US EPA ARCHIVE DOCUMENT

UNDATED

REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Substance Name: Benomyl
CASRN: 17804-35-2

The Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis, but may not exist for other toxic effects such as carcinogenicity. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Oral RfD Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of compounds which are also carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in the Carcinogenicity Assessment Section of this file when a review of that evaluation is completed.

RfD ASSESSMENT SUMMARY TABLE

Crit. Dose: 5 mg/kg-day [Study 1 NOAEL(adj)]
UF: 100 MF: 1 RfD: 5E-2 mg/kg-day Confidence: High

Crit Effect: (1) Decreased pup weanling weights

	NOAEL (Study 1)	LOAEL (Study 1)
Reported	100 ppm diet	500 ppm
ADJ	5 mg/kg-day	25 mg/kg-day
Study Type	3-Generation Reproduction Rat Study	3-Generation Reproduction Rat Study
Reference	duPont, 1968a	duPont, 1968a

1) duPont, 1968a
3-Generation Reproduction Rat Study

Critical Effect: Decreased pup weanling weights

Defined Dose Levels:
NOAEL= 100 ppm diet
NOAEL(ADJ)= 5 mg/kg-day
LOAEL= 500 ppm
LOAEL(ADJ)= 25 mg/kg-day

Conversion Factors: 1 ppm = 0.05 mg/kg/day (assumed rat food consumption)

DISCUSSION OF PRINCIPAL AND SUPPORTING STUDIES

E.I. duPont de Nemours and Co., 1968a. MRID No. 00066773 Available from EPA. Write to FOI, EPA, Washington D.C. 20460.

Benomyl, 50 or 70% wettable powder (dose based on % active ingredient), was administered in the diet at 0, 100, 500, and 2500 ppm (0, 5, 25, and 125

(1)

mg/kg/day) to male and female ChR-CD rats for 3 generations (7 litters). Six males and females were mated for the first generation, 12 males and females for the second generation, and 20 males and females for the third generation. Histology was performed on F3bn weanlings. F3c pups were used for a post weaning growth curve study. No treatment-related effects were seen with the exception of pup weanling weights in F2b, F3b, and F3c litters at 500 and 2500 ppm as compared with control values. The NOEL was 100 ppm (5 mg/kg/day) and the LEL, based on decreased pup weanling weights, was 500 ppm (25 mg/kg/day).

UNCERTAINTY AND MODIFYING FACTORS

UNCERTAINTY FACTORS:

A UF of 100 includes uncertainties in extrapolation from laboratory animals to humans. The extrapolation from the teratology data was considered to be sufficiently covered by this UF, since the NOEL for teratogenic effects is 30 mg/kg/day, that is, 6 times higher than the NOEL of 5 mg/kg/day used to establish the RfD. Thus, there is an overall 600-fold margin between the teratogenic NOEL and the RfD.

ADDITIONAL COMMENTS / STUDIES

Data Considered for Establishing the RfD

1) 3-Generation Reproduction - rat: Principal study - see previous description; core grade minimum

2) 2-Year Feeding (oncogenic) - rat: Systemic NOEL=2500 ppm (125 mg/kg/day) (HDT); core grade minimum (E.I. du Pont de Nemours and Co., 1969)

3) 2-Year Feeding - dog: NOEL=500 ppm (12.5 mg/kg/day); LEL=2500 ppm (62.5 mg/kg/day) (biochemical alterations, hepatic cirrhosis, decreased weight gain and lower food consumption); core grade minimum (E.I. du Pont de Nemours and Co., 1970)

4) Teratology - rat: NOEL=30 mg/kg/day; LEL=62.5 mg/kg/day (microphthalmia); core grade minimum (E.I. du Pont de Nemours and Co., 1982)

5) Teratology - rat: Fetotoxic NOEL=30 mg/kg/day; Fetotoxic LEL=62.5 mg/kg/day (decreased fetal weight); Maternal NOEL=125 mg/kg/day (HDT); core grade minimum (E.I. du Pont de Nemours and Co., 1980a)

6) Teratology - mouse: Teratogenic NOEL=50 mg/kg/day; Teratogenic LEL=100 mg/kg/day (supra occipital scars, subnormal vertebral centrum, supernumary ribs, cleft palate); core grade minimum (Kavlock et al., 1982)

Other Data Reviewed:

1) Ocogenic - mice: CD-1 mice were given 0, 500, 1500, and 7500 (reduced to 5000 ppm after 37 weeks) ppm (0, 75, 225, 1125/750 mg/kg/day). High dose males had microscopic evidence of hepatocellular and testicular (and epididymal) degeneration; core grade minimum (E.I. du Pont de Nemours and Co., 1980b)

2) 90-Day Feeding - rat: NOEL=500 ppm (25 mg/kg/day); LEL=2500 ppm (125

REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

mg/kg/day) (increased relative and absolute liver weight in females and increased SGPT values in males); core grade minimum (E.I. du Pont de Nemours and Co., 1967)

3) 90-Day Feeding - dog: NOEL=500 ppm (12.5 mg/kg/day); LEL=2500 ppm (62.5 mg/kg/day) (depressed albumon/globulin, A/G ratio, and increased SGPT in males); core grade minimum (E.I. du Pont de Nemours and Co., 1968b)

Data Gap(s): None

CONFIDENCE IN THE RfD

Study: Medium Data Base: High RfD: High

The principal study is of adequate quality and is therefore given medium confidence. Since additional studies are of adequate quality, the data base is given high confidence. High confidence in the RfD follows.

EPA DOCUMENTATION AND REVIEW

Source Document: This assessment is not presented in any existing U.S. EPA document.

Other EPA Documention: Pesticide Registration Standard April, 1986; Special Review Position Document; Pesticide Registration Files

Agency Work Group Review: 03/26/86

Verification Date: 03/26/86

EPA CONTACTS

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E.I. du Pont de Nemours & Company. 1980a. EPA Accession No. 256575. Available from EPA. Write to FOI, EPA, Washington D.C. 20460.

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E.I. du Pont de Nemours & Company. 1982. MRID No. 00115674. Available from EPA. Write to FOI, EPA, Washington D.C. 20460.

Kavlock, R.J., N. Chernoff, L.E. Gray, Jr., J.A. Gray and D. Whitehouse. 1982. Teratogenic effects of benomyl in the Wistar rat and CD-1 mouse, with emphasis on the route of administration. Toxicol. Appl. Pharmacol. 62: 44-54.

REVISION HISTORY

03/88 RfD Confid: Confidence levels revised
03/89 RfD Data: Major text revisions

CHEMICAL: BENOMYL

UNDATED

DER #	STUDY TYPE - DOSE LEVELS	NOEL (mg/kg/day)	LEL (mg/kg/yay)
1	2-YR FEED/CARCINOGEN RAT (1972) 0, 100, 500, 2500 & 5000 ppm 0, 5, 25, 125 & 250 mg/kg/day	25 [Acceptable]	125
	The dietary levels were increased in the 2500 ppm group to a level of 10,000 ppm from weeks 20 to study termination at week 104.		
2	2-YR FEED/CARCINOGEN RAT (1969) 0, 100, 500 & 2500 ppm 0, 5, 25, & 125 mg/kg/day	125 [Acceptable]	---
3	2-YR FEED/CARCINO MOUSE (1982) 0, 500, 1500 & 7500/5000 ppm 0, 75, 225 & 1125/750 mg/kg/day	See DER [Acceptable]	
	The 7500 ppm dose level was reduced to 5000 ppm after week 37.		
4	2-YR FEEDING DOG (1970) [Benomyl] 0, 100, 500 & 2500 ppm (50% ai) 0, 2.5, 12.5 & 62.5 mg/kg/day	12.5 [Core grade Minimum]	62.5
5	2-YR FEEDING DOG (1972) [MBC, Carbendazim] 0, 100, 500 & 2500 ppm (53% ai) 0, 2.5, 12.5 & 62.5 mg/kg/day	2.5 [Acceptable]	12.5
6	2-GEN REPRODUCTION RAT (1991) 0, 100, 500, 3000 & 10,000 ppm P1 M: 0, 5, 28, 168 & 553 mg/kg P1 F: 0, 7, 35, 210 & 712 mg/kg F1 M: 0, 8, 38, 234 & 954 mg/kg F1 F: 0, 9, 47, 280 & 1168 mg/kg/day	28 (M - Reproduct) 35 (F - Reproduct) [Core grade Guideline]	168 (M - Reproduct) 210 (F - Reproduct)

CHEMICAL: BENOMYL

DER #	STUDY TYPE - DOSE LEVELS	NOEL (mg/kg/day)	LEL (mg/kg/yay)
7	REPRODUCTION RAT (1972) 0, 100, 500, 2500 & 5000 ppm 0, 5, 25, 125 & 250 mg/kg/day Rats from the 1972 2-Year Feeding Study were used for this study.* The dietary levels were increased in the 2500 ppm group to a level of 10,000 ppm from weeks 20 to study termination at week 104.	25 [Core grade Minimum]	250
8	3-GEN REPRODUCTION RAT (1968) 0, 100, 500 & 2500 ppm 0, 5, 25 and 125 mg/kg/day	5 [Core grade Minimum]	25
9	DEVELOPMENTAL TOX RAT (1980) 0, 3, 10, 30, 62.5 & 125 mg/kg	125 (Maternal) 30 (Developmental) [Core grade Minimum when considered with DER #10]	--- (Maternal) 62.5 (Developmental)
10	DEVELOPMENTAL TOX RAT (1982) 0, 3, 6.25, 10, 20, 30 & 62.5 mg/kg/day This study was conducted to evaluate a specific effect on the development of eyes in fetal rats.	30 [Core grade Minimum when considered with DER #9]	62.5
11	DEVELOPMENTAL TOX RABBIT (1995) 0, 15, 30, 90 & 180 mg/kg/day	90 (Maternal) 180 (Developmental) [Acceptable]	180 (Maternal) --- (Developmental)
12	DEVELOPMENTAL TOX RABBIT (1968) 0, 100 & 500 ppm (dietary)	500 [Core grade Supplementary]	
13	90-DAY FEEDING RAT (1967) 0, 100, 500 & 2500 ppm M: 0, 9, 45 & 214 mg/kg/day F: 0, 9, 46 & 234 mg/kg/day	45 (Male) 46 (Female) [Core grade Minimum]	214 (Male) 234 (Female)

CHEMICAL: BENOMYL

DER #	STUDY TYPE - DOSE LEVELS	NOEL (mg/kg/day)	LEL (mg/kg/yay)
14	90-DAY FEEDING DOG (MBC) (1970) 0, 100, 500 & 1500 ppm M: 0, 2.7, 14.4 & 40.7 mg/kg F: 0, 2.7, 11.3 & 35 mg/kg/day	14.4 (Male) 11.3 (Female) [Core grade Minimum]	40.7 (Male) 35 (Female)
	The HDT group was gradually given increasing amounts of MBC using the following schedule: 500 ppm (3 days); 1000 ppm (2 days); 1500 ppm (2 days); 2500 ppm for a short time before the dose was lowered to 1500 ppm (week 3) due to decreased food consumption and weight loss.		
15	28-DAY FEEDING MOUSE (1970) 0, 100, 500, 3750 & 7500 ppm 0, 15.7, 85.4, 586 & 1180 mg/kg	85.4 [Core grade Supplementary]	586
16	ACUTE NEUROTOXICITY RAT (1993) 0, 500, 1000 & 2000 mg/kg	2000 [Acceptable]	---
17	95-DAY NEUROTOXICITY RAT (1994) 0, 100, 2500 & 7500 ppm M: 0, 6, 158 & 456 mg/kg/day F: 0, 8, 199 & 578 mg/kg/day	158 (Male) 199 (Female) [Core grade Guideline]	456 (Male) 578 (Female)

Benomyl: 2-Year Feeding/Carcinogenicity Study in Rats
E. I. du Pont de Nemours & Company. 1972. MRID No. 00088333.
HED Doc. No. 000721, 004678, 004679, ?.

Reviewer: Melba S. Morrow, D.V.M. MSM 10/30/90
Secondary reviewer: Joycelyn E. Stewart, Ph.D. JS 10/30/90

Study Type: Two year feeding study - rat

Tox. Chem.: 79C

Chemical: 2-benzimidazole carbamic acid, methyl ester

Synonyms: MBC, INE-965, Carbendazim

Haskell Lab Report Nos.: 195-72

Sponsor: E.I. duPont

Study Performed at: Haskell Lab for Toxicology and Industrial Medicine
Wilmington, Delaware

Authors: H. Sherman, S.B. Fritz, L.S. Wasileski

Date: 1972

Addendum to ~~Study Report~~: DER

~~Executive Summary - Benomyl - Chronic/Oncogenicity, Rat~~

MBC (methyl ester, 53 or 72.2%) was administered in the diets of male and female CRL: CD1 rats at dietary levels of 0, 100, 500, 2500 or 5000 ppm (equivalent to 0, 50, 250, 1250 or 2500 mg/kg/day). The dietary levels were increased in the 2500 ppm group to a level of 10,000 ppm from weeks 20 to study termination at week 104.

There were no apparent treatment related signs of toxicity nor were there any effects on mortality, food consumption or feed efficiency.

In females in the highest dose group, there was a decrease in body weight gain when compared to controls at both 15 (14% lower) and 24 months (24% lower).

At 2500 and 500 ppm, there were statistically significant decreases in red blood cell counts, hemoglobin and hematocrit values reported in females at 24 months. Transient, non-significant elevations in SGPT were reported in males and females receiving 10000 ppm at 12 months, but these findings were not apparent at 24 months and were therefore not considered to be related to the administration of benomyl.

In males and females receiving 2500 ppm and greater levels of benomyl, there was an increase in the incidence and severity of cholangiohepatitis and pericholangitis. There was no evidence of carcinogenicity reported in this study.

The NOEL in this study was 500 ppm, based on statistically significant decreases in red blood cell parameters and histological lesions in the liver (cholangiohepatitis and pericholangitis) at both 2500 ppm and 5000 ppm.

The study is acceptable and satisfies the requirement for a chronic/onco study in rats (83-5), although deficiencies were cited in an earlier review. Based on the observed decreases in body weight at the highest dose tested and the observations of liver lesions and decreases in hematology measurements, the study appears to have been conducted at adequate dose levels.

* It is noted that the microfische did not produce legible copies of the report such that tables could be readily reconstructed.

004679

STUDY TYPE: Two-year Feeding study-Rat

TOX. CHEM. NO.: 79C

HASKELL LAB. REPORT NO: 195-72
MR NO.: 1149

FICHE/MASTER: 00088333
ACCESSION NO.: 232870-C
232871

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

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

AUTHORS: H.Sherman, S.B.Fritz, L.S.Wasileski

DATE REPORT SUBMITTED: 1972

TEST MATERIAL: 2-benzimidazolecarbamic acid, methyl ester (50 or 70 % a.i., 53 or 72.2 % Tech.) (metabolite of Benomyl)

SYNONYMS: MBC
INE-965

MATERIAL AND METHODS: Male and female albino Charles River-CD strain rats were housed in pairs (by sex) and given food and water ad libitum. After a 12 day observation period (at 33 days of age) healthy rats were divided into groups based on equal average weight. The test compound was then added to the diet by the following scheme for either 1 or 2 years (see necropsy method):

Group	no. male	no. female	PPM (%a.i.)
group 1	36	36	0 (0)
group 1a	36	36	0 (0)
group II	36	36	100 (.01%)
group III	36	36	500 (.05%)
group IV	36	36	*2500 (.25%) - 10,000 (1.0 %)
group V	20	20	**5000 (.50%)

* level raised to .75% at 18 weeks and again to 1.0% two weeks later
** started treatment 3 weeks later (33 weeks of age) without preliminary hematology

Observations: Animals were observed and examined regularly (interval not specified) for behavioral and toxicological abnormalities.
Food Consumption and Weight: Animals were weighed once/week for 12 months than twice/month for the remainder of the study. Food consumption was monitored for the same intervals by sex and group (except group V).
Laboratory Studies: Hematology - Ten randomly selected rats/sex from groups I, Ia, and IV were tested at pretest (6/sex/group), 1, 3, 6, 9, 12, 18 and 24 months for hematocrit (HCT), hemoglobin (Hg), RBC count, WBC count and WBC differential count. Group V was tested at 18 and 24 months. Urinalysis (UA) - Urine was collected over a 24 hour period from the animals used for hematology (no pretest UA) and examined with respect to the following: protein, sugar, blood, pH, volume, solute concentration (mosmoles/l), color, appearance and microscopic abnormalities. Biochemistry-Ten randomly picked male and female rats from groups I, Ia and IV were tested/

† used for the first 8 weeks of the study
†† used for the remainder of the feeding study

after 1, 3, 6, 9, 12, 18 and 24 months for plasma alkaline phosphatase (AP) and serum glutamic-pyruvic transaminase (GPT). Group V was also tested at 12, 18 and 24 months.
Necropsy: There was an interm 1 year sacrifice with gross and microscopic pathologic examination reducing each sex/group to 30 animals. After 2 years, the surviving rats were also sacrificed. Animals that died or were sacrificed at other than the scheduled times were necropsied and tissues saved for histology when possible. Tissues were fixed in Bouin's solution, stained with hematoxylin-eosin and examined microscopically. All listed tissues from controls and group IV (12 and 24 months) were examined, while only the liver from groups II and III and liver, kidney and testes from group V were evaluated at 24 months.

†brain	†liver	thoracic aorta
†heart	†pituitary	thymus
†kidney	epididymis	bone marrow
†adrenal	lymph node	lumber spinal cord
ovaries	peripheral nerve	trachea
†stomach	Fallopian tube	†lung
eye	†spleen	pancreas
skeletal muscle	thyroid, parathyroid	duodenum
urinary bladder	prostate	cecum
salivary gland	††testes	colon
exorbital lacrimal gland	uterus	all masses and abnormal tissues

†organ weights, all groups, at 12 and 24 month sacrifice

RESULTS: Clinical Signs: There were no treatment related signs of toxicity noted in the study however individual animal data was not present to support conclusion.
Mortality: Mortality rate and mean age of death were not treatment related.
Body Weight Gain: There was a decrease in weight gain evident from 15 months until the end of the study in group V females and group IV males and females.

	weight as % of controls	
Group	15 months	24 months
V (0.5%) females	86	76
IV (1.0%) females	93	87
IV (1.0) males	94	84

Food Consumption and Efficiency: There were no treatment related differences between control and treatment groups for food consumption and efficiency.
Dose: Group II and III males and females received approximately the same dose (mg/kg) of MBC. Group IV females however received more MBC (mg/kg) than the males until day 644 at which time they received approximately the same level.

Laboratory Studies: Hematology -

twenty-four month values

Group	males			females		
	RBC	HGB	HCT	RBC	HGB	HCT
I (0%)	3.68	13.8	40	6.31	15.0	42
III (.05%)	3.40	13.2	40	6.33	14.1	39
V (0.5%)	3.14	13.2	38	5.23*	12.0*	36*
IV (1.0%)	2.92	12.1	36	5.17*	12.0*	36*

*significantly lower than controls - $p < 0.05$

At 18 months the HCT and HGB in group IV females were lower than controls Statistical significance was not mentioned. However, by 24 months the HCT, HGB and RBC in groups IV and V were significantly ($p < .05$) lower than controls. Males also had a decreased (not significant) RBC, HGB and HCT. Urinalysis - there were no treatment related abnormalities. Biochemistry - The registrant reported increased AP activity for both males and females at 6, 9 and 12 months in the 1.0% treatment group (12 month females $p < .05$). At 9 months the SGPT was elevated significantly for both males and females in the 1.0% group and at 12 months for the females.

SUMMARY OF BIOCHEMICAL MEASUREMENTS MADE ON RATS FED INE-965 FOR TWO YEARS

MALE

	% INE-965 in Diet	MONTHS ON TEST 1	3	6	9	12	18	24
Alkaline Phosphatase Bessey Units[1]	0	7.8	3.7	3.8	3.3	4.5	6.5	9.2
	0	7.2	3.7	3.1	3.1	4.5	8.8	-
	0.05	--	-	-	-	4.0	9.6	9.4
	1.0	6.9	3.9	4.7	4.1	4.8	8.3	8.8
	0.50	-	-	-	-	5.0	9.5	7.7
Transaminase Units	0	22	23	39	29	31*	26	27
	0	26	24	31	39	29*	42	-
	0.05	-	-	-	-	37	37	19
	1.0	29	26	31	54	31	57	27
	0.50	-	-	-	-	31	42	13

FEMALE

	% INE-965 in Diet	MONTHS ON TEST 1	3	6	9	12	18	24
Alkaline Phosphatase Bessey Units[1]	0	5.8	4.4	3.6	3.5	4.0	6.7	9.2
	0	5.7	3.4	3.3	3.2	3.7	5.6	-
	0.05	-	-	-	-	4.5	5.5	7.4
	1.0	6.8	3.7	4.0	4.0	6.1	7.1	10.4
	0.50	-	-	-	-	4.7	7.0	7.3
Transaminase Units	0	24	28	26	39	18*	47	40
	0	27	23	23	43	33*	60	-
	0.05	-	-	-	-	39	57	33
	1.0	24	30	29	81	49	62	45
	0.50	-	-	-	-	61	83	34

1) One through 12 months, units/ml blood; 18 and 24 months, units/ml plasma.

* Five animals/group examined.

Pathology: Organ weights - There was no apparant treatment related change in organ weights at either sacrifice time. Gross - Observations at necropsy were not reported in this study. Microscopic - There was a slight increase in incidence and severity of cholangiohepatitis and pericholangitis in the liver noted in group IV and V males and females. Prostatitis also appeared slightly increased in group IV males (21% incidence as compared to 11% in the controls). Intermediate group rats were not examined histologically for this lesion. There were no other treatment related lesions.

DISCUSSION: There appeared to be no treatment related change in clinical signs and mortality, however 2500 (10,000) ppm females and 5000 ppm males and females gained less weight than control groups. There was no related decrease in food consumption or feed efficiency at these levels. MBC appeared to affect the HGB, HCT and RBC in females at 2500 (10,000) and 5000 ppm and to a lesser extent, HGB and HCT in the high dose males. Although the registrant reported significant increases in AP and GPT in the 5000 ppm group these may not be biologically relevant since: 1) many of the values do not appear out of the normal range for these tests, 2) the elevated means appear only sporadically throughout the 2 year study period. No other biochemical tests were performed, limiting the usefulness of this study. Although AP and GPT were the only biochemical parameters tested there is no reason to expect other changes. Observations taken at necropsy were not noted in the report although they were listed as part of the procedure. Increased incidence and severity of "pericholangitis and cholangiohepatitis" although not serious, is a toxic effect of the compound. The significance of increased prostatitis in this study in not known. There may have been a slight increase over controls of pigment deposits in the spleen and bone marrow often observed with decreased hematologic parameters (due to hemolysis) but this was not consistant.

CONCLUSIONS:

NOEL = 500 ppm

LEL = 5000 ppm, based on: 1) decreased weight gain in females, 2) decreased HCT, HGB and RCB in females, 3) increased cholangiohepatitis and pericholangitis in males and females.

CORE-CLASSIFICATION:

Although lack of complete clinical chemistry data exists, sufficient histopathology and organ weight measurements permit a core-classification of minimum.

Review by M.P.Copley, D.V.M.
Tox. Br.
9/19/85

14

Benomyl: 2-Year Feeding/Carcinogenicity Study in Rats
E. I. du Pont de Nemours & Company. 1969. MRID No. 00097284.
HED Doc. No. 004679.

CASWELL FILE
004679

STUDY TYPE: Two-year Feeding/Onco study-Rat TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NO: 232-69(Path.No. 66-77) FICHE/MASTER: 00097284
MR NO.: 966 ACCESSION NO.: 050427-Q

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

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

AUTHORS: H.Sherman, J.R.Barnes, E.F.Stula, G.J.Stopps

DATE REPORT SUBMITTED: Aug. 15, 1969

TEST MATERIAL: Benomyl, 50 or 70% wettable powder; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester; (72.2%† or 51.5-52.0%†† tech.)

SYNONYMS: a.i. Benomyl
Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazol-2-yl)-methyl ester
INT-1991
NB- 5409-91
DPX-3866
N.B. 8084-166B

MATERIAL AND METHODS: Male and female albino Charles River-CD strain rats were housed in pairs (by sex) and given food and water ad libitum. After a nine day observation period, healthy rats were divided into groups based on equal average weights and administered test compound in their diet by the following scheme for either 1 or 2 years (see necropsy method):

Group	No./sex/group	Dose	PPM (%a.i.)
group 1	36	control	0 (0)
group 1a	36	control	0 (0)
group V	36	LDT	100 (.01%)
group VI	36	MDT	500 (.05%)
group VII	36	HDT	2500 (.25%)

Observations: Animals were observed and examined regularly (interval not specified) for behavioral and toxicological abnormalities. Food Consumption and Weight: Animals were weighed once/week for 12 months than twice/month for the remainder of the study. Food consumption was monitored for the same intervals by sex and group. Laboratory Studies: Hematology - Six randomly selected rats/sex/group were tested at pretest, 1, 3, 6, 9, 12, 18 and 24 months for hematocrit (HCT), hemoglobin (Hg), RBC count, WBC count and WBC differential count*. Urinalysis (UA) - Urine was collected over a 24 hour period from the animals used for hematology (no pretest UA) and examined with respect to the following: protein, sugar, blood, pH, ketone bodies, volume, solute concentration (mosmoles/l), color, appearance and microscopic abnormalities. Clinical Chemistry Ten randomly picked male and females rats in the 0, 500 and 2500 ppm groups were tested after 1, 3, 6, 9, 12, 18 and 24 months for plasma alkaline phosphatase. Serum glutamic-pyruvic transaminase (GPT)

† used for the first 8 weeks of the study
†† used for the remainder of the feeding study
* only on controls, .05% and .25% animals

was tested only in the control and HDT groups unless elevated levels were detected.

Necropsy: There was an interm 1 year sacrifice with gross and microscopic pathologic examination reducing each sex/group to 30 animals. After 2 years, the surviving rats were also sacrificed. Tissues were fixed in Bouin's solution, stained with Haskell quadrichrome and examined microscopically. All the listed tissues from the control and HDT (12 and 24 months) rats were examined initially, while only liver, kidney and testes were evaluated in the LDT and MDT at 24 months. Subsequently however, all 24 month tissues were examined histologically and reported in Supplemental Pathology Report No. 66-77.

†brain
†heart
†kidney
†adrenal
ovaries
†stomach
eye
skeletal muscle
urinary bladder
salivary gland
exorbital lacrimal gland
†liver
pituitary
epididymis
lymph node
peripheral nerve
fallopian tube
†spleen
thyroid, parathyroid
prostate
††testes
uterus
thoracic aorta
thymus
bone marrow smea
lumber spinal cc
trachea
†lung
pancreas
duodenum
cecum
colon

†organ weights, all groups, 12 and 24 months

RESULTS: taken from original review of March 25, 1970 by M.Quaife.

Chronic toxicity studies

Rat, 2-year feeding.*

No. of Animals. 36 M and 36 F/group.
Feeding Levels.* 0, 0 (second control group), 100, 500, and 2,500 ppm.
Duration. 2 years.
Mortality. No effect.
Body Weight. No significant effect. (None on food consumption or food efficiency, either.)
General Behavior. No effect. No clinical signs of toxicity attributed to effect of benomyl.
Organ Weight. No effect on weights (either absolute or relative to body weight) of brain, heart, lungs, liver, spleen, kidneys, testes, stomach, adrenals, and pituitary.
Clinical Laboratory Tests. No effect on alkaline phosphatase or serum glutamic-pyruvic transaminase determined in rats of both control

* Test substance was 70% or 50% wettable powder formulated as given, above, for either INT-1991-30 or INT-1991. Dietary levels based on active ingredient.

17

groups and those at 500 or 2,500 ppm at 0, 1, 3, 6, 9, 12, 18, and 24 months on test. No effect on hematologic values (same ones as determined in rat 90-day study at time intervals given in preceding sentence). No effect on results of urinalysis (also done at same time intervals): Volume; solute concentration; levels of sugar, protein, and ketone bodies; color; pH; presence of occult blood; and microscopic appearance of urinary sediment.

Histopathology. Tissues examined histologically, in addition to those listed under "organ weight," above, are: Ovary, epididymus, fallopian tube, prostate, uterus, urinary bladder, duodenum, cecum, colon, skeletal muscle, peripheral nerve, bone marrow, eye, thoracic aorta, lumbar spinal cord, trachea, thymus, pancreas, thyroid, parathyroid, salivary gland, lymph node, and exorbital lacrimal gland. These tissues from control groups and from 2,500-ppm group examined at both 1 and 2 years. At 2 years, liver, kidney, and testis of 100- and 500-ppm groups also studied. No significant findings believed related to intake of test compound, benomyl, were made. Validity of this opinion is verified by K. Davis, DVM, Pathologist (told to M. Quaife on January 20, 1970). We note that only certain tissues of animals in one of the control groups were studied. In male rats of the other control group (IA), there was a very high incidence of pituitary tumors and chronic nephritis (> 85% each); such incidence of pituitary tumors is not matched in the 2,500-ppm male rats. Liver changes were of frequent occurrence but about equally spread between control and test groups. Likewise, for testicular degeneration in male rats.

Neoplasms. No effect.

"No-Effect Level," 2,500 ppm.

Results in the supplementary pathology report with histology for all rats on test indicated no increased incidence of either neoplasti or non-neoplastic lesions.

DISCUSSION: AP and GPT were the only clinical chemistries performed on the rats making it difficult to confirm the NOEL for toxicity. There was however, no reason to expect chemistry changes since there were no compound related organ weights or histopathologic changes in any of the groups tested at either sacrifice time.

CONCLUSIONS:

NOEL chronic feeding > 2500 ppm
NOEL oncogenicity > 2500 ppm

CORE-CLASSIFICATION:

Chronic feeding - minimum
Oncogenicity - supplementary since no MTD was established

Original review evaluated and addendum added by M.P.Copley, D.V.M.
Tox. Br.
9/12/85

18

Benomyl: 2-Year Feeding/Carcinogenicity Study in Mice
E. I. du Pont de Nemours & Company. 1982. MRID No. 00096514, 00151267.
HED Doc. No. 003726, 004679.

Reviewer: Melba S. Morrow, D.V.M. MSM 10/31/96
Secondary reviewer: Joycelyn E. Stewart, Ph.D. JS 10/31/96

Study Type: Oncogenicity_ mouse

Tox. Chem.: 75A

Chemical: 2-benzimidazole carbamic acid, ~~methyl ester~~

Synonyms: Benomyl, INT-1991

Haskell Lab Report Nos.: 20-80

Sponsor: E.I. duPont

Study Performed at: Haskell Lab for Toxicology and Industrial Medicine
Wilmington, Delaware

Title: Long Term Feeding Study with Methyl-1-butylcarbamoyl)-2-benzimidazole carbamate in Mice

Authors: P.W. Schneider, Jr., B.E. Wiechman, T. Dilworth, et.al.

Date: January 26, 1982

Addendum to Study Report 20-80:

Executive Summary - Oncogenicity - Mouse

Benomyl (99%, 99.2%) was administered in the diets of CD-1 mice at levels of 0, 500, 1500 and 7500 ppm (the 7500 ppm dose level was reduced to 5000 ppm after week 37). This is equivalent to 0, 75, 225 or 1125 (750) mg/kg/day. At 500 ppm, there was a significant increase in hepatocellular carcinomas in both males and females. There was also an increase in the combined incidence of adenomas and carcinomas in mid and high dose females. At the highest dose tested, the testes and epididymides showed degenerative changes, which were characterized by degeneration of the seminiferous tubules, atrophy and tubular degeneration.

There was a statistically significant ($p < 0.05$) decrease in body weight reported for both males and females in the highest dose tested. The body weights were approximately 10% lower than controls in both sexes from weeks 13 to study termination at week 104. Sporadic decreases in body weights were also reported at one or two weighing intervals in mice receiving 1500 ppm but did not appear to be statistically or biologically significant.

This study is acceptable and satisfies the requirement for a carcinogenicity study in mice. Based on the reported decreases in body weight and the increase in incidence of liver tumors, the dose selection in this study appears to be adequate.

2

004679

Reviewed by: Dynamac - P. Wennerberg, W. McLellan, I.C. Felkner
Secondary reviewers: Marion P. Copley, D.V.M. MPCopley 6/21/85
Jane Harris, PhD, Section Head JH 6/21/85
Section 6 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity - mice TOX. CHEM. NO.: 75A

ACCESSION NUMBER: 246948A, 246949,246950 MRID NO.: 00096514

TEST MATERIAL: Benomyl

SYNONYMS: Methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate

STUDY NUMBER: Haskell No. 20-80

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

TESTING FACILITY: Haskell Lab. for Toxicology and Industrial Medicine, Newark, Del.

TITLE OF REPORT: Long-term feeding study with Methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate (INT-1991, Benomyl, Benlate®) in mice.

AUTHOR(S): P.W. Schneider, Jr., B.E. Wiechman, T. Dilworth; et al.

REPORT ISSUED: Jan. 26, 1982

CONCLUSION: NOEL for carcinogenicity < 500 ppm (LDT)
Carcinogenic at 500 ppm (LDT):
hepatocellular adenoma and carcinoma in males and female
pulmonary alveologenic carcinomas in males,
Degenerative changes in the testes and epididymides at 5000-7500 ppm (HDT)

Classification: Core-minimum

MATERIALS: Benomyl, 99-99.2% pure, lot #s INT-1991-366, INT-1991-414, grey crystalline material.

SEE ATTACHED REVIEW

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

CASWELL FILE

EPA: 68-01-6561
TASK: 81
June 13, 1985

DATA EVALUATION RECORD

BENOMYL

Oncogenicity in Mice

CITATION: Schneider, P.W., Jr.; Wiechman, B.E.; Dilworth, T.; et al. Long-term feeding study with methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate, (INT-1991, Benomyl, Benlate®) in mice. (Unpublished study, Report No. 20-82 by Haskell Laboratory for E.I. Du Pont De Nemours & Co., Inc., Wilmington, DE; dated January 26, 1982.)

REVIEWED BY:

Paul Wennerberg, D.V.M., M.S.
Project Scientist
Dynamac Corporation

Signature: [signature]
Date: 6-17-85

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APPROVED BY:

Marion Copley, D.V.M., M.S.
EPA Scientist

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Date: 6/17/85

Jane Harris, Ph.D.
EPA Section Head

Signature: [signature]
Date: 6/21/85

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity in mice.

CITATION: Schneider, P.W., Jr.; Wiechman, B.E.; Dilworth, T.; et al. Long-term feeding study with methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate, (INT-1991, Benomyl, Benlate®) in mice. (Unpublished study, Report No. 20-82 by Haskell Laboratory for E.I. Du Pont De Nemours & Co., Inc., Wilmington, DE; dated January 26, 1982.)

ACCESSION NUMBER: 246948-A, 246949, 246950.

MRID NUMBER: 00096514.

LABORATORY: Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, Newark, Delaware 19711.

QUALITY ASSURANCE STATEMENT: Chronological summary present and signed but not dated.

TEST MATERIAL: Methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate was supplied in two lots (INT-1991-366 and INT-1991-414) as a grey crystalline material which was stated to be 99% and 99.2% pure, respectively. It was used to prepare test diets from 8-29-78 to 9-9-80. Throughout the study, INT-1991 was refrigerated until used.

PROCEDURES:

1. Three hundred and twenty male and 320 female 4 week old CD®-1 mice were used from Charles River Breeding Laboratories, Wilmington, Massachusetts. After a thirteen day acclimation period, they were divided using computerized stratification to randomize by sex into four groups of 80 animals per sex, each group having approximately equal mean body weights. The mice were caged individually in stainless steel wire-mesh cages.

2. Diets were freshly prepared each week and stored under refrigeration until used. Ground Purina Laboratory Chow diet was mixed with test compound in corn oil to achieve the following concentrations: 0, 500 ppm, 1,500 ppm, 7,500 ppm. After 37 weeks on the diet, the highest concentration, 7,500 ppm, was reduced to 5,000 ppm. All diets contained 1% (w/w) Mazola® Corn Oil. Throughout the study, all mice received the appropriate test diet and tapwater ad libitum. Samples

of diet containing test material were collected for analysis during the following times: 1) at the time of preparation; 2) after storage at room temperature for 24 hours and 7 days; 3) after storage under refrigeration for 7 days. These samples were collected four times during the study and analyses showed no degradation of test compound. Data for test diet homogeneity were not presented.

3. All mice were examined daily for clinical signs of toxicity and palpated at least once every two weeks for tissue masses. Mice were weighed weekly (weeks 1-26), biweekly (weeks 26-52) and monthly (weeks 52-104). Recorded during the same times were body weight gains, food consumption, food efficiency and intake of test compound. Mortality was also recorded.

4. Ten mice per sex, per group had hematological examinations at intervals of approximately 1, 3, 6, 12, 18, and 24 months after the start of the study. The following parameters were examined: RBC, WBC, and differential WBC counts, hemoglobin, hematocrit, total plasma protein, MCV, MCH, and MCHC. Blood smears were prepared from all surviving mice at study termination.

5. Gross necropsy was performed on all mice used in the study regardless of time of death. Organ weights and relative organ weights (per final body weights) were obtained from all animals at terminal sacrifice for the following organs: brain, heart, lungs, liver (with gallbladder), spleen, kidneys (with adrenals attached), testes (with epididymides), and thymus. All Guideline-required organs except the rectum were examined histologically by "conventional methods."

6. The following statistical procedures were performed by the study authors: body weight and organ weight data were analyzed by one-way ANOVA. Hematological data were analyzed by crossed and tested ANOVA. The least significant difference or Dunnett's test was used to analyze differences between treatment groups. Survival was subjected to Kaplan Meier methods[1]. Comparisons of survival distributions and tumor incidences were analyzed by the Mantel-Haenszel method[2]. Comparisons of absolute proportion of survival and incidences of tumors and clinical observations were analyzed by Fisher's Exact test. Dose responses in tumor incidence were analyzed by the chi-square test for trends. The level of statistical significance was $p < 0.05$.

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[1] Kaplan, F.L., and Meier, P. 1958. Nonparametric estimation for incomplete observations, Journal of the American Statistical Association, Vol. 53, 457-481. (reference not presented by authors)

[2] Mantel, N. and Haenszel, W. 1959. Statistical aspects of the analysis of data from retrospective studies of disease, Journal of the National Cancer Institute, Vol. 22, No. 4, 719-748. (reference not presented by authors)

Unless otherwise noted, the word "significant" in this review has statistical connotations ($p < 0.05$).

RESULTS:

Clinical Observations and Mortality: No clinical observations in any treatment group were reported to be significantly different from controls. Individual and summary data showed that there was no increase in the number of treated animals with palpable masses as compared to controls.

Body Weight and Food Consumption: Table 1 presents mean body weight data for male and female mice at selected intervals during the study. Both male and female high-dose mice showed a significant reduction in mean body weight throughout the course of the study. The mid-dose groups showed a significant reduction in mean weights at 60% of the weighing intervals for males (32/53) and 40% for the females (21/53) when compared to controls. There were only 2 instances of significant weight reduction in both male and female low-dose groups. Mean body weight gains showed significant decreases from controls in about 50% of the mid- and high-dose male weights and about 25% of the mid- and high-dose female weights. Food consumption was slightly decreased in males and females at the mid- and high-dose groups compared to controls; however, statistical analyses of the data were not provided and could not be validated by our reviewers without individual data.

Hematology: According to the report, there were no dose-related alterations in hematologic parameters. Mean hematocrit, erythrocyte count, and hemoglobin concentration were slightly but significantly lower in mid-dose males than in controls from months 3-24. A very slight but significant decrease in erythrocyte count and increase in mean corpuscular volume and mean corpuscular hemoglobin concentration observed from months 3 to 24 in females receiving the high dose of benomyl were not considered compound related when compared with controls. Mid-dose females also showed a significant increase in the mean corpuscular volume and a significant decrease in the mean corpuscular hemoglobin concentration.

Organ Weights: There were significant increases in mean liver weight in mid-dose males and in liver-to-body weight ratios in mid- and high-dose males and in high-dose females when compared to controls (see Table 2). Brain-to-body weight ratios were significantly increased in low- and high-dose males and in high-dose females. Mean testes weight was significantly lower in high-dose males than in controls and kidney weights were significantly lower in high-dose females than in controls. Thymus weights were decreased in all dosed males when compared to controls. The increased liver weights and decreased testes weights were correlated with histopathological changes, and considered of biological significance by the authors. The other changes in organ weights were considered to be of equivocal biological significance in the absence of a dose-related trend and histopathological changes.

TABLE 1. Mean Body Weights of Mice Fed Benomyl for 104 Weeks At Selected Time Intervals.

Group/Dose (ppm)	Mean Body Weight (gm) Week 0	13	56	80	104
Males					
0	26.6	38.2	47.1	47.7	43.5
500	26.6	38.9	47.6	46.6	42.5
1500	26.6	37.3*	45.7	45.6	41.2*
5000-7500[a]	26.5	34.4*	42.3*	42.4*	39.7*
Females					
0	21.0	30.3	37.8	38.9	36.5
500	21.0	30.3	37.2	38.0	34.0
1500	21.0	30.1	36.8	36.6*	35.7
5000-7500[a]	21.0	27.9*	33.4*	34.3*	33.4*

* Significantly different from controls value ($p < 0.05$) when analyzed by ANOVA by study authors.

[a] Reduced from 7500 to 5000 ppm after week 37.

2c

TABLE 2. Selected[a] Mean Absolute and Relative Organ Weights at Terminal Sacrifice from Mice Fed Benomyl for 104 Weeks

Group/Dose (ppm)	MALES						
					Relative		
	Body Weight	Liver	Thymus	Testes	Brain[c]	Liver	Thymus
Control	44.35	2.58	0.07	0.43	1.14	5.86	0.16
500	42.30	2.64	0.05*	0.41	1.21*	6.26	0.12*
1500	42.13	3.29*	0.05*	0.44	1.19	7.80*	0.13*
5000-7500[b]	40.34*	3.06	0.05*	0.38*	1.24*	7.54*	0.14
	FEMALES						
					Relative		
	Body Weight	Brain	Kidney	Brain	Liver	Thymus	
Control	38.54	0.48	0.69	1.26	5.39	0.15	
500	36.30	0.48	0.64	1.35	5.67	0.15	
1500	37.25	0.50*	0.67	1.37*	6.14	0.18	
5000-7500	34.44*	0.48	0.62*	1.40*	7.08*	0.19*	

a (*) Significantly different from control value ($p < 0.05$) when analyzed by study authors.
b 7500 ppm changed to 5000 ppm after week 37.
c Organ:body weight ratio.

Gross Pathology: Individual animal gross necropsy findings were reported but summary data with statistical analysis were not provided nor were the gross findings discussed by the authors.

Histopathology: Significant incidences of non-tumor histopathological changes are presented in Table 3. Tissues of dosed animals showing significantly increased incidence of lesions as compared to controls were: thymus in males at 5,000 ppm (atrophy), thymus in females at 1,500 ppm (cysts), liver in males at 5,000 ppm (5 parameters showing hepatocellular alteration), spleen in females at 5,000 ppm (hemosiderosis), trachea in females at 1,500 and 5,000 ppm (lymphocytic infiltrates in the submucosa), testes in males at 500 and 5,000 ppm (atrophy and tubule degeneration), epididymides in males at 5,000 ppm (aspermia), prostate in males at 5,000 ppm (focal distended acini), thyroid in males at 500 and 5,000 ppm (distended colloid follicles), and nasal cavity in males at 5,000 ppm (interstitial fibrosis and amyloidosis).

Significant incidences of neoplastic changes are presented in Table 4. In the males, the incidences of hepatocellular carcinomas, combined hepatocellular adenomas and carcinomas, and pulmonary alveologenic carcinomas in the 500 and 1,500 ppm groups were significantly higher than controls. In the females, the incidences of hepatocellular carcinomas in the 500 and 5,000 ppm groups and combined adenomas and carcinomas in the 1,500 and 5,000 ppm groups were significantly higher than controls. The same five parameters showed a significant trend ($p < 0.05$) when analyzed by our reviewers using the Cochran-Armitage Trend test.

The mean-time-to-, and median-day-of-tumor discovery were stated by the study authors not to be significantly different between treated and control groups. Individual animal data (in the form of time to death with tumors present) were provided.

DISCUSSION:

The authors concluded that benomyl, fed at a minimum of 500 ppm, produced a significant increase in hepatocellular carcinomas in male and female mice. There was a significant dose response to treatment in females for hepatocellular carcinomas and combined hepatocellular neoplasms. Our review of the study substantiated these conclusions; however, several conclusions were not supported.

When we reanalyzed the data, we found several significant compound or treatment effects that were not discussed by the authors. There was a significant dose-related trend in the incidence of male pulmonary alveologenic carcinomas, hepatocellular carcinomas, and combined hepatocellular neoplasms in males. There was also a significant histopathological dose-response effect in male epididymides and thyroid. When the mean-time-to, and median-days-of-death, with lung alveolar cell carcinomas present, were analyzed by these reviewers using Kruskal-Wallis ANOVA, $p < 0.05$, all male treated groups were significantly lower than control (Table 5).

TABLE 3. Selected[a] Incidences of Non-Neoplastic Histopathologic Lesions in Mice Fed Benomyl for 104 Weeks

Tissue	Dose Level (ppm)							
	Male				Female			
	0	500	1500	5000-7500[b]	0	500	1500	5000-7500
Thymus	(58)[c]	(40)	(38)	(48)	(62)	(62)	(52)	(57)
-atrophy	7	6	2	12*	d			
-cyst					2	4	9*	7
Liver	(77)	(80)	(79)	(80)				
-foci of hepatocellular alteration	1	3	2	8*				
-karyomegaly and cytomegaly	9	5	12	21*				
-foci of ceroid, microgranuloma	22	26	32	38*				
-foci of hepatocellular ballooning, degeneration	0	1	0	6*				
-lymphocytic foci/inflammatory infiltrates	38	48	45	52*				
Spleen					(76)	(79)	(78)	(74)
-hemosiderosis					1	5	6	7*
Trachea					(77)	(79)	(78)	(77)
-lymphocytic infiltrates, submucosa					0	0	7*	6*
Testes	(78)	(79)	(79)	(79)				
-degenerated seminiferous tubules	10	19	15	27*				
-active seminiferous tubule degeneration	7	17*	10	17*				
-atrophy	12	12	8	31*				
-interstitial cell hyperplasia	4	4	7	18*				
Epididymides	(78)	(78)	(79)	(79)				
-aspermia	18	11	12	30*				
-distended tubules/ tubules filled with degenerated sperm	9	5	11	17[e]				
Prostate	(73)	(73)	(76)	(77)				
-distended acini, focal	1	0	0	7*				
Thyroid	(65)	(74)	(73)	(71)				
-distended colloid follicles	4	13*	6	18*[e]				
Nasal cavity	(72)	(68)	(71)	(69)				
-interstitial fibrosis and amyloidosis	1	0	2	7*				

[a] (*) Significantly different from control value ($p < 0.05$) when analyzed by study authors.
[b] 7500 ppm changed to 5000 ppm after week 37.
[c] No. of animals examined.
[d] No data entry signifies a non-significant finding.
[e] Significant trend ($p < 0.05$) using Cochran - Armitage trend test by our reviewers.

TABLE 4. Selected[a] Incidences of Neoplasams in Mice Fed Benomyl for 104 Weeks

Tissue	Dose Level (ppm)							
	Male				Female			
	0	500	1500	5000[b] 7500	0	500	1500	5000–7500
Liver	(77)[c]	(80)	(79)	(80)	(77)	(80)	(79)	(77)
–hepatocellular adenoma	9	9	11	10	2	2	7	7
–hepatocellular carcinoma	16	26*	41*	17[d]	2	7*	6	14*[d]
–combined adenomas and carcinomas	25	35*	52*	27[d]	4	9	13*	21*[d]
Lung	(79)	(79)	(79)	(80)	(77)	(79)	(78)	(74)
–alveologenic carcinoma	13	24*	23*	16[d]	16	7	4	6

[a] (*) Significantly different from control value ($p < 0.05$) when analyzed by study authors.
[b] 7500 ppm changed to 5000 ppm after week 37.
[c] No. of animals examined.
[d] Significant trend ($p < 0.05$) using Cochran-Armitage Trend test by our reviewers.

30

TABLE 5. Mean-Time-to, and Median-Day-of Death, When Lung Alveolar Cell Carcinomas were Present in Rats Fed Benomyl for 104 Weeks

Dose (ppm)	Days	
	Male	Female
0	736.8[a]	674.0
	23.4	101.9
	743	740
500	665.4*	674.4
	94.1	84.6
	728	715
1500	688.9*	719.5
	96.6	33.0
	741	736
5000-7500[b]	702.2*	730.7
	54.9	11.3
	739	737

[a] Upper value is the mean, the middle value is the standard deviation, the bottom value is the median-day-of-death.

[b] 7500 ppm changed to 5000 ppm after week 37.

* Significantly different from control ($p < 0.05$) when analyzed by these reviewers using Kruskal-Wallis ANOVA.

The mean weight gain over the course of the study was significantly decreased for mid- and high-dose males (14.3 and 13.3 g, respectively as compared to 17.1 g for controls) and high-dose females (12.5 g as compared to 15.5 g for controls), when analyzed by ANCOVA, $p < 0.05$. Statistical analyses for mean daily food consumption, food efficiency and daily intake of benomyl were not reported and individual animal data were not available, hence, these data could not be statistically analyzed by our reviewers. The summary data provided by the authors showed either no change from controls or a slight compound-related decrease. The latter was especially true for the high-dose female daily mean food consumption with a lesser decrease for high-dose male daily mean food consumption.

The administration of test compound caused no statistically significant increase in mortality in dosed animals when compared to controls at 78, 91, and 103 weeks of the study. At terminal sacrifice (105-106 weeks), the mid-dose female group had significantly fewer animals alive (23 (29%) vs 33 (41%) for control), but the low- and high-dose groups equaled the control value. The total number per group per sex for "found dead" or "moribund sacrifice" were not significantly different from controls except for the female mice found dead. The low-, mid-, and high-dose values were significantly greater (10/80, 12/80, and 11/80 respectively), than the control (2/80) when we analyzed the data using the Fisher exact test.

The authors stated that the hematologic changes were not of biological significance. However, the authors used a method of statistical analysis of the hemotological data that they did not adequately describe; therefore, the analyses could not be reproduced. The findings by the study authors however, allow a clinical diagnosis of toxicological importance when the authors' following significant findings are combined in the high-dose (5,000 ppm) females: 1) hemosiderosis in the spleen, 2) decreased red blood cell counts, 3) increased mean corpuscular volume, 4) increased mean corpuscular hemoglobin, 5) hepatocellular alterations (neoplasms). This information is indicative of regenerative hemolytic anemia. Using the more traditionally employed methods (Bartlett's test for homogeneous variance followed by ANOVA or Kruskal-Wallis test depending on whether a parametric or non-parametric test was appropriate) we found that the only significant hemotological parameter to change from controls was mean corpuscular hemoglobin values in the high-dose females.

The majority of the significant non-tumorous histopathological observations were not considered by the author to be compound related. Our assessment is that several of the changes are commonly seen in aged rats, however, the occurrence in only the high-dose group may imply a compound-related effect.

There were two reporting deficiencies. The clinical observation summary table provided for alopecia/dermatitis (the most prominent observation) was slightly under-reported when compared with the individual animal data. When we reanalyzed this data, none of these parameters were found to be significantly different from controls.

When we summarized and statistically analyzed individual animal necropsy data, no compound-related effect was seen with respect to the number of masses or nodules when treated groups were compared to controls. The number of masses seen at gross necropsy were about 30% of the number seen histologically.

Our criticisms of this study do not alter the general conclusions of the authors that under the study conditions, benomyl was carcinogenic at the lowest dose tested. There were no additional major deficiencies in the study.

CONCLUSIONS:

Under the conditions of this study, benomyl fed at a minimum of 500 ppm was carcinogenic in the liver and lung of CD-1 mice. Hepatocellular carcinomas were induced in both males (low and mid doses) and females (low and high doses). The combined incidence of hepatocellular adenomas and carcinomas were statistically increased in the mid- and high-dose females. Pulmonary alveologenic carcinomas were induced in males at the low and mid dose. The testes and epididymides showed degenerative changes at the highest dose tested.

CORE CLASSIFICATION: Minimum.

Reviewed by: Melba S. Morrow, D.V.M. 12/14/93
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Joycelyn E. Stewart, Ph.D.
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity Supplement (Re-read of liver slides)

GUIDELINE #: 83-2

TOX. CHEM. #: 75A

MRID #: 416070-04

TEST MATERIAL: Benomyl and MBC

STUDY NUMBERS: 3194 and 3207 (Original studies-20-82 and 70-82)

SPONSOR: E.I.Dupont

TESTING FACILITY: Haskell Labs
Newark, Delaware 19714

TITLE OF REPORT: Oncogenicity Studies with Benomyl and MBC in Mice (Supplemental Peer Review)

AUTHORS: S.R.Frame, DVM, PhD
C.S. VanPelt, DVM, PhD

REPORT ISSUED: June 28, 1990

CONCLUSIONS: In a two year study in CD-1 mice, Benomyl was administered at doses of 0, 500, 1500 and 5000 ppm. The compound was reported to induce the following tumor incidences (1) increased incidences of hepatocellular carcinoma in low and mid dose males and in low and high dose females, (2) increased incidences of combined hepatocellular neoplasms in low and mid dose males and in mid and high dose females. Likewise, MBC administered to CD-1 mice at doses of 0, 500, 1500 and 3750 ppm was reported to be associated with the following tumor incidences (1) increased incidence of hepatocellular carcinomas in mid dose males and in mid and high dose females and (2) increased incidences of hepatocellular adenomas in all treated females. Based on these findings, Benomyl and MBC were classified as C carcinogens in 1985, 1986 and 1989 by the agency.

A histopathology peer review was conducted by the registrant (using pathologists from their own Haskell Labs in collaboration with an independent pathologist from Experimental Pathology Laboratories, Inc.) on slides from liver samples collected in carcinogenicity studies with both benomyl and MBC. It was concluded that both chemicals were associated with an increase in

the number of benign liver tumors in CD-1 mice. Earlier studies concluded that there was an association with the use of these chemicals and hepatic neoplasia; however, the lesions have been reclassified in this peer review provided by the sponsor. A decrease in the number of hepatocellular carcinomas was apparent when the original results were compared to the results of the peer review. These malignant tumors were reclassified, resulting in an increase in the number of hepatocellular adenomas, which are considered benign tumors.

Re-review of the liver slides do not demonstrate that Benomyl and MBC are not carcinogens. For Benomyl, re-review of the data demonstrate an increase in hepatocellular adenomas at low and mid dose levels in male mice and at the high dose in female mice. For MBC, the number of hepatocellular adenomas was significantly increased at all dose levels in female mice and at the mid dose level in male mice. A decrease in tumor incidence was reported at the high dose level in male mice; however, this was associated with increased mortality.

There was also an increase in the number of animals with foci of cellular alteration in mid and high dose males and in all females receiving Benomyl. Similar increases were observed for both sexes of low and mid dose mice which received MBC. These foci of cellular alterations are often considered preneoplastic and would imply that there is a progression of hepatic lesions.

With regard to the carcinogenic potential of the compounds in question, the results of the re-evaluation would not support a reclassification of either compound. The compounds were classified as C carcinogens by the SAP on May 21, 1986 after they had been evaluated by the Cancer Peer Review Committee on two previous occasions (10/3/85 and 1/7/86). At the January 1986 Peer Review Committee meeting, it was decided that Benomyl met the criteria for classification as a group C carcinogen. This decision was upheld by the SAP. The decision to quantify the risks was made at the third Cancer Peer Review Committee meeting of January 25, 1989. It was determined that the quantification of risk was warranted based on the presence of a malignant response in two strains of mice for MBC and in one strain for Benomyl; the presence of a malignant response in both sexes and the association of the compound with genotoxicity.

The change in the ratio of malignant to benign liver tumors may warrant a reassessment of the decision to quantify the risk and this new information may need to be presented to the Committee for their consideration.

CLASSIFICATION: Supplementary. The information provided in this submission supplements and supercedes the results in the original submission. With regard to the carcinogenic potential of the compounds in question, the results of the re-evaluation would not support a reclassification of either Benomyl or MBC because both are associated with an increase in benign liver tumors.

MATERIALS: Eighty slides (per sex/group/chemical) from the livers of mice in the benomyl and MBC bioassay studies were examined microscopically. Slides were collected from bioassay studies in CD-1 mice in which benomyl was adminisetred at doses of 0, 500, 1500 or 5000 ppm and MBC was administered at doses of 0, 500, 1500 and 3750 ppm.

METHODS: The pathology results were re-reviewed by two pathologists from Haskell Labs in order to determine whether liver neoplasms were present and to classify all neoplasms that were reported. The results of this reclassification were further reviewed by a third pathologist from Experimental Pathology Laboratories, Inc. (EPL). Differences between the Haskell pathologists and the EPL pathologist were discussed and a consensus was reached by the two factions on tumor diagnosis and classification.

QUALITY ASSURANCE: A statement of QA (6/29/90 and a statement of compliance with Good Laboratory Practices were included in the submission.

STATISTICAL ANALYSIS: A one-tailed Fisher's exact test was conducted to compare lesions in treated and control groups. Significance was at $p < 0.05$.

RESULTS: From the previous evaluations of these two studies it was concluded that both compounds were associated with an increased incidence of liver carcinomas in one or both sexes of CD-1 mice. Increased incidences of combined (malignant and benign) neoplasms was also reported for both of these chemicals. A NOEL for systemic effects was not provided in this report; however, based on the information from the Third Peer Review Document dated May 1985, the systemic NOEL for Benomyl was 1500 ppm and the LOEL was 5000, based on decreased weight gain and liver and testicular pathology. For MBC, the systemic NOEL was 500 ppm and the LOEL was 1500 ppm based on hepatotoxicity and lymphoid depletion in the thymus.

The reevaluation demonstrated a significant increase in the number of adenomas in mid and low dose males receiving Benomyl and a corresponding decrease in the number of male mice with liver carcinomas. In females receiving MBC, the most notable change was in the number of carcinomas and the number of adenomas, with the former being decreased and the latter being increased in all treated groups as a result of the re-evaluation. (See Table I).

When the slides were reviewed by the third pathologist, the overall results were similar, with only slight differences in diagnoses being recorded (See Table II). The differences in interpretation were primarily in the borderline lesions that were difficult to classify. A consensus from the three pathologists is reflected in the figures appearing in Table I.

36

For Benomyl, the number of adenomas increased for males from 9 to 17, 9 to 31, 11 to 49 and from 10 to 21 for control, low, mid and high dose groups, respectively. It should be noted that the tumor incidence at 5000 ppm (HDT) was lower in male mice than in the low and intermediate groups. In females receiving Benomyl, the number of adenomas increased from 2 to 4, 2 to 8, 7 to 11 and 7 to 18 for control, low, mid and high dose groups, respectively.

In males receiving MBC, the number of adenomas increased from 14 to 19 for mid dose males, only, with all other male groups showing either a decrease or no change. In females the number of adenomas increased from 5 to 8, 5 to 20 and 3 to 15 for low, mid and high dose groups, respectively. No change was reported for control animals. At the highest dose tested in males, the decrease in tumor incidence was associated with an increase in mortality.

In high dose males receiving Benomyl, there did not appear to be a treatment related increase in liver tumors of any type; however, an increase was apparent in females receiving the same dose level. In males receiving MBC at the highest dose tested, the decrease in tumor incidences was due to increased mortality.

DISCUSSION: Based on the results of the re-evaluation of the pathology slides, both benomyl and MBC have been associated with an increase in the incidence of benign liver tumors in both sexes of CD-1 mice which is significant at the low and mid dose levels in male mice. In females, significant increases are seen at the high dose, only. In addition, the data for Benomyl demonstrated increased incidences of multiplicity of adenomas and foci of cellular alteration at all dose levels in females and at the mid and high dose levels in males. Although the number of malignant tumors has decreased, based on other observations in this supplement, there appears to be the potential for the progression of lesions, specifically, the multiplicity of adenomas and the foci of cellular alteration.

The submission that addressed the re-evaluation of the slides for benomyl and MBC also contained literature pertaining to the significance of hepatoblastomas that occured in Swiss mice following the administration of MBC. This study was conducted using MBC at dose levels of 0, 150, 300 or 5000 ppm, with the incidences of hepatoblastomas being reported as 0/50, 1/50, 1/50 and 7/10 for the respective groups of male animals only.

Several pieces of literature were cited to address the significance of mouse hepatoblastomas in human risk assessment. (See attached reference list). The literature suggested that diet, the strain of mouse and the sex of the animal may all play a role in the development of hepatoblastomas. It was also hypothesized that hepatoblastomas are variants of hepatocellular adenomas or carcinomas. Hepatoblastomas are often found within the structure of the more predominant tumor types and may be formed as a result of the effects of promoting agents on these

37

more frequently existing tumors.

The information in the literature would not result in a change in the conclusions made from data generated in the study with MBC in Swiss mice. The compound was associated with an increase in the incidence of hepatoblastomas in high dose males and no evidence has been offered to refute this earlier finding.

TABLE I
COMPARISON OF RESULTS FROM INITIAL STUDY TO PEER REVIEW RESULTS

BENOMYL				
Males/group	78	80	79	80
		Dose (ppm)		
	0	500	1500	5000
# Animals w/liver tumors				
original	25	35	52*	27
re-review	25	34	49*	25
Adenomas				
Original	9	9	11	10
Re-review	17	31*	49*	21
Carcinomas				
Original	16	26	41*	17
Re-review	10	7	11	4
Focus/foci of alteration				
Original	1	3	2	8
Re-review	2	3	5	10*
Females/group	79	80	80	79
Animals w/liver tumors				
Original	4	9	13*	21*
Re-review	4	9	12*	18*
Adenomas				
Original	2	2	7	7
Re-review	4	8	11	18*
Carcinomas				
Original	2	7	6	14*
Re-review	0	3	2	2
Focus/foci of alteration				
Original	1	1	1	4
Re-review	1	2	2	5

-Data taken from Tables 1 and 2 on pages 12 and 13 of report.
* = $p < 0.05$

Hemangiosarcomas and hepatoblastomas not included in table, but reflected in total number of animals with tumors.

35

RfD/PEER REVIEW - FY 94 SCHEDULE AND STATUS REPORT

PDATED: 11/15/93
DTE: This schedule is subject to change

CHEMICAL	REG. GROUP SPECIFIC ACTION OTHER ISSUES	PACKAGE DUE TO SAB	PACKAGE IN SAB	PROJECTED MEETING DATE	DRAFT REPORT OUT	FINAL REPORT OUT
None scheduled				10/07/93	Meeting canceled at Div. Dir. request	
Ethion	RED	N/A	09/09/93	10/14/93		
Propargite	OLD - New data	N/A	07/08/93	10/21/93	Meeting canceled	
Thiazopyr	New chemical	N/A	06/30/93			
Carbaryl	Special Review	10/14/93	10/14/93	10/28/93		
Thiazopyr	New chemical	N/A	06/30/93			
Desmedipham	New chemical	N/A	09/24/93	11/04/93		
Ethofumesate						

40

TABLE I (Cont.)

MBC				
Males/group	80	80	79	80
		Dose (ppm)		
	0	500	1500	3750
# Animals w/liver tumors				
original	13	20	23*	3
re-review	11	13	19	3
Adenomas				
Original	11	15	14	3
Re-review	10	12	19*	3
Carcinomas				
Original	2	5	9*	0
Re-review	1	2	3	0
Focus/foci of alteration				
Original	2	4	3	3
Re-review	2	7	6	1
Females/group	80	78	79	78
Animals w/liver tumors				
Original	1	9*	21*	15*
Re-review	1	8*	21*	17*
Adenomas				
Original	0	5*	5*	3
Re-review	0	8*	20*	15*
Carcinomas				
Original	1	4	15*	12*
Re-review	1	1	6	3
Focus/foci of alteration				
Original	3	2	3	14
Re-review	1	3	5	3

-Data taken from Tables 3 and 4 on pages 14 and 15 of report.

* = $p < 0.05$

Tumors like hemangiosarcomas, hepatoblastomas not included in this table, but are reflected in the total number of animals with tumors.

41

TABLE II
COMPARISON OF RESULTS BETWEEN LABORATORY PATHOLOGISTS AND EPL PATHOLOGIST

BENOMYL

Males ID #	Dose	Original Diagnosis	ELP Comments
6515	C	carcinoma	Hemangiosarcoma also present
6593	L	Adenoma	Foci of alteration
6654	M	Adenoma	No lesion in tissue section
6690		adenoma	Foci
6693		Adenoma	Foci
6705	H	Adenoma	Foci
6737		Adenoma	Foci
6768		Adenoma	Foci
Females			
6828	C	Adenoma	Foci
6944	M	Adenoma	Hemangiosarcoma also present
6949		No lesion	Hematopoetic tumor
6997		Sarcoma	sarcoma-absent, adenoma-present
7044	H	Adenoma	Foci
7088		Adenoma	Foci

MBC

Males ID #	Dose	Original Diagnosis	ELP Comments
7917	C	No lesion	Hemangiosarcoma
7932		Adenoma	Foci
8000	L	Adenoma	Foci
8001		"	"
8043		"	"
8055		"	"
8078	M	No Lesions	Adenoma
8083		Adenoma	Foci of alteration
8100		Adenoma	Foci
8116		Adenoma	Foci
Females			
8378	L	Adenoma	Lesion not present
8426	M	Adenoma	Lesion not present
8595	H	Foci	Adenoma
8536		Foci	adenoma

C= control, L = low, M = mid, H = high

Benomyl: 2-Year Feeding Study in Dogs
E. I. du Pont de Nemours & Company. 1970. MRID No. 00061618, 00081913, 0097305, 00097318, 00097326. HED Doc. No. 000721, 004678, 004679.

Reviewer: Melba S. Morrow, D.V.M. 10/3
Secondary reviewer: Joycelyn E. Stewart, Ph.D. 10/30/91

Study Type: Two year feeding study -dog

Tox. Chem.: 75A

Chemical: 2-benzimidazole carbamic acid, methyl ester

Synonyms: Benomyl, MBC, INT-1991, Carbendazim

Haskell Lab Report Nos.: 48-70, 129-69, 53-71, 54-71, 74-77

Sponsor: E.I. duPont

Study Performed at: Haskell Lab for Toxicology and Industrial Medicine
Wilmington, Delaware

Authors: H. Sherman, J.R. Barnes, E.F.Stula, G.J. Stopps

Date: 1970

Addendum to ~~Study Report~~: DER
Executive Summary - Benomyl - Two Year Feeding Study in Dogs

Beagles (4/sex/group) were administered benomyl (50%, a.i.) at dietary levels of 0, 100, 500 or 2500 ppm for two years. This is equivalent to 0, 2.5, 12.5, or 62.5 mg/kg/day.

There were no compound related clinical signs of toxicity and there were no effects on body weight, food consumption, hematology or urinalysis. At 2500 ppm (62.5 mg/kg/day) there was an increased incidence of hepatic cirrhosis and there was proliferation of the bile duct in 4/6 dogs which received the test material for two years. (One dog/sex from each dose level was sacrificed after one year).

Although earlier reports concluded that there were compound related effects (increases) on cholesterol, alkaline phosphatase and SGPT, these values were not altered to a biologically or statistically significant level.

Under the conditions of this study, the NOEL for benomyl was 500 ppm and the LOEL was 2500 ppm, based on liver damage. The study satisfies the requirement for a chronic non-rodent toxicity study (83-1b).

004679

STUDY TYPE: Two-year Feeding study-Dog

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NO: 48-70
129-69
53-71
54-71
74-77

FICHE/MASTER: 00097305
00081913
00097318
00097326
00061618

MR NO.: 966

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

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

AUTHORS: H.Sherman, J.R.Barns, E.F.Stula, G.J.Stopps

DATE REPORT SUBMITTED: 3/17/70, supp. path. report 11/22/77

TEST MATERIAL: 1-butylcarbamoyl-2-benzimidazolecarbamic acid, methyl ester (approx. 50 % a.i.)

SYNONYMS: benomyl
INT-1991
Banlate®wettable powder

Review by Bruce Jaeger in the 1983 WHO report:

"Groups of beagle dogs (four males and females/group) one to two years of age, were administered benomyl (50% active ingredient) in the diet at dosage levels of 0, 100, 500 and 2500* ppm for 2 years. Food consumption and body weight data were obtained weekly, and animals were examined daily for clinical signs of toxicity. Hematological, biochemical and urinalysis examinations were performed periodically* throughout the study. Interim sacrifice after one year was performed on one male and one female from control and high dose groups. Organ weights, gross necropsy and histopathological evaluations* were performed at the conclusion of the study. Only the livers and testes were examined histologically in the 100 and 500 ppm dose groups.

There was no mortality related to treatment. Body weight changes and food consumption values were similar among all groups, except the high dose which demonstrated both decreased food intake and body weight gain. The average daily dose was 55-58 mg/kg body wt. (initially, M & F), 74-79 mg/kg (at one year) and 45-55 mg/kg (at 2 year). One dog at the high dose lost its appetite and was replaced. No other clinical signs of toxicity were observed. Hematological evaluations and urinalyses were similar to control. Males in the 2500 ppm group had increased cholesterol, alkaline phosphatase and GPT values (initially), as well as decreased total protein and albumin/globulin (A/G) ratio. There were similar, but less

46

* see addendum for clarification and additional information.

marked effects in high dose females. Cholesterol and total protein were similar to controls among the females examined.

The biochemical determinations were supportive of adverse liver effects, demonstrated as liver cirrhosis among high dose group animals. There was also slight to marked bile duct proliferation in 4/6 dogs at the 2500 ppm level. Hemosiderosis, evident in one dog in the 2500 ppm group at one year, was not evidence in other dogs examined at 2 years after staining specifically for iron. Preparation of preserved wet tissue with oil red 0 and sudan black for hepatocyte vacuolation confirmed that benomyl was not hepatotoxic at 100 and 500 ppm in the diet."

Addendum:

MATERIAL AND METHODS: Food was offered ad libitum between 3:00 PM and 7:00 AM; there was free access to water. Hematological tests, biochemical tests and urinalysis were performed 3 times pretest, and 1, 2, 6, 9, 12, 15, 18, 21 and 24 months after test initiation.

Hematology - Red blood cell count, hemoglobin, hematocrit, total and differential leukocyte counts.

Biochemistry - Glucose, urea-nitrogen, cholesterol, alkaline phosphatase (APase), glutamic-pyruvic transaminase activity (GPT), total protein (TP), and albumin/globulin ratio (A/G).

Urinalysis - pH, volume, osmolality, protein, sugar, urobilinogen, acetone, bilirubin, occult blood and microscopic sediment examination.

Tissues from the control and 2500 ppm groups were fixed in Bouin's and stained with hematoxylin and eosin for histologic examination included:

†brain	†adrenal	mammary gland	tonsil
†heart	prostate	esophagus	trachea
†lung	†pituitary	cecum	gall bladder
†liver	pancreas	colon	spinal cord
†spleen	urinary bladder	trachea	salivary gland
†kidney	epididymis	skeletal m.	
†testis	Fallopian tubes	peripheral n.	
thymus	uterus	bone marrow	
†stomach	ovary	eye	
†thyroid	duodenum	thoracic aorta	

RESULTS:

	MALES (PPM)				FEMALES (PPM)			
	0	100	500	2500	0	100	500	2500
Chol.(2 mon.)	125	121	106	182	151	147	132	145
(2 yr)	116	128	111	166	161	141	135	179
APase(2 mon.)	2.0	1.9	1.2	8.9	2.5	1.5	1.9	2.6
(2 yr)	1.6	1.8	1.3	4.0	4.2	0.9	0.9	1.8
GPT (2 mon.)	22	19	22	163	18	70	21	39
(2 yr)	23	20	23	23	21	16	14	29
Alb/G(2 mon.)	.80	.80	.91	.72	.99	1.20	0.92	0.88
(2 yr)	.98	1.02	1.11	.81	1.14	0.92	0.96	0.77
Tot. (2 mon.)	5.80	6.64	5.78	5.57	no treatment related			
Prot.(2 yr)	6.32	6.36	6.27	5.75	change			

47

†organ weights were taken

Increases in (male) chol. and APase started as early as 1 month and remained elevated throughout most of the study in the 2500 ppm group. GPT (male) increased by 1 month but returned to normal levels within the 15 months in the 2500 ppm group. Alb/G ratios (males) decreased within 2 months and remained low throughout the study in the high dose group. Total protein (male) was slightly decreased within 1 month and remained low throughout the study in the high dose. Correlation coefficient analysis of chol., APase, GPT, Alb/G and total protein indicated a relation between the level of compound in the food and the change in blood levels. An F test also indicated differences between the treatment groups for these parameters (2 standard deviations were considered significant). Hepatic cirrhosis was evident grossly and microscopically in one male sacrificed at 1 year and 2 males and 1 female sacrificed at 2 years.

Sacrifice time	1yr	2 yr
Incidence of hepatic cirrhosis	1/2 male 0/1 female	2/3 male 1/3 female

Focal testicular degeneration was present in all treatment groups, with marked testicular degeneration (reduced testes weight, absence of spermatozoa and spermatic giant cells) in 1/3 dogs at 2500 ppm.

DISCUSSION: The report indicates that treatment was temporarily witheld from 2 male dogs that lost weight (high dose). One of these was off the compound for weeks at a time. There was no mention of frequency or duration of these occurrences. Although individual body weights were presented, there were no summaries present in the report which made weight gain difficult to analyze. Hepatic cirrhosis observed in the high dose males and females was probably treatment related. As reported by the previous reviewer and the registrant, the histologic and biochemical changes indicate possible liver damage in males at 2500 ppm. An outbreak of an inflammatory disease causing orchitis in beagle colonies at that time may have contributed to the unusually high level testicular lesions in the controls and treated groups. K. Davis, D.V.M. (Tox. Br. pathologist) of EPA examined the testes slides (see report of April 30, 1971) and concluded that "Certainly neither the degree or the distribution indicate that testicular changes are related to chemical ingestion." Data from the 2 year dog study on carbendazim, a primary metabolite of benomyl, provide additional confirmatory results for the absence of testicular effects at dietary levels of at least 100 ppm carbendazim. There were no other treatment related changes evident in this study.

Although the previous reviewer (WHO review) listed the NOEL as 100 ppm, the data supports a NOEL of 500 ppm and a LEL of 2500 ppm based on hepatic cirrhosis and clinical chemistry alterations.

48

CONCLUSION:

NOEL = 500 ppm

LEL = 2500 ppm based on biochemical and histological alterations indicating liver damage as well as decreased weight gain and food consumption.

CORE-CLASSIFICATION: minimum

Original review evaluated and addendum added by M.P.Copley, D.V.M.
Tox. Br.
9/19/85

10/18/85

MBC (Carbendazim): 2-Year Feeding Study in Dogs
E. I. du Pont de Nemours & Company. 1972. MRID No. 00088333.
HED Doc. No. 004679.

50

Reviewer: Melba S. Morrow, D.V.M. 10/30/96
Secondary reviewer: Joycelyn E. Stewart, Ph.D. 10/30/96

Study Type: Two year feeding study -dog

Tox. Chem.: 79C

Chemical: 2-benzimidazole carbamic acid, methyl ester

Synonyms: MBC, INE-965, Carbendazim

Haskell Lab Report No.: 195-72

Sponsor: E.I. duPont

Study Performed at: Haskell Lab for Toxicology and Industrial Medicine
Wilmington, Delaware

Authors: H. Sherman, S.B.Fritz , L.S. Wasileski

Date: 1972

Addendum to Study No.195-72

Executive Summary - Carbendazim, Two Year Dog Study

Beagles (4M, 4F) were administered carbendazim (53%) at dietary dose levels of 0, 100, 500 or 2500 ppm for two years. This is the equivalent of 0, 2.5, 12.5, or 62.5 mg/kg/day. The NOEL was 100 ppm (2.5 mg/kg) based on the presence of liver pathology at 500 ppm (12.5 mg/kg/day). The liver injury was characterized by swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis. At 500 ppm, there were also reported increases in cholesterol, total protein, SGPT and alkaline phosphatase, none of which were biologically or statistically significant but were in past reviews, correlated to chemically induced hepatic injury.

At 2500 ppm (62.5 mg/kg/day), anorexia, distended abdomens and poor nutritional condition were reported.

The study is acceptable and satisfies the requirement for a chronic non-rodent study (83-1(b)).

STUDY TYPE: Two-year Feeding study-Dog

TOX. CHEM. NO.: 79C

HASKELL LAB. REPORT NO: 195-72
MR NO.: 1149

FICHE/MASTER: 00088333
ACCESSION NO.: 232870-C
232871

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

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

AUTHORS: H.Sherman, S.B.Fritz, L.S.Wasileski

DATE REPORT SUBMITTED: 1972

TEST MATERIAL: 2-benzimidazolecarbamic acid, methyl ester (50 or 70 % a.i., 53 or 72.2 % Tech.) (metabolite of Benomyl)

SYNONYMS: MBC
INE-965
carbendazim

Review by Bruce Jaeger in the 1983 WHO report:

"Groups of beagle dogs (four males and 4 females/group) were administered carbendazim (53% active ingredient) in the diet at dosage levels of 0, 100, 500 and 2500* ppm for 2 years. Dogs were one to two years of age at the start of the test. Some dogs in the high dose group received only 1500 ppm. Food consumption and body weight data were obtained weekly, and animals were examined daily for clinical signs of toxicity. Hematological, biochemical and urinalysis examinations were performed periodically* throughout the study. Interim sacrifice after one year was performed on one male and one female from control and 500 ppm groups, as well as one female from the high dose group. One male from the high dose group was sacrificed in extremis after 42 weeks on test diet. Organ weights, gross necropsy and histopathological evaluations* were performed at the conclusion of the study. Only the livers and testes were examined histologically in the 100 and 500 ppm dose groups.

There was no mortality reported for the control or 100 and 500 ppm dose groups. However, three males in the high dose group were sacrificed after 22 and 42 weeks because of poor nutrition. No females in the high dose group died. Body weight and food consumption were all adversely effected in the high dose group animals, but not at lower levels. The average daily intake for the 500 ppm dose group was 15.0-20 mg/kg (initially, M & F), 14-18 mg/kg (1 year) and 10-16 mg/kg (2 years). Dogs in the highest dose group developed anorexia, distended abdomens and overall poor nutritional condition. Hematological evaluations and urinalyses were not apparently affected by treatment. The dogs in 500 ppm and 1500/2500 ppm dose groups had increased cholesterol, BUN, total protein, GPT and APase levels while similarly presenting evidence of a decreased A/G ratio throughout

* see addendum for clarification and additional information. 52

the study. This biochemical evidence of liver effect was supported by liver pathology, with incidences of hapatic cirrhosis, swollen vacuolated hepatic cells and mild chronic hepatitis in dogs fed 500 ppm or more of carbendazim. There were no noticeable effects on organ weights and organ-to-body weight ratios. Diffuse testicular atrophy (which was marked) and aspermatogenesis were observed in 2/4 males at 100 ppm but were not present in other dose groups or control males. Based on the lack of supporting data in the other dose group males, these findings are not considered compound related.

The NOEL in this study appears to be 100 ppm, based on the liver effects noted at 500 ppm and greater."

Addendum:

MATERIAL AND METHODS: Food was offered ad libitum between 3:00 PM and 7:00 AM. The compound was introduced gradually into the diet of group IV (2500 ppm); 500 ppm for 2 days, 1000 ppm for 3 days, 1500 ppm for 2 days then 2500 ppm. Due to weight loss and decreased appetite the compound level for several dogs was reduced to 1500 ppm. Hematological tests, biochemical tests and urinalysis were performed 3 times pretest, and 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 months after test initiation. Hematology - Red blood cell count, hemoglobin, hematocrit, total and differential leukocyte counts. Biochemistry - Glucose, urea-nitrogen, cholesterol, alkaline phosphatase (APase), glutamic-pyruvic transaminase activity (GPT), total protein (TP), and albumin/globulin ratio (A/G), albumin concentration. Urinalysis - color, appearance, pH, volume, osmolality, protein, sugar, urobilinogen, acetone, bilirubin, occult blood and microscopic sediment examination. Tissues from the control and 2500 ppm groups were fixed in Bouin's and stained with hematoxylin and eosin for histologic examination included:

†brain	†adrenal	ileum	mammary gland
†heart	†prostate	jejunum	esophagus
†lung	†pituitary	cecum	gall bladder
†liver	pancreas	colon	spinal cord
†spleen	urinary bladder	rectum	trachea
†kidney	epididymis	skeletal m.	salivary gland
†testis	Fallopian tubes	peripheral n.	tonsil
†thymus	uterus	bone marrow	lymph node
†stomach	ovary	eye	skin
†thyroid	duodenum	thoracic aorta	

†organ weights were taken

RESULTS:

	MALES (PPM)				FEMALES (PPM)			
	0	100	500	1500/2500	0	100	500	1500/2500
Chol.(2 mon.)	132	127	174	175	164	157	167	239
(2 yr)	152	150	200	250	211	162	411	171
APase(2 mon.)	1.5	1.2	2.7	13.	2.5	2.9	2.5	7.9
(2 yr)	2.4	2.3	3.3	4.3	3.0	2.8	4.6	6.1
GPT (2 mon.)	18	24	16	138	11	15	22	134
(2 yr)	13	12	17	18	13	10	15	18
Alb/G(2 mon.)	.90	1.01	.86	.70	no treatment related			
(2 yr)	.90	.98	1.05	.69	change			

53

Increases in (male and female) chol. and APase started as early as 1 month and remained elevated throughout most of the study in the 500 and 2500 ppm groups. GPT (male and female) increased by 1 month but returned to normal levels within the first year in the 2500 ppm group. Alb/G ratios (males) decreased within 1 month and remained low throughout the study in the high dose group. Regression analysis of chol., APase, GPT, Alb/G and Alb indicated a relation between the level of compound in the food and the change in blood levels. An F test also indicated differences between the treatment groups for these parameters (2 standard deviations were considered significant).

DISCUSSION: The report discussed problems with nutrition at 2500 ppm and 1500 ppm but did not state: 1) which dogs received less than 2500 ppm; 2) how long and how often they were switched to control diets for recovery. This information would be necessary to adequately assess toxicity at the high dose. This deficiency will not change the results of the study however, since major signs of toxicity were also observed at the 500 ppm level. The registrant discussed regression analysis of biochemical values but did not mention which type of analysis was used. The previous reviewer reported increases in BUN and TP, however these changes do not appear biologically relevant because they were within the range of pretest levels or had no dose response. As reported by the previous reviewer and the registrant. the histologic and biochemical changes indicated liver damage at 500 ppm and above. There were no other treatment related changes evident in this study.

CONCLUSION:

NOEL = 100 ppm

LEL = 500 ppm based on biochemical and histological alterations indicating liver dammage.

CORE-CLASSIFICATION: minimum

Original review evaluated and addendum added by M.P.Copley, D.V.M.
Tox. Br.
9/19/85

Benomyl: 2-Generation Reproduction Study in Rats
E. I. du Pont de Nemours & Company. 1991. MRID No. 41887901.
HED Doc. No. 009992.

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

JAN 29 1993

009992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: ID. No.: 099101, Benomyl Reproduction Study for California

Tox. Chem. No.: 075A
DP barcode No.: D177061
Record No. : S416212

FROM: Melba S. Morrow, D.V.M. MSM 1/25/93
Review Section II, Toxicology Branch I
Health Effects Division (H7509C)

TO: Susan Cerrelli/ Linda Propst PM 73
Reregistration Division (H7508W)

THRU: Karl P. Baetcke, Ph.D.
Chief,
Toxicology Branch I
Health Effects Division (H7509C)

Karl P. Baetcke 1/27/93

CONCLUSIONS:

When DPX-T1991-529 was administered to Crl: CDBR rats at dietary levels of 0, 100, 500, 3000 and 10000 ppm (for males:0, 5-8, 28-38, 168-234 and 553-954 mg/kg, and for females: 0. 7-9, 35-47, 210-280 and 712 - 1168 mg/kg), the reproductive NOEL was 500 ppm and the LOEL was 3000 ppm. The LOEL was based on decreases in the body weights of F2a and F2b offspring on days 14 and 21 of lactation and decreases in sperm counts reported for F1 parental males.

At doses of 3000 ppm and higher there was testicular pathology in both generations which consisted of atrophy and degeneration of the seminiferous tubules. Oligospermia was also reported at these doses.

At 10000 ppm, there were decreases in the birth weights of F1, F2a and F2b pups, decreased testicular weights in P1 and F1 males and decreases in body weights, body weight gains and food consumption in parental animals. In the F2b offspring, there was also an increase in the number of pups with partially opened or unopened eyes and a reported nonsignificant decrease in pup survival.

56

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Reviewed by: Melba S. Morrow, D.V.M. MSM 1/25/93
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Joycelyn E. Stewart, Ph.D. JES 1/27/93
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 2 Generation Reproduction

GUIDELINE #: 83-4

TOX. CHEM. #: 075A

MRID #: 418879-01

TEST MATERIAL: Carbamic acid, methyl ester

SYNONYMS: Benomyl (99.24%), INT1991-529, DPX-T1991-529
Methyl 1-(butylcarbarmoyl)-2-benzimidazole carbamate

STUDY NUMBERS: 765-90

SPONSOR: E.I. DuPont
Wilmington, Delaware

TESTING FACILITY: Haskell Laboratory
Newark, Delaware

TITLE OF REPORT: Reproduction and Fertility Effects with DPX-T1991-529 (Benomyl) Multigeneration Reproduction Study in Rats

AUTHORS: Charles A. Mebus

REPORT ISSUED: February 21, 1991

CONCLUSIONS:

When DPX-T1991-529 was administered to Crl CDBR rats at dietary levels of 0, 100, 500, 3000 and 10,000 ppm (for males 0, 5-8, 28-38, 168- 234 and 553- 954 mg/kg and for females, 0, 7-9, 35-47, 210 -280 and 712 - 1,168 mg/kg) the reproductive NOEL was 500 ppm and the LOEL was 3000 ppm. The LOEL was based on decreases in the body weights of F2a and F2b offspring on days 14 and 21 of lactation and on decreases in sperm counts reported for F1 parental males. At doses of 3000 ppm and higher, there was testicular pathology in both generations which consisted of atrophy and degeneration of the seminiferous tubules. Oligospermia was also reported at these higher doses.

At 10000 ppm, there were statistically significant decreases in the birth and lactation weights of F1, F2a and F2b pups, decreased testicular weights in P1 and F1 males and decreases in body weights, body weight gains and food consumption in parental

animals. In the F2b offspring, there was an increase in the number of pups with partially opened or unopened eyes and a nonsignificant decrease in pup survival.

CLASSIFICATION: Guideline

MATERIALS: DPX-T1991-529 (99.24%, Lot number F60317K) was the test material. The test animals were male and female Crl: CDBR rats, received from Charles River Laboratories in Raleigh, N.C. The males were 36 days of age at the time of receipt and weighed 122.2 to 191.2 grams. The females were 33 days of age and weighed 72.9 to 115.6 grams.

METHODS:

The doses selected for this study were based on the results of a pilot rat feeding study in which 10 males and 10 females received the test material at 0, 5000, 10000 and 15000 ppm for 32 days. Results included decreased body weights at the mid (88% of controls) and high (81% of controls) dose levels and decreased testicular sperm count at all three dietary levels. The decreased sperm count was not accompanied by a concurrent decrease in testicular weight.

Prior to assignment to treatment groups, animals were weighed and observed daily for clinical signs of disease and/or injury. The acclimation period was approximately 20 days. One hundred fifty animals per sex were selected to serve as the P1 generation; these animals were randomly assigned to 5 treatment groups per sex. (See Table I).

TABLE I
Treatment Groups

Group #	Dose (ppm)	Mean Dose(mg/kg)		# Animals*	
		P1 dose	F1 dose	M	F
I	0	0	0	30	-
II	0	0	0	-	30
III	100	5	8	30	-
IV	100	7	9	-	30
V	500	28	38	30	-
VI	500	35	47	-	30
VII	3000	168	234	30	-
VIII	3000	210	280	-	30
IX	10000	553	954	30	-
X	10000	712	1168	-	30

* The number of animals at each dose level was the same for the F1 parental animals.

58

After assignment to treatment groups, P1 animals were individually housed and received the compound in the diet for 71 days. After this period, male and female rats were housed in pairs that were formed with animals from the same dietary exposure level. Animals were kept together until either the female exhibited evidence of copulation (copulatory plug) or until a three week period had elapsed without evidence of copulation. After either of these events, females were removed from the males and were housed individually.

Females were observed twice daily as they approached the delivery date. After delivery, the number of live and dead pups were determined and body weights and sex were recorded for the pups. On day 4 post-partum, the litters were culled to eight (4/sex, where possible). No examinations were performed on culled offspring. Assessments for behavioral abnormalities and appearance were conducted on days 0, 4, 7, 14 and 21 post-partum. Body weights were measured at these intervals also.

On day 21 of lactation, 30 pups/sex/group from the F1 litters were selected to serve as the parents for the F2a and F2b generations. If possible only 1 rat per sex per group was selected from each litter. Adjustments were made by additional random selection when there were less than 30 litters per group.

On day 21 of lactation, an additional 20 F1 weanlings per group were sacrificed with carbon dioxide and subjected to a gross pathological examination.

The F1 animals that were selected to be parents to the next generation received the test material for at least 105 days . At the end of the 105 day treatment, animals were paired with nonsiblings of the same treatment group and housed and mated in the same manner that was described for the P1 animals. After the last F2 litters were weaned, F1 females were mated to a different nonsibling male from the same treatment group. This resulted in the F2b litters.

The test material was administered daily in the diet for the specified length of time. Diets were prepared weekly and refrigerated until they were used; however, during the final three weeks of the study, the diet was prepared every 10 to 11 days.

Samples were collected on test day one to verify concentration, homogeneity and stability of the test material in the diet. On test day 20, additional samples were collected. Samples for the determination of homogeneity were collected from three locations in the mixer. These samples were frozen until the time of analysis. Stability samples were collected and stored at room temperature for 7 and 14 days before analysis. Samples of the control diets were also frozen upon collection. Concentrations

59

were determined for each dietary level for samples collected from the feeders. Analysis for dietary concentrations of the test material was performed using HPLC methodology.

OBSERVATIONS:

Animals were observed for clinical signs of toxicity, morbidity and mortality at least once daily throughout the study. Moribund animals were sacrificed. Parental animals in both generations were handled at least once weekly and their behavior and appearance were assessed.

Body weights were recorded weekly for all animals during the pre-mating period. Non-pregnant females, those with unknown start of gestation and males continued to be weighed weekly. Pregnant females were weighed on days 0, 7, 14 and 21 of gestation and lactation. Food consumption was determined weekly for all animals during the pre-mating period, and on days 0, 7 and 14 for pregnant rats. Mean daily food intake, intake of the test material and feed efficiency were calculated from food consumption and body weight data.

P1 and F1 adults were sacrificed by exsanguination while under chloroform anesthesia. Scheduled sacrifice was as follows:

Group	Days on treatment
P1 males -	122 to 123
P1 females	115 to 135
F1 males	192 to 212
F1 females	210 to 245

The following tissues were collected from all parental animals: pituitary, testes/ovaries, prostate, seminal vesicles, coagulating glands, uterus, vagina and all gross lesions. Testes were also weighed. Fifteen males per treatment group were randomly selected for determination of sperm counts in the right testicle. The left testicle was subjected to histopathology. For males not selected to undergo this process both testicles were subjected to microscopic examination.

Histopathology was conducted on tissues from the control and high dose groups. Gross lesions and target organs from the low and mid dose groups were also examined histologically.

Any offspring dying during the lactation period were necropsied. Culled pups were discarded without gross examination. Gross examinations were conducted on 20 F1, 20 F2a and 20 F2b weanlings per sex per group.

60

The indices of reproductive performance calculated for P1 and F1 adults were as follows:

mating index(%) = # copulated/ # cohoused X 100

fertility index(%) = # bearing litters/ # copulating X 100

gestation index(%) = # litters with 1 live pup/ # litters

percentage of pups born alive(%) = # pups born alive/ # born

viability index (%) = 3 pups alive d4(precull)/ 3 born alive

lactation index (%) = # alive @ D21/ # alive D4(postculling)

litter survival (%) = # litters weaned/ # viable litters

STATISTICAL ANALYSIS:

ANOVA was conducted on body weights, body weight gain, food consumption, organ weights, spermatid counts and gestation length. Dunnett's test was applied to further analyze these parameters when significant differences among test group means existed. Bartlett's test for homogeneity of variances was performed on organ weights, ($p = 0.005$). Fisher's exact test with Bonferroni correction was used to analyze incidences of gross and microscopic pathological lesions and for reproductive and lactaional performances. The Mann Whitney U test with Bonferroni's correction was used to analyze the number of pups, survival weights, viability index and lactation index. Significance was at $p = 0.05$.

QUALITY ASSURANCE:

A Quality Assurance Statement and a statement of compliance with Good Laboratory Practices were included in the submission.

RESULTS:

Dietary Analysis

Based on the results from the analytical studies conducted to evaluate homogeneity, stability and concentration, it was determined that at all four dose levels, the test material was homogeneously mixed in the diet. Stability was confirmed for 7 and 14 days when the test material was kept at room temperature for dose levels up to 3000 ppm. Concentrations of the test material were greater than 90% of the nominal when the test diet was freshly frozen or refrigerated. At 10,000 ppm, stability studies on samples that were fresh frozen and on samples that were kept at room temperature for 7 and 14 days revealed concentrations of 83%, 80% and 88% of nominal values,

61

respectively. Only the 14 day refrigerated samples at the highest dose level had greater than 90% of the nominal concentration of the test compound.

At doses up to 3000 ppm, feeder samples had dietary concentrations of test material greater than 90%. At the highest dose tested, feeder samples had concentrations that ranged from 76 to 83% of nominal. These figures represented averages of duplicate analyses. Although the lowest concentration in the feed was 76%, it did not compromise the study since it occured at only one measuring interval. (See Table II for summary of dietary analysis).

P1 Generation:

No clinical signs of toxicity were observed in animals receiving the test material during the premating period. Alopecia was reported in the females at the 3000 ppm dose level during gestation, but was not believed to be related to the administration of the test compound. A total of 5 deaths were reported in the various treatment groups, but were not related to the administration of the test material. Dystocia was reported as the cause of death in 3 females (1 control, 2 @ 3000 ppm). One male at 500 ppm had glomerulonephritis and another which received 10,000 ppm had a urinary tract obstruction.

Slight decreases in body weight gains were reported in males at 3000 ppm at weeks 1, 3, 9, 16 and 17. These decreases were sporadic in their occurence and did not appear to be biologically significant. There were no significant differences in food consumption and feed efficiency when these animals were compared to controls. For pre and post-mating measurements at 10,000 ppm, body weights were 81% of controls and body weight gain was 57% of controls. Food consumption was lower in the high dose males beginning on the first week of dosing and continuing throughout the premating period. (See Table III for data on body weight and body weight gain).

In P1 females, there were significant decreases in body weight and body weight gain when the high dose group was compared to controls. At pre-mating, body weights were 17% lower than controls; at gestation, body weights were 19% lower than controls and at lactation, 18% lower. Body weight gain in the high dose group was also significantly lower in P1 females during pre-mating and gestation (43% and 30% lower than controls, respectively). During lactation, the body weight gain was higher than that reported for controls. Food consumption was also less than controls for high dose females during the pre-mating and gestation periods.

There were no statistically significant decreases in the mating and fertility indices or in the length of gestation. In the 3000

62

ppm and the 10000 ppm groups, males had sperm counts that were 21 and 41% lower (respectively) than controls when expressed as the number of sperm per gram of testes weight. No lowered fertility accompanied these reported decreases in sperm count. (See Table V for sperm data and Table VI reproductive and fertility data).

There were no significant differences in litter size and pup survival in the various treatment groups. At the highest dose tested, body weights were lower for both sexes of F1 pups; body size was also smaller for these pups when compared to controls.

At 500 ppm, the number of female pups at post-culling was lower than the number reported for controls; however, this finding was not believed to be associated with the administration of the test material because similar findings were not present at subsequent higher dose levels.

Pathology

The mean final body weights of P1 parental animals in the high dose group was significantly lower than that reported for controls (20% for males and 14% for females). Testicular weights for males at this dose level were also significantly lower than controls. The level of significance was at $p < 0.005$.

Grossly, small testicles were reported in high dose males and small spleens were reported in high dose females. Microscopically, testicular pathology was present at both 3000 and at 10000 ppm and was characterized by atrophy and degeneration of the seminiferous tubules. Oligospermia was evident in the epididymides of P1 males in the high dose group. There were no microscopic lesions that accompanied the small spleens in high dose females. This finding was believed to be related to the small size of the animals at the termination of the study.

F1 Generation

As stated earlier in the report, the birth weights of high dose pups in this F1 generation were significantly lower than controls. Lower body weights were also reported for pups in the 3000 ppm group. Nine deaths (5M and 4F) were reported in the group of high dose animals that were selected to serve as parents for the F2a and F2b generations. These deaths occured when the animals were between days 23 and 25 of age and death was attributed to the small size and weakened condition of the animals. One female at 500 ppm had metritis and one at 3000 ppm had a brain tumor. Both of these adult animals were sacrificed *in extremis*.

In adult F1 males lower body weights and body weight gains were reported at the two highest dose levels. At 3000 ppm the differences between control and treated groups was not statistically significant. At 10000 ppm, the body weights were 39% lower than controls and the body weight gain was 43% lower than controls. Food consumption was lower than controls in these high dose animals and food efficiency was significantly lower during the first four weeks of the study.

In female animals, the body weight in the high dose group was significantly lower than controls (53% lower than controls). Body weight gain was significantly lower than controls up to day 21 of the premating period. During both gestation periods, the body weights were approximately 30% lower than controls in the high dose females. These lower body weights were also carried over into the lactation periods (31 and 24% lower than controls). Decreases in mean body weight gain were also reported during both gestation periods (30 and 36% lower than controls). Weight gain was greater than controls for both lactations, with significant increases being recorded during the second lactation for both the 10000 ppm group and for the 3000 ppm group. (See Table IV for body weight and body weight gain data).

Food efficiency did not appear to be affected by the test material in females during premating or gestation. No measurement was made during lactation.

Reproductive Parameters

There were no significant differences reported for mating and fertility indices and for gestation length when dosed groups were compared to controls. Decreased sperm counts, of a greater magnitude than those reported in the P1 males, were evident in the F1 males. The lower sperm counts were reported in both the 3000 ppm and the 10000 ppm group (30 and 71% lower than controls, respectively); however, the lower counts did not affect fertility. (See Table V for sperm data and Table VII for reproductive and fertility data).

Body weights of male and female pups in the high dose group were significantly lower than the body weights reported for controls. This was true for both the F2a and the F2b offspring. At 3000 ppm, body weight was significantly less than controls on lactation days 14 and 21 in both sexes in the F2a offspring and in females of the F2b generation. In the F2a offspring from the high dose group, partially opened or unopened eyes were observed.

At the highest dose level, there was a decrease in the number of F2b offspring that were alive prior to culling and the survival index was lower (nonsignificantly) than controls for the F2b offspring at the highset dose tested.

Pathology:

In the high dose group, the final body weights were 27 and 40% lower than controls for F1 males and females, respectively. At this dose level, testicular weights were also significantly lower than controls. Grossly, the testicles of these animals were small and microscopically, there was atrophy and degeneration of the seminiferous tubules. Similar gross and microscopic findings were present at 3000 ppm. Additionally, at the two highest dose levels, oligospermia was evident in the epididymides of these males.

DISCUSSION: This study demonstrates a NOEL for reproductive effects of 500 ppm and a LOEL of 3000 ppm based on the decreases in body weights of F2a and F2b offspring on days 14 and 21 of lactation and based on the decrease in sperm count reported in both P1 and F1 males. At 3000 ppm, there were also decreases in body weights and body weight gains in F1 males during the premating period. Testicular pathology was present in both generations at doses of 3000 ppm and higher. The testicular lesions consisted of atrophy and degeneration of the seminiferous tubules. Oligospermia was also reported in the epididymides of F1 males at doses of 3000 ppm and higher.

In addition to the testicular effects reported at 10000 ppm, there were also decreases in the birth weights of offspring in F1 and both F2 generations, reports of small body size in F1 and F2a and F2b generations, decreased testicular weights in P1 and F1 males and decreases in body weights, body weight gains and food consumption in parental animals. In the F2b offspring, at the highest dose level, there was also an increase in the number of offspring that were observed to have partially opened or unopened eyes when compared to controls and a nonsignificant decrease in pup survival.

It should be noted that the reported decreases in sperm count at the two highest dose levels did not affect the fertility of the animals. It is quite possible that fertility in this strain of rats will not be affected until the sperm count falls lower than that which was reported for these animals. Nevertheless, the effects on sperm count are of toxicological significance and are in line with observations that have been previously made in spermatogenic studies conducted with benomyl.

The study is classified as core guideline. It satisfies the requirement for a two generation reproduction as set forth in the Subdivision F Guidelines 83-4.

65

TABLE II
Stability, Homogeneity and Concentration

Stability Sample Average Conc. ppm (% nominal)	Nominal Concentration 100	500	3000	10000
Fresh frozen	95(95)	495(99)	2852(95)	8258(83)
Room temp 7 days	95(95)	497(99)	3083(101)	8025(80)
Room temp 14 days	95(95)	500(100)	2977(99)	8849(88)
Refrig 14 days	96(96)	493(99)	3055(102)	9079(91)

Homogeneity

Layer	100	500	3000	10000
Top	111(111)	494(99)	3057(102)	8465(86)
Middle	113(113)	498(100)	3100(103)	8369(85)
Bottom	114(114)	487(97)	3071(102)	8356(85)

Concentration

Sample Date				
12/14/89	111(111)	469(94)	3049(102)	8269(83)
4/18/90	115(115)	519(104)	3037(101)	7574(76)
7/30/90	115(115)	529(106)	3029(107)	7927(79)

66

TABLE III
Body Weight (g) and Body Weight Gain (g) in P1 Animals

Males Days on Test	Body Weight 0 ppm	Body Weight 10000 ppm	Weight Gains 0 ppm	Weight Gains 10000 ppm
0	348.6	348.8	-	-
7	387.4	347.5*	38.8	-1.3*
14	423.2	375.5*	35.8	28.0*
21	452.4	400.8*	29.2	25.3
28	478.0	415.4*	25.6	14.6*
40	514.6	442.3*	13.2	6.9*
56	543.9	465.1*	19.3	12.0*
70	570.2	478.8*	7.7	0.7*
84	573.2	483.6*	9.0	9.1
98	591.7	498.7*	9.8	11.0
112	615.8	504.7*	10.9	5.5
119	624.6	506.5*	8.8	1.8*
		Day 0-70	221.6	130.0*
		Day 70-119	54.3	27.7*
Females				
Pre-mating				
0	212.9	211.9	-	-
7	238.7	210.2*	25.7	-1.7*
14	253.9	225.6*	15.3	15.3
21	266.4	242.7*	12.4	17.1
40	296.6	259.7*	-0.7	3.5
56	311.3	270.9*	7.8	5.3
70	321.9	274.4*	-1.0	-3.3
		Day 0-70	108.9	62.5*
Gestation				
0	314.6	273.6*	-	-
7	352.7	306.4*	38.1	32.8
14	382.2	324.3*	29.4	17.9*
21	473.0	385.0*	90.0	60.3*
		Day 0 -21	156.7	110.0*
Lactation				
0	368.7	286.3*	-	-
7	366.2	298.9*	-2.5	12.6*
14	370.3	295.1*	4.1	-3.8
21	351.4	308.6*	-17.2	13.5*
		Day 0-21	-15.2	22.3*

No significant differences were found at other dose levels.

* p = 0.05

67

TABLE IV
Mean Body Weight and Body Weight Gain for F1 Adults

Males	Body Weight (g)			Weight Gains (g)		
Conc. (ppm):	0	3000	10000	0	3000	10000
Days						
0	62.2	59.1	30.6*	-	-	-
7	111.5	105.0	47.8*	49.3	45.9	15.8*
14	177.0	165.9*	73.7*	65.5	60.9*	25.9*
21	244.6	228.9*	99.2*	67.6	63.1*	25.5*
28	312.3	291.2*	125.7*	67.5	62.3*	26.5*
42	417.2	394.6*	191.1*	45.3	44.1	32.4*
126	630.2	603.4	374.2*	6.2	11.5	14.9
154	675.7	641.7	408.8*	17.5	11.3	-0.6*
			Day 0-105:	553.6	531.8	316.4*

Females
Pre-mating

	Body Weights (g)		Weight Gain (g)	
Conc. (ppm)	0	10000	0	10000
0	58.8	27.9*	-	-
7	99.0	43.1*	40.3	14.1*
14	146.4	64.8*	47.4	21.7*
21	183.4	87.1*	37.0	22.3*
28	207.5	109.8*	24.1	21.7
56	273.1	173.9*	11.7	13.1
84	305.0	208.8*	5.5	4.4
105	324.7	226.3*	6.1	6.9
		Day 0-105:	265.9	197.3*
Gestation I				
0	322.0	227.7*	-	-
7	358.4	252.9*	36.5	25.2*
14	389.4	274.5*	30.9	21.6*
21	467.8	329.2*	78.4	54.7*
		Day 0 -21	145.8	101.5*
Lactation I				
0	357.6	238.3*	-	-
7	368.9	252.0*	11.8	12.6*
14	373.3	255.8*	4.4	3.8
21	359.4	261.2*	-13.9	5.4*
		Day 0-21	1.7	21.5*

* p = 0.05
Data from the second gestation and lactation periods were similar to the first.

No significant differences were observed at other dose levels

68

TABLE V
Sperm Indices

P1 Conc. (ppm):	0	3000	10000
Parameter			
Mean Testes Weight (g)	1.7	1.7	1.4(18)*
Mean sperm Count (10^6/testis)	123.1	96.5	62.8(51)*
Mean sperm count (10^6/gram testes)	71.9	57.1(79)	42.6(59)*

F1 Conc. (ppm):	0	3000	10000
Parameter			
Mean Testes Weight (g)	1.9	1.8	1.1(58)*
Mean sperm Count (10^6/testis)	138.7	97.3(70)*	40.5(29)*
Mean sperm count (10^6/gram testes)	74.6	57.4	34.1(46)*

* p = 0.05

The numbers in parentheses are percents of the control value.

Data was extracted from Table 49 of the report.

TABLE VI
Reproductive and Fertility Data - P1

Concentration (ppm) Parameters	0	100	500	3000	10000
Mating Index (%)	96.7	93.3	100	100	100
Fertility Index	89.7	78.6	83.3	76.7	76.7
Gestation Length	22.5	22.5	22.5	22.7	22.6
Gestation Index	100.0	100.0	100.0	100.0	100.0
Mean pups/litter					
Born	13.9	13.2	13.5	16.1	13.5
Born alive	13.7	12.9	13.2	15.8	13.5
Preculling	13.6	12.9	13.2	14.9	12.9
Postculling	8.0	7.6	7.6	8.0	7.7
Day 7	8.0	7.5	7.6	8.0	7.7
Day 14	8.0	7.5	7.6	8.0	7.6
Day 21	8.0	7.5	7.6	8.0	7.6
Sex ratio (males)	0.47	0.48	0.58*	0.54	0.50
% Born alive	99.0	97.9	98.1	98.0	99.7
Viability	98.9	99.7	99.7	96.3	96.0
Lactation Index	100.0	98.9	100.0	99.5	98.3
Litter Survival	100.0	100.0	100.0	100.0	100.0
Pup Weights (g)					
Day 0	7.0	6.9	6.8	6.9	5.9*
Day 4	12.0	11.9	11.8	11.3	8.7*
Day 7	19.3	19.3	19.1	18.1	12.7*
Day 14	38.1	38.1	38.7	35.9	21.6*
Day 21	61.4	61.0	62.8	56.9*	30.5*

* p= 0.05, but value is not indicative of reproductive toxicity.

Data tabulated from several tables 39,40 and 43 in the study report.

70

TABLE VI
Reproductive and Fertility Data - F1

Concentration (ppm) Parameters	0	100	500	3000	10000
Mating Index (%)	90.0	100.0	96.7	90.0	96.2
Fertility Index	63.0	76.7	75.9	88.9*	88.0*
Gestation Length	22.7	22.8	22.9	22.9	22.9
Gestation Index	100.0	95.7	100.0	100.0	100.0
Mean pups/litter					
Born	14.0	14.5	14.4	13.7	11.3*
Born alive	13.6	13.3	14.1	13.2	11.1*
Preculling	13.5	13.0	13.5	12.7	10.3*
Postculling	7.6	7.9	7.6	7.8	7.8
Day 7	7.6	7.8	7.6	7.8	7.7
Day 14	7.6	7.8	7.6	7.8	7.4
Day 21	7.6	7.7	7.6	7.8	7.3
Sex ratio (males)	0.44	0.50	0.46	0.54	0.48
% Born alive	95.8	88.5	96.2	96.8	98.8
Viability	99.6	94.0	97.6	92.9	93.2
Lactation Index	100.0	98.3	99.4	100.0	94.0
Litter Survival	94.1	100.0	100.0	95.7	95.5
Pup Weights (g)					
Day 0	7.1	6.7	7.0	6.9	6.0*
Day 4	12.1	11.7	11.8	11.5	8.8*
Day 7	19.5	19.1	19.1	18.4	12.2*
Day 14	39.5	38.5	38.6	35.3*	19.8*
Day 21	64.2	63.3	64.6	56.6*	27.5*

* p = 0.05

Data tabulated from tables 39,41 and 44 in the study report.

TABLE VII
Reproductive and Fertility Data -
F1 Second Gestation

Concentration (ppm) Parameters	0	100	500	3000	10000
Mating Index (%)	96.7	90.0	79.3	96.6	100.0
Fertility Index	69.0	74.1	82.6	82.1	69.2
Gestation Length	22.4	22.7	22.8	22.7	22.8
Gestation Index	100.0	100.0	100.0	100.0	100.0
Mean pups/litter					
Born	14.1	14.4	14.4	14.5	10.6*
Born alive	13.9	14.1	14.2	14.3	10.4*
Preculling	13.7	13.4	13.8	13.7	10.4*
Postculling	7.3	7.5	7.5	7.4	7.5
Day 7	7.3	7.5	7.5	7.4	7.5
Day 14	7.3	7.5	7.5	7.4	7.4
Day 21	7.3	7.5	7.5	7.4	6.9
Sex ratio (males)	0.52	0.50	0.52	0.47	0.51
% Born alive	99.2	97.3	98.5	99.1	96.4
Viability	96.8	94.1	97.8	93.3	94.4
Lactation Index	100.0	95.0	100.0	100.0	92.6
Litter Survival	100.0	95.0	100.0	100.0	88.9
Pup Weights (g)					
Day 0	7.0	6.7	7.3	6.8	6.2*
Day 4	12.0	11.9	12.2	10.9	9.4*
Day 7	19.3	19.6	19.8	18.2	13.0*
Day 14	39.2	39.9	40.0	35.3*	19.9*
Day 21	64.4	65.1	65.9	56.4*	28.9*

* = p = 0.05

Data taken from tables 39, 42 and 45 of the submission.

Benomyl: Reproduction Study in Rats
E. I. du Pont de Nemours & Company. 1972. MRID No. 00088333.
HED Doc. No. 004679.

STUDY TYPE: Reproduction - rat

TOX. CHEM. NO.: 79C

HASKELL LAB. REPORT NO: 195-72
MR NO.: 1149

FICHE/MASTER: 00088333
ACCESSION NO.: 232870-A
232871

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

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

AUTHORS: H.Sherman, S.B.Fritz, L.S.Wasileski

DATE REPORT SUBMITTED: 1972

TEST MATERIAL: 2-benzimidazolecarbamic acid, methyl ester (50 or 70 % a.i., 53 or 72.2 % Tech.) (metabolite of Benomyl)

SYNONYMS: MBC
INE-965
carbendazim

MATERIALS AND METHODS: ChR-CD rats from the 2 year chronic feeding study (HLR 195-72) were used for this study. At 21 days of age they were treated with:

Group	PPM (%a.i.) of MBC
group 1	0 (0)
group II	100 (.01%)
group III	500 (.05%)
group IV	*2500 (.25%) - 10,000 (1.0 %)
group V	5000 (.50%)

*level raised to .75% at 18 weeks and again to 1.0% two weeks later

The diets were prepared weekly and refrigerated until used.
Mating procedure: Sixteen females and 16 males per group were used for the F_0 generation. Each female was mated to each of 3 males for 5 days. Three weeks after mating they were observed twice daily for birth of the F_{1A} litters. All litters were reduced to 10 pups 4 days after birth. The F_{1A} litters were examined at weaning (21 days) and sacrificed. One week later, the F_0 rats were remated to produce the F_{1B} litters. The F_{1B} rats were fed the above mentioned diets after weaning. At 110 days of age 16 males and 16 females were mated using the procedure described previously producing the F_{2A} and F_{2B} litters. F_{3A} and F_{3B} litters were produced using the same procedure with the F_{2B} generation.
Parameters examined: Fertility index*, gestation index**, viability index***, lactation index****, weanling pup weight, live/dead ratio.

* % of matings resulting in pregnancy
** % of pregnancies resulting in birth of live litters
*** % of pups that survived 4 days
**** # pups surviving 4 days / # pups surviving 21 days (weaning)

74

Histopathologic examination of kidney, liver, trachea, heart, lung, brain, testis, bone marrow, spleen, thymus, gastrointestinal tract, adrenal, thyroid, pancreas, sciatic nerve and muscle was performed on 2 males and 2 females from each of 5 litters (20 pups total) from the control, 500 and 10,000 ppm groups in the F_{3B} generation at weaning.

RESULTS: All parameters were the same as the controls except average weight of weanlings.

MBC (ppm)	group	Ave. wt of weanlings (g) F_{1A}	F_{1B}	F_{2A}	F_{2B}	F_{3A}	F_{3B}
0	I	57	60	53	57	53	58
100	II	60	62	53	62	51	63
500	III	60	61	55	60	53	62
2,500*	IV	56	--	--	--	--	--
5,000	V	46	52	38	49	39	46
10,000*	IV	--	41	36	41	39	43

*see note about diet change in group IV on previous page.

No histologic or necropsy changes were observed in the study.

DISCUSSION: It cannot be determined from the description when the females were checked for pregnancy, if at all, and if they were all mated to 3 males or only those who were not already pregnant. There were no litter (or fetal) weights taken at birth, only at weaning. It therefore cannot be determined if the pup weight decrease at levels of 5000 ppm and greater is due to toxicity during the prenatal or lactation period. The number of dams in the test was only 16 (20 for group V), resulting in only 10-16 litters per group rather than the 20 litters recommended in the guidelines. This study, however, did have 6 matings (3 generations) per group which excedes the requirements. Due to the known testicular effect of MBC and Benomyl, special attention should have been given this organ such as organ weights. Nevertheless, histopathological examination of weanlings in the F_{3B} generation (500 and 10,000 ppm groups) showed no testicular lesions.

CONCLUSION: NOEL = 500 ppm
LEL = 5000 ppm based on toxic signs of decreased pup weight noted at weaning.

CORE-CLASSIFICATION: minimum

Review by M.P.Copley, D.V.M.
Tox. Br.
9/21/85

25

Benomyl: 3-Generation Reproduction Study in Rats
E. I. du Pont de Nemours & Company. 1968. MRID No. 00066773.
HED Doc. No. 004679.

004679

STUDY TYPE: Reproduction study - rats TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 264-68(11-67) FICHE/MASTER: 00066773
MR NO.: 966

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

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

§ AUTHORS: H. Sherman

§ DATE REPORT SUBMITTED: November 18, 1968

TEST MATERIAL: Benomyl, 50 or 70% wettable powder; 1-(Butylcarbamoy 2-benzimidazolecarbamic acid, methyl ester; (72.2%† or 51.5-52.0%†† tech.)

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazol 2-yl)-methyl ester
INT-1991
NB- 5409-91
DPX-3866
N.B. 8084-166B

" Reproduction study

Rat, 3-generation, 7-litter.

No. of Animals. 6 M and 6 F/group, F_0 parents (animals left from 90-day study); 12 M and 12 F/group, F_{1b} parents; and 20 M and 20 F/group, F_{2b} parents.

Feeding Levels.* 0, 100, 500, and 2,500 ppm.
Duration. Time to produce 3 generations, 7 litters in all.
Mortality. No effect on numbers of stillborn or on survival to 4 days or to weaning.
Body Weight. Pups from parents at 500 and 2,500 ppm weighed less, at weaning, than control or "100-ppm" pups in the F_{2b}, F_{3a}, F_{3b}, and F_{3c} litters. (See Table, below.) However, the various groups of F_{3c} pups kept on test for 9 weeks post-weaning and for a further 6 weeks on control diets had growth curves of similar slope.
Histopathology. No effect on F_{3b} weanlings. Tissues studied were: Pituitary, thyroid, parathyroid, adrenal, skeletal muscle, sciatic nerve, brain, spinal cord, eye, exorbital lacrimal gland, mammary gland, bone marrow, spleen, thymus, lung, upper trachea, heart, stomach, duodenum, cecum, salivary gland, pancreas, liver, testis or ovary, epididymus or fallopian tube, uterus or prostate, urinary bladder, and kidney.
"No-Effect Level." Conservatively, 100 ppm; since average weanling weights in F_{2b}, F_{3a}, F_{3b}, and F_{3c} litters are low for "500-ppm" and "2,500-ppm" pups, as compared to corresponding control and "100-ppm" values.

rch 25, 1970
M. Quaife, Ph.D.

77

§ Information obtained from the WHO bibliography (Nov.-Dec./83) on Benomyl.
† used through week 10 of the F_{1B} generation
†† used for the remainder of the reproduction study

Table from original review:

" PP Nos. OFO-906
and OGO-936 -8- March 25, 1970

Parameters in this reproduction study are tabulated:

ppm Benomyl	Average Litter Size	Average No. Born Alive	F.I. (%)	G.I. (%)	V.I. (%)	L.I. (%)	Average Weanling Weight (g)
			F_{1a} Litter				
0	11.7	11.2	100	100	94	98	48
100	11.2	11.2	67	100	100	100	54
500	10.2	10.0	83	100	98	98	59
2,500	13.0	12.8	100	100	97	93	57
			F_{1b} Litter				
0	12.5	10.8	100	100	87	98	57
100	13.6	13.2	83	100	97	100	58
500	11.6	10.6	83	100	91	93	62
2,500	13.2	12.[illegible]	100	100	91	100	54
			F_{2a} Litter				
0	10.8	10.4	83	100	95	96	51
100	11.8	11.6	92	100	97	95	56
500	10.6	10.5	83	100	98	97	52
2,500	11.3	11.2	91	100	96	98	48
			F_{2b} Litter				
0	10.8	10.0	92	91	90	99	60
100	13.6	13.6	92	100	100	100	59
500	11.1	10.6	67	100	89	97	52
2,500	12.9	12.6	91	90	96	100	51
			F_{3a} Litter				
0	9.5	8.9	85	100	93	99	56
100	11.3	10.7	75	93	90	98	57
500	9.6	9.5	70	100	98	100	52
2,500	11.9	11.7	80	100	98	99	51
			F_{3b} Litter				
0	13.1	12.6	80	100	95	99	58
100	13.5	13.3	68	92	97	100	59
500	11.1	10.7	70	100	94	99	52
2,500	11.9	10.4	85	100	84	98	54
			F_{3c} Litter				
0	11.6	10.0	65	92	87	100	60
100	11.9	10.5	67	100	87	100	62
500	9.5	8.5	55	100	88	93	52
2,500	13.0	10.6	75	93	79	96	51

78

Addendum to review for clarification of material and methods by M. Copley.

Dietary levels of INT-1991 (using 50% WP):

Group	Treatment
Control (I)	food + 1% CO
Low dose (V)(LDT)	food + 1% CO + 100 ppm INT-1991 (0.01% formulation)
Mid dose (VI)(MDT)	food + 1% CO + 500 ppm INT-1991 (0.05% formulation)
High dose (VII)(HDT)	food + 1% CO + 2500 ppm INT-1991 (0.25% formulation)

CO - corn oil

Species: ChR-CD rats.
Mating procedure: Each F_0 female was exposed sequentially to 3 F_0 males (from the same dietary group) for 5 days. After mating (15 d total) the females were separately housed and examined twice daily till parturition.

F_{1A} were sacrificed at weaning.
F_{1B} - Twelve males and females from each group were mated at 3 months.
F_{2A} were sacrificed at weaning.
F_{2B} - Twenty males and females from each group were mated at 3 months
F_{3A} were sacrificed at weaning.
F_{3B} - Two of each sex from each of five litters/group were examined at necropsy. Those from the control and HDT were examined histologically.

F_{3C} - used for reassessment of growth curve.

All litters were reduced to 10 when necessary. Parameters measured were: No. of pregnancies; no. of survivors at birth, 4, 12 and 21 days; body weight at weaning (21 days).

CONCLUSION: NOEL = 100 ppm
LEL = 500 ppm (decrease in pup weights)

CORE-CLASSIFICATION: core minimum

Original review evaluated and addendum added by M.P.Copley, D.V.M.
Tox. Br.
9/12/85

Benomyl: Developmental Toxicity Study in Rats
E. I. du Pont de Nemours & Company. 1980. MRID No. 00148393.
HED Doc. No. 004679.

004679

STUDY TYPE: Teratology - Rats

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 649-80
M.R.: 3501-001

FICHE/MASTER:
ACCESSION: 256575
GS0119-009

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

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Newark, Del.

AUTHOR: R.E.Staples, J.G.Aftosmis

DATE REPORT SUBMITTED: 9/18/80

TEST MATERIAL: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester; 99.2% a.i.; Lot #71008A.

SYNONYMS: Methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate
Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazol-2-yl)-methyl ester
INT-1991
DPX-3866

MATERIAL AND METHODS: Pregnant ChR-CR® strain rats, 9 weeks old, weighing from 156.4 to 206.1 gm were obtained from Charles River Breeding Labs, Inc. on days 2 and 3 of gestation (2G, 3G). Day 1G was determined by the presence of sperm in the vagina. They were individually housed, assigned unique animal numbers and fed standard rodent chow and water ad libitum. They were randomly assigned to treatment groups on day 6G such that group mean body weights were similar. Treatment with the test compound* was by gavage from days 7 through 16G. The groups were as follows:

Dose level (mg/kg/day)	No. of females
0	60
3.0	27
10.0	27
30.0	27
62.5	27
125.0	27

Observations - Dams were weighed upon arrival and days 6, 7, 11, 16, 18, and 21G (maternal weight gain was calculated for days 7G through 10G) and observed daily.
Sacrifice and necropsy examination - On day 21G, prior to sacrifice by chloroform inhalation, the dams were coded to eliminate bias. The uterus was removed and examined for: number of implantation sites, number and position of live, dead and resorbed fetuses; and number of corpora lutea. The uterus of "non-pregnant" rats was stained with ammonium sulfide to detect very early resorptions.
External alterations - All live and dead fetuses were sexed, weighed and examined for external alterations (2.5x magnification).
Visceral alterations - About half of the live fetuses per litter were fixed in Bouins and examined viscerally for soft tissue

81

* Benomyl suspension was prepared daily and given at a rate of 1 ml/rat/day

alterations using the method of Barrow and Taylor (J. Morph., 127: 291-305(1969). Micro-ophthalmia was determined by measuring the intact globes (eyelids of both eyes removed). Selected eyes were examined microscopically using hematoxylin and eosin stained sections.
Skeletal alterations - The remaining fetuses were prepared for examination 1) eviscerated, 2) fixed in 70 % ethanol, 3) macerated in 1 % aqueous KOH solution, 4) stained with alizarin red S.
Statistical evaluation - Incidence of pregnancy, maternal death and individual alterations were examined using the Fisher exact test. Maternal body weight and weight gain were tested for significance using a 1 way analysis of variance and Dunnett's test. Jonckheere's test was used to test for dose responses. The remaining fetal parameters were tested using the Mann-Whitney U test. $P < 0.05$ was considered the level of significance.

RESULTS: There were no overt clinical signs of maternal toxicity.
Maternal, reproductive and fetal toxicity - There were no significant differences in: maternal body weight or body weight gain, incidence of pregnancy, incidence of corpora lutea, implantation sites, fetal sex ratio, and stunted fetuses between treated and control groups. Fetuses in the 62.5 and 125.0 mg/kg/day groups were significantly lighter than control fetuses (Table 1).
Teratogenicity -
Malformations - There was a significant increase in malformed fetuses (table II) in both 62.5 and 125.0 mg/kg/day groups due mostly to ocular (microphthalmia and anophthalmia) effects and brain (distended lateral ventricles, hydrocephaly) lesions (high dose only). Fused ribs, sternebrae and arches occurred only at the high dose (see table II).
Variations - Several skeletal variations (see table III) including: sternebrai, -hemi, -misaligned, -partially to unossified; and centra, -bipartite, -hemi were significantly increased over controls in the high dose group. Visceral variations were not treatment-related.

DISCUSSION: There was no observed maternal toxicity at any dose; however, Benomyl caused decreased fetal weight at 62.5 mg/kg/day and 125 mg/kg/day. Fetal malformations included hydrocephaly (high dose) and microphthalmia (62.5 and 125.0 mg/kg/day). Haskell laboratories determined their background incidence of microphthalmia between 3/8/76 and 8/20/80 (22 studies). Only 1 case of bilateral anophthalmia and 3 cases of microphthalmia (not litter-mates) were confirmed in 530 litters (with 4,935 fetuses). There were two additional cases of suspected microphthalmia. They concluded that the background incidence for this lesion was 4-6/4935 or 1/1000 fetuses. The study authors speculated that the compound effect on the eye may have also extended to the 10 mg/kg/day dose due to the severity of the microphthalmia in the 2 affected fetuses at this dose and the low background for this lesion. Further studies would be needed to confirm this. There appeared to be

no correlation between microphthalmia and reduced fetal weight.

CONCLUSION: Maternal toxic NOEL > 125 mg/kg/day (HDT)

Fetotoxic NOEL = 30 mg/kg/day
Fetotoxic LEL = 62.5 mg/kg/day based on decreased fetal weight

Teratogenic NOEL remains undetermined until the lowest effect level demonstrating increased microphthalmia is determined. A treatment-related increase of microphthalmia was clearly evident at 62.5 and 125 mg/kg/day.

CORE-CLASSIFICATION: Supplementary until the microphthalmia question is resolved.

This study is Core-Minimum when combined with Haskell study number 587-82 which more clearly defines the NOEL and LEL.

1501-001
A-649-80

TABLE

BENOMYL: TERATOGENICITY STUDY IN THE RAT
WHEN GIVEN BY GAVAGE FROM DAYS 7 THROUGH 16 OF GESTATION

MATERNAL AND REPRODUCTIVE EFFECTS

	mg/kg/day[a]					
	Control	3.0	10.0	30.0	62.5	125.0
Females						
No. mated	60	27	27	27	27	27
No. deaths	0	0	0	0	0	0
No. pregnant -at sacrifice[b]	50	26	24	26	23	20
-by stain only[c]	0	0	1	0	0	1
-total %	50(83)	26(96)	25(92)	26(96)	23(85)	21(78)
Body weight (g±S.D.)[d] -Day 6	220.6±13.84	219.9±12.31	219.0±12.89	219.8±13.97	218.0±13.87	221.7±10.18
-Day 21	367.9±28.31	371.1±31.91	385.0±26.76	367.8±29.80	362.8±27.00	366.0±21.58
Body weight gain (g±S.D.)[d] -Day. 7-18	95.5±13.09	98.5±16.34	107.1±12.16	98.5±13.76	93.1±13.22	94.2±9.74
Implants[d]						
No. corpora lutea	657	317	317	346	296	252
-No./dam(±S.E.M.)	13.1±2.97	13.0±3.14	13.2±3.06	13.1±3.22	12.9±3.48	12.6±2.26
No. implants	520	237	272	250	227	214
-No./dam(±S.E.M.)	10.4±2.49	9.1±3.66	11.3±1.40	9.9±3.17	9.9±2.28	10.7±1.08
No. embryo-fetal deaths (in no. dams)	25(18)	20(13)	21(14)	20(12)	14(8)	33(12)
Avg. % embryo-fetal deaths/dam	4.6±7.64	10.3±15.44	7.9±9.10[f]	9.9±1.17	7.4±17.71	15.4±21.26[e]
No. dams with only resorptions	0	0	0	0	0	0
Fetuses						
No. alive	495	217	251	230	213	181
-No./litter(±S.D.)	9.9±2.40	8.3±3.61[e]	10.0±1.74	9.2±3.33	9.3±2.73	9.0±2.58[f]
Sex -no. males/females	246/249	109/108	115/136	115/123	107/106	88/93
-% males	49.7	50.2	45.8	48.3	50.2	48.6
No. stunted	0	0[e]	1	1	1	0
Fetal weight(g±S.D.)[g]	4.2±0.84	4.1±0.37	4.2±0.25	4.1±0.31	3.8±0.31[e]	3.5±0.51[e,f]

[a] dose based on average body weight on Day 6 of gestation

[b] females with visible sign of pregnancy evident at autopsy

[c] identified as having been pregnant only by ammonium sulfide staining of the uterus; data from these females used only for calculation of "total % pregnant"

[d] includes data only from females with visible sign of pregnancy at autopsy

[e] stunted and dead fetuses were excluded; expressed as average of mean fetus weight/litter

[f] significant dose-response as determined by Jonckheere's test

[e] significantly different from control incidence by two-tailed Mann-Whitney U test

[f] significantly different from control incidence by Mann-Whitney U test but only by one-tailed test

MR-3501-001
HLR-649-80

TABLE II

BENOMYL: TERATOGENICITY STUDY IN THE RAT
WHEN GIVEN BY GAVAGE FROM DAYS 7 THROUGH 16 OF GESTATION

FETAL MALFORMATIONS

	mg/kg/day[a]				
	Control	3.0	10.0	30.0	62.5	125.0
External						
No. examined -fetuses/litters	495/50	217/26	251/24	238/26	213/23	181/20
No. malformed -fetuses/litters	1/1	b		1/1		6/4
Visceral[c,d]						
No. examined -fetuses/litters	237/50	102/23[e]	120/24	113/25[e]	101/22[e]	89/20
No. malformed -fetuses/litters	3/3		2/2	1/1	12/6	23/9
Skeletal[d]						
No. examined -fetuses/litters	258/49[e]	115/26	131/24	125/26	112/23	92/20
No. malformed -fetuses/litters	1/1					4/4
Total no. malformed -fetuses/litters	5/5		2/2	1/1	12/6	26/9
Avg. % malformed fetuses per litter(±S.D.)[f]	0.9±3.2	0.0	0.7±2.3	0.4±2.0	5.1±13.5*	19.0±32.4*ϕ
No. affected fetuses/no. affected litters						
External						
Cleft palate	1/1	b				
Hydrocephaly						2/2
Edema						1/1
Multiple malformations[g]				1/1		
Micrognathia						3/2
Upturned snout						2/2
Visceral[c]						
Microphthalmia/anophthalmia	1/1		2/2	g	10/4[h]	21/8*ϕ
Lateral ventricles - distended						6/5*
Hydronephrosis	2/2				2/2	1/1
Skeletal						
Nasal and premaxilla-short						1/1
Sternebrae-fused						1/1*
Ribs-fused						3/3*
Arches-cervical-spread						1/1
-thoracic-fused						2/2
-cervical-reduced number						1/1
Centra-fused	1/1					

[a] dose based on average body weight on Day 6 of gestation

[b] blanks represent zero incidence of affected fetuses

[c] includes alterations detected in the heads of the fetuses examined for visceral alterations; in the high dose group also includes 2 fetuses with microphthalmia that were examined skeletally

[d] in any fetus includes only malformations at sites other than those detected externally

[e] difference from no. litters examined externally because one or more litters consisted of only one fetus which was examined for only visceral or skeletal alterations

[f] $100 \times \bar{x}\left[\frac{\text{No. malformed fetuses in the litter}}{\text{Total no. of fetuses in the litter}}\right]$ /total no. of litters

[g] F ϕ 257992, f_2 - edema lower neck and jaw, gastroschisis, meningocele, bilateral microphthalmia, 1 digit missing and 1 curved, laterally displaced lower limbs and tail, 3 chambered heart, fused pulmonary artery and aortic arch

[h] 7 of these 10 fetuses were from 1 litter (F ϕ 258027)

* significantly different from control incidence by two-tailed Mann-Whitney U test

ϕ significant dose-response as determined by Jonckheere's test

MR-3501-001
HLR-049-80

TABLE III

BENOMYL: TERATOGENICITY STUDY IN THE RAT
WHEN GIVEN BY GAVAGE FROM DAYS 7 THROUGH 16 OF GESTATION

FETAL VARIATIONS

	mg/kg/day[a]					
	Control	3.0	10.0	30.0	62.5	125.0
Total no. fetuses with variations/litters[b]	293/50	130/25	135/24	140/25	118/22	102/18
Avg. % fetuses with variations/litter (±S.D.)[c]	61 (±20.4)	61 (±26.0)	53 (±21.4)	57 (±25.4)	53 (±20.7)	52 (±22.7)
No. affected fetuses/no. affected litters						
External						
Hematoma	23/18	15/10	14/8	17/14	8/7	9/9
Petechiae	12/11	4/4	7/5	2/2	8/7	5/4
Visceral						
Liver -peliosis		1/1	1/1			
Lens -small pocket	6/5	2/2				1/1
Renal papilla-reduced	91/34	37/13	44/15	33/14	33/15	16/10
Renal pelvis-enlarged		1/1		2/2		
Skeletal						
Skull partially ossified						
-interparietal	4/3	5/3	1/1			
-parietal	1/1					
-supraoccipital	1/1	1/1			1/1	
-squamosal	1/1	2/2		1/1	1/1	
Sternebrae[d]						
-hemi		1/1			2/2	5/3 $^{\phi\dagger}$
-bipartite	1/1				1/1	2/2
-misaligned (2)[e]	7/6	4/3	1/1		4/4	11/9 $^{\#**}$
-misaligned (1)[f]	6/5	5/4	3/3	2/2	4/4	5/4
-unossified	17/10	11/5	9/5	5/5	12/6	11/8 $^{\phi}$
-partially unossified	86/38	31/15	32/15	47/19	36/16	50/17 *
Ribs						
-missing[g]					1/1	
-wavy		1/1		1/1	1/1	
-calloused						1/1
-partially ossified	1/1					
-extra ossification site(s)[h]	112/45	51/20	48/20	50/17 $^{\phi}$	40/17	39/17
-rudimentary[i]	19/12	6/6	6/4	18/12 $^{\phi}$	11/8	12/7
-extra[j]	4/3	1/1	1/1	4/2	5/3	4/3
Centra						
-dumbbelled	14/11	6/5	9/7	9/7	10/9	15/8
-bipartite	3/3	3/3	2/2	3/3	8/7 *	16/9 $^{\#*}$
-fused	1/1					
-hemi						3/3 *
-misaligned						1/1
-unossified					1/1	2/1
-partially ossified						1/1
-displaced						1/1
Arch -partially ossified					1/1	2/2

[a] dose based on average body weight on Day 6 of gestation

[b] the number of fetuses examined were identical to that listed in Table II except that fetuses with malformations were excluded

[c] $100 \times \Sigma \left[\frac{\text{No. malformed fetuses in the litter}}{\text{Total no. of fetuses in the litter}} \right]$ /total no. of litters

[d] variations in sternebra V were excluded

[e] at least two sternebra with lateral halves misaligned by one-third or more of their length

[f] same as for (e) except only one sternebra misaligned

[g] did not appear to be due to technical error

[h] the presence of a fourteenth rib as a round or oval center of ossification

[i] the fourteenth rib was termed rudimentary if its length exceeded twice its width

[j] the fourteenth rib was termed extra if its length was greater than or equal to the length of the preceding rib

[#] significant dose-response as determined by Jonckheere's test

[*] significantly different from control incidence by two-tailed Mann-Whitney U test

[ϕ] significantly different from control incidence by Mann-Whitney U test but only by one-tailed test

[†] significant dose-response as determined by Cochran-Armitage test

[**] significantly different from control incidence by Fisher's exact test

86

Benomyl: Developmental Toxicity Study in Rats
E. I. du Pont de Nemours & Company. 1983. MRID No. 00115674, 00126522.
HED Doc. No. 004679.

004679

STUDY TYPE: Teratology - Rats (microphthalmia) TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 587-82

FICHE/MASTER: 00115674
ACCESSION: 248563
249749

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

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

AUTHOR: R.E.Staples

DATE REPORT SUBMITTED: 1982

TEST MATERIAL: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazol-2-yl)-methyl ester; (99.1% purity)
INT-1991-474
DPX-3866
N.B. 5103-109

Reviewed by Roger Gardner, June 30, 1983

Materials and Methods:

Test substance: Benomyl (99.1% purity, contaminants were not identified) was used. The sample was numbered INT-1991-474, N.B. 5103-109, Lot F00117E

Test Species: Pregnant Crl: CD® (SD) BR rats were used. Day 1 of gestation was the day sperm were detected in vaginal smears.

Experimental Procedure:

Benomyl was suspended in stripped corn oil and administered by gavage at dosages of 0, 3, 6.25, 10, 20, 30, or 62.5 mg/kg. The dosages were administered in 1 ml of vehicle daily from day 7 through day 16 of gestation. There were 46, 47, 47, 48, 47, 47, or 19 animals in the 0, 3, 6.25, 10, 20, 30, or 62.5 mg/kg/day dose groups, respectively.

On day 21 of gestation dams were sacrificed and examined for gross pathological signs. Ammonium sulfide solution was used to determine the incidence of pregnancy in uteri of apparently non-pregnant dams.

Maternal body weights were obtained on day 5 of gestation for the purpose of dosage preparation.

88

At sacrifice the numbers of implantation sites, resorptions, live fetuses and dead fetuses were determined. Fetuses were individually weighted and mean live fetus weights per litter were calculated.

Fetal examinations were limited to the determination of the incidence of external hydrocephaly and microphthalmia. Eye diameters were measured in cases of suspected asymmetrical or small eyes. One measurement was made from the pinna through the center of the eye, and the other was made through the center and perplendicular to the first. The criteria for identification of microphthalmia considered the smaller of the two measurements. If both measurements were at least 0.4 mm less than those in the alternate eye, the smaller eye was classified as microphthalmic. Both eyes of a fetus were classified microphthalmic if the measurement was less than 1.8mm (the smallest diameter found in the control group). A transverse section through the center of both eyes was made freehand, and the eyes were examined for microphthalmia. All measurements and examination were made under magnification (10X).

A transverse section was made throught the widest portion of the head which was then examined for signs of internal hydrocephaly.

The author noted that the litter was considered the experimental unit for statistical analyses. The analyses included the Fisher's exact test for incidence of maternal and fetal mortality and occurrence of fetal effects, the Mann-Whitney U test for significant differences in maternal body weights, one-way analysis of variance and Dunnett's tests for maternal body weights after censoring those animals without live fetuses, dying before scheduled sacrifice, or those bred on the wrong date, and Jonckheere's test for significance of dose-response relationships.

Reported Results:

The author noted that no statistically significant differences between group mean maternal body weights were found.

One dam died on day 11 of gestation because of dosing error (30 mg/kg/day group). Other dams were excluded from the study because of errors in breeding date estimation (detected on the basis of unusually light or heavy litter weights). There were 4, 2, 3, 2, 3, 3, or 1 eliminated from the 0, 3, 6.25, 10, 20, 30 or 125 mg/kg/day groups, respectively.

Pregnancy rate varied from 84.2% (16/19) in the highest dose group to 95.7% (44/46) in the control group. No statistically significant differences were noted by the authors. Only one fetus was found dead (10 mg/kg/day). The highest dose group was reported to contain 1 fetus with microphthalmia and 1 fetus with hydrocephaly in separate litters). The number of litters containing fetuses with hematomas was comparable in the control and treated groups with the exception of the highest dose. In that dose group 11 of 16 litters contained an average of 11 (+ 89)) % fetuses with hematomas (1 to 2 per litter) while 15 of control group litters contained fetuses with hematomas (1 to 2 per litter). Mean fetal weight in each litter was statistically

significantly less an that reported in controls (3.9 + 0.08 g in the highest dose group compared with 4.1 + 0.04 g in the control group; p less than 0.05, Mann Whitney U test, two tailed). The author stated that no statistically significant dose-related effects were detected with respect to these observations as well as the other parameters measured.

Discussion and Conclusions:

This study is intended to evaluate a specific effect on the development of the eyes in fetal rats. The data presented by the author supports the stated conclusion that the lowest teratogenic effect level (LEL) is 62.5 mg/kg/day and that under the conditions of the study described herein a no-observed effect level (NOEL) is 30 mg/kg/day..

Core Classification. Supplementary.* The study was intended to evaluate a specific effect noted in previous studies.

References

Kavlock R.J., N. Chernoff, L.E. Gray, Jr., J. Gray and D. Whitehouse. 1980.

Report on the teratogenic potential of benomyl administered via the oral and dietary routes in the Wistar rat. Health Effects Research Laboratory. Experimental Biology Division, Develpment Biology Branch, U.S. EPA, Research Triangle Park, North Carolina.

Staples, R.E. 1980. Benomyl: Teratogenicity in the rat after administration by gavage. Medical Research Project No. 3501-001. Haskell Laboratory Report No. 649-80."

Roger Gardner 6-27-83

Roger Gardner
Toxicology Branch
Hazard Evaluation Division
(TS-769C)

for WPS 6/30/83

8/18/83

* When these are combined with prior data from same study, the overall CORE grade is minimum

Roger Gardner 8-18-83

90

Benomyl: Developmental Toxicity Study in Rabbits
E. I. du Pont de Nemours & Company. 1995. MRID No. 43788301.
HED Doc. No. 012058.

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

012058

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ID. No. 099101, Reregistration Benomyl, Developmental Toxicity Study in Rabbits 6(a)2

Tox. Chem. No.: 099101
DP Barcode #: D219996
Record No. : S495160

FROM: Melba S. Morrow, D.V.M. SLN 9/13/96
Review Section II, Toxicology Branch I
Health Effects Division (H7509C)

TO: Linda Propst/Susan Cerrelli, Team 73
Reregistration Division (H7508W)

THRU: Joycelyn E. Stewart, Ph.D. 9/16/96 KB 9/14/96
Head Section II,
Toxicology Branch I
Health Effects Division (H7509C)

Registrant: duPont Agriculture Products
Wilmington, Delaware 19880

SUBMISSION:

A developmental toxicity study conducted in rabbits was submitted to EPA . The initial intent of the study was to satisfy data requirements for the United Kingdom.

EXECUTIVE SUMMARY:

When DPXT-1991-529 (benomyl) was administered by gavage to pregnant Hra(NZW)SPF 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**

92

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significance of small renal papillae (in the absence of other renal developmental alterations) is unclear. furthermore, no dose response was present for this finding.

While the incidence of stained tails was statistically significant and could possibly be indicative of maternal toxicity at this high dose level. However in the offspring, the findings were not readily attributable to the administration of the test material. Small papillae were present in 4 control fetuses and involved one litter and in 2 high dose fetuses from two litters . 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 occured as a result of incomplete maturation.

The study is acceptable and satisfies the requirement for a developmental toxicity study in rabbits, in spite of the fact that the dosing interval exceeded that which is recommended in the Guidelines. A copy of the review is attached for your reference.

The study was submitted as a 6(a)2 study; however the results do not warrant such a characterization. The NOEL for developmental toxicity (180 mg/kg) is greater than the NOEL of 30 mg/kg reported for developmental toxicity in rats and the NOEL of 50 mg/kg for developmental toxicity in mice. Although the maternal NOEL of 90 mg/kg is lower than the maternal NOEL reported for rats and mice, it is based on the presence of stained tails, only. No additional data will be needed to address this study as there are no additional concerns relative to the developmental toxicity of the test material, benomyl.

Reviewed by: Melba S. Morrow, D.V.M. MSM 9/13/96
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Joycelyn E. Stewart, Ph.D. JES 9/16/96
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 occured 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 lighest 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 ± 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

Stability and Concentration

Sample site	Concentration (mg/mL) Nominal	Measured	% Nominal
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.

Table III
Developmental Data

Dose Levels (mg/kg)	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
Visceral					
Kidney					
Small papilla					
Size 1	4(1)	1(1)	2(1)[a]	--	--
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 occuring 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.

79

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 occured 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 sternebral 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.

Benomyl: Developmental Toxicity Study in Rabbits
E. I. du Pont de Nemours & Company. 1968. MRID No. 00035352.
HED Doc. No. 004679.

UU 4679

STUDY TYPE: Teratology - Rabbits

TOX. CHEM. NO.: 75A

HAZLETON LAB. REPORT NUMBER: 210-214
MR NO.: 1079

FICHE/MASTER: 00035352
ACCESSION: 091750-G

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

STUDIES PERFORMED AT: Hazleton Lab., Falls Church, Va.

AUTHOR: W.M.Busey

DATE REPORT SUBMITTED: 7/15/68

TEST MATERIAL: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester (50% a.i.)

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazol-2-yl)-methyl ester; (99.1% purity)
INT-1991-99
DPX-3866
N.B. 5103-109

Review by M.L. Quaife, 5/3/71

"We judge following summary to be a fair appraisal of results of study. We add selected values, below (in footnotes), to illustrate findings.

The purpose of this study was to evaluate the potential of fungicide 1991 (Benomyl, Code No. INT-1991-99, powder, approximately 50% active ingredient) for embryotoxic and/or teratogenic effects in (New Zealand White) albino rabbits. The test material was administered in the diet (Purina Rabbit Chow, available ad lib.) at dose levels of 0, 100, and 500 ppm (to 15 each artificially impregnated does/group on days 8-16, of gestation). (Seven or eight does in eachgroup were sacrificed on day 29 or 30 of gestation and the remainder allowed to hutch normally.)†

There were no maternal deaths during the study. One abortion occurred in the low level group. Tissue masses which were apparent fetuses and dead pups were found in the cage pans of one low-level doe and one high-level doe prior to initiation of the treatment period. Both of these animals were sacrificed on Day 6 and were excluded from the study. A total of 34 of 43 does used in this study (excluding the two does which were sacrificed) became pregnant (12 control, 13 low level, and nine high level).

The appearance, behavior, body weight gain*, and food consumption of the test animals were, in general, comparable to the controls. No evidence of a compound-related effect was noted in the following criteria: Findings from gross necropsies performed on the does; the number and placement of implantation sites,** resorption sites,*** or live and dead fetuses**** from Ceasarean deliveries; weight and length of fetuses, fetal external appearance, and gross visceral anatomy; the number of live and dead pups from full-term litters,***** pup weight and length, external appearance, and gross visceral

anatomy. The development and structure of test fetal and pup skeletons (studied after alizarin staining and clearing) were comparable with the control animals and with accumulated control data.

Dietary administration of Fungicide 1991 (benomyl) to female albino rabbits from Day 8 through Day 16 of gestation (at 100 or 500 ppm in the diet) had no discernible effect on fetal development.

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* Mean weight gain during 3-week period for controls, 100-, and 500-ppm females is 413, 421 and 369 g, respectively.

** Implantation sites, 7.3, 7.3, and 8.0/maternal rabbit--control to high level groups.

*** Resorption sites, 0.3, 0.9, and 0.2/maternal rabbit (0- to 500-ppm.

**** Live fetuses, 6.8, 6.0, and 6.8/maternal rabbit and dead fetuses, 0.2, 0.4, and 1.0/maternal rabbit--same progression.

***** Live pups, 6.8, 6.0, and 5.5/maternal rabbit and dead pups, 2.0, 0.6, and 0.3/maternal rabbit--same progression as above."

CONCLUSION: NOEL = 500 ppm (HDT)

CORE-CLASSIFICATION: Supplementary - due to dietary treatment rather than gavage and no maternal or fetal toxicity was evident at the high dose tested.

original review evaluated by M.P.Copley, D.V.M.
Tox. Br.
9/18/85

()† added by M.P.Copley to clarify the original review

Benomyl: 90-Day Feeding Study in Rats
E. I. du Pont de Nemours & Company. 1967. MRID No. 00066771.
HED Doc. No. 004679.

004679

STUDY TYPE: 90 day feeding study - Rats TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 11-67 FICHE/MASTER: 00066771
MR NO.: 924

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

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

AUTHORS: H.Sherman, J.R.Barnes, W.C.Krauss, J.W.Clayton

DATE REPORT SUBMITTED: Jan. 31, 1967

TEST MATERIAL: 70 % wettable powder (72.2% tech.) Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazol-2-yl)-methyl ester
INT-1991
NB- 5409-91
DPX-3866
N.B. 8084-166B

MATERIAL AND METHODS: Weanling albino ChR-CD rats were housed in pairs by sex, given food (with 1 % corn oil) and water ad libitum and observed for abnormal behavior, food consumption and weight gain for 8 days prior to test initiation. Sixteen rats per sex were placed into the following four treatment groups:

Group	Treatment
Control (I)	food + 1% CO
Low dose (V)(LDT)	food + 1% CO + 100 ppm INT-1991 (0.0143% formulation)
Mid dose (VI)(MDT)	food + 1% CO + 500 ppm INT-1991 (0.0714% formulation)
High dose (VII)(HDT)	food + 1% CO + 2500 ppm INT-1991 (0.357% formulation)

CO - corn oil

mg/kg/day = 9, 45 or 214 mg/kg/d for males and 9, 46 or 234 mg/kg/d for females

Observations - Animals were observed at unspecified intervals for toxic signs, mortality and behavior throughout the study.
Body weight - Animals were weighed prior to the test and twice per week thereafter.
Food consumption - Food consumption was measured prior to the test and once per week thereafter, by sex per group.
Laboratory tests - They were done of 6 randomly selected rats per sex per group at 30, 60 and 90 days. Hematology was also done prior to test initiation.
Hematology - White blood cell counts*, hemoglobin conc.*, hematocrit* and differential white blood cell count.
Urinalysis - 24 hr urine vol., conc.(m.osmols/l), protein conten sugar, ketones, pH, color, appearance and presence of occult blood. Pooled samples were used for microscopic examination.

105

* also performed during the pretest examination

Clinical chemistries - plasma alkaline phosphatase and glutamic-pyruvic transaminase activity (GPT), only tested on control and HDT.

Sacrifice - Ten male and 10 female rats* were euthanized with chloroform after 96-103 days of continuous feeding. Tissues were fixed in Bouin's solution and stained with Haskell quadrichrome. The following organs were removed for weight, fixation and staining: brain, heart, lungs, liver, spleen, kidney, testis, stomach, thymus, adrenal and pituitary. The following additional tissues were removed for fixation and staining: ovary, epididymis, Fallopian tubes, prostate, uterus, urinary bladder, duodenum, cecum, colon, skeletal muscle, peripheral nerve, bone marrow, eye, thoracic aorta, spinal cord, tracnea, pancreas, thyroid, parathyriod, salivary gland, and exorbital lacrimal gland.

RESULTS: Mortality - One LDT male died after 39 days, however the the registrant does not attribute the death to treatment. Observations - There was no change in body weight, food consumption, feed efficiency, clinical signs, hematology, urinalysis, alkaline phosphatase, SGPT - except female, organ weights (except liver - female) and histopathology from control values. The HDT female liver weights were elevated over controls by 22 %:

Dose	liver (gm)	liver/body wt. %
cont.	9.50	3.40
LDT	9.04	2.68
MDT	9.40	3.14
HDT	11.60	3.91

The amount of test compound consumed by the rats was similar on a body weight basis for males and females. The rats consumed slightly more then one third the amount at the end of the study then at the start (mg/kg/day).

Select average daily intake of INT-1991 (mg/kg/day)

	males				females			
Days	cont.	LDT	MDT	HDT	cont.	LDT	MDT	HDT
0-6	0	14	73	348	0	14	66	345
41-48	0	7	36	169	0	8	39	195
83-90	0	5	26	124	0	6	32	162

DISCUSSION: The lesions observed in the one mortality in the LDT were not attributed to the compound as no other rats had similar signs at that or higher doses. Although there were no histologic alterations in the liver, the elevated SGPT in HDT males at $p < 0.001$ may be biologically relevant considering the increased liver weight in the HDT females. This is difficult to assess since there were no intermediate groups tested for hepatic enzymes. Several tests required by our (Toxicology Branch) current guidelines were not performed, and no individual animal data was presented in the report. The study, however appears to be well done and the information necessary to set a NOEL is present.

106

*The remaining 6 rats per sex per group were used for a reproduction study reviewed separately. See Haskell report #264-68, MR #966, MRID #66773.

CONCLUSIONS: NOEL 500 ppm
LEL 2500 ppm based on increased absolute and relati liver weights (female) and elevated SGPT levels (males).

CORE-CLASSIFICATION: minimum

Reviewed by M.P.Copley, D.V.M.
Tox. Br.
9/12/85

This study has been reviewed previously by M. Quaife, 3/25/70 and L.B.Dale, 10/23/68.

MBC (Carbendazim): 90-Day Feeding Study in Dogs
E. I. du Pont de Nemours & Company. 1970. MRID No. 00099130.
HED Doc. No. 004679.

CASWELL FILE

004679

STUDY TYPE: 90 day feeding study - Dogs TOX. CHEM. NO.: 79C

HASKELL LAB. REPORT NUMBER: 283-70
MR NO.: 1270 FICHE/MASTER: 00099130

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

STUDIES PERFORMED AT: Haskell Lab. for Toxicology and Industrial Medicine, Wilmington, Del.

AUTHORS: H. Sherman, K.S. Carrol, C.W.Eddy

DATE REPORT SUBMITTED: 1970

TEST MATERIAL: 2-benzimidazolecarbamic acid, methyl ester; 50 % wettable powder (53% tech.), (metabolite of Benomyl)

SYNONYMS: MBC
INE-965

MATERIAL AND METHODS: One year old beagle dogs were given food and water ad libitum (between 4 pm-7 am), observed daily for behavior and weighed weekly for a month prior to test initiation. During this period, blood and urine samples were checked for the parameters listed in the lab. test section. Four males and 4 females were randomly assigned to each of the following treatment groups:

Group	Treatment (based on %a.i.)
Control (I)	food
Low dose (II)(LDT)	food 100 ppm MBC (.01 %)
Mid dose (III)(MDT)	food 500 ppm MBC (.05 %)
High dose (IV)(HDT)	food 1500 ppm MBC (.15 %)**

**Lowered from 2500 ppm due to weight loss

Diets were prepared weekly. The HDT group was gradually given increasing amounts of MBC using the following schedule: 500 ppm - 3 days; 1000 ppm - 2 days; 1500 ppm - 2 days; 2500 ppm for a short time before the dose was lowered to 1500 ppm (week 3) due to decreased food consumption and weight loss.

Observations - Animals were observed daily for toxic signs, mortality and behavior throughout the study.

Body weight and Food consumption - Animals were weighed and food consumption measured weekly.

Laboratory tests - The following tests were done three times during the pretest period and again after 30, 60 and 90 days of treatment.

Hematology - red blood cell count, white blood cell counts (total and differential), hemoglobin conc. and hematocrit.

Urinalysis - Urine vol. (24 hr), osmolality, protein, sugar, acetone, bilirubin, appearance, color, pH, presence of occult blood and microscopic examination for sediment.

Clinical chemistries - Glucose, urea nitrogen, cholesterol, alkaline phosphatase (AP), glutamic-pyruvic transaminase activity (GPT), total protein and albumin/globulin (A/G) ratio.

Sacrifice - All dogs were euthanized by electrocution after 90-105 days of continuous feeding and were examined for gross and microscop changes. Tissues were fixed in Bouin's solution and stained with Haskell quadrichrome. The following organs were removed for weight, fixation and staining: brain, heart, lungs, liver, spleen, pancreas, kidney, testis, prostate, stomach, thyroid, adrenal, thymus and pituitary. The following additional tissues were removed for fixation and staining: ovary, epididymis, Fallopian tubes, uterus, urinary bladder, duodenum, ileum, jejunum, cecum, colon, rectum, muscle, sciatic nerve, bone marrow, eye, aorta, mammary gland, gall bladder, spinal cord, trache, salivary gland, lymph node and skin. All tissues in the control and HDT were examined microscopically however only the liver, kidney and testes were examined at the LDT and MDT.

RESULTS: All animals survived the treatment period. Body weight in the high dose males decreased about 6.8% while all other groups, male and female, gained weight similar to the controls. Food consumption was decreased when the MBC was 2500 ppm, however it returned to control values after the level was lowered to 1500 ppm. The ave. daily dose in mg/kg of MBC received by the dogs was:

	males	females
100 ppm	2.7	2.7
500 ppm	14.4	11.3
1500/2500 ppm	40.7	35.0

There were no treatment related clinical signs or changes in hematologic or urinalysis parameters. Clinical chemistry - AP and GPT were elevated in the HDT males. Albumin was decreased in HDT males and females. Cholesterol appeared elevated at the mid and high levels in males and females.

	Treatment level (ppm)			
	0	100	500	1500
AP (males)	1.0	1.3	1.3	3.5
GPT (males)	14	15	15	78
Alb (males)	3.01	3.12	3.00	2.66
" (females)	3.45	3.35	3.25	2.88
Chol. (males)	136	140	175	189
" (females)	166	147	195	208
Testes wt. (gm)	.0018	.0018	.0017	.0015

There were no treatment related changes in the other clinical chemistry tests. Organ weights - The testes appeared lighter in the high dose dogs (see above table). No other changes were observed. Gross pathology and microscopic pathology - 1 HDT male (#948) and 1 HDT female (#1019) had evidence of hepatic cirrhosis with hepatic cell necrosis, tubular collapse and increased fibrous connective tissue around the triads. One of the HDT males also had diffuse testicular degeneration.

110

DISCUSSION: Toxic signs included weight loss in the HDT males, decreased food consumption only at 1500/2500 ppm, alteration in liver function tests in the: HDT males - AP (incr.),GPT (incr.); HDT males and females - Alb. (decr.); MDT, HDT males and females - Chol. (incr.). The elevated AP and GPT were altered in only 1 (#948) of the four male HDT animals which also had advanced hepatic cirrhosis. These changes as well as the increased Chol. in the mid and high dose dogs (male and female) indicate, as the registrant suggests, liver injury probably due to treatment. Testes weights were decreased in 3 out of the 4 HDT males with one of these dogs having mild diffuse testicular degeneration, also suggestive of a treatment related effect. The registrant considered this degeneration reversible but provided no evidence for this conclusion. HDT Males appeared to be more sensitive to treatment with MBC however, this may be a result of receiving about 14 % more MBC per kg body weight than the HDT females.

CONCLUSION: NOEL 500 ppm (.05 %) (14 mg/kg)
LEL 1500 ppm (.15 %) (41 mg/kg), toxicity consisted of increased alkaline phosphatase, cholesterol and GPT. The target organs demonstrated by histopathological changes at the high dose in 1 out of 4 males appeared to be liver and testes. This is consistent with lesions observed with Benomyl (the parent compound).

CORE-CLASSIFICATION: minimum

Reviewed by M.P.Copley, D.V.M.
Tox. BR.
9/19/85

111

Benomyl: 28-Day Feeding Study in Rats
E. I. du Pont de Nemours & Company. 1990. MRID No. 41607903.
HED Doc. No. 010723.

010723

Reviewed by: Melba S. Morrow, D.V.M. MSM 11/15/93
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Joycelyn E. Stewart, Ph.D. JES 11/22/93
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Twenty -eight day Feeding Study -Mice

GUIDELINE #: N/A

TOX. CHEM. #: 075A

MRID #: 416079-03

TEST MATERIAL: Carbamic acid methyl ester (96.1%)

SYNONYMS: Benomyl, DPX T1991-529, INT1991-529

STUDY NUMBERS: 324-90

SPONSOR: E.I. Dupont
Newark, Delaware

TESTING FACILITY: Haskell Laboratories
Newark, Delaware

TITLE OF REPORT: 28- Day Feeding Study with Benomyl in Mice

AUTHORS: C.S. Van Pelt, DVM, PhD

REPORT ISSUED: June 27, 1990

CONCLUSIONS: When administered to male CD-1 mice at dietary levels of 0, 100, 500, 3750 and 7500 ppm (0, 15.7, 85.4, 586.0 and 1180.0 mg/kg, respectively), benomyl was associated with increases in relative and absolute liver weights, an increase in the incidence of cellular hypertrophy and increased cell proliferation at the two highest doses tested. At 7500 ppm, there was evidence of nuclear hypertrophy and proliferation of the smooth endoplasmic reticulum when sections were examined under electron microscopy. An increase in Cyt P450 activity was also reported after high dose mice had received the test material for 14 and 28 days. The NOEL was 500 ppm (85.4 mg/kg).

In the bioassay study conducted with CD-1 mice, 500 ppm Benomyl was associated with a statistically significant increase in the number of hepatocellular adenomas in males when compared to controls.

CLASSIFICATION: Supplementary. This study was not conducted to satisfy toxicity data requirements.

113

MATERIALS: The test material was a methyl ether of carbamic acid that was 96.1% pure. The compound was incorporated in the diets of the test animals. Male CD-1 rats, 30 days of age and weighing between 18 and 26.5 grams, were the test species.

METHODS: After completing a quarantine period of 29 days, 100 male mice were randomly assigned to one of 5 test groups (20 animals/group). Animals were individually housed in stainless steel cages and were exposed to temperatures of 23° ± 2° C. The relative humidity in the facility was 50 ± 10%. All animals were exposed to a 12 hour light/dark cycle.

The test groups were as follows:

Group #	Concentration (ppm)	Mean Dose (mg/kg)
I	0	0
II	100	15.7
III	500	85.4
IV	3750	582.0
V	7500	1180.0

The test material was administered via the diet to the test animals. Controls received a ration that had been subjected to the same mixing procedures as the other groups.

Concentration, Homogeneity and Stability:

The test material was mixed with a Purina Rodent Chow diet for 20 minutes in a low speed mixer. Diets were prepared weekly and refrigerated. Samples were collected prior to the start of the study and analyzed for concentration, homogeneity and stability of benomyl. Samples were collected from the diet mixer and from the feed jars and stability was determined for samples stored at room temperature for 7 and 14 days. Stability was also determined for samples that were refrigerated. At least one sample was collected for each dose level and an analysis was performed.

Parameters for Evaluation:

Body weights were measured every fourth day and food consumption was determined at each weighing interval. Food consumption and body weight data were used to determine mean individual food consumption, food efficiency and test material intake. Animals were observed daily for clinical signs of toxicity.

On day 14 and on day 28 of dosing, 5 animals from each group were sacrificed by exsanguination while under anesthesia with pentobarbital. Approximately 2 hours prior to sacrifice, animals were intraperitoneally injected with 100 mg/kg of BRDU, in order to evaluate cell proliferation in the liver. Following

sacrifice, animals were necropsied and the livers were weighed. Liver to body weight ratios were determined.

Livers were fixed in 10% neutral buffered formalin (NBF) and several sections of the liver were processed. One section was stained with hematoxylin and eosin for microscopic evaluation; another was immunohistochemically stained for BRDU incorporation into the nuclei of hepatocytes for the purpose of evaluating cell proliferation. Additional sections were fixed in modified Karnovsky's solution for evaluation under the electron microscope in order to determine whether there was proliferation of peroxisomes and smooth endoplasmic reticulum.

An additional five mice per group were selected from the low and both intermediate groups for peroxisomal beta-oxidation and cytochrome P-450 measurements. These animals were sacrificed by exsanguination while under CO_2 anesthesia after receiving the test material for 13 or 27 days. On days 14 and 28 of exposure, the same number of animals in the control and high dose groups were sacrificed for the same purpose. The livers were removed and homogenized and the homogenate was separated by centrifugation into peroxisomal and microsomal fractions. Protein concentrations were determined for both fractions; cytochrome P450 contents were measured and the peroxisomal fractions were assayed for B-oxidation activity using labelled palmitoyl CoA as the substrate.

STATISTICAL ANALYSIS:

Body weight, weight gain, P-450 content, B-oxidation and liver weights were analyzed using ANOVA. Dunnett's was used on these same parameters for pair-wise comparison between test and control groups. Fisher's Exact, Bonferroni correction and Cochran Armitage (if needed) were used to evaluate clinical observations. Bartlett's Test for homogeneity of variance was conducted on organ weight, Cyt-P-450 content and B-oxidation data. Wilk-Shapiro, F-test and Student's T test or Mann-Whitney U were used to analyze cell proliferation data. Significance was at $p = 0.05$.

QUALITY ASSURANCE:

A statement of compliance with GLPs and a signed Quality Assurance Statement were included in the document.

RESULTS:

The test material was found to be stable under the following storage conditions: fresh frozen, 7 and 14 days at room temperature and 14 days of refrigeration. It was determined that the test material was homogeneously mixed in the diet and cage site feeder samples were all within 20% of the nominal concentration after the initiation of the study. In samples taken before the start of the study, there was an apparent mixing

error that resulted in less than half of the nominal concentration being accounted for in the 100 ppm group. (See Table I for results).

There was no effect on body weight or on body weight gain at doses up to 7500 ppm. No compound related clinical signs were reported and all animals survived until they were designated for sacrifice. No gross abnormalities were reported at necropsy.

Liver weights were increased in the two highest dose groups at 14 days (relative) and at 28 days (relative and absolute). Animals in the two highest dose groups had an increase in the incidence of centrilobular hepatocellular hypertrophy and at the highest dose tested, nuclear hypertrophy and proliferation of the smooth endoplasmic reticulum were observed under electronmicroscopy.

After 4 weeks of benomyl in the diet, there was an increase observed in the labeling index (cell proliferation) at the two highest doses tested. This finding was not considered statistically significant, but may be biologically significant when considered with the other hepatic alterations.

No compound related increases or decreases in peroxisomal activity were reported; however, Cyt-P450 content was significantly increased in the highest dose tested at both 14 and 28 days on the test diet. The level of induction at these intervals was 35 and 29% higher than controls, respectively. At 100 and 500 ppm, there appeared to be a decrease in Cyt P450 activity. The decreases were observed at 28 days and were 73% of the control activity for animals receiving 100 ppm and 64% of control activity for the 500 ppm animals.

DISCUSSION:

At dietary levels of 3750 and 7500 ppm, benomyl was associated with toxicological changes in the liver which included increases in relative and absolute liver weights, increases in the incidence of centrilobular hypertrophy and increased cell proliferation as indicated by the labeling index. At the highest dose tested (7500 ppm), nuclear hypertrophy and proliferation of the smooth endoplasmic reticulum were observed under electrom microscopy and cytochrome P450 activity was significantly increased at days 14 and 28 of dosing.

Although the cell proliferation increases were not statistically significant, when these results are considered with the other hepatic changes there appears to be a correlation between increased cell proliferation, increased liver weights and induction of cytochrome P450 activity. All of these findings would be the type of changes that would be suggestive of an adaptive response of the liver following exposure to high levels of benomyl in the diet.

While cytochrome P450 activity was increased at the two highest dose levels, the activity was markedly depressed at the two lowest dose levels (100 and 500 ppm) at the 28 day evaluation only. The authors suggested that the observed depression in Cyt P450 activity may have been related to some inhibitory effect of the compound at lower dose levels and that the inhibitory effect is probably masked when high concentrations of the test material are available to the system.

This suggestion may or may not be valid. The observed decrease in activity occured only at the 28 day evaluation period, with no similar findings being present at the 14 day interval. However, the real biological significance of this finding can not be determined since it appears to be incidental in nature.

The study is classified core- supplementary. It was not conducted to addresss any of the toxicology data requirements established under 40 CFR 158. The study was conducted to evaluate the possible mechanisms of liver tumor induction in male mice receiving the test material. Based on the results, the investigators concluded that benomyl was not a direct acting (genotoxic) carcinogen, but acted indirectly through physiological mechanisms of alteration to produce liver cancer in CD-1 mice.

TABLE I
STABILITY, CONCENTRATION, HOMOGENEITY

Stability

Sample	Nominal Dose (ppm)	Average Dose Measured (ppm)	% Nominal
fresh frozen	100 *	28	28
7-Day RT	100	37	37
14-Day RT	100	43	43
14 day refrig.	100	39	39
fresh frozen	500	511	102
7 day RT	500	479	96
14 Day RT	500	476	95
14 day refrig.	500	485	97
fresh frozen	3750	3708	99
7 day RT	3750	3598	96
14 day RT	3750	3648	97
14 day refrig.	3750	3769	101
fresh frozen	7500	6395	85
7 day RT	7500	6387	85
14 day RT	7500	6656	89
14 day refrig.	7500	6957	93

* At 100 ppm, for samples that were analyzed prior to the start of the study, a mixing was reported at the lowest dose level.

Concentration (from feeder samples)

Date of Sample	Nominal Conc.(ppm)	Average Dose Measured (ppm)	% Nominal
3/1/90	100	116	116
3/23/90	100	110	110
3/1/90	500	492	98
3/23/90	500	519	104
3/1/90	3750	3678	98
3/23/90	3750	3762	100+
3/1/90	7500	6760	90
3/23/90	7500	6971	93

TABLE I (Con't.)

Homogeneity

Sample	Nominal Dose (ppm)	Average Dose Measured (ppm)	% Nominal
Top	100	27	27
Middle	100	27	27
Bottom	100	26	26
Top	500	481	96
Middle	500	481	96
bottom	500	467	93
Top	3750	3603	96
Middle	3750	3607	96
Bottom	3750	3549	95
Top	7500	6542	87
Middle	7500	6743	90
Bottom	7500	6367	85

TABLE II
PARAMETERS for EVALUATION of HEPATIC EFFECTS

Parameter	Benomyl (ppm) 0	100	500	3750	7500
Mean body weight (g)	36.6	36.2	34.3	37.3	35.7
Mn. body wt. gain (g)	3.6	3.6	3.6	3.8	3.5
Mn. abs. liver wt @ 14d (g)	1.66	1.79	1.92	1.93	1.84
Mn. rel. liver wt @ 14d (g)	4.83	5.00	5.16	5.50*	5.48*
Mn. abs. liver wt @ 28d (g)	1.83	1.85	1.80	2.28*	2.31*
Mn. rel. liver wt. @ 28d (g)	4.99	5.11	5.27	6.11*	6.40*
Incidence of hypertrophy @14d	0/5	0/5	0/5	5/5	5/5
Incidence of hypertrophy @28d	0/5	0/5	0/5	4/5	5/5
Labeling index[a] @ 2 weeks	0.12	0.08	0.14	0.12	0.20
Labeling index @ 4 weeks	0.22	0.16	0.16	0.34	0.42
P450 @ 2 wks (nmol/mg protein)	0.80	0.87	0.87	0.79	1.08*
P450 @ 4 wks (nmol/mg protein)	1.21	0.88*	0.77*	0.96	1.56*
Peroxisome B oxidation @14d	30.04	28.90	23.70	23.11	23.94
Peroxisome B oxidation @28d	24.53	26.09	25.41	28.61	23.30

* p = 0.05

a = % BrdU positive hepatocytes per 100 hepatocytes evaluated

Data in this table compiled from several Tables throughout the submission.

Benomyl: Acute Neurotoxicity Study in Rats
E. I. du Pont de Nemours & Company. 1993. MRID No. 42817003.
HED Doc. No. 010625.

075A

CASWELL FILE

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

010625

OCT 12

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: ID. No. 099101, Benomyl, Acute Neurotoxicity Study

Tox. Chem. No.: 075A
DP Barcode #:D193152
D193405 (duplicate)
Record No. : S444607
S444975 (duplicate)

FROM: Melba S. Morrow, D.V.M. MSM 9/23/93
Review Section II, Toxicology Branch I
Health Effects Division (H7509C)

TO: Linda Propst/Susan Cerrelli PM 73
Reregistration Division (H7508W)
and
TO: Carl Grable, Team 21
Registration (H7505C)

THRU: Joycelyn E. Stewart, Ph.D. JS 9/30/93 KB 10/5/93
Section Head, Review Section II
Toxicology Branch I
Health Effects Division (H7509C)

CONCLUSIONS: When DPX-T1991-529 was administered to Crl:CDBR, VAF plus rats (Sprague Dawley) at doses of 0, 500, 1000 or 2000 mg/kg body weight, no signs of neurotoxicity were observed. Food consumption was significantly decreased for all treated males and for mid and high dose females on the first day following treatment. Loose stools were observed in male animals receiving 500 mg/kg and higher. Testicular lesions were present in the low and mid dose males which were expressed as changes in size (large and small testes reported) and flaccidity. While no dose response was evident in this study, testicular atrophy has been reported in other studies with this test material.

Females receiving 2000 mg/kg had reduced motor activity on the day of dosing which appear to be associated with the administration of the test compound, but which by itself is not indicative of neurotoxicity.

The NOEL for acute neurotoxic effects is > 2000 mg/kg.

A copy of the DER is attached for your reference.

122

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Reviewed by: Melba S. Morrow, D.V.M. MSM 7/27/93
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Joycelyn E. Stewart, Ph.D. JES 9/27/93
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Neurotoxicity

GUIDELINE #: 81-8

TOX. CHEM. #: 075A

MRID #: 42817003

TEST MATERIAL: DPX-T1991-529 (purity 97.4)

SYNONYMS: Benomyl, MBC, Carbamic acid (ester)

STUDY NUMBERS: HLO 825-92

SPONSOR: E.I. DuPont
Newark, Delaware
&2
TESTING FACILITY: Argus Research Labs
Horsham, Pa.

TITLE OF REPORT: Acute Neurotoxicity Study of DPX-T1991-529 (Benomyl) Administered Orally Via Gavage to Crl:CDBR VAF/Plus Rats

AUTHORS: John H. Foss, Ph.D.

REPORT ISSUED: June 14, 1993

CONCLUSIONS: When DPX-T1991-529 was administered to Crl:CDBR VAF plus rats (Sprague Dawley) at doses of 0, 500, 1000 or 2000 mg/kg body weight, no signs of neurotoxicity were observed. Food consumption was significantly decreased for all treated males on the first day following treatment and for mid and high dose females. Loose stools were observed in male animals receiving doses of 500 mg/kg and higher. Females receiving 2000 mg/kg had reduced motor activity on the day of dosing that was believed to be associated with the administration of the compound but was not indicative of neurotoxicity, because it occurred only on dosing day 1 and activity returned to levels comparable to activity levels in the control groups. Additionally, there was no neuropathology associated with the observation and no other clinical signs indicative of neurotoxicity were present. Testicular lesions were present in the low and mid dose males and were expressed as changes in size (large and small testes reported) and flaccidity. While no dose response was present, testicular atrophy has been reported in other studies with this test material.

123

The NOEL for acute neurotoxic effects is > 2000 mg/kg body weight.

CLASSIFICATION: Acceptable
TOX. CATEGORY: N/A

MATERIALS: DPX-T1991-529, a white coarse powder was dissolved in 0.5% methyl cellulose (Lot # F60317K, 94.7% pure) and served as the test material. Crl: CDBr (Sprague Dawley) rats were the test species. At the time of their arrival to the test facility, males were 52 days of age and females were 51 days of age. Males weighed from 165 to 231 grams; females weighed from 140 to 179 grams.

METHODS: After a one week acclimation period, ten animals per sex were randomly assigned to one of the following dosage groups. Males were assigned to groups I - IV, females to groups V to VII.

Doses

GROUP	# Animals	DOSAGE		
		Vol (ml/kg)	Conc (mg/ml)	Dose (mg/kg)
I	10M	10	0	0
II	10M	10	50	500
III	10M	10	100	1000
IV	10M	10	200	2000
V	10F	10	0	0
VI	10F	10	50	500
VII	10F	10	100	1000
VIII	10F	10	200	2000

Doses were selected based on the results of a pilot study and on an LD 50 of 10,000 mg/kg reported in the literature. The preliminary study was conducted at 2000 mg/kg (limit dose) with no clinical signs of toxicity being reported. This resulted in the selection of test doses of 0, 500, 1000 and 2000 mg/kg. Control animals received the vehicle, aqueous methyl cellulose as a single dose that was administered orally by gavage. The study was run in two replicates. Half of the animals in each dose group were assigned to each replicate.

Homogeneity and concentration of the test material were determined from samples that were collected from each dose group from top, middle and bottom portions of the the mixture containing the active ingredient and the and the vehicle. Analysis of the amount of active ingredient was conducted using spectophotometry and homogeneity was determined by calculating the coefficient of variance for average concentrations measured in the top, middle and bottom samples.

Animals were housed individually in stainless steel cages and each animal remained in the same cage until sacrifice on days 20 to 23 of the study. The room temperature was 70 to 78° F and the relative humidity was 40 to 70%. Animals were maintained on a 12 hour light/dark cycle. Food and water were provided ad libitum.

Animals were observed for viability twice daily. Body weights were recorded weekly, prior to dosing, on the day of dosing and and on the days when the FOB and the motor activity were assessed. Physical exams were conducted on the day of dosing, and daily during the post treatment period. Feed consumption was also measured at these same intervals. The Functional Operational Battery (FOB) was assessed on five separate occasions (prior to dosing, 2-hours post-dosing, and on days 1, 7 and 14 post-dosing). Motor activity was evaluated after sets of 10 males and 10 females were evaluated in the FOB. Body temperatures for each rat were recorded following completion of FOB.

Parameters for evaluation in the FOB included:

1. Autonomic function: lacrimation, salivation, palpebral reflexes, pupillary reaction to light, prominence of the eyes, piloerection, urination and defecation.
2. Sensorimotor responses to visual auditory tactile and painful stimuli.
3. Excitability- open field reactions to handling, behavior
4. Gait -open field, any abnormalities, righting reflexes, placing response and landing foot splay
5. Forelimb and hind limb grip strength
6. Abnormalities and clinical signs

The FOB scoring system was numerically based , whereby 1's were indicative of less intense or absent responses to stimuli and 4's and higher were indicative of unusual or extreme reactions. Pre-dosage values were provided for all animals for both the FOB and motor activity assessments.

Observations were blinded. Motor activity sessions were 1.5 hours in duration and activity was monitored by infrared sensors. Assessments were made for the number of movements and the time in movement for each 5 minute block. At the termination of the study, 14 days post-dosing, animals were sacrificed and necropsied. <u>In situ</u> perfusion was accomplished by 10% neutral buffered formalin. Gross examinations were conducted on nervous tissue (peripheral nerves, brain, spinal column) of animals not selected for neurohistology.

Rats selected for neurohistology had the following tissues trimmed for microscopic assessment after being immersed in 10% Neutral Buffered Formalin (NBF): Gasserian ganglia, spinal cord (3 sections with dorsal root ganglia and nerve roots, sciatic, tibial, fibular and sural nerves and the brain. Peripheral nerves were embedded in plastic; other tissues were embedded in paraffin. Tissues were appropriately stained with hematoxylin and eosin, luxol fast blue/cresyl violet or toluidine blue.

Study Validation and Positive Control Information:

The results of a study conducted with several positive control substances (acrylamide, carbaryl, DDT, IDPN and d-amphetamine were provided for validation of the test facility's FOB, motor activity and neuropathology procedures. The FOB for these positive control chemicals included the same parameters that were evaluated in the pivotal study with DPX T-1991-529. Doses for the positive control chemicals were based on literature and on preliminary observations made at the test facility. The results from these studies indicated that neurotoxicity could be detected using the procedures outlined for DPX-T1991-529.

Both FOB and motor activity assessments were conducted for all positive control compounds except D-amphetamine. This compound had only the FOB evaluated. Rats administered acrylamide, IDPN and their vehicles were perfused <u>in situ</u> with formalin and the central nervous tissues were fixed for histological examination. Animals receiving all of the positive control substances were subjected to clinical and necropsy observations and measurements of both body weights and food consumption were recorded. Validation studies were not run concurrently with the main study.

QUALITY ASSURANCE:

A statement of QA dated June 14, 1993 was included along with a statement of compliance with GLPs dated (6/21/93).

STATISTICS:

The FOB was analyzed using Bartlett's test followed by ANOVA, if indicated. For non- parametric data, Kruskall Wallis was used to analyze the data, followed by Dunn's test to compare groups. Motor activity was analyzed using an analysis of variance with repeated measures. Where appropriate ($p \leq 0.05$) the analysis of variance was followed by Dunnett's Test. All tests were evaluated at $p \leq 0.01$ and $p \leq 0.05$.

RESULTS:

The test material was homogeneously distributed in all dose groups and the concentration of active ingredient in each of the dose groups reached expected levels for both replicates. The percent of the nominal concentration was > 90% for all dose groups in both replicates for all (top, middle and bottom) sampling sites.

All rats survived to scheduled sacrifice. No clinical signs were visible in any of the treated groups and there were no significant differences reported in the body weights for either of the sexes. In treated males, there was an increase in the the incidence of soft stools following treatment (0/10, 1/10, 3/10 and 7/10 for control, low, mid and high dose groups, respectively) on study day 2 only. Three high dose animals had urine or fecal stained fur that was probably secondary to the soft stools. Food consumption was also significantly lower for all treated males on day 1 following treatment. Miosis was reported in 3/10 high dose males prior to treatment. This condition persisted in one of the affected animals but the condition was not considered treatment related because of its detection prior to dosing.

Testicular abnormalities were reported for low and mid dose males (3/10 and 5/10, respectively) and consisted of changes in size and flaccidity.

Food consumption was decreased in females in the mid and high dose groups (35% and 32%, respectively) when compared to controls.

FOB Results:

Under the major headings for components of the FOB, no treatment related abnormalities were reported for either sex at any reporting time.

Motor Activity Results:

No abnormalities in the number of movements or the time in movement were reported for males. Females in the high dose group had decreased motor activity in several of the time periods (blocks) on the day of treatment. The decreased activity in the different blocks was reflected in an overall decrease in motor activity for the first day only and returned to a level that was consistent with animals in the other treated groups. (See Table I for motor activity results in females).

Neurohistology:

No lesions were observed in peripheral nerves, spinal column sections, ganglia or the brain tissue of animals selected for microscopic evaluation. In males, absolute brain weights were decreased by approximately 6% at 500 and 2000 mg/kg. Relative brain weights were comparable for all dose groups.

DISCUSSION:

No neurotoxicity was observed in the study as indicated by the lack of clinical signs (no abnormalities in gait or posture, FOB normal, no tremors or convulsions) gross observations (no gross abnormalities of peripheral nerves, spinal cord, brain or ganglia) and neuropathology. The author states that the NOAEL in this study was < 500 mg/kg based on decreases in body weight gains in males (MD and HD); decreases in food consumption in both sexes on the first day following treatment (all males and MD and HD females) and the observation of soft stools in all treated male groups.

Body weight changes in males represented only a 2 gram reduction over the baseline mean body weight of 315.1 grams for the mid dose group and a 5.3 gram reduction for the high dose group, which had a baseline mean body weight of 317.5 grams. These reductions in body weight, while being statistically significant when compared to controls, are not considered biologically meaningful.

Food consumption on the day following treatment (designated as day 1-2) was 17%, 34% and 34% lower than controls for low, mid and high dose males, respectively. In females the food consumption was 35 and 31% lower than controls for mid dose and high dose groups. Food consumption returned to a level that was comparable to controls and occasionally exceeded controls.

Soft stools were observed in all treated males. The incidence of this clinical observation increased with the administration of an increasing dose. In females, there was a decrease in the motor activity at the high dose level at the first reporting period. While this finding may have been related to the administration of the compound, it is not considered to be a neurotoxic effect. The finding was a single observation on the day of dosing only, and was not associated with either clinical signs, changes in the FOB or histological lesions of the CNS or of the peripheral nerves. In subsequent assessments, motor activity was comparable to that observed in other groups and occasionally exceeded the activity reported in the other groups.

In males, the most notable finding that was associated with the administration of the test material was the observation of testicular and epididymal lesions following a single dose of benomyl. Changes in size and testicular flaccidity were reported at incidences of 0/10, 3/10, 5/10 and 1/10 for control, low, mid

and high dose males, respectfully. While there does not appear to be an actual dose response, the association between the test material and testicular lesions (specifically atrophy) has been established in at least one other toxicity study and should not be dismissed as being an incidental finding in this study.

The study is acceptable and satisfies the requirement for an acute neurotoxicity study.

TABLE I
MOTOR ACTIVITY IN FEMALES

DOSE (mg/kg)	0	500	1000	2000
Interval	Mean Number of Movements			
Predose	476.1	504.8	502.8	502.2
SD	122.5	162.6	135.4	114.6
Day 1	553.4	569.1	622.2	337.0
SD	160.8	206.3	275.6	120.7
Day 2	482.3	464.7	466.3	412.4
SD	125.5	122.2	212.3	116.2
Day 8	450.3	499.7	497.6	537.0
SD	111.1	174.0	208.8	169.1
Day 15	516.9	578.9	505.8	482.3
SD	139.5	196.5	200.9	214.1
Interval	Mean Time in Movement (seconds)			
Predose	771.4	884.0	842.8	956.8
SD	189.2	306.8	241.4	377.3
Day 1	1014.0	972.2	1056.6	630.2
SD	344.3	423.5	498.0	279.3
Day 2	750.0	742.2	687.6	610.3
SD	210.8	171.8	333.3	222.5
Day 8	762.8	854.5	866.9	984.0
SD	315.9	259.2	408.1	380.3
Day 15	843.3	1017.7	836.9	860.6
SD	244.6	340.3	361.3	462.9

Values taken from Appendix C, Table C3.

Benomyl: 95-Day Neurotoxicity Study in Rats
E. I. du Pont de Nemours & Company. 1994. MRID No. 43277901.
HED Doc. No. 011558.

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

011555

#075A

MAY 19 1995

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: ID. No. 099101, Subchronic Neurotoxicity Study for Benomyl

Tox. Chem. No.:075A
DP Barcode #:D206051
Record No. : S470972
PC CODE : 099101

FROM: Melba S. Morrow, D.V.M. MM 3/28/95
Review Section II, Toxicology Branch I
Health Effects Division (H7509C)

TO: Linda Propst/Susan Cerrelli, Team 73
Registration Division (H7505C)

THRU: Joycelyn E. Stewart, Ph.D. JES 5/11/95
Head, Section II
Toxicology Branch I
Health Effects Division (H7509C) KB 5/17/95

EXECUTIVE SUMMARY

Toxicology Branch I has completed our review of the Subchronic Neurotoxicity Study in Rats for Benomyl. (MRID 432779-01). The following conclusions have been made with regard to the study.

DPX T1991-529 (97.4%, lot # F60317K) was administered for 92 to 95 consecutive days in the diets of male and female Crl:CDBR rats (Sprague Dawley) at levels of 0, 100, 2500 or 7500 ppm. These dietary levels correspond to calculated doses of 0, 6, 158 and 456 mg/kg in males and 0, 8, 199 and 578 mg/kg in females. There were no compound related deaths and no biological or statistical differences between control and treated animals with respect to the pretest FOB parameters. Administration of benomyl at the stated doses did not result in any unusual behavior, alterations in gait or other clinical observations during the FOB assessment. Terminal body weight and body weight gain were both decreased at the highest dose tested in both sexes. Body weight was 15% lower for males and 12% lower for females when compared to controls and body weight gain was approximately 25% lower than controls for both sexes. Motor activity was also increased at 7500 ppm for males and females. No neuropathological lesions attributable to

132

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the administration of benomyl were observed when tissues were subjected to histological examination.

The NOEL was 2500 ppm and the LOEL was 7500 ppm based on decreased terminal body weight (15% for males and 12% for females), decreased body weight gain (approximately 25% for both sexes) and increased motor activity. Based on the results, the compound is not considered a neurotoxicant because the increase in motor activity was observed at a dose level where systemic toxicity occurred.

The study satisfies guideline requirements for a subchronic neurotoxicity study for 85-2.

Note: This study was submitted as 6(a)(2) data; however, after screening by the 6(a)(2) Team, it was forwarded to Toxicology Branch I with the recommendation for a non-expedited review. Based on review of the data, the 6(a)(2) classification is not warranted.

011558

Reviewed by: Melba S. Morrow, D.V.M. MSM 3/28/95
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Joycelyn E. Stewart, Ph.D. JES 5/1/95
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Neurotoxicity

GUIDELINE #: 82-5

TOX. CHEM. #: 75A

MRID #: 432779-01

TEST MATERIAL: DPXT1991-529 (97.4%)

SYNONYMS: Methyl 1-butylcarbamoyl-2-benzimidazole carbamate
Benomyl, Benlate

STUDY NUMBERS: HLO551-93

SPONSOR: E.I. duPont
Newark, Delaware

TESTING FACILITY: Argus Research
Horsham, Pa.

TITLE OF REPORT: Subchronic Neurotoxiciy Study of DPXT1991-529 (Benomyl) Administered Orally via the diet to Crl:CDBR VAF/Plus Rats

AUTHORS: John A. Foss

REPORT ISSUED: June 13, 1994

EXECUTIVE SUMMARY: DPX T1991-529 (Benomyl, 97.4%, lot # F60317K) was administered for 92 to 95 consecutive days in the diets of male and female Crl:CDBR rats (Sprague Dawley) at levels of 0, 100, 2500 or 7500 ppm. These dietary levels correspond to calculated doses of 0, 6, 158 and 456 mg/kg in males and 0, 8, 199 and 578 mg/kg in females. There were no compound related deaths and no biological or statistical differences between control and treated animals with respect to the pretest FOB parameters. The administration of benomyl at the stated doses did not result in any unusual behavior, alterations in gait or other clinical observations during the FOB assessment. Terminal body weight and body weight gain were both decreased at the highest dose tested in both sexes. Body weight was 15% lower for males and 12% lower for females when compared to controls and body weight gain was approximately 25% lower than controls for both sexes. Motor activity was also increased at 7500 ppm for

134

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CLASSIFICATION: Guideline. This study satisfies guideline requirements for a subchronic neurotoxicity study as set forth in 82-5.

MATERIALS: Animals were male and female Crl: CDBR rats, that were 38 days of age at arrival to the testing facility and weighed from 100 to 142 grams for males and 76 to 120 grams for females. DPX T1991-529 (Lot # F603 17K), a white powder with a purity of 97.4% was the test material.

METHODS: Animals were acclimated for a period of 1 week prior to being assigned to dose groups which consisted of 11 animals/sex/group. They were individually housed and kept in an environment that had a temperature range of 70 to 78°F and a relative humidity of 40 to 70%. Animals were maintained on a 12 hour light/dark cycle and food and water were available ad libitum.

The test material was administered in the diet for 92 to 95 days. The test groups were as follows:

Group	Concentration (ppm)	Dose mg/kg	# Animals M	F
Control	0	0	11	11
Low	100	6(M), 6(F)	11	11
Mid	2500	158(M), 199(F)	11	11
High	7500	456(M), 578(F)	11	11

Control diets were formulated with the vehicle.

Analysis for stability and homogeneity of the test material in the feed were conducted on the first day of exposure to the test material. Stability was analyzed under various storage conditions and homogeneity was determined with samples collected from top, middle and bottom portions of the feeders. Samples were also collected on days 43 and 85 of exposure and were stored at room temperature for a maximum of 7 days prior to freezing. Homogeneity was determined by calculating the coefficients of variation of the average measured concentrations of active ingredients for the top, middle and bottom samples from each dietary concentration level.

135

Animals were assessed for viability twice daily. Clinical condition and general health were assessed weekly during the acclimation period and daily during the study. Body weights were recorded weekly, on days that the Functional Operation Battery and motor activity assessments were made, and at sacrifice. Food consumption was recorded weekly.

The Functional Operation Battery (FOB) was conducted prior to exposure to the test material, and at 4, 8 and 13 weeks of exposure. The FOB was conducted in a blind fashion and included evaluation of the following:

- autonomic function (lacrimation, salivation, palpebral closure, eye prominence, pupillary reflex, piloerection, urination, respiration and defecation),

- sensorimotor responses to visual, auditory , tactile and painful stimuli

- reactions to handling in open field

- gait patterns in open field and gait and sensorimotor coordination, including righting reaction, visual placing and landing foot splay

- grip strength

- abnormal clinical signs including convulsions, tremors, behavioral abnormalities, hyper/hypotonia, dehydration and presence of oral, ocular and nasal deposits

Motor activity was evaluated after the rats had been examined in the FOB. Motor activity was monitored by infrared sensors mounted outside the stainless steel cages and each session recorded the number of movements over 1.5 hours. During the sessions, the number of movements and the time spent in each movement were recorded every 5 minutes. The sensors were tested twice annually for their accuracy and adjustments were made to reduce variation.

The motor tests were conducted in sets comprised of 11 males and 11 females. All animals in all of the groups were tested and each sex was assigned to a different block of cages. Groups were counter balanced across locations so that at least one rat from each group was assigned to each block of cages. Cages were not changed for individual rats for subsequent motor activity sessions.

At the end of the study, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital and sacrificed by whole body perfusion with heparinized saline followed by a solution of 3.0% paraformaldehyde, 3.0% glutaraldehyde and 0.5% picric acid in a phosphate buffer.

136

Gross lesions observed at necropsy were preserved in 10% neutral buffered formalin (NBF). Sciatic and tibial nerves were examined in 6 rats per sex in the controls and high dose groups. Other tissue samples, from these two groups, selected for histological processing included gastrocnemius muscle, brain, gasserion ganglion, dorsal root ganglia, ventral root fibers and spinal cord.

STATISTICAL ANALYSIS: Bartlett's, Analysis of Variance, Dunnett's, Kruskall Wallis and Dunn's tests were used to analyze parameters in the FOB ($p < 0.05$). Data from motor activity tests were analyzed using ANOVA and Dunnett's ($p < 0.05$). Clinical incidence data were analyzed using the Variance Test for Homogeneity of Binomial Distribution

QUALITY ASSURANCE: A statement of quality assurance (dated June 13, 1994) and a statement of compliance with GLPs (dated June 21, 1994) were included in the submission.

Study Validation and Positive Control Information:

The results of a study conducted with several positive control substances (acrylamide, carbaryl, DDT, IDPN and d-amphetamine were provided for validation of the test facility's FOB, motor activity and neuropathology procedures. The FOB for these positive control chemicals included the same parameters that were evaluated in the pivotal study with DPX T-1991-529. Doses for the positive control chemicals were based on literature and on preliminary observations made at the test facility. The results from these studies indicated that neurotoxicity could be detected using the procedures outlined for DPX-T1991-529.

Both FOB and motor activity assessments were conducted for all positive control compounds except D-amphetamine. This compound had only the FOB evaluated. Rats administered acrylamide, IDPN and their vehicles were perfused <u>in situ</u> with formalin and the central nervous tissues were fixed for histological examination. Animals receiving all of the positive control substances were subjected to clinical and necropsy observations and measurements of both body weights and food consumption were recorded. Validation studies were not run concurrently with the main study.

RESULTS:

Homogeneity, Stability and Concentration:
When preparations were analyzed for concentration and homogeneity, it was determined that the diets were within the targeted concentration range (± 20% of the nominal value). The coefficients of variation ranged from 1.2 to 2.0%, indicating a homogeneous mix of the test material in the diet. The test substance was also determined to be stable under a variety of storage conditions such as 14 day refrigeration and room temperature for up to 7 days. (See Table I).

137

Clinical Observations

There were no compound related deaths reported in any of the dose groups for either sex. No behavioral alterations were reported for either sex that could be attributed to the test material.

In males, there was a significant ($p < 0.05$) decrease in body weight reported on weeks 4, 8 and 13 (high dose, only). See Table II. There were also reports of transient reductions in feed intake in the high dose male group. During the period of exposure to DPX-T1991-529, the food consumption for this dose group was 14% lower than that reported for controls, and may have resulted in the reported body weight decrements in this sex.

Pretreatment FOB in males did not reveal any differences between groups for the parameters that were assessed. Additionally, after dosing, there were no observations in the FOB that could be associated with the administration of the test material. In high dose animals, observations included alopecia, chromodacryorrhea, chromorhinorrhea and stained fur; however, there was no significant increase in frequency and these observations were for the most part, singular occurances.

In the pretreatment assessment of motor activity in males, there was one statistically significant increase in activity reported in high dose males at 55 minutes and one incidence of statistically significant activity increase in low dose males at 35 minutes. With regard to motor activity assessment, DPX T1991-529 appeared to have an effect on the motor activity recorded in high dose males. High dose males had increased movements and a non-significant increase in the time spent in motion at 4, 8 and 13 weeks. In these animals, the patterns of movement (in blocks 6 -10 and block 13) reported at week 13 were significantly different from controls ($p < 0.05$, $p < 0.01$). No other significant differences in motor activity were reported for the other groups of males following dosing. (See Table III for total number of movements and total time spent in motion).

There were no gross or microscopic lesions that were associated with the administration of the test material in male rats of any dose group.

In females, there were no treatment related effects reported in the FOB. Body weight gains were significantly lower ($p < 0.01$) at various intervals during the study and at termination in high dose females. The body weight gain reported for the high dose group was 25% lower than that reported for controls at the termination of the study. During the in-life phase of the study, body weight gain for the high dose group ranged from 39 to 60% lower than that reported for controls at specific intervals. No effects on body weight or on body weight gain were reported at the two lower dose groups. (See Table II). Lower body weight gain corresponded to lower feed consumption in the highest dose group for days 1 to 8 of the study. Feed consumption was also

significantly lower than that reported for controls for days 1 to 92 of the study.

In the motor activity assessment, the number of movements reported for the 7500 ppm females was increased when compared to controls for weeks 4, 8 and 13; however, statistical significance ($p < 0.05$) was only reported at week 4. The increase in movements observed in females at the high dose was similar to that reported for high dose males. No other compound related effects were reported for females receiving 100 or 2500 ppm of the test material. There were no reported differences in motor activity between groups of females in the pre-exposure phase of the study. (See Table III).

In females, there were no gross lesions and no histological lesions that suggested nerve damage.

DISCUSSION:

Based on the results of this subchronic study, DPX-T1991-529 was not considered to be a specific neurotoxicant. Statistically significant increases in motor activity were reported in males and females at the highest dose administered, 7500 ppm. However, at this dose level, systemic toxicity was present as evidenced by decreases in both body weight and body weight gain (at termination, 25% lower than controls for both males and females). The systemic NOEL was 2500 ppm and the systemic LOEL was 7500 ppm based on decreases in body weight and body weight gain and increases in motor activity in both sexes.

The study satisfies the guideline requirements for a Subchronic Neurotoxicity study. (82-5(b)

The results reported in this study are consistent with those in the previously conducted neurotoxicity study in Crl:CDBR rats, in which benomyl did not produce neurotoxic sugns when tested at doses as high as 2000 mg/kg body weight.

TABLE I
Concentration, Stability and Homogeneity

	Nominal Dietary Concentration (ppm)		
	100	2500	7500
Homogeneity			
Top Sample	96.3	2590	7110
%	(96.3)	(104)	(94.8)
Middle Sample	98.4	2510	6920
	(98.4)	(100)	(92.3)
Bottom Sample	98.3	2560	7200
	(98.3)	(102)	(94.5)
Stability			
7 Day Room temperature	95.0	2560	6720
	(95.0)	(102)	(89.6)
14-Day refrigeration	112.0	2730	8670
	(112)	(109)	(116)
7 Day room temperature#	113.0	2520	7090
	(113)	(101)	(94.5)

second sample taken after start of study.

Data taken from Table I of the submission.

140

TABLE II
Mean Body Weight and Body Weight Gain (g)

	Dose (ppm)			
	0	100	2500	7500
Males				
Day				
1	241.0	238.4	236.1	236.4
8	309.3	308.6	300.8	277.3**
15	352.5	352.8	341.6	320.8**
22	399.8	401.1	382.6	359.6**
36	454.5	455.6	431.1	405.8**
50	494.9	492.8	464.9	432.6**
64	540.5	531.3	505.3	468.6**
78	565.0	559.4	531.5	488.3**
92	590.4	578.8	548.4	504.2**
Gain				
1-8	68.3	70.3	64.7	40.8**
8-15	43.3	44.2	40.8	43.5
15-22	47.3	48.3	41.0	38.8*
36-43	19.5	18.5	17.0	17.8
57-64	24.4	21.4	19.0	18.4
78-85	10.5	7.6*	9.9	5.9*
85-92	14.8	11.7	6.9**	10.3*
1-termin.	358.6	350.4	321.4	270.6**
Females				
1	173.4	169.4	168.7	170.9
8	207.5	202.7	202.0	191.8**
15	230.4	224.7	224.3	216.7
22	254.4	244.5	243.9	230.7**
36	276.5	265.7	266.0	256.3
50	293.4	289.4	287.2	269.5
64	314.4	302.7	299.8	280.2**
78	323.8	318.4	310.7	287.6**
92	331.0	323.8	320.3	292.3**
Gain				
1-8	34.1	33.3	33.3	20.9**
8-15	22.9	22.0	22.3	24.9
15-22	23.9	19.8	19.6	14.0
36-43	9.7	13.1	12.1	12.1
57-64	11.6	6.4	6.8	4.6
78-85	0.3	0.6	5.2	1.4
85-92	6.9	4.8	4.4	3.2
1-termin.	163.1	156.8	151.5	121.6**

* $p < 0.05$
** $p < 0.01$
Data taken from Tables B5, B6, C5 and C6 of report.

141

TABLE III
Motor Activity

Males	Dose (ppm)			
	0	100	2500	7500
Pre-exposure				
Total movements	507.0	634.1	501.7	635.9
+ SD	159.4	161.0	141.9	174.1
Time in motion	866.0	1076.5	887.9	1013.3
± SD (sec)	293.2	204.9	287.8	275.1
Week 4				
Total movements	583.4	571.5	596.6	813.0
+ SD	273.6	262.3	172.0	272.7
Time in motion	1127.5	1071.4	1115.5	1463.3
± SD	455.1	457.7	339.2	576.6
Week 8				
Total movements	481.2	532.0	604.4	748.9**
+ SD	138.8	195.9	202.4	207.7
Time in motion	821.8	887.1	1028.4	1267.9
± SD	231.2	326.7	401.0	551.1
Week 13				
Total movements	442.9	519.7	448.8	656.4**
+ SD	105.1	153.0	125.4	223.8
Time in motion	725.4	789.1	784.4	1021.7
± SD	244.9	291.9	216.0	383.0

Motor Activity (cont'd)
Females

	Dose (ppm)			
	0	100	2500	7500
Pre-exposure				
Total movements	753.4	701.6	651.5	694.4
+ SD	135.5	162.5	186.0	236.2
Time in motion	1062.0	992.8	1046.9	1004.1
±SD	188.4	210.9	413.2	296.8
Week 4				
Total movements	647.4	703.0	662.2	938.9
+ SD	160.3	344.0	180.8	353.6
Time in motion	1120.4	1152.2	1113.5	1599.4
±SD	175.5	458.7	272.7	562.2*
Week 8				
Total movements	593.5	667.1	666.4	796.8**
+ SD	286.9	235.1	207.7	207.0
Time in motion	891.8	1016.2	1032.5	1371.0
±SD	438.6	390.7	321.4	515.2
Week 13				
Total movements	612.8	586.6	689.4	762.2
+ SD	241.2	237.5	225.5	261.4
Time in motion	897.9	776.1	1040.4	1248.1
±SD	417.1	287.7	387.2	565.0