J. Pesticide Sci. **14**, 407–413 (1989)

Technical Information

Summary of Toxicity Studies on Tricyclazole

Eli Lilly Japan K.K. and Eli Lilly Research Laboratories

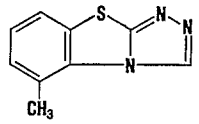
(Received May 20, 1989)

DESCRIPTION OF THE TEST CHEMICAL

Tricyclazole is a systemic fungicide for the control of rice blast desease caused by the fungus *Pyricularia oryzae*. The effectiveness of tricyclazole has been demonstrated in many areas of the world where rice blast is a commercial problem and tricyclazole has provided prolonged rice blast control.

Tricyclazole has the following chemical and physical properties.

Structural formula:



Chemical name: 5-Methyl-1, 2, 4-triazole(3, 4-b)benzothiazole

Molecular formula: $C_9H_7N_3S$

Molecular weight: 189.24

Appearance: crystalline solid

Melting point: 187–188°C

Vapor pressure: 2×10^{-7} mmHg (25°C)

Partition coefficient: 25.0 (*n*-octanol/water)

Solubility (*g/l* at 25°C): water 1.6, chloroform >500, methylene chloride 33, methanol 25, ethanol 25, acetone 10.4, acetonitrile 10.4, cyclohexanone 10, benzene 4.2, xylene 2.1, hexane <0.1

ACUTE TOXICITY STUDIES

1. Technical Material

Animal species, strain	Administration route	LD_{50} (mg/kg)		Testing facility,[a] reporting year
		Male	Female	
Rat, Wister	Oral	338	290	LRL, 1974
Rat, SD	Oral	358	223	IET, 1978
Mouse, ICR	Oral	247	244 338	LRL, 1974
Mouse, ICR	Oral	545	500	IET, 1978
Rat, SD	Dermal	>5000	>5000	IET, 1978

[a] Abbreviations: LRL, Lilly Research Laboratories; IET, Institute of Environmental Toxicology.

2. Formulated Product (BEAM)

Product (content %)	Animal species, strain	Administration route	LD_{50} (mg/kg)		Testing facility,[a] reporting year
			Male	Female	
BEAM G(4)	Rat, Wister	Oral	>5000	>5000	KU, 1979
	Mouse, ICR	Oral	>5000	>5000	KU, 1979
	Rat, Wister	Dermal	>2000	>2000	BRI, 1986

BEAM SG(4)	Rat, F344	Oral	>2500	>2500	LRL, 1986
	Mouse, ICR	Oral	>2500	>2500	LRL, 1986
BEAM SOL (20)	Rat, Wister	Oral	1340	1210	KU, 1979
	Mouse, ICR	Oral	3290	2830	KU, 1979
	Rat, Wister	Dermal	>2000	>2000	BRI, 1986
	Rat, SD	Inhalation	LC_{50} >2 mg/*l*	LC_{50} >2 mg/*l*	MITES, 1986
BEAM WP(20)	Rat, Wister	Oral	1500	1510	KU, 1979
	Mouse, ICR	Oral	2440	2620	KU, 1979
BEAM WP(75)	Rat, Wister	Oral	459	399	BRI, 1987
	Mouse, ICR	Oral	444	525	BRI, 1987
	Rat, Wister	Dermal	>2000	>2000	BRI, 1987
	Rat, F344	Inhalation	LC_{50} >2.33 mg/*l*	LC_{50} >2.33 mg/*l*	LRL, 1982

a) Abbreviations: KU, Keio University; BRI, Biomedical Research Institute; MITES, Mitsubishi Kasei Institute of Toxicological and Environmental Sciences.

PRIMARY IRRITATION STUDIES

1. Primary Ocular Irritation Studies

A group of 3 male and 3 female New Zealand albino rabbits was selected for testing. One eye of each animal was treated with 78 mg of technical grade tricyclazole and individual animal scores were graded at 1 hr, 1, 2, 3 and 7 days after treatment based on Draize scale. One hour after treatment, all treated eyes had slight conjunctival hyperemia and two exposed eyes had slight chemosis (conjunctiva edema). At twenty-four hours after dosing, two animals exhibited slight iritis and a corneal dullness that stained positive with sodium fluorescein dye. All eyes treated with tricyclazole appeared normal 72 hr following treatment.

(Lilly Research Laboratories, 1978)

Similarly, the test was carried out by treating with 38 mg of BEAM WP75 per eye. Corneal dullness and slight irritis developed in five of six treated eyes and mild conjuctivitis developed in all treated eyes within 1–24 hr after treatment. A positive response to sodium fluorescein dye was observed in all treated eyes at 24 hr after treatment and a negative response on three days postexposure.

(Lilly Research Laboratories, 1982)

BEAM G and BEAM SOL were tested using Japanese albino rabbits. Average grades (in 6 animals), based on the Draize score, were 1.7 in conjunctivae and 1.5 in chemosis at 1 hr after the treatment with 0.1 g of BEAM G. These disappeared on day 2. When treated with 0.1 ml of BEAM SOL, the average Draize grades were 1.0 in conjunctivae and 1.7 in chemosis at 1 hr after the treatment and cleared on day 3. No irritation was observed in cornea and iris in BEAM G and BEAM SOL.

(Biomedical Research Institute, 1986)

2. Primary Dermal Irritation Studies

Three male and three female albino rabbits were prepared for treatment by clipping their backs free of hair and one-half the animals were abraded with a stiff nylon brush. A 2.0 g/kg topical application of technical grade tricyclazole was applied and covered with an occlusive dressing for 24 hr. Signs of dermal irritation were observed for 14 days. No dermal irritation was noted during this study.

(Lilly Research Laboratories, 1978)

Dermal irritation of Beam WP75 was tested with the same method as described above. Slight to well-defined erythema and slight edema were observed 24 hr after treatment with BEAM WP75 and cleared withing 48–72 hr in

5 animals. The remaining animal developed moderate to severe erythema and moderate edema by test day 5. Concomitant with healing were dehydration and desquamation. The irritation index was 1.8 based on a scale having a maximum of 8.0. The treated skin of all animals appeared normal 14 days after treatment.

(Lilly Research Laboratories, 1982)

BEAM G and BEAM SOL were also examined. The test substance, 0.5 g BEAM G in paste with distilled water or 0.5 ml BEAM SOL, was applied to a 2 cm×3 cm area of the skin of Japanese albino rabbits (6 males). The exposure duration was 4 hr and, based on Draize method, skin reaction was evaluated at 1 hr, 1, 2, 3, 4 and 5 days after. In the test with BEAM G, very slight erythema in 5 animals, well-defined erythema in 1 animal and very slight edema in all animals were observed at 1 hr after the 4-hr exposure. The erythema and edema disappeared on day 5 and day 1, respectively. BEAM SOL exhibited very slight erythema in all 6 test animals and very slight edema in 2 animals at 1 hr after, then the erythema and edema cleared on day 5 and day 1, respectively. BEAM G and BEAM SOL gave weak irritation to the skin of the rabbits.

(Biomedical Research Institute, 1986)

DERMAL SENSITIZATION STUDIES

The potential of tricyclazole (as formulated products) to induce delayed contact hypersensitivity was tested in guinea pigs by Bueler's method. BEAM SOL induced weak dermal sensitization with a sensitization rate of 7%, while BEAM WP75 did not induce dermal sensitization at all.

(Biomedical Research Institute, 1986, 1987)

A previous study with technical tricyclazole resulted in no evidence of delayed hypersensitivity and dermal response was negative throughout the initial and challenge treatments.

(Lilly Research Laboratories, 1974)

SUBCHRONIC TOXICITY STUDIES

1. Three-month Toxicity Study in Rats

Rats (Wister derived) were maintained on diets containing 0, 282, 635 or 1640 ppm tricyclazole for 3 months. Clinical signs of toxicity and mortality related to tricyclazole administration were not observed. Decreases in mean body weight, average food consumption and efficiency of food utilization were noted for male and female rats given either 635 or 1640 ppm tricyclazole diets. Significant effects on hematologic and clinical chemistry parameters were limited to the 1640 ppm group and included an elevated lymphocyte ratio and GPT concentration for males and an elevated BUN concentration and decreased prothrombin time and monocyte ratio for females. Significant decreases in absolute and increases in relative organ weights were observed for several organs from both males and females given 282, 635 or 1640 ppm tricyclazole diets. At 282 ppm dose, observed changes were significant decrease in absolute organ weight of prostate and increases in relative organ weights of liver and heart for male and liver for female rats.

Since tricyclazole related histopathologic lesions were not found in most of these organs, it was concluded that the effects on organ weights were related to the impaired growth of these animals. Histopathological findings related to growth retardation and impaired nutrition included immature testes and small prostates in males given 1640 tricyclazole diets and a lower incidence of fatty metamorphosis of liver and fatty degeneration of kidneys in rats maintained on diets containing 635 or 1640 ppm tricyclazole. Tricyclazole related effects on the liver included a dose related increase in relative weight and *in vitro* *p*-nitroanisole metabolism in males and females and the microscopic finding of slight centrilobular hypertrophy of hepatocytes in 6 or 15 males from the 1640 group.

Because of the effect on organ weight, a maximal no-effect level was not established in this 3-month feeding study.

(Lilly Research Laboratories, 1978)

2. Three-month Toxicity Studies in Mice

1) Groups of 15 male and 15 female mice (ICR) were maintained on diets containing 0, 400, 1000, 2500 or 3600 ppm tricyclazole for 3 months. Nine mice did not survive during the study. All deaths occurred in groups receiving

diets equal to or greater than 1000 ppm tricyclazole. Thinness and piloerection were observed for several mice from the 1000, 2500 and 3600 ppm groups. Significant decreases in body weight were noted for male (2500 and 3600 ppm) and female (2500 ppm) mice during the initial month.

The only changes in hematologic or clinical chemistry parameters possibly related to tricyclazole administration were the finding of increased platelets in males from the 2500 and 3600 ppm groups and increased GPT in males from the 1000 and 3600 ppm groups. Significant alterations in organ weights apparently related to tricyalzole administration included the dose-related increase in absolute and relative liver weight for males from the 2500 and 3600 ppm groups and females from the 400, 1000, 2500 and 3600 groups and the increased absolute ovary weight for animals from the 1000, 2500 and 3600 ppm groups. The only treatment related histopathologic finding was that of focal slight proliferation of small bile ducts in the livers of 7 of 16 males and 8 of 14 females from the 3600 ppm group.

Based upon the aforementioned effects on survival, body weight, water consumption and liver parameters (GPT, absolute and relative weights, proliferation of small bile ducts), a no-effect level was not established.

(Lilly Research Laboratories, 1978)

2) Groups of 20 male and 20 female mice (ICR-JCL) were maintained on diets containing 0, 40, 200, 1000 and 5000 ppm tricyclazole for 3 months. During the study there were no noted clinical signs and no death. Differences in food consumption and water intake between control and any treated groups were not observed. However, significant decrease in efficiency of food utilization and reduction in body weight gain were noted for male and female rats given 5000 ppm tricyclazole diets.

In hematologic and clinical chemistry parameters, significant changes were elevated ALP, GOT and GPT and decreased hematocrit, hemoglobin concentration, erythrocyte count and blood glucose level in male rats and elevated total protein, BUN concentration, GOT and GPT and decreased leucocyte count in female rats from the 5000 ppm group; elevated total protein and decreased leucocyte count in male rats and elevated blood glucose and GOT and decreased ALP in female rats from the 1000 ppm group; and elevated blood glucose and decreased ALP in female rats from the 200 ppm group. Significant alterations in organ weights apparently related to tricyclazole administration included in dose related increase in absolute and relative liver weight for males from the 1000 and 5000 ppm groups and females from the 200, 1000 and 5000 ppm groups. The treatment related histopathological findings were diffuse hepatocellular proliferation accompanied by periportal acidophilic degeneration of hepatocytes, in all males and females of the 5000 ppm group.

At 1000 ppm dose, periportal acidophilic degeneration of hepatocytes were observed in 14 male and 10 female rats. No histopathologic alternation related to tricyclazole administration was observed in 200 and 40 ppm groups.

Based upon this study, maximum no-effect levels were 200 ppm (23.9 mg/kg) for males and 40 ppm (5.58 mg/kg) for females.

(Institute of Environmental Toxicology, 1978)

CHRONIC TOXICITY AND ONCOGENICITY STUDIES

1. Two-year Toxicity Study in Rats

Chronic toxicity and oncogenicity study in rats carried out at Lilly Research Laboratories (1977) was presented elsewhere.[1] Based on the effects seen in rats maintained on diets containing 0, 50, 100, or 275 ppm of tricyclazole for two years, a no-effect level of 275 ppm (8.1–14.4 and 12.0–23.7 mg/kg for male and female, respectively) was supported. Oncogenicity of tricyclazole was not observed in this rat study.

2. Two-year Toxicity Study in Mice

The toxicological evaluation of tricyclazole in mice for two years (Lilly Research Laboratories, 1978) was also presented elsewhere.[2] No evidence of any treatment-related effect was noted in mice maintained on diets containing 0, 50, 140 and 400 ppm or tricyclazole.

3. Twenty-two-month Toxicity Study in Mice

Groups of 64 male and female SPF ICR (JCL: ICR) mice were maintained on diets containing 0, 25, 75, 250, or 1000 ppm of

tricyclazole for 95 weeks. Ten males and ten females from each group were sacrified after 52 weeks of the treatment to examine hematology, blood biochemistry and pathology.

At 1000 ppm dose, male mice showed significant decreases or decreasing trends in the values of hematocrit (Ht), hemoglobin (Hb), erythrocyte count (RBC), and lymphocyte count after 52 weeks of treatment. Both male and female mice exhibited significant increases or increasing trends in absolute and relative liver weights after 52 and 95 weeks of treatment. Histopathological examination revealed fatty change and acidophilic degeneration of periportal hepatocytes in both sexes.

At 250 ppm dose, male mice showed significant decreases in Ht, Hb, and RBC, and significant increases in absolute and relative liver weights after 52 weeks of treatment. Histopathologically, the same types of hepatic lesions as those observed in the 1000 ppm group were also noted in both sexes.

From 75 and 25 ppm groups, no treatment-related changes were observed in either sex.

Based on the results, the maximum no-effect level, minimum toxic level, and sure toxic level of tricyclazole to ICR-JCL mice were 75 ppm (7.98 mg/kg/day for male and 6.67 mg/kg/day for female), 250 ppm (24.9 mg/kg/day for male and 21.8 mg/kg/day for female) and 1000 ppm (101 mg/kg/day for male and 91 mg/kg/day for female), respectively. Carcinogenicity of tricyclazole was negative in mice.

(Institute of Environmental Toxicology, 1985)

4. One-year Toxicity Study in Dogs

Beagle dogs, 4/sex/dose, were administered daily oral doses of 0, 5, 15, or 45 mg/kg of tricyclazole for one year. All dogs survived the one year period. Vomiting and soft, runny, and/or mucoid stools were noted in animals from all dose groups. The occurrence of these signs was sporadic, of low incidence and short duration, and was considered of no toxicological significance. There were no toxicological significant effects on body weight, body weight gain, hematology and clinical chemistry parameters, or urinalysis. Tricyclazole was undetectable in the serum of dogs treated with 5 or 15 mg/kg and reached levels of up to 5 μg/ml in the 45 mg/kg group. The peak serum levels were observed between 0 and 3 hr after dosing returning to minimal or undetectable levels by 4 hr after dosing. Decreases in *p*-nitroanisole metabolism and hepatic cytochrome P-450 content were noted in males treated with 15 and 45 mg/kg. Slight but statistically significant increases in relative and absolute kidney and liver weights were seen in males and females from the 45 mg/kg dose group. There were no gross or histopathological treatment-related lesions in these organs or any other organs examined. Data in this study support a no-effect dose of 5 mg/kg/day for tricyclazole in dogs.

(Lilly Research Laboratories, 1986)

REPRODUCTION STUDY IN RATS

Tricyclazole was administered continuously as a dietary component to 3 parental generations of rats (Harlan Wister derived) at levels of 50 or 275 ppm. The compound was well tolerated without significant mortality or apparent effect on the reproductive capacity of the animals. Slight reductions in progeny survival (first mating trial, F_1 generation) and progeny weight (F_2 generation) of tricyclazole treated rats were not substantiated by data of other generations. No abnormalities attributable to treatment were seen in either parents or offspring.

In conclusion, tricyclazole at dietary levels of 50 and 275 ppm did not impair the reproductive process of rats or affect growth or survival of the offspring.

(Lilly Research Laboratories, 1977)

TERATOLOGY STUDIES

1. Teratology Study in Rats

Tricyclazole was administered continuously as a dietary component to three generations of Wister rats at levels of 50 and 275 ppm. In each generation 20 control females and 20 females from each tricyclazole regimen were sacrificed on gestation day 20. No deaths or signs of toxicity occurred among the females assigned to the teratology studies. Nor was there any evidence of reproduction impairment; fertility, litter size, implantation, and fetal viability values were normal.

It was concluded that there was no evidence

of teratogenic effect when Wister rats were continuously fed diets containing 50 or 275 ppm tricyclazole throughout three generations.
(Lilly Research Laboratories, 1977)

2. Teratology Study in Rabbits

Tricyclazole was administered orally by gavage to a group of 15 female Dutch Belted rabbits in daily doses of 2, 10, or 50 mg/kg on gestation days 6 through 18, a total of 13 treatments. There were signs of respiratory tract infection, anorexia, and weight loss in control rabbits as well as rabbits assigned to the tricyclazole groups. One control rabbit, 1 rabbit in the 2 mg/kg group, and 4 rabbits in the 10 mg/kg group died; 2 control rabbits, 1 rabbit in the 2 mg/kg group, and 1 rabbit in the 10 mg/kg group aborted. Resorption occurrence was slightly higher in the tricyclazole treatment groups than in the current control group; these findings did not occur in a dose-related manner. Mean fetal weights of the tricyclazole treatment groups were lower than the control values. In most instances the reduced fetal weights were seen in conjunction with maternal toxicity (*i.e.*, anorexia, weight loss). No differences of toxicological significance were found between the control and tricyclazole treatment groups in the type or incidence of fetal defects. With the exceptions of 13 rib occurrence and sternal defects, which are common deviations in our Dutch Belted rabbit fetuses, abnormalities were confined to 1 or 2 litters in each group. On the basis of these findings, it was concluded that tricyclazole, administered in doses as great as 50 mg/kg/day on gestation days 6 through 18, did not produced a teratogenic effect in the Dutch Belted rabbit.
(Lilly Research Laboratories, 1977)

MUTAGENICITY STUDIES

1. Bacterial Reverse Mutation Assay

The Ames test was employed, with and without metabolic activation by an S9 liver microsomal fraction from Aroclor 1254-induced rats, using five histidine auxotrophs of *Salmonella typhimurium* (TA98, TA1537, TA1538, TA100 and TA1535) and one triptophan auxotrouph (WP2uvrA) of *Escherichia coli*. The concentrations of tricyclazole adapted in this test were 10–5000 μg/plate. The result indicated that, in both mutation tests with or without microsomal activation and each tester strain, there was no evidence of induced mutation under the condition tested.
(Institute of Environmental Toxicology, 1978).

2. Bacterial DNA-Repair Test

Recombination-wild (Rec^+) strain H17 and recombination-deficient (Rec^-) strain M45 of *Bacillus subtilis* were used in this test. The concentration of tricyclazole ranged 20 to 2000 μg/disk. The results concluded that tricyclazole had no DNA-damaging capability.
(Institute of Environmental Toxicology, 1978)

3. In Vitro Mammalian Cytogenetics Test

An *in vitro* chromosomal aberration test with tricyclazole was conducted with cultured Chinese hamster lung fibroblasts and was done both in the presence and in the absence of an exogenous metabolic activation system(phenobarbital/5, 6-benzoflavone induced rat liver S9).

The results showed that chromosome aberrations were not induced in the test without metabolic activation at doses of 12.5, 25 and 50 μg/ml following treatment for 6 hr; and at doses of 70, 140 and 180 μg/ml following 48 hr of treatment. After a 24-hr treatment period, doses of 70 and 140 μg/ml did not result in the induction of chromosome aberrations; however, chromosome aberrations were evident in the 280 μg/ml dose group. This treatment dose was more than three-fold higher than the IC_{50} (50% growth inhibition concentration), and the toxicity associated with this treatment would be expected to compromise the validity of the response observed.

In the presence of metabolic activation, substantial dose-related increases in the frequency of aberrant cells were observed at doses of 12.5, 25, and 50 μg/ml (which were equivalent to 1/4, 1/2 and 1× the IC_{50}). It is concluded that, under these conditions, tricyclazole produce chromosomal aberrations in cultured cells.
(Chemical Inspection and Testing Institute, 1987)

4. In Vivo Induction of Sister Chromatid Exchange

Tricyclazole was tested for the *in vivo* induc-

tion of sister chromatid exchange (SCE) in bone marrow of Chinese hamsters. Chinese hamsters were treated intraperitoneally with either 170, 85, 42.5 and 21.25 mg/kg of tricyclazole or 25 mg/kg of cyclophosphamide, and the induction of SCEs was scored 19 hr following treatment. Cytotoxicity resulted from treatment with 170 mg/kg of tricyclazole.

A positive response for chemical-induced SCE was obtained in hamsters treated with the pro-mutagen cyclophosphamide, which served as the positive control in this study. The frequency of SCE formation in tricyclazole-treated animals was not different from controls. It was therefore concluded that tricyclazole does not induce SCE *in vivo* in bone marrow of Chinese hamsters.

(Lilly Research Laboratories, 1981)

5. Micronucleus Test

The *in vivo* induction of micronuclei was measured in polychromatic erythrocytes in bone marrow of male and female Crl: ICR (CD-1) mice following single oral gavage doses of 300, 200 and 100 mg/kg of tricyclazole. The presence of micronuclei was evaluated in polychromatic erythrocytes of the bone marrow 24, 48 and 72 hr following administration. The pro-mutagen cyclophosphamide served as the positive control for this study and was administered by oral gavage at a dose level of 100 mg/kg. A 10% acacia solution served as the vehicle control and was administered orally at 0.02 ml/g body weight.

The frequency of micronucleated polychromatic erythrocytes in tricyclazole-treated male and female mice was not different from vehicle controls at 24, 48, or 72 hr after treatment. A positive response for induction of micronuclei was obtained in animals treated with cyclophosphamide, demonstrating the sensitivity of the test system for the detection of a chemical clastogen. It was concluded that tricyclazole did not induce micronuclei *in vivo* in bone marrow of CD-1 mice.

(Lilly Research Laboratories, 1988)

PHARMACOLOGICAL STUDIES

1. General Pharmacological Study

1.1 Multidimensional observation of rabbits

Clinical signs of three to four rabbits/group were multidimensionally observed after the oral administration at doses of 160, 320 and 640 mg/kg. No abnormality was observed at a dose of 160 mg/kg. Diarrhea was noted one day after the administration at a dose of 320 mg/kg. Decreases in spontaneous activity, abdominal muscle tone and leaping, in addition to motor incoordination and miosis were observed 0.5–4 hr after the administration at a dose of 640 mg/kg. No deaths were noted in the 160 mg/kg group. Two out of four rabbits in the 320 mg/kg group and all 5 rabbits in the 640 mg/kg group died.

1.2 Effects of tricyclazole on respiration, blood pressure and electrocardiogram in rabbits

Effects of tricyclazole on respiration, blood pressure and electrocardiogram in rabbits anesthetized with urethane were investigated for 4 hr after the oral administration at doses of 5, 20, 80 and 320 mg/kg. Three to four rabbits /group were used. One out of three rabbits in the 80 mg/kg group or one out of four rabbits in the 320 mg/kg group died. Decreases in blood pressure were noted at 20 mg/kg or above. Tricyclazole-induced changes were not observed in respiration and electrocardiogram at any dose.

From these results, decreases in blood pressure were noted as one of acute toxic effects of tricyclazole.

(Institute of Environmental Toxicology, 1985)

REFERENCES

1) L. C. Haward, H. M. Worth, N. V. Owen, S. S. Young, D. M. Morton & L. Golberg: *Toxicol. Appl. Pharmacol.* **45**, 322 (1978)
2) L. C. Howard, N. V. Owen, S. S. Young, D. M. Morton & L. Golberg: *Toxicol. Appl. Pharmacol.* **48**, A194 (1979)

Contact
Dr. M. Kawai, Eli Lilly Japan K. K., 4–2–20, Gokodori, Chuo-ku, Kobe 651, Japan
問合せ
日本イーライリリー株式会社　河合睦文
〒651 神戸市中央区御幸通 4-2-20