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

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MAR 22 1990

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT Review of Some of the Toxicity Data on Accent

TO: Robert Taylor, PM-25
Registration Division TS-7505C

FROM Marcia van Gemert, Ph.D. M van Gemert 2/20/90
Chief, HFAS Toxicology Branch, HED TS-7509C

THRU. Penny Fenner-Crisp, Ph.D. Penelope A. Fenner-Crisp 3/22/90
Director, Health Effects Division, TS-7509C

Chemical: Accent

Firm: Dupont

Caswell No: 359J

A number of toxicology studies on the herbicide Accent were submitted early, before the full chronic/oncogenicity and reproduction studies had been submitted. This change in SOP was the result of discussions between Dupont and Linda Fisher, Assistant Administrator for OPTS. Normally all the toxicology studies would be submitted together, passing a screen before review. In addition, this chemical was given expedited status by Linda Fisher, citing a "safer pesticide" policy. It is impossible to predict which chemical will be considered "safer" before the toxicology data have been thoroughly reviewed. Accent is an excellent example. This chemical is not completely "innocuous" has had been stated by Dupont. There are toxicological effects evident in the subchronic studies. Most of the mutagenicity studies are unacceptable and two of the three subchronic studies are considered supplementary because of missing information. Summaries of the conclusions reached on the submitted studies are attached below.

1. Subchronic oral toxicity: 90-day study with IN V9360-7 feeding in mice. Study #: 8102-001, March 30, 1988.
Conclusions: Hematologic parameters were effected by treatment with Accent in both males and females. White blood cells, and specifically neutrophils, lymphocytes, monocytes and eosinophils were decreased with treatment. The most significant effects occurred in neutrophils in females at 90 days, where decreases were seen at 1500 ppm and all doses above. Absolute spleen weights were decreased in females at the top dose of 10,000 ppm, relative spleen weights were decreased as well, but not statistically significantly. No other effects were noted. The study text mentions that bone smears were performed to clarify the WBC

effects, but no results were apparent in the appendix. Gross necropsy tables and individual data were missing from the study text as well. LEL = 1500 ppm (234.0 mg/kg males, 323.0 mg/kg females). NOEL = 300 ppm (43.9 mg/kg males, 62.0 mg/kg females). This study does not satisfy the guideline requirements for a 90-day rodent study (82-1a). The protocol has not met guidelines. it is missing gross necropsy data, and is therefore classified as supplementary. This study is not acceptable for regulatory purposes.

2. Subchronic oral toxicity· 90-day study with IN V9360-7 feeding study in dogs. Study # 8281-001, Sept. 8, 1988.
Conclusions 4 dogs/sex/dose were fed Accent at doses of 0, 250, 5000 and 20,000 ppm for 90 days Blood and urine samples were taken at 45 and 90 days for analyses. A slight decrease in neutrophils was seen at 90 days in the top dose females. No other effects were noted with the compound. LEL = 20,000 ppm (710 mg/kg males, 683 mg/kg females). NOEL = 5000 ppm (172 mg/kg males, 171 mg/kg females). This study satisfies the guideline requirements for a 90-day non-rodent study (82-1(b)). The protocol met guidelines and can be classified as core Minimum. This study is acceptable for regulatory purposes.

3. Subchronic oral toxicity: 90-day study with IN V9360-7 feeding study and one-generation reproduction study in rats. Study# 8101-001. March 11, 1988.
Conclusions: Doses of Accent in the diet were 0, 300, 1500 and 7500 and 20,000 ppm. Primary effects wre on the hematological, clinical chemistry and urinalysis parameters of the study. There was a decrease in female rat platelet counts at all doses tested at both 45 and 90 days. There was a decrease in calcium and bilirubin in male rats at 1500 ppm and above at 90 days calcium was additionally decreased at 45 days at 20,000 ppm. Urinalysis revealed an increase in ketones in male rats at all doses in a dose-related fashion at 90 days. LEL = 300 ppm (22.2 mg/kg males, 26.0 mg/kg females). NOEL not found. This study does not satisfy the guideline requirements for a 90-day rodent study (82-1a) since no NOEL was found. Since the protocol met guidelines it can be classified as core minimum, however, it is not acceptable for regulatory purposes.

4. Teratogenicity study of IN V9360-27 in rats. Study # 8400-001. Dec. 9, 1988.
Conclusions No treatment-related effects were noted on maternal or developmental toxicity up to and including the highest dose, 6 gms per kg (the limit of the suspension capability).
Maternal NOEL >or = 6000 mg/kg/day
Maternal LOEL > 6000 mg/kg/day
Developmental Toxicity NOEL >or= 6000 mg/kg/day
Developmental toxicity LOEL > 6000 mg/kg/day.
Classification: Core guideline, this study satisfies the guideline 83-3, developmental toxicity (teratology) study in rats.

5. Teratogenicity study in IN V9360-27 in rabbits. Study # 8401-001 Dec. 8, 1988.
Conclusions: Maternal toxicity was evidenced at 500 mg/kg/day and above by an increase in clinical signs, gross pathological observations, abortions, postimplantation loss and a decrease in body weight gain during the dosing period. There was evidence of developmental toxicity at 1000 mg/kg/day in the form of reduced mean fetal body weights and the apparent increase in postimplantation loss at 500 mg/kg/day and above.
Maternal NOEL = 100 mg/kg/day
Maternal LOEL = 500 mg/kg/day
Developmental Toxicity NOEL = 500 mg/kg/day
Developmental Toxicity LOEL = 1000 mg/kg/day
Classification: Core guideline. This study satisfies guideline 83-3 developmental toxicity (teratology) study in rabbits.

6. Reproductive and fertility effects in IN V9360-27 Multigeneration reproduction study in rats (first generation interim report) Study # 8277-001 Dec. 15, 1988.
Conclusions: There was a slight increase in food consumption for the F_0 rats in the 20,000 ppm dose group (statistically significant for females only). A statistically smaller mean litter size after culling was observed in the 5,000 and 20,000 ppm dose groups. An increase in bilateral testicular degeneration (not statistically significant) was observed in the 20,000 ppm males. These observations will be evaluated in the final report when data from the F_1 parental rats and F_{2a} and F_{2b} litters are available for comparison.
Classification: Core supplementary data since this is an interim report.

7. Metabolism of [pyridine-2-^{14}C] and [pyrimidine-2-^{14}C] DPX-V9360 by the laboratory rat. Study # 717-88, Dec. 16, 1988.
Conclusions: The metabolism of 2-(4,6-dimethoxy-2-pyrimidinyl) amino-carbonylaminosulfonyl-N,N-dimethyl-3-pyrimidinecarboxamide (Accent) was studied in male and female Sprague-Dawley Crl:CDBR rats. [pyrimidine-2-^{14}C] Accent was administered orally at 10 mg/kg or 1000 mg/kg, at 10 mg/kg following oral administration of unlabeled Accent at 10 mg/kg/day for 14 days, and intravenously at 10 mg/kg. [pyrimidine-2-^{14}C] Accent was administered orally at 1000 mg/kg. Total recovery of administered radioactivity 4 days postdosing accounted for 90 to 109 percent of the dose. Most of the radioactivity was excreted unchanged within 24 hours postdosing. With oral dosing, there were no apparent differences between sexes or dose groups, although a slightly greater percentage of the administered radioactivity was detected in feces of males receiving the high dose than in animals receiving the low dose. Following oral dosing, elimination in the feces accounted for 80 to 95 percent of the dose, and elimination in the urine accounted for 9 to 20 percent. The average total cumulative excretion indicated half-lives between 12 and 24 hours. Following intravenous administration, approximately 76 to 80 percent of the dose was eliminated in the urine and 27 to 30 percent in the feces. Residues in tissues accounted for from 0.05 to 0.5 percent of the dose. The major excretion product in urine and feces was unchanged parent compound. In addition, pyrimidinesulfonamide (N,N-dimethyl-2-sulfonamide pyrimidine-3-carboxamide) was detected in the urine and accounted for 1.1 to

5.7 percent of the dose. Pyrimidine acid sulfonamide (2-sulfon-amidepyridine-3-carboxylic acid) was tentatively identified as a minor metabolie in the feces of orally dosed rats and urine of intravenously dosed rats. Based on the available data, a metabolic pathway was proposed.
Classification: Acceptable.

8. UDS Assay in rat primary hepatocytes. Study # 302-88, May 6, 1988.
Conclusions: IN V9360-27 did not cause any DNA damage or inducible repair in the rat hepatocyte unscheduled DNA synthesis assay under the conditions tested. Classification: unacceptable. Deficiency inadequate highest dose tested.

9. Mammalian cells in culture gene mutation assay in Chinese hamster ovary cells. Study # 429-88. July 14 1988.
Conclusions: IN V9360-27 was nonmutagenic in the in-vitro CHO/HPRT mutation assay (by measuring induction of resistance to 6-thioguanine) with or without metabolic activation at the concentrations tested.
Classification: unacceptable.

10. In vivo micronucleus assay in mouse bone marrow. Study # 428-88. July 18. 1988.
Conclusions: IN v9360-27 had negative response in the mouse micronucleus test at all of the intervals (24,48,72 hours) evaluated. Classification: acceptable.

11. Mammalian cells in culture cytogenetics assay in human lymphocytes. Study # 470-88. July 26, 1988.
Conclusions: IN V9360-27 induced no significant damage to chromosomal structure of cultured human lymphocytes in the presence and absence of metabolic activation under the conditions tested.
Classification: unacceptable.

12. Salmonella/mammalian activation gene mutation assay. Study # 734-88. Nov. 23 1988.
Conclusions: IN V9360-7 was nonmutagenic in TA97A, TA98, TA100, and TA1535 strains of Salmonella typhimurium with or without metabolic activation at the concentrations tested.
Classification unacceptable.

Reviewed by: Marcia van Gemert, Ph.D. M van Gemert 2/15/90
Chief, Toxicology II Branch (H-7509C)
Secondary Reviewer: Penny Fenner-Crisp, Ph.D. Penelope A. Fenner-Crisp 3/22/90
Director, HED (H-7509C)

DATA EVALUATION REPORT

007830

STUDY TYPE: 3-month oral, mice
Guideline 82-1(a)

CASWELL NO: 359J

ACCESSION NUMBER:

MRID NO: 410826-05 thru 19

TEST MATERIAL: 3-Pyridinecarboxamide, 2-[[[[(4,6-dimethoxy-2-pyridinyl)amino]carbonyl]amino]sulfonyl]-N,N-dimethyl-

SYNONYMS: Accent

STUDY NUMBER: 8102-001

SPONSOR: Agricultural Products Dept. Dupont
E.I. duPont de Nemours & Co. Inc.
Wilmington, Delaware, 19898

TESTING FACILITY: Haskell Laboratory Elkton Rd. P.O. Box 50, Newark Delaware, 19714

TITLE OF REPORT: Subchronic Oral Toxicity: 90-day study with IN V9360-7 Feeding study in mice

AUTHORS: Jon C. Cook, Ph.D.

REPORT ISSUED: March 30, 1988

CONCLUSIONS: Hematologic parameters were effected by treatment with Accent in both males and females. White blood cells, and specifically neutrophils, lymphocytes, monocytes, and eosinophils were decreased with treatment. The most significant effects occurred in neutrophils in females at 90 days, where decreases were seen at 1500 ppm and all doses above. Absolute spleen weights were decreased in the females at the top dose of 10,000 ppm, relative spleen weights were decreased as well, but not statistically significantly. No other effects were noted. The study text mentions that bone smears were performed to clarify the WBC effects, but no results were apparent in the appendix. Gross necropsy tables and individual data were missing from the study text as well.

-LEL- 1500 ppm (234.0 mg/kg males, 323.0 mg/kg females

-NOEL- 300 ppm (43.9 mg/kg males, 62.0 mg/kg females)

CLASSIFICATION: This study does not satisfy the guideline requirements for a 90-day rodent study (82-1a). The protocol has not met guidelines, it is missing gross necropsy data, and is classified as supplementary. This study is not acceptable for regulatory purposes.

5 X

A. MATERIALS:

1. Test Compound: V9360-7
   Description. Not given.
   Batch #: 16,607
   Purity 94.9%

2. Test Animals:
   Species: mice
   Strain: Crl:CD-1(ICR) BR
   Age 29 days old
   Weight: 15-23 gms.
   Source: Charles River Laboratories. Inc. Kingston, N.Y.

B. STUDY DESIGN: Dose levels are based on a 10-day subchronic study in female mice with a top dose of 2,200 mg/kg b.w.

1. Animal Assignment - Animals were assigned to the following test groups:

Test Group	Doses in diet (ppm)	# on test Males	Females
1	0	15	15
2	300	15	15
3	1,500	15	15
4	7,500	15	15
5	10,000	15	15

Mice were housed individually in suspended cages.

2. Diet Preparation: Diets were prepared weekly and were refrigerated until use. Before test initiation (test day -8) diets were analyzed for concentration, homogeneity and stability. Stability was also measured under the following conditions: 1) fresh frozen, 2) 24-hour, 3) 10-day or 11-day room temperature. and 4) 20-day refrigerated. Additional samples for stability were taken near the completion of the study (test day 90).

   Results: Homogeneity and stability data are attached on appended page 1 and 2. Homogeneity, stability and test chemical recovery from the diet appear very close to target levels.

3. Animals received food and water ad libitum.

4. Statistics: One-way analysis of variance was used for body weights, body weight gains organ weights, and clinical laboratory measurements. When differences were seen that were significant, then pairwise comparisons were used: for differences in body weights, body weight gains, and clinical laboratory measurements were seen, then the Dunnett's test was used. When the differences were seen in organ weights then comparisons were made using the least significant difference (LSD) test as well as the Dunnett's test. A Bartlett's test was used to analyze clinical laboratory data.

variance was used for organ weights and clinical laboratory data. When the Bartlett's test was significant, the Kruskal-Wallis test was employed using the Mann-Whitney U test to compare means from the control and the treated groups.

5. Compliance: A signed statement of compliance with EPA's GLP was provided.

C. Methods and Results:

1. Observations: Animals were inspected for signs of toxicity and mortality at least once daily. At each weighing, each mouse was individually handled and examined.

Results: No adverse compound-related effects were seen clinically. 8 mice died on study, 4 males and 4 females. 5 appear to have been killed during or as a consequence of bleedings. The three remaining (1 control male, 1 low-dose female, and 1 high-dose female were found dead. These deaths were not compound-related.

2. Food Consumption and Compound Intake: Food consumption was determined weekly throughout the study. With these data and body weight data, individual mean daily diet consumption, food efficiency, and intake of test compound were determined.

Results: Food consumption and food efficiency appear unaffected by compound administration. (Note on pages 58-61 of the study text). Mean daily compound intake is summarized below from 62 and 63 of the study text.

Dose	Compound Intake (mg/kg/day)	
ppm	Males	Females
0	0	0
300	43.9	62.0
1,500	234.0	323.0
7,500	1164.0	1537.0
10,000	1509.0	2016.0

3. Body Weights: All mice were weighed weekly.

Results: Body weights were unaffected by compound administration. Body weight gains for both males and females were slightly but not statistically different from controls. These results are appended from the study text on appended pages 3 and 4.

4. Ophthalmology: Two examinations were performed, one pretest exam and the second on all surviving mice at study termination.

Results: No ophthalmological changes could be attributed to the compound.

5. Clinical Laboratory Evaluations: Blood was collected at 45 days and 90 days after study initiation. Samples were taken from the orbital sinus under light CO_2 anesthesia. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc.
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements	X	Total Plasma Protein
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		
	Red blood cell morphology		

*Required for subchronic and chronic studies.
Bone marrow smears were prepared on all mice at final sacrifice. Slides from male mice exposed to 0, 7,500 and 10,000 ppm and female mice from 0, 1500, 7,500, and 10,000 ppm were evaluated for appropriate neutrophil production and an adequate bone marrow storage pool of neutrophils.

Results: A number of hematologic parameters have been effected by treatment in both the males and females. These data are appended from the study text on pages 5-8. In males, neutrophils are significantly decreased at 7500 and 10,000 at 45 days. Neutrophils were decreased at 90 days also but the decrease was not statistically significant (appended page 5). White blood cells, Monocytes and eosinophils in males were depressed in a dose-related fashion at both 45 and 90 days with statistical significance seen at 90 days at 7500 ppm dose for monocytes (appended page 6). In female mice white blood cells were also effected by treatment, with a dose-response decrease in number at 45 and 90 days. Statistical significance was attained at 1500 and 10,000 ppm at 45 days and at 1500 ppm at 90 days. Neutrophils were also depressed with dose with statistical significance attained at 10,000 ppm at 45 days and at 1500 ppm and above at 90 days. Lymphocytes were decreased with dose, with statistical significance attained at 1500 and 10,000 ppm at 45 days and at 1500 ppm at 90 days (appended page 7). In females, monocytes were also depressed with dose, with statistical significance attained at 90 days at 1500 and 10,000 ppm. Eosinophils were also depressed with dose, most significantly at 90 days, where the 10,000 ppm dose attained statistical significance (appended page 8).

The study text states that the bone smears were reviewed at 90 days to see if there was adequate neutrophil and monocyte production. The data were reported in the study text to be located in Appendix F, however, the data are not readily apparent for examination.

7. Sacrifice and Pathology All mice that died before 90-day sacrifice were necropsied. After 90 days, 10 randomly selected mice from each group were sacrificed and necropsied. The remaining animals were discarded without pathological exam. The CHECKED (X) tissues were collected for histological examination. the (XX) organs in addition were weighed.

All tissues were fixed and stained in appropriate agents. Tissues collected from the high-dose and control groups and those found dead on study were processed to slides and examined microscopically. Liver, kidneys, lungs and all organs with gross lesions from the other three groups were also examined microscopically.

X	Digestive system	X	Cardiovasc./Hemat.	X	Neurologic
	Tongue	X	.Aorta*	X	.Brain*†
X	.Salivary glands*	XX	.Heart*	X	Periph. nerve*#
X	.Esophagus*	X	.Bone marrow*	X	Spinal cord (3 levels)*#
X	.Stomach*	X	.Lymph nodes*[3]	X	.Pituitary*
X	.Duodenum*	XX	.Spleen*	X	Eyes (optic n.)*#
X	.Jejunum*	X	.Thymus*		Glandular
X	.Ileum*		Tonsil	X	.Adrenals*
X	Cecum*	XX	.Kidneys*†	X	Lacrimal gland#
X	.Colon*	X	.Urinary bladder*	X	Mammary gland*#
X	.Rectum*	X	.Testes*†	X	.Parathyroids*††
XX	.Liver*†	X	Epididymides	X	.Thyroids*††[2]
X	Gall bladder*#	X	Prostate	X	Harderian glands
X	.Pancreas*	X	Seminal vesicle	X	Bone*# [1]
	Respiratory	X	Ovaries*†	X	Skeletal muscle*#
X	.Trachea*	X	.Uterus*	X	Skin*#
X	.Lung* [1]		Cervix		Sternum
	Nose°	X	Vagina	X	All gross lesions and masses.
	Pharynx°				
	Larynx°				

* Required for subchronic and chronic studies
° Required for chronic inhalation
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weights required for non-rodent studies

RESULTS:

a. Organ Weights: There was a decrease in absolute spleen weights at the top dose in females. Spleen to body weight was also decreased at this dose, but not statistically significantly. (see table for results)

SPLEEN (females)

Dose	Absolute weight (gms)	Relative weight (% of B.W.)
0	0.103 (0.018)[a]	0.3501 (.0546)
300	0.114 (0.028)	0.3947 (.0857)
1500	0.089 (0.013)	0.3132 (.0423)
7,500	0.105 (0.031)	0.3704 (.0986)
10,000	0.083 (0.015)*	0.2951 (.0531)

[a] Group means and standard deviations (SD)
* Significantly different from control at 5% level by Dunnett criteria

b. Gross Necropsy. No gross necropsy summary table accompanied this report. So nothing can be said about the gross findings.

c. Microscopic Pathology: No histopathologic effects could be attributed to treatment.

DISCUSSION: The primary effect was on hematologic parameters which were effected in both males and females. White blood cells, and specifically neutrophils, lymphocytes, monocytes, and eosinophils were decreased with treatment. The most significant effects occurred in neutrophils in females at 90 days where decreases were seen at 1500 ppm and all doses above. Absolute spleen weights were decreased in females at the top dose of 10,000 ppm, relative spleen weights were decreased as well, but not statistically significantly. No other effects were noted. The study text mentions that bone smears were performed to clarify the WBC effects, but no results were apparent in the appendix. Gross necropsy tables and individual data were missing from the study text as well.

Accent toxicology review

Page ______ is not included in this copy.

Pages 11 through 18 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.

Reviewed by: Marcia van Gemert, Ph.D *M van Gemert 2/27/90*
Chief. Toxicology II Branch (H-7509C)
Secondary Reviewer: Penny Fenner-Crisp Ph.D. *Penelope A. Fenner-Crisp 3/22/90*
Director, HED (H-7509C)

007830

DATA EVALUATION REPORT

STUDY TYPE 3-month oral, dog
Guideline 82-1(b)

CASWELL NO: 359J

ACCESSION NUMBER:

MRID NO: 410826-05 thru 19

TEST MATERIAL: 3-Pyridinecarboxamide, 2-[[[[(4,6-dimethoxy-2-pyridinyl) amino]carbonyl]amino]sulfonyl]-N,N-dimethyl-

SYNONYMS Accent

STUDY NUMBER: 8102-001

SPONSOR: Agricultural Products Dept. Dupont
E.I. duPont de Nemours & Co. Inc.
Wilmington, Delaware, 19898

TESTING FACILITY Haskell Laboratory Elkton Rd. P.O. Box 50, Newark Delaware, 19714

TITLE OF REPORT. Subchronic Oral Toxicity: 90-day study with IN V9360-7 Feeding study in mice

AUTHORS: Jon C. Cook, Ph.D.

REPORT ISSUED Completed September 8, 1988

CONCLUSIONS: 4 dogs/sex/dose were fed Accent at doses of 0, 250 5000 and 20,000 ppm for 90 days. Blood and urine samples were taken at 45 and 90 days for analyses. A slight decrease in neutrophils was seen at 90 days in the top dose females. No other effects were noted with the compound.

-LEL = 20,000 ppm (710 mg/kg males, 683 mg/kg females)

-NOEL = 5,000 ppm (172 mg/kg males, 171 mg/kg females)

CLASSIFICATION This study satisfies the guideline requirements for a 90-day non-rodent 82-1(b). The protocol met guidelines and can be classified as core minimum. This study is acceptable for regulatory purposes.

A. MATERIALS:

1. Test Compound: V9360-27
   Description:
   Batch #: 16,925-02
   Purity: 94.5%

2. Test Animals:
   Species: male and female beagle dogs
   Strain: beagle
   Age: 4-5 months old
   Weight: not given
   Source: Marshall Farms U.S.A. Inc. North Rose, N.Y.

B. STUDY DESIGN:

1. Animal Assignment - Animals were assigned to the following test groups:

Test Group	Doses in diet (ppm)	# on test Males	Females
1	0		
2	250	4	4
3	5,000	4	4
5	20,000	4	4

2. Diet Preparation: Diets were prepared weekly and refrigerated until use. Before test initiation, diets were analyzed for concentration, homogeneity and stability. Stability was also measured under the following conditions: 1) fresh frozen; 2) 24-hour and 10-day room temperature; Additional samples of diet for stability , homogeneity and concentration were taken near the end of the study (test day 90). Individual diet allotments of approximately 350g/dog were given to each dog/day.

   Results: Homogeneity and stability data are attached on appended page 1,2 and 3. Homogeneity, stability and test chemical recovery from the diet appear good.

3. Animals received food and water ad libitum.

4. Statistics: One-way analysis of variance was used for body weights, body weight gains, organ weights, food consumption and clinical laboratory measurements. When differences were seen that were significant, then pairwise comparisons were used: least significant difference (LSD)test and/or Dunnett's test was used. Fisher's exact test was used to evaluate clinical observations. Bartlett's test for homogeneity of variance was used for organ weights and clinical laboratory data. When the Bartlett's test was significant, the Kruskal-Wallis test was employed and the Mann-Whitney U test was used to compare means from the control and the treated groups.

5. Compliance: A signed statement of compliance with EPA's GLP was provided.

C. Methods and Results:

1. Observations: Animals were inspected for signs of toxicity and mortality at least once daily.

   Results: No treatment-related signs of toxicity were noted. No deaths were attributed to the compound. Clinical observations are summarized on pages 63 and 64 of the study text.

2. Food Consumption and Compound Intake: Food consumption was determined weekly throughout the study. With these data and body weight data, individual mean daily diet consumption, food efficiency, and intake of test compound were determined.

   Results: Food consumption and food efficiency appear unaffected by compound administration. (Note on pages 57-60 of the study text). Mean daily compound intake is summarized below from 61 and 62 of the study text.

Dose ppm	Compound Intake (mg/kg/day) Males	Females
0	0	0
250	9.23	9.14
5,000	172.0	171.0
20,000	710.0	683.0

3. Body Weights: All dogs were weighed weekly.

   Results: No compound-related changes in body weight or body weight gain were evident. (See pgs. 53-56 of study text)

4. Ophthalmology: Examinations on all animals were done pretest (test day -27) and on all surviving dogs in the study (test day 85).

   Results: No ophthalmological changes could be attributed to the compound.

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5. Clinical Laboratory Evaluations: Blood was collected twice pretest (test days -23 and -9) and 48 and 87 days after initiation of the study. Each dog was fasted 16 hours before blood collection. The CHECKED (X) parameters were examined.

a. Hematology

|X| Hematocrit (HCT)*
|X| Hemoglobin (HGB)*
|X| Leukocyte count (WBC)*
|X| Erythrocyte count (RBC)*
|X| Platelet count*
| | Blood clotting measurements
(Thromboplastin time)
(Clotting time)
(Prothrombin time)
| | Red blood cell morphology

|X| Leukocyte differential count*
|X| Mean corpuscular HGB (MCH)
|X| Mean corpuscular HGB conc.
|X| Mean corpuscular volume (MCV)
| | Reticulocyte count

*Required for subchronic and chronic studies.
Bone marrow smears were prepared from all dogs at termination of study but were not required for evaluation.

Results: Results are summarized in the study text on pages 65-70. There was a slight decrease in neutrophils at 90 days in females at the top dose. It is not clear if this is a treatment-related phenomen. The data are summarized below.

Neut. WBCx%	ppm	Pretest-1	pretest-2	45-days	90-days
	0	5387(684)[a]	5098(1083)	5242(1902)	6179(831)
	250	5718(445)	5374(1158)	5926(2132)	5090(1240)
	5000	6521(515)	6156(2271)	4705(1305)	5349(542)
	20,000	7848(3810)	5706(1950)	5688(1822)	4312(177)*

[a] Group means and standard deviations (SD)
* Significantly different from control at 5% level by Dunnett criteria.

b. Clinical Chemistry

X Electrolytes:		X Other:	
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*	X	Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
Enzymes		X	Total Bilirubin*
X	Alkaline phosphatase	X	Total Serum Protein*
	Cholinesterase#		Triglycerides
X	Creatinine kinase*°		Serum protein electrophoresis
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	gamma glutamyl transferase		
	glutamate dehydrogenase		

* Required for subchronic and chronic studies
\# Should be required for OP
° Not required for subchronic studies

Results: There was a slight increase in BUN in female dogs at 90 days at the highest dose tested. This dose had high BUN values for pretest samples, and does not appear to be a treatment-related phenomen. No other clinical chemistry parameters appear to be changed with treatment. (see summary tables on pages 71-76).

6. Urinalysis - Urine was collected during the periods of fasting pretest and at 48 and 87 days. The CHECKED (X) parameters were examined.

X		X	
X	Appearance *	X	Glucose*
X	Volume *	X	Ketones*
	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen
X	Osmolality		Hemoglobin

* Required for chronic studies.

Results: No treatment-related effects were evident at 45 days or at 90 days.

7. Sacrifice and Pathology: After 101 days of feeding, all surviving dogs were sacrificed by Bio-tal anaesthesia and exsanguinated and necropsied (test days 102-105). The CHECKED (X) tissues were collected for histological examination. the (XX) organs in addition were weighed.

All tissues were fixed and stained in appropriate agents, processed and histologically examined.

X	Digestive system	X	Cardiovasc./Hemat.	X	Neurologic
	Tongue	X	.Aorta*	XX	.Brain*†
X	.Salivary glands*	XX	.Heart*	X	Periph. nerve*#
X	.Esophagus*	X	.Bone marrow*	X	Spinal cord (3 levels)*#
X	.Stomach*	X	.Lymph nodes*3	X	.Pituitary*
X	.Duodenum*	X	.Spleen*	X	Eyes (optic n.)*#
X	.Jejunum*	X	.Thymus*		Glandular
X	.Ileum*	X	Tonsil	X	.Adrenals*
X	.Cecum*	XX	.Kidneys*†		Lacrimal gland#
X	.Colon*	X	.Urinary bladder*	X	Mammary gland*#
X	.Rectum*	XX	.Testes*†	XX	.Parathyroids*††
XX	.Liver*†	X	Epididymides	XX	.Thyroids*††2
X	Gall bladder*#	X	Prostate		Harderian glands
X	.Pancreas*		Seminal vesicle	X	Bone*# 1
	Respiratory	X	Ovaries*†	X	Skeletal muscle*#
X	.Trachea*	X	:Uterus*	X	Skin*#
X	.Lung* 1	X	Cervix	X	Sternum
	Nose°	X	Vagina	X	All gross lesions and masses.
	Pharynx°				
	Larynx°				

* Required for subchronic and chronic studies
° Required for chronic inhalation
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weights required for non-rodent studies
[1] Rib with costochondral junction

RESULTS:

a. Organ Weights: No changes in organ weights were noted.(see pgs. 81 and 82 of the study text)

b. Gross Necropsy: No compound-related changes in gross pathology were seen. (see pages 83 and 84 of the study text)

c. Microscopic Pathology: No treatment-related changes in microscopic pathology were seen. (see pages 85-90 of the study text)

DISCUSSION: The only effects seen with Accent were in hematological parameters, with a slight decrease in neutrophils at the top dose at 90 days in females. No other significant effects were seen with treatment.

TABLE I

RECOVERY OF IN V9360 ADDED TO CONTROL DIET

NOMINAL DIETARY CONCENTRATION (ppm IN V9360)	MEASURED DIETARY CONCENTRATION (ppm IN V9360)	PERCENT OF NOMINAL
250	226	90
20,000	18,394	92

MR-8281

TABLE II

IN V9360 HOMOGENEITY SAMPLES PREPARED 10/28/87

TYPE OF SAMPLE	NOMINAL DIETARY CONCENTRATION (ppm IN V9360)	MEASURED DIETARY CONCENTRATION (ppm IN V9360)	PERCENT OF NOMINAL
Top	250	230	92
Middle	250	209	84
Bottom	250	214	86
Top	5000	4709	94
Middle	5000	4819	96
Bottom	5000	4697	94
Top	20,000	18,394	92
Middle	20,000	18,026	90
Bottom	20,000	18,271	91

MR-8281

Appended pg 2

TABLE III

IN V9360 STABILITY IN STORAGE SAMPLES PREPARED 10/28/87

TYPE OF SAMPLE	NOMINAL DIETARY CONCENTRATION (ppm IN V9360)	MEASURED DIETARY CONCENTRATION (ppm IN V9360)	PERCENT OF NOMINAL
Fresh Frozen	0	N.D.(a)	---
Fresh Frozen	250	223	89
24 h Room Temperature	250	213	85
10 Day Room Temperature	250	218	87
10 Day Refrigerated	250	214	86
Fresh Frozen	5000	4819	96
24 h Room Temperature	5000	4292	86
10 Day Room Temperature	5000	4611	92
10 Day Refrigerated	5000	4697	94
Fresh Frozen	20,000	18,087	90
24 h Room Temperature	20,000	17,964	90
10 Day Room Temperature	20,000	17,903	90
10 Day Refrigerated	20,000	18,516	93

(a)No peak was detected in the 0 ppm chromatograms at the retention time of IN V9360.

MR-8281

9/21

Reviewed by: Marcia van Gemert, Ph.D. *M van Gemert 1/31/90*
Chief, Toxicology II Branch (H-7509C)
Secondary Reviewer: Penny Fenner-Crisp, Ph.D. *Penelope A. Fenner-Crisp 3/22/90*
Director, HED (H-7509C)

DATA EVALUATION REPORT 007830

STUDY TYPE: 3-month oral, rat
Guideline 82-1(a)

CASWELL NO: 359J

ACCESSION NUMBER:

MRID NO: 410826-05 thru 19

TEST MATERIAL: 3-Pyridinecarboxamide, 2-[[[[(4,6-dimethoxy-2-pyridinyl) amino]carbonyl]amino]sulfonyl]-N,N-dimethyl-

SYNONYMS: Accent

STUDY NUMBER: 8101-001

SPONSOR: Agricultural Products Dept. Dupont
E.I. duPont de Nemours & Co. Inc.
Wilmington, Delaware. 19898

TESTING FACILITY: Haskell Laboratory Elkton Rd. P.O. Box 50, Newark Delaware, 19714

TITLE OF REPORT: Subchronic Oral Toxicity: 90-day study with IN V9360-7 Feeding study and one-generation Reproduction study in rats.

AUTHORS: Jon C. Cook, Ph.D.

REPORT ISSUED: Completed March 11, 1988

CONCLUSIONS: Doses of Accent in the diet were 0, 300, 1500, 7500 and 20,000 ppm. Primary effects were on the hematological, clinical chemistry and urinalysis parameters of the study. There was a decrease in female rat platelet counts at all doses tested at both 45 and 90 days. There was a decrease in calcium and bilirubin in male rats at 1500 ppm and above at 90 days, calcium was additionally decreased at 45 days at 20,000 ppm. Urinalysis revealed an increase in ketones in male rats at all doses in a dose-related fashion at 90 days.

-LEL- 300 ppm (22.2mg/kg males, 26.0 mg/kg females), lowest dose tested.
-NOEL- Not found

CLASSIFICATION: This study does not satisfy the guideline requirements for a 90-day rodent study (82-1a) since no NOEL was found. Since the protocol met guidelines it can be classified core minimum. However, it is not acceptable for regulatory purposes.

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A. MATERIALS:

1. Test Compound: V9360-7
   Description:
   Batch #: 16,607
   Purity: 94.9%

2. Test Animals:
   Species: male and female rats
   Strain: Crl:CD©BR
   Age: Approximately 32 days old
   Weight: 42.4-61.8 gm males, 28.5-50.9 gm. females
   Source: Charles River Breeding Laboratories, Kingston, N.Y.

B. STUDY DESIGN:

1. Animal Assignment - Animals were assigned 1 per cage to the following test groups:

Test Group	Doses in diet (ppm)	# on test Males	Females
1	0	20*	20*
2	300	20*	20*
3	1,500	20*	20*
4	7,500	20*	20*
5	20,000	20*	20*

* The first ten rats assigned to each group were used for the subchronic toxicity part of the study, the remaining ten rats were used in a one-generation, one-litter reproduction study.

2. Diet Preparation: Diets were prepared every two weeks and refrigerated until use. Before test initiation, diets were analyzed for concentration, homogeneity and stability. Stability was also measured under the following conditions: 1) fresh frozen; 2) 24-hour and 10-day room temperature; 3) 10- and 20- day refrigeration. Additional samples of diet for stability were taken near the end of the study (test day 83).

   Results: Homogeneity and stability data are attached on appended pages 1 and 2.

3. Animals received food and water ad libitum.

4. Statistics: One-way analysis of variance was used for body weights, body weight gains, organ weights, and clinical laboratory measurements. When differences were seen that were significant, then pairwise comparisons were used: least significant difference (LSD)test was used for body weights and body weight gains; Dunnett's test was used for clinical laboratory measurements, and organ weights; Bartlett's test for homogeneity of variance was used for organ weights and clinical laboratory data. When the Bartlett's test was significant ($p < 0.005$), the Kruskall-Wallis test was employed and the Mann-Whitney U test was used

to compare means between groups. Fisher's Exact and the Mann-Whitney U tests wre used in the reproduction and lactation portions of the study.

5. Compliance: A signed statement of compliance with EPA's GLP was provided.

C. Methods and Results:

1. Observations: Animals were inspected for signs of toxicity and mortality at least once daily. During the 90-day toxicity study, animals were individually handled and examined for behavior and appearance at the time of body weighings.

   Results: No treatment-related signs of toxicity were noted. No deaths were attributed to the compound.

2. Food Consumption and Compound Intake: Food consumption was determined weekly throughout the study. With these data and body weight data, mean daily diet consumption, food efficiency, and intake of test compound were determined.

   Results: Based on the test analyses for stability and compound intake, the compound appears to have met the targeted concentrations for the study, judging from the dietary stability values in Appendix B of the report on pgs 147 and 154-5. It isn't clear what the differences are between the tables on 147 and 154-5. No dates were given as to when these samples were prepared. Mean compound intake is listed below. These data were extracted from tables 10 and 11, pgs. 69 and 70.

Dose	Compound Intake (mg/kg/day)	
ppm	Males	Females
0	0	0
300	22.2	26.0
1,500	107.0	133.0
7,500	555.0	685.0
20,000	1495.0	1830.0

   Food consumption and food efficiency were uneffected by compound intake. (Tables 6 thru 9, pgs 65 through 68).

3. Body Weights: Body weights were measured weekly during the 90-day phase of the study.

   Results: Body weights and body weight gains were not significantly effected by treatment. (see pgs 61-65 of the study text)

4. Ophthalmology: Examinations on all animals were done pretest and on all surviving rats in the subchronic portion of the study.

   Results: No ophthalmological changes could be attributed to the compound.

5. Clinical Laboratory Evaluations: Blood was collected for evaluation at 45 and 90 days. The rats sampled were the ten/sex/group designated for the subchronic study. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc.
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		
	Red blood cell morphology		

*Required for subchronic and chronic studies.

Results: There was a statistically significant decrease in platelets at all doses tested both at 45 days and at 90 days. The data are extracted from Table 15, pg. 78 of the study text. No other hematological parameters were significantly affected.

Platelets $x10^3$/ul	Dose	Sampling time 45-day	90-day
	0	1251 (143)[a]	1402 (338)
	300	1046 (176)*	1116 (133)#
	1500	1183 (207)	1169 (245)#
	7500	1046 (107)*	1080 (104)#
	20,000	1065 (88)*	1102 (98)#

[a] Group means and standard deviations
* Significantly different from controls at 5% level by Dunnett criteria
# Significantly different from controls at 5% level by Mann-Whitney U criteria

b. Clinical Chemistry

X Electrolytes:
- [X] Calcium*
- [X] Chloride*
- [ ] Magnesium*
- [X] Phosphorous*
- [X] Potassium*
- [X] Sodium*

Enzymes
- [X] Alkaline phosphatase
- [ ] Cholinesterase#
- [ ] Creatinine phosphokinase*°
- [ ] Lactic acid dehydrogenase
- [X] Serum alanine aminotransferase (also SGPT)*
- [X] Serum aspartate aminotransferase (also SGOT)*
- [ ] gamma glutamyl transferase
- [ ] glutamate dehydrogenase

X Other:
- [X] Albumin*
- [X] Blood creatinine*
- [X] Blood urea nitrogen*
- [X] Cholesterol*
- [X] Globulins
- [X] Glucose*
- [X] Total Bilirubin*
- [X] Total Serum Protein*
- [ ] Triglycerides
- [ ] Serum protein electrophoresis

* Required for subchronic and chronic studies
\# Should be required for OP
° Not required for subchronic studies

Results: Calcium and Bilirubin were both depressed in males at 90 days in the 1500 ppm group and above. In addition, calcium was depressed at the 20,000 ppm dose in males at 45 days. The data are detailed below from page 82 of the study submission. It is not clear if these are treatment-related, however, both effects, while not dose-response related, were statistically significant.

TESTS	CCN (ppm)	SAMPLING TIME 45-day	Males 90-day
Calc. mg/dl	0	11.3 (0.2)[a]	11.3 (0.2)
	300	11.2 (0.4)	11.0 (0.4)
	1500	11.0 (0.5)	10.8 (0.4)*
	7500	11.2 (0.4)	10.9 (0.4)*
	20,000	10.6 (0.3)*	10.8 (0.4)*
BILRN mg/dl	0	0.16 (0.03)	0.23 (0.05)
	300	0.14 (0.04)	0.23 (0.09)
	1500	0.16 (0.02)	0.14 (0.04)*
	7500	0.17 (0.04)	0.15 (0.04)*
	20,000	0.16 (0.02)	0.15 (0.04)*

[a] = Group means and standard deviations (SD)
*Significantly different from controls at 5% level by Dunnett test

6. Urinalysis - Urine was collected during the periods of fasting at 45 and 90 days. The CHECKED (X) parameters were examined.

X		X	
X	Appearance *	X	Glucose*
X	Volume *	X	Ketones*
	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen
X	Osmolality	X	Hemoglobin

* Required for chronic studies.

Results: Ketones were present at 90 days in all treated male groups in a dose-related manner. Females showed a very minimal increase in ketones, and the effects were not dose-related. Results are presented below, and were extracted from pgs 90 and 92 of the study text.

URINALYSIS- Males

Ketones	Dose ppm	Sampling Time 45-days	90-days
	0	0/10	0/10
	300	0/10	3/10
	1500	0/10	5/10
	7500	0/10	6/10
	20,000	0/10	10/10

\- Females

Ketones	Dose ppm	Sampling Time 45-days	90-days
	0	0/10	0/8
	300	0/10	0/7
	1500	0/10	1/9
	7500	0/10	0/9
	20,000	0/8	1/9

7. Sacrifice and Pathology: After 90 days of feeding, all surviving rats were sacrificed by chloroform anaesthesia and exsanguinated and necropsied. The CHECKED (X) tissues were collected for histological examination. the (XX) organs in addition were weighed.

All tissues in the high-dose and control groups, as well as those found dead, were examined microscopically. Liver. Lungs and kidneys and all gross lesions from rats in the low and low-intermediate and high-intermediate groups were also examined microscopically. Bone marrow smears were prepared from all rats that were sacrificed by design. Rats used for the Repro study that were found dead were necropsied and discarded.

X	X	X
Digestive system	Cardiovasc./Hemat.	Neurologic
X Tongue	X .Aorta*	XX .Brain*†
X .Salivary glands*	XX .Heart*	X Periph. nerve*#
X .Esophagus*	X .Bone marrow*	X Spinal cord (3 levels)*#
X .Stomach*	X .Lymph nodes*[3]	XX .Pituitary*
X .Duodenum*	XX .Spleen*	X Eyes (optic n.)*#
X .Jejunum*	X .Thymus*	Glandular
X .Ileum*	Urogenital	X .Adrenals*
X .Cecum*	XX .Kidneys*†	Lacrimal gland#
X .Colon*	X .Urinary bladder*	X Mammary gland*#
.Rectum*	XX .Testes*†	.Parathyroids*††
XX .Liver*†	X Epididymides	X .Thyroids*††[2]
Gall bladder*#	X Prostate	X Harderian glands
X .Pancreas*	X Seminal vesicle	X Bone*#
Respiratory	X .Ovaries*†	X Skeletal muscle*#
X .Trachea*	X .Uterus*	X Skin*#
X .Lung* [1]		X All gross lesions and masses*
Nose°		
Pharynx°		
Larynx°		

* Required for subchronic and chronic studies
° Required for chronic inhalation
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weights required for non-rodent studies

RESULTS:

a. Organ Weights: No changes in organ weights were noted.(see pgs. 93 and 94 of the study text)
b. Gross Necropsy: No compound-related changes in gross pathology were seen. (see pages 95 and 96 of the study text)
c. Microscopic Pathology: No treatment-related changes in microscopic pathology were seen. (see pages 97-104 of the study text)

REPRODUCTIVE STUDY:

1. Mating and Gestation: After 90 days of test compound feeding a one-generation, one-litter reproduction study was performed. During this reproduction phase, all animals were fed their respective treatment diets. Females were housed for 7 days with similar diet males. Observations were made daily until appearance of a vaginal plug which was considered day 0 of gestation. If a vaginal plug was not detected within 7 days the female was housed for a second seven day period with a proven male.

2. Lactation: Delivery day was considered day 0 postpartum. Culling to 8/litter was performed on day 4 postpartum with one litter culled to 9. Weaning was day 21 postpartum.

3. Study Design:

   a. Body weights: Female rats were weighed on days 0 and 21 of lactation. male and female offspring were weighed on day 21 postpartum.

   b. Litter weights: All pups within each litter were counted and weighed collectively on days 0, 4 (pre and post-culling) 7 and 14.

   c. Clinical observations (parental): Parents were checked twice daily for signs of moribundity, morbidity and abnormal behavior.

   d. Clinical observations (offspring): After delivery, number of live and dead offspring were recorded and sex determined and checked for signs of abnormalities. Offspring were individually handled and examined on days 4, 7, 14 and 21 postpartum. Onsite observation for other signs was made twice daily.

   e. Sacrifice, Necropsy: Offspring were sacrificed on day 21 by carbon dioxide asphyxiation and discarded without pathological exam. Parental males and females were sacrificed and discarded at the end of the individual dam weaning period.

   f. Reproduction and Lactation Indices: The following reproduction and lactation indices were calculated; fertility index; gestation index; Number of Pups born alive; viability index; lactation index; litter survival; average # of pups/litter.

RESULTS: No compound-related effects were evident on reproduction indices, pup count, pup weights, maternal weight and pup or maternal clinical observations. (results summarized on pages 105-110 of the study text).

DISCUSSION: The primary effects were on the hematological clinical chemistry and urinalysis parameters of the study. There was a decrease in female rats in platelet counts at all doses tested at both 45 and 90 days which was statistically significant. In the 1500 group alone at 45 days there was no statistical significance. In males there was a decrease in calcium and bilirubin at 1500 ppm and above at 90 days which was statistically significant. Calcium was significantly decreased at 45 days only at 20,000 ppm. Urinalysis revealed an increase in ketones present in a dose-related fashion at all doses tested in males at 90 days. This may be attributable to some pharmacologic action of the sulfonylurea on glucose metabolism, although no changes in blood glucose were evident. There is still no NOEL for two parameters in the study, platelets and urinary ketones.

Appended p81

TABLE I

RECOVERY OF INV-9360 ADDED TO CONTROL DIET

NOMINAL DIETARY CONCENTRATION (ppm INV-9360)	MEASURED DIETARY CONCENTRATION (ppm INV-9360)	PERCENT OF NOMINAL
300	294	98
20,000	21,951	110

MR-8101

TABLE II

INV-9360 HOMOGENEITY SAMPLES PREPARED 1/21/87

TYPE OF SAMPLE	NOMINAL DIETARY CONCENTRATION (ppm INV-9360)	MEASURED DIETARY CONCENTRATION (ppm INV-9360)	PERCENT OF NOMINAL
Top	300	290	[illegible]
Middle	300	286	95
Bottom	300	282	94
Top	1500	1551	103
Middle	1500	1477	98
Bottom	1500	1500	100
Top	7500	7517	100
Middle	7500	7308	97
Bottom	7500	7412	99
Top	20,000	20,922	105
Middle	20,000	20,770	104
Bottom	20,000	22,066	110

MR-8101

Appended pg 2

TABLE III

INV-9360 STABILITY IN STORAGE SAMPLES PREPARED 1/21/87

TYPE OF SAMPLE	NOMINAL DIETARY CONCENTRATION (ppm INV-9360)	MEASURED DIETARY CONCENTRATION (ppm INV-9360)	PERCENT OF NOMINAL
Fresh Frozen	0	N.D.(1)	---
Fresh Frozen	300	304	101
24 h Room Temperature	300	293	98
10 Day Room Temperature	300	285	95
10 Day Refrigerated	300	302	101
20 Day Refrigerated	300	288	96
Fresh Frozen	1500	1561	104
24 h Room Temperature	1500	1583	106
10 Day Room Temperature	1500	1549	103
10 Day Refrigerated	1500	1559	104
20 Day Refrigerated	1500	1574	105
Fresh Frozen	7500	7565	101
24 h Room Temperature	7500	7288	97
10 Day Room Temperature	7500	7803	104
10 Day Refrigerated	7500	7517	100
20 Day Refrigerated	7500	7555	101
Fresh Frozen	20,000	22,180	111
24 h Room Temperature	20,000	21,570	108
10 Day Room Temperature	20,000	21,684	108
10 Day Refrigerated	20,000	22,142	111
20 Day Refrigerated	20,000	20,922	105

(1)None detected, based on a minimum detectable limit of 0.49 μg/mL INV-9360 in the extraction solvent.

MR-8101

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 1/9/90
Pharmacologist, Review Section I, TB-HFAS (H7509C)

Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. YMI 1/9/90
Section Head, Review Section I, TB-HFAS (H7509C)

007830

DATA EVALUATION RECORD

Study Type: Developmental Toxicity - Teratology
Species - Rat
Guideline 83-3

EPA ID Numbers: EPA MRID (Accession) No. 410826-08
EPA ID No. 9F3763
EPA Record No. 244953
Caswell No. 359J
HED Project No. 9-2188

Test Material: IN V9360-27

Synonyms: 3-Pyridinecarboxamide, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-N,N-dimethyl-

Sponsor: Agricultural Products Department
E.I. du Pont de Nemours & Company, Inc.
Wilmington, Delaware 19898

Study Numbers: Medical Research No. 8400-001
Laboratory Project ID 611-88

Testing Facility: E.I. du Pont de Nemours & Company, Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road
P.O. Box 50
Newark, Delaware 19714

Title of Report: Teratogenicity Study of IN V9360-27 in Rats.

Author: Louis Alvarez, B.A.

Report Issued: December 9, 1988

Conclusions: No treatment-related effects were noted on maternal or developmental toxicity up to and including the highest dose tested, 6 grams per kilogram (the limit of the suspension capability).

Maternal NOEL >or= 6000 mg/kg/day
Maternal LOEL > 6000 mg/kg/day
Developmental Toxicity NOEL >or= 6000 mg/kg/day
Developmental Toxicity LOEL > 6000 mg/kg/day

Core Classification: Core Guideline Data

This study satisfies the Guideline 83-3, Developmental Toxicity (Teratology) Study in rats.

A. **Materials**:

A copy of the "Materials and Methods" section from the investigators report is appended.

Test Compound: Purity: 97.4%
Description: Solid white powder.
Lot No.: Not provided.
Contaminants: not provided

Vehicle: 0.5% aqueous suspension of methylcellulose.
Methylcellulose obtained from Fisher Scientific, Fair Lawn, NJ.
Lot No. 870743.

Test Animals: Species: Rat, Female, nulliparous
Strain: Crl:CD BR
Source: Charles River Breeding Laboratories, Inc. Kingston, NY
Age: 63 days (females); 84 days (males)
Weight: Females, 169.5 to 214.8 grams (mean 196.4 ± 0.67 grams)
Males, 222.6 to 378.6 grams (mean 337.1 ± 2.53 grams)

B. **Study Design**

This study was designed to assess the developmental toxicity potential of IN V9360-27 when administered by gavage to rats on gestation days 7 through 16, inclusive.

Mating

Natural, 1:1. Presence of copulation plug in the vagina was considered as confirmation of copulation and designated as Gestation Day 1.

Group Arrangement

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	25
Low Dose	200	25
Low Mid Dose	1000	25
High Mid Dose	2500	25
High Dose	6000	25

Dosing

All doses were in a volume of 20 ml/kg of body weight/day prepared daily during the dosing period. The dosing solutions were analyzed for concentration and stability. Dosing was based on the most recent body weight.

Observations

The animals were checked for mortality or abnormal condition daily. Dams were sacrificed on day 22 of gestation. Examinations at sacrifice consisted of: Looking for evidence of gross pathological changes. The liver and gravid uterus were weighed and recorded. The uterus was opened, the contents examined, the position of live and dead fetuses, and resorptions were noted and recorded. Apparent "non-pregnant" uteri were stained with ammonium sulfide to detect resorption sites. Corpora lutea were counted and recorded for each ovary.

The fetuses were examined in the following manner: Live fetuses were weighed, sexed, and examined for external alterations. "The first live fetus and thereafter every other fetus in each litter were decapitated and examined for visceral alterations" by the Staples technique, and the sex of the fetus was verified at this time. "The heads were fixed in Bouin's fluid and examined" by the the method of Barrow and Taylor. The remaining fetuses and all the decapitated fetuses were fixed in 70% ethanol, eviscerated (if not done before), cleared in 1% aqueous potassium hydroxide and stained with alizarin red s and then examined for skeletal alterations.

Some historical control data were provided to allow for comparison with concurrent controls; however, data were not provided for all measured parameters.

Statistical Analysis

The following statistical analysis methods were employed (extracted from the investigator's report):

> The litter (i.e., the proportion of affected fetuses per litter or the litter mean) was considered the experimental unit for the purpose of statistical evaluation. The level of significance selected was alpha = 0.05. When appropriate, the parameters listed in the following tabulation were analyzed by the statistical tests indicated.

Parameter	Test for Linear Trend	Pair-wise Test Between Groups
Incidence of pregnancy Clinical observations Maternal mortality Litters with total resorptions	Cochran-Armitage	Fisher's exact
Maternal weight Maternal weight change Maternal feed consumption Maternal liver weight	Linear combination of dose ranks from ANOVA	Dunnett's when one-way ANOVA was significant
Live fetuses Dead fetuses Resorptions Nidations Corpora lutea Fetal weight Incidence of fetal alterations	Jonckheere's	Mann-Whitney U

When more than 75 percent ties occurred in reproductive and fetal parameters, the Cochran-Armitage test replaced Jonchkeere's test to detect trend and the Fisher's exact test was applied instead of the Mann-Whitney U test to detect a significant difference between groups.

The use of the words "significant" or "significantly" in this report indicates a statistically significant difference between the control and the experimental groups.

Compliance

A signed "Statement of No Data Confidentiality Claims" was provided.

A signed "Good Laboratory Practice Compliance Statement" was provided.

A signed "Quality Assurance Documentation" statement was provided.

A signed statement of "Flagging of Studies for Potential Adverse Effects" was provided.

C. Results:

Analyses of Suspensions

Methods and results of analyses were provided. The nominal concentrations of the 200, 1000, 2500, and 6000 mg/kg suspensions were considered to be 10, 50, 125, and 300 mg/ml. At 5 hours at room temperature, the mean concentrations ranged from 96 to 106 % of nominal; concentrations immediately after preparation ranged from 96 to 107 % of nominal.

1. Maternal Toxicity:

a. Mortality

No animals were reported to have died.

b. Clinical Observations

High-dose animals were reported to have a change in the color of the feces (light brown), however, this is not considered compound toxicity. No treatment-related effects were noted. Two control animals had evidence of gavage error; however, they were kept on study along with two additional control animals (in case of the death of these animals).

c. Body Weight

The investigators supplied the following data: group mean and individual animal data. Corrected body weights were not provided. The following table presents body weight gains.

Table I: Body Weight Gains (grams)[a]

Group	Prior to Dosing Period	Dosing Period	Postdosing Period	Entire Gestation Period
Control	29.2	57.7	82.4	170.7
LDT	27.2	59.5	79.3	164.6
LMDT	31.2	55.3	75.3	161.7
HMDT	28.6	61.5	86.2	173.9
HDT	30.7	60.4	83.1	174.9

[a] = Data extracted from Report MR-8400-001, Table 1 & Appendix D.

No treatment-related effects were noted.

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d. Food Consumption

The investigators supplied the following data: group mean and individual animal data. The following table presents the mean food consumption data.

Table II: Food Consumption Data (g/day)[a]

Group	Prior to Dosing Period	Dosing Period	Postdosing Period
Control	21.6	24.0	28.0
LDT	21.6	23.8	27.1
LMDT	21.3	24.1	28.1
HMDT	21.0	24.1	29.1
HDT	21.4	25.1	29.6

a = Data extracted from Report MR-8400-001, Table 2.

No treatment related effects were noted.

e. Gross Pathological Observations

The investigators supplied the following data: individual animal data, including absolute and relative liver weights.

No treatment-related gross abnormalities were noted in the data provided. The following table presents the mean maternal liver weights.

Table III: Mean Maternal Liver Weights[+]

Group[a]	Daily Dose (mg/kg)	Body Weight[b] Final	Body Weight[b] Adjusted	Absolute (g)	Relative[c] (g/100 g)
Control	0	426.7	330.6	16.0	4.8
LDT	200	418.6	325.9	16.2	5.0
LMDT	1000	414.6	326.6	15.8	4.8
HMDT	2500	425.5	330.0	16.2	4.9
HDT	6000	427.8	331.0	16.1	4.8

a = Data from females that were not pregnant, delivered early, or had gavage trauma were excluded from calculations.
b = Final weight (g) is the weight of the intact animal on Day 22 of gestation. Adjusted weight is the weight on Day 22 excluding the products of conception.
c = Calculation of relative weights is based on adjusted body weights.
+ = Data extracted from Report MR-8400-001, Table 4.

The investigators found "No significant trend (linear combination of dose ranks from ANOVA) or significant differences from control means (Dunnett's test)".

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f. Cesarean Section Observations

Table III: Cesarean Section Observations[a]

Dose	Control	LDT	LMDT	LHDT	HDT
No. Animals Assigned	27	25	25	25	25
No. Animals Mated/ Inseminated	25	25	25	22	23
Pregnancy Rate (%)	93	100	100	88	92
Maternal Wastage					
No. Died	0	0	0	0	0
No. Died/Pregnant	0	0	0	0	0
No. Nonpregnant	2	0	0	0	0
No. Aborted	0	0	0	0	0
No. Premature Delivery	1	1	0	0	0
Total # of Litters	22[+]	24	25	22	23
Total Corpora Lutea	367	392	402	352	379
Corpora Lutea/Dam	16.7	16.3	16.1	16.0	16.5
Total Implantations	333	351	351	327	352
Implantations/Dam	15.1	14.6	14.0	14.9	15.3
Total Live Fetuses	319	334	336	313	332
Live Fetuses/Dam	14.5	13.9	13.4	14.2	14.4
Total Resorptions	14	17	15	14	20
Early	13	16	15	14	19
Late	1	1	0	0	1
Resorptions/Dam	0.6	0.7	0.6	0.6	0.9
Total Dead Fetuses	0	0	0	0	0
Dead Fetuses/Dam	0.0	0.0	0.0	0.0	0.0
Total Stunted Fetuses	2	1	2	0	1
Preimplantation Loss(%)	9.3	10.5	12.7	7.1	7.1
Postimplantation Loss(%)	4.2	4.8	4.3	4.3	5.7
Mean Fetal Weight (g)	5.26	5.23	5.21	5.24	5.26
Mean Sex Ratio (M/F)	6.6/7.9	6.5/7.4	6.7/6.8	7.0/7.2	7.3/7.1

[+] = Two dams not included due to "gavage trauma."
[a] = Data extracted from Report MR-8400-001, Table 5 & Appendix I.

No biologically relevant differences were noted in the above-cited data.

2. Developmental Toxicity

a. External Observations

No treatment-related observations were noted.

b. Visceral Observations

Table IV: Visceral Examinations[+]

Observations[a]	Control	Low Dose	Low Mid Dose	High Mid Dose	High Dose
# pups examined	181	174	175	163	171
# litters examined	24	24	25	22	23
Brain - Hydrocephaly	0/0[b]	0/0	2/2	0/0	0/0
No innominate artery	0/0	0/0	1/1	0/0	0/0
Common Trunk pulmonary arteries	0/0	1/1	1/1	1/1	0/0
Kidney-Small papilla					
Size 1	6/6	5/5	10/7	9/6	13/7
Size 2	19/14	38/15	16/12	30/15	28/17
Ureter - Distended	42/22	56/19	31/16	46/14	60/21

[a] = Some observations may be grouped together.
[b] = Fetal (litter) incidence.
[+] = Data extracted from Report MR-8400-001 Tables 6 and 7.

No treatment-related observations were noted in the above data. Historical control data were provided for incidence of small papillas and distended ureters.

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Table V: Skeletal Examinations[+]

Observations[a]	Control	Low Dose	Low Mid Dose	High Mid Dose	High Dose
# pups examined	351	334	336	313	332
# litters examined	24	24	25	22	23
Ribs:					
Fused	0/0[b]	1/1	1/1	0/0	0/0
Rudimentary -					
Thoracic 13	11/6	11/5	3/3	7/3	17/9
Cervical	2/2	1/1	3/3	3/1	6/4
Lumbar	7/3	6/4	4/3	6/3	8/5
Wavy	1/1	0/0	2/2	3/2	2/2
Partially Ossified	4/2	0/0	0/0	2/1	2/1
Vertebra:					
Fused	0/0	0/0	2/2	0/0	1/1
Accessory Structure (Lumbar)	1/1	0/0	0/0	0/0	0/0
5 Lumbar or 12 Thoracic	3/2	3/2	0/0	3/1	2/2
Extra Lumbar	1/1	0/0	0/0	0/0	0/0
Dumbelled Centrum	6/5	2/2	6/5	2/2	3/2
Partially Ossified	4/2	1/1	2/2	7/5	6/6
Sternebra:					
Misaligned (1)	1/1	1/1	1/1	2/2	5/5
Misaligned (2+)	1/1	0/0	2/2	0/0	1/1
Partially Ossified	0/0	2/2	0/0	0/0	1/1
Unossified	3/3	3/1	1/1	2/1	0/0
Ileum					
Unilateral anterior shift	0/0	0/0	0/0	0/0	2/1
Skull:					
Partially Ossified: Frontal	1/1	0/0	0/0	0/0	0/0
Interparietal	16/11	8/6	14/6	13/6	16/12
Maxilla	6/6	2/2	2/2	9/4	15/8
Parietal	3/2	2/2	1/1	6/4	6/3
Nasal	1/1	0/0	0/0	0/0	0/0
Squamosal	3/2	1/1	2/2	8/5	3/2
Supraoccipital	7/6	2/2	4/4	8/4	5/3
Zygoma	4/3	4/4	2/1	7/4	2/2
Hyoid-Unossified	7/5	4/3	4/4	5/4	9/7
Ischium - Partially Ossified	0/0	0/0	0/0	1/1	1/1
Pubis - Partially Ossified	0/0	0/0	0/0	2/2	2/1

a = Some observations may be grouped together.
b = Fetal (litter) incidence.
+ = Data extracted from Report MR-8400-001, Tables 6 and 7.

No treatment related findings were noted.

D. <u>Discussion/Conclusions</u>:

1. <u>Maternal Toxicity</u>

No treatment-related effects were noted on maternal body weight gain, mortality, clinical observations, food consumption, and gross pathological observations (including liver weights) up to and including the highest dose tested, 6 g/kg (the limit of the suspension capability).

2. <u>Developmental Toxicity</u>

a. <u>Deaths/Resorptions</u>

No treatment-related effects were noted.

b. <u>Altered Growth</u>

No treatment-related effects were noted.

c. <u>Developmental Anomalies</u>

No treatment-related effects were noted.

d. <u>Malformations</u>

No treatment-related effects were noted.

E. <u>Study Deficiencies</u>:

The protocol stated that gravid uterus weights were taken; however, these data were not provided to the Agency.

F. <u>Core Classification</u> - Core-Guideline Data.

Maternal NOEL >or= 6000 mg/kg/day
Maternal LOEL > 6000 mg/kg/day
Developmental Toxicity NOEL >or= 6000 mg/kg/day
Developmental Toxicity LOEL > 6000 mg/kg/day

G. <u>Risk Assessment</u>:

None necessary at this time.

Accent toxicology review

Page ______ is not included in this copy.

Pages 48 through 54 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.

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 1/9/90
Pharmacologist, Review Section I, TB - HFAS (H7509C)
Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T., Section Head, Review Section I, TB - HFAS (H7509C) YMI 1/9/90

DATA EVALUATION RECORD

007830

Study Type: Developmental Toxicity - Teratology
Species: Rabbit
Guideline: 83-3

EPA ID Numbers: EPA MRID (Accession) No. 410826-09
EPA ID. No. 9F3763
EPA Record No. 244953
Caswell No. 359J
HED Project No. 9-2188

Test Material: IN V9360-27

Synonyms: Haskell No. 16,925
3-Pyridinecarboxamide, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-N,N-dimethyl-

Sponsor: Agricultural Products Department
E.I. du Pont de Nemours & Company, Inc.
Wilmington, Delaware 19898

Study Numbers: Medical Research No. 8401-001
Laboratory Project ID 694-88

Testing Facility: E.I. du Pont de Nemours & Company, Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road
P.O. Box 50
Newark, Delaware 19714

Title of Report: Teratogenicity Study of IN V9360-27 in Rabbits.

Author: Mark E. Hurtt, Ph.D.

Report Issued: December 8, 1988

Conclusions: Maternal toxicity was evidenced at 500 mg/kg/day and above by an increase in clinical signs, gross pathological observations, abortions, postimplantation loss and a decrease in body weight gain during the dosing period. There was evidence of developmental toxicity at 1000 mg/kg/day in the form of reduced mean fetal body weights and the apparent increase in postimplantation loss at 500 mg/kg/day and above.

Maternal NOEL = 100 mg/kg/day
Maternal LOEL = 500 mg/kg/day
Developmental Toxicity NOEL = 500 mg/kg/day
Developmental Toxicity LOEL = 1000 mg/kg/day

Core Classification: Core Guideline Data
This study satisfies Guideline 83-3 Developmental Toxicity (Teratology) study in rabbits.

A. **Materials**:
A copy of the "Materials and Methods" section from the investigators' report is appended.

Test Compound: Purity: 97.4%
Description: Solid white powder.
Lot No.: Not provided.
Contaminants: none provided.

Vehicle: 0.5% aqueous suspension of methylcellulose.
Methylcellulose obtained from Fisher Scientific, Fair Lawn, NJ.
Lot No. 870743.

Test Animals: Species: Rabbit, Female, nulliparous
Strain: (NZW) SPF
Source: Hazleton Research Products, Inc.
Denver, PA
Age: 5.5 months
Weight: 2858.6 to 4237.1 grams
(mean 3495.9 $\pm$ 29.49 grams)

B. **Study Design**:

This study was designed to assess the developmental toxicity potential of IN V9360-27 when administered by gavage to rabbits on gestation days 7 through 19, inclusive.

Mating

Artificial insemination with the day of insemination considered as gestation day 0 (see attached materials and methods for more detail; includes age and weight of proven males used).

Group Arrangement

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	20
Low Dose	100	20
Low Mid Dose	500	20
High Mid Dose	1000	20
High Dose	2000	20

Dosing

All doses were in a volume of 10 ml/kg of body weight/day prepared daily during the dosing period. The dosing solutions were analyzed for concentration and stability. Dosing was based on the most recent body weight.

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Observations

The animals were checked for mortality or abnormal condition daily. Dams were sacrificed on day 29 of gestation. Examinations at sacrifice consisted of: Looking for evidence of gross anatomical abnormalities. The liver and gravid uterus were weighed and recorded. The uterus was then opened, and the position of live and dead fetuses and resorptions were noted and recorded. Empty uterine weights were recorded. "Non-pregnant" uteri were stained with ammonium sulfide to detect resorptions sites. Corpora lutea were counted and recorded for each ovary.

The fetuses were examined in the following manner: Each fetus was weighed and examined for external abnormalities and then examined for visceral abnormalities and sexed. "The brain was examined by making a transverse section between the parietal and frontal bones of the unfixed fetal head". They then removed the eyelids and examined the eyes. Finally, all fetuses were then fixed in 70% ethanol, cleared in a potassium hydroxide solution and stained with alizarin red S for skeletal anomalies.

Historical control data were not provided to allow for comparison with concurrent controls.

Statistical Analysis

The following statistical analysis methods were employed (extracted from the investigator's report).

> The litter (i.e., the proportion of affected fetuses per litter or the litter mean) was considered the experimental unit for the purpose of statistical evaluation. The level of significance selected was alpha = 0.05. When appropriate, the parameters listed in the following tabulation were analyzed by the statistical tests indicated.

Parameter	Test for Linear Trend	Pair-wise Test Between Groups
Incidence of pregnancy Clinical observations Maternal mortality Does with total resorptions	Cochran-Armitage	Fisher's exact
Maternal weight Maternal weight change Feed consumption Maternal liver weight	Linear combination of dose ranks from ANOVA	Dunnett's when one-way ANOVA was significant
Live fetuses Dead fetuses Resorptions Corpora lutea Fetal weight Incidence of fetal alterations	Jonckheere's	Mann-Whitney U

When more than 75 percent ties occurred in reproductive and fetal parameters, the Cochran-Armitage test replaced Jonchkeere's test to detect trend and the Fisher's exact test was applied instead of the Mann-Whitney U test to detect a significant difference between groups.

The use of the words "significant" or "significantly" in this report indicates a statistically significant difference between the control and the experimental groups.

Compliance

A signed statement of "No Data Confidentiality Claims" was provided.

A signed "Good Laboratory Practice Compliance Statement" was provided.

A signed "Quality Assurance Documentation" statement was provided.

A signed "Flagging of Studies for Potential Adverse Effects" statement was provided.

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C. Results:

Analyses of Test Suspensions

The investigators used nominal concentrations of 10, 50, 100, and 200 mg/ml for the 100, 500, 1000, and 2000 mg/kg dosing solutions. Concentrations at the first two timepoints tested ranged from 96 to 117% of the nominal concentration. For the final timepoint, concentrations ranged from 69 to 151%. The means and standard deviations for all combined timepoints were 9.9 ± 1.4, 56 ± 6.8, 114 ± 13, and 224 ± 37 for the 10, 50, 100, and 200 mg/ml concentrations. They also determined "uniformity" of dosing suspensions for the 10 and 200 mg/ml solutions, the coefficient of variation was 10 and 3%, respectively.

1. Maternal Toxicity

a. Mortality

Five pregnant females were reported to have died. Two animals, one each in the low and high dose group died of gavage error. Another female in the low mid dose group was found dead on gestation day 23, one day after aborting. Two females in the high dose group were found dead, one on gestation day 19 and the other on gestation day 26 following abortion.

b. Clinical Observations

An increase in the number of animals with diarrhea was noted in the high-dose group during the dosing period; there was also an increase in a discharge believed to be vaginal in origin. No other observations appeared related to treatment.

c. Body Weight

The investigators supplied the following data: group mean and individual animal data. Corrected body weight gains were not provided.

Table I: Body Weight Gains (grams)[a]

Group	Prior to Dosing Period	Dosing Period	Post-Dosing Period	Entire Gestation Period
Control	116.5	137.1[+]	159.7	413.5
LDT	53.7	98.0	132.0	283.7
LMDT	92.9	69.6	196.8	359.3
HMDT	108.3	-15.2*	211.2	304.3
HDT	102.0	51.5	131.7	285.3

* = $p < 0.05$ as compared to control (Dunnett's test).
[+] = $p < 0.05$ linear trend analysis (ANOVA).
[a] = Data extracted from Report MR-8401-001, Table 1 & Appendix D.

There appears to be a linear downward trend in body weight gain during the dosing period, however, this is not maintained following the dosing period. Body weights and body weight gains in rabbits do not necessarily support evidence of true toxicity due to the feeding habits of rabbits.

d. Food Consumption

The investigators supplied the following data: group mean and individual animal data.

Table II: Food Consumption Data (mean grams)[a]

Group	Prior to Dosing Period	Dosing Period	Postdosing Period
Control	146.9	146.1[+]	137.0
LDT	141.3	134.3	120.4
LMDT	146.7	139.3	139.1
HMDT	146.0	128.2	139.5
HDT	149.4	131.2	140.2

[+] = $p < 0.05$, linear trend analysis (ANOVA).
[a] = Data extracted from Report MR-8401-001, Table 2.

A decreasing linear trend was noted in food consumption during the dosing period, however, due to the feeding habits of rabbits, this may be more of a palatability problem than true toxicity.

e. Gross Pathological Observations

The investigators supplied the following data: individual gross observations, including absolute and relative liver weights.

The only observation was the staining of hair in the rectal and vaginal areas consistent with what was observed during the clinical observations. No other compound-related observations were noted.

The following table presents the absolute and relative mean maternal liver weights.

Table III: Mean Maternal Liver Weights[+]

Group[a]	Daily Dose (mg/kg)	Body Weight[b] Final	Body Weight[b] Adjusted	Liver Weight Absolute (g)	Liver Weight Relative[c] (g/100 g)
Control	0	4038.1	3694.8	109.1*	3.0
LDT	100	3919.4	3571.8	104.1	2.9
LMDT	500	4008.3	3632.4	100.9	2.8
HMDT	1000	3905.7	3545.2	100.6	2.8
HDT	2000	3857.7	3561.4	99.7	2.8

[a] = Data from females that were not pregnant, aborted, delivered early, had total resorptions, died prior to scheduled sacrifice or whose uterine weight was not recorded were excluded. No significant difference from controls (Dunnett's test; $p \leq 0.05$).
[b] = Final weight (g) is the weight of the intact animal on Day 22 of gestation. Adjusted weight is the weight on Day 22 excluding the products of conception.
[c] = Calculation of relative weights is based on adjusted body weights.
* = Significant trend (linear combination of dose ranks ANOVA; $p = \leq 0.05$).
[+] = Data extracted from Report MR-8401-001, Table 4.

A linear downward trend was noted in the absolute liver weights, and although the relative liver weights exhibited a similar pattern, they did not achieve statistical significance. Further information on this observation should be obtained from the chronic feeding studies.

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f. Cesarean Section Observations

Table IV: Cesarean Section Observations[a]

Dose	Control	LDT	LMDT	HMDT	HDT
No. Animals Assigned	20	20	20	20	20
No. Animals Mated/ Inseminated	19	17	18	18	19
Pregnancy Rate (%)	95	85	90	90	95
Maternal Wastage					
No. Died	0	1	1	0	3
No. Died/Pregnant	0	1	1	0	3
No. Nonpregnant	1	3	2	2	1
No. Aborted	0	0	3[+]	2	6[+]
No. Premature Delivery	0	0	0	1	0
Resorptions Only	0	0	0	1	0
Total # of Litters	19	16	15	14	11
Total Corpora Lutea	182	139	166	146	108
Corpora Lutea/Dam	9.6	8.7	11.1	10.4	9.8
Total Implantations	117	108	117	101	70
Implantations/Dam	6.2	6.8	7.8	7.2	6.4
Total Live Fetuses	116	105	105	94	67
Live Fetuses/Dam	6.1	6.6	7.0	6.7	6.1
Total Resorptions	1	3	12	10	3
Early	0	2	10	4	0
Late	1	1	2	6	3
Resorptions/Dam	0.1	0.2	0.8**	0.5	0.3
Total Dead Fetuses	0	0	0	0	0
Dead Fetuses/Dam	0	0	0	0	0
# stunted fetuses	0	0	0	0	0
Mean Fetal Wgt (g)	48.00	44.21	44.96	43.25**	44.45
Preimplantation Loss(%)	35.7	22.3	29.5	30.8	35.2
Postimplantation Loss(%)	0.9	2.8	10.3	6.9	4.3
Sex Ratio (Mean M/F)	2.8/3.3	3.1/3.4	4.1/2.9	3.1/3.6	3.7/2.4

[+] = One animal died and aborted.

** = $p < 0.05$ as compared to controls (Mann-Whitney U Test).

[a] = Data extracted from Report MR-8401-0001, Table 5 and Appendix I.

Maternal toxicity was evidenced by the increase in abortions from the LMDT (500 mg/kg/day) and above, there was also an apparent increase in percent postimplantation loss at 500 mg/kg and above. There was possible evidence of slight developmental toxicity in the

form of reduced mean fetal body weights at the HMDT (1000 mg/kg/day). The high dose did not show a similar decrease, but this may be due to the reduced number of litters available as a result of the number of abortions. No other parameter exhibited biologically relevant differences.

2. Developmental Toxicity

External Observations

Table V: External Examinations[+]

Observations[a]	Control	Low Dose	Low Mid Dose	High Mid Dose	High Dose
# pups examined	116	105	105	94	67
# litters examined	19	16	15	14	11
Abdomen - Gastroschisis	0/0[b]	0/0	3/1	0/0	0/0
Paw - Clubbed	0/0	0/0	0/0	1/1	0/0
Subcutis-Hemorrhage	1/1	0/0	0/0	0/0	0/0

a = some observations may be grouped together.
b = Fetal (litter) incidence.
+ = Data extracted from Report MR-8401-001 Tables 6 and 7.

No treatment related observations were noted.

3. Visceral Observations

Table VI: Visceral Examinations[+]

Observations[a]	Control	Low Dose	Low Mid Dose	High Mid Dose	High Dose
# pups examined	116	105	105	94	67
# litters examined	19	16	15	14	11
Head:					
Eye-Hemorrhage	1/1[b]	0/0	2/2	0/0	1/1
Brain-Hydrocephaly	0/0	0/0	0/0	1/1	0/0
Visceral:					
Great Heart Vessels:					
Persistent Truncus Arteriosus	0/0	1/1	0/0	0/0	0/0
Lt Carotid Off Innominate	19/11	27/13	13/7	17/7	11/8
Heart:					
Septal Defect	0/0	0/0	0/0	1/1	0/0
Large	0/0	0/0	1/1	0/0	0/0
Bladder-Hemorrhage	9/7	7/6	9/6	6/3	1/1
Kidney:					
Small papilla - Size 2	0/0	1/1	0/0	0/0	0/0
Supernumerary Vessel-extra	6/6	4/3	6/4	5/4	4/3

a = Some observations may be grouped together.
b = Fetal (litter) incidence.
+ = Data extracted from Report MR-8401-001, Tables 6 & 7.

No treatment-related observations were noted.

4. Skeletal Observations

Table VII: Skeletal Examinations[+]

Observations[a]	Control	Low Mid Dose	High Mid Dose	Mid Dose	High Dose
# pups examined	116	105	105	94	67
# litters examined	19	16	15	14	11
Ribs:					
Absent	0/0[b]	0/0	1/1	0/0	0/0
Rudimentary Cervical	0/0	0/0	1/1	0/0	0/0
Partially Ossified	2/2	2/2	2/2	0/0	1/1
Skull:					
Parietal Fused	0/0	0/0	0/0	0/0	1/1
Bone Island	2/2	8/4	1/1	9/5	4/2
Interparietal-Absent	0/0	0/0	1/1	0/0	0/0
Parietal Partially Ossified	0/0	1/1	0/0	1/1	0/0
Sternebrae:					
Fused	1/1	0/0	0/0	0/0	0/0
Partially Ossified	3/3	5/3	1/1	1/1	2/2
Unossified	3/1	1/1	0/0	0/0	0/0
Vertebrae:					
Hemi	0/0	0/0	1/1	0/0	0/0
Partially Ossified	0/0	0/0	2/2	2/2	0/0
Hyoid:					
Bent	2/1	0/0	3/3	0/0	0/0

[a] = Some observations may be grouped together.
[b] = Fetal (litter) incidence
[+] = Data extracted from Report MR-8401-001, Tables 6 and 7.

No treatment-related observations were noted.

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D. Discussion/Conclusions:

1. Maternal Toxicity

Maternal toxicity was evidenced at 500 mg/kg/day and above by an increase in the number of animals with diarrhea (high dose), possible vaginal discharge, abortion, a decrease in body weight gain during the dosing period, increase in gross pathological observations and an apparent increase in percent postimplantation loss.

2. Developmental Toxicity

a. Deaths/Resorptions

There was an apparent increase in percent postimplantation loss in the 500 mg/kg/day dose group and above.

b. Altered Growth

There was slightly reduced mean fetal body weight at the HMDT (1000 mg/kg/day). The high dose did not show a similar decrease, but this may be due to the reduced number of litters available as a result of the number of abortions.

c. Developmental Anomalies

No treatment-related observations were noted.

d. Malformations

No treatment-related observations were noted.

E. Study Deficiencies:

The protocol stated that gravid uterine weights were taken; however, these data were not provided to the Agency. Historical control data for all measured parameters were not provided.

F. Core Classification - Core-Guideline Data

Maternal NOEL = 100 mg/kg/day
Maternal LOEL = 500 mg/kg/day
Developmental Toxicity NOEL = 500 mg/kg/day
Developmental Toxicity LOEL = 1000 mg/kg/day

G. Risk Assessment:

None necessary at this time

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Accent toxicology review 007830

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Pages 66 through 73 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

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___ 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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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.

Primary Review By: Stephen C. Dapson, Ph.D. Stephen C. Dapson 1/22/90
Pharmacologist, Review Section I, TB-HFAS/HED (H7509C)
Secondary Review By: Yiannakis, M. Ioannou, Ph.D., D.A.B.T. YMI 1-23-90
Section Head, Review Section I, TB-HFAS/HED (H7509C)

DATA EVALUATION RECORD

007830

Study Type: Multigeneration Reproduction - Rat
Guideline 83-4

MRID No.: 410826-10

Test Material: Technical Grade IN V9360-27 (94.5%)
(Batch No.) Haskell No. 16,925

Synonyms: 3-Pyridinecarboxamide, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-N,N-dimethyl-

Study Nos.: Medical Research Project No. 8277-001
Haskell Laboratory Report No. 631-88

Sponsor: Agricultural Products Department
E.I. du Pont de Nemours & Company, Inc.
Wilmington, Delaware 19805

Testing Facility: E.I. du Pont de Nemours & Company, Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road
P.O. Box 50
Newark, Delaware 19714

Title of Report: Reproductive and Fertility Effects with IN V9360-27 Multigeneration Reproduction Study in Rats (First Generation Interim Report).

Author: Linda S. Mullin, M.A.

Date Report Issued: December 15, 1988

Conclusions: There was a slight increase in food consumption for the F_0 rats in the 20,000 ppm dose group (statistically significant for females only). A statistically smaller mean litter size after culling was observed in the 5,000 and 20,000 ppm dose groups. An increase in bilateral testicular degeneration (not statistically significant) was observed in the 20,000 ppm males. These observations will be evaluated in the final report when data from the F_1 parental rats and F_{2a} and F_{2b} litters are available for comparison.

Core Classification: Core-Supplementary Data since this is an interim report.

I. PROTOCOL

A copy of the materials and methods section from the investigators' report is included as an appendix.

A. Materials:

1. Test Species: 43-day-old male and female Crl:CD BR strain rats were obtained for the first parental generation of the study from Charles River Laboratories, Inc., Kingston, New York on October 13, 1987. The rats were acclimated for a period of 9 to 14 days before they were placed into the study. They received Ground Certified Purina Laboratory Chow (GCPLC) and tap water *ad libitum*.

2. Diet Preparation: Test diets were analyzed for homogeneity of mixtures and chemical stability in dietary mixtures (see attached materials and methods for more detail).

B. Procedures and Study Design:

1. Mating: One male was caged with one female from the same test group until sperm cells were observed in vaginal smears taken daily during the mating period. If sperm was not found after 7 days observation, the first male was removed and replaced by another male from a group of males with proven fertility in the same test group for a period of 7 days. It was not indicated if brother-sister matings were avoided.

After successful mating, each pregnant female was individually housed in a cage with a wire mesh bottom until gestation day 14 when all females were housed individually in a cage with a solid bottom and bedding where they were kept through gestation and lactation.

2. Mating Schedule: The F_0 parental animals were given test diets for 70 days before they were mated. Selection of parents for the F_1 generation was made when the pups were 21 days of age.

3. Animal Assignment: F_0 animals were randomly assigned to test groups as follows:

Test Groups		Dose	Animals per Group**	
No.	Designation	(ppm)*	Males	Females
1	Control	0	30	30
2	Low (LDT)	250	30	30
3	Mid (MDT)	5000	30	30
4	High (HDT)	20000	30	30

*Diets were administered from the beginning of the study until the animals were sacrificed.

**The same number of animals were picked from the F_1 litters as parents for the F_2 generation.

C. Observation Schedule:

1. Parental Animals: Observations and the schedule for those observations are summarized from the report as follows:

Type of Observation	Number of Animals per Sex per Group	Frequency
Mortality and signs of toxicity	All	Once a day during premating and growth periods.
Detailed clinical observations	All	Once a week during growth and breeding periods.
Body weight	All	At beginning of study and weekly through growth and mating periods.
	Maternal animals	Days 0, 7, 14, and 21 of gestation; days 0, 7, 14, and 21 postpartum; and weekly until sacrifice.
	Maternal animals	Weekly post-mating until sacrifice.
Food consumption	All	Weekly during premating period.

2. Reproductive Performance: Parental reproductive performance was assessed from breeding and parturition records of animals in the study. A mating was considered successful if there was evidence of intravaginal or extruded copulation plug. The following indices were calculated:

$$\text{Mating Index} = \frac{\#\ \text{copulated}}{\#\ \text{cohoused}} \times 100$$

$$\text{Female Fertility Index} = \frac{\#\ \text{females pregnant}}{\text{Total}\ \#\ \text{females mated}} \times 100$$

$$\text{Gestation Index} = \frac{\#\ \text{live litters born}}{\#\ \text{pregnancies}} \times 100$$

3. **Litter Observations**: According to the report, the following litter observations were made:

Observation	Time of Observation (Lactation Day)				
	Birth	Day 4[+]	Day 7	Day 14	Day 21
Number of live pups	x	x	x	x	x
Group pup weight	x	x	x	x	x[++]
External alterations	x	x	x	x	x
Number of dead pups[+++]	x	x	x	x	x
Sex of each pup	x	x	x	x	x

[+]Litters were culled randomly to 8 animals (4 per sex if possible) on postpartum day 4. Extra animals were sacrificed and discarded. Litters were counted and weighed before and after culling.

[++]Individual pup weights were also determined.

[+++]Dead pups were examined grossly for external and internal abnormalities, and a possible cause of death was determined for pups born or found dead.

The following indices were calculated:

$$\text{Gestation Index} = \frac{\text{\# of females bearing litters with at least one live pup}}{\text{\# of females bearing litters}} \times 100$$

$$\text{Viability Index} = \frac{\text{Total \# of pups alive on Postpartum Day 4 (before culling)}}{\text{Total \# of pups born alive}} \times 100$$

$$\text{Pups Born Alive} = \frac{\text{\# of live pups}}{\text{Total \# of pups born}} \times 100$$

$$\text{Lactation Index} = \frac{\text{\# alive at day 21}}{\text{\# alive at day 4}} \times 100$$

$$\text{Litter Survival} = \frac{\text{\# of litters weaned}}{\text{\# of litters delivered}} \times 100$$

4. **Necropsy**

a. **Parental Animals**: All surviving parental males were sacrificed as soon as possible after the last litters in each generation were produced. Maternal animals were sacrificed within 2 days of weaning of litters. These animals were subject to postmortem examinations as follows:

Animals Examined	Macroscopic	Microscopic
Found dead	x	
Unscheduled sacrifice	x	
Scheduled sacrifice	x	x+

+see section c below

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b. Offspring: Extra offspring after culling at day 4 were sacrificed and discarded without pathological examination. Remaining F_1 weanlings not chosen to serve as parents for the F_2 generation were also sacrificed and discarded without pathological evaluation.

c. Necropsy Observations: Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.

The following tissues were prepared for microscopic examination.

X	Ovaries	X	Epididymides
X	Uterus	X	Prostate
X	Unusual lesions	X	Seminal vesicles
X	Vagina/cervix	X	Testes (weighed)

Additional tissues prepared for microscopic examination included coagulating gland in the male, and the pituitary in both sexes.

According to the investigators, "except for the testes, epididymides, and gross lesions, histopathological examination of the tissues was conducted only for the control and 20,000 ppm group of both sexes".

D. Data Analyses:

Statistical Analyses: According to the investigators:

Body weights, body weight gains, food consumption, gestation length, and organ weights were analyzed by a one-way analysis of variance. When the test for differences among test group means (F test) was significant, pairwise comparisons between test and control groups were made with the Dunnett's test. Incidence of clinical observations were evaluated by the Fisher's Exact test with a Bonferroni correction and the Cochran-Armitage test for trend. Mating index, fertility index, gestation index, and litter survival were evaluated with the Fisher's Exact test. Pup numbers, pup weights, viability index, and lactation index were analyzed with the Mann-Whitney U test. Significance for all tests was judged at alpha = 0.05.

II. REPORTED RESULTS

A. Analysis of Test Diets:

The initial "purity" of the sample was 97.4 %. Prior to the start of the study, a sample was analyzed and found to have a "purity" of 94.5 %. The investigators used this for diet calculations. Reanalysis 1 month later found a "purity" of 95.5 %.

Analysis of diet mixes found a range of "approximately 95-97% of nominal dietary concentration". The F_0 rats received average concentrations of 242, 4734, and 18,963 ppm for the low, mid, and high dose levels, respectively. The investigators reported that the mixtures were homogeneous and stable when tested.

B. Parental Animals:

1. Mortality and Clinical Signs: The investigators noted no specific treatment related observations in F_0 males and females.

No animals were reported to have died.

2. Body Weight and Food Consumption: The report noted that "individual food consumption was determined weekly throughout the premating feeding period for the F_0 rats", and additionally for females it was "recorded on day 0, 7, and 14 of gestation".

Reported body weight and selected food consumption results are summarized as follows:

Observation and Study Week	Dose Group Control	Low	Mid	High
F_0 Generation Males - Premating				
Mean Body Weight (g)				
0	284.1	285.2	285.1	284.4
10	585.1	589.0	603.0	585.8
Mean Weight Gain (g)				
0 - 1	301.0	303.9	317.9	301.4
Mean Food Consumption (g/rat/day)				
1	27.5	27.9	27.9	28.7
2	26.0	26.9	26.4	27.5
10	27.7	28.3	29.4	28.7
0 - 10	27.1	27.0	27.7	28.2
F_0 Generation Females - Premating				
Mean Body Weight (g)				
0	189.7	187.8	191.0	189.2
10	305.4	302.6	317.8	313.4
Mean Weight Gain (g)				
0 - 10	115.8	114.7	126.8	124.2
Mean Food Consumption (g/rat/day)				
1	19.6	19.6	20.1	21.0
5	18.5	20.2	19.8	19.9
6	19.2	20.3	20.3	22.5*
7	18.9	19.1	19.4	24.7*
8	20.3	18.8	20.0	21.4
10	19.5	20.1	20.0	20.6
0 - 10	19.0	19.7	20.0	21.2*

*Statistically significantly different from control, $p < 0.05$.

There were no biologically relevant differences between body weight, body weight gains, and food consumption for male and female rats during the premating period.

Selected group mean body weights and food consumption values for pregnant or nursing dams were summarized in the report as follows:

Observation and Study Week	Dose Group Control	Low	Mid	High
F_1 Generation Females - Gestation				
Mean Body Weight (g)				
0	303.9	302.7	322.5	313.8
1	331.2	333.0	353.2	347.1
2	358.7	362.3	382.6	377.1
3	431.0	437.3	454.9	439.4
Mean Body Weight Gains (g)				
0 - 1	27.4	30.4	30.8	33.2
1 - 2	27.4	29.2	29.3	30.0
2 - 3	71.9	75.0	72.3	66.6
0 - 3	127.3	134.6	132.4	129.3
Mean Food Consumption (g/rat/day)				
1	22.4	22.3	22.9	23.7
2	23.2	23.6	24.2	25.5
0 - 2	22.8	23.0	23.5	24.6
F_1 Generation Females - Lactation				
Mean Body Weights (g)				
0	330.2	324.2	350.4	346.9
1	324.8	321.5	335.7	331.1
2	340.2	330.8	345.0	351.7
3	314.8	316.3	328.8	331.0
Mean Body Weight Gains (g)				
0 - 1	-5.5	-2.7	-14.8	-13.4
1 - 2	15.5	9.3	9.4	20.7
2 - 3	-25.4	-14.4	-16.2	-20.8
0 - 3	-15.4	-7.8	-21.6	-15.0

No statistically significant differences were noted and no biologically relevant differences were noted in the above data.

3. **Test Substance Intake**: Based on food consumption, body weight, and dietary analyses results, the doses expressed as mg test substance/kg body weight/day were as follows during the premating period:

	Dose Levels (ppm)					
	Males			Females		
	250	5000	20,000	250	5000	20,000
Week	F_0 Generation					
0 - 1	20.7	410	1707	23.3	468	1943
1 - 2	17.3	340	1438	19.9	423	1690
2 - 3	15.8	308	1346	19.1	367	1657
3 - 4	13.5	289	1227	21.4	434	1582
4 - 5	13.9	288	1106	19.1	358	1442
5 - 6	13.3	264	1114	18.6	359	1584
6 - 7	12.9	255	1060	17.0	330	1685
7 - 8	12.0	242	1026	16.6	332	1437
8 - 9	12.4	251	1029	17.0	317	1355
9 - 10	12.0	244	980	16.8	314	1330
0 - 10	14.4	289	1203	18.9	370	1571

As can be seen from the above data, the test substance intake declined over the premating period; however, no biologically relevant differences were noted.

The test substance intake for females during gestation is as follows:

Week	250	5000	20,000
0 - 1	16.5	327	1356
1 - 2	16.3	319	1348
0 - 2	16.4	323	1352

4. **Reproductive Performance**: The investigators noted the following effects on reproductive performance: "After culling on day 4 until weaning, the mean number of pups in the 5,000 ppm group was significantly less than the mean number of control pups. The mean number of pups in the 20,000 ppm group was less from postnatal day 7 until weaning. However, there were no significant differences between groups in the lactation index or litter index or litter survival. The statistically significant difference from controls in pup numbers for the 5,000 and 20,000 groups after culling is considered to be due in part to several small litters which were born in these groups. That is, some differences in mean litter size were present from birth, but did not become statistically significant (for the 5,000 ppm group) until after the litters were reduced on day 4. For the 20,000 ppm group, the difference in litter size did not become statistically significant until postnatal day 7 when, in addition to slightly smaller litter size from birth, some pup deaths also occurred. The lower mean litter size in these groups is on the low end of control values typically seen at this laboratory" [however, no historical control data were provided].

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Results for the parental animals are summarized from the report as follows:

Observation	Dose Group Control	Low	Mid	High
F_0 Generation - Litter F_1				
Median precoital interval (days)	Not provided			
Males				
Mated	5	2	4	4
Fertile	5	2	4	4
Fertility not determined	-	-	-	-
Intercurrent deaths	0	0	0	0
Mating Index (%)	96.7	100.0	96.7	93.3
Females				
Number mated	30	30	30	30
Number fertile	28	24	23	23
Fertility not determined	1	1	2	0
Intercurrent deaths	0	0	0	0
Fertility Index (%)	96.6	80.0	79.3	82.1
Median gestation interval (days)	22.3	22.2	22.6	22.2
Number of litters	28	24	23	23
Total litter losses	0	0	0	0
Mean litter size				
At Birth	13.8	14.5	12.3	13.2
Born Alive	13.8	14.3	12.3	13.1
Day 4 Precull	13.6	14.0	12.1	13.0
Day 4 Postcull	8.0	7.8	7.5*	7.5
Day 7	8.0	7.8	7.5*	7.4*
Day 14	8.0	7.8	7.5*	7.4*
Day 21	8.0	7.8	7.5*	7.4*
Pup deaths (Day 1-29)	6	11	7	10
Mean pup weight (g)				
Postpartum Day 0	6.6	6.4	6.9	6.5
Precull 4	10.5	10.2	11.1	10.5
Postcull 4	10.5	10.3	11.2	10.5
7	16.5	15.9	17.0	15.7
14	34.6	33.7	36.5	34.3
21	57.2	56.1	60.6	55.3

*Statistically significantly different from control, $p < 0.05$.
**Statistically significantly different from control, $p < 0.01$.

As can be seen in the above data, the mean litter size of the mid and high dose groups was statistically significantly lower than the control from Day 4 Postcull on, however, the biological relevance of this observation is unclear and will require data from the second generation for complete evaluation. No other differences were noted.

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5. **Necropsy Results**

a. **Organ Weights**: The report noted no specific effects on testes weight. The data for male rats are summarized from the report as follows:

Observation	Dose Group			
	Control	Low	Mid	High
F_0 Generation - Males				
Final body weight (g)	629.2	640.9	656.8	635.0
Organ weight (g) - Testes	3.556	3.563	3.690	3.571
Relative to body weight (g) - Testes	0.5710	0.5613	0.5658	0.5658
F_0 Generation - Females				
Final body weight (g)	314.6	319.7	334.8	334.8

b. **Pathology**

1) **Macroscopic Examination**: The report noted no specific treatment related observations.

2) **Microscopic Examination**: The investigators noted "a small (not statistically significant) incidence of bilateral testicular degeneration".

Observation	Dose Group			
	Males			
	Control	Low	Mid	High
Number examined	30	30	30	30
Testes				
Degeneration, seminiferous (atrophy), bilateral	0	1	3	4
Degeneration, seminiferous (atrophy), unilateral	0	0	0	1
Epididymides				
Epithelial degeneration, focal vacuolar	3	1	1	1
Immature Sperm, increased bilateral	0	1	1	3
Inflammation, focal interstitial	3	2	5	5
Sperm granuloma, unilateral	1	1	1	1

Other observations in males and those in females did not exhibit a dose response. The above observation in testes will require the second generation for a more complete evaluation.

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C. Offspring:

Viability and Clinical Signs: The authors noted that no differences were seen in the viability indices. Viability results from pups during lactation are summarized from the report as follows:

Observation and Study Time	Dose Group Control	Low	Mid	High
F_0 Generation				
Litter F_1				
Gestation Index (%)	100.0	100.0	100.0	100.0
No. Pups Born Alive (%)	99.5	98.3	99.5	99.3
Viability Index (%)	99.0	98.4	98.5	98.7
Lactation Index (%)	100.0	100.0	99.4	98.4
Litter Survival (%)	100.0	100.0	100.0	100.0

Changes in mean litter sizes were summarized in the report as follows:

Observation and Study Time	Dose Group Control	Low	Mid	High
F_0 Generation - Litter F_1				
Mean litter size				
At Birth	13.8	14.5	12.3	13.2
Born Alive	13.8	14.3	12.3	13.1
Day 4 Precull	13.6	14.0	12.1	13.0
Day 4 Postcull	8.0	7.8	7.5*	7.5
Day 7	8.0	7.8	7.5*	7.4*
Day 14	8.0	7.8	7.5*	7.4*
Day 21	8.0	7.8	7.5*	7.4*

*Statistically significantly different from control, $p < 0.05$.

The report stated that no differences were seen in the clinical observations.

III. DISCUSSION

A. Investigators' Conclusions:

The following is from the investigators' report:

No effects were observed in this study at 250 ppm of IN V9360-27 in the diet. A slight increase in food consumption by the F_0 rats was observed in the 20,000 ppm groups (statistically significant for females only). There was a statistically smaller mean litter size after culling the 5,000 and 20,000 ppm groups. There were significantly heavier final body weights for females (pregnant and nonpregnant combined) in the 5,000 and 20,000 ppm groups. An increase in bilateral testicular degeneration (not statistically significant) was observed in the 20,000 ppm males.

It is inconclusive whether these effects are compound related and the importance of all these findings will be evaluated in the final report when data from the F_1 parental rats and F_{2a} and F_{2b} litters are available for comparison.

B. Reviewer's Discussion:

The observations noted in this study cannot be adequately assessed until all data from F_1 parental animals and the additional litters are received and reviewed.

Accent toxicology review

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Pages 87 through 93 are not included in this copy.

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___ 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

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NATIONAL SECURITY INFORMATION

EPA No.: 68D80056
DYNAMAC No.: 262-A
TASK No.: 2-62A
January 24, 1990

007830

DATA EVALUATION RECORD

ACCENT

Metabolism Study in Rats

STUDY IDENTIFICATION: Hundley, S.G. Metabolism of [pyridine-2-^{14}C] and [pyrimidine-2-^{14}C]DPX-V9360 by the laboratory rat. (Unpublished report No. 717-88 prepared by Haskell Laboratory for E.I. duPont de Nemours and Company, Inc., Wilmington, DE; dated December 16, 1988.) MRID No. 410826-17.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: [signature]

Date: 1/24/90

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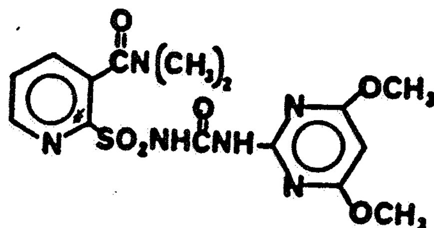
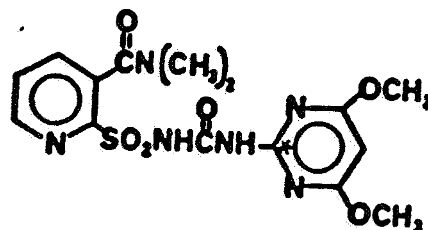
1. CHEMICAL: Accent; 2-(4,6-dimethoxy-2-pyrimidinyl)aminocarbonylaminosulfonyl-N,N-dimethyl-3-pyridinecarboxamide; DPX-V9360.

2. TEST MATERIAL: [Pyridine-2-^{14}C]Accent (I) had a specific activity of 62.9 μCi/mg and a radiochemical purity of 99 percent; [pyrimidine-2-^{14}C]Accent (II) had a specific activity of 62.2 μCi/mg and radiochemical purity of 98.8 percent. Unlabeled test material had a chemical purity of approximately 99.5 percent. The chemical structures and radiolabeled carbons (denoted by asterisks) are as follows:



I



II

3. STUDY/ACTION TYPE: Metabolism in rat.

4. STUDY IDENTIFICATION: Hundley, S.G. Metabolism of [pyridine-2-^{14}C] and [pyrimidine-2-^{14}C]DPX-V9360 by the laboratory rat. (Unpublished report No. 717-88 prepared by Haskell Laboratory for E.I. duPont de Nemours and Company, Inc., Wilmington, DE; dated December 16, 1988.) MRID No. 410826-17.

5. REVIEWED BY:

Nicolas P. Hajjar, Ph.D. Signature: *Nicolas P. Hajjar*
Principal Reviewer
Dynamac Corporation Date: *January 22, 1990*

William L. McLellan, Ph.D. Signature: *William L. McLellan*
Independent Reviewer
Dynamac Corporation Date: *January 24, 1990*

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Roman J. Pienta, Ph.D. Signature: *Roman J. Pienta*
Department Manager
Dynamac Corporation Date: *Jan 24, 1990*

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Mike Ioannou, Ph.D., D.A.B.T.
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Toxicology Branch II
(H-7509C)

Signature: [signature]
Date: 1-29-90

96
B

7. CONCLUSIONS:

The metabolism of 2-(4,6-dimethoxy-2-pyrimidinyl)aminocarbonylaminosulfonyl-N,N-dimethyl-3-pyridinecarboxamide (Accent) was studied in male and female Sprague-Dawley Crl:CDBR rats. [pyridine-2-^{14}C]Accent was administered orally at 10 mg/kg or 1000 mg/kg, at 10 mg/kg following oral administration of unlabeled Accent at 10 mg/kg/day for 14 days, and intravenously at 10 mg/kg. [pyrimidine-2-^{14}C]Accent was administered orally at 1000 mg/kg. Total recovery of administered radioactivity 4 days postdosing accounted for 98 to 109 percent of the dose. Most of the radioactivity was excreted unchanged within 24 hours postdosing. With oral dosing, there were no apparent differences between sexes or dose groups, although a slightly greater percentage of the administered radioactivity was detected in feces of animals receiving the high dose than in animals receiving the low dose. Following oral dosing, elimination in the feces accounted for 80 to 95 percent of the dose, and elimination in the urine accounted for 9 to 20 percent. The average total cumulative excretion indicated half-lives between 12 and 24 hours. Following intravenous administration, approximately 76 to 80 percent of the dose was eliminated in the urine and 27 to 30 percent in the feces. Residues in tissues accounted for from 0.05 to 0.5 percent of the dose. The major excretion product in urine and feces was unchanged parent compound. In addition, pyridinesulfonamide (N,N-dimethyl-2-sulfonamide pyridine-3-carboxamide) was detected in the urine and accounted for 1.1 to 5.7 percent of the dose. Pyridine acid sulfonamide (2-sulfonamidepyridine-3-carboxylic acid) was tentatively identified as a minor metabolite in the feces of orally dosed rats and urine of intravenously dosed rats. Based on the available data, a metabolic pathway was proposed.

These studies are acceptable and fulfill EPA requirements.

Items 8 to 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOL):

A. Materials and Methods:

1. Appropriate amounts of [pyridine-^{14}C] and unlabeled Accent were mixed in aqueous 0.05 M phosphate buffer at pH 9.0. The test material was then administered by oral gavage. For the intravenous experiments, [pyridine-2-^{14}C]Accent was formulated in 0.05 M potassium phosphate at pH 7.4 and administered via surgically inserted jugular vein cannulas.

[1]Only the items appropriate to this DER have been included.

Four experiments were conducted with [pyridine ^{14}C]Accent as follows: a single low oral dose of 10 mg/kg; a single high oral dose of 1000 mg/kg; a single low oral dose following oral administration of unlabeled test material at 10 mg/kg/day for 14 days, and a single low intravenous dose. All animals received at least 10 μCi of radioactivity. A fifth group of animals received a single 1000 mg/kg oral dose of [pyrimidine-^{14}C] Accent.

2. Male and female Crl:CD BR rats were obtained from Charles River Breeding Laboratories, Kingston, NY, or Raleigh, NC. The animals were 6 to 9 months old and were acclimated to laboratory conditions for 1 week prior to dosing. Five males and five females were used per group.

3. Following dosing, each rat was placed in a glass metabolism cage designed for separate collection of urine and feces. Expired air was also collected from one rat in each of the high-dose groups. Excreta were collected 12, 24, 36, 48, 72, and 96 hours postdosing and stored at -20°C until assayed. Carbon dioxide trapping solutions were changed at the same intervals.

   Animals were sacrificed 96 hours postdosing, and the following organs and tissues were collected: blood, heart, lungs, liver, kidneys, spleen, gonads, uterus, brain, and samples of skin, muscle, fat, and bone marrow.

   Aliquots of urine, dosing solutions, cage washes, CO_2 trapping solutions, fecal extracts, and high-pressure liquid chromatography (HPLC) eluant fractions were radioassayed directly by liquid scintillation counting (LSC). Blood, tissues, feces, and residual feed were homogenized and samples were combusted prior to radioassay by LSC. The gastrointestinal tract was washed and its contents radioassayed.

4. Pooled urine samples from all collection intervals were analyzed by HPLC. Aliquots of 0.5 mL urine were mixed with a 1:1 mixture of acetonitrile:0.05 M potassium phosphate buffer and then centrifuged. The liquid layer was then analyzed by HPLC. Feces (0.5-g samples) were freeze dried and extracted three times with methylene chloride:methanol (1:1, v/v), three times with methanol, and three or four times with a mixture

of methanol:0.05 M potassium phosphate buffer (3:1, v/v) at pH 9.0. The extracts were pooled and evaporated to dryness under nitrogen. The residues were then reconstituted in 0.05 M potassium phosphate buffer and acetonitrile prior to HPLC analysis. Urinary and fecal metabolites were also analyzed by mass spectrometry.

B. Protocol: A protocol was not presented.

12. REPORTED RESULTS:

A. Total recovery of administered radioactivity 4 days postdosing accounted for 98 to 109 percent of the dose (Table 1). Most of the radioactivity following oral dosing was eliminated in the feces (80 to 95 percent) with only 9 to 20 percent eliminated in the urine. Most of the radioactivity was eliminated within 24 hours of dosing. Little, if any, radioactivity (<0.01 percent of the dose) was eliminated as $[^{14}C]CO_2$. There were no apparent differences between sexes or between orally dosed test groups, although animals receiving the high dose excreted slightly higher amounts of radioactivity in the feces than animals receiving the low dose.

Following intravenous administration, approximately 76 to 80 percent of the radioactivity was eliminated in the urine of dosed rats and 27 to 30 percent was eliminated in the feces. The average total cumulative excretion following oral dosing indicated excretion half-lives between 12 and 24 hours, whereas following intravenous dosing the excretion half-life less than 12 hours.

B. Total radioactivity recovered in the carcass, tissues, and gastrointestinal contents ranged from 0.05 to 0.5 percent of the dose (Table 1). The highest residue levels were detected in the liver (1 μg/g) and kidneys of animals receiving 1000 mg/kg of [pyridine-2-^{14}C]Accent (Table 2).

C. An average of 97 percent or more of the radioactivity contained in the feces of dosed animals was extracted. The major excretion product in urine and feces was unchanged Accent, which accounted for 87 to 98 percent of the dose (Table 3). Pyridine sulfonamide was detected in urine from all rats dosed orally with [pyridine-2-^{14}C]Accent and accounted for about 1.1 to 5.7 percent of the recovered radioactivity. Unknown A was tentatively identified as pyridine acid sulfonamide and was detected only in the feces of orally dosed rats. However, it was also detected in urine from females receiving repeated dosing. Another minor metabolite (Unknown 1) was also detected in the feces of rats receiving a single dose of 10 or 1000 mg/kg [pyridine-2-^{14}C]Accent.

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TABLE 1. Biodisposition of Radioactivity 4 Days Following the Administration of [^{14}C]Accent to Rats

Test Group, Dose	Percent of Dose Recovered: Urine	Feces	Carcass and tissues	Cage Wash	Total
Single oral dose, 10 mg/kg[a]					
Males	19.9	80.5	0.09	0.34	101
Females	13.7	87.9	0.13	0.66	102
Single oral dose, 1000 mg/kg[a]					
Males	8.79	89.0	0.06	0.14	97.9
Females	9.65	94.0	0.12	0.57	104
Single oral dose, 1000 mg/kg[b]					
Males	11.30	92.50	0.05	0.08	104
Females	9.14	95.2	0.06	0.14	105
Repeated oral dosing, 10 mg/kg[c]					
Males	12.7	86.6	0.10	0.23	99.6
Females	18.7	85.1	0.25	0.40	104
Single iv dose, 10 mg/kg[a]					
Males	76.3	29.6	0.32	1.03	107
Females	79.9	27.0	0.48	1.55	109

[a] [pyridine-2-^{14}C]Accent.

[b] [pyrimidine-2-^{14}C]Accent.

[c] Single oral dose of [pyridine-2-^{14}C]Accent following oral administration of unlabeled test material at 10 mg/kg/day for 14 days.

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TABLE 2. Radioactive Residues in Tissues of Rats 4 Days Following the Administration of [^{14}C]Accent

Tissue	Tissue Radioactive Residue Labels (µg/g) in rats Receiving (mg/kg):									
	10[a]		1000[a]		1000[b]		10[c]		10[d]	
	M	F	M	F	M	F	M	F	M	F
Blood	0.003	0.003	<0.3	<0.4	<0.1	<0.2	0.004	0.004	0.006	0.007
Heart	0.006	0.005	0.3	0.5	<0.04	ND[e]	0.008	0.007	0.010	0.012
Lungs	0.006	0.003	<0.3	<0.4	<0.04	<0.1	0.006	0.004	0.007	0.006
Liver	0.017	0.015	0.92	1.2	0.3	0.4	0.017	0.019	0.020	0.022
Spleen	<0.007	<0.004	0.06	<0.1	ND	ND	<0.005	<0.006	0.006	0.006
Kidneys	0.008	0.009	0.5	0.7	<0.2	<0.2	0.009	0.011	0.024	0.032
Testes	<0.003	<0.004	<0.3	ND	<0.04	ND	0.004	0.003	0.005	0.004
Fat	<0.015	<0.002	<0.14	ND	<0.2	<0.1	<0.002	<0.002	<0.006	<0.004
Skin	0.009	0.005	<0.28	0.6	0.3	<0.3	0.008	0.007	0.011	0.010
Muscle	0.009	0.003	0.3	<0.4	ND	ND	0.005	0.004	0.006	0.007
Brain	0.004	0.003	<0.3	<0.4	ND	ND	0.005	0.004	0.006	0.006
Marrow	<0.019	ND	<0.28	ND	ND	ND	<0.003	<0.001	<0.004	<0.006
GI[f]	0.003	0.004	0.3	0.92	0.3	<0.3	0.005	0.021	0.010	0.024

[a]Single oral dose of [pyridine-2-^{14}C]Accent.

[b]Single oral dose of [pyrimidine-2-^{14}C]Accent.

[c]Single oral dose of [pyridine-2-^{14}C]Accent following oral administration of unlabeled test material at 10 mg/kg/day for 14 days.

[d]Single iv dose of [pyridine-2-^{14}C]Accent.

[e]Not detected.

[f]Gastrointestinal tissue.

TABLE 3. Percent Recovery of Radioactive Compounds in Urine Samples and Fecal Extracts from Rats Dosed with [^{14}C]Accent

Sample/Metabolite	Percent of Total Recovered Radioactivity:									
	10[a]		1000[a]		1000[b]		10[c]		10[d]	
	M	F	M	F	M	F	M	F	M	F
Urine										
Accent	14.0	11.0	8.0	8.5	8.9	6.9	9.7	14.0	70.0	71.0
Pyridine sulfonamide	5.7	2.7	1.3	1.1	--	--	3.0	3.5	--	--
5-Hydroxy pyrimidine amine	--	--	--	--	1.9	1.9	--	--	--	--
Unknown A[e]	--	--	--	--	--	--	--	0.5	0.7	2.4
Feces										
Accent	73.0	79.0	81.0	85	89	91	87	82	19.0	20.0
Unknown A[e]	--	2.1	--	--	--	--	--	--	1.3	3.4
Unknown 1	6.6	4.9	9.8	5.4	--	--	--	--	7.5	1.8

[a]Single oral dose of [pyridine-2-^{14}C]Accent.

[b]Single oral dose of [pyrimidine-2-^{14}C]Accent.

[c]Single oral dose of [pyridine-2-^{14}C]Accent following oral administration of unlabeled test material at 10 mg/kg/day for 14 days.

[d]Single iv dose of [pyridine-2-^{14}C]Accent.

[e]Tentatively identified as pyridine acid sulfonamide.

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Rats dosed with [pyrimidine-2-^{14}C]Accent excreted 5-hydroxy pyrimidine amine in the urine, which accounted for 1.9 percent of the recovered radioactivity. Pyridine sulfonamide was not detected in the urine of rats dosed intravenously. However, unknown A was detected in the urine at about 0.7 to 2.4 percent of the dose, and in the feces at about 1.3 to 3.4 percent of the dose. The identities of Accent and pyridine sulfonamide were confirmed by mass spectrometry. Based on the available data, a metabolic pathway was proposed for Accent (Figure 1).

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

A. The metabolism data clearly suggest limited intestinal absorption of Accent following any of the oral dosing routines, as demonstrated by the high fecal excretion of unchanged Accent. Even when dosed intravenously, there was minimal biotransformation of Accent. Excretion half-lives were between 12 and 24 hours following any of the oral dosing routines for either sex and less than 12 hours after the iv dose. In addition, all the tissue concentration data indicated low residual levels at sacrifice, even following the 1000-mg/kg dose. No organ or tissue from either sex exhibited selective retention or potential for accumulation of dosed radioactivity. The sum total of metabolic data support the observation of minimal toxicity, both acute and chronic.

B. A quality assurance statement was signed and dated December 15, 1988.

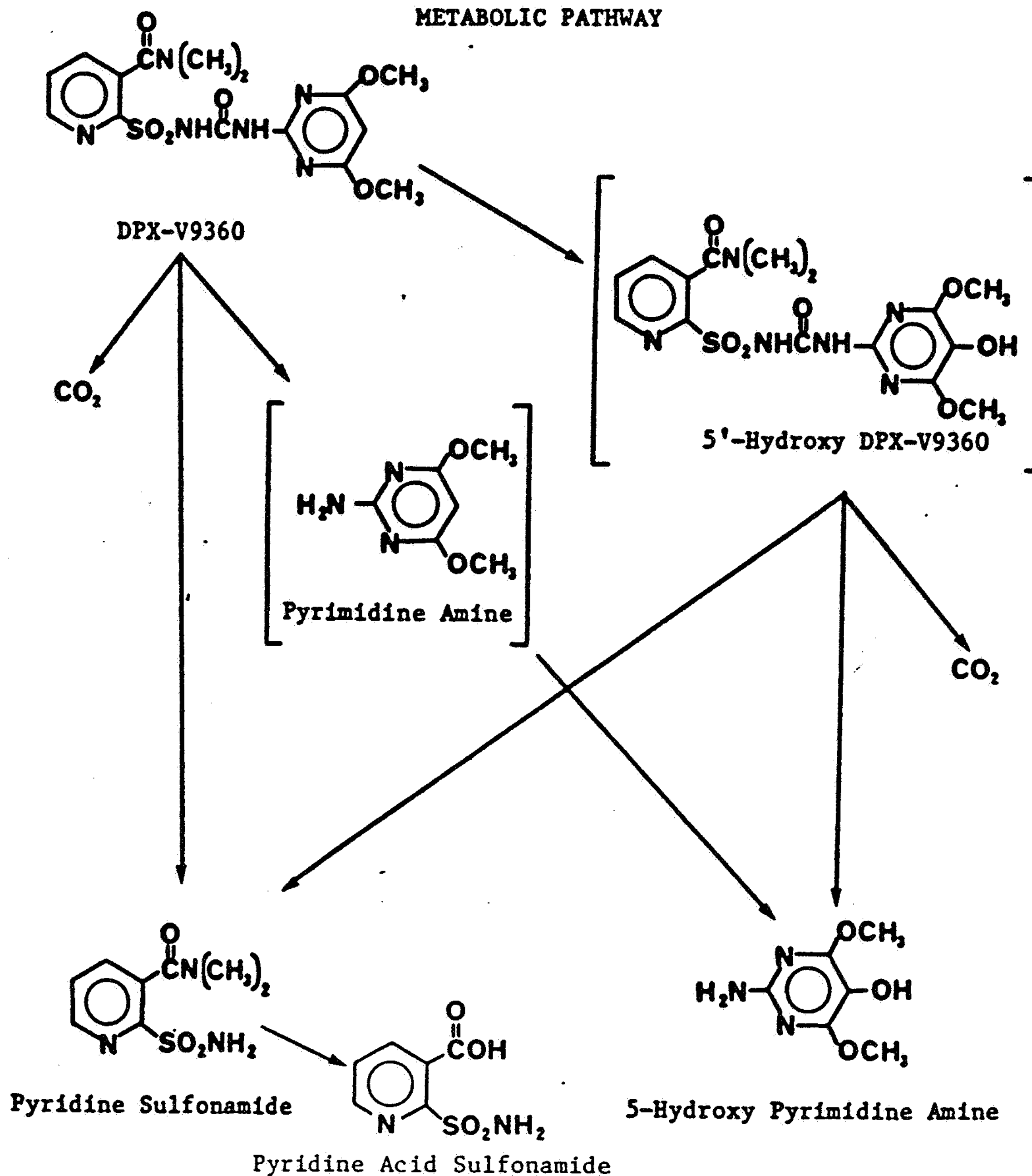
14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This study was adequately conducted, and the conclusions of the author are supported by the data presented. Adequate numbers of male and female rats were used in each group. The test material used in four experiments was labeled in the pyridine moiety and an additional experiment was conducted with the test material labeled in the pyrimidine moiety. The results support and corroborate each other. The analytical procedures used were adequate, and the identity of the parent compound and a metabolite were confirmed by mass spectrometry. These studies also conform with EPA's guidelines.

Items 15 and 16--see footnote 1.

Figure 1

METABOLIC PATHWAY



Source: CBI Figure 24, CBI page 79

Tox Chem No. 359 J DPX-V9360 (Accent) File Last Update: ________ Current Date ________

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Metabolism Species : Rat Haskell Laboratory 717-88 ; 12/16/88	DPX-V9360 (99.5% ai)	410826-17	^{14}C-Accent (labeled either in the pyridine or pyrimidine moeity) was administered to Sprague-Dawley rats at 10 mg/kg as a single oral dose, single intravenous dose or 14 day repeated oral dose. A high dose of 1000 mg/kg was also administered to rats as a single oral dose. Results indicate that through the oral route very little Accent is absorbed from the gastrointestinal tract and is excreted in the feces (80-95% of the dose) as parent compound. IV administration resulted in the excretion of 76-80% of the dose in the urine as unchanged parent compound. Two metabolites were identified in the urine of male and female rats as: pyridine sulfonamide and 5-hydroxy pyrimidine; another metabolite found in small quantities in the urine and feces of both sexes was tentatively identified as pyridine acid sulfonamide		Guideline

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Guideline Series 84: MUTAGENICITY

Reviewed by: John H.S. Chen, D.V.M. John H.S. Chen 12/21/89
Section I, Toxicology Branch II - HFAS (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D. YMI 1/4/90
Section I, Toxicology Branch II - HFAS (H7509C)

007830

DATA EVALUATION REPORT

CHEMICAL: 3-Pyridinecarboxamide

Tox. Chem. No.:

EPA File Symbol:

STUDY TYPE: UDS Assay in Rat Primary Hepatocytes
MRID No.: 410826-13
ACCESSION NUMBER:

SYNONYMS/CAS No.: IN V9360-27

SPONSOR: E.I. du Pont Nemours and Company, Inc., Wilmington, DE

TESTING FACILITY: Haskell Laboratory, Newark, DE

TITLE OF REPORT: Assessment of IN V9360-27 in the In-Vitro Unscheduled DNA Synthesis Assay in Rat Primary Hepatocytes

AUTHOR(S): Karin S. Bentley

STUDY NUMBER(S): 302-88

REPORT ISSUED: May 6, 1988

CONCLUSION(S) - Executive Summary:

IN V9360-27 did not cause any DNA damage or inducible repair in the rat hepatocyte unscheduled DNA synthesis assay under the conditions tested.

Dose levels tested: 0.04, 0.4, 1.2, 4.1, 12, 41, 122, 409 & 470 ug/ml

Study: Unacceptable
(Deficiency: inadequate highest dose tested)

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I. Materials and Methods:

1. Test Materials

The test compound, IN V9360-27 (97.4% Purity; Batch No. E-51077-87-1-B1) dissolved in DMSO was used in this study. 2-Acetylaminofluorene (1-AAF, 0.22 ug/ml) was used as the positive control.

2. Medium

Williams' medium E containing 10% fetal bovine serum, 292 mg/L L-glutamine and 50 ug/ml gentomycin.

3. Indicator Cells

Primary hepatocytes were isolated from adult male rats (Crl: CD BR) by in situ-collagenase perfusion according to protocol UDS-99, Edition 2, Attendant Standard Operating Procedures on files with the Quality Assurance Section of Haskell Laboratory (Reference: Kreamer et al., In Vitro Cellular and Developmental Biology, 22: 201-211, 1986).

Monolayer cultures were established on chamber slides for initiation of the UDS assay. All cultures were maintaining at about 37° C in a humidified atmosphere with 5% CO_2 (4 cultures for each treatment).

4. Preliminary Cytotoxicity Test

A cytotoxicity test was performed to determine the highest concentration to be used in this UDS assay. Attached primary cells were exposed to 9 concentrations of the test material (i.e., 0.04, 0.4, 1.2, 4.1, 12, 41, 122, 409 & 470 ug/ml) for 18 hours. After exposure, cytotoxicity was determined by elevation of lactate dehydrogenase activity (LDA). Based on the results obtained, there was no cytotoxicity observed at any of the test concentrations (See results in attached Table 1).

5. UDS Assay

The freshly isolated liver cells attached on chamber slides (5 X 10^5 viable cells) were used. Following the addition of IN V9360-27 and ^{3}H-thymidine (5 uCi/ml) in the culture chamber, the cultures were then incubated for 18 hours at 37° C. After incubation, the treated cultures were rinsed with WME, swelled with 1% sodium citrate and fixed with ethanol:glacial acetic acid (3:1). The slides were dipped into Kodak Autoradiographic Emulsion, Type NTB-2, dried for 2 hours, and stored in desiccated slide box at -70° C for 4 to 5 days to expose the emulsion. The slides were then developed, and stained with methyl-green pyronin Y.

6. Grain Counting

Counting of silver grains over the nuclei and cytoplasm of the hepatocytes was carried out with the aid of a remote TV camera to a microscope. From each of the treatment groups and from the positive and the negative controls, 100 nuclei in altogether 4 slides (25 cells/slide) were scored. Cytoplasmic grains were substracted from the nuclear grains to determine the net nuclear grains of each cell. The mean net nuclear grains from all slides of the same test concentration were averaged to determine the UDS response for that treatment in each trial..

7. Evaluation Criteria

The test compound is generally considered to be active in the DNA repair test, if one of the following conditions is met:

A. The average UDS response for any concentration of the test material from both trials is 5 net nuclear grains or more above the control response, and this increase is at least 3 standard deviations above the control response.

B. There is a positive correlation between increasing concentrations of test material and the average UDS response in the absence of a negative correlation between the test concentration and average cytoplasmic grains.

II. Reported Results:

1. Cytotoxicity determined by elevating LDH activity was not observed at any of the concentrations tested (0.04 through 470 ug/ml).

2. UDS was not observed in either trial as a result of treatment of primary rat hepatocytes with IN V9360-27 and at no concentration of IN V9360-27 was the mean net nuclear grain of trials 1 and 2 greater than three standard deviations above control. Therefore, the test material was negative in this assay.

III. Reviewer's Discussion and Conclusion:

1. The positive control compound (2-AAF) induced significant increase in the net nuclear grains per nucleus when compared to that of the corresponding controls (See Results in attached Tables II and III). These results indicated that the cell population employed was adequate for the detection of UDS in rat hepatocytes.

2. However, based on the results of cytotoxicity test, the highest concentration (470 ug/ml) used in this study did not demonstrate any cytotoxic effect (expressed by the elevation of LDA) for all of the experiments evaluated. It is questionable whether an appropriate highest dose of the test material (greater than 470 ug/ml and less than 10 mg/ml; Reported limit of solubility - 47 mg/ml) was chosen for this study. Therefore, this study is unacceptable in the present form.

Accent toxicology review

Page ______ is not included in this copy.

Pages _110_ through _115_ 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.

Guideline Series 84: **MUTAGENICITY**

Reviewed by: John H.S. Chen, D.V.M. [signature] 12/21/89
Section I, Toxicology Branch II (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D. [signature] 1/4/90
Section I, Toxicology Branch II (H7509C)

007830

DATA EVALUATION REPORT

CHEMICAL: 3-Pyridinecarboxamide

Tox. Chem. No.:

EPA File Symbol:

STUDY TYPE: **Mammalian cells in culture gene mutation assay in** the Chinese hamster ovary cells

ACCESSION NUMBER:
MRID No.: 410816-14
SYNONYMS/CAS No.: IN V9360-27

SPONSOR: E.I. du Pont de Nemours and Company, Inc., Wilmington, Delaware

TESTING FACILITY: Haskell Laboratory, Newark, Delaware

TITLE OF REPORT: Mutagenicity Evaluation of IN V9360-27 in the CHO/HPRT Assay

AUTHOR(S): Karin S. Bentley

STUDY NUMBER(S): 429-88

REPORT ISSUED: July 14, 1988

CONCLUSION(S) - Executive Summary:

IN V9360-27 was nonmutagenic in the in-vitro CHO/HPRT mutation assay (by measuring induction of resistance to 6-thioguanine) with or without metabolic activation at the concentrations tested.

Concentrations tested: 4, 20, 40, 200, & 465 ug/ml

Study: Unacceptable

(See deficiencies identified in the attached DER)

MAMMALIAN CELLS IN CULTURE GENE MUTATION

A. MATERIALS

1. Test Material: Name: IN V9360-27
   Description (e.g. technical, nature, color, stability):
   White solid
   Batch #: E-51077-87-1B1 Purity: 97.4%
   Contaminants: if reported, list in CBI appendix
   Solvent used: DMSO
   Other comments:

2. Control Materials:
   Negative: DMSO
   Solvent/final concentration:
   Positive: Non-activation (concentrations, solvent):
   62.1 ug/ml Methanesulfonic Acid Ethylester (EMS) in phosphate buffered saline

   Activation (concentrations, solvent):
   3.8 ug/ml 9,10-dimethyl-1,2-benzanthracene (DMBA) in DMSO

3. Activation: S9 derived from
   X Aroclor 1254 X induced male rat X liver
   ___ phenobarbital ___ non-induced ___ mouse ___ lung
   ___ none ___ hamster ___ other
   ___ other ___ other
   If other, describe below
   Describe S9 mix composition (if purchased, give details):

   Cofactor solution was not included

4. Test Cells: mammalian cells in culture
   ___ mouse lymphoma L5178Y cells
   X Chinese hamster ovary (CHO) cells
   ___ V79 cells (Chinese hamster lung fibroblasts)
   ___ other (list):

   Properly maintained? (Y) / N (circle one)
   Periodically checked for Mycoplasma contamination?
   (Y) / N (circle one)
   Periodically checked for karyotype stability?
   Y / (N) (circle one)
   Periodically "cleansed" against high spontaneous background?
   Y / (N) (circle one)

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5. Locus Examined:

___ thymidine kinase (TK)
selection agent: ___ bromodeoxyuridine (BrdU)
(give concentration) ___ fluorodeoxyuridine (FdU)
___ trifluorothymidine (TFT)

_X_ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
Selection agent: ___ 8-azaguanine (8-AG)
(give concentration) 1×10^{-5} M 6-thioguanine (6-TG)

___ Na^+/K^+ ATPase
Selection agent: ___ ouabain
(give concentration)

___ other (locus and/or selection agent; give details):

6. Test compound concentrations used:
Non-activated conditions: 4, 20, 40, 200, & 465 ug/ml

Activated conditions: 4, 20, 40, 200, & 465 ug/ml

B. TEST PERFORMANCE

1. Cell treatment:
a. Cells exposed to test compound for:
18-19 hours (non-activated) _5_ hours (activated)

b. Cells exposed to positive controls for:
18-19 hours (non-activated) _5_ hours (activated)

c. Cells exposed to negative and/or solvent controls for:
18-19 hours (non-activated) _5_ hours (activated)

d. After washing, cells cultured for _7_ days (expression period) before cell selection

e. After expression, cells cultured for 6-8 days in selection medium to determine numbers of mutants and for 6-8 days without selection medium to determine cloning efficiency

2. <u>Protocol</u> **(brief description, or attach copy to appendix, if appropriate; include e.g. number of cell cultures; medium; incubation times; cell density during treatment; number of cells seeded for treatment and selection; subculture and feeding schedules, if necessary):**

The protocol used in this study was adopted from the method described by Hsie et al (Mutation Res. 86: 193-214, 1981). The detailed procedures described on pages 7, 8, 9, 10, and 11 of the original laboratory report are attached.

3. **<u>Preliminary cytotoxicity assay</u> (include concentration ranges, activation and nonactivation; reported results, e.g. cytotoxicity and solubility):**

In this assay, cultured cells were exposed to 5 concentrations of test material in 25 cm^2 flasks for 5 hours with and without metabolic activation (4 , 20, 40, 200, & 465 ug/ml). After the treatment, the treated cells were washed with culture medium, followed by reincubation in fresh growth medium for 21-25 hours, subcultured, and incubated for further 6-8 days. The colonies were stained and counted. The highest concentration of IN V9360-27 tested (465 ug/ml) produced little or no toxicity to the cultured CHO cells in both the noncativated and activated studies. Tables 1, 2, 4 and 5 are attached.

4. <u>Mutagenicity assay</u> **(reported results, e.g. induction of mutant colonies - individual colony counts and/or summary given; mutant frequencies per 10^6 survivors; positive and background mutant frequencies; inclusion of concentration levels used; number of cultures per concentration; levels of cytotoxicity obtained; appropriateness of cloning efficiencies; include representative table, if appropriate):**

Based on the mutagenicity results obtained from two trials (Tables 1 2, 4, & 5) with and without metabolic activation, no significant increase in mutant frequency was observed at any of the test concentrations tested (i.e., 4, 20, 40, 200, & 465 ug/ml) and no positive dose-response relationship was present in all of these two trials (Tables 3 & 6 attached). Therefore, IN V9360-27 showed no evidence of mutagenic activity in the CHO/HPRT mutation assay.

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5. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions):

A. The positive control compounds (EMS & DMBA) induced significant increases in the mutation frequency with respect to the corresponding solvent control by a mutation factor at least 36.3 (i.e., DMBA, 36.3-53.2; EMS, 40.6-41.9), indicating the assay system was sensitive to detect known mutagens in the presence and absence of metabolic activation.

B. However, the evaluation of mutagenicity of IN V9360-27 in the cultured CHO cells cannot be accomplished due to the following reported deficiencies:

i. The actual composition of the metabolic activation system including a cofactor solution was not included in the report.

ii. Since the highest concentration (465 ug/ml) used in this study did not reduce the survival to 10% of that seen in the corresponding solvent control for all the experiments (See Tables 1, 2, 4, & 5), it is questionable that the highest concentration of IN V9360-27 was appropriately selected for this study (Reference Hsie et al., Mutation Res. 86: 193-214, 1981)

iii. As a rule the S9 concentration which gives the greatest cytotoxicity should be chosen for the cultured mammalian cell gene mutation assay (References: Hsie et al., Mutation Res. 86: 193 -214, 1981; Li et al.. Mutation Res. 119: 387-392, 1983). It is unclear whether a specific study was performed to establish the optimal S9 concentration for this study.

Therefore, the study is unacceptable in the present form.

6. Was test performed under GLPs (is a quality assurance statement present)? (Y) / N (circle one)

7. CBI appendix attached (Y) / N (circle one)

Accent toxicology review

Page ______ is not included in this copy.

Pages 122 through 132 are not included in this copy.

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___ The product confidential statement of formula

___ Information about a pending registration action

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Guideline Series 84: **MUTAGENICITY**

Reviewed by: John H.S. Chen, D.V.M.
Section I, Toxicology Branch II - HFAS (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D.
Section I, Toxicology Branch II - HFAS (H7509C)

007830

DATA EVALUATION REPORT

CHEMICAL: 3-Pyridinecarboxamide

Tox. Chem. No.:

EPA File Symbol:

STUDY TYPE: **In vivo micronucleus assay in** mouse bone marrows
MRID No. 410826-16
ACCESSION NUMBER:

SYNONYMS/CAS No.: IN V9360-27

SPONSOR: E.I. du Pont de Nemours and Company, Inc., Wilmington, Delaware

TESTING FACILITY: Haskell Laboratory, Newark, Delaware

TITLE OF REPORT: Mouse Bone Marrow Micronucleus Assay of IN V9360-27

AUTHOR(S): Demetra A. Vlachos

STUDY NUMBER(S): 428-88

REPORT ISSUED: July 18, 1988

CONCLUSION(S) - Executive Summary:

IN V9360-27 had negative response in the mouse micronucleus test at all of the intervals (24, 48, & 72 hrs) evaluated.

Dose levels tested: 500, 2500 & 5000 mg/kg

Study: Acceptable

A. MATERIALS

1. Test Material: Name: IN V9360-27
Description (e.g. technical, nature, color, stability):
White solid,
Batch #: E-51077-87-1B1 Purity: 97.4%
Contaminants: if reported, list in CBI appendix
Solvent used: Corn oil
Other comments:

2. Control Materials:
Negative/Route of administration:

Vehicle/Final concentration/Route of administration:
Corn oil/ Single oral intubation in a volume of 15 ml/kg b.w.

Positive/Final concentration/Route of administration:
Cyclophosphamide/ 40 mg/kg b.w. / Single oral intubation in a volume of 10 ml/kg b.w.

3. Test compound:
Route of administration: Single oral intubation in a volume of 15 ml/kg

Dose levels used: 500, 2500, & 5000 mg/kg b.w.

4. Test animals:
a. Species mouse Strain Crl:CD-1(ICR)BR Age 42 days old
Weight 23.8(F)-33.5(M) g Source: Charles River Breeding Lab., N.Y.
b. No. animals used per dose: 5 males 5 females
c. Properly maintained? (Y) / N (circle one) (6 males and 6 females for 5000 mg/kg group)

B. TEST PERFORMANCE

1. Treatment and Sampling Times:
a. Test compound
Dosing: X once ____ twice (24 hr apart)
____ other (describe):

Sampling (after last dose): ____ 6 hr ____ 12 hr
X 24 hr X 48 hr X 72 hr (mark all that are appropriate)
____ other (describe):

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b. Negative and/or vehicle control
Dosing: __X__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
__X__ 24 hr __X__ 48 hr __X__ 72 hr (mark all that are appropriate)
_____ other (describe):

c. Positive control
Dosing: __X__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
__X__ 24 hr _____ 48 hr _____ 72 hr (mark all that are appropriate)
_____ other (describe):

2. **Tissues and Cells Examined:**

__X__ bone marrow _____ other (list):

No. of polychromatic erythrocytes (PCE) examined per animal: 1000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: 1000
Other (if other cell types examined, describe):

3. **Details of slide preparation:**

At the end of the specified intervals, the animals were sacrificed and bone marrow samples were obtained from both femurs of the test animals. The cells were resuspended in fetal bovine serum. Following centrifugation to pellet the cells, the supernatant was removed, leaving a small amount of serum with the remaining cell buttom. A Miniprep Automatic Blood Smearing Instrument was used to make the bone marrow smears. At least two slides per animal were prepared and fixed in methanol and stained in acridine orange.

4. **Preliminary cytotoxicity assay (reported results, e.g. include dose range, signs of toxicity - e.g. MTD considerations, clinical signs; no. animals):**

No cytotoxicity assay was conducted in this study. However. IN V9360-27 had an approximate lethal dose estimated at 11000 mg/kg. Therefore, a dose of 5000 mg/kg was selected as the highest dose for the micronucleus study. Additional levels of 2500 and 500 mg/kg were selected to complete a log dose range.

5. <u>Micronucleus assay</u> **(reported results, e.g. include induction of micronuclei; appropriateness of negative, solvent and positive control micronucleus frequencies; ratio of PCE/NCE; sex differences (if any); appropriateness of dose levels and route; statistical evaluation; include representative table, if appropriate):**

There were no statistically significant differences in micronucleated polychromatic erythrocyte (PCE) frequencies (Mean % Micronucleated PCEs) found between IN V9360-27 treated mice and concurrent solvent control groups at any sampling interval nor were any dose-related trend observed (Table 2 attached). The ratios of PCE to NCE observed in groups treated with the test material were not significantly different from concurrent solvent control mice. In contrast, the positive control compound (CP) induced significant increase in the frequencies of micronucleated PCEs in the bone marrow of mice of both sexes ($P < 0.001$) (See also Table 2).

6. <u>Reviewer's discussion/conclusions</u> **(include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions):**

A. The positive control compound, cyclophosphamide, apparently induced marked increase of the PCE with micronuclei ($P < 0.001$) in the bone marrows of both males and females, indicating the sensitivity of the assay system to detect a known clastogen.

B. The spontaneous rates of micronuclei in the PCE of the vehicle control mice (treated with corn oil) were found from 0.02% (males) to 0.04% (females) in this study. These results are within the normal range for performing the mouse micronucleus test as described by Heddle et al (Mutation Res. 123: 61-118, 1983).

C. Although there was no evidence of clinical toxicity or body weight change occurred in the mouse groups treated with IN V9360-27, the test material has been tested up to 5000 mg/kg.

D. Under the conditions tested, IN V9360-27 had negative response in the mouse micronucleus assay at all of the sampling intervals (24, 48, & 72 hrs) evaluated. The study is acceptable.

7. Was test performed under GLPs (is a quality assurance statement present)? (Y) / N (circle one)

8. CBI appendix attached (Y) / N (circle one)

TABLE 2: MICRONUCLEUS DATA SUMMARY

IN V9360-27 MOUSE MICRONUCLEUS ASSAY

IN V9360-27 Treatment (mg/kg)	Sacrifice Time (hrs)	Mean % Micronucleated PCEs ± S.E. (1000 PCEs Scored)				Mean Ratio PCE:NCE ± S.E.	
		N	Males	N	Females	Males	Females
0	24	5	0.18 ± 0.07	5	0.36 ± 0.08	0.85 ± 0.13	0.90 ± 0.15
500	24	5	0.16 ± 0.08	5	0.10 ± 0.06	0.61 ± 0.08	1.00 ± 0.13
2500	24	5	0.24 ± 0.12	5	0.14 ± 0.05	0.73 ± 0.07	0.86 ± 0.09
5000	24	6	0.13 ± 0.02	6	0.32 ± 0.08	0.63 ± 0.04	0.82 ± 0.09
Trend Analysis			N.S.		N.S.	N.S.	N.S.
0	48	5	0.12 ± 0.05	5	0.22 ± 0.09	1.04 ± 0.22	1.36 ± 0.19
5000	48	6	0.23 ± 0.07	6	0.32 ± 0.09	1.08 ± 0.12	1.07 ± 0.20
0	72	5	0.20 ± 0.07	5	0.16 ± 0.08	1.07 ± 0.34	1.05 ± 0.15
5000	72	6	0.17 ± 0.07	6	0.15 ± 0.04	0.79 ± 0.19	1.60 ± 0.40
CP 40 mg/kg	24	5	2.10 ± 0.27***	5	1.56 ± 0.17***	0.85 ± 0.17	0.73 ± 0.07

IN V9360-27 = H-16,925-02
N.S. = not significant
*** $p < 0.001$

MR 4581-554

Guideline Series 84: MUTAGENICITY

Reviewed by: John H.S. Chen, D.V.M. *[signature]* 1/10/90
Section I, Toxicology Branch II (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D. *[signature]* 1/11/90
Section I, Toxicology Branch II (H7509C)

007830

TA EVALUATION REPORT

CHEMICAL: 3-Pyridinecarboxamide

Tox. Chem. No.:

EPA File Symbol:

STUDY TYPE: **Mammalian cells in culture cytogenetics assay in** human lymphocytes

ACCESSION NUMBER:
MRID No. : 410826-15

SYNONYMS/CAS No.: IN V9360-27

SPONSOR: E.I. du Pont de Nemours and Company, Inc., Wilmington, DE

TESTING FACILITY: Haskell Laboratory, Newark, DE

TITLE OF REPORT: In Vitro Evaluation of IN V9360-27 for Chromosome Aberrations in Human Lymphocytes

AUTHOR(S): Demetra A. Vlachos

STUDY NUMBER(S): 470-88

REPORT ISSUED: July 26, 1988

CONCLUSION(S).- Executive Summary:

IN V9360-27 induced no significant damage to chromosomal structure of cultured human lymphocytes in the presence and absence of metabolic activation under the conditions tested.

Dose Levels tested: 40, 200, 400 & 470 ug/ml

Classification: Unacceptable

(See the deficiencies identified in the attached DER)

A. MATERIALS

1. Test Material: Name: IN V9360-27
   Description (e.g. technical, nature, color, stability):
   White solid
   Batch #: E-51077-87-1-B1 Purity: 97.4%
   Contaminants: if reported, list in CBI appendix
   Solvent used: DMSO
   Other comments:

2. Control Materials:
   Negative: DMSO
   Solvent/final concentration:
   Positive: Non-activation (concentrations, solvent):
   0.35 ug/ml Mitomycin-C dissolved in distilled deionized water

   Activation (concentrations, solvent):
   10 ug/ml Cyclophosphamide dissolved in distilled deionized water

3. Activation: S9 derived from
   _X_ Aroclor 1254 _X_ induced _male_ rat X___ liver
   ___ phenobarbital ___ non-induced ___ mouse ___ lung
   ___ none ___ hamster ___ other
   ___ other ___ other
   If other, describe below
   Describe S9 mix composition (if purchased, give details):

   The S9 mix composition used in this study was not given.

4. Test compound concentrations used:
   Non-activated conditions: 40, 200, 400 & 470 ug/ml

   Activated conditions: Same as above

5. **Test Cells**: mammalian cells in culture
   Describe cell line, cell strain or primary cell culture (if human lymphocytes, describe conditions of subjects) used:

   Human blood was drawn aseptically from healthy donors and diluted RPMI 1640 culture medium containing 1.5% PHI, 15% dialyzed heat-inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (42 or 50 IU/ml), streptomycin (42 or 50 ug/ml) and HEPES buffer (0.021 Or 0.025 M). Appropriately labelled duplicate cultures were initiated for each test condition, including controls, and positive indicators. The cultures were incubated at 37°C.

   Properly maintained? (Y) / N (circle one)

   Cell line or strain periodically checked for Mycoplasma contamination? Y / N (circle one) Not Applicable

   Cell line or strain periodically checked for karyotype stability? Y / N (circle one) Not Applicable

B. **TEST PERFORMANCE**

1. **Cell treatment:**
   a. **Cells exposed to test compound for:**
      3 **hours (non-activated)** 3 **hours (activated)**

   b. **Cells exposed to positive controls for:**
      3 **hours (non-activated)** 3 **hours (activated)**

   c. **Cells exposed to negative and/or solvent controls for:**
      3 **hours (non-activated)** 3 **hours (activated)**

2. **Protocol (brief description, or attach copy to appendix, if appropriate; include e.g. number of cell cultures; medium; incubation times; if lymphocytes, nature of mitogen and when added; cell density during treatment; harvest times; spindle inhibitor and when used; chromosome preparation and analysis; number of cells/culture analyzed; statistics used):**
   The protocol used in this study was provided on the pages 8-12 of the original laboratory report (attached).

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3. **<u>Preliminary cytotoxicity assay</u> (include concentration ranges, activation and nonactivation; reported results, e.g. cytotoxicity and solubility; rationale for determining harvest times (e.g. alterations in cell cycle) and concentration levels, if reported):**

   In the preliminary cytotoxicity assay, seven concentrations of test material (i.e., 4, 50, 100, 200, 300, 400 & 470 ug/ml) were exposed to cultured cells for 3 hours. IN V9360-27 was not cytotoxic at concentrations up to 470 ug/ml. No cell cycle delay was seen under either nonactivated or activated conditions (See attached results in Table 1). Therefore, a harvest time of approximately 18-hour post-treatment was chosen for this study. Test concentrations selected for the cytogenetic assay were 470, 400, 200, and 40 ug/ml.

4. <u>Cytogenetics assay</u> **(reported results, e.g. induction of aberration frequency; types of aberrations, e.g. whether gaps are included in analysis or not, chromatid vs. chromosomal events, complex aberrations; positive and background aberration frequencies; number of cultures per concentration; levels of cytotoxicity obtained, e.g. effect on mitotic index or cell survival, if examined; include representative table, if appropriate):**

(A) The positive control compounds (Mitomycin-C & CP) induced statistically significant positive responses (expressed by % aberrations per cell) in the presence and absence of metabolic activation ($P < 0.001$) (See attached results in Tables 2 & 3).

(B) IN V9360-27 caused no statistically significant increase in the incidence c chromosomal damages (expressed by % aberrations per cell) at any dose level when tested either in the presence or absence of metabolic activation system (See resuls in Tables 2 & 3).

(C) Based on these results, IN V9360-27 did not exhibit clastogenic effect in cultured human lymphocytes either with or without S9 mix activation.

5. **Reviewer's discussion/conclusions** (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions; remember, do not include gaps in final aberration frequency analysis):

(A) The positive control compounds (MMC & CP) adequately demonstrated the sensitivity of the cultured human lymphocytes to detect a clastogenic effect under either the activated or nonactivated system.

(B) However, the evaluation of clastogenic actiivity of IN V9360-27 in this study cannot be accomplished due to the following reported deficiencies:

i. The actual composition of the metabolic activation system including a cofactor solution was not reported for this study.

ii. There was no indication that slides were coded prior to scoring.

iii. Since the highest dose level of test material used in this study (470 ug/ml) did not demonstrate cytotoxicity to dividing lymphocytes resulting in reduction of mitotic index by approximately 50% (i.e., Nonactivated assay: Trial 1, 68.2% ; Trial 2, 102.8% of the solvent control; Activated assay: Trial 1, 88.9%; Trial 2, 100% of the solvent control), it is questionable whether an appropriate highest dose of the test material (greater than 470 ug/ml and less than 10 mg/ml; Reported limit of solubility-47 mg/ml) was chosen for this study.

iv. The study is judged unacceptable in the present form.

6. **Was test performed under GLPs (is a quality assurance statement present)? (Y) / N (circle one)**

7. **CBI appendix attached (Y) / N (circle one)**

Accent toxicology review

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007830

Guideline Series 84: MUTAGENICITY

Reviewed by: John H.S. Chen, D.V.M. [signature] 12/21/89
Section I, Toxicology Branch II - HFAS (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D. [signature] 1/4/90
Section I, Toxicology Branch II - HFAS (H7509C)

DATA EVALUATION REPORT

CHEMICAL: 3-Pyridinecarboxamide **Tox. Chem. No.:**

EPA File Symbol:

STUDY TYPE: Salmonella/mammalian activation gene mutation assay

ACCESSION NUMBER:
MRID No.: 410826-12
SYNONYMS/CAS No.: INV9360-7

SPONSOR: E.I. de Pont de Nemours and Company, Inc., Wilmington, Delaware

TESTING FACILITY: Haskell Laboratory, Newark, Delaware

TITLE OF REPORT: Mutagenicity Testing of INV9360-7 in the Salmonella typhimurium Plate Incorporation Assay

AUTHOR(S): Vincent L. Reynolds

STUDY NUMBER(S): 734-88

REPORT ISSUED: November 23, 1988

CONCLUSION(S) - Executive Summary:

INV-9360-7 was nonmutagenic to TA97A, TA98, TA100, and TA1535 strains of Salmonella typhimurium with or without metabolic activation at the concentrations tested.

Concentrations tested: 0.1, 0.25, 0.5, 0.75 & 1 ug/plate for TA98 and TA1535; 0.02, 0.04, 0.06, 0.08 & 0.1 ug/plate for TA97A; 0.1, 0.5, 1, 5, & 10 ug/plate for TA100

Study: Unacceptable
(Deficiency: lack of toxicity data for strains TA97A, TA98 & TA1535)

A. MATERIALS

1. Test Material: Name: INV-9360-7
   Description (e.g. technical, nature, color, stability):

   Batch #: E-47940-71 Purity: 94.9%
   Contaminants: if reported, list in CBI appendix
   Solvent used: DMSO
   Other comments:

2. Control Materials:
   Negative: DMSO
   Solvent/final concentration:
   Positive: Non-activation:
   Sodium azide ___2___ ug/plate TA100, TA1535
   2-Nitrofluorene ___25___ ug/plate TA98, ######
   ICR-191 Acridine ################ ___10___ ug/plate TA97, ######
   Other (list):

   Activation:
   2-Aminoanthracene (2-anthramine) ___1___ ug/plate
   usually all strains
   Other (list):

3. Activation: S9 derived from
   _X_ Aroclor 1254 _X_ induced male___ rat _X_ liver
   ___ phenobarbital ___ non-induced ___ mouse ___ lung
   ___ none ___ hamster ___ other
   ___ other ___ other
   If other, describe below
   Describe S9 mix composition (if purchased, give details):
   The S9 mix consisted of the following: 8 mM $MgCl_2$, 33 mM KCl; 5mM Glucose-6-phosphate, 4mM NADP, 100mM Sodium phosphate (pH 7.4), and 1.6 mg S9 fraction/1 ml of S9 mix.

4. Test organisms: S. typhimurium strains
   _X_ TA97 _X_ TA98 _X_ TA100 ___ TA102 ___ TA104
   _X_ TA1535 ___ TA1537 ___ TA1538 ; list any others:
   Properly maintained? (Y) / N (circle one)
   Checked for appropriate genetic markers (rfa mutation, R factor)? Y / N (circle one)

5. Test compound concentrations used:
   Non-activated conditions: 0.1, 0.25, 0.5, 0.75 & 1 ug/plate for TA98 and TA1535; 0.02, 0.04, 0.06,0.08&0.1 ug/plate for TA97A; 0.1, 0.5, 1, 5, 10 ug/plate for TA100.
   Activated conditions
   Test compound concentrations used under activated conditions are identical as described above.

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B. TEST PERFORMANCE

1. Type of Salmonella assay:
 - X standard plate test
 - ___ pre-incubation (___ minutes)
 - ___ "Prival" modification (i.e. azo reduction method)
 - ___ spot test
 - ___ other (describe in a.)

a. Protocol (brief description, or attach copy to appendix, if appropriate; e.g. include mediums used, incubation times, assay evaluation):

The study was conducted according to the method described by Ames and coworkers (Mutation Res. 113: 173-215, 1983). The detailed procedures used in this study were provided on pages 8-9 of the original laboratory report (attached).

2. **Preliminary cytotoxicity assay** (**include concentration ranges, activation and nonactivation; strain(s) used; reported results, e.g. cytotoxicity indices (effect on background lawn; reduction in revertants) and solubility):**

INV-9360-7 was tested for cytotoxicity in strain TA100. The results of this study indicated that the test material was toxic to strain TA100 at dose level of 10 ug/plate with and without activation. Therefore, the concentration of 10 ug/plate was chosen for the initial mutagenicity trial (Table 1 attached). The test material was also tested for mutagenicity in Salmonella typhimurium strains TA1535, TA97A, and TA98 with and without activation. Toxicity was observed in a preliminary mutagenicity trial in strains TA97A, TA98, and TA1535 with and without activation at dose levels of 0.5, 5, and 5 ug/plate, respectively. Therefore, the maximum doses with and without activation were adjusted in subsequent mutagenicity trials as follows: for TA97A, 0.1 ug/plate; for TA98 and TA1535, 1 ug/plate.

3. Mutagenicity assay (reported results, e.g. induction of revertants - individual plate counts and/or summary given; appropriateness of positive and background (concurrent and/or historical) revertant levels; number of concentration levels used; number of cultures per concentration; include representative table, if appropriate):

The mutagenicity of INV-9360-7 in this study was evaluated using four tester strains of Salmonella typhimurium (TA97A, TA98, TA100 & TA1535) at the concentrations previously recommended either in the presence or absence of metabolic activation.

Results for the activated and nonactivated mutation assays showed that counts of revertant colonies for each tester strain treated with INV-9360-7 were not different from that of the corresponding solvent controls at the concentrations tested (See Tables II & III for TA1535; Tables IV & V for TA97A; Tables VI & VII for TA98; Tables VIII & IX for TA100). The strain specific control compounds (2NF, NAAZ & ICR191 Acridine) and the positive control compound (2-AA) to ensure the efficacy of the activation system have given the positive responses as expected.

4. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions):

A. The spontaneous revertant colonies for each of the four tester strains of Salmonella typhimurium are found within the normal ranges of revertants recommended by Ames et al. (Mutation Res. 31: 347-364, 1975).

B. The significantly positive responses from the strain specific control compounds (2NF, NAAZ & ICR191 Acridine) and the positive control compound (2-AA) to ensure the efficacy of the activation system demonstrated the sensitivity of the assay systems with and without metabolic activation.

C. The rationale used to determine the maximum dose level for each of these tester strains of Salmonella typhimurium for this mutagenicity tests is considered adequate.

D. Since no statistically significant increases (less than 2-fold increase) in the number of revertant colonies for any of the four tester strains were observed following exposure to the test material either in the presence or absence of activation, we assess that the results of the two independently performed assays with INV-9360-7 did not suggest a positive effect.

E. Since the detailed data for the toxicity study in a preliminary mutagenicity trial in strains TA97A, TA98 and TA 1535 were not included in this report, the study is not fully acceptable in the present form. However, this study may be upgraded on resolution of the reported deficiency.

5. Was test performed under GLPs (is a quality assurance statement present)? (Y) / N (circle one)

6. CBI appendix attached (Y) / N (circle one)

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