Caswell 573S

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

004384

APR 11 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Review of EUP Proposal No. 352-EUP-RER and Temporary Tolerance Proposal No. 4G3138 for Barley and Wheat.

From: Thomas Edwards, Pharmacologist
Hazard Evaluation Division (TS-769)

TO : Robert Taylor, PM25
Registration Division (TS-767)

THRU: Clint Skinner PhD, Toxicologist
Section Head, Review Section III

Chemical: DPX-M 6316

Caswell No. 573S

Accession No. 072848, 072849, 073010

The experimental use permit and the temporary tolerances can be toxicologically supported. The requested temporary tolerances are for barley grain 0.05 ppm, for barley straw 0.10 ppm, for wheat grain 0.05 ppm, and for wheat straw 0.10 ppm.

The results of the reviews are as follows:

DPX M6316 technical:

Acute oral LD50 Rat: 5000 mg/kg, IV, Guideline
4 Hour inhalation LC50, rat: 7.9 mg/L III Minimum
Acute Dermal LD50, Rabbit: 2000mg/kg III Minimum
90 Day Feeding Rat : NOEL 100 ppm
LEL 2500 ppm (Body and organ weight)
MTD 7500 ppm
Minimum
One generation Reproduction rat: Acceptable as range finder only

13 Week Feeding Study -Dog : NOEL 1500 ppm
LEL 7500 ppm (Body and adrenal weight reduced-Males)
Classification: Minimum

Teratology Rat : NOEL Fetotoxicity and teratogenesis 159 mg/kg/d
LEL Fetotoxicity and teratogenesis 725 mg/kg/d
Maternal toxicity was not evidenced
Classification: Minimum

DPX- M6316

Acute Oral LD50, RAT : 5000 mg/kg IV , Guideline
Acute Dermal LD50, Rabbit: 2000 mg/kg, III , Minimum
Dermal Sensitization: Not a Sensitizer, Minimum

A PADI of 0.005 mg/kg/d was based on a NOEL of 10 mg/kg/d from a 90 day rat subchronic feeding study with a safety factor of 2000.

The proposed tempoory tolerance of 0,05 ppm on barely and wheat resulted in a TMRC of 0.0078 mg/day using 2.60 % of the MPI of 0.3000 mg/kg/day.

Data requirements for registration in addition to the usual must include a statement of total triazine residues.

Also note that the following while not demonstrating hazard have deficiencies which must be corrected prior to full registration.

1. Mutagenicity in Salmonella typhimurium

2. Unscheduled DNA synthesis in rat hepatocytes

The CHO/HGPRT assay will be analyzed seperately.

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute oral toxicity, rat

Accession Number: 072847 (4)

MRID Number:

Sponsor: DuPont, No. 320-84

Contracting Lab:

Date: 7-24-84

Test Material: DPX-M6316, technical (96.5%)
2-Thiophenecarboxylic acid, 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]-sulfonyl]-, methyl ester

Protocol: "Male and female, 7-week-old, Crl:CD® rats were received from Charles River Breeding Laboratories, Kingston, New York. Rats were housed singly in suspended, stainless steel, wire-mesh cages. Each rat was assigned a unique identification number which was recorded on a card affixed to the cage. Purina Certified Rodent Chow® #5002 and water were available ad libitum. Rats were quarantined, weighed, and observed for general health for approximately one week prior to testing. Animal rooms were maintained on a timer-controlled, 12/hour/12 hour light/dark cycle; target humidity and temperature were 50 ± 10% and 74 ± 2°F.

"The EPA 1982 guidelines for pesticide registration were followed. Rats were fasted 24 hours prior to dosing with food being returned one hour after dosing. Single oral doses of the test material, as a 300 mg/mL suspension in Mazola® corn oil, were administered to a group of 5 male and 5 female rats at a dose level of 5,000 mg/kg. Individual dose amounts were calculated by using fasted body weights. After dosing and later in the day, rats were checked for clinical signs of toxicity. Rats were weighed once daily and observed twice daily (weekends excluded) during a 14-day recovery period. Following the recovery period, all rats were sacrificed and given a gross pathologic examination."

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Results:

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"Data:

Dose (mg/kg)	Fasted Mean Body Weight (g)	Suspension Concentration (mg/mL)	Mean Dose (mL)	Mortality Ratio
Males:				
5,000	217	300	3.6	0/5
Females:				
5,000	152	300	2.5	0/5

"Clinical Signs: Clinical signs were similar for male and female rats. They included a white crust on the external genitalia of several rats, diarrhea, and yellow-stained perineum for 1-3 days after dosing. Slight sporadic weight loss (Appendix I) was observed in females and 1 male rat had moderate initial weight loss followed by weight gain.

"Pathological Changes: Gross pathology revealed no abnormalities in either male or female rats."

Conclusions:

Oral LD50 (male and female rats): more than 5000 mg/kg

Acute oral toxicity category: IV

Core Classification: Guideline

W. Thomas Edwards

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TOXICOLOGY BRANCH
DATA REVIEW

Study: Acute oral toxicity, rat

Accession Number: 072847 (6)

MRID Number:

Sponsor: DuPont, No. 321-84

Contracting Lab:

Date: 7-26-84

Test Material: DPX-M6316, 75DF (75%)

Protocol: "Male and female, 7-week-old, Crl:CD® rats were received from Charles River Breeding Laboratories, Kingston, New York. Rats were housed singly in suspended, stainless steel, wire-mesh cages. Each rat was assigned a unique identification number which was recorded on a card affixed to the cage. Purina Certified Rodent Chow® #5002 and water were available adlibitum. Rats were quarantined, weighed, and observed for general health for approximately one week prior to testing. Animal rooms were maintained on a timer-controlled 12/hour/12 hour light/dark cycle; target humidity and temperature were 50 ± 10% and 74 ± 2°F.

"Rats were fasted 24 hours prior to dosing with food being returned one hour after dosing. Single oral doses of the test material, as a 300 mg/mL suspension in Mazola® corn oil, were administered to a group of 5 male and 5 female rats at a dose level of 5,000 mg/kg. Individual dose amounts were calculated by using fasted body weights. After dosing and later in the day, rats were checked for clinical signs of toxicity. Rats were weighed once daily and observed twice daily (weekends excluded) during a 14-day recovery period. Following the recovery period, all rats were sacrificed and given a gross pathologic examination."

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Results:

"Data:

Dose (mg/kg)	Fasted Mean Body Weight (g)	Suspension Concentration (mg/mL)	Mean Dose (mL)	Mortality Ratio
Males:				
5,000	224	300	3.8	0/5
Females:				
5,000	151	300	2.5	0/5

"Clinical Signs. Diarrhea (1 rat on the day of dosing was the only clinical sign observed in male rats. In female rats, diarrhea (1 rat) was also observed on the day of dosing. Other clinical signs included wet and/or yellow-stained perineum and slight sporadic weight loss.

"Pathological Changes. Gross pathology revealed no abnormalities in male rats. In 2/5 female rats, gross pathology revealed lungs that were discolored and contained diffuse foci (<0.5 mm) and/or grey patchy areas. These effects have been observed historically in untreated rats and are not considered compound related."

Conclusions:

Acute oral LD50 (male and female rats): more than 5000 mg/kg.

Acute oral toxicity category: IV

Core Classification: Guideline

W Thomas Edwards

TOXICOLOGY BRANCH
DATA REVIEW

Study: Acute dermal toxicity, rabbit

Accession Number: 072847 (7)

MRID Number:

Sponsor: DuPont

Contracting Lab: Hazleton Labs., No. 201-728

Date: 4-12-84

Test Material: DPX-M6316, technical
2-thiophenecarboxylic acid, 3-[[[[(4-methosy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-amino] sulfonyl]-, methyl ester

Protocol: "New Zealand White rabbits were received from Hazleton Dutchland, Denver, Pennsylvania. Five rabbits of each sex were assigned to this study.

"Prior to initiation, the hair was closely clipped from the back of each rabbit. Just prior to compound application, the skin of all animals in each group was abraded with minor incisions which were sufficiently deep to penetrate the stratum corneum, but not deep enough to disturb the derma or to produce bleeding. The test material, mixed with approximately 3 ml tap water to form a paste, was applied to the skin of each rabbit at a dose level of 2000 mg/kg. The test material remained in contact with the skin for 24 hours by means of nonabsorbent binder composed of rubber damming. Plastic collar restrainers were placed on the animals at time of treatment and removed on Day 7.

"Twenty-four hours following application, the binders were removed, the residual amount of the test material was estimated, and the exposure sites were wiped with gauze to preclude further exposure of the animals to the test material. The test material was administered dermally because potential human exposure is by the dermal route.

"All of the rabbits were observed for mortality and signs of toxic and pharmacologic effects once daily for 14 consecutive days. Dermal responses were graded and scored on Day 1 according to the system of Draize (1959).

"At termination (Day 14), all rabbits were sacrificed with T-16® Euthanasia Solution (Taylor Pharmacal Company, Decatur, Illinois) without necropsy."

Results: "When tested by this procedure, no mortality, toxicity or dermal effects were observed."

Conclusions:

Acute dermal LD50 (rabbit): more than 2000 mg/kg.

Acute dermal toxicity category: III

Core Classification: Minimum

W Thomas Edwards

TOXICOLOGY BRANCH
DATA REVIEW

Study: Acute dermal toxicty, rabbit

Accession Number: 073010 (7)

MRID Number:

Sponsor: DuPont, No. 201-726

Contracting Lab:

Date: 4-12-84

Test Material: DPX-M6316, 75DF

Protocol: "New Zealand White rabbits were received from Hazleton Dutchland, Denver, Pennsylvania."

"Prior to initiation, the hair was closely clipped from the back of each rabbit. Just prior to compound application, the skin of all animals in each group was abraded with minor incisions which were sufficiently deep to penetrate the stratum corneum, but not deep enough to disturb the dermal or to produce bleeding. The test material, mixed with approximately 3 ml tap water to form a paste, was applied to the skin of each rabbit at a dose level of 2000 mg/kg. The test material remained in contact with the skin for 24 hours by means of nonabsorbent binder composed of rubber damming. Plastic collar restrainers were placed on the animals at time of treatment and removed on Day 7.

"Twenty-four hours following application, the binders were removed, the residual amount of the test material was estimated, and the exposure sites were wiped with gauze to preclude further exposure of the animals to the test material."

"All of the rabbits were observed for mortality and signs of toxic and pharmacologic effects once daily for 14 consecutive days. Dermal responses were graded and scored on Day 1 according to the system of Draize (1959). Individual body weights were recorded at initiation, Day 7, and at termination."

Results: "When tested by this procedure, the test material produced no mortality or signs of toxicity other than dermal erythema which cleared by seven days.

"All animals exhibited well-defined erythema at Day 1. On Day 3, one of each sex showed very slight erythema. The remaining animals were clear of erythema."

Conclusions:

Acute dermal LD50 (rabbit): more than 2000 mg/kg.

Acute dermal toxicity category: III

Core Classification: Minimum

W Thomas Edwards

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TOXICOLOGY BRANCH
DATA REVIEW

Study: Primary eye irritation, rabbit

Accession Number: 072847 (8)

MRID Number:

Sponsor: DuPont

Contracting Lab: Hazleton Labs., No. 201-724

Date: 3-28-84

Test Material: DPX-M6316, 75DF (75% a.i.), a formulation of 2-thiophenecarboxylic acid, 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-amino] sulfonyl]-, methyl ester

Protocol: "New Zealand White rabbits were received from Hazleton Dutchland, Inc., Denver, Pennsylvania." Six anmials were used.

"Prior to instillation of the test material, both eyes of each rabbit were examined following staining with 2% fluorescein sodium solution (Alcon Laboratories, Inc., Fort Worth, Texas) to confirm the absence of corneal defects. Only rabbits initially free of corneal defects were used in this study. A 48 mg aliquot of the test solution was placed into the conjunctival sac of the left eye of each rabbit. The eye was gently held closed for approximately one second following instillation in six eyes. In three eyes, the eyes were held closed for one second and then 10 seconds after instillation the treated eyes were flushed with lukewarm water for one minute. The right eye of each rabbit was not treated, and thus served as a negative control.

"Eye irritation was scored and graded at 24, 48, and 72 hours according to the system of Draize (1959). A copy of the scale used in scoring the ocular lesions is presented on page 6. The treated eye of each rabbit was examined using fluorescein dye at 24 hours. Any corneal defects were reconfirmed by examination at 48 and 72 hours."

Results: "Corneal opacity (Grade 1) covering one-quarter of the cornea was observed in four unwashed eyes at 24 hours postapplication, persisting through 48 hours in two eyes. Iritis (Grade 1) was observed in two unwashed eyes at 24 hours only. Conjunctive redness (Grades 1 or 2) was observed in all unwashed eyes at 24 hours, persisting through 48 hours in four eyes. Conjunctival chemosis (Grade 1) was observed in four unwashed eyes at 24 hours only. Conjunctive discharge (Grade 1) was observed in five unwashed eyes at 24 hours only. All ocular irritation cleared by 72 hours in the unwashed eyes.

"No ocular irritation was observed in any of the washed eyes. Fluorescein examinations were negative in all washed eyes.

"DPX-M6316-24 75DF Weed Killer in the unwashed eyes produced moderate iritis in two of six animals, small scattered corneal opacities in four, and slight to moderate conjunctival irritation in six. All unwashed eyes were cleared of all effects by 72 hours. The test material produced no effects in the washed eyes."

Conclusions:

Primary eye irritation was moderate.

Primary eye irritation Category: III

Core Classification: Guideline

W Thomas Edwards

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TOXICOLOGY BRANCH
DATA REVIEW

Study: Primary dermal irritation, rabbit

Accession Number: 072847 (9)

MRID Number:

Sponsor: DuPont

Contracting Lab: Hazleton Labs., No. 201-725

Date: 4-2-84

Test Material: DPX-M6316, 75DF (75%)

Protocol: "New Zealand White rabbits were received from Hazleton Dutchland, Inc., Denver, Pennsylvania. Six male rabbits were assigned to this study.

"Prior to treatment, the dorsal area of each rabbit was clipped free of hair, and four sites were chosen for application of Haskell No. 15,299. The skin of two sites of all animals was abraded with minor incisions which were sufficiently deep to penetrate the stratum corneum, but not deep enough to produce bleeding. Two sites remained intact. A 0.5 gram aliquot of the test material was introduced to skin premoistened with water under an approximate one inch square gauze patch which was secured in place with transparent tape. The trunk of each rabbit was wrapped with impervious rubber damming. The rabbits were then immobilized in stocks for 24 hours without food and water.

"Twenty-four hours following application, the binders and patches were removed and the exposure sites were wiped with a dry towel to preclude further exposure of the animal to the test material.

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"Dermal responses were graded and scored at 24 and 48 hours according to the system of Draize (1959). A copy of the scale used in scoring erythema and edema as well as a key to other dermal effects are presented on page 5. All animals were observed once daily for mortality.

"After termination (48 hours postapplication), all rabbits were sacrificed with T-61® Euthanasia Solution (Taylor Pharmacal Company, Decatur, Illinois) and discarded without necropsy."

Results: "The primary irritation score was zero in all six animals. No erythema, edema, or other dermal effects were observed during the study."

Primary dermal category: IV

Case Classification: Guideline

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TOXICOLOGY BRANCH
DATA REVIEW

Study: Dermal sensitization study, guinea pig

Accession Number: 072847 (10)

MRID Number:

Sponsor: DuPont

Contracting Lab: Hazleton Labs. No. 201-727

Date: 7-9-84

Test Material: DPX-M6316, 75DF

Protocol: "Twenty-three young male albino guinea pigs of the Hartley strain were received from Hazleton Dutchland, Inc., Denver, Pennsylvania.

"Three guinea pigs were used in determining the induction and challenge dose levels, and the remaining 20 guinea pigs were divided into two test groups (10/group) and used in testing for primary irritation and sensitization.

"Based on the range finding study results, concentrations of 5 and 50% were used for the next phase of this study."

"Primary Irritation

Ten test and 10 control guinea pigs were treated in the primary irritation phase. The dorsal skin was clipped free of hair and two sites on each animal were chosen for a single application. Two concentrations (5% and 50% suspensions in distilled water) of the test material were applied to separate test sites on each animal's back in an area approximately 25 mm in diameter. The resulting irritation scores were compared to the challenge scores as determined at the end of the sensitization period."

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"Intradermal Induction of Sensitization

The same 20 animals were used in the primary irritation phase were treated during the induction phase. The sacral/hip area of each animal was clipped free of hair and an intradermal injection of a 0.1 ml aliquot of the test material suspension (1% in distilled water) was given. Control animals received a single 0.1 ml aliquot intradermal injection on the vehicle. Injections were repeated weekly on alternating sides for an additional three weeks for a total of four injections."

"Challenge Phase

Thirteen days after the final sensitization application was administered, the backs of all animals (both test and control groups) were shaved. The following day, a 0.05 ml aliquot of the challenge test suspension at 5% and 50% was applied to the assigned two test sites on each animal in an area approximately 25 mm in diameter. The 10 test animals, as well as the 10 control animals, were exposed to the same challenge doses."

"Observations and Records

At 24 and 48 hours after each application in the range-finding primary irritation, and challenge phases, the test sites were examined and scored according to the method of Draize (1959). After each induction phase treatment, the test sites were observed for necrosis and erythema at 24 hours only.

"Throughout the study, all animals were observed for mortality and moribundity."

"Sacrifice and Gross Pathology

At termination, all surviving animals were sacrificed with T-16® Euthanasia Solution (Taylor Pharmacal Company, Decatur, Illinois) and discarded without necropsy."

Results

Mortality - no mortality was observed

Sensitization

As seen in table 1 (attached) six animals exhibited slight erythema after administration of the primary phase in site 2.

Slight erythema was also seen in most control and treated animals 24hrs after each induction injection.(Table 2)

Slight erythema was also noted in one treated and one control animal 24 hrs after administration of the challenge phase (Table 3).

Conclusions:

The data support the authors conclusion that DPX-M6316 was not shown to be a sensitizing agent in guinea pig.

Core Classification: Minimum

Table 1 - Continued
Individual Dermal Irritation Scores
Primary Irritation and Sensitization Study
of Haskell No. 15,299 in Guinea Pigs
Primary Phase - Control Animals

Animal Number	Site Number	Observations			
		24 Hours		48 Hours	
		Erythema	Necrosis	Erythema	Necrosis
H05955	1	0	0	0	0
H05956	1	0	0	0	0
H05957	1	0	0	0	0
H05958	1	0	0	0	0
H05959	1	0	0	0	0
H05960	1	0	0	0	0
H05961	1	0	0	0	0
H05962	1	0	0	0	0
H05963	1	0	0	0	0
H05964	1	0	0	0	0

Table 1
Individual Dermal Irritation Scores
Primary Irritation and Sensitization Study
of Haskell No. 15,299 in Guinea Pigs
Primary Phase - Test Animals

Animal Number	Site Number	Dose Level (%)	Observations			
			24 Hours		48 Hours	
			Erythema	Necrosis	Erythema	Necrosis
H05945	1	5	0	0	0	0
	2	50	1	0	0	0
H05946	1	5	0	0	0	0
	2	50	1	0	0	0
H05947	1	5	0	0	0	0
	2	50	1	0	0	0
H05948	1	5	0	0	0	0
	2	50	0	0	0	0
H05949	1	5	0	0	0	0
	2	50	0	0	0	0
H05950	1	5	0	0	0	0
	2	50	1	0	0	0
H05951	1	5	0	0	0	0
	2	50	0	0	0	0
H05952	1	5	0	0	0	0
	2	50	1	0	0	0
H05953	1	5	0	0	0	0
	2	50	0	0	0	0
H05954	1	5	0	0	0	0
	2	50	1	0	0	0

Table 2
Individual Dermal Irritation Scores
Primary Irritation and Sensitization Study of Haskell No. 15,299 in Guinea Pigs

Animal Number	Week 1 - Left Side Injection 1		Week 2 - Right Side Injection 2		Week 3 - Left Side Injection 3		Week 4 - Right Side Injection 4	
	Erythema	Necrosis	Erythema	Necrosis	Erythema	Necrosis	Erythema	Necrosis
Induction Phase - Test Animals								
H05945	1	0	1	0	1	0	1	0
H05946	1	0	1	0	2	0	1	0
H05947	1	0	1	0	1	0	1	0
H05948	1	0	1	0	1	0	1	0
H05949	0	0	1	0	1	0	1	0
H05950	1	0	1	0	1	0	1	0
H05951	1	0	1	0	2	0	1	0
H05952	1	0	1	0	1	0	1	0
H05953	1	0	1	0	1	0	1	0
H05954	1	0	1	0	1	0	1	0
Induction Phase - Control Animals								
H05955	1	0	1	0	1	0	1	0
H05956	1	0	1	0	1	0	1	0
H05957	1	0	1	0	1	0	1	0
H05958	1	0	1	0	1	0	1	0
H05959	1	0	1	0	1	0	1	0
H05960	1	0	1	0	1	0	1	0
H05961	1	0	1	0	1	0	1	0
H05962	1	0	1	0	1	0	1	0
H05963	1	0	1	0	1	0	1	0
H05964	1	0	1	0	1	0	1	0

Table 3
Individual Dermal Irritation Scores
Primary Irritation and Sensitization Study
of Haskell No. 15,299 in Guinea Pigs
Challenge Phase - Test Animals

Animal Number	Site Number	Dose Level (%)	Observations			
			24 Hours		48 Hours	
			Erythema	Necrosis	Erythema	Necrosis
H05945	1	5	0	0	0	0
	2	50	1	0	0	0
H05946	1	5	0	0	0	0
	2	50	0	0	0	0
H05947	1	5	0	0	0	0
	2	50	0	0	0	0
H05948	1	5	0	0	0	0
	2	50	0	0	0	0
H05949	1	5	0	0	0	0
	2	50	0	0	0	0
H05950	1	5	0	0	0	0
	2	50	0	0	0	0
H05951	1	5	0	0	0	0
	2	50	0	0	0	0
H05952	1	5	0	0	0	0
	2	50	0	0	0	0
H05953	1	5	0	0	0	0
	2	50	0	0	0	0
H05954	1	5	0	0	0	0
	2	50	0	0	0	0

Table 3 - Continued
Individual Dermal Irritation Scores
Primary Irritation and Sensitization Study
of Haskell No. 15,299 in Guinea Pigs
Challenge Phase - Control Animals

Animal Number	Site Number	Dose Level (%)	Observations			
			24 Hours		48 Hours	
			Erythema	Necrosis	Erythema	Necrosis
H05955	1	5	0	0	0	0
	2	50	0	0	0	0
H05956	1	5	0	0	0	0
	2	50	0	0	0	0
H05957	1	5	0	0	0	0
	2	50	0	0	0	0
H05958	1	5	0	0	0	0
	2	50	0	0	0	0
H05959	1	5	0	0	0	0
	2	50	0	0	0	0
H05960	1	5	0	0	0	0
	2	50	0	0	0	0
H05961	1	5	0	0	0	0
	2	50	0	0	0	0
H05962	1	5	0	0	0	0
	2	50	1	0	0	0
H05963	1	5	0	0	0	0
	2	50	0	0	0	0
H05964	1	5	0	0	0	0
	2	50	0	0	0	0

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TOXICOLOGY BRANCH
DATA REVIEW

Study Type: 4-hour inhalation toxicity, rats

Accession Number: 072847 (5)

MRID Number:

Sponsor: DuPont, No. 340-84

Contracting Lab:

Date: 7-30-84

Test Material: DPX-M6316, technical, INM-6316-22, 2-Thiophenecarboxylic acid, 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-, methyl ester

Protocol:

"Exposure Protocol

Groups of ten male and ten female rats were exposed for four hours to atmospheres containing INM-6316-22 dust. During exposures, rats were restrained in perforated, stainless steel holders which were positioned in the 38 L glass chamber so that only the rats' heads were exposed to the test atmosphere. A similar group of control rats was exposed to air alone. Rats were weighed and observed daily during a two-week recovery period except for the Saturday and Sunday of the second week post exposure.

"Animals

Male and female Crl:CD® rats were obtained from Charles River Breeding Laboratories, Kingston, New York. Male rats used in these studies were eight weeks old and weighed between 260 and 268 grams; females were nine weeks old and weighed between 180 and 189 grams. Rats were housed in pairs (sexes separate).

"Atmospheric Generation

Atmospheres containing INM-6316-22 dust were generated by passing pressurized air through a 2-stage hourglass-shaped system. The bottom stage served as the dust reservoir; the top acted as an elutriator. Air introduced at the bottom carried particles upward to the elutriator. Dilution air was added to the elutriator. Vibrators and a stirring rod were used to reduce dust buildup on the walls of the generator. Dust concentration was controlled by varying the two air flows.

"Analytical

Calibrated volumes of chamber atmosphere were drawn through preweighed, glass-fiber filters, usually at 15- to 20-minute intervals. Filters were weighed on a Cahn® 26 Automatic Electrobalance. Atmospheric concentration of test material was determined from filter weight gain.

"Data for calculating mass median diameter and percent of respirable particulate were obtained with a Sierra® Model 210 cascade impactor. Chamber temperatures were monitored with a mercury thermometer. Chamber oxygen levels were monitored with a BioMarine® Model 225 oxygen analyzer. Relative humidity was measured with a Bendix® Model 566 psychrometer.

"Pathology

Fourteen days after exposure three male and three female rats from each group were necropsied and the lungs, kidneys, and liver examined microscopically."

"Exposure Data

Rats were exposed to a mean INM-6316-22 dust concentration (± SD) of 7.0 ± 2.7 mg/L. The mass median aerodynamic diameter of the dust was 6.8 um; 64% of the dust was respirable (< 10 um aerodyanamic diameter). Chamber conditions during the exposures were:

- temperature: 24-25° test, 23°C control;
- oxygen: 20% test, 21% control;
- relative humidity: 50% test and control.

Results:

Body weights decreased in males and females for the day following exposure but recovered by two days post exposure.

Clinical Observations

Mortality - No mortality was observed

Symptoms - Red occular discharge (Stress response) was observed in some treated animals.

Gross and Microscopic Patholog - (lung,kidney,liver)

No macroscopic or microscopic alterations were reported.

Conclusions:

4-hour inhalations LD50 (rat): more than 7.9 mg/L

Inhalation toxicity category: III

Core Classification: Minimum

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TOXICOLOGY BRANCH
DATA REVIEW

Study Type: 90-Day feeding and one-generation reproduction, rat

Accession Number: 072847 (11)

MRID Number:

Sponsor: Du Pont, No. 89-84

Contracting Lab:

Date: July 20, 1984

Test Material: DPX-M6316, technical

Protocol:

A. 90-Day Feeding

"Seventy-eight male and 78 female CD® rats, born on April 4, 1983, were received from Charles River Breeding Laboratories, Kingston, NY, on April 26, 1983."

"After release by a laboratory animal veterinarian at the end of the pretest period and on the basis of body weight gain and freedom from any grossly apparent clinical signs of disease or injury, 64 rats of each sex were divided by computerized, stratified randomization into four groups of 16 male and four groups of 16 female rats such that the mean body weights of each group of rats within a sex were approximately equal. The order of rats within each group was then randomized. The first ten rats assigned to each group were designated to be sacrificed at the end of the 90-day feeding period. The remaining six rats assigned to each group were designated for the reproduction phase of the study."

Groups and dosages were as follows:

"Treatment Group		Dietary Concentration of INM-6316* (ppm)
Male	Female	
I	II	0 (Control)
III	IV	100
V	VI	2,500
VII	VIII	7,500

* weight/weight concentration active ingredient, INM-6316 (adjusted for purity)"

"During the 90-day feeding period of the study all rats were weighed once weekly."

"The amount of food consumed by each group was determined weekly. Food consumption and body weight data were used to calculate mean individual daily food consumption, food efficiency (utilization of food for weight gain), and daily intake of INM-6316."

"Throughout the study all rats were observed at least once daily for abnormal behavior or appearance and moribundity. In addition, during the 90-day feeding phase of the study, all rats were handled individually once weekly and examined for abnormal appearance or behavior. Mortality during the study was recorded."

"Approximately one, two, and three months after initiation of the study, hematological, clinical chemical, and urologic analyses were conducted. For these analyses, the ten rats from each treatment group that had been designated to be sacrificed at the end of the 90-day feeding period were fasted for approximately 16 hours prior to collection of blood. Urine from each rat was collected during the fasting period. Peripheral blood for hematological and clinical chemical evaluations was then collected on each rat via tailcut.

Hematological parameters examined at each interval consisted of erythrocyte, leukocyte, differential leukocyte, and platelet counts, hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). Blood smears were prepared for reticulocyte counts, but were not read because results of hematological analyses did not warrant their evaluation."

"Clinical chemistry evaluations consisted of measurement of serum alkaline phosphatase (AP), alanine-aminotransferase (ALT), and aspartateaminotransferase (AST) activities, blood urea nitrogen (BUN), total serum protein, albumin, globulin (calculated), creatinine, cholesterol, glucose, calcium, sodium and potassium.

Urinalyses consisted of quantitative measures of urine volume, pH, and osmolality, and semi-quantitative measures of glocose, protein, bilirubin, urobilinogen, ketone, and occult blood. Urine color and transparency were recorded and sediment from each urine sample was examined microscopically."

"On test days 92 and 93, the surviving rats designated for the 90-day phase of the study were sacrificed and necropsied. For the sacrifice, each group of ten rats was divided into two subgroups of five rats each. The order of sacrifice was

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determined by computer randomization of the resulting eight subgroups within each sex. For rats in the 90-day sacrifice, brain, heart, liver, spleen, kidneys, and testes were weighed and organ weight/final body weight ratios calculated. All rats that died before the end of the study were also necropsied. The following tissues, taken from rats designated to be evaluated for subchronic toxicity that died prior to the end of the 90-day feeding period (tissue integrity permitting) and from high-dose and control group rats in the 90-day sacrifice, were examined microscopically: thymus, spleen, femoral bone marrow, lymph nodes, heart, thoracic aorta, trachea, lungs, salivary glands, esophagus, stomach, small intestine (duodenum, jejunum and ileum), large intestine (colon and cecum), liver, pancreas, kidneys, bladder, pituitary, thyroid - parathyroids, adrenals, testes, epididydmies, prostate, ovaries, corpus and cervix uteri, vagina, brain, eyes, muscle (thigh), bone (femur), and all gross lesions. All gross lesions and the heart, liver, and kidneys from rats in the low- and intermediate-level groups that have been subjected to the 90-day sacrifice were also examined histopathologically. However, since microscopic findings in the high-level group did not reveal any target organs, the other tissues listed above which had been collected from rats in the low- and intermediate-dose groups were not evaluated histologically. Bone marrow smears were prepared from all rats sacrificed by design at the end of the 90-day feeding period, but experimental findings did not justify their evaluation."

"Tissue (brain, liver, kidney, spleen, muscle, testis, and fat) and blood samples were collected from rats at the 90-day sacrifice and pooled by test group. Urine and feces were also collected from these rats just prior to the 90-day sacrifice. All samples were frozen and sent to the Agricultural Chemicals Department for residue analyses. The results of any of these analyses will appear in a separate Agricultural Chemicals Department report."

B. "<u>Reproduction Substudy</u>"

Survivors among the six designated rats in each test group were used in a one-generation, two-litter reproduction substudy. Throughout the reproduction substudy all rats received their respective treatment group's diets. Each female (F_0) rat was housed with a randomly selected F_0 male from the same treatment level for a period of fifteen days. During the fifteen-day mating period, females were checked daily for the presence of copulation plugs and the date of first observation of plugs was recorded. After the fifteen-day mating period, each female was housed individually in polypropylene cages with Bed-O-Cob® cage bedding. Starting six days later, female rats were examined at least three times daily for the birth of pups (F_{1a}). Live and

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dead pups in F_{1a} litters were counted as soon after delivery as possible, and 1, 4, 12, and 21 days postpartum. Litters containing more than ten pups were reduced to this number on day 4 postpartum by including in the litter an equal number of male and female pups (when possible) that were, by gross appearance, representative of the health status of all pups in the litter. Extra pups were sacrificed and discarded without pathological evualation. The total pup weight for each of the F_{1a} litters was determined approximately 24 hours and four days after birth (prior to litter size reduction on day 4). F_{1a} pups were individually weighed, sexed, sacrificed, and discarded without pathological evaluation on day 21 postpartum. Pups found dead before 21-days postpartum were discarded without pathological evaluation. Mortality of F_0 rats and pups were recorded. Fertility, gestation, viability, and lactation indices were calculated.

Because of unusually low fertility and viability in the control-group rats after the first mating, all rats within each treatment group were remated seven days after the last F_{1a} litter was weaned. Females were mated to different males than for the first mating. Procedures for the second mating were the same as described for the first. Surviving F_0 female rats were weighed, sacrificed, and discarded without pathological evaluation six days after the last litter from the second mating (F_{1b}) was weaned. Surviving F_0 males were also sacrificed and discarded without pathological evaluation at this time.

Statistical Analyses

Body weights, body weight gains, organ weights, and clinical laboratory measurements were analyzed using a one-way analysis of variance. When the test for differences among test group means (F-test) was significant, pairwise comparisons were made between test and control groups. For body weights and body weight gains these comparisons were made with the least significant difference (LSD) test. For organ weights, the comparisons were made using both LSD and Dunnett's tests. Barlett's test for homogeneity of variances was calculated on the organ weight and clinical laboratory data. Significance for the comparison of means was judged at the $p \leq 0.05$ level."

Results

A. 90 Day Feeding

Mortality - No dose relation in mortality was observed.

Body Weights - Mean body weights in the 2500 and 7500 ppm groups were significantly lower than controls throughout the study.

Body Weight Gains - Mean body weight gains were significantly reduced in 2500 and 7500ppm males and females.

Clinical Pathology - Significant increases in BUN,and decreases in serum globulins and total protein occured in the high level (7500ppm) males at 3 months of treatment.
Alterations in females were not meaningful.

Organ Weight - Spleen weight was decreased in a dose related manner (p=.o5) in 2500 and 7500 ppm males. Liver and heart weight were reduced at 7500 ppm.

Relative brain weight was significantly increased in 2500 &7500 ppm males as were relative kidney and testes weight in 7500ppm males.

In 7500ppm females relative brain and heart weight were increased.

Pathological Evaluation - No gross or microscopic findings related to treatment were reported.

Conclusion - A NOEL was demonstrated at 100 ppm.
The LEL was 2500 ppm (Body and orgnan weights)
An MTD was achieved at the HDT 7500 ppm.

Core Classification - Minimum

Results

B. Reproduction Substudy

Only one surviving litter was produced in the control group. Of the treated groups two produced no litters and one dam produced litters with no live pups.

Another mating produced only one control litter. This litter was compared to the surviving treated litters and reproduction indices were derived.

Conclusion

Due to the lack of sufficient numbers of litters this study is acceptable only as a pilot study.

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: 13-Week feeding study, dog

Accession Number: 072848 (12)

MRID Number:

Sponsor: Du Pont

Contracting Lab: Hazleton Labs., No. 201-661

Date: 7-23-84

Test Material: DPX-M6316, technical

Protocol:

"Thirty-two, healthy, young adult (25 to 26 weeks old at the initiation of treatment) purebred beagles were selected for this study. The dogs were received from Hazleton Research Animals, Inc., Cumberland, Virginia, on November 17, 1983, and were quarantined for 32 days following receipt. During this time, each dog was observed daily with respect to appearance, behavior, appetite (qualitative) and fecal elimination.

"The 16 males and 16 females used in this study were stratified by weight and assigned to the following groups using a table of random permutations of nine (Cochran and Cox, 1957).

Group Number	Number of Animals		Treatment Level
	Males	Females	ppm
1	4	4	0
2	4	4	75
3	4	4	1500
4	4	4	7500

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"During the study, all of the dogs were observed twice daily for mortality and moribundity and once daily for appearance, behavior, fecal elimination, and signs of toxic and pharmacologic effects. These observations were recorded daily. Individual food consumptions were recorded daily and individual body weights are reported weekly beginning one week prior to the initiation of treatment.

"Clinical Pathology

The following clinical laboratory studies were performed on all dogs twice prior to treatment (Weeks -4 and -1) and during study Weeks 4, 8, and 13.

Hematology:
- Hemoglobin (HGB)
- Hematocrit (HCT)
- Erythrocyte count (RBC)
- Platelet count (PLATELET)
- Leukocyte count (WBC)
- Differential leukocyte count
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Leukocyte and erythrocyte morphology

Serum Chemistry:
- Urea nitrogen (BUN)
- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Alkaline phosphatase (ALK PHOS)
- Total protein (T PROT)
- Glucose (GLUCOSE)
- Total bilirubin (T BILI)
- Total cholesterol (T CHOL)
- Lactate dehydrogenase (LDH)
- Albumin (ALBUMIN)
- Albumin/globulin ratio (ALB/GLOB RATIO)
- Globulin (GLOBULIN)
- Uric acid (URIC AC)
- Creatinine (CREAT)
- Sodium (SODIUM)
- Potassium (POTAS)
- Calcium (CALCIUM)

Urinalysis:
- Appearance (APPEAR)
- Specific gravity (SP GR)
- Protein (PROTEIN)
- pH (PH)
- Glucose (GLUCOSE)
- Bilirubin (BILI)
- Ketones (KETONES)
- Microscopic examination of sediment
- Volume (U VOL)
- Occult blood (OC BLD-U)
- Urobilinogen (UROBIL)
- Osmolality (U OSMOL)
- Reducing substances (RED SUBS)

The clinical pathology samples were collected by jugular puncture (hematology and clinical chemistry) or cage pan run-off (urinalysis). The dogs were food and water fasted overnight prior to collection."

"Sacrifice and Necropsy

All animals were sacrificed following 13 weeks of treatment by exsanguination while under the influence of sodium thiamylal anesthesia. A complete gross examination was performed on each dog and necropsies were performed on each by appropriately trained personnel using procedures approved by board-certified pathologists.

The terminal body weight and following organ weights were recorded from each dog. From these data, the organ/body weight ratios were calculated.

- Brain (including brainstem)
- Heart
- Liver with gallbladder
- Thyroid with parathyroids
- Adrenals
- Spleen
- Kidneys
- Testes with epididymides
- Ovaries
- Pituitary

The following tissues from each dog were preserved in 10% neutral buffered formalin.

Brain (fore-, mid-, hind-)
Pituitary
Spinal cord (thoracic and lumbar)
Eyes (both)
Salivary glands (mandibular)
Thyroid with parathyroids
Trachea
Tongue
Esophagus
Heart and thoracic aorta
Spleen
Adrenals
Prostate
Ovaries
Uterus (one section from each horn)
Femur
Bone marrow (sternum)
Lung (sections from left diaphramatic and right apical)
Liver (two lobes)
Kidneys
Pancreas
Gallbladder
Nerve (sciatic)
Mammary gland
Cervix
Vagina
Tonsil
Small intestine (duodenum, jejunum, and ileum)
Large intestine (cecum, colon)
Urinary bladder
Testes with epididymides
Stomach (cardiac, fundic, and pyloric)
Skin
Muscle
Lymph nodes (mesenteric, mediastinal & mandibular)
Rib with osteochondral junction
Rectum
Unusual lesions

Bone marrow smears (prepared from rib marrow) were prepared from each dog and stained for possible future evaluation."

"Residue Analysis

Samples of tissue, blood, urine, and feces, pooled by sex and group, were collected from all dogs sacrificed at termination of the study, frozen, and shipped to the sponsor for possible future residue analysis. Samples collected included the following: feces (collected the day prior to the first day of sacrifice), blood (collected the first day of sacrifice), urine (aspirated from the bladder at the time of sacrifice), and tissues (consisting of brain, liver, kidney, spleen, muscle [rectus femoris], testis, and fat)."

"Histopathology

All of the preserved tissues were embedded in Paraplast®, sectioned, stained with hematoxylin and eosin, and examined microscopically."

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"Statistical Analyses

Mean body weight changes for Weeks 0-4, 8, and 13; total food consumption for Weeks 1-4, 1-8, and 1-13; clinical pathology data (excluding differential leukocyte counts, erythrocyte morphology, and qualitative urinalysis data); and organ weight data of the control group were compared statistically to the data of the treated groups of the same sex.

Tests for homogeneity of variances and ANOVA were evaluated at the 5.0% one-tailed probability level. Control vs. compound-treated group mean comparisons of the above data were evaluated at the 5.0% two-tailed probability level."

Results:

Mortality: There were no mortalities in the study

Body Weight: Male group 4 animals exibited a trend toward decreased body weight.

Organ Weight : A reduction in mean absolute and relative adrenal weight was seen in 7500 ppm males.

Clinical Chemistry: An apparent decrease in LDH was seen in 1500 and 7500 ppm males and females in weeks 4 and 13 respectively.

Hematology : A trend to decreased WBC was observed in male 7500 ppm animals at 13 weeks.

Histopathology: No treatment related alterations were observed.

Conclusion :

A NOEL was attained at 75 ppm.
An MTD was attained at 7500 ppm.

Core Classification : Minimum

1. CHEMICAL: INM-6316-7,2-Thiophenecarboxylic acid, 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-amino]carbonyl]amino]-sulfonyl]-methyl ester.

2. TEST MATERIAL: INM-6316-7 (DPX-M6316), 93.4%.

3. STUDY/ACTION TYPE: Mutagenicity-reverse mutation in Salmonella.

4. STUDY IDENTIFICATION: Russell, J.F., Arce, G.T., Sariff, A.M., et al. Mutagenicity evaluation in Salmonella typhimurium. (Unpublished study No. HLR 235-83 by Haskell Laboratory, Newark, DE for E.I. du Pont de Nemours and Co. Inc.; dated June 17, 1983.) Accession No. 072849.

5. REVIEWED BY:

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Principal Author
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Date: 3-14-85

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Genetic Toxicology
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Signature: I Cecil Felkner
Date: 3-19-85

W. Thomas Edwards
EPA Reviewer

Signature: _____
Date: _____

Clint Skinner, Ph.D.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. Under the conditions of the assay INM-6316-7 (DPX-M6316) was not mutagenic in Salmonella typhimurium strains TA1535, TA97, TA98, or

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TA100 with or without S9 activation at dose levels ranging from 0.1 to 20 µg/plate. However, the dose range selected was inappropriate to demonstrate a mutagenic response.

8. RECOMMENDATIONS:

The test material should be retested at a dose range that is verifiable as adequate based on maximum solubility and/or cytotoxicity. At a minimum, the highest dose of the test material should reduce the bacterial population by 63% since survival of 37% would represent an average of one lethality per cell exposed, and is the ideal dose for maximum mutagenic response.

9. BACKGROUND: Not applicable.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES: Not applicable.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. INM-6316-7 was described as a white solid with a purity of 93.4%. The test material was dissolved in dimethylsulfoxide (DMSO), also the solvent control, to final concentrations of 0.1, 0.5, 1.0, 5.0, 10, or 20 µg/plate.

2. The following strains of Salmonella typhimurium were used: TA1535, TA97, TA98, and TA100.

3. Toxicity of the test material was tested with strain TA1535 from an unspecified concentration up to 10,000 µg/plate.

4. The Ames[1] assay for mutagenicity was performed. The four tester strains were dosed at test material concentrations of (0.1, 0.5, 1, 5, 10, or 20 µg/plate nonactivated or with S9 activation) and plated in 4 replicates, except at the highest dose, which was only plated in duplicate. The solvent and positive controls were also plated in 4 replicates.

5. A test material is considered mutagenic when the probability that the number of revertants at one or more concentrations is greater than the number of revertants in the solvent control is > 0.01 and the probability that there is a positive correlation between the number of revertants and increasing concentrations of the test material is > 0.01.

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[1]Ames et al. Mutation Res. 31:347-364, 1975.

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6. Statistics: The mean number of revertants/plate were transformed by $Y = X^{0.2}$. The two analyses were performed. The first analysis compared the response observed at each concentration to the control by a t-test to determine significant increases in mutation frequency. The second analysis tested the significance of a dose-response relationship using a F-test.

B. Protocol:

A complete protocol was not reported. See Appendix A for details.

12. REPORTED RESULTS:

Toxicity Assay: Cytotoxicity was assessed with strain TA1535, using the test material at unspecified lower concentrations up to 10,000 µg/ plate. Cytotoxicity was reported at dose levels $\geq$ 5000 µg/ plate with metabolic (S9) activation and $\geq$ 500 µg/plate without S9 activation. Based on these results, nontoxic and slightly toxic concentrations of 0.1 to 20 µg/plate were chosen by the authors for the mutagenicity assay.

Mutagenicity Assay: In the solvent control the number of revertants for each tester strain was within the accepted range relative to published data[1]. The positive controls confirmed that the assay had an appropriate level of sensitivity (Table 1).

The number of revertant colonies reported for the various doses of test material were not increased over the solvent control (DMSO, Table 1), and there was no significant dose response.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "under the conditions tested" INM-6316-7 was not mutagenic with or without S9 activation.

B. Quality assurance measures were not reported.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that under the assay conditions, the dose range selected in this study for INM-6316-7 was not supported by the appropriate cytotoxicity data. Therefore, the data are unacceptable for the assessment of mutagenicity of INM-6316-7 in Salmonella typhimurium.

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[1]Ames et al. Mutation Res. 31:347-364, 1975.

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TABLE 1. Controls Used in Salmonella/Microsome Assay with INM-6316-7

Substance	Conc. (µg/plate)	S9 Activation	Strains/Revertants/Plate[a] TA1535	TA97	TA98	TA100
DMSO (100 µl)	-	-	22 96	17	94	
		+	14	129	22	101
MNNG	4	-	2875			
2-AA	2	+	200			
9-AA	50	-		936		
2-AA	1	+		457		
2-NF	25	-			1545	
2-AA	2	+			1669	
MNNG	4	-				2845
2-AA	1	+				647

[a]Average count of 4 plates, calculated by reviewers.
MNNG = N-methyl-N'-nitro-N-nitrosoguanidine.
2-AA = 2-aminoanthracene.
9-AA = 9-aminoacridine.
2-NF = 2-nitrofluorene.

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The authors did not state what criteria were used to evaluate toxicity. They reported that "cytotoxicity was observed at ≥ 5000 µg/plate in the presence of an activation system and ≥ 500 µg/plate in the absence of an activation system." Since no cytotoxicity data were reported for doses below 500 µg/plate and the dose range selected was 0.1 to 20 µg/plate, there is no scientific basis for the doses selected to assess the mutagenicity of INM-6316-7. It is our assessment that INM-6316-7 was not tested at the limit of its solubility or to the limits of its cytotoxicity.

15. COMPLETION OF ONE-LINER FORM FOR STUDY: Not applicable.

16. CBI APPENDIX: Appendix A, Methods and Materials, CBI pp. 1-4.

APPENDIX A

(Materials and Methods)

Harmony Reviews

Page _____ is not included in this copy.

Pages 43 through 46 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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EPA: 68-01-6561
TASK: 86
March 14, 1985

DATA EVALUATION RECORD

INM-6316-20

Unscheduled DNA-Synthesis/Rat Hepatocytes In Vitro

STUDY IDENTIFICATION: McCooey, K.T. Unscheduled DNA synthesis/rat hepatocytes in vitro. (Unpublished study No. 337-84 by Haskell Laboratory, Newark, DE for E.I. du Pont de Nemours and Co.; dated July 27, 1984.) Accession No. 072849 (T6).

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-14-85

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1. CHEMICAL: INM-6316-20 (DPX-M6316).

2. TEST MATERIAL: The test material was referred to as INM-6316-20 and was identified as 2-thiophenecarboxylic acid, 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-amino]carbonyl]amino]sulfonyl]-methyl-ester. Purity was estimated to be 95.6 percent and the identities of contaminants were not reported. No further description of the test material was reported.

3. STUDY/ACTION TYPE: Mutagenicity: unscheduled DNA synthesis in rat hepatocytes in vitro.

4. STUDY IDENTIFICATION: McCooey, K.T. Unscheduled DNA synthesis/rat hepatocytes in vitro. (Unpublished study No. 337-84 by Haskell Laboratory, Newark, DE for E.I. du Pont de Nemours and Co.; dated July 27, 1984.) Accession No. 072849 (16).

5. REVIEWED BY:

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Clint Skinner, Ph.D.
EPA Section Head

Signature: _____
Date: _____

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7. CONCLUSIONS:

A. Our conclusion is that under the conditions of this study INM-6316-20 did not induce significant increases in unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes. However, in the absence of data correlating the release of lactic dehydrogenase and percent cytotoxicity, it cannot be determined if the doses of INM-6316-20 samples tested were adequately high. Therefore, the study is unacceptable.

8. RECOMMENDATIONS:

A. Our recommendations are that the study authors should:

1. Clearly define the cytotoxicity parameters.

2. Report historical data generated by the photometric analysis.

3. Report correlation data for the photometric analysis vs. standard methods.

9. BACKGROUND: Not applicable.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES: Not applicable.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

See Appendix A for details.

1. INM-6316-20 dissolved in DMSO was tested in duplicate at nine concentrations in the range of 0.001 to 7.0 mM. There were 6 replicate DMSO solvent controls and 4 replicate DMBA positive controls (0.1, 0.5, or 1.0 mM in the treatment medium) in each trial. Only 2 DMSO slides and 2 DMBA slides from the 1.0 mM treatment were analyzed for UDS. Duplicate cultures treated with benzo(a)pyrene [B(a)P] at concentrations of 5, 25, or 50 µM were also included in each trial to investigate the suitability of B(a)P as a positive control.

2. Freshly isolated hepatocytes from the livers of eight-week old male Charles River/Sprague-Dawley rats were used to start primary hepatocyte cultures. Hepatocyte suspensions with viabilities greater than or equal to 70% were used for the assay.

The level of lactate dehydrogenase activity released into the medium was used to assess cytotoxicity. Incorporation of

[methyl-^{3}H]-thymidine into the DNA of the hepatocytes was detected as silver grains in the developed emulsion layer over cell nuclei. Grains were not counted, but quantitated using a photometer to measure the intensity of reflected light. Photometric readings were taken over the nuclei, and an adjacent nuclear-sized cytoplasmic area for 25 cells/slide. The photometric reading of each cytoplasmic area (background) was subtracted from its corresponding nuclear reading to obtain a net nuclear reading for each scored cell. Conclusions were based on the results of 2 test trials.

3. For analysis of the results the authors used a two variable Analysis of Variance (ANOVA) model and a t-test to evaluate dose response relationships and the significance of an increase in UDS over the control values, respectively. Linear, quadratic, and higher order effects were tested by an F-test of significance. Significance was judged at the $p < 0.05$ level.

B. Protocol: Refer to Appendix A.

12. REPORTED RESULTS:

Cytotoxicity: INM-6316-20 at concentrations of 2.5 mM in trial 1 and 5.0 and 70 mM in trials 1 and 2 were cytotoxic as measured by the release of lactate dehydrogenase into the treatment medium. Levels of lactate dehydrogenase released into the treatment medium of DMBA (1.0 mM) treated cells were increased over the solvent control in both trials (Table 1). It was reported that the three highest doses of INM-6316-20 (2.5, 5.0, and 7.0 mM) precipitated at the start of treatment. No data on the cytotoxicity of B(a)P were presented.

UDS Assay: The test material did not cause any increase in nuclear photometer readings compared to control at any dose tested. The average readings ranged from -4.5 to -1.7 in dosed groups. The average reading for the DMSO was -1.7 and for the 1.0 mM DMBA (positive control) was 38.1 and 25.1 in trials 1 and 2, respectively. B(a)P did not give a positive result at the highest dose tested (0.05 mM), however, data on lower doses of B(a)P were not included in the report.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author's conclusion was:

"INM 6316-20 did not induce unscheduled DNA-synthesis in primary cultures of rat hepatocytes when assayed under the conditions described."

B. There was no quality assurance statement or other related information associated with the study report.

TABLE 1. Lactate Dehydrogenase Activity

Sample	Concentration (mM)	Average Activity (U/L)[a] Trial 1	Trial 2
INM-6316-20	0 (DMSO)	210.3	332.1
"	0.001	230.8	298.6
"	0.010	221.6	311.0
"	0.033	184.5	276.2
"	0.10	161.8	279.4
"	0.33	158.2	233.6
"	1.0	211.6	243.6
"	2.5	260.8	344.3
"	5.0	338.6	514.4
"	7.0	473.6	525.3
DMBA	1.0	470.8	811.3

[a]One International Unit (U) of an enzyme will transform 1 micromole of substrate per minute.

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14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Our assessment is that the author's conclusion is not adequately supported by the data, since the sensitivity of this assay compared to conventional assays of UDS (Kilbey, et al., 1979)[1] has not been defined. While lactic dehydrogenase release into the medium is used to monitor cytotoxicity, the levels of lactic dehydrogenase are not correlated with percent killing of cells. The conventional method for measuring cytotoxicity is microscopic evaluation of trypan blue exclusion.

Microscopic grain counting rather than photometer measurement is the conventional method for assessing unscheduled DNA synthesis (Kilbey, et al., 1979)[1]. A compound is considered active if the increase in mean grain count per nucleus is 6 above the concurrent control or the percent of nuclei having 6 or more grains is 10% greater than the concurrent control, or the percent of cells with greater than 20% grains/nucleus is 2% greater than the control. Using the photometric method the author has not defined equivalent evaluation criteria.

15. COMPLETION OF ONE-LINER FORM FOR STUDY: Not applicable.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 2-4.

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[1] Kilbey, B.J., Legator, M., Nichols, W., and Ramel, C. Handbook of Mutagenicity Test Procedures. Elsevier, NY. 1979.

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APPENDIX A

Materials and Methods

Harmony Reviews

Page _____ is not included in this copy.

Pages 54 through 57 are not included in this copy.

The material not included contains the following type of information:

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___ Information about a pending registration action

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Data Evaluation Report 004384

Compound:

2-Thiophenecarboxylic acid, 3-(((((4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino)carbonyl)amino)sulfonyl)-, methyl ester; INM-6316, DPX M6316 Herbicide

Study type:

Teratology - rat

Citation:

Solomon, H. M., Alvarez, L., Staples, R. E., Krauss, W. C., Parks, A. E. Developmental toxicity study in rats given INM-6316 by gavage on days 7-16 of gestation. Medical research project no. 4490-001; Haskell laboratory report no. 146-84; dated issued: August 6, 1984. Study conducted at the E. I. Du Pont de Nemours & Co. Inc. Haskell Laboratory. Study received at EPA 10/31/84 as part of 4G3138; in Acc. 073010.

Reviewed by:

Byron T. Backus
Toxicologist
Toxicology Branch

Approved by:

Clint Skinner, Ph.D.
Section Head
Review Section III
Toxicology Branch

Core Classification:

Minimum

Conclusions:

1. The fetotoxic and teratogenic NOEL's are 159 mg/kg/day. The fetotoxic and teratogenic LEL's are 725 mg/kg/day. Fetotoxic effects at 725 mg/kg/day were slight (2.7%) but significantly lower mean fetal body weight, and a significant increase in the incidence of small renal papillae. An equivocal teratogenic effect was the absence of renal papillae in 5/168 viscerally examined fetuses at 725 mg/kg/day. P = 0.086 for the increased incidence of this finding (which was also present in 1/180 control and 1/156 26 mg/kg/day fetuses which were examined viscerally).

2. There is no evidence for any maternal toxicity at the highest dose tested. A dose-related decrease in mean weight gains is reported for the first 24-hour period after dosing (controls gained 4.1 g, low dose rats 3.7 g, mid-dose rats 2.9 g, and high dose rats 1.7 g, but statistically these values were not significantly different). If this response was actually dose-related, the implication would be that a NOEL was not

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observed in this study.

Materials:

Nulliparous female rats, Crl:CD®(SD)BR, about 60 days old, from Charles River Breeding Laboratories, Inc., Kingston, New York. Weights ranged from 153.8 to 202.0 g (mean 177.9 g) one day after arrival.

Test material: INM-6316-18 (Notebook number 9802-13-3), 95.6% pure.

Procedure:

Six days after the females arrived, they were mated to males of the same strain and source. Females were checked for copulation plugs during the next few days. As females were determined to have mated they were assigned to the control and 3 dosage level groups "using a randomized block design based on body weights." Each group was composed of 25 females.

The test material was administered in an 0.5% aqueous suspension of methyl cellulose. Administration was once daily (each morning) of days 7-16 of gestation at a volume of 5 ml/kg at nominal dosage levels of 0 (vehicle only), 30, 200 and 800 mg/kg/day. The respective analytically determined dose levels (mean of 6 samples ± S.D.) were 0, 26.3 ± 1.45, 159.2 ± 17.15 and 725.2 ± 37.7 mg/kg. (Note: a correction factor of 1.03x, based on a recovery study, was apparently applied to the means of the values given on p. 55 to obtain the values on p. 11). Dose levels had been selected on the basis of a prior pilot study in which no adverse maternal or fetal effects had been found at 300 or 600 mg/kg/day.

Females were weighed the day after arrival, before mating, and on the mornings of days 1, 7-17, and 21 of gestation. They were observed for possible clinical signs each morning from day 1 through 21, and each afternoon from day 7 through 16 of gestation. Individual food consumptions were measured for the periods of days 1-6, 7-16, and 17-21 of gestation.

All females were sacrificed on day 21 by cervical dislocation. They were examined for gross pathologic changes and the liver and gravid uterus were removed and weighed. The uterus was opened and the number and position of viable and non-viable fetuses and resorptions were recorded. The empty uterine weight was then recorded. The uterus of each "nonpregnant" rat was stained with ammonium sulfide to detect early resorptions. The numbers of corpora lutea were recorded.

Live fetuses were weighed, sexed and examined for external "alterations." About half of the live fetuses of each litter and all stunted or malformed fetuses were examined for visceral alterations and verification of sex. The heads from viscerally examined fetuses were fixed in Bouin's fluid and examined. Also, the eyes of Bouin's fixed fetal heads from seven litters in the control group and seven litters of the high dose group, as well as those of fetuses classified as malformed, stunted, or with small or no visible eye bulge were measured with an ocular micrometer after eyelids were removed. Except for the heads fixed in Bouin's fluid all fetal material was fixed in

70% ethanol, eviscerated (if not already done), macerated in 1% aqueous KOH, stained in alizarin red S, and examined for skeletal alterations.

Statistical tests were applied to determine if possible linear (dose-related) trends were present, as well as to compare results between groups. The following parameters were examined:

- Incidence of pregnancy
- Clinical signs
- Maternal death
- Litters with total resorptions
- Maternal body weight
- Feed consumption
- Liver weights (absolute & relative)
- Nidations
- Live fetuses
- Dead fetuses
- Resorptions
- Corpora lutea
- Male fetuses
- Female fetuses
- Fetal weight
- Percent resorptions
- Incidence of fetal alterations

Results:

There were no spontaneous mortalities among the dams. There were no significant differences in incidences of adverse clinical signs between the control group dams and those receiving the test material either before (days 1-6 of gestation), during (days 7-16) or after (days 17-21) dosage.

The mean maternal body weight gains are given in Table 4 (p. 18):

Mean maternal body weight gains (in grams)

Daily Dose (nominal) mg/kg/day	DAYS OF GESTATION 1-6	7-9	10-12	13-16	7-16	17-21
0	28.2	12.0	17.4	30.3	59.7	60.0
30	25.4	11.9	17.1	29.4	58.5	59.1
200	25.8	10.2	17.6	28.9	56.7	58.2
800	23.8	10.7	16.4	29.7	56.8	57.1

There were no significant differences between groups.

The only sign of possible maternal toxicity in the 800 (nominal) mg/kg/day dams was "a transient decrease in body weight gain that was not statistically significant, but was dose-related ($p \leq 0.05$)...noted among dams in the 800 mg/kg group on the first day of dosing. No other adverse effects were noted among dams at this level or at the 30 mor 200 mg/kg levels."

The following was calculated from the data in Appendix E (p. 76-87):

Mean maternal body weight gains (in grams)

Daily Dose (nominal) mg/kg/day	DAYS OF GESTATION 7-8	8-9	9-10	10-11	11-12	12-13
0	4.1	3.4	4.6	4.4	8.0	4.9
30	3.7	4.5	3.7	6.0	6.0	5.1
200	2.9	4.6	2.7	5.7	6.7	5.2
800	1.7	5.1	3.8	5.4	6.1	5.0

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Two dams in the control group and 2 in the 800 (nominal value) mg/kg/day group were found to have hydronephrosis of the kidney at termination. There were no statistically significant differences between groups with respect to mean absolute and mean relative liver weights at termination.

There were no dead fetuses and no litters where total resorption took place. There were no significant differences between controls and the dosage groups, or significant dose-related trends involving such parameters as number of pregnant females in each group, litters in each group, and group means for corpora lutea, nidations, live fetuses, resorptions or stunted fetuses.

At the two highest dose levels, there was a significant reduction in the mean number of males/pregnant animal, however, this was not accompanied by a significant decrease in number of live fetuses/dam, resorptions per pregnant animal, or significant increase in mean number of females/pregnant animal. At the highest dose tested there was a slight (about 2.7%), but statistically significant drop in mean fetal body weight. From table 5, p. 19:

	DAILY DOSE (MG/KG) -(NOMINAL VALUES)			
	0	30	200	800
Live fetuses/pregnant dam	13.8	13.5	13.2	13.4
Mean number of resorptions/dam	0.6	1.0	1.2	0.6
Females/pregnant dam	6.6	6.9	7.3	7.4
Males/pregnant dam	7.2	6.6	5.9**	6.0*
Mean fetal body weight (grams) (stunted fetuses excluded).	3.7	3.7	3.7	3.6*

*Significantly different from control value at $p \leq 0.05$
**Significantly different from control value at $p \leq 0.01$

Individual fetal weights are not reported. However, by using the mean fetal weight for the litter from each dam, multiplying by the number of fetuses from that dam, obtaining a total fetal weight summation from the group, and dividing through by the total number of fetuses in that group, a fetal mean body weight was calculated which was similar to that reported by taking the means of the mean fetal weights from each litter within a group.

Appendix I indicates there were no litters in the 800 mg/kg/day group with mean fetal weight > 4 grams. The only litter in which the mean fetal weight was < 3 grams was in the 800 mg/kg/day group, however there was no significant difference between groups with respect to incidences of litters with a mean fetal weight < 3.5 grams.

Daily Dose (nominal) mg/kg/day	Mean fetal weight* (grams)	Incidence of litters with mean fetal weight >4 g	Incidence of litters with mean fetal weight <3 g	Incidence of litters with mean fetal weight <3.5 g
0	3.71	2/25	0/25	2/25
30	3.66	1/22	0/22	6/22
200	3.72	3/23	0/23	6/23
800	3.62	0/24	1/24	5/24

*(Summation of the total litter weights from the group)/(number of fetuses in that group).

There were no externally malformed fetuses.

The following incidences of visceral and skeletal malformations among fetuses are reported in table 6 (p. 20):

	Nominal daily dose (mg/kg/day)			
Visceral	0	30	200	800
Number examined	180	156	159	168
Number affected	3	1	2	8
Renal papilla - absent	1	1	0	5
Transposition of Great Vessels	1	0	0	0
Malformed cerebrum	1	0	0	0
Microphthalmia	0	0	1	2
Situs inversus	0	0	1	0
Hydrocephaly	0	0	0	1
Skeletal				
Number examined	346	297	303	322
Number affected	1	0	0	0
Fused vertebral arch and rib	1	0	0	0

The incidence of fetuses with no renal papilla was higher in the 800 mg/kg/day group than in the controls, but the increase is reported (p. 14) as not being significant, with p = 0.086, and "borderline" (p = 0.054) for a dose-relationship. The increased (but not significantly so) incidence of visceral malformations in the 800 mg/kg/day group was essentially due to the 5 fetuses (in 5 separate litters) with no renal papilla.

The following incidences of fetal variations are among those reported in table 7 (p. 21-24):

	Nominal daily dose (mg/kg/day)			
Visceral	0	30	200	800
Number examined	180	156	159	168
renal papilla - small	0	0	1	4*
renal pelvis - large	8	13	14	19
Number examined with heads	179	156	159	167
Skull bones partially ossified				
-parietal	1	0	1	10
-interparietal	25	12	17	34
-supraoccipital	12	10	7	12
-squamosa	0	1	1	2
-zygoma	0	0	2	2
Total with variations	135	114	145	163
Mean % affected per litter† (± SEM)	38.7± 4.31	39.0± 3.29	48.0± 4.77	49.1*± 4.81

† Significant dose-related response, $p \leq 0.05$
* Significantly different from control value, $p \leq 0.05$

There were no significant differences between controls and groups exposed to the test material with respect to such skeletal variations as misaligned sternebra, bipartite sternebra, bipartite or dumbelled centrum, or an extra ossification center in the rib.

Discussion:

As the mean analytically determined dosages were 0, 26, 159 and 725 mg/kg/day these must be considered the dosage levels, rather than the nominal values of 0, 30, 200 and 800 mg/kg/day.

The dose-related trend in maternal body weights during the 24 hours following the first dose must be considered as a random event. Given a control group and 3 dosage groups the probability that a random, seemingly dose-related trend will occur from a higher to lower value is 1/4! or 1/24. As no similar dose-related trend was observed at any other time, and as the mean weight increases for the maternal groups were never statistically

different, this isolated "dose-related trend" must be considered a random event.

Fetotoxicity occurred at the highest dose level (725 mg/kg/day), as indicated by the slightly (but significantly) lower mean fetal weight, along with the significantly increased incidence of fetuses with slight variations. These slight variations can be ascribed in delays in development.

Although the increased incidence of fetuses at the highest dosage level with no renal papilla was not statistically significant ($p = 0.086$) from the controls, and although this was not necessarily an anomalous condition, it appears appropriate at this time to be conservative and to classify this as a possible teratogenic effect, thereby setting both the teratogenic and fetotoxic NOEL's at 159 mg/kg/day.

004384

EPA: 68-01-6561
TASK: 86
March 14, 1985

DATA EVALUATION RECORD

INM-6316-20 (DPX-M6316)

Mutagenicity-Chromosome Aberration in Rats

STUDY IDENTIFICATION: Ullman, D.V., Sariff, A.M., Poet, L., et al. In vivo assay for chromosome aberrations in rat bone marrow cells. (Unpublished study No. 302-84 by Haskell Laboratory, Newark, DE for E.I. du Pont de Nemours and Co.; dated July 16, 1984). Accession No. 072849(17).

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-14-85

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1. CHEMICAL: INM-6316-20 (DPX-M6316). 2-Thiophenecarboxylic acid, 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-amino]carbonyl]amino]-sulfonyl]-methyl ester.

2. TEST MATERIAL: INM-6316-20 (DPX-M6316), an off-white solid with a purity of 95.6%.

3. STUDY/ACTION TYPE: Mutagenicity-chromosome aberration.

4. STUDY IDENTIFICATION: Ullman, D.V., Sariff, A.M., Poet, L., et al. In vivo assay for chromosome aberrations in rat bone marrow cells. (Unpublished study No. 302-84 by Haskell Laboratory, Newark, DE for E.I. du Pont de Nemours and Co.; dated July 16, 1984). Accession No. 072849(17).

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EPA Reviewer

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Date: 3-16-85

Clint Skinner, Ph.D.
EPA Section Head

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Date: _____

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7. CONCLUSIONS:

A. Under the conditions of the assay INM-6316-20 (DPX-M 6316) did not induce a clastogenic response when administered by gavage to male or female Sprague-Dawley rats at a dose level of 5000 mg/kg.

8. RECOMMENDATIONS: Not applicable.

9. BACKGROUND:

Dose selection was based on information from sponsor. INM-6316-20 had a relatively low oral toxicity in rats; the lethal dose was estimated at > 11,000 mg/kg. The dose selected for the study represented the maximum dose which could be administered to the animals. Solubility of test material and tolerance of the animals for vehicle precluded testing at a higher dose.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES: Not applicable.

11. MATERIALS AND METHODS (PROTOCOLS):

See Appendix A for details.

A. Materials and Methods:

1. INM-6316-20 (DPX-M6316) is an off-white solid with a purity of 95.6%. The dose level tested was 5000 mg/kg of the test material in corn oil (vehicle).

2. Male and female Sprague-Dawley CD rats (43 days old) were used on study. Groups of 15 rats/sex were given a single intragastric dose of 5000 mg/kg. Five males and five females were sacrificed at 6, 24, and 48 hours. The animals received a single ip dose of cochicine (1 mg/kg) two hours prior to sacrifice. Negative control animals received corn oil, and groups of 5 were sacrificed at same intervals as the dosed groups. Positive controls (5/sex) received a single dose of 20 mg/kg cyclophosphamide and were sacrificed 24 hours later. Bone marrow smears were prepared for cytogenetic analysis by the method of Killian.[1] Fifty metaphase cells/rat were analyzed for chromosomal aberrations. The mitotic index was determined on 500 cells. The percent abnormal cells (total), the percent of abnormal cells with

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[1] Killian, D.J., et al. Handbook of Muta. Test Procedures, pp. 243-260, 1977.

more than 1 aberration, and the number of aberrations/cell were tabulated for each animal and for each test group.

B. Protocol:

See Appendix A for detailed protocol.

12. REPORTED RESULTS:

A. Clinical Observations: The clinical findings in the dosed animals were mild and nonspecific, and were not considered to be dose related.

The findings reported were: wet perianal area in 3 dosed females at the 24-hr sacrifice, one dosed male at the 6-hr sacrifice, and one control male rat at the 48-hr sacrifice; soft feces were noted in 2 control and 2 dosed males at the 6-hr sacrifice; red anal discharge was observed in one dosed male at the 48-hr sacrifice.

A significant ($p < 0.05$) decrease in body weight was observed in the dosed males at the 6-hr sacrifice, and at the 24-hr sacrifice in the males and females combined ($p < 0.01$) when compared to the corresponding negative control groups. There was a significant ($p < 0.05$) body weight decrease in the male and female positive control groups at the 24-hr sacrifice.

B. Mitotic Index: There was no significant difference among the dosed groups when compared to the negative control. The mean mitotic index (M.I.) for the rats in the negative controls, considering all times of sacrifice, ranged from 28 to 35 in the males and 22.6 to 31.2 in the females. In the dosed groups, the M.I. ranged from 31.2 to 33.6 in the males and 20.4 to 32.0 in the females. There was a significant ($p < 0.01$) decrease in the M.I. in the male positive control (18.4 ± 2.4^a) compared to the negative control (35.0 ± 3.3^a). Although the female positive control had an M.I. of (14.0 ± 2.4^a), it was not significantly decreased compared to the negative control (23.4 ± 4.5^a); however; the combined M.I. of the male and female positive control groups (16.2 ± 1.7^a) showed a significant decrease when compared to the combined negative control (29.2 ± 3.3^a) (Table 1).

C. Chromosomal Aberrations: A clastogenic response was demonstrated in the male and female positive controls. There was a significant ($p < 0.001$) increase in percent abnormal cells, percent cells with > 1 aberration, and average number of aberrations per cell (Table 1). There were no significant differences in male/female dosed groups at 6, 24, or 48-hr sacrifices when compared to their concurrent negative controls.

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[a] Standard error.

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TABLE 1. Results at the 24-hr Sacrifice of the Controls Used for Chromosome Aberrations Assay in Rats with INM-6316-20[a]

Substance	Sex	Number of Cell/Group	% Abnormal Cells/Group	% Cells with >1 Aberration/Group	Average # of Aberrations/Cells	Average M.I./ 500 Cells (S.E.)
Negative Control						
Corn Oil	M	250	0.0	0.0	0.000	35.0(3.3)
	F	250	0.4	0.0	0.004	23.4(4.5)
	Combined	500	0.2	0.0	0.002	29.2(3.3)
Positive Control						
Cyclophosphamide (20 mg/kg)	M	250	17.6***	10.8***	0.404***	18.4** (2.4)
	F	250	31.2***	21.6***	0.688***	14.0 (2.4)
	Combined	500	24.4***	16.2***	0.546***	16.2** (1.7)

[a]Five rats/sex/group.

**Different from negative control value ($p < 0.01$).

***Different from negative control value ($p < 0.001$).

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13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that INM-6316-20 did not induce chromosome aberrations in bone marrow cells of rats. INM-6316-20 was non-clastogenic in this in vivo assay.

B. A quality assurance statement was present stating that the study design complied with the published guidelines of the U.S. EPA Office of Toxic Substances (U.S. EPA 1983) and that the study was performed according to the Good Laboratory Practice Standards of the EPA Pesticide Program.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. It is our assessment that the authors correctly interpreted their data. The positive control (cyclophosphamide) caused a clastogenic effect and the sensitivity of the assay was appropriate. Our assessment is that INM-6316-20 was not clastogenic in male or female Sprague-Dawley rats at a dosage of 5000 mg/kg under the conditions of the study.

15. COMPLETION OF ONE-LINER FORM FOR STUDY: Not applicable.

16. CBI APPENDIX: Appendix A, Materials and Methods (Protocol), CBI pp. 2-8.

APPENDIX A

Materials and Methods (Protocol)

Harmony Reviews

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Pages 72 through 77 are not included in this copy.

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___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ The document is a duplicate of page(s) _____

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Unverified Printout

[illegible] DAILY INTAKE [illegible] **DRAFT**

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[illegible]	[illegible]	S.F.	[illegible]	[illegible]
[illegible]/Kg	ppm		mg/kg/day	mg/day(60kg)
10.000	200.00	2000	0.0050	0.3000

Current Action 4G3138

Crop	Tolerance	Food Factor	mg/day(1.5kg)
Barley(3)	0.050	0.03	0.00002
Wheat(170)	0.050	10.36	0.00777

MPI	TMRC	% ADI
0.3000 mg/day(60kg)	0.0078 mg/day(1.5kg)	2.60

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