Summary of Toxicological Studies on Prodiamine

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TEST CHEMICAL

Prodiamine has been developed by Sandoz Crop Protection Corporation since 1986. Prodiamine is a long residual broad spectrum preemergence herbicide with a good selectivity in many agronomic and horticultural crops. In Japan, the chemical was registered as a herbicide for the control of annual grasses in 1992.

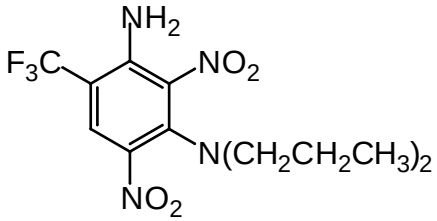
Identity, physical and chemical properties, solubility and stability are as follows.

Common name : Prodiarnine (ISO)

Trade name : Kusablock

Chemical narme : 5-Dipropylamino-α,α,α-trifluoro-4,6-dinitro-*o*-toluidine

Structural formula :



Molecular formula : $C_{13}H_{17}F_3N_4O_4$

Molecular weight : 350.3

Appearance : Yellow crystalline powder

Specific gravity : 1.47 (25˚C)

Melting point : 124˚C

Vapor pressure : 2.5 x $l0^{-8}$ mmHg (25˚C)

Solubility : Water, 0.03 ppm (25˚C); ethanol; slightly soluble; chloroform, benzene, acetonitrile, xylene, soluble; DMSO, acetone, very soluble

Stability : Heat, acid, alkali, stable ; photo, unstable

ACUTE TOXICITY STUDIES

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

Pilo-election and hunched posture were observed in the rat after dosing orally of each prodiamine technical or 65% water dispersible granule (65 WDG) formulation. No signs of systemic toxicity were observed in the mouse administered orally and in the rat administered dermally. In the inhalation toxicity studies in the rat, closing or

partial closing of eyes, abnormal breathing and abnormal body posture were observed during and after exposure of technical and closing or partial closing of eyes, shallow breathing, pilo-election and hyper activity were observed in the case of 65 WDG formulation.

Table 1 The results of acute toxicity studies.

Test substance	Route	Species	Sex[f]	LD_{50} (mg/kg)
Technical	Oral[b]	Mouse	M	> 5000
			F	> 5000
	Oral[c]	Rat	M	> 5000
			F	> 5000
	Dermal[c]	Rat	M	> 2000
			F	> 2000
	Inhalation[d]	Rat	M	> 256 [g]
			F	> 256 [g]
65WDG[a] formulation	Oral[b]	Mouse	M	> 5000
			F	> 5000
	Oral[e]	Rat	M	> 5000
			F	> 5000
	Dermal[e]	Rat	M	> 2000
			F	> 2000
	Inhalation[e]	Rat	M	> 1810 [h]
			F	> 1810 [h]

[a] 65WDG: 65% water dispersible granule. [b] Safepharm Laboratories Ltd., 1988. [c] Huntingdon Research Centre Ltd., 1984. [d] Huntingdon Research Centre Ltd., 1985. [e] Huntingdon Research Centre Ltd., 1986. [f] M=male, F= female. [g] LC_{50} (mg/m^3), as actual prodiamine concentration. [h] LC_{50} (mg/m^3), as total dust concentration.

IRRITATION STUDIES

1. Primary Eye lrritation

Six New Zealand White rabbits were used in each prodiamine technical or 65WDG formulation study. A 0.1 ml of technical or 65WDG formulation was placed into the lower everted lid of one eye of each animal. The eyelids were then gently held together for one second before releasing. The contralateral eye remained untreated and served as a control. Examination of the eyes was made after 1 hr and 1, 2, 3, 4, 7

days after instillation.

Instillation of prodiamine technical resulted n that none of the animals gave a "positive" response and temporary mild conjunctival reaction only were observed in all six animals. The eyes were normal in four days.

In the case of prodiamine 65WDG formulation, five of the animals gave a "positive" response but no corneal damage or iridial inflammation was observed in any of the animals. A diffuse crimson red colouration of the conjunctivae (score 2 in 3) accompanied by considerable swelling with partial eversion of the eyelids (score 2 in 4) was seen in five animals at the one-hour reading only and ocular discharge was seen in all six animals. The eye were normal two, three or four days after instillation.

(Huntingdon Research Centre Ltd., 1984, 1986)

2. Primary Dermal lrritation

Six New Zealand White rabbits were used in each prodiamine technical or 65WDG formulation study. A 0.5g amount of technical or 65WDG formulation was applied under a 2.5 cm square gauze moistened with 0.5 ml distilled water to one intact skin site on each animal. After 4 hr the bandages were removed and the application sites were washed. Observations were made on day 1 (*i.e.* 30 min after removal the bandages) and days 2, 3, 4.

Following removal of the bandages after application of prodiamine technical, any evidence of erythema or oedema could not be found. None of animals showed any observable response to treatment during the following three days.

In the case of prodiamine 65WDG formulation, transient very slight erythema was observed in one animal only. Five animals did not show any observable response to treatment throughout the four days observation period. Therefore prodiamine 65WDG was considered to be non-irritant to skin.

(Huntingdon Research Centre Ltd., 1984, 1986)

DERMAL SENSITIZATION STUDIES

The Buehler method was employed for a dermal sensitization study. A 0.5 ml of 20% prodiamine technical in acetone or 60% Prodiamine 65WDG formulation in distilled water was applied to 10 guinea pigs. And a negative control group of 10 animals and a positive control (formalin) group of 10 animals were provided.

Following challenge application of prodiamine technical, no dermal reactions

were observed in any of the test or control animals. According to these results, prodiamine technical was considered to be a non-sensitizer.

In the case of prodiamine 65WDG formulation, the dermal reactions observed in four test animals were more marked and persistent than those seen in animals of the control group. An inconclusive result was obtained in a further two test animals. No evidence of delayed contact hypersensitivity was seen in the remaining four test animals. Therefore prodiamine 65WDG formulation might be considered to be a mild sensitizer.

In addition the positive control, formalin, produced evidence of delayed contact hypersensitivity in both studies. (Huntingdon Research Centre Ltd., 1984, 1986)

SUBCHRONIC TOXICITY STUDY

A group of 20 male and 20 female Sprague-Dawley rats per dose level was given the diet containing prodiamine technical at a concentration of 0, 400, 1200 or 4000 ppm for 13 weeks .

There was a yellow discolouration of the fur and tails of rats receiving 4000 ppm. Other treated rats were not affected. There were no deaths. Bodyweight gains and food intake of males receiving 4000 ppm were less than the controls, with a similar effect on body weights also noted for females of this dosage group. Other treated rats were not affected. A greater intake of water was noted in week 11 for males receiving 4000 ppm when compared with the controls. Lower haemoglobin values were noted among rats receiving 4000 or 1200 ppm and males receiving 400 ppm, compared to the controls. Greater values relating to cholesterol and protein were noted among treated rats, compared to the controls. A greater protein content of urine was noted for males receiving 4000 ppm when compared to the controls. Greater liver and kidney weights were noted for males treated with 4000 ppm and greater liver and spleen weights were noted for females of this dosage group when compared with the controls. No macroscopic and microscopic findings attributable to treatment with prodiamine were observed.

In conclusion, rats treated with prodiamine for 13 weeks at a concentration of 4000 ppm in their diet had a retarded bodyweight gain and minor changes in their blood and, in males only, urine profiles. In addition liver, kidney and spleen weights did appear to be affected by treatment at this level. There were, however, no changes noted histologically which were considered to be a reaction to the test compound.

Treatment-related change at 1200 and 400 ppm were confined to minor changes in the blood profile. (Huntingdon Research Centre Ltd., 1985)

TERATOGENICITY STUDIES

1. Teratogenicity Study in Rats

Groups of 25 Sprague-Dawley rats were administered prodiamine technical with corn oil by oral gavage at a dose level of 0, 100, 300, or 1000 mg/kg daily for 10 consecutive days, from day 6 to 15 of gestation. The animals were sacrificed for Cesarean section on day 20 of gestation and fetuses were removed and examined.

Clinical observations included yellow urogenital staining for a few animals in the 1000 mg/kg group and orange colored urine for some animals in the 100, 300 and 1000 mg/kg groups at approximately one-hour post-dosing. Maternal toxicity in the 300 and 1000 mg/kg group was expressed as statistically reduced body weight gain over the entire treatment period, the effect was dose-related and due primarily to a statistically significant decrease during the first three days of treatment. There was no effect on intrauterine survival at all dose levels tested, nor on fetal weights. Fetal developmental findings as assessed by external, visceral and skeletal examination did not suggest a test material relationship.

In conclusion, prodiamine was not considered to be teratogenic nor embryotoxic, although maternal toxicity was expressed at dosages of 300 and 1000 mg/kg. The 100 mg/ kg dosage in the present study probably represents a "no-effect" level for maternal toxicity. (WIL Research Laboratories, Inc., 1985)

2. Teratogenicity Study in Rabbits

Groups of 18 New Zealand White rabbits were administered prodiamine technical with 0.5% aqueous methylcellulose by gavage at a dose level of 0, 100, 300 or 500 mg/kg from day 6 to 18 of gestation. On day 29 of gestation, the fetuses were removed by cesarean section for examination.

Maternal toxicity was expressed in both the 300 and 500 mg/kg groups by a statistically significant mean body weight loss during the treatment period. Parallel reductions in food consumption also occurred at the 300 and 500 mg/kg levels during the period of administration. There were no sign of embryotoxicity in any dose group and no indication of teratogenic response at any dose level investigated in this study.

In conclusion, prodiamine was not considered to be teratogenic nor embryotoxic.

Based on the results of this study, the 300 and 500 mg/kg dosages produced maternal toxicity. The 100 mg/kg dosage in the present study probably represents a "no-effect" Ievel for maternal toxicity. (WIL Research Laboratories, Inc., 1985)

MUTAGENICITY STUDIES

1. Reverse Mutation Assay

Five strains of *Salmonella typhimurium,* TA 1535, TA1537, TA1538, TA98 and TA100 and *Escherichia coli* WP2 *uvr*A were used to evaluate the mutagenic potential of prodiamine technical. The study of 5 strains of *S. typhimurium* was carried out at a dose level of 0, 100, 500, 2500, 5000, or 10,000 μg/plate of prodiamine with or without metabolic activation. The study of *E. coli* WP2 *uvr*A was carried out at a dose level of 0, 313, 625, 1250, 2500 or 5000 μg/plate of prodiamine with or without metabolic activation.

Prodiamine induced no appreciable increases in the numbers of revertant colonies of any strain at any dose with or without metabolic activation, compared with those of the cor-responding control. Based on the result, prodiamine was not considered to have capability of inducing reverse mutation.

(Microbiological Associates Inc., 1985)
(BML, Inc., 1990)

2. Chromosome A berration Assay

Prodiamine technical was tested in the chromosbme aberration assay using Chinese hamster ovary cells. The assay was conducted of prodiamine with or without metabolic activation at a dose level of 0, 4, 8, 15, 30, or 60 μg/ml.

Prodiamine technical did not induce a significant level of chromosome aberrations and should be considered to be negative in this assay system.

(Microbiological Associates Inc., 1985)

3. DNA Repair Test (Rec-assay)

The DNA-damaging potential was tested at a dose level of 0, 275, 550, 1100, 2200, or 4400 μg/disk of prodiamine technical using the recombination-wild (H-17) and -deficient (M-45) strains of *Bacillus subtilis.*

In the absence of metabolic activation system, the differences of growth inhibitory zone induced by prodiamine technical between strain H-17 and M-45 were 0.5mm or

less. In the presence of metabolic activation system, prodiamine technical did not cause any inhibitory in either strain, even at the highest dose of 4400 μg/disk.

It was considered that prodiamine had no DNA-damaging capability.

(Chemicals Inspection & Testing Institute, l 990)

4. Unscheduled DNA Synthesis

Prodiamine technical was tested in the unscheduled DNA synthesis test using rat primary hepatocytes. Based on the results of an initial toxicity test, prodiamine technical was tested at a dose level of 0, 0.3, 1.0, 3.3, l0, 20, 30 or 100 μg/ml.

The results of the unscheduled DNA synthesis assay indicated that under the test conditions, prodiamine technical did not cause a significant increase in the mean number of net nuclear grain counts (*i.e.*, an increase of at least 5 counts over the control) , at any dose level: Therefore, prodiamine technical was considered to be negative in this study. (Microbiological Associates Inc., 1985)

SUMMARY

Prodiamine was considered to be a low toxic substance since its LD_{50} values of acute toxicity in rats and mice were over 5000 mg/kg in oral test and 2000 mg/kg in dermal test. Prodiamine 65WDG formulation had a "positive" response to eye in rabbits. Its technical, however, was non-irritant to eye and both of 65WDG and technical were considered to be non-irritant to skin. Prodiamine technical was considered to be a non-sensitizer. Its 65WDG formulation might be considered to be a mild sensitizer.

The high dose level of prodiamine produced retarded body weight gain and minor change in the blood in subchronic dietary toxicity study. However, middle and low dose level of prodiamine produced almost no changes in the study.

Prodiamine had no teratogenic potential in the studies of rats and rabbits, and no potential revealed in mutagenic studies.

Prodiamine has been one of important herbicides for golf course since it registered in 1991.

要　約

プロジアミンの毒性試験の概要

株式会社エス･ディー･エスバイオテック農薬対策室

プロジアミンはラットおよびマウスの急性経口毒性試験でのLD_{50}値がいずれも5000mg/kg以上であること，および，ラットの急性経皮毒性試験でのLD_{50}値が2000mg/kg以上であることからきわめて毒性の低い物質であると考えられた．本剤の65WDG製剤はウサギの眼に対して刺激性があるものの，原体は眼に刺激性がないと考えられ，皮膚に対しては原体および65WDGともに刺激性がないと考えられた．皮膚感作性は原体では認められなかったが，65WDG製剤では弱い感作性があると考えられた．亜急性経口毒性試験において，プロジアミンは高用量において体重増加の抑制，血液学的および血液生化学検査項目のわずかな変化がみられた．しかしながら，中および低用量ではほとんど変化を認めなかった．プロジアミンはラットおよびウサギにおいて催奇形性は認められず，また，変異原性試験においても陰性の結果を得た．プロジアミンは1991年に登録され，ゴルフ場における除草剤として有用である。

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