Summary of Toxicity Studies On Pyrimidifen

Sankyo Company, Limited; Ube Industries, Limited
(Accepted February 20, 1999)

DESCRIPTION OF THE TEST CHEMICALS

Pyrimidifen (Miteclean®) is a new acaricide and insecticide jointly developed by Sankyo Company, Limited and Ube Industries, Limited.

This chemical is remarkably effective against a wide range of mites which are harmful to fruits, vegetables and tea and against the diamondback moth which is harmful to brassica vegetable. Official tests have been conducted in Japan since 1988, leading to the pesticide registration of this chemical approved by Japanese authority in April, 1995 .

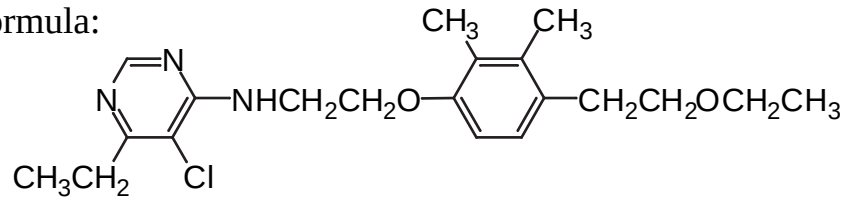
This article provides a toxicological feature of the technical material and 4% SC-formulation of pyrimidifen.

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

Common name: pyrimidifen (ISO name)

Chemical name: 5-chloro-*N*-[2-[4-(2-ethoxyethyl)-2,3-dimethylphenoxy]ethyl]-6-ethylpyrimidin-4-amine

Structure formula:



Molecular formula: $C_{20}H_{28}ClN_30_2$

Molecular weight: 377.92

Appearance: white crystalline solid or powder

Specific gravity: 1.22 (20˚C)

Melting point: 69.4-70.9˚C

Solubility (g/*l*, 25˚C): water, 0.00217; acetonitrile, 97; methanol, 276; *n*-hexane, 34; xylene, 364; dimethyl sulfoxide, 307

Vapor pressure: 1.2 x $l0^{-9}$ mmHg (25˚C)

ACUTE TOXICITY STUDIES

Table 1 shows, results of acute toxicity studies with pyrimidifen technical and 4% SC-formulation at different routes of administration. There were no distinctive signs of toxicity throughout any route of administration.

(The Institute of Environmental Toxicology, 1989; Huntingdon Research Centre Ltd., 1993)

Table 1 Summary of acute toxicity.

	Route	Species	LD_{50} (mg/kg)
Technical	Oral[1)]	Rat	M[a)]: 148 F[a)]: 115
	Oral[2)]	Mouse	M: 245 F: 229
	Dermal[3)]	Rat	M, F: >2.000
Formulation (4%-SC)	Oral[4)]	Rat	M, F: > 5,000
	Oral[5)]	Mouse	M: 3,000, F: 5,100
	Dermal[6)]	Rat	M, F: >2,000
	Inhalation[7)]	Rat	M, F: >2.56 mg/*l*

[a)] M=Male, F= Female. [1), 2), 3)] The Institute of Environmental Toxicology, 1989. [4),5),6),7)] Huntingdon Research Centre Ltd., 1993.

IRRITATION STUDIES

1. Primary Eye Irritation on Rabbits

Instillation of pyrimidifen technical (about 46 mg/eye) into the eyes of one male and five female New Zealand White rabbits caused slight irritant effects of transient conjunctival inflammation, being, however, reversible within 3 days. Formulation (4%-SC, 0.1 ml/eye, six male New Zealand White rabbits) caused slight conjunctival irritation. Full recovery was observed within 2 days. Furthermore, there were no positive response, corneal damage or iridial inflammation in 1000-fold dilution of 4%-SC formulation (0.1 ml/eye, one male and five female New Zealand White rabbits) which is a practical dosage. It's instillation elicited temporary mild conjunctival irritation only. (Huntingdon Research Centre Ltd., 1991 and 1993)

2. Primary Dermal Irritation on Rabbits

Pyrimidifen technical (0.5 g) or it's formulation (4%-SC, 0.5ml) were applied to the intact skin of New Zealand White rabbits (technical; three males and three females, formulation; six males). Both of them showed no response to treatment in any animal throughout the observed period. There was no dermal irritation.

(Huntingdon Research Centre Ltd., 1991 and 1992)

3. Dermal Sensitization Study on Guinea Pigs

Three induction applications of pyrimidifen technical (0.5 ml, 65% w/w in acetone) or it's formulation (0.5 ml of 4%-SC) were made once a week for 3 weeks. Ten female Dunkin/Hartley guinea pigs were challenged using pyrimidifen technical (0.5 ml, 65% w/w in acetone) or it's formulation (0.5 ml of 4%-SC and 50% v/v in distilled water) after the 3rd induction application respectively. Their potential to induce delayed contact hyper-sensitivity was evaluated according to Buehler test. Both pyrimidifen technical and it's formulation (4%-SC) showed no evidence of dermal sensitization potential. (Huntingdon Research Centre Ltd., 1991 and 1992)

SUB-CHRONIC TOXICITY STUDIES

1. Thirteen-Week Feeding Study on Rats

Groups of 12 male and 12 female Fischer (F344/ DuCrj) rats were fed diet containing 0, 10, 30, 100 and 150 ppm of pyrimidifen for 13 weeks. Treatments in 100 ppm or higher male and female caused the depression or the depressing tendency of bodyweight gain, reduced food consumption, some changes in hematology and blood biochemistry and increased kidney weight. Liver weight was increased in 10 ppm or higher female and 100 ppm or higher male. Therefore, the NOEL for this study was considered to be 30 ppm (1.794 mg/kg/day) in male and less than l0 ppm (0.693 mg/kg/day) in female. (The Institute of Environmental Toxicology, 1990)

2. Thirteen- Week Feeding Study on Mice

Groups of 12 male and 12 female ICR (Crj: CD-1) mice were fed diet containing 0, 10, 30, 100 and 300 ppm of pyrimidifen for 13 weeks.

Increased liver and kidney weights were noted at 100 ppm or higher females. Centrilobular hepatocellular swelling of the liver was observed at 300 ppm of male and female. A decreasing tendency of food consumption and slight anemia were also observed at 300 ppm female. In males in the 100 ppm group and in females and males in 30 and 10 ppm groups, there were no treatment-related changes in the examinations.

Therefore, the NOEL for this study was considered to be 100 ppm (13.4 mg/kg/day) in male and 30 ppm (5.33 mg/kg/day) in female.

(The Institute of Environmental Toxicology, 1990)

3. Thirteen-Week Oral Toxicity Study on Dogs

Groups of 4 male and 4 female beagle dogs were administered orally *via* gelatin capsule at dose levels of 0, 0.15, 0.5, 1.5 and 4.5 mg/kg/day of pyrimidifen for 13 weeks. Increased frequency of vomiting and liquid faeces were noted at 0.5 mg/kg/day or higher. Reduced bodyweight gain, reduced food intake and occasional incidence of salivation were also noted at 4.5 mg/kg/day. The NOAEL for this study was considered

to be 0.15 mg/kg/day in male and female.

(Huntingdon Research Centre Ltd., 1992)

CHRONIC TOXICITY AND ONCOGENICITY STUDIES

1. Combined Chronic Toxicity and Oncogenicity Study on Rats

Groups of 50 male and 50 female Fischer (F344/ DuCrj) rats were fed diet containing 0, 3, 10, 30 and 100 ppm of pyrimidifen for 104 weeks.

Treatments in 30 ppm or higher males and females caused the depression of bodyweight gain and reduced food consumption. The decrease of hemoglobin and the increase of relative liver weight were observed in 30 ppm or higher females. In 100 ppm females, the dark-coloured kidneys were observed and kidney weight and brown pigment deposition (lipofuscin) in the tubular epithelium of the kidneys was increased. On the other hand, increased pheochromocytoma (benign) in the adrenal was noted in 100 ppm males. In addition, there was no significant difference between the total number of neoplastic lesions (especially malignant tumors) nor the number of animals having the neoplastic lesion of the treated groups and those of the control group. In 10 ppm and 3 ppm groups, neither male nor female showed findings attributable to the treatment in any examinations.

Therefore, the NOEL for this study was considered to be 10 ppm in both male and female (male: 0.338 mg/kg/ day, female: 0.427 mg/kg/day) and no apparent oncogenicity was indicated. (The Institute of Environmental Toxicology, 1993)

2. Oncogenicity Study on Mice

Groups of 50 male and 50 female ICR (Crj: CD-1) mice were fed diet containing 0, 10, 30, 100 and 300 ppm of pyrimidifen for 86 weeks.

In 100 ppm or higher males and females, decrease or decreasing tendency of bodyweight gain was observed. Males and females in the 300 ppm group showed lower food consumption, lower food efficiency and so on. In 30 and 10 ppm groups, there were no toxic effects related to the test substance. In addition, there were no significant increases in the incidence of neoplastic lesions in any treated group.

Therefore, the NOEL for this study was considered to be 30 ppm in both male and female (male: 2.839 mg/kg/day, female: 2.638 mg/kg/day) and no oncogenicity was indicated. (The Institute of Environmental Toxicology, 1993)

3. Chronic Toxicity Study on Dogs

Groups of 4 male and 4 female beagle dogs were administered orally via gelatin capsule at dose levels of 0, 0.15, 0.75 and 3.75 mg/kg/day of pyrimidifen for 52 weeks.

In 0.75 mg/kg/day or higher male and female, clinical signs of liquid faeces, vomiting and salivation prior to dose administration were noted throughout the treatment

period. In 0.15 mg/kg/day, the incidence of liquid faeces was slightly greater than that of control but individual incidence was almost same as that of control. There was no difference between the treatment groups and the control one in any other investigations.

Therefore, the NOAEL for this study was considered to be 0.15 mg/kg/day in male and female. (Huntingdon Research Centre Ltd., 1993)

4. Chronic Toxicity Study on Dogs for 26 Weeks (Additional Study)

Groups of 4 male and 4 female beagle dogs were administered orally *via* gelatin capsule at dose levels of 0, 0 (lactose), 0.l0, 0.12, 0.15 and 0.75 mg/kg/day of pyrimidifen for 26 weeks to ensure a NOEL in beagle dogs. Only in 0.75 mg/kg/day group, the incidence of liquid faeces and vomiting were increased. In any other treated groups, there were no changes related to the test substance. According to the results in both the above mentioned chronic toxicity study on dogs and this study, 0.15 mg/kg/day can be considered to be the NOEL.

(Huntingdon Research Centre Ltd., 1 993)

REPRODUCTION STUDY

Groups of 32 male and 32 female Crl: CD (SD) rats were fed diet containing 0, 10, 30 and 100 ppm of pyrimidifen throughout two generations. In 100 ppm parental animals, reduced food consumption and reduced bodyweight gain, increased liver weight and some changes in histopathological examination were noted. For litters, reduction of bodyweight gain and differences of organ weights were noted and there was no influence of fertility in any group. At neither 10 or 30 ppm, there were no apparent treatment-related effects and changes.

Therefore, the NOEL for parent animals was considered to be 30 ppm (male: 2.2-2.5 mg/kg/day, female: 2.5-2.7 mg/kg/day) and that for reproduction to be 100 ppm (male: 7.6-8.4 mg/kg/day, female: 8.3-9.5 mg/kg/day)

(Huntingdon Research Centre Ltd., 1992)

TERATOGENICITY STUDIES

l. Teratogenicity Study on Rats

Pyrimidifen was administered by oral gavage at dose levels of 0, l, 5 and 25 mg/kg/day to groups of 24 pregnant Crl: CD (SD) rats for 10 days. In 25 mg/kg/ day parental females, reduced bodyweight gain and reduced food consumption were noted during the early phase of the treatment. For the developing foetus of this dose level, their mean weight was slightly reduced and some retardation of ossification and an increase in the incidence of foetuses with 14 th rib were noted. Administration of this substance at 1 or 5 mg/kg/day had no discernible effects on either the mother or the developing foetus.

The NOEL for the mother or the developing foetus was considered to be 5 mg/kg/day. Administration of pyrimidifen at the highest level examined, 25 mg/kg/day, had no teratogenic effect on the developing foetus. (Life Science Research Ltd.,1990)

2. Teratogenicity Study on Rabbits

Groups of at least 15 pregnant New Zealand White rabbits were administered by oral gavage at dose levels of 0, 1, 4, 20 mg/kg/day of pyrimidifen for 14 days. In 20 mg/kg/day parental females, reduced bodyweight gain and reduced food consumption were noted and one of them was killed. For the developing foetus of this dose level, incidence of foetuses with reduced ossification of the distal bones of the limbs was increased but with this exception foetal development was similar in all groups. There were no changes in any other examinations. In 4 or 1 mg/kg/day, maternal performance, foetal survival, growth and development were similar to those of the control.

Therefore, the NOEL for the mother or the developing foetus was considered to be 4 mg/kg/day. Administration of pyrimidifen at the highest level examined, 20 mg/kg/day, had no teratogenic effects on the developing foetus.

(Life Science Research Ltd., 1990)

MUTAGENICITY STUDIES

1. Bacterial Reverse Mutation Assay

Pyrimidifen was tested in Ames plate incorporation assays using the histidine auxotrophic *Salmonella typhimurium* (TA 98, TA 100, TA 1535 and TA 1537) and tryptophane auxotrophic *Escherichia coli* (WP2uvrA) in both presence and absence of rat liver metabolic activation system (S9) at a dose level of 313 to 5000 μg/plate.

Increase of a number of revertant colonies of any strain was not caused at any dose with or without metabolic activation. Therefore, pyrimidifen is considered to be no induction of reverse mutation in this test system.

(Chemical Inspection and Testing Institute, 1989)

2. Chromosome Aberration Assay

Pyrimidifen was tested in chromosomal aberration assay using Chinese hamster lung fibroblast (CHL cell). Dose levels used were 22 to 50 μg/ml for 24 hr treatment, 0.009 to 0.020 μg/ml for 48 hr treatment by the direct method (without metabolic activation) and 67 to 150 μg/ml with metabolic activation respectively.

Pyrimidifen did not induce structural chromosomal aberrations and polyploids at any dose with or without metabolic activation. Therefore, pyrimidifen is considered to be negative in this test system. (Chemical Inspection and Testing Institute, 1989)

3. Bacterial DNA Repair Test (Rec-Assay)

Pyrimidifen was tested in a bacterial recombination assay using *Bacillus subtilis*

strains (H17 and M45) in both presence and absence of metabolic activation at a dose level of 175 to 2800 μg/disk. There was no inhibition of any strains with or without metabolic activation. It was considered that pyrimidifen had no DNA-damaging capability in this test system. (Chemical Inspection and Testing Institute, 1989)

GENERAL PHARMACOLOGY STUDIES

Seven kinds of pharmacological studies of pyrimidifen were carried out as follows:

l . Effects on the central nervous system (behavioral signs in rats, hexobarbital-induced sleeping times in mice, tilting plane test in rats)
2. Effects on the respiratory and cardiovascular systems in dogs
3. Effects on the autonomic nervous system in cats
4. Effects on the gastro-intestinal function (charcoal propulsion in mice, gastric secretion in rats)
5. Effects on motor co-ordination in mice
6. Effects on blood parameters (blood coagulation in rats, haemolysis *in vitro*)
7. Effects on renal function in rats

Pyrimidifen caused some effects, that was, effects on the general behavior (moderate apathy, decreased respiration, hunched posture and abnormal gaits) in rats, effects on the respiratory and cardiovascular systems (changes in all the parameters measured at higher doses) in dogs, effects on the gastric secretion (decrease in the volume of the gastric sample produced and decrease in the secretion of Na^+, K^+, Cl^- and H^+) in rats, effects on haemolysis in vitro and effects on renal function (inhibition of urinary output and increase in urinary electrolyte excretion) in rats at higher dose levels. Lower dose levels had no significant changes related to the test substance.

(Huntingdon Research Centre Ltd., 1992)

SUMMARY

A lot of toxicology studies were carried out in order to investigate toxicological properties of technical pyrimidifen and it's formulation (4%-SC). The results of acute toxicity tests indicate that pyrimidifen technical displays moderate toxicity and it's formulation display much lower toxicity than pyrimidifen technical, Pyrimidifen should be handled carefully because of eye irritation and inhalation toxicity. In chronic toxicity studies, some effects as depression of body weight gain, lower food consumption and small changes in organs were generally observed at higher dose levels. In dogs, clinical signs of liquid faeces and vomiting were also observed at higher dose. In any test and any animal, the NOELs Were confirmed.

Pyrimidifen is not genotoxic or oncogenic and does not interfere with normal reproduction and development. When the rules for usage of pyrimidifen are kept, it is not only an effective acaricide and insecticide but a safe one.

Pyrimidifen is considered to be one of the most useful agricultural materials.

要　約

ピリミジフェンの毒性試験の概要

三共株式会社農薬開発部；宇部興産株式会社研究開発本部

ピリミジフェンの安全性評価のために各種毒性試験を実施した。

ピリミジフェン原体の急性経口毒性は、ラット、マウスとも劇物相当であったが、製剤での毒性は弱く、両種とも普通物に相当した。急性経皮毒性は、原体、製剤とも低かった。眼粘膜一次刺激性については、一過性の軽度の刺激性が認められたが、２～３日後には正常に回復した。皮膚一次刺激性については、原体、製剤で刺激性は全く認められず、また、皮膚感作性についても原体、製剤共に陰性であった。マウス、ラットおよびイヌでの亜急性毒性、慢性毒性及び発癌性試験で、高濃度投与群で飼料摂取量の低下や体重の増加抑制などが認められ、イヌでは水様便、嘔吐などの頻度が増加した。しかしながら、ピリミジフェン投与に起因する腫瘍性病変の発現はなかった。また、各種変異原性試験においても陰性であった。繁殖や次世代に対する悪影響や催奇形性は認められなかった。

本剤は、1995年以降、リンゴ、ミカン、ナシなどの果樹やチャのハダニ類、及びキャベツのコナガ等に対して登録を取得している。

ピリミジフェンは定められた使用基準を遵守すれば、安全性が高い薬剤であり、農業資材の一つとして有用であると考えられる。

問合せ

三共株式会社農薬開発部

〒104－8113東京都中央区銀座二丁目7番12号

宇部興産株式会社研究開発本部農薬開発部

〒140－8633東京都品川区東刷11二丁目3番11号