Summary of Toxicity Studies on Imazapyr

Technical Department, Cyanamid (Japan) Ltd.
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DESCRIPTION OF THE TEST COMPOUND

Imazapyr is a nonselective herbicide developed by American Cyanamid Company. Through a number of field trials for the biological activity, it has been confirmed that imazapyr isopropylamine has been effective for perennial and annual weeds in non-crop field. The product was registered in 1984 in the U.S.A.

In Japan, we started the field trials with the aqueous solution of 25% imazapyr isopropylamine salt (AS) <tradename : ARSENAL*> as a herbicide for non-crop use in 1982 and registered it in 1987. Field trials with a l% AS <tradename : CHOPPER*> were also started for weed growth suppression for railway and road uses with the encouraged performance for perennial weeds. We registered it in 1993.

This article provides a toxicological feature of imazapyr obtained from toxicological studies with the imazapyr technical grade, its isopropylamine salt technical grade (49% water solution) and the formulation of aqueous solution (AS) containing 25% imazapyr isopropylamine salt.

The chemical structure and physical properties of imazapyr isopropylamine salt are given below :

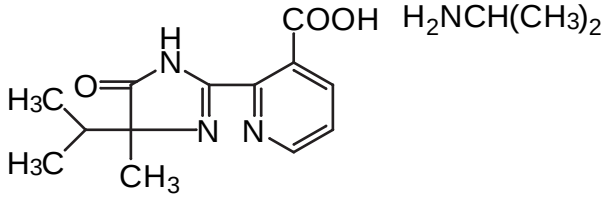
Common name : Imazapyr isopropylamine salt

Code name : AC252,925, CL252,925

Product name : ARSENAL, CHOPPER

Chemical name : Isopropylammonium=(*RS*)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinate (IUPAC)

Structural formula :



Molecular formula : $C_{13}H_{15}N_3O_3$ C_3H_9N

Molecular weight : 320.4

Appearance : Yellowish green to green liquid as water solution

Melting point : 128-130˚C

Solubility (g/*l* solvent) : Water > 600, methanol > 500, isopropanol approx. 10, xylene < 5, cyclohexane < 5

Partition coefficiant (*n*-octanol/water) : 1.3 as the acid tech.

Stability : No decomposition at 37˚C for 9 months, 45˚C for 3 months

Table 1

Test substance	Animal species	Administration route	LD_{50} (mg/kg)		Tesing facility, reporting year
Imazapyr isopropylamine technical (49.3%)	Rat	Oral	Male	> 10,000	Medical Scientific Research, Laboratory, 1983
			Female	> 10,000	
		Intraperitoneal	Male	4,200	
			Female	3,700	
		Subcutaneous	Male	> 5,000	
			Female	> 5,000	
		Dermal	Male	> 2,000	
			Female	> 2,000	
	Mouse	Oral	Male	> 10,000	
			Female	> 10,000	
		Intraperitoneal	Male	3,450	
			Female	3,000	
		Subcutaneous	Male	> 5,000	
			Female	> 5,000	
Imazapyr technical	Rat	Oral	Male	> 5,000	American Cyanamid Company, 1983
			Female	>5,000	
	Rabbit	Dermal	Male	> 2,000	
			Female	> 2,000	
	Rat	Inhalation	Male	> 1.3 mg/*l*	Food and Drug Research Laboratories, 1983
			Female	> 1.3 mg/*l* (analytical)	
Imazapyr isopropylamine 25% AS	Rat	Oral	Male	> 5,000	American Cyanamid Company, 1983
			Female	> 5,000	
	Mouse	Oral	Male	> 5,000	American Cyanamid Company, 1986
			Female	>5,000	
	Rabbit	Dermal	Male	> 2,148	American Cyanamid Company, 1983
			Female	>2,148	
	Rat	Inhalation	Male	>0.2 mg/*l*	Food and Drug Research Laboratories, 1983
			Female	>0.2 mg/*l* (analytical)	

ACUTE TOXICITY STUDIES

Results of acute toxicity studies with imazapyr technical, imazapyr isopropylamine technical, and imazapyr isopropylamine 25% AS formulation at different routes of administration are shown Table l.

After oral administration with imazapyr technical, no signs of toxicity were observed in rats. Decreased activity was observed in both rats and mice after oral administration with imazapyr isopropylamine technical and also with imazapyr isopropylamine 25% AS formulation.

By dermal administration, no toxic signs were observed.

After intraperitoneal administration of the imazapyr isopropylamine technical, decreased activity, blepharoptosls, ataxia, sedation, cyanosis, convulsion, and decreased body weight were observed in both rats and mice. After subcutaneous administration of imazapyr isopropylamine technical, decreased activity, blepharoptosls, and sedation were observed in mice, but only decreased activity was observed in rats.

In inhalation study with imazapyl technical, slight amount of nasal discharge was observed on the first day. No toxic signs were observed In the study with imazapyr isopropylamine 25% AS.

IRRITATION STUDIES

1. Primary Eye Irritation Studies in Rabbits

1) Nine male New Zealand White rabbits were dosed with 100 mg of imazapyr technical instilled into the conjunctival sac of the right eye. The eyes of three of the nine rabbits were flushed with 200 ml of tap water after being exposed to the test material for approximately 20 sec (20-sec group). After 24 hr of exposure, the treated eyes of the remaining six animals were rinsed with tap water (24 hr group) and examined for irritation using fluorescein. The eyes were then scored at 24, 48 and 72 hr, and at 4 and 7 days post-dosing according to the "Draize Method."

In the 20-sec group, mild to moderate irritation responses were observed in the conjunctiva within 24 hr ; however they had disappeared by 72 hr. In the 24-hr group, mild to moderate irritation responses were observed in the cornea within 24 hr, which disappeared by 72 hr; and in the conjunctiva within 24 hr, which disappeared by 7 days.

Based upon the results, the product was considered to be irritating to the rabbit eye with complete recovery within 7 days of exposure.

(American Cyanamid Company, 1983)

2) Nine male New Zealand White rabbits were dosed with 0.1 ml of imazapyr isopropylamine 25% AS instilled into the conjunctive sac of the right eye. The eyes of three of the nine rabbits were flushed with 200 ml of tap water after being exposed to the test material for approximately 20 sec (20-sec group). After 24 hr of exposure, the treated eyes of the remaining six animals were rinsed with tap water (24-hr group) and examined for irritation using fluorescein. The eyes were then scored at 24, 48, 72 hr, and 4 and 7 days post-dosing according to the "Draize method."

In the 20-sec group, mild to moderate irritation responses were observed in the conjunctiva within 24 hr ; however they had disappeared after 72 hr. In the 24-hr group, mild to moderate irritation responses were observed in the cornea within 24 hr (2 of 6 animals), which disappeared by 7 days ; and in the conjunctiva within 24 hr (6 of 6 animals), which disappeared by 7 days.

Based upon the results, the product was considered to be irritating to the rabbit eye with complete recovery within 7 days of exposure.

(American Cyanamid Company, 1983)

2. Primary Skin Irritation Studies in Rabbits

1) Six male New Zealand White rabbits were dosed dermally with an aqueous paste of imazapyr technical that was applied to an intact site and an abraded site on the same animal. The test material was left in contact with the skin for 24 hr. Sites

were scored for irritation after the 24 hr contact period and again at 72 hr postdosing according to the "Draize Method."

Slight erythema was observed on the abraded site (2 of 6 animals) at 24 hr post-dosing. No other reactions were observed.

Based upon the results, the product was considered to be mildly irritating to rabbit skin. (American Cyanamid Company, 1983)

2) Six male New Zealand White rabbits were dosed dermally with an aqueous paste of imazapyr isopropylamine 25% AS that was applied to an intact site and an abraded site on the same animal. The test material was left in contact with the skin for 24 hr. Sites were scored for irritation after the 24-hr contact period and again at 72 hr post-dosing according to "Draize method."

Slight erythema was observed on the intact site and abraded site at 24 and 72 hr post-dosing. Slight edema was observed on the abraded site at 24 hr post-dosing. Based upon the results, the product was considered to be mildly irritating to rabbit skin. (American Cyanamid Company, 1983)

3. Dermal Sensitization Study in Guinea Pigs

The study was performed according to the Buehler's Method.

Male Hartley guinea pigs were dermally dosed with imazapyr isopropylamine 25% AS to an intact site on the back of each animal for 6 hr. These inductive applications were administered once per week for three consecutive weeks. Following these three applications, test animals were rested for 14 days and were then given a challenge dose of imazapyr isopropylamine 25% AS to an intact site on the right flank of each animal. The site was examined 24 and 48 hr postdosing.

No skin reactions were observed after challenge doses. It was concluded that imazapyr isopropylamine 25% AS was not a skin sensitizer in the male Hartley guinea pig under the conditions of this study. (Toxicology Pathology Services, 1983)

SUBACUTE TOXICITY

1. 21-Day Dermal Toxicity Study in Rabbits

Imazapyr technical was applied to the closed-cripped intact or abraded skin on the back of ten male and ten female New Zealand White rabbits at dose levels of 0, 100, 200 and 400 mg/kg/day for five days each week over a three week period. The test site was covered with a gauze patch, which was moistened with saline, and occluded for 6 hr per day.

There were no consistent or distinct adverse treatment-related effects indicative of systemic toxicity in evaluation of body weights, food consumption, hematology, serum chemistry, clinical observations, necropsy observations, and histopathology including skin reactions. Microscopic evaluation of all tissues from control and high dose rabbits and all remarkable tissues from low and middle dose rabbits did not reveal any consistent or distinct effects related to topical treatment with imazapyr

technical. (Toxicology Pathology Services, 1983)

SUBCHRONIC TOXICITY

1. 13- Week Feeding Toxicity Study in Rats

CD rats received imazapyr isopropylamine in the diet at levels of 0, 1000, 5000 and l0,000 ppm for 13 consecutive weeks.

All rats survived the dosing period. Weight gains and food efficiency of male rats were depressed at 10,000 ppm in 7th week and 8th week, respectively. No test substance-related changes were observed in food intake, water intake, urinalysis, hematological or biochemical parameters, or organ weights (absolute and relative). There were no gross or microscopic changes attributed to ingestion of imazapyr isopropylamine.

It was considered that the maximum no-effect level of imazapyr isopropylamine in the rat diet for this study was 5000 ppm (325 mg/kg/day in males and 370 mg/kg/day in females). (Medical Scientific Research Laboratory, 1983)

TERATOGENICITY STUDIES

1. Teratogenicity Study in Rats

Imazapyr technical was administered orally, *via* gavage, to groups of 22-24 female CD rats during gestation days 6-15 at dose levels of 0, 100, 300, or 1000 mg/kg/day.

All females survived to terminal sacrifice. Maternal body weights and body weight gains for treated females were similar to the control group. Salivation was observed at 1000 mg/kg group. No gross pathologic changes were noted which were considered related to maternal exposure to imazapyr technical. Reproductive data for treated dams were similar to the control group. Mean fetal body weight and crown-rump length data for fetuses from treated dams were comparable to the control group.

Examination of fetal external, skeletal and visceral development revealed no aberrant structural changes which appeared related to maternal exposure to imazapyr technical. In conclusion, imazapyr technical was not teratogenic to CD rats under these test conditions. (Toxigenics, 1983)

2. Teratogenicity Study in Rabbits

Imazapyr technical was administered orally, *via* gavage, to groups of 16-18 female New Zealand White rabbits during gestation days 6-18 at dose levels of 0, 25, 100 or 400 mg/kg/day. The dose levels were set up based upon a preliminary test. In the test, imazapyr technical was administered orally at dose levels of 0, 250, 500, 1000 or 2000 mg/ka/day to five rabbits during gestation days 6-18. Mortality occurred in the preliminary test (2 of 5 animals at 250 mg/kg/day, 4 of 5 animals at 1000 mg/kg/day, and 5 of 5 animals at 2000 mg/kg/day died) ; resulting in the choice of 400 mg/kg/day as the highest dose for the definitive study.

In the definitive study, maternal body weight data for treated rabbits were similar

to the control group and no antemortem observations were noted which could be correlated with exposure to imazapyr technical. Two control and two high dose animals died during the dosing period ; gross pathologic findings were limited to pulmonary change. Maternal reproductive data, and fetal body weight and crown-rump length data for the treated groups were comparable to the control group.

There were no consistent, deleterious findings noted during the evaluations of the fetal internal, skeletal, and internal head development which were considered related to exposure to imazapyr technical.

In conclusion, imazapyr technical was not teratogenic to New Zealand White rabbits under these test conditions. (Toxigenic, 1983)

MUTACENICITY STUDIES

1. DNA Repair Test

DNA repair was determined in the *in vitro* rec-assay using *Bacillus subtilis* strains H-17 (Rec+) and M-45 (Rec-). Imazapyr isopropylamine dissolved in water, was tested at dose levels of 0.27 to 26.6 mg/disk.

Negative results were obtained in all assays, indicating that the test substance did not cause DNA damage. (Japan Food Research Laboratories, 1984)

2. Reverse Mutation Assay

Imazapyr isopropylamine was tested in the Bacterial/Microsome (Ames) assay at concentrations up to 5000 μg per plate using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, and *Escherichia coli* strain WP2uvrA with and without metabolic activation.

Negative results were obtained in all assays, indicating that the test substance did not cause genetic damage.

(Food and Drug Safety Center, Hitano Research Institute, 1982)

3. Chromosomal Aberration Assay

Chinese hamster ovary cells were exposed to 5000, 1700, 500, 170 and 50 μg/ml of imazapyr technical for either 2 hr in the presence of metabolic activation followed by a 1, 6 or 10 hr expression period or 3, 8 or 12 hr in the absence of metabolic activation.

There was no significant increase in the frequency of chromosomal aberrations at any dose level tested, at any sampling time in the presence or absence of metabolic activation. Under the conditions of this test, imazapyr technical was not clastogenic at any dose level. (Hazleton Laboratories America, 1984)

GENERAL PHARMACOLOGY STUDIES

Male mice or male rabbits were orally administered imazapyr isopropylamine at levels of 1000, 3000, and l0,000 mg/kg to define the effect on gross behavior, central

nervous system, and digestive system. Male rabbits or male rats were administered intravenously imazapyr isopropylamine at 100, 300, and 1000 mg/kg to define the affect on respiratory and circulatory system, and levels of 300, 1000, 3000 mg/kg for the effect on skeletal muscle.

Imazapyr isopropylamine produced a stimulant effect on gross behavior and increased the sleeping time induced by hexobarbital at high dose in mice, slightly increased muscle contractility in rats, depressed gross behavior at high dose in rabbits, slightly changed respiratory rate, blood pressure, and heart rate in rabbits, and increased the volume of urine at high dose in both mice and rabbits. No effect on the digestive system was observed. (Medical Scientific Research Laboratory, 1992)

CONCLUSION

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

The studies show that imazapyr is extremely low acute toxicity or subchronic toxicity, no mutagenic or teratogenic potential. Irritant effect in eyes and slight irritant effect on skin are noted, but no dermal sensitization is observed.

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

要　　約

イマザピルの毒性試験の概要

日本サイアナミッド株式会社技術開発部

イマザピルはアメリンカン・サイアナミッド社が開発した多年生及び一年生雑草を対象とする非農耕地用の非選択性除草剤である．またそのユニークな作用特性により1%液剤は多年生雑草を対象とした抑草剤として開発に成功した．イマザピルは各種毒性試験の結果，きわめて安全性の高い薬剤であることが示された．急性毒性は非常に弱く普通物に相当した．変異原性，催奇形性は認められず，顕著な薬理作用も認められなかった．眼に対する刺激性，皮膚に対する弱い刺激性はみられたが，皮膚感作性は陰性であった．ラットを用いた亜急性毒性試験では，高用量群（10,000 ppm）の雄ラットで一時的に体重増加抑制，食餌効率の低下がみられたのみで非常に毒性が低かった．以上より，イマザピルは，その使用方法，使用上の注意事項を厳守すればきわめて安全性の高い薬剤であると考えられる．

Contact
Technical Department, Cyanamid（Japan）Ltd.
1-4-30，Roppongi，Minato-ku，Tokyo106，Japan

問合せ
日本サイアナミッド株式会社技術開発部
〒106　東京都港区六本木1-4-30　六本木25森ビル23階