

Discovery and Development of a Novel Insecticide “Clothianidin”

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Clothianidin is a novel neonicotinoid insecticide possessing a thiazolyl ring which has been developed and commercialized by Sumitomo Chemical Takeda Agro Company, Ltd. The characteristics of neonicotinoids include a good systemic action and high insecticidal activity against sucking insect pests such as Hemiptera and Thysanoptera. Clothianidin is even effective for Diptera, Coleoptera and Lepidoptera pests and can be applied by a wide variety of treatment methods. This report describes the details of development, biological activity, safety and methods for synthesizing clothianidin.

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Introduction

It is a commonly known fact that agricultural chemicals have made a large contribution to the improvement of productivity in modern agriculture. At the same time, however, the ecological influence of such chemicals has been a matter of much concern. Furthermore, close attention is being paid to the problem of resistance in pathogenic insects, fungi and weeds because of the large amounts of limited types of agricultural chemicals used. If we limit ourselves to insecticides, there have been many reports of individual insect pests having high levels of resistance to widely used pesticides including organophosphor, carbamate and pyrethroid pesticides. Under these circumstances, compounds that are highly active for pests that are resistant to current pesticides with low toxicity to mammals and beneficial arthropods and that have little impact on the environment are preferable.

Clothianidin is a new neonicotinoid insecticide possessing a thiazolyl ring which was invented and developed by Sumitomo Chemical Takeda Agro Company, Ltd. (Takeda Chemical Industries Ltd, Agro Company at the time) (Fig. 1). This compound exhibits great biological efficacy in small amounts for a wide variety of pests such as Hemiptera, Thysanoptera, Coleoptera, Lepidoptera and Diptera, and a high level of opera-

tional safety has been confirmed for grains such as paddy rice, fruit trees and vegetables. A variety of application methods, such as spraying, nursery box application, planting hole application, root application and seed application are possible as the chemical application method. In addition, as a result of safety research, it has been discovered to be an insecticide that has low toxicity for mammals, birds and aquatic species with a high level of safety.

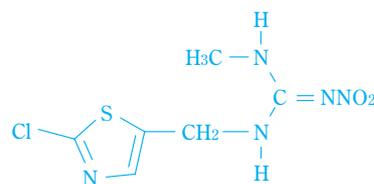


Fig. 1 Structure of clothianidin

In Japan, “Fullswing®” was registered as an agricultural chemical for lawn grass in December 2001, and “Dantotsu®” was registered as an agricultural chemical for food in April 2002.

In addition, it is being sold overseas starting with South Korea and Taiwan and in the U.S.A. and England as well as Hungary and the Ukraine through joint development with Bayer CropScience. In principle

countries other than these, progress is being made with practical testing with Hemiptera, Thysanoptera and Coleoptera as the principle targets.

History of Development

1. Neonicotinoid Compounds

There exist nicotinic acetylcholine receptors (nAChR) in the parts of the postsynaptic membrane connecting nerves in insects. Nereistoxin based compounds, which are antagonists, have long been known as agents that act on this location. A typical example is cartap hydrochloride (product name : Padan[®])¹⁾ (Fig. 2).

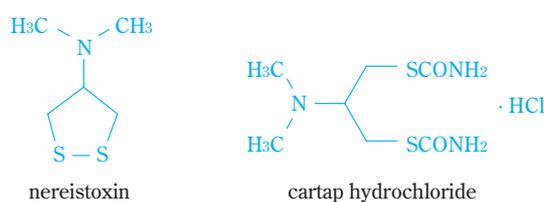


Fig. 2 Nereistoxin and cartap hydrochloride

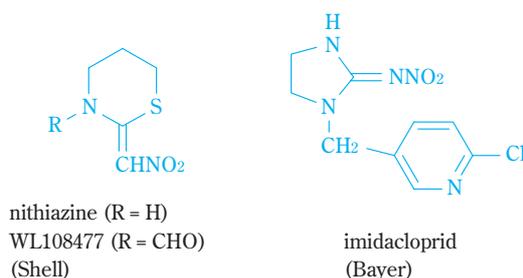


Fig. 3 Nithiazine and imidacloprid

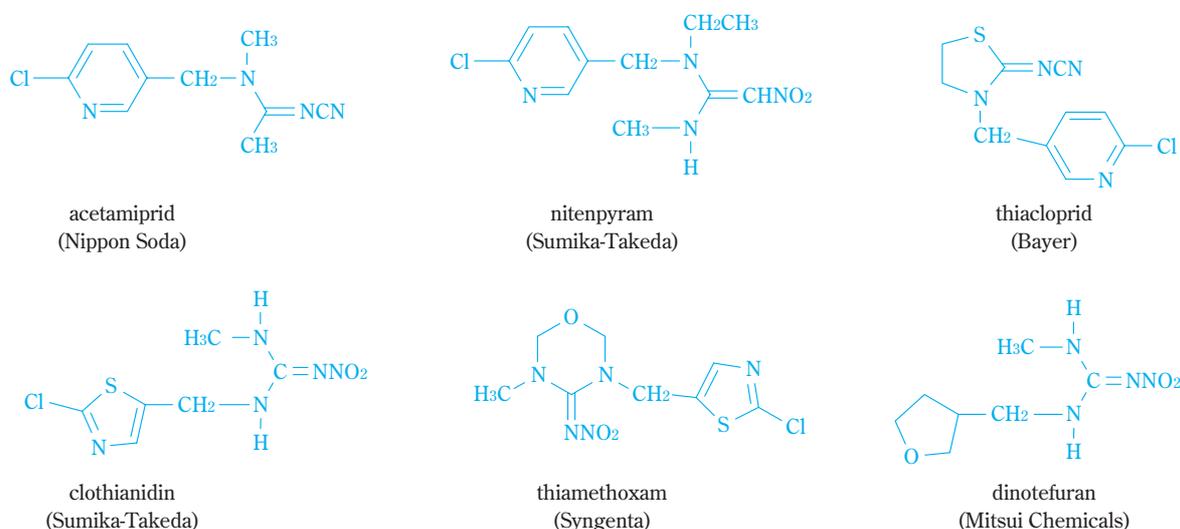


Fig. 4 Neonicotinoid insecticides developed after imidacloprid

In 1978, Shell discovered that nithiazine, which is a derivative of 1,3-thiazine that has a nitromethylene group, has strong insecticidal activity and clarified the fact that its mechanism is being an acetylcholine agonist.^{2), 3)} Since this substance has poor light stability, attempts were made at making it practical in the formyl form WL108477³⁾, but this did not make it onto the market. Taking this research as a hint, Nihon Tokushu Noyaku Seizo K.K. at the time (currently Bayer CropScience) discovered imidacloprid⁴⁾ with greatly improved activity against Hemiptera and chemical stability, and this was commercialized in 1991 (Fig. 3). Furthermore, various companies have been energetically carrying out research, and at present six insecticides containing clothianidin have been developed and marketed (Fig. 4). These compounds are all acetylcholine agonists similar to nicotine and nithiazine, and they have come to be called neonicotinoid (also called nitromethylene or chloronicotinyl) compounds in general. The use of neonicotinoids has rapidly increased because of their superior characteristics, and they have reached sales of \$1.4 billion worldwide and make up 18% of insecticides (2004).

2. Development of Nitenpyram

In the middle of the 1980's, the former Nihon Tokushu Noyaku Seizo K.K. published a large number of patent applications for heterocyclic compounds having nitromethylene groups.⁵⁾⁻⁹⁾ The structures of these are given by the typical Formula 1 in Fig. 5. At around the same time, Takeda Chemical Industries,

Ltd., discovered that noncyclic compounds **2a** and **2b** having nitromethylene groups exhibited insecticidal action against Hemiptera. This fact differed from the Shell conclusions^{2), 3)} that it was necessary to have a cyclic structure at the bonding part for the nitrometh-

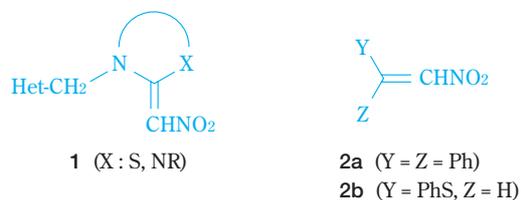


Fig. 5 Precursors for nitenpyram

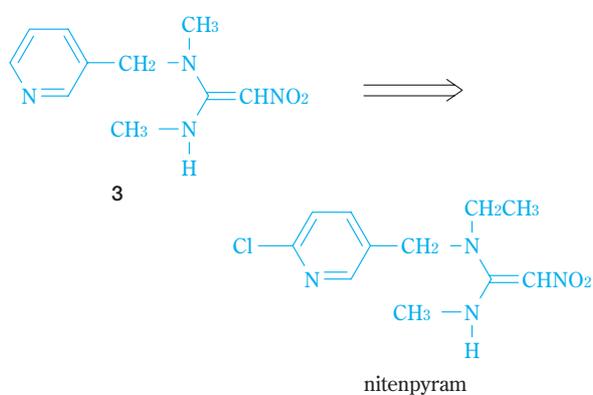


Fig. 6 Nitenpyram and the lead compound

ylene group. From this fact, it was surmised that a cyclic structure was unnecessary, and open ring compound **3** (Fig. 6) was synthesized. As a result of this, it was found that these compounds had comparatively strong insecticidal activity, so structural optimization was carried out, and nitenpyram (code number : TI-304; product name : Bestguard[®]) was selected.¹⁰⁾ The main targets of nitenpyram are Hemiptera and Thysanoptera.

3. From Nitenpyram to Clothianidin

Nitenpyram has several excellent features, such as high activity against Hemiptera, low toxicity for non-target species, systemic action and no cross resis-

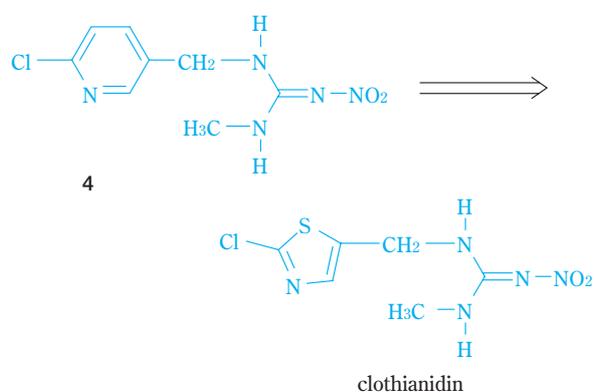


Fig. 7 Lead compound for clothianidin

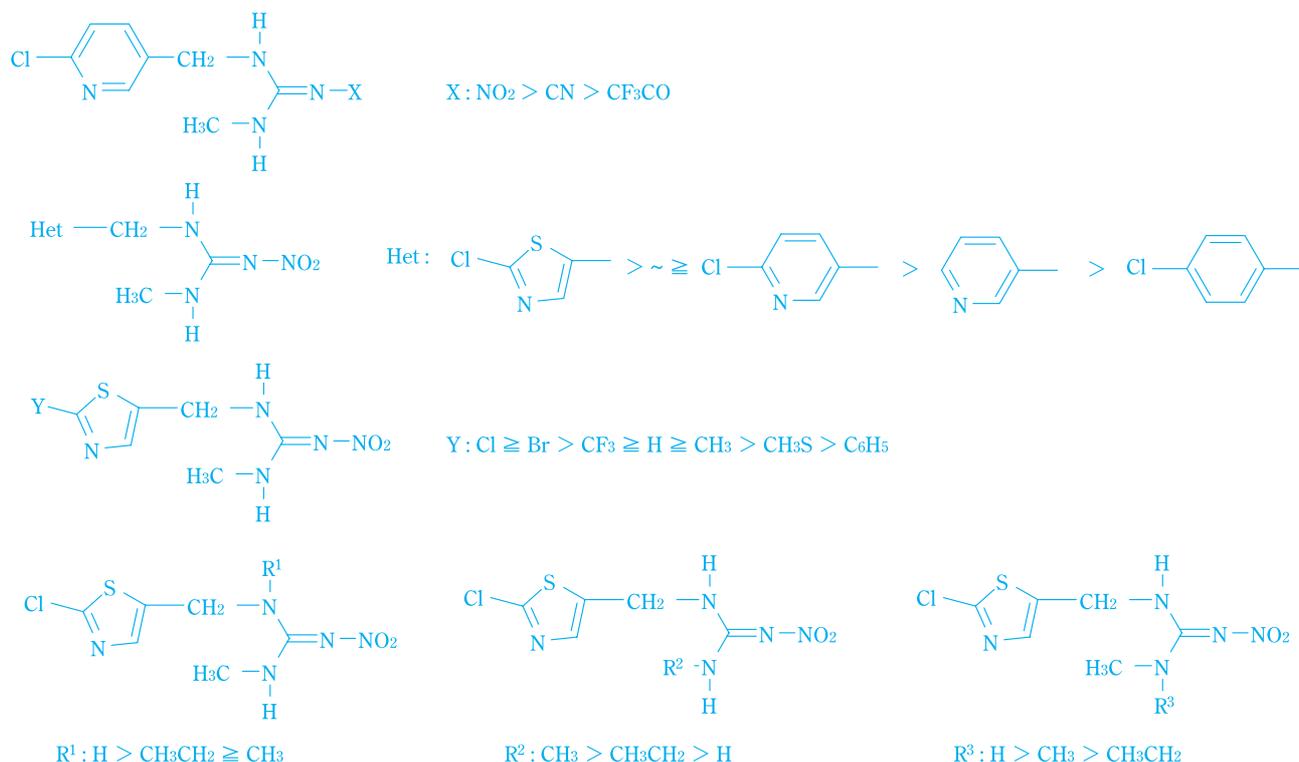


Fig. 8 Structure-activity relationships of nitroguanidine derivatives

tance with conventional insecticides. However, since the width of its insecticidal spectrum was not always satisfactory, research was continued further.

For compounds where the methylene group in nitenpyram was replaced with a nitroimino group, that is, nitroguanidine derivative **4** (Fig. 7), it was found that the effect on Hemiptera was maintained, and insecticidal activity was also exhibited on Lepidoptera, so this was optimized as the lead compound. The rough structure-activity-relationships of the main substituents are shown in Fig. 8. In the end, clothianidin was selected as the candidate compound based on basic activity on various pests, the results of field trials

and safety test data.¹¹⁾ Starting in 1995, work began on full-scale development of tests consigned to the Japan Plant Protection Association and in 2001 clothianidin made it to registration as an agricultural chemical.

Characteristics of the Biological Efficacy

1. Insecticidal Properties

Clothianidin is highly effective against a wide variety of insect species as described in Table 1. It shows good activity against Hemiptera, Coleoptera, Thysanoptera, Lepidoptera and Diptera species. When compared with fenitrothion (Sumithion®) which is a

Table 1 Insecticidal activity of clothianidin

Insect species	Stage ^{a)}	Methods ^{b)}	LC ₅₀ (mg a.i./L)		Ratio b/a
			clothianidin (a)	fenitrothion (b)	
Hemiptera					
<i>Nilaparvata lugens</i>	N3	LS	0.015	41	2700
<i>Laodelphax striatellus</i>	N3	LS	0.025	4.1	180
<i>Nephotettix cincticeps</i>	N3	LS	0.0006	4.5	7500
<i>Aphis gossypii</i>	A	LS	0.011	0.87	92
<i>Myzus persicae</i>	A	LS	0.21	> 20	> 95
<i>Bemisia argentifolii</i>	N1	LS	0.3	> 100	> 330
<i>Trigonotylus caelestialium</i>	A	IS	0.21	8.3	40
Coleoptera					
<i>Henosepilachna vigintioctopunctata</i>	L2	FD	0.051	> 1	> 19
<i>Diabrotica undecimpunctata</i>	L1	SI	0.16	—	—
Thysanoptera					
<i>Thrips palmi</i>	L1	LS	5.4	300	69
<i>Frankliniella occidentalis</i>	L1	LS	6.1	110	31
Lepidoptera					
<i>Chilo suppressalis</i>	L3	LS	0.28	6.6	37
<i>Spodoptera litura</i>	L3	LS	2.9	7.7	16
<i>Plutella xylostella</i>	L2	LD	59	3.1	13
<i>Carposina niponensis</i>	E	ED	0.24	1.2	18
Diptera					
<i>Liryomyza triflorii</i>	L1	LS	1.2	> 100	> 86

a) E : eggs, N : nymph, L : larva, A : adult, the numeral indicates the instar number.

b) LS : leaf spray, IS : insect spray, LD : leaf dipping, FD : fruit dipping, ED : egg dipping, SI : soil incorporation



Fig. 9 Target insect pests of clothianidin in citrus

wide spectrum agent, the activity is more than ten times higher for all taxonomical groups. Beside these insect groups, the compound is very effective against termites and fleas, and is used in practical situations in termite control agents (TakeLock[®] and Ariatol[®]AX). The wide spectrum insecticidal activity makes it possible to control many pest species simultaneously with a single application. Nursery box application of a granule formulation of clothianidin (Dantotsu[®]) effectively controls many kinds of rice insect pests such as rice plant hoppers and leaf hoppers (Hemiptera); rice water weevils and leaf beetles (Coleoptera); rice-stem borers and green rice caterpillars (Lepidoptera); and small rice leaf miners (Diptera) without any additive chemical application. In the fields of horticulture and fruit trees, practical control effects have been found for many important pest insects by foliar application. The pest insects shown in Fig. 9 are the target pests for clothianidin listed in the recommendation table for citrus trees.

2. Mode of Action

Neonicotinoid insecticides exhibit excellent insecticidal activity with a high level of safety for vertebrates. It has been shown that neonicotinoids act as agonists on nicotinic acetylcholine receptors (nAChR).^{12), 13)} Whereas neonicotinoids, including clothianidin, were ineffective on the chicken $\alpha 4\beta 2$ nAChR, they show high agonist actions at low concentrations on the *Drosophila* $D\alpha 2$ (SAD)/chicken $\beta 2$ nAChR. In general, the maximum response of the $D\alpha 2\beta 2$ nAChR to neonicotinoids is smaller than that to acetylcholine. However, the maximum response induced by clothianidin is greater than the acetylcholine-induced response (Fig. 10).¹⁴⁾ The "super agonist" action of clothianidin leads to its

characteristic insecticidal properties. Since the mode of action of clothianidin differs from that of organophosphates, carbamates, pyrethroids and IGRs, it can display a high level of activity against pest insects that have developed resistance to these existing compounds.

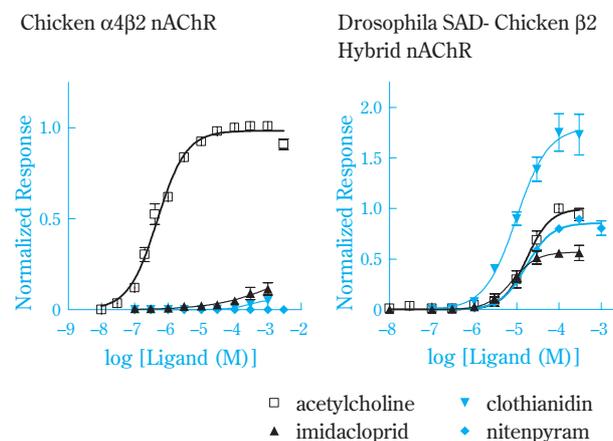


Fig. 10 The agonistic action of clothianidin and related chemicals against hybrid nicotinic acetylcholine receptors expressed in *Xenopus laevis* oocytes

3. Control Effects

While clothianidin is easily absorbed and transported in plants, it is very safe for crops. Making use of this characteristic, it is possible to select a variety of application methods.¹⁵⁾ In addition, long-term control effects can be obtained with a low dosage through its high insecticidal activity and proper chemical stability.

In paddy fields, nursery box application, foliar spray and paddy water application have been used to practical effect (Fig. 11).

Through soil applications for vegetables, such as

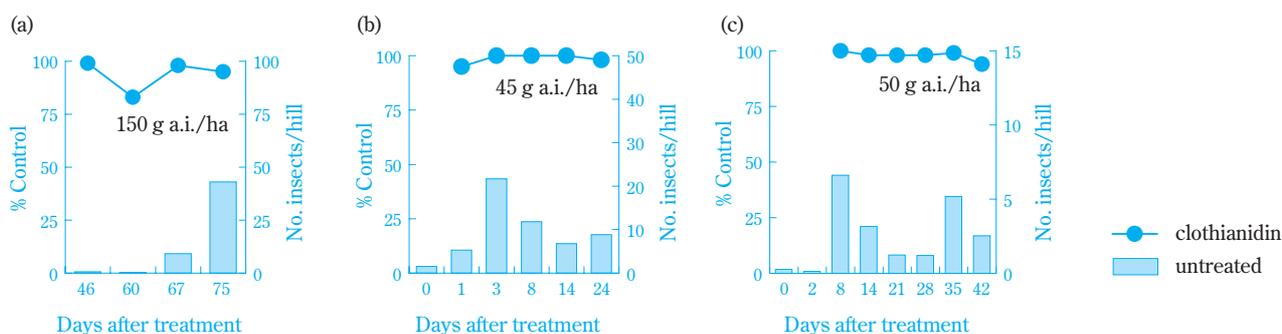
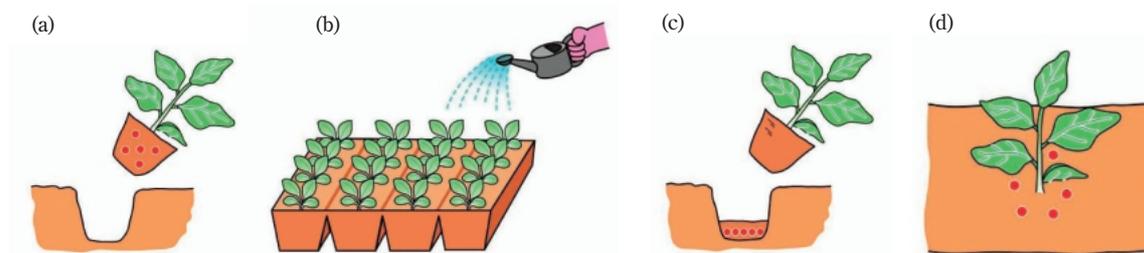


Fig. 11 Control of brown rice planthopper, *Nilaparvata lugens* by (a) nursery box application, (b) foliar spray, and (c) paddy water application



(a) nursery soil incorporation, (b) drench application, (c) planting hole application, (d) plant foot application

Fig. 12 Various soil application methods for vegetables

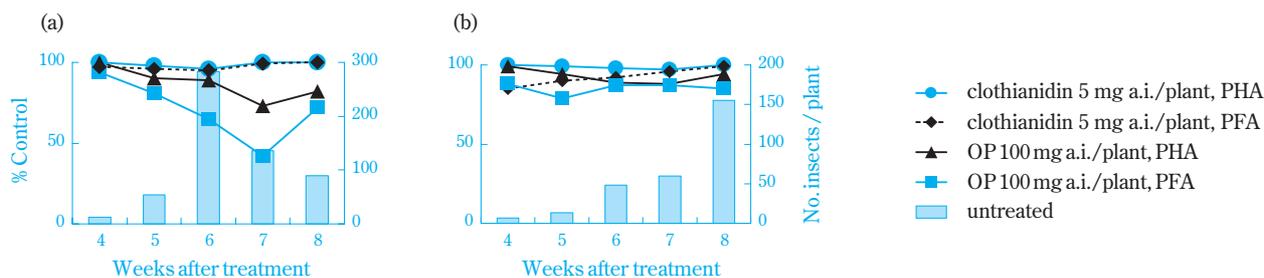


Fig. 13 Control of (a) cotton aphid, *Aphis gossypii*, and (b) green peach aphid, *Myzus persicae*, by planting hole application (PHA) and plant foot application (PFA)

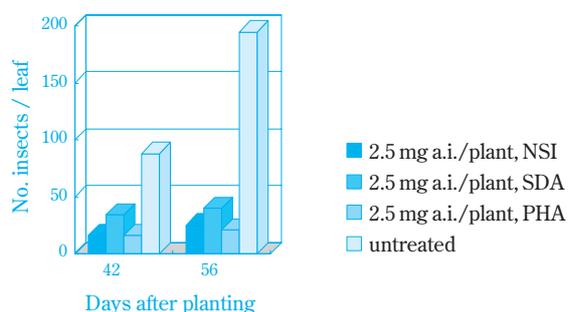


Fig. 14 Control of Silverleaf whitefly, *Bemisia argentifoli* by nursery soil incorporation (NSI), soil drench application (SDA), and planting hole application (PHA) of clothianidin

nursery soil incorporation, soil drench application before planting, planting hole and plant foot application during plant growth, aphids and whiteflies can be successfully controlled for approximately two months (Fig. 12, 13 and 14).

With fruit trees, it is possible to control citrus leaf miner by applying a highly-concentrated solution to the trunks of citrus trees (Fig. 15), and the labor for pesticide application can be reduced.

Furthermore, long-term control of aphids which infest the ears of wheat can be obtained by seed application of clothianidin (Fig. 16). The application

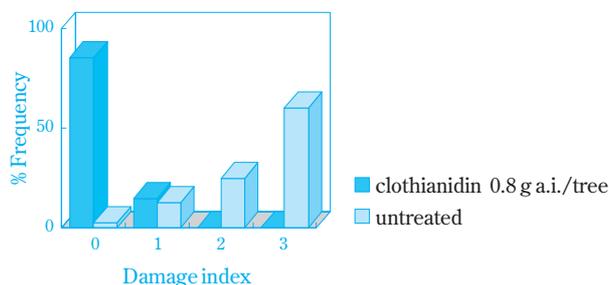


Fig. 15 Control of citrus leafminer, *Phyllocnistis citrella* by trunk spray application

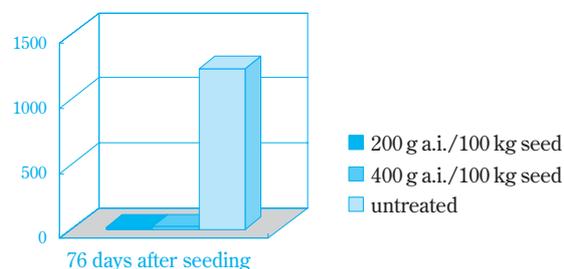


Fig. 16 Control of wheat aphid, *Rhopalosiphum padi* by seed treatment of clothianidin

method has been made practical by Bayer CropScience in the United States, and it has contributed to the control of major pests such as the corn rootworm (Coleoptera) that causes serious damage to corn.

Manufacturing Methods

When clothianidin was first synthesized, there was no method for obtaining the important intermediate 2-chloro-5-chloromethylthiazole (CCT) in a good yield, and this was the same as for the typical synthesis methods for nitroguanidine derivatives. However, a superior manufacturing route where industrial production was possible was discovered through subsequent investigations into manufacturing methods. Since other companies have applied for many patents for manufacturing methods for CCT and nitroguanidine derivatives, we will give an introduction that includes these.

1. 2-Chloro-5-chloromethylthiazole (CCT)

(1) Investigations by Sumitomo Chemical Takeda Agro Company, Ltd.

When clothianidin was first synthesized, only the production method¹⁶⁾ described by Fig. 17 where allyl isothiocyanate **5** and sulfuryl chloride or chlorine are reacted as the method for producing CCT was publicly known. Because this method requires large amounts of a chlorinating agent, a large number of impurities are produced as byproducts. The authors discovered a new manufacturing method¹⁷⁾ that uses 2-chloro-2-propenyl ester **6** instead of allyl ester.

(2) Manufacturing Methods by Other Companies

Since the discovery by Sumitomo Chemical Takeda Agro Company, Ltd. described above, there have been many patent applications by multiple companies

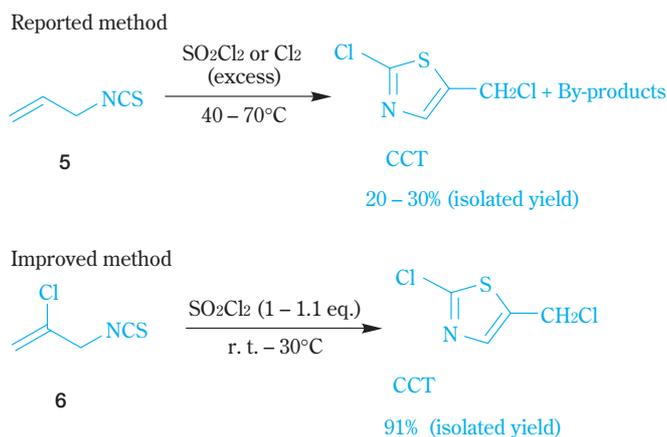


Fig. 17 Synthetic methods of CCT (1)

regarding CCT manufacturing methods. The main ones^{18)–21)} are noted in Fig. 18. Among these, the method in A is thought to have the highest level of practicality.

2. Forming the Guanidine Skeleton

(1) Investigations by Sumitomo Chemical Takeda Agro Company, Ltd.

Research was conducted with the goal of establishing a route for the synthesis of nitroguanidines for the synthesis of clothianidin and a series of its derivatives. As a result of keen examinations, we were able to establish the three routes^{22)–24)} shown in Fig. 19.

(2) Manufacturing Methods by Other Companies

Fig. 20 shows the routes published by patent appli-

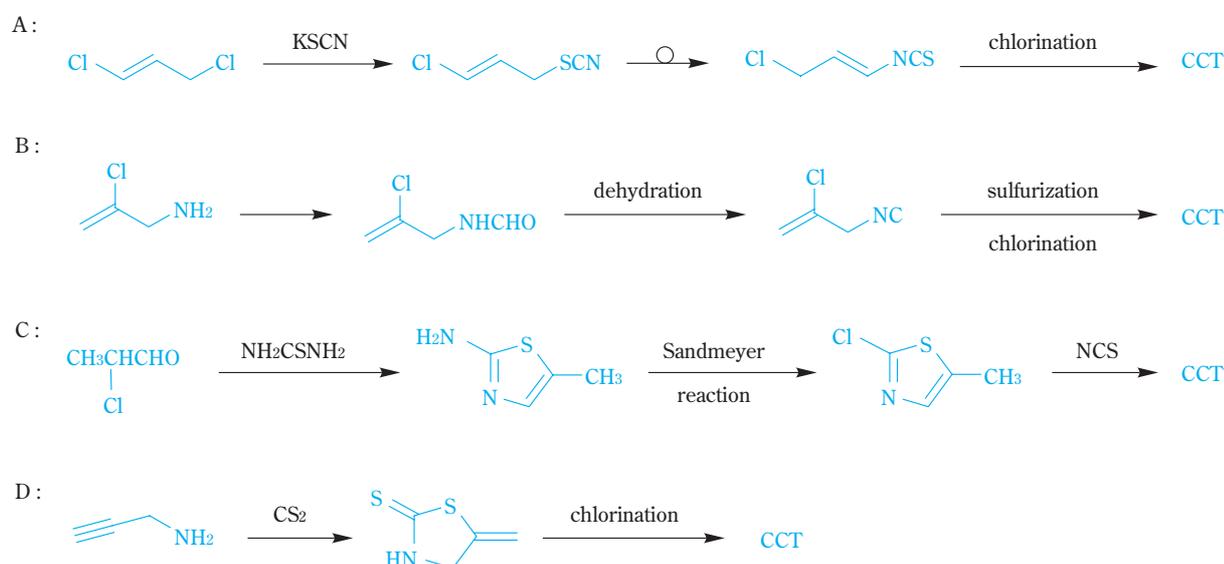


Fig. 18 Synthetic methods of CCT (2)

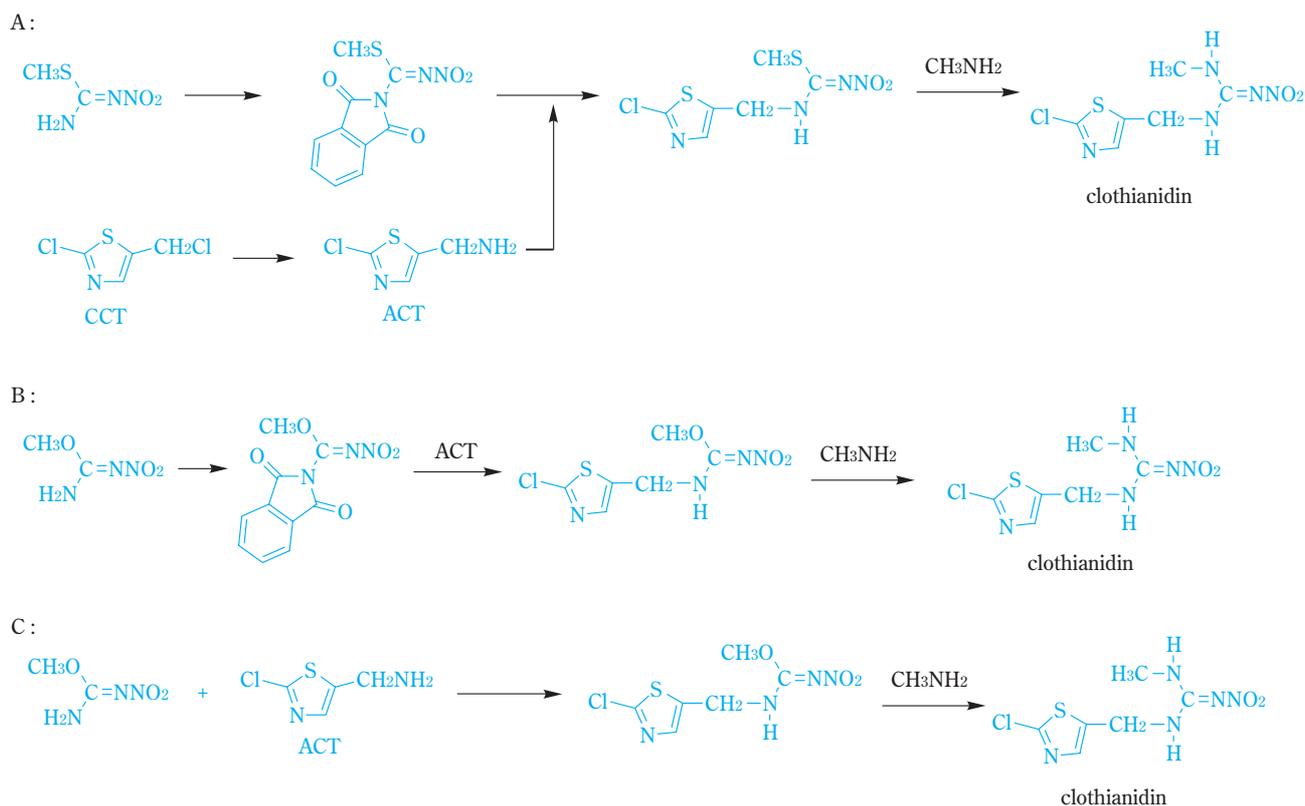


Fig. 19 Formation of guanidine moiety (1)

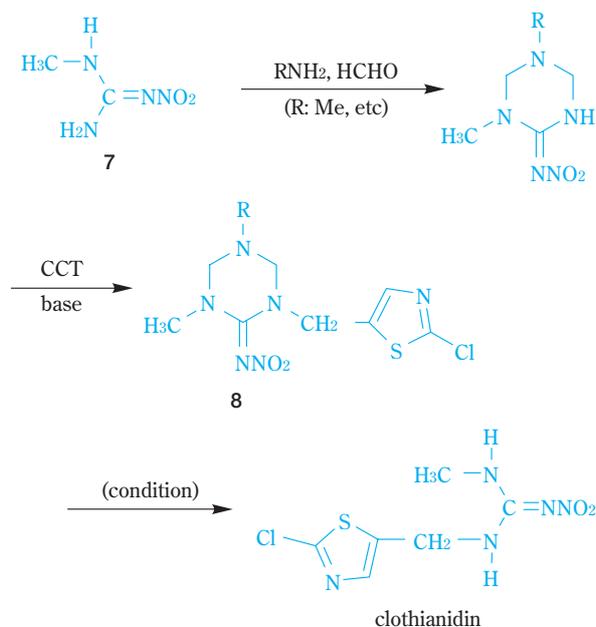


Fig. 20 Formation of guanidine moiety (2)

cations.²⁵⁾ The characteristics of these routes include reacting the compound obtained by the ring closure of methylisothiocyanate **7** with CCT and then opening the ring by hydrolysis of the resulting triazine **8**. There are multiple patent applications concerning processes for producing clothianidin from **8**.^{26)–29)}

Physical Properties and Formulation

1. Chemical Properties

The physical and chemical properties of clothianidin are given in **Table 2**. Clothianidin is a colorless and odorless solid having a vapor pressure of 1.3×10^{-10} Pa (25°C).

2. Stability

The results of stability tests performed on clothianidin are given in **Table 3**. Clothianidin was found to be stable under all of the following storage conditions: after 6 months at a temperature of 40°C ; and after 1 year at 25°C .

3. Analysis

The active ingredient in clothianidin can be precisely analyzed with high accuracy using a liquid

Table 2 Physical and chemical properties of clothianidin

ISO Name	clothianidin
Code Number	TI-435
Chemical Name (IUPAC)	(<i>E</i>)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine
Trade Name	DANTOTSU®
CAS Registry Number	210880-92-5
Molecular Formula	C ₆ H ₈ ClN ₅ O ₂ S
Color and physical state	Clear and colorless solid powder
Odor	Odorless
Density	1.61 g/ml (20°C)
Melting Point	176.8°C
Vapor Pressure	1.3 × 10 ⁻¹⁰ Pa (extrapolated, 25°C)
Solubility (Water)	0.327 g/L (20°C)
Dissociation constant (pKa)	11.09 (20°C)
Partition coefficient (log Pow)	0.7 (25°C)

Table 3 Stability of clothianidin technical grade

Storage conditions	Storage period	Remaining content (%)
25°C	3 months	99.2
	6 months	100.0
	9 months	100.2
	12 months	99.2
40°C	3 months	100.1
	6 months	100.4

chromatography-based standard internal method that utilizes an ODS based column, 0.1% acetic acid / acetonitrile (75 : 25) for the mobile phase. In addition, impurities within the clothianidin can also be analyzed using a liquid chromatography-based method that utilizes the same type of column.

4. Formulation

The chemical stability of clothianidin is relatively high, and its compatibility with inert ingredients, such

as carriers and surfactants, is also good. Additionally, there are few disadvantages of clothianidin in the physical properties, and therefore, a variety of formulations has been already designed. Many kinds of formulations are commercially available in Japan, and for solo products, eight formulations, namely 50% wettable powder, 16% water soluble granule, 1.5% granule for nursery box, 1.0% granule, 0.5% granule, 0.15% dust, 0.5% dust and 20% suspension concentrate agents have been marketed for agricultural use.

Safety for Mammals and the Environment

1. Safety for Mammals

(1) Acute Toxicity, Irritation and Skin Sensitization

The oral, dermal and inhalation toxicity for clothianidin in rats were all weak (Table 4). Slight irritation was observed for clothianidin for the eyes, but no irritation was observed for the skin and skin sensitization was negative in the maximization test.

Table 4 Acute toxicity studies with clothianidin

Animal	Administration Route	LD ₅₀ (mg/kg)	
		Male	Female
Rat	Oral	> 5000	> 5000
	Dermal	> 2000	> 2000
	Inhalation *)	> 6141	> 6141

*) LC₅₀ (mg/m³), 4 hours inhalation from nose

(2) Mutagenicity

Table 5 shows the results of mutagenicity tests for clothianidin. In *in vitro* chromosomal aberration tests using Chinese hamster lung cells (CHL), the result was weakly positive, but reverse mutation tests (Ames tests) using bacteria, micronucleus tests using mice,

Table 5 Mutagenicity studies with clothianidin

Study	Test System	Study Condition	Result
Reverse mutation (Ames test)	<i>S. typhimurium</i> : TA98, TA100, TA1535, TA1537, TA102	-S9mix : 16-5000 µg /plate +S9mix : 16-5000 µg/plate	Negative
<i>In vitro</i> gene mutation (V79-HPRT test)	Chinese hamster lung cells (V79)	-S9mix : 156-5000 µg/mL +S9mix : 156-5000 µg/mL	Negative
<i>In vitro</i> chromosomal Aberration	Chinese hamster lung cells (CHL)	-S9mix : 156.25-1250 µg/mL +S9mix : 625-1875 µg/mL	Weakly positive
Micronucleus	CD-1 Mouse, 5 animals/sex/group	25, 50 & 100 mg/kg (single oral administration), 24 hr, 48 hr & 72 hr	Negative
<i>In vivo</i> / <i>in vitro</i> Unscheduled DNA synthesis	Wistar Rat, 4-6 males/group	2500 & 5000 mg/kg (single oral administration), 4 hr & 16 hr	Negative

gene mutation tests using Chinese hamster lung cells (V79) and *in vivo/in vitro* unscheduled DNA synthesis (UDS) tests using rats were all negative. Based on the weight of the evidence presented above, clothianidin does not pose a genotoxic concern.

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

Table 6 shows the results of the short term toxicity, long term toxicity and carcinogenicity studies using rats, mice and dogs. The NOAEL for each study is also shown in Table 6.

In 13-week subchronic toxicity studies using rats, reduced body weight gain was observed in both sexes at a dosage of 3000 ppm, and an increase of hepatic drug-metabolizing enzyme activity (cytochrome P-450, etc.) was observed in males at a dosage of 3000 ppm. There were no treatment-related effects on organ weights at any dosage and the only treatment-related histopathological finding was spleen pigmentation in males at a dosage of 3000 ppm. In 13-week subchronic toxicity studies using dogs, decreased white blood cell counts and lymphocytes counts were observed in both sexes at a dosage of 2250 ppm, reduced body weight gain, decreased Ht values, segmented neutrophils and ALT values were observed in males at a dosage of 2250 ppm, and decreased total protein was observed in females at the same dosage. In addition, the increased incidence of thinness in both sexes and decreased albumin and ALT values in females were also observed at dosages of 1500 ppm and above.

In 24-month chronic toxicity and carcinogenicity studies using rats, reduced body weight gain and food consumption were observed in both sexes at dosages

of 1500 and 3000 ppm and in the females at a dosage of 500 ppm. Increased inorganic phosphorous and increased incidences of pelvic mineralization, hyperplasia of the pelvic transitional epithelium, hemorrhage and edema in the glandular stomach and eosinophilic foci of the liver were observed in the males at a dosage of 3000 ppm. In addition, increased incidences of erosion and edema of the glandular stomach and eosinophilic foci of the liver were observed in the females at a dosage of 3000 ppm. Furthermore, increased incidences of hyperplasia of ovarian interstitial glands were observed in the females at dosages of 500, 1500 and 3000 ppm. It was concluded in this study that there was no oncogenicity for clothianidin. In 18-month carcinogenicity studies in mice, there was a reduced body weight gain in both sexes at dosages of 1250 ppm and 2000 (males) / 1800 (females) ppm, and reduced food consumption was observed at dosages of 2000 (males) / 1800 (females) ppm. In addition, in histopathological examinations, increased incidences of hepatocellular hypertrophy were observed at dosages of 100 (males only), 1250 (males and females) and 2000 (males) / 1800 (females) ppm. These findings could be attributed to the induction of hepatic drug-metabolizing enzyme activities, which are a common pharmacological response to a xenobiotic and are not considered to be an adverse effect of clothianidin. It was concluded in this study that there was no evidence of oncogenicity for clothianidin. In 12-month chronic toxicity studies using dogs, increased incidences of localized erythema inside the ears and transient body weight loss during the first week of treatment were observed in males at a dosage of 2000 ppm, and reduced food consumption, decreased leuko-

Table 6 Short term, long term and carcinogenicity studies with clothianidin

Species	Administration period	Administration route	Dose	NOAEL
Dog (Beagle)	13 weeks	Oral (dietary)	325, 650, 1500, 2250 ppm	Male : 19.3 mg/kg/day (650 ppm) Female : 21.2 mg/kg/day (650 ppm)
Dog (Beagle)	52 weeks	Oral (dietary)	325, 650, 1500, 2000 ppm	Male : 36.3 mg/kg/day (1500 ppm) Female : 15.0 mg/kg/day (650 ppm)
Rat (SD)	13 weeks (Recovery group +7wks)	Oral (dietary)	150, 500, 3000 ppm	Male : 27.9 mg/kg/day (500 ppm) Female : 34.0 mg/kg/day (500 ppm)
Rat (SD)	104 weeks	Oral (dietary)	150, 500, 1500, 3000 ppm	Male : 27.4 mg/kg/day (500 ppm) Female : 9.7 mg/kg/day (150 ppm)
Mouse (CD-1)	78 weeks	Oral (dietary)	100, 350, 1250, 2000 (male) /1800 (female) ppm *)	Male : 47.2 mg/kg/day (350 ppm) Female : 65.1 mg/kg/day (350 ppm)

*) The initial dose level was 100, 350, 700, 1250 ppm. Animals received 700 ppm for Weeks 1 through 4; 2000 ppm for Weeks 5 through 10; 2500 ppm for Weeks 11 through 34; and 2000 ppm for males and 1800 ppm for females for Weeks 35 through termination.

cytes, neutrophils, red blood cell counts, Ht values and Hb values and an increase in adrenal to body weight ratio were observed in females at a dosage of 2000 ppm. The increased incidences of localized erythema inside the ears were also observed in females at dosages of 1500 ppm and above, and decreased ALT values were observed in both sexes at dosages of 650 ppm and above. The increase in adrenal to body weight ratio seen in females at a dosage of 2000 ppm was not considered to be a treatment-related change since no significant difference in absolute adrenal weight was observed and no related histopathological changes were observed. In addition, the decreased ALT values in both sexes at the dosages of 650 ppm or above were also not considered to be a treatment-related effect since no related histopathological changes were observed.

(4) Developmental and Reproductive Toxicity

Table 7 describes the results of developmental and reproductive toxicity studies. In teratogenicity studies in rats, reduced maternal bodyweight gain and food consumption were observed during the entire gestation period at a dosage of 125 mg/kg/day. There was also reduced maternal body weight gain and food consumption during gestation days 6–9 at a dosage of 40 mg/kg/day. On the other hand, there were no adverse effects on fetal development as evaluated in this study, and it was concluded that clothianidin was not a developmental toxicant.

In teratogenicity studies in rabbits, reduced maternal body weight gain and increased incidences of abortions were observed at a dosage of 100 mg/kg/day, and increased incidences of scant feces and discol-

ored urine were observed at dosages of 75 mg/kg/day and above. With respect to fetal toxicity, reduced fetal body weight, and increased incidences of small kidneys and fusion of the caudal vertebrae were observed in both sexes at a dosage of 100 mg/kg/day, and increased incidences of absent intermediate lung lobe and retardation in ossification were also observed at dosages of 75 mg/kg/day and above. Since the small kidney occurred only in one litter and the rates of incidences of absent intermediate lung lobe and fusion of the caudal vertebrae were within the range of the historical control data of the testing facility, they were not considered to be treatment-related effects.

In a 2-generation reproductive study in rats, as effects on the parental animals, there were reduced body weight gain and reduced thymus weight in the F₀ and F₁ generations at a dosage of 2500 ppm, and reduced body weight gain was also observed during the lactation period in the F₀ generation at a dosage of 500 ppm. As neonatal toxicity, reduced body weight gain and reduced spleen weight were observed in the F₁ and F₂ generations at a dosage of 2500 ppm, and reduced body weight gain was also observed during the lactation period in the F₁ generation at a dosage of 500 ppm. There were no reproductive effects in either generation at any dosage level.

(5) Pharmacological Study

To investigate the effect of clothianidin on biofunction, the effects on the central nervous system, circulatory system, autonomic nervous system, digestive system, the skeletal muscle and blood coagulation system were examined. With respect to the pharmacological actions of clothianidin, there were suppres-

Table 7 Development and reproductively toxicity studies with clothianidin

Study	Species	Administration Period	Administration route	Dose	NOAEL
Developmental Toxicity	Rat (SD)	Organogenesis (GD 6-19)	Oral (gavage)	10, 40, 125 mg/kg/day	No teratogenicity P : 10 mg/kg/day F ₁ : 125 mg/kg/day
Developmental Toxicity	Rabbit (Hra : NZW)	Organogenesis (GD 6-28)	Oral (gavage)	10, 25, 75, 100 mg/kg/day	No teratogenicity P : 25 mg/kg/day F ₁ : 25 mg/kg/day
2-Generation Reproductive Toxicity	Rat (SD)	10 Week pre-mating, 3 weeks mating, 3 weeks gestation and 3 weeks lactation (F ₀ and F ₁), 6 weeks (F ₂)	Oral (dietary)	150, 500, 2500 ppm	F ₀ : 150 ppm F ₁ : 150 ppm (Male F ₀ ; 9.8, F ₁ ; 10.7, Female F ₀ ; 11.5, F ₁ ; 12.2 mg/kg/day) Reproduction : 2500 ppm

sive effects on the central nervous system and the digestive system, and there was also a suppressive effect on skeletal muscle even though it was slight.

2. Metabolism in Animals and Plants

(1) Metabolism in Mammals

To examine the absorption, distribution, metabolism and excretion of clothianidin, rats were given a single oral dose of clothianidin labeled with ^{14}C at the nitroguanidine moiety or the thiazolyl ring at 5 or 250 mg/kg. The orally administered clothianidin was rapidly absorbed and distributed throughout the entire body, and most of the administered ^{14}C was excreted into urine within two days after administration. In addition, the residual ^{14}C levels in the tissues 7 days after administration were low ($\leq 0.25\%$: percent of the dose), and no accumulation was observed in specific tissues.

The metabolic reactions of clothianidin in rats were (1) demethylation, (2) denitration, (3) formation of urea by hydrolysis, (4) cleavage of the carbon-nitrogen bond between the thiazolylmethyl and nitroguanidine moieties and, after that, further metabolic reactions via glutathione conjugation of the thiazolyl ring (Fig. 21). The primary metabolites were TZNG produced in (1)

and MNG and MTCA produced in (4). No gender differences were found in either metabolism or pharmacokinetics.

(2) Metabolism in Plants

Metabolism studies were conducted on three different crops, using ^{14}C -labeled clothianidin. In all cases, no differences were observed in the metabolism and translocation of clothianidin and its metabolites, and the metabolic reactions in plants were (1) demethylation, (2) denitration, (3) formation of urea by hydrolysis, (4) cleavage of the carbon-nitrogen bond between the thiazolylmethyl and nitroguanidine moieties (Fig. 21).

3. Safety Toward the Environment

(1) Environmental Fate and Residue

(i) Degradation in Water

Clothianidin was stable in sterile buffers over a pH range of 4 to 7 at 25°C .

On the other hand, it underwent photolysis with a half-life of approximately 30 minutes in distilled water and 40 minutes in natural water by exposure to simulated sunlight whose irradiance was equivalent to that in Tokyo in April–June. The main photodegradation

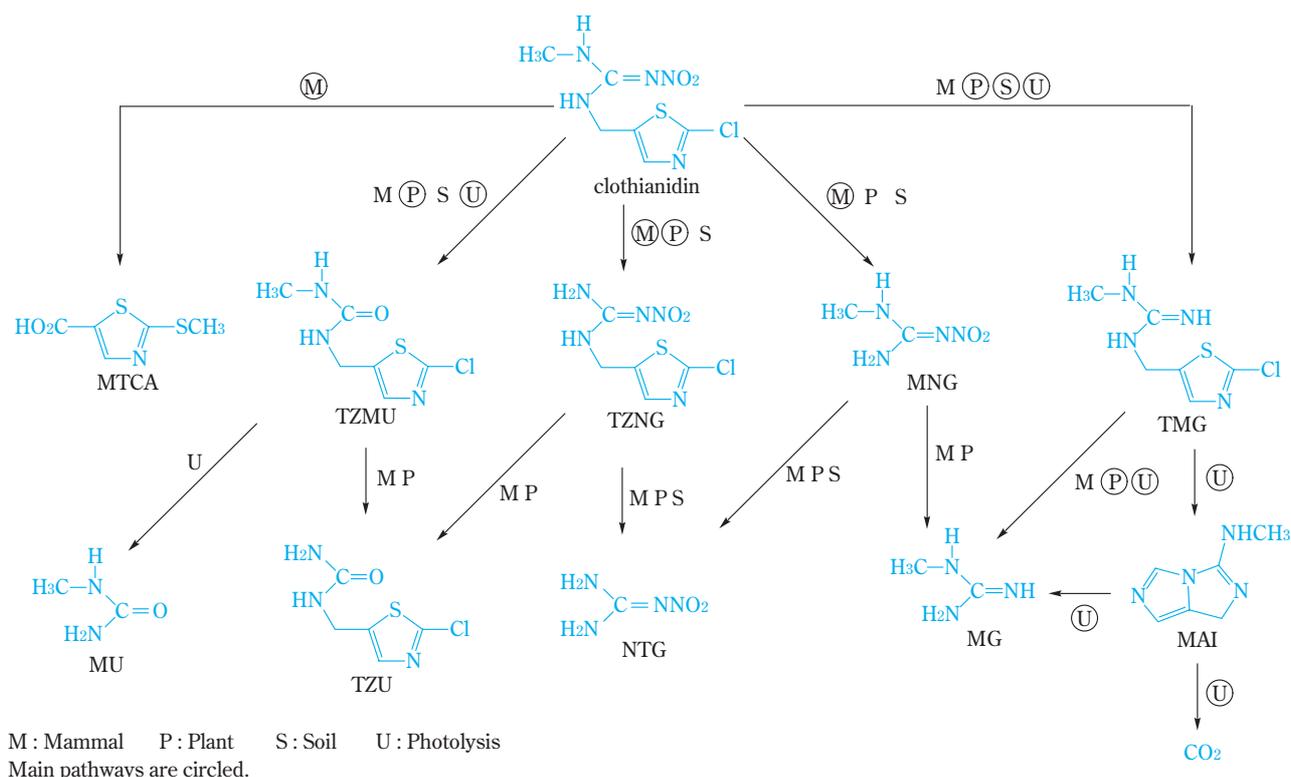


Fig. 21 Proposed metabolic and degradation pathways of clothianidin

pathways in water were as follows : (1) formation of urea by hydrolysis, (2) denitrification, (3) cyclization of TMG produced in (2), and (4) formation of MG and mineralization to carbon dioxide through ring cleavage of the MAI produced in (3).

(ii) Metabolism in Soil

Clothianidin labeled with ^{14}C was applied to flooded soil at the maximum proposed application rate on a dry soil basis and the treated soil was then incubated in the dark at 25°C . Clothianidin gradually degraded via denitrification (TMG) finally with mineralization to carbon dioxide or formation of unextractable bound residues.

In upland conditions at 25°C , clothianidin underwent gradual cleavage of the carbon-nitrogen bond between the thiazolylmethyl and nitroguanidine moieties, and finally mineralized to carbon dioxide or formed unextractable bound residues.

(iii) Field Dissipation

Field dissipation studies under flooded conditions were conducted in two locations, Ibaraki and Kochi in Japan, following a nursery box treatment (content : 1.5%, 1.25 kg/10a) and three applications of granules (content : 1.0%, 1 kg/10a) at 7-day intervals. The maximum residues of clothianidin were found in the range of 0.14 to 0.42 ppm on 0 day and 3 days after the last application. The DT_{50} values were estimated to be 7 to 8 days.

Field dissipation studies under upland conditions were conducted in two locations, Ibaraki and Miyazaki in Japan, following a granule treatment (content : 0.5%, 10 kg/10a) and three applications with the spray solutions where clothianidin 16% wettable powder was diluted by a factor of 2000. The application interval was 7 days and the application rate per treatment was 200L/10a. The maximum residues of clothianidin were found in the range of 1.00 to 1.96 ppm on 0 day and 7 days after the last application and clothianidin was dissipated with the DT_{50} values of 27 to 65 days.

(iv) Mobility within Soil

A high correlation was observed between the adsorption of clothianidin and organic carbon content of soil, and the soil adsorption coefficient on an organic carbon content basis (K_{oc}) was 90–250 ml/g.

(v) Residue in Crops

The residue trials of clothianidin were conducted for 15 types of crops, including paddy rice, fruit vegetables, fruit trees and root vegetables, and the maximum residual level was found not exceed 3 ppm

except for tea.

(2) Effects on Non-target Species

The results of ecotoxicological tests performed on aquatic organisms and birds are summarized in **Table 8**.

Table 8 Summary results of toxicity tests on non-target organisms

Species	Study	Results
Carp	Acute	96 hr LC_{50} > 100 mg/L
Alga *)	Acute	72 hr EC_{50} = 177 mg/L
<i>Daphnia magna</i>	Acute	48 hr EC_{50} = 40 mg/L
Bobwhite quail	Acute	LD_{50} > 2000 mg/kg

*) *Pseudokirchneriella subcapitata*

(i) Effects on Aquatic Organisms

Clothianidin showed low toxicity in carp : 96 hr LC_{50} > 100 mg/L. Clothianidin also demonstrated low toxicity toward algae (72 hr EC_{50} : 177 mg/L) and *Daphnia magna* (48 hr EC_{50} : 40 mg/L). These results show that the effects of clothianidin on aquatic organisms are low.

(ii) Effects upon Birds

The LD_{50} value on Bobwhite quail was > 2000 mg/kg, indicating that clothianidin has relatively insignificant effects on birds.

Conclusion

As has been discussed above, clothianidin is a neonicotinoid insecticide with a broad insecticidal spectrum, and in Japan it has been registered for application techniques including nursery box application, foliar spraying, paddy water application (paddy rice field) ; application of the granules during seeding, seedling growth, permanent planting and during the growing period (horticultural fields) and foliar spraying of water dissolved agent (horticulture and fruit tree fields). It is an agent that is winning high acclaim already, but it can be assumed that a variety of application methods that make use of its superior systemic action are possible. Using its characteristics, we plan to establish new application techniques aimed at simplification and reduction of the labor requirements for application of the insecticide.

In addition, there is a plan to register it in many more countries overseas, and we think that because of

the performance of clothianidin, it can contribute to the development of agricultural production worldwide.

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References

- 1) H. Uneme, "Nihon no Nouyaku-kaihatsu", Ed. by Pesticide Science Society of Japan, Soft Science (2003), p. 117.
- 2) S. B. Soloway, A. C. Henry, W. D. Kollmeyer, W. M. Padgett, J. E. Powell, S. A. Roman, C. H. Tieman, R. A. Corey, and C. A. Horne, "Advances in Pesticide Science", Part 2, Pergamon Press (1978), p. 206.
- 3) M. Harris, R. N. Price, J. Robinson, and T. E. May, *British Crop Protection Council Conference – Pests and Diseases*, 2B-4 (1986).
- 4) S. Kagabu, *J. Synth. Org. Chem., Jpn.*, **58**, 606 (2000).
- 5) Nihon Tokusyu Noyaku, JP60-172976 (1985).
- 6) Nihon Tokusyu Noyaku, JP61-178981 (1986).
- 7) Nihon Tokusyu Noyaku, JP61-178982 (1986).
- 8) Nihon Tokusyu Noyaku, JP61-183271 (1986).
- 9) Nihon Tokusyu Noyaku, JP61-267561 (1986).
- 10) A. Akayama and I. Minamida, "Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor", Ed. by I. Yamamoto and J. E. Casida, Springer (1999), p. 127.
- 11) H. Uneme, K. Iwanaga, N. Higuchi, Y. Kando, T. Okauchi, A. Akayama, and I. Minamida, *Pestic. Sci.*, **55**, 202 (1999).
- 12) D. Bai, S. C. R. Lumis, W. Leicht, H. Breer, and D. B. Sattelle, *Pestic. Sci.*, **33**, 197 (1991).
- 13) I. Yamamoto, G. Yabuta, M. Tomizawa, T. Saito, T. Miyamoto, and S. Kagabu, *J. Pestic. Sci.*, **20**, 33 (1995).
- 14) M. Ihara, K. Matsuda, M. Shimomura, D. B. Sattelle, and K. Komai, *Biosci. Biotechnol. Biochem.*, **68**, 761 (2004).
- 15) Y. Ohkawara, A. Akayama, K. Matsuda, and W. Andersch, *British Crop Protection Council Conference – Pests and Diseases*, 51 (2002).
- 16) Bayer, JP63-83079 (1988).
- 17) Takeda Chem. Ind., JP4-234864 (1992) ; H. Uneme, N. Higuchi, H. Ishizuka, M. Konobe, Y. Kamiya, Y. Kandoh, and I. Minamida, Abstracts of Papers, 77th Annual Meeting of The Chemical Society of Japan, p. 424 (1999).
- 18) A : Fine Organics and Agro-Kanesho, JP9-110844 (1997) ; Kuraray, JP10-36360 (1998) ; Reilly Industries, WO98/45279 (1998).
- 19) B : Ciba-Geigy, WO97/10226 (1997).
- 20) C : Kureha Chem. Ind., JP9-202778 (1997).
- 21) D : Novartis, WO97/23469 (1997) ; Bayer, JP9-176140 (1997).
- 22) Takeda Chem. Ind., JP3-157308 (1991) ; Y. Kandoh, H. Ishizuka, H. Uneme, S. Itoh, and I. Minamida, Abstracts of Papers, 77th Annual Meeting of The Chemical Society of Japan, p. 425 (1999).
- 23) Takeda Chem. Ind., JP9-67342.
- 24) Takeda Chem. Ind., JP10-120666.
- 25) Agro-Kanesho, JP3-291267 (1991) ; Ciba-Geigy, JP4-330049 (1992).
- 26) Novartis, WO98/56764 (1998).
- 27) Bayer, WO98/42690 (1998).
- 28) Mitsui Chemicals, JP11-236381 (1999).
- 29) Bayer, WO01/46160 (2001).

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