Development of a Novel Paddy Rice Herbicide Propyrisulfuron (ZETA-ONE®)

Sumitomo Chemical Co., Ltd.
Health & Crop Sciences Research Laboratory
Hajime IKEDA
Shigeyuki ITO
Yukio OKADA
Environmental Health Science Laboratory
Kazuki MIKATA
Mariko ENDO
Organic Synthesis Research Laboratory
Ichiro KOMOTO

Propyrisulfuron is a novel sulfonylurea herbicide with a fused heterocyclic moiety, developed by Sumitomo Chemical Co., Ltd. Propyrisulfuron is used as a rice herbicide to control annual and perennial paddy weeds, including *Echinochloa* spp., sedges and broadleaf weeds. Propyrisulfuron controls weed biotypes that have developed resistance to the commercialized sulfonylurea herbicides. Propyrisulfuron shows safer profiles for human health and the environment. The granular formulation, suspension concentrate formulation and jumbo formulation (granules packed in water-soluble film) are available in Japan with the brand name of ZETA-ONE®.

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Introduction

Herbicides have made a large contribution to reducing the workload of crop production. While herbicides with various modes of action are currently used, sulfonylurea herbicides, which were developed starting in the 1970s, are in widespread use worldwide, and they hold the central position in weed control in Japan as paddy rice herbicides. However, as is the fate of agricultural chemicals that are used intensively, the problem of resistance to sulfonylurea herbicides began to appear in the latter half of the 1990s.

While propyrisulfuron (**Fig. 1**) is a compound that has a sulfonylurea skeleton, it has the unique characteristic of exhibiting herbicidal activity against weeds that show resistance to commercialized sulfonylurea herbicides. This chemical controls a wide spectrum of paddy weeds at low dose rates. In addition, because of its physical and chemical properties, it can be provided in the three major formulations used in paddy rice culture in Japan, namely granular formulation, suspension concentrate and jumbo formulation. Furthermore, it shows a high level of safety for mammals, birds and aquatic organisms.

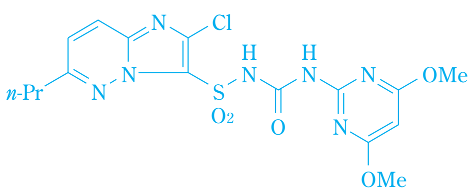


Fig. 1 Structure of propyrisulfuron

Herbicide products containing this compound were registered as agricultural chemicals in Japan on December 13, 2010. Moreover, development of this compound is also moving forward in South Korea, and practical evaluations are being conducted in other parts of the Asian region. In the following, we will report on the discovery of propyrisulfuron, its history of development and the knowledge obtained through the processes involved.

History of Development

1. Sulfonylurea herbicides

Sulfonylurea herbicides[1)–4)], which were discovered by Levitt with DuPont, have strong points such as (i) low toxicity for untargeted organisms (ii) a much lower application rate than conventional herbicides, (iii) a broad herbicidal spectrum ranging from broadleaf weeds to grasses, and (iv) safety to various crops that can be imparted by structural modifications. It has come to hold a foremost position among selective herbicides.

Up to now, several agricultural chemical companies have developed and marketed large numbers of sulfonylurea herbicides with selectivity for a wide range of crops. Among them, bensulfuron methyl (DuPont) and pyrazosulfuron ethyl (Nissan Chemical Industries, Ltd.) were developed as paddy rice herbicides. At the beginning of the 1980s, Takeda Chemical Industries, Ltd. (now Takeda Pharmaceutical Company Ltd.) also focused on that chemical family and developed and marketed the paddy rice herbicide imazosulfuron[5)-8)] and the wheat herbicide sulfosulfuron[9)]. These chemicals have a structure with a condensed heterocycle that have a nitrogen atom at the bridgehead position, a configuration which was not familiar in agricultural chemicals at the time. (**Fig. 2**). These two compounds hold an important position among the herbicides in Sumitomo Chemical Co., Ltd..

The features of sulfonylureas make it possible to develop one-shot herbicides which can control weeds in paddy fields with a single treatment. Sulfonylurea herbicides save much labor in the cultivation of paddy rice and are used in almost all paddy fields in Japan.

2. Development of propyrisulfuron

Since single treatments using one-shot herbicides have spread among rice growers in Japan, some weeds have begun to show resistance to sulfonylurea herbicides.[10)] This problem is unavoidable when herbicides that have the same mode of action are used every year.

Sumitomo Chemical was concerned about these weeds that show resistance to commercialized sulfonylurea herbicides (hereinafter called SU-resistant weeds), and has investigated the herbicidal activity of various sulfonylurea compounds on SU-resistant weeds collected from paddy fields. As a result, compound (A) (**Fig. 3**) was found in imazosulfuron analogs as a lead compound that exerts a herbicidal effect even on SU-resistant weeds.

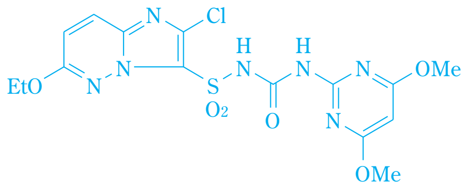


Fig. 3 Structure of compound (A)

The resistance to sulfonylurea herbicides found up until now has been reported to be caused by mutations in acetolactate synthase (EC 2.2.1.6, ALS), which is the site of action, rather than improvements in the detoxification capacity of plants.[11)-13)] However, it became clear that compound (A) was active on the enzymatic level from investigations using ALS which was isolated from SU-resistant *Schoenoplectus juncoides*. Commonly, compounds in the same chemical family exhibit cross-resistance for mutations at the site of action, but compound (A) was a rare example that did not follow this pattern, and this was the impetus for starting an investigation into a sulfonylurea compound which was effective against SU-resistant weeds.

The structural differences between imazosulfuron and compound (A) are in the structure of the condensed heterocycle portion and the substituent at position 6. According to tests of the inhibitory activity of ALS, it became clear that both of them affected the herbicidal efficacy. The results are shown in **Table 1**.

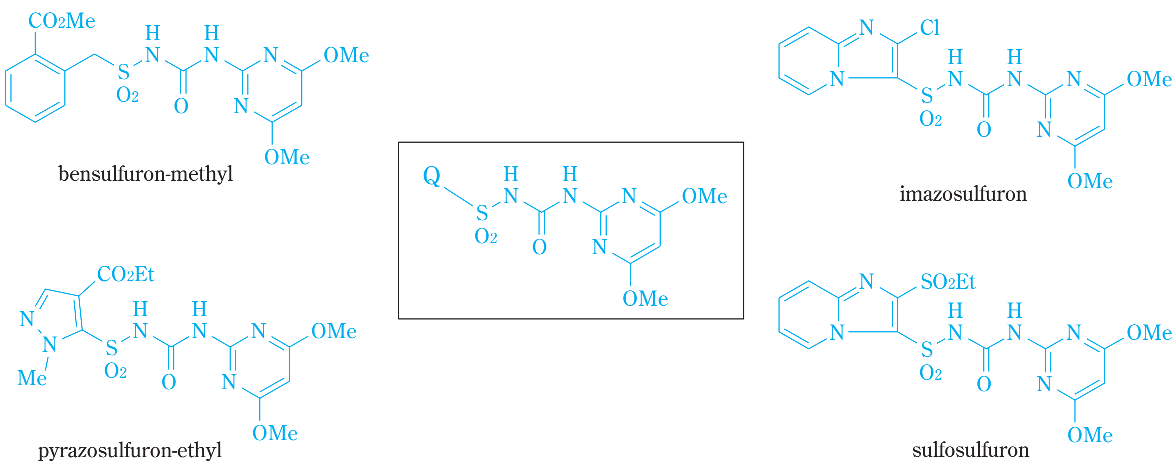
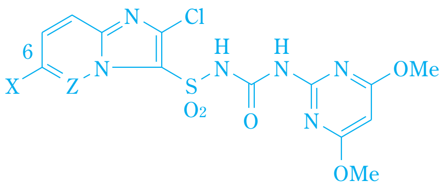


Fig. 2 Sulfonylurea herbicides

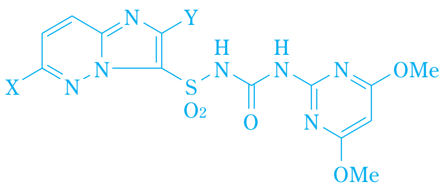
For the next step, the effect of substituents on the imidazopyridazine ring was investigated. The results are shown in **Table 2**.

Table 1 Inhibition of *Schoenoplectus juncoides* ALS by sulfonylurea compounds



X	Z		IC50 (nM) S	IC50 (nM) R
H	C	imazosulfuron	15	>10000
EtO	C		0.6	185
H	N		53	7818
EtO	N	Compound (A)	4.1	47

Table 2 Inhibition of *Schoenoplectus juncoides* ALS by sulfonylurea compounds



No.	X	Y	IC50 (nM) S	IC50 (nM) R
1	H	Cl	53	7818
2	Me	Cl	352	5338
3	Et	Cl	21	47
4	*n*-Pr	Cl	20	16
5	*i*-Pr	Cl	36	67
6	*n*-Bu	Cl	24	66
7	*cyclo*-Pr	Cl	2	9
8	vinyl	Cl	42	427
9	Allyl	Cl	73	1643
10	MeO	Cl	11	1056
11	EtO	Cl	4	47
12	*n*-PrO	Cl	2	520
13	MeS	Cl	2	24
14	EtS	Cl	1	14
15	*n*-PrS	Cl	1	13
16	EtO	Me	5	42
17	EtO	Et	0.2	33
18	EtO	*n*-Pr	6	40
19	EtO	Cl	4	47
20	EtO	SO_2Me	10	374
21	H	Me	181	3195
22	Me	Me	79	6224
23	Et	Me	18	6
24	*n*-Pr	Me	8	20
25	*i*-Pr	Me	28	16
26	MeO	Me	30	624
27	EtO	Me	5	42

Regarding X, the substituents having a chain length of 2–3 atoms are favorable for the inhibition of ALS from SU-resistant *Schoenoplectus juncoides*, and among these, the ethyl group and the propyl group are best for inhibition activity. Regarding Y, the methyl group and the chlorine atom exhibited a high level of activity. Among all compounds investigated, propyrisulfuron (No. 4)[14] was selected not only for its herbicidal activity for SU-resistant weeds, but also for its herbicidal spectrum and selectivity for rice.

Manufacturing Methods

The synthesis of a sulfonylurea compound having an imidazo[1,2-*b*]pyridazine ring is based on the methods for synthesizing[5)–7),9)] condensed heterocycle sulfonylurea compounds in the development of imazosulfuron and sulfosulfuron, and the method shown in **Fig. 4** is carried out.

In addition, diligent investigations into the various manufacturing routes including that described above were carried out, and the method for producing high-purity propyrisulfuron in high yields was chosen and established.

Table 3 Weed spectrum for propyrisulfuron at 90 g a.i./ha

	Weed species	Application window
Grasses	*Echinochloa oryzicola*	~ 3-leaf
	Echinochloa crus-galli	~ 3-leaf
	Echinochloa crus-galli var. formosensis	~ 3-leaf
Sedges	*Eleocharis kuroguwai*	~ 14 cm
	Cyperus difformis	~ 2-leaf
	Cyperus serotinus	~ 3-leaf
	Schoenoplectus juncoides	~ 3-leaf
	Schoenoplectus nipponicus	~ 17 cm
	Bolboschoenus koshevnikovii	~ 12 cm
Broadleaf weeds	*Monochoria vaginalis*	~ 2-leaf
	Sagittaria pygmaea	~ 2-leaf
	Sagittaria trifolia	~ 3-leaf
	Alisma canaliculatum	~ 2-leaf
	Lindernia angustifolia	~ 1-leaf
	Lindernia procumbens	~ 1-leaf
	Lindernia dubia	~ 1-leaf
	Rotala indica	~ 2-leaf
	Elatine triandra	~ 2-leaf
	Bidens tripartita	~ 2-leaf
	Bidens frondosa	~ 3-leaf
	Eclipta thermalis	~ 2-leaf
	Aeschynomene indica	~ 2-leaf
	Oenanthe javanica	at emergence
	Potamogeton distinctus	~ 6-leaf

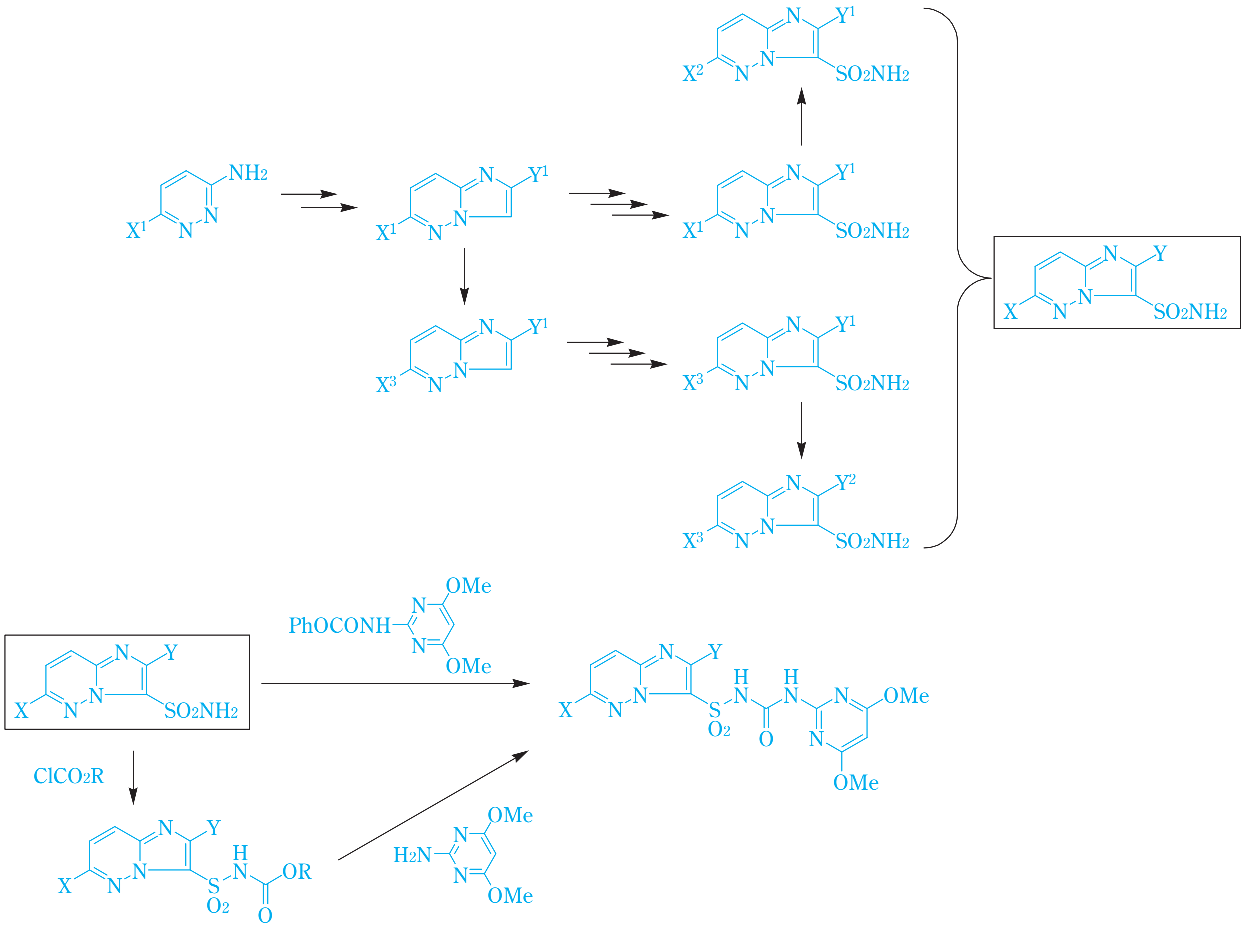


Fig. 4 Synthetic route of sulfonylureas contain imidazo[1,2-*b*]pyridazine moiety

Table 4 Weed control efficacy of propyrisulfuron at the application timing of 2.5-leaf stage of *Echinochloa* in a field trial

Herbicide	Dose	Herbicidal activity*1					
		ECHss	SCPJO	MOOVA	BLWs	CYPSE	OENJA
	g a.i./ha	Weed control (0 - 100, 6WAA*2)					
Propyrisulfuron 0.9 % granule	90	99	99	100	100	99	99
Imazosulfuron 0.9 % + benzobicyclon 2.0 % + cafenstrole 2.0 % granule	90 + 200 + 200	99	100	100	100	99	100

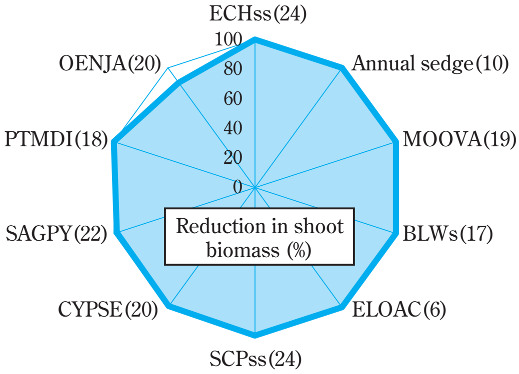
Field trial was conducted at Kasai Experimental Farm in Hyogo, 2006.
Tested weeds were: ECHss, *Echinochloa* spp.; SCPJO, *Schoenoplectus juncoides*; MOOVA, *Monochoria vaginalis*; BLWs, broadleaf weeds (*Lindernia* spp., *Rotala indica*, *Elatine triandra*, etc); CYPSE, *Cyperus serotinus*; OENJA, *Oenanthe javanica*.
*1 Visually evaluated by the rating of 0 (no effect) to 100 (complete kill)
The trial was conducted in two replications and average data were presented.
*2 WAA, weeks after application.

Biological Effects

1. Spectrum

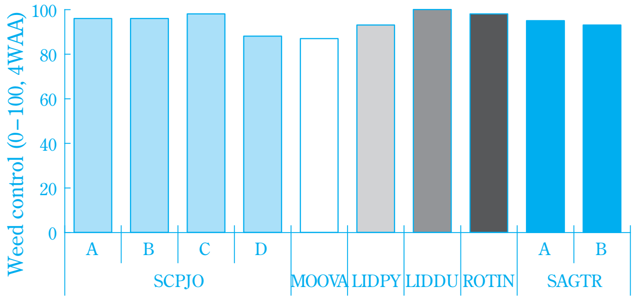
Propyrisulfuron shows high herbicidal activity against a wide variety of weeds by submerged application at a dose rate of 90 g/ha, which is the application rate registered in Japan (**Table 3**). Unlike conventional sulfonylurea herbicides for rice, propyrisulfuron shows herbicidal activity against not only sedges and broadleaf weeds but also *Echinochloa* spp. This makes it possible to provide a product that was conventionally unavailable as a one-shot herbicide with a single active ingredient.

The results from an in-house field trial are given in **Table 4**. Propyrisulfuron controlled all paddy weeds



The dose of propyrisulfuron was 90 g a.i./ha. Application timing was 3-leaf stage of *Echinochloa* spp. The shoot biomass was evaluated at 21–55 days after the application. Medians of several trials were presented. The numbers in parentheses are those of trials. ECHss, *Echinochloa* ssp.; MOOVA, *Monochoria vaginalis*; BLWs, broadleaf weeds; ELOAC, *Eleocharis acicularis*; SCPss, *Schoenoplectus* spp.; CYPSE, *Cyperus serotinus*; SAGPY, *Sagittaria pygmaea*; PTMDI, *Potamogeton distinctus*; OENJA, *Oenanthe javanica*.

Fig. 5 Summary of trials of propyrisulfuron 0.9 % granule conducted by JAPR (Japan Association for Advancement of Phyto-Regulators) in 2004–2009



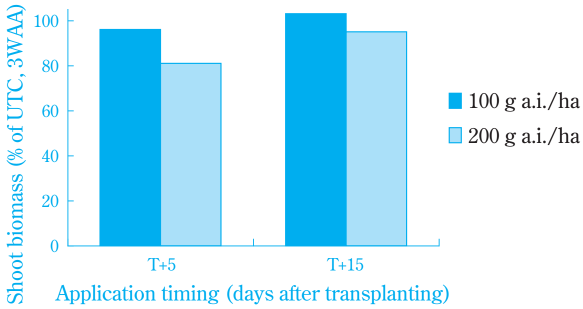
The dose of propyrisulfuron was 90 g a.i./ha. The trial was conducted in three replications and average data were presented. Water leaching treatment at 3 cm / day was conducted and continued for two days after the application. Tested weeds and their growth stage at the application were: SCPJO, *Schoenoplectus juncoides* at 1.5-leaf stage; MOOVA, *Monochoria vaginalis* at 1–1.5-leaf stage, LIDPY, *Lindernia procumbens* at 1-leaf stage; LIDDU, *Lindernia dubia* at 1-leaf stage; ROTIN, *Rotala indica* at 1-leaf stage; SAGTR, *Sagittaria trifolia* at 2–3-leaf stage. A–D were different biotypes. All tested weeds showed resistance to imazosulfuron.
Weed control were visually evaluated.
WAA, weeks after application.

Fig. 6 Herbicidal activity of propyrisulfuron against 'sulfonylurea-resistant' weeds

tested in this trial, and its practicality was confirmed.

Official trials by the Japan Association for Advancement of Phyto-Regulators also proved that it showed high herbicidal activity up to the application timing of 3-leaf stage of *Echinochloa* (**Fig. 5**).

As mentioned above, propyrisulfuron shows herbicidal activity against SU-resistant weeds (**Fig. 6**). However, mutations at the site of action, enhancement in the detoxification capacity for the agent, absorption of the agent, changes in the migration within the plant and various other things concerning the mechanism of acquiring resistance are known, and it is also important to manage the resistance of SU-resistant weeds for which propyrisulfuron shows herbicidal activity at present.



The trial was conducted in three replications and average data were presented. The cultivar of rice was Koshihikari. The growth stage of rice at the application was: T+5, 2.6-leaf stage; T+15, 4.4-leaf stage.

Fig. 7 Influence of propyrisulfuron on transplanted rice

Table 5 Influence to transplanted rice of propyrisulfuron at the application timing of five days after transplanting of rice.

Herbicide	Dose	Plant height*1		Number of culms*1	
		28DAA*2	50DAA	28DAA	50DAA
	g a.i./ha	cm		/ hill	
Propyrisulfuron 0.9 % granule	90	33.2	62.1	14.5	20.9
	180	33.2	63.1	15.7	22.9
Imazosulfuron 0.9 % granule	90	32.8	61.7	16.0	20.8

Field trial was conducted at Makabe Experimental Farm in Ibaraki, 2009.
The cultivar of rice was Koshihikari.
*1 The trial was conducted in three replications and the data were collected from 10 hills in each replication. Average data were presented.
*2 DAA, days after application.

2. Crop selectivity

Propyrisulfuron exhibits a high level of selectivity for rice plants (**Fig. 7**). When propyrisulfuron was applied at 90 g/ha, which is the registered rate of application in Japan, and twice that amount five days after transplanting, there was no significant difference in rice growth from that treated with imazosulfuron, which is already on the market (**Table 5**).

3. Mode of action

As mentioned above, the site of action for propyrisulfuron is ALS, which is the same as that for commercialized sulfonylurea herbicides. ALS is involved in the first step in the synthesis of branched-chain amino acids such as leucine, isoleucine and valine. ALS inhibitors starve affected plants of the amino acids mentioned above, and in the end lead to death (**Fig. 8**). Since ALS is not present in animals, ALS inhibitors have animal-plant selectivity at the mode of action level.

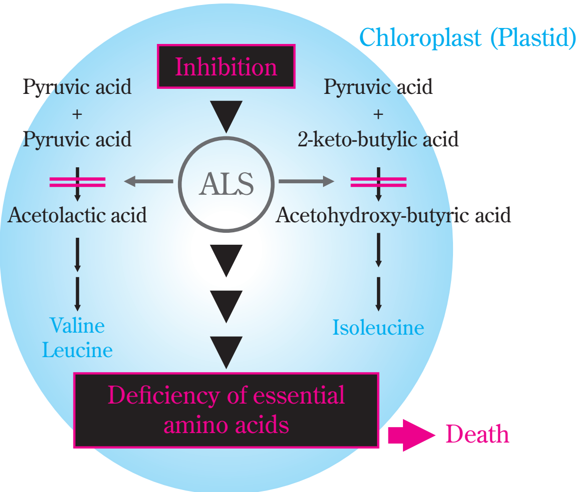


Fig. 8 Mechanism of ALS inhibition

Physical Properties and Formulation

1. Physical and chemical properties

The physical and chemical properties of the propyrisulfuron technical product are given in **Table 6**. The technical grade (TG) of propyrisulfuron is an odorless white solid (powder). Water solubility is 0.98 mg/L [20°C], and the octanol-water partition coefficient is 2.9; therefore, it is less hydrophilic than other sulfonylurea herbicides. On the other hand, it is a somewhat acidic substance with a dissociation constant of 4.89 [20°C], and the water solubility increases in high pH environments. The density is 1.775 g/mL [20°C], and it is in a large category among organic agricultural chemical technical products.

Table 6 Physical and chemical properties of propyrisulfuron

General Name (ISO)	Propyrisulfuron
Chemical Name (IUPAC)	1-(2-chloro-6-propylimidazo [1,2-*b*] pyridazin-3-ylsulfonyl)-3-(4,6-dimethoxypyrimidin-2-yl)urea
CAS RN	570415-88-2
Molecular Formula	$C_{16}H_{18}ClN_7O_5S$
Molecular Weight	455.88
Physical Form	Solid (Ta)
Color	White (Ta)
Odor	Odorless (Ta)
Density	1.775 g/mL (20°C)
Melting Point	> 193.5°C (degradation)
Solubility	Water : 0.98 mg/L (20°C)
	Hexane : < 0.01 mg/L (20°C)
	Toluene : 0.156 g/L (20°C)
	Chloroform : 28.6 g/L (20°C)
	Ethyl acetate : 1.61 g/L (20°C)
	Acetone : 7.03 g/L (20°C)
	Methanol : 0.434 mg/L (20°C)
Octanol-water partition coefficient (logPow)	2.9
Acid dissociation constant (pKa)	4.89 (20°C)

2. Analysis

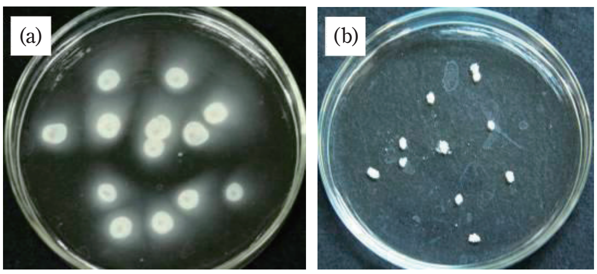
The active ingredient and impurities in the propyrisulfuron TG can be accurately analyzed by high performance liquid chromatography (HPLC) using L-column ODS for the column, a 0.1% aqueous solution of acetic acid for the mobile phase and a 0.1% acetic acid/acetonitrile solution (gradient method). In addition, the active ingredient in the propyrisulfuron formulation can be analyzed with good precision by HPLC-internal standards using ODS-based Wakosil Ⅱ 5C18 for the column, and water/acetonitrile/methanol (4/3/3, pH adjusted to 3.1 by phosphoric acid) for the mobile phase.

3. Formulation

In Japan, most paddy rice herbicides are applied from the time directly after rice transplanting to approximately one month thereafter, and one-shot herbicides, which greatly reduce the labor of weed control operations, hold the principal position among rice herbicides. One-shot herbicides are required to control a wide variety of weed species; therefore, they are normally formulated by combining two to five active ingredients. As noted previously, propyrisulfuron itself has a sufficient herbicidal spectrum, and it was possible to move forward with investigations into formulations under a development concept for a one-shot herbicide with a single active ingredient or a mixture of two active ingredients.

There are three main forms of formulation for one-shot herbicides, namely “1 kg granule” (granular formulation), “flowable” (suspension concentrate, SC) and “jumbo” (jumbo formulation), and these made up approximately 80% of the herbicide products for paddy rice when the investigation into developing propyrisulfuron began. The application practice, where a comparatively small amount of a product is applied directly into flooded rice fields, is common to the three formulations, and a mechanism that rapidly defuses the active ingredient in the water must be designed for all of the formulations.

The 1 kg granule is a literally granular formulation which is applied at dose of 1 kg per 10 a. This formulation reduces the burden on the person who used to apply 3–4 kg of granules per 10 a using a conventional backpack type power applicator. Furthermore, methods have been developed in recent years for treatment at the same time as transplanting using specialized attachments on rice planting machines (treatment during transplanting). Clay minerals that have a swelling property when water is included are mixed into the 1 kg granules of propyrisulfuron, and as is shown in **Fig. 9**, a single granule disintegrates and disperses greatly in water when compared with normal granules. In addition, in order to handle treatment during transplanting, the granule size is set smaller than granules that are not for treatment during transplanting, and it has been confirmed that there is no problem with the producibility of the formulation and the physiochemical properties of the formulation in such cases in industrialization studies.



(a) ZETA-ONE® 1kg granule, (b) Existing product of granular insecticide.

Fig. 9 Disintegrativity of granule in water on 30 minutes after treatment

Fig. 10 Application of flowable herbicide

The flowable formulation is a liquid formulation with the active ingredient suspended in water, and roughly 500 mL per 10 a is applied. As is shown in **Fig. 10**, the method of use is direct application of the formulation by swinging a bottle with the hand, and the salient feature is not requiring a special machine for application. Therefore, in formulation studies, the formulation is given suitable viscosity so that precipitation does not arise after long-term storage, and it is necessary to maintain suitable flowability so that there is no hindrance to the discharge from the bottle. In addition, it is also necessary to give the preparation itself the capability for self-diffusion in rice paddies. Furthermore, from the standpoint of preventing chemical damage to rice plants, it is necessary to control the surface tension of the formulation so that it does not adhere even if the formulation comes into contact with rice plants by hand movements during the application. By overcoming these problems, it was possible to bring to fruition a formulation exhibiting excellent herbicidal effects even at locations 20 m away from the point of treatment as is shown in **Fig. 11** and **Table 7**.

The jumbo is a formulation that is used by throwing approximately several tens of grams directly into flooded paddy fields from the ridges between rice fields at 10–20 g per 10 a, and at present, the main current is a packaged agent where the granular formulation is wrapped in a water-soluble film (**Fig. 12**). The most important problem in formulation for the jumbo is giving it superior self-diffusion capabilities similar to those of the flowable formulation, but it is difficult to make the jumbo, which is a solid and granular, migrate through the flooded rice paddy in the same manner as the flowable formulation. Therefore, it is made to migrate by having the granules containing the active ingredient float on the water surface where there is generally little

Table 7 Results of the field spreading test (See **Fig. 11**)

Herbicide	Herbicidal activity*1							
	Echinochloa oryzicola				*Schoenoplectus juncoides*			
	5m*2	10m	15m	20m	5m	10m	15m	20m
	Weed control (0-100, 42DAA*3)							
Propyrisulfuron 1.7 % SC	100	100	100	98	100	100	100	100
Commercial standard-1	100	100	94	98	100	100	100	99
Commercial standard-2	90	93	97	93	100	100	85	75

Field trial was conducted at Makabe Experimental Farm in Ibaraki, 2010.
The herbicides were applied at seven days after transplanting of rice.
The dose of herbicides was 500 mL product/10a.
*1 visually evaluated by the rating of 0(no effect) to 100(complete kill)
The trial was conducted in two replications. Average data were presented.
*2 The distance from the application area (**Fig. 11**).
*3 DAA, days after application.

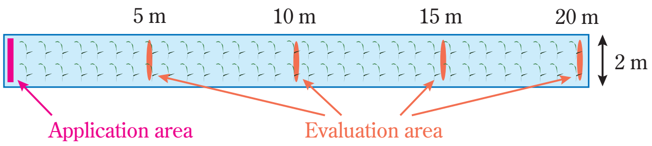


Fig. 11 Schematic diagram of the field spreading test

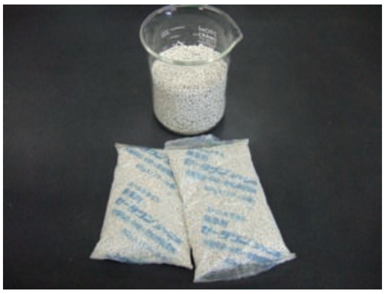


Fig. 12 Jumbo formulation

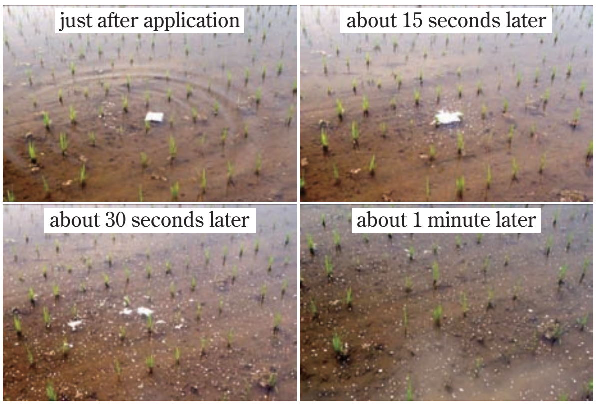


Fig. 13 Spreading behavior of jumbo formulation

resistance to movement, and by allowing the active ingredient to spread over the entire surface of the soil in the flooded rice paddy by gradually disintegrating during migration and after migration. In formulation studies for the jumbo formulation of propyrisulfuron, the most appropriate surfactants (auxiliary agents for spreading on the water surface), binders and carriers that impart floating properties were selected. In addition, these granules are disintegratable, and there are cases of deterioration in manufacturability, but auxiliary agents were optimized with caution given to this point. Furthermore, a water-soluble film that was optimized for solubility is used for packing the granules. As a result, the formulation is one in which the film dissolves approximately 15 seconds after it is thrown into the flooded paddy field as shown in **Fig. 13**, and next, the granules that have come out of the film spread out uniformly over the entire paddy field, gradually disintegrating after they have spread out.

ZETA-ONE® 1 kg granule, ZETA-ONE® flowable and ZETA-ONE® jumbo, which are single agent propyrisulfuron formulations shared as described above, as well as MEGAZETA® 1 kg granule, MEGAZETA® flowable and MEGAZETA® jumbo, which are a mixture of two agents that also include pyraclonil from Kyoyu Agri Co., Ltd., were put on the market in March 2011.

Toxicity, Metabolism and Persistence

1. Main toxicity to mammals

(1) Acute toxicity, irritation and potential skin sensitization

In the acute oral, dermal and inhalation toxicity studies for rats with propyrisulfuron TG, 0.9% granule and 1.7% SC, no toxic symptoms were observed by any routes of administration (**Table 8**). A very weak irritation was observed for propyrisulfuron TG, 0.9% granule and 1.7% SC in eye irritation studies for rabbits,

Table 8 Acute toxicity summary of propyrisulfuron

Study	Species	Result
Propyrisulfuron TG		
Acute oral	rat	LD_{50} > 2000 mg/kg
Acute dermal	rat	LD_{50} > 2000 mg/kg
Inhalation	rat	LC_{50} > 4300 mg/kg (4-hour, nose only exposure)
Eye irritation	rabbit	Minimally irritant
Skin irritation	rabbit	Non-irritant
Skin sensitization	guinea pig	Non-sensitizer
Propyrisulfuron granule (0.9%)		
Acute oral	rat	LD_{50} > 2000 mg/kg
Acute dermal	rat	LD_{50} > 2000 mg/kg
Eye irritation	rabbit	Minimally irritant
Skin irritation	rabbit	Non-irritant
Skin sensitization	guinea pig	Non-sensitizer
Propyrisulfuron SC (1.7%)		
Acute oral	rat	LD_{50} > 2000 mg/kg
Acute dermal	rat	LD_{50} > 2000 mg/kg
Eye irritation	rabbit	Minimally irritant
Skin irritation	rabbit	Non-irritant
Skin sensitization	guinea pig	Sensitizer

although there was no skin irritation. Skin sensitization was negative for propyrisulfuron TG and 0.9% granule, but the 1.7% SC showed potential for skin sensitization from a Buehler test.

(2) Subacute toxicity, chronic toxicity and carcinogenicity

In the results of subacute and chronic toxicity tests (**Table 9**), anemia and toxic effects on liver were mainly observed in dogs. Of these, an increase in the reticulocyte count and induction of hemopoiesis were both observed with anemia; therefore, the anemia was classified as regenerative, and was not thought of as a serious condition. In addition, in terms of the effects on the liver, the only changes were an increase in weight and centrilobular heptocyte hypertrophy, thus these were thought of as adaptive responses. Otherwise, a relative increase in weight of the kidneys and the heart were observed in rats, but these alterations were slight and not accompanied by histopathological changes and, in the same manner, were not thought of as severe findings. In addition, no significant increase in incidence of tumors was observed in rats and mice.

(3) Reproductive and developmental toxicity

In the developmental toxicity studies in rats and rabbits, no increase in the frequency of malformation in fetuses was found. In the rat study, there was a significant increase in the number of fetuses having supernumerary ribs as a skeletal variation. However, it is known that supernumerary ribs not accompanied by changes in the number of presacral vertebra will degenerate and disappear after birth. Therefore, the increase in the frequency of supernumerary ribs was not considered as an adverse effect. In the rat reproduction studies, no effects on reproductive performance were found (**Table 10**).

(4) Genotoxicity

The results of the genotoxicity study, including the reverse mutation test with strains of *Salmonella typhimurium* and *Escherichia coli*, the *in vitro* chromosomal aberration test using Chinese hamster lung cells and the micronucleus test using mice, were all negative (**Table 11**).

Table 9 Subacute and chronic toxicity summary of propyrisulfuron

Species	Administration route and duration	Dose (ppm)	NOAEL (mg/kg/day)
Dog	Oral (in diet), 13 weeks	400, 4000, 40000	Male: 10 (400 ppm) Female: 12 (400 ppm)
	Oral (in diet), 12 months	40, 350, 3500, 35000	Male: 8.45 (350 ppm) Female: 1.11 (40 ppm)
Rat	Oral (in diet), 13 weeks	200, 2000, 20000	Male: 1365 (20000 ppm) Female: 148 (2000 ppm)
	Oral (in diet), 24 months	200, 2000, 6000, 20000	Male: 74.2 (2000 ppm) Female: 309 (6000 ppm) No carcinogenicity
Mouse	Oral (in diet), 13 weeks	200, 2000, 7000	Male: 268 (2000 ppm) Female: 1064 (7000 ppm)
	Oral (in diet), 18 months	70, 700, 7000	Male: 761 (7000 ppm) Female: 693 (7000 ppm) No carcinogenicity

Table 10 Developmental and reproductive toxicity summary of propyrisulfuron

Study	Species	Administration route and duration	Dose		NOAEL (mg/kg/day)
Two-generation reproductive toxicity	Rat	Oral (in diet)	200, 2000, 20000 (ppm)	Parental	Systemic NOAEL Male: 135 (2000 ppm) Female: 202.5 (2000 ppm) Reproductive NOAEL Male: 1405.5 (2000 ppm) Female: 2062.5 (2000 ppm)
				Offsprings	Systemic NOAEL Male: 135 (2000 ppm) Female: 202.5 (2000 ppm)
Developmental toxicity	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: 1000 Developmental NOAEL: 1000
				Fetal	1000

Table 11 Mutagenicity summary of propyrisulfuron TG

Study	Study design	Results
Reverse mutation (Ames test)	*S. typhimurium*: TA98, TA100, TA1535 and TA1537 *E. coli*: WP2*uvr*A −/+S9 mix: 156–5000 µg/plate	Negative
In vitro chromosomal aberration	Chinese hamster CHL/IU −/+S9 mix: 1250–5000 µg/mL	Negative
Micronucleus	CD-1 mice 500, 1000, 2000 mg/kg	Negative

2. Metabolism in animals and plants

(1) Metabolism in animals

Propyrisulfuron was rapidly metabolized in rats, and the ^{14}C concentration in various tissues decreased with time at the same rate as the ^{14}C in plasma. No tendency for persistence of ^{14}C in specific tissues was observed. Excretion of propyrisulfuron and its metabolites from the body was substantially complete within five days after administration, and the main route of primary excretion was feces. From the results of bile excretion tests, the oral absorption rate of propyrisulfuron was 88.0–93.7% at a dose of 5 mg/kg and 21.3–23.2% at a dose of 1000 mg/kg. The major metabolic reaction for propyrisulfuron is as follows. (i) Hydroxylation of pyrimidine ring, propyl groups and imidazopyridazine ring, (ii) O-demethylation, (iii) opening pyrimidine ring and (iv) conjugation reaction (glucuronidation and sulfate conjugation).

(2) Metabolism in plants

Rice plants received two applications of ^{14}C-propyrisulfuron to the surface water of the pots. The major metabolic pathway of propyrisulfuron in the rice plants was *O*-demethylation and cleavage of the sulfonylurea linkage followed by conjugation of the resultant amine derivative. Successively, propyrisulfuron was 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-propyrisulfuron was conducted in sterilized buffer solutions at pH4, pH7 and pH9. The results demonstrated that propyrisulfuron was degraded *via* cleavage of the sulfonylurea linkage with half-lives of 6.3–6.7 days, 77.0–90.0 days and 100.4 days (25°C), respectively. The aqueous photolysis study of ^{14}C- propyrisulfuron was conducted in natural water and distilled water. Propyrisulfuron was rapidly photodegraded *via* cleavage of the sulfonylurea linkage, with estimated half-lives of 10.9 and 10.7

days respectively, under natural sunlight at Tokyo in spring.

(2) Metabolism in soil

^{14}C- propyrisulfuron was aerobically applied to the flooded soil and maintained under aerobic conditions in the dark at 25°C. Propyrisulfuron was rapidly metabolized with a half-life of 4.8 days *via O*-demethylation, opening of the pyrimidine ring and cleavage of sulfonylurea linkage. Subsequently, it was decomposed and eventually mineralized to $^{14}CO_2$ or firmly bounded as the soil residue.

(3) Field dissipation

Field dissipation studies were conducted in two paddy fields in Ibaragi and Osaka prefectures with two treatments of propyrisulfuron 0.9% granule at a rate of 5 kg/10 a. The half-lives were estimated to be 5–22 days with the maximum residues of 0.694–1.25 mg/kg.

(4) Adsorption to soil

The soil adsorption coefficient $K_{Foc(ads)}$ calculated using the Freundlich adsorption isotherm ranged from 138 to 410 mL/g.

(5) Paddy field lysimeter

A paddy field lysimeter study was conducted with a single paddy-water application of propyrisulfuron 0.9% granule at a rate of 1 kg/10 a. The maximum concentration was 0.0996–0.128 mg/L. Propyrisulfuron rapidly dissipated with a half-life of 2.1–2.8 days.

(6) Residue in crops

Residue trials for rice were conducted with two applications at 15 days after transplanting and 60–91 days before harvest. The applications were performed using propyrisulfuron 0.9% granule at a rate of 1 kg/10 a or 1.7% SC at a rate of 500 mL/10 a. All of the residues in the rice grain and straw were below the limit of quantification (< 0.01 ppm).

4. Effects on non-target organisms

Table 12 summarizes the test results for effect levels on aquatic organisms and birds.

(1) Effects on aquatic organisms

The acute 96-hour LC_{50} value in carp for propyrisulfuron TG was greater than 10 mg/L, and the 48-hour EC_{50} value in *Daphnia magna* was greater than 10 mg/L. The 72-hour EC_{50} value in freshwater green algae was greater than 0.011 mg/L.

In addition, for propyrisulfuron 0.9% granule, the acute 96-hour LC_{50} value in carp was greater than 1000 mg/L, and the 48-hour EC_{50} value in *Daphnia magna* was greater than 1000 mg/L. The 72-hour EC_{50} value in freshwater green algae was 1.5 mg/L. For propyrisulfuron 1.7% SC, the 96-hour LC_{50} value in carp was greater than 1000 mg/L and the 48-hour EC_{50} value in *Daphnia magna* was greater than 1000 mg/L. The 72-hour EC_{50} value in freshwater green algae was 0.90 mg/L.

(2) Effects on birds

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

From the above it can be assumed that propyrisulfuron has low acute and chronic toxicity for mammals, and even if there is long-term exposure, there are no

Table 12 Eco-toxicological summary of propyrisulfuron on non-target organisms

Test substance	Test species	Test type	Results
Propyrisulfuron TG	Carp	Acute (96 hr)	$LC_{50} > 10$ mg/L
	Daphnia magna	Acute (48 hr)	$EC_{50} > 10$ mg/L
	Green alga *1	Acute (72 hr)	$EC_{50} > 0.011$ mg/L
	Bobwhite quail	Acute oral	$LD_{50} > 2250$ mg/kg
Propyrisulfuron granule (0.9%)	Carp	Acute (96 hr)	$LC_{50} > 1000$ mg/L
	Daphnia magna	Acute (48 hr)	$EC_{50} > 1000$ mg/L
	Green alga *1	Acute (72 hr)	$EC_{50} = 1.5$ mg/L
Propyrisulfuron SC (1.7%)	Carp	Acute (96 hr)	$LC_{50} > 1000$ mg/L
	Daphnia magna	Acute (48 hr)	$EC_{50} > 1000$ mg/L
	Green alga *1	Acute (72 hr)	$EC_{50} = 0.90$ mg/L

*1 *Pseudokirchneriella subcapitata*

bad effects of carcinogenicity, teratogenicity or fertility in the next generation. Based on the environmental fate and the effects on non-target organisms, the usage of propyrisulfuron is unlikely to pose an unacceptable risk to the environment.

Conclusion

In Japan, the agricultural workforce is steadily decreasing each year, and according to the Ministry of Internal Affairs and Communications statistics, there were 6.363 million agricultural workers in 1985, and in 2008 that had fallen to 2.986 million. Furthermore, the aging of agricultural workers is also an important problem, and the proportion of all agricultural workers aged 65 years or older exceeded 60% in 2008. In the setting of paddy rice production, more efficient and labor saving practices will be required in the future. On the other hand, according to Food and Agricultural Organization (FAO) statistics, the worldwide area of cultivation for the rice paddies in 2009 was 158 million ha, and of that 89% was in the Asian region. In most of these areas, rice production is directly connected to food security, and stable and efficient production is required. As has been reported above, propyrisulfuron has superior characteristics as a herbicide for paddy rice, and we are confident that this herbicide is an effective resource that will contribute to paddy rice production in Japan and, furthermore, throughout the world.

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PROFILE

Hajime IKEDA

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

Kazuki MIKATA

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

Shigeyuki ITO

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

Mariko ENDO

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

Yukio OKADA

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

Ichiro KOMOTO

Sumitomo Chemical Co., Ltd.
Organic Synthesis Research Laboratory
Senior Research Associate
Ph.D.