Summary of Toxicology Studies With Dithiopyr

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This report summarizes the results of laboratory toxicology studies conducted with dithiopyr. All studies were conducted in compliance with Good Laboratory Practice standards and study protocols followed internationally recognized pesticide testing guidelines. For all tests conducted with dithiopyr, technical grade material was used.

DESCRIPTION OF THE TEST CHEMICAL

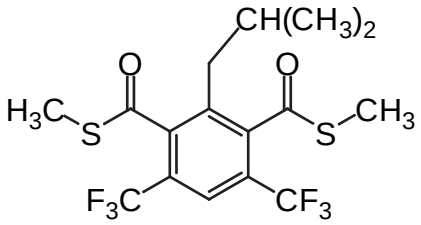
Dithiopyr is a new herbicide developed by Monsanto Company for the control of annual grasses and selected broadleaf weeds in established turf and rice paddies. Dithiopyr has the following physical and chemical properties :

Common name : Dithiopyr

Synonyms: MON 7200, MON 15100

Chemical name : *S,S*-Dimethylester-2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridinedicarbothioic acid

Chemical structure :



Molecular formula : $C_{15}H_{16}F_5NO_2S_2$

Molecular weight : 401.4

Physical state : Pale yellow crystalline solid

Melting point : 65˚C

Vapor pressure: 4 x 10^{-6} mmHg (25˚C)

Stability : Stable to heat, light, acid and alkali

Octanol/water partition coefficient : 5.625 x $l0^4$

Water solubility : 1.38 mg/*l* (20˚C)

ACUTE TOXICITY STUDIES

1. Dithiopyr Technical Material

The results of acute toxicity studies with dithiopyr are displayed in Table 1. In these tests, no distinctive signs of toxicity were observed following exposure *via* the different routes.

2. Tests with Dimension® Turf Herbicide

The results of acute toxicity studies with the formulated product, Dimension Turf Herbicide, are displayed in Table 2. Dimension is an organic solvent-based liquid formulation containing approximately 13% dithiopyr and is registered for use in the United States.

3. Tests with Dictran® EC Herbicide

The results of acute toxicity studies with the formulated product, Dictran EC Herbicide, are displayed in Table 3. Dictran EC is an organic solvent-based liquid formulation containing approximately 33% dithiopyr and is registered for use in Japan.

IRRITATION STUDIES

1. Primary Dermal Irritation

Topical semi-occlusive exposure at 2 sites on the shaved backs of 6 New Zealand white (NZW) albino rabbits to 0.5 g of dithiopyr for 4 hr resulted in very slight erythema. All evidence of dermal irritation was gone by 72 hr. The primary irritation index was calculated to be 0.2 on a scale of 8.0. (Bio/dynamics Inc., 1988)

Table 1 Results of acute toxicity tests with dithiopyr.

Species, strain	Route	Sex	Vehicle[a),b)]	Median lethal dose
Rat, S-D	Oral[c)]	M	1 % Tween 80	> 5000 mg/kg
		F	1 % Tween 80	> 5000 mg/kg
Mouse, CD-1	Oral[c)]	M	1 % Tween 80	> 5000 mg/kg
		F	1 % Tween 80	> 5000 mg/kg
Rat, S-D	Dermal (24-hr)[c)]	M	-	>5000 mg/kg
		F	-	> 5000 mg/kg
Rat, F-344	Inhalation (4-hr)[c)]	M	DMSO	> 5.98 mg/*l*
		F	DMSO	> 5.98 mg/*l*

[a)] Oral dosing solutions were prepared as aqueous suspensions containing 1% Tween 80.
[b)] DMSO : dimethylsulfoxide.
[c)] The Institute of Environmental Toxicology, 1988.

Table 2 Results of acute toxicity tests with Dimension®[a] turf herbicide.

Species, strain	Route	Sex	Vehicle	Median lethal dose
Rat, S-D	Oral[b]	M	-	4100 mg/kg
		F	-	3000 mg/kg
Rabbit, NZW	Dermal (24-hr)[b]	M	-	> 5000 mg/kg
		F	-	> 5000 mg/kg
Rat, S-D	Inhalation (4-hr)[c]	M	-	11 mg/*l*
		F	-	8.9 mg/*l*

[a] Dimension is a registered trademark of the Monsanto Company.
[b] Bio/dynamics Inc., 1987.
[c] Bio/dynamics Inc., 1989.

Table 3 Results of acute toxicity tests with Dictran®[a] EC herbicide.

Species, strain	Route	Sex	Vehicle	Median lethal dose
Rat, S-D	Oral[b]	M	-	3397 mg/kg
		F	-	4129 mg/kg
Mouse, CD-1	Oral[b]	M	-	1996 mg/kg
		F	-	1672 mg/kg
Rabbit, NZW	Dermal (24-hr)[b]	M	-	> 5000 mg/kg
		F	-	> 5000 mg/kg
Rat, S-D	Inhalation (4-hr)[c]	M	-	> 4.3 mg/*l*[d]
		F	-	> 4.3 mg/*1*[d]

[a] Dictran is a registered trademark of Monsanto Japan Ltd. and Dainippon Ink & Chemicals, Inc.
[b] Bio/dynamics Inc., 1989.
[c] Monsanto Environmental Health Laboratory, 1989.
[d] Represents maximum attainable atmospheric concentration.

2. Primary Ocular Irritation

Mild conjunctival irritation was observed up to 24 hr following instillation of 64 mg of dithiopyr into the conjunctival sac of 6 NZW albino rabbits. No iridial or corneal involvement was apparent, All evidence of conjunctival irritation was gone by. 48 hr. (Bio/dynamics Inc., 1988)

DERMAL SENSITIZATION STUDIES

The potential of dithiopyr to induce delayed contact hypersensitivity was evaluated in a modified Buehler assay.[1, 2] Induction comprised topical occlusive exposure of 5 male and 5 female Hartley albino guinea pigs to undiluted dithiopyr (moistened with

saline) for 6 hr, once per week for 3 weeks. Treated and naive guinea pigs (5 per sex) were challenged 14 days after the last induction dose with undiluted dithiopyr (moistened with saline). Dithiopyr exhibited no evidence of dermal sensitization potential.

(Bio/dynamics Inc., 1988)

SUBCHRONIC STUDIES

1. Four-week Feeding Study in Rats

Dithiopyr was administered *via* the diet to groups of 6 male and 6 female Fischer-344 (F-344) rats for 4 weeks at concentrations of 0, 300, 1000, 3000, 10,000 and 30,000 ppm. Decreased body weight and food consumption, as well as clinical signs of toxicity were observed at the higher dose levels. Liver enlargement and discoloration, thymic atrophy, emaciation, and increased liver, kidney and thyroid/parathyroid weights were noted on gross postmortem examination. Due to an elevation in liver and kidney weights at the low dose level, the no observable effect level (NOEL) is considered to be less than 300 ppm. (The Institute of Environmental Toxicology, 1986)

2. Thirteen-week Feeding Study in Rats

Dithiopyr was administered *via* the diet to groups of 12 male and 12 female F-344 rats for 13 weeks at concentrations of 0, 10, 100, 1000 and 5000 ppm. Decreased body weight gain, decreased feed efficiency and mild anemia were observed at the higher dose level. Post-mortem examination revealed primarily cholestasis and renal disease. Elevated plasma alkaline phosphate (AP), γ-glutamyl trans-peptidase (γ-GT), urea nitrogen, total bilirubin, and cholesterol levels, along with liver enlargement and discoloration, hepatocellular swelling and bile duct proliferation, were indicative of an obstruction in bile flow. Evidence of renal toxicity included : increased organ weight, organ discoloration, increased urine protein and specific gravity, and an increased incidence or severity of focal tubular atrophy. Other findings included : multiple organ weight effects, thyroid follicular hypertrophy, adrenal cortical hypertrophy, and pulmonary foam cell aggregation. The subchronic NOEL in rats is considered to be 10 ppm. (The Institute of Environmental Toxicology, 1988)

3. Four-week Feeding Study in Mice

Dithiopyr was administered *via* the diet to groups of 6 male and 6 female CD-1 mice for 4 weeks at concentrations of 0, 300, 1000, 3000, 10,000 and 30,000 ppm. Mortality, body weight depression, decreased food consumption, and clinical signs of toxicity were observed at the higher dose levels. Liver enlargement and discoloration, adrenal enlargement, and atrophy of the thymus, spleen, seminal vesicles, ovaries and uterus were noted on gross post-mortem examination. Due to an elevation in liver weight in females at the low dose level, the NOEL is considered to be less than 300 ppm. (The Institute of Environmental Toxicology, 1987)

4. Thirteen-week Feeding Study in Mice

Dithiopyr was administered *via* the diet to groups of 12 male and 12 female CD-1 mice for 13 weeks at concentrations of 0, 10, 100, 1000 and 5000 ppm. Mortality, decreased body weight gain, reduced feed efficiency, and mild anemia were observed at the higher dose level. By study termination many high dose animals appeared jaundiced, emaciated and had distended abdomens. Postmortem examination revealed primarily liver and kidney toxicity. Elevated plasma AP, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen and cholesterol levels were indicative of cholestasis and liver toxicity. These biochemical changes were accompanied by a significant increase in liver weight, hepatocellular swelling, vacuolation and necrosis, dissociation of hepatocellular cords, and bile duct proliferation. Increased kidney weights, decreased urine protein and specific gravity, and acidophilia of proximal tubular cells were indicative of mild renal toxicity. Other findings included : multiple organ weight effects, adrenal cortical hypertrophy and ovarian atrophy. The subchronic NOEL in mice is considered to be 10 ppm.

(The Institute of Environmental Toxicology, 1989)

5. Two-week Oral Study in Dogs

Dithiopyr was administered orally *via* gelatin capsule to 1 male and 1 female beagle dog for 2 weeks at dose levels of 0, 10, 30, 60, 100 and 200 mg/kg/day. Emesis and decreased body weight were observed at the higher dose levels. Biochemical and histopathological evidence of liver toxicity, manifest as increased AP, AST, and γ-GT levels in plasma, along with hepatocellular swelling, pigment deposition, and focal necrosis, were apparent on postmortem examination. The NOEL is considered to be 10 mg/kg/day. (The Institute of Environmental Toxicology, 1988)

6. Thirteen-week Oral Study in Dogs

Dithiopyr was administered orally *via* gelatin capsule to groups of 6 male and 6 female beagle dogs for 13 weeks at dose levels of 0, 1, 10 and 30 mg/kg/day. Postmortem examination revealed liver toxicity and moderate to severe cholestasis at the higher dose levels. Specific findings included : increased liver weights, increased AP, AST and γ-GT levels in plasma, along with liver swelling, distended gallbladders with sandy stones, pigment deposition in bile canaliculi and Kupffer cells, and mononuclear cell infiltration of the liver. The subchronic NOEL in dogs is considered to be 1 mg/kg/day. (The Institute of Environmental Toxicology, 1988)

7. Three-week Dermal Study in Rats

Dithiopyr was applied under semi-occlusive dressing to the intact skin of 5 male and 5 female Sprague-Dawley (S-D) rats at dose levels of 0, 50, 500 and 1000

mg/kg/day for 6 hr/day, 5 days/week for 3 weeks. Mild transient dermal irritation was observed. Increased liver weight at the high dose level was the only indication of a systemic effect. The NOEL is considered to be 500 mg/kg/day.

(Bio/dynamics Inc., 1989)

CHRONIC TOXICITY AND ONCOGENICITY STUDIES

1. Twenty-four-month Feeding Study in Rats

In a combined chronic toxicity and oncogenicity study, dithiopyr was administered *via* the diet to groups of 90 male and 90 female F-344 rats for 104 weeks at concentrations of 0, 3, 10, 100 and 300 ppm. Animal appearance, behavior, survival, body weight gain and food consumption were comparable in treated and control groups. Postmortem examination provided evidence of liver and kidney toxicity. There were no statistically significant or biologically significant increases in neoplastic lesions.

Increased AP, AST, ALT, urea nitrogen and cholesterol levels in plasma were indicative of mild liver toxicity and cholestasis. These biochemical changes were accompanied by a significant increase in liver weight, focal hepatocellular necrosis, spongiosis hepatis and bile duct proliferation. Renal effects included : increased urinary protein and an increased severity of glomerulonephropathy. The chronic NOEL in rats is considered to be 10 ppm (equivalent to a daily intake of 0.36 mg/kg bodyweight in males and 0.43 mg/kg b.w. in females) .

(The Institute of Environmental Toxicology, 1989)

2. Eighteen-month Feeding Study in Mice

In an oncogenicity study, dithiopyr was administered *via* the diet to groups of 70 male and 70 female CD-1 mice for 78 weeks at concentrations of 0, 3, 30 and 300 ppm. Animal appearance, behavior, survival, body weight gain and food consumption were comparable in treated and control groups. Postmortem examination revealed primarily liver toxicity. There were no statistically significant or biologically significant increases in neoplastic lesions.

Liver enlargement and discoloration, hepatocellular swelling, bile duct proliferation, and pigment deposition in hepatocytes, Kupffer cells and the bile canaliculi were indicative of mild liver toxicity and cholestasis. Other findings included : increased adrenal weights, adrenal cortical swelling, spleen enlargement and increased splenic extramedullary hematopoiesis. The chronic NOEL in mice is considered to be 3 ppm (equivalent to a daily intake of 0.31 mg/kg b.w. in males and 0.37 mg/kg b.w. in females). (The Institute of Environmental Toxicology, 1989)

3. Twelve-month Oral Study in Dogs

Dithiopyr was administered orally *via* gelatin capsule to groups of 6 male and 6 female beagle dogs for 52 weeks at dose levels of 0, 0.5, 5 and 25 mg/kg/day. Other

than an increased frequency of vomiting early in the study, there were no clinically overt signs of toxicity observed. Animal survival, body weight gain and food consumption were comparable in the treated and control groups. Postmortem examination revealed liver toxicity and cholestasis. Specific findings included : increased AP levels in plasma, liver enlargement and discoloration, the presence of black sandy materials in the gallbladder, hepatocellular necrosis and fibrosis, pseudo-bile duct formation, bile duct proliferation, and increased mucoidal secretion in the gallbladder. Brown pigment deposition was also found in the bile canaliculi, Kupffer cells and the kidneys. The chronic NOEL in dogs is considered to be 0.5 mg/kg/day.

(The Institute of Environmental Toxicology, 1989)

REPRODUCTION AND DEVELOPMENTAL TOXICITY STUDIES

1. Two-generation Reproduction Study in Rats

Dithiopyr was administered *via* the diet to groups of 24 male and 24 female S-D rats over 2 consecutive generations at concentrations of 0, 25, 250 and 2500 ppm. Parental animal appearance, behavior and survival were comparable in the treated and control groups. Parental body weight gain during the premating growth phases was depressed by 7-10% at the high dose level ; food consumption was slightly depressed in high-dose females. Dithiopyr administration had no effect on animal mating, fertility, length of gestation, parturition, litter size, sex ratio or pup viability. The appearance and behavior of off-spring during the lactation period was comparable in the treated and control groups. Throughout the lactation period, body weights of F_1 and F_2 generation pups and weanlings were 6-21% lower than control at the high dose level.

Postmortem examination of parental animals revealed primarily liver toxicity, consisting of organ enlargement and discoloration, hepatocellular swelling and necrosis, and bile stasis. Other findings included : increased kidney weight, focal renal tubular atrophy, thyroid follicular hypertrophy, and adrenal cortical hypertrophy. Postmortem examination of the offspring revealed liver enlargement and the appearance of white spots at the periphery of the liver lobes. These findings were accompanied by histopathological evidence of hepatocellular swelling and localized areas of hepatocellular necrosis, fibrosis and mineralization. The white liver spots are considered a reversible lesion due to their declining incidence with increasing animal age and their complete absence in adults. The NOELS for systemic and reproductive toxicity in rats are considered to be 25 and 2500 ppm, respectively.

(The Institute of Environmental Toxicology, 1989)

2. Developmental Toxicity Study in Rats

Dithiopyr was administered daily *via* gastric intubation in an aqueous suspension to groups of 24 mated female S-D rats on days 6 through 15 of gestation at dose

levels of 0, 30, 300 and 1000 mg/kg/day. Maternal toxicity in the form of a slight reduction in food consumption during the first few days of dosing was observed at the high dose level. Dithiopyr administration had no effect on the incidence of viable fetuses, resorptions, postimplantation loss, fetal sex ratio or fetal body weight. There were no significant differences between treated and control group incidences of fetal malformations or developmental variations. The NOELS for maternal and developmental toxicity in the rat are considered to be 300 and 1000 mg/kg/day, respectively. (The Institute of Environmental Toxicology, 1987)

3. Developmental Toxicity Study in Rabbits

Dithiopyr was administered daily *via* gastric intubation in an aqueous suspension to groups of 18 mated female NZW rabbits on days 7 through 19 of gestation at dose levels of 0, 150, 500 and 1000 mg/kg/day. Maternal toxicity in the form of a slight reduction in body weight during the first few days of dosing was observed at the high dose level. Dithiopyr administration had no effect on the incidence of viable fetuses, resorptions, postimplantation loss, fetal sex ratio or fetal body weight. There were no significant differences between treated and control group incidences of fetal malformations or developmental variations. The NOELS for maternal and developmental toxicity in the rabbit are considered to be 500 and 1000 mg/kg/day, respectively. (Hazleton Laboratories America, Inc., 1989)

GENETIC TOXICITY STUDIES

1. Gene Mutation Assays

The potential of dithiopyr to induce reverse gene mutations was evaluated in Ames' plate incorporation assays employing the histidine auxotrophic *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 in both the presence and absence of an Arochlor 1254-induced rat liver metabolic activation system. Dithiopyr was tested to its limit of solubility at dose levels ranging from 0.01 to 5.0 mg/plate. No mutagenic effect was observed. (SRI International, 1987)

The potential of dithiopyr to induce reverse gene mutations was also evaluated in the tryptophan auxotrophic *Escherichia coli* strain WP2*uvr*A in both the presence and absence of an Arochlor 1254-induced rat liver metabolic activation system. Dithiopyr was tested at dose levels ranging from 0.05 to 5.0 mg/plate. No mutagenic effect was observed. (The Institute of Environmental Toxicology, 1986)

The potential of dithiopyr to induce gene mutations was evaluated at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) gene locus of cultured Chinese hamster ovary cells in both the presence and absence of an Arochlor 1254-induced rat liver metabolic activation system. Dithiopyr was tested at concentrations ranging from 10 to 100 μg/ml in the presence of metabolic activation and at concentrations ranging from 10 to 300 μg/ml in the absence of metabolic activation. No mutagenic effect

was observed. (Monsanto Environmental Health Laboratory, 1987)

2. Chromosomal Damage Assay

The potential of dithiopyr to produce chromosome damage was evaluated *in vitro* using Chinese hamster lung cells in both the presence and absence of an Arochlor 1254-induced rat liver metabolic activation system. Dithiopyr was tested at concentrations ranging from 0.01 to 1.0 mM. No mutagenic effect was observed.

(The Institute of Environmental Toxicology, 1986)

3. DNA Damage Assays

The potential of dithiopyr to damage DNA was evaluated in vitro in a rat hepatocyte DNA repair test. Cultured hepatocytes from F-344 rats were exposed to dithiopyr at concentrations ranging from 0.1 to 1000 μg/ml. DNA damage was measured as unscheduled DNA synthesis. No genetic damage was observed.

(SRI International, 1987)

The potential to induce DNA damage was also evaluated *in vitro* in a bacterial recombination assay using *Bacillus subtilis* strains H17 (rec^+) and M45 (rec^-) both in the presence and absence of an Arochlor 1254-induced rat liver metabolic activation system. Dithiopyr was tested at dose levels ranging from 0.2 to 20 mg/disc. No genetic damage was observed. (The Institute of Environmental Toxicology, 1986)

PHARMACOLOGY STUDIES

Acute intraperitoneal injections of dithiopyr in 3 male and 3 female ICR mice at dose levels of 0, 19.5, 78, 313, 1250 and 5000 mg/kg resulted in animal deaths at the high dose level and weak effects on motor activity, muscle tone and autonomic nervous system function at levels of 313 mg/kg and above. The pharmacological NOEL in mice is considered to be 78 mg/kg. Acute oral administration of dithiopyr to 3 male albino rabbits at dose levels of 0, 313, 1250 and 5000 mg/kg resulted in no behavioral or neurological effects.

Acute oral administration of dithiopyr to 3 anesthetized male albino rabbits at dose level of 0, 1250 and 5000 mg/kg resulted in no measurable effects on respiration or cardiovascular function. The pharmacological NOEL in rabbits is considered to be 5000 mg/kg. (The Institute of Environmental Toxicology, 1988)

CONCLUSION

A comprehensive toxicology database has been developed to support the use of dithiopyr products worldwide. The results of laboratory animal tests indicate that dithiopyr displays low mammalian toxicity following acute oral, dermal or inhalation exposure. Dithiopyr has low dermal and ocular irritation potential and does not produce allergic skin reactions. Subchronic and chronic exposure produces primarily liver and

kidney toxicity, effects for which threshold exposure levels have been established. Dithiopyr is not genotoxic or oncogenic and does not interfere with normal reproduction and development.

Human expdsure to dithiopyr is expected to be very low. Field application rates are low, typically in the range of 0.1 to 0.5 kg a.i./ha. Residues of dithiopyr are not detectable in rice (unpublished Monsanto data from residue trials in rice). Dermal pharmacokinetics studies with rhesus monkeys have shown that only 0.08% of a topically-applied dose of ^{14}C-dithiopyr is systemically absorbed.[3)] Human biological monitoring studies have shown that systemic exposure to dithiopyr resulting from field application averages 1.01 x 10^{-4} mg dithiopyr/kg b.w./kg a.i. applied.[3)]

Granular formulations of dithiopyr for rice paddy use and an emulsifiable concentrate (EC) formulation for turf use were registered in Japan in April of 1991. A withholding limit for pesticide registration was established at 0.1 ppm for rice.* EC formulations of dithiopyr for turf use were registered in the United States in June of 1991. When used in accordance with label directions, these products will not adversely affect human health.

* Withholding hmits are estabhshed by the Environment Agency for food crop registrations of pesticides in Japan and serve the same purpose as maximum residue hmits.

REFERENCES

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2) H. Ritz & E. Buehler: "Current Concepts in Cutaneous Toxicity," ed. by V. Drill & P. Lazar Academic Press, New York, pp. 25-40, 1980
3) J. Cowell, C. Lottman & M. Manning: *Arch. Environ. Contam. Toxicol.* **21**, 195 (1991)

要　　約

ジチオピルの毒性試験の概要

モンサント社アグリカルチュラル・グループ毒性部
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ジチオピルは，モンサント社が開発した一年生イネ科雑草および一部の広葉雑草を対象とする芝地および水田用除草剤である．動物実験の結果，本剤の哺乳動物に対する急性経口，経皮，吸入毒性はいずれも軽微で，眼および皮膚一次刺激性は低く，皮膚感作性も認められなかった．亜急性および慢性毒性試験の結果，主として肝および腎に検体投与による影響が認められたが，これらの影響には閾値が存在した．また本剤には，遺伝毒性および催腫瘍性も認められず，正常な繁殖や発生過程を阻害することもなかった．実使用条件における使用薬量が低く，皮膚浸透性が低いことっさらに食品中の残留が検出限界以下で

あることから，ジチオピルの人間への暴露の可能性は極めて低いものと考えられる．

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