

(8-31-95)

Reviewed by: Melba S. Morrow, D.V.M.  
Section II, Tox. Branch I (H7509C)  
Secondary Reviewer: Joycelyn E. Stewart, Ph.D.  
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity Study in Rabbits

GUIDELINE #: 83-3

CHEM. #: 099101 DPBarcode: D219996

MRID #: 43788301 Submission #: S495160

TEST MATERIAL: DPXT1991-529 (Benomyl) 97.4%

SYNONYMS: Carbamic Acid, methyl ester

STUDY NUMBERS: 164-95

SPONSOR: E.I. duPont Agriculture Products  
Wilmington, Delaware

TESTING FACILITY: Haskell Laboratories

TITLE OF REPORT: Developmental Toxicity Study DPXT1991-529

AUTHORS: Susan Munley

REPORT ISSUED: August 31, 1995

**EXECUTIVE SUMMARY:**

When DPXT-1991-529 (benomyl) was administered by gavage to pregnant does (20/group) at dose levels of 0, 15, 30, 90 or 180 mg/kg on days 7 to 28 of gestation, the compound was associated with a significant increase in the incidence of small renal papillae in the fetuses from the highest dose tested. In maternal animals, clinical signs of toxicity (stained tails and reduced feed consumption) were also present at the high dose, only. The NOEL for both maternal and developmental toxicity was 90 mg/kg as determined by the registrant; however, it is the opinion of this reviewer that the NOEL for developmental toxicity is 180 mg/kg. This is based on the fact that the biological significance of small renal papillae (in the absence of other renal developmental alterations) is unclear. Furthermore, no dose response was present for this observation.

The incidence of stained tails was statistically significant and could possibly be indicative of maternal toxicity at this high dose level. However the findings in the offspring were not

readily attributable to the administration of the test material. Small papillae were present in 4 control fetuses and involved one litter. Only the litter incidence was increased by one when doses of 0 and 180 mg/kg are compared. The small renal papillae that were discussed are considered visceral variations and not malformations and may have occurred as a result of incomplete maturation.

The study is acceptable and satisfies the requirement (83-3) for a developmental toxicity study in rabbits, in spite of the fact that the dosing interval exceeded that which is recommended in the current Guidelines.

#### **MATERIALS:**

The test material was DPXT-1991-529, a 97.4% pure white solid that was formulated in 0.5% methyl cellulose. The test animals were 100 Hra(NZW)SPF female rabbits. The animals were nulliparous and were time mated upon receipt.

#### **METHODS:**

Animals were individually housed under conditions that provided a 12 hour light and dark cycle an ambient temperature of 20 degrees C and a relative humidity of 50 ± 10%. Water was provided ad libitum and 150 grams of Rabbit Chow were provided daily.

Animals were randomly assigned to one of the following dose groups and were designated to receive the test or control material by gavage on days 7 through 28 of gestation.

Group	Dose (mg/kg)	Conc. (mg/mL)	# does
I	0	0.0	20
II	15	7.5	20
III	30	15.0	20
IV	90	45.0	20
V	180	90.0	20

Control animals received the methyl cellulose vehicle. Test material and vehicle were administered at a volume of 2 mL/kg. Samples of the dosing solution were analyzed for verification of concentration, homogeneity and stability at three times during the study. The samples were collected on January 31, February 9 and February 23, 1995.

#### **DOSE SELECTION:**

The doses selected for this study were based on the results of earlier developmental studies with both carbendazim and benomyl. In one study carbendazim was administered by gavage to rabbits on days 7 -19 of gestation. The doses in this study ranged from 0 to 125 mg/kg. The maternal NOEL was 20 mg/kg based on the presence of abortions and decreases in body weight and food

intake; the developmental NOEL was 10 mg/kg based on decreased implantations, increased resorptions and a reduction in the number of live fetuses.

In another developmental toxicity study in which carbendazim was administered by gavage to rats on gestation days 7 thru 16, the maternal NOEL was 20 mg/kg based on decreases in weight and food consumption at the next highest dose of 90 mg/kg. The developmental NOEL was 10 mg/kg based on reduced fetal weights and increased variations. The percentages of weight gain and food consumption and the description of the fetal variations were not provided in the report.

Two developmental toxicity studies were conducted in rats with benomyl. In both studies the developmental NOEL was 30 mg/kg and the maternal NOEL was 125 mg/kg.

#### **OBSERVATIONS:**

All animals were observed daily for morbidity, mortality and clinical signs. Body weights were recorded on day 4 and on days 7 thru 29 of gestation. Food consumption was recorded for intervals that encompassed the period of days 4 thru 29 of gestation.

On day 29, all does were euthanised with an injection of Euthanasia 5 solution. Does were examined grossly for changes in the thoracic and abdominal cavities. The uteri were removed, weighed and opened and the contents (implants, fetuses) were examined. Each uterus was emptied and re-weighed and stained with ammonium sulfide to detect early resorptions.

Live fetuses were weighed, sexed and examined. All fetuses were injected with Sodium pentobarbitol and examined for visceral alterations. Fetuses were then fixed in ethanol, macerated in potassium hydroxide and stained with alizarin red and finally examined for skeletal abnormalities. To identify stunted fetuses, for each litter the maximum stunted weight was calculated by subtracting the lightest weight from the total weight, dividing the remaining number of fetuses, and multiplying by 0.666.

#### **QUALITY ASSURANCE:**

Statements of Quality Assurance and statements of compliance with GLPs are provided in the submission along with the data confidentiality statement.

#### **STATISTICS:**

ANOVA was performed on maternal weight and food consumption parameters, Jonkheere's test was applied to fetal data, resorptions, nidations, corpora lutea and fetal alterations; Cochran Armitage analysis was used to analyze pregnancy

incidence, clinical signs, maternal mortality, abortions, number of females with resorptions and early deliveries. The ANCOVA was used to evaluate fetal weights and sex ratios. The level of significance for all evaluations was  $p < 0.05$

**RESULTS:**

Concentration, Stability and Homogeneity

An analysis of the suspension by spectrophotometry revealed that the concentration was within  $\pm 18\%$  of nominal at most sampling intervals and that the suspension was homogeneous. Stability at room temperature was demonstrated for 5 hours. (See Table I below).

TABLE I

Sample site	Stability and Concentration		% Nominal
	Concentration (mg/mL)		
	Nominal	Measured	
Low Dose			
Top	7.5	7.98	106
Middle	7.5	8.28	110
Bottom	7.5	8.58	114
Mid Dose			
Top	15.0	17.4	116
Middle	15.0	17.6	117
Bottom	15.0	18.2	121
Mid Dose			
Top	45.0	45.6	101
Middle	45.0	46.4	103
Bottom	45.0	47.7	106
High Dose			
Top	90.0	103.0	114
Middle	90.0	101.0	112
Bottom	90.0	97.2	108
Stability After 5 hours at Room Temperature			
	7.5	8.2	109
	15.0	16.7	111
	45.0	48.0	107
	90.0	91.8	102

Developmental and Maternal Toxicity

No compound related mortality except that resulting from dosing trauma was reported. The deaths due to this were reported in control (1), 30 mg/kg (2), 90 mg/kg (1) and 180 mg/kg groups (2). In addition to these deaths, one animal in the 30 mg/kg group was sacrificed in extremis due to complications from gavage trauma.

There were no reported effects on maternal body weight, body weight change or adjusted body weight (See Table II).

Table II  
Maternal Weight Gain (g)

Dose Level (mg/kg)	No.	Corrected weight gain* During dosing period
0	18	-82.2
15	19	-128.3
30	17	-110.8
90	17	-88.6
180	15	-94.4

\* Body weight gain minus the gravid uterine weight at study termination.

Data taken from Table I of the study report.

Maternal food consumption was reduced at 180 mg/kg on days 7 to 13 and on days 25 to 27 of gestation. Clinically, stained tails were reported to be significantly increased in the high dose dams (6/20) when compared to controls (1/20). Although abortions were reported there was no compound related increase in the incidence. No effects were reported on pregnancy rate, resorptions, the number of corpora lutea or the number of implants.

Fetal results indicated that there was a greater number of resorptions in the group receiving 180 mg/kg, but the incidence was not considered to be significant. The compound had no effects on fetal mortality, fetal body weight or the number and type of malformations.

At 180 mg/kg, there was a significant increase in the incidence of small renal papillae. Sternebral ossification was reported to be significantly increased for all groups; however, the author states that control incidence of this variation was lower in this study than that reported for the historical controls.

Dose Levels (mg/kg)	Table III Developmental Data				
	0	15	30	90	180
Endpoints					
# mated	20	20	20	20	20
# pregnant	20	20	20	19	19
# aborted	1	1	0	1	2
# killed (gavage)	1	0	3	1	2
Mean CL	9.1	9.8	8.9	9.0	9.3
# implants (mean)	8.8	9.6	8.6	8.4	8.9
total resorptions	0.1	0.4	0.4	0.4	0.3
dead fetuses (mean)	0.0	0.1	0.0	0.0	0.0
Live fetuses (mean)					
Total	8.7	9.2	8.2	7.9	8.7
Males	4.6	4.9	3.8	3.7	4.5
Females	4.1	4.3	4.4	4.2	4.1
Fetal weight (g)	39.03	36.79	38.26	37.95	36.97

TABLE IV  
SELECTED FETAL VARIATIONS

Dose group	0	15	30	90	180
<u>Visceral</u>					
Kidney					
Small papilla					
Size 1	4 (1)	1 (1)	2 (1) <sup>a</sup>	--	--
Size 2	--	--	1 (1)	--	2 (2)*

Skeletal

Sternebral

Retarded Ossif. 7(5) 26(10)\* 19(11)\* 20(7)\* 20(8)\*

a = all animals were from same litter for this dose group.

\* p < 0.05, () = litter incidence

**DISCUSSION:**

Based on the results of this study, the maternal and developmental NOEL is 90 mg/kg according to the study author. This is based on clinical signs of stained tails and decreased food consumption in maternal animals and the increased incidence of renal papillae in the fetuses, both occurring at 180 mg/kg.

While the incidence of stained tails was statistically significant and could possibly be indicative of maternal toxicity at this high dose level, the biological significance of small renal papillae (in the absence of other urinary tract developmental alterations) that affected a total of two fetuses and involved two litters at the highest dose tested, is unclear. Small papillae were present in 4 control fetuses and involved one

litter. Only the litter incidence was increased by one when doses of 0 and 180 mg/kg are compared. The small renal papillae that were discussed are considered visceral variations and not malformations and may have occurred as a result of incomplete maturation.

The fetal and litter incidence of retarded ossification was significantly increased for all groups receiving the test substance; however, this finding does not appear to be treatment related because there is no dose response. Furthermore, the registrant states that the control incidence for this variation is below the historical control incidence. Historical control data were not provided in this submission; however, ranges for sternbral ossification were reported from control groups from the test facility (3 to 41 fetuses affected and 1 to 8 litters affected).

It is the opinion of this reviewer that the NOEL for developmental toxicity could be increased to 180 mg/kg.

Based on the results, benomyl is not considered a reproductive toxicant under the conditions of this study. The study satisfies the requirement for a developmental toxicity study.

Sign-off date: 09/20/96  
DP Barcode: d219996  
HED DOC Number: 012058  
Toxicology Branch: tb1