Summary of Toxicity Studies on Imazaquin and it's Ammonium Salt

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DESCRIPTION OF THE TEST COMPOUND

Imazaquin-ammonium is an imidazolinone herbicide developed by American Cyanamid Company. It has been confirmed that imazaquin-ammonium has been effective for some perennial and annual weeds.

In the U.S.A., through a number of field trials for the biological activity from 1981, this product was registered for soybean use in 1986, and for turf use in 1987 by the trade names of "SCEPTER*" and "IMAGE*," respectively, In Japan, we started the field trials with the aqueous solution of 20% imazaquin-ammonium (AS) <tradename: TONE-UP*> as a herbicide for turf use in 1986, and it has been confirmed that this product has been effective for annual and cyperaceous weeds. The product was registered on October 31 , 1990.

This article provides a toxicological feature of imazaquin obtained from toxicological studies with the imazaquin technical grade and the formulation of aqueous solution (AS) containing 20% imazaquin-ammonium. The chemical structure of imazaquin-ammonium are given below:

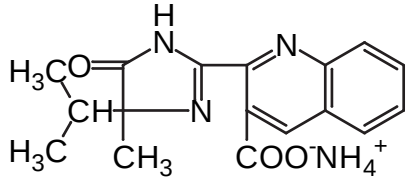
Common name: imazaquin-ammonium

Code name: AC252,214

Product name: TONE-UP*

Chemical name: ammonium salt of (*RS*)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) quinoline-3-carboxylate

Structural formula:



Physical properties of imazaquin are given below:

Molecular formula: $C_{17}H_{17}N_3O_3$ Molecular weight: 311.34

Appearance: off-white to light tan solid Specific gravity: 1.383 (22˚C)

Melting point: 219-224˚C

Vapor pressure: < 2 x 10^{-8} mmHg (45˚C)

Solubility (g/100 g, 25˚C): water, 60 ppm, methanol, 0.5; ethanol, 0.3; acetone, 0.3; toluene, 0.04; heptane, 0.02; methylene chloride, 1.36;

dimethylsulfoxide, 15.9

Partition coefficient (*n*-octanol/water): 2.2 (22˚C)

pH: 3.8 (23˚C)

ACUTE TOXICITY STUDIES

Results of acute toxicity studies with imazaquin technical and the 20% AS formulation by different routes of administration are shown Table l.

In acute oral studies with imazaquin technical, decreased spontaneous motor activity, sedation, prone posture, diarrhea, lacrimation and salivation in Wistar rats and ICR mice were observed from 10 minutes to Day 5 after administration. By oral administration of imazaquin 20% AS formulation to rats, salivation and diarrhea were observed from 30 minutes after dosing, and had resolved by 2 hr. After oral administration with imazaquin 20% AS formulation, no toxic signs were observed in mice.

By dermal administration of imazaquin technical and imazaquin 20% AS to rats, no toxic signs were observed in either study. In an inhalation study in Sprague-Dawley rats, salivation and slight nasal discharge were observed just after the exposure. All animals appeared normal by Day 4.

Table l

Test substance	Animal species	Administration route	LD_{50} (mg/kg) or LC_{50} (mg/*l*)		Testing facility, reporting year
Imazaquin technical (acid)	Rat	Oral	Male Female	4457 4073	Medical Scientific Research Laboratory (1989)
		Dermal	Male Female (1989)	> 2000 > 2000	
		Inhalation	Male Female (analytical)	>5.7 mg/*l* >5,7 mg/*l*	Food and Drug Research Laboratories (1984)
	Mouse	Oral	Male Female	1752 1790	Medical Scientific Research Laboratory (1989)
Imazaquin ammonium 20% AS	Rat	Oral	Male Female	> 5000 >5000	
		Dermal	Male Female	> 2000 > 2000	
<TONE-UP*>	Mouse	Oral	Male Female	> 5000 > 5000	

IRRITATION AND DERMAL SENSITIZATION STUDIES

Results of irritation and dermal sensitization studies with imazaquin technical and the 20% AS formulation by different routes of administration are shown in Table 2.

Table 2

Test substance	Animal species	Administration route	Result	Testing facllity, reporting year
Imazaquin ammonium 20% AS	Rabbit	Eye	Non-irritating	Medical Scientific Research Laboratory (1989)
		Dermal	Mildly irritating	
<TONE-UP*>	Guinea pig	Dermal sensitization	Non-sensitizer	
Imazaquin technical (acid)	Guinea Pig	Dermal sensitization	Non-sensitizer	Hazleton Laboratories America (1984)

1. Primary Eye Irritation Study in Rabbits

Nine male Japanese White rabbits were dosed with 0.1 ml of imazaquin-ammonium 20% AS instilled into the conjunctival sac of the right eye; the left eye served as a control. The eyes of three rabbits were flushed with physiological saline 2 minutes after application of the test material. After 24, 48, and 72 hours the eyes were observed for effects on the cornea, iris, and conjunctivae and scored according to the criteria described in Japanese MAFF guideline.

No clinical signs of toxicity were noted in any animals.

No irritation was observed in either group, with irrigation or without irrigation. Based upon the results, imazaquin-ammonium 20% AS was considered to be non-irritating to the rabbit eyes. (Medical Scientific Research Laboratory, 1989)

2. Primary Skin Irritation Study in Rabbits

A 4 hr topical application of 0.5 ml imazaquin-ammonium 20% AS was applied to the shorn skin of six male Japanese White rabbits. Following exposure, residual test material was removed. Erythema and edema were scored according to the criteria described in Japanese MAFF guideline.

Very slight erythema was observed in all six animals and edema was observed in three animals at the one-hour observation period. These reactions were resolved within 6 days of treatment, and no other clinical signs were observed. Based upon the results, imazaquin-ammonium 20% AS was considered to be non-irritating to the rabbit skin. (Medical Scientific Research Laboratory, 1989)

3. Dermal Sensitization Studies in Guinea Pigs

(1) Imazaquin technical

The study was performed according to the Buehler Method.

For the induction phase, male Hartley guinea pigs were dermally dosed with 0.4 ml of 50% w/v imazaquin technical or positive control material (DNCB) to an intact site on the back of each animal for 6 hr. These induction applications were applied once per week for three consecutive weeks. Challenge was carried out 2 weeks after the last induction in the same manner. This site was examined 24 and 48 hr postdosing and evaluated according to the Draize Method.

No positive responses in any animal in the test group were shown at either 24 or 48 hr after removal of the challenge patches. Based upon the results, imazaquin technical was considered to be a non-sensitizer.

(Hazleton Laboratories America, 1984)

(2) lmazaquin-ammonium 20% AS formuration

The study was performed according to the Buehler Method.

For the induction phase, male Hartley guinea pigs were dermally dosed with 0.5 ml of imazaquin-ammonium 20% AS, as received, or positive control material (DNCB) to an intact site on the back of each animal for 6 hr. These induction applications were applied once per week for three consecutive weeks. Challenge with 0.5 ml of 2% dilution (in purified water) of imazaquin-ammonium 20% AS, was performed 2 weeks after the last induction. The challenge application was a 24 hr exposure.

This site was evaluated 24 and 48 hr after removal of the patch.

No positive responses were observed in any animal of the test group. Based upon the results, imazaquin-ammonium 20% AS was considered to be a non-sensitizer.

(Medical Scientific Research Laboratory, 1989)

SUBCHRONIC TOXICITY STUDY

Thirteen-Week Feeding Toxicity Study in Rats:

Sprague-Dawley rats received imazaquin technical in the diet at levels of 0, 250, 1000, 5000, or l0,000 ppm for 13 consecutive weeks.

All rats survived the dosing period. Absolute liver and kidney weights were increased at 5000 ppm in females; however, these findings were not supported by any pathological changes and were not considered to be significant toxicological effects. Relative liver and kidney weights were comparable to those of the untreated controls at all treatment levels. No test substance-related changes were observed in clinical signs, body weight gain, food intake, hematological and biochemical parameters, or urinalysis. There were no gross or microscopic changes attributed to ingestion of imazaquin technical.

It was considered that the no-observable effect level of imazaquin technical in the rat diet for this study was l0,000 ppm (799.5 mg/kg in males and 861.6 mg/kg in females). (American Cyanamid Company, 1983)

TERATOGENICITY STUDIES

1. Teratogenicity Study in Rats

Imazaquin technical was administered orally as a suspension in corn oil, via gavage, to groups of 25 female CD (SD) BR rats during gestation days 6-15 at dose levels of 0, 250, 500, or 2000 mg/kg/day. Clinical signs, including salivation following treatment, alopecia, and urogenital staining, were observed in all treated groups, with increased frequency at 2000 mg/kg/day. Two animals in the high dose group died on test days 16 and 17, respectively, of gestation. Dark-colored fur around mouth, nose, and forelegs, ocular discharge and lethargy were observed at the dose of 2000 mg/kg/day in dams. In necropsy and litter observations, mean fetal body weights were decreased and incomplete ossification induced by delays in development were increased. Test material did not affect the number of implantations, live fetuses, or resorptions.

There were no effects which were considered related to exposure to imazaquin technical at the dose level of 500 mg/kg/day. In conclusion, imazaquin technical was not embryo-fetal toxic or teratogenic to CD rats up to the level of 500 mg/kg/day. The maternal no-observable effect level for imazaquin technical in the rat was 500 mg/kg/day. (Hazleton Laboratories America, 1983)

2. Teratogenicity Study in Rabbits

Imazaquin technical was administered orally as a suspension in 0.5% aqueous methyl cellulose, via gavage, to groups of 19 female New Zealand White rabbits during gestation days 6-18 at dose levels of 0, 100, 250, and 500 mg/kg/day.

There were no clinical signs of toxicity observed in any of the treated groups. Treatment-related findings for the maternal animals were observed only at the 500 mg/kg dose level. These findings included reduced mean body weights and the death of one dam at 500 mg/kg. Test material did not affect the number of implantations, live fetuses, resorptions, fetal body weights, or sex ratio. There were no effects on incidence of malformations for fetuses at any dose level related to exposure to imazaquin technical.

In conclusion, imazaquin technical was not teratogenic at the dose level of 500 mg/kg/day, and it was considered that the maternal no-observable effect level for imazaquin technical in the rabbit was 250 mg/kg/day.

(WIL Research Lab., USA, 1984)

MUTAGENICITY STUDIES

1. DNA Repair Test (Rec-Assay)

DNA repair was determined in the in vitro rec-assay using *Bacillus subtilis* strains H-17 (rec^+) and M-45 (rec^-) at dose levels of 187.5 to 3000 μg/disk.

Test substance caused no growth inhibition in either strain at any dose level. It was concluded that imazaquin technical did not induce DNA repair.

(Chemical Inspection & Testing Institute, Hita Research Lab., 1990)

2. In Vitro UDS Assay in Rat Primary Hepatocytes

DNA repair was assessed by measuring unscheduled DNA sysnthesis (UDS) in cultures of rat primary hepatocytes treated with imazaquin technical. Cell cultures were exposed to the test substance and tritiated thymidine at dose levels of 0.2 to 6000 μg/well. The amount of radioactivity incorporated into the nucleus of exposed cells (nuclear grain count) was measured and compared to unexposed cells. Doses above 600 μg/well were cytotoxic and could not be evaluated.

The number of mean nuclear grains were similar between control and treated groups. It was concluded that imazaquin technical did not induce DNA synthesis.

(Pharmaco Research International USA, 1984)

3. Reverse Mutation Assay

Imazaquin technical was evaluated for mutagenic potential at dose levels of 313 to 5000 μg/plate using four strains of *Salmonella typhimurium* (TA1535, TA100; base pair substitution type, and TA98, TA1537; frame-shift type), and *Echerichia coli* strain WP2 *uvrA* in the presence and absence of a metabolic activation system.

Toxicity was not observed in any of the treated groups.

Increase in the number of revertants was not observed for any strains at any dose level with or without metabolic activation. It was concluded that imazaquin technical was non-mutagenic. (B.M.L, 1989)

4. Chromosomal Aberration Assay

Chinese hamster ovary calls were exposed to imazaquin technical at dose levels of 450 to 1000 μg/ml, limited by solubility, in the presence and absence of a metabolic activation system. Cells were harvested and analyzed after 10 and 20 hours. These was no increase in the frequency of chromosomal aberrations at any dose level with or without metabolic activation. It was concluded that imazaquin was not clastogenic.

(Litton Bionetics, USA, 1985)

GENERAL PHARMACOLOGY STUDIES

A general pharmacological study of imazaquin technical was conducted in ICR mice, dogs, Hartley guinea pigs, and Wistar rats. Result of these studies are as follows.

(1) Effect on central nervous system:

Mice were treated orally at doses of 100 to 3000 mg/kg. These studies showed that imazaquin produced a muscle relaxant effect and depressant effect on the central nervous system in mice.

(2) Effects on respiration and circulation system:

Dogs were treated intraperitoneally at the dose of 1000 mg/kg. Dogs given the test substance exhibited decreases of blood flow and blood pressure, and increases in heart rate and respiration.

(3) Effects on the isolated guinea-pig ileum:

The isolated guinea-pig ilia were treated at doses of 1 x 10^{-7} to 1 x $l0^{-4}$ g/ml. This study showed that no effect on the autonomic nervous system was observed.

(4) Effects on digestive system:

Mice were treated orally at doses of 100, 300 and 1000 mg/kg. This study showed that imazaquin produced an increase in the propulsion of charcoal meal in the small intestines of mice.

(5) Effects on skeletal muscle:

Rats were treated intraperitoneally at the dose of 1000 mg/kg. In this study imazaquin technical inhibited the twitch response of the gastrocnemius muscle induced by electrical stimulation of the sciatic nerve.

(6) Effects on blood:

Rats were treated orally at doses of 300, 1000 and 3000 mg/kg. This study showed that no effect on blood coaglation was observed.

(Medical Scientific Research Laboratory, 1989)

CONCLUSION

A number of toxicological studies with imazaquin were conducted to define the toxicological profile.

The studies show that imazaquin has an extremely low acute toxicity or subchronic toxicity, and no mutagenic or teratogenic potential. A mild irritant effect on skin is noted, but no irritant effect in eyes and no dermal sensitization are observed.

Imazaquin is a product with a wide safety margin for humans according to the use recommendations established.

要　　約

イマザキン及びそのアンモニウム塩の毒性試験の概要

日本サイアナミッド株式会社技術開発部

イマザキン及びそのアンモニウム塩（商品名トーンナップ＊液剤）はアメリカン・サイアナミッド社が開発したイミダゾリノン系化合物の一種であり、植物に特有な必須アミノ酸の生合成（AHAS）を阻害することにより除草効果を発揮する。本成分は広範囲の一年生雑草および多年生カヤツリグサ科雑草に茎葉兼土壌処理により優れた効果をあらわし、芝草用除草剤として登録され、普及されている。

各種毒性試験の結果、イマザキン及びそのアンモニウム塩は低毒性であることが確認されている。急性経口、経皮、吸入毒性試験においてこれらの毒性は非常に低く、普通物相当であった。刺激性についても、ウサギの皮膚に対しては軽度な刺激を示したものの、眼に対する刺激性はなく、皮膚感作性も認められなかった。ラットを用いた13週間投与によ

る亜急性経口毒性試験では、5000ppm投与群の雌のみに肝臓ならびに腎臓の絶対重量の低下がみられたが、その他のいずれの群ではいかなる異常も認められず、本試験における最大無作用量は試験実施最高用量である10,000ppmであると判断された。催奇形性、変異原性は認められず、薬理試験においても顕著な毒性作用はみられなかった。

以上の結果より、イマザキン及びそのアンモニウム塩はその使用方法、使用上の注意事項を厳守することにより、人畜に対して影響の少ない薬剤であるものと考えられる。

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