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

007939

MAY 21 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ACCENT® (IN V9360) - Data Evaluation Reports for Studies Submitted by the Registrant.

Caswell No.: 359J
HED Project No.: 0-0565
Record Nos.: 258654, 258657 and 258656
Identifying Nos.: 9F3763, 352-LGL and 352-LGU
MRID Nos.: 413601-01 through 413601-05

FROM: Alan C. Levy, Ph. D., Toxicologist Alan C. Levy 5-16-90
Review Section I, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

TO: Robert J. Taylor PM 25
Registration Division (H7505C)

THRU: Yiannakis M. Ioannou, Ph. D., Section Head Y M Ioannou 5/16/90
Review Section I, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph. D., Branch Chief
Toxicology Branch II (HFAS)
Health Effects Division (H7509C) Marcia van Gemert 5/17/90

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

Action Requested: Review the following toxicology documents with ACCENT® (IN V9360):

Rat Two-Generation Study
Dog One-Year Study
Mouse Oncogenicity Study
Rat Toxicity/Oncogenicity Study
Rat Acute Inhalation Study

Addendum Regarding Purity Analyses

MULTIGENERATION REPRODUCTION - RAT Guideline §83-4

MRID No.: 413601-01
Test Material: IN V9360-27; 94.5% purity; Haskell Batch No. 16,925
Study Nos.: Medical Research Project No. 8277-001
Haskell Laboratory Report No. 429-89
Report Title: Reproduction and Fertility Effects with IN V9360-27; Multigeneration Reproduction Study in Rats
Concentrations (ppm): 0, 250, 5,000 and 20,000
Results:
decreased body weight gain in females in both generations during the final week of gestation - 20,000 ppm (1269 mg/kg/day)
reduced litter size at birth in the F_{2a} generation - 20,000 ppm (1269 mg/kg/day)
decreased pup weights postpartum days 14 through 21 in F_{2a} generation - 20,000 ppm (1269 mg/kg/day)

NOTE: Reduced fertility in groups (including control) was not considered to be test article related.

Systemic Toxicity NOEL = 5,000 ppm (287 mg/kg/day)
Systemic Toxicity LOEL = 20,000 ppm (1269 mg/kg/day)
Reproductive Toxicity NOEL = 5,000 ppm
Reproductive Toxicity LOEL = 20,000 ppm

Classification: Core Minimum

This study satisfies the data requirement for a reproduction study in rats (Guideline §83-4).

CHRONIC ORAL TOXICITY -DOG Guideline §83-1

MRID No.: 413601-02
Test Material: IN V9360-27; 90.6% purity; Haskell Batch No. 16,925
Study Nos.: Medical Research Project No. 8430-001
Haskell Laboratory Report No.: 390-89
Report Title: Chronic Toxicity Study with IN V9360-27 - One-Year Feeding Study in Dogs
Concentrations (ppm): 0, 250, 5,000 and 20,000
Results:
decrease in body weight gain - male: 20,000 ppm
female: 250, 5,000 and 20,000 ppm (28-40% from control value)
increase in relative liver and kidney weights - male at 20,000 ppm

Systemic Toxicity NOEL = male: 5,000 ppm (125 mg/kg/day)
female: not attained
Systemic Toxicity LOEL = male: 20,000 ppm (500 mg/kg/day - decreased body weight gain as well as increased relative liver and kidney weights
female: 250 ppm (6.25 mg/kg/day) - lowest dose tested - decreased body weight gain

2

Classification: Core Supplementary (no NOEL)

This study does not satisfy the guideline requirements (§83-1) for a chronic oral dog toxicity study.

ONCOGENICITY STUDY - MOUSE Guideline §83-2

MRID No.: 413601-03
Test Material: IN V9360-27; 94.5% (revised to 90.6%); Batch No. 16,925-02
Study Nos.: Medical Research Project No. 8313-001
Haskell Laboratory Report No. 645-89
Report Title: Oncogenicity Study with IN V9360-27; Eighteen-Month Feeding Study in Mice
Concentrations (ppm): 0, 25, 250, 2,500 and 7,500
Results:
no apparent test article effects observed

Systemic Toxicity NOEL = 7,500 ppm (mg/kg/day) was 993 for males and 1312 for females

The Agency Limit Dose of 1,000 mg/kg/day was tested. Carcinogenicity potential has been adequately assessed for the mouse. There was no apparent effect of the test article on tumor incidence.

Classification: Core Minimum.

For the record, historical control data should be submitted on the incidence of hepatocellular adenomas observed in the mouse at the testing facility.

This study satisfies the data requirement for an oncogenicity study in mice (Guideline §83-2).

COMBINED CHRONIC TOXICITY/ONCOGENICITY - RAT Guideline §83-5

MRID No.: 413601-04
Test Material: IN V9360-27; 97.4% purity revised to 90.6%; Haskell Batch No. 16,925
IN V9360-29; 92.75%; Haskell Batch No. 17,499
Study Nos.: Medical Research Project No. 8269-001
Haskell Laboratory Report No. 637-89
Report Title: Combined Chronic Toxicity/Oncogenicity Study with IN V9360 - Two-Year Feeding Study in Rats
Concentrations (ppm): 0, 50, 1,500, 7,500 and 20,000
Results:
no apparent test article effects observed

Systemic Toxicity NOEL = > 20,000 ppm (mg/kg/day: 786 for males and 1098 for females)
Systemic Toxicity LOEL = not attained - > 20,000 ppm (mg/kg/day: 786 for males and 1098 for females)

The Agency Limit Dose of 1,000 mg/kg/day was tested. The test article did not appear to increase the number of any tumors over control values.

Classification: Core Minimum

This study satisfies the guideline requirements (§83-5) for a combined chronic toxicity/oncogenicity rat study.

ACUTE INHALATION STUDY - RAT Guideline §81-3

MRID No.: 413601-05
Test Material: DPX-V9360-45 (milled); 75% purity (active ingredient); Haskell Batch No. 18,001
Study Nos.: Medical Research Project No. 4581-758
Haskell Laboratory Report No. 704-89
Report Title: Acute Inhalation Toxicity Study with DPX-V9360-45 (Milled) in Rats
Concentrations (mg/m^3): 2,600, 4,800 and 5,600 (nose-only exposure)
Results:
no apparent test article effects observed

LC_{50} = > 5,600 mg/m^3

Classification: Core Supplementary. Particle sizes were too large (25% were not < 1 um in diameter). The reason for not generating an atmosphere with smaller particles was not given. The study may be upgraded to Core Minimum if an acceptable response to the "particle size" inquiry is provided.

Toxicity Category: IV

This study does not satisfy the data requirement for an acute inhalation toxicity study in rats (Guideline §81-3).

ADDENDA TO PREVIOUSLY SUBMITTED REPORTS (no Guideline No.)

The Registrant submitted additional data regarding the "purity analyses" for the test article IN V9360-27 and IN V9360-7.

IN V9360-27: original purity = 97.4%; reanalysis purity = 90.6%
IN V9360-7: original purity = 94.9%; reanalysis purity = 90.4%

The addendum refers to the following studies:

Acute Oral - Rat - MRID No. 413601-12
Acute Dermal - Rabbit - MRID No. 413601-13
Acute Inhalation - Rat - MRID No. 413601-14
Primary Eye Irritation - Rabbit - MRID No. 413601-15
Primary Dermal Irritation - Rabbit - MRID No. 413601-16
Dermal Sensitization - Guinea Pig - MRID No. 413601-17
Subchronic Oral Toxicity and One-Generation Reproduction - Rat - MRID No. 413601-18
Subchronic Oral Toxicity - Mice - MRID No. 413601-19

Subchronic Oral Toxicity - Dog - MRID No. 413601-20
Teratogenicity - Rabbit - MRID No. 413601-21
Teratogenicity - Rat - MRID No. 413601-22
Mutagenicity - CHO/HPRT Assay - MRID No. 413601-23
Mutagenicity - Salmonella typhimurium - MRID No. 413601-24
Mutagenicity - Unscheduled DNA Synthesis in Rat Primary Hepatocytes - MRID No. 413601-25
Mutagenicity - Mouse Bone Marrow Micronucleus - MRID No. 413601-26
Mutagenicity - Chromosome Aberrations in Human Lymphocytes - MRID No. 413680-01

Primary Review By: Stephen C. Dapson, Ph.D. Stephen C. Dapson 5/11/90
Pharmacologist, Review Section I, TB-HFAS/HED (H7509C)
Secondary Review By: Yiannakis, M. Ioannou, Ph.D., D.A.B.T. 5/11/90
Section Head, Review Section I, TB-HFAS/HED (H7509C)

DATA EVALUATION RECORD

Study Type: Multigeneration Reproduction - Rat (Crl:CD BR)
Guideline 83-4

MRID No.: ~~410826-10~~ 413601-01 007939

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. 429-89

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

Author: Linda S. Mullin, M.A.

Date Report Issued: December 13, 1989

Conclusions: The No Observed Effect Level (NOEL) for Systemic Toxicity is 5000 ppm (287 mg/kg/day) with a Lowest Observed Effect Level (LOEL) of 20000 ppm (1269 mg/kg/day), based on F_1 (first mating) females with a lower body weight gain during the final week of gestation and a similar pattern in the F_0 females during the same period of gestation. The No Observed Effect Level (NOEL) for Reproductive Effects is 5000 ppm with a Lowest Observed Effect Level (LOEL) of 20000 ppm based on a minimal reduction on litter size at birth and in pup weights at postpartum day 14 through 21 in the F_{2a} high dose group. The other litters showed a similar tendency, but not a statistically significant difference. Doses tested: 250, 5000 and 20000 ppm. Strain:Crl: CD BR (Charles River).

Systemic Toxicity NOEL = 5000 ppm (287 mg/kg/day)
Systemic Toxicity LOEL = 20000 ppm (1269 mg/kg/day)
Reproductive Toxicity NOEL = 5000 ppm
Reproductive Toxicity LOEL = 20000 ppm

Core Classification: Core-Minimum Data
THIS STUDY SATISFIES THE DATA REQUIREMENT FOR A REPRODUCTION STUDY IN RATS (GUIDELINE NO. 83-4).

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 Purina Cetified Rodent Chow #5002 (PCRC) 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 evidence of copulation was observed (intravaginal or extruded copulation plug). If evidence of copulation was not detected after 7 days observation, the female was housed with 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 (from interim report). Selection of parents for the F_1 generation was made when the pups were 119 days of age. According to the investigators: "Due to low fertility on the first F_1 mating, a second set of litters was produced. F_1 rats were remated one week after the last F_{2a} litters were weaned."

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 the study.
Detailed clinical observations	All	Once a week during premating, gestation and lactation periods.
Body weight	All	At beginning of study and weekly through premating.
	Maternal animals	Days 0, 7, 14, and 21 of gestation; days 0, 7, 14, and 21 postpartum; and weekly until sacrifice.
	Paternal animals	Weekly post-mating until sacrifice.
Food consumption	All	Weekly during premating period.
	Maternal animals	Days 0, 7 and 14 of gestation.

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:

	Time of Observation (Lactation Day)				
Observation	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(only for the F_2 pups), and a possible cause of death was determined for pups born or found dead.

The following indices were calculated:

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

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

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

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

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

4. Necropsy

a. Parental Animals: According to the investigators: "All F_0 males were sacrificed after siring litters (days 112 and 114). F_1 males were sacrificed near the end of the study. F_0 females were sacrificed within two days after weaning litters. F_1 females were sacrificed after weaning F_{2b} pups. All F_0 and F_1 parental rats, including females not bearing litters, were subjected to gross pathological examination." 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

b. Offspring: All "extra" offspring for both generations, 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. Randomly selected F_2 generation offspring received a gross pathological evaluation with those not selected sacrificed and discarded without further evaluation. According to the investigators: "F_1 offspring that died during the lactation period were discarded without pathological evaluation. F_2 offspring that died during the lactation period were necropsied." The investigators randomly chose twenty F_{2a} and F_{2b} weanlings per sex per dose group for a complete pathological examination conducted on day 21 of lacatation. All gross lesions were preserved but not examined further.

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".

For selected F_2 weanlings (20 per sex per dose group), the following tissues were collected and those marked with an * were weighed (and relative organ to body weights determined):

Thymus*, spleen*, bone marrow (sternum), mesenteric lymph node, heart*, trachea, lungs*, esophagus, stomach, small intestine (duodenum, jejunum and ileum), large intestine (colon, cecum and rectum), liver*, pancreas, kidneys*, bladder, thyroid, adrenals, testes*, epididymides, ovaries, uterus, vagina, brain, eyes, bone (sternum), and all gross lesions.

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 %. At the end of the study reanalysis found a triplicate sample mean of 93.7 $\pm$ 2.5%. Therefore the investigators considered the sample as "stable". The sponsor, however, stated that the compound had a purity of 90.6% (8/89). This is believed to be due to the differences in hydration of the sample at different sites.

Analysis of diet mixes at the start of the study, the beginning of the F_1 feeding phase , and at the end of the study found a range of "approximately 88-108 % of nominal dietary concentration. The F_0 rats received average concentrations of 245, 4914, and 19,733 ppm for the low, mid, and high dose levels, respectively. The investigators reported that the mixtures were homogeneous and stable for up to 14 days.

B. Parental Animals:

1. Mortality and Clinical Signs: The investigators noted no specific treatment related observations in F_0 males, but they found a statistically significant decrease in incidence of "colored nasal discharge" in the F_1 male mid dose group as compared to control. This was not considered to be compound related, and the biological relevance of such a finding is unclear. No other relevant observations were noted for the F_1 males. The investigators reported that there were no relevant observations in the F_0 or F_1 females during the premating, gestation or lactation periods. Data are presented on attached Tables 24 through 29.

No animals were reported to have died in the F_0 groups. For the F_1 males, two low dose animals died, one of unknown causes and the other as a result of trauma from a fall. Two F_1 males were sacrificed early, one low dose sacrificed on day 195 with malignant lymphoma and the other on day 225 due to spinal cord trauma. For the F_1 females, two animals were reported to have died, one low dose one day after delivery of a litter of two pups, the other was a high dose animal which was found to have malignant lymphoma. None of these deaths were treatment related.

2. Body Weight and Food Consumption: The investigators determined individual food consumption weekly throughout the premating feeding period for the F_0 and F_1 rats, and additionally for all maternal animals it was recorded on day 0, 7, and 14 of gestation.

12

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 - 10	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	27.1	27.6	28.3
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	19.1	19.7	20.0	21.2*

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

There were no biologically relevant differences between body weight, and body weight gains for F_0 male and female rats during the premating period. Although food consumption for high dose F_0 females was occasionally statistically significantly greater than controls, the differences are slight and the biological relevance of this observation is unclear.

Observation and Study Week	Dose Group: Control	Low	Mid	High
F_1 Generation Males - Premating				
Mean Body Weight (g)				
0	58.2	56.8	61.2	55.8
17	618.7	645.6	654.1	620.6
Mean Weight Gain (g)				
0 - 17	560.5	588.7	592.8	564.8
Mean Food Consumption (g/rat/day)				
1	14.3	14.4	14.7	14.3
8	26.8	27.3	28.8	27.1
17	25.7	26.3	27.0	26.6
0 - 17	25.0	25.6	26.4	25.8
F_1 Generation Females - Premating				
Mean Body Weight (g)				
0	56.6	55.9	59.3	54.4
17	330.4	330.2	347.1	330.3
Mean Weight Gain (g)				
0 - 17	273.8	274.3	287.8	275.8
Mean Food Consumption (g/rat/day)				
1	14.2	13.4	14.4	13.6
4	16.4	17.3	18.0	17.8
8	19.8	20.4	20.1	19.6
12	20.0	20.3	19.8	19.5
17	18.5	19.5	19.0	18.3
0 - 17	18.4	18.8	18.9	18.8

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

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_0 Generation Females				
GESTATION				
Mean Body Weights (g)				
0	303.9	302.7	322.5	313.8
-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

Observation and Study Week	Dose Group Control	Low	Mid	High
F_0 Generation Females LACTATION				
Mean Body Weights (g)				
0	330.2	324.2	350.4	346.9
3	314.8	316.3	328.8	331.0
Mean Body Weight Gains (g)				
0 - 1	-5.5	-2.7	-14.8	-13.4
0 - 3	-15.4	-7.8	-21.6	-15.0
F_1 Generation Females 1ST GESTATION				
Mean Body Weights (g)				
0	329.5	326.8	329.0	319.6
3	470.1	460.6	459.0	442.6
Mean Body Weight Gains (g)				
0 - 1	27.0	30.4	23.5	32.6
1 - 2	26.3	25.0	27.2	25.2
2 - 3	86.0	77.6	79.2	65.2*
0 - 3	138.5	133.1	129.8	123.1
Mean Food Consumption (g/rat/day)				
1	20.2	22.4	20.2	22.2
2	22.7	23.1	21.7	24.0
0 - 2	21.4	22.8	21.0	23.1
F_1 Generation Females 1ST LACTATION				
Mean Body Weights (g)				
0	361.8	361.0	355.1	359.4
3	341.1	339.1	345.8	349.0
Mean Body Weight Gains (g)				
0 - 1	-5.2	-10.9	-6.9	-2.9
0 - 3	-18.4	-21.9	-9.3	-10.4
F_1 Generation Females 2ND GESTATION				
Mean Body Weights (g)				
0	350.7	332.9	326.7	344.3
3	491.7	478.6	475.8	489.5
Mean Body Weight Gains (g)				
0 - 1	32.7	30.4	33.8	33.1
1 - 2	29.3	31.4	29.0	28.8
2 - 3	78.9	88.9	86.3	83.3
0 - 3	140.9	149.3	149.1	145.2
Mean Food Consumption (g/rat/day)				
1	26.5	25.4	26.4	28.3
2	26.5	26.2	24.9	27.2
0 - 2	26.5	25.8	25.6	27.8

Observation and Study Week	Dose Group Control	Low	Mid	High
F_1 Generation Females 2ND LACTATION				
Mean Body Weights (g)				
0	391.6	374.5	368.2	380.9
3	360.9	357.8	353.3	360.1
Mean Body Weight Gains (g)				
0 - 1	-17.9	-6.9	-2.0	-5.8
0 - 3	-30.8	-16.7	-14.9	-20.8

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
Week				F_1 Generation		
0 - 1	35.6	678	2836	35.3	716	2868
1 - 2	30.5	604	2489	31.1	624	2547
2 - 3	26.1	516	2115	24.7	500	2077
3 - 4	21.4	433	1809	22.4	446	1804
4 - 5	19.1	390	1589	20.3	412	1670
5 - 6	16.9	355	1446	19.9	395	1771
6 - 7	15.9	323	1348	17.7	363	1550
7 - 8	14.4	302	1201	19.5	368	1464
8 - 9	13.1	282	1128	18.0	354	1428
9 - 10	13.0	266	1089	18.3	348	1454
10 - 11	12.0	246	1035	17.6	328	1333
11 - 12	12.2	249	1041	17.2	320	1297
12 - 13	11.4	236	972	16.7	299	1245
13 - 14	11.3	225	946	16.3	299	1247
14 - 15	10.9	219	901	14.6	288	1204
15 - 16	10.7	219	901	15.7	290	1201
16 - 17	10.2	206	857	14.9	275	1115
0 - 17	16.7	338	1428	20.0	390	1604

The test substance intake (in mg/kg/day) for females during gestation is as follows:

	Dose Levels (ppm)		
	250	5000	20,000
Week		F_0 Generation	
0 - 1	16.5	327	1356
1 - 2	16.3	319	1348
0 - 3	16.4	323	1352
		F_1 Generation - 1st Gestation	
0 - 1	15.7	287	1265
1 - 2	15.2	286	1274
0 - 3	15.5	287	1269
		F_1 Generation - 2nd Gestation	
0 - 1	17.2	360	1500
1 - 2	16.3	320	1340
0 - 3	16.8	339	1420

There were no biologically relevant differences in the above presented data.

4. Reproductive Performance: The investigators noted the following effects on reproductive performance:

There were no statistically significant differences in F_0 mating or fertility indices or gestation length between controls and any compound-treated groups. Fertility ranged from 96.6% in the control group to 79.3% in the 5,000 ppm group. This variation is within the range of biological variability of fertility observed with this strain of rat at this laboratory [no historical control data were provided].

Fertility was low for all study groups in the F_1 generation, ranging from 46.4% in the 5,000 ppm group to 70.4% in the 20,000 ppm group for the first mating. Although a number of possibilities were investigated as to the reason for the low fertility (including a new shipment of bedding, new water bottles, and age of rats), no specific cause was identified. For this reason, a second set of litters was produced and again fertility remained low 37.5% in the 5,000 ppm group to 70.8% in the 20,000 ppm group. The low fertility is not considered a compound-related effect since no groups were statistically different from the controls and the highest fertility on either mating occurred in the 20,000 ppm group. In spite of the low fertility, it is considered that a sufficient number of litters were produced to adequately assess any potential litter effects in offspring, particularly since two sets of litters were produced and litter sizes were large in all groups.

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

Observation	Dose Group Control	Low	Mid	High
F_0 Generation				
Median precoital interval (days)		Not provided		
Males				
Mated	30	30	30	30
Fertile	29	30	29	28
Fertility not determined	1	0	1	2
Intercurrent deaths	0	0	0	0
Mating Index (%)	96.7	100.0	96.7	93.3
Females				
Number mated	29	30	29	28
Number fertile	28	24	23	23
Fertility not determined	1	6	6	5
Intercurrent deaths	0	0	0	0
Fertility Index (%)	96.6	80.0	79.3	82.1
Median gest. 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)	4	6	5	8
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, $p < 0.05$.

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, this was not seen at statistically significant levels in subsequent matings of the second generation during the postcull period, although a trend was seen in the F_{2b} litters, also the differences were minimal, involving 0.3 to 0.4 pups, therefore the biological relevance of this observation is unclear. No other differences were noted.

Observation	Dose Group Control	Low	Mid	High
F_1 Generation - 1st Gestation (F_{2a})				
Median precoital interval (days)	Not provided			
Males				
Mated	30	30	30	30
Fertile	28	27	28	27
Fertility not determined	2	3	2	3
Intercurrent deaths	0	3	0	1
Mating Index (%)	93.3	90.0	93.3	90.0
Females				
Number mated	28	27	28	27
Number fertile	16	15	13	19
Fertility not determined	12	12	15	8
Intercurrent deaths	0	1	0	1
Fertility Index (%)	57.1	55.6	46.4	70.4
Median gest. interval (days)	22.2	22.1	22.1	22.2
Number of litters	16	15	13	19
Total litter losses	0	0	0	0
Mean litter size				
At Birth	13.6	13.1	12.8	10.8*
Born Alive	13.4	12.7	12.8	10.6*
Day 4 Precull	12.4	12.6	12.8	11.0
Day 4 Postcull	7.5	7.6	8.0	7.6
Day 7	7.5	7.5	8.0	7.6
Day 14	7.5	7.5	8.0	7.6
Day 21	7.5	7.5	8.0	7.6
Pup deaths (Day 1-29)	7	2	0	2
Mean pup weight (g)				
Postpartum Day 0	6.4	6.4	6.6	6.8
Precull 4	11.0	10.7	10.6	11.5
Postcull 4	11.0	10.7	10.6	11.5
7	17.9	17.4	16.9	18.2
14	36.4	34.6	34.4	34.2*
21	59.8	57.8	56.5	55.4*

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

The fertility in all groups was low compared to the previous generation (not dose related). The investigators conducted a second mating of this generation to assess this observation. The mean litter size and the mean pup weight at postpartum days 14 and 21 of the high dose group were statistically significantly lower than the control.

Observation	Dose Group Control	Low	Mid	High
F_1 Generation - 2nd Gestation (F_{2b})				
Median precoital interval (days)	Not provided			
Males				
Mated	30	30	30	30
Fertile	25	25	24	25
Fertility not determined	5	5	6	5
Intercurrent deaths	0	3	0	1
Mating Index (%)	83.3	83.3	80.0	83.3
Females				
Number mated	25	25	24	24
Number fertile	14	15	9	17
Fertility not determined	13	10	15	7
Intercurrent deaths	0	1	0	1
Fertility Index (%)	56.0	60.0	37.5	70.8
Median gest. interval (days)	22.5	22.0	22.2	22.0
Number of litters	16	15	13	19
Total litter losses	0	0	0	0
Mean litter size				
At Birth	14.1	14.5	12.9	12.6
Born Alive	13.4	14.3	12.7	12.4
Day 4 Precull	13.2	14.3	12.7	12.3
Day 4 Postcull	7.8	7.8	7.8	7.5
Day 7	7.8	7.8	7.8	7.5
Day 14	7.8	7.8	7.8	7.5
Day 21	7.8	7.8	7.8	7.5
Pup deaths (Day 1-29)	3	1	0	2
Mean pup weight (g)				
Postpartum Day 0	6.4	6.1	6.6	6.6
Precull 4	10.9	10.3	10.9	11.5
Postcull 4	11.0	10.3	11.0	11.6
7	17.5	16.6	17.5	18.2
14	35.4	34.0	35.9	35.2
21	57.0	55.3	59.5	57.1

The fertility in all groups was still low compared to the F_0 generation (not dose related). The previously noted statistically significant differences in mean litter size and the mean pup weight at postpartum days 14 and 21 of the high dose group were not apparent in the second mating, although there appears to be a slightly reduced litter size in the F_{2b} litters.

5. Necropsy Results

a. Organ Weights: The report noted no specific effects on final body weight or absolute or relative testes weight of treated F_0 or F_1 males when compared to control. 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_1 Generation - Males			
Final body weight (g)	736.2	772.6	794.6	746.1
Organ weight (g) - Testes	3.687	3.650	3.863	3.646
Relative to body weight (g) - Testes	0.5057	0.4783	0.4922	0.4937

Data for the final body weight for female rats are summarized from the report as follows:

Observation	Dose Group			
	Control	Low	Mid	High
	F_0 Generation - Females			
Final body weight (g)	314.6	319.7	334.8	334.8
	F_1 Generation - Females			
Final body weight (g)	383.2	386.8	412.3	387.8

The investigators stated: "..., final body weight data for females represents a heterogeneous population of nonpregnant and lactating females, and since the effect was not found in the second generation, it is not considered compound related."

b. Pathology

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

2) Microscopic Examination: The investigators noted:

> ...a low (not statistically significant) incidence of bilateral testicular degeneration... Since the incidence of this lesion for the same dietary levels was (lower) in F_1 males who were exposed to IN V9360-27 longer (including *in utero*) and since it was not seen in other studies conducted with this material, it is not considered a compound-related effect.

The observed incidence is presented on the following table:

Observation	Dose Group Males Control	Low	Mid	High
F_0 Generation - Males				
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
Immature Sperm, increased unilateral	0	0	0	0
Inflammation, focal interstitial	3	2	5	5
Sperm granuloma, unilateral	1	1	1	1
F_1 Generation - Males				
Number examined	30	30	30	30
Testes				
Degeneration, seminiferous (atrophy), bilateral	1	1	0	1
Degeneration, seminiferous (atrophy), unilateral	3	1	0	4
Epididymides				
Epithelial degeneration, focal vacuolar	7	8	12	9
Immature Sperm, increased bilateral	0	0	0	0
Immature Sperm, increased unilateral	1	0	0	0
Inflammation, focal interstitial	12	13	16	11
Sperm granuloma, unilateral	0	0	0	0

Other observations in males and those in females did not exhibit a dose response. The above observations in testes do not appear to be related to treatment, although apparently dose related in the F_0 generation, this observation did not repeat, as a dose-related condition, in the F_1 generation.

C. Offspring:

Viability and Clinical Signs: The authors noted that:

In the F_1 offspring of the F_0 rats, there were no statistically significant differences between controls and treatment groups in the number of pups born, born alive, alive on day 4 before litter reduction, or on any indices of litter survival. The mean litter sizes were significantly less than mean control litter size after culling on day 4 in the 5,000 ppm group and from day 7 on in the 20,000 ppm group. When analyzed separately by sex, mean number/litter was significantly reduced only in the 5,000 ppm females from day 4 postculling until weaning.

Among the F_{2a} pups, there were significantly fewer pups born and born alive in the 20,000 ppm group. However, the percent born alive and other indices of litter survival were not affected. By day 4, the difference in litter size was no longer statistically significant. When analyzed separately by sex, there were no differences in mean litter size found. The 0-4 day viability of the 5,000 ppm group was significantly better than that of controls. No other differences in indices of litter survival were found in the F_{2a} pups.

The mean size of compound-treated F_{2b} litters was never significantly less than that of controls, although litter size was again smallest in the 20,000 ppm group. There were no differences between groups in indices of litter survival. No sex-related differences were found.

The smaller litter size of the 5,000 and 20,000 F_1 pups after culling is attributed to a vary high mean control litter size (8.0) and thus is not considered biologically significant or compound related. The effect was not observed in the F_{2a} or F_{2b} litters. The greater 0-4 day viability in the 5,000 ppm F_{2a} pups is not considered a compound-related effect.

The mean litter sizes at birth of the 20,000 ppm F_{2a} litters may be a minimal compound-related effect since litter size in the 20,000 ppm group was always smaller than for controls, although the effect was significant only for F_{2a} pups at 20,000 ppm. This smaller litter size in the 20,000 ppm group is considered a contributing factor in the lower weight gain of F_1 dams during the final week of gestation.

Viability results from pups during lactation are summarized from the report as follows:

Observation and study time	Dose group: Control	Low	Mid	High
	F_1 Generation			
Gestation index	100	100	100	100
Mean % Born Alive	99.5	98.3	99.5	99.3
0-4 Day Viability	99.0	98.4	98.8	98.7
Lactation Index	100	100	100	100
Litter Survival	100	100	100	100
	F_2 Generation			
Litter A				
Gestation index	100	100	100	94.7
Mean % Born Alive	98.8	95.8	99.4	93.4
0-4 Day Viability	96.9	99.5	100*	98.6
Lactation Index	100	99.2	100	100
Litter Survival	100	100	100	100
Litter B				
Gestation index	100	100	100	100
Mean % Born Alive	95.5	99.1	98.6	98.8
0-4 Day Viability	98.4	99.6	100	99.1
Lactation Index	100	100	100	100
Litter Survival	100	100	100	100

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

Changes in mean litter sizes are presented on pages 13 through 15 of this document. As was mentioned previously, the mean litter size of the mid and high dose groups for the F_1 litters was statistically significantly lower than the control from Day 4 Postcull on, this was not seen in the same time period in subsequent litters. The mean litter size for the F_{2a} litters at postpartum days 14 and 21 of the high dose group was statistically significantly lower than the control and for the F_{2b} litters there appears to be a slightly reduced litter size in the high dose group (not statistically significant). However, all these differences were minimal, involving 0.3 to 0.4 pups, therefore the biological relevance of this observation is unclear.

The report stated that no significant differences in clinical observations were noted in the F_1, F_{2a}, or F_{2b} pup in any of the treatment groups, attached Tables 37 through 39 present the reported observations. Evaluation of the supplied data noted no treatment related effects.

2. Body weight: Selected group mean body weights are summarized from the report as follows:

Observation and study time	Dose group Control	Low	Mid	High
F_1 Generation				
Males				
Body weight (g) - Day 0	6.8	6.7	7.4	6.7
Body Weight (g) - Day 21	58.4	57.0	61.9	56.8
Females				
Body weight (g) - Day 0	6.4	6.7	7.1*	6.4
Body Weight (g) - Day 21	56.0	55.2	59.4	54.1
F_2 Generation				
Litter A				
Males				
Body weight (g) - Day 0	6.5	6.6	6.9	7.0
Body Weight (g) - Day 21	61.1	59.0	58.6	56.7*
Females				
Body weight (g) - Day 0	6.2	6.2	6.4	6.4
Body Weight (g) - Day 21	57.8	56.4	54.6	53.0*
Litter B				
Males				
Body weight (g) - Day 0	6.7	6.3	6.7	6.7
Body Weight (g) - Day 21	57.9	56.4	60.1	58.6
Females				
Body weight (g) - Day 0	5.9	5.9	6.4	6.5
Body Weight (g) - Day 21	55.8	54.8	58.9	55.1

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

3. Necropsy results

a. Organ Weights:

Organ weight data were not provided by the investigators, although stated as being measured in the protocol.

b. Pathology

The investigators reported "...no compound-related lesions in found dead pups which were necropsied."

The investigators randomly selected 20 weanlings per sex per dose group from the F_{2a} and F_{2b} litters amd subjected them to gross pathological examination.

The mean final body weights are as follows:

Observation and study time	Dose group: Control	Low	Mid	High
	F_2 Generation			
Litter A				
Males				
Body Weight (g) - Day 21	58.9	57.9	59.3	58.0
Females				
Body Weight (g) - Day 21	58.0	56.6	54.3	52.4*
Litter B				
Males				
Body Weight (g) - Day 21	58.7	57.0	60.1	59.1
Females				
Body Weight (g) - Day 21	55.5	53.8	58.6	54.9

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

The investigators noted a statistically significant decrease in the mean body weight of high dose females of the F_{2a} generation; however, they stated that this was "...attributed to a high weanling weight for controls and this is not considered to be compound related." They further reported that "There were no significant differences between control and IN V9360-27 treated weanlings in final body weight in male F_{2a} or male and female F_{2b} pups."

The investigators reported "no compound-related lesions in either male or female F_{2a} or F_{2b} weanlings."

III. DISCUSSION

A. Investigators' Conclusions:

The following is from the investigators' report:

No effects observed in this study at 250 or 5,000 ppm were attributed to administration of IN V9360-27. The minimal effects found at 20,000 ppm that may have been compound related were lower weight gain of dams during the final week of gestation and smaller litter size at birth.

B. Reviewer's Discussion:

No effects attributable to the addition of IN V9360-27 in the diet were noted on parental animals in the low (250 ppm) or mid (5000 ppm) dose groups. At the high dose (20000 ppm) the F_1 (first mating) females exhibited a lower body weight gain during the final week of gestation than that observed in the controls. Although this may be due to the lower litter size as suggested by the investigators, a similar, but not statistically significant, pattern occurred in the F_0 females during the same period of gestation. Therefore, the No Observed Effect Level (NOEL) for Systemic Toxicity is 5000 ppm (287 mg/kg/day) with a Lowest Observed Effect Level (LOEL) of 20000 ppm (1269 mg/kg/day).

Reproductive parameters were unaffected in the low and mid dose groups. There was a minimal decrease in litter size at birth and in pup weights at postpartum day 14 through 21 in the F_{2a} high dose group. The other litters showed a similar tendency, but not a statistically significant difference. Therefore, the No Observed Effect Level (NOEL) for Reproductive Effects is 5000 ppm with a Lowest Observed Effect Level (LOEL) of 20000 ppm.

The reduced fertility noted in the two matings of the F_1 was not considered related to treatment as all groups were involved, including control, with no dose response apparent, in fact, the high dose presented with the greatest fertility. The investigators were unable to discern the cause for the reduction of fertility.

Systemic Toxicity NOEL = 5000 ppm (287 mg/kg/day)
Systemic Toxicity LOEL = 20000 ppm (1269 mg/kg/day)

Reproductive Toxicity NOEL = 5000 ppm
Reproductive Toxicity LOEL = 20000 ppm

Accent toxicology review

Page _____ is not included in this copy.

Pages 28 through 45 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.

Raeviewed by: Alan C. Levy, Ph. D., Alan C. Levy 5-8-90
Section I, Tox. Branch II (H7509C)

Secondary Reviewer: Yiannakis M. Ioannou, Ph. D. JMI 5-8-90
Section I, Tox. Branch II (H7509C)

DATA EVALUATION REPORT 007939

STUDY TYPE: Chronic Oral Toxicity - Dog (§83-1)

TEST MATERIAL: IN V9360-27 TOX. CHEM. NO.: 359J

SYNONYMS: Accent® MRID NO.: 413601-02

STUDY NUMBER: Laboratory Project 390-89; Medical Research Project 8430-001

SPONSOR: Agricultural Products Department
E. I. du Pont de Nemours and Company, Inc.
Wilmington, DE 19805

TESTING FACILITY: E. I. du Pont de Nemours and Company, Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
Newark, DE 19714

TITLE OF REPORT: Chronic Toxicity Study with IN V9360-27 - One-Year Feeding Study in Dogs

AUTHOR: Jon C. Cook

REPORT ISSUED: November 2, 1989

CONCLUSIONS:

In males, a concentration of 20,000 ppm appeared to cause a decrease in body weight gains and a concomitant increase in relative liver and kidney weights. All dosed female groups (250, 5,000 and 20,000 ppm) appeared to have decreased body weight gains over the 364 days of the study (28-40% decrease from the control group mean value).

The No Observed Effect Level (NOEL) =
males: 5,000 ppm (approximately 125 mg/kg)
females: not attained

The Lowest Observed Effect Level (LOEL) =
males: 20,000 ppm (approximately 500 mg/kg) - decreased body weight gain as well as increased relative liver and kidney weights
females: = or < 250 ppm (approximately 6.25 mg/kg); lowest dose tested

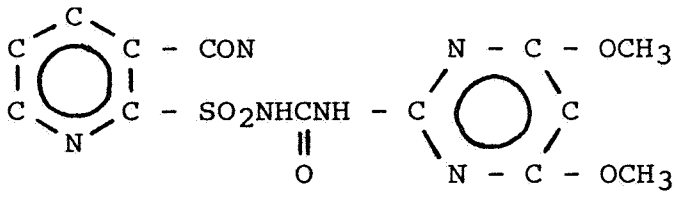
Classification: Core-Supplementary

This study does not satisfy the guideline requirements (§83-1) for a Chronic Oral Dog Toxicity Study.

I. Materials and Methods

A. Test Article

Name: IN V9360-27; 3-Pyridinecarboxamide, 2-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino] carbonyl] amino] sulfonyl]-N,N-dimethyl-



Lot Number and Purity:

IN V9360-27 (Haskell No. 16,925); purity = 90.6%

B. Compound Purity and Diet Analyses

Purity had originally been stated as being 94.5% and this value was varified by Haskell Laboratory. Later analyses determined the purity to be 90.6%. Concentrations in the diet were adjusted for 94.5% purity and not 90.6%.

Table 1 shows percents of desired amounts of test article measured as follows: pure test article, added to control feed, homogeneity and cage feeder samples. [The values/ ranges were deerived from a number of assay values.]

The majority of the analyses indicated that at least 90% of the desired amount was attained. Even though the test article analyses later showed 90.6% purity (as opposed to a previous purity value of 94.5%) it is considered that the amount of IN V9360-27 in the diet was sufficient to allow the study to be acceptable.

C. Animals

Male and female (22 of each) beagle dogs, about four months of age, were received from Marshall Farms U.S.A., Inc.,.North Rose, NY. Body weights were 5.5-7.3 kg for males and 4.9-6.5 kg for females. During a 45-day pretest period, the dogs were:

- fed about 350 g ground Purina® Certified Canine Diet #5007 each day; tap water ad libitum
- weighed seven times
- observed
- given two clinical laboratory examinations
- given an ophthalmic examination

Table 1

ANALYSES OF PURE IN V9360-27 AS WELL AS CONCENTRATIONS IN CONTROL FEED, HOMOGENEITY AND CAGE FEEDERS

	Desired Conc. (ppm)	Percent of Nominal
IN V9360-27 sample analysis (ug/ml)	10	93 - 101 (12)†
Added to Control Feed	250	85 - 100 (4)
	20,000	93 - 101 (4)
Homogeneity (Top, Middle, Bottom)	250	88 - 106 (6)
	5,000	94 - 104 (6)
	20,000	93 - 97 (6)
Cage Feeder Samples	250	90 - 97 (4)
	5,000	100 - 104 (4)
	20,000	96 - 103 (4)
Fresh Frozen	250	94-100
	5,000	97-98
	20,000	97
24-Hour Room Temperature	250	92-95
	5,000	97-100
	20,000	91-106
10-Day Room Temperature	250	90-98
	5,000	96-101
	20,000	95-97
10-Day Refrigerator	250	90-100
	5,000	97-105
	20,000	95-98

† = Number of values

Data extracted from report Appendix B, pages 135-159.

Table 2

BODY WEIGHTS AND WEIGHT GAINS FOR DOGS FED V9360-27 FOR ONE YEAR

Days on Test	Males †				Females †			
ppm =	0	250	5,000	20,000	0	250	5,000	20,000
GROUP MEAN BODY WEIGHTS (kg)								
0	8.1	8.3	8.1	8.1	7.0	7.2	7.2	7.3
7	8.3	8.6	8.4	8.4	7.3	7.4	7.4	7.8
14	8.5	8.8	8.5	8.7	7.4	7.5	7.5	7.9
21	8.8	9.0	8.8	8.9	7.7	7.8	7.7	8.1
28	9.0	9.4	8.9	9.1	7.9	8.1	7.9	8.3
56	9.8	10.2	9.7	9.8	8.7	8.6	8.3	9.0
84	10.3	10.8	10.4	10.4	9.1	8.9	8.6	9.6
112	10.8	11.3	10.8	10.7	9.4	9.3	9.0	9.6
140	11.1	11.7	11.1	10.7	9.8	9.6	9.3	9.9
168	11.2	11.8	11.2	10.6	10.1	9.7	9.4	10.0
196	11.0	11.6	11.2	10.7	10.1	9.4	9.3	10.0
224	10.9	11.7	11.4	10.4	10.0	9.6	9.5	10.1
252	11.0	11.7	11.3	10.2	10.0	9.7	9.5	9.9
280	11.1	11.8	11.3	10.1	10.1	9.8	9.6	10.4
308	11.0	11.8	11.5	10.2	10.4	9.9	9.6	10.3
336	11.2	11.7	11.4	9.9	10.7	10.1	9.7	10.3
364	11.0	11.6	11.4	9.8	11.0	10.1	9.6	10.2
GROUP MEAN BODY WEIGHT GAINS (kg)								
0 - 91	2.5	3.0	2.5	2.4	2.2	1.9	1.6	2.4
91 - 182	0.3	0.4	0.7	0.1	0.8	0.4	0.6	0.3
182 - 364	0.0	-0.1	0.1	-0.9	1.1	0.6	0.2	0.1
0 - 364	2.9	3.4	3.3	1.7	4.0	2.9	2.4	2.9
Percent from control (days 0-364)	-	+17	+14	-41	-	-28	-40	-28

† = 5 dogs/sex/group

Data extracted from report Tables 2-5, pages 54-61.

After release from quarantine by the veterinarian, the dogs were computer randomized into four groups of 5 males and 5 females. Targeted room temperature and relative humidity values were 23 ±2 °C and 50%±10%, respectively. Weather permiting, dogs were placed in outdoor fenced runs for 2-6 hours each day (separated by group and sex).

Dietary admixes were prepared fresh weekly in Purina® Certified Canine Diet #5007 and were refrigerated until used. The concentrations utilized were: 0 (control), 250, 5,000 and 20,000 ppm.

D. General Observations

1. Mortality and Clinical Observations - All dogs were observed at least once daily.

   There was no mortality. No clinical signs considered attributable to test article administration were observed.

2. Body Weights - All animals were weighed pre-dosing and weekly thereafter.

   There appeared to be a non-statistically significant decrease in group mean body weights and body weight gains in the 20,000 ppm males (Table 2). Utilizing the selected data in Table 2, the control group mean weights remained about the same during the last 200+ days of the study (10.9-11.2 kg); whereas, in the 20,000 ppm males, the group mean decreased from 10.7 to about 9.8 kg. The four groups gained the following kg during the year study: 0 = 2.9, 250 = 3.4, 5,000 = 3.3 and 20,000 = 1.7. There did not appear to be a no effect level in body weight gains for females as there were 28-40% lower gains in all treated groups compared with controls.

3. Food Consumption - The amount of food consumed by each dog was determined on a daily basis.

   There was little or no difference in mean daily food consumed between any of the male groups: 310-314 g. In females, the control mean daily average was 302 g; whereas, the three dose levels were 293, 270 and 284 g at 250, 5,000 and 20,000 ppm, respectively.

   Food efficiency in the 20,000 ppm male group was 0.015 versus 0.026 in controls (kg body weight gain/kg food consumed). In females, the three treated groups had a slightly lower food efficiency compared with the control group (0.036 for control and 0.024-0.027 for treated).

4. Ophthalmoscopic Examinations - All dogs were examined on days -32 and 350 by James M. Clinton, V.M.D. (veterinary ophthalmologist) using focal illumination and indirect ophthalmoscopy.

Dr. Clinton concluded that there was no evidence to suggest that the test article produced ocular changes.

E. Clinical Pathology

Evaluations were made on test days -31, -11, 77, 174, 272 and 361. The animals were fasted for about 16 hours during which time urine was collected. At the end of the fast, blood samples were collected from the jugular vein of each dog.

1. Hematology - The CHECKED (x) parameters were examined.

X		X	
x	Hematocrit (HCT)*	-	Total plasma protein (TP)
x	Hemoglobin (HBG)*	x	Leukocyte differential count
x	Leukocyte count (WBC)*	x	Mean corpuscular HGB (MCH)
x	Erythrocyte count (RBC)*	x	Mean corpuscular HGB conc. (MCHC)
x	Platelet count*	x	Mean corpuscular volume (MCV)

x Reticulocyte counts (prepared, not evaluated)

* = EPA Guideline Requirement "-" = Not examined

There were no apparent test article induced changes in any of the parameters examined.

2. Blood Chemistry - The CHECKED (x) parameters were examined.

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	Globulin
x	Sodium*	x	Glucose*
		x	Total Bilirubin*
	Enzymes	x	Total Protein*
x	Alkaline phosphatase	-	Triglycerides
-	Cholinesterase		
-	Creatinine phosphokinase*		
-	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		

x Creatine kinase

* = EPA Guideline Requirement "-" = Not examined

There were no apparent test article induced changes in any of the parameters examined.

3. Urinalysis - The CHECKED (x) parameters were examined.

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

* = EPA Guideline Requirement "-" = Not examined

There were no apparent test article induced changes in any of the parameters examined.

F. Sacrifice and Pathology

All dogs were sacrificed and necropsied at the end of the study (days 367-371). There was at least a 16 hour fast prior to sacrifice. The animals were anesthetized by intravenous injection of a barbiturate and euthanized by exsanguination. A rib section was collected from each dog for bone marrow collection. The CHECKED (x) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X	Digestive system	X	Respiratory	X	Urogenital
-	Tongue	x	Trachea*	xx	Kidneys*
x	Salivary glands*	x	Lung*	x	Urinary bladder*
x	Esophagus*			xx	Testes*
x	Stomach*		Cardiovasc./Hemat.	x	Epididymides
x	Duodenum*	x	Aorta*	x	Prostate
x	Jejunum*	xx	Heart*	-	Seminal vesicle
x	Ileum*	x	Bone marrow*	x	Ovaries
x	Cecum*	x	Lymph nodes*	x	Uterus*
x	Colon*	x	Spleen*		
x	Rectum*	x	Thymus*		
xx	Liver*				
x	Gallbladder*				
x	Pancreas*				

X	Neurologic	X	Glandular	X	Other
xx	Brain*	x	Adrenals*	x	Bone*
x	Periph. Nerve*	-	Lacrimal gland	x	Skeletal muscle
x	Spinal cord (3 levels)*	x	Mammary gland*	x	Skin
x	Pituitary*	xx	Parathyroids*	x	All gross lesions and masses
x	Eyes (optic n.)*	xx	Thyroids*		

x Vagina x Tonsil x Cervix

* = EPA Guideline Requirement "-" = Not examined

1. Macroscopic

There were no findings which were considered related to test article administration.

2. Organ Weights

The following organs were weighed and the weights expressed as absolute (g) and relative (organ weight/ body weight ratio - %): liver, kidneys, heart, thyroids/ parathyroids brain and testes.

Group mean relative liver and kidney weights in 20,000 ppm males only were higher (statistically significant - $p < 0.05$) than controls. As the absolute weights were similar (study author states not statistically significant increase) in treated and control groups, the relative increase was considered to be due to a lower group mean body weight. Group mean final body weights as well as liver and kidney weights are presented in Table 3.

Table 3

FINAL BODY†, LIVER AND KIDNEY WEIGHTS OF MALE DOGS ADMINISTERED IN V9360-27 IN THE DIET FOR ONE YEAR

ppm	Final body weight (g)	Absolute (g) liver	Absolute (g) kidney	Relative (%) liver	Relative (%) kidney
0	11020±1484††	307±52	53±10	2.8±0.4	0.48±0.04
250	11700± 600	317±42	59±10	2.7±0.4	0.50±0.07
5,000	11360± 891	317±34	53± 8	2.8±0.4	0.46±0.03
20,000	9960±1246	342±60	58±12	3.4±0.3*	0.58±0.05*

(Table 3 Continued)

Individual absolute liver weights

0 ppm = 227, 298, 303, 350, 358 (mean = 307)

20,000 ppm = 273, 301, 349, 356, 430 (mean = 342)

==========

† = after About a 16 hour fast - all study dogs sacrificed days 367-371
†† = Standard Deviation
* = $p < 0.05$ (LSD and Dunnett's Test)

Data extracted from report Tables 20-21 and Appendix G, pages 98-99, 396 and 399.

---

3. Microscopic

There were no findings which were considered to be related to test article administration.

The Reviewer has no comments regarding the Methods and Materials section.

A copy of the statistical analyses is attached.

A Good Laboratory Practice Compliance Statement and a list of Quality Assurance Inspections were provided.

II. Discussion

Test article effects appeared to be as follows: a non-statistically significant decrease in body weight gains in males at 20,000 ppm; a statistically significant ($p<0.05$) increase in relative liver and kidney weights in males at 20,000 ppm; and a decrease in body weight gains in all treated female groups (250, 5,000 and 20,000 ppm). The increase in relative organ weights was probably due to a decrease in body weight gain as there were little differences between treated and control absolute weights.

Control females gained a group mean of 4.0 kg during the one year study (days 0-364). The dosed groups gained as follows (% from control in parentheses): 250 = 2.9 kg (28%); 5,000 = 2.4 kg (40%); and 20,000 = 2.9 kg (28%). A review of all individual body weight and body weight gain data appears to substantiate the difference. [NOTE: In the 90-day dog study, no compound-related changes in body weights or body weight gains were observed.]

III. Conclusions

In males, a concentration of 20,000 ppm appeared to cause a decrease in body weight gains and a concomitant increase in relative liver and kidney weights. All dosed female groups (250, 5,000 and 20,000 ppm) appeared to have decreased body weight gains over the 364 days of the study (28-40% decrease from the control group mean value).

The No Observed Effect Level (NOEL) =
males: 5,000 ppm (approximately 125 mg/kg)

females: not attained

The Lowest Observed Effect Level (LOEL) =
males: 20,000 ppm (approximately 500 mg/kg) - decreased body weight gain as well as increased relative liver and kidney weights

females: = or < 250 ppm (approximately 6.25 mg/kg); lowest dose tested

Classification: Core-Supplementary

This study does not satisfy the guideline requirements (§83-1) for a Chronic Oral Dog Toxicity Study.

STATISTICAL ANALYSES

Body weights, body weight gains, food consumption data, organ weights, and clinical laboratory measurements 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. The Bartlett's test for homogeneity of variances was performed on the organ weight and clinical laboratory data. When the results of the Bartlett test were significant the Kruskal-Wallis test was employed and the Mann-Whitney U test was used to compare means from the control groups and each of the groups exposed to IN V9360-27. Except for Bartlett's test, all other significance was judged at alpha = 0.05.

007939
00 7443

REVIEWED BY: LINDA L. TAYLOR, PH.D. Linda Lee Taylor 5/17/90
TOX. BRANCH II, SECTION II, HED (H7509C)
SECONDARY REVIEWER: K. CLARK SWENTZEL K. Clark Swentzel 5/17/90
SECTION II HEAD, TOX. BRANCH, HED (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: ONCOGENICITY STUDY - MOUSE TOX. CHEM. NO.: 359J

MRID NO.: 413601-03

TEST MATERIAL: 3-PYRIDINECARBOXAMIDE, 2[[[[(4,6-DIMETHOXY-2-PYRIMIDINYL) AMINO]CARBONYL]AMINO]SULFONYL]-N,N-DIMETHYL-

SYNONYMS: ACCENT, IN V9360-27

STUDY NUMBER: MEDICAL RESEARCH PROJECT # 8313-001;
HASKELL LABORATORY REPORT # 645-89

SPONSOR: DUPONT

TESTING FACILITY: HASKELL LABORATORY FOR TOXICOLOGY AND INDUSTRIAL MEDICINE

TITLE OF REPORT: ONCOGENICITY STUDY WITH IN V9360-27; EIGHTEEN-MONTH FEEDING STUDY IN MICE

AUTHOR: JON C. COOK, PH.D.

REPORT ISSUED: DECEMBER 7, 1989

QUALITY ASSURANCE: A QUALITY ASSURANCE STATEMENT WAS PROVIDED.

CONCLUSIONS: THERE WERE NO EFFECTS ON BODY WEIGHT, BODY-WEIGHT GAIN, FOOD CONSUMPTION, SURVIVAL, CLINICAL PATHOLOGY, OPHTHALMOLOGY, ORGAN WEIGHT, GROSS OR MICROSCOPIC PATHOLOGY, TUMOR INCIDENCE NOTED IN EITHER SEX FOLLOWING EXPOSURE TO FOUR DOSE LEVELS OF ACCENT IN THE DIET FOR 18 MONTHS. SINCE THE HIGHEST DOSE LEVEL TESTED REPRESENTS A MAXIMUM LEVEL RECOMMENDED BY THE AGENCY FOR CARCINOGENICITY STUDIES, THE CARCINOGENICITY POTENTIAL OF ACCENT HAS BEEN ADEQUATELY ASSESSED FOR THE MOUSE. UNDER THE CONDITIONS OF THE STUDY, THE NOEL FOR BOTH SEXES OF MOUSE CAN BE SET AT 7500 PPM (993 AND 1312 MG/KG/DAY FOR MALE AND FEMALE MICE, RESPECTIVELY).

CLASSIFICATION: CORE: MINIMUM. FOR THE RECORD, HISTORICAL CONTROL DATA SHOULD BE SUBMITTED ON THE INCIDENCE OF HEPATOCELLULAR ADENOMA OBSERVED IN THE MOUSE AT THE TESTING FACILITY. THIS STUDY SATISFIES THE GUIDELINE REQUIREMENTS (83-2) FOR A MOUSE ONCOGENICITY STUDY.

A. MATERIALS:

1. TEST COMPOUND: IN V9360-27, DESCRIPTION: NONE PROVIDED, BATCH #: NONE STATED AS SUCH - HASKELL No. 16,925-02, PURITY: 94.5% (90.6%)*.

*THE CONCENTRATIONS OF IN V9360-27 IN THE DIETS WERE ADJUSTED FOR A PURITY OF 94.5%; SUBSEQUENT PURITY ANALYSES BY SPONSOR FOUND PURITY TO BE 90.6%, WHICH WAS CONFIRMED AT THE TESTING LABORATORY. NO CHANGE IN CALCULATION FOR DOSE WAS MADE, HOWEVER. IT IS STATED THAT HYDRATION OF THE TEST MATERIAL (ANALYTICAL SAMPLE) DURING STORAGE MAY HAVE CONTRIBUTED TO THE VARIATION IN PURITY OBSERVED AT THE TESTING FACILITY.

2. TEST ANIMALS: SPECIES: MOUSE, STRAIN: CRL: CD®-1(ICR)BR, AGE: 44 DAYS OLD, WEIGHT: MALES: 27.0-27.3 GRAMS, FEMALES: 20.9-21.7 GRAMS, SOURCE: CHARLES RIVER LABORATORIES, KINGSTON, NEW YORK.

3. STATISTICS: AS STATED IN THE REPORT, BODY WEIGHTS, BODY-WEIGHT GAINS, ORGAN WEIGHTS, AND CLINICAL LABORATORY MEASUREMENTS WERE ANALYZED BY A ONE-WAY ANALYSIS OF VARIANCE, AND 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 AND TUMORS WAS EVALUATED BY THE FISHER'S EXACT TEST (WITH A BONFERRONI CORRECTION FOR CLINICAL OBSERVATIONS) AND THE COCHRAN-ARMITAGE TEST FOR TREND. SURVIVAL PROBABILITIES WERE ESTIMATED WITH THE KAPLAN-MEIER PROCEDURE, AND SURVIVAL AMONG THE GROUPS WAS COMPARED WITH THE MANTEL-HAENSZEL TEST AND THE FISHER'S EXACT TEST.

B. STUDY DESIGN:

1. METHODOLOGY

MICE (80/SEX/GROUP; HOUSED INDIVIDUALLY) WERE ADMINISTERED IN V9360-27 IN THE DIET AT DOSE LEVELS OF 0, 25, 250, 2500, AND 7500 PPM FOR 18 MONTHS. THE ANIMALS WERE PLACED INTO FIVE GROUPS PER SEX BY COMPUTERIZED, STRATIFIED RANDOMIZATION SO THAT THERE WERE NO STATISTICALLY SIGNIFICANT DIFFERENCES AMONG GROUP BODY WEIGHT MEANS WITHIN A SEX. THE DOSE LEVELS WERE CHOSEN BASED ON THE 90-DAY SUBCHRONIC STUDY AND ON THE EXPECTED APPLICATION RATES AND LOW RESIDUE LEVELS.

2. DIET PREPARATION

TEST DIETS WERE PREPARED WEEKLY, INITIALLY, AND THEN EVERY TWO WEEKS (STORED IN REFRIGERATOR). SAMPLES WERE COLLECTED TO DETERMINE THE CONCENTRATION, STABILITY, AND HOMOGENEITY OF THE TEST MATERIAL IN THE DIET PRE-TEST AND AT VARIOUS INTERVALS DURING THE STUDY.

RESULTS

THE DIET SAMPLES WERE SHOWN TO BE HOMOGENEOUSLY MIXED AND STABLE OVER THE TIME INTERVAL OF USE. THE CONCENTRATIONS ATTAINED WERE STATED TO BE WITHIN 20% OF THE NOMINAL VALUE (SEE TABLE 1, COPY ATTACHED), WITH THE EXCEPTION OF THE LOWEST CONCENTRATION. THE AUTHOR STATED THAT THE LOWER VALUES AT THE 25 PPM LEVEL (61-95% OF NOMINAL VALUE) MAY BE DUE TO INCOMPLETE EXTRACTION RATHER THAN A REAL DEVIATION FROM THE TARGETED CONCENTRATION.

THE OVERALL MEAN DAILY INTAKE VALUES OF ACCENT BY THE MICE ON A MG/KG BASIS WERE LISTED AS FOLLOWS:

PPM	MALES (MG/KG/DAY)*	FEMALES
25	3.33	4.37
250	32.7	44.8
2500	327	438
7500	993	1312

*NOTE: THE INTAKE VALUES LISTED ABOVE ARE BASED ON DIETS PREPARED USING A PURITY VALUE OF 94.5%. BASED ON THE LOWER PURITY VALUE FOUND BY THE SPONSOR, THE MEAN DAILY INTAKE VALUES ARE ACTUALLY 4% LESS THAN THOSE LISTED ABOVE.

3. FEED (PURINA® CERTIFIED IRRADIATED CHOW® #5002M (PCIC) AND WATER WERE PROVIDED AD LIBITUM.

C. METHODS AND RESULTS:

1. CLINICAL OBSERVATIONS AND PALPATIONS

THE ANIMALS WERE OBSERVED DAILY FOR SIGNS OF ABNORMAL BEHAVIOR, MORTALITY AND MORBIDITY, AND APPEARANCE, AND AT EVERY WEIGHING, EACH MOUSE WAS INDIVIDUALLY HANDLED AND CAREFULLY EXAMINED FOR ABNORMAL BEHAVIOR AND APPEARANCE.

RESULTS

TOXICITY/MORTALITY (SURVIVAL)

THERE WAS NO TREATMENT-RELATED EFFECT ON SURVIVAL. THE NUMBER OF ANIMALS DYING ON STUDY (% SURVIVAL) IS LISTED BELOW FOR BOTH SEXES.

	DOSE LEVELS (PPM)				
	0	25	250	2500	7500
MALES	32(60)	35(56)	34(58)	32(60)	34(58)
FEMALES	23(71)	35(56)	25(69)	33(59)	27(66)

THE MOST COMMON CAUSE OF DEATH IN EITHER SEX WAS FROM AMYLOIDOSIS. CLINICAL OBSERVATIONS WERE COMPARABLE AMONG THE GROUPS FOR BOTH SEXES.

59
B

2. Body Weight and Food Consumption

Body weight (individual) was determined once a week for the first 6 months and once every other week thereafter. The amount of food consumed by each group over each weighing interval was determined throughout the study. From these data, mean individual daily food consumption, food efficiency, and test material intake were calculated.

RESULTS

There was no compound-related effect on body weight or body-weight gain for either sex, although there were statistically significant decreases noted at various time points in female body weight at the three highest dose levels. The differences were never greater than 6% below control value. The overall mean daily food consumption and food efficiency for both sexes were similar to their respective controls. Test material intake was discussed under Diet Preparation RESULTS, above. NOTE: The initial body weight for the highest dose females was statistically significantly lower than control value (contrary to Protocol).

3. Hematology: Blood samples (collected from the orbital sinus) were obtained from 10 fasted rats/sex/dose (randomly selected for each evaluation) at approximately 3, 6, 12, and 18 months after study initiation. The checked (X) parameters were examined.

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpuscular volume (MCV)
X	Platelet count	X	Reticulocyte count (blood smear all animals)
		X	Est. total plasma protein conc.

RESULTS

No compound-related effects were reported. There were no consistently significant and/or dose-related differences observed in either sex for any of the parameters measured. NOTE: There were no baseline data provided for any of the parameters measured, and different mice were used at each time point (as per protocol); however, no explanation was provided why different mice were used for each time point when most of the animals used for the first measurement were available throughout the study. For example, 7 out of 10 of the male and female control animals used for the 3-month sample survived to study termination; 8 out of 10 high-dose males and 6 out of 10 high-dose females sampled at 3 months survived to termination. Due to the inherent variability among animals in general, this practice results in data of little value.

4. OPHTHALMOLOGICAL EXAMINATIONS

OCULAR EXAMINATIONS WERE PERFORMED ON ALL ANIMALS PRIOR TO STUDY INITIATION AND ON ALL SURVIVORS PRIOR TO SACRIFICE. BOTH EYES WERE EXAMINED BY FOCAL ILLUMINATION AND INDIRECT OPHTHALMOSCOPY. A 1% ATROPINE SOLUTION WAS PLACED IN EACH EYE AT LEAST ONE HOUR PRIOR TO EXAMINATION.

RESULTS

THE INCIDENCE OF EYE LESIONS (MOST CONSIDERED TO BE AGE-RELATED BY THE AUTHOR) WAS COMPARABLE AMONG THE GROUPS. IN SOME ANIMALS, PALE OCULAR FUNDI WERE OBSERVED, AND THIS WAS ATTRIBUTED TO ANEMIA BY THE AUTHOR. THIS COULD NOT BE SUBSTANTIATED, HOWEVER, SINCE CLINICAL PATHOLOGY WAS NOT PERFORMED ON ALL ANIMALS BUT ON 10 RANDOMLY-SELECTED ANIMALS PER GROUP. OF THOSE EXAMINED, NO EVIDENCE OF ANEMIA WAS OBSERVED. ADDITIONALLY, THERE WAS NO DOSE-RELATED TREND IN THE INCIDENCE AND, IT WAS CONCLUDED THAT THERE WERE NO COMPOUND-RELATED EFFECTS OBSERVED.

5. GROSS PATHOLOGY: ALL ANIMALS THAT DIED, WERE SACRIFICED IN EXTREMIS, OR WHO WERE KILLED ACCIDENTALLY WERE NECROPSIED. ALL SURVIVORS WERE SACRIFICED AND NECROPSIED ON TEST DAYS 551 TO 561. THE BRAIN, LIVER, SPLEEN, HEART, KIDNEYS, AND TESTES WERE WEIGHED AND ORGAN-TO-BODY WEIGHT RATIOS WERE DETERMINED.

RESULTS

THERE WERE NO STATISTICALLY SIGNIFICANT DIFFERENCES IN ABSOLUTE OR RELATIVE ORGAN WEIGHT AMONG THE GROUPS OF EITHER SEX. ADDITIONALLY, THE INCIDENCE OF GROSS OBSERVATIONS WAS COMPARABLE AMONG THE GROUPS OF EITHER SEX, ALTHOUGH THERE WERE A FEW STATISTICALLY SIGNIFICANT DIFFERENCES.

	MALES					FEMALES				
	0	25	250	2500	7500	0	25	250	2500	7500
LIVER MASSES	10	10	10	19*	11	5	1	2	1	0*
KIDNEY (CYST)	1	2	3	4	7*					
OVARIES (CYST)						35	35	47*	43	37
THYMUS (LARGE)						9	8	7	6	2*

* $p<0.05$; FISHER'S EXACT TEST

6. HISTOLOGY: THE FOLLOWING CHECKED (X) ORGANS/TISSUES WERE COLLECTED FROM ALL ANIMALS. MICROSCOPIC EXAMINATION WAS PERFORMED ON THE TISSUES FROM THE HIGH-DOSE AND CONTROL ANIMALS AND FROM MICE THAT WERE FOUND DEAD OR WERE SACRIFICED IN EXTREMIS (TISSUE INTEGRITY PERMITTING). ADDITIONALLY, THE LIVER, KIDNEYS, LUNGS, AND ALL ORGANS WITH GROSS LESIONS WERE EXAMINED FROM ALL ANIMALS.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	TONGUE	X	AORTA	X	BRAIN
X	SALIVARY GLANDS	X	HEART	X	PERIPH. NERVE (SCIATIC)
X	ESOPHAGUS	X	BONE MARROW	X	SPINAL CORD
X	STOMACH	X	LYMPH NODES*	X	PITUITARY
X	DUODENUM	X	SPLEEN	X	EYES
X	JEJUNUM	X	THYMUS		GLANDULAR
X	ILEUM		UROGENITAL	X	ADRENALS
X	CECUM	X	KIDNEYS		LACRIMAL GLAND
X	COLON	X	URINARY BLADDER	X	MAMMARY GLAND
X	RECTUM	X	TESTES	X	PARATHYROIDS
X	LIVER	X	EPIDIDYMIDES	X	THYROIDS
X	GALL BLADDER	X	PROSTATE		OTHER
X	PANCREAS	X	SEMINAL VESICLE	X	BONE (FEMUR)
	RESPIRATORY	X	OVARIES	X	SKELETAL MUSCLE
X	TRACHEA	X	UTERUS	X	SKIN
X	LUNG**		CERVIX	X	ALL GROSS LESIONS AND MASSES
X	NOSE		OVIDUCT		HEAD+
	PHARYNX	X	VAGINA	X	HARDERIAN GLAND
	LARYNX			X	EXORBITAL LACRIMAL GLAND

* MANDIBULAR AND MESENTERIC

RESULTS

THE LIVER MASSES OBSERVED ON GROSS EXAMINATION IN THE 2500 PPM MALES WERE CONFIRMED ON MICROSCOPIC EXAMINATION TO BE HEPATOCELLULAR ADENOMAS. HOWEVER, SINCE THE INCIDENCE OF THIS LESION IN THE 7500 PPM (HIGHEST DOES) MALE GROUP, AS WELL AS IN THE TWO OTHER MALE DOSE GROUPS AND IN THE FEMALE DOSE GROUPS, WAS SIMILAR TO THE CONCURRENT CONTROL INCIDENCE, THIS INCREASE IN HEPATOCELLULAR ADENOMAS WAS CONSIDERED TO BE UNRELATED TO TEST MATERIAL ADMINISTRATION.

	MALES				
	0	25	250	2500	7500
HEPATOCELLULAR ADENOMA	4	6	5	16*	6
(MULTIPLE)	1	1	1	1	-
HEPATOCELLULAR CARCINOMA	1	4	3	2	-
	FEMALES				
	0	25	250	2500	7500
HEPATOCELLULAR ADEMONA	2	-	-	1	-
HEPATOCELLULAR CARCINOMA	-	-	-	-	-

* $P<0.05$; FISHER'S EXACT TEST

NO HISTORICAL CONTROL DATA WERE PROVIDED FOR THE INCIDENCE OF HEPATOCELLULAR ADENOMA AT THE FACILITY WHERE THE STUDY WAS CONDUCTED. BECAUSE THE INCIDENCE IN THE 2500 PPM MALE GROUP EXCEEDS THAT OF SOME PUBLISHED HISTORICAL DATA, AS WELL AS THAT OF THE OTHER GROUPS IN THIS STUDY, THE REGISTRANT SHOULD SUBMIT HISTORICAL CONTROL DATA OF THE TESTING FACILTITY, FOR THE RECORD.

DISCUSSION

THERE WERE NO EFFECTS ON BODY WEIGHT, BODY-WEIGHT GAIN, FOOD CONSUMPTION, SURVIVAL, CLINICAL PATHOLOGY, OPHTHALMOLOGY, ORGAN WEIGHT, GROSS OR MICROSCOPIC PATHOLOGY, AND TUMOR INCIDENCE NOTED IN EITHER SEX FOLLOWING EXPOSURE TO FOUR DOSE LEVELS OF ACCENT IN THE DIET FOR 18 MONTHS. SINCE THE HIGHEST DOSE LEVEL TESTED REPRESENTS A MAXIMUM LEVEL RECOMMENDED BY THE AGENCY FOR CARCINOGENICITY STUDIES, THE CARCINOGENICITY POTENTIAL OF ACCENT HAS BEEN ADEQUATELY ASSESSED FOR THE MOUSE. ALTHOUGH THE INCIDENCE OF HEPATOCELLULAR ADENOMA IN THE 2500 PPM MALES WAS UNUSUALLY HIGH, A SIMILAR OCCURRENCE WAS NOT OBSERVED IN THE FEMALES AND NO OTHER PARAMETER SHOWED A DIFFERENCE AMONG THE VARIOUS GROUPS TO SUPPORT THIS INCREASE AS RELATED TO TEST MATERIAL EXPOSURE.

AT THE PRESENT TIME, THERE IS NO OVERWHELMING EVIDENCE THAT THE UNUSUALLY HIGH INCIDENCE OF HEPATOCELLULAR ADENOMA IN THE MALES AT THE 2500 PPM DOSE LEVEL IS RELATED TO TEST MATERIAL ADMINISTRATION. A METABOLISM STUDY IN THE MOUSE AT THE DOSE LEVELS UTILIZED IN THIS STUDY WOULD CLARIFY WHETHER A DIFFERENCE EXISTS BETWEEN THE METABOLISM OF THE 2500 PPM AND LOWER DOSE LEVELS AND THE 7500 PPM DOSE LEVEL TO ACCOUNT FOR THE DIFFERENCE OBSERVED. IN THE METABOLISM STUDY ON ACCENT IN SPRAGUE-DAWLEY RATS, IT WAS SHOWN THAT MOST OF THE RADIOACTIVITY WAS EXCRETED UNCHANGED WITHIN 24 HOURS OF AN ORAL DOSE (EITHER 10 OR 1000 MG/KG). ELIMINATION IN THE FECES WAS 80-95% OF THE DOSE AND ELIMINATION IN THE URINE ACCOUNTED FOR 9-20% OF THE DOSE. THE MAJOR EXCRETION PRODUCT IN THE FECES AND URINE WAS UNCHANGED PARENT. REPEATED DOSING AT THE HIGH DOSE WAS NOT PERFORMED. A HIGHER PERCENTAGE OF THE DOSE WAS EXCRETED IN THE FECES AFTER THE SINGLE HIGH DOSE THAN AFTER THE SINGLE LOW DOSE, WHICH COULD BE INTERPRETED TO INDICATE THAT THE EXCRETORY MECHANISM HAD BEEN OVERLOADED AND ANOTHER MECHANISM FOR ELIMINATION WAS BEING USED AT THE HIGH DOSE.

CONCLUSION

UNDER THE CONDITIONS OF THE STUDY, THE NOEL FOR BOTH SEXES OF MOUSE CAN BE SET AT 7500 PPM (993 AND 1312 MG/KG/DAY FOR MALE AND FEMALE MICE, RESPECTIVELY).

COMMENT: NO HISTORICAL CONTROL DATA WERE PROVIDED FOR THE INCIDENCE OF HEPATOCELLULAR ADENOMA IN CD-1 MICE AT THE FACILITY WHERE THE STUDY WAS CONDUCTED. BECAUSE THE INCIDENCE IN THE 2500 PPM MALE GROUP EXCEEDS THAT OF SOME PUBLISHED HISTORICAL DATA, AS WELL AS THAT OF THE OTHER GROUPS IN THIS STUDY, THE REGISTRANT SHOULD SUBMIT HISTORICAL CONTROL DATA ON MOUSE HEPATOCELLULAR ADENOMA INCIDENCE AT THE TESTING FACILTITY, FOR THE RECORD.

Reviewed by: Alan C. Levy, Ph. D. Alan C. Levy 5-9-90
Section I, Tox. Branch II (H7509C)

Secondary reviewer: Yiannakis M. Ioannou, Ph. D. JMI 5/9/90
Section I, Tox. Branch II (H7509C)

DATA EVALUATION REPORT 007939

STUDY TYPE: Combined Chronic Toxicity/Oncogenicity (rats) (§83-5)

TEST MATERIAL: IN V9360 TOX. CHEM. NO.: 359J

SYNONYMS: Accent® MRID NO.: 413601-04

STUDY NUMBER: 637-89

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

TESTING FACILITY: E. I. du Pont de Nemours and Company, Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
Newark, DE

TITLE OF REPORT: Combined Chronic Toxicity/Oncogenicity Study with IN V9360 - Two-Year Feeding Study in Rats

AUTHOR: Jon C. Cook

REPORT ISSUED: December 27, 1989

CONCLUSIONS:

There did not appear to be any effect of the dietary administration of IN V9360 at concentrations of 50, 1,500, 7,500 and 20,000 ppm on any toxicological parameters examined in rats given the test article for about two years.

The No Observed Effect Level (NOEL) = or > 20,000 ppm (males, 786 mg/kg; females, 1098 mg/kg) - HDT - no toxicological effects observed

The Lowest Observed Effect Level (LOEL) > 20,000 ppm (males, 786 mg/kg; females, 1098 mg/kg) - HDT - no toxicological effects observed

The Maximum Tolerated Dose (MTD) > 20,000 ppm (males, 786 mg/kg; females, 1098 mg/kg - HDT - not attained

The test article did not appear to increase the number of any tumors over control values.

This study is classified Core Minimum.

This study satisfies the guideline requirements (§83-5) for a combined chronic toxicity/oncogenicity rat study.

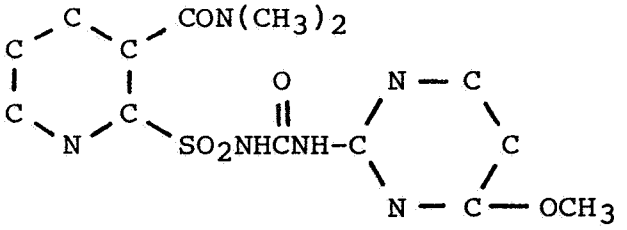
M. Statistical Analyses

Body weights, body weight gains, organ weights, and clinical laboratory measurements 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. When the results from the Bartlett's test were significant ($p < 0.005$) for clinical pathology data, the Kruskal-Wallis test was employed and the Mann-Whitney U test was used to compare means from the control groups and each of the group's exposed to IN V9360. Incidences of clinical observations were evaluated by the Fisher's Exact test with a Bonferroni correction and the Cochran-Armitage test for trend. Survival probabilities were estimated with the Kaplan-Meier procedure. Survival among groups were compared with the Mantel-Haenszel test and the Fisher's Exact test. Tumor incidence was evaluated by the Fisher's Exact test. Significance was judged at alpha = 0.05.

1. MATERIALS, METHODS AND RESULTS

A. Test Article

Name: IN V9360; 3-Pyridinecarboxamide, 2-[[[[[4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-N,N-dimethyl-



Lot Numbers and Purity:

IN V9360-27 (Haskell No. 16,925); Purity = initially stated as 97.4%, revised to 90.6%

IN V9360-29 (Haskell No. 17,499); Purity = 92.75%

B. Compound Purity and Diet Analyses [See Table 1]

IN V9360-27: The sponsor (du Pont) initially stated a purity of 97.5%. Haskell Laboratory (different laboratory analysis) determined the purity to be 94.5%. From test days 1 through 17, a value of 97.5% was used. From test day 18 (stated on report page 26; report page 18 states from day 19) through day 490, a value of 94.5% was used. Both the sponsor and Haskell Laboratories subsequently (in August, 1989, when the study was completed) determined purity to be 90.6%.

IN V9360-29: The sponsor stated the purity to be 92.75% (no indication that Haskell Laboratory analyzed this lot). This lot was used on test days 491-724.

NOTE: Regarding the purity of IN V9360-27, the report author stated, "No correction was made in this report for the effect of this difference in purity value (97.4 or 94.5% versus 90.6%) on either the concentration of the test material in the diet or the daily intake of test material by the rats."

The author attributed the variations observed to the degree of hydration.

Table 1 presents homogeneity, stability and feeder-sample values for day 18 of the study. Homogeneity ranged from 85-95% of the desired concentration. Ten-day room temperature stability values were 80-94% of desired concentrations. Mean cage-site feeder sample values (7 intervals)

66

B

Table 1

SUMMARY OF DAY 18 DIETARY ANALYSES - RATS ADMINISTERED V9360 IN THE DIET FOR TWO YEARS (a)

Desired Concentration (ppm)	50	1,500	7,500	20,000
HOMOGENEITY[b]				
Top	45 (90)c	1,421 (95)	6,495 (87)	18,711 (94)
Middle	45 (90)	1,398 (93)	6,397 (85)	18,395 (92)
Bottom	43 (86)	1,406 (94)	6,633 (88)	18,671 (93)
STABILITY[b]				
Fresh Frozen	45 (90)	1,402 (93)	6,554 (87)	19,263 (96)
1-Day Room Temperature	42 (84)	1,409 (94)	6,475 (86)	18,789 (94)
10-Day Room Temperature	40 (80)	1,406 (94)	6,732 (90)	18,079 (90)
10-Day Refrigerated	45 (90)	1,441 (96)	6,712 (89)	18,711 (94)
CAGE-SITE FEEDER SAMPLES				
Test Day 144[b]	42 (84)	1,443 (96)	7,130 (95)	19,028 (95)
Test Day 221[b]	38 (77)	1,534 (102)	7,150 (95)	19,715 (99)
Test Day 354[b]	32 (64)	1,479 (99)	8,061 (107)	19,851 (99)
Test Day 445[b]	38 (77)	1,471 (98)	7,052 (94)	19,307 (97)
Test Day 522[d]	30[e] (61)	1,364 (91)	7,210 (96)	18,079 (90)
Test Day 606[d]	44 (89)	1,410 (94)	6,953 (93)	19,381[e] (97)
Test Day 704[d]	40[e] (80)	1,557 (104)	7,582 (101)	19,524 (98)
Mean ± S.D.	38+5 (7$\bar{5}$)	1,465+67 (9$\bar{8}$)	7,305+387 (9$\bar{7}$)	19,269+590 (9$\bar{6}$)

a = Values are not corrected for recovery which ranged from 90-108% of the nominal concentration at 50 ppm and 80-98% at 20,000 ppm.
b = Values are from diets prepared with IN V9360-27.
c = Number in () is mean % of nominal concentration.
d = Values are from diets prepared with IN V9360-29.
e = Values represent the mean of repeat analyses.
Data extracted from report Table 1, pages 69-70.

67

showed 75-98% of desired concentrations.

Detailed results for compound purity and dietary analyses are presented in report Appendix B, pages 280-324.

Reviewer's Comment: Homogeneity and stability appeared to be relatively consistant, particularly at the highest desired concentration of 20,000 ppm (90-96%). For cage-site feeder samples, the mean of the 7 intervals for the 20,000 ppm concentration was 96% of the desired concentration with individual interval values ranging from 95-99%.

This Reviewer feels that, in spite of the reported variations in the results of the purity testing of IN V9360, the concentrations in the diet were sufficient to indicate that the test article (at the proposed concentration of 20,000 ppm) had little if any effect on any of the toxicological parameters examined. [The Agency's LIMIT DOSE for a chronic toxicity/oncogenicity rat study is considered to be 20,000 ppm; 1,000 mg/kg.]

It is felt that reliable purity analyses should have been performed prior to use of the lots of IN V9360.

C. Animals

Male and female (358 of each sex) Crl:CD®BR rats (21 days of age) were received from Charles Rivér Laboratories, Inc., Kingston, NY. The animals were housed 3/stainless steel wire mesh cage during a 13 day quarantine period. On test day -10, all rats were examined by a veterinary ophthalmologist. On test day -7 (following quarantine), 310 rats/sex were selected for the study based on body weight gain (5 weighing intervals and daily observations) and freedom from disease or injury. Computerized, stratified randomization divided the animals into 5 dose groups of 62 each. After assignment to treatment groups, the rats were housed individually in stainless steel wire mesh cages.

Separate racks were used for each sex with the racks being rotated within the room each week. Cages were repositioned on the cage racks every two weeks. There was a 12 hour light/dark cycle. Targeted temperature and humidity values were 23 + 2°C and 50 + 10%, respectively. Tap water was available ad libitum (no mention of water bottles or automatic watering system).

Dietary admixes were prepared fresh weekly in Purina® Certified Irradiated Chow® #5002 and were refrigerated until used. The concentrations utilized were: 0 (control), 50, 1,500, 7,500 and 20,000 ppm.

D. General Observations

1. Mortality and Moribundity - Cage-site examinations were conducted at least once daily.

Selected survival intervals are presented in Table 2. The study was originally scheduled for 104 weeks, but due to a greater than anticipated projected mortality rate in males at 1,500 ppm, the Registrant requested (the Agency agreed) that the Agency allow the terminal sacrifice to begin after week 102 (letter from T. E. Catka of du Pont to R. J. Taylor of EPA dated June 1, 1989; EPA memorandum from A. C. Levy to C. Giles dated June 8, 1989).

As noted in Table 2, the percent survival for males was 37-38 in all dose groups except at 1,500 ppm where it was 21%. In females, survival was 38% in controls, 37% at 20,000 ppm and 50-60% in the other three dose groups. Therefore, test article administration did not appear to have an effect on the survival of male or female rats in this study. The lower rate in 1,500 ppm males is considered to be a normal biological variation and there is no apparent reason to attribute this to IN V9360 administration.

2. Clinical Observations - Cage-site examinations were conducted at least once daily. In addition, each rat was closely examined for abnormal behavior and appearance at every weighing (once weekly during the first 6 months and once every other week for the remainder of the study).

No test article-related clinical observations were reported.

3. Body Weights - Individual body weights were recorded weekly during the first 6 months of the study and every other week thereafter.

Table 3 presents selected intervals of mean group body weights. Except for an occasional statistically significant difference, there did not appear to be a test article related difference between the control and treated groups of either sex. In addition, group mean body weight gains (Table 4) were similar for all study groups. Administration of IN V9360 up to 20,000 ppm did not appear to effect group mean body weights or group mean body weight gains.

4. Food Consumption and Test Article Intake - Food consumption was calculated as g/rat/day and was presented in the report as weekly periods for the first six months and as two week periods for the remainder of the study (fresh food was supplied weekly throughout the study). Group food consumption was determined by weighing the amount of diet prepared at the start of the interval and subtracting the amount of diet remaining (plus spillage) at

Table 2

SURVIVAL OF RATS ADMINISTERED V9360 IN THE DIET FOR TWO YEARS

	Males					Females				
ppm =	0	50	1500	7500	20000	0	50	1500	7500	20000
Days on Test										
84-91	62	61	62	62	62	61	62	62	61	62
175-182	61	60	61	62	62	61	62	62	61	62
259-273	60	60	61	62	62	60	62	62	61	62
357-371	56	59	59	59	61	57	60	60	60	61
441-455	45	46	45	47	47	45	47	50	46	45
539-553	40	38	36	39	40	42	43	42	37	36
623-638	25	30	20	28	28	32	37	34	33	26
693-707	20	19	11	19	20	20	31	26	30	19
Total Rats:										
Study Start	62	62	62	62	62	62	62	62	62	62
Scheduled Deaths[a] ...	10	10	10	10	10	10	10	10	10	10
Unscheduled Deaths[b] ...	32	33	41	33	32	32	21	26	22	33
Percent Survival	38	37	21	37	38	38	60	50	58	37

a = Includes interim 12-month sacrifice.
b = Includes rats accidentally killed, found dead, and sacrificed in extremis.

NOTE: No statistically significant differences were found when compared to the controls by the Fisher's Exact test using a Bonferroni correction or by the Cochran-Armitage test for trend at alpha = 0.05.

Data extracted from report Tables 14 and 15, pages 96-101.

Table 3

GROUP MEAN BODY WEIGHTS OF RATS ADMINISTERED V9360 IN THE DIET FOR TWO YEARS

Days on Test	Males					Females				
ppm =	0	50	1500	7500	20000	0	50	1500	7500	20000
0	182	183	183	183	182	136	135	137	135	136
28	385	388	389	385	389	214	214	217	216	215
56	500	505	505	499	508	261	263	263	265	263
84	567	574	578	564	574	284	288	290	294	290
182	698	710	716	697	704	345	355	355	369	352
273	777	785	790	779	776	397	414	411	428	404
371	846	838	822	832	822	445	461	465	474	443
455	859	877	819	849	856	480	506	512	517	473
553	854	857	771*	842	849	493	528	529	543	507
638	814	815	795	822	831	498	570	550	576	524
707	759	779	836	778	793	515	584	547	556	522

Group mean body weights expressed in grams.
* = Statistically significant difference from control at alpha = 0.05.
Data extracted from report Tables 2 and 3, pages 71-74.

Table 4

GROUP MEAN BODY WEIGHT GAINS OF RATS ADMINISTERED V9360 IN THE DIET FOR TWO YEARS

Days on Test	Males					Females				
ppm =	0	50	1500	7500	20000	0	50	1500	7500	20000
0-7	59	61	62	61	63	26	25	25	28	28
28-35	36	36	33	33	34	13	13	14	15	15
56-63	21	23	23	21	20	8	6	10	10	10
84-91	10	11	11	12	10	6	5	4	2	1
182-189	8	11	10	12	10	8	7	5	7	8
273-287	12	9	7	3*	7	7	8	11	6	3
371-385	3	1	0	6	5	7	5	7	4	2
455-469	-5	1	6	4	3	8	12	10	11	5
553-567	-19	-17	-25	-26	-9	-6	-2	2	5	-6
638-651	-17	-20	-32	-13	-18	-12	2	4	-1	-5
693-707	-40	-41	-26	-35	-30	-15	-6	-8	0	-2
0-371	665	655	639	649	640	309	326	328	339	307
371-707	-56	-30	44	-22	-11	79	125	83	90	83
0-707	573	598	559	597	613	378	447	412	423	386

Group mean body weight gains expressed in grams.
* = Statistically significant difference from control at alpha = 0.05.

Data extracted from report Tables 4 and 5, pages 75-78.

the end of the interval. Test article intake was calculated from food consumption and body weight data.

Group mean food consumption and food efficiency were similar for the control and all dosed groups.

As indicated in the "Compound Purity and Diet Analysis" section, the purity of IN V9360 was reported to be different at various times during the study. The following paragraph from page 28 of the report addresses this subject:

> "The overall mean daily intake values (test days 0-707) of IN V9360 by male rats in the 50, 1,500, 7,500, and 20,000 ppm groups were 1.90, 58.1, 289, and 786 mg/kg/day, respectively (Table 10). In the female 50, 1,500, 7,500, and 20,000 ppm groups, the overall mean daily intake values (test days 0-707) of IN V9360 were 2.59, 77.1, 382, and 1,098 mg/kg/day, respectively (Table 11). The fluctuations in these values between sexes were related to normal differences in body weight gain and to the variability in food consumption by each sex during the study. The mean daily intake values from test days 0-18 and 19-490 are based on diets prepared using purity values of 97.4% and 94.5%, respectively. Subsequent purity analysis by the sponsor found the purity of IN V9360-27 to be 90.6%. Based on the lower purity value, the mean daily intake values are approximately 7% and 4% less than the stated values on test days 0-18 and 19-490, respectively."

5. Ophthalmoscopic Examinations - These were performed on all animals on test days -10, 354 and 710 by James M. Clinton, V.M.D., veterinary ophthalmologist. His detailed findings were included in the study report.

Dr. Clinton concluded that he did not believe the test article produced ocular changes.

E. Clinical Pathology - Hematology, blood chemistry and urine parameters were examined from 10 randomly selected rats/sex/group at approximately 3, 6, 12, 18 and 24 months. These animals were designated prior to the 3-month interval and were used for all subsequent evaluations (those that died/sacrificed in extremis were replaced by randomly selected alternates). At the 24 month evaluation, a 20,000 ppm female died and was not replaced. Two days prior to collection of blood samples, the selected rats were placed in metabolism cages. There was a 16 hour fast prior to blood collection. Urine was collected during that period. Blood samples were taken from the orbital sinus (under light carbon dioxide anesthesia) at the end of the fast.

1. Hematology - The CHECKED (x) parameters were examined:

X	
x	Hematocrit (HCT)*
x	Hemoglobin (HGB)*a
x	Leukocyte count (WBC)*
x	Erythrocyte count (RBC)*
x	Platelet count *

X	
-	Total plasma protein (TP)
x	Leukocyte differential count
x	Mean corpuscular HGB (MCH)
x	Mean corpusc. HGB conc. (MCHC)
x	Mean corpusc. volume (MCV)

* = EPA Guideline Requirement "-" = Not examined

Although there were some isolated statistically significant differences between treated and control groups, there was no indication that test article administration had an effect on any hematological parameters.

2. Blood Chemistry - The CHECKED (X) parameters were examined:

X	Electrolytes
x	Calcium*
x	Chloride*
-	Magnesium*
x	Phosphorous*
x	Potassium*
x	Sodium*

X	Other
x	Albumin*
x	Blood creatinine*
x	Blood urea nitrogen*
x	Cholesterol*
x	Globulins
x	Glucose*
x	Total Bilirubin*
x	Total Protein*
-	Triglycerides

	Enzymes
x	Alkaline phosphatase
-	Cholinesterase
-	Creatinine phosphokinase*
-	Lactic acid dehydrogenase
x	Serum alanine aminotransferase (also SGPT)*
x	Serum aspartate aminotransferase (also SGOT)*

* = EPA Guideline Requirement "-" = Not examined

The administration of IN V9360 did not appear to have an effect on any of the blood chemistry parameters examined.

3. Urinalysis - The CHECKED (X) parameters were examined:

X	
x	Appearance*
x	Volume*
x	Specific Gravity (Osmolality)*
x	pH
x	Sediment (Microscopic)*
x	Protein*

X	
x	Glucose*
x	Ketones*
x	Bilirubin*
x	Blood*
-	Nitrate
x	Urobilinogen

* = EPA Guideline Requirement "-" = Not examined

No urinary parameters appeared to be affected by IN V9360.

F. Sacrifice and Pathology - All animals that died or were sacrificed in extremis before the end of the study were necropsied. Ten rats/sex/group were randomly selected at about 12 months (excluding clinical laboratory evaluation animals) for an interim sacrifice/necropsy. CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed. All rats in the control and 20,000 ppm groups as well as all rats that died or were sacrificed in extremis in the 50, 1,500 and 7,500 ppm groups had all tissues examined microscopically. Only liver, kidneys, lungs and gross lesions were examined from rats in the 50, 1,500 and 7,500 ppm groups at scheduled sacrifices.

Blood smears from all rats sacrificed in extremis or by design and bone marrow smears from all rats sacrificed by design were prepared to clarify morphologic findings, if necessary. [It was reported that evaluation of these smears was not needed.]

All tissues were fixed in 10% neutral buffered formalin except for testes, epididymides, eyes, skin (male) and skin with mammary gland (female) which were fixed in Bouin's solution.

X	Digestive system
-	Tongue
x	Salivary glands*
x	Esophagus*
x	Stomach*
x	Duodenum*
x	Jejunum*
x	Ileum*
x	Cecum*
x	Colon*
x	Rectum*
xx	Liver*
x	Pancreas*

X	Respiratory
x	Trachea*
x	Lung
	Cardiovasc./Hemat.
x	Aorta*
xx	Heart*
x	Bone marrow*
x	Lymph nodes*
xx	Spleen*
x	Thymus*

X	Urogenital
xx	Kidneys*
x	Urinary bladder*
xx	Testes*
x	Epididymides
x	Prostate
x	Seminal vesicle
x	Ovaries
x	Uterus*

X	Neurologic
xx	Brain*
x	Periph. Nerve*
x	Spinal cord (3 levels)*
x	Pituitary *
x	Eyes (optic n.)*

X	Glandular
x	Adrenals*
x	Lacrimal gland
x	Mammary gland*
x	Parathyroids*
x	Thyroids*

X	Other
x	Bone*
x	Skeletal muscle
x	Skin
x	All gross lesions & masses

x Nose x Vagina x Harderian glands

* = EPA Guideline Requirement "-" = Not examined

NOTE: Spinal Cord - No mention of 3 levels examined

1. Macroscopic -

Interim (12 month) Sacrifice: There were no macroscopic observations which were attributed to IN V9360 administration.

Terminal Sacrifice: There were no macroscopic observations which were attributed to IN V9360 administration.

2. Organ Weights -

Interim (12 month) Sacrifice: There were no absolute (g) or relative (% of body weight) organ weight differences between treated and control groups which were considered related to IN V9360 administration.

Terminal Sacrifice: There were no absolute (g) or relative (% of body weight) organ weight differences between treated and control groups which were considered related to IN V9360 administration.

3. Microscopic -

Interim (12 month) Sacrifice: There were no apparent increases in the incidence of microscopic lesions in males or females which were considered attributable to IN V9360 administration.

Terminal Sacrifice: No apparent increases in the numbers of rats with lesions were noted in the treated groups versus the control group.

The study author indicated, "The only statistically significant increases in tumor incidences noted occurred as a result of combining benign and malignant mammary tumors and it occurred in the 50 ppm concentration group. The increase was not considered significant." The following data are extracted from report Table 33, page 185:

Table 4

MAMMARY GLAND BENIGN (B) AND MALIGNANT (M) TUMORS IN FEMALE RATS TREATED WITH IN V9360 (ACCENT ®) FOR TWO YEARS

ppm =	0	50	1,500	7,500	20,000
No. examined/total number	47/47	46/46	44/44	39/39	51/51
B: Adenoma	2	1	3	3	1
B: Adenoma, intraductile, single/multi..	-	1	-	1	2
B: Fibroadenoma, multiple	6	8	5	3	5
B: Fibroadenoma, single	10	15	18	14	11
M: Adenocarcinoma, multiple	3	3	1	1	4
M: Adenocarcinoma, single	4	12	8	7	8
M: Carcinosarcoma	1	1	-	-	1

The following table (extracted from report tables 30-33, pages 154, 169, 176 and 188) presents the number of rats with microscopically observed tumors:

Table 5

INCIDENCES OF MICROSCOPICALLY OBSERVED TUMORS IN RATS ADMINISTERED IN V9360 FOR TWO YEARS

	Males					Females				
ppm =	0	50	1500	7500	20000	0	50	1500	7500	20000
NECROPSIED:	THROUGH DAY 373					THROUGH DAY 374				
Number in group	16	13	13	13	11	15	12	12	12	11
Total rats with primary	3	1	2	3	3	6	2	2	1	5
Total primary tumors	5	1	2	3	4	6	2	2	1	8
Total rats with benign	3	1	1	2	3	3	1	2	1	3
Total benign tumors	4	1	1	2	4	3	1	2	1	4
Total rats with malignant	1	0	1	1	0	3	1	0	0	4
Total malignant tumors	1	0	1	1	0	3	1	0	0	4
Total rats with secondary	1	0	1	1	0	1	0	0	0	1
Total secondary tumors	1	0	9	2	0	5	0	0	0	4
NECROPSIED:	FROM DAYS 374 - 722					FROM DAYS 375 - 724				
Number in group	46	49	49	49	51	47	50	50	50	51
Total rats with primary	41	37	40	42	48	45	46	47	46	48
Total primary tumors	74	66	66	72	90	92	101	97	93	99
Total rats with benign	37	37	38	37	46	42	42	46	43	45
Total benign tumors	61	55	56	54	75	73	74	81	78	77
Total rats with malignant	12	10	7	18	14	16	18	15	15	20
Total malignant tumors	13	11	10	18	15	19	27	16	15	22
Total rats with secondary	2	4	5	4	4	6	5	2	3	3
Total secondary tumors	2	5	15	31	9	10	5	8	4	12

There does not appear to be any lesion observed during the course of this study which is considered attributable to test article administration.

NOTE: The Registrant (du Pont's Haskell Laboratory's standard operating procedures) had a "review pathologist" examine (peer review) all tissues from 5/sex/group (randomly selected by computer) plus all neoplasms. The "review pathologist" evaluated the quality of histological preparations in addition to the interpretation of the morphology. The "review pathologist" stated, "I am in essential agreement with the study pathologist's reports including his diagnosis, interpretation, and conclusion."

The Reviewer has no comments regarding the Methods and Materials section.

A description of the statistical analyses employed was included in the report and a copy is attached.

A Good Laboratory Practice compliance statement and documentation of Quality Assurance inspections were included.

II. DISCUSSION

Purity

There were apparent discrepancies regarding the reported purity of the test article (IN V9360-27) which was incorporated into the diet from the start of the study through day 490. A purity of 97.5% was reported by the sponsor (du Pont); 94.5% was the result determined by the performing laboratory (Haskell); and a purity of 90.6% was noted by both of the above mentioned sources at about the time the study was terminated (about days 722-724). The variations were attributed (by the report author) to the degree of hydration.

The Registrant included extensive descriptions and results of analyses (report Appendix B, pages 280-324). IN V9360-29 (used from days 491-study termination at day 724) was reported by du Pont (not Haskell Laboratory) to have a purity of 92.75%.

The EPA Limit Dose of 20,000 ppm (1,000 mg/kg) was the highest concentration tested. No apparent test article related differences from control values were noted regarding any parameter examined. Although the overall mean daily intake of the test article by the male rats administered the high dose was only 786 mg/kg/day (thus, not a limit dose), it is believed that a repeat study (to establish the MTD) with 1,000 mg/kg/day will not result in major changes in any of the toxicological parameters measured. Therefore, this study is considered to be adequate at the dose levels tested.

It is felt by this Reviewer that, even though there were purity discrepancies and no compensation for the active ingredient being less than 100% pure, the overall integrity of the study is not compromised.

Toxicology Parameters

At the proposed dietary concentrations of 50, 1,500, 7,500 and 20,000 ppm, there did not appear to be any effect of the administration of IN V9360 on any of the toxicological parameters examined (mortality, clinical signs, body weights/gains, food consumption, food efficiency, ophthalmoscopy, hematology, blood chemistry, urinalyses, gross pathology, organ weights or microscopic pathology).

In a 90-day rat study, IN V9360 resulted in statistically significant changes in some hematology, clinical chemistry and urinalysis parameters at all dose levels tested (300, 1,500, 7,500 and 20,000 ppm.) None of these effects were reported in the present two-year study.

III. CONCLUSIONS

There did not appear to be any effect of the dietary administration of IN V9360 at concentrations of 50, 1,500, 7,500 and 20,000 ppm on any toxicological parameters examined in rats given the test article for about two years.

The No Observed Effect Level (NOEL) = or > 20,000 ppm (males, 786 mg/kg; females, 1098 mg/kg) - HDT - no toxicological effects observed

The Lowest Observed Effect Level (LOEL) > 20,000 ppm (males, 786 mg/kg; females, 1098 mg/kg) - HDT - no toxicological effects observed

The Maximum Tolerated Dose (MTD) > 20,000 ppm (males, 786 mg/kg; females, 1098 mg/kg - HDT - not attained

The test article did not appear to increase the number of any tumors over control values.

This study is classified Core Minimum.

This study satisfies the guideline requirements (§83-5) for a combined chronic toxicity/oncogenicity rat study.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

007939

MEMORANDUM

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Evaluation of an Acute Inhalation Toxicity Study with DPX-V9360-45 (Milled) in Rats.

FROM: Jess Rowland, Toxicologist Jess Rowland 5/29/90
Section II, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

TO: C. Giles
Product Manager (25)
Registration Division

THRU: K.Clark Swentzel, Section Head K. Clark Swentzel 5/29/90
Section II, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)
and
Marcia van Gemert, Ph.D., Chief Marcia van Gemert 5/17/90
Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

STUDY IDENTIFICATION: MRID No. 413601-05; Haskell Lab.Report No. 704-89; Medical Research No. 4581-758; HED Project No. 0-0565

ACTION REQUESTED: Review and Evaluation of the study.

SUMMARY: Nose-only exposure of male and female rats for 4-hours to a particulate atmosphere of milled DPX-V9360-45 in air to concentrations of 2600, 4800, or 5600 mg/m^3 did not cause mortality or toxicologically significant effects on body weight, clinical signs, or gross pathology during a 14-day observation period. The 4-hour LC_{50} of this test material was greater than 5600 mg/m^3. The particle sizes of the test atmospheres were too large i.e, 25% of the particles were not less than 1 um in diameter. The reason for not generating an atmosphere with smaller particles was not given. Therefore, this study is classified as CORE SUPPLEMENTARY and can possibly be upgraded to CORE MINIMUM following review of the reason for not generating an atmosphere with small particles.

CORE CLASSIFICATION: Supplementary;(81-3) due to questions regarding particle size.

Primary Reviewer: Jess Rowland, Toxicologist 3/29/90
Section II, Tox.Branch II

Secondary Reviewer: K. Clark Swentzel, Section Head R. Clark Swentzel 3/29/90
Section II, Tox.Branch II

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation (Guideline 81-3) TOX.CHEM.No:359J

MRID No.: 413601-05 I.D.No.:352-LGL-LGU PROJECT No.: 0-0565

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

SYNONYMS: DPX-V9360-45; IN V-9360 75DF; IN V9360-45

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

TESTING LABORATORY: E.I.du Pont de Nemours and Company, Inc. Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware.

STUDY IDENTIFICATION: **Medical Research No.4581-758; Haskell Laboratory Report No. 704-89**

TITLE OF REPORT: **Acute Inhalation Toxicity Study with DPX-V9360-45 (Milled) in Rats.**

AUTHOR: **Dolores E. Malek**

REPORT DATE: December 8, 1989

CONCLUSION: Groups of five male and five female Crl:CD®BR rats were exposed via inhalation (nose-only) for 4-hours to a particulate atmosphere of milled DPX-V9360-45 in air at concentrations of 2600, 4800, or 5600 mg/m^3. Test atmospheres were generated by suspension of the milled test material in air using a jet mill and cyclone elutriator. Animals were sacrificed following a 14-day observation period. No deaths or toxicologically significant effects were observed on body weight, clinical signs, or gross pathology. Under the conditions of this study, the LC_{50} of DPX-V9360-45 (milled) was > 5600 mg/m^3.

CORE CLASSIFICATION: Supplementary; can possibly be upgraded following review of rationale for not generating an atmosphere of smaller (25% < 1 um) particles.

TOXICITY CATEGORY: IV

80
D

I. INTRODUCTION

This Data Evaluation Report summarizes the experimental procedures and results of an acute inhalation toxicity study in rats with DPX-V9360-45 (milled).

II. MATERIALS AND METHODS

1. TEST MATERIAL:

Designation: DPX-V9360-45 (milled)
Composition: 75% DPX-V9360 (active ingredient
25% inert ingredients
Type: Formulation
Purity: 75%
Physical Form: Off-white milled powder

2. TEST ANIMALS:

Species: Rat
Strain: Crl:CD BR
Sex: Males and females
Weight: Males - 243-288 g; females - 200-236 g
Age: 8-weeks old

3. ANIMAL HUSBANDRY:

Housing: Individually during quarantine and in pairs during study in suspended wire mesh cages
Food: Purina Certified Rodent Chow #5002
Water: tap water ad libitum.
Environment: Temperature - 23 $\pm$ 2° C; Humidity - 50 $\pm$ 10%
Light/dark cycle: 12 hr. light/dark cycle

4. STUDY DESIGN:

Group	No./Sex/Group	Atmospheric concentration
1	5 M & 5 F	2600 $\pm$ 340 mg/m^3
2	5 M & 5 F	4800 $\pm$ 890 mg/m^3
3	5 M & 5 F	5600 $\pm$ 1800 mg/m^3

5. STUDY PROTOCOL:

Rats were exposed for a single 4-hour period and were weighed and observed daily during a 14-day observation period (excluding weekends) at which time they were sacrificed for gross pathological examination.

6. EXPOSURE CONDITIONS

a. Nose-only Exposure: Rats were placed in perforated stainless steel cylinders with conical nose pieces which were inserted into the face plate of a 38-L cylindrical glass exposure chamber so that only the rat's nose protruded into the chamber. Rats were exposed for a single 4-hour period to a particulate atmosphere of milled DPX-V9360-45 in air at concentrations of 2600, 4800, or 5600 mg/m^3.

b. Atmospheric Generation: Atmospheres of the test material were generated by suspension of the DPX-V9360-45 with an air stream. Prior to testing, the test material was milled on three separate occasions to obtain respirable particulates; the median diameter was 2.2 um based on volume %. During generations, the test material was metered into a Fluid Energy Process Equipment Model OO Jet-O-Mizer with a K-Tron Model T-20 twin-screw volumetric feeder and K-Tron Series 6300 digital speed controller. The jet mill disrupted the larger particles with high pressure air jets and discharged the milled test material into a 4-L glass cyclone elutriator. The elutriator retained the larger particles and allowed the smaller particles to enter the 38-L cylindrical glass exposure chamber. Particles in the chamber were disbursed by a baffle for more uniform distribution.

c. Distribution Assessment: In a generation trial, the homogeneous distribution of total particulate concentration and particle size distribution within the exposure chamber operating at a flow rate greater than approximately 45 L/min was assessed. An atmosphere of the test material was generated under conditions similar to those used in animal exposure. This trial lasted for approximately 30 minutes and no animals were exposed.

d. Range Finding: A range finding trial was conducted to establish generation conditions that would produce the most respirable atmospheres (at acceptable airflows and concentrations) that could be achieved with the test material. Atmospheres of the test material were generated under 3 sets of conditions similar to those used in animal exposures. The trials had flow rates ranging from 40 to 63 L/min; no animals were exposed and the trials ranged from 0.5 to 4 hours in duration.

e. Analytical: Gravimetric analysis was used to determine the atmospheric concentration of total particulate at approximately 30-minute intervals during each exposure. Known volumes of chamber atmosphere were drawn from the rat's breathing zone through pre weighed filters. The atmospheric concentration was determined from the difference in the pre- and post-sampling filter weights. Since the actual chamber concentration of the test material was measured, the determination of nominal concentration was not considered to be a useful parameter. Particle size distribution (mass median aerodynamic diameter and percent particles < 1, 3, and 10 um diameter) was determined at least once during each exposure. In addition, chamber airflow, temperature, oxygen concentration and relative humidity were measured at approximately 30-minute intervals during the exposure period.

III. RESULTS

a. Exposure assessment: The atmospheric concentrations generated during exposure, particle size characterization, and the chamber environmental conditions during exposure are presented in Tables 1, 2 , and 3, respectively.

Data obtained during the distributions assessment trial indicated a homogeneous distribution of concentration and particle size distribution within the exposure chamber (Table 4). All animals within an exposure concentration were assumed to be subject to the same atmospheric concentration and particle size distribution.

Data from the rangefinding trials are presented in Table 5. In these trials, when airflow into the jet mill and cyclone were altered to affect particle grinding and separation, the mass median diameters were reduced (3.0 to 2.1) and the fraction of small particles < 1 um were increased (13 to 18%). However, these particle size distribution changes were coincident with an increase in total airflow (40 to 63 L/min) and a reduction in total particulate concentration form 4500 to 2400 mg/m^3. The study author indicates that although particles < 1 Um in diameter (greater than 25%) is desirable at a limit test concentration of $\geq$ 5000 mg/m^3,"such an atmosphere was not possible to generate with the subject test material" but failed to give a rationale for the deficiency.

b. Toxicity assessment: No mortality occurred during the study. On day 1 post exposure, rats exhibited moderate transient body weight depressions (5.8% ± 2.4% in males and 4.2 ± 2.0% in females). Although 1 male rat exposed to 2600 mg/m^3 and all females regardless of exposure group, exhibited intermittent slight body weight losses of less than 5 % , in general rats gained weight throughout the 14-day recovery period. The dense particulate atmospheres in the chamber prevented visual observation of rats during exposure. However, immediately following exposure, commonly observed clinical signs were lethargy, and red ocular and red or brown nasal discharge. Rats at the 4800 and 5600 mg/m^3 concentrations had their faces covered with the test material. One female rat at 4800 mg/m^3 and 2 rats of each sex at 5600 mg/m^3 concentrations exhibited red or brown nasal discharge up to study-day 4. One female rat at the 5600 mg/m^3 had lung noise on study-days 2 and 3. Gross necropsy revealed no evidence of organ-specific toxicity in rats sacrificed 14 days after exposure.

IV. CONCLUSION

Under the conditions of this study, DPX-v9360-45 (milled) exerted no acute toxicity in male and female rats. The 4-hour LC_{50} was greater than 5600 mg/m^3. There was no mortality or adverse clinical signs of toxicity with only sporadic slight body weight loss (mainly shortly after exposure). The particle size ranged from 2.3 to 3.0 um and approximately 90 to 96% of the test material was < 10 um and 10 to 16 % < 1 Um in diameter. The registrant stated that smaller particles were unattainable with the test material but did not provide rationale for not generating an atmosphere of smaller particles.

V. RECOMMENDATION

a. This study is to be considered SUPPLEMENTARY because no rationale for provided for not being able to generate 25% of the particles that are smaller than 1 um in diameter.

b. This study may be upgraded to CORE MINIMUM provided the response to the above is acceptable, even though the particle sizes were considered too large. This is based upon the relatively low toxicity of the chemical and an apparent problem with the physical nature of the test material.

84
6

Table 1. Atmospheric Concentration of DPX-V9360-45 During Animal Exposure.

Total Particle Concentration (mg/m³)[a]			
Mean	S.D.	Range	n
2600	340	2100-3200	8
4800	890	3500-5800	8
5600	1800	4100-8600	8

[a] Total particulate concentration during each 4-hour exposure is based on gravimetric analyses and is expressed on a weight/volume basis. All values are reported to 2 significant figures.

Table 2. Particle Size characterization of DPX-V9360-45 Atmospheres During Animal Exposure.

Mean Particle Concentration (mg/m³)	n	MMAD um[a]	S.D.[b]	% Particles <1 um[c]	% Particles <3 um[d]	% Particles <10 um[e]
2600	1	2.3	2.3	16	64	96
4800	1	3.0	2.5	12	63	90
5600	1	3.0	2.5	10	48	91

[a] Mass median aerodynamic diameter in micrometers.

[b] Geometric standard deviation.

[c,d] Percent by weight of particles with aerodynamic diameter less than 1 and 3 um.

[e] Percent by weight of particles with aerodynamic diameter less than 10 um.

85
4

Table 3. Chamber environmental Conditions During Animal Exposure.

Total Particle Concentration (mg/m^3)	Total Chamber Airflow (L/min)			Temperature (°C)			Relative Humidity (%)			Oxygen (%)
	Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n	
2600	65	0	8	22	0	8	40	0.93	8	21
4800	62	0	8	22	0.52	8	51	1.3	8	21
5600	40	0	8	22	0.32	8	53	1.0	8	21

Values are reported to 2 significant figures

Table 4. Exposure Chamber Distribution: Concentration and Particle Size Distribution.

Sample	Port No. Sampled[a]	Total Particulate Concentration[b] (mg/m^3)	MMAD (um)[c]	% Particles <1 um	% Particles <3 um	% Particles <10 um
I	1	1870	2.2	16	66	97
II	5	1820	2.1	19	66	97
III	6	1550	2.0	20	68	97
		mean ± SD = 1747 ± 172				

[a] Refer to Figure C-1 for position of Port No. indicated.

[b] A cascade impactor was used. Concentration based on cumulative weight of particles from 7 stages. A 3.8 L sample was drawn.

[c] Mass median aerodynamic diameter in micrometers.

Generation was 30 minutes in duration and total airflow was 63 L/min.

Table 5. Rangefinding Generation Conditions and Analytical Results.

Generation Number[a]	Total Particulate Concentration[b] (mg/m³)	n[c]	MMAD (um)[d,f]	n[e]	% Particles <1 um	% Particles <3 um	% Particles <10 um	Total Airflow L/min[g]
I	4500	9	3.0	2	13	51	90	40
II	4100	7	2.5	2	12	67	96	60
III	2400	4	2.1	3	18	67	97	63

[a] Generations ranged from 0.5 to 4 hours in duration.

[b] Mean concentration based on gravimetric analysis.

[c] Number of gravimetric samples drawn during generation.

[d] Mass median aerodynamic diameter in micrometers.

[e] Number of cascade impactor samples drawn for particle size distribution evaluation during generation.

[f] Values for MMAD and percent particles less than 1, 3 or 10 um are the means of n cascade impactor samples.

[g] Total airflow resulted from adjustment of 3 separate airflows through the jetmill.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

007939

MEMORANDUM

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: ACCENT - Review of "Addendum to Report" submitted by E.I.duPont de Nemours Inc.

FROM: Jess Rowland, Toxicologist Jess Rowland 3/27/90
Section II, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

TO: C. Giles
Product Manager (25)
Registration Division

THRU: K.Clark Swentzel, Section Head K. Clark Swentzel 3/29/90
Section II, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)
and
Marcia van Gemert, Ph.D., Chief Marcia van Gemert 5/14/90
Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

ACTION REQUESTED: Review the "addendum to report" information submitted for 16 studies with ACCENT (11 studies with IN V9360 - 27 and 5 studies with IN V9360 - 7). HED Project No. 0-0565.

RESPONSE: The new information submitted by the registrant reflects updated purity values for the test materials IN V9360 - 27 (original- 97.4% versus reanalysis- 90.6%) and IN V9360 - 7 (original- 94.9% versus reanalysis- 90.4%).

The original analyses of the test material were performed using the best available analytical material. The test material was reanalyzed following the discovery that hydrate and nonhydrate form of IN V9360 existed. The difference in purity values between the analyses was believed to be due mainly to use of a standard for the original assay that was in the hydrate form, and was compounded by some rehydration of the sample during storage.

Except for reducing the no-observed-adverse-effect (NOAEL) levels in Teratology studies in Rabbits (MRID NO.413601-21) and Rats (413601-22) with IN V9360-27, the lower purity levels had no effect on the results or the conclusion of the 16 studies, because the test results were based on nominal test concentration, not corrected for sample purity.

Primary Reviewer: Jess Rowland, Toxicologist 3/27/90
Section II, Tox.Branch II (H7509C)

Secondary Reviewer: K. Clark Swentzel 3/29/90
Section Head, Tox. Branch II

HED PROJECT No.: 0-0565 REVIEW OF ADDENDUM TO REPORTS

1). Study Title : Acute Oral Toxicity with IN V9360-27 in Male and Female Rats.

Study Identification: MRID No. 413601-12; Haskell No.16925; HLR No. 737-88; Med.Res.No. 4581-554

Addendum: Test material purity: Original- 97.4%; Reanalysis- 90.6%

Result: The change in purity had no affect on the results or the conclusion of this study since the test results were based on nominal concentration, not corrected for sample purity.

2). Study Title: Acute Dermal Toxicity Study of IN V9360-27 in Rabbits.

Study Identification: MRID No. 413601-13; Haskell No.16925; HLR No. 582-87; Med.Res.No. 4581-554

Addendum: Test material purity: Original- 97.4%; Reanalysis- 90.6%

Result: The change in purity had no affect on the results or the conclusion of this study since the test results were based on nominal concentration, not corrected for sample purity.

3). Study Title: Acute Inhalation Toxicity Study with IN V9360-27 in Rats.

Study Identification: MRID No. 413601-14; Haskell No.16960; HLR No. 81-88; Med.Res.No. 4581-554

Addendum: Test material purity: Original- 97.4%; Reanalysis- 90.6%

Result: The change in purity had no affect on the results or the conclusion of this study since the test results were based on nominal concentration, not corrected for sample purity.

89

4). Study Title: Primary Eye Irritation Study with IN V9360-7 in Rabbits.

Study Identification: MRID No. 413601-15; Haskell No.16607 HLR No. 146-87; Med.Res.No. 4581-469

Addendum: Test material purity: Original- 94.9%; Reanalysis- 90.4%

Result: The change in purity had no affect on the results or the conclusion of this study since the test results were based on nominal concentration, not corrected for sample purity.

5). Study Title: Primary Dermal Irritation Study with IN V9360-27 in Rabbits.

Study Identification: MRID No. 413601-16; Haskell No.16925; HLR No. 647-87; Med.Res.No. 4581-554

Addendum: Test material purity: Original- 97.4%; Reanalysis- 90.6%

Result: The change in purity had no affect on the results or the conclusion of this study since the test results were based on nominal concentration, not corrected for sample purity.

6). Study Title: Closed-Patch Repeated Insult Dermal Sensitization Study (Buehler Method) with IN V9360-7 in Guinea Pigs.

Study Identification: MRID No. 413601-17; Haskell No. 16607 HLR No. 429-87; Med.Res.No. 4581-469

Addendum: Test material purity: Original- 94.9%; Reanalysis- 90.4%

Result: The change in purity had no affect on the results or the conclusion of this study since the test results were based on nominal concentration, not corrected for sample purity.

90 B

7). Study Title: Subchronic Oral Toxicity : 90-Day Study with IN V9360-7 Feeding Study and One-Generation Reproduction Study in Rats.

Study Identification: MRID No. 413601-18; Haskell No.16607; HLR No. 15-88; Med.Res.No.8101-001

Addendum: Test material purity: Original- 94.9%; Reanalysis- 90.4%

Result: Based on the lower purity, the mean daily compound intake values were 4% less (94.9% versus 90.4%) than originally reported. This change, however, did not affect the results or the conclusion of the study.

8). Study Title: Subchronic Oral Toxicity: 90-Day Study with IN V9360-7 Feeding Study in Mice.

Study Identification: MRID No. 413601-19; Haskell No.16607; HLR No. 16-88; Med.Re.No. 8102-001

Addendum: Test material purity: Original- 94.9%; Reanalysis- 90.4%

Result: Based on the lower purity, the mean daily compound intake values were 4% less (94.9% versus 90.4%) than originally reported. This change, however, did not affect the results or the conclusion of the study.

9). Study Title: Subchronic Oral Toxicity: 90-Day study with IN V9360-27 Feeding Study in Dogs.

Study Identification: MRID No. 413601-20; Haskell No.16925-002; HLR No. 332-88; Med.Res.No.8281-001

Addendum: Test material purity: Original- 97.4%; Subsequent analysis- 94.5%; Reanalysis- 90.6%.

Result: Based on the lower purity, the mean daily compound intake values were 4% less (94.5% used for diet preparations versus 90.6%) than originally reported. This change, however, did not affect the results or the conclusion of the study.

10). Study Title: Teratogenicity Study of IN V9360-27 in the Rabbit.

Study Identification: MRID NO. 413601-21; Haskell No.16925; HLR No. 694-88; Med.Res.No. 8401-001

Addendum: Test material purity: Original-97.4%; Reanalysis-90.6%

Result: (i) Using a purity of 90.6%, the recalculated dose levels are:

	Daily Dose (mg/kg)*	
Group	Reported	Amended
1	0	0
2	100	93
3	500	465
4	1000	930
5	2000	1860

* administered by gavage on Days 7-19 of gestation, at a dosing volume of 10 mL/kg.

(ii) The no-observed-adverse-effect-level (NOAEL) was lowered from 100 to 93 mg/kg/day for the dams and from 500 to 465 mg/kg/day for the conceptus.

Other than decreasing the doses administered and reducing the NOAELs, the change in the purity had no effect on the conclusion of the study.

9V5

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

Study Identification: MRID No. 413601-22; Haskell No.16925; HLR No. 611-88; Med.Res.No. 8400-001

Addendum: Test material purity: Original-97.4%; Reanalysis-90.6%

Result: (i) Using a purity of 90.6%, the recalculated dose levels are:

	Daily Dose (mg/kg)*	
Group	Reported	Amended
1	0	0
2	200	186
3	1000	930
4	2500	2325
5	6000	5581

* administered by gavage on Days 7-16 of gestation, at a dosing volume of 20 mL/kg

(ii) Theno-observed-adverse-effect-level (NOAEL) was lowered from 6000 to 5581 mg/kg/day.

Other than decreasing the doses administered and reducing the NOAEL, the change in the purity had no effect on the conclusion of the study.

12). Study Title: Mutagenicity Evaluation of IN V9360-27 in the CHO/HPRT Assay.

Study Identification: MRID No. 413601-23; Haskell No.16925-02; HLR No.429-88; Med.Res.No. 4581-554

Addendum: Test material purity: Original- 97.4%; Reanalysis-90.6%

Result: The change in purity had no affect on the results or the conclusion of this study since the test results were based on nominal concentration, not corrected for sample purity.

93 16

13). Study Title: Mutagenicity Testing of IN V9360-7 in the Salmonella typhimurium plate incorporation assay.

Study Identification: MRID No. 413601-24; Haskell No.16606; HLR No. 734-88; Med.Res.No. 4581-469

Addendum: Test material purity: Original- 94.9%; Reanalysis- 90.4%

Result: The change in purity had no affect on the results or the conclusion of this study since the test results were based on nominal concentration, not corrected for sample purity.

14). Study Title: Assessment of IN V9360-27 in the in vitro Unscheduled DNA Synthesis Assay in the Rat Primary Hepatocytes.

Study Identification: MRID No. 413601-25; Haskell No.16925-02; HLR NO. 302-88; Med.Res.No. 4581-554

Addendum: Test material purity: Original-97.4%; Reanalysis-90.6%

Result: The change in purity had no affect on the results or the conclusion of this study since the test results were based on nominal concentration, not corrected for sample purity.

15). Study Title: Mouse Bone Marrow Micronucleus Assay of IN V9360-27.

Study Identification: MRID No. 413601-26; Haskell No.16925-02; HLR No. 428-88; Med.Res.No. 4581-554

Addendum: Test material purity: Original- 97.4%; Reanalysis-90.6%

Result: The change in purity had no affect on the results or the conclusion of this study since the test results were based on nominal concentration, not corrected for sample purity.

16). Study Title: In vitro Evaluation of IN V9360-27 for Chromosome Aberrations in Human Lymphocytes.

Study Identification: MRID No. 413680-01; Haskell No.16925-02; HLR NO. 470-88; Med.Res.No. 4581-554

Addendum: Test material purity: Original- 97.4%; Reanalysis-90.6%

Result: The change in purity had no affect on the results or the conclusion of this study since the test results were based on nominal concentration, not corrected for sample purity.

95 8