B-8706

CONFIDENTIAL

Final Report

(English Version)

SYJ-310: Acute oral toxicity study in rats (Up-and-Down-Procedure)

Study Number B-8706

Study Period
29 May 2020 to 13 October 2020

Test Facility
Gotemba Laboratory, BoZo Research Center Inc.
1284, Kamado, Gotemba-shi, Shizuoka 412-0039, Japan

Sponsor
Syngenta Japan K.K.
Office Tower X 21F, 1-8-10, Harumi, Chuo-ku, Tokyo 104-6021, Japan

Contractor
BoZo Research Center Inc.
36-7, Oyama-cho, Shibuya-ku, Tokyo 151-0065, Japan

1. GLP Compliance Statement

Study Number: B-8706

Study Title: SYJ-310: Acute oral toxicity study in rats (Up-and-Down-Procedure)

This study was conducted in compliance with the following GLP standards.

- "Ministerial Ordinance on Good Laboratory Practice for Agricultural Chemicals", Ordinance of the Ministry of Agriculture, Forestry and Fisheries No.76, 2018
- "OECD Principles of Good Laboratory Practice", OECD:, 26 November 1997

(Signed in the original) 13 October 2020

Toyohisa Katsumata, BSc Date
Study Director
Gotemba Laboratory, BoZo Research Center Inc.

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3. Outline of Study

3.1 Study Number

B-8706

3.2 Study Title

SYJ-310: Acute oral toxicity study in rats (Up-and-Down-Procedure)

3.3 Objective

The objective of the study was to assess the acute oral toxicity of the SYJ-310 when administered in a single oral dose to rats.

3.4 Sponsor

Syngenta Japan K.K.

Office Tower X 21F, 1-8-10, Harumi, Chuo-ku, Tokyo 104-6021, Japan

3.5 Contractor

BoZo Research Center Inc.

36-7, Oyama-cho, Shibuya-ku, Tokyo 151-0065, Japan

3.6 Test Facility

Gotemba Laboratory, BoZo Research Center Inc.

1284, Kamado, Gotemba-shi, Shizuoka 412-0039, Japan

3.7 Study Director

Toyohisa Katsumata, BSc

Toxicology Division, Gotemba Laboratory, BoZo Research Center Inc.

3.8 Main Contributor

Test substance manager: Chie Yamane

3.9 Study Schedule

Initiation of study: 29 May 2020

Receipt of test substance: 1 May 2020[Note]

Receipt of animal: 1 June 2020

Administration

First administration (Experimental starting date):
9 June 2020

Second administration: 12 June 2020

Third administration: 15 June 2020

Fourth administration: 17 June 2020

Fifth administration: 19 June 2020

Scheduled necropsy

First necropsy: 23 June 2020

Second necropsy: 26 June 2020

Third necropsy: 29 June 2020

Fourth necropsy: 1 July 2020

Fifth necropsy (Experimental completion date):
3 July 2020

Completion of study: 13 October 2020

Note: The test substance was received before the initiation of study and it was stored and controlled by the person responsible for test substance management. The test substance was distributed to the study director on 29 May 2020.

3.10 Environmental Factor That Might Have Affected the Credibility of Study Data

There were no factors that might have affected the credibility of study data.

3.11 Archives

The original Protocol and Protocol Amendments, recording documents, raw data and reports (including the original of the Final Report) are retained in the scientific archives of Gotemba Laboratory, BoZo Research Center Inc. They will be retained for a period of 10 years after the completion of study. At the end of this period, Syngenta Japan K.K. and BoZo Research Center Inc. will discuss and determine the disposition of the said materials.

3.12 Signature of Study Director and Date

(Signed in the original) 13 October 2020

Toyohisa Katsumata, BSc Date
Gotemba Laboratory, BoZo Research Center Inc.

4. Summary

The acute oral toxicity of SYJ-310 was examined in Sprague-Dawley rats [Crl:CD(SD), 1 female per one administration] at 8 to 9 weeks of age. The dose level was set at 2000 mg/kg, one animal was administered first, and since no death was observed, another 4 animals were sequentially administered. The administration interval was 48 hours or longer. The animals were observed for 14 days after administration.

1) Mortality
   No deaths occurred.
2) Clinical Observations
   There were no abnormalities in any animal.
3) Body Weights
   There were favorable body weight changes in any animal.
4) Gross Pathology
   There were no gross lesions in any animal.

Based on the above results, it was estimated that the LD_{50} value of SYJ-310 was greater than 2000 mg/kg when the test substance was administered to rats once orally.

5. Introduction

As a part of the safety assessment of SYJ-310, an acute oral toxicity study was conducted in rats at BoZo Research Center Inc. as a contract research on behalf of Syngenta Japan K.K. and the results are reported in the following. This study was conducted in compliance with the following standards and in accordance with the following guideline. This study was conducted under the approval of the Institutional Animal Care and Use Committee (IACUC) of BoZo Research Center Inc. (Approval Number: G200106).

1) GLPs
   - "Ministerial Ordinance on Good Laboratory Practice for Agricultural Chemicals", Ordinance of the Ministry of Agriculture, Forestry and Fisheries No.76, 2018
   - "OECD Principles of Good Laboratory Practice", OECD:, 26 November 1997
2) Toxicity Study Guidelines
   - "Data requirements for registration of agricultural chemicals", The notification No.30-shouan-6278, issued on 29 March 2019 by Director-General, Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries
   - "OECD Guideline for Testing of Chemicals 425: Acute Oral Toxicity - Up-and-Down-Procedure (UDP)", OECD: 3 October 2008
3) Animal Welfares
   - "Act on Welfare and Management of Animals", Act No. 105, 1 October 1973
   - "Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain", Notification No. 88 of the Ministry of the Environment, Japan, 28 April 2006
   - "Guidelines for Proper Conduct of Animal Experiments", Science Council of Japan, 1 June 2006

6. Materials and Methods

6.1 Test Substance

The test substance and the information below were supplied by Syngenta Japan K.K.

Name:	SYJ-310
Lot number:	CRS2240
Amount supplied:	50 g (including related study)
Active ingredient and content:	
	Cyantraniliprole 75 g/L, Isotianil 200 g/L,
	Penflufen 200 g/L
Description:	Flowable
Storage condition:	Room temperature (actual values: 19.7°C to 24.0°C)

Expiration date: End of March 2023
Stability: Stable under the room temperature
Storage place: Test substance storage room at Gotemba Laboratory and test substance preparation room
Disposition of remaining test substance:
After completion of related study, all test substance remaining after use was returned to the supplier.

6.2 Preparation of Dose Formulation

Just before use, the requisite amount of the test substance was weighed and diluted in vehicle (distilled water, Japanese Pharmacopoeia, Otsuka Pharmaceutical Factory, Inc., Lot No.: 9G91) to make the specified concentration.

6.3 Justification for Choice of Test Species and Strain

Rats were chosen as they are widely used in the toxicity studies of agrochemicals. The strain which were used for this study was chosen as their biological characteristics are well-known. Females were chosen in accordance with the toxicity study guidelines since there is no information on sex difference in sensitivity.

6.4 Test Animals and Select of Animals

Animal species: Rat (SPF)
Strain: Sprague-Dawley [Crl:CD(SD)]
Source: Atsugi Breeding center, Charles River Laboratories Japan Inc.
Age at receipt: 7 weeks of age
Number of animals purchased:
8 females
Duration of quarantine/acclimation:
Over a week
Age at administration: 8 to 9 weeks of age
Number of animals used: 5 females
Observations during the quarantine/acclimation period:
For all animals, body weights were recorded three to seven times, and clinical observations, including any abnormality in the external appearance, nutritional condition, posture, behavior, and excretions were observed once a day. There were no

observable abnormalities in clinical observations or body weights in any animal.

Selection of test animals: Based on the above observations/measurement, healthy animals were selected as the candidates for the test animal.

Select of animals: All animals were selected on the day before each administration. The first time, all the animals used for selection were weighed, and one animal with the larger weight was selected. The weights of all the animals used for selection were measured from the second time onward, and one animal was selected so that the body weight range on the administration day and the average body weight of all the test animals did not exceed the following rules.

Body weight variation on the day of administration:

Individual body weights at the time of administration ranged from 193 g to 211 g, and were within ± 20% of the mean body weight for all animals used.

Animals remaining after group allocation:

The animals not used were excluded from the study on day 3 after administration of the Fifth administration and transferred to the animal breeding/management section.

6.5 Animal Husbandry

Environmental conditions in animal room:

The animals were reared in an animal room (No. 506) under the following conditions: temperature at 23°C ± 3 °C (actual values: 20 to 22°C); relative humidity at 50% ± 20% (actual values: 50% to 56%); air ventilation: 10 to 15 times per hour, lighting: 12 hours per day (7:00 a.m. to 7:00 p.m.). The animals were housed individually in plastic solid-floored cages (W 440 × D 275 × H 180 mm: Hanyu Co.) with bedding (ConfiNest, Falma Co., Ltd., Lot No. 2019-05).

Feed: The animals were allowed free access to pelleted diet CR-LPF (γ-irradiated, Oriental Yeast Co., Ltd., Lot No. 191216) using stainless steel feeders.

Drinking water: The animals were allowed free access to tap water (Gotemba City Water) via using water bottles.

Environmental enrichment:

Appropriate environmental enrichment was provided in accordance with guideline of the IACUC.

6.6 Contaminants in Feed, Bedding, and Drinking Water

It was confirmed that the results of the following analytical reports on contaminants were within the acceptance criteria.

- Analytical reports on the feed (Eurofins Food and Product Testing Japan K.K., for the lot used)
- Analytical reports on the bedding (Japan Food Research Laboratories, for the lot used)
- Analytical reports on the drinking water in accordance with the Waterworks Law (Shibaura Semtek Co., Ltd., on the quarterly basis)

6.7 Animal Identification

Ear tags for small animals:

Ear tags, on which a serial number from 1 to 999 was engraved, were attached following animal receipt and individuals were identified uniquely throughout the experiment.

Animal number: After group allocation, a 4-digit number was assigned to each animal. The first digit indicated the dose level (1 for 2000 mg/kg), the second digit sex (1 for females), and the last 2 digits individual numbers.

Cage label: After group allocation, each cage had a label color-coded according to the date of administration, indicating the study number, administration route, dose level, sex, animal number, ear tag number and the date of administration.

6.8 Route of Administration, Observation Period and Justification for Their Choice

In accordance with the toxicity study guidelines, the oral route was selected, and the length of the observation period was 14 days after administration.

6.9 Method of Administration

Oral administration was performed by gavage, which is the normal method to oral administration to rodents.

The dose volume was set at 10 mL/kg body weight. The dose formulation was administered once orally by gavage using a stomach tubes to the rats which were fasted overnight (approximately 18 hours) before administration. Individual dose volume was calculated from the body weight of the animals on the day of administration (Day 0 of administration). Animals were allowed free access to feed again after observing the clinical signs at 4 hours after administration.

6.10 Dose Levels and Test Procedure

Since the acute oral toxicity of the test substance was expected to be low, the dose level was set at 2000 mg/kg was selected according to the limit test of the toxicity test guideline. A total of 5 animals were administered, but one animal was administered first, and since no death was observed, 4 animals were sequentially administered. The administration interval was 48 hours or longer. Group composition is shown below.

Study group	Dose level (mg/kg)	Sex	Number of animal	Animal number
First	2000	Female	1	1101
Second	2000	Female	1	1102
Third	2000	Female	1	1103
Fourth	2000	Female	1	1104
Fifth	2000	Female	1	1105

6.11 Method of Observation, Measurement and Examination

The day of administration was designated as day 0 of administration and the following observation, measurement and examination were conducted.

6.11.1 Clinical Observations

All animals were observed frequently for the first 6 hours after administration (from immediately after administration to 5 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 6 hours after administration), and once daily thereafter for 14 days (between 8:48 a.m. and 10:29 a.m.), for clinical signs such as abnormalities of external appearance, nutritional condition, posture, behavior and excretions.

6.11.2 Body Weights

Body weight was measured on the day of administration (before administration) and on days 1, 7 and 14 after administration (between 8:46 a.m. and 10:25 a.m.).

6.11.3 Gross Pathology

All animals were euthanized by exsanguination via the abdominal aorta under isoflurane anesthesia at the end of the 14-day observation period, and external appearance and organs/tissues in the cranial, thoracic and abdominal cavities were examined macroscopically. No organs/tissues were preserved because necropsy revealed no gross lesions in any animal.

6.12 Estimation of LD_{50} value and Data Handling

6.12.1 Estimation of LD_{50} value

According to the toxicity study guideline "Up-and-Down-Procedure", approximate LD_{50} value was estimated based on the mortality during the 14-day observation period after administration.

6.12.2 Data Handling

Statistical analysis was not performed.

7. Results

7.1 Mortality

Mortality and LD_{50} value is shown in Table 1.

There were no deaths in animals were administered at the 2000 mg/kg. Therefore, the LD_{50} value was estimated to be greater than 2000 mg/kg.

7.2 Clinical Observations

The results are shown in Table 2.

There were no abnormal clinical observations during the observation period in any animal were administered at the 2000 mg/kg.

7.3 Body Weights

The results are shown in Table 3.

Animals were administered at the 2000 mg/kg, showed a favorable body weight gain during the observation period after administration.

7.4 Gross Pathology

The results are shown in Table 4.

No animals were administered at the 2000 mg/kg, showed any abnormality in the external appearance or organs/tissues in the cranial, thoracic or abdominal cavity.

8. Discussion

The acute oral toxicity of SYJ-310 was examined in Sprague-Dawley rats [Crl:CD(SD), 1 female per one administration] at 8 to 9 weeks of age. The dose level was set at 2000 mg/kg, one animal was administered first, and since no death was observed, another 4 animals were sequentially administered. The administration interval was 48 hours or longer. The animals were observed for 14 days after administration.

No deaths occurred at 2000 mg/kg.

There were no test substance-related changes in the clinical observations, body weights or gross pathology in any animal.

Based on the above results, it was estimated that the LD_{50} value of SYJ-310 was greater than 2000 mg/kg when the test substance was administered to rats once orally.

Table 1 SYJ-310: Acute oral toxicity study in rats (Up-and-Down-Procedure)

Mortality and LD_{50} value

Sex : Female

Dose (mg/kg)	Number of animals	Distribution of death																						Mortality	LD_{50} (mg/kg)
		minutes			hours				days																
		i~5	15	30	1	2	4	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
2000	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5	>2000

i : Immediately after administration

Table 2 SYJ-310: Acute oral toxicity study in rats (Up-and-Down-Procedure)

Clinical signs

Sex : Female

Dose (mg/kg) : 2000

Animal No.	minutes			hours				days													
	i~5	15	30	1	2	4	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1105	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

i : Immediately after administration
- : No abnormality

Table 3 SYJ-310: Acute oral toxicity study in rats (Up-and-Down-Procedure)

Body weight

Sex : Female

Dose (mg/kg) : 2000

Animal No.	Day after administration 0	1	7	14
1101	211	229	259	299
1102	205	223	245	268
1103	205	222	240	256
1104	208	227	246	264
1105	193	216	232	248

Unit : g

Table 4 SYJ-310: Acute oral toxicity study in rats (Up-and-Down-Procedure)

Gross pathological findings

Sex : Female

Dose (mg/kg) : 2000

Organs	Findings	Animal No.				
		1101	1102	1103	1104	1105
External appearance		-	-	-	-	-
Cerebrum		-	-	-	-	-
Cerebellum		-	-	-	-	-
Pituitary		-	-	-	-	-
Submandibular gland		-	-	-	-	-
Sublingual gland		-	-	-	-	-
Submandibular lymph node		-	-	-	-	-
Thyroid		-	-	-	-	-
Thoracic cavity		-	-	-	-	-
Thymus		-	-	-	-	-
Heart		-	-	-	-	-
Lung		-	-	-	-	-
Abdominal cavity		-	-	-	-	-
Liver		-	-	-	-	-
Spleen		-	-	-	-	-
Pancreas		-	-	-	-	-
Kidney		-	-	-	-	-
Adrenal		-	-	-	-	-
Esophagus		-	-	-	-	-
Stomach		-	-	-	-	-
Intestine		-	-	-	-	-
Mesenteric lymph node		-	-	-	-	-
Bone marrow		-	-	-	-	-
Femoral muscle		-	-	-	-	-
Urinary bladder		-	-	-	-	-
Ovary		-	-	-	-	-
Uterus		-	-	-	-	-
Vagina		-	-	-	-	-
Other tissues or organs		-	-	-	-	-

- : No abnormality

Quality Assurance Statement (1/2)

Study Number: B-8706

Study Title: SYJ-310: Acute oral toxicity study in rats (Up-and-Down-Procedure)

I, the undersigned, hereby declare that this study was conducted in compliance with the following GLPs.

- "Ministerial Ordinance on Good Laboratory Practice for Agricultural Chemicals", Ordinance of the Ministry of Agriculture, Forestry and Fisheries No.76, 2018
- "OECD Principles of Good Laboratory Practice", OECD:, 26 November 1997.

Inspections were conducted and reported as follows.

Study-Based Inspections

Items	Inspectors	Dates of Inspection	Dates of Report to Study Director and Management
Protocol	Y. Inaba	29 May 2020	29 May 2020
Protocol amendment (1)	Y. Inaba	8 June 2020	8 June 2020
Preparation / Storage of the test substance	Y. Nakano	9 June 2020	9 June 2020
Administration / Clinical observation	Y. Nakano	9 June 2020	9 June 2020
Necropsy	A. Hosoda	23 June 2020	23 June 2020
Raw data (Animal experiment, necropsy, animal care)	M. Miyao	8 September 2020	8 September 2020
Draft final report / Tables	M. Miyao	8 September 2020	8 September 2020
Raw data (Test substance)	M. Miyao	15 September 2020	15 September 2020
Final report	M. Miyao	13 October 2020	13 October 2020

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Quality Assurance Statement (2/2)

Process-Based Inspections

Items	Study Numbers	Inspectors	Dates of Inspection	Dates of Report to Study Director and Management
Receipt of animals	B-8695	H. Ozawa	13 May 2020	13 May 2020
Quarantine / Acclimatization	B-8695	H. Ozawa	13 May 2020	13 May 2020
	B-8695	M. Miyao	19 May 2020	19 May 2020
Measurement of body weight	B-8693	Y. Inaba	11May 2020	11May 2020

(Signed in the original) 13 October 2020

Minoru Izutsu, M. Sc. Date
Manager, Quality Assurance Unit
BoZo Research Center Inc.

Study Number: B-8706

Study Title: SYJ-310: Acute oral toxicity study in rats (Up-and-Down-Procedure)

This English version is an accurate translation of the original Japanese Final Report.

Toyohisa Katsumata 16 October 2020

Toyohisa Katsumata Date

Translator/Study Director

Gotemba Laboratory, BoZo Research Center Inc.