Applied Development of a Novel Fungicide Isotianil (Stout®)

Sumitomo Chemical Co., Ltd.
Health & Crop Sciences Research Laboratory
Masaomi OGAWA
Atsushi KADOWAKI
Environmental Health Science Laboratory
Tomoya YAMADA
Orie KADOOKA

Isotianil (Stout®) is a novel fungicide which induces systemic acquired resistance in plants. It has excellent preventive effects at low dosages against rice blast which is one of the most serious diseases in rice. We started a study on its mixture formulation in 2005 with our insecticide, clothianidin, and in May 2010 acquired pesticide registration approval for Stout® Dantotsu® granule for nursery-box treatment and Stout® Dantotsu® 08 granule for nursery-box treatment which have excellent efficacy against rice pests and leaf blast in paddy fields. Its low phytotoxicity to rice provides us with a wide range of application timing from seeding to transplanting. These properties enable rice farmers to distribute work and to improve work efficiency. The basic property of efficacy against rice blast, granule formulation properties, mammalian toxicity, residues, environmental fate and behavior and ecotoxicity of isotianil are reviewed in this report.

This paper is translated from R&D Report, "SUMITOMO KAGAKU", vol. 2011-I.

Introduction

Rice blast (*Magnaporthe grisea*) is the most serious rice disease in Japan, and since long ago it has brought frequent, significant yield reductions to farmers. To date, several agents having inhibitory activity on the specific sites of pathogens have been developed as fungicides against blast. However, frequent use of such agents poses the risk of emergence and development of resistant strains. For that reason, researchers have recently begun paying attention to those agents which do not show antimicrobial activity directly against the pathogenic microbe but instead prevent the outbreak of the disease by inducing systemic acquired resistance (SAR) in the host plant (rice plant). These agents are categorized as "plant activators."

The plant activators against rice blast that have been developed and registered as pesticides in Japan include probenazole, acibenzolar S-methyl and tiadinil. Although these compounds demonstrate no antifungal activity against *M. grisea*, it is known that they induce the accumulation of mRNA coding for pathogenesis-related protein (PR-protein) and of PR-proteins in the treated plant, which have resulted in various resistance activities of the plant.[1)]

Given such circumstances, isotianil (Stout®) has been developed as a novel plant activator against rice blast. The outstanding characteristics of isotianil are that its effect lasts for a long period of time with a lower dosage relative to the other existing plant activators, and that it can be applied to various types of treatment methods. This paper describes its basic characteristics, preventive properties, physical properties, biological safety, metabolism in animals and plants, and environmental behaviors.

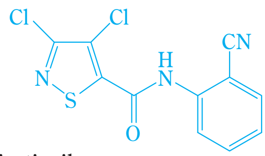
Development Process

Isotianil was discovered by Bayer AG (currently Bayer CropScience AG) in Germany as the result of an exhaustive search for isothiazole-based compounds. As with the known plant activators against rice blast, isotianil does not show antimicrobial activity against phytopathogenic fungi and bacteria, and it is considered that it demonstrates the action (plant disease resistance inducing activity) to activate the plant's own protective function against *Magnaporthe grisea*.

In Japan Sumitomo Chemical Co., Ltd. and Bayer CropScience K.K., the Japanese affiliate of Bayer CropScience AG, began internal investigations in 2003 into the dosages, treatment method and formulation of isotianil for the use in protection against rice blast. Consequently, they have elucidated that isotianil possesses greater preventive effectiveness against the disease than other existing plant activators, and that it does so with a lower dosage. The investigation of the treatment methods, i.e., spraying the agent directly onto the rice leaf sheath and applying the agent via the plant roots (seed treatment, nursery-box treatment, treatment of paddy water) have also revealed that the dosage (environmental load) could be reduced more with the plant-root treatment than with the spray treatment. Subsequently, in 2005 the two companies (Sumitomo Chemical Co., Ltd. and Bayer CropScience Group) agreed to conduct the joint development of isotianil, aiming for the registration of the pesticide, and each company conducted several types of safety evaluations and efficacy/phytotoxicity tests using the active ingredient development codes of S-2310 (Sumitomo Chemical Co., Ltd.) and BYF1047 (Bayer CropScience K. K.).

Physicochemical Properties

Unlike the existing plant activators probenazole, acibenzolar S-methyl and tiadinil, the structural characteristics of isotianil are such that it possesses an isothiazole ring (**Fig. 1**). Of all the agents that have systemic properties, LogPow has relatively high systemic properties and low aqueous solubility as well. These properties can minimize the loss of ingredients into the environment and thereby allow the agent to maintain a steady preventive effect.



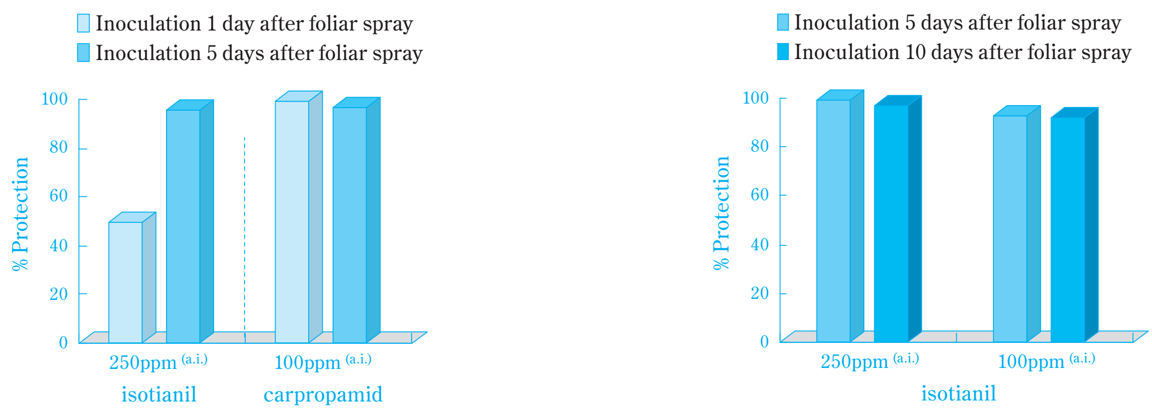
Common name : isotianil
IUPAC name : 3,4-dichloro-2'-cyano-1,2-thiazole-5-carboxanilide
Molecular formula : $C_{11}H_5Cl_2N_3OS$
Molecular weight : 298.15
Appearance : White powder
LogPow : 2.96 (25°C)
Solubility in water : 0.5 mg/L (20°C)
Vapor pressure : 2.36×10^{-7}Pa (25°C)

Fig. 1 Chemical and physical properties of isotianil

Mode of Action

Isotianil showed no antimicrobial activity against fungus and bacteria, including *M. grisea* and *Xanthomonas oryzae* pv. *oryzae* on media *in vitro* (**Table 1**). However, the results of pot and field tests indicated the excellent preventive effects on such diseases via the host plants.[2), 3)] (**Table 2**) In the spray test isotianil showed better preventive effects on rice plants inoculated five days after spraying than on those inoculated one day after spraying, thus suggesting that it requires a few days for the efficacy to manifest.[2), 3)] (**Fig. 2**)

Regardless of the inoculation or non-inoculation of *M.grisea,* isotianil treatment induced the accumulation of plant disease resistance related enzymes such as lipoxygenase, phenylalanine ammonia-lyase and chitinase that are contained endogenously in rice tissues.[4)] (**Table 3**) Research into the gene expression has also revealed that isotianil induces disease-resistance related genes, including *WRKY45*.



Plant: Rice plant (2-3 leaf stage) cv. Koshihikari
Rice plants were inoculated with a spore suspension of *M. grisea* 1 or 5 days after foliar spray. Disease area was observed 7 days after inoculation.

Plant: Rice plant (2-3 leaf stage) cv. Koshihikari
Rice plants were inoculated with a spore suspension of *M. grisea* 5 or 10 days after foliar spray. Disease area was observed 7 days after inoculation.

Fig. 2 Efficacy against leaf blast on rice by foliar spray/Pot test[3)]

Table 1 Antimicrobial spectrum

Filamentous fungus	Antifungal activity
Alternaria mali	–
Aspergillus niger	–
Botrytis cinerea	–
Gibberella zeae	–
Phytophthora cryptogea	–
Magnaporthe grisea	–
Pythium aphanidermatum	–
Rhizoctonia solani	–
Septoria tritici	–
Ustilago avenae	–

Spore suspension of fungi except for hyphal fragments of *P. cryptogea, P. aphanidermatum* or *R.solani* was inoculated to potato dextrose broth containing isotianil at 0.1–100 ppm. Two days after incubation, light transmittance of the medium was measured for evaluation of antifungal activity.

Bacteria	Antibacterial activity
Acidovorax avenae avenae	–
Burkholderia glumae	–
Clavibacter michiganensis michiganensis	–
Erwinia carotovora carotovora	–
Pseudomonas aeruginosa	–
Pseudomonas syringae pv. *lachlymans*	–
Xanthomonas campestris	–
Xanthomonas campestris pv. *citri*	–
Xanthomonas campestris pv. *pruni*	–
Xanthomonas oryzae pv. *oryzae*	–

Bacteria were incubated on potato dextrose agar containing isotianil at 500 ppm and incubated for 2 days. The diameter of colony was measured and an antibacterial activity was evaluated.

Efficacy +++ : 100–80% control ++ : 79–70% + : 69–50% (Virus : 69–20%) – : 50% >

Table 2 Efficacy against plant diseases [3]

Target crops	Target Diseases	Pathogen	Efficacy
Rice	Rice blast	*Magnaporthe grisea*	+++
	Sheath blight	*Rhizoctonia solani*	–
	Bacterial leaf blight	*Xanthomonas. oryzae* pv. *oryzae*	+++
	Brown spot	*Cochliobolus miyabeanus*	–
	Seedling blight	*Rhizopus chinensis*	–
Wheat	Powdery mildew	*Blumeria graminis* f.sp *tritici*	+++
	Yellow spot	*Pyrenophora tritici-repentis*	–
Potato	Late blight	*Phytophthora infestans*	–
Cucumber	Downy mildew	*Pseudoperonospora cubensis*	–
	Anthracnose	*Colletotrichum orbiculare*	+++
	Bacterial spot	*Pseudomonas syringae* pv. *lachlymans*	+++
Pumpkin	Powdery mildew	*Sphaerotheca fuliginea*	++
Strawberry	Anthracnose	*Glomerella cingulata*	++
	Powdery mildew	*Sphaerotheca aphanis*	+
Eggplant	Powdery mildew	*Sphaerotheca fuliginea*	+
Tomato	Powdery mildew	*Oidiopsis sicula*	+
	Late bligth	*Phytophthora infestans*	+
Soybean	Purple stain	*Cercospora kikuchii*	–
Chinese cabbage	Alternaria leaf spot	*Alternaria brassicae*	++
	Bacterial soft rot	*Erwinia carotovora* subsp. *carotovora*	–
Citrus	Melanose	*Diaporthe citri*	–
Tobacco	Spotted wilt	TSWV (tomato spotted wilt virus)	+

Foliar spray to a plant in a pot or a field. Spray concentration : 100–250ppm
Efficacy +++ : 100–80% control ++ : 79–70% + : 69–50% (Virus : 69–20%) – : 50% >

Table 3 Effect of isotianil and pathogen infection on enzyme activities

Chemicals	With/without inoculation of *M. grisea*	POX*	LOX	PPO	PAL	CHT
isotianil	No	–	⬆⬆	–	↗	↗
	Yes	–	⬆⬆	–	–	–
probenazole	No	–	↗	–	⬆	–
	Yes	–	↗	–	↗	–
untreated	Yes	–	↗	–	⬇	–

Ratio to untreated and no-inoculation control < 0.5 ; ⬇, 0.5~1.9 ; –, 2~3.9 ; ↗, 4~7.9 ; ⬆, 8 < ; ⬆⬆

* POX : Peroxidase, LOX : Lipoxygenase, PPO : Polyphenol oxidase, PAL : Phenylalanine ammonia-lyase, CHT : Chitinase

Isotianil was mixed with water culture medium at 5 ppm.
A spore suspension of *M. grisea* was sprayed to the foliage of rice 5 days after application.
Leaves were collected and assess enzyme activity over time.

Based on the above findings, it has been concluded that isotianil was a plant activator.

Special Features of Isotianil

1. Manifesting High Preventive Effect with Low Dosage

Pot tests in which nursery boxes were treated with chemicals were conducted in order to investigate the preventive effects of isotianil against leaf blast per dosage. The efficacy of isotianil at dosages of 0.5g a.i. to 2.0g a.i./box (10g a.i. to 40g a.i./10a when converted from 20 nursery boxes/10a) was equivalent to that of existing plant activators at a dosage of 12.0g a.i./box (240g a.i./10a when converted from 20 nursery boxes/10a) (**Fig. 3**). In the paddy field test isotianil treatment of nursery boxes at 1.0g a.i. to 1.5g a.i./box showed an excellent efficacy equivalent to that of commercially-used plant activators at 12g a.i./box (**Fig. 4**).

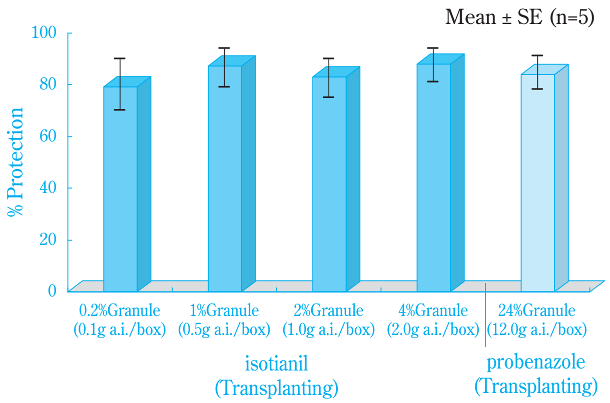


Fig. 3 Efficacy against leaf blast on rice in pots

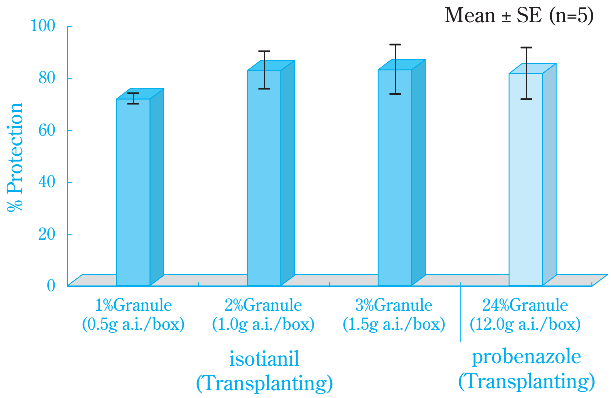


Fig. 4 Efficacy against leaf blast on rice in paddy field [3]

(C) Pesticide Science Society of Japan, reproduced with the permission. (Further reproduction is prohibited without permission.)

Currently, while isotianil products are registered as a pesticide at dosages of 20g a.i. to 30g a.i./10a, the existing plant activators probenazole and tiadinil are used at 100g a.i. to 240g a.i./10a and 120g a.i. to 180g a.i./10a, respectively. Thus isotianil demonstrates an excellent preventive effect against leaf blast at lower dosages, which results in less environmental load than the similar, existing agents do.[5)]

2. Applicable Treatment Methods

The trend in many other nursery-box treatment agents currently undergoing development is that their proper treatment periods have been expanded from the conventional period (which is three days before transplanting) to either the greening period or the time of seeding for the purpose of dispersing farming labor (weeding of the ridges, plowing and irrigating the fields, applying manure, maintaining tractors and rice transplanting equipment, and conducting the water management of nursery boxes and paddy fields) during the busy season, which is immediately before the transplanting of rice seedlings. In order to accommodate various treatment methods, the applicability of isotianil to the following treatment methods was also investigated: treatment at the time of transplanting; treatment at the time of seeding before soil-covering; and mixing treatment with bed soil or covering soil prior to seeding. Consequently, isotianil demonstrated excellent efficacy with all of the above treatment methods (**Fig. 5**). Moreover, one can assume that isotianil alone can be used in a wide range of proper treatment periods (from the period before seeding to the day of transplantation) without adversely affecting the rice plants during the period of raising seedlings nor affecting the

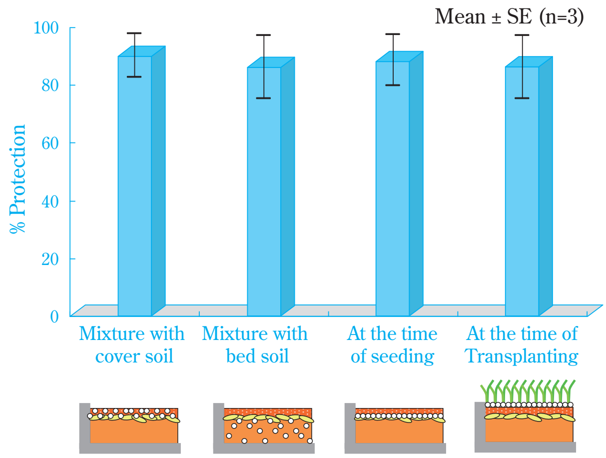
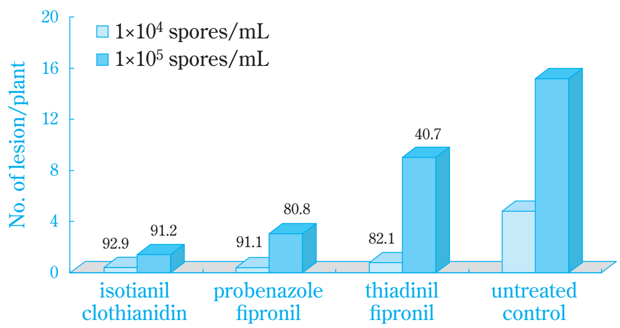


Fig. 5 Efficacy of isotianil against leaf blast with different application timings

growth of rice plants after transplanting, thus contributing to the dispersion of the workload. Additionally, many manufacturers have recently developed and launched various types of pesticide applicators that enable farmers to simultaneously conduct transplantation operations and nursery-box treatment. The combined use of such pesticide applicators will enable farmers to perform treatment as part of the seeding process, thus reducing the workload significantly.[6)]

3. Stability of Efficacy

The outbreak of rice blast can be greatly affected by external factors (climate, environment, fertilization practice, etc.). In order to determine whether the leaf blast outbreak conditions would affect the efficacy of isotianil, the correlation between the infectious pressure and the preventive effect against leaf blast was investigated through paddy field tests. Spore suspensions of *M. grisea* (from 1×10^4 to 1×10^5 spores/mL) as inocula were sprayed onto the rice plants 30 days after transplanting, and as a result the number of lesions per plant treated with isotianil was less at both concentrations of the spore suspension, thus confirming isotinial's excellent efficacy (**Fig. 6**). Furthermore, the analysis of data from the special field trials provided by The Japan Plant Protection Association (JPPA) on isotianil granules for nursery-box treatment conducted throughout Japan from Hokkaido to Kyushu during the period of 2008 till 2009 confirmed the agent's stable efficacy under all disease severity conditions, from slight to severe disease occurrences, with treatment at the time of seeding through to transplanting (**Fig. 7**). Based on these results, one can assume that isotianil possesses a stable efficacy against leaf blast without being largely affected by the rice blast outbreak conditions.

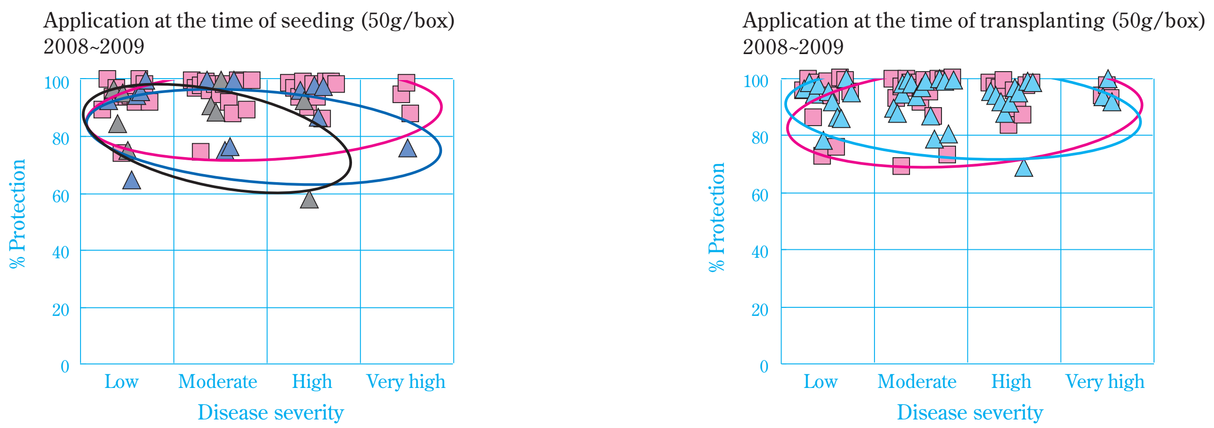


The number above each bar indicates the protection value

Fig. 6 Efficacy against leaf blast with different inoculation pressure

4. Long-lasting Effects

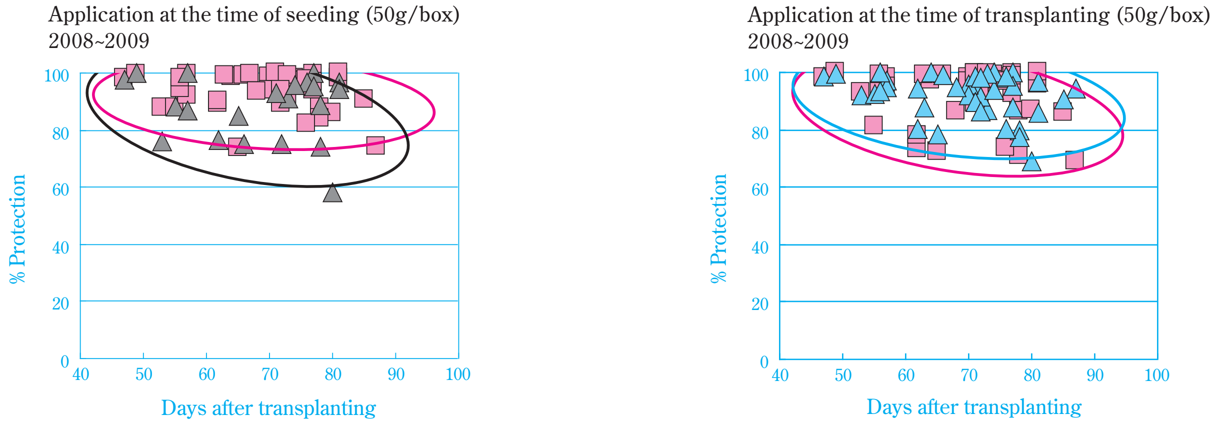
Because the growth and progress status of rice vary depending on the regional environment, the required period of lasting efficacy of the pesticide varies significantly according to the region. However, the results of the special field trials provided by JPPA in 2008 and 2009 on isotianil granules for nursery-box treatment have revealed that stable efficacies can be achieved regardless of the region throughout Japan at either time of treatment (seeding or transplanting) (**Fig. 8**). One can therefore assume that when isotianil is applied to rice plants through nursery-box treatment it can



The contour in each graph is bivariate density ellipse (p=0.95). All statistical analyses were carried out with JMP software version 5.0.1J (SAS Institute, Tokyo, Japan).

- ■ isotianil2% GR (at the time of seeding or transplanting)
- △ probenazole · fipronil GR (at the time of transplanting)
- ▲ probenazole · fipronil GR10H (at the time of seeding)
- △ probenazole · fipronil GR6 (at the time of seeding)

Fig. 7 Efficacy against leaf blast on rice after transplanting to the paddy field (Efficacy and disease severities of untreated control)



The contour in each graph is bivariate density ellipse (p=0.95). All statistical analyses were carried out with JMP software version 5.0.1J (SAS Institute, Tokyo, Japan).

- isotianil2% GR (at the time of seeding or transplanting)
- probenazole · fipronil GR (at the time of transplanting)
- probenazole · fipronil GR6 (at the time of seeding)

Fig. 8 Long-lasting efficacy against leaf blast on rice after transplanting to the paddy field.

demonstrate an excellent efficacy that is applicable throughout nearly the entire the leaf blast infection period in any region of Japan, as long as it is a normal rice blast outbreak year.

5. Safety to Rice Plants

It is known that the existing plant activators used for rice plants can cause chemical injuries such as the yellowing of lower leaves and growth inhibition when applied excessively or under extreme climatic conditions.

Over the past eight years from 2003 through 2010, Sumitomo Chemical Co., Ltd. and Bayer CropScience K. K. have conducted phytotoxicity tests of isotianil under various conditions. As a results of repeated studies conducted, taking the following various conditions into account, no phytotoxic symptom that could cause practical problems was recognized in terms of compatibility with the major rice cultivars (e.g. Koshihikari, Hinohikari, Akitakomachi, etc.); compatibility with the major nursery soils (e.g. zeolite soil, Biwako-baido, Nobateron, etc.); seeding methods aiming to achieve a reduction of workloads and lighter weight (e.g. pool nursing, rock wool nursing, etc.); seed disinfectants for the healthy growth of seedlings (e.g. Prochloraz-oxolinic acid SE, thiuram-benomyl water dispersible powder, etc.); and soil-drench treatment agents (e.g. Benomyl, Benomyl-chlorothalonil, etc.).[7)–9)] (**Table 4**) On rare occasions, symptoms by which leaves appeared to have an accelerated aging process (yellowing, early earing, brown spots on the leaves. etc.) could be recognized. However, even in such cases no adverse effects on the growth or yield of rice plants have been observed. Although the results of the surveys pertaining to the yield conducted during the period of 2003 till 2006 in various regions under the conditions in which the effects of pests were eliminated also contained cases that showed brown spots, in no test was a significant

Table 4 Phytotoxicity on rice plant

Results of several trials

Chemicals	Application timing	Application weight	Nursery box			Paddy field
			Germination	Foliage	Root growth	Foliage
isotianil 2% granule	Sowing*2	50g/box	–*1	–	–	–
		100g/box	–	–	– ~ ±	– ~ ±
	Transplanting	50g/box	Not Tested			–
		100g/box				– ~ ±

*1 – : No phytotoxicity, ± : Slight phytotoxicity, + : Severe

Rice cultivar : Koshihikari, Hinohikari, Kinuhikari, Nihonbare, Akitakomachi
Bed soil : zeolite soil, biwako-baido2, Nobateron, Inaho-baido, Honen-baido, Biosumi
Seeding : Nursery box, Nursery box on a tray filled with water, rock wool used as a bed soil, Karukaru-new-line

Additional treatment : Seed treatment (Prochloraz · oxolinic acid, Ipconazole-C, Benomyl · thiuram)
: Soil drench in a nursery box (Benomyl, Benomyl · TPN, Hymexazol · metalaxyl)

*2 Application at the time of seeding before cover with soil,Mixture with bed soil, Mixture with cover soil difference recognized between the non-treated regions and the yield.[5)]

6. Efficacy Against Major Rice Diseases Other Than Blast

In many paddy field tests isotianil showed a favorable efficacy against death of rice panicles caused by *Xanthomonas campestris* pv. *oryzae* and *Cochliobolus miyabeanus* as well as an efficacy against bacterial grain rot caused by *Pseudomonas glumae*, and thus it has already been registered for these diseases. In addition to those diseases, isotianil also demonstrates a plant disease resistance inducing action against various bacterial diseases including bacterial seedling rot caused by *Burkholderia glumae*, bacterial seedling blight caused by *Pseudomonas plantarii* and bacterial palea browning caused by *Erwinia herbicola*. Thus a development plan is currently in progress for the expansion of its target diseases (**Table 5**).

Table 5 Efficacy of isotianil against diseases on rice

Target Diseases	Efficacy	Developmental status
Bacterial leaf blight	Excellent	Registered (May 19, 2010)
Helminthosporium blight (Panicle)	Good~Excellent	Registered (Nov. 10, 2010)
Bacterial grain rot (*P.glumae*)	Good~Excellent	Registered (Nov. 10, 2010)
Bacterial seedling rot (*P.glumae*)	Good	Under evaluation
Bacterial seedling blight	Good	Under evaluation
Bacterial palea browning	Good	Under evaluation
Bacterial foot rot	Poor	None
Sheath blight	Poor	None

7. Efficacy Against Seedling and Leaf Blast in Nursery Boxes

Rice blast that breaks out during the period of raising seedlings can be classified into seed-borne seedling blast and airborne leaf blast. Regarding plant activators, it is known that it requires a few days for the plant to express disease resistance after the application of the inducer. When the plant is treated with isotianil at the time of seeding, it also expresses an efficacy against blast that breaks out during the period of raising seedlings as time elapses. However, although the efficacy of isotianil is superior to the existing plant activators, it is less effective than the basic pesticides such as diclocymet and seed disinfectants (**Fig. 9** and **Fig. 10**).

Therefore, in order to prevent rice blast from breaking out during the period of raising seedlings, the following actions are crucial: (1) thorough replacement of the seeds every year; (2) using completely healthy seeds that have not been infected by the disease; (3) selecting seeds in a saline solution with a specific gravity; and (4) not leaving damaged straw and rice hulls that can cause infection inside and around the nursery during the period of raising seedlings.[10)]

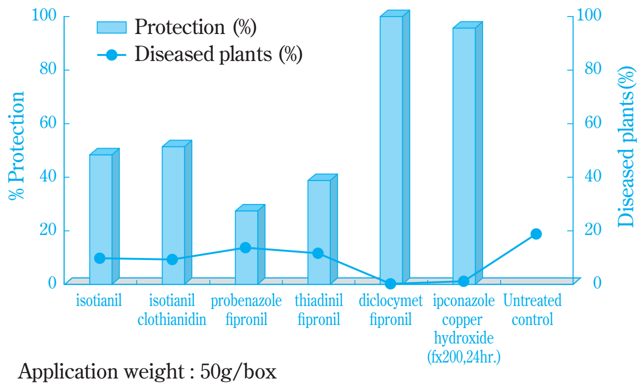


Fig. 9 Efficacy against seed-borne leaf blast in nursery box

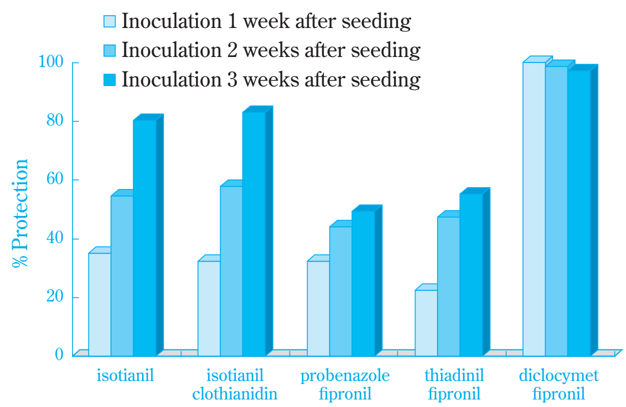
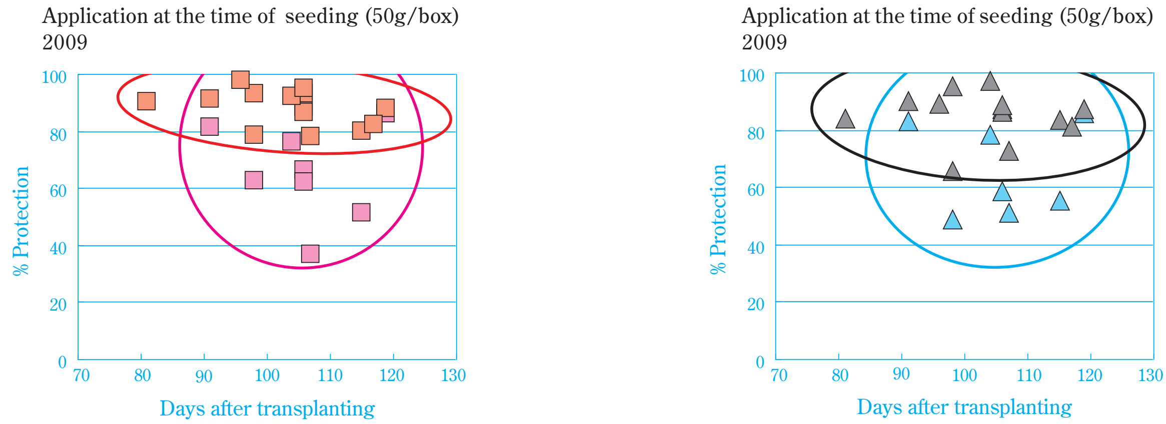


Fig. 10 Efficacy against leaf blast in nursery box with an application at the time of seeding

8. Efficacy Against Panicle Blast

Regarding the results of internal tests conducted thus far by Sumitomo Chemical Co., Ltd. and Bayer CropScience K. K., as well as the practical application tests for new agricultural agents conducted by JPPA, it cannot be said that all the plant activators (including isotianil) show adequate efficacies against panicle blast. One can surmise that this is because the disease-resistance-induction system does not function sufficiently in the panicle tissues. Taking into account the result of



The contour in each graph is bivariate density ellipse (p=0.95). All statistical analyses were carried out with JMP software version 5.0.1J (SAS Institute, Tokyo, Japan).

- isotianil 2%GR
- isotianil GR + Foliar spray
- probenazole 24% or 20%GR
- probenazole GR + Foliar spray

Fig. 11 Efficacy against panicle blast with nursery box application and foliar spray

special field trials provided by JPPA regarding isotianil granules for nursery-box treatments conducted in 2009, isotianil's efficacy against panicle blast has been recognized in many tests. One can assume that this effect was achieved by eliminating the cause of panicle infection through protection against leaf blast. By systematically applying both isotianil in a nursery-box treatment and panicle blasticides with sprays in paddy fields, excellent efficacy against panicle blast was observed (**Fig. 11**).

The fact that pathogens can migrate more often from adjacent fields in a year when many serious leaf blast outbreaks occur raises a concern that the morbidity of panicle blast may increase. One can assume that if this happens it will be necessary to conduct the systematic application of isotianil together with blasticides to counter panicle blast.[11)]

Applicable Diseases and Direction for Use

Tables 6 and **7** show the pests to which Stout® Dantotsu® granules for nursery-box treatment (isotianil 2.0%, clothianidin 1.5%) and Stout® Dantotsu® 08 granules for nursery-box treatment (isotianil 2.0%, clothianidin 0.8%) were applied, as well as the directions for the use of those formulations.

The main products in the mixture formulation, for Stout® Dantotsu, can be classified into two types depending on the content of Dantotsu® (clothianidin[12), 13)]): Stout® Dantotsu® granules and Stout® Dantotsu® 08 granules. These granules can be used depending on the types of target insects. For both formulations, once the agricultural registration (50g/nursery box, from the time before covering with soil upon seeding to the date of transplanting) was acquired on May 19, 2010, an

Table 6 Directions for use (isotinil 2.0% · clothianidin 1.5%GR)

Target crops	Target Pests & Diseases	Application weight	Application timing	Number of application	Application method
rice (nursery box)	rice blast bacterial leaf blight rice water weevil rice leaf beetle planthoppers green rice leafhopper green rice caterpillar rice stem borer	50g/box	Sowing (before cover with a soil) ~ Transplanting	1	Drop granule uniformly from above in a nursery box
			Before Sowing		Mixture with bed soil or cover soil in a nursery box.
	rice leafminer		Three days before transplanting ~ Transplanting		Drop granule uniformly from above in a nursery box

Registration situation as of Nov. 10, 2010.

Table 7 Directions for use (isotinil 2.0% · clothianidin 0.8%GR08)

Target crops	Target Pests & Diseases	Spray volume	Application timing	Number of application	Application method
rice (nursery box)	rice blast bacterial leaf blight rice water weevil rice leaf beetle planthoppers	50g/box	Sowing (before cover with a soil) ~ Transplanting	1	Drop granule uniformly from above in a nursery box
			Before Sowing		Mixture with bed soil or cover soil in a nursery box.

Registration situation as of Nov. 10, 2010.

additional registration request was made on May 20, 2010, thus resulting in the expansion of the applicable pests and approval for registration of the soil mixture process (bed soil and covering soil) on November 10, 2010. Both formulations can be applied during any period from the time before seeding through the time immediately before transplanting, targeting the major paddy rice diseases such as leaf blast and bacterial leaf blight, as well as a wide range of major pests. They also demonstrate outstanding efficacies regardless of the treatment period.

Furthermore, of all the existing pesticides containing plant activators, Stout® Dantotsu® was the first to be applicable at any period of treatment from the time before seeding to the date of transplanting. Thus we believe that the products will contribute to the reduction of the farming workload and will improve the efficiency of farming work.

Analysis Method and Formulation

1. Analysis Method

The active ingredients in isotianil can be accurately analyzed through the liquid chromatograph-internal standard method, using an ODS-based column and water/acetonitrile/phosphoric acid (500:500:1) for the mobile phase. Furthermore, impurities in isotianil TG can be analyzed through liquid chromatography and other methods using the same column described above.

2. Formulation

The main goals of the investigation of the formulation were to achieve granules of a non-disintegrated type, which will not become deformed when dropped into water in order to avoid the phytotoxicity caused by dirt on the rice plants and excess elusion of active ingredients due to morning dew; and to select and granulize the raw material which shows high safety toward the rice plants and persistence for a long time, even during the seeding period (rice plants become most susceptible to chemical injury during this period). The recipe for a formulation that has the above performance has been successfully established through screening evaluations in the laboratory and in the paddy fields. Furthermore, the storage stability of the formulation was verified, revealing that there was no problem with either its active ingredients or its physical properties. Additionally, given the fact that isotianil has low solubility in water, particles pulverized into a few micrometers were used, taking into account the aspects of efficacy, chemical injury and manufacturing.

It is important to establish an efficient manufacturing process for the industrialization of isotianil. However, all the products thus obtained must possess the target quality.

The major physical properties required for these newly developed box granules include the handling performance upon application. The application methods for box granules vary according to the scale of farming. While on some occasions they are applied manually, on other occasions a commercially available applicator is used. When performing manual application, uniformity can be achieved with a sufficient volume of granules. However, when using an applicator it is necessary to continuously supply the product from the hopper without any delay between the applications. Given such circumstances, the fluidity of the formulation within the applicator must be taken into account. It is therefore crucial to particularly control the shape of the granules. Because the newly developed box granules are manufactured through an extruding granulation method, it is necessary to design the manufacturing process in such a way that the granule lengths can be controlled, particularly aiming to reduce the contamination rate of long granules. For the purpose of reference, the difference between application methods will be explained as follows:

Fig. 12 Application to nursery box

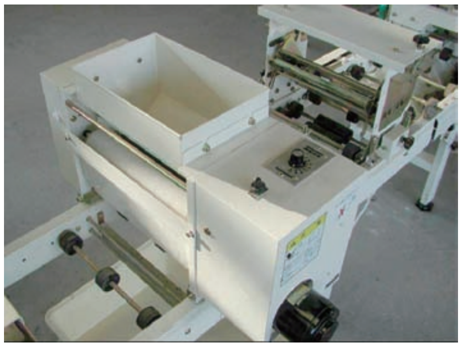


Fig. 14 Feed hopper unit for applying pesticide granules onto nursery box

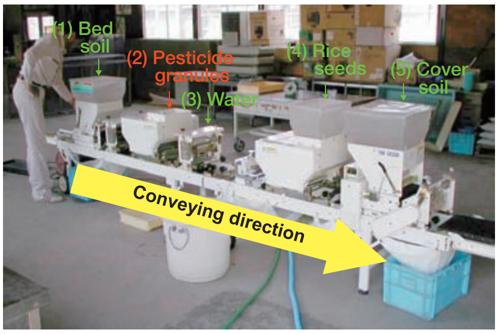
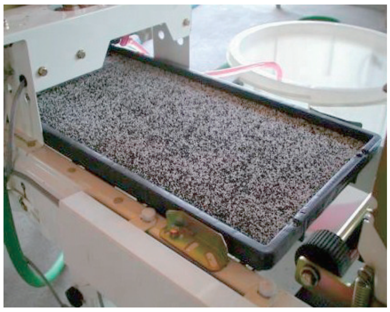


Fig. 13 Automatic sowing system for nursery box

Granules falling from the feed hopper unit onto bed soil.



Granules applied uniformly on bed soil.

Fig. 15 Granular application part of automatic sowing system for nursery box (Stout®Dantotsu® box granule was used)

As shown in **Fig. 12**, the general application method for the formulation for paddy rice nursery-boxes is that 50g of the pesticide is applied to the nursery box (30 cm × 60 cm) immediately before rice transplantation. However, for medium- to large-scale farmers it is necessary to treat a large number of nursery boxes ranging from several hundred to several thousand, thereby incurring a heavy workload during the busy season which is immediately before rice transplantation. **Fig. 13** shows a picture of general seeding work using a fully automatic sowing system. A nursery box is first inserted from the left side of the system. The application process is completed after going through the five processes in the following order: provision of bed soil; application of pesticide; irrigation; sowing; and covering the seeds with soil. Nursery boxes are fed to the system one after another without interruption. Although the processing speed varies according to the equipment model, it can be set to process 200 to 1,500 boxes per hour. **Fig. 14** and **Fig. 15** show enlarged images of the commonly used hopper and granular application part, respectively. The pesticide granules with a fixed volume are poured into the hopper, and the granules naturally fall through the opening valve (the ratio of valve opening is adjustable), installed at the exit of the hopper, onto the nursery box. The possible problems that may occur are clogging in the hopper and insufficient discharge from the opening valve. In most cases, the cause of such problems is excessive contamination by long granules.

One of the original purposes of creating granules was to reduce the risk that the user would be exposed to powder dust when using dust formulations or wettable powder formulations. Thus, for extruding granulation, the raw material is often granulated after sufficient kneading is conducted in order to give the granules adequate hardness as well as to minimize the production of fine particles. However, if kneading is

Table 8 Physical properties of Stout®Dantotsu® box granule and Stout®Dantotsu® box granule 08

Properties	Stout®Dantotsu® Box Granule	Stout®Dantotsu® Box Granule 08
clothianidin content	1.5%	0.80%
isotianil content	2.0%	2.0%
Appearance	Whitish fine granule	Whitish fine granule
Bulk Density	0.92	0.91
Moisture	0.6%	0.5%
Hardness	0.6%	0.6%
pH	9.4	9.4
Disintegrability in Water	No disintegration	No disintegration
Granule Number	984/g	974/g
Granulometry		
> 1190μm	0%	0%
500~1190μm	99.8%	99.8%
< 500μm	0.2%	0.2%

excessive it can produce a higher percentage of long granules. Therefore, optimizing the amount of water used for kneading and the duration of kneading are among the important factors in a proper manufacturing process. Furthermore, although it is possible to control granule size in the pulverizing process, if the pulverization intensity is too high during this process, it will increase powder generation during pulverization and thereby decrease not only the product quality but also the product yield. Based on the above factors, the manufacturing process for nursery-box granules with high product efficiency and yield has been successfully established by adjusting and optimizing all the conceivable parameters in the manufacturing process. **Table 8** indicates the typical physicochemical properties of the products obtained through this new manufacturing process. The hardness (powder generation rate through ball mill attrition) of both products was high at less than 1%, and no granule having a size larger than 1190 μm was present in either product. As shown in **Fig. 15**, the granules were sprayed uniformly. Furthermore, bridging or clogging did not occur in the hopper even under continuous operation. Also, in the official trials for registration conducted by the Japan Plant Protection Association, no handling issue was observed, and satisfactory efficacy and phytotoxicity prevention effects were achieved.

Consequently, although there were some issues in the development of nursery-box granules containing the novel antimicrobial substance isotianil, they were all resolved, whereupon the recipe and manufacturing process for high-quality nursery-box granules that can be safely supplied were successfully established.

Toxicity, Metabolism and Residue

1. Mammalian Toxicity

(1) Acute Toxicity, Irritation and Skin Sensitization Potential

The acute toxicity of isotianil TG and isotianil 3% granules was weak as neither death nor toxic symptoms was observed atthe applied dose in the acute oral, dermal and inhalation studies (**Table 9**). In the eye irritation studies for rabbits, the isotianil TG was demonstrated to be practically non-irritant and 3% granules were minimally irritant, while neither the isotianil TG nor the 3% granules showed skin irritation. Although the result of the maximization tests on the isotianil TG showed potential for skin sensitization, the result of the Buehler test on the 3% granules was negative.

(2) Subacute Toxicity, Chronic Toxicity and Carcinogenicity

As a result of the subacute and chronic toxicity studies (**Table 10**), although suppressed body weight gain was observed after the administration of the isotianil TG, no noteworthy toxicity such as neurodoxic symptoms was observed, and the toxic effects were seen mainly in the stomach, liver and kidney.

Mucosal cell hyperplasia was observed in the limiting ridge of the forestomach in rats. The result of the study separately conducted to examine the mode of action of producing this finding revealed that the isotianil TG possessed cell-proliferation activity toward the mucosal cells concerned. However, the effect had a threshold and did not cause neoplastic alteration. A human does not have

Table 9 Acute toxicity summary of isotianil

Study	Species	Dose	Result
isotianil TG			
Acute oral	rat	2000 mg/kg	LD_{50} > 2000 mg/kg
Acute dermal	rat	2000 mg/kg	LD_{50} > 2000 mg/kg
Inhalation	rat	5000 mg/m^3	LC_{50} > 4750 mg/m^3 of air (4-hour, nose only exposure)
Eye irritation	rabbit	0.043g (0.1mL)/eye	Practically non-irritant
Skin irritation	rabbit	0.5g/skin	Non-irritant
Skin sensitization	guinea pig		Sensitizer
isotianil granule (3.0%)			
Acute oral	rat	2000 mg/kg	LD_{50} > 2000 mg/kg
Acute dermal	rat	2000 mg/kg	LD_{50} > 2000 mg/kg
Eye irritation	rabbit	0.1g /eye	Minimally irritant
Skin irritation	rabbit	0.5g/skin	Non-irritant
Skin sensitization	guinea pig		Non-sensitizer

Table 10 Subacute and chronic toxicity summary of isotianil TG

Species	Administration route and duration	Dose (ppm)	NOAEL (mg/kg/day)
Rat	Oral (in diet), 13 weeks	20, 500, 2500, 20000	500ppm (Male: 29.7, Female: 35.1)
Rat	Oral (in diet), 12 months	60, 600, 6000, 20000	60ppm (Male: 2.83, Female: 3.70)
Rat	Oral (in diet), 24 months	2000, 6000, 20000	< 2000ppm (Male: < 79.2, Female: < 105) No carcinogenicity
Dog	Oral (in diet), 13 weeks	500, 2000, 8000	500ppm (Male: 12.2, Female: 13.4)
Dog	Oral (in diet), 12 months	200, 1000, 5000/3000	200ppm (Male: 5.22, Female: 5.33)
Mouse	Oral (in diet), 18 months	70, 700, 7000	7000ppm (Male: 706, Female: 667) No carcinogenicity

such a forestomach. Furthermore, although the human and dog esophagus have similar histological structures as that of the rat forestomach[14) ; the administration of isotianil TG did not affect the dog esophagus. Based on these findings, it was concluded that this toxicological finding is not relevant to humans under practical use of the chemicals. With respect to the effects on the liver, the increase in the liver weight and the alterations of blood parameters suggesting liver dysfunction were observed in both dogs and rats. Histologically, these alterations were accompanied by centrilobular or diffuse hepatocellular hypertrophy. In addition, bile-duct hyperplasia was also observed in dogs. However, no degeneration, necrosis nor neoplastic alteration was observed in hepatic cells. Regarding the effects on the kidney, although an increase in kidney weight and renal tubular dilatation were observed in dogs and rats, those alterations had thresholds. Regarding carcinogenicity, no significant increase in tumor incidence was observed in any kind of organ or tissue in rats and mice, indicating that there was no carcinogenicity.

(3) Reproductive/Developmental Toxicity

Neither lethal effects nor teratogenicity on embryos-fetuses was observed in rats or rabbits at the maximum dose level specified by the guideline (1000 mg/kg/day). At this dose, suppressed body weight-gain was observed in rabbit dams. Additionally, with the administration of 1000 mg/kg/day, suppressed body weight gain was observed in fetuses. In the reproduction study using rats, although suppressed body weight gain was observed in F1 fetuses to which 1000ppm or more of isotianil TG was administered, no effect on reproduction was observed (**Table 11**).

Table 11 Developmental and reproductive toxicity summary of isotianil TG

Study	Species	Administration route and duration	Dose		NOAEL (mg/kg/day)
Developmental toxicology	Rat	Oral (gavage) Days 6-19 of gestation	100, 300, 1000 (mg/kg/day)	Maternal	Systemic NOAEL: 1000 Developmental NOAEL: 1000
				Fetal	1000
	Rabbit	Oral (gavage) Days 6-27 of gestation	100, 300, 1000 (mg/kg/day)	Maternal	Systemic NOAEL: 300 Developmental NOAEL: 1000
				Fetal	300
Two-generation reproductive toxicology	Rat	Oral (in diet) Male: 10 week before mating to termination (18 weeks) Female: 10 week before mating to weaning (18weeks)	50, 1000,10000 (ppm)	Parental	Systemic NOAEL: 50 ppm (Male: 3.35, Female: 4.16) Reproductive NOAEL:10000 ppm (Male: 662, Female: 831)
				Offsprings	Systemic NOAEL:50 ppm (Male: 3.35, Female: 4.16)

(4) Genotoxicity

The results of reverse mutation tests using Salmonella typhimurium, *in vitro* chromosome aberration tests using Chinese hamster lung-derived cells and mouse micronucleus tests were negative (**Table 12**).

Table 12 Mutagenicity summary of isotianil TG

Study	Study design	Results
Reverse mutation (Ames test)	*S. typhimurium*: TA98, TA100, TA102, TA1535 and TA1537 –/+S9 mix: 16–5000 µg/plate	Negative
In vitro chromosomal aberration	Chinese hamster V79cells –/+S9 mix: 7–28 µg/mL	Negative
Micronucleus	CD-1 mice (8-week old) 500, 1000, 2000 mg/kg (orally twice)	Negative

2. Metabolism in Animals/Plants

(1) Metabolism in Animals

Isotianil was rapidly metabolized in rats. The half-life of isotianil labeled with ^{14}C in each tissue ranged from 10 to 100 hours, and not much residue in a specific tissue was observed.

The excretion of isotianil and its metabolites was nearly completed 48 hours after administration, and defecation was the main excretion route.

The major metabolic reactions were as follows: (1) hydroxylation of phenyl groups; (2) cleavage of amide bonds; and (3) conjugation reaction (glucuronic acid and sulfuric acid conjugations). No sex-related difference was observed in the metabolism and pharmacokinetics. From the results of biliary excretion studies, the absorption rate of isotianil administered orally in rats ranged from 72.5% to 85.9%.

(2) Metabolism in Plants

Rice plants were applied with ^{14}C-isotianil at the growth stage of seedling, followed by two treatments to the surface water on the pots. The major metabolic pathway of isotianil in rice plants was cleavage of the amide linkage to produce the corresponding carboxylic acid and aniline. Successively, isotianil was extensively metabolized into small molecules and $^{14}CO_2$ which were reincorporated into glucose units in starch and cellulose and other natural products in the rice plants.

3. Environmental Fate and Residue

(1) Degradation in Water

The hydrolysis study of ^{14}C-isotianil was conducted in sterilized buffer solutions at pH4, pH7 and pH9. The results demonstrated that isotianil was stable at pH4 while it was degraded *via* cleavage of the amide linkage at pH 7 and pH 9 with half-lives of 60.8–71.4 days and 53.7–55.0 days, respectively. The aqueous photolysis study of ^{14}C-isotianil was conducted in natural water and distilled water. Isotianil was rapidly photodegraded with estimated half-lives of 7.4–9.4 days and 7.9 days respectively under natural sunlight at Tokyo in spring, ,*via* cleavage of the amide linkage and gradually mineralized to $^{14}CO_2$ (half-life: 1.8–2.3 days).

(2) Metabolism in Soil

^{14}C-isotianil was aerobically applied to flooded soil and maintained under aerobic conditions in the dark at

25°C. The half-lives in the aqueous and soil layers and whole system were 0.3–3.3, 69.3–92.4 and 61.9–73.7 days, respectively. Isotianil was metabolized principally by amide bond cleavage, followed by reductive dechlorination. Subsequently, it was decomposed and eventually mineralized to $^{14}CO_2$ or firmly bound into the soil residue.

(3) Field dissipation

Field dissipation studies were conducted in two paddy fields in Ibaragi and Kochi prefectures with three treatments of isotianil 3% granules at the rate of 1 kg/10a. The half-lives were estimated to be 0.5 and 13 days with the maximum residues of 2.30 and 0.38 mg/kg, respectively.

(4) Adsorption into soil

The soil adsorption coefficient (KFoc) (ads) calculated using the Freundlich adsorption isotherm ranged from 497 mL/g to 1596 mL/g.

(5) Paddy field lysimeter

A paddy field lysimeter study was conducted with a single paddy-water application of isotianil 3% granules at the rate of 1 kg/10a. The maximum concentration was 0.36–0.042 mg/L. Isotianil rapidly dissipated with a half-life of 0.8–3.3 days.

(6) Crop Residue

Residue trials for rice were conducted with three applications of isotianil 3% granules. One treatment was performed at the rate of 50 g per nursery box, and then the others were applied to transplanted rice in paddy fields at the rate of 1 kg/10a with a one-week interval between the treatments. The maximum residues in the rice grain and straw were 0.08 and 0.84 ppm, respectively.

(7) Transfer to Milk

Dairy cattle were orally administered isotianil at the rate of 27 mg/cow/day for seven consecutive days. All of, the residues were below the limit of quantification (< 0.01 ppm) in milk collected from the beginning of the administration to five days after the last administration.

4. Effects on Non-Target Organisms

Table 13 summarizes the test results of effect levels on aquatic organisms and birds.

(1) Effects on Aquatic Organisms

The acute toxicity of isotianil TG in aquatic organisms was found to be low. The acute 96-hour LC_{50} value in carp, 48-hour EC_{50} value in *Daphnia magna* and 72-hour EC_{50} value in freshwater green algae were all > 1.0 mg/L, which was the highest concentration tested based on the water solubility of isotianil.

Furthermore, isotianil 3% granules had low acute toxicity in the three organisms. The acute 96-hour LC_{50} value in carp, the 48-hour EC_{50} value in *Daphnia magna* and the 72-hour EC_{50} value in freshwater green algae were all > 1000 mg/L.

(2) Effects on Birds

Bobwhite quails were orally administered with isotianil TG and the acute LD_{50} value was > 2250 mg/kg.

Consequently, it is likely that the low toxicity of isotianil in mammals induces no adverse effect on succeeding generations, such as carcinogenicity, teratogenicity and reproductive toxicity, even if it is taken for a long period of time. Furthermore, due to its rapid degradation in the environment and low toxicity in non-target organisms, the usage of isotianil is unlikely to pose an unacceptable risk to the environment.

Table 13 Eco-toxicological summary of isotianil on non-target organisms

Test substance	Test species	Test type	Results
isotianil TG	Carp	Acute (96 hr)	LC_{50} > 1.0 mg/L
	Daphnia magna	Acute (48 hr)	EC_{50} > 1.0 mg/L
	Green alga*	Acute (72 hr)	EC_{50} > 1.0 mg/L
	Bobwhite quail	Acute oral	LD_{50} > 2250 mg/kg
isotianil granule (3.0%)	Carp	Acute (96 hr)	LC_{50} > 1000 mg/L
	Daphnia magna	Acute (48 hr)	EC_{50} > 1000 mg/L
	Green alga*	Acute (72 hr)	EC_{50} > 1000 mg/L

* : *Pseudokirchneriella subcapitata*

Conclusion

Isotianil is a highly convenient agent that can accommodate various treatment methods and periods due to its properties as a chemical substance. Furthermore, it can be considered as an agent with a low environmental load based on the facts that it can maintain efficacy against rice blast for a long period of time with extremely low dosage for a plant activator (20 g to 30 g/10a as an active ingredient) and that there are no significant effects in terms of soil residue or non-target organisms.

This report has summarized the findings obtained during the joint development conducted by Sumitomo Chemical Co., Ltd. and Bayer CropScience K. K Both Stout® Dantotsu® granule and Stout® Dantotsu® 08 granule formulations, which were registered as agricultural pesticides on May 19, 2010, make the most of the aforementioned advantages, as a consequence of which they are highly versatile. We therefore expect that they will be applied to broad areas for a long period of time as efficient, labor-saving protection resources against blast. Under the circumstances in which the problems of an aging society with fewer children and successors are accumulating, one can assume that the level of hardship in rice cropping in Japan will continue to rise. Thus we will continue to develop products containing isotianil in order to further contribute to rice cultivation in Japan, taking into account the changes in farmers' work situations, the cultivation technology and the trend in paddy rice pest outbreaks.

Acknowledgement

We want to express our deep appreciation to the Japan Plant Protection Association, the prefectural and independent administrative agencies and the testing/research institutes such as universities, all of which helped us to evaluate the practical utility of isotianil agents and provided us with valuable advice. At the same time, we request that those organizations continue to teach us and provide us with further guidance.

References

1) T. Arie and H. Nakashita *Plant Protection* (*Shokubutsu Boeki*), **61** (10), 531 (2007).
2) H. Sakuma, Y. Araki, K.Tanaka, T. Kinbara, K. Imanishi, T. Shigyo, Y. Kuchii, M. Ogawa, R. Ishikawa and H. Sawada *Jpn. J. Phytopathol.*, **74**, 267 (2008).
3) H. Sakuma 26th *Symposium by the Bioactivity of Pesticides Committee*, 1 (2009).
4) Y. Kuchii, Y. Araki and H.Sakuma *Jpn. J. Phytopathol.*, **75**, 216 (2009).
5) H. Sawada *Sponsored by Jap. Plant Prot. Assoc. "New development of pest control measures by pesticide" Abstract* (2008).
6) M. Ogawa, M. Urakawa *SUMITOMO KAGAKU,* **2004-I***,* 31
7) M. Ogawa, H. Sakuma, H. Sawada, R. Ishikawa *Jpn. J. Phytopathol.*, **74**, 267 (2008).
8) M. Ogawa, Y. Kuchii, R. Ishikawa *Ann.Rept. Plant Prot. North Japan* **61**, 261 (2010).
9) M. Ogawa, Y. Kuchii, N.Kimura, R. Ishikawa 62nd *Proc. Assoc. Pl. Prot. Hokuriku* 51 (2010).
10) M. Ogawa, R. Ishikawa *Jpn. J. Phytopathol.*, 77 (in press) (2011).
11) R. Ishikawa *Tenth Evidence-based-Control Workshop*, **6**, (2010).
12) H. Uneme, M. Kohnobe, A. Akayama, A. Yokota and K. Mizuta, *SUMITOMO KAGAKU,* **2006-II**, 20 (2006).
13) Y. Ohkawara, A. Akayama, K. Matsuda and W. Andersch, *British Crop Protection Council Conference-Pests and Diseases*, **51** (2002).
14) D. Proctor, N. Gatto, S. Hong and K. Allamneni. *Toxicol. Sci.*, **98**, 313 (2007).

PROFILE

Masaomi OGAWA

Sumitomo Chemical Co., Ltd.
Health & Crop Sciences Research Laboratory
Senior Research Associate

Tomoya YAMADA

Sumitomo Chemical Co., Ltd.
Environmental Health Science Laboratory
Senior Research Specialist

Atsushi KADOWAKI

Sumitomo Chemical Co., Ltd.
Health & Crop Sciences Research Laboratory
Senior Research Associate

Orie KADOOKA

Sumitomo Chemical Co., Ltd.
Environmental Health Science Laboratory
Senior Research Associate