Research and Development of a Novel Fungicide, Inpyrfluxam

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Inpyrfluxam is a new succinate dehydrogenase inhibitor (SDHI) fungicide discovered by Sumitomo Chemical Co., Ltd. This compound shows robust activity against Asian soybean rust, apple scab and other important phytopathogenic fungi on various crops. Inpyrfluxam has good fungicidal properties, such as preventive efficacy, curative efficacy and translaminar activity. Inpyrfluxam also has safer profiles for human health and the environment. In Japan, a formulated product, KANAME® flowable, has been launched in 2020. Inpyrfluxam is under commercial development in many other countries, including Brazil, Argentina, the United States, Canada, Europe and South Korea.

This paper is translated from R&D Report, "SUMITOMO KAGAKU", vol. 2020.

Introduction

Soybean is cultivated worldwide as a major crop; in Brazil, in particular, the planted area of soybean, which is one of the most economically important crops in this country, reaches 35 million ha.[1] In Brazil, since Asian soybean rust (caused by *Phakopsora pachyrhizi*), which seriously reduces soybean yield, was identified in 2001, the disease has been controlled mainly with sterol demethylation inhibitor (DMI) fungicides and quinone outside inhibitor (QoI) fungicides, making Brazil the world's largest fungicide market, with current product sales exceeding USD 2 billion. However, in recent years, soybean rust having acquired resistance to these major fungicides is widely spreading, and the stable production of soybean is becoming difficult.[2] Thus, the development of a novel fungicide with high efficacy against this disease has been requested by soybean growers.

Inpyrfluxam[3] (the ISO common name for the compound was obtained in April 2017) is a novel fungicide uniquely developed by Sumitomo Chemical Co., Ltd. This compound shows excellent efficacy against various plant diseases, including the abovementioned soybean rust which has resistance to several fungicides (**Fig. 1**).

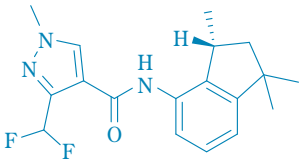


Fig. 1 Chemical structure of Inpyrfluxam

The pesticide registration application for this compound has been submitted in Brazil, Argentina, the United States, and Canada in 2017, and the review process for registration is currently ongoing in these countries. In Japan, we obtained pesticide registration as KANAME® flowable (Inpyrfluxam 37% (w/w) suspension concentrate (SC)) in the fields of fruit trees and garden plants in September 2019, and the product has been launched in March 2020. Outside Japan, multiple trade names, including "Excalia™," are planned to be used, depending on the region and intended use.

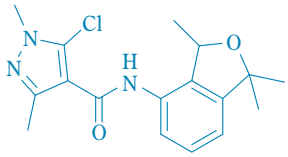
In this article, we will report the history of discovery, manufacturing method, mode of action, biological efficacy, formulation, and safety for mammals and the environment of Inpyrfluxam.

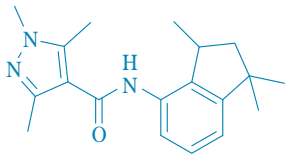
History of discovery

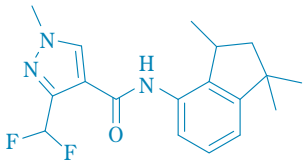
Sumitomo Chemical launched furametpyr (Limber®) which has high activity against Basidiomycetes, including the causal agent of rice sheath blight disease, in 1996,[4] and the Health & Crop Sciences Research Laboratory of Sumitomo Chemical has a large group of peripheral compounds synthesized during its developmental process. *P. pachyrhizi*, a fungal species devastatingly affecting soybean production in Brazil, belongs to Basidiomycetes. Therefore, we decided to reevaluate the activity of the group of these peripheral compounds against this disease.

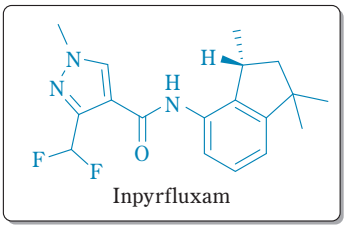
In 2008, the efficacy evaluation system for soybean rust was reconstructed, and the abovementioned compound group was evaluated. In this evaluation, Compound A was found to have high efficacy against soybean rust.

Subsequently, the peripheral compounds of Compound A were synthesized, and their activity was evaluated. In this evaluation, Compound B was found to have a certain level of efficacy also in the field. Further evaluation of Compound B revealed that, compared with racemic Compound B, higher efficacy against soybean rust was observed when the proportion of an enantiomer (*R*) was high (**Fig. 2**). It was also demonstrated


Furametpyr


Compound A

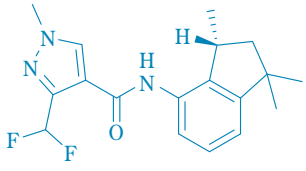

Compound B

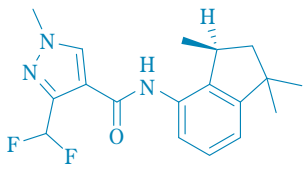

Inpyrfluxam

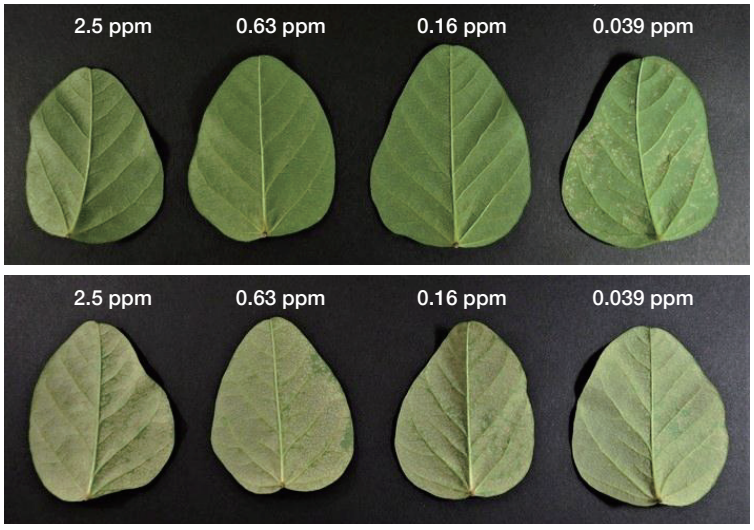
	P. pachyrhizi % control of the given concentration in mg/L	
	50	25
Compound A	+	–
Compound B	++	+
Inpyrfluxam	+++	++

+++ 100% control, ++ ≥ 90% control, + ≥ 50% control, – < 50% control

Fig. 2 Discovery of Inpyrfluxam


R-enantiomer


S-enantiomer



Application: Detached primary soybean leaf was dipped in each test compound amended solution for 3 seconds.
Inoculation: 1 day after treatment, uredospore suspension of *P. pachyrhizi* was inoculated to the treated leaves.
Assessment: 12 days after inoculation, the percentage of infected leaf area was evaluated. (UTC mean: 86%)

Fig. 3 Activity of *R*-enantiomer and *S*-enantiomer against *P. pachyrhizi* by detached leaf assay

that the other enantiomer (*S*) had little efficacy against soybean rust (**Fig. 3**). From these results, Inpyrfluxam, the *R*-enantiomer of Compound B, was selected as the candidate compound for development.

Manufacturing method

Inpyrfluxam is an amide composed of an optically active amine and a carboxylic acid having a pyrazole structure. For the optically active amine, a few possible synthesis routes exist, as shown in **Fig. 4**, and after vigorous investigation, an effective manufacturing method was established. This enabled the manufacturing of Inpyrfluxam on an industrial scale.

Mode of action (MoA)

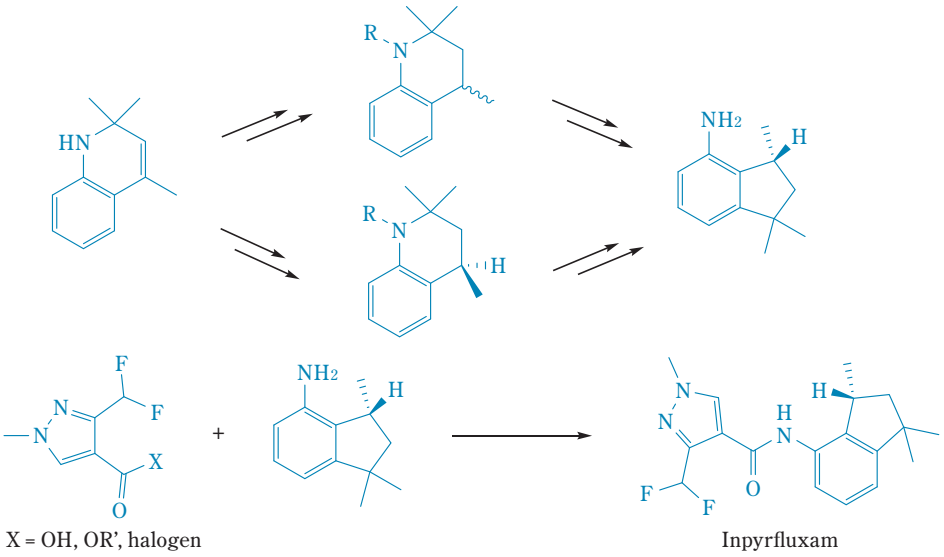
Inpyrfluxam has structural similarity with other mitochondrial complex II (succinate dehydrogenase) inhibitors. To clarify the MoA of Inpyrfluxam, effects on the electron transport system of *P. pachyrhizi* were examined. For enzymatic assay, the crude sub-mitochondrial fraction was extracted from germinated spore of *P. pachyrhizi*. (1) NADH dehydrogenase activity reflects electron transfer from NADH to complex III *via* complex I, and (2) succinate-cytochrome *c* reductase (SCR) activity reflects electron transfer from succinate to cytochrome *c* *via* complex II and complex III. Inpyrfluxam showed a significant inhibitory activity against SCR (**Table 1**). These results demonstrated that Inpyrfluxam is a succinate dehydrogenase inhibitor (SDHI) fungicide.

To further investigation of physiological status under Inpyrfluxam treatment, metabolome analysis by using capillary electrophoresis-mass spectrometer (CEMS) was performed. Germinated spore of *P. pachyrhizi* was treated with Inpyrfluxam, and hydrophilic metabolites of germinated spore were extracted one hour after treatment, and extracted fractions were analyzed by CEMS. As a result of this CEMS analysis, obvious increase of succinic acid and decreases of malic acid, fumaric acid, and glucose-6-phosphate (G6P) were observed, and all of these changes were in dose dependent manner (**Fig. 5**). From above enzyme assay and metabolome analysis, it is considered that the primary target site of Inpyrfluxam is complex II in the mitochondrial electron transport chain of phytopathogenic fungi, and Inpyrfluxam disrupts energy metabolism-related metabolic pathways, *i.e.*, the citric acid cycle and glycolysis system, thereby exerting fungicidal activity (**Fig. 6**).

Table 1 Mitochondrial electron transport chain inhibitory assay (*P. pachyrhizi*)

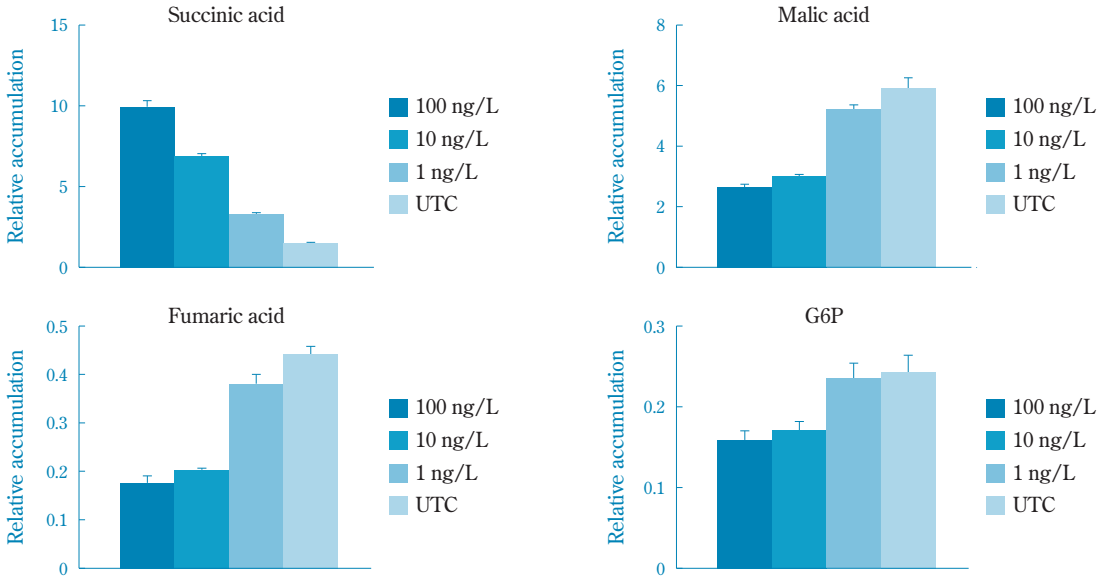
Compound	IC_{50} (mg/L)		Target site
	SCR assay	NADH assay	
Inpyrfluxam	0.000057	> 3	Complex II
Benzovindiflupyr	0.00013	> 3	Complex II
Azoxystrobin	0.0033	0.0037	Complex III
Tolfenpyrad	> 3	0.074	Complex I

Submitochondrial fraction of *P. pachyrhizi* was extracted from germinated spores in 1/2 MS buffer.
The succinate-cytochrome *c* reductase (SCR) assay and NADH assay were carried out as previously described[5].
The inhibitory activity of each chemicals was determined as the concentration required for 50% inhibition (IC_{50}).



X = OH, OR', halogen

Fig. 4 Synthesis route of Inpyrfluxam



Total extract from Inpyrfluxam-treated *P. pachyrhizi* was analyzed by CEMS, and 4 internal metabolites were picked up by statistical analysis.
CE-MS experiments were performed using a CE capillary electrophoresis system (Agilent Technologies, Inc.) and separations were carried out using a fused silica capillary (50 μm id × 100 cm total length) filled with 1 M FA or 20 mM ammonium formate (pH 10.0) as the electrolyte for cation and anion analyses, respectively.
Exact mass data were acquired at a rate of 1.5 cycles/s over a 50–1000 m/z range.

Fig. 5 Internal metabolite affected by Inpyrfluxam treatment in *P. pachyrhizi*

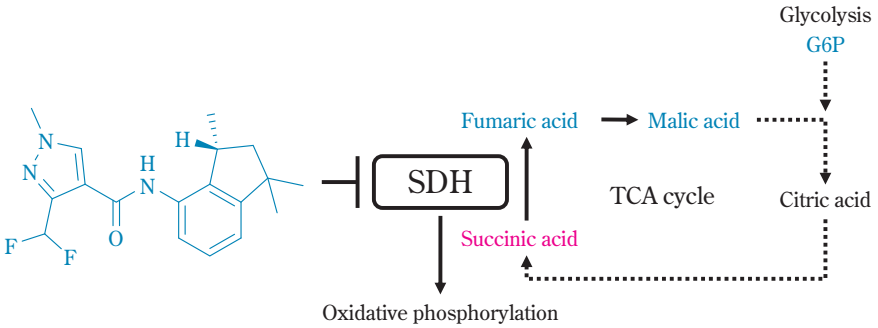


Fig. 6 Mode of action of Inpyrfluxam

Biological efficacy

1. Antifungal spectrum

Inpyrfluxam has a broad antifungal spectrum and exhibits antifungal activity against numerous important phytopathogenic filamentous fungi on crops. In particular, Inpyrfluxam showed potent antifungal activity against species in the division Basidiomycota and those in the class Dothideomycetes and class Leotiomycetes of the division Ascomycota (**Table 2**).

2. Characteristics of action

(1) Preventive efficacy

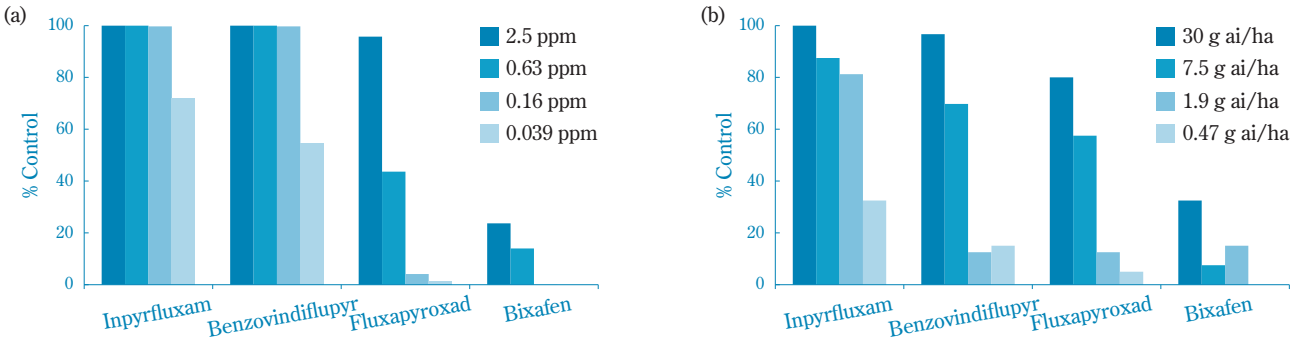
In the laboratory pot tests conducted to evaluate the preventive efficacy of Inpyrfluxam against soybean rust, Inpyrfluxam provided 100% control at 0.16 ppm, indicating an effect similar to or higher than that of major SDHIs used in Brazil (**Fig. 7**(a)). Inpyrfluxam was also highly effective against apple scab, with 100% control at the registered dose for fruit trees (100 ppm) in Japan (**Fig. 8**).

(2) Post-infection efficacy

In the test conducted to evaluate the post-infection efficacy of Inpyrfluxam (a test by applying the fungicide after phytopathogenic fungal infection), Inpyrfluxam provided 100% control against soybean rust at 30 g ai/ha, which was superior to that of other major SDHIs (**Fig. 7**(b)). The efficacy of Inpyrfluxam against apple scab at 100 ppm were also 100%, which was similarly superior to that of other major SDHIs (**Fig. 8**). These results indicate that Inpyrfluxam is able to prevent disease progress when applied at an early stage of phytopathogenic fungal infection.

Table 2 Antifungal activity of Inpyrfluxam

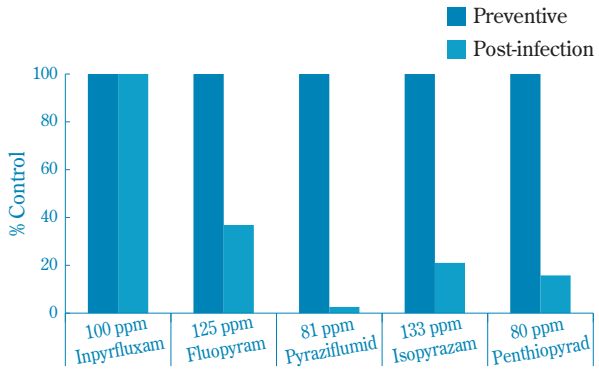
Division	Class	Species	EC_{50} (mg/L)
Ascomycota	Dothideomycetes	*Zymoseptoria tritici*	0.015
		Cercospora zeae-maydis	0.030
		Pyrenophora teres	0.027
		Corynespora cassiicola	0.0095
		Venturia inaequalis	0.0011
	Leotiomycetes	*Botrytis cinerea*	0.0040
		Sclerotinia sclerotiorum	0.015
	Sordariomycetes	*Colletotrichum gloeosporioides*	7.2
		Pyricularia oryzae	2.2
Basidiomycota	Agaricomycetes	*Rhizoctonia solani* (AG1)	0.00077
		Rhizoctonia solani (AG2-1)	0.0061
		Rhizoctonia solani (AG2-2 III B)	0.0018
		Rhizoctonia solani (AG2-2 IV)	0.0029
		Rhizoctonia solani (AG3)	0.0093
		Rhizoctonia solani (AG4)	0.00089
		Rhizoctonia solani (AG5)	0.0014
		Sclerotium rolfsii	0.0046
	Ustilaginomycetes	*Ustilago maydis*	0.00027
Oomycota	Oomycetes	*Phytophthora infestans*	> 10
		Pythium ultimum	> 10



Fungicides were applied 1 day before *P. pachyrhizi* inoculation.
Disease severity in the untreated control: 89%

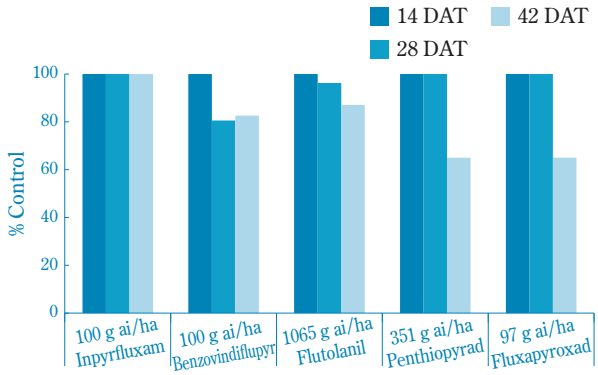
Fungicides were applied 4 days post *P. pachyrhizi* inoculation.
Disease severity in the untreated control: 100%

Fig. 7 The (a) preventive efficacy on detached soybean leaf and (b) post-infection efficacy on soybean seedlings of Inpyrfluxam against soybean rust



Preventive: *V. inaequalis* was inoculated 2 days after fungicide application.
Post-infection: *V. inaequalis* was inoculated 2 days before fungicide application.

Fig. 8 The preventive and post-infection efficacy of Inpyrflucam against apple scab



DAT: *Corticium rolfsii* was inoculated 14, 28, 42 days after fungicide treatment

Fig. 9 The residual activity of Inpyrflucam against peanut white mold

(3) Residual activity

In the 42-day residual activity test, Inpyrfluxam showed high efficacy against peanut white mold, with 100% control at 100 g ai/ha (**Fig. 9**).

(4) Rainfastness

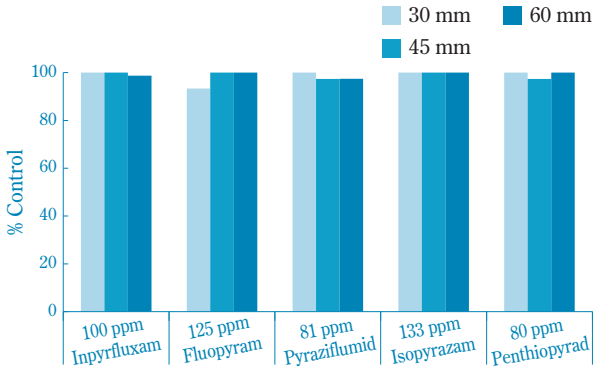
In the rainfastness test against apple scab, Inpyrfluxam showed high efficacy, with 99% control at 100 ppm, under conditions of artificial rainfall treatment for a total of 60 min (30 mm/hour) at 2 hours after fungicide spraying (**Fig. 10**).

(5) Translaminar activity

In the translaminar test (a test conducted by applying fungicide on the abaxial side of leaves and inoculating fungi on the adaxial side of leaves), Inpyrfluxam showed high efficacy against apple scab, with 100% control at 100 ppm (**Fig. 11**). This result suggests that Inpyrfluxam is rapidly absorbed into the plant body and moves also to the unapplied side of the plant.

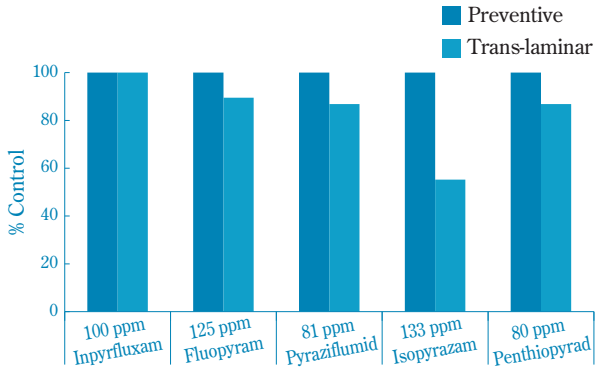
(6) Systemic action

^{14}C-labeled Inpyrfluxam was applied by spotting on



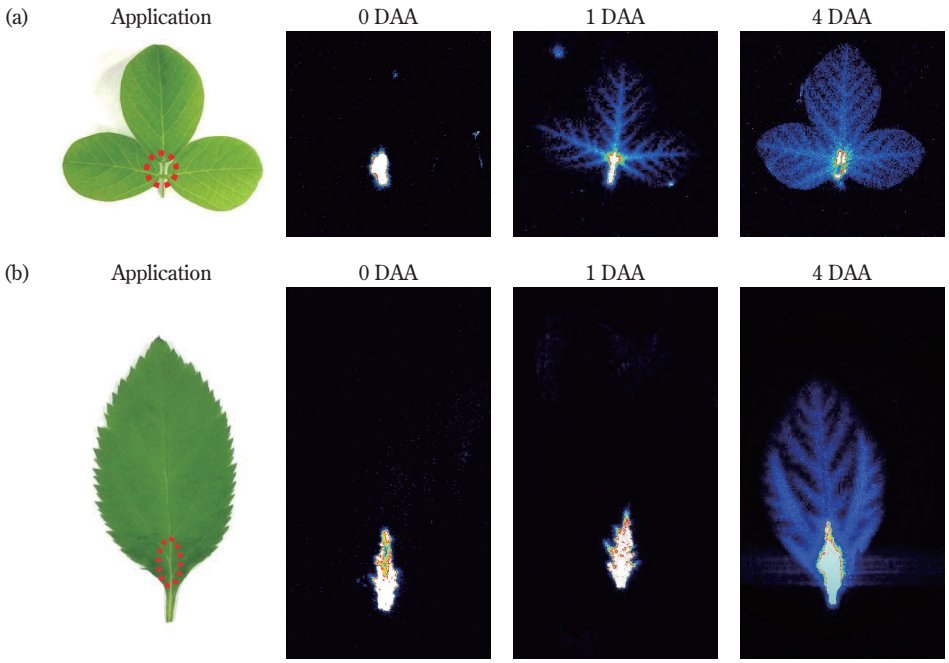
Rainfall: Artificial rainfall was conducted 1 to 2 hours at 2 hours after fungicide application with an intensity of 30 mm/hour.
Inoculation: *V. inaequalis* was inoculated 2 days after artificial rainfall.

Fig. 10 The rainfastness of Inpyrfluxam against apple scab



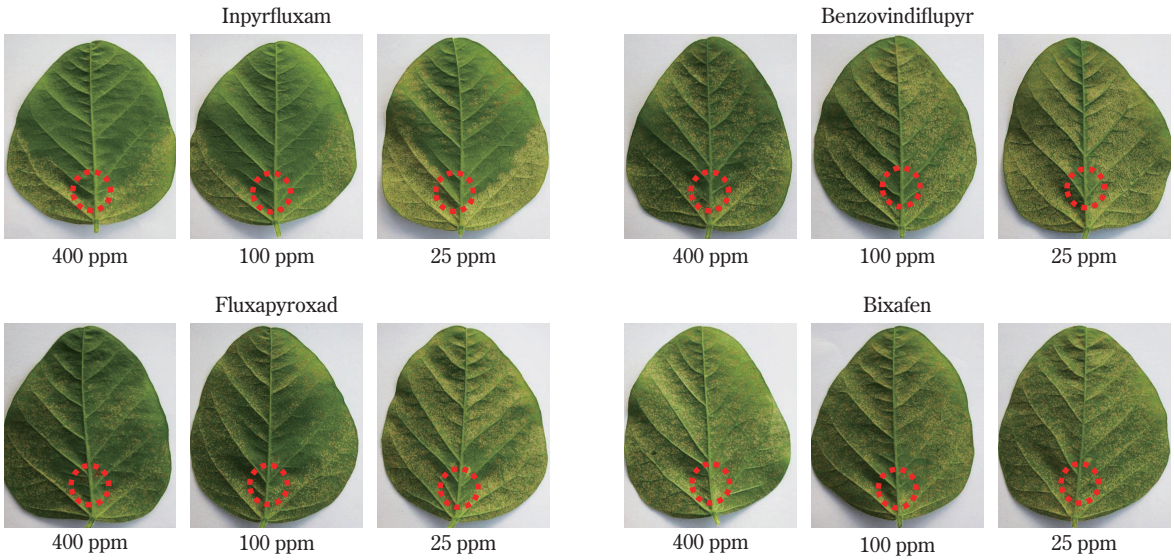
Preventive: *V. inaequalis* was inoculated on adaxial side of leaves 2 days after fungicide application on adaxial side of leaves.
Trans-laminar: *V. inaequalis* was inoculated on adaxial side of leaves 1 day after fungicide application on abaxial side of leaves.

Fig. 11 The translaminar activity of Inpyrfluxam against apple scab



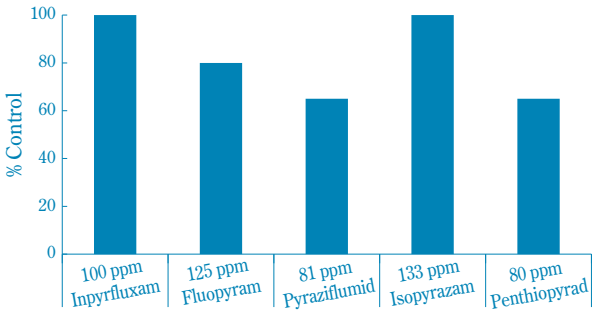
Application: Radio labeled Inpyrfluxam suspension with adjuvant was applied on the petiole (red circle).
Assessment: 0–4 days after application (DAA), radioactivity was visualized by imaging analyzer.

Fig. 12 Autoradiograph of (a) soybean and (b) apple treated with radio labeled Inpyrfluxam



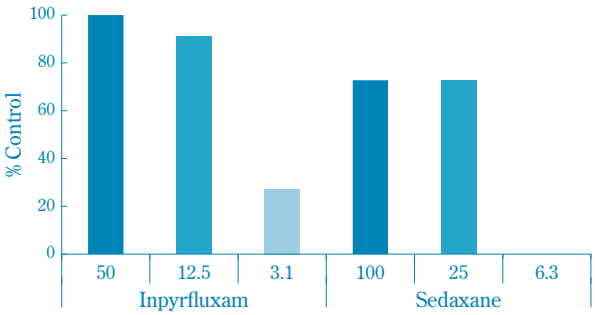
Fungicide suspension was applied on the petiole (red circle) 1 day before *P. pachyrhizi* inoculation.

Fig. 13 Systemic action of Inpyrfluxam against soybean rust



Fungicide suspension was applied on the petiole 5 days before *V. inaequalis* inoculation.

Fig. 14 Systemic action of Inpyrfluxam against apple scab



Treatment: Fungicide solution and soybean seeds were shaken well in plastic bag. (dose: mg/kg seed)
Inoculation: 1 day after treatment, the seeds were sown in soil with *R. solani*.

Fig. 15 Seed treatment of Inpyrfluxam against corn Rhizoctonia root rot

the petiole of soybean and apple, and its distribution was examined by autoradiography. At 1 to 4 days after application, the movement of Inpyrfluxam to the tip of the leaf was observed, demonstrating its systemic action (**Fig. 12**). In fact, by spotting on the petiole, high efficacy against soybean rust and apple scab was observed in the unapplied parts (**Figs. 13** and **14**). In practical use, uneven spraying tends to occur due to thick growth of crops; thus, the movement of a fungicide to unapplied parts is sometimes desired for disease control. The translaminar activity/systemic action of Inpyrfluxam is inferred to contribute to the stable, superior efficacy even in such a situation.

(7) Seed treatment

In the seed treatment test against corn Rhizoctonia root rot, Inpyrfluxam showed high efficacy, with 100%

Application: Radio labeled Inpyrfluxam suspension was applied on corn seed.
Assessment: 7 days after application, radioactivity was visualized by imaging analyzer.

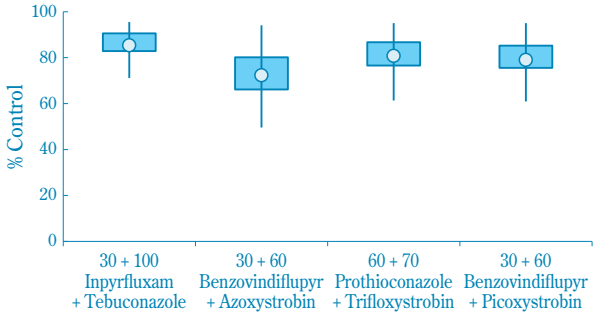
Fig. 16 Autoradiograph of corn seed treated with radio labeled Inpyrfluxam

control at 50 mg/kg seeds treatment (**Fig. 15**). When the distribution of ^{14}C-labeled Inpyrfluxam treated on corn seeds was examined by autoradiography, the movement of Inpyrfluxam not only to the vicinity of seeds but also to the mesocotyls and coleoptiles was observed 7 days after seeding (**Fig. 16**).

3. Practical evaluation

(1) Soybean rust (Brazil)

As mentioned above, soybean rust occurring in Brazil in recent years is resistant to DMIs and QoIs, and its control is becoming difficult. In addition, in the 2015 to 2016 season, fungal strains with a point mutation that reduces SDHI sensitivity (a nucleobase mutation in the SdhC gene causing the substitution of isoleucine, the 86th amino acid, to phenylalanine) were detected, with a report of actual decrease in efficacy in the fields.[6] In such a situation, Inpyrfluxam showed a stable, high efficacy against soybean rust in multiple practical application trials (**Fig. 17**). The results suggest that Inpyrfluxam is highly useful for controlling soybean rust with multiple fungicide resistance.

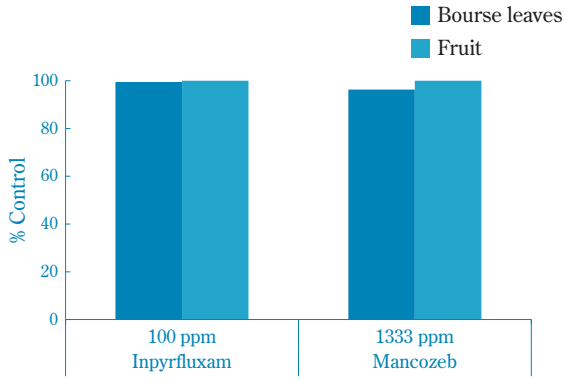


Soybean rust field trials in the 2017–18 season (total 14 trials).
Dose: g ai/ha
Box, interquartile range; Dot, mean value; Edge of the line, the highest and lowest value

Fig. 17 Efficacy against soybean rust in field trial

(2) Apple scab (Japan)

Similar to soybean rust in Brazil, a part of apple scab occurring in Japan in recent years is resistant to the key fungicides, DMIs and QoIs, and its control is becoming difficult. Inpyrfluxam showed high efficacy against apple scab in a field trial (**Fig. 18**). The result suggests that Inpyrfluxam is highly useful for controlling apple scab and is a promising disease control agent in this situation.



Location: Aomori prefecture, Japan
Application: Foliar application. First application in flowering and second and third applications in abscission period
Assessment: Conducted 24 days after final fungicide application. Assessed the disease incidence of 299–367 leaves and 150 fruits/tree
Replication: 3 trees/treatment

Fig. 18 Efficacy against apple scab in field trial

Formulation

As mentioned above, KANAME® flowable (Inpyrfluxam 37% (w/w) SC) has been launched in March 2020 in Japan (**Fig. 19**).

The formulation study for KANAME® flowable was focused on the optimization of the combination of auxiliary materials and the amount of each material, and this formulation is satisfied the physical and chemical properties desired for spraying and also has good storage stability for a long term.

Table 3 shows representative physical and chemical properties of KANAME® flowable.

In addition to KANAME® flowable, MILLIONAIRE flowable, which is treated for various crops, including sugar beet, potato, onion, and beans in Hokkaido, is to

Fig. 19 KANAME® flowable

Table 3 Physical and chemical properties of KANAME® flowable

Items	Typical value (Methods)
Appearance	Whitish viscous liquid (Visual observation)
Density	1.08 g/mL (20 °C)
pH	8.3 (Electric pH meter, neat)
Viscosity	1435 mPa·s (Brookfield viscometer, Spindle No. 2, 6 rpm, 25 °C)
Suspensibility	98% (250 mL cylinder, 53.6 ppm hard water, 500 times dilution, 20 °C, 15 min)
Persistent foam	20 mL (250 mL cylinder, 342 ppm hard water, 500 times dilution, 20 °C, 1 min)
Stability	Physical and chemical properties after storage at ambient temperature for 4 years were very stable

be launched at the end of 2020. Furthermore, the development of formulation for nursery-box application and foliar application on paddy fields against sheath blight is ongoing.

Outside Japan, a mixture flowable concentrate with tebuconazole (trade name: Excalia Max™) against stem and leaf diseases, such as soybean rust, is to be launched for South American countries, including Brazil. In addition, the development of an emulsifiable concentrate against wheat diseases (*e.g.*, Septoria leaf blotch and rust) and barley diseases (*e.g.*, net blotch and rust) in Europe is ongoing. Furthermore, the formulation study of a mixture flowable concentrate for seed treatment against soil diseases (*e.g.*, damping off caused by *Rhizoctonia solani*) in South America is also in progress.

Toxicity/Metabolism/Persistence

1. Toxicity in mammals

(1) Acute toxicity, irritation, and skin sensitization

The LD_{50} value of Inpyrfluxam technical product was >50 mg/kg body weight (bw) and <300 mg/kg bw in rats for oral administration; >2000 mg/kg bw in rats for dermal application; and >2610 mg/m^3 in rats for inhalation exposure. The major clinical signs observed in rats following oral administration and inhalation exposure included decrease of spontaneous activity, ataxic gait, and lateral position; no noteworthy changes were observed following dermal application. The LD_{50} value of 37% SC was >300 mg/kg bw and <2000 mg/kg bw in rats for oral administration and >2000 mg/kg bw in rats for dermal application. Following oral administration, clinical signs similar to those seen after administration of Inpyrfluxam technical product were observed; no noteworthy changes were observed following dermal application. The acute toxicity of 3% granules (3% GR) was extremely low, with no deaths or toxic signs observed following oral administration at 3000 mg/kg bw or dermal application at 2000 mg/kg bw. Eye irritation potential of Inpyrfluxam technical product and 3% GR was minimal and was reduced after washing. There was no eye irritation potential of 37% SC. None of Inpyrfluxam technical product, 37% SC, and 3% GR caused skin irritation or skin sensitization (**Table 4**).

(2) Subacute/chronic toxicity and carcinogenicity

In the subacute toxicity, chronic toxicity, and carcinogenicity studies in rats, dogs, and/or mice, repeated administration of Inpyrfluxam technical product resulted in decreased body weight gain and decreased food consumption, with toxic effects mainly seen in the liver and thyroid (**Table 5**). In the liver, increased organ weight and hepatocellular hypertrophy were

Table 4 Acute toxicity summary of Inpyrfluxam

Test type	Inpyrfluxam	Inpyrfluxam 37%SC	Inpyrfluxam 3%GR
Rat acute oral (LD_{50})	50 mg/kg < LD_{50} < 300 mg/kg	300 mg/kg < LD_{50} < 2000 mg/kg	> 3000 mg/kg
Rat acute dermal (LD_{50})	> 2000 mg/kg	> 2000 mg/kg	> 2000 mg/kg
Rat inhalation (LC_{50})	> 2610 mg/m^3 of air (4 hours, nose only exposure)	—	—
Eye irritation (Rabbit)	Minimally irritant	Non-irritant	Minimally irritant
Skin irritation (Rabbit)	Non-irritant	Non-irritant	Non-irritant
Skin sensitization (Guinea pig)	Non-sensitizer	Non-sensitizer	Non-sensitizer

Table 5 Subacute and chronic toxicity summary of Inpyrfluxam

Species	Administration route and duration	Dose (ppm)	NOAEL (mg/kg/d)
Rat	Dermal, 28 days	100, 300, 1000	Male: 1000 Female: 1000
Rat	Oral (in diet), 13 weeks	150, 500, 2000, 4000	Male: 31.7 (500 ppm) Female: 37.5 (500 ppm)
Rat	Oral (in diet), 24 months	Male: 150, 500, 2000 Female: 150, 500, 1500/1000*	Male: 19.4 (500 ppm) Female: 25.5 (500 ppm) No carcinogenicity
Dog	Oral (in capsule), 13 weeks	40, 160, 700/500* mg/kg/d	Male: 40 Female: 40
Dog	Oral (in capsule), 12 months	2, 6, 30, 160 mg/kg/d	Male: 6 Female: 6
Mouse	Oral (in diet), 13 weeks	200, 800, 3500, 7000	Male: 111 (800 ppm) Female: 130 (800 ppm)
Mouse	Oral (in diet), 18 months	700, 2000, 7000/5000*	Male: 77.0 (700 ppm) Female: 69.3 (700 ppm) No carcinogenicity

*: The highest dose was reduced during the treatment period due to severe effects.

observed. These hepatic changes were accompanied by follicular cell hypertrophy in the thyroid. Interstitial gland vacuolation in the ovary was observed in rats and cortical cell vacuolation in the adrenal was observed in rats and dogs; however, they were not considered to be the toxic effects attributable to the direct effect of Inpyrfluxam technical product on the endocrine system or serious changes that impair the function of these organs. No carcinogenicity was found in rats or mice.

(3) Developmental and reproductive toxicity

In the developmental toxicity (teratogenicity) studies in rats and rabbits, no teratogenicity was found in fetuses. In the two-generation reproductive toxicity study in rats, no effect was found on reproductive performance or nursing behavior (**Table 6**).

(4) Neurotoxicity

In either acute neurotoxicity or subacute neurotoxicity study in rats, no specific neurotoxic effect was found (**Table 7**).

(5) Mutagenicity

In the reverse mutation study in *Salmonella typhimurium* and *Escherichia coli*, the chromosomal aberration study in Chinese hamster lung CHL/IU cell line, the gene mutation study in Chinese hamster lung V79 cell line, and the micronucleus study in mice, the results were all negative (**Table 8**).

Table 6 Developmental and reproductive toxicity summary of Inpyrfluxam

Study	Species	Administration route and duration	Dose (mg/kg/d)	NOAEL (mg/kg/d)	
Developmental toxicity	Rat	Oral (gavage) Days 6–20 of gestation	(1) 10, 25, 80 (2) 90	Maternal Fetal	25 25
	Rabbit	Oral (gavage) Days 6–27 of gestation	20, 60, 200	Maternal Fetal	60 200
Two-generation reproductive toxicity	Rat	Oral (in diet)	Male: 150, 500, 2000 ppm Female: 150, 500, 1250 ppm	Parental	Systemic Male: 31.3 (500 ppm) Female: 35.5 (500 ppm) Reproductive Male: 124 (2000 ppm) Female: 86 (1250 ppm)
				Offspring	Systemic 31.3 (500 ppm)

Table 7 Neurotoxicity summary of Inpyrfluxam

Study	Species	Administration route and duration	Dose	NOAEL (mg/kg/d)
Neurotoxicity	Rat	Acute oral (gavage)	30, 100, 200 mg/kg/d	Male: 200 Female: 30
	Rat	Oral (in diet), 13 weeks	Male: 500, 2000, 4000 ppm Female: 500, 1000, 2000 ppm	Male: 118.9 (2000 ppm) Female: 35.2 (500 ppm)

Table 8 Mutagenicity summary of Inpyrfluxam

Study	Study design	Results
Reverse mutation (Ames test)	*S. typhimurium*: TA98, TA100, TA1535 and TA1537 *E. coli*: WP2uvrA –/+S9 mix: 1.50–5000 µg/plate	Negative
In vitro gene mutation	Chinese hamster V79 –/+S9 mix: 6.5–78.0 µg/mL	Negative
In vitro chromosomal aberration	Chinese hamster CHL/IU –S9 mix (6 h): 32.5–130 µg/mL +S9 mix (6 h): 42.5–170 µg/mL –S9 mix (24 h): 0.188–1.50 µg/mL	Negative
Bone marrow micronucleus	CD-1 mice 200, 400, 800 mg/kg	Negative

2. Animal/plant metabolism

(1) Metabolism in animals

Following oral administration of a ^{14}C-labeled Inpyrfluxam to rats, Inpyrfluxam was rapidly absorbed and distributed to the whole body. Inpyrfluxam was then rapidly metabolized, and a similar amount was excreted in the urine and feces. The oral absorption rate was estimated to be ≥95.7%. No persistence or accumulation was observed in the tissue.

The major metabolic reactions for Inpyrfluxam were N-demethylation, hydroxylation of the methyl group in position 1 of the indane ring and formation of a carboxylic acid metabolite, and glucuronidation. In addition, hydroxylation in position 3 or 7 of the indane ring was also observed.

(2) Metabolism in plants

Plant metabolism studies using ^{14}C-labeled Inpyrfluxam with four different types of crops (paddy rice, soybean, potato and apple) demonstrated that the metabolic pathway of Inpyrfluxam was similar in all. Inpyrfluxam was metabolized *via* hydroxylation at the 3-position of the indane ring, hydroxylation of the methyl group at the 1-position of the indane ring, N-demethylation and cleavage of the amide bond. The metabolites produced were considered to be further conjugated with sugars and others, and finally incorporated into the constituents of plants.

3. Environmental behavior and residue

(1) Degradation in water

In the hydrolysis study, ^{14}C-labeled Inpyrfluxam was stable in buffer solutions at pH 4, 7 and 9 with a half-life of ≥1 year (25 °C). The photodegradation half-life of Inpyrfluxam was also ≥1 year in a buffer solution (pH 7), whereas degradation was accelerated by light irradiation in natural water (pH 7.5) with a half-life of 223 to 549 days (equivalent to the natural sunlight at Tokyo in spring) *via* hydroxylation at the 3-position of the indane ring and cleavage of the amide bond, and finally mineralized to carbon dioxide.

(2) Metabolism in soil

In the flooded aerobic soil and aerobic soil metabolism studies, the degradation half-life (25 °C) of ^{14}C-labeled Inpyrfluxam was ≥1000 days and ≥827 days, respectively. Degradation on the soil surface was slightly accelerated by exposure to light, and the half-life (20 °C) was ≥763 days (equivalent to the natural sunlight at Tokyo in spring). The major degradation pathway in soil was hydroxylation at the 3-position of the indane ring and finally bound to soil matrix.

(3) Residue in soil

Following a single spray application of 3% GR to rice paddies in Ibaraki and Kochi at 3 kg/10 a, the maximum residue was 1.69 to 3.20 mg/kg, with a half-life of 7.9 to 10.9 days. Following a single spray application of a 750- or 1500-fold diluted 37% SC to upland fields in Ibaraki, Saitama, Kochi, Kumamoto, and Miyazaki at 300 L/10 a, the maximum residue was 0.74 to 2.82 mg/kg, with a half-life of 11.1 to 69.8 days.

(4) Mobility in soil

The absorption coefficient $K_{Foc(ads)}$ and desorption coefficient $K_{Foc(des)}$ of Inpyrfluxam corrected with the organic carbon content were calculated using the Freundlich adsorption isotherm and were 500 to 891 and 682 to 1264, respectively.

(5) Residue in crops

Following a single rice nursery-box application of Inpyrfluxam 3% GR 50 g with or without submerged applications twice during the growing season at 1 kg/10 a, the residue was below the limit of quantification in rice grain and unhulled rice and 0.02 to 0.61 mg/kg in the rice straw.

Following application by dipping, irrigation, or spraying of a 40- to 4000-fold diluted 37% SC to 20 crops* in addition to paddy rice shown below, the maximum mean residue was below the limit of quantification to 6.08 mg/kg.

* Crops in the residue studies: paddy rice, wheat, barley, soybean, kidney bean, potato, sugar beet, onion, green onion, snow pea, string bean, edamame (green soybean), satsuma mandarin (*Citrus unshiu*), Chinese citron, *Citrus sudachi*, *Citrus sphaerocarpa*, apple, Japanese pear, peach, grape, and persimmon.

(6) Residue in succeeding crops

Because the half-life of Inpyrfluxam in the soil residue studies was 7.9 to 69.8 days, the effects on succeeding crops were considered to be small.

4. Effects on non-target species

The study results in aquatic plants and animals, honeybees, silkworms, natural enemy insects, and birds are summarized in **Table 9**.

(1) Effects on aquatic plants and animals

The acute toxicity values for Inpyrfluxam technical product in fish, *Daphnia magna*, and freshwater green algae (LC_{50}/EC_{50}/ErC_{50}) were 0.031, 1.1, and >23 mg/L, respectively. The corresponding toxicity values for Inpyrfluxam 3% GR and 37% SC were 5.0, 29, and >1000 mg/L and 0.18, 3.2, and 130 mg/L, respectively. Because these values are sufficiently higher than the concentrations expected for water in the environmental from practical use, the effects of Inpyrfluxam on aquatic plants and animals are considered to be low.

Table 9 Ecotoxicological summary of Inpyrfluxam on non-target organisms

Test substance	Test species		Test type	Results
Inpyrfluxam	Aquatic organisms	Rainbow trout	Acute (96 hours)	LC_{50} = 0.031 mg/L
		Daphnia magna	Acute (48 hours)	EC_{50} = 1.1 mg/L
		Green alga*	Acute (72 hours)	ErC_{50} > 23 mg/L
	Honeybee	*Apis mellifera*	Acute oral (48 hours)	LD_{50} > 111.3 µg/bee
		Apis mellifera	Acute contact (48 hours)	LD_{50} > 100.0 µg/bee
	Bird	Bobwhite quail	Acute oral	LD_{50} > 2250 mg/kg
Inpyrfluxam 3%GR	Aquatic organisms	Carp	Acute (96 hours)	LC_{50} = 5.0 mg/L
		Daphnia magna	Acute (48 hours)	EC_{50} = 29 mg/L
		Green alga*	Acute (72 hours)	ErC_{50} > 1000 mg/L
Inpyrfluxam 37%SC	Aquatic organisms	Carp	Acute (96 hours)	LC_{50} = 0.18 mg/L
		Daphnia magna	Acute (48 hours)	EC_{50} = 3.2 mg/L
		Green alga*	Acute (72 hours)	ErC_{50} = 130 mg/L
	Silkworm	*Bombyx mori*	Acute oral (23 days)	Mortality 24% (at 185 mg ai/L)
		Bombyx mori	Residual toxicity test	< 3 days
	Natural enemy	*Neoseiulus californicus* (adult)	Acute contact (7 days)	Mortality ≤ 2.0% (at 185 mg ai/L)
		Orius strigicollis (adult)	Acute contact (7 days)	Mortality ≤ 2.0% (at 185 mg ai/L)
		Aphelinus asychis (adult)	Acute contact (7 days)	Mortality ≤ 2.0% (at 185 mg ai/L)

*: *Raphidocelis subcapitata*

(2) Effects on honeybees, silkworms and natural enemy insects

The LD_{50} values for oral administration and contact administration of Inpyrfluxam technical product in western honeybees were >111.3 and >100.0 μg/bee, respectively. The mortality of silkworms following acute oral administration of 37% SC was 24%; however, in the residual toxicity study with mulberry leaves sprayed with 37% SC, no effect was observed on silkworms at three days post-application and thereafter, and the residual toxicity period was considered to be <3 days. Regarding natural enemy organisms, the mortality of *Neoseiulus californicus*, *Orius strigicollis*, and *Aphelinus asychis* following contact administration was ≤2.0%. From these results, the effects of Inpyrfluxam on honeybees, silkworms, and natural enemy insects are considered to be low in practical use.

(3) Effects on birds

The acute toxicity of Inpyrfluxam technical product in bobwhite quail was low, with a LD_{50} value for oral administration of >2250 mg/kg. From this result, the effects of Inpyrfluxam on birds are considered to be low in practical use.

In summary, although the acute toxicity of Inpyrfluxam technical product and 37% SC in mammals following oral administration is relatively strong, that of 3% GR is very weak, and long-term intake of Inpyrfluxam technical product is unlikely to cause carcinogenicity or adverse effects on the next generation such as teratogenicity or impaired fertility. In addition, from the evaluation of the environmental behavior and the effects on non-target organisms, the effects on the environment are considered to be low when used according to the methods applied for registration.

Conclusion

Inpyrfluxam is a novel SDHI fungicide with high efficacy against soybean rust, a disease devastatingly affecting soybean production in Brazil. Excalia Max™, a mixture formulation containing Inpyrfluxam, has shown excellent performance, such as the best efficacy against soybean rust and the maximum soybean yield for three consecutive years, in the field studies conducted by the Brazilian Agricultural Research Corporation (Embrapa) to evaluate the performance of fungicides being marketed or developed,[7)–9)] and its early launch is desired by local groups of soybean producers and others. In addition, Inpyrfluxam is quite useful for controlling apple scab and other important phytopathogenic fungi on major crops. It would be possible to use Inpyrfluxam as the key fungicide in various disease control scenes. However, in order to prevent the development of resistance to this product, it is desirable to avoid continuous application of Inpyrfluxam and use it in a disease controlling program in combination with fungicides with other mode of action.

In future studies, for example, field trial data for disease controlling programs using Inpyrfluxam will be further accumulated, and taking into consideration risk management against resistance development, we will promote effective use of this product for better crop disease control to worldwide farmers.

Acknowledgments

We would like to express our deep gratitude to the Japan Plant Protection Association, research organizations in various prefectures, universities, and the National Research and Development Agencies for the conduct of practical evaluation studies and plenty of advice given during the development of Inpyrfluxam.

Reference

1) United States Department of Agriculture, "World Agricultural Production, 2019", https://www.fas.usda.gov/data/world-agricultural-production (Ref. 8/6/2019).
2) S. H. Furlan *et al.*, Outlooks Pest Manage., 29, 120 (2018).
3) Sumitomo Chemical Co., Ltd., JP 6246768 B2 (2017).
4) T. Mori *et.al.*, SUMITOMO KAGAKU, 1997-II, 12 (1997).
5) S. Kataoka *et al.*, J. Pestic. Sci., 35, 99 (2010).
6) K. Simões *et al.*, J. Plant Dis. Prot., 125, 21 (2018).
7) C. V. Godoy *et al.*, Embrapa soja - circular tecnica, 129 (2017).
8) C. V. Godoy *et al.*, Embrapa soja - circular tecnica, 138 (2018).
9) C. V. Godoy *et al.*, Embrapa soja - circular tecnica, 148 (2019).

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