Technical Information

Summary of Toxicological Studies with Chromafenozide

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GENERAL INFORMATION ON CHROMAFENOZIDE

Chromafenozide (Product name: MATRIC® Flow-able and MATRIC® Dust DL), a novel insecticide against lepidopteran larvae with a similar mode of action to ecdysone known as an insect-specific ecdysis hormone, was discovered and developed under cooperative works by Nippon Kayaku Co., Ltd. and Sankyo Co., Ltd.

Chromafenozide is one of the most suitable plant protection products for the Integrated Pest Management (IPM) to which growing attentions have been attracted with recent global needs for the sustainable agriculture.

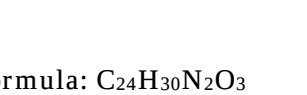
Chromafenozide on the plant is ingested orally by insect larvae to exhibit an ecdysis-promoting activity like ecdysone and lead to death. The activity is selective only to lepidopteran larvae that damage rice, tea, fruit trees, vegetables or other agricultural plants.

The chemical structure and physical and chemical properties of chromafenozide are as follows:

Common name: chromafenozide
(ISO common name)

Chemical name: 2'-tert-butyl-5-methyl-2'-(3,5-xyloyl)-chromane-6-carbohydrate

Chemical structure:



Chemical formula: $C_{24}H_{30}N_2O_3$

Molecular weight: 394.51

Physical state: White solid powder

Melting point: 186.4 °℃

Solubility (g/l, 20℃): Toluene: 0.32 Dichloromethane: ＞336 Acetone: 186 Ethyl acetate: 50.6 Ethanol: 173 Diisopropylether: 0.35 Water: 1.12 mg/l

Partition coefficient (n-octanol/water): log P_{ow}＝2.7

ACUTE TOXICITY STUDIES

Summary of acute toxicity studies via various administration routes is presented in the following table.

IRRITATION STUDIES

1. Primary Eye Irritation Studies

Primary eye irritation potential of chromafenozide technical, MATRIC® Flowable (5% SC), 1/500 aqueous dilution (the highest field application rate) of 5% SC, and MATRIC® Dust DL (0.3% D) was assessed with female or male New Zealand White rabbit and evaluated according to the Draize's scoring system. One eye of the rabbit was applied 0.1 g or 0.1 ml of the test substance and assessed irritation appeared on cornea, iris and/or conjunctiva. In addition, if irritation was observed, an eye irrigation effect with water was assessed on the group.

Chromafenozide technical: Mild irritation observed was recovered by 48 hr after application. The irrigation was effective at 2 min after application.

(The Institute of Environmental Toxicology, 1995)

5% SC: Mild irritation observed was recovered by 72 hr after application. The irrigation was effective at 30 sec after application.

(The Institute of Environmental Toxicology, 1998)

Aqueous dilution (1/500) of 5% SC (the maximum field application rate): No irritation was observed.

(Safepharm Laboratories, 1998)

0.3% D: Minimum irritation observed was recovered by 24 hr after application. The irrigation was effective at 30 sec after application.

(The Institute of Environmental Toxicology, 1998)

2. Primary Dermal Irritation Studies

Primary dermal irritation potential of chromafenozide technical, 5% SC, and 0.3% D was assessed with female New Zealand White rabbit and evaluated according to the Draize's scoring system. The test substance was applied on a 2.5 cm × 2.5 cm area of the shaved trunk skin of a rabbit at 0.5 g or 0.5 ml and the appearance of the irritation responses (erythema, eschar and edema) was assessed over 72 hr.

Chromafenozide technical: No irritation was observed.

(The Institute of Environmental Toxicology, 1995)

5% SC: No irritation was observed.

(The Institute of Environmental Toxicology, 1998)

0.3% D: No irritation was observed.

(The Institute of Environmental Toxicology, 1998)

DERMAL SENSITIZATION STUDIES

Dermal sensitization potential of chromafenozide technical, 5% SC and 0.3% D was assessed with female Hartly guinea pig.

Chromafenozide technical: Mild sensitization reaction was observed after an intradermal inductive injection at 5% suspension and a topical inductive application at 25% in white petrolatum followed by a challenge application at 25% in white petrolatum (Maximization test).

(The Institute of Environmental Toxicology, 1995)

5% SC: No sensitization reaction was observed after three topical inductive applications and a challenge application of the intact test substance (Buehler test).

(The Institute of Environmental Toxicology, 1998)

0.3% D: No sensitization reaction was observed after three topical inductive applications and a challenge application of 50% aqueous suspension of the test substance (Buehler test).

(The Institute of Environmental Toxicology, 1998)

SUBCHRONIC TOXICITY STUDIES

1. 13-Week Oral Subchronic Toxicity Study in Rats

Chromafenozide technical mixed with a rodent diet at 0, 300, 1000, 3000, 10,000 or 20,000 ppm was administered to 12 male and 12 female Fischer rats per group for 13 weeks.

Decreased body weight, anemia, increased creatine phosphokinase, increased weights of the liver and spleen, periportal hepatocellular swelling, and increased incidence of dark in color and brown pigment deposition of the spleen were noted in males and females at 20,000 ppm, the highest dose level. Decreased total cholesterol and triglyceride in males and increased blood urea nitrogen in females were also observed at 20,000 ppm. No test substance-related findings were observed in males at 1000 ppm or below and in females at 3000 ppm or below.

Based on these findings, under the conditions of this study, the no observed adverse effect levels (NOAELs) of chromafenozide were determined to be 1000 ppm (64.4 mg/kg/day) for males and 3000 ppm (207.7 mg/kg/day) for females. (The Institute of Environmental Toxicology, 1996)

2. 13-Week Oral Subchronic Toxicity Study in Mice

Chromafenozide technical mixed with a rodent diet at 0, 310, 1250, 5000, 20,000 or 30,000 ppm was administered to 12 male and 12 female ICR mice per group for 13 weeks.

Darkening in color and brown pigment deposition of the spleen in males and females and centrilobular hepatocellular swelling in males were noted at 20,000 and 30,000 ppm. Increased spleen weight and extramedullary hematopoiesis in the spleen in males and females and decreased erythrocyte count and increased relative weight of the liver in males were observed at 30,000 ppm, the highest dose level.

Based on these findings, under the conditions of this study, the NOAEL of chromafenozide was determined to be 5000 ppm for both males and females (624.7 mg/kg/day for males and 723.1 mg/kg/day for females).

(The Institute of Environmental Toxicology, 1996)

3. 13-Week Oral Subchronic Toxicity Study in Dogs

Chromafenozide technical mixed with a diet at 0, 2000, 10,000 or 30,000 ppm was administered to 4 male and 4 female beagle dogs per group for 13 weeks.

At 30,000 ppm, increased platelet count, darkening in color of the spleen and congestion in the spleen in both sexes, mild anemia, increased mean corpuscular volume, increased relative weight of the liver, increased absolute and relative weight of the spleen in males, increased hematopoiesis in the bone marrow and increased triglyceride in females were observed. Darkening in color of the spleen and congestion in the spleen in both sexes, increased platelet count in males and increased total bilirubin and increased absolute and relative weight of the spleen in females were noted at 10,000 ppm.

Based on these findings, under the conditions of this study, the NOAEL of chromafenozide was determined to be 2000 ppm for both sexes (52.2 mg/kg/day for males and 52.4 mg/kg/day for females).

(The Institute of Environmental Toxicology, 1996)

CHRONIC TOXICITY AND ONCOGENICITY STUDIES

1. 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats

Chromafenozide technical mixed with a rodent diet at 0, 250, 1000, 4000 or 15,000 ppm was administered to 85 male and 85 female Fischer rats per group for 24 months.

Lower body weight gain at 15,000 ppm, mild anemia and periportal hepatocellular

hypertrophy, increased liver weight, and increased weight, darkening in color, and brown pigment deposition to be considered hemosiderin in the spleen at 4000 and 15,000 ppm were noted in both sexes.

Based on these findings, under the conditions of this study, the NOAEL of chromafenozide was determined to be 1000 ppm for both males and females (35.8 mg/kg/day for males and 44.0 mg/kg/day for females). It was considered that there was no tumorigenic potential in the test substance. (The Institute of Environmental Toxicology, 1998)

2. 18-Month Oral Oncogenicity Study in Mice

Chromafenozide technical mixed with a rodent diet at 0, 800, 5000 or 30,000 ppm was administered to 50 male and 50 female ICR mice per group for 18 months.

Lower body weight gain in females and increased incidence of brown pigment deposition in the spleen in both sexes were noted at 30,000 ppm

Based on these findings, under the conditions of this study, the NOAEL of chromafenozide was determined to be 5000 ppm for both males and females (553.3 mg/kg/day for males and 484.8 mg/kg/day for females). It was considered that there was no tumorigenic potential in the test substance. (The Institute of Environmental Toxicology, 1998)

3. 12-Month Oral Chronic Toxicity Study in Dogs

Chromafenozide technical mixed with a diet at 0, 1200, 6000 or 30,000 ppm was administered to 4 male and 4 female beagle dogs per group for 12 months.

Decreased erythrocyte count, increasing tendency of reticulocyte count and increased platelet count in females, and increased total bilirubin in plasma in both sexes were observed at 30,000 ppm. In addition, increased organ weight, swelling, darkening in color, congestion and brown pigment (hemosiderin) deposition of the spleen, increased hemosiderin deposition in the Kupffer cells of the liver, and increased hematopoiesis in the bone marrow were also observed at the same dose level. Increased total bilirubin in plasma in males, swelling, darkening in color, and congestion of the spleen, increased hemosiderin deposition in the Kupffer cells of the liver, and increased hematopoiesis in the bone marrow were noted at 6000 ppm.

Based on these findings, under the conditions of this study, the NOAEL of chromafenozide was determined to be 1200 ppm for both males and females (27.9 mg/kg/day for males and 27.2 mg/kg/day for females).

(The Institute of Environmental Toxicology, 1998)

REPRODUCTION STUDY IN RATS

Chromafenozide technical mixed with a rodent diet at 0, 200, 2000 or 20,000 ppm was administered to 24 male and 24 female SD rats per group to assess potential toxicity and effects on the reproduction performance over successive 2 generations.

Decreased body weight gains in F1 male parental animals were observed at 20,000 ppm. Darkening in color and brown pigment deposition of the spleen and periportal hepato-cellular hypertrophy in both sexes, increased relative weight of the liver in males, increased absolute and relative weight of the liver in females and increased absolute and/or relative weight of the spleen in females were also observed in 20,000 ppm F0 and F1 parental animals. No test substance-related adverse effects were noted in any of the parameters tested for reproductive performance of F0 and F1 parental animals up to the highest dose level. Temporary decreased body weight gains observed in F1 and F2 offsprings at 20,000 ppm were not observed at 2000 ppm and 200 ppm.

Based on these findings, under the conditions of this study, the NOAEL of chromafenozide was determined to be 2000 ppm (128 - 152 mg/kg/day for males and 144 - 165 mg/kg/day for females) for the general toxicity to the parental animals and offsprings. The NOAEL for the reproductive performance was determined to be 20,000 ppm (1284 - 1549 mg/kg/day for males and 1416 - 1666 mg/kg/day for females).

(The Institute of Environmental Toxicology, 1997)

TERATOLOGY STUDIES

1. Teratogenicity Study in Rats

Chromafenozide technical suspended in 1% CMC aqueous solution was orally administered daily at 0, 100, 300 and 1000 mg/kg to 24 copulated female SD rats per group for 10 days from 6 to 15 of gestation.

No test substance-related effects on the maternal rats were noted and any induction of the fetal malformation or variation was caused at dose levels up to 1000 mg/kg.

Based on these findings, under the conditions of this study, the NOAEL of chromafenozide was determined to be 1000 mg/kg for the maternal rats and the fetuses. Therefore, the teratogenic potential of chromafenozide was determined to be negative.

(The Institute of Environmental Toxicology, 1997)

2. Teratogenicity Study in Rabbits

Chromafenozide technical suspended in 1% CMC aqueous solution was orally administered daily at 0, 100, 300 and 1000 mg/kg to 18 copulated female Kbl: JW rabbits per group for 13 days from 6 to 18 of gestation.

No test substance-related effects on the maternal rabbits were noted and any induction of the fetal malformation or variation was caused at dose levels up to 1000 mg/kg.

Based on these findings, under the conditions of this study, the NOAEL of chromafenozide was determined to be 1000 mg/kg for the maternal rabbits and the fetuses. Therefore, the teratogenic potential of chromafenozide was determined to be negative.

(The Institute of Environmental Toxicology, 1997)

MUTAGENICITY STUDIES

1. Reverse Mutation Assay

Gene mutation potential of chromafenozide technical treated to histidine auxotrophic strains of Salmonella typhimurium (TA98, TA100, TA1535 and TA1537) and a tryptophan auxotrophic strain of Escherichia coli (WP2 uvrA) with or without S-9 Mix at 0, 313, 1250 and 5000 μg/plate was assessed using the reverse mutation assay (Ames test).

No increased revertant colonies were noted on the treated plates up to 5000 μg/plate, the highest concentration, comparing with control plates regardless with or without S-9 Mix. Reverse mutation potential of chromafenozide was considered to be negative. (BML, 1994)

2. In Vitro Cytogenetics Test

Chromosome aberration potential of chromafenozide was assessed using cultured Chinese hamster lung (CHL) cells by the direct method and the metabolic activation method. Dose levels of chromafenozide technical were 0, 78, 156, 313 and 625 μg/mL for the 24 hr-treatment of the direct method and the metabolic activation method with and without S-9 Mix, and 0, 39, 78, 156 and 313 μg/ml for the 48 hr-treatment of the direct method.

No increased incidence of abnormal cells was observed in treated cultures comparing with control cultures. Consequently, chromosome aberration potential of chromafenozide was determined to be negative.

(The Environmental Toxicology Institute, 1995)

3. DNA Repair Test (Rec-Assay)

DNA-damaging activity of chromafenozide was assessed with the DNA repair test (rec-assay) using the recombination-proficient strain (H17, rec+) and the recombination-deficient strain (M45, recE−) of Bacillus subtilis at 0, 313, 1250, 2500, 5000 and 10,000 μg/disc.

Comparable growth inhibitory zones were not observed in both strains of B. subtilis up to the highest dose, 10,000 μg/disc, indicating a negative DNA-damaging activity of chromafenozide.

(The Environmental Toxicology Institute, 1995)

GENERAL PHARMACOLOGICAL STUDIES

1. Effects on Central Nervous System

1.1 Clinical signs and body weight in mice

Behavior of 3 male and 3 female ICR mice per group injected intraperitoneally with chromafenozide technical in 1% Tween 80 aqueous solution at 0, 320, 800, 2000 or 5000 mg/kg was examined according to the Irwin's method.

Slightly reduced body muscle tone was observed in both sexes received 5000 mg/kg at 1 hr after treatment, and decreased body weight in males received 5000 mg/kg on day 1 of the study.

1.2 Toxicological signs and body weight in rats

Observation for acute toxicological signs after oral administration of chromafenozide technical suspended in 1% Tween 80 aqueous solution at 0, 2000 and 5000 mg/kg was performed with 6 male SD rats.

No abnormal findings and body weight changes were observed up to 5000 mg/kg, the highest dose level.

1.3 Effects on the hexobarbital sleep in mice

Effects of chromafenozide technical on the sleeping time induced by subcutaneous injection of hexobarbital at 100 mg/kg were assessed with 8 male ICR mice per group which were received intraperitoneal injection of the test substance suspended in 1% Tween 80 aqueous solution at 0, 128, 320, 800, 2000 and 5000 mg/kg at 1 hr before the hexobarbital treatment.

Prolonged sleeping time was noted at dose levels of 2000 and 5000 mg/kg.

1.4 Effects on body temperature of rats

Body temperature was measured after oral administration of chromafenozide technical suspended in 1% Tween 80 aqueous solution at 0, 2000 and 5000 mg/kg to 6 male SD rats per group.

No treatment-related changes were observed.

2. Effects on Respiratory and Cardiovascular Systems

Maximum blood pressure and heart rates were measured after oral administration of chromafenozide technical suspended in 1% Tween 80 aqueous solution at 0, 2000 and 5000 mg/kg to 6 male SD rats per group.

No treatment-related changes were observed.

3. Effects on Autonomic Nervous System

Pupillometry was performed after oral administration of chromafenozide technical suspended in 1% Tween 80 aqueous solution at 0, 2000 and 5000 mg/kg to 6 male SD rats per group.

No treatment-related changes were observed.

4. Effects on Dygestive System

Transportability of charcoal meal administered orally at 10 ml/kg through the small intestine was assessed with 8 male ICI mice per group into which chromafenozide technical suspension had been injected intraperitoneally in 1% Tween 80 aqueous solution at 0, 128, 320, 800, 2000 and 5000 mg/kg at 1 hr before the charcoal meal administration.

Suppressed transportability of charcoal meal was noted at dose levels of 320 mg/kg or more.

5. Effects on Skeletal Muscle

Forelimb grip strength was measured after oral administration of chromafenozide technical suspended in 1% Tween 80 aqueous solution at 0, 2000 and 5000 mg/kg to 6 male SD rats per group.

No treatment-related changes were observed.

6. Effects on Blood

6.1 Effects on in vivo hemolysis and hemocoagulation

Prothrombin time, activated partial thromboplastin time and hemoglobin

concentration were measured with blood samples collected after oral administration of chromafenozide technical suspension in 1% Tween 80 aqueous solution at 0, 2000 and 5000 mg/kg to 6 male SD rats per group.

No treatment-related changes were observed.

6.2 Effects on in vitro hemolysis and hemocoagulation

Prothrombin time, activated partial thromboplastin time and hemoglobin concentration were measured by adding chromafenozide technical solved in DMSO and diluted with 1% Tween 80 saline solution at 0, 10^{-7}, 10^{-6} and 10^{-5} g/ml to plasma and 10% erythrocyte suspension.

No treatment-related changes were observed.

(The Environmental Toxicology Institute, 1998)

SUMMARY

Results of various toxicology studies conducted for characterization of effects on human health demonstrate that chromafenozide is a pesticide with extremely low risk properties.

The acute toxicity of chromafenozide to rats, mice and rabbits corresponds to the low toxic substance group specified in the Japanese Toxic Substance Regulation Act. In irritation studies, mild eye irritation induced by the technical substance, 5% SC and 0.3% D can be remedied by eye irrigation. No eye irritation was occurred with 5% SC at field application rate (1/500 dilution). Neither dermal irritation nor dermal sensitization was observed.

Subchronic toxicity, chronic toxicity and oncogenicity studies with chromafenozide in rats, mice and dogs were revealed decreased body weight gain, mild anemia, hepatocellular hypertrophy, increased weight of the liver and spleen, and darkening in color and brown pigment deposition of the spleen without any tumorigenesis in animals received the high dose of the test substance. In the reproduction study in rats and the teratology studies in rats and rabbits, no test substance-related effects were noted. In the three mutagenicity studies chromafenozide was demonstrated to be negative. In general pharmacology studies, prolonged hexobarbital sleeping time and suppressed transportability of charcoal meal in the small intestine were characteristic of chromafenozide.

MATRIC® Flowable (5% SC) and MATRIC® Dust DL (0.3% D) were registered as insecticides containing chromafenozide as the active ingredient by Japanese Ministry of

Agriculture, Forestry and Fisheries on December 27, 1999.

Contact

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Table Acute toxity studies.

Chroma-fenozide	Species	Dosing route	LD50 (mg/kg) Male	Female	Laboratory (year)
Technical	Rat	Oral	>5000	>5000	IET[d] (1995)
	Mouse	Oral	>5000	>5000	
	Rabitt	Dermal	>2000	>2000	
	Rat	Inhalation	>4.68 (mg/l)	>4.68 (mg/l)	
5% SC[a]	Rat	Oral	>5000[c]	>5000[c]	IET[d] (1998)
		Dermal	>2000[c]	>2000[c]	
	Mouse	Oral	>5000[c]	>5000[c]	
	Rat	Inhalation	>5.4[c]* (mg/l)	>5.4[c]* (mg/l)	
0.3% D[b]	Rat	Oral	>5000[c]	>5000[c]	
		Dermal	>2000[c]	>2000[c]	
	Mouse	Oral	>5000[c]	>5000[c]	

[a]MATRIC® Flowable; [b]MATRIC® Dust DL; [c]Value as product; [d]The Institute of Environmental Toxicology; *LC_{50}.