Summary of Toxicological Studies on Quinoclamin

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DESCRIPTION OF QUINOCLAMIN

Quinoclamin, a naphthoquinone derivative, is a chemical that acts as an inhibitor of photo-synthesis when absorbed by the stems and leaves of grasses.

The chemical was first developed by Uni-Royal Co. as an algaecide for industrial use in the 1960s. In Japan, it is being developed by Agro-Kanesho Co. since 1968 as a herbicide with excellent activity against aquatic weeds, including annual and perennial weeds in paddy fields as well as against mosses in lawns and potted plants.

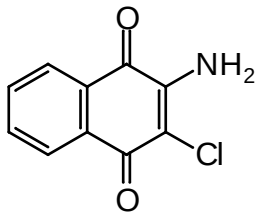
The chemical structure and physicochemical properties of Quinoclamin are given below;

Trade name : Mogeton

Chemical name : 2-amino-3-chloro-1,4-naphthoquinone (IUPAC)

Common name : Quinoclamin (ISO)

Structural formula :



Molecular formula : $C_{10}H_6ClNO_2$

Molecular weight : 207.62

Appearance : Orange colored crystal powder

Specific gravity : 1.66

Melting point : 197-200˚C

Solubility (g/*l* at 20˚C) : 0.022 in distilled water, 1.48 in ethyl acetate, 25.6 in acetone, 6.6 in methanol, and 37 in nitrobenzene.

Partition coefficient (log P_{ow}) : 1.53

Vapour pressure (at 20˚C) : 2.3×10^{-7}

Stability : Stable in acid, unstable on exposure to sun light or in hot alkalis

ACUTE TOXICITY STUDIES

The results of acute toxicity studies are summarized in Table 1.

Table 1

Test substance	Species	Route of administration	Sex[a]	LD_{50} (mg/kg)	Testing facility (Reporting year)
Quinoclamin technical (>95%)	Rat	Oral	M	1360	Tokyo Women's Medical College
			F	1600	(1972)
		Dermal	M	>5000	Tokyo Dental College
			F	>5000	(1978)
		Intraperitoneal	M	315	Tokyo Dental College
			F	375	(1978)
		Inhalation (4 hr)	M	> 0.79[b]	Huntingdon Research Centre
			F	> 0.79	(1986)
	Mouse	Oral	M	1350	Tokyo Women's Medical College
			F	1260	(1972)
		Dermal	M	>2000	Tokyo Dental College
			F	>2000	(1978)
		Intraperitoneal	M	412	Tokyo Dental College
			F	375	(1978)
Wettable powder (25%)	Rat	Oral	M	>5000	Toxicol Laboratories
			F	>5000	(1985)
		Dermal	M	>2000	Toxicol Laboratories
			F	>2000	(1985)
		Inhalation (4 hr)	M	>1.73[b]	Huntingdon Research Centre
					(1986)
	Mouse	Oral	M	>5000	Toxicol Laboratories
			F	>5000	(1985)
Granules (9%)	Rat	Oral	M	>5000	MECT Corporation
			F	>5000	(1989)
		Dermal	M	>2000	MECT Corporation
			F	>2000	(1989)
	Mouse	Oral	M	9694	MECT Corporation
			F	6450	(1989)

[a] M:Male, F:Female.
[b] LC_{50} (mg/*l*) as actual concentration.

On oral injection, Quinoclamin technica (hereinafter TECH) and 25% Wettable Powder (WP) is unlikely to prove particularly to toxic to rats and mice, however 9% Granule (GR) caused soft feces in rats, and depression, hunching and convulsion in mice.

There were no adverse skin reactions nor toxic signs by dermal administration with TECH, WP and GR.

By intraperitoneal administration with TECH in rats and mice, depression, labored respiration, urine brown stains, rough coat were observed at all dosing levels, and hunching, convulsion and anischuria were also noted at high dose levels.

In inhalation toxicity studies in rats with TECH and WP, some opacity in the eyes, salivation and infiammation of the penis were observed during the observation period.

IRRITATION STUDIES

1. Primary Eye lrritation Studies in Rabbits

1) 0.06 g each of TECH and WP was instilled into the conjunctival sac of the left eye of 9 New Zealand White adult rabbits. The eyes of 3 rabbits which constituted the rinsed group, were washed using distilled warm water 2 min after dosing. The eyes of the other 6 rabbits which constituted the unrinsed group, were not washed. Irritant responses were evaluated according to "Draize method."

Mild to moderate irritant responses were observed in the cornea, iris and conjunctival mucosa within 24 hr after dosing ; however, 168 hr later they. had disappeared. The irritant responses in the rinsed group were milder than those in the unrinsed group administered TECH, however the responses were almost the same in both groups when WP was used.

The results suggested that TECH and WP nduced moderate irritation to rabbit eyes. Eye-wash proved effective in case of TECH.

(Toxicol Laboratories Limited, 1985)

2) A similar test was carried out using 0.1 g of GR.

Mild to moderate irritant responses were observed in the cornea, iris and conjunctival mucosa 24 hr after dosing; however, 11 days later they were no longer observed except for 2 rabbits. The responses in the rinsed group were milder than those observed in the unrinsed group.

The results suggested, that GR induced a mild irritation to the rabbit .eyes. Eye-wash

proved effective in both cases. (MECT Corporation, 1989)

2. Primary Skin lrritation Studies in Rabbits

0.5 g each of TECH, WP and GR was applied to the intact skin of 6 New Zealand White adult rabbits over an area of 6 cm X 6 cm during 4 hr.

Slight erythema was observed on the treated skin of one rabbit 1 hr after dosing with TECH and GR. No other responses were noted.

These results suggested that TECH, WP and GR induced no irritation to rabbit skin. (Toxicol Laboratories Limited, 1985)

SENSITIZATION STUDIES

1. Dermal Sensitization Studies in Guinea Pigs with TECH, WP and GR

1) The studies were carried out according to the open epicutaneous method using 8 female Hartley strain guinea pigs per group.

0.1 ml of ethanol solution containing 0.3, 1, 3, 10 or 30% of TECH and WP was applied for 21 consecutive days on the right clipped flank of each animal. Twenty four hours and 14 days after the end of the induction treatment, all animals were challenged by applying 0.025 ml of ethanol solution containing 1, 3, 10 and 30% of the test substances on the left clipped flank.

In case of TECH, a slight erythema was observed in one animal when challenged with a 30% Solution in the 10% induction group 24 hr after both challenges. No other reactions were observed after each challenge in either group. As for in WP, no skin reactions were observed after either challenge.

The results suggested that neither TECH nor WP induced sensitization to the skin of guinea pigs. (Toxicol Laboratories Limited, 1985)

2) The study was performed according to Buehler's method using 10 Hartley strain guinea pigs per group. For the induction of sensitization, 0.5 ml of 50% suspended solution of GR was applied for 6 hr to the clipped left flank on day 0, 7 and 14. On day 28, twenty test animals were challenged with 0.5 ml of 50% suspended solution applied for 6 hr to the clipped right flank. The skin was observed 24 and 48 hr after the end of the challenge.

No skin reactions were noted after the challenge in the test animals.

The results suggested that GR did not induce sensitization to the skin of guinea pigs. (Safepharm Laboratories, 1989)

SUBACUTE TOXICITY STUDIES

1. Ninety-Day Toxicity Studies in Rats

1) Quinoclamin was administered *via* the diet to groups of 10 male and 10 female SD strain rats at the dose levels of 0, 50, 200 and 1000 ppm for 90 days.

Increase of kidney weight and hemosiderosis in the spleen were observed in males and females at the 200 and 1000 ppm dose levels. At the 1000 ppm dose level, inhibition of body weight gain and increased liver and spleen. weight were also observed.

The NOEL (maximum non-effect level) in this study was considered to be 50 ppm (3 mg/kg/day in males and females). (Tokyo Women's Medical College, 1972)

2) Quinoclamin was administered *via* the diet to groups of 10 male and 10 female SD strain rats at the dose levels of 0, 60, 300 and 1500 ppm for 90 days.

Anemia, inhibition of body weight gain, decrease of hemoglobin, hematocrit and erythrocyte count, increase of total protein and AIP activity, and increased liver weight were observed at the 300 and 1500 ppm dose levels. In addition to these adverse effects, increase of the leukocyte count, GOT and weight of almost all organs except lungs, adrenals and uterus were observed at the 1500 ppm dose level.

The NOEL in this study was considered to be 60 ppm (4 mg/kg/day in males and 5 mg/kg/ day in females). (Juntendo Medical College, 1972)

2. Ninety-Day Toxicity Studies in Mice

1) Quinoclamin was administered *via* the diet to groups of 10 male and 10 female ICR strain mice at the dose levels of 0, 50, 200 and 1000 ppm for 90 days.

Inhibition of body weight gain, changes in submaxillary glands and adrenals weight, as well as hemosiderosis in the spleen were observed at the 200 and 1000 ppm dose levels. In addition to these adverse effects, changes in liver and spleen weight were observed at the 1000 ppm dose level.

The NOEL in this study was considered to be 50 ppm (7 mg/kg/day in males and females). (Tokyo Women's Medical College, 1972)

2) Quinoclamin was administered *via* the diet to groups of 10 male and 10 female ICR strain mice at the dose levels of 0, 50, 200 and 1000 ppm for 90 days.

Anemia was observed at the 200 ppm dose level. Anemia, increase of the leukocyte count, neutrophil ratio, GOT and AIP activity, increased weight of the heart, liver, kidneys and spleen, and hemosiderosis in the spleen were observed at the 1000 ppm dose level.

The NOEL in this study was considered to be 50 ppm (8 mg/kg/day in males and 12 mg/kg/day in females). (Juntendo Medical College, 1972)

CHRONIC TOXICITY AND CARCINOGENICITY STUDIES

1. Chroleic Toxicity/Carcinogenicity Studies in Rats

1) Quinoclamin was administered *via* the diet to groups of 30 male and 30 female SD strain rats at the dose levels of 0, 1, 5, 25, 125 and 500 ppm for 104 weeks. At week 52, 5 males and 5 females per group were sacrified and subjected to detailed examinations.

The only dose-related finding in this study was a slight inhibition of body weight at the 500 ppm dose level.

The NOEL in this study was considered to be 125 ppm (5.7 mg/kg/day in males and 7.3 mg/kg/day in females). There were no indications of carcinogenic potential even at the highest dose level. (Hazleton Laboratories America, 1976)

2) Quinoclamin was administered *via* the diet to groups of 100 male and 100 female SD strain rats at the dose levels of 0, 4, 52 and 676 ppm for 104 weeks. Ten males and 10 females per group were killed for clinical and pathological investigation at week 27, 53 and 79 and other surviving rats were killed for clinical and pathological examinations at the end of dosing.

A dose-related reduction in food consumption and body weight gain were noted at the 676 ppm dose Ievel. In the pathological examination, atrophy of pancreatic acinus, epithelial hyperplasia of urinary tracts, and transitional cell papilloma (benign tumor) in urinary bladder developed from epithelial hyperplasia were observed at the 676 ppm dose level. At the 52 ppm dose level, atrophy of pancreatic acinus and epithelial hyperplasia of urinary. tracts were observed.

The NOEL in this study was considered to be 4 ppm (0.21 mg/kg/day in males and 0.28 mg/kg/day in females). Quinoclamin did not induce , any specific malignant

tumor even at the highest dose level. (Toxicol Laboratories, 1991)

2. Chronic Toxicity Study in Dogs

Quinoclamin was administered *via* the diet to groups of 4 male and 4 female Beagle dogs at the dose levels of 0, 2, 10, 50, 250 and 1000 ppm for 104 weeks.

Inhibition of body weight gain, decrease of the erythrocyte count, hematocrit and hemoglobin concentration, increase of GOT, GPT and AIP activity suggestive of liver disorders, increase of spleen weight and decrease of thymus, testes and prostate weight, and pathological changes in adrenals, Iiver, gallbladder, kidneys and spleen were observed at the 1000 ppm dose level. At the 250 ppm dose level, increase of GOT, GTP and AIP activity, and pathological changes in the adrenals, Iungs spleen, Iiver, urinary bladder and testes were observed.

The NOEL in this study was considered to be 50 ppm (1.4 mg/kg/day in males and 1.3 mg/kg/day in females). (Hazleton Laboratories America, 1976)

REPRODUCTION AND TERATOGENICITY STUDIES

1. Three-Generation Reproduction Study in Rats

Quinoclamin was administered *via* the diet to groups of 25 male and 25 female SD strain rats at the dose levels of 0, 1, 25 and 500 ppm throughout three successive generations. The parents were mated twice after feeding a diet containing Quinoclamin for at least 9 weeks. The offspring from the first mating trials (F_{1a}, F_{2a}) were sacrificed at weaning and those from the second mating trials were taken by cesarean section at the end of the gestation period, or delivered and maintained through weaning or for 5 weeks (F_{1b}) or 3 months (F_{2b}) postweamng.

A slight inhibition of body weight gain of the P-generation and the weights of pups (F_{1a} and F_{2a}) at weaning were observed at the 500 ppm dose level, but no extra-scheduled death, abnormal clinical signs or decreased food consumption were observed during the dosing period in either group. In the Fl-generation, the incidence of gestation, litter size at parturition and body weight of the offspring at weaning in the highest dose Ievel were lower than those of the control.

The NOEL in this study was considered to be 25 ppm (1.7mg/kg/day in males and 2.0 mg/kg/day in females). (Hazleton Laboratories America, 1975)

2. Teratogenicity Study in Rats

Quinoclamin was administered orally to groups of 24 mated female SD strain rats at the dose levels of 0 (vehicle), 5, 20 and 75 mg/kg/day during organogenesis from day 7 to 17 of gestation . Cesarean section was performed on day 20 and all fetuses were examined for external characteristics. Half of fetuses were subjected to skeletal examination and the others for visceral examination by Wilson's method.

A slight inhibition of body weight gain and decreased food consumption were observed in dams at the 75 mg/kg/day dose level except in the non-treated period, but none of the dams, in either groups, showed abnormal clinical signs, death or abortion. Significant decrease of ossification suggestive of growth retardation of the fetuses in relation to the maternal effects of Quinoclamin was observed at the 25 and 75 mg/kg/day dose levels.

The NOEL in this study was considered to be 5 mg/kg/day, but Quinoclamin did not induce fetal abnormalities even at the highest dose level.

(Toxicol Laboratories Limited, 1986)

3. Teratogenicity Study in Rabbits

Quinoclamin was administered orally to groups of 16 mated female New Zealand White rabbits at the dose levels of 0 (vehicle), 2.5, 7.5, and 22.5 mg/kg/day during organogenesis from day 6 to 18 of gestation. The cesarean section was performed on day 28, and all fetuses were examined for external, visceral and skeletal abnormalities.

At the 22.5 mg/kg/day dose level, slight inhibition of body weight gain was observed in dams only at the early phase of the treatment period, and a slight decrease of fetal weight accompanied by retardation of ossification.

It was concluded that the NOEL in this study was 7.5mg/kg/day, and that Quinoclamin did not induce fetal abnormalities at the highest dose level.

(Toxicol Laboratories Limited, 1986)

MUTAGENICITY STUDIES

1. Reverse Mutatian Study

Reverse mutation was investigated using 5 strain of *Salmonella typhimurium* and WP2*hcr* strain of *Escherichia coli*, according to the method of Ames in the presence

and absence of S9. All strains were incubated in,agar plates containing 0.1 to 500 /μg of Quinoclamin per plate.

A number of revertant colonies did not increase when cultured with or without S9 at any concentration of the test substance. The results suggested that Quinoclamin did not induce the gene mutation. (Institute of Environmental Toxicology, 1978)

2. DNA Repair Study

DNA repair ability was investigated using H-17 and M-45 strains of *Bacillus subtilis*, according to the method of streak at the concentration of 0.1 to 20 μg of Quinoclamin per disk.

No differences on growth inhibition were observed at any concentration of the substance. The results suggested that Quinoclamin did not affect the DNA repair ability. (Institute of Environmental Toxicology, 1978)

3. Metaphase Analysis of Chromosome from Human Lymphocytes

Human lymphocytes were obtained from 2 different donors. Cells were cultured at dose levels of 2.25 to 18 μg/ml in the presence of S-9, or at dose levels of 1.125 to 9 μg/ml in the absence of S-9.

No changes were observed in the incidence of chromosomal aberrations at any dose level of Quinoclamin in the absence of S-9. However, the number of chromosomal aberrations increased in the cells from one donor in the presence of S-9 at dose levels of 9 and 18 μg/ml.

The results suggested that Quinoclamin did not induce chromosomal aberrations in human lymphocyte in the absence of S-9, however the possibility of chromosomal aberration induction in the presence of S-9 cannot be denied.

(Toxicol Laboratories Limited, 1987)

4. Micronucleus Test in Mice

Quinoclamin was administered intraperitoneally to groups of 15 male and 15 female LACA strain mice at the dose levels of 0, 125, 250 and 500 mg/kg. Bone marrow smears were prepared 24, 48 and 72 hr after dosing.

There were no increases in the number of micronucleated polychromatic erythroblasts or in the proportion of polychromatic erythroblasts to the orthochromatic

erythroblasts 24 to 72 hr after dosing.

These results suggested that Quinoclamin did not induce the formation of micronucleated erythroblasts. (Toxicol Laboratories Limited, 1987)

SUMMARY

A wide variety of toxicological studies on Quinoclamin have been conducted to assess its safety. The results of these studies support the view that this herbicide is safe in practical use when employed as recommended.

Mogeton Granule herbicide which contains Quinoclamin as its active ingredient was registered by Ministry of Agriculture, Forestry, and Fisheries in Japan in 1968 and the "Standard for withholding registration" was established with 0.03 ppm for rice and vegetables.

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