Research and Development of a Novel Insecticide 'pyridalyl'

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Pyridalyl was discovered and has been under worldwide development by Sumitomo Chemical Co., Ltd. It was already registered in some Asian countries in 2004, including Japan. This novel insecticide exerts excellent control against various lepidopterous and thysanopterous pests on cotton and vegetables. Many existing insecticide-resistant strains of lepidopterous pests can be adequately controlled by pyridalyl as well as susceptible strains. Since pyridalyl develops quite unique insecticidal symptoms, it is considered that pyridalyl has a different mode of action from any other existing insecticide. Its excellent safety to mammals and various beneficial arthropods would provide us with an important tool in IPM (Integrated Pest Management).

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Introduction

Agricultural chemicals have proven to be indispensable in protecting crops from pests and weeds, improving productivity and reducing the amount of farm operations. Some reports have shown that the production of paddy rice, apple and cabbage decreased by 27.5%, 97% and 63.4% without using any insecticide, respectively.[1] On the other hand, existing insecticides are getting less effective due to insects and pathogenic bacteria, which are resistant to them. These resistance problems are recently becoming more serious than before. Furthermore, public concerns about safety and environmental impact of agricultural chemicals have arisen in recent years. Thus, in Europe and the USA, as well as in Japan, governments have implemented policies designed to achieve environmentally friendly agricultural productions (i.e., sustainable agriculture). Integrated Pest Management (IPM) can be thought to be one of the useful tools for such productions. It requires that highly selective insecticides should be used for pest control. Moreover, much safer insecticides need to be developed in order to decrease the burden on the environment by using less chemicals in comparison to conventional insecticides.

Pyridalyl (common name) is a novel insecticide invented and developed by Sumitomo Chemical Co., Ltd. This novel insecticide exerts excellent control against lepidopterous and thysanopterous pests on cotton, vegetable and fruits.[2), 3)] It is also effective on pests that have developed resistance to existing insecticides, indicating a different mode of action from any other conventional insecticide (**Fig. 1**). Furthermore, pyridalyl is highly safe to various kinds of important beneficial arthropods, which makes it compliant with IPM. Pyridalyl was first registered in 2004 as an agricultural chemical in Japan and Korea, and is sold under the name "Pleo® flowable." Sumitomo Chemical is subsequently developing the compound in many

other countries. Here we would like to report on its invention, efficacy, manufacturing process, physical and chemical properties, analytical methods, formulations and safety data for mammals and the environment.

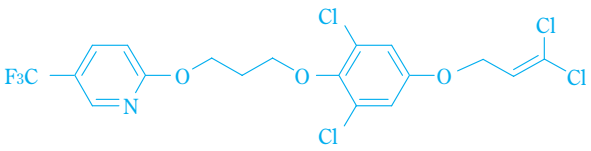


Fig. 1 Structure of pyridalyl

Discovery of Pyridalyl

1. Lead Generation

The discovery of a new insecticide begins from generating a suitable lead compound. This lead compound is structurally modified to higher active compounds against the target insects. Although a variety of approaches may be used for creating an appropriate lead compound, in the case of pyridalyl, we utilized particular known compounds for it. We first chose lepidopterous pests, which have developed resistance to existing insecticides on cotton, vegetable and fruits, as the target insects. Then we carefully screened a number of scientific reports pertaining to bioactive substances. As a result, we were able to locate "dichloroallyl alcohol derivatives 1 and 2", which had been reported to regulate insect growth, as a potential chemical species.[4), 5)] We assumed that the "3,3-dichloro-2-propenyl group" as a common structure in both compounds would contribute moderately to the biological activity. Therefore, we began to design some 3,3-dichloro-2-propenyl compounds to generate a new lead compound.

Each of the compounds 1 and 2 contains a 3,3-dichloro-2-propenyl moiety in the form of an alkyl ester and an alkyl ether. In the syntheses of them, we had noticed that these compounds were somewhat chemically unstable, thus we attempted to stabilize them by introducing a phenyl ether into their molecular structures. We synthesized a variety of derivatives using 4-phenoxy-2-substituted phenols, synthetic intermediates related to our previous research on insect growth regulator (IGR). Among these derivatives, the 4-phenoxy-2-(trifluoromethyl)phenyl(3,3-dichloro-2-propenyl) ether (3) gave 60% control of tobacco cutworm (*Spodoptera litura*) larvae at 500ppm. Furthermore, the compound 3 demonstrated certain other interesting characteristics such as unique lethal symptoms. This result prompted us to modify the structure of the lead compound 3 (**Fig. 2**).[6), 7)]

2. Optimization of the Lead Compound

We initiated an opitimization program on the lead structure 3, which can be divided into 3 parts. Then structural modifications were performed on the propenyl side chain, as well as on the right-side benzene ring. As a result, the "3,3-dichloro-2-propenyl group" was required for the activity on the side chain and "substituents at the 3- and 5-positions" on the benzene ring were the most favorable for the activity. This effect of the introduction of the substituents at the 3- and 5-positions on the benzene ring suggested the possibility of a correlation between biological activity and molecular conformation. In addition to further changes on the left-side benzene ring, the linker section was also optimized, which appear to have influenced the molecular conformation. We found that the use of a pyridine or a benzene ring for the left-side and a 1,3- or 1,4- alkylene dihydroxy linker would lead us to achieve greater insecticidal activity.[6), 7)]

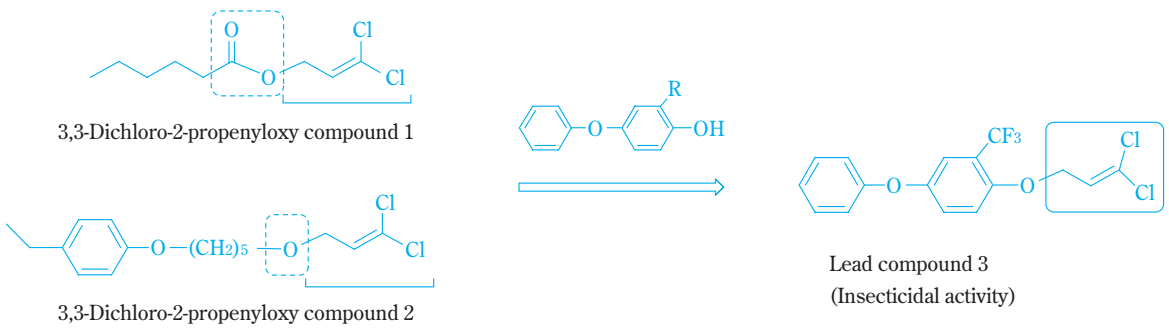


Fig. 2 Discovery of the lead compound 3

Among highly active compounds, pyridalyl was finally selected as the compound for development, based on overall considerations of efficacy, safety, environmental impact and manufacturing cost (**Fig. 3**).

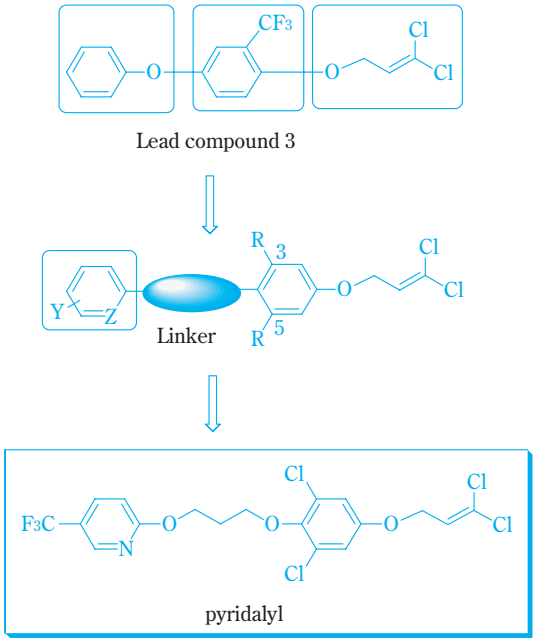


Fig. 3 Optimization of the lead compound 3

Efficacy

1. Insecticidal Activity

As shown in **Table 1**, pyridalyl possesses excellent insecticidal activity against numerous lepidopterous pests. In particular, it demonstrates a high level of insecticidal activity against important vegetable crop pests, such as *Helicoverpa armigera*, *Spodoptera exigua* and the tobacco cutworm. In addition, pyridalyl also shows strong insecticidal activity against *Tysanopteran* insects, such as *Thrips palmi*, as well as against *Dipteran* insect, such as the tomato leaf miner (fly).[8)]

Table 1 Insecticidal activity of pyridalyl against lepidopterous pests

Scientific name	Stage*1	Test method	DAT*2	LC_{50} (mg a.i./litre)
Cnaphalocrosis medinalis	L3	Foliar spray	5	1.55
Helicoverpa armigera	L3	Leaf dip	5	1.36
Helicoverpa zea	L2	Leaf dip	5	3.23
Heliothis virescens	L2	Leaf dip	5	4.29
Mamestra brassicae	L3	Foliar spray	5	1.98
Spodoptera exigua	L3	Leaf dip	5	0.93
Spodoptera litura	L3	Foliar spray	5	0.77
Pieris rapae	L2	Foliar spray	5	3.02
Plutella xylostella	L3	Leaf dip	3	4.48

*1 L2 and L3 means 2nd and 3rd instar larva, respectively.
*2 Days after treatment

2. Functional Characteristics

(1) Cross Resistance

Many lepidopterous pests have developed resistance to insecticides that have been in use for relatively long periods of time, such as organo-phosphates and pyrethroids. This phenomenon is particularly obvious in insects that have relatively short generation periods. More specifically, throughout Japan *Plutella xylostella* has developed a high resistance to organo-phosphates, synthetic pyrethroids, benzoylphenyl urea and chlorfenapyr. Pyridalyl shows strong insecticidal activity against *Plutella* strains, both those having resistance to the abovementioned existing insecticides and those that are susceptible (**Table 2**). Furthermore, pyridalyl has also demonstrated a high level of insecticidal activity against resistant strains of *Heliothis virescens*, which has developed a high resistance to organo-phosphates and pyrethroids. *Heliothis virescens* is now proving to be a serious problem in the USA, as it is the main lepidopterous pest that infests cotton plants.[8)] Thus, since pyridalyl has excellent insecticidal activity, even against lepidopterous pests that have developed high resistance to existing insecticides, it is expected to be an ideal pest control compound for use in Insect Resistance Management (IRM).

Table 2 Insecticidal activity of pyridalyl against insecticide resistant strain of *P. xylostella*

Insecticide	Class	LC_{50} (mg a.i./litre)	
		resistant strain	susceptible strain
pyridalyl		2.6	4.5
cyfluthrin	synthetic pyrethroid	> 500	3.7
pyrimifos methyl	organic phosphate	> 450	12
chlorfluazuron	benzoyl phenylurea	> 25	3.4

(2) Rain Fastness and Residual Activity

Fig. 4 shows the rain fastness and residual activity of pyridalyl. In our experiment, pyridalyl (100 ppm) was sprayed on potted cabbage plants. Immediately after the spraying a one-hour artificial rainfall was applied, at a rate of 20 mm/h, using a rainfall simulator. The plants were subsequently left inside the room. Tobacco cutworms were then released onto these plants on each of the following days: the day of the treatment; 7

days after the treatment and 14 days after the treatment. The cutworm mortality was then observed 4 days after each insect release. Pyridalyl demonstrated a mortality of 100% on the day of treatment and even 14 days after the treatment.[9] These results clearly indicate that pyridalyl still demonstrates high pest control efficacy, even after an occurrence of rainfall immediately after application, thus indicating that it possesses adequate rain fastness and appropriate residual activity. We therefore conclude that pyridalyl can be applied without concern for rainfall, thus improving farm efficiency by decreasing the need for frequent applications.

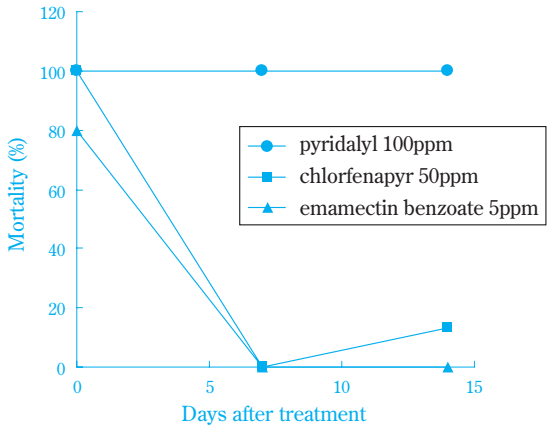


Fig. 4 Residual activity and rain fastness of pyridalyl

(3) Impact Upon Beneficial Arthropods

Along with the widespread understanding of the importance of Integrated Pest Management, IPM, a concept that involves the combination of several pest control methods with agricultural chemicals, has become increasingly popular. In order to implement IPM, the most critical issue is to ensure the usage of appropriate agricultural chemicals that will not interfere with the activity of the pests' natural predators. To achieve this goal, a high level of safety for such natural predators is required for the agricultural chemicals themselves. **Table 3** indicates that pyridalyl, applied at a labeled concentration of 100 ppm, has minimal impact upon predaceous arthropods and parasitic wasps.[8] It also has minimal impact upon beneficial insects, such as bumble bees and honey bees, which are important crop pollinators. Furthermore, it has been observed that when pyridalyl 10% flowable was sprayed on mulberry trees (1:1000 dilute solution, a sufficient amount), it had a residual toxicity period of approximately 15 days after initial treatment against 4th instar *Bombyx mori* (silkworm) larvae. Since pyridalyl has an insignificant impact upon beneficial arthropods, including natural predators, we conclude that it is an extremely effective material for usage in IPM.

Table 3 Beneficial arthropods not affected by pyridalyl at 100 mg a.i./litre

Scientific name	beneficials	Stage*1	Test method
Trichogramma japonicum	Egg parasitic wasp of lepidoptera	Adult	Foliar spray
Chrysoperla carnea	Predatory Chrysopidae	L2-3	Insect dip
Harmonia axyridis	Predatory Coleoptera	L2-3	Foliar spray
Orius sauteri	Predatory Hymenoptera	Adult/Nymph	Foliar spray
Phytoseiulus persimilis	Predatory Acarina	Adult	Foliar spray
Apis mellifera	Pollinator	Worker	Direct spray
Bombus terrestris	Pollinator	Worker	Direct spray

*1 L2 -3 means 2nd to 3rd instar larvae.

3. Results of Field Trials

The characteristics and basic activity of pyridalyl have been described in previous sections. This section describes the effects of pyridalyl in actual field trials. **Fig. 5** shows the results of trials performed in Japan's Hyogo prefecture against Diamondback moth (*Plutella xylostella*) on cabbage. In the region, *Plutella xylostella* had developed resistance to many existing insecticides. As shown in Fig. 5, while pyrethroids (permethrin) did not demonstrate adequate pest control efficacy against *Plutella xylostella*, pyridalyl demonstrated high efficacy at concentrations of 100 ppm, which was far more effective than chlorfenapyr, a widely utilized insecticide. Thus, as pyridalyl possesses excellent pest control efficacy toward lepidopterous pests that have developed high resistance

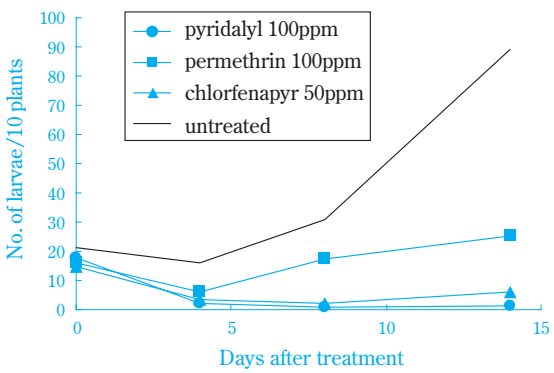


Fig. 5 Field trial against Diamondback moth, *Plutella xylostella* on cabbage

to existing insecticides, it is also expected to function as an ideal pest control compound useful in IRM. In fact, pyridalyl is already enjoying widespread popularity from farmers in areas where insecticide resistance has become a serious problem.

Fig. 6 shown the results of field trials performed on the tobacco cutworm (*Spodoptera litura*) and on Thrips, which infest the green pepper. These field trials investigate the impact of pyridalyl upon the pirate bug (*Orius strigicollis*), a natural predator of *Thrips palmi*, as well as against the tobacco cutworm (*Spodoptera litura*) and Thrips, which are target pests for pyridalyl. As shown in Fig. 6, pyridalyl demonstrated as high an insecticidal efficacy against the tobacco cutworm (*Spodoptera litura*) and Thrips as that of chlorfuruazuron, a reference insecticide. As well, in regions treated with pyridalyl, the population of pirate bugs (*Orius strigicollis*) was maintained at the same level as, or greater than that of the untreated areas. These results prove that pyridalyl possesses high pest control efficacy against pests such as Lepidoptera and Thrips, yet has minimal impact upon the natural predators of these pests. Therefore, we have concluded that pyridalyl is an excellent compound for use in IPM, which can control lepidopterous pests, while at the same time not inhibiting the predacious behavior of the natural predators that are native in the field. In addition, it is possible to conduct pest control activities by combining the application of pyridalyl with natural enemy products.

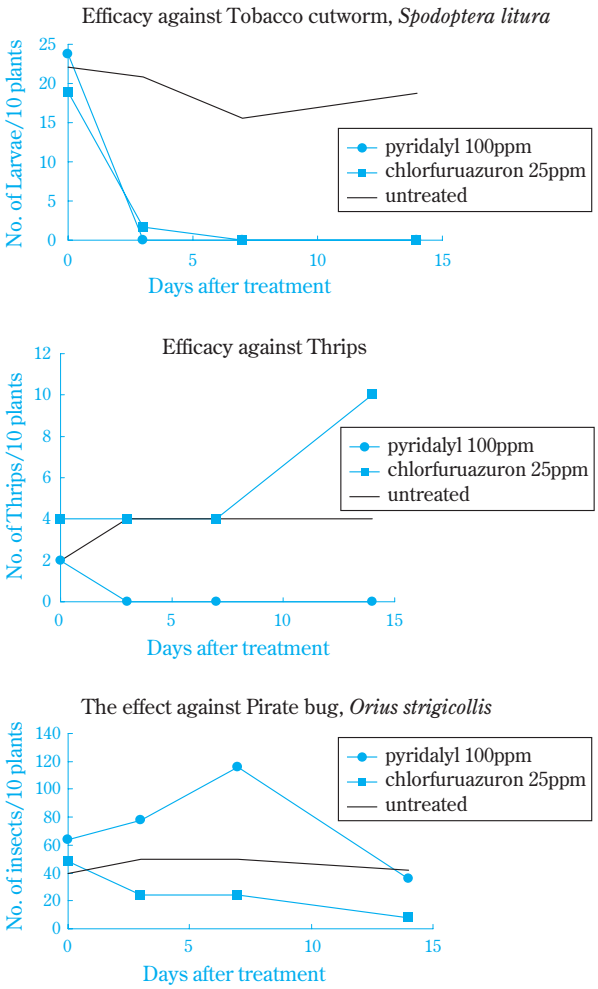
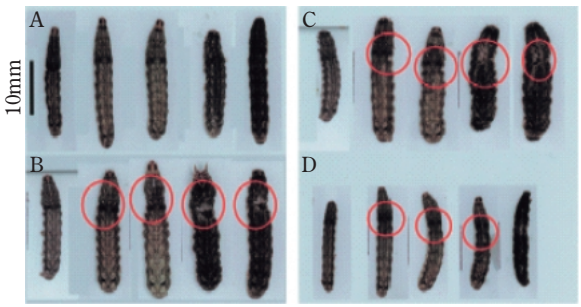


Fig. 6 The efficacy against Tobacco cutworm, *Spodoptera litura*, and Thrips with effect against the population of Pirate bug, *Orius strigicollis* of pyridalyl

4. Action Mechanisms

As a result of comparisons between the action mechanisms of pyridalyl and those of existing insecticides, we have confirmed that pyridalyl, does not act upon the nervous system, as do organo-phosphoric pesticides and synthetic pyrethroids; does not inhibit insect growth, as do IGR agents; and does not inhibit the respiratory system.

After 100 ppm of pyridalyl was locally applied to the surface of a tobacco cutworm (*Spodoptera litura*) larva, it died a few hours later. However, after application the larva did not vomit up body fluids or go into convulsions, which are both typical symptoms of lethality. Instead, the entire body of the larva merely appeared to go flaccid. Furthermore, when pyridalyl is applied topically at sub-lethal doses, the treated skin becomes blackened, indicating necrosis (**Fig. 7**).



Development of *S. Iitura* larvae in the control (A) and sub-lethal symptoms caused by lower dosages of pyridalyl (B, C, D). Each larva was photgraphed 1 to 5 days after treatment (left to right). The larvae in the control group (A) developed normally. B, C and D shows symptoms (circled area) occurring in larvae treated with pyridalyl at 1.56, 6.25 and 25ng, respectively. The larvae treated with 25ng of pyridalyl turned black and died 5 days after treatment (D).

Fig. 7 The effect of topical application of pyridalyl on Tobacco cutworm, *Spodoptera litura*

From this phenomenon, we infer that pyridalyl possesses a certain type of toxicity for insect cells.[10] We therefore investigated pyridalyl's action upon cultured insect cells (Sf9) and found that pyridalyl inhibited the cell growth. It appeared as though this action was caused by the artificial inhibition of proteins. Thus, we conducted an experiment using the uptake of [^{3}H] leucine as an index. In this experiment, pyridalyl demonstrated a concentration-dependent inhibitory effect and showed obvious protein inhibitory action within a short period of time, even at very low concentrations of under 0.1μM. The activity levels for pyridalyl were significantly higher than that for cycloheximide, which inhibits protein synthesis in cultured mammalian cells. When this same experiment was repeated using mammalian cells, both the inhibitory effect on cell growth and the protein synthesis inhibitory effects of pyridalyl were significantly lower than those of cycloheximide.[11] From these findings, we have concluded that pyridalyl demonstrates insecticidal activity by selectively inhibiting protein synthesis in insect cells.

Method of Manufacture

Since pyridalyl possesses 3 ether linkages, a number of different manufacturing routes can be considered, depending upon the order of condensation of the 4 units that are separated by each ether linkage. **Fig. 8** depicts some typical methods of manufacture. These methods include one in which the pyridine skeleton section is bonded at the end; one in which the dichloropropenyl group is bonded at the end; and one in which the ether linkage in the middle of the pyridalyl molecule is formed at the end. After a thorough evaluation of these methods, we then established a commercial method of manufacture that enables the production of high purity pyridalyl at high yields.[12)–15)]

Physical Properties and Formulations

1. Physical and Chemical Properties

Table 4 depicts the physical and chemical properties of pyridalyl. Pyridalyl is an odorless liquid having a vapor pressure of 6.24×10^{-8} Pa (25°C). Although soluble in most organic solvents, it is not readily soluble in water.

Table 4 Physical and chemical properties of pyridalyl

ISO Name	pyridalyl
Code Number	S-1812
Chemical Name (IUPAC)	2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether
Trade Name	Pleo
CAS RN	179101-81-6
Molecular Formula	$C_{18}H_{14}Cl_4F_3NO_3$
Molecular Weight	491.1
Physical Form	Liquid (20°C)
Odor	Odorless (20°C)
Density	1.445 g/cc (20°C)
Melting Point	< –17°C
Vapor Pressure	6.24×10^{-8} Pa (25°C)
Solubility	Water : 0.15 ppb (20°C)
	Organic solvents: soluble in most

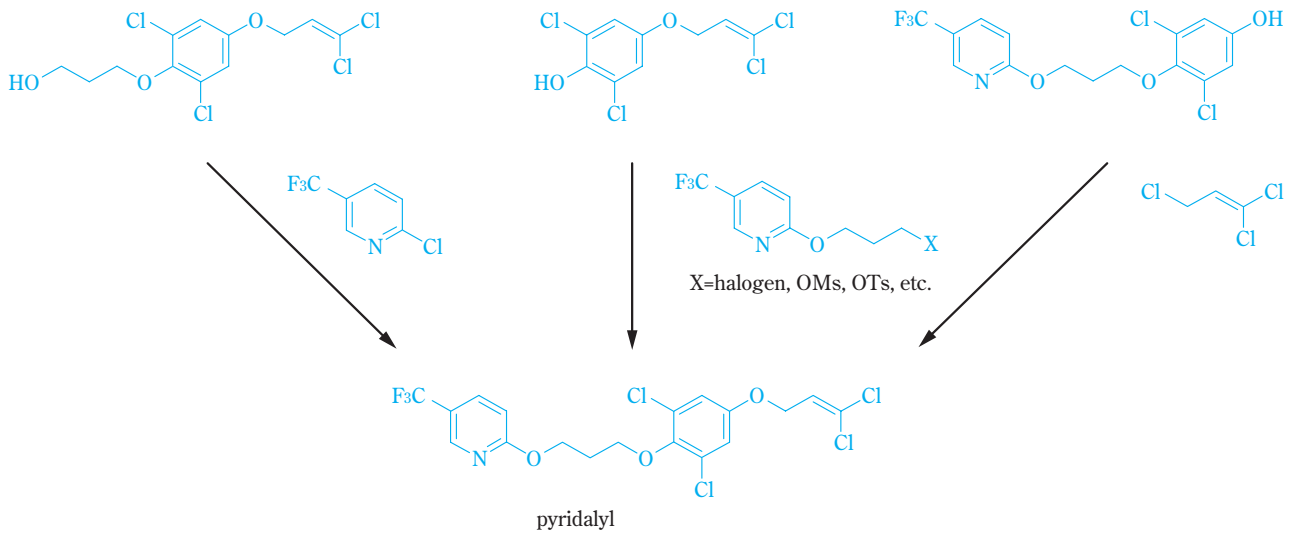


Fig. 8 Synthetic routes to pyridalyl

2. Stability

Table 5 depicts the results of stability tests performed on pyridalyl. Pyridalyl was found to be stable under all of the following conditions: after 2 weeks at a temperature of 54°C; after 3 months at a temperature of 40°C; and after 3 years at ambient temperatures.

Table 5 Stability of pyridalyl technical grade

Storage conditions	Storage period	Remaining content (%)
Ambient temperature	6 months	100.1
	12 months	99.6
	18 months	99.9
	24 months	99.7
	36 months	99.6
40°C	1 month	100.8
	2 months	100.0
	3 months	100.4
54°C	1 week	100.0
	2 weeks	100.1

3. Formulations

In Japan, pyridalyl formulation progressed primarily for the purpose of controlling pests that feed upon vegetable crops. As a result of this development, "Pleo®flowable" (pyridalyl: 10%) was formally registered and launched on the market in August 2004. This product's special features include being odorless, non-irritant, safe and environmentally friendly.

"Pleo®flowable" also has lower viscosity than that of conventional "flowable" insecticides, allowing it to be easily discharged from containers. Furthermore, when diluted with water it demonstrates excellent dispersibility. Since little foaming occurs during dilution, the resulting solution is readily sprayable, indicating outstanding formulation design. In addition, during the design process for "Pleo®flowable," special measures were undertaken to optimize insecticidal efficacy against target pests. **Table 6** depicts typical physical and chemical properties of "Pleo®flowable," indicating that it possesses an excellent combination of properties and outstanding preservation stability.

Table 6 Physical and chemical properties of Pleo®flowable

Items	Typical value (Methods)
Appearance	Whitish viscous liquid (Visual observation)
Density	1.0 g/cc (CIPAC MT3.3.2)
pH	6.2 (Electric pH meter, without dilution)
Viscosity	1070 mPa·S (Brookfield viscometer, Spindle No.2, 6 rpm, 25°C)
Suspensibility	98 % (CIPAC MT41, 1000 times, 20°C, 15 min)
Stability	Physical and chemical properties after storage at ambient temperature for 4 years were very stable

As for overseas markets, in 2004 a 10% flowable product was launched in Korea. During 2005, a 10% emulsifiable concentrate (EC) was put onto the market, primarily within South East Asian countries. A 50% emulsifiable concentrate (EC) is planned for launch in many Middle Eastern countries during 2005, as well as in Australia during 2006. Furthermore, in the US, development is currently proceeding on a 35% wettable powder (WP), and a 10% flowable product is being formulated in Europe, for an early market launch.

4. Methods of Analysis

The active ingredients in pyridalyl and its formulations can be precisely analyzed with high accuracy using a liquid chromatography-based standard internal method that utilizes L-column ODS for the column and water / acetonitrile for the mobile phase (20:80). Impurities within the pyridalyl can also be analyzed using a liquid chromatography-based method that utilizes the same type of column.

Safety for Mammals and the Environment

1. Safety for Mammals

(1) Acute Toxicity, Irritation and Dermal Sensitizing Potential

(i) Pyridalyl (Progenitor)

When tested using rats, pyridalyl was found to have very low oral, dermal and inhalation toxicities (**Table 7**). Although the dermal sensitizing potential of pyridalyl was positive under the Maximization test, the skin and eye irritating potentials were non-irritating and minimally irritating, respectively.

Table 7 Acute toxicity studies with pyridalyl and Pleo®flowable

Compound	Administration route	LD_{50} (mg/kg) Rat (SD)	
		Male	Female
pyridalyl	Oral	>5000	>5000
	Dermal	>5000	>5000
	Inhalation[a)]	>2010	>2010
Pleo®flowable	Oral	>2000	>2000
(10% pyridalyl formulation)	Dermal	>2000	>2000

a) LC_{50} (mg/m^3), 4 hours inhalation from nose

(ii) "Pleo®flowable" (10% pyridalyl formulation)

"Pleo®flowable" was found to have very low oral and dermal toxicities (Table 7). Although the dermal sensitizing potential was positive under the Buehler method, the skin and eye irritating potentials were mildly irritating and minimally irritating, respectively.

(2) Mutagenicity

Table 8 depicts the results of mutagenicity tests of pyridalyl. The result of *in vitro* chromosomal aberration test using Chinese hamster lung cells (CHL/IU) was weakly positive in the presence of a drug-metabolizing enzyme system (S9mix). However, negative results were obtained in all of the following tests: reverse mutation test (Ames test) using bacteria; micronucleus test using mice; gene mutation test using cultured mammalian cells; and *in vivo / in vitro* unscheduled DNA synthesis (UDS) test using rats. From these results, we have concluded that pyridalyl possesses no concern in mutagenicity.

(3) Short Term Toxicity, Long Term Toxicity and Carcinogenicity

Table 9 depicts the studies of pyridalyl for short term toxicity, long term toxicity and carcinogenicity using rats, mice and dogs, at the non-toxic dosages specified. The various toxicity studies indicate that the organs primarily affected by pyridalyl are the liver, kidney, lung, adrenal glands and ovaries. The effects upon each organ are briefly described below.

Liver: in dog, the following effects were observed at dosages of 80 mg/kg/day and higher: vacuolation of midzonal hepatocytes; hypertrophy accompanied with increased liver weight; and increased liver enzyme activity. In rats, the following effects were observed at dosages of 1,000ppm and higher: hypertrophy accompanied with increased liver weight; increase in the number of single cell necrosis of hepatocytes; increased total cholesterol; and increased γ-glutamyl peptidase. In mice, increased liver weight was observed at dosages of 2,500 ppm.

Ovaries: In rats, at dosages of 1,000ppm and higher, vacuolation of interstitial gland cell was observed.

Lung: in dog, the following effects were observed at dosages of 100 mg/kg/day and higher, increased lung weight; hyperplasia of arterial and arteriolar walls; and lymphocytes infiltration around blood vessels. In rats, foamy cell aggregation was observed at dosages of

Table 8 Mutagenicity studies with pyridalyl

Study	Test System	Study Condition	Result
Reverse mutation (Ames test)	*S.typhimurium* : TA100, TA98, TA1535, TA1537 E.coli : WP2*uvrA*	–S9mix : 9.77 ~ 313μg/plate +S9mix : 39.1 ~ 1250μg/plate	Negative
In vitro gene mutation	Chinese hamster ovary cells (CHO-K1-BH4)	–S9mix : 9.4 ~ 300μg/mL +S9mix : 2.0 ~ 10.0μg/mL	Negative
In vitro chromosomal aberration	Chinese hamster lung cells (CHL/IU)	–S9mix : 20 ~ 1250μg/mL +S9mix : 15 ~ 25μg/mL	Weakly positive (+S9mix)
Micronucleus	Mouse (CD-1), 5 males/group	500, 1000, 2000mg/kg (single oral administration), 24hr (all doses) & 48h (2000mg/kg)	Negative
In vivo/in vitro unscheduled DNA synthesis	Rat (SD), 3 males/group	500, 1000, 2000mg/kg (single oral administration), 2 ~ 4hr & 15 ~ 16hr	Negative

Table 9 Short term, long term and carcinogenicity studies with pyridalyl

Species	Administration period	Administration route	Dose	NOAEL
Dog (Beagle)	13 weeks	Oral (capsule)	10, 100, 1000 (300)mg/kg/day	10mg/kg/day
Dog (Beagle)	52 weeks	Oral (capsule)	1.5, 5, 20, 80mg/kg/day	20mg/kg/day
Rat (SD)	13 weeks	Oral (dietary)	100, 1000, 2000ppm	Male: 5.56mg/kg/day (100ppm), Female: 6.45mg/kg/day (100ppm)
Rat (SD)	104 weeks	Oral (dietary)	30, 100, 500, 1000ppm	Male: 3.40mg/kg/day (100ppm), Female: 4.10mg/kg/day (100ppm)
Mouse (CD-1)	78 weeks	Oral (dietary)	15, 50, 1000, 2500ppm	Male: 5.04mg/kg/day (50ppm), Female: 4.78mg/kg/day (50ppm)

Table 10 Developmental and reproductive toxicity studies with pyridalyl

Study	Species	Administration period	Administration route	Dose	NOAEL
Developmental Toxicity	Rat (SD)	Organogenesis (GD 6-19)	Oral (gavage)	10, 50, 250 mg/kg/day	No teratogenicity P: 10mg/kg/day F1: 250mg/kg/day
Developmental Toxicity	Rabbit (JW)	Organogenesis (GD 6-27)	Oral (gavage)	15, 50, 150 mg/kg/day	No teratogenicity P: 50mg/kg/day F1: 50mg/kg/day
2-Generation Reproductive Toxicity	Rat (SD)	10 weeks pre-mating, 2 weeks mating, 3 weeks gestation and 3 weeks lactation (P and F1), 5 weeks (F2)	Oral (dietary)	40, 200, 1000ppm	P: 40ppm (Male: 3.10mg/kg/day) (Female: 3.37mg/kg/day) F1: 40ppm Reproduction: 40ppm

1,000 ppm and higher.

Adrenal Gland: in dogs, vacuolation of zona fasciculate in adrenal cortex was observed in the adrenocortical fasciculate zone at dosages of 300 mg/kg/day. In rats, an increase in vacuolation of zona reticulate in adrenal cortex was observed at dosages of 2,000 ppm.

Kidney: in dogs, kidney weight increased and lipofuscin pigmentation occurred on renal proximal tubules, at 100 mg/kg/day and higher, however, no abnormalities were observed for related parameters. From these studies, it can be concluded that pyridalyl has low toxicological significance. In mice, an increase in kidney weight was observed at dosages of 2,500 ppm.

In the functional observation battery in Combined chronic toxicity and carcinogenicity study in rats, an increase of locomotor activity was observed at the highest dosages. However, we have concluded that pyridalyl does not posses specific neurotoxicity, due to the following reasons: no effects were observed in histopathlogical examination for brain and nervous system and no findings suggested the neurotoxicity were observed in other studies. The following findings suggested that pyridalyl does affect the metabolism of lipids: vacuolations in liver, ovary and adrenal gland cells, as well as lung foamy cell aggregation in both dogs and rats; and an increase in total cholesterol in rats. No carcinogenicity was observed for pyridalyl.

(4) Developmental and Reproductive Toxicity

Table 10 describes the results of developmental and reproductive toxicity studies. In teratology studies using rats and rabbits, neither teratogenicity nor embryo-fetal lethality were observed, even at dosages that had suppressed bodyweight gain and so on in the maternal animals. In a 2-generation reproduction study using rats, the following effects were observed in the P generation at dosages of 200 ppm and higher: suppressed bodyweight gain and food consumption; increased organ weights (thyroid, lung, testis, ovary); histopathological changes (thyroid and ovary); and decreased pup body weight. As well, in the F1 generation, the following effects were observed at dosages of 200 ppm and higher: delayed vaginal opening ; as well as suppressed bodyweight gain and food consumption, increased organ weights (thyroid, testis, ovary), histopathological changes (thyroid, ovary), and decreased pup body weight. In the F2 generation (female only), although some delayed vaginal opening was observed in the 1,000 ppm group, it was marginal and within the normal range for historical control data. No treatment-related abnormalities were observed in other reproductive parameters. Based on these results, it is concluded that 40 ppm (male: 3.10 mg/kg/day; female: 3.37 mg/kg/day) correspond to the no-observed-adverse-effect level (NOAEL) for parental animals. For reproductive performance and offsprings, 40 ppm was also NOAEL.

(5) General Pharmacological Study

To investigate the effect of pyridalyl on biofunction, effects on clinical signs, behavior, and the respiratory and circulatory systems were examined. No effects on clinical signs and behavior in rats were observed following oral administration of pyridalyl at 600 and 2,000 mg/kg. In anesthetized dogs intraduodenally given pyridalyl at 80, 400 and 2,000 mg/kg, tendency

of increased respiratory rate at 400 mg/kg and higher, and tendency of decreased respiratory systolic, diastolic, and mean blood pressure at 2000 mg/kg were observed. No abnormalities were observed in heart rate or electrocardiograms (PR interval, QRS duration, QT interval, and QTc). These results revealed that intraduodenal administration of pyridalyl causes increased respiratory rate and decreased blood pressure at dosed of 400 mg/kg and higher, but showed no effects on heart rate, electrocardiograms, clinical signs or behavior.

(6) Effects upon the Endocrine System

Since the results of above studies had suggested that the pyridalyl has the effect on the mammalian endocrine system (increases in the relative weights of the ovaries and the adrenal glands, slightly delayed vaginal dilation, etc.), we performed further investigations into the effects of pyridalyl upon mammalian hormonal biosynthesis, through both *in vitro* and *in vivo* testing. As a result of experiments using primary cell cultures from the testes and the ovaries, pyridalyl was noted to cause slight inhibition of 17β-HSD, which is a type of enzyme used in steroid hormones biosynthesis. Experiments were then performed in which pyridalyl was administered repeatedly to both male and female rats for 4 weeks, to examine the compound's effects upon the endocrine system (dosage: 100 ppm, 500 ppm, 1,000 ppm and 2,000 ppm). The results of these experiments made it clear that pyridalyl has no severe effect upon the endocrine system, as no effects were observed on hormone concentrations in the serum and in other organs, with only slight effects being observed in the prostate gland (weight of dorsum lobe) and in the ovaries (vacuolation of ovary interstitial gland cells). In addition, the NOAEL was obtained for the effect on the endocrine system. Furthermore, since pyridalyl demonstrated neither agonistic nor antagonistic effects at concentrations ranging from 10 nM ~ 1 mM using a variety of hormone receptors (ERα, AR and TRα) in reporter gene assays, we conclude that pyridalyl does not disrupt endocrine function via these receptors.

2. Animal / Plant Metabolism

(1) Pyridalyl Metabolism in Mammals

Pyridalyl labeled at ^{14}C of either the phenyl ring, the pyridyl group or the propenyl group (abbreviated as Ph-, Py- and Pr-^{14}C respectively) was prepared. Rats were given a single oral dose of these 3 compounds at dosages of 5 and 500 mg/kg to examine the absorption, distribution, metabolism and excretion. The administered pyridalyl was immediately absorbed, distributed throughout the entire body, metabolized and then excreted. The ^{14}C levels in the tissues 7 days after administration were as follows: 1.1% ~ 5.2% of the total dose of Ph- and Py-^{14}C; and 5.4% ~ 10.0% of the total dose of Pr-^{14}C. Relatively high concentrations of ^{14}C was observed in adipose tissue, the adrenal glands and the ovaries, since a relatively high concentration of unchanged pyridalyl was distributed to these tissues. In addition, as for Pr-^{14}C, since ^{14}C was incorporated into biological macromolecule, such as triglycerides and amino acids, it was observed that the residual concentrations of ^{14}C were higher than those for Ph- and Py-^{14}C.

The accumulation of ^{14}C in tissues, after oral administration of Ph-^{14}C to rats over a consecutive 14-day period was examined. Although the ^{14}C concentrations in adipose tissue were elevated to quite high concentration, they were constant at the 14 days and once administration had ceased, ^{14}C concentrations decreased with the biological half-life time of 10 ~ 15 days. In other organs and tissues, ^{14}C concentrations became constant within 10 days after initial administration, disappeared immediately once administration had ceased. From these results, we have concluded that pyridalyl accumulation does not pose a problem.

We have determined that pyridalyl was metabolized by following reactions in rats, and then they were excreted: (1) cleavage of the ether linkage between the benzene ring and the propenyl group; (2) oxidation of the propenyl group; (3) hydroxylation of the pyridine ring at position 3; (4) cleavage of the ether linkage between the trimethylene chains and the pyridine ring; (5) cleavage of the ether linkage between the trimethylene chains and the benzene ring; (6) glucuronic acid conjugation and sulfate conjugation of the phenol hydroxy group generated in (1), as well as the pyridine hydroxy groups generated in (3) and (4); and N-methylation of the pyridine ring (**Fig. 9**). The primary metabolites are S-1812-DP produced in (1) and CO_2 created in (2). No sex differences were observed in either metabolism or pharmacokinetics.

(2) Pyridalyl Metabolism in Plants

Metabolism study was conducted on 3 different

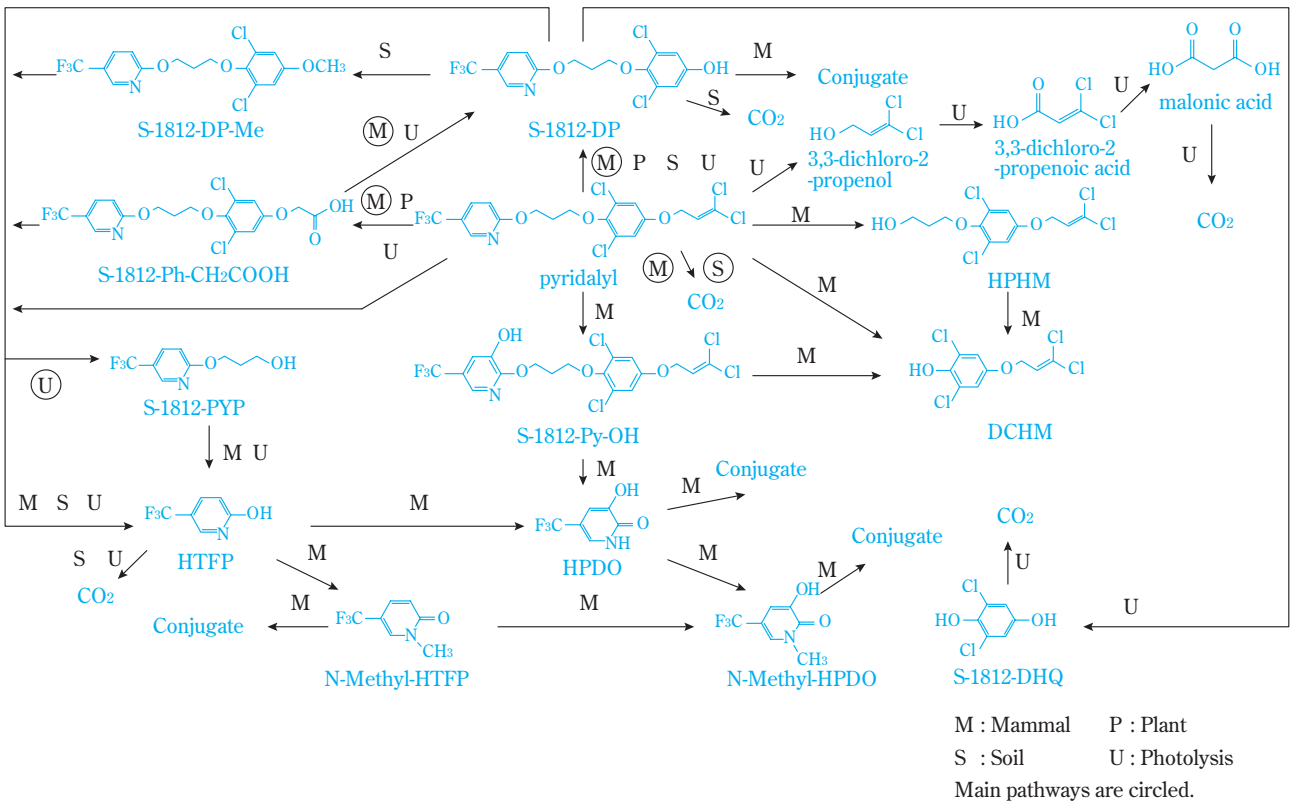


Fig. 9 Proposed metabolic and degradation pathways of pyridalyl

crops, using pyridalyl labeled with ^{14}C. In all cases, pyridalyl was metabolized to S-1812-DP due to cleavage of the propenyl ether linkage. Furthermore, the production of S-1812-Ph-CH_2COOH was also observed. This compound was formed via the oxidative cleavage of the double bond in the propenyl group. In addition, none of the metabolism studies showed the presence of metabolites that could have indicated cleavage of the pyridyl ether linkage. The propenyl group was further metabolized into a low molecular weight compound and converted into several polar metabolites, including cellular components, such as glucose. No uptake of either pyridalyl or its soil metabolites was observed (Fig. 9).

3. Safety Toward the Environment

(1) Environmental Fate and Residue

(i) Degradation in Water

Pyridalyl is stable in water and shows only slight degradation when dissolved in sterile buffer solutions at pHs 4, 7 and 9, at 25°C. On the other hand, pyridalyl undergoes photolysis in water with a half-life of approximately 9 days in buffer and only 4 days in humic water by exposure to simulated sunlight whose irradiance was equivalent to that in Tokyo in April-June. The primary degradation pathways were as follows: production of S-1812-Ph-CH_2COOH due to cleavage of the double bond in the propenyl group; production of S-1812-DP, S-1812-PYP and HTFP due to cleavage of ether linkages at 3 locations. Pyridalyl ultimately mineralized to carbon dioxide.

(ii) Degradation in Soil

Pyridalyl labeled with ^{14}C was applied to Ushiku soil at the maximum proposed rate on dry soil basis. The soil was then incubated in a dark at 25°C, where it underwent gradual degradation via the following pathways: S-1812-DP was produced from cleavage of the propenyl ether linkage. Subsequently, the hydroxy group of phenol underwent methylation (S-1812-DP-Me) and the pyridyl ether linkage underwent cleavage to form 2-hydroxy-5-trifluoromethylpyridine (HTFP). In the soil, pyridalyl ultimately mineralizes to carbon dioxide or produces unextractable bound residues.

(iii) Field Dissipation

Field dissipation studies were conducted in two locations, Kochi and Iwate in Japan following 4 applications with the spray solutions where pyridalyl 10% flowable was diluted by a factor of 1000. The interval of applications was 7 days and the application rate per treatment was 200L/10a. The maximum residues of pyridalyl

were found in the range of 0.68 ppm to 1.64 ppm on 0 day and 3 days after the last application. The values of DT_{50} were 3 to 8 months.

(iv) Mobility within Soil

As pyridalyl possesses high lipophilicity, it is strongly adsorbed to the soil. Thus, we have concluded that it possesses very little soil mobility.

(v) Residue in Crops

Residue trials were conducted in 9 crops including 3 crop groups from root, fruit and leaf vegetables following 2 or 4 foliar applications with the spray solution where pyridalyl 10% flowable was diluted by a factor of 1000. The interval of applications was 7 days and the application rate per treatment was 100 - 250L/10a. The crops were harvested on 1-14 days after the last applications. The maximum residues were below approximately 3 ppm.

(vi) Residues in Rotational Crops

Rotational crop residue study were conducted using chinese cabbage and Japanese radish in field after tomatoes previously treated with pyridalyl were harvested. The residues in all rotatinal crops tested were < 0.01 ppm.

(2) Effects upon Non-Target Organisms

The effects of pyridalyl upon beneficial arthropods are as described in the previous section (Table 3). The results of toxicity testing performed upon certain aquatic organisms and birds are summarized in **Table 11**.

Table 11 Summary results of toxicity tests on non-target organisms

Species	Study	Results
Carp	Acute	96hrLC_{50} > 10 mg/L
Alga [1)]	Acute	72hrEC_{50} > 10 mg/L
Daphnia magna	Acute	48hrEC_{50} = 3.8 μg/L
Bobwhite quail	Acute	LD_{50} > 2250 mg/kg

1) *Selenastrum capricornutum*

(i) Effects upon Aquatic Organisms

Pyridalyl was found to have low toxicity in carp: 96hr LC_{50}, > 10 mg/L. Pyridalyl also demonstrated low toxicity toward algae (*Selenastrum capricornutum*): 72 hr EC_{50}, > 10 mg/L. In contrast, pyridalyl was highly toxic to *Daphnia magna*: 48hr EC_{50}, 3.8μg/L.

Since the aqueous solubility of pyridalyl is extremely low (0.15μg/L), the maximum concentrations in actual water systems would not exceed this value, thus providing an adequate margin of safety, even when compared to the toxicity level for *Daphnia magna*. Thus, we can conclude that pyridalyl has relatively insignificant effects upon aquatic organisms.

(ii) Effects upon Birds

The results of toxicity test performed on the Bobwhite quail indicated an LD_{50} > 2,250 mg/kg. Therefore, we conclude that pyridalyl also has relatively insignificant effects upon birds.

As described above, only *Daphnia magna* demonstrated susceptibility to low concentrations of pyridalyl. However, based on the environmental behavior of pyridalyl, its effects upon ecosystems are expected to be extremely minor and we conclude that pyridalyl can be safely utilized in natural environments

Conclusion

Pleo®flowable (active ingredients: pyridalyl, test code: S-1812) is a proprietary novel insecticide developed by Sumitomo Chemical Co. Ltd. It functions via new action mechanisms that are completely different from those utilized by existing agents, such as organophosphates, carbamates, synthetic pyrethroids and IGRs .

The results of a variety of experimental tests performed by Sumitomo Chemical show that Pleo®flowable demonstrates outstanding insecticidal efficacy toward lepidopterous pests, including the Diamondback moth (*Plutella xylostella*), *Helicoverpa armigera* and the tobacco cutworm, as well as against *Tysanopteran* insects, including *Thrips palmi*. In addition, its practical usage as an insecticide has been well-proven through numerous official efficacy trials conducted since 1998 at several public research institutions, sponsored by the Japan Plant Protection Association. Furthermore, the efficacy of Integrated Pest Management (IPM) systems that utilize Pleo®flowable have also been proven through a series of special synergistic tests conducted over a 2-year period beginning in 2002. In particular, Pleo®flowable has been verified as an environmentally friendly insecticide that is suitable for use in IPM systems, for the following reasons: it possesses a high level of safety for humans, animals and fish; and it has minimal impact upon natural pest predators, such as parasitic wasps, pirate bugs (*Orius strigicollis*), lacewingladybugs, predatory mites and spiders,

as well as on pollinating insects, such as the honey bee and the bumble bee.

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