alcohol (3-PBalc), 3-phenoxybenzaldehyde (3-PBald), and 3-phenoxybenzoic acid (3-PBacid), were formed. However, only 3-PBalc was formed in the absence of cofactors. The microsome activities of rat liver and small intestine were inhibited by bis-*p*-nitrophenyl phosphate, an inhibitor of carboxylesterases (CESs). ES-3 and ES-10, isoforms of the CES 1 family, exhibited significant hydrolytic activities toward *trans*-permethrin. 3-PBald and 3-PBacid were formed from 3-PBalc with rat liver microsomes in the presence of NADPH. The oxidizing activity was inhibited by SKF 525-A (CYP P450 inhibitor). Rat recombinant CYP2C6 and 3A1 exhibited significant oxidase activities with NADPH. 3-PBacid was formed from 3-PBald with the microsomes in the presence of NADPH. CYP1A2, 2B1, 2C6, 2D1, and 3A1 exhibited significant oxidase activities in this reaction. Thus, permethrin was hydrolyzed by CES, and the 3-PBalc formed was oxidized to 3-PBald and 3-PBacid by the CYP isoforms or alcohol dehydrogenase in rats.

4.2 Esterases

Carboxylesterases (CESs) catalyze hydrolysis of pyrethroids. The expression of CESs is ubiquitous in mammals. The highest hydrolase activity is present in liver.

Table 1 Cytochrome P450s and carboxylesterases responsible for metabolism of pyrethroids

Pyrethroids	Cytochrome P450s (CYP)	Carboxylesterases (CES)	Others	References
Baythrin		hCE1, hCE2	Activated hPXR (> 3X), rPXR (> 3X)	[29]
Bifenthrin		(hCE1), hCE2		[29]
	Rat 1A1,1A2,2B1,2C6,2C11,2C12,3A1,3A2			[20]
	Human 2C8,2C9, 2C19			
Bioallethrin		hCE1, hCE2		[29]
S-Bioallethrin	Rat 1A1,2A1,2C6,2C11,3A1,3A2			[20]
	Human 2C8,2C9,2C19,3A4			
Bioresmethrin		hCE1, hCE2		[29]
	Rat 1A1,1A2,2C6,2C11,2D2,3A1,3A2			[20]
	Human 1A2,2B6,2C8,2C9,2C19,3A4			
β-Cyfluthrin	Rat 1A1,2A1,2C6,2C12,2D1,3A1,3A2			[20]
	Human 1A1,1A2,2C8,2C9,2C19,3A4			
λ-Cyhalothrin		hCE1, hCE2	Activated hPXR (6.3X), rPXR (> 3X)	[29]
	Rat 1A1,2C6,2C11,2D1,3A1,3A2			[20]
	Human 1A1,1A2,2B6,2C8,2C9,2C19,3A4			
Cypermethrin	Rat 1A1,1A2,2A1,2B1,2C6,2C11,2D1,2D2,3A1,3A2			[20]
	Human 1A2,2C8,2C9,2C19,3A4			
Deltamethrin		hCE1, hCE2	Activated hPXR (> 4X), rPXR (> 3X)	[29]
	Rat 1A1,2C6,2C11,3A2			[21]
	Human 2C8,2C19,3A5			
Esfenvalerate		hCE1, (hCE2)	Activated hPXR (> 3.5X), rPXR (> 4X)	[29]
	Rat 1A1,2C6,2C11,3A2			[21]
	Human 2C8,2C9,2C19,3A5			
τ-Fluvalenate		hCE2	Activated hPXR (3.5X), rPXR (> 3X)	[29]
cis-Permethrin		hCE1, hCE2		[29]
	Rat 1A1,1A2,2C6,2C11,3A2			[20]
	Human 1A1,1A2,2C8,2C9,2C19			

trans-Permethrin	-	hCE1, hCE2		[29]
	Rat 1A1,1A2,2C6,2C11,3A1,3A2			[20]
	Human 1A1,1A2,2C8,2C9,2C19,3A4			
		rat CES1		[22]
Resmethrin	Rat 1A1,1A2,2A1,2C6,2C11,2C13,2D2,3A1,3A2			[20]
	Human 2C8,2C9,2C19,3A4			
Tetramethrin	3A4	hCE1, hCE2	Activated hPXR, $>$ 3.5X	[29]
3-PBalc	Rat 1A1,1A2,2C6,2D1,2E1,3A1			[22]
3-PBald	Rat 1A1,1A2,2B1,2C6,2C9,2D1,2E1,3A1			[22]

CESs are also detected in small intestine, kidney, and lung [23]. Ross and Crow [24] showed that two major CESs expressed in human liver, hCE1 and hCE2, are different gene products, and conducted phenotyping of these proteins. There was little variation in hCE1 protein expression in human liver microsome samples from 11 individuals. hCE2 protein expression in individual human liver microsomes was only slightly more variable than hCE1. However, amount of hCE1 protein was 46-fold higher in the microsomes than that of hCE2 protein (64 μg hCE1/mg microsomal protein compared to 1.4 μg hCE2/mg microsomal protein). hCE1 is highly expressed in the liver and also detected in macrophages, lung epithelia, heart, and testis [25]. hCE2 is found in the small intestine, colon, kidney, liver, heart, brain, and testis [26]. Although these two enzymes are present in various tissues, hCE1 and hCE2 contribute predominantly to the hydrolase activity of liver and small intestine, respectively. It has also been shown that CESs exhibit species differences. For example, Li et al. [27] demonstrated that human plasma contains no CES activity; in contrast, the mouse, rat, rabbit, horse, cat, and tiger have high levels of plasma CESs.

Pure human CESs (hCE1 and hCE2), a rabbit CES (rCE), and two rat CESs (Hydrolases A and B) were used to study the hydrolytic metabolism of the following pyrethroids: 1*R-trans*-resmethrin (bioresmethrin), 1*RS-trans*-permethrin, and 1*RS-cis*-permethrin [28]. hCE1 and hCE2 hydrolyzed *trans*-permethrin 8- and 28-fold more efficiently than *cis*-permethrin (when k_{cat}/K_m values were compared), respectively. In contrast, hydrolysis of bioresmethrin was catalyzed efficiently by hCE1, but not by hCE2. The kinetic parameters for the pure rat and rabbit CESs were qualitatively similar to the human CESs when hydrolysis rates of the investigated pyrethroids were evaluated. Further, a comparison of pyrethroid hydrolysis by hepatic microsomes from rats, mice, and humans indicated that the rates for each compound were similar between species.

Yang et al. [29] reported structure-selective hydrolysis of several pyrethroids by human liver microsomes and recombinant hCE1 and hCE2. The type of pyrethroids may be served as a better indicator for the overall hydrolysis in the liver, with the one exception of deltamethrin. Type I pyrethroids were generally hydrolyzed at high rates by liver microsomes. On the other hand, the majority of the pyrethroids hydrolyzed at lower rates belong to Type II compounds. hCE1 preferentially hydrolyzed bioallethrin, bioresmethrin, deltamethrin, esfenvalerate, and λ-cyhalothrin; however, hCE2 showed higher or similar hydrolysis activity toward baythroid, bifenthrin, *cis*-permethrin, τ-fluvalinate, and tetramethrin than hCE1 (Table 2).

The serum CES was purified to homogeneity to determine its contribution to pyrethroid metabolism in the rat [30]. Both *trans*-permethrin and bioresmethrin were effectively cleaved by this serum CES, but deltamethrin, esfenvalerate, α-cypermethrin, and *cis*-permethrin were slowly hydrolyzed. Two model lipases produced no hydrolysis products from pyrethroids. These results demonstrated that extrahepatic esterolytic metabolism of specific pyrethroids might be significant.

Yang et al. demonstrated deltamethrin metabolism by chymotrypsin [31]. Four crude products were formed following incubation of deltamethrin with

Table 2 Specific activity of hCE1 and hCE2 for various authentic pyrethroids

Substrate	hCE1 (nmol/min per mg)	hCE2 (nmol/min per mg)
Baythroid	9.7	13
Bifenthrin	2.0	5.9
Bioallethrin	64	20
Bioresmethrin	71	11
λ-Cyhalothrin	11	3.3
Deltamethrin	61	17
Esfenvalerate	11	0.3
trans-Permethrin	48	56
cis-Permethrin	3.6	25
τ-Fluvalinate	0.4	1.8
Tetramethrin	59	68

The figures in the table were estimated from the data in the report by Yang et al. [29]

α-chymotrypsin from bovine pancreas. The V_{max} and K_m were 98 nmol/L/min and 7.8 μM, respectively.

Pyrethroids and pyrethroid-like fluorescent substrates exhibited a consistent pattern of stereoselective hydrolysis by a recombinant murine hepatic CES [32]. All hepatic CESs tested displayed a consistent pattern of stereoselective hydrolysis: the chiral center(s) in the acid moiety more strongly influenced stereoselective hydrolysis than the chiral center in the alcohol moiety. For cypermethrin analogs with a cyclopropane ring in the acid moiety, *trans*-isomers were generally hydrolyzed faster than the corresponding *cis*-isomers. For fenvalerate analogs without a cyclopropane ring in the acid moiety, 2R-isomers were better substrates than 2S-isomers. These general hydrolytic patterns were examined by modeling the pyrethroid-like analogs within the active site of the crystal structure of hCE1. Stereoselective steric clashes were found to occur between the acid moieties and either the catalytic Ser loop (residues 219–225) or the oxyanion hole (residues 140–144). These clashes appeared to explain the stereopreference between *trans*- and *cis*-isomers of cypermethrin analogs, and the 2R- and 2S-isomers of fenvalerate analogs by hCE1.

4.3 Species, Age, Sex Differences of Enzymes

The species differences in biotransformation pathways, rates of elimination, and intrinsic hepatic clearance of esfenvalerate and deltamethrin using rat and human liver microsomes were examined [33]. Esfenvalerate was eliminated primarily via NADPH-dependent oxidative metabolism in both rat and human liver microsomes. The CL_{int} of esfenvalerate was estimated to be threefold greater in rodents than in humans on a per kg body weight basis. Deltamethrin was also eliminated primarily via NADPH-dependent oxidative metabolism in rat liver microsomes; however, in human liver microsomes, deltamethrin was eliminated almost entirely via

NADPH-independent hydrolytic metabolism. The CL_{int} for deltamethrin was estimated to be twice as rapid in humans as in rats on a per kg body weight basis. Metabolism by purified rat and human CESs was used to examine further the species differences in hydrolysis of deltamethrin and esfenvalerate. Results of CES metabolism revealed that hCE1 was markedly more active toward deltamethrin than the Class I rat CESs, hydrolase A and B, and the Class II human CES, hCE2; however, hydrolase A metabolized esfenvalerate twice as fast as hCE1, whereas hydrolase B and hCE1 hydrolyzed esfenvalerate at equal rates. These studies demonstrated a significant species difference in the in vitro pathways of biotransformation of deltamethrin in rat and human liver microsomes, which was due in part to differences in the intrinsic activities of rat and human CESs.

It was reported that the distribution and activities of esterases that catalyze pyrethroid metabolism using several human and rat tissues, including small intestine, liver, and serum, were examined [30]. The major esterase in human intestine was hCE2. *trans*-Permethrin was effectively hydrolyzed by pooled human intestinal microsomes (five individuals), while deltamethrin and bioresmethrin were not. This result correlated well with the substrate specificity of recombinant hCE2. In contrast, pooled rat intestinal microsomes (five animals) hydrolyzed *trans*-permethrin 4.5 times slower than the human intestinal microsomes. Furthermore, pooled samples of cytosol from human or rat liver were ca. half as hydrolytically active as the corresponding microsome fraction toward pyrethroids; however, the cytosolic fractions had significant amounts (ca. 40%) of the total hydrolytic activity. Moreover, a sixfold interindividual variation in hCE1 protein expression in human hepatic cytosols was observed.

Yang et al. [34] reported that the liver contains the highest CES activity and expresses two major CESs: hCE1 and hCE2. Individual liver samples (104) were divided into three groups for analysis of the expression patterns of both CESs: adults ($\geq$ 18 years of age), children (0 days–10 years), and fetuses (82–224 gestation days). In general the adult group expressed significantly higher hCE1 and hCE2 levels than the child group, which expressed significantly higher levels than the fetal group. The age-related expression was confirmed by RT-PCR and Western immunoblotting. To determine whether the expression patterns reflected the hydrolytic activity, liver microsomes were pooled from each group and tested for the hydrolysis of deltamethrin. Consistent with the expression patterns, adult microsomes were approximately four times as active as child microsomes and ten times as active as fetal microsomes in hydrolyzing these chemicals. Within the same age group, particularly in the fetal and child groups, a large inter-individual variability was detected in mRNA (430-fold), protein (100-fold), and hydrolytic activity (127-fold).

The age dependence of deltamethrin metabolism in vitro, and toxic signs and blood levels of the neurotoxic parent compound following administration of deltamethrin at 10 mg/kg p.o. was investigated [35]. Metabolism was quantified in vitro by monitoring the disappearance of the parent compound from plasma (via CESs) and liver microsomes (via CESs and CYPs) obtained from 10-, 21-, and 40-day-old male SD rats. Mean intrinsic clearances (V_{max}/K_m) in these respective

age groups by liver CYPs (4.99, 16.99, and 38.45) and by liver CESs (0.34, 1.77, and 2.53) and plasma CESs (0.39, 0.80, and 2.28) increased significantly with age, because of progressive increases in V_{max}. CL_{int} of deltamethrin by plasma CESs and liver CYPs reached adult levels by 40 days, but clearance by liver CESs did not. Hepatic CYPs played the predominant role in deltamethrin biotransformation in young and adult rats. The incidence and severity of neurotoxic effects varied inversely with age. Correspondingly, blood deltamethrin areas under the concentration vs time curve (AUCs) and C_{max} values progressively decreased with increasing age. Internal exposure to deltamethrin (blood AUCs) was closely correlated with toxic signs such as salivation and tremors.

No explicit sex-related difference in ester cleavage of pyrethroids was reported in pyrethroid metabolism in mammals.

5 Human Metabolism

When evaluating the safety of chemicals in humans, it is very important to know the fate of chemicals in the human body and the amounts of exposure in daily activity. This section reviews the metabolic reactions of pyrethroids in humans, and the biomonitoring of pyrethroid metabolites in human urine for the exposure assessment. Mathematical modeling is a useful tool to predict the fate of chemicals in humans. This section also deals with the recent advance of mathematical modeling of pyrethroids to predict the pharmacokinetics of pyrethroids.

5.1 Metabolic Profiles

Relatively few in vivo metabolism studies of pyrethroids have been published for humans, though the metabolism of many kinds of pyrethroids in animals has been investigated in detail, as described above (Fig. 5). After oral administration of cypermethrin (*cis:trans* = 1:1, w/w) to six male volunteers at 3.3 mg per person, its four major metabolites from the acid and alcohol moieties were analyzed in urine. Equal amounts of (*cis*-DCCA + *trans*-DCCA) and (3-PBacid + 4'-hydroxylated 3-PBacid) were excreted, with the peak of excretion occurring between 8 and 24 h after administration. Between 27% and 57% of the dosed cypermethrin was excreted as DCCA, and the ratio of *trans*- to *cis*-DCCA was about 2:1. These data suggested that ester hydrolysis was the major metabolic pathway of cypermethrin in humans, and that the cleavage of ester bond occurred more easily for the *trans*-isomer than the *cis*-isomer, as is the case with rats. On the other hand, dermal application of cypermethrin at 31 mg/800 cm^2 (*cis:trans* = 56:44) resulted in a different ratio of metabolites (the ratio of *trans*- to *cis*-DCCA is 1:1.2) as compared with the oral administration. The dermal study demonstrated that cypermethrin could be significantly metabolized before reaching the systemic circulation,

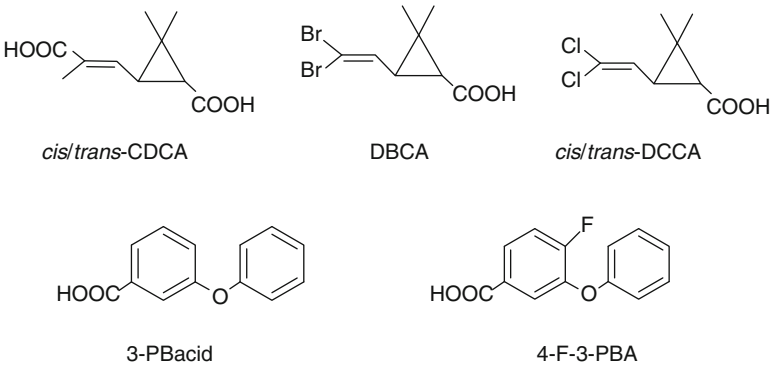

Fig. 5 Metabolites of some pyrethroids

resulting in a metabolite profile that was not formed to a significant extent following oral administration [36].

Four male volunteers were exposed to cyfluthrin through inhalation at 160 μg/m3 for 60 min. The study was performed in an exposure room, where an aerosol containing cyfluthrin was sprayed to obtain an atmosphere with the mean target concentration. Of the metabolites, 93% was excreted within 24 h after dose with half-lives of 6.9 h for *cis*-DCCA, 6.2 h for *trans*-DCCA, and 5.3 h for 4-fluoro-3-PBacid [37]. These results were in good agreement with that of oral administration of cyfluthrin [38]. Measurement of metabolites in human urine after exposure to (*S*)-bioallethrin indicated that *trans*-CDCA was suitable as a biomarker, which was in close agreement with the results of numerous animal studies. *Trans*-CDCA was rapidly excreted, with maximum peak within the first 24 h after exposure [39]. Takaku et al. reported comparative metabolic profiles of *trans*-permethrin between humans and rats, and identified the UDP-glucuronyltransferase (UGT) isoform responsible for the glucuronidation of 3-PBacid, which is known as a common metabolite of several pyrethroids in mammals [40]. Major metabolic reactions of *trans*-permethrin in humans were the same as those in rats for not only phase 1 (oxidation and hydrolysis) but also phase 2 biotransformation (glucuronidation). ^{14}C-*trans*-permethrin was incubated with hepatic microsomes or CYP isoforms of humans and rats. *trans*-Permethrin mainly underwent the rapid enzymatic cleavage of ester linkage to give 3-PBalc, followed by oxidation to 3-PBacid. 3-PBalc was also monohydroxylated at the 4-position of phenoxy moiety to 4'-OH-3-PBalc mainly by CYP 2E1 for humans. It was demonstrated that there was no difference in glucuronyltransferase activity of 3-PBacid between humans and rats, and that only UGT1A9 catalyzed the glucuronidation of 3-PBacid among human UGTs (1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, and 2B17). As there are UGT1A9 variants that have poor glucuronidation activity in humans, exposure to *trans*-permethrin or other pyrethroids that give 3-PBacid as metabolite might provide another excretion profile of 3-PBacid in individuals with a UGT1A9 deficiency or polymorphism.

These in vivo and in vitro human metabolism studies indicate that pyrethroids undergo rapid metabolism and elimination as observed in rats, and qualitative metabolic profiles (e.g., kinds of metabolites) of pyrethroids are assumed to be almost the same between humans and rats, suggesting that a large database of animal metabolism of pyrethroids could provide useful information for the evaluation of behavior of pyrethroids in humans. Nowadays, human pesticide dosing studies for regulatory propose are severely restricted in the US, and thus detailed comparison of in vitro metabolism (e.g., metabolic rate constants of pathways on a step-by-step basis) using human and animal tissues could be an appropriate method to confirm the similarity or differences in metabolism between humans and animals.

When elimination of a chemical from the body occurs via a single metabolic pathway, the difference in metabolic rate for the pathway can lead to large differences in the chemical and its metabolite concentrations in the body (blood and/or tissues) and urine or feces. Enzyme induction and inhibition by other chemicals or genetic polymorphisms could change the metabolic rates in some cases. In vitro studies using human enzyme isoforms, which is a rapidly evolving area for understanding in vivo metabolism, will allow a comprehensive characterization of metabolism of the pyrethroids in humans. The Sect. 4 shows that pyrethroids are metabolized by several CYP isoforms and CESs in humans, indicating that it is unlikely that there are poor metabolizers of pyrethroids in humans.

5.2 Bio-Monitoring

Bio-monitoring means the measurement of chemicals and their metabolites in human specimens such as blood or urine for investigating exposure to chemicals. Bio-monitoring is useful for evaluating exposure to chemicals, and also a significant tool for integrating environment and health for risk assessment. To obtain useful data in bio-monitoring, it is important to select appropriate target analytes for measurement to understand the exposure to chemicals. In humans exposed to pyrethroids, parent compounds are not excreted in urine due to their high lipophilicity and rapid metabolism in the body. Table 3 shows urinary metabolites of pyrethroids frequently used as biomarkers for bio-monitoring. Some metabolites are common for several pyrethroids. For example, CDCAs are derived from allethrin, bioallethrin, resmethrin, phenothrin, tetramethrin, and pyrethrins. 3-PBacid is derived from cyhalothrin, cypermethrin, deltamethrin, fenvalerate, and permethrin. Some pyrethroids give a specific metabolite unique to their structure.

To date, analytical techniques have advanced greatly and, accordingly, very low levels of urinary metabolites can be used as biomarkers to assess exposure to pyrethroids that can occur not only for occupational cases but also normal daily lives. Leng et al. reported a highly sensitive simultaneous determination of *trans*-CDCA, *cis*-/*trans*-DCCA, DBCA, 3-PBacid, and 4-F-3-PBacid in human urine

Table 3 Specific and nonspecific metabolites of pyrethroids

	cis/*trans*-CDCA	DBCA	*cis*/*trans*-DCCA	3-PBacid	4-F-3-PBacid
Allethrin/bioallethrin	*	–	–	–	–
Cyfluthrin	–	–	*	–	*
Cyhalothrin	–	–	–	*	–
Cypermethrin	–	–	*	*	–
Cyphenothrin	–	–	*	*	–
Deltamethrin	–	*	–	*	–
Fenvalerate	–	–	–	*	–
Fenpropathrin	–	–	–	*	–
Permethrin	–	–	*	*	–
Phenothrin	*	–	–	*	–
Pyrethrins	*	–	–	–	–
Resmethrin	*	–	–	–	–
Tetramethrin	*	–	–	–	–

*:present, –:absent

using a gas chromatographic–high resolution mass spectrometric (GC-HRMS) method. These metabolites are biomarkers for an exposure to pyrethrum, allethrin, cyfluthrin, cypermethrin, deltamethrin, permethrin, phenothrin, pyrethrins, resmethrin, and tetramethrin, as shown in Table 3. With this method, a complete assessment of exposure to several pyrethroids became possible for the first time. In this method, urine samples undergo acid hydrolysis and extraction with *tert*-butyl-methyl-ether. After removal of the solvent, the residue is derivatized with 1,1,1,3,3,3-hexafluoroisopropanol and analyzed by GC/HRMS in electron impact mode (detection limits < 0.1 μg/L urine) as well as in negative chemical ionization mode (detection limit < 0.05 μg/L urine) [41].

Instrumental analyses using GC/MS have made it possible to detect very low levels of urinary metabolites; however, they require several sample pretreatment processes, such as hydrolysis, extraction, derivatization, and clean-up procedures. Chuang et al. reported alternative lower-cost bioanalytical approaches using enzyme-linked immunosorbent assay (ELISA) for determination of 3-PBacid in human urine samples. The optimized coating antigen concentration was 0.5 ng/mL with a dilution of 1:4000 for the 3-PBacid antibody and 1:6000 for the enzyme conjugate. Urine samples were hydrolyzed with concentrated hydrochloric acid, extracted with dichloromethane, and solvent-exchanged into a methanol/buffer solution, prior to analysis in a 96-microwell plate immunoassay. Quantitative recoveries of $92 \pm 18\%$ for 3-PBacid were obtained for fortified urine samples. Analytical results from over 100 urine samples showed that the ELISA and GC/MS data were highly correlated, with a correlation coefficient of 0.95. At the 10 ng/mL comparative concentration level, the false positive rate was 0% and the false negative rate was 0.8% for ELISA when using GC/MS as the reference method. This ELISA method indicated a sufficiently low detection limit with 0.5 ng/mL for bio-monitoring of 3-PBacid [42].

Assessments of pyrethroid exposure levels by monitoring urinary metabolites of pyrethroids in large-sized general populations have been reported.

The National Report on Human Exposure to Environmental Chemicals (National Exposure Report), by the Centers for Disease Control and Prevention (CDC), is a series of ongoing assessments of exposure to environmental chemicals in the US population by measuring chemicals in blood and urine from a random sample of participants from the National Health and Nutrition Examination Survey (NHANES). NHANES is a program of studies designed to assess the health and nutritional status of adults and children in the United States, and includes 24-h diet data and information on household pesticide use, activities, occupation, demographics, and other exposure factors. In the fourth National Exposure Report (2009), it was described that the finding of a measurable amounts of DBCA, *cis*/*trans*-DCCA, 3-PBacid and 4-F-3-PBacid in human urine does not mean that the level causes an adverse health effect [43]. 3-PBacid was detected in 75% of urine samples analyzed for pesticides in NHANES (1999–2002). Urinary levels of 3-PBacid indicated that household pesticide use was not significantly associated with urinary 3-PBacid in any age groups of children (179 persons: 6–10 years of age), teens (603 persons: 11–18 years), or adults (1087 persons: $\geq$19 years). Diet exposure was significant for all three groups. Among adults, tobacco use was positively associated with 3-PBacid ($p = 0.0326$), and positive associations were suggested with the number of CYP–inhibiting medications taken ($p = 0.0652$) and minutes spent gardening ($p = 0.0613$) in the previous month [44]. *cis*- and *trans*-DCCA were highly correlated with each other and with 3-PBacid, suggesting that urinary 3-PBacid was derived primarily from exposure to permethrin, cypermethrin, or their degradates [45].

In Japan, the relationships between urinary 3-PBacid concentrations and diets have been examined. Spot urine samples were taken from a total of 535 persons (184 men and 351 women) who attended a healthcare checkup program (Hokkaido, Japan, 2005) to measure the 3-PBacid levels. Investigation regarding the intake frequency of 12 food items was also conducted. Food items were as follows: green-leafy vegetables, carrots, squash, tomatoes, cabbage and lettuce, Chinese cabbage, beans, potatoes, edible wild plants, citrus fruits, other fruits, and vegetable juice. Urinary 3-PBacid was detected in 98.3% of all samples. Both a significant association and a significant positive linear trend between the 3-PBacid concentrations and the frequency of tomato consumption were found in female subjects; however, the 3-PBacid levels, even among those subjects with the highest consumption of tomatoes, were far below the levels of toxicological significance. In contrast, no such association was found in the male subjects. The frequency of tomato consumption was confirmed to predict strongly the urinary pyrethroid metabolite levels in the general population–presumably because tomatoes are most often consumed raw and unpeeled (more so than all other vegetables and fruits analyzed in the current study) [46].

In Germany, the Human Biomonitoring Commission of the German Federal Environmental Agency continuously establishes the reference values of pyrethroid metabolites in urine, as there is a need for reference values to characterize the

exposure to pyrethroids in the population. Based on the reliable data that have been reported for about 2,100 children and adults in total, the reference values derived were as follows: *cis*-DCCA 1 ng/mL, *trans*-DCCA 2 ng/mL, and 3-PBacid 2 ng/mL. Reference values were defined as the 95th percentile, rounded to within the 95% confidence interval of the population studied, and showed no significant age-dependence [47].

The human body is continuously exposed to a variety of natural and synthetic substances in normal daily activities. It is therefore not surprising that a large number of substances, including metabolites of pyrethroids, can be detected in urine. Detection of some substances does not automatically mean health risk or hazard. Many studies and programs have been conducted to measure metabolites of pyrethroids as a biomarker to assess environmental exposure, and it was indicated that some metabolites were detected widely in human urine at very low levels. These data will help in the understanding of the exposure routes and amounts, and the toxicological significance. It has to be emphasized that bio-monitoring data should be accompanied with toxicological evaluation when it is used in exposure assessment for health.

5.3 Simulation and Risk Assessment

PBPK (Physiologically Based PharmacoKinetic) modeling is a mathematical technique used to simulate the behavior of chemicals in animal bodies. The models are composed of several compartments corresponding to actual anatomical structures of the animal body (Fig. 6), and each compartment contains mathematical descriptions of the absorption, distribution, metabolism, and elimination of a chemical. Essential data needed to develop a PBPK model include key physiological parameters (e.g., tissue volumes, blood flow rates) and chemical-specific parameters (metabolic rate constants, absorption rate constants, tissue partition constants) for the species. PBPK models are commonly used to predict concentrations of an internal dose at target sites following external exposure via different routes and durations, and predict human internal concentration at target sites based on the animal data. In toxicology and risk assessment, PBPK models have been used particularly to extrapolate between species (e.g., rats to humans) [48].

In chemical-specific parameters used for PBPK modeling, the metabolic rate constant is crucial to the accuracy of modeling results in many cases. For some pyrethroids, hydrolysis in intestine and serum has a significant role in the metabolism of the compound in mammals besides oxidation and ester cleavage in liver, which is the most important organ for detoxification of many chemicals.

trans-Permethrin is effectively hydrolyzed by human intestinal microsomes, while deltamethrin and bioresmethrin are not. In contrast, rat intestinal microsomes hydrolyze *trans*-permethrin 4.5 times slower than those of humans. Human serum was shown to lack pyrethroid hydrolytic activity, but rat serum has hydrolytic activity.

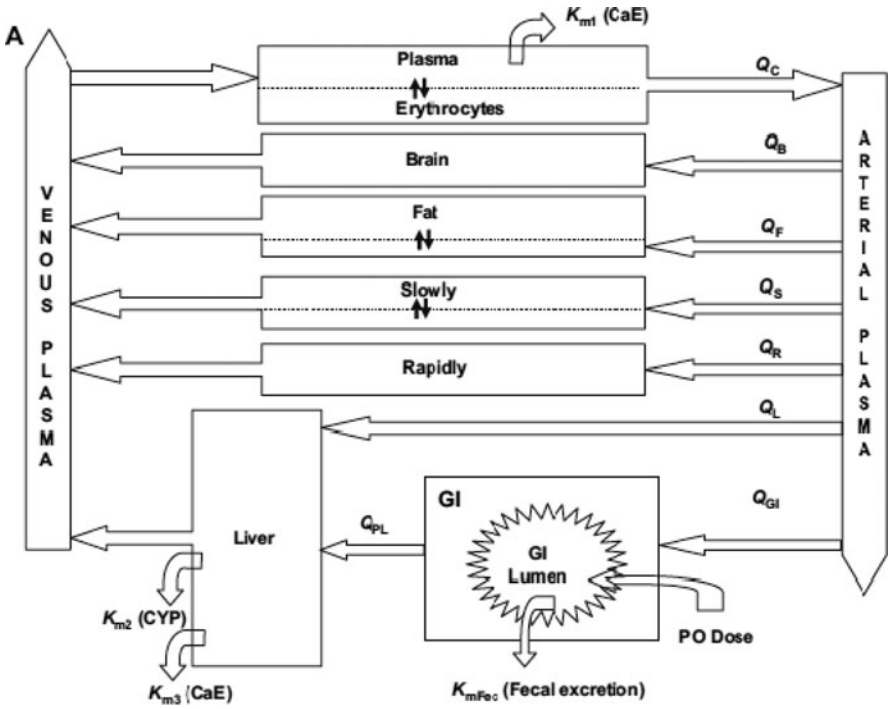

Fig. 6 Seven-compartment PBPK model for deltamethrin K_{m*} represents metabolic rate constant (K_{m1}: carboxylesterase in blood, K_{m2}: cytochrome P450 in liver, K_{m3}: carboxylesterase in liver, K_{mFec}: rate constant in feces) [49]

Both *trans*-permethrin and bioresmethrin were effectively cleaved by rat serum CES; on the other hand, deltamethrin, esfenvalerate, α-cypermethrin, and *cis*-permethrin were slowly hydrolyzed. These results suggest that PBPK models of some pyrethroids may require the parameter of esterase activity to calculate the concentrations in the intestinal tract, liver, and serum if it is shown that the compounds in the model are appreciably hydrolyzed within these tissues. Such data for human and animal tissues will help to improve the accuracy of extrapolation between species (e.g., rats to humans) and thus enable better predictions of tissue and blood concentrations in humans following exposure to pyrethroids [30].

The US EPA has developed PBPK models for pyrethroids, starting with a model for deltamethrin in adult rats reported by Mirfazaelian et al. [49]. Godin et al. improved the Mirfazaelian model to extrapolate it to humans [50]. The Mirfazaelian model was expanded to include age-dependent parameters for rats by Tornero-Velez et al. The Tornero-Velez model described age-dependent organ weights and oxidative and hydrolytic metabolic rate constants of deltamethrin using a generalized Michaelis–Menten model for growth. The deltamethrin concentrations calculated by the model were in agreement with experimental

time-course values in plasma, blood, brain, and fat for the four age groups evaluated (10, 21, 40, and 90 days old) [51].

The US EPA has been concerned with neurotoxicity and sensitivity of children to pyrethroids (Sect. 5.1.2), and demanded pyrethroid registrants in the US to conduct developmental neurotoxicity (DNT) studies. However, the EPA concluded that a DNT study was inadequate for the evaluation of age-dependent sensitivity for pyrethroids, and asked interested parties to submit study protocols voluntarily to understand better the age-dependent sensitivity for pyrethroids (February 2010). To deal with this issue, the pyrethroid registrants in US formed a task force group, and proposed a new study design including PBPK modeling to the US EPA.

In the near future, the pyrethroid PBPK model will possibly be applied in the regulation of pyrethroids in the US, reflecting significant advances in the predictability of pharmacokinetics over the past 10 years. The application of PBPK models will enhance the assessment of exposure to pyrethroids for regulatory purposes. However, there still remain significant challenges to utilize PBPK modeling as a tool for assessment of comparable toxicity. The relative toxic sensitivity to pyrethroids between species or ages depends not only on pharmacokinetics, which could be simulated with PBPK, but also on pharmacodynamic differences between species or ages. In order to assess the comparable sensitivity to pyrethroids, characterization of species and age-dependent differences in the pharmacodynamic responses to pyrethroids is also required, in addition to development of PBPK modeling.

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Ecotoxicology of Synthetic Pyrethroids

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Abstract In this chapter we review the ecotoxicology of the synthetic pyrethroids (SPs). SPs are potent, broad-spectrum insecticides. Their effects on a wide range of nontarget species have been broadly studied, and there is an extensive database available to evaluate their effects. SPs are highly toxic to fish and aquatic invertebrates in the laboratory, but effects in the field are mitigated by rapid dissipation and degradation. Due to their highly lipophilic nature, SPs partition extensively into sediments. Recent studies have shown that toxicity in sediment can be predicted on the basis of equilibrium partitioning, and whilst other factors can influence this, organic carbon content is a key determining variable. At present for SPs, there is no clear evidence for adverse population-relevant effects with an underlying endocrine mode of action. SPs have been studied intensively in aquatic field studies, and their effects under field conditions are mitigated from those measured in the laboratory by their rapid dissipation and degradation. Studies with a range of test systems have shown consistent aquatic field endpoints across a variety of geographies and trophic states. SPs are also highly toxic to bees and other nontarget arthropods in the laboratory. These effects are mitigated in the field through repellency and dissipation of residues, and recovery from any adverse effects tends to be rapid.

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Contents

1 Introduction

Synthetic pyrethroids (SPs) represented a breakthrough in pest insect control compared to earlier insecticide chemistries (e.g., organochlorines, carbamates, and organophosphates). SPs give broad-spectrum control coupled with relatively low use rates; for earlier analogs, use rates are typically around 100–200 g active ingredient (a.i.)/ha, and as the chemistry was refined, use rates fell below 10 g a.i./ha for some pests. Due to their cost-effectiveness, SPs gained rapid and widespread adoption in agriculture, and applications also extended to nonagricultural pests, including forestry, animal health, and vector control. Overall, SPs have provided substantial benefits to society through the effective control of arthropod pests. However, widespread use in agriculture also means that significant amounts of the compounds are released into the environment, and there may be potential exposure of nontarget organisms. Consequently, assessment of the potential for effects on nontarget organisms is needed. A substantial body of work is available on the effects of SPs on nontarget organisms, and in this chapter we provide an overview of the ecotoxicological profile of the SPs. We focus on bifenthrin, cyfluthrin, cypermethrin, *lambda*-cyhalothrin, Deltamethrin, esfenvalerate, and permethrin since these are among the major commercial compounds globally and are also well-studied.

Laskowski [1] has thoroughly reviewed the physico-chemical properties of the SPs, and these are summarized briefly below. SPs are typically of low water solubility (in the low microgram per liter range) and are highly nonpolar (logarithmic octanol:water partition coefficients of around 6–7), indicating potential for bioaccumulation. Fish bioconcentration factors (BCF) of several hundred to several thousand are reported; however metabolism limits the amount of bioaccumulation,

and depuration is therefore rapid. High lipophilicity also means that SPs readily adsorb to soils and sediments, and soil organic carbon adsorption constants (K_{oc}) are in the range of 100,000–700,000. SPs generally degrade readily in soils and sediment, with aerobic soil half lives in the range of 20–100 days, and similar aerobic aquatic half lives. SPs are generally hydrolytically stable at neutral or acidic pH, but the ester bridge is hydrolyzed at alkaline pHs, and for certain compounds this results in rapid degradation, with half-lives of a matter of days at pH 9. SPs are not photolytically labile (unlike natural pyrethrins).

The SP mode of action is via disruption of sodium channels in neurons [2]. Delay in the closure of the channel results in multiple action potentials firing in the nerve, which causes neurological disruption. Unlike organophosphate or carbamate insecticides, pyrethroids do not inhibit acetyl cholinesterase. SPs are sometimes subcategorized as type I or type II, depending on their toxicological profile and structure [3]. Type I compounds (permethrin, bifenthrin) induce tremors and have no cyano group in the alcohol moiety. Type II compounds (cypermethrin, cyfluthrin, *lambda*-cyhalothrin, Deltamethrin, esfenvalerate) induce seizures and contain a cyano group in the alcohol moiety.

Despite their extensive use around the world in many different applications, there are very few reported cases of human pyrethroid poisoning [4]. Mammals tend to be less sensitive than arthropods to SPs. The reasons for this difference are because insects have increased sodium channel sensitivity, smaller body size, and lower body temperature (resulting in lower rates of SP metabolism, i.e., detoxification). SPs are generally moderately to highly acutely toxic to mammalian wildlife, but risk assessments typically raise no concerns for these organisms from environmental exposure. Pyrethroids are generally of much lower toxicity to birds (Table 1) than mammals (probably due to higher metabolic rates in birds compared to mammals), and pose negligible risks to avian wildlife. SPs are of low toxicity to terrestrial oligochaetes like earthworms (Table 1) and have no adverse effects on plants (due to the lack of relevance of the mode of action). These nontarget organism groups are therefore not considered further in this chapter.

Not surprisingly, considering their highly efficacious and broad-spectrum insecticidal activity, SPs are highly toxic to honeybees and other nontarget arthropods. High levels of toxicity to aquatic arthropods have been reported [5]. Although of relatively low toxicity to birds, fish are also sensitive, probably because of a less

Table 1 Acute toxicity of SPs to birds and earthworms (http://ec.europa.eu/sanco_pesticides/public/index.cfm)

Compound	Acute oral LD_{50} bird (mg/kg body weight)	Acute toxicity earthworm LC_{50} (mg/kg soil)
Bifenthrin	1,800	>8
Cyfluthrin	>2,000	>1,000
Cypermethrin	>10,000	>100
Lambda-cyhalothrin	>3,950	>1,000
Deltamethrin	>2,250	>1,290
Esfenvalerate	1,312	10.6
Permethrin	9,800	1,440

pronounced ability to metabolize the compounds [6]. Based on their inherent toxicity in laboratory studies, pyrethroids are among the most aquatic ecotoxicologically active of all pesticides, with effect concentrations in standard acute and chronic studies with fish and arthropods ranging from the microgram to nanogram per liter level [7]. However, the effects of SPs under field conditions are mitigated by their environmental behavior, as we shall describe below.

The remainder of the chapter will review in more detail the ecotoxicology of synthetic pyrethroids to fish, aquatic invertebrates (including sediment organisms), honeybees, and other nontarget arthropods in both laboratory and field ecotoxicological studies. We also consider the potential interaction of pyrethroids with the endocrine system of nontarget organisms. The focus of this chapter is on the ecotoxicological hazard profile of the SPs. To assess the potential risks for the wide range of uses of SPs, it is also necessary to conduct an exposure assessment, considering the use pattern and environmental fate properties of the compounds. Ecological risk assessment of SPs is not covered in detail here, since each use needs to be considered on a case-by-case basis. Finally we identify some areas for further research on the ecotoxicology of SPs.

2 Toxicity to Aquatic Organisms

Substantial amounts of data on the effects of SPs on aquatic organisms are available and have been summarized in earlier reviews [5–7]. Many of these data were generated to register SPs (see for example the EU database http://ec.europa.eu/sanco_pesticides/public/index.cfm) and originate from laboratory studies following standard test guidelines with organisms from the three key groups: fish, aquatic invertebrates, and aquatic plants/algae. These studies assess survival (in acute tests), reproduction and growth or development (in chronic tests) at known concentrations of the test substance, and results are reported as median lethal concentrations (LC_{50}s), median effect concentrations (EC_{50}s), no observed effect concentrations (NOECs), and lowest observed effect concentrations (LOECs). The majority of the available data on SPs are from acute tests. Typically acute tests with standard aquatic invertebrate species have a 48 h exposure duration (e.g., with *Daphnia magna*) up to 96 h, and for fish the duration again is typically 96 h. For chronic tests where sublethal effects such as growth and reproduction are studied, durations are longer. For example, in the standard growth and reproduction studies, the exposure duration for *D. magna* is 21 days, and for fish, growth and development studies involve exposure for between 30 and 60 days, and fish full life cycle studies evaluating reproduction can take up to 300 days.

Increasingly over recent years, data covering a wider range of species and data from nonstandard test species and systems have been published in the scientific literature. Much of these data can be found on publically available databases such as the US EPA's ECOTOX database (http://www.epa.gov/ecotox/) and a comprehensive evaluation has recently been conducted by Giddings (Giddings

Table 2 Ranges of acute toxicity (μg/L) of SPs to fish, various groups of aquatic invertebrates, and algae (Giddings JM (2006) Compilation and evaluation of toxicity data for synthetic pyrethroids. Unpublished report of Compliance Services International, Rochester)

Pyrethroid	Range of fish LC_{50} values	Range of crustacean $E(L)C_{50}$ values	Range of insect $E(L)C_{50}$ values	Range of mollusk $E(L)C_{50}$ values[a]	Range of algae EC_{50} values[a]
Bifenthrin	0.1–17.8	0.00397–5.7	0.39–9.1	285	–
Cyfluthrin	0.0247–4.05	0.00246–0.344	3.4	3.42–>100,000	>991
Cypermethrin	0.4–6.3	0.0036–1.37	0.0069–9.8	>5 to >2,270	>1,300
Deltamethrin	0.048–5.13	0.0016–0.44	0.02–0.71	8.2–445	>9,100
Esfenvalerate	0.172–5	0.008–53	0.13–80	>12.5 to >10,000	>1,000
λ-Cyhalothrin	0.078–2.3	0.0023–3.3	0.0028–0.13	>590	>1,000
Permethrin	1.5–246	0.018–2.29	0.027–45	14.9–1,740	12.5–1,600

[a]These values are well above water solubilities

JM (2006) Compilation and evaluation of toxicity data for synthetic pyrethroids. Unpublished report of Compliance Services International, Rochester).

The ranges in acute ecotoxicity of SPs to fish, various groups of aquatic invertebrates, and algae are shown in Table 2. Algae are not sensitive to pyrethroids and reported ecotoxicity values tend to be at or above their water solubility, which is in the region of 0.01–2 μg/L [1]. However, from the available data for pyrethroids it is clear that these chemicals are as a group highly toxic to fish and to certain groups of aquatic invertebrates, notably arthropods. Mollusks are not sensitive.

A useful technique for analyzing the variability in species sensitivity is to construct a species sensitivity distribution (SSD). The use of SSDs in ecotoxicology has been described by Postuma et al. [8]. From the SSD it is possible to derive the exposure concentrations at which a given proportion of the species are affected. These concentrations are referred to as the hazardous concentration (HC_x) where x is the given proportion of species. In this way, species sensitivity distributions can be useful in assessing the ecological risk posed by the use of pesticides (see for example [9, 10]). As cypermethrin has one of the largest aquatic ecotoxicological datasets, the data for this pyrethroid are used here to examine the distribution in aquatic species sensitivity. The cypermethrin data (Giddings JM (2006) Compilation and evaluation of toxicity data for synthetic pyrethroids. Unpublished report of Compliance Services International, Rochester) covers 68 different species. The data are log10 transformed, ranked, and the ranks converted to proportions. The inverse cumulative distribution function of the normal distribution is then obtained by transforming the proportions to probit values. The ranked cypermethrin aquatic ecotoxicity data are presented as a cumulative distribution function in Fig. 1.

The distribution of aquatic species sensitivities to cypermethrin is typical of SPs [7] (Giddings JM (2006) Compilation and evaluation of toxicity data for synthetic pyrethroids. Unpublished report of Compliance Services International, Rochester). Crustacean and insect species (from the phylum Arthropoda) tend to be more sensitive to pyrethroids compared to other invertebrates such as worms and mollusks, and fish tend to be less sensitive than arthropods. These sensitivities are

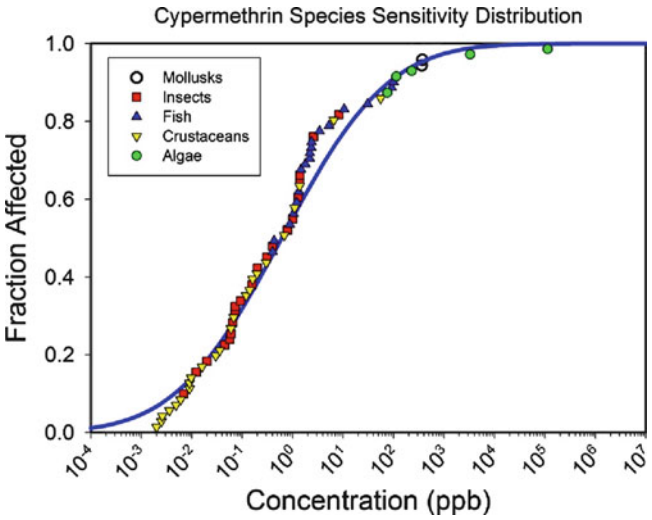

Fig. 1 Species sensitivity distribution for aquatic organisms exposed to cypermethrin

not surprising considering the taxonomic relationship to the target pest. Where tested, *Americamysis bahia* (marine mysid, crustacean), *Hyallela azteca* (freshwater amphipod, crustacean) and *Asellus aquaticus* (freshwater isopod, crustacean) and the insect *Chaoborus obscuripes* (freshwater dipteran, insect) tend to show the greatest inherent sensitivity to SPs. There is little difference in sensitivity of species from different microhabitats (water column, epibenthic or benthic) in water-only studies. For example from the cypermethrin dataset, the acute endpoints for the water column isopod *Asellus aquaticus*, the epibenthic dwelling amphipods, *H. azteca* and *Gammarus pulex*, and the benthic species like Chironomus are very similar with E(L)C_{50} values range between 0.004 and 0.009 μg/L.

It should be noted that the aquatic toxicity data referred to here have been generated in water-only laboratory test systems, and for the most part test exposure concentrations have either been maintained using water flow-through systems or exposure concentrations have been analyzed during the course of the test and the test endpoints adjusted to reflect the mean measured concentration throughout the test duration. For pyrethroids, these laboratory-based ecotoxicity values are likely to be over-estimates of effects in the field due to differences in exposure to the pyrethroids in natural water bodies. This is for two key reasons. First, pyrethroids are extremely lipophilic (see Sect. 1). As natural water contains suspended particulates and dissolved organic matter, this means that not all of the pyrethroid in the water column will be dissolved in the water phase and a significant proportion will be sorbed to the particulates or associated with the organic matter. Day [11] found the addition of dissolved organic carbon (DOC) in the form of humic acid to test solutions at up to 15.5 mg/L decreased the acute toxicity of fenvalerate to *D. magna* by up to a factor of 17. She also showed that the *Daphnia* exposed to pyrethroid in the presence of humic acid were only able to accumulate the chemical

that was truly free in aqueous solution. Yang et al. [12] also reported a decrease in toxicity of permethrin to *Ceriodaphnia dubia* with increasing DOC from natural sources. These researchers found that the measured permethrin LC_{50} values increased from 0.48 to 0.56 μg/L in DOC-free controls with increasing DOC and were statistically increased ($\alpha = 0.05$) in a pond water sample with >5 mg DOC/L and in a lake water sample and compost extract with 10 mg DOC/L. Natural water bodies would typically contain DOC at levels of 1–10 mg/L or more [11] and so these findings indicate that the toxicity in the environment would tend to be lower than that predicted from standard laboratory tests.

The second difference between the laboratory tests and exposure under realistic environmental conditions is that in the laboratory exposure concentrations are maintained, or the ecotoxicological endpoints are adjusted to account for any decline. Under natural conditions a combination of the pyrethroids' tendency to partition rapidly and extensively to organic matter, coupled with their susceptibility to degradation in aquatic systems where algae and macrophytes are present [13, 14], means their overall dissipation rate from the water phase is generally relatively rapid. Water column dissipation half-lives tend to be around 1 day (see Sect. 5). This behavior means that it is unlikely that aquatic organisms will be exposed to pyrethroids in the water phase for prolonged periods in natural water bodies.

Studies with *lambda*-cyhalothrin and the freshwater crustacean *G. pulex* have shown that shorter durations of exposure result in substantially less severe effects than maintained, long-term exposures [15]. In this case there was a significant reduction in toxicity with decreasing exposure times, with an 18-fold reduction in toxicity with exposure for 1 h compared to that determined after exposure for 96 h. Given this inverse correlation with exposure duration and toxicity, effects under natural environmental conditions where water column concentrations of pyrethroids are expected to decrease relatively rapidly are again likely to be less than would be predicted from estimates of toxicity under standard laboratory conditions with maintained concentrations (see Sect. 5).

For similar reasons, chronic toxicity tests are probably less relevant for use in environmental risk assessment because of the significant discrepancy between the duration of exposure in the laboratory (many days to weeks) and field (usually 1 day or less – see Sect. 5). Sensitivity profiles for chronic toxicity are similar to acute toxicity, although, as would be expected, chronic endpoints are lower than the acute endpoints. Chronic toxicity endpoints for SPs are summarized in Table 3.

3 Sediment Toxicity

The SPs have been shown to be highly toxic to fish and aquatic invertebrates, particularly arthropods, with toxicities as low as the nanogram per liter range in laboratory studies (see Sect. 3). However, due to their high lipophilicity and octanol: water partition coefficient (K_{oc}) values, they are rapidly adsorbed to suspended and bottom sediments [1], effectively limiting the exposure of water column organisms.

Table 3 Chronic toxicity of SPs in laboratory studies

Pyrethroid	Fish	*Daphnia*	Chironomid	Mysid
Bifenthrin	0.012	0.0011	320	0.0015
Cyfluthrin	0.025	0.020	11	0.00017
Cypermethrin	0.077	0.0075	–	0.00044
Deltamethrin	0.017	0.0041	0.010	–
Esfenvalerate	0.018	0.052	0.13	0.00037
Lambda-cyhalothrin	0.031	0.00198	0.16	0.00022
Permethrin	0.14	0.048	–	0.0078

Source: Giddings JM (2006) Compilation and Evaluation of Toxicity Data for Synthetic Pyrethroids. Unpublished report of Compliance Services International, Rochester and http://ec.europa.eu/sanco_pesticides/public/index.cfm. Values presented are lowest reported no observed effect concentrations in μg/L.

Early studies focused on how adsorption to sediments affected the toxicity of SPs to aquatic organisms, through modifying exposure. It was shown for example that when permethrin and cypermethrin were adsorbed to sediments, the apparent toxicities to the water flea (*D. magna)*, the mayfly nymph (*Cloeon dipterum*), and bluegill sunfish (*Lepomis macrochirus*) were significantly reduced [5].

Whilst adsorption of SPs to sediments in the environment greatly reduces the exposure to organisms in the water column, this in turn raises the question of exposure and potential toxicity to sediment-dwelling organisms. A number of routes of exposure in sediments need to be considered, including via overlying and interstitial (pore) water, contact with the sediment, and through sediment ingestion. SPs in sediments may be adsorbed to both the organic and inorganic fractions or associated with dissolved organic carbon. Although their water solubility values are very low, some fraction of SPs will be freely dissolved in the interstitial water, and the freely dissolved SP is thought to be responsible for most, perhaps all, sediment SP toxicity. Sediments differ with respect to inorganic and organic content; the relative mass of each fraction as well as the sorption coefficient can vary greatly among sediments, which will affect the potential toxicity of SPs (e.g., [12]).

Since persistence in sediments is longer than that in the water column, the relevant toxicity studies are those that consider longer term, chronic exposures. A number of standard tests have been developed for assessing sediment toxicity and the bioassay of field collected sediments (e.g., [16–24]). The most commonly tested freshwater species are arthropods, including the amphipod shrimp *H. azteca* and chironomid midge larvae, both *Chironomus dilutus* (formerly *C. tentans*) and *C. riparius*. Water-only studies have demonstrated that *H. azteca* are particularly sensitive to SPs (see Sect. 3) and in the published literature, this is the most commonly tested species for assessing the sediment toxicity of SPs.

A variety of laboratory studies conducted over the last decade have evaluated the bioavailability and toxicity of SPs in sediments. Maund et al. [25] studied the partitioning, bioavailability, and toxicity of cypermethrin to *H. azteca* and *C. dilutus* using three sediments with organic carbon contents of 1, 3, and 13%. Bioavailability was assessed by measuring the body burden in *C. dilutus* and results demonstrated that bioavailability decreased with increasing organic carbon content

Table 4 Observed sediment toxicity values for *H. azteca* and *C. dilutus* and pore water concentrations predicted by equilibrium partitioning [25]

Sediment OC content (%)	K_{oc}	*H. azteca* 10 days LC_{50}			*C. dilutus* 10 days LC_{50}		
		µg/kg dry weight	ng/L estimated water-phase concentration	µg/g sediment organic carbon	µg/kg dry weight	ng/L estimated water-phase concentration	µg/g sediment organic carbon
1	239,000	3.6	1.5	0.36	13	5.5	1.3
3	503,000	18	1.6	0.61	67	4.3	2.2
13	178,000	23	1.0	0.17	62	2.6	0.47

of the sediment. Measurements of pore water concentrations seemed to overestimate bioavailability, which was presumed to be due to the presence of dissolved organic carbon and colloidal material, which has been demonstrated to reduce the toxicity of SPs in water [11, 12]. However the estimated freely-dissolved concentration, using equilibrium partitioning (EqP) theory [26], gave a reliable indication of the bioavailable fraction. EqP predicts that, for nonionic, organic chemicals, bioavailability is determined by a chemical equilibrium amongst the water, sediment, and organism phases, with bioavailability, and thus toxicity, best predicted from the concentration of the freely-dissolved chemical. Sediment toxicity studies with *H. azteca* and *C. dilutus* confirmed this by determining that estimated water phase concentrations at the 10-day LC_{50} were similar across sediments with different organic contents (see Table 4). Furthermore, these water-phase concentrations were similar to the 48-h water-only LC_{50} of 5.3 ng/L generated previously in the same laboratory with *H. azteca*. The authors suggested that reasonable predictions of cypermethrin toxicity in sediment could be made by estimating the concentration of cypermethrin in the aqueous phase and comparing that to toxicity data from water-only studies. These findings have recently been corroborated in 10-day *H. azteca* toxicity tests with cypermethrin in four other natural sediments that ranged from 0.6 to 6% OC (Giddings JM (2009) Pyrethroid Working Group Sediment Toxicity Testing Program: Overview of Part 1 (Comparison of sediments with different organic contents). Study 09817, Unpublished report of Compliance Services International, Rochester). Expressing SP toxicity in sediments in terms of pore water concentration or normalized to sediment OC may provide more consistent endpoints than expressing toxicity in terms of bulk sediment concentrations, though there are also other influencing factors (see below).

The bioavailability and bioconcentration of *lambda*-cyhalothrin in *C. riparius* was investigated in five field soils and five aquatic sediments, with organic carbon contents ranging from 0.3 to 4.4% [27]. Based on sediment concentrations, the bioconcentration factor (concentration in the organism/concentration in the sediment – BCF) ranged from 0.11 to 0.84, with a coefficient of variation (CV) of 61%. BCFs calculated based on predicted water-phase concentrations, using measured K_{oc} values for the individual soils/sediments, showed less variability, ranging from 1,300 to 3,400 (mean 2,300) with a CV of 25%. Furthermore, these BCF values were similar to the BCF determined in water alone of 2,000. These results are a

further indication that the bioavailability and toxicity of SPs in sediment can be predicted by EqP to the pore water fraction. The calculation of pore water concentrations can be particularly useful for pyrethroids due to the challenges of measuring these compounds in the small volumes of pore water typically available from laboratory studies.

Comparison of the relative sediment toxicity of different SPs can be difficult as there are a variety of different test methods and endpoints evaluated, in addition to other confounding factors relating to sediment quality. Amweg et al. [28] determined the toxicity of six SPs to *H. azteca* in 10-day studies at 23 °C in natural sediments containing 1–6% OC. Toxicity data were reported as bulk sediment concentrations and normalized to the organic carbon content (Table 5). The results indicated that normalization removed some, but not all, of the variability between sediments. Other factors such as sediment texture may also affect bioavailability and hence apparent toxicity in sediment studies.

Studies with *H. azteca* have also demonstrated that, in common with previously reported effects on target insects [29] and fish [30], the toxicity of bifenthrin, esfenvalerate, *lambda*-cyhalothrin, and permethrin increased with decreasing temperature [31]. Ten-day LC_{50}s for these four compounds at 23 °C (the standard test temperature) were similar to those previously reported (Table 5). At lower test temperatures of 18 and 13 °C, all became more toxic, approximately doubling in toxicity at 18 °C and tripling in toxicity at 13 °C. When temperature was raised to 28 °C, the responses were inconsistent, with esfenvalerate and *lambda*-cyhalothrin less toxic and bifenthrin and permethrin showing similar toxicity. The increase in toxicity with decreasing temperature is believed to be a result of reduced metabolism at lower temperatures [31].

Table 5 Results of 10-day sediment toxicity tests with *Hyalella azteca* [28]

Chemical	Sediment OC (%)	LC_{50}		Growth NOEC µg/g OC
		µg/kg sediment dry wt.	µg/g OC	
Bifenthrin	1.4	8.6	0.63	0.23
	1.1	6.6	0.57	0.60
	6.5	23.5	0.37	0.23
Cyfluthrin	1.4	14.9	1.07	0.28
	1.1	12.5	1.09	0.46
Deltamethrin	1.4	9.8	0.71	0.12
	1.1	10.0	0.87	2.62
Esfenvalerate	1.4	24.3	1.76	0.30
	1.1	17.9	1.59	0.50
	6.5	83.1	1.28	0.30
Lambda-cyhalothrin	1.4	6.0	0.43	0.14
	1.1	5.2	0.46	0.08
Permethrin	1.4	249	17.9	7.0
	1.1	127	11.1	7.0
	6.5	226	3.51	<1.5

In summary, the bioavailability and observed toxicity of synthetic pyrethroids in sediment–water systems is influenced by a number of physicochemical factors, including the quantity and type of organic and inorganic matter in sediment and in water, as well as by temperature. The use of equilibrium partitioning calculations can be a useful tool for estimating the dissolved and potentially bioavailable fraction of pyrethroids.

4 Aquatic Field Studies

The high aquatic toxicity of SPs in the laboratory (see Sects. 2 and 3) led to concerns about the potential effects on aquatic ecosystems during agricultural use. This concern was balanced however by the knowledge that, due to their highly lipophilic nature, when pyrethroids enter the aquatic environment, exposures in the water phase would be rapidly reduced through processes of adsorption and degradation. Hence the predicted levels of effects based on standard laboratory toxicity data (where exposure concentrations are maintained throughout the course of the experiment) would probably be expected to overestimate the effects observed in the field due to the mitigating impact of reduced exposure.

In order to investigate this phenomenon, during the 1980s, manufacturers of pyrethroids and other researchers performed many aquatic field studies to investigate the impact of SPs under natural conditions. These included farm pond monitoring studies (where pyrethroids were treated in a pond catchment, and residues and impacts monitored) and replicated large-scale pond mesocosm studies to compare pyrethroid-treated ponds with untreated controls. By the late 1980s, there was a substantial database of studies which had investigated the potential effects of SPs under laboratory and field conditions. During the 1990s and into the new century, aquatic field experiments in outdoor microcosms were more commonplace than farm pond or mesocosm studies as these test systems offered more cost-effective methods and better options for experimental design [32].

Over the subsequent decades, the database has continued to grow, and reviews of the available field data have been produced periodically [33–36]. In general, the initial effects that are observed in field studies with pyrethroids are consistent with predictions based on the laboratory data – those organisms that are observed to be among the most sensitive in the laboratory also tend to be among the most sensitive in the field.

Similarly, the reductions in toxicity observed in laboratory toxicity tests where exposure is modified (either through the addition of sediment or by removal to clean water) are also apparent in the field. Field effect concentrations are generally observed to occur at concentrations around three to ten times above those based on standard laboratory data. Dissipation and degradation are therefore clearly the critical factors in mitigating effects of pyrethroids under field conditions. This provides reassurance that preliminary ecological risk assessments based on

laboratory data (with safety factors applied) will be protective. Similar findings have been observed with a range of insecticides and herbicides [37, 38].

The methodology for conducting aquatic model ecosystem studies was well established by the late 1990s. However, the use of the data in risk assessments raised a number of uncertainties regarding their interpretation and implementation [32]. Four of the uncertainties that were identified were the extent to which aquatic model ecosystem data generated in one location could be applied to another situation, the potential influence of mixtures of chemicals or stressors, whether the timing (season) of application would influence the outcome of the study, and whether differences in ecosystem properties (e.g., trophic status) might influence the results.

For SPs, it is noteworthy that for microcosm and mesocosm studies conducted with a range of different communities, in a range of geographic locations, under a variety of trophic conditions, in different seasons and by different research teams, the exposure concentrations at which certain response types and the extent of the responses seen are generally similar, and recovery of sensitive endpoints usually occurred within 2 months of the last application [35]. Similarly, a review of a series of eight indoor and outdoor microcosm and mesocosm experiments of differing trophic status and size with *lambda*-cyhalothrin reported consistent results [36]. These studies showed that the fate and effect profiles observed were consistent irrespective of the study design. Dissipation from the water column was rapid, with median dissipation times of less than a day. Effects thresholds for no to slight effects were consistent across studies with a threshold initial nominal treatment concentration of 10 ng/L. As might be expected (due to differences in the ecosystem tested), thresholds for effects with recovery were a little more variable, with thresholds between initial nominal treatment concentrations of 16 and 50 ng/L.

Although mesocosm studies provide a reliable method for predicting effects in the field, there are limitations to their ability to predict the longer-term consequences of those effects, particularly for organisms which do not have nonaquatic dispersal mechanisms. This is because the closed nature and absence of untreated areas in the test system means that ecological processes which may be important influences on recovery under natural conditions (e.g., avoidance, autochthonous or allochthonous immigration) are often not well-represented. One particular case in point is for the amphipod crustaceans, which have often been shown to be affected in SP mesocosm studies whilst showing little signs of recovery (due to their limited ability to recolonize isolated test systems).

Better estimates of recovery potential can be made either through empirical or modeling studies. Maund et al. [39] showed that reintroducing amphipods (and other organisms) to microcosms following exposure to cypermethrin resulted in significantly more rapid recovery than systems where there was no reintroduction. Similar results have been observed elsewhere in mesocosms with Deltamethrin [40, 41] and also in natural systems [42]. While such experimental studies are useful for demonstrating this point, they are limited by the logistical constraints of experimentation, in that usually only a limited number of recovery scenarios can be investigated. Consequently, ecological modeling approaches may in the future provide an alternative tool for investigating likely recovery rates under a range of

conditions. Even relatively simple modeling approaches have been demonstrated to provide much better estimates of recovery potential than predictions based on toxicity alone [43]. Developments of such models will allow a broader variety of scenarios to be explored in the future [44].

5 Interaction of Pyrethroids with Wildlife Endocrine Systems

The potential for chemicals to interact with wildlife endocrine systems, and in some cases disrupt those processes, has become a subject of great debate since the early 1990s (see, e.g., [45]). An endocrine disrupter (ED) is defined as: "... an exogenous substance that causes adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function. ..." [46], although there is still much debate as to how to quantify such an evaluation in practice [47]. The potential endocrine activity of SPs has undergone some investigation over the last decade, and a number of publications are considered below. However, a full evaluation of the potential endocrine properties of a compound requires a fully integrative assessment of all information from both mammalian toxicity, ecotoxicity, and mechanistic studies. This should combine the nature of any adverse effects detected in studies which give concern for endocrine toxicity with an understanding of the mode of action of toxicity so that adverse effects can be explained scientifically [47]. Such a detailed evaluation is beyond the scope of this chapter, but a brief review of the existing data is considered below.

Tyler et al. [48] investigated the in vitro ED potential of a selected number of pyrethroids, and certain metabolic and environmental degradation products in recombinant yeast assays expressing the human estrogen and human androgen receptors. For permethrin and fenpropathrin, weak estrogenic and antiandrogenic activity was observed. Cypermethrin, allethrin, and bioallethrin showed weak antiestrogenic and antiandrogenic activity. Breakdown products of permethrin, some common to other pyrethroids, also exhibited low levels of activity, with the 3-phenoxybenzyl alcohol showing estrogenic and antiandrogenic activities and the 3-phenoxybenzoic acid and the cyclopropane acid being antiestrogenic. Consequently the authors state that the antiandrogenic activities of the active substances may be the result of derivative contamination or occur after metabolism in the test system. Though these and other studies have demonstrated weak in vitro activity, it is unclear whether this translates into effects at the in vivo level potentially leading to adverse effects.

One of the challenges for ED is that the general level of understanding of invertebrate endocrinology is limited [49] and consequently there are few data in the literature that explore the link between potential endocrine action and adverse effects in invertebrates. However, from an ecological risk assessment perspective this is not an issue, considering the wealth of invertebrate data available that assesses population relevant endpoints (see Sect. 4). Selected pyrethroids have been used in basic invertebrate research, particularly in the investigation of vitellogensis (generation of female egg yolk protein that is under hormonal control).

From these studies, some patterns of effect potentially mediated by the endocrine system have been observed. These include the accumulation of vitellogenin in ticks [50–52] and accelerated ovarian development in beetles and ticks [51, 53]. Different researchers have attributed these effects to a range of different mechanisms, including induced excitation of neurosecretory cells releasing juvenile hormone [50], and ecdysteroid disruption either by blocking the neuropeptide itself or at the epidermal site of synthesis [51]. Other researchers have not corroborated these results and have even found contradictory effects, e.g., suppressed ovarian development [52]. Therefore at present for SPs, there is no clear evidence for adverse population-relevant effects with an underlying endocrine mode of action.

Considering fish, Moore and Lower [54] and Jaensson et al. [55] performed a series of experiments with male salmonids (*Salmo salar* and *Salmo trutta* respectively) pre-exposed to cypermethrin before measuring their subsequent response to pheromonal priming. Both report instances of a compromised capacity to respond to priming. Normal pheromonal priming in male salmonids resulted in increased concentrations of various steroid hormones in plasma and bile (e.g., T, 11-KT, and 17,20β-P) and stimulated the production of milt. Across both studies, effects were reported on concentrations of T, 11-KT, and 17,20β-P (in plasma and bile), volume of expressible milt, and number of spawning occasions after pre-exposure to cypermethrin. Whilst there are several inconsistencies between the two studies in terms of the sensitivity of salmonids to cypermethrin (e.g., Moore and Lower report significant effects at concentrations of cypermethrin <0.004 μg/L, which were not replicated by Jaensson et al.), these studies provide some evidence for reduced capacity to respond to reproductive pheromones. Whilst the exact mechanism for this disruption has yet to be elucidated, these data suggest that it is the neuroendocrine element of the HPG axis in salmonids which is sensitive to cypermethrin, rather than agonistic or antagonistic interactions with hormone receptors in reproductive organs. This is supported by an experiment reported in Moore and Lower [54] that found steroid metabolism in the testes of *S. salar* to be unaffected after exposure to cypermethrin. These effects are mechanistically interesting but their population relevance is yet to be established. Therefore, a full evaluation requires interpretation in light of apical studies looking at population level adverse effects, such as fish full lifecycle tests.

In summary, some indicators of endocrine activity are reported in the literature for some pyrethroids; however, the underlying mechanisms are not clear or consistent amongst in vitro and in vivo data. This suggests that there is noise in the data which can only be evaluated following a weight of evidence evaluation of all data including regulatory ecotoxicology and toxicology data packages for individual active substances. These packages contain a wealth of mammalian toxicology data useful for assessing potential endocrine activity in vertebrates that, with care, may be extrapolated across to wildlife species (mammals, birds, and fish). Further, many active substances with fish full lifecycle studies will be useful in assessing the population relevance of effects in fish.

Looking to the future, some pyrethroids will be tested in the battery of endocrine screening assays being developed for regulatory programs in the US and EU.

For example, bifenthrin, cyfluthrin, cypermethrin, esfenvalerate, and permethrin have been chosen (based entirely on exposure considerations and not any underlying ED concerns) for testing in the US Environmental Protection Agency endocrine disruptor screening program. Though this battery of 11 in vitro and in vivo mammalian, fish, and amphibian screens are not without technical and interpretative issues [56], it is likely that the data in combination with regulatory apical studies will help clarify the endocrine potential of specific pyrethroids. From the limited data with these assays currently available in the literature (Hershberger and uterotrophic assays for esfenvalerate, fenvalerate, and permethrin), (anti-) androgenic or estrogenic effects in vivo with SPs have not been demonstrated [57].

6 Bees

Honeybees (*Apis mellifera*) are recognized as extremely important economic insects, being the primary insect pollinator of many crops. Levin estimated the value of crops in the USA that benefit directly and indirectly from honeybee pollination approaches $20 billion annually [58]. The importance of wild bees and other insect pollinators in crops is largely unknown but likely to be considerable. In order to protect such an important resource, extensive laboratory and field study methodologies have been developed and are routinely undertaken to evaluate the toxicity of various pesticides to honeybees [59], and before a pesticide is registered the risk to bees should be fully understood [60]. Integrated pest management strategies or mitigation approaches have been employed to minimize the exposure and impact of pesticides on bees, including reduced numbers or rates of application, avoiding applications when plants are in bloom, safer formulations, and evening applications after bee foraging [61].

6.1 Laboratory Data

In the determination of intrinsic toxicity of a pesticide to honeybees, laboratory based dose–response studies are first carried out to provide an estimate of median lethal dose (LD_{50}) of the pesticide in question [62]. Data from such studies can then be used as the basis for further testing using methods of increasing complexity and applicability to practical situations [63, 64]. Usually a tiered (stepwise) testing program is followed, progressing from laboratory based studies, through cage and tunnel tests, to small-plot field studies and large-scale field trials designed to investigate short-term and long-term effects [65, 66].

Based on laboratory dose response data, pyrethroids are considered to be either highly toxic (an LD_{50} of 0.1–1.0 μg a.i./bee) or extremely toxic (an LD_{50} of <0.1 μg a.i./bee) to honeybees (Table 6), according to the classification proposed by the International Commission for Bee Botany [67, 68].

Table 6 Oral and contact acute toxicity of pyrethroids to honeybees

Compound	Acute oral LD_{50} (μg a.i./bee)	Acute contact LD_{50} (μg a.i./bee)
Bifenthrin	0.01	0.002
Cyfluthrin	0.05	0.001
Cypermethrin	0.03	0.02
Lambda-cyhalothrin	0.91	0.038
Deltamethrin	0.08	0.001
Esfenvalerate	0.21	0.06
Permethrin	0.03	0.1

Toxicity classification based on acute toxicity alone has long been an accepted practice for honeybees [69]. The hazard posed by a formulated pesticide however depends not only on its toxicity but also on amount applied or field rate, the proportion of dose that is available for transfer to bees, and the behavior of the bee itself [60]. For example, the pyrethroid insecticide cypermethrin has a topical LD_{50} of approximately 0.02 μg a.i./bee and a recommended field rate of 25 g a.i./ha [70]. Triazophos has a very similar LD_{50} of 0.05 μg a.i./bee, but has a suggested field rate of 400 g a.i./ha. A higher number of potential LD_{50} doses per unit area of triazophos are applied compared to cypermethrin, thus presenting a substantially increased risk to bees in the field. Considering these arguments, the classification of pesticide toxicity to bees was optimized by introducing the "hazard ratio," now used in many sequential pesticide testing schemes. The ratio between application rate and toxicity gives an approximation of how close the likely exposure of bees is to a toxicologically significant level. In calculating the hazard ratio (dose ha^{-1}/LD_{50}), dose per hectare is the highest recommended application rate in grams a.i./hectare, and the LD_{50} is measured in micrograms a.i./bee from the lowest of the acute oral or contact laboratory study. If a pesticide has a hazard ratio of <50 it is not considered to be hazardous; if the ratio is over 2,500, then the pesticide is classified as dangerous. These upper and lower thresholds are determined on the basis of bee toxicity, application rate, and an independent classification of risk verified by extensive data and experience of poisoning incidents with pesticides. If the ratio is between 50 and 2,500 then further testing with cage and field trials should be undertaken to establish whether or not the pesticide poses a significant risk in practice, or if the risk can be mitigated [60].

Pyrethroids, although highly toxic to bees in the laboratory, generally have a lower hazard ratio compared to organophosphate and carbamate insecticides because they have lower application rates. Pyrethroid hazard ratios usually fall into the 50–2,500 category and therefore trigger further semifield cage studies and full field trials to enable a full understanding of potential risk to bees.

6.2 Cage and Semifield Studies

The use of field cages or semifield tunnel tests was originally devised by Gerig [71] and has constituted a useful and cost-effective part of the hazard evaluation

Fig. 2 Semifield tunnels built over a sunflower crop

program. The primary advantage of tunnel tests over full scale field trials is a greater degree of experimental flexibility and reduced cost. Exposure in a cage or tunnel (Fig. 2) is more intensive than the field. The active ingredient or formulated product is therefore regarded as presenting low risk if the effects on colony survival and development are similar to those in the control tunnel, provided the environmental conditions are suitable for detection of effects [72].

There have been many cage studies performed with pyrethroids and these have been reviewed by Inglesfield [63]. Results of these tunnel studies generally show that the compounds tested have no effect on mortality at the hive entrance, or in some cases a short transient minor increase in mortality was observed immediately after application at field dose rates. Foraging activity observations on treated plots demonstrated transient decreases in foraging for a number of days following application. These mortality and foraging effects have therefore triggered the requirement to conduct higher tier investigations with large-scale field trials designed to simulate commercial conditions of use.

6.3 Field Studies

Full scale field trials may be required to assess the risk to honeybees for a number of reasons, e.g., the Tier 1 risk assessment based on hazard quotients, brood effects, systemic activity (though this is not the case for SPs), or based on the results of tunnel trials [73]. Trials are normally conducted by placing colonies in or on the edge of oilseed rape, mustard, *Phacelia*, or another attractive flowering crop, and the treatment is applied at the highest application rate during full flowering or as intended on the label. Plots are usually large (approximately 1 ha) and well

Table 7 Field trial results investigating effects of pyrethroids on honeybees

Compound	Dose rate (g a.i./ha)	Crop	Mortality	Effects	
				Foraging activity	Colony development
Cypermethrin	25	Oilseed rape	NE	−ve	NE
Lambda-cyhalothrin	10	Oilseed rape	NE	−ve	NE
Deltamethrin	6.25	Mustard	NE	−ve	–
Esfenvalerate	30	Oilseed rape	NE	−ve	–
Permethrin	70	Oilseed rape	NE	–	–

NE no effect, −ve short-term suppression, – not evaluated

separated to avoid bees foraging on the wrong plot (2–3 km depending on local conditions). Mortality and foraging assessments are made before and at least 7 days after application. In hive assessments are also conducted to investigate potential brood and food storage effects.

Although SPs are not systemic, their high toxicity to bees in lab tests has triggered field studies and many field studies have been conducted [63]. The majority of studies demonstrate low or no effects on mortality and a transient suppression of foraging activity after SP application at typical field rates (Table 7). This widely reported suppression of foraging activity with pyrethroids is often referred to as "repellency," and is a key reason why the high mortality evident in the laboratory is not translated into field effects. The reduced foraging evident in field trials varies from a number of hours to several days after application; this limits the exposure of bees to the treated plant surface, therefore reducing risk.

6.4 Monitoring Data

The final tier of information available from which to evaluate the risk of pesticides to honeybees is monitoring data from various schemes available from various Europe countries. These schemes confirm the safety of pyrethroids to bees following in-field use and have reported very few significant bee toxicity incidences where products have been used according to the label [74]. Higher than expected bee mortalities have however been reported in cases where pyrethroids have been tank-mixed with ergosterol biosynthesis inhibitor (EBI) fungicides (e.g., triazoles), but this is a specific case, well documented and understood [75]. The mechanism behind this is inhibition of the bees' mixed function oxidase enzymes by EBI fungicides, thus reducing the metabolism and detoxification of the pyrethroid and increasing its toxic effects [76]. These specific mixture effects are mitigated by directing the user not to tank-mix pyrethroids and EBI fungicides in field uses where bees may be exposed.

There are fewer reports in the literature on the impact of pyrethroids on non-*Apis* species, although one review is available that focuses on species in North America [77]. Tasei gives examples of studies conducted on *Nomia melanderi* (alkali bee),

Megachile rotundata (lucerne leafcutting bee), and *Bombus terrestris* (bumble bee). In general results showed that intrinsic susceptibility of non-*Apis* bees measured by oral and topical LD_{50} varied between species and also from *A. mellifera*. A number of studies have demonstrated the toxicity of pyrethroids to *B. terrestris* in the laboratory [78]; however, management practices including removal of the colonies during application in greenhouses, for example, significantly reduce the exposure and consequent risk.

7 Nontarget Arthropods (Other than Bees)

As synthetic pyrethroids are broad spectrum insecticides, the early concerns, and resulting research, into effects on nontarget arthropods (NTAs), concentrated on investigating the potential impact of their use within established Integrated Pest Management (IPM) programs. In IPM systems such as in some vineyards and top fruits, effects of pesticides on natural enemies of pests are considered undesirable. This is because reducing the natural biological control offered by such natural enemies can result in pest resurgence problems. As a consequence there is an extensive data set within the literature outlining the effects of synthetic pyrethroids on natural enemies (e.g., predators and parasitoids) of pests in both laboratory and field studies. Typically such studies were conducted on selected natural enemy species using single application rates of formulated product, with species and application rates selected based on the crop of interest and associated field recommended application rates.

A good example of how these data are used in IPM schemes is within the IOBC (International Organization for Biological and Integrated Control of Noxious Animals and Plants) Working Group on Pesticides and Beneficial Organisms guidelines [79]. In these IOBC guidelines, pesticides are classified based on either laboratory or field effects data as either harmless (0–30% – lab; 0–50% – field), moderately harmful (30–79% – lab; 51–75% – field), or harmful (>80% – lab; >75% – field). These IOBC classifications of pesticides are published in the IOBC side effects to beneficial organisms database (http://www.iobc-wprs.org/ip_ipm/index). An analysis of the pyrethroid data within this published IOBC database not surprisingly shows that the synthetic pyrethroids – when classified based on laboratory toxicity data – are all classified as harmful to NTAs. This is entirely consistent with the fact that the pyrethroids are broad spectrum insecticides being tested under worst case laboratory conditions. However, when the same pyrethroids were classified according to available IOBC semifield or field data, then classifications of moderately harmful and harmless were often reported for some species. This indicates that the effects of the pyrethroids on NTAs at recommended application rates under field conditions is significantly less and can be more selective than reported under worst case laboratory conditions. For an additional comprehensive review of published IPM field data on effects of pyrethroids on

NTAs see Inglesfield [63], which includes a helpful overview by representative crop types (e.g., cereals, pome fruit, cotton, etc.).

In 1995, the new European Union Plant Protection Product Directive 91/414/EEC was implemented. Consequently, it became a regulatory requirement to demonstrate no unacceptable effects on NTAs before a plant protection product could be registered for use. For guidance on NTA testing requirements and risk assessment, 91/414/EEC referred to the Society of Environmental Toxicology and Chemistry ESCORT Guidance Document on Regulatory Testing Procedures for Pesticides with Nontarget Arthropods [80] and European and Mediterranean Plant Protection Organization and Council of Europe Arthropods Natural Enemies Risk Assessment Scheme [81]. The ESCORT Guidance Document concluded that, whilst such regulatory testing had different objectives to historical IPM testing, the valuable experience and data gained from pesticide IPM testing on beneficial arthropods, by the IOBC Working group, should be utilized within the regulatory framework.

To this end, the ESCORT Guidance Document concluded that the parasitic wasp *Aphidius rhopalosiphi* and the predatory mite *Typhlodromus pyri* should be selected as sensitive and representative nontarget arthropod indicator species to be tested for all pesticides. In addition, it was recommended that two additional crop relevant species from different functional groups should also be tested, all using the existing IOBC IPM based single dose study designs. The original ESCORT Guidance Document was then superseded in 2001 by the publication of ESCORT2 Guidance Document [82], which introduced a step change in NTA regulatory testing and risk assessment. This step change was the introduction of laboratory dose response testing for the selected indicator species [83], similar to the approach used for other nontarget organisms (e.g., fish, *Daphnia*, birds, etc.), along with new semifield and field study methods [84, 85] and a new field validated hazard quotient approach for risk assessment [86].

As a consequence of these EU regulatory requirements for NTA testing, a significant database of regulatory nontarget arthropod data has been generated during the last 10 years and much of this data is published on the EU Pesticides Database http://ec.europa.eu/sanco_pesticides/public/index.cfm. This regulatory database provides a good data source to review the more recent regulatory focused nontarget arthropod pyrethroid toxicity data, conducted to internationally agreed test guidelines and good laboratory practice. Toxicity data on selected pyrethroids are summarized in Table 8.

The laboratory data summarized in Table 8 indicate that, for single dose laboratory studies, high mortality is reported at field application rates. The only consistent exception to this is the lower sensitivity of carabid beetles reported for cyfluthrin, esfenvalerate, and *lambda*-cyhalothrin. The data are consistent with the published data of a similar nature from the IOBC database and reflect the worst case exposure of the laboratory test design. In the more recent laboratory dose response studies, LR_{50}s ranged from 0.017 g a.i./ha for cyfluthrin to *Coccinella septumpunctata* to 8.14 g a.i./ha for bifenthrin to *Aphidius rhopalosiphi*. As can be seen from the bifenthrin and cyfluthrin data, sensitivity can vary over two orders of magnitude between different

Table 8 Summary of laboratory nontarget arthropod toxicity endpoints

SP	Indicator test species	Study type	Endpoint	Toxicity
Bifenthrin	*Aphidius rhopalosiphi*	Limit test	Mortality	7.5 g a.i./ha – 100%
	Poecilus cupreus	Limit test	Mortality	60 g a.i./ha – 90%
	Chrysoperla carnea	Limit test	Mortality	60 g a.i./ha – 100%
	Episyrphus balteatus	Limit test	Reproduction	7.5 g a.i./ha – 61%
	Typhlodromus pyri	Limit test	Mortality	60 g a.i./ha – 100%
	Aphidius rhopalosiphi	LR_{50}	Mortality	8.14 g a.i./ha
	Typhlodromus pyri	LR_{50}	Mortality	0.11 g a.i./ha
	Chrysoperla carnea	LR_{50}	Mortality	5.13 g a.i./ha
	Coccinella septumpunctata	LR_{50}	Mortality	0.08 g a.i./ha
Cyfluthrin	*Coccinella septumpunctata*	Limit test	Mortality	62.5 g a.i./ha – 100%
	Pterostichus melanarius	Limit test	Mortality	56 g a.i./ha – 100%
	Poecilus Cupreus	Limit test	Mortality Sublethal	15 g a.i./ha −26.7% 15 g a.i./ha – 100%
	Phytoseiulus persimilis	Limit test	Mortality	2–250 g a.i./ha – 100%
	Encarsia Formosa	Limit test	Mortality	17.5 g a.i./ha – 100%
	Typhlodromus pyri	LR_{50}	Mortality	0.42 g a.i./ha
	Aphidius rhopalosiphi	LR_{50}	Mortality	1.63 g a.i./ha
	Aleochara bilineata	ER_{50}	Parasitization	6.31 g a.i./ha
	Coccinella septumpunctata	LR_{50}	Mortality	0.017 g a.i./ha
Deltamethrin	*Coccinella septumpunctata*	Limit test	Mortality	13.5 g a.i./ha – 100%
	Chrysoperla carnea	Limit test	Mortality	13.5 g a.i./ha – 98–100%
	Trichogramma cacoeciae	Limit test	Parasitism	13.5 g a.i./ha – 100%
Esfenvalerate	*Typhlodromus pyri*	Limit test	Mortality/ reproduction	15 g a.i./h – 10% 27 g a.i./ha – 48.8% 150 g a.i./ha – 90.7%
	Chrysoperla carnea	Limit test	Mortality	12.5 g a.i./ha – 10%
	Poecilus Cupreus	Limit test	Mortality/ sublethal	12.5 g a.i./ha – 3.3%
	Liniphiid Spiders	Limit test	Mortality	12.5 g a.i./ha – 100%
Lambda-cyhalothrin	*Pterostichus melanarius*	Limit test	Mortality	7.5 g a.i./ha – 23%
	Poecilus Cupreus	Limit test	Mortality	7.5 g a.i./ha – 0 to 10%
	Episyrphus balteatus	Limit test	Mortality	9 g a.i./ha – 27%
	Pardosa spp	Limit test	Mortality	7.5 g a.i./ha – 83 to 90%
	Typhlodromus pyri	LR_{50}	Mortality	0.2 g a.i./ha

test species for the same pyrethroid, whilst variability in toxicity between different pyrethroids to the same test species varies significantly less, e.g., LR_{50} data for *A. rhopalosiphi*, *T. pyri*, and *C. septumpunctata*. However, these differences in laboratory toxicity data between species and pyrethroids are not significant in terms of the ultimate regulatory risk assessment, since the calculated hazard quotients (application rate g a.i./ha divided by LR_{50} g a.i./ha) for at least one of the tested species for all pyrethroids would be greater than the ESCORT2 recommended trigger value of 2 [82], indicating the need to conduct higher-tier field studies.

The field nontarget arthropod data for pyrethroids summarized in Table 9 indicate that for all pyrethroids there was an initial reduction in abundance for some species shortly after application. There was a trend of more marked effects at the higher full field application rates with less marked affects at the lower drift rates tested (e.g., Deltamethrin, bifenthrin, and esfenvalerate). Also there was a trend of greater selectivity (i.e., fewer taxa affected) at lower drift rates (e.g., *lambda*-cyhalothrin and Deltamethrin). For nearly all the pyrethroid field studies, either full or partial recovery of affected taxa was reported by the end of the field study or growing season, and in some cases for certain taxa, recovery occurred within 1–3 weeks.

These field data are consistent with the IOBC "side effects to beneficial organisms" database (http://www.iobc-wprs.org/ip_ipm/index) in that they indicate that the reported impact of pyrethroids in nontarget arthropods field studies is significantly less that can be predicted from laboratory toxicity data. In the current EU regulatory guidelines, the acceptability criteria currently applied to in-crop nontarget arthropod field studies is recovery of affected taxa within duration of field study or crop season (ESCORT and ESCORT2 Guidance Documents [80, 82]). The field data indicate that most pyrethroids would meet these regulatory criteria indicating no unacceptable effects on NTAs.

A third ESCORT Workshop was held in 2010 (ESCORT3) which was organized to provide new guidance in the following areas: protection goals, off-crop effects, recovery, and interpretation of field studies [84, 85]. This workshop further recommended that for in-field risk assessment the most relevant protection goal should be to preserve maintenance of relevant functions, e.g., pollination, control of pest arthropods (including IPM), and food sources for wildlife. For the off-field environment, maintaining nontarget arthropod biodiversity was recommended as the appropriate protection goal. Following this workshop, the regulatory challenges facing the pyrethroids and other insecticides in EU will be how to assess off-crop effects, where exposure and species present may differ significantly from those in the existing in-field studies and risk assessments.

8 Summary and Research Needs

As the review described above demonstrates, the ecotoxicology of the pyrethroids has been extensively evaluated in a variety of laboratory and field studies with a wide range of nontarget taxa. As would be expected for a highly efficacious group

Table 9 Summary of nontarget arthropod field/semifield studies (app = application)

SP	Crop	Application rates	Reported effects	Recovery
Bifenthrin	Orchard	2 app at either 20, 30, or 50 g a.i./ha with 21 day spray interval	Effects reported on all groups of predators at all application rates but with more marked effect at 50 g a.i./ha	Full recovery not reported at 50 g a.i./ha by end of study. Signs of recovery were reported by end of study at 20–30 g a.i./ha
	Orchard	1 app of 30 g a.i./ha	Effects reported in all groups of predators	Recovery after 33–40 days
	Wheat	1 app of 5 g a.i./ha or 1 app of 5 g a.i./ha followed by 2nd app of 7.5 g a.i./ha	Conclusions only possible for Microhymenoptera and lacewings, with moderate population effects reported for lacewings	Effects on Lacewings were reversible but full recovery not reported during study
Cypermethrin	Winter Wheat	0.595 (drift rate) and 25 g a.i./ha (2 app at 14 day intervals)	Effects reported on a wide range of species studies at both 0.595 and 25 g a.i./ha	Recovery reported for all taxonomic groups 38–40 days after 2nd application of both 0.595 and 25 g a.i./ha
Deltamethrin	Cereal	0.0125, 0.125 and 12.5 g a.i./ha	No reported effects at 0.0125 g a.i./ha. Temporary effects on spiders and possibly collembola at 0.125 a.i./ha. Significant effects on spiders, beetles and collembolan which persisted for >3 weeks	3 weeks after 12.5 g a.i./ha
	Orchard	0.1, 0.6, 2 and 12.5 g a.i./ha	Short lasting effects on few taxa at 0.1 g a.i./ha. Reduced populations of larger number of taxa at 0.6 and 2 g a.i./ha. Reduced population of most taxa at 12.5 g a.i./ha	Recovery within season at 0.6 and 2 g a.i./ha. No recovery during season for some taxa at 12.5 g a.i./ha
Esfenvalerate	Summer Cereal	2 app at 7.5 g a.i./ha and 15 g a.i./ha	No significant effects on Carabids and Staphylinid beetles and short-lived effects on Lycosidae, Dipterans, and Aphids	Recovery after 3 weeks

(continued)

Table 9 (continued)

SP	Crop	Application rates	Reported effects	Recovery
	Orchard	3 app at 2 week intervals of 1.5 (drift rate at 5 m), 7.5, and 15 g a.i./ha	No effects reported at 1.5 g a.i./ha. 25–35% reduction in mite abundance and reported effects on aphid predators and parasitoids at 7.5 g a.i./ha 22–43% reduction of mite abundance and effects on aphid predators and parasitoids and other predators at 15 g a.i./ha	Recovery evident after 30 days for all species at both 7.5 and 15 g a.i./ha
Lambda-cyhalothrin	Autumn Cereals	5 g a.i./ha	Mean depression in abundance of affected species was 20–60% And lasted 4–5 weeks	Recovery after 4–5 weeks
	Summer Cereals	7 g a.i./ha	Mean depression in abundance of affected species was 75% And lasted 27 ± 6 days	Recovery after 27 ± 6 days
	Summer Cereals	2.4, 5 and 10 g a.i./ha	Depression in abundance of affected species lasted 1–7 weeks. No statistically significant effects remaining after 7 weeks. Increased selectivity reported at reduced rates	Recovery after 1–7 weeks

of insecticides, laboratory toxicity to aquatic and terrestrial nontarget arthropod species tends to be high, and fish are also sensitive. However, due to differences in exposure in the field (either through degradation, dissipation, or repellency), use of pyrethroids would not be anticipated to lead to long-lasting effects on nontarget ecosystems.

Given this extensive database, there are relatively few further research needs. Some questions remain concerning potential for interaction with the endocrine systems, and as new assays to evaluate these aspects of ecotoxicology are conducted, further information will become available. However, at present, there is no clear evidence for adverse population-relevant effects with an underlying endocrine mode of action. In addition, whilst the apparent differences in sensitivity between aquatic arthropod species are clear, the reasons for these differences are far from certain. It would be interesting in future research to develop our understanding of why different arthropod taxa may differ in their inherent sensitivity, perhaps based on their ecological traits as has been explored for the organophosphate insecticide chlorpyrifos [87]. Similarly, predicting the recovery of species following environmental exposure to pyrethroids using ecological models would allow for further refinement of ecotoxicological profile of these insecticides. Developments in this area in the future will provide some interesting insights [44].

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Environmental Behavior of Synthetic Pyrethroids

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Abstract New experimental approaches together with recent progress in spectroscopic technologies have given useful information to understand better the environmental fate of synthetic pyrethroids. The successive transformation of intermediate free radicals by using spin-trapping reagents and fluorophores enables their easier detection in aqueous photolysis. Chiral chromatographic analyses have shown stereo-selective metabolism of pyrethroids in soil. The knowledge on relevant enzymes in soil and plant being involved in hydrolysis, oxidation, and glucose conjugation of pyrethroids has been accumulated. Utilization of either iron–porphyrin with an oxidant or isolated leaf cells as model systems can give more information on metabolism of pyrethroids.

Keywords Chirality · Hydrolysis · Metabolism · Mobility · Occurrence · Photolysis

Contents

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1 Introduction

Pesticides as formulations applied to the field are concurrently distributed to plants, soil, water, biota, and air, depending on its physico-chemical properties, type of formulation, and application method. Pesticides reaching the ground would be then adsorbed into the soil, partially move downwards by leaching, and be subjected to various chemical and microbial degradations. A fraction is possibly transported to neighboring water bodies via runoff and drainage, as well as contamination via spray drift. In order to assess the impact of pesticides on the environment, including various kinds of terrestrial and aquatic species, we first have to know how the applied pesticide is distributed and transformed and how much residue of pesticides and degradates finally remains in each compartment such as crop, air, soil, and water bodies.

Synthetic pyrethroids are basically carboxylic esters whose acid and/or alcohol moieties have geometrical isomerism and/or optically active center(s) in most cases (see Appendix) and they are used as mixtures of stereoisomers except for the cases when the most biologically active enantiomers or diastereomers have been commercially developed. The basic physico-chemical properties and chemical reaction profiles such as hydrolysis are considered to be the same between enantiomers, while many processes controlling their environmental behavior after application are most likely to depend on stereoisomerism, similarly to biological efficacy, especially when enzymes participate in their transformation. Furthermore, these stereoisomers may be converted to each other via photochemical and chemical processes occurring in the environment. Since the environmental profiles of a chemical can be conveniently estimated just from its chemical structure by using structure–activity (property) relationships represented by the USEPA EPI-Suite program [1], it was conveniently applied to ten pyrethroids, as summarized in Table 1. One of the typical features is a higher hydrophobicity as represented by *n*-octanol/water partition coefficient (log K_{ow}), resulting in low water solubility (WS), high adsorptive ability (K_{oc}) to soil, and high bioconcentration factor (BCF) to biota. Based on the Level III Fugacity model, pyrethroids are considered to be mainly distributed in soil and sediments with much less distribution in a water phase. The above approach is very convenient for grasping a rough idea of the behavior of pyrethroids but is too simplified for use in considering complexity of their environmental fate and relevant compartments. In order to refine the estimated (or predicted) environmental concentration (EEC or PEC) of pesticide in a focused compartment, many kinds of elaborate mathematical models have been developed based on various parameters collected through laboratory studies, and some of them

Table 1 Physico-chemical properties of synthetic pyrethroids by the USEPA EPI-Suite program

No.	Pyrethroid	logK_{ow}[a]	WS[b]	Vp[c] $\times 10^{-6}$	K_{oc}[d] $\times 10^{4}$	DT_{50} (air)[e]		BCF[f]	Level III fugacity model[g]			
						$OH^{\bullet}$	O_3		Air	Water	Soil	Sediment
1	Allethrin	4.78	4.6	35.3	0.500	0.578	0.298	36.45	0.01	17.3	79.6	3.1
12	Imiprothrin	2.90	93.5	0.157	0.0242	1.18	0.640	2.096	<0.01	15.3	84.5	0.15
14	Metofluthrin	5.52*	0.104*	20.1	0.664	1.74(t),1.94(c)	1.38(t),2.12(c)	111.6	0.01	4.72	76.9	18.4
16	Phenothrin	7.54*	<0.0097	1.92	12.2	1.21	0.640	399.9	0.017	11.4	60.8	27.8
19	Tetramethrin	4.73	1.83	0.0125	0.280	1.01	0.546	33.79	0.02	18.5	80.7	0.806
15	Permethrin	6.50	0.006	0.826	3.24	5.61	1,182	497.3	0.16	5.99	51.3	42.6
2	Bifenthrin	8.15*	0.1	0.391	23.3	4.33	169	199.6	0.039	1.85	50.7	47.4
8	Fenpropathrin	6.00	0.33	3.13	4.23	7.18	–	232.6	0.281	10.2	73.0	16.6
5	Cypermethrin	6.05	0.004	0.130	4.57	5.99	1,182	254.9	0.051	3.16	69.4	27.4
9	Fenvalerate	6.20	0.024	0.0414	5.60	5.76	–	5901	0.077	3.19	44.8	51.9

[a]Experimental values cited in the program. *Estimated values
[b]Water solubility in ppm at 25 °C. *Estimated from log K_{ow}
[c]Vapor pressure in mmHg estimated by the modified Grain method
[d]Soil adsorption coefficient in L/kg estimated by log K_{ow}
[e]Estimated degradation half-life in air in h. "t" and "c" indicate the *trans* and *cis*-isomer, respectively
[f]Fish bioconcentration factor in L kg^{-1} estimated by log K_{ow} by considering metabolism
[g]Distribution (%) in the environment

such as EU FOCUS surface water and USEPA EXAMS-PRZM models are used for regulatory purposes [2, 3]. The reliability of estimated EEC or PEC depends greatly not only on physico-chemical properties and environmental fate parameters such as half-life and soil adsorption coefficient but also on the use pattern of pesticides and environmental factors such as climate and geology of sites to be assessed. For example, the model to describe the behavior of pyrethroids has been developed by using the fate data in mesocosms simulating farmland ponds [4]. Shamim et al. [5] have recently evaluated the risk of synthetic pyrethroids in the USA to nontarget species using EECs through characterization of their use, exposure, and effects.

In this review, the recent progress in knowledge of the environmental fate of synthetic pyrethroids is focused with some basic knowledge for better understanding of their behavior in the environment when more realistic and precise assessment is conducted. Through a survey of the literature, some issues to be further investigated are raised to enable a better understanding of the environmental behavior of pyrethroids more in detail.

2 Physico-Chemical Properties

2.1 Basic Nature

Most synthetic pyrethroids having a molecular weight around 400 are esters of a substituted chrysanthemic acid with a hydrophobic alcohol moiety such as a 3-phenoxybenzyl group. A U-shaped molecular geometry has been proposed in a lower-energy state conformation for some pyrethroids by using quantum chemical calculations [6, 7]. A less hydrophobic alcohol moiety such as a cyclopentenoyl group was first introduced by analogy to pyrethrins, while more hydrophobic fluorinated benzyl groups have recently been introduced (see Appendix). These structural features result in log K_{ow} values greater than 6 as reported [8]. This means their low WS values at a ppb level and high soil K_{oc} above approximately 10^4 [9] for most synthetic pyrethroids for agricultural usage causes not only their rapid dissipation from water bodies due to association and sorption to suspended and dissolved matters as well as bottom sediment but also reduction of their bioavailability to aquatic organisms [10]. Their extremely lower WS and higher log K_{ow} values are usually difficult to be determined precisely even by the generator column method and hence, the group combination methods appearing in the EPI-Suite program [1] have been conveniently used. The adsorption of pyrethroid onto a glass surface even in the presence of cosolvent should be kept in mind due to its high adsorptive nature [11–14].

The adsorption and desorption of pyrethroids to and from soil and sediment are usually described either by the linear or Freundlich isotherm by using the following equations [9]:

Linear isotherm: $Q_e = K_d \times C_e$,
Freundlich isotherm: $\ln Q_e = \ln K_F + (1/n) \ln C_e$,

Organic-carbon normalization: $K_{oc} = K_d/f_{oc}$,

where Q_e, C_e, K_d, and K_F are the amount of pyrethroid per unit weight of an adsorbent, concentration of pyrethroid in water, and linear and Freundlich adsorption coefficients, respectively. The exponent term $1/n$ can be a joint measure of both the relative magnitude and diversity of energies associated with a particular adsorption process. Since an organic matter content of soil and sediment is one of the important factors to determine the adsorption of pyrethroid via hydrophobic interactions [15], the adsorption coefficient can be normalized (K_{oc}) against a fraction of organic carbon (f_{oc}). The particle size of constituents in soil and sediment is also an important determinant as demonstrated by the adsorption of bifenthrin (**2**) to sediments [16]. Furthermore, Liu et al. [17] have proposed the hydrogen-bonding mechanism in soil adsorption of pyrethroid by the shift of infrared absorption due to an OH stretching. The presence of copper ion reduced the adsorption of λ-cyhalothrin (**4a**) and cypermethrin (**5**), possibly via competition for the same adsorption sites. The presence of suspended particles and dissolved organic matters (DOM) in a bulk aqueous phase and pore water is known to affect significantly the adsorption coefficient of hydrophobic chemicals such as pyrethroids [9, 18]. The hydrophobicity-dependent adsorption to suspended clay minerals was reported for several pyrethroids [13] and much higher concentration of cypermethrin (**5**) was observed in pore water than in the overlying water [19]. The structural features of DOM such as humic substances have been shown to modulate markedly the sorption of esfevalerate (**9a**) [20]. The recent progress in saturation transfer double difference NMR [21] may help greatly to understand how pyrethroid molecules interact with humic substances at a molecular level. In addition to hydrophobic and hydrogen-bonding interactions, Keiluweit and Kleber [22] have emphasized the importance of polar interactions between aromatic π-systems of a chemical and adsorbents, which may operate in the adsorption of pyrethroid to soil and sediment. By the way, Yang et al. [23] have reported the affinity of black carbon in sediment to various pyrethroids. Not only black carbon but also kerogen in soil and sediment is considered to provide spaces for small hydrophobic organic solutes to be trapped, which results in nonlinearity in an adsorption isotherm for bifenthrin (**2**) and hysteresis in desorption as evaluated by the ratio of the $1/n$ values in adsorption and desorption [24]. As with aging, the fraction of rapid desorption is known to be reduced with a concomitant increase of the fraction resistant to desorption by the kinetic study of pyrethroid adsorption to sediment using the Tenax® or polydimethylsiloxane (PDMS) fiber extraction methods [25, 26].

Most agricultural pyrethroids have a very low vapor pressure (Vp) – around 10^{-8} mmHg at an ambient temperature – which is usually measured by the gas saturation method [8] and, therefore, its distribution to an air compartment is considered less important, as listed in Table 1. Tsuzuki [27] has improved the modified Watson method to estimate the vapor pressure of pyrethroids with reasonable precision just from their chemical structures. The volatilization from water can be conveniently evaluated by the Henry's law constant defined as vapor pressure divided by water solubility [28] and the small values of synthetic pyrethroids

indicate a lower tendency of volatilization. However, azeotropic codistillation may be possible under certain conditions as demonstrated for other pesticides dissipating from a model plant surface [29]. The volatilization from soil surface has been conveniently estimated by the classical Dow method using Vp, WS, and Koc [28], but an improved method has recently been proposed by Voutsas et al. [30].

2.2 Stereoisomerism

Synthetic pyrethroids usually consist of several isomers due to the presence of chiral center(s) and geometrical isomerism, as shown in the Appendix. Enantiomers of synthetic pyrethroids should give the same physico-chemical properties, but they are reasonably assumed to exhibit different biological activity and toxicity. The importance of considering stereoisomerism through assessment of various pesticides was raised from a regulatory viewpoint [31] and the recent revision of Directive 91/414/EEC prescribes that the plant protection product containing a significant proportion of nonactive isomers shall be approved as a candidate of substitution [32]. In order to investigate differences of stereoisomers not only in their biological activity but also their environmental fate and ecological toxicity including wild terrestrial and aquatic species, many kinds of chromatographic separation and stereo-selective analytical methods have been developed [33, 34]. A specially developed column having a chiral stationary phase is used to analyze each isomer of pyrethroid in gas and high-performance liquid chromatographies (GC and HPLC) with an appropriate detection interface [35, 36]. The separation of each isomer from racemic mixtures of permethrin (**15**) enables one to determine their absolute configuration by the aid of spectroscopies and X-ray crystallography [37]. In the case of a chiral GC analysis, reduction of matrix and temperature effects is indispensable to avoid artificial epimerization at the α-cyanobenzyl carbon during analysis [38, 39]. The same epimerization in the alcohol moiety of pyrethroids having the α-cyanobenzyl moiety is known to proceed by base-catalyzed hydrolysis [40] and alcohols such as methanol and ethanol have been reported to enhance this epimerization [36, 41, 42]. As an alternative analytical methodology, immunoassay has been developed to detect pyrethroid in the environmental samples [43, 44] but only the partial selectivity of each isomer was reported for esfenvalerate (**9a**) [45].

3 Abiotic Processes

3.1 Mobility in Soil

The movement of synthetic pyrethroids in soil and sediment is basically controlled by diffusion, convection, and dispersion. When entering a water–sediment system

by spray drift, pyrethroid molecules dissolved in overlying water are considered to be rapidly partitioned to the surface layer of bottom sediment. The algal/bacterial biofilm materials covering the sediment surface play a dominant role in adsorbing permethrin (**15**) in river bed-sediments in lotic flume channels [46]. Colloidal matters in pore water are known to control the diffusion of a hydrophobic chemical, leading to its depth-dependent distribution in sediment [9]. Concerning the mobility of pyrethroids in soil with or without aging, a column leaching study has been conducted using soil-packed columns or intact undisturbed soil cores being treated with radio-labels to examine the possibility of groundwater contamination in accordance with the OECD guideline [47]. Pyrethroids themselves have been found to reside mostly in the treated portion of agricultural soil columns with a minimal downward movement, as reported for bifenthrin (**2**) [24] and cyfluthrin (**3**) [48]. The insignificant movement is in good agreement with their high adsorption coefficients and higher K_{oc} in soil with higher organic matter content results in lower mobility in a column leaching study, as demonstrated for permethrin (**15**) in Malaysian soils [49]. The metabolites formed via cleavage of an ester linkage during aging were detected in leachates but mostly less than several percent of the applied dose.

The mobility of pyrethroids has been more extensively examined for deltamethrin (**6**) under a constant saturated water flow by the miscible displacement technique in packed soil columns showing the symmetrical breakthrough curve (BTC) of ^3H_2O [50]. The extremely asymmetrical BTCs with tailing were observed for (**6**), some of which showed a maximum concentration at an earlier stage of elution than expected from a chromatographic flow. The facilitated transport via association of (**6**) with DOM in soils was most probable as demonstrated for hydrophobic chemicals and some pesticides [51, 52]. The modification of soil–pesticide interactions is also known to be caused by the presence of surfactants used in formulation [53] and, hence, more research on the effect of these factors on mobility of pyrethroid needs to be conducted. Based on accumulated evidence, the mobility of synthetic pyrethroids in soil is rationally considered to be very limited by their high soil adsorptivity and moderate metabolic degradation in soil. Although limited information on a field leaching study for pyrethroids is available, λ-cyhalothrin (**4a**) and α-cypermethrin (**5a**) were not detected in groundwater and very rarely detected in vulnerable zones at trace levels [54, 55].

3.2 Hydrolysis in Water

Most pyrethroids undergo acid- and base-catalyzed hydrolysis to form the corresponding acid and alcohol (Fig. 1a), typically with U-shaped pH–rate profiles [8, 40]. The hydrolysis of pyrethroids in water basically obeys first-order kinetics with a half-life simply calculated from hydrolysis rate constant (k_{obs}) as $0.693/k_{obs}$. Pyrethroids are generally stable under the acidic and neutral conditions at pH 4–7,

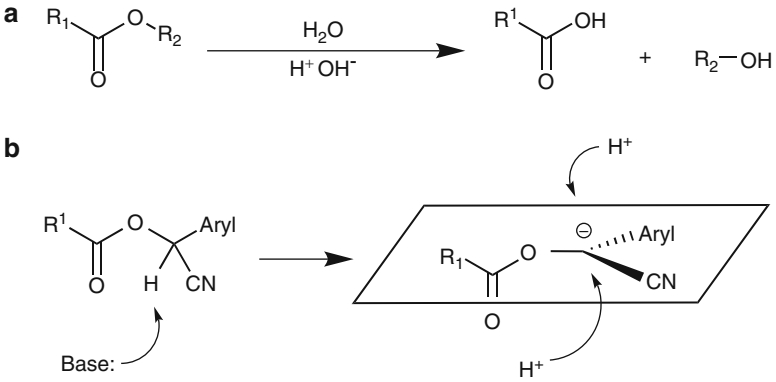


Fig. 1 Hydrolytic degradation pathway of synthetic pyrethroids

while they are hydrolyzed in alkaline conditions with the half-life greatly dependent on chemical structure via $B_{AC}2$ mechanism where the initial attack of OH^- at the acyl carbon to form a tetrahedral intermediate is followed by elimination of an alcohol moiety. The negative activation entropy of −28 to −23 cal mol^{-1} K^{-1} for alkaline hydrolysis of *cis* isomers of cypermethrin (**5**) and esfenvalerate (**9a**) indicates a bimolecular nucleophilic reaction [40]. The slightly faster alkaline hydrolysis of *trans* isomers was reported for cypermethrin (**5**) and permethrin (**15**) than their *cis* ones. Much faster alkaline hydrolysis of (**5**) and deltamethrin (**6**) than fenpropathrin (**8**) shows the effect of the electron-withdrawing dihalovinyl group in the acid moiety. Furthermore, the tetrafluoro substitution at the aromatic hydrogens of the alcohol moiety enhanced the alkaline hydrolysis of metofluthrin (**14**), tefluthrin (**18**), and transfluthrin (**20**) [40, 56]. Base-catalyzed isomerization at the α-cyanobenzyl carbon is one of the major pathways for pyrethroids such as (**6**). Ruzo et al. [57] have clearly shown the epimerization of (**6**) as a main dark reaction through the extensive photolysis study. Proton abstraction at the α-cyanobenzyl carbon by a base is considered to form a planar carbanion which can be attacked by proton from both sides of the plane, resulting in epimerization (Fig. 1b). Extremely higher susceptibility of tetramethrin (**19**) to hydrolysis at 25 °C was reported for its (1*R*)-*trans* isomer with a half-life of less than 1 day at pH 7–9, as compared with other pyrethroids. The LC-MS analysis of hydrolysates has shown that the imide ring of (**19**) is rapidly opened at pH > 7 to give an unstable intermediate whose ester linkage is first cleaved to form *N*-hydroxymethyltetrahydrophthalamic acid being successively degraded via an intramolecular acid-catalyzed cleavage of the amide linkage by the adjacent carboxyl group to form tetrahydrophthalamic acid (Fig. 2) [40].

These hydrolytic profiles have been obtained mostly in homogeneous buffered aqueous solutions, but the natural water body contains many kinds of dissolved and suspended matters such as humic substances, metal oxides, and clay particles. The distribution of nonionic pyrethroid molecules in these matters can be explained in terms of a partition model. The association with these matters reduces the fraction of

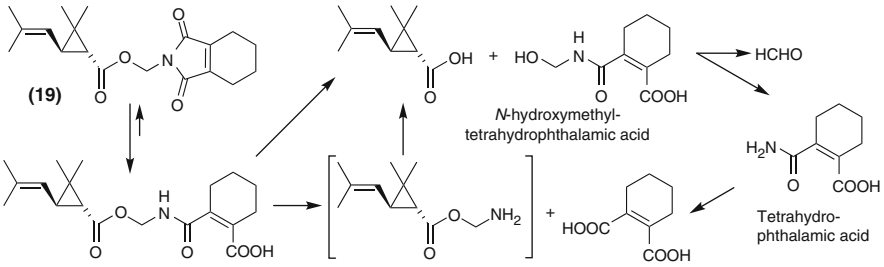


Fig. 2 Hydrolysis of (1*R*)-*trans* isomer of tetramethrin (**19**)

pyrethroid dissolved in an aqueous phase, causing less opportunity for hydrolysis. Furthermore, the microenvironment of these matters such as condensed surface charge and base character may inhibit or accelerate a hydrolytic reaction [40]. Bondarenko and Gan [58] have recently measured freely dissolved pyrethroids in sediment pore water containing DOM by the solid-phase microextraction (SPME) technique using a polydimethylsiloxane (PDMS) fiber and reported that most of the pyrethroid fraction is associated with DOM due to its high affinity with partition coefficients around 10^5–10^6 L kg^{-1}, similarly to clay minerals [13]. Since the local concentration of OH^- around these materials is considered to be reduced by an electrostatic repulsion against the negative surface charge of many humic substances and clay minerals [40, 59], alkaline hydrolysis of pyrethroids is likely to be retarded. By the way, the similar local condensation of pyrethroid molecules may be postulated in the presence of surfactant micelles in formulation. The micellar-catalyzed alkaline hydrolysis has been reported not only for substituted phenyl esters but also for carbamate, imide, and organophosphorus pesticides, while no information is available for pyrethroids [53].

3.3 *Photolysis in Air and Water and on Solid Surfaces*

Synthetic pyrethroids in the environment are exposed to natural sunlight and various reactive oxygen species (ROS), leading to formation of many unique degradates [60]. Sunlight irradiance near the ground exhibits a maximum at 440–460 nm with a cut-off below 290–295 nm mainly due to UV absorption by O_3 in the atmosphere. Since synthetic pyrethroids usually have a measurable UV absorption at wavelengths up to around 300 nm, photolysis is considered to play a role in their dissipation in the environment. Pyrethroid molecules absorbing light photo-physically undergo excitation to singlet state (S_1) which is either deactivated via nonradiative internal conversion or emission of fluorescence, or changes to an excited triplet state (T_1) via spin-forbidden intersystem crossing followed by slow radiationless deactivation or emission of phosphorescence. During these processes, two types of photochemical reactions known as “direct” and “indirect” photolysis

possibly occur. The former means the photo-reaction proceeding by absorbing light energy, while the latter is defined as reactions of a ground-state molecule with the other excited molecule or photochemically produced reactive species such as ROS. Direct photolysis of synthetic pyrethroids by absorbing sunlight energy mainly consists of homolytic cleavage of a certain bond to form intermediate radicals and photo-induced ester hydrolysis (Fig. 3a). The *cis–trans* isomerization in the cyclopropyl moiety is of importance in considering not only efficacy but also ecotoxicological impact of chrysanthemate-type pyrethroids since the fraction of a potent isomer decreases with isomerization. The isomerization is considered to be initiated via cleavage of the C_1–C_3 bond by light absorption, followed by internal

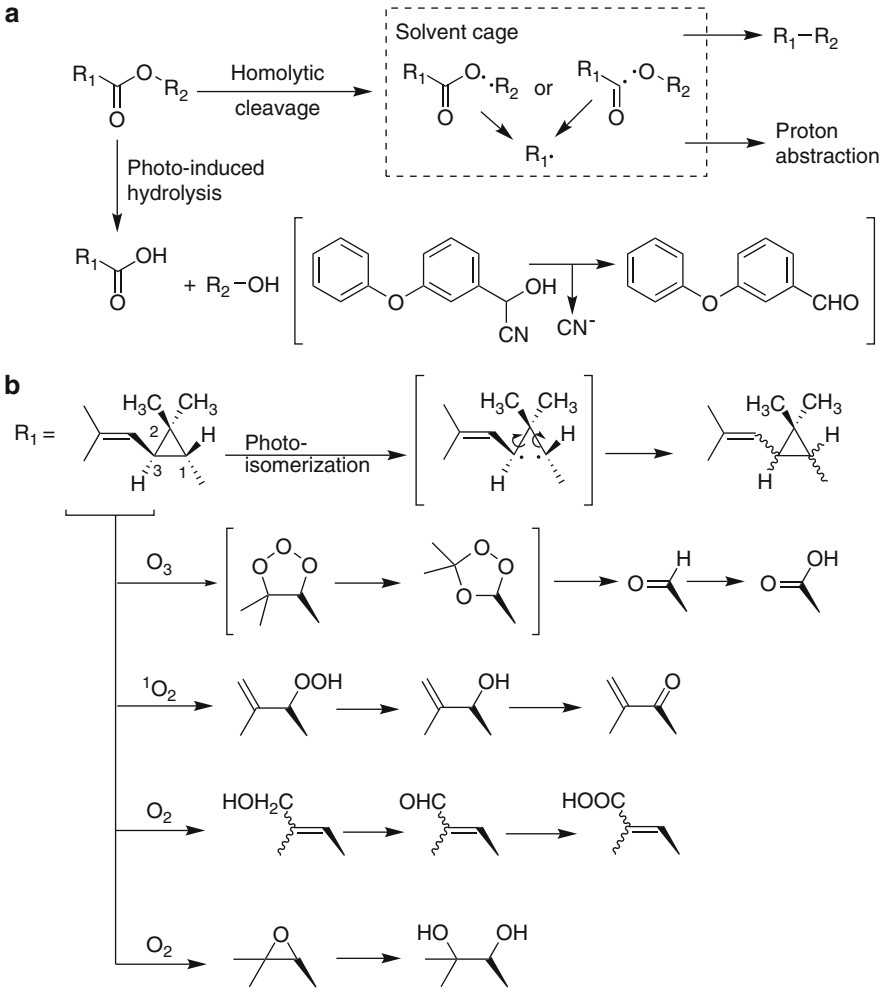


Fig. 3 Photolytic degradation pathway of synthetic pyrethroids

rotation along C_2–C_1 or C_2–C_3 bonds and recombination of biradicals (Fig. 3b). Photo-induced dehalogenation at the dihalogenated side chain of the cyclopropyl ring is also known for deltamethrin (**6**) and permethrin (**15**). These photochemical processes have been theoretically explained by quantum chemical calculations [60]. In the case of kadethrin (**13**), the complex isomeric mixtures were obtained by isomerization both in the cyclopropyl ring and its side chain [61]. In contrast, any isomerization at the benzyl carbon in the acid moiety of fenvalerate (**9**) has never been observed during photolysis. Most alcohol moieties in pyrethroids are considered to act as light absorbing chromophores and to be resistant to direct photolysis.

One of the most unique reactions is the photo-induced decarboxylation typically known for (**9**). The reaction is considered to be initiated by the homolytic cleavage of the ester linkage followed by an elimination of CO_2 and the resultant radicals are recombined in a solvent cage (Fig. 3a). These intermediate radicals were first confirmed by electron spin resonance spectroscopy using spin-trapping agents [62] and their transient absorption has recently been obtained through laser flash photolysis study [63]. Furthermore, Suzuki and Katagi [64] have trapped these radical intermediates originating from (**9**) with 3-amino-2,2,5,5-tetramethyl-1-pyrrolidinyloxy free radical followed by derivatization with fluorescamine (Fig. 4). The structures of the resulting fluorescent adducts (FL-3AP-R) were

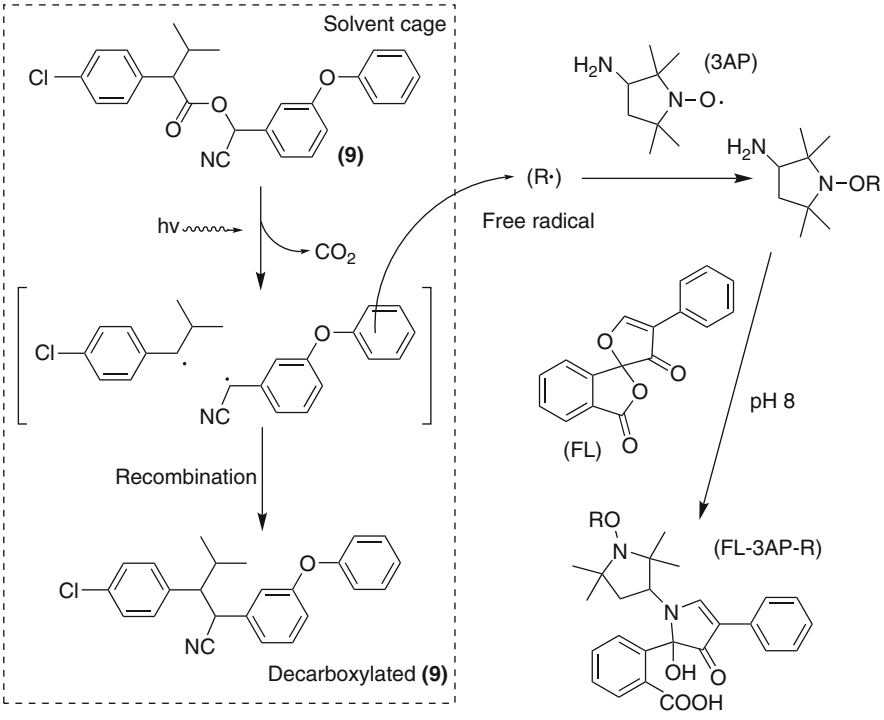


Fig. 4 Trapping of free radicals generated in photodegradation of fenvalerate (**9**). *3-AP* 3-amino-2,2,5,5-tetramethyl-1-pyrrolidinyloxy free radical, *FL* fluorescamine

further confirmed by LC-MS. It was also clarified that the lower solvent viscosity causing more escape of radicals from a solvent cage produced higher amounts of adducts. Photo-induced decarboxylation experiments using model esters together with quantum chemical calculations have shown the ease of bond cleavage at the ester linkage as the important controlling factor [65]. The other type of photo-induced cleavage-recombination reaction was reported for etofenprox (**7**) with elimination of CH_2O [66], but the photo-oxidation via proton abstraction at the benzyl carbon followed by reaction with molecular oxygen to form the ester derivative was found dominant through photodegradation in water and on soil surface [67].

Indirect photolysis of pyrethroids mainly proceeds via reaction with ROS such as hydroxyl radical ($OH^{\bullet}$), ozone (O_3), and singlet oxygen ($^1O_2$) (Fig. 3b). The concentration of $OH^{\bullet}$ is $1–10 \times 10^6$ molecules cm^{-3} in air [60] and $10^{-17}–10^{-15}$ mol L^{-1} in natural water, the latter of which is dependent on the concentration of NO_3^-, NO_2^-, and DOM [68]. The concentration of O_3 in air is 20–90 ppb varying by site and season [60] and recently its convenient method of measurement with a fluorogenic probe has been developed [69]. The relevant experimental information on photolysis of pyrethroids in air is very limited and, hence, a group contribution method first proposed by Atkinson [70] has been used as an alternative to estimate its degradation. Recently a similar approach has been proposed for the reaction with $OH^{\bullet}$ in an aqueous phase [71]. In aqueous photolysis of pyrethroids having the chrysanthemic acid moiety, ozonization of the isobutenyl side chain was found to proceed not only in water but also on plant foliage to give the corresponding aldehyde and acid derivatives as reported for phenothrin (**16**) [72]. Recently, the real-time monitoring FT-IR technique has revealed formation of the corresponding acid derivative of cypermethrin (**5**) via reaction with O_3 in air [73]. In addition to ozonization, aqueous photolysis of metofluthrin (**14**) gave the diol derivative possibly formed through epoxidation of the prop-1-enyl side chain in the acid moiety followed by its hydrolysis [56].

Photodegradation of pyrethroids on the surface of plant foliage has been less investigated than that in water but the photo-induced *cis–trans* isomerization and decarboxylation reactions were similarly observed for cypermethrin (**5**), deltamethrin (**6**), and fenvalerate (**9**), with lesser extents as compared with those in water [60, 74]. The short dissipation half-lives of pyrethroids on plant foliage seem to originate at least in part from their penetration into plant tissues through cuticles. In the case of soil, not only the photic depth of around 1 mm from the surface but also the high adsorptivity of pyrethroid to soil components causing less movement are considered to limit its photodegradation [60]. Graebing and Chib [75] have shown through photodegradation in dry and moist soils that the fraction of esfenvalerate (**9a**) existing at the top of soil is only photo-degradable and its extent increased in moist soil due to more movement to the photic zone. As a result, the half-lives under the light are considered to be mostly similar to or slightly shorter than those in darkness [8]. In addition to the products formed via ester cleavage, the stepwise hydration of the α-cyano group to $CONH_2$ and COOH is known as unique reactions for fenpropathrin (**8**), fenvalerate (**9**), flucythrinate (**10**),

and fluvalinate (**11**), and is accelerated by sunlight irradiation in some cases [60]. The *O*-dephenylation of (**9a**) on soil and clay surfaces was enhanced on exposure to UV light at >300 nm and about half of ^{18}O was found by MS analysis to be incorporated into this photoproduct from the clay prepared using $H_2{}^{18}O$ [76]. Both the same product formed with Fenton's reagent and photochemical reactions in clay [77] indicate the involvement of OH· photochemically produced on a clay surface.

4 Biotic Processes

4.1 Metabolism in Soil and Sediment

Many factors other than microbial metabolism are considered to control the dissipation of pyrethroids in the field and, hence, the half-lives generally fluctuate depending on site and season of application; 17–49 weeks [bifenthrin (**2**)] [78], <1–6 weeks [λ-cyhalothrin (**4a**)] [79], <2–16 weeks [α-cypermethrin (**5a**)] [80], 1–4 weeks [deltamethrin (**6**)] [81], and 9–18 weeks [esfenvalerate (**9a**)] [82]. The high soil adsorptivity of pyrethroids results in their rapid dissipation from the water phase of a water–sediment system with a half-life in the region of 1 h to 1 day, but the degradation rates in total, including a sediment layer, seem comparable to those in aerobic soil metabolism [9]. By the way, the metabolic studies are usually conducted in darkness and, therefore, the contribution of photolysis cannot be evaluated when the behavior of pyrethroids in a real shallow water body with less coverage of macrophytes is investigated. This is especially important in assessing the ecotoxicological impact of a photoproduct rapidly formed to a larger extent in water and/or on the surface of bottom sediment, which was raised as a possible concern by the Health and Consumer Directorate – General of European Commission [83]. In line with this consideration, Kodaka et al. [84] have examined the degradation profiles in illuminated water–sediment systems for esfenvalerate (**9a**), rapidly forming the decarboxylated derivative via photolysis in water. The rapid partition of (**9a**) from overlying water to bottom sediment greatly reduced the formation of this photoproduct (Fig. 5), which demonstrated practically no impact of this unique photoproduct on aquatic species. The isomer (**9b**) via epimerization at the α-cyanobenzyl carbon was detected in both water and sediment phases. The main metabolite in darkness was the 4′-OH derivative of (**9a**) via oxidation, while 3-phenoxybenzoic acid (PBacid) was dominantly produced instead under illumination. It was considered that cyanohydrin derivative formed via photolysis (Fig. 3a) was finally oxidized to PBacid via elimination of hydrogen cyanide.

In contrast to hydrolysis and photolysis, an enantioselective and/or regioselective degradation of pyrethroids is expected since various kinds of enzymes in microbes participate in their metabolism in soil and sediment. The diastereomers should be separated by using a chiral GC or HPLC to examine the fate of each isomer [85]. Sakata et al. [86] have conducted the first extensive aerobic soil metabolism study

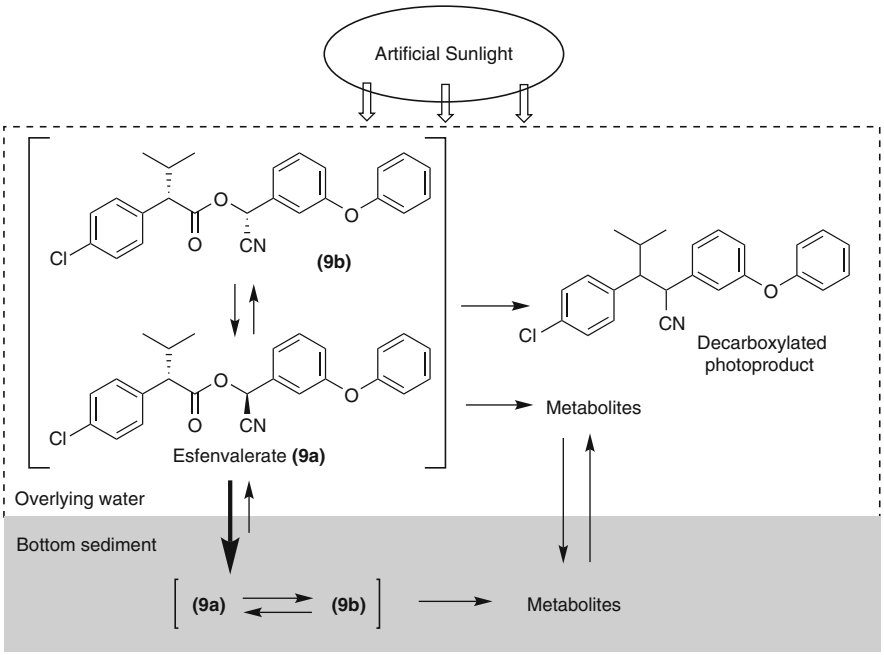


Fig. 5 Distribution and degradation of esfenvalerate (**9a**) in the water–sediment system under illumination

on each ^{14}C-labeled isomer of cypermethrin (**5**), deltamethrin (**6**), fenvalerate (**9**), and permethrin (**15**), as well as racemic mixtures of fenpropathrin (**8**), and clearly demonstrated the faster degradation of *trans* isomers than *cis* ones. The (*S*) diastereomers, due to the optical isomerism at the α-cyanobenzyl carbon, are more favorably metabolized than the (*R*) ones, especially in alkaline soil, but without any clear difference between isomerism in the acid moiety [86–88]. The epimerization proceeded in sterile soils but not in acidic ones, indicating chemical epimerization most probable as described before. This epimerization was also reported for *β*-cyfluthrin (**3a**) and *β*-cypemethrin (**5b**) in alkaline soils [89] but not in acidic and neutral soils and sediments. Many researchers have confirmed faster aerobic metabolism of *trans* isomers of chrysanthemate pyrethroids than *cis* ones in soil, sediment, and microbial isolates by using chiral GC and HPLC methods [89–93]. The same trend was also reported for the field dissipation study of (**5**), (**8**), (**9**), and (**15**) [94]. Concerning the optical isomerism at the 1-position of the cyclopropyl ring, most studies have shown more favorable metabolism of (*S*) isomers as compared with (*R*) ones but characteristics of soil and sediment with aerobicity seem to affect this relationship [95–97].

The typical metabolic reactions of pyrethroids are hydrolysis of an ester linkage and oxidation of an alkyl group or an aromatic ring in either acid or alcohol moiety, as shown in Fig. 6. The oxidative cleavage of the C=C bond of the prop-1-enyl

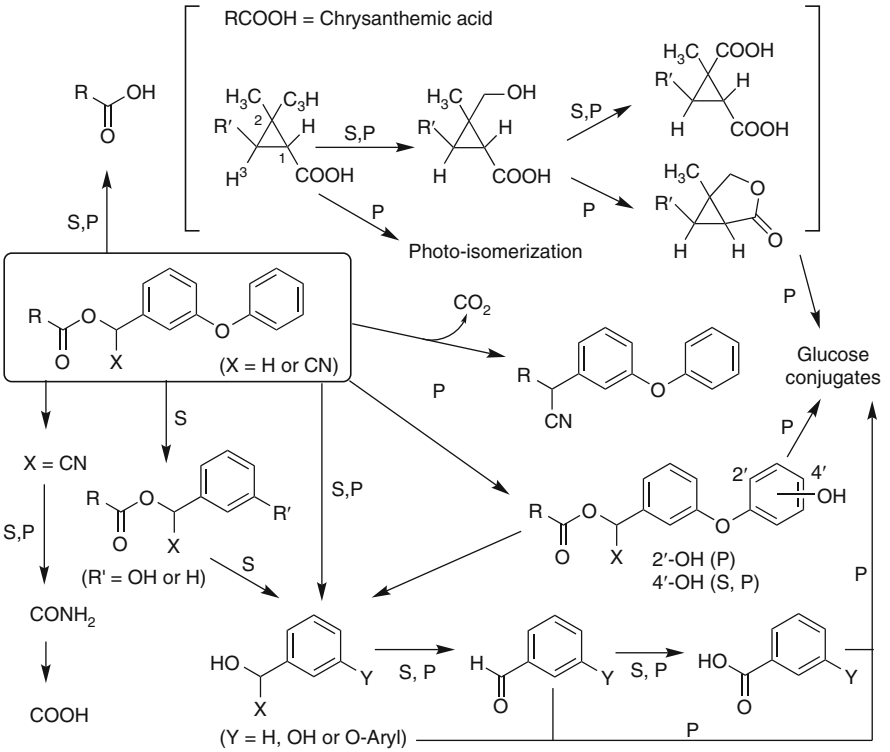


Fig. 6 Metabolic pathway of synthetic pyrethroids in soil (S) and plants (P). R, aryl or cyclopropyl derivative; R′, alkyl or isobutenyl group

group to form aldehyde or COOH derivative has been demonstrated for metofluthrin (**14**) [98]. For the alcohol moiety, the ring oxidation dominantly proceeds at the 4′-position of the 3-phenoxybenzyl moiety. The *para* position of the biphenyl moiety is similarly hydroxylated for bifenthrin (**2**) [78]. The type of linkage between acid and alcohol moieties seems not to affect the regioselectivity as demonstrated by formation of the similar hydroxylated product from etofenprox (**7**) in an aerobic water–sediment system but the benzyl oxidation more favorably proceeded in aerobic soil [67]. The arylmethyl oxidation and oxidative *O*-demethylation in the alcohol moiety after hydrolysis have been also reported for (**14**) [98]. Although the metabolic information on nonaromatic alcohol moiety is limited, Suzuki et al. [99] have shown that 1-propargylimidazolidine-2,4-dione is rapidly formed via hydrolysis of imiprothrin (**12**) in aerobic soil followed by ring opening and decarbamoylation. The cyano group at the α-benzyl carbon of 3-phenoxybenzyl moiety is successively hydrolyzed to $CONH_2$ and COOH for cypermethrin (5), fenpropathrin (**8**), and fenvalerate (**9**) in parallel with ester hydrolysis. The unique ether cleavage in the 3-phenoxybenzyl moiety has been reported for (**9**) and phenothrin (**16**) with the corresponding 3-hydroxybenzyl esters being obtained as

products. However, Saikia and Gopal [100] have recently confirmed the ether cleavage via different mechanism in biodegradation of *β*-cyfluthrin (**3a**) by fungi since the corresponding ester product lacked the hydroxyl group. The same product was also obtained in its biodegradation by bacteria [101].

Various kinds of microbes participate in the transformation of pyrethroids in soil and sediment [102–105]. Many bacterial strains isolated from sediments had the ability to degrade bifenthrin (**2**) and permethrin (**15**), but the strong adsorption of (**2**) to sediments retarded microbial activity of some strains. By the way, Xia and Wang [106] have recently reported the importance of coexisting sediment together with its particle size in biodegradation of polycyclic hydrocarbons, indicating a water–sediment interface as reaction sites. Therefore, bioavailability of pyrethroid molecules in the neighborhood of sediment surface is considered to be important. Not only bacteria but also fungi are known to degrade pyrethroids along the same pathways reported in metabolism studies. Sakata et al. [107] have isolated crude esterases having a different stereoselectivity on cypermethrin (**5**) and fenvalerate (**9**). Permethrin (**15**) was metabolized by three pure bacterial cultures with faster rates in the *trans* isomer [108]. The similar degradation was confirmed for (**5**) by Tallur et al. [109] who measured the activity of several participating enzymes and also showed the involvement of mono- and dioxygenases in its degradation. Allethrin (**1**) underwent hydrolysis followed by oxidation and reduction of the resultant acid and alcohol, respectively, in biodegradation by *Acidomonas* sp [110]. Two fungal species isolated from the egg masses of root knot nematodes hydrolyzed *β*-cyfluthrin (**3a**) to the corresponding acid and aldehyde, the latter being degraded from the cyanohydrin with a release of cyanide [111]. In addition to these metabolites, the unique metabolite 4-fluorobenzyl derivative of (**3a**) was identified through biodegradation of other fungal strains [100, 101].

Recently, the enzymology relevant to these metabolic transformations of pyrethroids has been investigated. Liang et al. [112] have isolated the hydrolase efficiently hydrolyzing *p*-nitrophenyl esters from *Aspergillus niger* ZD11. The enzyme is a monomeric structure having a molecular weight of 56 kDa with optimal pH and temperature of 6.5 and 45 °C, and is also able to hydrolyze some pyrethroids. Permethrin (**15**) was much more hydrolyzed than deltamethrin (**6**) and fenvalerate (**9**) but without a selectivity of *cis*/*trans* isomerism. The inhibitory effect by Hg^{2+} and Ag^{+} indicated the involvement of a sulfhydryl group at its active site. The hydrolase isolated from *Sphingobium* sp JZ-2 having a monomer structure of 31 kDa most efficiently hydrolyzed fenpropathrin (**8**) among the several pyrethroids with optimal conditions of pH 7.5 and 40 °C and its activity was inhibited by the above metal ions, malathion, and phenylmethylsulfonyl fluoride [113]. These enzymatic profiles are in accordance with those of carboxyesterases (CaEs) [10]. Esterases are classified depending either on substrate specificity or inhibition by organophosphorus (OP) compounds [114]. CaEs being classified in B-esterases as well as cholinesterases are usually distributed in all tissues and known to hydrolyze pyrethroids. At their active site, histidine (His) and aspartate (Asp) residues concertedly play a role in effectively increasing nucleophilic reactivity of serine (Ser) residue towards the acyl carbon of a substrate. CaEs are

typically inhibited by OPs as a result of the extremely slow dephosphorylation of tetrahedral intermediates formed between OPs and a serine residue at their active sites [115]. Wang et al. [116] have confirmed some similarity in gene sequence of *Sphingobium* sp JZ-1 to many α/β-hydrolase fold proteins but this enzyme did not show any stereoselectivity for (**8**) and (**9**).

There is no concrete information on the enzymology relating to microbial oxidation of pyrethroid itself but monooxygenase is most probable by analogy to mammalian metabolism [117]. The dioxygenase-mediated cleavage of a diaryl ether bond has been reported for biodegradation by *Pseudomonas* and *Sphingomonas* strains and the MS analysis of products in the presence of $^{18}O_2$ have shown that the reaction favorably proceeds at 1,2-positions of a phenyl ring [118–120]. The similar reaction proceeded at the benzoyl ring of 3-phenoxybenzoate derivatives to form protocatechuate and the corresponding phenol, which partly explained the metabolism of some pyrethroids [121–123]. The catechol derivative should be formed if dioxygenase with the same regioselectivity is involved in aerobic soil metabolism of fenvalerate (**9**), but the identified product was the desphenyl one. The observed metabolism may be accounted for by the change of regioselectivity to 1,2-positions of the more electron-rich phenoxy ring of (**9**) as demonstrated by molecular orbital calculations [124] than that of 3-phenoxybenzoate, together with some steric hindrance imposed by an amino acid backbone of the enzyme. Alternatively, monooxygenation may also play a role in the ether cleavage of pyrethroid, as reported for microbial degradation of phenoxybutyrate herbicides [125]. Monooxygenases such as cytochrome P450 may be involved in the dephenoxylation of β-cyfluthrin (**3a**). By the way, the mineralization of the tetrafluoro-substituted phenyl ring in metofluthrin (**14**) and tefluthrin (**18**) has been observed [98, 126], which indicated the successive oxidative defluorination and ring destruction catalyzed by hydroxylases [127, 128]. The mechanism of the enzymatic opening of a cyclopropyl ring is still obscure, but the contribution of coenzyme A may be assumed from the biodegradation of cyclopropanecarboxylate in bacteria and fungi [129, 130].

4.2 Plant Metabolism

The metabolism of synthetic pyrethroids in plants has been extensively studied and many reviews are available [74, 117, 131]. After application as a formulation to plants, pyrethroid molecules are considered to be dissolved in epicuticular waxes followed by penetration to interior tissues where various chemical and enzymatic reactions proceed. The existing metabolism studies using ^{14}C-labeled pyrethroids clearly show insignificant translocation from treated sites to other parts of plants due to their hydrophobic nature. The reactions in plants can be generally classified into three types: photolytic and chemical reactions on plant surface and so-called phase I and II reactions successively proceeding in tissues [60]. Not only the photo-induced *cis–trans* isomerization for cypermethrin (**5**) and deltamethrin (**6**) but also

epimerization at the α-cyanobenzyl carbon for λ-cyhalothrin (**4a**) have been observed in plant leaves, together with photo-induced decarboxylation for fenvalerate (**9**) and flucythrinate (**10**) [132]. The phase I metabolic reactions include hydrolysis and oxidation to form more hydrophilic metabolites having OH, COOH, NH_2, and SH functional groups, which are then subject to conjugation with carbohydrates and amino acids in the phase II reactions. Although enantioselective metabolism has been found for some herbicides [133, 134], such observations have not been reported for pyrethroids. The typical metabolic pathways are shown in Fig. 6. Most of the reactions are common to those observed in microbial metabolism in soil and sediment, except for oxidation at the 2′-position of 3-phenoxybenzyl moiety and *gem*-methyl groups of the cyclopropyl ring with an ester linkage being preserved and less hydrolytic transformation of the α-cyano group, as well demonstrated for fenpropathrin (**8**) [74].

4.2.1 Phase I Reactions

Most synthetic pyrethroids have an ester linkage susceptible to enzymatic hydrolysis and the corresponding acid and alcohol moieties are generally detected as main residues in plants. The similar metabolism of some pyrethroids was also reported for aquatic macrophytes [10]. Multiple esterase isoenzymes in a monomeric form having a molecular weight of 40–45 kDa have been identified in many crops and weeds, some of which are considered to be serine hydrolases judging from their inhibition by paraoxon [135, 136]. The pyrethroids such as permethrin (**15**) seem to be more slowly hydrolyzed by these isoenzymes than other ester-type herbicides. Recently, B-type CaEs in plants have been further classified into three categories by the α,β-hydrolase structural fold and relation to bacterial GDS lipases in conjunction with their roles in biosynthesis and transformation of simple carboxylic esters and herbicides [137, 138]. The molecular interactions of a potent substrate and inhibitor with the catalytic triad residues Ser, Asp, and His in the enzyme active site have been demonstrated for methyl salicylate and paraoxon by X-ray crystallography [139, 140].

By the way, oxidation of pyrethroids is considered to be catalyzed by mixed function oxidases including cytochrome P450, peroxidases, and peroxygenases by analogy to metabolism of other pesticides in plants, though direct evidence on their contribution has not been clearly shown [74, 141, 142]. These enzymes either oxidize an alkyl group stepwise finally to carboxyl or hydroxylate an aromatic ring carbon. The enzymology of P450 has been extensively investigated and CYP 71–CYP99 gene families are known [143]. Fukushima and Katagi [124] have recently utilized synthetic iron(III) porphyrins having electron-withdrawing substituents at their *meso* (2,3,4,5,6-pentafluorophenyl) and β (Br) ring positions as models of the active species in P450 and examined oxidative reactions of fenvalerate (**9**), as shown in Fig. 7. The dominant formation of 4′-OH, desphenyl, and carbamoyl derivatives, commonly detected in plant and soil metabolism, was

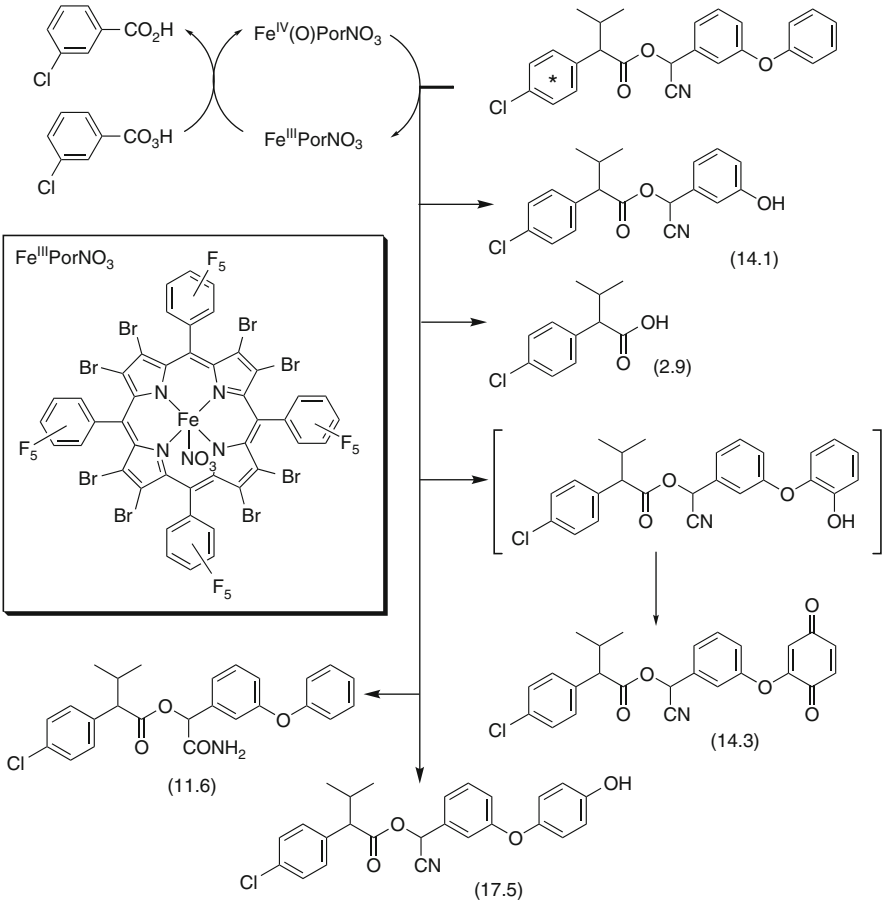


Fig. 7 Cytochrome P450-mimic oxidation of fenvalerate (**9**) by iron(III) porphyrin. The values in *parentheses* are percent of the applied ^{14}C (*Asterisk* labeled position) after 50 min

demonstrated. The quinone derivative was also detected possibly via further oxidation of 2′-OH derivative of (**9**). Since the product distribution was almost the same using the iron(III) porphyrin having hydrogens at β-positions and chloride as an axial ligand, the oxo-ferryl π-cation radical (quartet, $S = 3/2$) of this porphyrin and (**9a**) were subjected to AM1 semiempirical molecular orbital (MO) calculations with a full geometry optimization to explain the reaction mechanism [144]. The localization of unpaired electrons at iron, oxygen, and the porphyrin ring was well described by AM1 calculation. The energy levels of $d\pi$–$p\pi$ orbitals localized at the Fe^{IV}–O moiety (−9.09 and −9.15 eV) are very close to those of the highest occupied MO (HOMO) and its next one (HO + 1)MO of (**9a**), indicating that the oxygen of Fe^{IV}–O favorably attacked the carbon of (**9a**), having more electron density in these MOs (Fig. 8). The higher density at 2′- and 4′-positions as well as

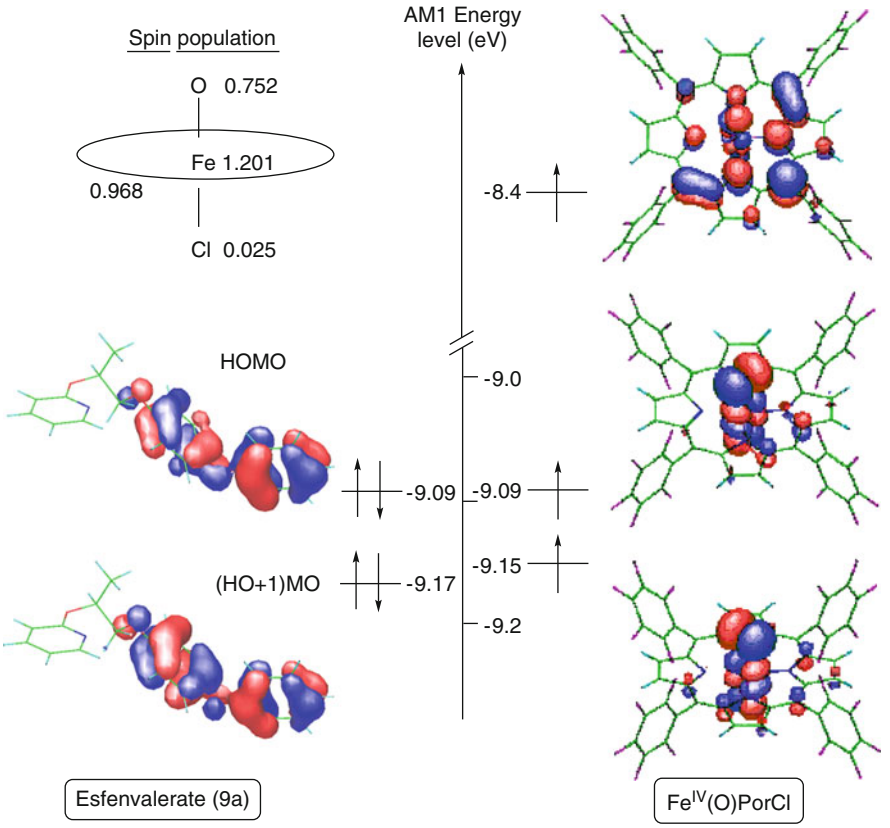


Fig. 8 AM1 semiempirical molecular orbital calculations of esfenvalerate (**9a**) and π-cation radical of oxoferryl(IV) porphyrin

the carbons adjacent to the ether oxygen well explained the dominant formation of the corresponding products.

4.2.2 Phase II Reactions

Transferase-catalyzed conjugation with glucose and higher saccharide derivatives predominates in plant metabolism of pyrethroids as well as its metabolites [131, 132]. However, amino acid conjugation as well as that of glutathione is of much less importance in metabolism of pyrethroids. The chemical structure of an intact conjugate of a pyrethroid itself is rarely reported but its existence can be confirmed by detecting an aglycon formed via enzymatic hydrolysis. Formation of higher saccharide conjugates in plant metabolism has been well documented for the corresponding acid and alcohol moieties of pyrethroids such as 3-phenoxybenzoic acid [131]. Malonylglucose conjugates are also detected for 2,2,3,3-

tetramethylcyclopropane carboxylic acid through metabolism of fenpropathrin (**8**) in several plants [145]. The relevant glucosyltransferases are soluble enzymes having a molecular weight of 45–60 kDa and catalyze the transfer of glucose from the corresponding uridine diphosphate derivative (UDPG) to oxygen, nitrogen, or sulfur atoms of a substrate [141, 146]. *O*-Glucosyltransferases are widely distributed in the plant kingdom, while higher enzyme activity of *N*-glucosyltransferases seem to be observed in marine algae [147]. The substrate specificity is not absolute but these enzymes exert regiospecificity for sugar. The highly conserved residues of glutamic acid and histidine are considered to be involved in a nucleophilic S_N2 attack of oxygen or nitrogen atoms in a substrate to an anomeric carbon of glucose at 1-position. The three-dimensional structure of the corresponding active site has been recently revealed by Offen et al. [148] by X-ray crystallography for red grape UDP-glucose:flavonoid 3-*O*-glycosyltransferase VvGT1. Although the investigation of *O*-glucosyltransferase activity on metabolites of pyrethroid has not been reported, the contribution of these enzymes is most likely by analogy to those on phenol and benzoate derivatives [149, 150]. Brazier et al. [151] have expressed sequences in the *Arabidopsis* genome as recombinant proteins in *Escherichia coli* and examined *O*-glucosyltransferase activity for hydroxylated benzoates. The 14 proteins showed the regioselective glucosylation of the hydroxyl group at *ortho*, *meta*, and *para* positions as well as a carboxyl group. Therefore, 2′- and 4′-OH derivatives of pyrethroids and their carboxylic acid derivatives originating from either acid or alcohol moieties are considered to be similarly glucosylated by these enzymes. Recently, possible phase II reactions of pyrethroid metabolites, acid moieties of fenvalerate (**9**) and permethrin (**15**) and 3-phenoxybenzoic acid (PBacid), have been examined in duckweed (*Lemna gibba*) by Fujisawa et al. [152]. The similar amounts were taken up by duckweed but only PBacid was susceptible to phase II reactions, giving the malonylglucoside and malate conjugates whose structures were confirmed by LC-MS/MS and NMR.

By the way, the investigation of metabolic pathway in intact plants is generally laborious due to isolation of an extremely small amount of each metabolite, especially for conjugates from complex components of plant tissues. The usage of plant cells and cultures can be considered as an alternative method to isolate potential metabolites more easily [153]. Fujisawa et al. [154] have prepared the leaf cell suspensions from seedlings of cabbage and tomato and incubated them with potential phase I metabolites of pyrethroids to investigate phase II reactions (Fig. 9). The cabbage leaf cells were found to transform efficiently chrysanthemic acid and 3-phenoxybenzyl alcohol to the corresponding malonylglucosides through the corresponding glucosides, while no reaction was observed for carrot cells. In the case of PBacid, much more metabolic activity was detected in cabbage leaf cells than carrot cells. The malate conjugate was uniquely produced in addition to the usual 4′-OH derivative and glucose conjugate, all of which were chemically identified by using LC-MS/MS.

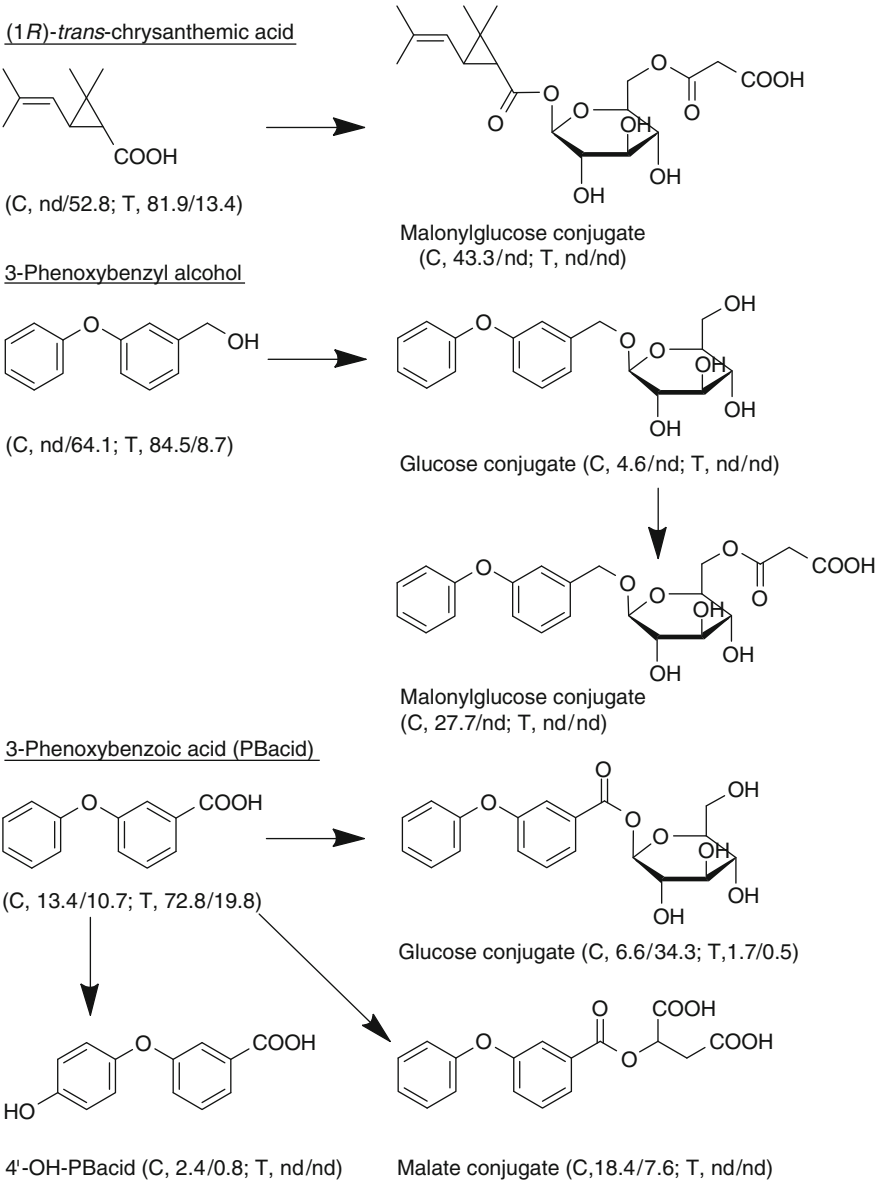


Fig. 9 In vitro metabolism of pyrethroid metabolites in leaf cell suspension of cabbage (C) and tomato (T). The values in *parentheses* are percent of the total radioactive residues in "leaf cell extract/medium" after 1-day incubation. *nd* not detected

4.3 Metabolism and Bioconcentration in Aquatic Organisms

High hydrophobicity of pyrethroids is likely to result in their bioconcentration in biota which is mostly controlled by lipid content. On the other hand, the high hydrophobicity would reduce bioavailability of pyrethroids due to association with DOM and/or adsorption to suspended and bottom sediments [10]. The bioconcentration factor (BCF) from water to organisms is conveniently defined by

$$\mathrm{BCF} = C_\mathrm{B}/C_\mathrm{W} = k_\mathrm{U}/k_\mathrm{E}.$$

Here C_B, C_W, k_U, and k_E are the concentrations of a chemical in biota and water, and rate constants of uptake and elimination, respectively. The BCF values of pyrethroids have been reported to be approximately 500–6,000 in fish [8] and a wider range of 25–10,000 is known for other aquatic organisms [10]. The effect of an optical isomerism on BCF has rarely been examined, but bifenthrin (**2**) in fish [155] and flucythrinate (**10**) in oysters [156] have been shown to be stereoselectively bioconcentrated. By the way, bottom sediments serve as important habitats for many aquatic organisms such as molluscs, larvae of chironomids and oligochaetes and hence the bioaccumulation of a chemical dissolved in an interstitial water phase that is bioavailable to organisms should be considered. Based on the equilibrium partitioning of a chemical between sediment carbon and interstitial water phases, DiToro et al. [157] have proposed the biota-sediment accumulation factor (BSAF) by using the concentration of a chemical in an organism (C_pb) and sediment (C_ps) as follows:

$$\mathrm{BSAF} = (C_\mathrm{pb}/f_\mathrm{b})/(C_\mathrm{ps}/f_\mathrm{oc}) \quad C_\mathrm{pb} = K_\mathrm{L} \times f_\mathrm{b} \times C_\mathrm{d} \quad C_\mathrm{ps} = f_\mathrm{oc} \times K_\mathrm{oc} \times C_\mathrm{d}.$$

Here K_L, f_b, and C_d are lipid–water partition coefficient, weight fraction of lipid in an organism, and concentration of a chemical dissolved in interstitial water, respectively. The BASF values determined for pesticides in aquatic organisms are quite variable. Cypermethrin (**5**) was found to exhibit very low BASF values for *Daphnia magna* (0.08–0.31) and *Chironomus tentans* (0.08–0.63), indicating a low possibility of bioaccumulation [19]. Since pyrethroids in interstitial water are most likely to be in equilibrium with DOM, more appropriate BSAF value would be estimated by measuring their truly dissolved fraction with Tenax® resin or solid-phase microextraction (SPME) glass fiber techniques. You et al. [158] have reported higher BSAF value (2.2–3.8) of permethrin (**15**) for *Lumbriculus variegatus*.

Many investigations of relevant enzymes in transformation of xenobiotics by aquatic species have shown that the similar enzymes observed in metabolism in soil, plant, and mammals play a role, such as esterases and oxidases [10, 159, 160]. Metabolism of pyrethroids has been most extensively studied in fish for cypermethrin (**5**) and permethrin (**15**). Aromatic hydroxylation at the 4′-position of the 3-phenoxybenzyl moiety followed by conjugation with glucuronic acid

predominates for *cis* isomer in carp and rainbow trout, while an ester cleavage is a main pathway for *trans* isomer. The oxidation of *gem*-methyl group in the cyclopropyl ring was also detected for (**5**). The acid and alcohol moieties formed via hydrolysis of (**5**), (**15**), and their 4′-OH derivatives were conjugated with glucuronic acid at carboxyl or phenolic hydroxyl groups by glucuronyltransferases. The similar metabolism has been observed for bifenthrin (**2**) [161] and fenvalerate (**9**) [162] in bluegill and rainbow trout. The hydroxyl group was conjugated with sulfate by sulfotransferases and the carboxylic acids originating from either moiety were also conjugated with taurine by acyltransferases. The taurine conjugate of a carboxylate is known for fish, crayfish, and lobster [10], and that of a hydroxylated pyrethroid having an ester linkage has been reported for prallethrin (**17**) in bluegill [163]. By the way, much less information on metabolism of pyrethroids in aquatic species except fish is available [10]. Hydrolysis would be a main degradation pathway as observed in algae, macrophytes, and mussel. The *trans* isomer of (**5**) was metabolized more quickly than the *cis* isomer in the mussel *Mytilus edulis* and P450 was found to be involved in metabolism of (**5**) and deltamethrin (**6**) in sea lice. Although the laboratory model ecosystem using (2*S*) isomer of (**9**) has shown formation of metabolites via hydration of the α-cyano group in snail and daphnia, the coexistence of bottom sediment made this transformation difficult to be interpreted as metabolism in aquatic species.

5 Occurrence in Environment

The high reactivity of synthetic pyrethroids with $OH^{\bullet}$ together with its very low vapor pressure should result in a short residence time in air as supported by the EPI Suite estimation (Table 1) and, therefore, they would be rarely detected in air, for example, a day after application. This is indirectly demonstrated by no detection of some pyrethroids such as cypermethrin (**5**) in rain water collected at agricultural lands in four states of the United States during crop growing seasons [164]. Runoff loads of permethrin (**15**) to rivers have been assessed by using PRZM as an aid to geographical information systems to identify the sites being more contaminated [165]. Since pyrethroids are moderately resistant to hydrolysis at an environmental pH around 7 and an immediate association with suspended matters in water may reduce the contribution of photolysis, it is reasonable to predict the higher concentrations in suspended matters and bottom sediment than that truly dissolved in water. In order to determine correctly the residues in each phase of the environment, a solid-phase microextraction (SPME) method has been more favorably utilized with GC and LC equipped with electron-capture or mass detector equipment. Hladik and Kuivila [166] have examined the residues of four pyrethroids in five natural waters collected from drains and creeks in California. Laboratory study indicated 20–38% of the applied pyrethroid was truly dissolved, the remaining fraction being associated with suspended sediment or adsorbed to the glass wall. Similarly, most pyrethroids were associated with suspended sediments in natural

waters and more residues of bifenthrin (**2**) and cyfluthrin (**3**) were detected than cypermethrin (**5**) and permethrin (**15**). Less concentration in the water phase of rivers and streams were also reported for esfenvalerate (**9a**) and (**15**) in California, UK, and Denmark [167–169], but the timing of pyrethroid application and monitoring as well as an application rate and its frequency should always be taken into account for the interpretation of residue data. By the way, Weston and Lydy [170] have recently reported the residues of eight pyrethroids in waters collected from urban runoff, publicly owned treatment works (POTWs), and agricultural drains in California. In addition, (**2**) and (**15**) were more frequently detected in any water with higher residues. More residues of the several pyrethroids were detected in water collected from POTWs equipped with organic-rich biosolids in plants, as compared with those from agricultural drains. Since the concentration of suspended sediments was low, these high values were considered to originate from pyrethroids in sewer disposal of household insecticides, pet products, etc. In the case of agricultural drains, pyrethroids in water might be removed by association with suspended matters followed by sedimentation, direct adsorption to bottom sediments, and/or various kinds of transformation during transport from application sites.

More residues of pyrethroids were found in bottom sediments of streams, rivers, and creeks than in overlying waters. Among the several pyrethroids, bifenthrin (**2**) and permethrin (**15**) appear to be detected with more frequencies in sediments at the highest level of 0.1–0.5 ppm. The residue levels are highly variable depending on the monitored sites [171–174]. For example, the highest residue of (**2**) was detected in Sacramento, California but with no residue in Nashville, Tennessee, and more cypermethrin (**5**) was reported for the latter site. Although more runoff is considered to cause more transport of contaminated soils into a water body leading to increased concentrations of pyrethroids in sediment, Weston et al. [175] have reported through the analysis of sediment-associated pyrethroids in waters at California's Central Valley that the concentrations of pyrethroids tended to be greater shortly after their use rather than after heavy rain events.

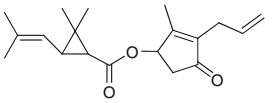
6 Conclusion

The basic information to examine the environmental behavior of pyrethroids has been extensively collected through various laboratory and field studies. Among the physico-chemical properties, investigation of adsorption coefficient with soil and sediment had better be revisited using the SPME method, since the concentration of pyrethroid truly dissolved in water might have been overestimated by the usual liquid–liquid partition method due to its association with dissolved organic matters and suspended particles. Information on the effect of stereoisomerism both in acid and alcohol moieties still seem to be limited to hydrolysis and soil metabolism of some pyrethroids. From the viewpoint of less load of chemical to environment and avoidance of unfavorable ecotoxicological impact originating from less or no

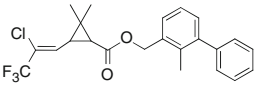
biologically active stereoisomers, pesticide commercialized as racemic mixtures tends to become a candidate for replacement with a newer one in regulation. Therefore, the effect of stereoisomerism on biological transformation in soil, plants, and many kinds of aquatic species should be investigated more in relation to stereo- and regio-selectivity of relevant enzyme systems. Since metabolic transformation of pyrethroids in plants and aquatic species has been investigated before the recent progress in LC-MS/MS technology, the chemical identities of various conjugates have not been well investigated. The clarification of their structures together with investigation of relevant enzymology at a gene level helps us to understand the environmental fate of pyrethroids in more detail. At the same time, newer methodology to simulate or investigate metabolic and chemical transformations needs to be developed further. Metabolism study using isolated cells or chemical reactions using metalloporphyrins would be one of the convenient and promising approaches for this purpose. Finally, bioconcentration, bioaccumulation, and metabolism in aquatic species, especially in sediment dwelling organisms, should be investigated more to assess the effect of pyrethroids in parallel with more collection of ecotoxicological data and their actual residues in the aquatic environment.

Appendix. Chemical Structures of Synthetic Pyrethroids

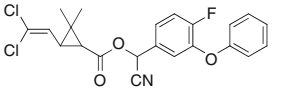
1) allethrin; racemic



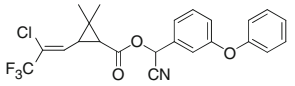
2) bifenthrin; (*Z*)-(1*RS*)-cis



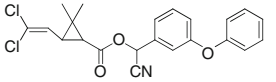
3) cyfluthrin; racemic



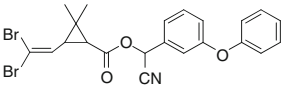
4) cyhalothrin; (*Z*)-(1*RS*)-cis, (*αRS*)



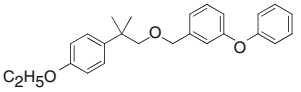
5) cypermethrin; racemic



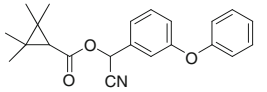
6) deltamethrin; (1*R*)-cis, (*αS*)



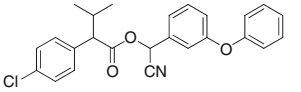
7) etofenprox



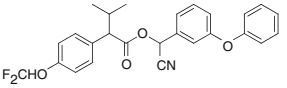
8) fenpropathrin; racemic



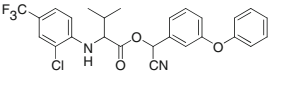
9) fenvalerate; racemic



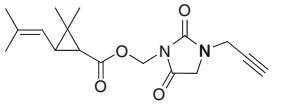
10) flucythrinate; (2*S*),(*αRS*)



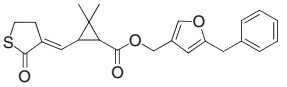
11) fluvalinate; (2*R*),(*αRS*)



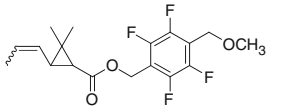
12) imiprothrin; (1*R*)-*trans*/*cis* (4:1)



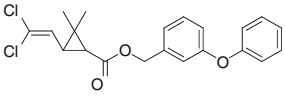
13) kadethrin; (*E*)-(1*S*)-*cis*



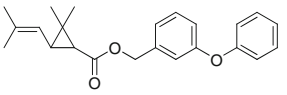
14) metofluthrin; racemic, (*Z*)-rich



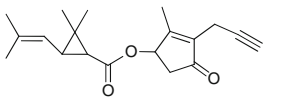
15) permethrin; racemic



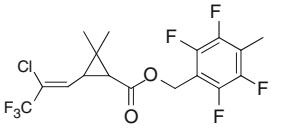
16) phenothrin; racemic



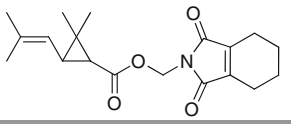
17) prallethrin; racemic



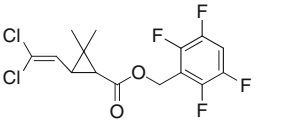
18) tefluthrin; (*Z*)-(1*RS*)-*cis*



19) tetramethrin; racemic



20) transfluthrin; (1*S*)-*trans*



(**3a**) *beta*-cyfluthrin; (1*S*)-*cis*/*trans*,(αR) + (1*R*)-*cis*/*trans*,(α*S*) (1:1). (**4a**) *lambda*-cyhalothrin; (*Z*)-(1*R*)-*cis*,(α*S*). (**5a**) *alpha*-cypermethrin; (1*R*)-*cis*,(α*S*) + (1*S*)-*cis*,(α*R*) (1:1). (**5b**) *beta*-cypermethrin; (1*R*)-*cis*/*trans*,(α*S*) + (1*S*)-*cis*/*trans*,(α*R*) (*cis*/*trans* = 2:3). (**5c**) *theta*-cypermethrin; (1*R*)-*trans*, (α*S*) + (1*S*)-*trans*,(α*R*) (1:1). (**5d**) *zeta*-cypermethrin ; (1*RS*)-*cis*/*trans*,(α*S*) (*cis*/*trans* = 1:1). (**9a**) esfenvalerate; (2*S*),(α*S*). (**9b**) (2*S*),(α*R*) isomer. (**14**) *Z*/*E* = 9:1

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The Biological Activity of a Novel Pyrethroid: Metofluthrin

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Abstract Metofluthrin (commercial name: SumiOne®, Eminence®) is a novel pyrethroid insecticide developed by Sumitomo Chemical Co., Ltd. Metofluthrin has extremely high insecticidal activity to various pest insects, especially to mosquitoes. In addition, Metofluthrin has relatively high volatility and low mammalian toxicity. Metofluthrin is therefore suitable for use not only in conventional mosquito control formulations such as coils and liquid vaporizers, but also in a variety of novel devices that do not require heating, such as fan vaporizers and paper and resin emanators. Here we describe the insecticidal activity of Metofluthrin mainly against mosquitoes in various formulations in both laboratory and field trials.

Keywords Biting inhibition · Field · Insecticidal activity · Metofluthrin · Mosquito

Contents

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1 Introduction

Since the olden days, mosquitoes have been one of the insects most troublesome to mankind because they are not only nuisance pests but also vectors of various diseases. Malaria, which is transmitted by the *Anopheles* mosquito, is still a threat to people, particularly those in African countries. The intermediate for West Nile fever, which appeared in New York in 1999, is the house mosquito (*Culex pipiens*) and other mosquito species. Besides Japanese encephalitis due to *Culex tritaeniorhynchus* and filariasis in dogs due to *C. pipiens*, *Aedes* mosquitoes are vectors for various diseases such as dengue fever, yellow fever, and Chikungunya fever. Furthermore, recent investigations have suggested that the areas inhabited by mosquitoes are expanding because of global warming.

The main devices used for mosquito protection in households have been mosquito coils, electric mosquito mats, and liquid vaporizers, all of them methods that vaporize insecticides into the air using heating by means of fire or electricity to control the insects. In recent years, new anti-mosquito products have been commercialized such as fan vaporizers, paper strip type emanators, and resin net type emanators which vaporize insecticides without heating. In all of these products pyrethroid insecticides are used as active ingredients because they are superior in what is called "knockdown effect," where noxious insects are rapidly paralyzed and cannot bite, and have a high level of safety for humans.

Metofluthrin (SumiOne®: 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (*EZ*(*E*/*Z* = 1/8))-(*1R, 3R*)-2,2-dimethyl-3-(prop-1-enyl)cyclopropanecarboxylate, Fig. 1) is a novel pyrethroid insecticide discovered by Sumitomo Chemical Co., Ltd. [1]. Metofluthrin was registered in more than 20 countries including Japan and USA and is currently under worldwide development for a variety of environmental health applications.

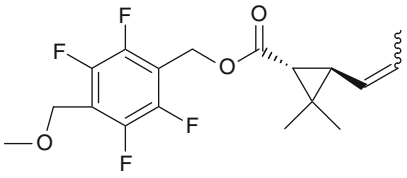

Fig. 1 Structure of Metofluthrin (SumiOne®)

Metofluthrin is a suitable active ingredient for various formulations and devices due to its high lethal/knockdown activity against pest insects (especially mosquitoes), as well as having low mammalian toxicity and favorable physico-chemical properties. This report summarizes its outstanding efficacy in various mosquito control formulations in both laboratory tests and field trials [2].

2 Basic Insecticidal Activity of Metofluthrin in Laboratory Studies

2.1 Technical Grade

The lethal efficacy (topical application) of Metofluthrin for medically important pests is given in Table 1.

The LD_{50} value of Metofluthrin for common house mosquito (*Culex pipiens pallens*) adults was 0.0015μg/female adult, and the relative lethal efficacy of Metofluthrin was approximately 25 times higher compared to D-allethrin and approximately 4 times higher than prallethrin. In addition, it had approximately twice the efficacy of permethrin, which is a representative killing agent. The LD_{50} value of Metofluthrin for Asian tiger mosquito (*Aedes albopictus*) adults was 0.00047 μg/female adult, and the relative lethal efficacy of Metofluthrin was approximately 50 times that of D-allethrin, approximately 10 times that of prallethrin and approximately 4 times that of permethrin.

On the other hand, the lethal efficacy for adult house flies (*Musca domestica*) is about the same as D-allethrin and approximately 0.5 that of prallethrin. In addition, the efficacy for female adult German cockroaches (*Blattella germanica*) is approximately twice that of D-allethrin and approximately 0.5 that of prallethrin. From the results mentioned above, we can see that Metofluthrin has an extremely high lethal efficacy for mosquitoes in particular.

The lethal efficacy of Metofluthrin against four medically important mosquito species (*C. pipiens pallens*, *Culex quinquefasciatus*, *Aedes aegypti*, and *A. albopictus*) is summarized in Fig. 2. The efficacy of Metofluthrin was found to be very high against all these 4 species and it was between 19 and 49 times higher than that of D-allethrin.

Table 1 Lethal efficacy of Metofluthrin against sanitary pests: topical application method, LD_{50}(μg/female adult)

Compounds	*Culex pipiens pallens*	*Aedes albopictus*	*Musca domestica*	*Blattella germanica*
Metofluthrin	0.0015	0.00047	0.24	1.3
D-Allethrin	0.038	0.023	0.21	2.9
Prallethrin	0.0056	0.0050	0.13	0.59
d-Tetramethrin	0.0096	0.0036	0.28	7.8
Permethrin	0.0028	0.0012	0.013	1.5

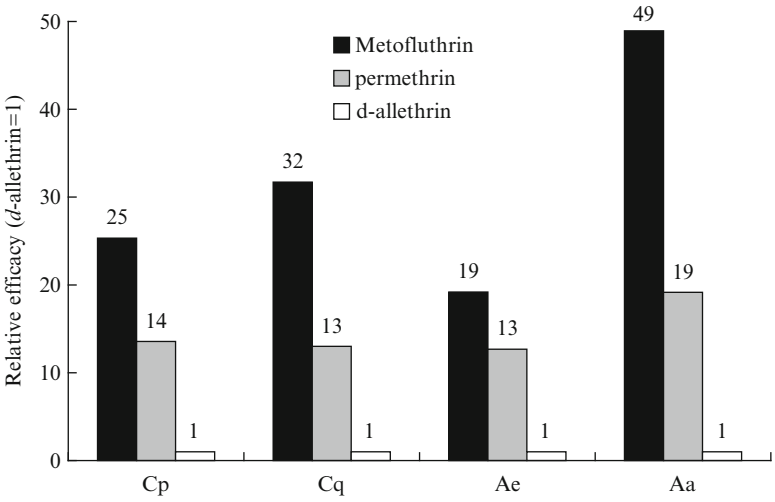

Fig. 2 Lethal efficacy of Metofluthrin against mosquitoes: topical application method, relative efficacy: the larger the number the more active the compound (Cp: *Culex pipiens pallens*, Cq: *Culex quinquefasciatus*, Ae: *Aedes aegypti*, Aa: *Aedes albopictus*)

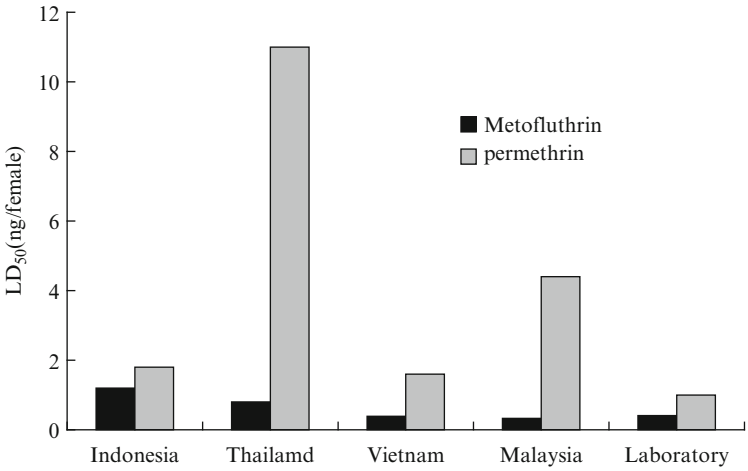

Fig. 3 Lethal efficacy of Metofluthrin against field strains of *Culex quinquefasciatus*: topical application method, LD_{50}(μg/female adult) ("Laboratory" strain is a susceptible strain) (reproduced from [3])

The lethal efficacy of Metofluthrin was also evaluated against various field strains of *C. quinquefasciatus* collected around Asia and results are shown in Fig. 3. Metofluthrin exhibited high efficacy against all strains, even against some strains (collected in Thailand and Malaysia) which displayed decreased susceptibility against permethrin [3].

2.2 Mosquito Coils

Mosquito coils, which were first invented in Japan during the nineteenth century, are widely used throughout the world. Especially popular in Southeast Asia, they are the most common method used to protect against biting mosquitoes. To evaluate the efficacy of Metofluthrin coils against various species of mosquitoes, we implemented evaluations using a large, 28 m^3 chamber containing free-flying mosquitoes.

The efficacy for the common house mosquito (*C. pipien pallens*), which is the main species in the temperate parts of Asia, including Japan, is given in Table 2. Coils containing 0.013% Metofluthrin had an efficacy somewhat exceeding that of coils containing 0.2% D-allethrin. Against southern house mosquito (*C. quinquefasciatus*), which is widely distributed in tropical and subtropical areas worldwide and is the most important target species in the field of mosquito control, coils containing 0.005% Metofluthrin exhibited an efficacy exceeding that of coils containing 0.2% D-allethrin and those containing 0.03% transfluthrin. Therefore, the relative efficacy is estimated to exceed 40 times that of D-allethrin and six times that of transfluthrin, as shown in Table 3.

In order to confirm efficacy against field strains, large chamber free-flying tests were conducted against *C. quinquefasciatus* collected in Bogor, Indonesia. The results are shown in Table 4. Since field strains of mosquitoes tend to have longer

Table 2 Knockdown efficacy of Metofluthrin coil against *Culex pipiens pallens* (laboratory strain) by large chamber free-flying method

A.I.	Conc. (wt%)	KT_{50} (min)
Metofluthrin	0.013	49
	0.02	35
	0.04	22
D-Allethrin	0.2	54

Table 3 Knockdown efficacy of Metofluthrin coil against *Culex quinquefasciatus* (laboratory strain) using the large chamber free-flying method

A.I.	Conc. (wt%)	KT_{50} (min)
Metofluthrin	0.005	60
	0.01	40
Transfluthrin	0.03	68
D-Allethrin	0.2	75

Table 4 Knockdown efficacy of Metofluthrin coils against *Culex quinquefasciatus* (Bogor, Indonesia, field strain) using the large chamber free-flying method, 1-h pre-fumigation

A.I.	Conc. (wt%)	KT_{50} (min)
Metofluthrin	0.005	25
	0.0075	16
D-Allethrin	0.3	27

knockdown time compared with laboratory strains, these tests were carried out by releasing insects into the chamber once the test coil inside had been allowed to burn for 1 h. Coils containing 0.005% Metofluthrin exhibited an efficacy almost equal to that of coils containing 0.3% D-allethrin. The relative efficacy is therefore approximately 60 times that of D-allethrin, which means an increase in the efficacy ratio when compared with the laboratory strain.

2.3 Liquid Vaporizers

Metofluthrin is a suitable active ingredient in liquid vaporizers due to its high knockdown activity, the suitable degree of vaporization, and the easy solubility in various solvents such as kerosene. The efficacy of the liquid vaporizer for *C. pipien pallens* (laboratory susceptible strain) is given in Table 5. A 45 mL liquid vaporization formulation containing 240 mg of Metofluthrin has the same efficacy as a 45 mL liquid vaporization formulation containing 600 mg of prallethrin. The efficacy of a Metofluthrin liquid vaporization formulation for *C. quinquefasciatus* (laboratory susceptible strain) was very high at five to six times that of prallethrin (Table 6). The efficacy of Metofluthrin liquid vaporization tested by the large chamber free flying method (28 m^3, insects released after 1 h pre-fumigation) on field strains of *C. quinquefasciatus* from Bogor, Indonesia is given in Table 7.

Table 5 Knockdown efficacy of Metofluthrin liquid vaporizer (60 days use formulation) against *Culex pipiens pallens* (laboratory strain) large chamber (28 m^3) cage method

A.I.	A.I. mg/45 mL	KT_{50} (min)
Metofluthrin	120	> 90
	240	70
	480	48
Prallethrin	600	74

Table 6 Knockdown efficacy of SumiOne® liquid vaporizer against *Culex quinquefasciatus* (laboratory strain) using the large chamber free-flying method

A.I.	AI mg/45 mL	Evaporation rate (mg/h)	KT_{50} (min)
Metofluthrin	120	0.17	35
	180	0.22	25
	240	0.35	21
Prallethrin	600	1.01	35

Table 7 Knockdown efficacy of Metofluthrin liquid vaporizer against *Culex quinquefasciatus* (Bogor field strain) by the large chamber free-flying method with 1 h pre-fumigation

A.I.	AI mg/45 mL	Evaporation rate (mg/h)	KT_{50} (min)
Metofluthrin	30	0.08	30
	60	0.13	12
Prallethrin	300	0.66	31

In the case of the field strain, there was an increase in the efficacy ratio with prallethrin over the laboratory susceptible strain, and the relative efficacy ratio was estimated to be over eight times that of prallethrin.

2.4 Fan Vaporizers

One of the major characteristics of Metofluthrin is its vapor action at room temperature not seen in the existing pyrethroids D-allethrin and prallethrin. We will describe a fan type formulation ("a fan vaporizer") where a motor turns a fan and the active ingredient is vaporized by the airflow from it at room temperature.

Mosquito control mat and liquid vaporizer exhibit their effects by vaporizing the active ingredient through heating. However, since these heaters require a comparatively large amount of electric power, there has been a limit to use with normal batteries. On the other hand, the active ingredient in fan vaporizer is vaporized by the airflow from a fan at room temperature, but the power required to turn the fan is lower than that for heating, so it is possible to use them with normal batteries as power supply. Therefore, the fan vaporizer has the merit of making it possible to use them outdoors without need of electrical outlets.

With the goal of getting a grip on the basic activity of Metofluthrin as the active ingredient in a fan vaporizer, we investigated the knockdown efficacy for the common house mosquito (laboratory susceptible strain) in a semi-practical setting, with various amounts of vaporization using a disk capable of adjusting the amount of vaporization without changing the airflow, by large chamber free-flying method. Transfluthrin was used as the control chemical. The results are shown in Table 8. Based on the results of these tests, it was found that Metofluthrin had an efficacy of three to four times that of transfluthrin (Table 9).

Table 8 Knockdown efficacy of Metofluthrin fan vaporizer against *Culex pipiens pallens* (laboratory strain) by large chamber (28 m^3) free flying method

	Evolution rate (mg/h)	KT_{50} (min)	Linear regression expression
Metofluthrin	0.09	34	$\log (KT_{50}) = -0.89 \times \log$ (evaporation rate) + 0.61 $R^2 = 0.999$
	0.18	18	
	0.26	14	
Transfluthrin	0.20	38	$\log (KT_{50}) = -0.59 \times \log$ (evaporation rate) + 1.17 $R^2 = 0.973$
	0.36	29	
	0.54	21	

Table 9 Comparison of efficacy against *Culex pipiens pallens* (laboratory strain) between Metofluthrin and transfluthrin

	KT_{50}	
	20 min	30 min
Metofluthrin (mg/h)	0.17	0.11
Transfluthrin (mg/h)	0.61	0.31

We investigated the efficacy for the Asian tiger mosquito (laboratory susceptible strain) which is a representative striped mosquito (*Aedes*) in Japan using a test fan vaporizer by large chamber free-flying method. The results are shown in Table 10. In comparisons of the efficacies based on the amount of vaporization, we found that Metofluthrin exhibited an efficacy approximately five times that of transfluthrin. A carrier containing 160 mg of Metofluthrin was loaded into the test fan vaporizer (using two AA alkaline batteries, with battery replacement after 332 h), and we carried out residual effectiveness tests over 637 h of operation. As a result, Metofluthrin exhibited knockdown activity during the test period that was generally the same or greater than a commercial 60 day liquid vaporizer (Fig. 4). With the 60 day liquid vaporizer, the effectiveness just after starting it up was somewhat lacking, but the fan vaporizer using Metofluthrin exhibited immediate effects after it was started.

Due to the excellent efficacy of Metofluthrin described above, when Metofluthrin is used as the active ingredient in a fan type formulation, the size of the device can even be scaled down. Since one of the main merits of fan type formulations is their use as transportable devices, the reduction in size is essential for this formulation and

Table 10 Knockdown efficacy of Metofluthrin fan vaporizer against *Aedes albopictus* (laboratory strain) by large chamber (28 m^3) free flying method

A.I.	Evaporation rate (mg/h)	KT_{50} (min)
Metofluthrin	0.090	40
Transfluthrin	0.28	>60
	0.39	55
	0.50	55

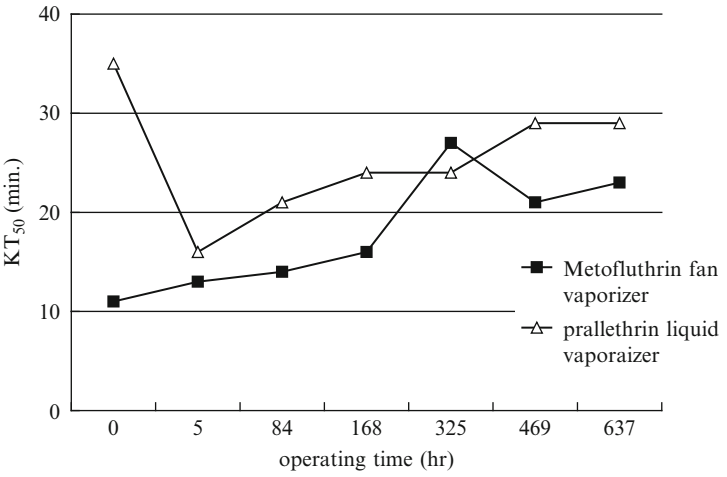

Fig. 4 Knockdown efficacy of Metofluthrin(=160 mg) fan vaporizer against *Culex pipiens pallens* (susceptible strain) compared to commercial liquid vaporizer (prallethrin = 600 mg)

we can assume that Metofluthrin is the only active insecticidal ingredient that can make that possible.

3 Practical Efficacy of Metofluthrin in Field Trials

3.1 Practical Efficacy of Mosquito Coils

To determine the practical effects of mosquito coils, field trials were conducted in tropical households against indoor night biting mosquitoes, particularly *C. quinquefasciatus* using the Human Bare-Leg technique [4]. The trials were conducted from 21:00 to 01:00 the next morning, in order to coincide with the peak activity period of *C. quinquefasciatus*.

When selecting houses for the trials, the size and ventilation (number of windows) of the living rooms were taken into consideration. One pre-treatment night catch was conducted in each test home to determine the relative density of each biting mosquito population. Houses with lower than 15 catches per hour were not used for the trial, while houses with 15 or more catches per hour were ranked and then arranged into two separate blocks (houses with high catches and houses with low catches). Each house was randomly assigned a treatment and a human volunteer as bait. During treatment nights, the test samples and control rotated through each house on their assigned block. The houses used as the control, on the other hand, were fixed and not rotated during the subsequent treatment nights. The volunteers assigned to each house for the pre-treatment night remained with the same house for each subsequent treatment night.

Over the pre-treatment night, each house was assessed with the absence of test samples. For the treatment nights, all test samples were assigned to one house of each block. Two were then selected as control houses to monitor the natural fluctuation of the mosquito population at the trial site. During trials, the temperature and relative humidity were recorded on the hour. All mosquitoes caught during the pre-treatment and treatment nights were brought back to the laboratory for species identification.

Bite reduction rate was calculated using the following formula in view of the nightly fluctuation in mosquito biting pressure:

$$\% \text{ bite reduction} = 100 - \frac{(C_1 \times T_2)}{(T_1 \times C_2)} \times 100.$$

C_1 = Number of mosquitoes in "control" houses during the pre-treatment night.
C_2 = Number of mosquitoes in "control" houses during the treatment night.
T_1 = Number of mosquitoes in "treated" houses during the pre-treatment night.
T_2 = Number of mosquitoes in "treated" houses during the treatment night.

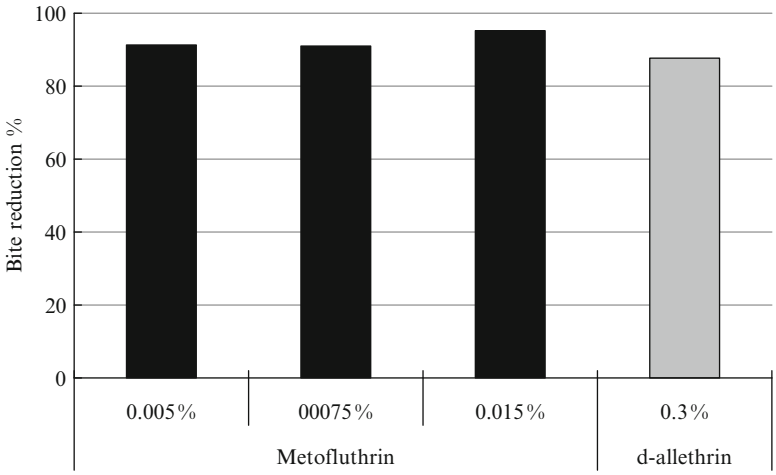

Fig. 5 Field efficacy of Metofluthrin coils against indoor night biting mosquitoes, evaluated in homes in Indonesia

The field trial for Metofluthrin mosquito coils was conducted by staff from the Faculty of Veterinary Medicine, Bogor Agricultural University (BAU), from 18 to 27 June 2005, in Cangkaura Wok Village, Darmaga, Bogor, West Java, Indonesia. The pre-treatment night catch indicated the predominance of *C. quinquefasciatus* (>95%). During the treatment nights, one mosquito coil was ignited and placed on the floor of the living room in each test home, approximately 1–1.5 m away from the human volunteer. The average living room size was 20 m^3. Results showed that the mosquito coil containing 0.005 wt% Metofluthrin (with 91.3% bite reduction) provided better protection against indoor night biting mosquitoes than coils containing 0.3 wt% D-allethrin (with 87.7% bite reduction). Therefore, when used in mosquito coils, the relative potency of Metofluthrin is estimated to be more than 60 times better than D-allethrin as shown in Fig. 5.

3.2 Practical Efficacy of Liquid Vaporizer

The field efficacy of Metofluthrin liquid vaporizers was tested with the same method as in the case of mosquito coils, by staff from the Vector Control Research Unit of Universiti Sains Malaysia (USM), in a residential area in Ujung Batu on the north-western coastal area of the mainland Peninsular Malaysia adjacent to Penang Island. The field trial was repeated in Cangkaura Wok Village, Darmaga, Bogor, West Java, Indonesia by staff from the Faculty of Veterinary Medicine, Bogor Agricultural University (BAU) from 27 August 2005 to 11 September 2007. The average sizes of living rooms used for USM and BAU field trials were 55 m^3 and 20 m^3, respectively. The pre-treatment night catches indicated that *C. quinquefasciatus* (> 95%) was the most predominant indoor night biting mosquito species at both trial sites.

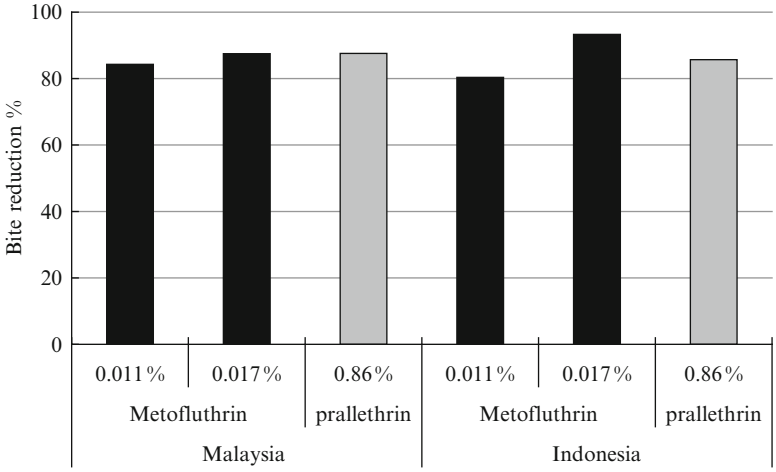

Fig. 6 Field efficacy of Metofluthrin liquid vaporizers against indoor night biting mosquitoes, evaluated in homes in Indonesia and in Malaysia

In the treated homes, one liquid vaporizer was switched on and placed on the floor in the living room, approximately 1–1.5 m away from the human bait. Results obtained from the two trial sites indicated that liquid vaporizers containing 0.11 wt % Metofluthrin were slightly inferior to those containing 0.86 wt% prallethrin. However, the percentage of bite reduction provided by 0.17 wt% Metofluthrin was similar to, or better than, that provided by 0.86 wt% prallethrin (Fig. 6). Therefore, the relative efficacy of Metofluthrin in liquid vaporizer formulations is at least five times better than prallethrin.

3.3 Practical Efficacy of Paper Emanator

Natural vaporization agents, where the active ingredient is formulated on paper or resin, and where it is vaporized without heating or use of power, are easy to use, so there is particular high potential for associated new developments in the field of mosquito control. Insecticides that can be used in formulations for this purpose must possess the characteristics of room temperature vapor action, high level activity, and a high degree of safety for mammals. Metofluthrin meets all of these requirements.

In the first stage of development of the emanator, we evaluated efficacy of a device using paper as a substrate (Fig. 7). Field trials were conducted in Malaysia. The testing was carried out according to the methods of Yap et al. [5]. The results are shown in Fig. 8. A paper emanator containing 100 mg of Metofluthrin exhibited high levels of preventative effects exceeding those of coil formulations containing 0.25% D-allethrin for *C. quinquefasciatus*.

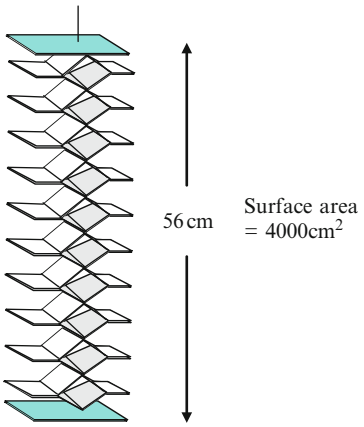

Fig. 7 Metofluthrin paper emanator

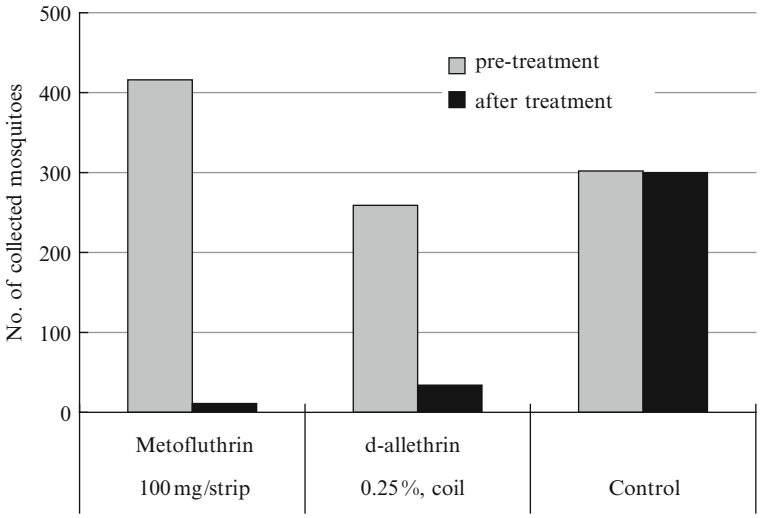

Fig. 8 Field efficacy of Metofluthrin paper emanator against night biting indoor mosquitoes (*Culex quinquefasciatus*) in Malaysia

Practical tests using similar paper emanators were conducted in Indonesia, Japan and USA.

In a house on Lombok Island in Indonesia, a paper emanator impregnated with 200 mg of Metofluthrin exhibited repellent effects of 80% or greater against *C. quinquefasciatus* and *Anopheles* mosquitoes over a period of 4 weeks [6]. In addition, in outdoor conditions on Lombok Island, it exhibited superior repellent effects against *C. quinquefasciatus* as well as *Anopheles balabaciensis* and *An. sundaicus*, which are vector mosquitoes for malaria [7].

A similar paper emanator impregnated with 200 mg of Metofluthrin exhibited almost complete repellent activity against *A. albopictus* in Japan [3].

In Washington State and Florida State in the USA, this emanator showed excellent repellent activity against *Aedes vexans* and *Ochlerotatus taeniorhynchus,* respectively [8].

From these results, we were able to confirm that Metofluthrin paper emanator had sufficient effects against various species of mosquitoes under practical conditions.

3.4 Practical Efficacy of Resin Emanators

After developing and evaluating paper emanator technology, we have continued to improve and develop new ambient temperature emanators by investigating alternative substrates on which Metofluthrin is impregnated. Polyethylene resin is one of the most promising candidate materials for improving the performance and handling characteristics of ambient temperature emanators. This type of resin has some advantages compared with paper, such as improved and prolonged release characteristics, as well as being waterproof and far more durable.

To confirm the efficacy of Metofluthrin resin emanators, we conducted a field trial in Japan. A polyethylene net incorporated with 185 mg Metofluthrin was folded and set in a plastic frame as shown in Fig. 9. The plastic frame was used

Fig. 9 Metofluthrin resin emanator

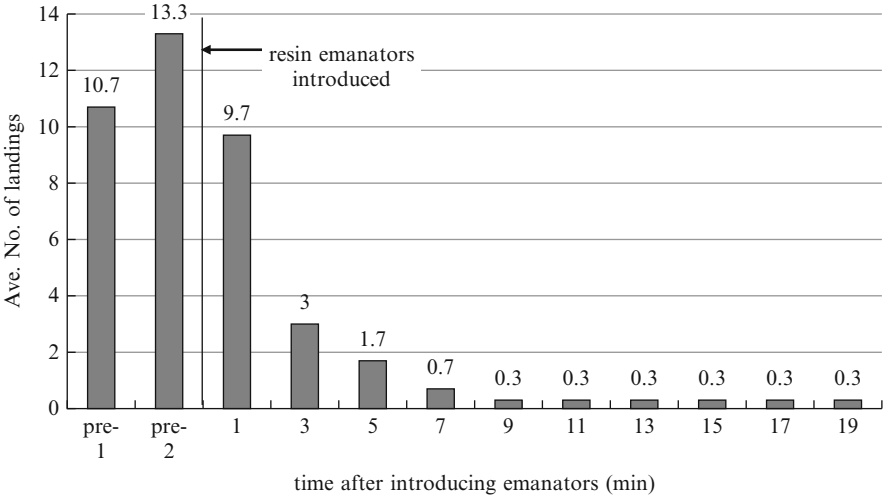

Fig. 10 Field efficacy of Metofluthrin resin emanator in Japan (two emanators)

for making the emanator more compact and for protecting the operators from direct contact with the insecticide treated net. Field trials were conducted in Nagasaki, Japan. The predominant mosquito species was *A. albopictus*. Human volunteers were used for this trial, wearing overall suits to prevent mosquito biting. Use of skin applied repellents (e.g., DEET) etc. was prohibited. Tow resin emanators were hung at 1.2 m height and 1.8 m distance from each other. The volunteer was standing between the two emanators and mosquito landing rates were counted at 2 min intervals until 19 min after setting emanators. Under these test conditions Metofluthrin resin emanators showed a dramatic decrease in mosquito landing rates (Fig. 10). These results demonstrate that the efficacy of resin emanators is similar to or better than that observed using paper emanators.

Further field trials of resin emanators were conducted in Nagasaki, Japan. The test sample was a fine meshed resin net impregnated with 200 mg of Metofluthrin and a fan vaporizer (commercial product in Japan) as reference. Test conditions in the field were as follows: temperature 28–31 °C, relative humidity 55–65%, and average wind velocity 0.48 m/s. One test resin sample was hung at 1.2 m in height and at 1.2, 2.4, or 3.6 m up-wind from the volunteer. A reference sample, a fan vaporizer, was attached to the waist of the volunteer according to the instructions for the product. Three replicates were conducted per sample and results are shown in Fig. 11. The Metofluthrin resin emanator at 1.2 m up-wind apart from the volunteer showed high repellent efficacy in field condition; the emanator even showed fairly good efficacy at 2.4 and 3.6 m distance from the volunteer.

In this trial, Metofluthrin aerial concentration was also measured and average concentration was roughly 0.2–0.5 $\mu g/m^3$.

Practical tests using Metofluthrin resin emanators with different shapes were also conducted in Vietnam. Resin emanators containing 1 g of Metofluthrin

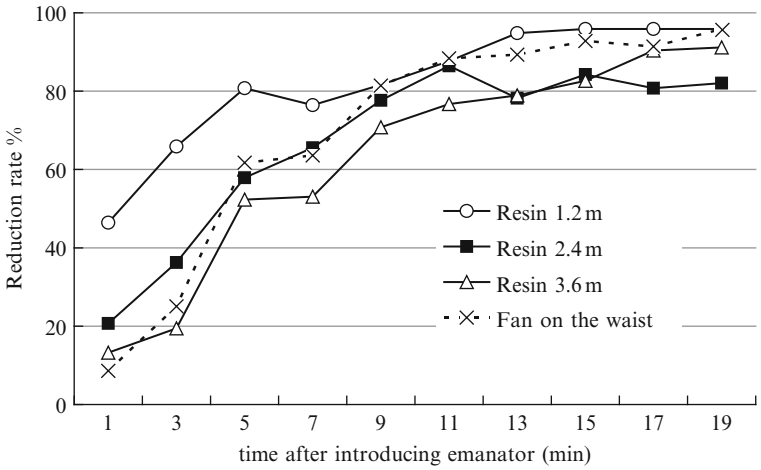

Fig. 11 Field efficacy of Metofluthrin resin emanator in Japan (one emanator, various distances)

exhibited excellent spatial repellent effects against both *C. quinquefasciatus* and *A. aegypti* for at least 6 weeks [9].

4 Biting Inhibition Activity of Metofluthrin in Laboratory Studies

4.1 Biting Inhibition Activity of Mosquito Coils

The knockdown activity is usually evaluated in the laboratory as the criteria by which the biological performance of mosquito-control-devices is compared. However, under more practical conditions, spatial repellency and biting inhibition activity are thought to reflect more accurately the actual performance than knockdown activity. In order to evaluate biting inhibition activity in the laboratory, we established the following test method. Two nylon-meshed cages, each containing a live chick, were hung 1.2 m above the floor in the test chamber (28 m^3), followed by a release of 100 adult unfed female *A. aegypti* into the chamber (Fig. 12). The number of insects attracted to each cage was counted 5 min post-release. A mosquito coil was then ignited and placed on the floor at a distance of 1.2 m from the test cages. The number of mosquitoes attracted to each cage was calculated at designated intervals over the following 60-min period. During this time, the mosquitoes which were knocked down onto the floor were also counted. This experiment was performed in accordance with the Sumitomo Chemical Co. Ltd *Guide for Animal Care and Use*.

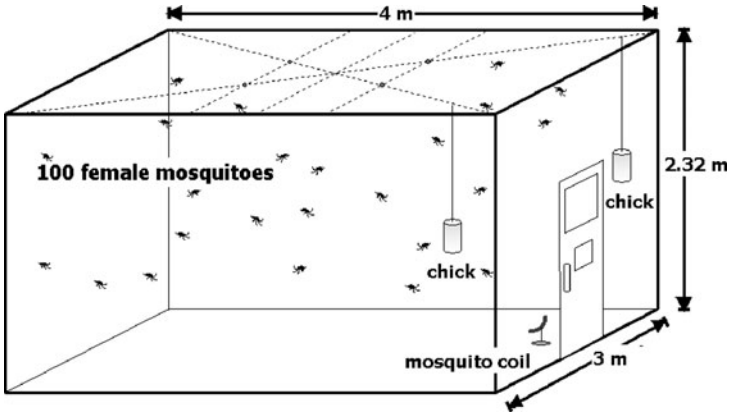

Fig. 12 Test method for determining in-laboratory biting inhibition against *Aedes aegypti*

Table 11 Comparison of biting inhibition activity and knockdown activity in mosquito coil

Active ingredient	Conc. (%w/w)	Relative ratio	Biting inhibition activity IT_{95} (min)	Knockdown activity KT_{50} (min)
Metofluthrin	0.01	1	7.2	16.4
D-Allethrin	0.3	30	12.9	15.9

IT = Inhibition time, IT_{95} (min): calculated time required for 95% biting inhibition, KT = Knockdown time, KT_{50} (min): calculated time required for 50% knockdown (lower IT and KT values mean higher efficacy)

The results shown in Table 11 indicate that the biting inhibition caused by Metofluthrin occurred before the insects were knocked down. The biting inhibition of Metofluthrin was much more than 30 times higher than D-allethrin. The efficacy ratio is higher than that estimated when using knockdown as a performance criterion, which indicates that Metofluthrin is approximately 30 times as active as *d*-allethrin.

4.2 Biting Inhibition Activity of Paper Emanator

Results of the same test using paper emanators are shown in Table 12, where evaporation rates of active ingredient were determined by weight loss. These results again indicate that biting inhibition caused by Metofluthrin occurred well before insects were knocked down. These data indicate that Metofluthrin possesses about 10 times higher bite inhibition efficacy than transfluthrin. This ratio is higher than that estimated when using knockdown as a performance criterion, which indicates that Metofluthrin is approximately only six times more active than transfluthrin. The relationship between IT_{95} and KT_{50} values for these paper emanators is shown in Fig. 13. This clearly demonstrates that, for a given KT value, Metofluthrin has

Table 12 Comparison of biting inhibition activity and knockdown activity in paper emanators

Active ingredient	Evaporation rate (mg/h)	Biting inhibition activity IT_{95} (min)	Knockdown activity KT_{50} (min)
Metofluthrin	0.02	22	57
	0.04	15	29
	0.08	8.4	23
Transfluthrin	0.11	33	50
	0.22	21	33
	0.44	13	20

IT = Inhibition time, IT_{95} (min): calculated time required for 95% biting inhibition. KT = Knockdown time, KT_{50} (min): calculated time required for 50% knockdown. (lower IT and KT values mean higher efficacy)

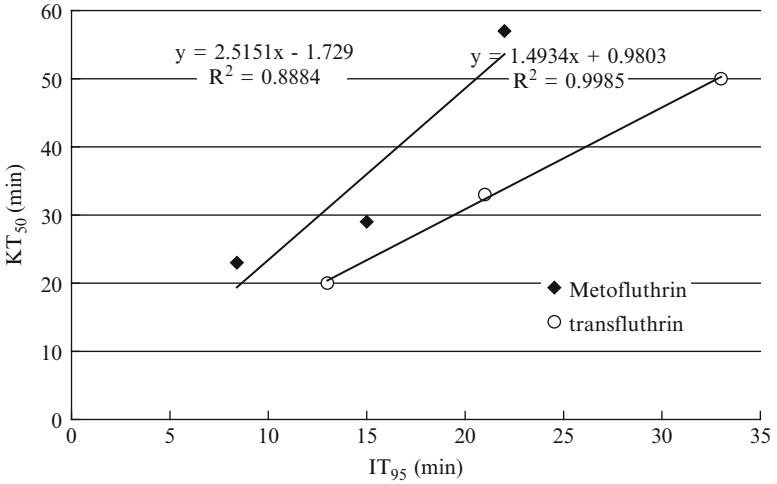

Fig. 13 Relationship between IT_{95} and KT_{50} values for Metofluthrin and transfluthrin paper emanators

a far smaller IT value, indicating the far superior biting inhibition activity of this chemistry when compared to the knockdown activity of transfluthrin.

These data support the excellent biting inhibition activity of Metofluthrin noted in field trials.

5 Conclusion

Metofluthrin is a newly developed highly effective pyrethroid which has excellent insecticidal activity against mosquitoes. Due to its superior characteristics, Metofluthrin is suitable for use in various types of mosquito control formulation, including mosquito coils and liquid vaporizers. Metofluthrin also has excellent

vapor action and is suitable for use in ambient temperature emanators, which can be made from substrates as simple as paper and plastic resin. In addition, excellent biting inhibition activity of Metofluthrin was demonstrated in both laboratory and field tests. This exciting new pyrethroid opens up new possibilities for the effective control of both nuisance biting insects and disease vectors.

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