



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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APR 18 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: EPA ID # 524-UGN: Dithiopyr - Review of Developmental
Toxicity and Mutagenicity Studies Submitted for New
Chemical Registration

Tox. Chem. Number: 717C
Project Number: 9-1879
Record Number: 240294

From: Paul Chin, PhD *Paul Chin* 4/13/90
Section 2
Toxicology Branch I
Insecticide and Rodenticide Support (IRS)
Hazard Evaluation Division (H7509C)

To: Joanne Miller, PM 23
Registration Division (H7505C)

Thru: Marion P Copley, DVM, DABT *Marion P Copley* 4/13/90
Section Head
Section 2, Toxicology Branch I (IRS)
Hazard Evaluation Division (H7509C) *MB 4/16/90*

NOTE: Final evaluation of the data requirements for the toxicity studies submitted for the new chemical registration of dithiopyr will be conducted following the review of a 21-day dermal toxicity study (MRID No. 413045-01) which is currently being reviewed by the HED.

Conclusions:

Developmental Toxicity Studies

Developmental toxicity studies in rats and rabbits studies with dithiopyr were reviewed by the Toxicology Branch I. Both developmental studies were core-graded supplementary. However, these studies may be either upgraded to core-guideline or downgraded to invalid pending reevaluation of the additional information on bioavailability of MON-15100 (See section on ADDITIONAL INFORMATION NEEDED at the end of this memorandum). Here are the conclusions of the evaluations. [The Data

Evaluation Reports of the developmental toxicity studies are appended to this memorandum.]

1. Developmental toxicity study in rats (MRID No. 41001507)

Maternal NOEL = 300 mg/kg/day
Maternal LEL = 1000 mg/kg/day (decreased food consumption)
Developmental Toxicity NOEL = 1000 mg/kg/day (the highest dose tested)
Developmental Toxicity LEL = not established
Dose levels tested: 0, 30, 300, or 1000 mg/kg/day.
Core classification: Core-supplementary.

2. Developmental toxicity study in rabbits (MRID No. 41001508)

Maternal NOEL = 500 mg/kg/day
Maternal LEL = 1000 mg/kg/day (reduced body weight gain)
Developmental Toxicity NOEL = 1000 mg/kg/day (the highest dose tested)
Developmental Toxicity LEL = not established
Dose levels tested: 0, 150, 500, or 1000 mg/kg/day
Core classification: Core-supplementary.

Mutagenicity Studies

Five mutagenicity studies (MRID Nos. 410015-09, -10, -11, -12, and -13) with dithiopyr were reviewed by Irving Mauer. Four studies (MRID No. 410015-09, -10, -11, and -13) were core-graded acceptable and one study (MRID No. 410015-12) was core-graded unacceptable because the study cannot be evaluated until essential procedural information and other unexplained items are provided. Here are the conclusions of the evaluations. [The DERS for the mutagenicity studies are attached to this memorandum (see memorandum from Irving Mauer to Joanne Miller, HED Project No. 9-1879, EPA Record No. 240294).]

1. Ames Assay/MON-7200/ML-87-11/EHL 87004

MRID number: 41001509
Conclusion: negative
Core classification: Acceptable

2. Ames Assay/MON-7200/SR-86-375/LSC-2755-1

MRID number: 41001510
Conclusion: negative
Core classification: Acceptable

3. CHO/HGPRT Mutation Assay/MON-7200/ML-87-10/EHL 87006

MRID number: 41001511
Conclusion: negative

Core classification: Acceptable

4. In Vitro Cytogenetics Test/MON-7200/ET-86-79

MRID number: 41001512

Conclusion: negative

Core classification: unacceptable

5. Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures/MON-7200/SR-87-9/LSC 3116

MRID number: 41001513

Conclusion: negative

Core classification: Acceptable

Action Requested:

Monsanto has submitted two developmental toxicity studies and five mutagenicity studies to satisfy the data requirements for registration of dithiopyr (also designated as MON-15100 or MON-7200, depending upon its registration status here or abroad).

Review and evaluate the above toxicity studies with dithiopyr.

ADDITIONAL INFORMATION NEEDED:

Bioavailability data of MON-7200 in test animal

The Agency must be assured that the apparent low toxicity of MON-7200 demonstrated in the developmental toxicity study in test animal (marginal maternal toxicity at 1 g/kg/day, HDT) is not the result of the poor availability of the test material in carboxymethylcellulose (CMC). Therefore, the registrant must provide the following data and information.

- a. The maternal liver weight from the developmental toxicity study in test animal.
- b. Data on the bioavailability of MON-7200 in the 0.5 - 1 % CMC, such as information on the degree of binding of the vehicle for MON-7200.
- a. Maternal Liver weight data

Liver weight data (liver weights and liver/body weight ratios) are needed because the liver was the common target organ in a 21-day dermal toxicity study in rabbits (see Background attached to this DER) with MON-7200 or the pilot dietary study in rats with MON-13200. The liver weight increase observed in these studies is apparently related to the absorbed dose of the test substance from 2 different routes of administration.

b. Bioavailability data

Carboxymethylcellulose (CMC), a widely used suspending agent, binds some chemicals. The CMC used in the developmental toxicity study may sufficiently bind MON-7200 and cause a decrease in the availability of test material for absorption in test animals. Therefore, the registrant should demonstrate the availability of MON-7200 for absorption when test material is suspended in CMC.

Data gaps:

1. A 21-day dermal toxicity study (82-2). This study (MRID No.413045-01) is currently being reviewed by the Toxicology Branch I.
2. Developmental toxicity studies in rats and rabbits.
3. Mutagenicity --Chromosome damage in vitro.
4. A 90-day feeding study is required to be consistent with what is currently being required under reregistration for the 1988 amendments to FIFRA.

Background:

Monsanto is requesting the registration of MON-15100 technical grade "dithiopyr" active ingredient for use in products to be applied to turf in both residential and nonresidential sites.

MON-15100 and MON-7200 are Monsanto designations for the same ingredient, ie., dithiopyr [3,5-pyridine-dicarbothioic acid, 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-S,S-dimethyl ester]. MON-15100 and MON-7200 are the designations for the active ingredient for registration in the United States and outside the United States, respectively.

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Section 2, Tox. Branch 1 (IRS) (H7509C). 4/11/90
Secondary reviewer: David Anderson, PhD. *David Anderson* 4/13/90
Section 2, Tox. Branch 1 (IRS) (H7509C).

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat

EPA Identification No.: EPA MRID No. 410015-07
Caswell No. 717C

Test Material: MON-7200, technical

Synonyms: Dithiopyr; MON-15100; MON-7200; 91% S,S-Dimethyl 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridine dicarbothioate.

Sponsor: Monsanto Japan Limited

Study Number(s): ET-86-208

Testing Facility: The Institute of Environmental Toxicology,
Tokyo, Japan

Title of Report: Teratogenicity Study in Rats with MON-7200

Author(s): R. Suzuki

Report Issued: October 19, 1987

Conclusions on effect levels and no effect levels

Maternal NOEL = 300 mg/kg/day
Maternal LEL = 1000 mg/kg/day (decreased food consumption)
Developmental Toxicity NOEL = 1000 mg/kg/day (the highest dose tested)
Developmental Toxicity LEL = not established
Dose levels tested: 0, 30, 300, or 1000 mg/kg/day.
Test species [strain]: rat [Charles River Crj:CD (SD)]
Route of administration: gastric intubation

Core classification: Supplementary. This study may be either upgraded to core-guideline or downgraded to invalid pending reevaluation of the additional information on bioavailability of MON-7200. (See ADDITIONAL INFORMATION NEEDED attached to this DER)

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A. MaterialsTest Compound: Purity: 91%

Description: a tan solid

Lot No.: NBP 3352929-B

Melting point: 48-51° C

Vapor pressure: 4×10^{-6} torr at 25° C

Solubility: 0.7 ppm in water. Soluble in toluene, ether, acetone, and chloroform

Vehicle(s):

<u>Material</u>	<u>Description</u>	<u>Purity</u>
Carboxymethyl-cellulose	White powder	100%

Compound preparation:

MON-7200 was suspended in 1% aqueous solution of carboxymethylcellulose to yield dose levels of 30, 300, and 1000 mg/kg/day. The control animals received the vehicle only at 10 ml/kg/day.

Test Animal(s):

Species: Rat

Strain: Charles River Crj:CD (SD)

Source: Charles River Japan Inc.

Age: 12 weeks

Weight: 256-259 g (mean body weight on gestation day 0)

Acclimation Period: 11 days

Environmental Conditions:

Temperature: 24±1° C

Humidity: 55±10%

Light:dark cycle: 14:10

B. Study Design

This study was designed to assess the developmental toxicity of MON-7200 when administered by gastric intubation to pregnant rats on gestation days 6 through 15.

Mating Procedure:

Each female was placed in the cage with a male rat until copulation was observed (1:1 basis). Observation of vaginal plugs and/or sperm was considered to be the evidence of copulations, and the day on which the evidence was found was designated as day 0 of gestation. The mating procedures were repeated for 6 successive days. Each day, the copulated females were assigned to 4 test groups so that mean and standard deviations of body weights would be equalized among the groups. Each group consisted of 24 copulated females.

Group Arrangement:

<u>Test Group</u>	<u>Dose Level (mg/kg/day)</u>	<u>Number Assigned</u>	<u>Dosing Schedule (gestation days)</u>
Control	0	24	6-15
Low Dose	30	24	6-15
Mid Dose	300	24	6-15
High Dose	1000	24	6-15

Dosing:

All doses were in a volume of 10 ml/kg of body weight/day. The volume of dosing solutions was calculated based on the individual body weights daily during the dosing period. The vehicle and test material suspensions were stirred during the dosing period. Doses of 30, 300, and 1000 mg/kg/day were given to groups of 24 rats. The dosing suspensions were analyzed for concentration and stability. Analyses of the concentration revealed that the test substance was found at the levels of 97-109% of the target levels in each dosing solution and the test substance was verified to be stable during the dosing period.

Food and Water

Food (Oriental Yeast Co., Ltd.) and local tap water were supplied ad libitum. The feed and water were routinely analyzed for suspected contaminants (the identities of the contaminants were not specified in the study report). The study reported that no contaminants were detected in such levels that could adversely affect the results of the study.

Observations:

The animals were checked daily for mortality or abnormal condition. Body weight determinations were made on gestation days 0, 6-15 (daily), and 20. Food consumption was measured on gestation days 0-6, 6-9, 9-12, and 15-20. Dams were killed with deep ether anesthesia on gestation day 20. They were examined grossly. Uteri were examined for the number of placement of implantation sites, number of live and dead fetuses, the number of resorption and any abnormalities. Ovaries were examined for corpora lutea.

Fetuses were sexed and their viscera were examined externally and about 1/2 of the fetuses were fixed in Bouin's fluid and examined for visceral abnormalities. All remaining fetuses were then fixed in 70% ethanol, macerated, stained with alizarin red S and examined for skeletal malformations. Historical control data were provided to allow comparison with concurrent controls.

Statistical analysis:

Results were analyzed statistically using the following tests. Bartlett's test for homogeneity of variance, analysis of variance, Kruskal-Wallis's H test, and Dunnett's or Scheffe's test for multiple comparison were used in analyzing the data on the body weights, body weight gains, and food consumption of maternal rats, on the numbers of corpora lutea, implants, and live fetuses, and on the fetal and placental weights. Fisher's exact probability test was used in analyzing the data on the incidences of maternal rats having anomalous fetuses, incidences of fetal malformations and variations, and fetal sex ratio, and Mann-Whitney's U-test in the data on the percent incidences of resorptions and fetal deaths.

Compliance:

A signed Statement of Confidentiality Claim was provided. A signed Statement of compliance with EPA GLP's was provided.

A signed Quality Assurance Statement was provided by the Chief of the Quality Assurance Unit, Masahiro Hirano, on 7/6/87.

C. Results

1. Maternal Toxicity:
Mortality

Survival and pregnancy status in maternal rats treated with test substance is summarized in Table 1 of the study report attached to this DER. One animal from the high dose group died on gestation day 20. Gross pathological examination performed on this animal exhibited hemorrhage in the uterus, hydropericardium, and cloudy enlargement of the liver. Histopathological examination performed on this animal revealed that the death was due to septicemia and probably unrelated to treatment. Another animal from the same dose group exhibited emaciation on gestation day 20. Gross pathological examination performed on this animal exhibited abnormalities such as retention of reddish pleural fluid, adhesion of yellowish white material to the mediastinum and pericardium. These findings were suggestive of a technical error in administering the test substance.

Clinical Observations

The appearance and behavior of treated animals were considered to be comparable to controls. No clinical signs were noted during the pretreatment period (gestation days 0-5).

During treatment and posttreatment periods (gestation days 6-20), clinical signs noted were as follows: scabs in the forearms (one animal in the mid dose group); slight nasal discharge (one animal in the high dose group); and hair loss in the limbs and thoracic regions (one or two animals in all groups).

Body Weight

A summary of the mean body weight changes during gestation is presented in Table I. In the low dose group, the body weight gain during gestation days 6-9 was significantly increased as compared with that in the control group. In the mid dose group, significant increases were found on gestation days 14, 15, and 20 and during gestation days 6-15 and 0-20. However, the changes in these groups were not considered to be treatment related since they occurred sporadically and no dose-response relationship was evident.

Significant increase in body weight gain in the high dose group (10 g) was found during gestation days 6-9 in comparison to the control group (6 g). The body weight gain in the high dose group was 166% of the control values on gestation day 6-9.

Food Consumption

A summary of food consumption during gestation is presented in Table II. The food consumption was significantly decreased in the high dose group during gestation days 6-9 and 6-15. All other mean food consumption values were not significantly different than the concurrent control values.

Gross Pathological Observations

Gross pathology findings noted were as follows:

1. Enlargement and coarse surface of the spleen and its adhesion to the peritoneum in 1 mid dose group female.
2. Accessory spleen in 1 high dose group female.

However, these findings were not considered to be related to test substance treatment.

Table I. Mean Body Weight Changes (grams) During Gestation^a

Dose Group (mg/kg/day)	0	30	300	1000
Day 0-6 Mean±S.D.	31±7	31±15	32±6	29±6
Day 6-15 Mean±S.D.	35±9	42±13	42±8 **	35±9
Day 15-20 Mean±S.D.	59±12	62±14	67±12	57±17
Day 0-20 Mean±S.D.	125±20	134±18	141±16 **	121±25
Day 6-9 Mean±S.D.	6±5	12±12 *	9±5	10±3 *

a = Data extracted from Study No. ET-86-208, Tables 3 and 4.

* Statistically significantly different from the control group (p< 0.05).

** Statistically significantly different from the control group (p< 0.01).

Table II. Food Consumption (g/day) during Gestation^a

Group	Prior to Dosing Period	Dosing Period (days 6-15)	Postdosing Period days (15-20)
Control	21	20	20
Low Dose	21	21	20
Mid Dose	22	20	20
High Dose	21	17 **	18 *

^a = Data extracted from Study number ET-86-208, Table 5 from the study report.

* Statistically significantly different from the control group (p< 0.05). Females that were not pregnant, delivered early, aborted or died were excluded from the mean and statistical calculations.

** Statistically significantly different from the control group (p< 0.01).

Cesarean section Observations

No statistically significant differences or trends were found between treated and control groups for the following parameters: pregnancy rate, mean number of corpora lutea, mean number of uterine implantation, fetal viability, sex ratio, mean fetal body weights or placental weights (see Table 6 from the study report attached to this DER).

The percent incidence of resorptions and fetal deaths in the low dose group (6.8%) was significantly higher than that in the control group (2.2%). However, this change is not considered to be related to the test substance because no dose-response relationship was evident and the incidence rate (6.8%) in the low dose group was within the range of historical control data (2.2-12.3%) for this strain of rat in the testing facility.

2. Developmental Toxicity:

External Examinations

External examinations of all fetuses showed no variations or malformations.

Visceral Examinations

Variations or malformations noted in the soft tissue did not indicate a treatment effect in any group. Summaries of soft tissue variations and malformations are shown in Table 8 of the study report attached to this DER.

The following visceral variations were reported in the study report:

1. Slight dilatation of renal pelvis with the presence of the renal papilla was observed in all groups. The percent incidences of fetuses (litters) were 2.5% (9.1%), 8.5% (19.0%), 6.3% (13.0%), and 6.7% (22.7%) in the control, low, mid, and high dose groups, respectively. The percent incidences of this variation in fetuses (litters) in the historical control as reported in the submission ranged from 0 to 2.1% (0 to 13.6%). The incidences of this variation in the low dose group was statistically significantly different from the control group. However, this change is not considered to be related to treatment because the incidence is not dose-related. Because dilatation of the renal pelvis is commonly observed in the normal stages of renal development (1), the toxicological significance of an increase

- in this finding is not clear.
2. Thymic remnant in the neck was observed in 4.7% to 14.1% of the fetuses in all groups including the control group. The incidence of this variation in the low dose group was significantly lower than that in the control group.
 3. Left umbilical artery was observed in one fetus from each of the low and mid dose groups.
 4. Y-shaped 3rd ventricle was observed in one fetus of the control group.

The following visceral malformations were reported in the study report:

1. Microphthalmia in one fetus from each of the control and high dose groups.
2. Situs inversus viscerum in one fetus of the low dose group.

Skeletal Examinations

Skeletal evaluation data from animals sacrificed at the scheduled cesarean sections are summarized in Table 8 of the study report attached to this DER.

Skeletal variations were noted in all groups and they did not occur in a pattern or frequency which indicated a treatment effect. The following skeletal variations were reported in the study report.

1. Lumbar ribs were observed in 1.5%, 0.7%, 4.5%, and 3.5% of the fetuses in the control, low, mid, and high dose groups, respectively. Although slightly higher incidences were found in the mid and high dose groups, the differences from the control value were not statistically significant and these values were within the range of the incidences in the historical control data (1.8-7.3%).
2. Shortened 13th rib was observed in 2, 1, 1, and 1 fetuses of the control, low, mid, and high dose groups, respectively.
3. Cervical ribs were observed in one fetus of the mid dose group.
4. 14th ribs with 27 presacral vertebrae were observed in one fetus of the high dose group.

Skeletal malformations noted did not indicate a treatment effect in any group. The following malformations were reported in the study report:

1. Splitting of ossification center of the thoracic vertebral body was observed in one fetus of the

- mid dose group.
2. Splitting of ossification center of the lumbar vertebral body in one fetus and fusion of the ribs in one fetus of the high dose group were observed.

D. Discussion/Conclusions

Groups of 24 pregnant rats were given daily doses of dithiopyr (MON-7200) by gastric intubation at 0, 30, 300, or 1000 mg/kg body weight on gestation days 6 through 15. Based on statistically significant decreased food consumption on day 6-15, maternal toxicity may have been demonstrated at the 1000 mg/kg/day dose level. The no-observed-effect level (NOEL) for maternal toxicity is 300 mg/kg/day. There was no compound related developmental toxicity (embryo/fetal toxicity or malformations) at any dose level, however, there was a nominal increased trend in malformations and skeletal variations. Since these incidences were comparable to the historical controls and were not statistically significant, they were not considered treatment related. (The study report noted that the historical control data base is comprised of only 307 to 328 fetuses from 4 studies). The NOEL for developmental toxicity is considered to be 1000 mg/kg/day. Since the guideline allows a limit dose of 1000 mg/kg/day, a repeat study is not required if the response to the section on ADDITIONAL INFORMATION NEEDED (below) is acceptable.

Reference

1. Woo, D.C. and R. M. Hoar, "Apparent Hydronephrosis" as a Normal Aspect of Renal Development in Late Gestation of Rats: The Effect of Methyl Salicylate., *Teratology*, 6: 191-196, 1972.

ADDITIONAL INFORMATION NEEDED:

Bioavailability data of MON-7200 in test animal

The Agency must be assured that the apparent low toxicity of MCN-7200 demonstrated in the developmental toxicity study in rats (marginal maternal toxicity at 1 g/kg/day, HDT) is not the result of the poor availability of the test material in carboxymethylcellulose (CMC). Therefore, the registrant must provide the following data and information.

- a. The maternal liver weight from the developmental toxicity study in rats.
- b. Data on the bioavailability of MON-7200 in the 1 % CMC, such as information on the degree of binding of the vehicle for MON-7200.

a. Maternal Liver weight data

Liver weight data (liver weights and liver/body weight ratios) are needed because liver was the common target organ in a 21-day dermal toxicity study in rabbits (see Background) with MON-7200 or the pilot dietary study in rats with MON-13200. The liver weight increase observed in these studies is apparently related to the absorbed dose of the test substance from 2 different routes of administration.

b. Bioavailability data

Carboxymethylcellulose (CMC), a widely used suspending agent, binds some chemicals. The CMC used in the developmental toxicity study may sufficiently bind MON-7200 and cause a decrease in the availability of test material for absorption in test animals. Therefore, the registrant should demonstrate the availability of MON-7200 for absorption when test material is suspended in CMC.

Background

Bioavailability of MON-7200 or MON-13200 (a structurally related substance to MON-7200) has been demonstrated in several studies listed below. See structures for MON-7200 and MON-13200 below.

Studies Conducted with MON-7200Developmental Toxicity Study in Rats

Document No.: EPA MRID No. 410015-07
Maternal NOEL = 300 mg/kg/day
Maternal LEL = 1000 mg/kg/day (decreased food consumption)
Developmental Toxicity NOEL = 1000 mg/kg/day (the highest dose tested)
Developmental Toxicity LEL = not established
Vehicle used: 1 % CMC

Developmental Toxicity Study in Rabbits

Document No.: EPA MRID No. 410015-08
Maternal NOEL = 500 mg/kg/day
Maternal LEL = 1000 mg/kg/day (decreased body weight gain)
Developmental Toxicity NOEL = 1000 mg/kg/day (the highest dose tested)
Developmental Toxicity LEL = not established
Vehicle used: 0.5 % CMC

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21-Day Dermal Toxicity Study in Rats

Document No.: EPA MRID No. 413056-01. This study is currently being reviewed by the Toxicology Branch I.

LEL: 1 g/kg/day based on increased liver weights and liver/body weight ratios.

Vehicle used: None. Applied as powder.

Note: The LEL in this dermal study is same as the dose level utilized in the developmental toxicity study (1 g/kg/day)

Studies Conducted with MON-13200

Pilot Dietary Study in Rats

Document: A letter from Dr. Joel Kronenberg, Monsanto to William Burnam, OPP, EPA, on July 18, 1988).

LEL: 1000 ppm (approximately equivalent to 0.05 to 0.1 g/kg/day using a conversion factor) based on increased liver weights and enlarged hepatocytes.

Vehicle used: the test substance was incorporated in the diet.

Note: The LEL (approximately 0.05 to 0.1 g/kg/day) in this pilot dietary study is approximately 1/10 to 1/20th of the dose level utilized in the above developmental toxicity study (1 g/kg/day).

Metabolism Study in Rats .

Reference: Results presented at a meeting held on December 11, 1989 between representatives of EPA and Monsanto Company, at room 1023, OPP, EPA.

Bioavailability: 25% of the orally administered dose was excreted in the urine

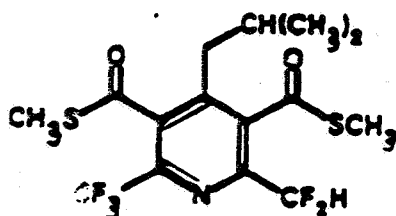
Vehicle used: Not specified.

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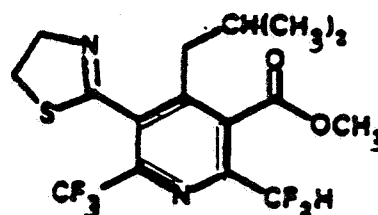
Note:

Based on the absorption of MON-13200, it is estimated that the absorption of MON-7200 from the gut in rats is about 25%.

Structures of MON-7200 and MON-13200



MON-7200
(Dithiopyr)



MON-13200
(Thiazopyr)

Dithiopyr Science Reviews

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Pages 17 through 20 are not included in this copy.

The material not included contains the following type of information:

- _____ Identity of product inert ingredients.
- _____ Identity of product inert impurities.
- _____ Description of the product manufacturing process.
- _____ Description of product quality control procedures.
- _____ Identity of the source of product ingredients.
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- _____ A draft product label.
- _____ The product confidential statement of formula.
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- X FIFRA registration data.
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GUIDELINE: 83-3
Primary reviewer: Paul Chin, PhD. *Paul Chin 4/13/90*
Section 2, Tox. Branch 1 (IRS) (H7509C).
Secondary reviewer: David Anderson, PhD. *David Anderson 4/13/90*
Section 2, Tox. Branch 1 (IRS) (H7509C).

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rabbit

EPA Identification No.s: EPA MRID (Accession) No. 410015-08
Caswell No. 717C

Test Material: MON-7200, technical

Synonyms: Dithiopyr; MON-15100; MON-7200; 93% S,S-Dimethyl 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridine dicarbothioate.

Sponsor: Monsanto Agricultural Company

Study Number(s): HL-88-110/HLA 241-218

Testing Facility: Hazleton Laboratories America, INC.
9200 Leesburg Turnpike, Vienna, VA 22180

Title of Report: Rabbit teratology Study with MON-7200
Monsanto Study Number HL-88-110

Author(s): S. A. Morseth

Report Issued: January 13, 1989

Conclusions on effect levels and no effect levels

Maternal NOEL = 500 mg/kg/day
Maternal LEL = 1000 mg/kg/day (reduced body weight gain)
Developmental Toxicity NOEL = 1000 mg/kg/day (the highest dose tested)
Developmental Toxicity LEL = not established
Dose levels tested: 0, 150, 500, or 1000 mg/kg/day
Test species (strain): Rabbit (New Zealand White)
Route of administration: oral gavage

Core classification: Supplementary. This study may be either upgraded to core-guideline or downgraded to invalid pending reevaluation of the additional information on bioavailability of MON-7200. (See ADDITIONAL INFORMATION NEEDED attached to this DER)

A. Materials

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Test Compound: Purity: 93%

Description: a yellow solid

Lot No.: Dayton 88-1

Melting point: 48-51° C

Vapor pressure: 4×10^{-6} torr at 25° C

Solubility: 0.7 ppm in water. Soluble in toluene, ether, acetone, and chloroform

Vehicle(s):

<u>Material</u>	<u>Lot No.</u>	<u>Description</u>	<u>Purity</u>
Tween 80	870326	Yellow, viscous liquid	100%
Carboxymethyl-cellulose	116F-0231	White powder	100%

Compound preparation:

MON-7200 was suspended in 0.5% carboxymethylcellulose and 0.1% Tween 80 to yield dose levels of 150, 500, and 1000 mg/kg/day. The control animals received the vehicle only at 5 ml/kg/day.

Test Animal(s):

Species: Rabbit

Strain: New Zealand White

Source: Hazleton Research Products Inc.,
Denver, PA

Age: 20 weeks

Weight: 2192 to 3365 g

Environmental conditions:

Temperature: 68-73° F

Humidity: 45-84%

Light: dark cycle: 12:12B.

Study Design

This study was designed to assess the developmental toxicity of MON-7200 when administered by gavage (oral intubation) to pregnant rabbits on gestation days 7 through 19.

Mating Procedure:

Each female was placed in the cage with a male rabbit until copulation was observed. After copulation was observed and confirmed by examination of the vulva, the female was injected with human chorionic gonadotropin (HCG). If mating was not confirmed after 20 minutes, the female was placed in the cage of another male rabbit and the procedure was repeated. The day mating was confirmed was designated as day 0 of gestation. The mating continued until 18 pregnant rabbits were assigned to each of the four treatment groups.

Group Arrangement:

<u>Test Group</u>	<u>Dose Level (mg/kg/day)</u>	<u>Number Assigned</u>	<u>Dosing Schedule (gestation days)</u>
Control	0	18	7-19
Low Dose	150	18	7-19
Mid Dose	500	18	7-19
High Dose	1000	18	7-19

Dosing:

All doses were in a volume of 5 ml/kg of body weight/day. The vehicle and test material suspensions were stirred during the dosing period and doses of 0, 150, 500, and 1000 mg/kg/day were given by oral gavage to groups of 18 does. The dosing suspensions were analyzed for concentration and stability. Analyses of the concentration revealed that the test substance was found at the levels of 97-103% of the target levels in each dosing solution and the test substance was verified to be stable during the dosing period. Dosing was based on the most recently recorded individual body weight values (individual body weight values on gestation day 0 ranged from 3086 to 3875 g).

Observations:

The animals were checked twice daily for mortality or abnormal condition. Body weight determinations were made on gestation days 0, 7, 9, 11, 15, 20, 24, and 29, and food consumption was measured throughout the study. Dams were sacrificed by intravenous injection of T-61 Euthanasia Solution on gestation day 29. They were examined grossly. Uteri were examined for the number of placement of implantation sites, number of live and dead fetuses, the number of early and late resorptions and any abnormalities. Ovaries were examined for corpora lutea.

Fetuses were sexed and their viscera were examined for variations and malformations. Fetuses were then fixed in 95% ethanol and macerated in 1.5% potassium hydroxide. The skeletons were then stained with alizarin red S and examined for skeletal malformations. All live fetuses were examined for external, visceral and skeletal abnormalities. Historical control data were provided to allow comparison with concurrent controls.

Statistical analysis:

See Appendix #1, taken from the study report.

Compliance:

007863

A signed Statement of Confidentiality Claim was provided.
A signed Statement of compliance with EPA GLP's was provided.
A signed Quality Assurance Statement was provided by Blair Wingard at the Quality Assurance Unit on 1/12/89.

C. Results

1. Maternal Toxicity:

Mortality

One control group female (No. 46921) and one low dose group female (No. 46936) died on gestation days 28 and 23, respectively. Animal No. 46921 contained 14 dead fetuses and animal No. 46936 contained 2 implantation sites with normally developing fetuses on left uterine horn. The cause of death of these 2 animals is unknown. One control group female aborted on gestation day 24 and one high dose group female delivered on gestation day 29.

Clinical Observations

The appearance and behavior of treated animals were considered to be comparable to controls. No clinical signs were noted during the pretreatment period (gestation days 0-7).

During the treatment period (gestation days 7-20), signs of lacrimation, diarrhea and alopecia in the area surrounding the tear ducts of both eyes were noted in one control group female that was found dead on gestation day 28. Diarrhea was noted in one low dose female. During the posttreatment period (gestation days 20-29), clinical signs noted were as follows: thin appearance (one in the high dose group); alopecia (one in the mid dose and one in the high dose groups); diarrhea (one in the mid dose and one in the high dose groups); hair loss (two in the mid dose and three in the high dose group); and clear gelatinous material found in the cage pan (one in the mid dose group). The above signs do not appear to be treatment related.

Body Weight

A summary of the mean body weight changes during gestation is presented in Table I below. Mean body weight gain values for the low and mid dose groups were similar to the respective control values at all intervals.

However, the high dose group exhibited statistically

significant decrease ($p < 0.05$) in body weight gain only during gestation days 7-9 in comparison to the control group. The high dose group lost 43 g (approximately 1.2% of the gestation day 0 body weight) while the control group gained 21 g (approximately 0.6% of gestation day 0 body weight) over the first 2 days of treatment. Therefore, body weight gain in the high dose group during gestation days 7-9 is approximately 3 fold less than that of the control group. Although food consumption was normal during gestation days 7-9 (see Table II below), the efficiency of food utilization was negative (-0.13) in comparison to control value (0.06) (see Table III below).

During the dosing period (gestation day 7-20), the body weight gain in this high dose group (18 ± 200 g) was lower than that of the controls (162 ± 81 g). However, the decrease was not statistically significantly different from the controls due to wide variation (mean $\pm$ standard deviation of 18 ± 200 g) of the body weight gain among the high dose group. The weight gains in the high dose group during posttreatment period was similar to those of the controls.

Food Consumption

A summary of food consumption during gestation is presented in Table II. The food consumption was significantly decreased in the low dose group during gestation days 7-9. In the high dose group it was significantly increased during gestation days 0-2 and 0-7. All other mean food consumption values were not significantly different than the concurrent control values.

Table I. Mean Body Weight Changes (grams) During Gestation^a

Dose Group (mg/kg/day)		0	150	500	1000
Day 0-7	Mean±S.D.	165±112	97±79	155±82	176±92
Day 7-20	Mean±S.D.	162±81	159±83	72±193	18±200
Day 20-29	Mean±S.D.	167±95	184±147	172±171	235±194
Day 7-29	Mean	330	344	245	255
Day 7-9	Mean±S.D.	21±30	10±49	-1±36	-43±
Gravid Uterine Weight		376	440	470	433
Corrected Body Weight gains					
GD 7-29 ¹		-46	-96	-225	-178
Entire Period ²					
Mean±S.D.		118±288	2±231	-71±299	-3±335

¹ = corrected body weight gain for dosing period = body weight gain for gestation day 7-29 minus gravid uterus weight.

² = corrected body weight gain for entire gestation period = body weight gain for entire gestation period minus gravid uterus weight.

a = Data extracted from HLA Study No. 241-218, Tables 4 and 6.

* Statistically significantly different from the control group (p < 0.05).

Table II. Food Consumption (g/dam) during Gestation^a

Group	Prior to Dosing Period (Day 0-7)	Dosing Period (Day 7-20)	Post- Dosing Period (Day 20-29)	Gestation Period (Day 7-9)
Control	1071	2048	1058	324
Low Dose	1010	1805	1021	265 *
Mid Dose	1126	1716	1083	326
High Dose	1221*	1701	1298	306

^a = Data extracted from Study Report number HLA 241-218, Table 5 from the study report.

* Statistically significantly different from the control group (p < 0.05). Females that were not pregnant, delivered early, aborted or died were excluded from the mean and statistical calculations.

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Table III. Efficiency of Food Utilization During Gestation
(g body weight gained/g food consumed)

Dose Group (mg/kg/day)	0	150	500	1000
Day 0-7	0.15	0.09	0.14	0.14
Day 7-9	0.06	0.04	-0.0003	-0.13

Gross Pathological Observations

Gross pathology findings noted were as follows:

1. Several dark red spots on all lobes of the lung in 1 control group female that aborted on gestation day 24.
2. Pale liver (all lobes) in 1 control group female (No. 46921 that was found dead on gestation day 28) and in 1 low dose group female.
3. Firm, fluid-filled mass within the muscle of the abdominal wall in 1 low dose group female (No. 46936) that was found dead on gestation day 23.
4. A large amount of clear, watery fluid within the thoracic cavity of 1 mid dose group female.
5. Accessory spleen(s) in all groups.

However, these findings were not considered to be related to test substance treatment.

Cesarean section Observations

No statistically significant differences or trends were found between treated and control groups for the following parameters: pregnancy rate (83, 89, 100, and 94% for controls to the highest dose level), mean number of corpora lutea, mean number of uterine implantation, efficiency of implantation, number of resorption, fetal viability, number of dead fetuses/litter, sex ratio, or mean fetal body weights (see Table 8 from the study report attached to this DER).

2. Developmental Toxicity:

External Examinations

External examinations of all fetuses showed no variations or malformations except for one low dose fetus. The fetus was reported to have bulging eyes and a small flap of skin on the head. A summary of external variations is presented in Table IV.

Table IV. External Variations

<u>Observations</u>	<u>Control</u>	<u>Low Dose</u>	<u>Mid Dose</u>	<u>High Dose</u>
#pups(litters) examined	75 (12)	100 (14)	138 (18)	101 (15)
#pups(litters) affected	0 (0)	1 (1)	0 (0)	0 (0)
Bulging eye(s)				
#pups(litters) affected	0 (0)	1 (1)	0 (0)	0 (0)
Flap of skin on head				
#pups(litters) affected	0 (0)	1 (1)	0 (0)	0 (0)

Visceral Examinations

Variations or malformations noted in the soft tissue did not indicate a treatment effect in any group. The following malformations were reported in the study report:

1. In low dose group, 1 fetus had opacities in both eyes, 1 fetus had gallbladder agenesis, and 1 fetus had dilated aortic arch with an intraventricular septal defect.
2. In mid dose group, 2 fetuses had malformations of the aortic arch and 1 fetus had an absent kidney and ureter.
3. No malformed fetuses were found in control and high dose groups.

Skeletal Examinations

Skeletal variations were noted in all groups and included variations in the stage of ossification and in the rib counts. They did not occur in a pattern or frequency which indicated a treatment related effect.

Skeletal malformations noted did not indicate a treatment effect in any group. The following malformations were reported in the study report:

1. Vertebral anomalies in either the thoracic or lumbar region were noted in 1 fetus of the control group, 4 fetuses of the low dose group, 3 fetuses of the mid dose group, and 5 fetuses of the high dose group. The vertebral anomalies are common in rabbit fetuses and did not occur in a dose-related pattern.
2. Fused sternbrae was noted in 1 fetus of the low dose group litter mate of one of the fetuses with a vertebral anomaly.

3. Forked ribs were noted in 1 fetus of the mid dose group litter mate of one of the fetuses with a vertebral anomaly.
4. The percent of fetuses (litters) with skeletal malformations was 1.3 (8.3), 4.0 (29), 2.9 (17), and 4.0 (20) for the control, low, mid, and high dose groups, respectively, including the litter that delivered early. These differences were not statistically significant.

D. Discussion/Conclusions

Groups of 18 pregnant New Zealand White rabbits were given daily doses of dithiopyr (MON-7200) by oral gavage at 0, 150, 500, or 1000 mg/kg body weight on gestation days 7 through 19. Based on statistically significant decrease in maternal body weight gain between gestation days 7 to 9 at the highest dose level, the lowest-effect-level (LEL) for maternal toxicity is 1000 mg/kg/day. The no-observed-effect level (NOEL) for maternal toxicity is 500 mg/kg/day. There was no compound related developmental toxicity (embryo/fetal toxicity or malformations) at any dose level. The NOEL for developmental toxicity is considered to be 1000 mg/kg/day. Since the guideline allows a limit dose of 1000 mg/kg/day, a repeat study is not required if the response to the section on ADDITIONAL INFORMATION NEEDED (below) is acceptable.

ADDITIONAL INFORMATION NEEDED:

Bioavailability data of MON-7200 in test animal

The Agency must be assured that the apparent low toxicity of MON-7200 demonstrated in the developmental toxicity study in rabbits (marginal maternal toxicity at 1 g/kg/day, HDT) is not the result of the poor availability of the test material in carboxymethylcellulose (CMC). Therefore, the registrant must provide the following data and information.

- a. The maternal liver weight from the developmental toxicity study in rabbits.
- b. Data on the bioavailability of MON-7200 in the 0.5 % CMC, such as information on the degree of binding of the vehicle for MON-7200.

a. Maternal Liver weight data

Liver weight data (liver weights and liver/body weight ratios) are needed because liver was the common target organ in a 21-day dermal toxicity study in rabbits (see

Background) with MON-7200 or the pilot dietary study in rats with MON-13200. The liver weight increase observed in these studies is apparently related to the absorbed dose of the test substance from 2 different routes of administration.

b. Bioavailability data

Carboxymethylcellulose (CMC), a widely used suspending agent, binds some chemicals. The CMC used in the developmental toxicity study may sufficiently bind MON-7200 and cause a decrease in the availability of test material for absorption in test animals. Therefore, the registrant should demonstrate the availability of MON-7200 for absorption when test material is suspended in CMC.

Background

Bioavailability of MON-7200 or MON-13200 (a structurally related substance to MON-7200) has been demonstrated in several studies listed below. See structures for MON-7200 and MON-13200 below.

Studies Conducted with MON-7200

Developmental Toxicity Study in Rats

Document No.: EPA MRID No. 410015-07
Maternal NOEL = 300 mg/kg/day
Maternal LEL = 1000 mg/kg/day (decreased food consumption)
Developmental Toxicity NOEL = 1000 mg/kg/day (the highest dose tested)
Developmental Toxicity LEL = not established
Vehicle used: 1 % CMC

Developmental Toxicity Study in Rabbits

Document No.: EPA MRID No. 410015-08
Maternal NOEL = 500 mg/kg/day
Maternal LEL = 1000 mg/kg/day (decreased body weight gain)
Developmental Toxicity NOEL = 1000 mg/kg/day (the highest dose tested)
Developmental Toxicity LEL = not established
Vehicle used: 0.5 % CMC

21-Day Dermal Toxicity Study in Rats

Document No.: EPA MRID No. 413056-01. This study is currently being reviewed by the

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Toxicology Branch I.

LEL: 1 g/kg/day based on increased liver weights and liver/body weight ratios.

Vehicle used: None. Applied as powder.

Note: The LEL in this dermal study is same as the dose level utilized in the developmental toxicity study (1 g/kg/day)

Studies Conducted with MON-13200

Pilot Dietary Study in Rats

Document: A letter from Dr. Joel Kronenberg, Monsanto to William Burnam, OPP, EPA, on July 18, 1988).

LEL: 1000 ppm (approximately equivalent to 0.05 to 0.1 g/kg/day using a conversion factor) based on increased liver weights and enlarged hepatocytes.

Vehicle used: the test substance was incorporated in the diet.

Note: The LEL (approximately 0.05 to 0.1 g/kg/day) in this pilot dietary study is approximately 1/10 to 1/20th of the dose level utilized in the above developmental toxicity study (1 g/kg/day).

Metabolism Study in Rats

Reference: Results presented at a meeting held on December 11, 1989 between representatives of EPA and Monsanto Company, at room 1023, OPP, EPA.

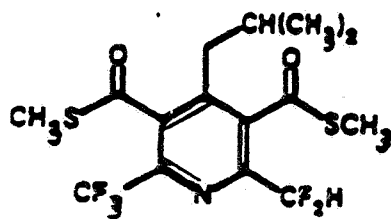
Bioavailability: 25% of the orally administered dose was excreted in the urine

Vehicle used: Not specified.

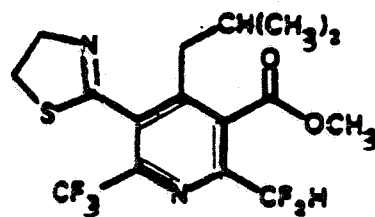
Note: Based on the absorption of MON-13200, it is estimated that the absorption of MON-7200 from the gut in rats is about 25%.

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Structures of MON-7200 and MON-13200



MON-7200
(Dithiopyr)



MON-13200
(Thiazopyr)

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APPENDIX #1

STATISTICAL ANALYSES
(Study Report pp. 15-20)

Dithiopyr Science Reviews

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Pages 34 through 41 are not included in this copy.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

Chemical (Caswell): 717C
RD Record No.: 240,294
HED Project No.: 9-1879

MEMORANDUM

SUBJECT: Dithiopyr - Submission of Tox Data (MRID Nos.
410015-09, -10, -11, -12, and -13)
EPA ID No. 524-UGN

FROM: Irving Mauer, Ph.D., Geneticist *J. Mauer* 04/04/90
Toxicology Branch I - Insecticide-Rodenticide Support
Health Effects Division (H7509C)

TO: Joanne I. Miller, Acting PM 23
Fungicide-Herbicide Branch
Registration Division (H7505C)

THRU: Karl P. Baetcke, Ph.D., Chief *Karl P. Baetcke* 4/10/90
Toxicology Branch I - Insecticide-Rodenticide Support
Health Effects Division (H7509C)

Registrant: Monsanto Agricultural Division, Monsanto
Chemical, St. Louis, MO

Request

Review and evaluate the following five mutagenicity studies with dithiopyr (also designated as MON-15100 or MON-7200, depending upon its registration status here or abroad):

1. Ames/Salmonella Mutagenicity Assay of MON-7200, unpublished study No. ML-87-11/EHL 87004, performed at Monsanto's Environmental Health

Laboratory, St. Louis, MO, Final Report issued July 17, 1987, and catalogued by Monsanto as submission R.D. #900, Volume 10 (EPA MRID No. 410015-09).

2. In vitro Microbiological Mutagenicity Assays with MON-7200, unpublished study performed at SRI International, Menlo Park, CA, SRI Project #LSC-2755-1, Final Report issued January 28, 1987, (Monsanto No. SR-86-375), and catalogued by Monsanto as submission RD #900 Volume 11 (EPA MRID No. 410015-10).
3. CHO/HGPRT Gene Mutation Assay with MON-7200, unpublished study No. ML-87-10/EHL 87006, performed at Monsanto's Environmental Health Laboratory, St. Louis, MO, Final Report issued September 11, 1987, and catalogued by Monsanto as submission R.D. #900, Volume 12 (EPA MRID No. 410015-11).
4. MON-7200: in vitro Cytogenetic Test, performed at the Institute of Environmental Toxicology, Tokyo (Japan), Study #ET-86-79, Final Report issued August 1, 1986, and catalogued by Monsanto as submission R.D. #900, Volume 13 (EPA MRID No. 410015-12).
5. Evaluation of the Potential of MON-7200 to Induce Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures, unpublished study performed by SRI International, Menlo Park, CA, SRI Project #LSC-3116 (Monsanto No. SR-87-9), Final Report issued July 6, 1987, and catalogued by Monsanto as submission RD #900 Volume 14 (EPA MRID No. 410015-13).

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TB Conclusion

[Detailed reviews are appended to this memorandum.]

Study (Monsanto No.)	Reported Results	TB Evaluation
1. Ames (ML-87-11)	Negative for reverse mutation in Salmonella (Ames) TA strains up to the dose limit of solubility (3000 <u>ug</u> /plate), w/without activation.	ACCEPTABLE
2. Ames (SR-86-375)	Negative for reverse mutation in Ames Salmonella strains exposed w/without activation up to the limit of solubility (5000 <u>ug</u> /plate).	ACCEPTABLE
3. CHO/HPRT (ML-87-10)	Negative for inducing forward mutation at the HGPRT locus of Chinese hamster ovary cells exposed w/without activation up to cytotoxic dose levels (300 <u>ug</u> /mL/-S9; 30 <u>ug</u> /mL/+S9).	ACCEPTABLE
4. CHL/Chrom. Ab (ET-86-79)	Although reported as negative for inducing structural chromosome aberrations in Chinese hamster lung cells exposed to the limit of solubility (0.33 and 1.0 mM), essential procedural information was lacking.	UNACCEPTABLE
5. HPC/UDS (SR-87-9)	Negative for UDS (genotoxicity) unscheduled DNA synthesis (UDS) in rat hepatocytes (HPC), as measured by silver grain counts indicative of DNA damage/repair.	ACCEPTABLE

ATTACHMENTS (DERs)

Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

Irving Mauer
04/04/90

Karl P. Baetcke
4/10/90

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DATA EVALUATION REPORT

I. SUMMARY

MRID No.: 410015-09
ID No.: 524-UGN
RD Record No.: 240,294
Caswell No.: 717C
Project No.: 9-1879

Study Type: Mutagenicity - Bacterial gene mutation (Ames Assay)

Chemical: Dithiopyr [3,5-pyridine-dicarbothioic acid, 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-S,S-dimethyl ester]

Synonyms: MON-15100; MON-7200

Sponsor: Monsanto Company, St. Louis, MO

Testing Facility: Environmental Health Laboratory (EHL),
Monsanto Company, St. Louis, MO

Title of Report: Ames/Salmonella Mutagenicity Assay
of MON-7200.

Author: Annette M. Kirk

Study No.: ML-87-11/EHL 87004 (RD No. 900, Volume 10)

Date of Issue: July 17, 1987

TB Conclusions:

The test article was assayed in replicate experiments with the standard battery of Ames strains (Salmonella typhimurium LT2) up to its limit of solubility in tissue culture medium (3000 μ g/plate), and was found negative for reverse gene mutation (his⁻ to his⁺), since test revertent colony counts were comparable to solvent and background values.

Classification (Core-Grace): ACCEPTABLE

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II. DETAILED REVIEW

A. Test Material - MON 7200 technical (Monsanto Agricultural Division)

Description: Light yellow solid
Batch (Lot): Dayton Batch 2 (EHL Code T870003)
Purity (%): 91.5
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Bacterial cultures

Species: Salmonella typhimurium LT2
Strains: TA98, TA100, TA1535, TA1537, TA1538
(all his⁻)
Source: Professor B.N. Ames (UCal, Berkeley)

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of dithiopyr when administered in vitro to cultures of Salmonella TA(his⁻) strains. Standardized procedures were employed, as referenced in published articles by Ames.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was provided.

D. Procedures/Methods of Analysis - Genetic integrity of the Ames bacterial strains (melted from frozen permanent stocks) was verified by standard techniques. The mammalian metabolic activation preparation (rat S9) used in this study was purchased from Litton Bionetics, and tested for activity before use (with benzo(a)pyrene, B(a)P).

A preliminary toxicity screen was conducted using (only) TA100 with/without S9 mix at concentrations of MON-7200 up to 10,000 $\mu\text{g}/\text{plate}$. In the main assay (employing the standard plate incorporation method of Ames, 1975 et seq.), triplicate plates of the five TA strains were exposed at 37 °C to each of five concentrations of the test substance, both in the absence and presence of 10% (v/v) S9 with the addition of the appropriate generating cofactors: NADP, magnesium and potassium chloride salts, and phosphates (=S9 mix). Known mutagens specific to each TA strain served as positive controls,* and DMSO as the solvent (negative) control.

* See footnote, p. 3

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Revertent colonies (his⁻ to his⁺) were counted after 48 hours incubation, either by eye (with/or without the use of a stereomicroscope), or electronically (using an Artek Model 88 Automatic Colony Counter). Revertent/plate values were transformed to log-10 values for statistical analysis, either by Bartlett's procedure, or a one-sided t-test; Grubbs Test was also run for the presence of outliers. Significance for any dose response was by regression analysis for the log-10 transformed data.

The entire assay was repeated once.

- E. Results - The test article was not toxic to TA100 cells (+S9) at doses up to 10,000 micrograms/plate, but precipitation was observed at both 3000 μ g and the HDT (Report Table 1). Hence the highest level selected for the main assay was 3000 μ g/plate; four other doses at one-third sequences were also chosen (1000, 300, 100, and 30 μ g/plate).

Both summary tabulations of mean plate incorporation test results (Appendix I) as well as individual plate counts for each experimental point in both assays (Appendix II) were presented (both attached to this DER).

In only one instance was there a significantly increased ($p < 0.01$) revertent count. The initial assay in activated (S9) TA100 treated at 100 μ g MON 7200 revealed a mean count of 129 revertents per plate vs. a solvent control value of 108 (Table 2). Retested in a narrower dose range (50, 100, 150 μ g), however, revealed negative results (Table 5).

All other test treatments in both assays were within (or below) negative (solvent) control (DMSO) responses. The strain-specific positive controls responded appropriately, with counts ranging from 8 to over 80 times DMSO values.

The authors concluded that the test article, MON-7200 (Dayton Batch 2) was not mutagenic in Ames testing under the conditions of this assay.

* -- Strain	-S9	+S9
TA98	4-NQO	2-AAF
TA100	4-NQO	B(a)P
TA1535	NaNO ₂	2-AAAnth.
TA1537	9-AAcri.	2-AAAnth.
TA1538	4-NQO	2-AAF

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- F. TB Evaluation - ACCEPTABLE. The test article was assayed in replicate experiments under adequate and standardized conditions up to the limit of solubility (3000 μ g/plate), and the negative results generated are judged valid.

Attachments (Data Tables)

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ATTACHMENT 1
(09) Data Tables

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Dithiopyr Science Reviews

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Pages 50 through 56 are not included in this copy.

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

Irving Mauer
04/04/80

Karl P. Baetcke
4/10/90

007863

DATA EVALUATION REPORT

I. SUMMARY

MRID No.: 410015-10
ID No.: 524-UGN
RD Record No.: 240,294
Caswell No.: 717C
Project No.: 9-1879

Study Type: Mutagenicity - Bacterial gene mutation
(Ames Assay)

Chemical: Dithiopyr [3,5-pyridine-dicarbothioic acid,
2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoro-
methyl)-S,S-dimethyl ester]

Synonyms: MON-15100; MON-7200

Sponsor: Monsanto Company, St. Louis, MO

Testing Facility: SRI International (SRI), Menlo Park, CA

Title of Report: In vitro Microbiological Mutagenicity
Assay with MON-7200.

Author: Edward S. Riccio

Study No.: Monsanto No. SR-86-375/SRI Project No. LSC-2755-1
(RD No. 900, Volume 11)

Date of Issue: January 28, 1987

TB Conclusions:

Negative for reverse mutation in repeat assays in Ames
strains of Salmonella exposed up to the limit of solubility
(5000 ug/plate), with/without activation.

Classification (Core-Grade): ACCEPTABLE

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II. DETAILED REVIEW

A. Test Material - MCN 7200 technical (from Monsanto Agricultural)

Description: Light, yellow powder
Batch (Lot): Dayton Batch 1
Purity (%): 93.7
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Bacterial cultures

Species: Salmonella typhimurium LT2
Strains: TA98, TA100, TA1535, TA1537, TA1538
(all his⁻)
Source: Dr. Bruce Ames (UCal, Berkeley)

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of MON 7200 when administered in vitro to Salmonella typhimurium cultures (TA strains). The procedures employed were derived from standardized techniques in peer reviewed scientific journals (and referenced in the Final Report).

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - Aliquots were thawed from frozen stocks of the five Ames tester strains and checked for their genotypic characteristics.

Following preliminary toxicity screening (employing strain TA100 only), triplicate plates of each strain were exposed for 48 hours to each of six concentrations of the test article, both in the absence and presence of two levels of a mammalian activation enzyme system consisting of the 9000X g supernatant fraction (S9) of liver homogenates from Sprague-Dawley male rats pretreated with a mixture of PCBs (specifically Aroclor 1254, to enhance hepatic synthesis of monooxygenase enzymes), plus generating cofactors: NADP, G-6-P, salts (=S9 mix). Strain-specific mutagens served as positive controls,* and the solvent DMSO as the negative (diluent) control, against which test responses were to be measured.

*Sodium azide (SA) for TA1535 and TA100 (-S9)
9-Aminoacridine (9-AAC) for TA1537 (-S9)
9-Nitrofluorene (9-NF) for TA1538 and TA98 (-S9)
2-Anthramine (2-Anth) for all tester strains (+S9).

After 48 hours, histidine revertent colonies (his⁻ to his⁺) were counted electronically (Biotran II Automatic Colony Counter), or manually (if test chemical precipitating interference was present), using an electric probe.

Data were analyzed statistically following log-10 transformation, employing the following methods:

1. Bartlett's Test, for significance among treatment variances;
2. One-tailed Dunnett's t-test, for comparing test treatments with controls; and
3. Regression analysis (log-log) for all treatments (dose-trend), and t-tests to evaluate significance of dose-response.

Other (non-statistical) guidelines for responses were also available at the discretion of the Study Director.

The following criteria were applied to define mutagenic responses:

"Positive. A test article is considered a mutagen when the values of three or more dose levels are reproducibly significantly greater than the control values ($p < 0.01$), and when there is a reproducible, significant positive dose-response ($p < 0.01$).

"Negative. A test article is considered a nonmutagen when no significant increase in the number of revertants is observed in at least two independent experiments. The highest dose level tested for nontoxic solid substances and pure liquids is usually 5000 ug/plate. The highest dose level tested with an extract and other than pure liquids is usually 200 ul/plate. However, occasionally test articles are evaluated at higher dose levels (10,000 ug/plate or 400 ul/plate) when no solubility problems are encountered or when there is some evidence of increase in revertant numbers. For toxic compounds, only the highest dose level tested should show evidence of toxicity.

"Inconclusive. When inconsistent results are obtained in two or more experiments, or when a test article cannot be identified clearly as a mutagen or nonmutagen, the results are classified as inconclusive." (SRI Final Report, page 8.)

The entire assay was repeated 2 weeks later.

- E. Results - In the preliminary toxicity tests, activated (+S9) and nonactivated (-S9) cultures of TA100 were treated up to the limit dose, 5000 $\mu\text{g}/\text{plate}$, without evidence of cytotoxic effect(s). Precipitation of test compound, however, was reported at this HDT. Hence, the highest concentration selected for the main assays with all five tester strains was 5000 $\mu\text{g}/\text{plate}$, plus five lower doses, namely: 1000, 500, 100, 50, and 10 $\mu\text{g}/\text{plate}$ (+S9).

In the first assay, no mutagenicity was evident in any test culture (see Report Data Tables 2 to 4, attached to this DER), either without activation or in the presence of 4 percent S9.

For the second assay, an increased level of activation was employed, namely 10 percent S9. Again, no consistent dose-related increased revertent counts were observed (Report Tables 5 to 7, attached here). However, several activated TA98 plates gave slightly elevated (fortuitous) counts, in the dose range 50 to 500 $\mu\text{g}/\text{plate}$ (and unrelated to dose), none of which reached the level of significance for mean values.

In order to settle the issue of such isolated increases, a third assay was run, but with only TA98 (+S9) over a range of seven doses, from 1 through 1000 $\mu\text{g}/\text{plate}$ (Report Table 8), with negative results (Report Table 10). Hence the author concluded that the apparent elevated counts seen in the second assay represented random fluctuations in the number of spontaneous revertents, i.e., were not compound nor treatment-related.

The general conclusion for this study was that MON 7200 was not mutagenic in Ames testing up to the limit of test substance solubility and dosing (5000 $\mu\text{g}/\text{plate}$).

- F. TB Evaluation - ACCEPTABLE. This study was well conducted with all appropriate controls and adequate procedures, and the negative results demonstrating MON 7200 not to be a mutagen in Ames testing are judged valid.

Attachments (Data Tables)

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ATTACHMENT I
(10) Data Tables

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

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04/04/90

Karl P. Baetcke
N/10/90

007863

DATA EVALUATION REPORT

I. SUMMARY

MRID No.: 410015-11
ID No.: 524-UGN
RD Record No.: 240,294
Caswell No.: 717C
Project No.: 9-1879

Study Type: Mutagenicity - Foreward mammalian gene mutation
in vitro (CHO/HPRT)

Chemical: Dithiopyr [3,5-pyridine-dicarbothioic acid,
2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoro-
methyl)-S,S-dimethyl ester]

Synonyms: MON-15100; MON-7200

Sponsor: Monsanto Company, St. Louis, MO

Testing Facility: Environmental Health Laboratory (EHL),
Monsanto Chemical, St. Louis, MO

Title of Report: CHO/HGPRT Gene Mutagenicity Assay with
MON-7200.

Author: Leonard J. Flowers

Study No.: ML-87-10/HL 87006 (RD No. 900, Volume 12)

Date of Issue: September 11, 1987

TB Conclusions:

Negative for inducing foreward gene mutation at the
hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) locus
in Chinese hamster ovary (CHO) cells treated up to cytotoxic
levels in the absence and presence of several concentrations
of metabolic activation (rat S9).

Classification (Core-Grace): ACCEPTABLE

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II. DETAILED REVIEW

A. Test Material - MON 7200 (Monsanto Agricultural)

Description: Light yellow solid
Batch (Lot): Dayton Batch 1
Purity (%): 93.7
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Established mammalian cell line

Species: Chinese hamster ovary (CHO)
Strains: Subclone K₁BH₄
Source: Dr. A.W. Hsie, ORNL (Oak Ridge, TN)

C. Study Design (Protocol) - This study was designed to assess the mutagenic potential of MON-7200 when administered in vitro to cultures of Chinese hamster ovary cells. The procedures employed were standardized techniques as found in the scientific literature, and referenced in the Final Report.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - A preliminary range-finding, cytotoxicity test was performed in which cell cultures were treated at 37.5 °C for 3 hours with nine concentrations of test material (ranging from 0.3 to 1000 ug/mL), in the absence as well as in the presence of four concentrations of a mammalian metabolic activation system (1, 2, 5 and 10%), purchased from Litton Bionetics, and consisting of the 9000X g supernatant of liver homogenates from Sprague-Dawley male rats (S9) pretreated with Aroclor 1254 (a mixture of PCB used to enhance hepatic metabolizing monooxygenase enzymes), plus appropriate generating cofactors (=S9 mix). Following discarding of the treatment media, cultures were assessed for cloning efficiency (CE = no. of colonies/no. of cells plated), returned to incubation for 6 to 9 days, following which relative survivals (relative to the solvent control = 100%) were determined (RS = CE (treated)/CE (DMSO control)). Doses for the mutagenesis assays were selected based on concentrations in this range-finder that (according to the investigator) "... induced significant cytotoxicity . . ." under the various activation conditions (i.e., no S9, or with 1, 2, 5, or 10 percent S9).

In an initial mutagenesis assay, CHO cell cultures were exposed in triplicate for 3 hours to the test article at each of three concentrations (selected from the preliminary toxicity test), or to DMSO (solvent, negative, control), or to the mutagens ethylmethanesulfonate (200 μ g/mL EMS, for a nonactivation positive control), or benzo(a)pyrene (1 μ g/mL B(a)P, serving as positive control for S9-supplementation).

Following removal of treatment media, cultures were processed as described above for cytotoxicity determination, except additional cells were cultured in hypoxanthine-free medium for 7 to 9 days in order for the mutant phenotype to be expressed, followed by 6 to 10 days in a selecting medium consisting of hypoxanthine-free F12 supplemented with 10 mM 6-thioguanine (TG) and serum, which would permit only mutant colonies to survive. After this selection period, colonies were fixed, stained and counted. Results were expressed as mutant frequency (MF), and calculated as:

$$MF = \frac{\text{No. mutant colonies}}{\text{No. cells plated}} \times \frac{1}{CE}$$

Data were analyzed according to the method developed specifically for the CHO/HGPRT mutation assay,* by which MF values are transformed by the equation: $Y = (X+1)^{0.15}$, following which Student's t-test is used to compare treatment data to solvent control values. The method also permits determination of dose-response relationships, using a computer program also developed by one of the authors.

The entire experiment was repeated once ("confirmatory assay").

- E. Results - [Individual test data from this study were provided, as APPENDIX II, as well as summarized in APPENDIX I.]

Results from the preliminary range-finder revealed "significant" cytotoxicity (defined by the investigator as an RS between 25 and 50 percent) for the various activation conditions beginning at 70 μ g/mL (-S9), 10

*Snee, R.D. and J.D. Irr (1981) Design of a statistical method for the analysis of mutagenesis at the hypoxanthine guanine phosphoribosyl transferase locus of Chinese hamster ovary cells. MUTATION RES. 85: 77-93.

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ug/mL (1, 2, and 5 percent S9) and 30 ug/mL (10 percent S9). (Report Table 1, attached to this DER). Hence, in the initial mutagenesis assay, the following concentrations of MON 7200 were employed: 30, 100, and 300 ug/mL without activation; 3, 10, and 30 ug/mL under all activation conditions (1, 2, 5, and 10 percent).

No statistically significant increases in MF were recorded in any test culture of this initial assay (Report Table 2).

A separate confirmatory experiment was conducted, with an expanded schedule of test doses (10, 30, 100, 200, and 300 ug/mL/-S9), but under an activation condition with 5 percent S9 only (at doses of 3, 7, 10, 20 and 30 ug/mL). Again, even at severely toxic concentrations, no significant increases in mutant colonies were observed (Report Table 3, attached here).

By contrast, in both assays, the positive controls yielded the expected positive responses, with increases 48 to 50X background for EMS, 3 to 20X control for B(a)P.

The investigator concluded that MON 7200 was not mutagenic at the HGPRT locus in CHO cells treated in repeat experiments up to cytotoxic levels.

- F. TB EVALUATION - ACCEPTABLE. This study was performed under adequate conditions with sufficient appropriate controls to validate the negative (nonmutagenic) conclusions drawn.

ATTACHMENTS (Data Tables)

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ATTACHMENT I
(11) Data Tables (APP. I)

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007863

DATA EVALUATION REPORT

I. SUMMARY

MRID No.: 410015-12
ID No.: 524-UGN
RD Record No.: 240,294
Caswell No.: 717C
Project No.: 9-1879

Study Type: Mutagenicity - Chromosome damage in vitro (CHL)

Chemical: Dithiopyr [3,5-pyridine-dicarbothioic acid,
2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoro-
methyl)-S,S-dimethyl ester]

Synonyms: MON-15100; MON-7200

Sponsor: Monsanto Company, St. Louis, MO

Testing Facility: Institute of Environmental Toxicology,
Tokyo (Japan)

Title of Report: MON 7200: In Vitro Cytogenetics Test.

Author: Y.F.X. Sasaki

Study No.: ET-86-79 (RD 900, Volume 13)

Date of Issue: August 1, 1986

TB Conclusions:

Reported as negative for inducing structural chromosome aberrations in activated and nonactivated Chinese hamster lung cell cultures exposed to concentrations at the limit of test substance solubility (0.33 and 1.0 mM).

Classification (Core-Grade): Cannot be evaluated until essential procedural information and other unexplained items are provided.

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II. DETAILED REVIEW

A. Test Material - MON 7200 (from Monsanto Japan, Tokyo)

Description: Light brown solid
Batch (Lot): npb 3097391
Purity (%): 97.6
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Established mammalian cell line

Species: Chinese hamster lung [strain not provided]
Source: Dr. M. Ishidate, National Institute of Hygienic Science, Tokyo

C. Study Design (Protocol) - This study was designed to assess the clastogenic (chromosome-damaging) potential of MON 7200 when administered in vitro to cultures of Chinese hamster lung cells. The procedures employed were stated to derive from guidelines provided by the Ministry of Agriculture, Forestry and Fisheries (Japan), the OECD, as well as the Agency.

A statement affirming compliance with Agency GLPs was provided. A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis: A preliminary range-finding cytotoxicity test (as determined by mitotic index) was conducted, cell cultures being exposed to increasing concentrations of test article (up to $1 \times 10^{-3}M$), for 48 hours in the absence of activation, but limited to 6 hours in the presence of S9 mix.* Based upon the results of this range-finder, five concentrations of MON 7200 were selected for the main assay with/without activation.

In the main assays, nonactivated cultures were exposed in duplicate for either 24 or 48 hours, whereas S9-supplemented cells were treated for only 6 hours, followed by treatment-free periods in fresh medium of either 12 or 18 hours. Two hours prior to harvesting, treatment and/or supplemental incubated cultures were exposed to the mitotic-arresting alkaloid, colchicine ($0.5 \mu g/mL$), in order to accumulate cells in c-metaphase for easier scoring of structural aberrations.

*A mammalian metabolic activation system consisting of the Aroclor 1254 (PCB)-stimulated 9000X g liver homogenate from male Sprague-Dawley rats (5% S9), plus NADPH-generating cofactors.

The solvent DMSO served as the reference negative control, and the clastogens mitomycin-C (MMC, $6 \times 10^{-7}M$) and benzo(a)pyrene (BaP, $1.5 \times 10^{-4}M$) as positive controls, respectively, for the nonactivated and S9-supplemented series. Duplicate cultures were employed for each experimental treatment.

After conventional harvesting and cytological preparation for microscopy, chromosome aberrations were scored and classified according to conventional types from 200 "complete" metaphases (i.e., typical of CHL cells) per experimental point for test groups, but only 100 from positive controls. A metaphase containing at least one structural chromosome aberration was considered an "aberrant" metaphase. An indirect measure of cytotoxicity was determined, namely, mitotic index, calculated as the number of metaphases per 1000 cells found at a particular sampling time.

According to this investigator's criteria of assay results, a "positive" is declared if 10 percent or more aberrant metaphases are found; "negative" if there are less than 5 percent, and "inconclusive" if the frequency is between 5 and 10 percent.

[No statistical methods for data analyses of the data were reported.]

- E. Results - In the preliminary range-finder, no cytotoxicity (suppression in mitotic indices) was found up to the HDT ($1 \times 10^{-3}M$, or 1 mM). However, moderate to marked precipitation of test article were observed at both the HDT and the next lower concentration, $3.3 \times 10^{-4}M$ (approximately 0.33 mM).

In both activated and nonactivated cultures treated with MON 7200 at at least two sampling periods and up to limits of solubility, only occasional chromatid gaps or simple breaks were found, in total far less than the 5 percent limit set by the lab as defining a "negative" (Report Tables 1 through 4, attached to this DER). As found in the preliminary range-finder, mitotic indices were unaffected at all dose levels.

By contrast, both clastogens induced marked increases in aberrant metaphases (including complex rearrangements), greater than 50 percent for both MMC and B(a)P.

The investigator concluded that MON 7200 was not clastogenic under conditions of this study when tested up to the limits of solubility.

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- F. TB Evaluation: Although this single assay was conducted with apparently sufficient procedural controls, providing presumptively valid negative results, no information on harvesting techniques and other constrictions, nor on cytological (slide) preparation was provided in the Final Report.

Additionally, some explanation is required as to the discrepancy in the appearance and purity of the lot of MON 7200 used in the Japanese study ("light brown solid," 97.6%) compared to the Dayton batches used in the other (U.S.) studies of this submission ("light yellow powder," 91.5 to 93.7%).

Hence, this study is unacceptable until these missing items are provided.

Attachments (Data Tables)

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ATTACHMENT I
(12) Data Tables (1-4)

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Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

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04/04/90

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4/10/90

DATA EVALUATION REPORT

007863

I. SUMMARY

MRID No.: 410015-13
ID No.: 524-UGN
RD Record No.: 240,294
Caswell No.: 717C
Project No.: 9-1879

Study Type: Mutagenicity - DMA damage/repair (Rat HPC/UDS)

Chemical: Dithiopyr [3,5-pyridine-dicarbothioic acid,
2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoro-
methyl,-S,S-dimethyl ester)]

Synonyms: MON-15100; MON-7200

Sponsor: Monsanto Company, St. Louis, MO

Testing Facility: SRI International (SRI), Menlo Park, CA

Title of Report: Evaluation of the Potential of MON-7200 to
Induce Unscheduled DNA Synthesis in Primary
Rat Hepatocyte Cultures.

Author: J.P. Bakke and J.C. Mirsalis

Study No.: Monsanto No. SR-87-9/SRI Project No. LSC-3116
(RD No. 900, Volume 14)

Date of Issue: July 6, 1987

TB Conclusions:

MON 7200 was negative in repeat assays in primary rat
hepatocytes exposed to limit concentrations at the level
of insolubility (1000 ug/mL).

Classification (Core-Grade): ACCEPTABLE

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II. DETAILED REVIEW

A. Test Material - MON 7200 (from Monsanto Agricultural)

Description: Light yellow solid
Batch (Lot): Dayton Batch 1
Purity (%): 93.7
Solvent/Carrier/Diluent: Acetone

B. Test Organism - Primary mammalian (rodent) cell cultures

Species: Rat
Strains: Fischer-344
Age: Adult (12 to 18 wk)
Weights - males: 290 to 310 g
Source: Harlan, Indianapolis, IN

C. Study Design (Protocol) - This study was designed to assess the DNA damage/repair (by measuring UDS) potential of MON 7200 when administered in vitro to primary cultures of rat hepatocytes. A protocol was included which appeared to employ methods standardized by experts in this type of assay, as recorded in the scientific literature referenced in this Final Report.

A statement affirming compliance with Agency GLPs was provided.

A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - Hepatocytes isolated from two male F-344 rats by the Williams procedure were allowed to attach for up to 2 hours to coverslips in WME medium, following which triplicate cultures were exposed for 18 to 21 hours simultaneously to each of 10 concentrations of the test material, as well as to a constant concentration of tritiated (radioactive) thymidine (10 μ Ci/ml 3 H-TdR, with sp. act. approximately 80 Ci/ μ M). Following exposure, cultures were washed free of treatment medium, and the cells treated in situ with hypotonic sodium citrate (to swell the cells for easier counting), then the coverslips treated by conventional cytological techniques and mounted on glass microscope slides. Slides were dipped in photographic emulsion (Kodak NTB-2) dried, then sealed in light-tight slide boxes at -20 °C for 7 days. After exposure, the emulsions were developed (to reveal silver grains, a measure of repair of genotoxic damage by unscheduled DNA synthesis) by standard photographic techniques, and stained with methyl-green Pyronin Y.

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Two negative controls were incorporated in each assay: solvent (acetone) and untreated medium, plus a positive control using 2-acetylaminofluorene (2-AAF, 3 $\mu\text{g/mL}$), a mutagen known to induce UDS repair after metabolic conversion by primary rat hepatocytes. Net nuclear silver grains per nucleus (NG) were determined in 30 morphologically normal cells per slide (90 per treatment) by subtracting cytoplasmic (background) grain counts from nuclear counts (representing unscheduled DNA synthesis, UDS, a measure of repair from genotoxic damage), using an automatic counter (Artek 880/890) interfaced to a Zeiss microscope with an attached TV monitor. Count data were fed directly into a VAX 8800 computer, programmed to provide frequency distributions of NG for each test concentration, as well as average and median grain counts compared to control values. The investigators' criteria for genotoxic responses were stated as follows (from page 5 of FINAL REPORT):

Positive. A test article is considered positive if UDS (amount of incorporated ^3H -thymidine) is markedly elevated above that in the solvent control. The presence of a dose response, a change in the frequency distribution of cellular responses, an increase in the percentage of cells in repair, and reproducibility of data are also considered in classifying the test article as a "positive" or "negative." If the data warrant, a test article may be classified as a "weak positive."

Negative. A material is considered negative if testing has been performed to the limits of solubility or cytotoxicity, or at 5000 $\mu\text{g/mL}$, and if UDS is not significantly elevated above that in the solvent control.

- E. Results - DNA repair to MON-7200 exposure (UDS, as measured by net nuclear grain counts) was assayed in an initial experiment at concentrations ranging between 0.1 and 1000 $\mu\text{g/mL}$ (the limit for solubility), with negative results (Report Table 1, attached to this DER). A repeat experiment at test concentrations between 10 and 1000 $\mu\text{g/mL}$ was also negative, with NG values comparable to both solvent and medium controls. By contrast, 2-AAF produced the expected strong positive response (38.3 and 20.1 NG per nucleus - see attached data table).

The authors concluded that MON-7200 was not genotoxic in the rat hepatocyte-UDS repair assay.

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F. TB Conclusion: ACCEPTABLE

This study was conducted with appropriate procedure and adequate control to yield a valid negative result.

Attachment (Data Table)

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ATTACHMENT I
(13) Data Table

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