[redacted]

313-1601
4679

[redacted]

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

004679

10/24/85

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Toxicology Branch Chapter of the Registration Standard for Benomyl

Tox. Chem. No.: 75A

TO: H. Jacoby (PM 21)
Registration Division (TS-767C)

FROM: Marion P. Copley, D.V.M.
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Jane Harris, Ph.D., Section Head
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)
and
Theodore M. Farber, Ph.D., Branch Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

J.P. Harris 10/21/85

WMB 10/24/85

Attached is the Toxicology Branch (TB) chapter for the registration standard for benomyl including the following six subparts.

Generic data tables
Policy discussion
Data gaps
Tolerance reassessment
Bibliography
Toxicology Branch "one-liners"

cc: Esther Saito, SIS, HED
Judy Heckman, HED
Robert Coberly, TOX, HED
Charles Frick, TOX, HED
Henry Jacoby, RD
Marion Copley, TOX, HED
Jane Harris, TOX, HED
Caswell file, TOX, HED

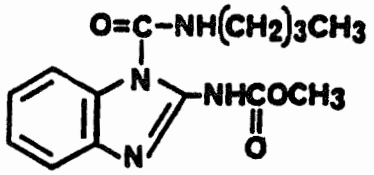
10/1(

004679

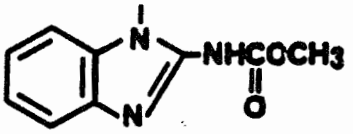
POLICY DISCUSSION

A. Introduction

Benomyl, a systemic fungicide used in many agricultural and non-agricultural sites. The structure of benomyl (methyl-1(Butylcarbamoyl)-2-benzimidazolecarbamate) and its major metabolite MBC (methyl 2-benzimidazole carbamate) are:



BENOMYL



MBC

The United States Environmental Protection Agency (EPA) issued a notice of Rebuttable Presumption Against Registration (RPAR) and Continued Registration of Pesticide Products containing Benomyl (Dec. 6, 1977) based on mutagenic effects and reduction in spermatogenic activity. The Position Document 2/3 (Aug. 39, 1979) determined that risks of concern included mutagenic effects, reduction in spermatogenic activity and acute toxicity to aquatic organisms. The Position Document 4 (Oct. 1, 1982) stated that new data indicated benomyl had oncogenic potential in mice.

As stated in the PD 1, 2/3 and 4, benomyl rapidly hydrolyses to MBC in an aqueous environment. MBC also appears to be the initial metabolite in mammalian systems. The acute and chronic toxicity of MBC is either similar or more severe than the parent compound, benomyl. For the above mentioned reasons, the Agency, in the PD 4 has used MBC data to confirm and supplement benomyl data where applicable.

The major regulatory action taken by the agency was to require the use of a dust mask by persons during mixing and loading of benomyl for aerial exposure. It was estimated that this would reduce the respiratory work-related component of exposure by 90 %. Dermal exposure was considered slight and not considered in the EPA risk estimate because of minimal dermal absorption (approximately 0.5 % per hour).

B. Use Summary

Benomyl is a systemic fungicide registered for many agricultural and non-agricultural uses. The crops and their respective tolerances will be discussed in the tolerance reassessment section. Non-agricultural uses include ornamentals and turf. Benomyl is available as a technical (95 %) and under the trade names of Benlate® and Tersan 1991® by E. I. duPont de Nemours and Co., Inc. Formulations include: formulation intermediate (FI; 50 %), granular (G; 1.1, 1.5, 1.57, 1.6 and 1.95 %), wettable powder (WP; 25 and 50 %), flowable concentrate (FlC; 0.25 lb/gal or 3 %, 75%(dry)), soluble concentrate/liquid (SC/L; 0.72 lb/gal or 10 %).

004679

C. Toxicology Profile

1. Acute Effects

a. Acute oral toxicity (00097277)

10,000 mg/kg of technical benomyl was given by gavage to 10 each of male and female ChR-CD rats. There were no mortalities. The acute LD_{50} was greater than 10,000 mg/kg.

Toxicity Category: IV

Core-Grade Classification: Minimum

The requirement for an acute oral study is satisfied.

b. Acute dermal toxicity (00064822)

Five each of male and female New Zealand white rabbits were treated dermally with 2000 mg/kg of Benlate* (75% a.i.). The substance was applied to shaved and abraded skin for 24 hr. There were no death within 14 days. The acute dermal LD_{50} was greater than 2000 mg/kg.

Toxicity Category: III

Core-Grade Classification: Guideline

The requirement for an acute oral study is satisfied.

c. 1. Acute inhalation toxicity (00097599)

Six male rats were treated with either 0, .27, 1.39 or 4.01 mg/l (actual conc.) of benomyl, 50 % wettable powder. There were no deaths in the treatment groups. All groups however, had slight to severe aspermatogenesis. The acute inhalation LC_{50} was greater than 4.01 mg/l. There is no reason to expect the toxicity category to change with females.

Toxicity Category: III

Core-Grade Classification: minimum

2. Acute inhalation toxicity (00097281)

This study was designed to determine the NOEL for aspermatogenesis due to acute inhalation exposure. Ten male ChR rats were exposed for 4 hr to benomyl, 50 % wettable powder in an inhalation chamber at

3

* Benomyl is the active ingredient

004679

0, .02, .12, .20 or .82 mg/l. There were no deaths at any treatment level. Aspermatogenesis was present only in the 0.82 mg/l group (HDT).

Core-Grade Classification: minimum

These studies satisfy the requirement for data on acute inhalation toxicity.

d. 1. Primary eye irritation (00064820)

Benlate®Dry Flowable* (75 % a.i.), when applied to rabbit eyes, produced corneal opacities which were reversible by 11 days without irrigation. Mild iritis and conjunctivitis were present for only 3 days after treatment. Washing was effective in decreasing the severity and duration of the lesions.

Toxicity Category: II

Core-Grade Classification: Guideline

2. Primary eye irritation (00084579)

Benlate®Dry Flowable* (75 % a.i.), when applied to rabbit eyes, produced corneal opacities which were reversible by 7 days without irrigation. Mild iritis and conjunctivitis were present for only 3 days after treatment. Washing was effective in decreasing the severity and duration of the lesions.

Toxicity Category: III

Core-Grade Classification: Minimum

The toxicity category for eye irritation for labelling purposes is II since one study had corneal opacities present at 8 days, reversible by 11 days. These studies satisfy the requirement for data on primary eye irritation.

e. Primary skin irritation (00064821)

Six New Zealand white rabbits were exposed to Benlate®Dry Flowable* (75 % a.i.), in an aqueous paste for 24 hr. There was mild irritation which was reversible by day 6. The day 1 PIS was .67.

Toxicity Category: IV

Core-Grade Classification: Guideline

4

This study satisfies the requirement for data on primary skin irritation.

f. Dermal sensitization (00097289)

Benomyl technical was applied to the skin of 10 male guinea pigs. It was a mild to moderate sensitizer.

Core-Grade Classification: minimum

This study satisfies the requirement for data on dermal sensitization.

2. Subchronic Toxicity (oral)

a. Subchronic oral rodent (00066771)

Benomyl, 70 % WP (wettable powder) (72.2 % a.i.) was incorporated into the diet of 4 ChR-CD rats per sex per group at the following concentrations; 0, 100, 500, and 2500 ppm for 90 days. The only toxic signs were increased relative and absolute liver weight (female) and increased SGPT (male) values, both in the high dose. The NOEL was 500 ppm. The LEL, based on SGPT and liver weight increases, was 2500 ppm.

Core-Grade Classification: minimum

b. Subchronic oral non-rodent (00066785)

Benomyl, 50 % WP (51.1 % tech.) was incorporated into the diets of 4 beagles per sex per group at the following concentrations, 0, 100, 500, and 2500 ppm for 90 days. Toxic signs included depressed albumen/globulin (A/G) ratio and increased SGPT in HDT males throughout the study. The NOEL was 500 ppm. The LEL, based on increased SGPT and a depressed A/G ratio, was 2500 ppm.

Core-Grade Classification: minimum

c. Subchronic oral non-rodent (00099130) (MBC)

MBC, 50 % WP (53 % tech.) was incorporated into the diets of 4 beagles per sex per group at the following concentrations, 0, 100, 500, and 2500 (lowered to 1500 at 3 weeks due to weight loss) ppm for 90 days. Toxic signs included increased alkaline phosphatase and SGPT in HDT males, increased cholesterol in mid and high dose males and females, decreased albumen in high dose males

* Benomyl is the active ingredient.

and females. Absolute testicular weight was decreased in high dose males. One high dose male and female had hepatic cirrhosis and necrosis. One high dose male had diffuse testicular degeneration. The NOEL was 500 ppm. The LEL, based on altered liver function tests, liver histology, decreased testicular weight and testicular degenerattion, was 2500 ppm.

Core-Grade Classification: minimum

These studies satisfy the requirement for data on subchronic oral toxicity in rodents and non-rodents.

3. Subchronic Toxicity (dermal) (00097287)

a. Benlate® [redacted] (a.i. 51.5 %) was applied daily (5 out of every 7 days) for 3 weeks to the skin of New Zealand rabbits. The following doses were used: 0, 50, 250, 500, 1000 and 5000 mg/kg (based on % a.i.). Testicular weights (relative and absolute) were nonstatistically decreased at 1000 mg/kg and above. The NOEL was 500 and the LEL, based on decreased testicular weights, was 1000 mg/kg.

Core-Grade Classification: minimum

This study satisfies the requirement for subchronic dermal toxicity testing.

4. Subchronic Toxicity (inhalation)

a. Subchronic inhalation

There is currently no subacute inhalation study. This study is required for registration because: 1) farm applicators may receive repeated exposure to benomyl since large fields or orchards may require multiple days to complete fungicide application. Custom applicators may be exposed daily for the length of the growing season. 2) The lowest effect level for spermatogenic inhibition in an acute rat inhalation study was 33 mg/kg (NOEL = 7.5 mg/kg). The margin of safety is only 21 since applicators (mixer/loader) may be exposed to as much as 0.35 mg/kg/day by the inhalation route (see PD4).

5. Neurotoxicity (GS0119-007)

a. Benomyl was tested in hens at 500, 2500 and 5000 mg/kg. There was no indication of delayed neurotoxicity.

This study satisfies the requirement for data on neurotoxicity.

004679

6. Reproduction and Fertility Effects (feeding)

a. 3 Generation Reproduction Study - rats (00066773)

Benomyl 50 or 70 % WP (dose based on % a.i.) was administered in the diet at 0, 100, 500, and 2500 ppm 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 F_{3b} weanlings. F_{3c} pups were used for a post weaning growth curve study. No treatment related effects were seen with the exception of pup weanling weights in F_{2b}, F_{3b} and F_{3c} litters at 500 and 2500 ppm as compared to control values. The NOEL was 100 ppm and the LEL, based on decreased pup weanling weights, was 500 ppm.

Core-Grade Classification: minimum

This study satisfies the requirement for data on reproduction effects.

b. 3 Generation Reproduction Study - rats (00088333) (MBC)

MBC 50 or 70 % WP (dose based on % a.i.) was administered in the diet at 0, 100, 500, 2500 (raised to 10,000 ppm at 20 weeks), and 5000 ppm to male and female ChR-CD rats for 3 generations (6 litters). Sixteen males and females were mated for each generation. Histology was performed on F_{3b} weanlings from 0, 500 and 10,000 ppm litters. No treatment related effects were seen with the exception of decreased weanling weights at 5000 and 10,000 ppm as compared to control values. The NOEL was 500 ppm (25 mg/kg) and the LEL, based on decreased pup weanling weights, was 500 ppm (250 mg/kg).

Core-Grade Classification: minimum

7. Teratogenicity

a. Rabbits (00035352)

Benomyl (50 % purity) was added to the diets of 15 New Zealand white albino rabbits at each of the following doses: 0, 100 and 500 ppm for days 8 through 16 of gestation. Fetuses were examined either after sacrifice on days 29 or 30 of gestation or after natural parturition. There were no treatment related fetal or maternal toxic effects

004679

or teratogenic effects observed in any group. This study can not be used for regulatory purposes however, due to the dietary route of exposure and the absence of fetal or maternal toxicity at the high dose tested.

Core-Grade Classification: supplementary

This study does not satisfy the requirement for data on teratogenic effects in one species.

b. rat (GS0119-009)

Benomyl (99.2 % a.i.) was administered by gavage to 27 ChR-CD strain rats at each of the following doses: 0, 3, 10, 30, 62.5, and 125 mg/kg/day for days 7 through 16 of gestation. Dams were sacrificed on day 21 of gestation and the fetuses examined. There were no treatment related maternal or fetal toxic effects except for decreased fetal weight in the 62.5 and 125 mg/kg/day groups. There were significant increases in microphthalmia and anophthalmia at 62.5 and 125 mg/kg/day and distended lateral ventricles and hydrocephaly at 125 mg/kg/day. Two cases of microphthalmia also occurred at 10 mg/kg day. The registrant felt that this may be due to treatment since the background incidence of this lesion at the testing laboratory was 1/1000. The NOEL for maternal toxicity was greater than 125 mg/kg/day and the fetal toxic NOEL was 30 mg/kg/day. The teratogenic NOEL, however remains undetermined until the LEL for microphthalmia is determined. It is at least 62.5 mg/kg/day however.

Core-Grade Classification: supplementary

c. rat (00115674, 00126522)

Benomyl (99.1 % purity) was administered by gavage to 46-48 Crl:CD® (SD)BR rats at each of the following doses: 0.3, 6.25, 10, 20, 30 and 62.5 (only 19 dams) mg/kg/day for days 7 through 16 of gestation. Dams were sacrificed on day 21 of gestation and the fetuses were examined specifically for ocular effects. There were no treatment related signs of maternal toxicity noted. The high dose fetuses were significantly lighter than the controls. There was 1 fetus with microphthalmia present in the 16 litters in the high dose, no other ocular abnormali ties were reported.

8

Core-Grade Classification: supplementary

004679

Studies 7.b. and 7.c. , when considered together, are core-minimum. Together, they satisfy the requirement for data on teratogenic effects of benomyl on one species. Th combined NOEL was 30 mg/kg/day and the LEL was 62.5 mg/kg/

d. mouse (GS0119-017)

Benomyl technical was administered by gavage to 25 pregnant CD-1 mice at each of the following doses: 0, 50, 100 and 200 mg/kg/day for days 7 through 17 of gestation. Dams were sacrificed on day 18 of gestation and the fetuses were examined. Doses as high as 200 mg/kg/day did not affect maternal viability or growth. Doses of 200 (significant from controls $p < .05$) and, to a much lesser extent, 100 mg/kg/day adversely affected fetal development including: decreased fetal weight, and delayed skeletal and visceral (including subnormal vertebral centrums, enlarged cerebral ventricles, and renal pelves) development, and increased supernumerary ribs. The incidence of major anomalies observed in the fetuses was 1.3, 1.0, 16.8 and 47.3 % at 0, 50, 100 and 200 mg/kg/day. The incidences of major fetal and litter anomalies were significant at the $p < .001$ in the mid and high dose groups. Anomalies included: short and/or kinky tail, fused vertebrae, fused ribs and cleft palate. The NOEL was 50 mg/kg/day and the LEL, based on teratogenic effects, was 100 mg/kg/day.

Core-Grade Classification: minimum

This study satisfies the requirement for data on the teratogenic effects of benomyl in one species.

The requirement for data on teratogenic effects in two species has been satisfied.

8. Mutagenicity

a. Gene Mutation

1. (GS0119-001) Benomyl (99.6 % a.i.), at levels of 500 and 10,000 ug/plate was mutagenic in S. typhimurium strains TA1535 and TA98 with activation. Classification: acceptable

2. (00038808) Chinese hamster ovary cells (HGPRT) were treated with from 17 to 172 uM of benomyl (99.9-100 % a.i.) with and without S-9 activation. Benomyl was not mutagenic in this system.

Classification: acceptable

3. (GS0119-002) Benomyl was tested in mouse L5178Y TK$^{+/-}$ lymphoma cells with and without activation. The test material was mutagenic without activation at 50 ug/ml and with activation from 12 ug/ml to 25 ug/ml. At higher doses relative total cell growth was less than 10 %. At lower doses, mutagenic frequency was not consistently twice the control rate. Mutagenic activity was enhanced by activation.

Classification: acceptable

b. Chromosomal Aberrations

1. (GS0119-003) Micronucleus test - Benomyl (% a.i. not given) was administered by gavage to male mice on 2 consecutive days at the following doses: 0, 250, 500 and 1000 mg/kg/day. Animals were sacrificed 24, 48 or 72 hours after the second dose. Five hundred polychromatic erythrocytes (PCE) per rat from the femoral bone marrow were examined for micronuclei and the number of mature erythrocytes was counted until 200 PCEs were found. A compound is considered positive if the number of cells with micronuclei/500 PCEs is statistically increased over controls, in at least 2 dose-time groups. There were increased numbers of cells with micronucle in 4 groups including low and mid dose groups at 48 hr and the high dose group at 48 and 72 hr. Benomyl is considered positive in this test system.

Classification: acceptable

2. (GS0119-004) Sister chromatid exchange (SCE) - Chinese hamster ovary cells (in culture) were treated with from 0.625 to 10 ug/ml of benomyl (99 % a.i.) without activation or from 0.375 to 150 ug/ml of benomyl with activation. Cells underwent at least 2 cell divisions within 24 hr. Two samples of 25 cells were scored for SCE and chromosome number per treatment. There were not enough metaphases at concentrations of 5 ug/ml and above, without activation. Comparing the scores from 3 cytogeneticists using an analysis of variance indicated a small but statistically significant increase in SCEs with benomyl with and without activation. The authors concluded that benomyl was weakly positive in this test system.

Classification: acceptable

034879

c. Other mutagenic testing, DNA repair

1. (GS0119-005) Mouse - Benomyl (% a.i. was unspecified) was tested for mutagenic effects in I° mouse (B6C3F1) hepatocytes using tritiated thymidine as the label. Concentrations ranged from 0.5 to .00005 mg/ml and were tested in triplicate. Cells were fixed and examined microscopically for cytotoxicity then analyzed using autoradiography to determine tritium uptake. A net increase of 5 grains in each nucleus (above background) in all 3 replicates were considered a positive response. Level of benomyl 0.05 mg/kg and above, were cytotoxic. Benomyl did not induce DNA repair in this test system.

Classification: acceptable

2. (GS0119-006) rat - Benomyl (% a.i. was unspecified) was tested for mutagenic effects in I° rat (F344) hepatocytes using tritiated thymidine as the label. Concentrations ranged from 0.5 to 0.00005 mg/ml and were tested in triplicate. Cells were fixed and examined microscopically for cytotoxicity then analyzed using autoradiography to determine tritium uptake. A net increase of 5 grains in each nucleus (above background) in all 3 replicates were considered a positive response. Level of benomyl 0.05 mg/kg and above, were cytotoxic. Benomyl did not induce DNA repair in this test system.

Classification: acceptable

The requirements for mutagenic testing have been met.

9. Chronic Toxicity (feeding)

a. rat (00097284)

Thirty six albino Charles River CD rats of each sex were assigned to groups given 0, 100, 500, and 2500 ppm of 50 or 70 % benomyl WP (dose based on a.i.) for 2 years . There were no treatment related effects observed throughout the study. At sacrifice, there were no treatment related changes in neoplastic or non-neoplastic lesions, or changes in body weight or organ weights (including testicular weight). A maximum tolerated dose was not established. The NOEL for toxicity was established at 2500 ppm.

Core-Grade Classification: minimum for chronic toxici-

b. Non-rodent (00097305, 00081913, 00097318, 00097326, 00061618)

Four beagles of each sex were assigned to groups given 0, 100, 500, and 2500 ppm of Benomyl (50 % a.i.) for 1 or 2 years. A fifth male added to the high dose group when 1 dog became sick. Body weight gain and food consumption were decreased in the high dose males and females. The following effects occurred in the 2500 ppm males: elevated cholesterol and alkaline phosphatase from 1 month to the end of the study; elevated glutamic-pyruvic transaminase activity levels which returned to normal by 15 months; decreased albumin/globulin ratio and total protein from 1 to 2 months to the end of the study. Hepatic cirrhosis, observed with both micro- and macroscopic examination, was observed in 1/2 males at 1 year (in 0/1 females), and 2/3 males and 1/3 females at 2 years. Testicular lesions, after examination by the E.P.A. pathologist, were considered unrelated to ingestion of the chemical. The NOEL was 500 ppm and the LEL was 2500 ppm based on biochemical alterations, hepatic cirrhosis, decreased weight gain and lower food consumption.

Core-Grade Classification: minimum

c. rat (00088333) (MBC)

Thirty six albino Charles River CD rats of each sex were assigned to groups given 0, 100, 500, and 2500 (raised to 10,000 ppm after week 20) 5000 ppm of 50 or 70 % MBC (dose based on a.i.) for 2 years. There were no treatment related effects observed throughout the study. At sacrifice, there were no treatment related changes in neoplastic lesions or organ weights. Mid dose females and high dose males and females weighed less than controls. Mid and high dose males and females had increased incidence of pericholangitis and cholangiohepatitis. Hematocrit (HCT), hemoglobin (HGB), and red blood cell counts were decreased in mid and high dose females; HCT and HGB were not significantly decreased in high dose males. The NOEL was 500 ppm and the LEL based on decreased weight gain, altered hematology and liver histology was 5000 ppm.

Core-Grade Classification: minimum

d. Non-rodent (00088333) (MBC)

Four beagles of each sex were assigned to groups given 0, 100, 500, and 2500 lowered to 1500 ppm of MBC (53 % a.i.) for 2 years. Body weight gain and food consumption were decreased in the high dose males and females. The high dose males were sacrificed early due to poor nutrition. No females died early. High dose dogs developed anorexia, distended abdomens and poor condition. The following effects occurred in the 500 and 1500 ppm dogs: elevated cholesterol, BUN, total protien, SGPT and alkaline phosphatase. Hepatic cirrhosis, and mild chronic hepatitis was observed at 500 ppm and above. The NOEL was 100 ppm and the LEL was 500 ppm based on biochemical alterations, hepatic cirrhosis.

Core-Grade Classification: minimum

These studies satisfy the requirements for chronic toxicity testing in a rodent and non-rodent species.

10. Oncogenicity (feeding)

a. Rat (00097284) benomyl, see section 9.a.

Core-Grade Classification: supplementary for onco.

b. Rat (00088333) MBC, see section 9.c.

Core-Grade Classification: minimum for onco.

Although no maximum tolerated dose (MTD) has been established in a chronic/onco. rat study using benomyl (high dose of 2500 ppm), the MTD for the chronic/oncogenicity study with MBC was set at 5000 ppm. These studies satisfy part of the oncogenicity requirement for registration.

b. Mouse (00096514)

Eighty CD-1 mice of each sex were assigned to groups given 0, 500, 1500 and 7500 (reduced to 5000 ppm after 37 weeks) ppm of benomyl (99-99.2 % pure). There was decreased body weight throughout the study at the high dose and to a lesser extent the mid dose. There was increased relative liver weight in the high dose males and females as well were decreased in the high dose males. High dose males also had microscopic evidence of hepatocellular and testicular (and epididymal) degeneration. Under the conditions of this study, benomyl fed at a

minimum of 500 ppm demonstrated oncogenic potential in the liver and lung. Hepatocellular carcinomas were induced in both males (low and mid doses) and females (low, mid and high doses). The combined incidences of hepatocellular adenomas and carcinomas were statistically increased in the mid and high dose females,and low and mid dose males. Pulmonary alveologenic carcinomas were induced in males at the low and mid dose. Oncogenic potential at 500 ppm.

Core-Grade Classification: minimum

This study satisfies part of the oncogenicity requirements for registration.

The chronic rat and mouse oncogenicity studies satisfy the oncogenicity study requirements for registration.

c. Mouse (00096513) (MBC)

Eighty CD-1 mice of each sex were assigned to groups given 0, 500, 1500 and 7500 (males were reduced to 3750 ppm after 66 weeks due to mortality) ppm of MBC. There were no treatment related changes in body weight, food consumption or clinical signs of toxicity throughout the study. High dose males were sacrificed at 73 weeks due to high mortality. High dose females had reduced erythrocyte and a marginal decrease in hemoglobin concentration. Abs. liver weight (high dose females) and rel. liver weight (mid and high dose females) were increased. Mid and high dose males had microscopic evidence of hepatotoxicity and necrosis. Hepatocellular carcinomas were increased in the mid dose males; too few male mice survived to 18 months at the high dose to ascertain the oncogenic potential at this dose level in males. Mid and high dose males and females had lymphoid depletion of the thymus. Under the conditions of this study, MBC was associated with an increased incidence of liver carcinomas in mid and high dose females. Hepatocellular adenomas were marginally increased in the low and mid dose females. The systemic NOEL was 500 ppm and the LEL, based on increased incidence of lymphoid depletion of the thymus in males and females, hepatotoxic lesions in males, was 1500 ppm. MBC demonstrated oncogenic potential at 1500 ppm (hepatocellular carcinomas).

Core-Grade Classification: minimum

c. Mouse (GS0119-011) (MBC)

MBC was administered to 100-120 male and female NMRKf(SPF 71) mice per group at dosage levels of 0, 50, 150, 300 and 1000 (increased to 5000 after 8 weeks) ppm. Animals were sacrificed after 96 weeks. There was increased abs. and rel. liver weight, marked hepatocellular necrosis and cellular alterations indicative of hepatotoxicity at the high dose in both males and females. There was no increased incidence of hepatic neoplasia in treated as compared with control mice. The NOEL was 300 ppm and the LEL based on hepatotoxicity was 5000 ppm.

Core-Grade Classification: minimum

d. Mouse (GS0119-012) (MBC)

MBC was administered to 100 male and female SPF Swiss mice per group at dosage levels of 0, 150, 300, and 1000 (increased to 5000 ppm at 8 weeks) ppm for 80 weeks. Results were presented in summary form only. Body weight and condition were not affected by the compound. Rel. liver weights were "altered" in the high dose male and female groups. Clear cell and mixed cell foci were present in livers of the high dose males and females High dose females had an increase incidence of neoplastic nodules. High dose males however had an increased incidence of hepatoblastomas. The NOEL was 300 ppm and the LEL based on hepatic lesions and altered cell foci was 5000 ppm. MBC demonstrated oncogenic potential (hepatic neoplasia) at 5000 ppm.

Core-Grade Classification: supplementary

11. Metabolism - (00066776)

One male rat was treated with 2500 ppm benomyl (% a.i.unknown), for 12 days then given 7.7 mg/kg of ring labeled benomyl by gavage. The only urinary metabolites identified were sulphate and glucuronide conjugates of methyl 5-hydroxybenzimidazole-2-yl-carbamate (5-OH-MBC). Most of the label (greater than 99 %) was excreted by 72 hr, with the majority observed in the urine (86 %) and 13 % in the feces.

Core-Grade Classification: Unacceptable

Although some metabolic data in rodents is available (see policy discussion), there are currently no low dose, high dose or repeated dose metabolism studies meeting our core-minimum requirements of acceptability. These studies are needed to satisfy the requirements for registration.

12. Dermal absorption (GS0119-014)

Four rats/time point/dose were treated dermally (greater than 16 % of their surface area) with *Benlate®WP (50 % a.i.). The duration of exposures were 0.5, 1, 2, 4, and 10 hours, doses tested were 0.2, 2, 20, and 200 mg of ^{14}C-Benlate/rat (0.1, 1, 10, and 100 mg, respectively of benomyl). The concentration of benomyl in the blood increased (nonlinear) with increasing dose (see table 1). The percent of administered dose of benomyl in the urine decreased (nonlinear) with increasing dose and increased (nonlinea with duration. The percent absorption also had a nonlinear decrease with increasing dose and a nonlinear increase with duration (see table 2). The percent absorbed ranged from 0.031 (high dose) to 3.518 (low dose) for the maximum exposure of 10 hours. By 10 hours 96-99 percent of the absorbed dose at all treatment levels had been excreted in the urine.

Table 1. Concentration of benomyl in the blood (ug/ml)

Dose (a.i.)	Duration of exposure (hours)				
mg/rat	0.5	1.0	2.0	4.0	10
0.1	.001	.004	.004	.004	.003
1.0	.006	.009	.008	.008	.004
10	.026	.028	.034	.036	.024
100	.033	.054	.048	.070	.064

Table 2. Total percent of dose of benomyl absorbed

Dose (a.i.)	Duration of exposure (hours)				
mg/rat	0.5	1.0	2.0	4.0	10
0.1	.046	.174	.714	1.734	3.518
1.0	.016	.045	.118	.340	.491
10	.006	.011	.039	.046	.096
100	.001	.004	.010	.029	.031

Classification - acceptable

* a.i. is benomyl

D. Policy Discussions

The toxicity concerns have been evaluated in detail in the Position Document 2/3 (PD 2/3) (Dec. 1977) and 4 (Oct. 1982). The risks are based on the increase in tumors, as well as increased teratogenic, mutagenic and spermatogenic effects related to benomyl or its metabolite, MBC.

Although valid metabolism studies have not been performed to adequately describe the metabolism of benomyl in animals, one postulated pathway reported in an article by Douche (00036818) is as follows:

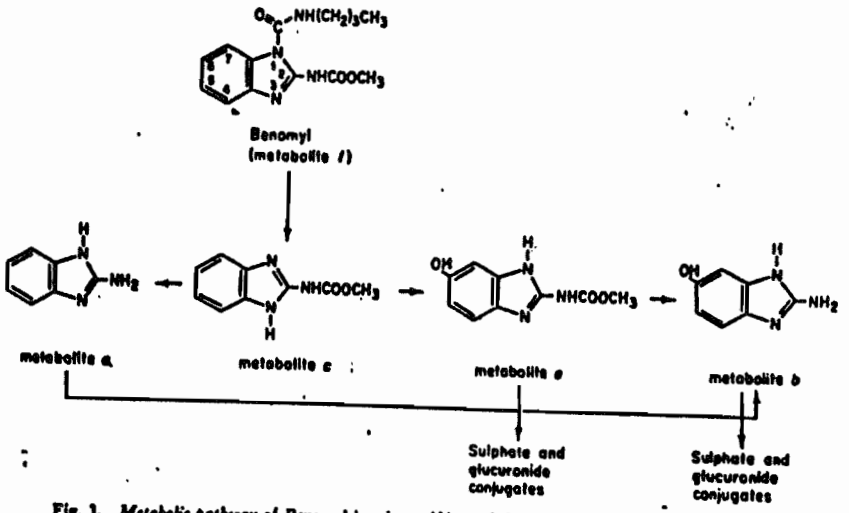


Fig. 3. *Metabolic pathway of Benomyl in mice, rabbits and sheep.*

a. 2-aminobenzimidazole (2AB)
b 5 hydroxy-2-aminobenzimidazole (5 OH-2-AB)
c. methyl benzimidazole-2ylcarbamate (MBC)
e. methyl 5-hydroxybenzimidazole-2-yl-carbamate (5 OH-MBC)
f. Benomyl (parent)

Animals all received 0.1 gm/kg (substantially higher than the estimated dietary exposures) of compound either by oral (P.O.) or intraperitoneal (I.P.) route of exposure. He reported that metabolite distribution in the mouse, rabbit and sheep was similar. It was also similar whether administered orally or peritoneally suggesting biliary secretion as part of the process. Ninety-four % of the label was excreted by mice within 96 hr with 20 % of the administered label excreted as conjugates (sulfate and glucuronide) of hydroxylated metabolites (44-71 % in urine; 21-46 % in feces). There was no parent compound found in either the urine or feces. The metabolic pathway with 0.1 gm exposure may not however, represent the pathway that would occur with lower dietary exposure levels.

In a separate study (00066776), a single male rat received 2500 ppm of cold benomyl for 12 days followed by a dose of ^{14}C labeled benomyl (7.7 mg/kg). The only metabolite found in the urine, after treatment with glucuronidase and sulfatase, was 5 OH-MBC. Again most of the label was observed in the urine (86 %), 13 % in the feces. Less than 1 % remained in the carcass.

The metabolism of benomyl needs to be more completely described. Studies need to be conducted in males and females using sufficient numbers of animals at: 1) expected exposure levels, 2) elevated levels and 3) with pretreatment, in order to have adequate information to satisfy the regulations.

Table 1. Incidence of Liver Tumors in Mice from Positive Studies

Study # Strain	liver neopl.	dose in ppm 0	150	300	500	1500	3750/ 7500**	5000	7500
Benomyl									
00096514									
CD-1-male	benign	9/77			9/80	11/79		10/80	
	malig.	16/77			26/80	41/79		17/80	
	total	25/77			35/80	52/79		27/80	
-female	benign	2/77			2/80	7/79		7/77	
	malig.	2/77			7/80	6/79		14/77	
	total	4/77			9/80	13/79		21/77	
MBC									
00096513	benign	11/80			15/80	14/80	3/80*		
CD-1-male	malig.	2/80			5/80	9/80	0/80*		
	total	13/80			20/80	23/80	3/80*		
-female	benign	0/79			5/78	5/80			3/78
	malig.	1/79			4/78	15/80			12/78
	Hb	0/79			0/78	1/80			0/79
	total	1/79			9/78	21/80			15/78
MBC									
GS0119-012	NN	9/100	6/98	13/100				9/100	
Swiss-male	malig.	1/100	1/98	2/100				1/100	
	Hb	1/100	1/98	1/100				7/100	
	total	10/100	8/98	16/100				17/100	
-female	NN	0/97	1/99	1/98				9/97	
	malig.	1/97	0/99	0/98				0/97	
	Hb	0/97	0/99	0/98				0/97	
	total	1/97	1/99	1/98				9/97	

liver neopl.
benign (adenomas)
malig. (adenocarcinomas)
N.N. (neoplastic nodules - benign)
Hb (hepatoblastoma - malignant)

*sacrificed at week 73 due to high mortality
**Males received 7500 ppm for 15 months, than 3750 ppm until sacrifice

004679

The positive oncogenicity studies discussed in the PD4 included one benomyl and two MBC mouse studies. Both the benomyl (00096514) and 1 MBC study in CD®1 mice (00096513) had an increased incidence of hepatocellular adenomas and carcinomas in males and females (see table 1).

Beems et al. (GS0119-012) reported in 1976 that SPF, albino, Swiss random bred mice treated with up to 5000 ppm for 80 weeks, had an increased incidence of malignant liver tumors (high dose males) and benign liver tumors (high dose females) (see table 1).

A recently submitted study tested MBC in NMRKf(SPF71) mice for 22 months (GS0119-011). A preliminary evaluation indicated no increase in incidence of neoplasia. However, there was an increased incidence in toxic liver damage consisting of hypertrophy of centrolobular and intermediary hepatocytes, liver necrosis and increased mitotic activity at the high dose (5000 ppm).

There was no increased incidence of neoplasms in dogs or albino Charles River CD rats. Although, there was no maximum tolerated dose (MTD) established in the chronic benomyl rat study (high dose 2500 ppm), the MBC chronic mouse study established an MTD at 5000 ppm. It is expected that the toxicity of MBC is either equal to, or greater than benomyl since benomyl rapidly hydrolyzes to MBC both in vitro and in vivo in an aqueous environment.

The Q_1^* of 2.065×10^{-5}, used in the PD4, was based on the hepatic neoplasms in CD-1 female mice treated with MBC since that study was considered to produce a more conservative assessme of risk than the benomyl study. The Toxicology Branch Peer Review Committee on Benomyl is currently evaluating the weight-of-the-evidence for assessing the oncogenic potential of benomyl.

The mutagenic activity of benomyl and MBC was discussed in detail in the PD 2/3 and 4. The PD 4 concluded that both compounds were spindle poisons which could result in nondisjunction and aneuploidy. Nondisjunction was reported in A. nidulans after exposure to benomyl and MBC (GS0119-013). Benomyl was associated with gene mutation in strains of S. typhimurium with activation (GS0119-001) at 10,000 ug/plate. It also was mutagenic in mouse lymphoma cells (L5178Y $TK^{+/-}$) (GS0119-002) with and to a lesser extent without activation. In chinese hamster ovary cells (HGPRT) (00038808) however, benomyl,even at levels of 172 uM, was not mutagenic with or without activation. Benomyl was not associated with DNA repair in primary mouse and rat hepatocyte cultures (GS0119-005, GS0119-006). There were no chromosome breaks in vivo with chinese hamster bone marrow cells treated with up to 1000 mg/ml MBC (GS0119-008). Although benomyl and MBC caused increased incidence of micronuclei in polychromatic erythrocytes in mouse bone marrow it is likely that this response was a result of spindle effects rather than chromosomal damage (GS0119-
-008). Benomyl was weakly positive for sister chromatid exchange (SCE) in vitro in chinese hamster ovary cells with and without

activation (GS0119-004). The PD4 concluded that "benomyl and the MBC metabolite of benomyl ... have been shown to cause effects to the cellular spindle apparatus. The impact of this effect to human health cannot be adequately assessed at this time. Therefore, mutagenic risk in the form of heritable spindle effects or point mutagenicity do not now lead to a recommendation for regulatory action. However, the data on mutagenicity are supportive of the qualitative determination of benomyl's potential teratogenic, spermatogenic and oncogenic effects."

The testes has been identified as a primary target of benomyl. It has been reported to affect the testes and epididymus in both acute and chronic studies. Decreased size of testes, depressed spermatogenesis or aspermatogenesis has been observed in rats, dogs, mice and rabbits by oral (gavage and dietary), dermal and inhalation routes. These studies are summarized in Table 2.

Table 2 Benomyl Studies With Testicular Effects

Oral - Dietary
- Dogs - no effect after 2 years at 2500 ppm (62.5 mg/kg/day) (00097305, 00081913, 00097318, 00097326, 00061618, 00068981).
- Rats (ChR-CD) - no effect in rats after 2 years at 2500 ppm (125 mg/kg/day)(00097284, 00068981).
- Mice (CD1) - testicular degeneration at 2 years at 5000 ppm (750 mg//kg/day)(00096514).

Oral - intubation
- Rats (ChR-CD) - single exposure - testicular effects after 14 days with 670 mg/kg (NOEL = 450 mg/kg) (00066779).
- Rats (ChR-CD) - 10 doses (in 14 days) - testicular effects at 200 mg/kg/day, the lowest dose tested (00097601).

Inhalation
- Rats (ChR-CD) - a single exposure at 33 mg/kg (0.82 mg/l) resulted in testicular effects (NOEL = 7.5 mg/kg (0.20 mg/l)) (00097281).
- Dogs - a single exposure at 82 mg/kg (1.65 mg/l) resulted in testicular effects (NOEL = 32 mg/kg (0.65 mg/l)) (00097275).

Dermal
- Rabbits - a 21 day exposure study resulted in decreased testicular weights at 1000 mg/kg, the NOEL was 500 mg/kg (00097287).

The teratogenic effects of benomyl were discussed in detail in the PD4. Microphthalmia, anophthalmia and hydrocephalia occurred when benomyl was administered by gavage to Wistar rats at 125 mg/kg/day on days 1-20 or 7-15 of gestation (NOEL = 62.5 mg/kg/day) (GS0119-015). In a separate gavage study with Wistar rats, lack of eye bulges and CNS herniations were observed at the low dose tested of 62.5 mg/kg/day (GS0119-016). Microphthalmia was also observed in the fetuses of ChR-CD rats given 62.5 mg/kg/day but not 30 mg/kg/day by gavage (00115674).

20

This study was used to set the NOEL of 30mg/kg/day for teratogenesis. Benomyl was also teratogenic (LEL 100 mg/kg/day) in CD-1 mice (GS0119-017) when administered by gavage. Studies in the rabbit and Wistar and ChR-CD rats have all been negative when the route of exposure to benomyl was dietary (00035352, GS0119-017, 00078620). The PD4 considered the different teratogenic results depending on exposure route as follows:

> "The FIFRA Scientific Advisory Panel (SAP) has acknowledged the contradictory results obtained from the two methods of dosing and have recommended that any NOEL established through the gavage method of administration of benomyl should be qualified by the essentially negative results obtained in dietary studies.
>
> The Agency considers that gavage administration of test material is scientifically more acceptable than dietary dosing for determining a NOEL for teratogenicity because it eliminates problems of palatability, drug stability, nutrient integrity and calculations of accurate dose levels. The Agency supports the ... opinion, that gavage assures relevance of treatment to the human condition for chemicals like benomyl (which are rapidly metabolized), since rodents, by preference, eat frequently during waking hours, whereas humans dine at relatively orderly intervals during the day. Therefore, the peaking of blood residues following gavage administration more nearly parallels the human situation than continuous uptake of the chemical in the rat diet."

The dermal absorption of benomyl is complex. Percent of benomyl absorbed/unit time decreased with increasing dose in a nonlinear fashion. It also increased with duration of exposure to a maximum at about 4 hours (see table). Blood concentrations of benomyl also increased with increasing dose (see table 3). Between 96 and 99 % of absorbed benomyl were excreted in the urine by 10 hours.

Table 3. Percent of the benomyl dose absorbed per hour, and blood levels at 4 hours

Dose (a.i.) mg/rat	Duration of exposure (hours) 0.5	1.0	2.0	4.0	10	ug/ml in blood
0.1	.090	.174	.357	.434	.352	.004
1.0	.032	.045	.059	.085	.049	.008
10	.012	.011	.020	.012	.010	.036
100	.002	.004	.005	.007	.003	.070

Benomyl has been shown to be a Toxicity Category II for eye irritation (00064820), a skin sensitizer (00097289) and a mild dermal irritant (toxicity category IV). The labels should have the appropriate warning statements.

Data Gaps for Benomyl

- Subchronic inhalation toxicity study - 90-day
- Metabolism studies: low dose, high dose, and repeated dose.

Tolerance Reassessment

Tolerances for benomyl in the CFR § 180.294 are for the parent compound and its metabolites containing the benzimidazole moiety (calculated as benomyl).

ADI

The five studies required to determine an acceptable daily intake (ADI) are listed in table 4.

Table 4. 5 required studies and NOELs for establishing an ADI

Study Type	NOEL		Reference
	ppm	mg/kg/day	
chronic rat feeding	2500	125	00097284, 00068981
chronic dog feeding	500	12.5	00097305, 00081913, 00097318, 00097326, 00061619
3 generation reproduction	100	5	00066773
rat teratology		30	00115674, 00126522
mouse teratology		50	GS0119-017

The no observable effect level (NOEL) of 5 mg/kg/day, obtained from the most sensitive study (see table 1), was divided by a safety factor of 100 to derive a ADI of 0.05 mg/kg/day. The maximal permissible intake (MPI) for a 60 kg adult is:

0.05 mg/kg/day x 60 kg = 3 mg/day

TMRC

Tolerances for the commodities in table 5 have been published in the federal register and have a theoretical maximal residual concentration (TMRC) of 1.9785 mg/day of benomyl which is 65.78 % of the ADI. The TMRC of 1.9785 mg/day would be 0.03289 mg/kg/day for a 60 kg adult. Table 6 lists commodities that have petitions pending for tolerances. These commodities, if approved, would add 0.2728 mg/day, raising the TMRC 13.79 % to 2.2513 mg/kg, which is 75.04 % of the ADI. The new TMRC of 2.2513 mg/day would be 0.03752 mg/kg/day for a 60 kg adult. The proceeding calculations used tolerances, the worst case for dietary exposure and did not consider per cent of crop treated and actual residue values (including processing).

The PD 4 listed percent of crop treated where the data was available (see table 5). When these values are taken into consideration the average residue level of benomyl (for crops with published tolerances) in the diet decreases to 1.00689 mg/day which is 33.56 % of the ADI.

004679

Teratogenesis

The NOEL for teratogenesis used for this analysis was 30 mg/kg/day (00115674, 00126522). Tolerance level residues were assumed for every commodity with published tolerances. Using the detailed acute analysis[1], with all statistics based on users daily consumption of food containing benomyl (for females 13 years of age and older) the weighted-average daily exposure was 0.039421 mg/kg/day. The MOS[2] based on the NOEL of 30 mg/kg/day was $\frac{30\text{mg/kg/day}}{0.039421\text{mg/kg/day}} = 761$.

The distribution of exposure for the population at risk (females greater than 13 years of age) using the TAS analysis, is summarized as follows. Forty four % of these women had a MOS at or above 1000. The NOEL was 200 times the exposure level reported for the individual with the highest potential estimated exposure.

Individual crops with high residues were tomatoes, brussel sprouts, grapefruit, oranges, peaches, nectarines, plums, pineapple.

Spermatogenic effects

Tolerance level residues were assumed for every commodity with published tolerances. The NOEL for spermatogenic effects used in the PD4 was 7.5 mg/kg/day was based on an acute rat inhalation study (00097281) because it was the most sensitive test for spermatogenic effects. The limiting dose used for comparison was 0.075 mg/kg/day (the NOEL of 7.5 mg/kg/day divided by a safety factor of 100). Using the TAS detailed acute analysis[1], with all statistics based on users daily consumption of food containing benomyl (for males 13 years of age and older) the weighted-average daily exposure was 0.035151 mg/kg/day. This was 46.87 % of the limiting dose of 0.075 mg/kg/day. The MOS[2] based on the NOEL of 7.5 mg/kg/day was

$$\frac{7.5\text{mg/kg/day}}{0.035151\text{ mg/kg/day}} = 213$$

The distribution of exposure for the population at risk (males greater than 13 years of age) using the TAS acute exposure analysis is summarized as follows. Thirteen % of these men had exposure at or above the limiting level. No individuals had exposures greater than 3 times the limiting dose. The MOS was 33 for the individual with the highest exposure.

Individual crops with residues which result in acute single serving dietary exposure to benomyl at or above 75 % of the limiting dose (0.075 mg/kg/day) were grapefruit, peaches, and pineapple.

The chronic dog feeding study (00097305) with a NOEL of 62.5 mg/kg/day (HDT) for spermatogenic effects, is the most conservative NOEL for this lesion, from a chronic feeding study (rat NOEL = 125 mg/kg/day; mouse NOEL = 225 mg/kg/day). The TMRC (calculated from the maximum published tolerances and

23

[1] Tolerance assessment system (TAS) was used for these calculations
[2] MOS = margin of safety

food factors) for chronic exposure to benomyl is 1.9735 mg/day (60 kg adult) or .03289 mg/kg/day. The resultant MOS using this NOEL (62.5 mg/kg/day) is

$$\frac{62.5 \text{ mg/kg/day}}{0.03289 \text{ mg/kg/day}} = 1900.$$

Oncogenesis

The potency estimator, Q_1^* for oncogenicity, presented in the PD 4 was 2.065×10^{-3} (mg/kg body weight/day^{-1}) (see policy discussion). The upper 95 % bound on cancer risk for dietary exposure is 6.8×10^{-5} (the Q_1^* multiplied times the TMRC). Table 5 gives the risks for the individual commodities. When the percent of crops treated is taken into consideration the risk decreases to 3.5×10^{-5}. Both values however, round to 10^{-5} to 10^{-4}. Therefore, current data concerning percent of crops treated, does not appreciably decrease the dietary risks of benomyl.

The 3C2B letter of May 14, 1985, required the registrant to submit real residue on apples, peaches, pineapples, rice, milk, soybeans, citrus and tomatoes. The potential residues for these commodities, when based on published tolerances, comprise 76 % of the TMRC. The resultant residue data may decrease the % ADI, teratogenic, spermatogenic and oncogenic risks from benomyl.

The risk to applicators and mixer/loaders was addressed in detail in the PD4. The Exposure Assessment Branch chapter has not yet been completed as of the date of this section. They have reported no major alterations in the use patterns of benomyl since the PD4 (verbal communication with Harry Day, EAB, 10/4/85). The worst case job related exposure would be for mixer/loaders for grapes/fruit crops with aerial application; 0.35 mg/kg/day without a dust mask. This results in a margin of safety of 21 for spermatogenic inhibition (NOEL from an acute rat inhalation study was 7.5 mg/kg). The exposure would decrease by 90 % with a dust mask. If alterations are reported in the Exposure Assessment chapter when completed, Toxicology Branch would need to consider their impact on the job related risk due to benomyl.

004679

TABLE 5. Dietary "Worst Case" Exposure
and Estimate of Upper Bound Risk (95 % Confidence Level)
Based on Tolerances for Benomyl

CROP	Tolerance (ppm)	Food factor	Daily Intake (mg/1.5kg diet/day)	Cancer Risk (100 % crop treated)	Percent of crop treated	Daily adjust % trea
:us fruits	10.	3.81	0.57179	10^{-5}	66.	0.377
les	7.	2.53	0.26565	10^{-5}	23.	0.061
atoes	5.	2.87	0.21561	10^{-6}-10^{-5}	18.	0.037
:hes	15.	0.90	0.20235	10^{-6}-10^{-5}	74.	0.149
eapple	35.	0.30	0.15560	10^{-6}-10^{-5}	33.	0.051
;es, not raisins	10.	0.45	0.06745	10^{-6}	29.	0.019
ns	2.	2.04	0.06120	10^{-6}	37.	0.022
< & dairy prod.	0.1	28.62	0.04292	10^{-6}	100.	0.042
e	5.	0.55	0.04139	10^{-6}	22.	0.009
at	0.2	10.36	0.03109	10^{-6}	100.	0.031
sins	50.	0.04	0.03066	10^{-6}	29.	0.008
ons	1.	2.	0.03005	10^{-6}	80.	0.024
ns, inc prunes	15.	0.13	0.02989	10^{-6}	17.	0.005
rs	7.	0.26	0.02683	10^{-6}	46.	0.012
icots	15.	0.11	0.02529	10^{-6}	73.	0.025
rries	15.	0.10	0.02299	10^{-7}-10^{-6}	36.	0.008
t, inc poultry	0.1	13.85	0.02077	10^{-7}-10^{-6}	100.	0.020
awberries	5.	0.18	0.01380	10^{-7}-10^{-6}	70.	0.009
ery	3.	0.29	0.01288	10^{-7}-10^{-6}	88.	0.011
ar, cane & beet	0.2	3.64	0.01091	10^{-7}-10^{-6}	100.	0.010
mbers, inc pickl	1.	0.73	0.01088	10^{-7}-10^{-6}	44.	0.004
ssel sprouts	15.	0.03	0.00675	10^{-7}	100.	0.006
tarines	15.	0.03	0.00675	10^{-7}	74.	0.005
nese cabbage	10.	0.03	0.00450	10^{-7}	100.	0.004
delion	10.	0.03	0.00450	10^{-7}	100.	0.004
nrooms	10.	0.03	0.00450	10^{-7}	50.	0.002
n, sweet	0.2	1.43	0.00429	10^{-7}	100.	0.004
anas	.2	1.42	0.00426	10^{-7}	100.	0.004
s	0.1	2.77	0.00416	10^{-7}	100.	0.004
ckberries	7.	0.03	0.00315	10^{-7}	50.	0.001
eberries	7.	0.03	0.00315	10^{-7}	55.	0.001
senberries	7.	0.03	0.00315	10^{-7}	50.	0.001
rants	7.	0.03	0.00315	10^{-7}	100.	0.003
berries	7.	0.03	0.00315	10^{-7}	50.	0.001
anberries	7.	0.03	0.00315	10^{-7}	50.	0.001
pberries	7.	0.03	0.00315	10^{-7}	27.	0.000
beans (oil)	0.2	0.92	0.00275	10^{-7}	2.6	0.000
nip greens	6.	0.03	0.00270	10^{-7}	100.	0.002
bage, sauerkraut	0.2	0.74	0.00221	10^{-8}-10^{-7}	100.	0.002
pkin, inc squash	1.	0.11	0.00169	10^{-8}-10^{-7}	90.	0.001
rots	0.2	0.48	0.00144	10^{-8}-10^{-7}	100.	0.001
cados	3.	0.03	0.00135	10^{-8}-10^{-7}	5.	0.000
goes	3.	0.03	0.00135	10^{-8}-10^{-7}	50.	0.000
ayas	3.	0.03	0.00135	10^{-8}-10^{-7}	100.	0.001

1. mg/1.5kg diet/day

25

Table 5 continued

CROP	Tolerance (ppm)	Food factor	Daily Intake (mg/1.5kg diet/day)	Cancer Risk	Percent of crop treated	Daily Inta adjusted f % treated
t potatoes	0.2	0.40	0.00120	10^{-8}-10^{-7}	100.	0.00120
	0.2	0.36	0.00107	10^{-8}-10^{-7}	100.	0.00107
ıts	0.2	0.36	0.00107	10^{-8}-10^{-7}	22.	0.00024
ɜr squash	1.	0.03	0.00045	10^{-8}	100.	0.00045
ɜrsquash	1.	0.03	0.00045	10^{-8}	100.	0.00045
ɜrs	0.2	0.12	0.00037	10^{-8}	100.	0.00037
ɔoli	0.2	0.1	0.00031	10^{-8}	100.	0.00031
	0.2	0.10	0.00031	10^{-8}	38.	0.00012
ırds	0.2	0.08	0.00025	10^{-8}	100.	0.00025
iflower	0.2	0.07	0.00021	10^{-9}-10^{-8}	100.	0.00021
ırd greens	0.2	0.06	0.00018	10^{-9}-10^{-8}	100.	0.00018
ıch	0.2	0.05	0.00015	10^{-9}-10^{-8}	100.	0.00015
ips	0.2	0.05	0.00015	10^{-9}-10^{-8}	100.	0.00015
ɜy	0.2	0.03	0.00009	10^{-9}-10^{-8}	100.	0.00009
lant	0.2	0.03	0.00009	10^{-9}-10^{-8}	100.	0.00009
ic	0.2	0.03	0.00009	10^{-9}-10^{-8}	100.	0.00009
	0.2	0.03	0.00009	10^{-9}-10^{-8}	100.	0.00009
ɜabi	0.2	0.03	0.00009	10^{-9}-10^{-8}	100.	0.00009
ɜ	0.2	0.02	0.00009	10^{-9}-10^{-8}	100.	0.00009
ɔagas	0.2	0.03	0.00009	10^{-9}-10^{-8}	100.	0.00009
	0.2	0.03	0.00009	10^{-9}-10^{-8}	100.	0.00009

ɟ/1.5kg diet/day

TABLE 6. Dietary "Worst Case" Exposure
and Estimate of Upper Bound Risk (95 % Confidence Level)
Based on Proposed Tolerances for Benomyl

CROP	Tolerance (ppm)	Food factor	Daily Intake (mg/1.5kg diet/day)	Cancer Risk
yams (yautia)	0.2	0.03	0.00009	10^{-9}-10^{-8}
lettuce	10.	1.31	0.19622	10^{-7}-10^{-6}
eggplant*	4.8	0.03	0.00216	10^{-8}-10^{-7}
peppers*	4.8	0.12	0.00883	10^{-7}-10^{-6}
escarole/endive	10.	0.03	0.00450	10^{-7}
beets	0.2	0.17	0.00052	10^{-8}
beet greens	15.	0.03	0.00675	10^{-7}
liver*	1.8	0.3	0.00081	10^{-8}
cabbage, sauerkraut*	4.8	0.74	0.05298	10^{-6}

*these represent additional tolerances over that previously published,
i.e. eggplant: previous 0.2 ppm; additional requested 4.8 ppm; total new tolerance requested 5.0 ppm.

Tox Chem No. Benomyl 75A File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
21-day dermal - rabbit; Haskell lab.;211-69; 7/20/69	52.5-53 % a.i. [redacted]	MRID 00097287	NOEL = 500 mg/kg LEL = 1000 mg/kg based on a non-statistically sig. decr. in rel.& abs. testes weight levels tested 50 to 5000 mg/kg		minimum 004679 -
Acute oral LD_{50} - rat Haskell labs; 17-69; 1/22/69	Technical	MRID 00097277	LD_{50} > 10,000 mg/kg	IV	000721 Minimum 004679 -
Acute inhalation LC_{50} - rat; Hazleton Lab; #201-220; 10/18/68	50% a.i. Fungicide 1991 Benomyl WP	MRID 00097599	LC_{50} > 4.01 mg/L (HDT)(testicular alterations noted at all levels tested: 0.27, 1.0 and 4.01 mg/L)	III	000721 004678 minimum 004679 -
Primary dermal irrit. - guinea pig; 84-69; 4/18/69	technical	Acc.# 050427-W MRID 00097289	mild skin irritation	IV	minimum 004679 -
Dermal sensitization- guinea pig; 84-69; 4/18/69	technical	Acc.# 050427-W MRID 00097289	mild to moderate sensitization		minimum 004679 -
90-Day feeding - rat; Haskell Lab.; #11-67; 1/31/67	70% WP (72.2% tech)	MRID 00066771	Systemic NOEL = 500 ppm LEL = 2500 ppm based on incr. SGPT (male),rel. & abs. liver wt.(female) dose levels:0, 100, 500, 2500ppm(ai)		000721 004678 minimum 004679 -
90-Day feeding - dog; Haskell Lab.; #269-68; 11/20/68	51% Technical 50 % WP	MRID 00066785	Systemic NOEL = 500 ppm LEL = 2500 ppm based on incr. SGPT, Alk.phos, A/G ratio (male) dose levels: 0, 100, 500, 2500ppm(ai)		000721 004678 minimum 004679 -
2-Year feeding - rat; Haskell Lab.; #232-69; 8/15/69 (supp. path. report 66-77; 2/9/78)	51 or 72.2 % Tech, 50 or 70% WP	MRID 00097284 00068981	Systemic NOEL > 2500 ppm Oncogenic NOEL > 2500 ppm No effect on sperm production Dosage levels = 100, 500, 2500 ppm in ChR-CD strain		000721 004678 minimum chronc suppl onco 004679 -

Tox Chem No. Benomyl 75A File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
2-Year feeding - dog; Haskell Lab.; #48-70 (129-69,74-77), 48-70,66-77; 3/7/70	51 or 72.2 % Tech, 50 or 70% WP	MRID 00097305 00081913 00097318 00097326 00061618 00068981	Systemic NOEL = 500 ppm Systemic LEL = 2,500 ppm (HDT, cirrhosis and body weight depression Dosage levels = 100, 500, 2500 ppm		000721 004678 minimum 004679 -
3-Generation reproduction - rat; Haskell Lab.; #264-68; 11/18/68	51 or 72.2 % Tech, 50 or 70% WP	MRID 00066773	Systemic NOEL = 100 ppm LEL = 500 ppm based on decreased pup weights dose levels:0, 100, 500, 2500 ppm(ai)		000721 004678 minimum 004679 -
Teratology - rabbit; Hazleton Lab.;Hazelton; 210-214; 1968	53.5% WP 50% a.i.	MRID 00035352	Terata NOEL = 500 ppm (HDT) NOEL fetal, maternal tox > 500 ppm Dose levels: 0, 100, 500 ppm by diet		000722 supplementary 004679 -
Teratology - rat; Schtenberg & Torchinsky; 1972	Technical	GS0119-015	Fetotoxic NOEL = 62.5 mg/kg Fetotoxic LEL = 125 mg/kg Terata NOEL = 62.5 mg/kg Terata LEL = 125 mg/kg (Brain hernias, hydrocephaly and microphtalmia) Dosage = 62.5, 125, 250, 500 mg/kg (gavage) in Wistar strain		000722
Acute dermal LD_{50} - rabbit; Haskell; #554-80, 7/23/80	Benomyl - 75% (Benlate DF)	Acc.# 243043 MRID 00064822	LD_{50} > 2000 mg/kg Severe skin irritation.	III	Guideline 000863 004679 -
Primary eye irritation - rabbit; Haskell; #497-80; 6/13/80	Benomyl - 75% (Benlate DF)	Acc.# 243043 MRID 00064820	Corneal opacity at 8 days. For the irrigated eyes, irritation cleared by day 8. PIS day 1 = 28, day 11 = 0	II	Guideline 000863 004678 004679 -
Primary dermal irritation - rabbit; Haskell; #367-80;5/12/80	Benomyl - 75% (Benlate DF)	Acc.# 243043 MRID 00064821	Slight edema and slight erythema at 24 hours; at 72 hours, only very slight erythema. PIS = 0.67 All scores were Ø by day 6.	IV	Guideline 000863 004679

Tox Chem No. Benomyl 75A File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology - rat; Haskell Labs.; report #587-82; E.I. DuPont de Nemours; 1982	Technical 99.1% Pure	Acc.# 248563-A 249749-A MRID 00115674	STUDY LIMITED TO MICROPHTHALMIA NOEL = 30 mg/kg LEL = 62.5 mg/kg (microphthalmia) Levels tested by gavage - (0, 3, 6.25, 10, 20, 30 & 62.5)ChR-CD rats		Supplementary 002578 Upgraded to Minimum 003042
2 Year feeding - mouse; Dupont Haskell Lab; 20-80; 1/26/82	Benomyl 99-99.2% pure	MRID 00096514	Oncogenic NOEL < 500 ppm male and female, significant increase in hepatocellular neoplasms in male and female, pulmon. alveol. carcin. in males; degen. of testes and epididymis at 5000 ppm. Dosage levels = 500, 1500, 5000 ppm (5000 lowered from 7500 ppm)in CD-1		003726 minimum 004679 -
Teratology - rat; Sherman et al.; 1975	Benomyl	GS0119-018 MRID 00078620	Terata NOEL = 5000 ppm (HDT) (inconclusive result since ingested dose not measured accurately)ChR-CD doses 0, 100, 500, 2500, 5000 ppm		003728
Teratology - rat; Midwest Res. Inst.; #68-02-2982 1979	Benomyl	GS0119-016	Terata NOEL < 62.5 mg/kg (LDT; CNS herniations, defects of extremities, lack of eye bulges) Dosage levels = 0 - 500 mg/kg/day by gavage in Wistar strain		003728
Teratology - rat; Health Effects Res. Lab; US. EPA; 1/11/80	Benomyl	GS0119-017	Terata NOEL = 31.2 mg/kg Terata LEL = 62.5 mg/kg (microphthalmia and increased fetal mortality; reduced fetal weight) Dosage levels = 15.6, 31.2, 62.5 and 125 mg/kg by gavage, Wistar rat		003728
Teratology - rat; Health Effects Res. Lab; US EPA ; 1/11/80	Benomyl	GS0119-017	NOEL = 169 mg/kg LEL = 298 mg/kg (weight decrease in fetuses). No dose related incidences of anomalies or malformations Dosage levels = 0 - 500 mg/kg in diet in Wistar rats		003728

Tox Chem No. Benomyl 75A File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology - rat; Haskell Lab; #649-80; 1980	Benomyl	Acc.# 256575 GS0119-009	Unilateral microphthalmia at 10 mg/ kg/day (2 animals) NOEL = 30 mg/kg LEL = 62.5 mg/kg (embryotoxicity) Dosage levels - 0, 3, 10, 62.5, 125 mg/kg/day by gavage in ChR-CD rats		003728 Supplementary 004689 Minimum when combined with 003042
Neurotoxicity - chicken; IRDC;125-039; 10/8/79	Benomyl in corn oil (99% Tech.)	Acc.# 241930 GS0119-007	No evidence of delayed neurotoxicity was found NOEL other neurotox.signs =2500mg/kg Dosage levels = 500, 2500,5000 mg/kg		003728 minimum 004679 -
Mutagenic- micronucleus test - mice; SRI Int., (Kirkhart; 1980);LSU-7553-19;2/12/80	Benomyl	GS0119-003	Mutagenic - significant dose related increase in micronuclei in bone marrow from femor bones at all doses Dosage levels = 250, 500, 1000 mg/kg		003744 acceptable 004679 -
Mutagenic - L5178Y TK+ (mouse lymphoma); SRI Int.;LSU-7558; Dec, 80	Benomyl (99% a.i.)	GS0119-002	Dose related increase in mutation frequency at TK locus of L5178Y cells, in vitro - weak mutagen with and without activation		003744 acceptable 004679 -
Mutagenic - SCE- chinese hamster ovary; SRI Int.; LSU-5778; Aug, 80	Benomyl (99% a.i.)	GS0119-004	Weakly positive for sister chromatid exchange, levels tested with activ. .375-150 ug/ml; without activ. .625-10 ug/ml		003744 acceptable 004679 -
Mutagenic - microorganisms; Donvan and Krahn; 1981	Benomyl		Not mutagenic in TA 1537, 1535, 98 and 100 up to dosage levels of 250 mg/plate		003744
Mutagenic - S.Typhim. Haskell; 560-80; 8/2/80	Benomyl (99.6% a.i.)	GS0119-001	Mutagenic for strains TA 1537 and 98 with activation (Dose levels = 100 - 10,000 ug)		003744 acceptable 004679 -
Mutagenic - ovary cells - chinese hamster; Haskell; 438-80; 6/16/80	Benomyl (99.9-100% a.i)	MRID 00038808	Not mutagenic at the HGPRT locus with or without activation, range tested 17-172 uM		003744 acceptable 004679 -

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic - mouse - DNA repair; Haskell; 741-81; 10/20/81 (Tong, 1981)	Benomyl	GS0119-005	Not a mutagen when tested for DNA repair using mouse hepatocyte cultures		003744 acceptable 004679 -
Teratology - mice; Kavlock et al; 1982 (Tox & Appl Pharm, 62: 44-54; 1982)	Benomyl		Dosage levels=0, 50, 100, 200 mg/kg given by gavage NOEL = 50 mg/kg LEL = 100 mg/kg (supra occipital scars, subnormal vertebral centrum, supernumerary ribs, cleft palate)		003744
Acute inhalation - rat; Haskell Lab; #95-69; 4/24/69	Benomyl 50% WP (50%a.i)	MRID 00097281	NOEL = 0.2 mg/L (7.5 mg/kg) LEL = 0.82 mg/L (reduction of spermatogenic activity)(33 mg/kg)ChR-CD LC_{50} > 0.82 doses tested: 0, 0.02, 0.12, 0.2, 0.82 mg/l	II	003744 minimum 004679 -
14-day intubation - rats Haskell labs; 100-66; 7/15/66	Benomyl 1991 (% unspecified)	MRID 00097601	Systemic NOEL > 200 mg/kg/day for spermatogenic effects LEL = 3400 mg/kg/day (4/6 deaths) levels tested; 200, 3400 mg/kg/day in ChR-CD strain		000720
Acute oral - rats; Haskell labs; 100-66; 7/15/66	Benomyl 1991 (% unspecified)	MRID 00097601	LD_{50} > 9590 mg/kg Levels tested: 500, 2250, 3400, 3600, 7500, 9590 mg/kg in ChR-CD rats 1 rat/dose, all had testicular alterations		supplementary 004679 -
Mutagenic micronucleus - mouse; J.P.Seiler; 1976	Benomyl MBC	GS0119-008	increased micronucleus formation in mouse bone marrow NOEL = 500 mg/kg benomyl LEL = 1000 mg/kg " NOEL = 50 mg/kg MBC LEL = 100 mg/kg " for serum concentration NOEL = 8 ug/kg MBC LEL = 11.5 ug/kg " no chromosome breaks <u>in vivo</u> in hamster bone marrow at up to 1000mg/kg		incomplete 004679 -

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute oral - rats; Haskell labs; 179-65; 12/15/65	Benomyl	MRID 00066779	No deaths, 1 rat/dose at 200,450, 670 and 1000 mg/kg. There was a dose response for decr. rel. testes weight. Tubular degeneration and necrosis of the testes was present at 670 and 1000mg/kg in ChR-CD rats		supplementary 004679 -
Acute inhalation - dogs; Hazelton labs; HLR#192-69; 7/14/69	Benomyl WP 50% 50 % a.i.	MRID 00097275	LC_{50} > 1.65 mg/l (HDT no deaths) NOEL = .65 mg/l (32 mg/kg) LEL 1.65 mg/l (82 mg/kg)based on reducedspermatogenic activity at 14 d (notpresent at 28 days)	II	minimum 004679 -
Metabolism - rat (1); DuPont; #?; 1968?	benomyl	MRID 00066776 Acc.# 091561-F	The major urinary metabolites of benomyl, after 12 days of 2500 ppm in the diet followed by a 7.7 mg oral dose of benomyl-$2^{14}C$, are conjugates of 5-OH-MBC.		unacceptable 004679 -
Metabolism; Douch, PGC; Xenobiotica; 3(6):367-380	benomyl	MRID 00036818	1) mouse, rabbit and sheep had similar metabolite distribution 2) oral and intraperitoneal routes were similar 3) most of label was excreted by 96 hr (majority in urine) 4) no parent in urine or feces.		supplementary RS DOC #
Dermal absorption - rat; duPont; #?; 3/9/79	benomyl 50%WP 50% a.i.	GS0119-014	Benomyl was absorbed in a nonlinear dose and duration related manner. % absorbed ranged form .031 (after high dose of 100 mg a.i.) to 3.518 (after low dose of 0.1 mg a.i.) after 10 hours.		acceptable 004679 -
Mutation - A. nidulans; Kappas, et al; Mutat. Res.; 26(1) 1974, 17-27	benomyl	GS0119-013	Benomyl and MBC induced genetic segregation in a heterozygous green diploid strain of A. nidulans.		PD4 #
Primary eye irritation - rabbits; Haskell labs; 179-81;4/6/81	Benlate DF 75 % a.i.	MRID 00084579	day 1 | 4 | 7 PIS 28 | 7.3 | 0	III	minimum 004679 -

004679

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Tox Category	Core Grade/ Doc. No.
90-day feeding-dog Haskell Lab.; #283-70; 1970	50% WP (53% tech) MBC	MRID 00099130	Systemic NOEL = 500 ppm (14 mg/kg/d) LEL = 1500 ppm (41 mg/kg/d) based on incr.alk. phos., chol, SGPT and minimal microscopic alterations in the liver (1/4 males and females) and testes (1/4 males) levels tested: 0, 100, 500, 2500/1500 ppm a.i.		minimum 004679 -
2 Year feeding/onco-rat; Haskell Lab.; 195-72; 1972	50 or 70 % ai (53 or 72.2 % tech.) MBC	Acc.# 232870-C 232871 MRID 00088333	Systemic NOEL = 500 ppm LEL = 5000 ppm based on decr. wt. gain, decr. HCT, HGB and RCB in females; incr. pericholangitis in males and females. Onco. NOEL > 10,000 ppm (HDT) Doses tested: 0, 100, 500, 5000, 2500/10000 ppm a.i.		minimum 004679 -
2 Year feeding - dog; Haskell Lab.; 195-72; 1972	50 or 70 % ai (53 or 72.2 % tech.) MBC	Acc.# 232870-C 232871 MRID 00088333	Systemic NOEL = 100 ppm LEL = 500 ppm based on biochem. and histological alterations indicating liver damage; levels tested: 0, 100, 500, 1500/2500 ppm a.i.		minimum 004679 -
3 generation repro - rat Haskell Lab.; 195-72; 1972	50 or 70 % ai (53 or 72.2 % tech.) MBC	Acc.# 232870-C 232871 MRID 00088333	NOEL = 500 ppm LEL = 5000 based on decr. pup weight at weaning		minimum 004679 -
2 Year Onco - mice; Woods et al, 1982 Haskell Lab.	MBC	GS0119-010	NOEL = 500 ppm LEL = 1500 ppm based on increased incidence of hepatocellular carcinomas, lymphoid depletion of the thymus in males and females. Hepatotoxic lesions were only present in the males. Dose levels: 0, 500, 1500, 7500(females), 7500 for 15 mo. then 3750 (males) ppm in CD-1 strain.		WHO review

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	Core Grade/ Doc. No.
			Not oncogenic at 5000 ppm		
96 Week onco - mice; Kramer and Weigand et et al., 1982.	MBC	GS0119-011	NOEL = 300 ppm LEL = 5000 ppm based on increased abs. and rel. liver weight, marked hepatocellular necrosis and cellular alterations indicative of toxicity. Dose levels: 0, 100, 300, 1000 (increased to 5000 at 8 weeks) ppm in NMRKf(SPF 71)strain		WHO review
80 Week onco - Mice Beems, 1976	MBC	GS0119-012	NOEL = 300 ppm LEL = 5000 ppm based on increased incidence of neoplastic nodules of the liver in females, and hepatoblastomas in males, altered rel. liver weight in males and females. Doses tested 0, 150, 300 and 1000 (increased to 5000 at 8 wks) in SPF Swiss mice.		WHO review

§158.135 - TOXICOLOGY - ACUTE TESTING :
- 81-1 - Acute Oral Toxicity - Rat

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester :

:PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[R]	YES	
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[R]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[R]	YES	
[X]	G. Forestry	[R]	YES	
[X]	H. Domestic Outdoor	[R]	YES	
[ ]	I. Indoor	[R]		

:STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

:CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

00097277 (S)

DATA REQUIREMENT FOOTNOTES:

§158.135 - TOXICOLOGY - ACUTE TESTING :
- 81-2 - Acute Dermal Toxicity

A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[R]	YES	
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[R]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[R]	YES	
[X]	G. Forestry	[R]	YES	
[X]	H. Domestic Outdoor	[R]	YES	
[ ]	I. Indoor	[R]		

STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

00064822 (S)

DATA REQUIREMENT FOOTNOTES:

158.135 - TOXICOLOGY - ACUTE TESTING :
- 81-3 - Acute Inhalation Toxicity - Rat

A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[R]	YES	
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[R]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[R]	YES	
[X]	G. Forestry			
[X]	H. Domestic Outdoor	[R]	YES	
[ ]	I. Indoor	[R]		

STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____

Months to Generate Additional Data _____

CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

00097599 (S)
00097281 (S)

DATA REQUIREMENT FOOTNOTES:

58.135 - TOXICOLOGY - ACUTE TESTING :
- 81-4 - Primary eye irritation - Rabbit

.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

'AGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[R]	YES	
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[R]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[R]	YES	
[X]	G. Forestry	[R]	YES	
[X]	H. Domestic Outdoor	[R]	YES	
[ ]	I. Indoor	[R]		

TATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

ITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful'

00064820 (S)
00084579 (S)

IA REQUIREMENT FOOTNOTES:

004679

4/

58.135 - TOXICOLOGY - ACUTE TESTING :
- 81-5 - Primary dermal irritation

.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

\GE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[R]	YES	
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[R]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[R]	YES	
[X]	G. Forestry	[R]	YES	
[X]	H. Domestic Outdoor	[R]	YES	
[ ]	I. Indoor	[R]		

ATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

TATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful)

00064821 (S)

\ REQUIREMENT FOOTNOTES:

3.135 - TOXICOLOGY - ACUTE TESTING :
- 81-6 - Dermal sensitization

:.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

:E 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[R]	YES	
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[R]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[R]	YES	
[X]	G. Forestry	[R]	YES	
[X]	H. Domestic Outdoor	[R]	YES	
[ ]	I. Indoor	[R]		

TUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

ATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

00097289 (S)

REQUIREMENT FOOTNOTES:

40

§158.135 - TOXICOLOGY - ACUTE TESTING :
- 81-7 - Acute delayed neurotoxicity - hen

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

:PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[R]	YES	
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[R]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[R]	YES	
[X]	G. Forestry	[R]	YES	
[X]	H. Domestic Outdoor	[R]	YES	
[ ]	I. Indoor	[R]		

:STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

:CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

GS0119-007 (S)

DATA REQUIREMENT FOOTNOTES:

§158.135 - TOXICOLOGY - SUBCHRONIC TESTING :
- 82-1 - 90-day feeding - rodent, non-rodent

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

:PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood			
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood			
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood			
[X]	G. Forestry			
[X]	H. Domestic Outdoor			
[ ]	I. Indoor	[R]		

:STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____

Months to Generate Additional Data _____

:CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

00066771 (P) (rat)
00066785 (P) (dog)

DATA REQUIREMENT FOOTNOTES:

§158.135 - TOXICOLOGY - SUBCHRONIC TESTING:
- 82-2 - 21-day dermal

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

:PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[CR]	yes	
[X]	B. Terrestrial - Nonfood	[CR]	yes	
[ ]	C. Aquatic - Food Crop	[CR]		
[ ]	D. Aquatic - Nonfood	[CR]		
[X]	E. Greenhouse - Food Crop	[CR]	yes	
[X]	F. Greenhouse - Nonfood	[CR]	yes	
[X]	G. Forestry	[CR]	yes	
[X]	H. Domestic Outdoor	[CR]	yes	
[ ]	I. Indoor	[CR]		

:STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

:CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

00097287 (rat) (S)

DATA REQUIREMENT FOOTNOTES:

§158.135 - TOXICOLOGY - SUBCHRONIC TESTING :
- 82-3 - 90-day dermal

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

:PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[CR]	no	
[X]	B. Terrestrial - Nonfood	[CR]	no	
[ ]	C. Aquatic - Food Crop	[CR]		
[ ]	D. Aquatic - Nonfood	[CR]		
[X]	E. Greenhouse - Food Crop	[CR]	no	
[X]	F. Greenhouse - Nonfood	[CR]	no	
[X]	G. Forestry	[CR]	no	
[X]	H. Domestic Outdoor	[CR]	no	
[ ]	I. Indoor	[CR]		

:STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____

Months to Generate Additional Data _____

:CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

DATA REQUIREMENT FOOTNOTES:

004879 4/85

§158.135 - TOXICOLOGY - SUBCHRONIC TESTING :
- 82-4 - 90-day inhalation - rat

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

:PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[CR]	yes	
[X]	B. Terrestrial - Nonfood	[CR]	yes	
[ ]	C. Aquatic - Food Crop	[CR]		
[ ]	D. Aquatic - Nonfood	[CR]		
[X]	E. Greenhouse - Food Crop	[CR]	yes	
[X]	F. Greenhouse - Nonfood	[CR]	yes	
[X]	G. Forestry	[CR]	yes	
[X]	H. Domestic Outdoor	[CR]	yes	
[ ]	I. Indoor	[CR]		

:STATUS OF DATA REQUIREMENTS

Satisfied _____ Partially Satisfied _____ Not Satisfied __X__

Months to Generate Additional Data 12 months

:CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

DATA REQUIREMENT FOOTNOTES:

§158.135 - TOXICOLOGY - SUBCHRONIC TESTING :
- 82-5 - 90-day neurotoxicity - hen

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

.PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[CR]	no	
[X]	B. Terrestrial - Nonfood	[CR]	no	
[ ]	C. Aquatic - Food Crop	[CR]		
[ ]	D. Aquatic - Nonfood	[CR]		
[X]	E. Greenhouse - Food Crop	[CR]	no	
[X]	F. Greenhouse - Nonfood	[CR]	no	
[X]	G. Forestry	[CR]	no	
[X]	H. Domestic Outdoor	[CR]	no	
[ ]	I. Indoor	[CR]		

:STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

:CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

DATA REQUIREMENT FOOTNOTES:

§158.135 - TOXICOLOGY - CHRONIC TESTING :
- 83-1 - Chronic feeding - (2 spp.) - rodent, non-rodent

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

:PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]		
[X]	B. Terrestrial - Nonfood	[CR]		
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[CR]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[CR]		
[X]	G. Forestry	[CR]		
[X]	H. Domestic Outdoor	[CR]		
[ ]	I. Indoor	[CR]		

:STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

:CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

00097284 (P) (rat)
00097305, 00081913, 00097318, 00097326, 00061618 (P) (dog)

DATA REQUIREMENT FOOTNOTES:

§158.135 - TOXICOLOGY - CHRONIC TESTING :
- 83-2 - Oncogenicity study - (2 spp.) - rat , mouse

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

:PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[CR]		
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[CR]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[CR]		
[X]	G. Forestry	[CR]		
[X]	H. Domestic Outdoor	[CR]		
[ ]	I. Indoor	[CR]		

:STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

:CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

00097284 (P) (rat)
00096514 (P) (mouse)

DATA REQUIREMENT FOOTNOTES:

004679

§158.135 - TOXICOLOGY - CHRONIC TESTING :
- 83-3 - Teratogenicity - (2 spp.) - rat, rabbit

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

:PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[CR]		
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[CR]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[CR]		
[X]	G. Forestry	[CR]		
[X]	H. Domestic Outdoor	[CR]		
[ ]	I. Indoor	[CR]		

:STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

:CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

00035352 (N) (rabbit) [1]
00115674, 00126522 (P) (rat)
GS0119-009 (P) (rat)
GS0119-017 (P) (mouse)

DATA REQUIREMENT FOOTNOTES:
1. a second species gavage teratology study is still required

§158.135 - TOXICOLOGY - CHRONIC TESTING :
- 83-4 - Reproduction - rat - 2 generation

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

:PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[CR]		
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[CR]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[CR]		
[X]	G. Forestry	[CR]		
[X]	H. Domestic Outdoor	[CR]		
[ ]	I. Indoor	[CR]		

:STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

:CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

00066773 (S)

DATA REQUIREMENT FOOTNOTES:

§158.135 - TOXICOLOGY - MUTAGENICITY TESTING
- 84-2 - Gene mutation

A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[CR]		
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[CR]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[CR]		
[X]	G. Forestry	[CR]		
[X]	H. Domestic Outdoor	[CR]		
[ ]	I. Indoor	[CR]		

STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____

Months to Generate Additional Data _____

CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful)

00038808 (S)
GS0119-001 (S)
GS0119-002 (S)

DATA REQUIREMENT FOOTNOTES:

§158.135 - TOXICOLOGY - MUTAGENICITY TESTING :
- 84-2 - Structural chromosomal aberration

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

:PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[CR]		
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[CR]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[CR]		
[X]	G. Forestry	[CR]		
[X]	H. Domestic Outdoor	[CR]		
[ ]	I. Indoor	[CR]		

:STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

:CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful

GS0119-003 (S)
GS0119-004 (S)

DATA REQUIREMENT FOOTNOTES:

158.135 - TOXICOLOGY - MUTAGENICITY TESTING :
- 84-4 - Other genotoxic effects

A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[CR]		
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[CR]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[CR]		
[X]	G. Forestry	[CR]		
[X]	H. Domestic Outdoor	[CR]		
[ ]	I. Indoor	[CR]		

STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____
Months to Generate Additional Data _____

CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful)

GS0119-005 (S)
GS0119-006 (S)

DATA REQUIREMENT FOOTNOTES:

158.135 - TOXICOLOGY - SPECIAL TESTING :
- 85-1 - General metabolism

A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[CR]		
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[CR]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[CR]		
[X]	G. Forestry	[CR]		
[X]	H. Domestic Outdoor	[CR]		
[ ]	I. Indoor	[CR]		

STATUS OF DATA REQUIREMENTS

Satisfied _____ Partially Satisfied _____ Not Satisfied X

Months to Generate Additional Data 24 months

CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Usefu

00066776 (N)

DATA REQUIREMENT FOOTNOTES:

§158.135 - TOXICOLOGY - SPECIAL TESTING :
- 85-2 - Dermal penetration

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), methyl ester

:PAGE 1 of 1 for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

Current Use	Use Category	Guideline Status	Are Data Required	Footnote Number
[X]	A. Terrestrial - Food Crop	[R]	YES	
[X]	B. Terrestrial - Nonfood	[CR]		
[ ]	C. Aquatic - Food Crop	[R]		
[ ]	D. Aquatic - Nonfood	[CR]		
[X]	E. Greenhouse - Food Crop	[R]	YES	
[X]	F. Greenhouse - Nonfood	[CR]		
[X]	G. Forestry	[CR]		
[X]	H. Domestic Outdoor	[CR]		
[ ]	I. Indoor	[CR]		

:STATUS OF DATA REQUIREMENTS

Satisfied X Partially Satisfied _____ Not Satisfied _____

Months to Generate Additional Data _____

:CITATIONS: (S= Fully satisfactory P= Partially Satisfactory N= Not Useful):

GS0119-014 (S)

DATA REQUIREMENT FOOTNOTES:

004679

4/85

§158.135 - TOXICOLOGY

:A.I.=Benomyl; Carbamic acid,(1-((butylamino)carbonyl)-1H-benzimidazole-2-yl), :
: methyl ester

:PAGE 2 of __ for this requirement::DATED __/__/__::Supercedes page dated __/__/__:

DISCUSSION OF DATA:

BENOMYL #75A

004679

doc 1

004679

STUDY TYPE: Acute oral LD_{50} - Rats TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 174-65 FICHE/MASTER: 00066779
MR NO.: 581

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

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

AUTHORS: G.M.Zwicker, H.Sherman

DATE REPORT SUBMITTED: December 15, 1965

TEST MATERIAL: 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: Young ChR-CD rats were given single doses of test compound in peanut oil, by stomach tube. The dosing schedule was as follows:

Dose (mg/kg)	14 day surv./Treated	Rel. Testis Wt (gm)
200	1/1	0.86
450	1/1	0.86
670	1/1	0.77
1000	1/1	0.79

The survivors were necropsied after 14 days and the testes weighed and compared to the final body weight. They were also evaluated for visual and microscopic alterations.

RESULTS: There was no apparent change in body weight. There may have been an absolute and relative testicular weight decrease however there were too few animals to confirm this. Both testes were small and soft in the 670 mg/kg treated rats. Up to 10 % of the seminiferous tubules had degeneration and necrosis of the germinal epithelium, absence of mature sperm in the tubules, and multinucleated germinal giant cells (670 and 1000 mg/kg treated animals).

DISCUSSION: There was definite evidence of macroscopic and microscopic testicular dammage at the two high doses tested. Too few animals were tested to determine the significance of the target organ weight changes. Although no other toxic signs were noted, the report did not say whether they were looked for. The report did not give the composition of the test compound (formulation or technical, and % a.i.).

CONCLUSIONS: The testis is a target organ with tubular degeneration and necrosis.

CORE CLASSIFICATION - supplementary

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

004679

STUDY TYPE: Acute oral LD_{50} - Rats TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 100-66 FICHE/MASTER: 00097601
MR NO.: 581

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

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

AUTHORS: H.Sherman, W.C.Krauss

DATE REPORT SUBMITTED: July 15, 1966

TEST MATERIAL: 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: Young adult male ChR-CD rats were given single doses of test compound in a 20-22.5 % suspension of peanut oil, by stomach tube. The dosing schedule was as follows:

Dose (mg/kg)	Dead/Treated	Rel. Testis Wt (gm)	% tubules affected
1500	0/1	0.83	< 10
2250	0/1	1.29	> 70
3400	0/1	0.69	> 70
5000	0/1	0.71	< 10
7500	0/1	0.48	> 70
9590	0/1	0.42	> 10 < 70

The survivors were necropsied after 14 days and the testes weighed and compared to the final body weight. They were also evaluated for visual and microscopic alterations.

RESULTS: There were no deaths or change in body weight. Although there appeared to be a 50 % decrease in absolute and relative testicular weight, there were too few animals to confirm this. Testes from rats given greater than 1500 mg/kg of material were small. See the above table for percent of seminiferous tubules with degenerated germinal epithelium, multinucleated giant cells and reduced to no sperm. Rats treated with 3400 mg/kg and greater also had reduced or no sperm in the epididymis.

DISCUSSION: There is definite evidence of testicular damage, both macroscopic and microscopic, at all doses tested. Too few animals were tested to determine the level of significance of the target organ weight changes. Although no other toxic signs were noted,

the report does not say whether they were looked for. The report also does not give the composition of the test compound (formulation or technical, and % a.i.).

CONCLUSIONS: There were too few animals/dose to determine LD_{50} (no death at 9590 mg/kg - 1 rat). The testis is a target organ with tubular degeneration and necrosis.

CORE CLASSIFICATION - supplementary

Reviewed by M.P.Copley, D.V.M.
Tox. Br.
9/12/85
(Original review by L.B.Dale, October 23, 1968)

STUDY TYPE: Acute oral LD_{50} - Rats TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 421-80 FICHE/MASTER: 64819
MR NO.: 581-867 ACC.: 243043

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

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

AUTHORS: J.A.Hall, G.L.Kennedy

DATE REPORT SUBMITTED: May 23, 1980

TEST MATERIAL: Benlate®, (75 % a.i.); active ingredient: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

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

Original review:
"Procedure: A group of (fasted)* 5M (ave.wt. 226 gm)*, 5F (ave.wt. 154 gm)* ChR-CD rats received oral application of the test substance by intubation. The substance used was "Benlate DF Fungicide" suspended (30% suspension)* in corn oil at a dosage rate of 5000 mg/k The animals were observed for 14 days. Survivors were sacrificed at the termination of the study; all animals were necropsied.

Results: No mortalities. Symptoms included stained face, stained an wet perineal area, chromodacryorrhea and weight loss. Necropsies revealed testes slightly-small to small, soft, grey with white subcapsular streaks and foci; livers - slightly heavy; lungs - pale red with grey foci throughout. LD_{50} is greater than 5000 mg/kg.

Toxicity Category: IV - CAUTION "

Original reviewer: M.L.Quafe (3/25/70)

Core Classification: minimum

CONCLUSION: LD_{50} > 5000 mg/kg (75 % a.i.)
The testes appears to be a target organ.

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

()* Facts from study inserted into original review by M.P.Copley for completeness.

STUDY TYPE: Acute oral LD_{50} - Rats TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 17-69 FICHE/MASTER: 97277
MR NO.: 581

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.A.Zapp

DATE REPORT SUBMITTED: Jan. 22, 1969

TEST MATERIAL: Benlate® [redacted] (53 % tech, % a.i. not given); active ingredient: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: a.i.:Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazol-2-yl)-methyl ester
INT-1991
NB- 6299-77
DPX-3866
N.B. 8084-166B
Haskell No. 5833

MATERIAL AND METHODS: A group of 10 male* (ave. wt. 254 gm) and 10 female (ave. wt. 181 gm) ChR-CD rats were fasted then treated with a 20 or 30 % suspension of compound in peanut oil at a dosage rate of 10,000 mg/kg**. The animals were observed for 14-16 days.

RESULTS: There were no mortalities. No other data was given.

CONCLUSION: LD_{50} is greater than 10,000 mg/kg (53 % tech.)

Core Classification: Minimum

Toxicity Category: IV

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

63

*Consisted of 2 groups of males with 5 each treated at different times. Female group of 10 was treated at ont time.
** Based on active ingredient.

[redacted]

004679

STUDY TYPE: Acute oral LD_{50} - Rats TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 17-69 FICHE/MASTER: 97277
MR NO.: 581

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.A.Zapp

DATE REPORT SUBMITTED: Jan. 22, 1969

TEST MATERIAL: Benomyl technical; % a.i. not given, active ingredient: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: a.i.:Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazol-2-yl)-methyl ester
INT-1991
NB- 6275-176
DPX-3866
N.B. 8084-166B
Haskell No. 5834

MATERIAL AND METHODS: A group of 10 male* (ave. wt. 245 gm) and 10 female (ave. wt. 180 gm) ChR-CD rats were fasted then treated with a 20 or 30 % aqueous suspension of compound at a dosage rate of 10,000 mg/kg**. The animals were observed for 14-16 days.

RESULTS: There were no mortalities. No other data was given.

CONCLUSION: LD_{50} is greater than 10,000 mg/kg (tech.)

Core Classification: Minimum

Toxicity Category: IV

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

*Consisted of 2 groups of males with 5 each treated at different ti
Female group of 10 was treated at ont time.
** Based on active ingredient.

64

[redacted]

STUDY TYPE: Acute dermal LD_{50} - Rabbits

TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 554-80

FICHE/MASTER: 000648

MR NO.: 0581-867

ACC. No.: 243043

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

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

AUTHORS: O.L.Dashiell, P.Ashley

DATE REPORT SUBMITTED: July 23, 1980

TEST MATERIAL: Benlate®, (75 % a.i.); active ingredient: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: Benomyl Dry Flowable concentrate
a.i.: 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: Five male (ave. wt. 2848 gm) and five female (ave. wt. 2071 gm) adult albino rabbits, New Zealand White strain, were clipped over the back and trunk and plastic collars attached. Two thousand mg/kg of test substance (moistened with physiological saline) was applied to abraded skin on each rabbit and covered with gauze pads and wrapped with plastic wrap and adhesive bandage. After 24 hr the material and wrapping were removed and the treated skin wiped dry. The animals were observed and weighed during a 14 day period then sacrificed. Only two rabbits per sex were necropsi at that time.

RESULTS: There were no deaths. Although there was sporatic weight loss in the males (from 1-14 days) and females (1-7 days) there was an average 14 day weight gain of 11 % for the males and 21 % for the females. Moderate to moderate-severe skin irritation was prese on all animals the day after dosing. All rabbits had returned to normal by day 14, except 1 male and 1 female. No compound related abnormalities were observed at necropsy.

CONCLUSIONS: LD_{50} > 2000 mg/kg (75% a.i.)

TOXICITY CATEGORY: III

CORE CLASSIFICATION: Guideline

Reviewed by M.P.Copley, D.V.M.
Tox. Br.
9/12/85
(Original review by Sherell Sterling, 11/19/

65

STUDY TYPE: Acute dermal LD_{50} - Rabbits TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 201-216 FICHE/MASTER: 00976(
MR NO.: 581-239

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

STUDIES PERFORMED AT: Hazleton Labs., Inc., Falls Church, Va.

AUTHORS: W.M.Busey

DATE REPORT SUBMITTED: June 21, 1968

TEST MATERIAL: Benomyl; 50 % wettable powder; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS:
a.i.: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: Abdomens of 4 rabbits (2.3-2.9 kg) per dose (2 abraded, 2 intact skin) were exposed for 24 hr to 464, 1000, 2150, 4640 mg/kg of test material covered with an occlusive dressin One rabbit each was exposed to 3430 and 10,000 mg/kg, also with an occlusive dressing for 24 hr. After treatment, the skin was washed with water to remove the remaining compound. Animals were given food and water ad libitum and observed daily for 14 days for death and toxicity. Body weights were taken before and at termination of the study. All survivors were necropsied and the testes were examined microscopically for several rabbits.

RESULTS: There were no deaths. One animal (out of four) in each o the first four treatment groups developed non-treatment related enteric problems and anorexia. Dermal irritation at 464, 1000, 215, 4640 mg/kg was slight to moderate (slight erythema and moderat edema), subsiding by day 4 or 5. There was also slight desquamatio on days 4 and 5. At 3430 and 10,000 mg/kg, there was marked erythe lasting 7 days and slight edema lasting 2 days. There was also slight desquamation observed by day 5. There were no treatment related lesions observed at necropsy or histopathologic changes in the testes.

CONCLUSIONS: LD_{50} > 4640 mg/kg (50% WP)

TOXICITY CATEGORY: III

CORE CLASSIFICATION: minimum

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

Originally reviewed by L.B.Dale, 10/23/68:
The original review listed the LD_{50} as > 10,000 mg/kg however M. Copley disagrees because only 1 rabbit was tested at 10,000 mg/kg.

66

004679

STUDY TYPE: Acute inhalation LC_{50} - Rats TOX. CHEM. NO.: 7

HAZELTON PROJECT NUMBER: 201-220 FICHE/MASTER: 000
MRO NO.: 1126

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

STUDIES PERFORMED AT: Hazelton Lab., Inc., Falls Church Va.

AUTHOR: W.M.Busey

DATE REPORT SUBMITTED: October 18, 1968

TEST MATERIAL: Benomyl; 50 % wettable powder; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester; 50 %

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

MATERIAL AND METHODS: Four groups of 6 albino rats (ave. wt. 29
gm) were exposed in an inhalation chamber to Benomyl for 4 hr at the following analytical concentrations:

mg/L	dead/treated	Aspermatogenesis (severity)	#/survivor
0	1/6		0/5
0.27	0/6	slight to moderate	1/6
1.39	0/6	slight	1/6
4.01	0/6	severe	2/6

The chamber was a 1000 L plexiglass and stainless steel container with aerosolization by means of a constant airflow generator or a Devolbiss powder blower. The analytical concentration was deter by gravimetric analysis at least twice during the exposure. The animals were individually housed in the exposure chamber, then by group for the duration of the 14 day observation period. The rat were observed frequently during the exposure period for toxic sig and death, then daily for the duration of the study. Necropsies performed on all animals either at death or at the 14 day sacrifi Gross lesions, lungs, trachea, liver, kidneys and testes were exa histologically. Other organs were saved in 10 % buffered formali

RESULTS: Mortality - There was 1 death in the control group on d due to a preexisting condition.
Observations - During exposure the 1.39 mg/L group animals were covered with a white powder within 15 min. After 45 minutes breathing became progressively more labored and shallow with gasp There was excessive lacrimation and salivation. The rats recover shortly after cessation of exposure. The 4.01 mg/L rats were not visable due to the high concentration of compound in the chamber. After 2 hr the aerosol was reduced momentarily to visualize the

67

animals. They were inactive, gasping and heavily coated with dus
They resumed normal activity and breathing patterns after removal
from the chamber. All animals were normal from day 1 till the en
of the study with the exception of 1 death in the control group
(noted earlier).

Necropsy - Most animals had lung discolorations and spots of vari
colors and sizes. There were no treatment related necropsy
observations.

Pathology - The report noted a slight increase in severity and
frequency of lung inflammatory lesions. There was also increased
severity over controls of intrabronchiolar epithelial hyperplasia
and perivascular infiltration of mononuclear cells and lymphocyte
There was also an increased severity over controls of tracheitis
with inflammatory cell infiltration into the tracheal submucosa i
the 4.01 mg/L. Aspermatogenesis was present in all treated group
(see table).

DISCUSSION: Although only 6 rats were used per group, the LD_{50} l
can be estimated as greater than the high dose tested (no deaths a
any treatment level). There is no mention of partical size. Sinc
heavy dust was reported on the animals and cage some of the expos
may have been oral due to preening. The background of lung lesion
in the controls due to using older animals, made it difficult to
determine a treatment related response. The increased lung inflam
lesions noted in the report's summary was not evident from the
histopathology tables. The major target of this compound appeared
to be the testes, characterized by aspermatogenesis. The report
mentions a treatment related decrease in sperm production however,
the average level of spermatogenic activity in those rats without
aspermatogenesis was the same for all groups.

CONCLUSIONS: LC_{50} > 4.01 mg/L (50% a.i.). Aspermatogenesis is
present at all levels tested (0.27 mg/L LDT).

TOXICITY CATEGORY: III

CORE CLASSIFICATION: Minimum

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

This study was originally reviewed by R.D.Coberly on 2/27/73.

[redacted]

STUDY TYPE: Acute inhalation LC_{50} - Rats TOX. CHEM. NO.: 75A

HASKELL PROJECT NUMBER: 95-69 FICHE/MASTER: 00097281
MR NO.: 1192

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

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

AUTHOR: C.S.Hornberger

DATE REPORT SUBMITTED: April 24, 1969

TEST MATERIAL: Benomyl; 50 % wettable powder; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester; 50 % a.i. (52.2% Tech.)

SYNONYMS: Benlate® dust (Fungicide)
a.i.: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: Ten male Charles River Caesarean Derived rats (64-65 days old) were exposed for 4 hr to Benlate® dust - nose only. The dust was generated by exposing a falling stream of powder to a pneumatic jet. A small cyclone head was used to recycle particles less than 10 um to the generator. The dust then passed through a cylinder into which the heads of the rats projected. Particle size was determined by a cascade impactor. Actual concentrations, determined gravimetrically, were; 0, 0.02, 0.12, 0.20, and 0.82 mg/L. After exposure, the rats were observed for 7 (5 per group) or 14 days then necropsied. Body weight and testicular weights were noted. All testis and lungs were examined histologically Lymph nodes, liver, spleen, and kidney were examined histologically in 1 rat per group per time period.

RESULTS:
There was no change in relative testicular weight due to the concentrations used. At 14 days 2/5 rats (0.82 mg/L) had decreased spermatogenesis. All other groups were normal except for one control rat who fell in the Benlate® dust for one hr and also had decreased spermatogenesis.

DISCUSSION: Oral exposure was minimized by using the nose only exposure. In the previous study at Hazelton (whole body) the low dose testicular effect may have been due in part to ingestion as the one control that fell into the dust also had decreased spermatogenesis.

CONCLUSIONS: LC_{50} > 0.82 mg/L (50% WP, 50% a.i.)
The NOEL for altered spermatogenesis (acute inhalation) is 0.20 mg/L (7.5 mg/kg)
The LEL for altered spermatogenesis (acute inhalation) is 0.82 mg/L (33 mg/kg)

TOXICITY CATEGORY: II

CORE CLASSIFICATION: minimum

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

004679

STUDY TYPE: Acute inhalation LC_{50} - Dogs TOX. CHEM. NO.: 75A

HAZELTON PROJECT NUMBER: HLR-192-69* FICHE/MASTER: 00097282
MR NO.: not given

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

STUDIES PERFORMED AT: Hazelton Lab., Inc., Falls Church, Va.

AUTHOR: N.A.Littlefield

DATE REPORT SUBMITTED: 7/14/69

TEST MATERIAL: Benomyl; 50 % wettable powder; 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester; (information obtained from summary MRID # 00097275)

SYNONYMS: Benlate® Dust (Fungicide)
a.i.: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: Animals - Sexually mature male beagle dogs were assigned to 3 groups of 10 dogs each.
Exposure - The animals were exposed for 4 hr to either; 1) Fungicide 1991 - Benlate Formulation at an actual concentration of 0.65 (LDT) or 1.65 (HDT) mg/l, or 2) filtered room air. They were in a 4000 liter stainless steel and glass chamber. The compound was aerosolized by a pneumatic-dust generator with a cyclone head. The air flow was 1500 l/min. for the LDT and 260 l/min for the HDT. Actual concentrations were determined by gravimetric analysis and particle sizes were determined with a cascade impactor. After exposure the dogs were rinsed in warm water and dried to decrease exposure by ingestion.
Observations - Animals were observed for toxicity and death. They were weighed prior to treatment and weekly thereafter. The testes were palpated at unspecified intervals.
Sacrifice - The dogs were necropsied after 14 days (5/group) or 28 days of observation. Testes from all animals were preserved in chilled Bouin's fluid. Other tissues were fixed in 10 % neutral buffered formalin. Trachea, lungs (all lobes), testes and liver were examined histologically for all animals. The following tissues were also fixed, however only one animal per group per time period had a complete histopathologic evaluation: brain, pituitary, thyroid glands, heart, gallbladder, spleen, kidney, eye, stomach, pancreas, small intestine, large intestine, lymph nodes, urinary bladder, bone, bone marrow, adrenal glands, thymus, cervical spinal cord, salivary gland, lacrimal glands, prostate gland, sciatic nerve, skeletal muscle, aorta, and any tumors. The testes, brain and liver were weighed for organ/body and organ/brain weight ratios as well as absolute organ weights.

* number obtained from bibliography in WHO review on Benomyl (Nov-Dec/83) by R. Jaeger

Statistics - All data were tested with the F-test or analysis of variance, at a probability level of 5 %. Bartlett's method was used to test for heterogeneity of variances. If the variances were different, samples were tested with the Sachs' test or the Fisher-Behrens modified "t"-test.

RESULTS: There were no deaths.
Clinical signs were as follows:

control — No observations were noted.

0.65 mg/l — The animal coats were coated with compound during the exposure period, no other observations were noted.

1.65 mg/l — During the exposure period there was an aerosol cloud of high density which hindered observations. Coats were heavily coated with test aerosol during exposure. One dog had gagging, mastication and an oral mucous discharge starting 2 hr into the exposure period. All dogs were lethargic and had a white oral and nasal discharge when the exposure was terminated. One dog vomited on day 3. The remainder of the dogs appeared normal throughout the 28 day observation period.

Body weight - Although the report states there was a significant loss of weight in the high dose by 28 days, this is not evident from the data (see discussion of this review).

HDT body weight (kg)	pretest	wk 1	wk 2	wk4
all 10 dogs	10.2	10.2	10.3	--
5 dogs from 28 day sac	9.6	9.7	9.7	9.5

Necropsy -There were no compound related effects observed at either the 14 or 28 day necropsy.
Organ weight - There were no organ weight changes at 14 days, however, at 28 days the HDT absolute liver weight, LDT and HDT liver to brain weight and LDT liver to body weight were significantly decreased from control values.

	control		.65 mg/l		1.65 mg/l	
	14d	28d	14d	28d	14d	28d
brain/body wt (%)	.757	.693	.733	.757	.781	.880
liver (g)	311	370	315	300	271	276*
liver/body wt (%)	2.86	3.22	2.74	2.60*	2.60	3.00
liver/brain (%)	378	468	375	346*	334	342*

* $p < .05$

Histology - At 14 days there was no alteration in the testes at the LDT. In the HDT animals 4/5 had reduced spermatic activity due to reduced spermatogenesis although at 28 days, no reduction was evident. There were no treatment related lesions in the other organs examined.

DISCUSSION: The high dose exposure appeared to cause mucosal irritation evidenced by a nasal and oral mucous discharge which abated after the exposure period. The only response mentioned in the report was a depressed body weight in the HDT. This however, is not evident when the initial weights of the HDT dogs is considered. Although the final (terminal sac.) 28 day weight for the HDT is 9.2 kg and the control weight is 11.5 kg (sig. at $p < 0.5$) the pretest weights for the 5 HDT dogs was 9.6 kg and the 5 controls was 11.7 kg. The terminal weights were less for all groups than the 4 wk weights, possibly due to a prenecropsy fast (not mentioned). At the 14 day necropsy the liver (relative or absolute weight) appeared lighter at the HDT. At 28 days the liver was statistically lighter in both the LDT and HDT groups. Histologically at 14 days the spermatogenic activity was reduced. By day 28, the testes were histologically normal indicating that the depression was reversible.

CONCLUSIONS: $LC_{50} > 1.65$ mg/l (HDT) (50% WP)
No deaths occured at HDT
Reduced spermatogenic activity occured at 14 days but not 28 days

TOXICITY CATEGORY: II

CORE-CLASSIFICATION: minimum

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

MRID 00066771

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

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 content, sugar, ketones, pH, color, appearance and presence of occult blood. Pooled samples were used for microscopic examination.

7.1

* 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, trachea, pancreas, thyroid, parathyriod, salivar 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 live 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.

75

*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.

004679

CONCLUSIONS: NOEL 500 ppm
LEL 2500 ppm based on increased absolute and rel. 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. Qualfe, 3/25/70 and L.B.Dale, 10/23/68.

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

HASKELL LAB. REPORT NUMBER: 269-68 FICHE/MASTER: 00066785
MR NO.: 1069

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, J.W.Clayton

DATE REPORT SUBMITTED: Nov. 20, 1968

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

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

MATERIAL AND METHODS: Beagle dogs, 7-9 months old, were given fo and water ad libitum (between 4 pm-7 am), observed daily for abno behavior, signs of toxicity 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
Control (I)	food
Low dose (II)(LDT)	food 100 ppm INT-1991 (.01 %)
Mid dose (III)(MDT)	food 500 ppm INT-1991 (.05 %)
High dose (IV)(HDT)	food 2500 ppm INT-1991 (.25 %)

Diets were prepared weekly and refrigerated. The HDT group was gradually given increasing amounts of INT-1991 using the followin schedule: 500 ppm - 2 days; 1000 ppm - 3 days; 1500 ppm - 2 days. than 2500 ppm for the remainder of the study.

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

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

Laboratory tests - They were done three times during the pretest period and again at 30, 60 and 90 days.

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

Urinalysis - Urine vol. (time unspecified), osmolality, protein, sugar, urobilinogen, acetone, bilirubin, 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) rat

Sacrifice - All dogs were euthanized by electrocution after 90-10 days of continuous feeding and were examined for gross and micros changes. Tissues were fixed in Bouin's solution and stained with Haskell quadrichrome. The following organs were removed for weig fixation and staining: brain, heart, lungs, liver, spleen, pancre kidney, testis, prostate, stomach, thyroid, adrenal and pitultary. The following additional tissues were removed for fixation and staining: ovary, epididymis, Fallopian tubes, uterus, urinary bladder, duodenum, cecum, colon, skeletal muscle, peripheral ner bone marrow, eye, thoracic aorta, mammary gland, esophagus, gall bladder, spinal cord, trachea, thymus, salivary gland, and tonsil.

RESULTS: All animals survived the treatment period. There were n treatment related changes in body weight, food consumption, clinic observations, urinalysis, organ weights, gross pathology, and his pathology. Several clinical chemistry values were reported to be significantly different ($p < .05$) from either control or pretest values: AP for HDT males was higher than control values, GPT for HDT males was higher than pretest values, A/G for HDT males and females was lower than pretest and control values. These values, however were averages of all three time periods. All other clini chemistry values were normal.

TEST	pretest(S.D.)	0%	0.01%	0.05%	0.25%
AP(male)	2.2(0.7)	1.8*	2.0	2.4	2.5
GPT(male)	16(3.2)	23	19	18	25
A/G(male)	1.02(.19)	1.08	0.95	0.84	0.68
A/G(fmale)	1.27(.27)	1.09	0.92	1.01	0.72

*values are average of all three time periods (30, 60, 90 days)

DISCUSSION: The study reports elevated AP, however when compared pretest values, the change was not meaningful. GPT was only elev during the third month while the A/G values were depressed during the entire 90 day period.

Males		pretest (range)	30 day	60 day	90 day
GPT	control	14-19	24	17	20
	HDT	12-20	20	20	29
A/G	control	.74-1.22	.98	1.10	1.16
	HDT	.77-1.02	.69	.69	.67

Although there appears to be a treatment related increase in cholesterol, the values are within the pretest values. Lesions reported at necropsy and histologicaly appeare to be non-treatment related.

004079

CONCLUSIONS: NOEL 500 ppm (.05%)
LEL 2500 ppm (.25%) consisting of increased alk phosphatase, serum glutamic-pyruvic transamainase and decreased A/G ratio.

CORE-CLASSIFICATION: minimum

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

This study has been reviewed previously by M. Qualfe, 3/25/70

STUDY TYPE: Acute Neurotoxicity-Hens TOX. CHEM. NO.: 75

HASKELL LAB. REPORT NUMBER: HLO 28-79 ACCESSION NO: 2419
MR NO.: 2837-001
IRDC No.: 125-028

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

STUDIES PERFORMED AT: International Research and Development Corporation, Mattawan, Michigan

AUTHORS: E.I.Goldenthal, R.G.Geil, D.C.Jessup, W.P.Dean, R.J.A

DATE REPORT SUBMITTED: Jan. 5, 1979

TEST MATERIAL: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarba acid, methyl ester (% a.i. not given)

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

Summary of study by original reviewer:
"In the first study with Benomyl, five groups of 10 White Leghorn were administered,by gavage,single doses of the following test ma in 20 ml corn oil/kg respectively: 0 mg/kg (vehicle control), 750 tri-o-tolyl phosphate (TOTP, positive control), and 500 mg/kg, 25 and 5000 mg/kg benomyl (experimentals). Animals were observed fo pharmacotoxic symptoms including neurotoxicity. Surviving animal sacrificed, autopsied and organs examined grossly and selected ne tissue from spinal cord and peripheral (sciatic) nerves examined histologically for microscopic neurotoxic effects. Weights of al were monitored during the study.

None of the vehicle controls showed any symptoms. High dose benom treated hens had one death, decreased activity and diarrhea, and s neurotoxic symptoms (altered behavior). The high dose and mid dos benomyl-treated hens (5000 mg/kg and 2500 mg/kg, respectively) sh some compound related effects on microscopic examination of spinal and peripheral nerves. Positive controls (TOTP treatment) displa appropriate spectrum of nerve tissue degeneration known to be pro that chemical. However, at the conclusion of the experiment, the was declared inconclusive, due to evidence of underlying disease i hens. The disease state was determined by a group of pathologist examined the histological slide preparations of nerve tissues and identified pathological characteristics of Marek's disease."
Original reviewer, M. Sochard, Oct. 1

CORE-CLASSIFICATION: supplementary

CONCLUSION: This study can not answer the question of neurotoxic potential due to underlying disease.

Evaluation of summary by M.P.Copley, D.V
Tox. Br.
9/12/85

80

004679

STUDY TYPE: Acute Neurotoxicity-Hens TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: HLO 674-79 ACCESSION NO: 24193(
IRDC No.: 125-039 GS0119-00

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

STUDIES PERFORMED AT: International Research and Development Corporation, Mattawan, Michigan

AUTHORS: W.P.Dean, D.C.Jessup, R.J.Arceo, E.J.F.Spicer

DATE REPORT SUBMITTED: 10/8/79 (original), 12/6/79 (addendum)

TEST MATERIAL: Benomyl; 1-(Butylcarbamoyl)-2-benzimidazolecarbam: acid, methyl ester (99 % Tech., % a.i. not given)

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

MATERIAL AND METHODS: Fasted White Leghorn hens (1305-1890 gm, 1 months old) were given, by gavage, single doses of the following materials in 20 ml corn oil/kg:

Compound	Dose (mg/kg)	# Treated	Mortality
0(vehical cont.)	0	10	0
TOTP(pos. cont.)	1200	10	0
Benomyl	500	10	0
"	2500	10	0
"	5000	10	5(day-

TOTP - tri-o-tolyl phosphate

All hens had been vaccinated against New Castle disease, Avian Encephalomyelitis, Bronchitis and Marek's disease. They were individually housed in environmentally controlled rooms and given water and food ad libitum. After treatment they were observed twice daily for pharmacotoxic signs including neurotoxicity and weighed pretest and days 7, 14, 21. Survivors were necropsied an examined grossly. Microscopic examination was performed on selec nerve tissue from the spinal cord (3 levels) and sciatic nerve.

RESULTS AND DISCUSSION: (from the summary by M. Sochard, 10/14/81 "Five of the 10 high dose benomyl treated (5000 mg/kg) hens died between 6-9 days following treatment. The deaths were considered to be the consequence of acute toxicity of benomyl. Acute neurotoxicity symptoms were seen in the high dose benomyl-treated survivors, and in TOTP-treated hens. Symptoms in high dose benom treated survivors were primarily of decreased activity. TOTP treated hens showed symptoms of delayed neurotoxicity, which benc treated hens did not. The neurotoxic behavior symptoms displayed

81

early in the 5000 mg/kg benomyl treated hens was attributed to acute toxic effect of the chemical. Hens treated with mid and doses of benomyl (2500 mg/kg and 500 mg/kg, respectively) appe normal and behavior was normal. No treatment related gross pathological effects were seen in any hen at sacrifice and aut in benomyl treated hens. Microscopic examination of spinal co and sciatic nerves showed a spectrum of (expected) positive fi characteristic of TOTP treatment in the TOTP treated hens. So degenerative changes were seen in negative control nerve tissu well as in some 500 mg/kg treated and 2500 mg/kg treated benom hens - and none were seen in similar tissues from 5000 mg/kg b treated hens."

CONCLUSION: Benomyl does not appear to have delayed neurotoxi potential

NOEL for other neurotoxic signs: 2500 mg/kg

CORE-CLASSIFICATION: minimum
There were only 5 survivers in the high dose group; however, it is unlikely that it has delayed neurotoxic potential since the high and mid doses (5000 mg/kg, 2500 mg/kg) showed no histopathologic changes related to delayed neurotoxicity and this compound is not an organophosphate.

Review and Evaluation of summary by M.P.Copley, D.V.M.
Tox. Br.
9/12/85

82

[redacted]

004679

STUDY TYPE: 21 day dermal study - rabbits TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NUMBER: 211-69 FICHE/MASTER: 00097287
MR NO.: 1191 Path No.(38-69)

MRID 00097287

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

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

AUTHORS: D.B.Hood, J.R.Barnes, E.F.Stula, J.A.Zapp

DATE REPORT SUBMITTED: July 30, 1969

TEST MATERIAL: Benlate®; 1-(Butylcarbamoyl)-2-benzimidazolecarbam
acid, methyl ester; powder; [redacted]

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

MATERIAL AND METHODS: Benlate was applied topically to healthy, mature (> 3 kg) rabbits (strain unspecified). All rabbits were fitted with plastic collars and their trunks clipped prior to treatment. The study lasted 21 days (5 days treatment followed b 2 days no treatment). Test material was applied as an aqueous pa (75%) to the clipped back (abraded daily), covered by a gauze pad taped in place (non-occlusive). After 6 hours of exposure the material was washed off and the backs were dried. The groups wer as follows:

Formulation	Dose (mg/kg)*	Males	Females
[redacted]	0**	5	5
	50	5	5
	250	5	5
	500	5	5
	1000	5	5
	1000	5	5
	5000	2	2

* based on a.i.
** inert control - volume same as for the 1000 mg/kg group
*** not part of the original design.

The animals were given food and water ad libitum except during th exposure period. They were observed frequently during the test period for clinical signs and weighed daily during the first week and three times a week thereafter. Prior to sacrifice, blood was taken from all animals for hemoglobin determinations, and red and white blood cell counts. The following organs were weighed: thymus, liver, kidneys, spleen, adrenal, and testes. The followi

tissues were examined histologically: brain, heart, lung, trachea, liver, kidney, spleen, duodenum, bone marrow, adrenal, pituitary, thyroid, gonads, thymus, mammary gland, uterus, muscle, eye, and skin.

RESULTS: Body weight Only the 1000 mg/kg [redacted] male group showed a decrease in weight during the first week which lasted throughout the study. The weight in the 5000 mg/kg male group was variable, although both lost weight initially, 1 weighed less and 1 more at the end of the study. There was no treatment related weight response in the females. Observations were as follows:

Dose (mg/kg)	Clinical Observations
0	mod. erythema - cracking, desquamation
50[S]	mild - mod. erythema
250[S]	mild - mod. erythema
500[S]	mild - mod. erythema, desquamation
1000[S]	strong skin irrit., desquamation, dry feces
1000[C]	mild - mod. erythema, dry feces
5000[S]	mod. erythema, severe sloughing, feces dry and scant

Blood cell counts were normal. Hemoglobin concentration however, was decreased in the males at 1000[S] and 5000[S] mg/kg.

Dose (mg/kg)	Hemoglobin (gm/100 ml)
0	13.1
50[S]	13.2
250[S]	13.4
500[S]	13.5
1000[S]	12.4
1000[C]	13.3
5000[S]	11.9

Organ weights - Absolute and relative testes weights were as follows:

	Testes Weights [redacted]					
Dose[S] (mg/kg)	0	50	250	500	1000	5000
individual	7.44	5.11	4.20	6.11	6.71	6.25
absolute	4.90	7.50	5.90	4.00	4.30	6.50
(gm)	7.20	6.50	5.81	6.96	3.90	
	6.20	5.60	7.70	4.39	4.13	
	7.10	4.28	6.70	5.20	4.10	
ave. abs. (gm)	6.57	5.80	6.06	5.33	4.63	6.38
ave. rel.	.185	.155	.169	.148	.140	.212

The sponsor reports a decrease in absolute testes weight only in the 1000[S] mg/kg group. Aside from the one 5000[S] mg/kg rabbit with with focal testicular degeneration, no treatment related histopathological changes were reported.

[redacted]

DISCUSSION: There was a treatment related decrease in male weight at the 1000^{S} mg/kg dose level. The lack of a consistant response at the higher dose (5000^{S} mg/kg) was probably due to the number of animals treated at that level (2), while the lack of response with the other 1000^{C} mg/kg group may be due to the difference in formulation [redacted] Skin irritation also was more severe at 1000^{S} and 5000^{S} mg/kg than 1000^{C}, although the inert control group did have evidence of some irritation as well. The altered feces at 1000^{S,C} and 5000^{S} mg/kg appeared to be treatment related although the significance of this was not addressed by the registrant. Testes weights (rel. and abs.) indicated a possible although not statistically significant, decrease at the 1000 mg/kg group with the [redacted] There were only two rabbits in the 5000^{S} mg/kg group, but no decreased testicular weights were observed. Once again the difference in formulation may be responsible for the lack of testes alterations in the [redacted] 1000^{C} mg/kg. Only one rabbit with the high dose [redacted] had histologic testicular alterations noted (degeneration). Although it is difficult to interpret whether this effect on the testes is treatment-related, this compound has been shown to cause inhibition of spermatogenesis in the rat by other routes of exposure (i.e. inhalation, oral). There are additional problems with the report as presented: 1) The blood data is difficult to interpret without either standard deviations or individual animal data, 2) The Table of organ weights for female groups 0, 50, 250 and 500 mg/kg is missing in the submitted report, however, the authors indicated no effects on organ to body weight ratios.

CONCLUSIONS: [redacted]

NOEL = 500 mg/kg
LEL = 1000 mg/kg based on decreased (not statistically) testes weights (rel. and abs.).

[redacted]

NOEL = 1000 mg/kg (only dose tested)

The [redacted] appears to be more toxic at equivalant doses than the [redacted] fromulation.

CORE-CLASSIFICATION: minimum

Review and Evaluation of summary by M.P.Copley, D.V.M.
Tox. Br.
9/12/85

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-(Butylcarbamoyl)-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.

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

86

§ 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

004679

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	12.1	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

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 days 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

MRID 00097284

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.: 05042

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-(Butylcarbamo/ 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 I	36	control	0 (0)
group Ia	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 (inter. not specified) for behavioral and toxicological abnormalities. Food Consumption and Weight: Animals were weighed once/week for 1. 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 Chemistr Ten randomly picked male and females rats in the 0, 500 and 2500 p; groups were tested after 1, 3, 6, 9, 12, 18 and 24 months for plasm 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

89

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 quad-richrome 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 smear
lumber spinal cor
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.

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, fallop 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 (LA), 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. Liv changes were of frequent occurrence but about equally spread betwee 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 neopl or non-neoplastic lesions.

DISCUSSION: AP and GPT were the only clinical chemistries performe 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

STUDY TYPE: Primary Eye Irritation - Rabbit TOX. CHEM. NO.: 75A

HASKELL LAB. REPORT NO: 497-80 FICHE/MASTER: 00064820
MR NO.: 0581-867 ACCESSION NO.: 243043-

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

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

AUTHORS: O.L.Dashiel, P.Dashley, G.L.Kennedy

DATE REPORT SUBMITTED: June 13, 1980

TEST MATERIAL: Benlate Dry Flowable (75% a.i.); 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

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

Original review

Eye Irritation in Rabbits; Haskell Report #497-80; June 13, 1980; Acc. No. 243043

Procedure: "Benlate DF Fungicide" was applied into one eye of each of 9 albino rabbits. In each case, 0.1 ml of the test substance was applied. Three of the animals' eyes were irrigated with tap water for one minute, 20 seconds post-treatment.

Results: In the non-irrigated eyes at 24 hours, corneal opacity observed in 2/6=5, 1/6=10, 1/6=20, 2/6=40; iris irritation in 3/6=5; conjunctival redness in 5/6=1; chemosis in 5/6=1 and discharge in 3/6=1. By day 8, the only irritation was slight corneal opacity (1/6=5, 1/6=10). All non-irrigated eyes were clear by day 11. Three animals showed injury when viewed with biomicroscope at 8 days. For the irrigated eyes at 24 hours, corneal opacity exhibited in 1/3=5, 2/3=10; conjunctival redness in 2/3=1. All irritation cleared by day 8.

Study Classification: Core Guideline Data.

Toxicity Category: II-WARNING. In this case 2/6 non-irrigated eyes exhibited corneal opacity, 3/6 animals showed corneal injury when viewed with biomicroscope all at day 8.

Original review by Sherell A. Sterling
FHB/TSS
11/14/80

(addendum on next page)

Addendum by M.P.Copley

MATERIAL AND METHODS:
Test material (0.1 ml, 51.4 gm) was instilled in the right eyes. Eyes were examined and scored according to the method of Draize o days 1, 2, 3, 4, 8, and 11.

CONCLUSIONS: Unwashed eyes: Benlate produced corneal opacities which were reversible by 11 days. Mild iritis and conjunctiviti were present for only 3 days. Washed eyes: Washing after 20 se was effective in decreasing corneal lesions and keeping conjunct to a minimum.

Eye irritation score:

	1d	2d	3d	4d	8d	11d	
unwashed	27	17	9	7	3	0	n=6
washed	10	9	7	2	0	0	n=3

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

STUDY TYPE: Primary Eye Irritation - Rabbit TOX. CHEM. NO.:

HASKELL LAB. REPORT NO: 179-81 FICHE/MASTER: 0
MR NO.: 0581-935 ACCESSION NO.: 2

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

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

AUTHORS: L.S.Silber, O.L.Dashiel, G.L.Kennedy

DATE REPORT SUBMITTED: April 6, 1981

TEST MATERIAL: Benlate Dry Flowable (75% a.i.); 1-(Butylcarbamo 2-benzimidazolecarbamic acid, methy' ester

SYNONYMS: a.i. Benomyl
Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimi 2-yl)-methyl ester
INT-1991
DPX-3866
N.B. 0759-85-2

MATERIAL AND METHODS: Test material (0.1 ml, 46.2 mg) was inst in the right eye of 9 male albino rabbits (strain unspecified). Twenty seconds later 3 eyes were washed with tap water for 1 mi The left eyes were used as untreated controls. Eyes were exam scored according to the method of Draize on days 1, 2, 3, 4 an

RESULTS: Unwashed eyes: Corneal opacities persisted 24 hr in animal, 2d in 2 rabbits and 3d in 3 rabbits. All unwashed eye were normal by 4 days. Minimal iritis occurred in 5 animals an disappeared after 4 days. Conjunctival irritation, characteri by minimal redness, chemosis and a bloody discharge (day 1 onl was present in all 6 eyes for no more than 2d. Individual anim scores ranged from 14-55 on day 1 and 0-29 on day 2. Biomicros corneal effects were moderate to slight on day 1 and disappear day 4. Washed eyes: Washing after treatment decreased lesions minimal corneal opacities lasting only 3d in 2 animals. The th rabbit had lesions lasting till day 4, most severe on day 3. Conjunctival redness occured in 1 rabbit lasting 2 days. Biomi corneal effects were slight in all 3 rabbits lasting through da

CONCLUSIONS: Unwashed eyes: Benlate produced slight to mild c opacities which were reversible by 7 days. Minimal iritis and conjunctivitis were present for 4 days. Washed eyes: Washing after 20 seconds was effective in decreasing corneal lesions an keeping conjunctivitis to a minimum in 2 out of 3 animals. On had lesions as severe as these without washing.

Eye irritation score:

	1d	2d	3d	4d	7d	
unwashed	28.1	14.2	4.2	7.3	0	n=6
washed	4.7	4.7	13.3	3.3	0	n=3

004679

Toxicity Category: III

CLASSIFICATION OF STUDY: core-minimum

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

STUDY TYPE: Skin Irritation - Rabbit

TOX. CHEM. NO.:

HASKELL LAB. REPORT NO: 367-80

FICHE/MASTER: 000

MR NO.: 0581-867

ACCESSION NO.: 24

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

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

AUTHORS: O.L.Dashiel, L.S.Silber

DATE REPORT SUBMITTED: May 12, 1980

TEST MATERIAL: Benlate Dry Flowable (75% a.i.); 1-(Butylcarbamoy 2-benzimidazolecarbamic acid, methyl ester

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

Original review

"

Skin Irritation Test on Rabbits; Haskell Report #367-80; May 12, 1980; Acc. No. 243043

Procedure: 6 New Zealand white rabbits were exposed to the "Benlate DF Fungicide" at 4 sites on each rabbit (2 abraded, 2 intact). The test substance was applied as a paste at 0.5g per site under occlusive wrap fo 24 hours. Animals were observed at 24, 72 hours, 6 days.

Results: At 24 hours intact sites showed erythema in 6/12=1, 2/12=2; no edema. Abraded sites at 24 hours exhibited erythema in 9/12=1, 2/12=2; edema in 6/12=1, 1/12=2. By 72 hours, only 1/12 showed very slight erythema at abraded sites; no irritation at intact sites. All scores wer 0 by day 6.

Study Classification: Core Guideline Data.

Toxicity Category: IV - CAUTION "

Original review by Sherell A. Sterling; FHB/TSS; 11/14/80

Addendum

CONCLUSION: Benomyl (75% a.i.) is a slight to mild irritant

PIS: 0.67 (range 0.25 - 1.0) for day 1

Original review evaluated and addendum added by M.P.Copley, D.V.M.
Tox. Br.
x9/12/85

96

004679

STUDY TYPE: Skin Irritation and sensitization - Guinea Pigs

TOX. CHEM. NO.: 7

HASKELL LAB. REPORT NO: 84-69
MR NO.: 0581

FICHE/MASTER: 0009
ACCESSION NO.: 504.

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

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

AUTHORS: C.W.Colburn, J.A.Zapp

DATE REPORT SUBMITTED: April 18, 1969

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

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidaz 2-yl)-methyl ester
INT-1991-202
DPX-3866
N.B. 6275-175

MATERIAL AND METHODS: Skin Irritation: Ten male albino guinea pi were treated with .05 ml each of a 10%, 25% and 40% paste of Beno in dimethylphthalate (DMP). The material was rubbed into shaved intact skin and scored 24 hr later using the following system:

0	- negative
+	- mild erythema
++	- moderate erythema
+++	- strong erythema
++++	- erythema with edema
Sensitized (S)	- presence of reaction beyond site of applicat

Sensitization: Induction - The same ten animals were used as foll 5 received 8 applications of the 40% and 1 of 26.6% paste rubbed on abraded skin over a 3 week period. The remaining 5 animals received 4 intradermal injections of a 1% solution in DMP (.1 ml) Challenge - The animals received the 1st challenge after a 2 week rest period. Twenty-five and 10% pastes were applied to both intact and abraded skin on the induced animals and scored 24 hr later. Challenge 2 was given 5 days later. Ten controls without prior induction also received both challenges.

RESULTS: The following are the results for the irritation and sensitization tests:

dose	irritation*			challenge 1**					challenge 2**				
level	0	+	++	+	++	+++	++++	S	0	+	++	+++	++++
10%	2	7	1	7/5	2/4	1/	/1	6	5/3	4/5	/1	1/1	
25%	1	8	1	3/1	5/5	/1	2/3		1/1	6/6	2/2		1/1
40%	7	3											

97

* number of animals affected
** number of intact sites/number of abraded sites

Control animals had:

dose level	intact 0	intact +	abraded 0	abraded +
10%	9	1	6	4
25%	6	4	8	2

DISCUSSION: This compound is a mild to moderate irritant in guin pigs. Although it is a moderate sensitizer the sensitization decreases rapidly. Individual animal data was not presented by t registrant.

CONCLUSIONS: Moderate sensitizer
Mild irritant

CORE-CLASSIFICATION: minimum

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

STUDY TYPE: Mutagenicity Evaluation in Salmonella Typhimurium

TOX. CHEM. NO.: 7

HASKELL LAB. REPORT NO: 560-80
MR NO.: 0581-881

FICHE/MASTER: GS01

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

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

AUTHORS: A.L.Horst , D.F.Krahn

DATE REPORT SUBMITTED: Aug. 22, 1980

TEST MATERIAL: Benomyl (99.6% a.i.); 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: a.i. Benomyl
Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimida-2-yl)-methyl ester
INT-1991
DPX-3866

Original review

"Horst and Krahn (1980) evaluated the mutagenic potential of technical grade benomyl (Polish;Cieck, 97.6% pure) in S. Typhimur Plates were treated with dosages ranging from 100 to 10,000 ug in the presence or absence of liver microsomal activation. The 5,00 and 10,000 ug doses with activation increased the number of rever plate over that of control plates by 2.6 and 7.6 times in strain TA 1537, respectively. The same two doses caused respective incr of 2 and 10 times (above)* controls in strain TA 98."

Original review by Roger Gardner, TB, 7/6/82

Addendum

MATERIAL AND METHODS: Strains TA 1535, TA 1537, TA 98 and TA 100 were tested in 2 independent trials each with duplicate plates. was no cytotoxicity observed in TA 1535 at concentrations up to 10,000 ug/plate.

CONCLUSION: Test material is mutagenic in S. typhimurium with activation.

CLASSIFICATION: Acceptable

Original review evaluated and addendum added by M.P.Copley, D.V.M.
Tox. Br.
x9/12/85

()*added to original review

STUDY TYPE: Chinese Hamster Ovary Cell Assay (HGPRT) TOX. CHEM. NO.: 7

HASKELL LAB. REPORT NO: 438-80 FICHE/MASTER: 0003
MR NO.: 581-852

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

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

AUTHORS: K.Fitzpatrick, D.F.Krahn

DATE REPORT SUBMITTED: May 16, 1980

TEST MATERIAL: Benomyl (99.9-100% a.i.); 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidaz 2-yl)-methyl ester
INT-1991
DPX-3866

Original review

"A report on a mutagenicity assay with a Chinese hamster ovar cell line which can demonstrate mutations at the gene locus coding for hypoxanthine-guanine phosphoribosyl transferase (HGPRT) was submitted (Fitzpatrick and Krahn, 1980). This study included benzo(a)pyrene and ethyl methane sulfonate as positive controls an a vehicle control (DMSO) were used, and benomyl was added to test cultures with or without metabolic activation by rat liver microso enzymes (S-9). Resistance of cells to 6-thioguanine was used as the indicator of mutagenic effects.

The authors reported a dose-related cytotoxic response which was more evident in cultures exposed to the chemical without activation. No statistically significant differences in mutation frequency were noted in cultures treated with activated or nonactivated benomyl. Concentrations ranged from 17 to 172 uM, an no statistically significant trends were noted. Positive controls demonstrated that the test system was sensitive, and cell survival was greater than 10% at most concentrations used. The authors concluded that benomyl was not mutagenic under these test conditio (Fitzpatrick and Krahn, 1980)."

Original review by Roger Gardner; TB; 7/6/82

Addendum

MATERIALS AND METHODS: Benomyl was tested in 2-5 independent trials each with duplicate plates.

CONCLUSION: Test material is not mutagenic in this test system

CLASSIFICATION: Acceptable

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

004679

STUDY TYPE: Mutagenesis - L5178Y TK$^{+/-}$ TOX. CHEM. NO.: 75

LAB. PROJECT NO: LSU-7558 FICHE/MASTER: GS0119-002

SPONSOR: Environmental Protection Agency

STUDIES PERFORMED AT: SRI International, Menlo Park, California

AUTHORS: M.M.Jotz, D.D.Rundle, A.D.Mitchell

DATE REPORT SUBMITTED: Dec. 1980

TEST MATERIAL: Benomyl (99% a.i.); 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazo 2-yl)-methyl ester
INT-1991
DPX-3866

Original review

"The ... study (Jotz, 1980) evaluated the ability of benomyl with and without metabolic activation (liver S-9 mix) to induce forward mutations at the thymidine kinase (TK) locus in mouse L5178Y lymphoma cells, ethylmethane sulfonate (EMS) and 3-methylcholanthrene were used as positive controls.

The authors concluded that benomyl is mutagenic in this test system since the mutation frequency was increased in a dose-relate manner, and some doses which had cytotoxicity increased the mutati frequency by more than twice that of the vehicle control (DMSO). The table summarizes the results for the controls and highest dose causing less than 90% cell death. The results indicated that metabolic activation enhanced benomyl's mutagenic activity."

Original review by Roger Gardner; TB; 7/6/82

Addendum

MATERIALS AND METHODS: Concentrations without activation were fro 2-25 ug/ml and with activation were 5-50 ug/ml, with higher doses causing less than 10 % growth. The range was determined based bot on relative total growth and precipitation. There was one trial with duplicate samples.

CONCLUSION: Increased mutation frequencies were observed at 50 ug without activation and at 12 and 25 ug/ml with activation. The te material is a weak mutagen in this system.

CLASSIFICATION: Acceptable

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

"The Effects of Benomyl on the Frequency of Forward Mutations at the TK Locus in L5178Y Mouse Lymphoma Cells (Jotz et al., 1980)[1]

Test Group	Relative Total Growth Percent	Mutation Frequency (X 10^{-6}),[2]
Without Activation		
Vehicle Control	107.2	38
(DMSO 1%)	93.1	37
Positive Control		
(EMS 1500	35.2	655
ug/ml)	48.2	449
Benomyl	18.8	122
(50 ug/ml)[3]	23.6	90
With Activation		
Solvent Control	103.4	66
(1% DMSO)	96.5	91
3-methylcholanthrene	50.8	316
(5 ug/ml)	58.3	342
Benomyl	9.1	531
(25 ug/ml)[4]	10.5	583

[1] Replicate results are reported.

[2] Determined by dividing the number of mutant colonies seen by the umber of potentially viable colonies per 3 x 10^6 cells plated.

[3] Higher doses caused relative total growth to be less than 10%, and lower doses did not consistently cause mutation frequencies of twice the control rate.

[4] The highest dose tested. Doses as low as 12 ug/ml doubled the mutation frequency when compared with controls."

STUDY TYPE: Micronucleus test

TOX. CHEM. NO.: 757

LAB. PROJECT NO: LSU-7558-19

FICHE/MASTER: GS0119-C03

SPONSOR: Environmental Protection Agency

STUDIES PERFORMED AT: SRI International, Menlo Park, California

AUTHORS: B. Kirkhart

DATE REPORT SUBMITTED: Feb. 12, 1980

TEST MATERIAL: Benomyl (% a.i. not given); 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidazo 2-yl)-methyl ester
INT-1991
DPX-3866

Original review

"In the ... study, groups of 24 male mice were given daily doses by gavage of 250, 500, or 1,000 mg benomyl per kg body weight on two consecutive days. A vehicle control (DMSO) group was also included. The authors stated that the highest dose presented a solubility problem which was corrected before the second dosage was administered, but they were uncertain about the amount given the first time. Eight animals from each group were sacrificed 24, 48, or 72 hours after the second dose was administered. Bone marrow from the femur of each animal was taken for examination. For each animal, 500 polychromatic erythrocytes (PCE) were examined for micronuclei, and the number of mature erythrocytes was counted until 200 PCE's were found.

The authors stated that a compound is considered to be positi in this assay if at least two dose-time groups had a statistically significant increase over controls in the number of cells with micronuclei per 500 PCE's. Before the study is considered negativ at least 4,000 PCE's per dose-time group must be examined at 24 to 96 hours after the first dose is given; the highest dose is a maximum tolerated dose; the average PCE to erythrocyte ratio is greater that 0.15 for each group and all groups do not have a statistically significant increase in the number of cells with micronuclei per 500 PCE's.

According to the authors, the statistical procedure used show that four groups had significantly increased numbers of cells with micronuclei. These groups included the low and mid dose groups at 48 hours (15/3500 and 16/4000, respectively as compared to 5/3000 cells from vehicle controls) and in the high dose group at 48 and 72 hours after treatment (20/3500 and 17/3500, respectively; respective control values are 5/3000 and 6/3500)."

Original review by Roger Gardner; TB; 7/6/82

Addendum

CONCLUSION: Test material is mutagenic in this system

CLASSIFICATION: Acceptable

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

STUDY TYPE: Sister Chromatid Exchange (CHO) TOX. CHEM. NO.: 7

LAB. PROJECT NO: LSU-7558 FICHE/MASTER: GS0119-004

SPONSOR: Environmental Protection Agency

STUDIES PERFORMED AT: SRI International, Menlo Park, California

AUTHORS: E.L.Evans, A.D.Mitchell

DATE REPORT SUBMITTED: Aug. 1980

TEST MATERIAL: Benomyl (99 % a.i.); 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

SYNONYMS: Carbamic Acid, (1-((Butylamino)-carbonyl)-1H-benzimidaz 2-yl)-methyl ester
INT-1991
DPX-3866

Original review

"The ... study (Evans and Mitchell, 1980) was conducted with Chinese hamster ovary cells in cultures containing varying concentrations of benomyl without metabolic activation (0, 0.625, 1.25, 2.5, 5 or 10 ug/ml) or metabolically activated benomyl (0, 0.375, 18.75, 37.5, 75 or 150 ug/ml).... These concentrations wer selected so that treated cells could undergo at least two divisio within 24 hours. Ethyl methane-sulfonate (EMS) and dimethylnitro (DMN) were used as positive controls and a vehicle control (0.95 ethanol in culture medium) was included. Two samples of 25 cells each were scored for number of sister chromatid exchanges and number of chromosomes. A total of 50 cells were scored for each group.

The 5 and 10 ug/ml dosages did not allow sufficient numbers second division metaphases to occur for an evaluation. The autho stated that there was a scoring discrepancy between the two cytogeneticists' observations. One found no effects, while the second noted an increase in sister chromatid exchanges (SCE) whic peaked at the 1.25 ug/ml concentration. A third cytogeneticist's observations were reported to show a plateau in the increased numb of SCE's at the three concentrations. Analysis of variance appli to all three sets of observations showed a statistically significa increase in SCE's for cells treated with benomyl without metaboli activation (variance between groups was significantly greater thar that within groups). Similar results were obtained when three se of observations were analyzed for metabolically activated benomyl.

The number of SCE's (per chromosome) in the EMS group was three to four-times that of the unactivated negative controls, while the three unactivated benomyl groups had one-third more SCE' (per chromosome) than controls. In the experiments with activated benomyl, the DMN positive controls had approximately twice the number of SCE's found in negative controls. The benomy groups had increased numbers of SCE's (by approximately 25 to 100

above controls). The number of SCE's per cell was increased by or sixth to one-half above that for negative controls for unactivate benomyl. The activated fungicide increased that number by approximately 50 to 100% over that seen in controls.

The authors concluded that results of this study were weakly positive (Evans and Mitchell, 1980)."

Original review by Roger Gardner; TB; 7/6/82

Addendum

CONCLUSION: Test mater al is weakly mutagenic in this system

CLASSIFICATION: Acceptable

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

004679

STUDY TYPE: DNA Repair (1° mouse hepatocytes) TOX. CHEM. NO.:

HASKELL LAB. REPORT NO: 741-81 FICHE/MASTER: GS0119-005
MR NO.: 4065-001

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

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

AUTHORS: C. Tong

DATE REPORT SUBMITTED: Oct. 20, 1981

TEST MATERIAL: Benomyl (% a.i. not given); 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

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

Original review

"The DNA repair assay in mouse hepatocyte primary cultures (HPC) was submitted (Tong, 1981). Benomyl ... was evaluated al with dimethylnitrosamine, dimethylformamide, fluorene and 2-amin fluorene which were used as positive controls. The liver was removed and primary cultures were started with hepatocytes from B6C3F1 mice benomyl and tritiated thymidine (19 uCi) were added to the culture medium. After 18 to 20 hours of incubating the treated cultures, they were fixed and examined microscopical for morphological changes and absence of s-phase nuclei indicati of cytotoxicity. Autoradiographic techniques were used to deter the number of nuclear grains induced by test chemicals. Backgro counts were obtained by evaluating three nuclear-sized areas in cytoplasm; these values were averaged and subtracted from the nu counted in the nucleus to obtain a net value for each nucleus. chemical is considered capable of inducing DNA repair when a net count of 5 grains or more is observed consistently in each of th nuclei examined in each of 3 replicate experiments.

Benomyl did not induce DNA repair in ... mouse hepatocytes (Tong, 1981) .... The dimethylnitrosamine and 2-amino fluorene increased the number of nuclear grains from 7 to 15 times the le set as the criterion for a positive response (5/nucleus; Tong, 1

Original review by Roger Gardner; TB; 7/6/82

Addendum

MATERIAL AND METHODS: Five log doses were tested in triplicate independent tests (.5, .05, .005, .0005, .00005 mg/ml). Only th non-toxic levels were counted.

RESULTS: Test 1: .5 and .05 mg/ml were toxic
Test 2: .5 and .05 mg/ml were toxic

107

CONCLUSION: Test material is not mutagenic in this system

CLASSIFICATION: Acceptable

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

C04679

STUDY TYPE: DNA Repair (1° rat hepatocytes) TOX. CHEM. NO.:

HASKELL LAB. REPORT NO: 741-82 FICHE/MASTER:
MR NO.: 4065-001 GS0119-006

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

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

AUTHORS: C. Tong

DATE REPORT SUBMITTED: Oct. 20, 1981

TEST MATERIAL: Benomyl (% a.i. not given); 1-(Butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester

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

Original review

"The DNA repair assay in rat hepatocyte primary cultures (HPC) was submitted (Tong, 1981). Benomyl ... was evaluated a with dimethylnitrosamine, dimethylformamide, fluorene and 2-ami fluorene which were used as positive controls. The liver was removed and primary cultures were started with hepatocytes from F344 rats benomyl and tritiated thymidine (19 uCi) were added t the culture medium. After 18 to 20 hours of incubating the tre cultures, they are fixed and examined microscopically for morphological changes and absence of s-phase nuclei indicative of cytotoxicity. Autoradiographic techniques are used to deter the number of nuclear grains induced by test chemicals. Backgr counts were obtained by evaluating three nuclear-sized areas in cytoplasm; these values were averaged and subtracted from the n counted in the nucleus to obtain a net value for each nucleus. chemical is considered capable of inducing DNA repair when a ne count of 5 grains or more is observed consistently in each of t nuclei examined in each of 3 replicate slides.

Benomyl did not induce DNA repair in ... rat hepatocytes (Tong, 1981) .... The dimethylnitrosamine and 2-amino fluorene increased the number of nuclear grains from 7 to 15 times the l set as the criterion for a positive response (5/nucleus; Tong,

riginal review by Roger Gardner; TB; 7/6/82

ddendum

MATERIAL AND METHODS: Five log doses were tested in triplicate independent tests (.5, .05, .005, .0005, .00005 mg/ml). Only t non-toxic levels were counted.

RESULTS: Test 1: .5 mg/ml was toxic
Test 2: .5 and .05 mg/ml were toxic

CONCLUSION: Test material is not mutagenic in this system

CLASSIFICATION: Acceptable

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

MRID 00115674

004679

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

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.

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.

111

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 (± 6.0) % fetuses with hematomas (1 to 2 per litter) while 15 of 43 control group litters contained fetuses with hematomas (1 to 2 per litter). Mean fetal weight in each litter was statistically

significantly less than 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.

004679

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)

Rf for CWS 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

004679

STUDY TYPE: Teratology - Rabbits TOX. CHEM. NO.: 75A

HAZLETON LAB. REPORT NUMBER: 210-214 FICHE/MASTER: 00035
MR NO.: 1079 ACCESSION: 091750-C

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 illustrat 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 consumptio 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.

---

* 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

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

* 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: sternebral, -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.

...WHICH 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±29.88	362.8±27.88	366.8±23.58
Body weight gain (g±S.D.)[d] -Day. 7-18	95.5±13.09	98.5±16.14	107.1±12.16	98.5±13.76	93.1±13.22	94.2±9.74
Implants[d]						
No. corpora lutea	657	337	317	346	296	252
-No./dam(±S.E.M.)	13.1±2.97	13.0±3.14	13.2±3.06	13.3±3.22	12.9±3.48	12.6±2.26
No. implants	520	237	272	258	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±9.18[f]	9.9±3.17	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	238	213	181
-No./litter(±S.D.)	9.9±2.48	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±0.17	4.2±0.25	4.1±0.31	3.8±0.11[e]	3.5±0.51[e]

[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
MLR-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[*#]
~~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	8	10/4[h]	21/8[*#]
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] $100 \times \Sigma \left[\frac{\text{No. malformed fetuses in the litter}}{\text{Total no. of fetuses in the litter}} \right] /\text{total no. of litters}$

[g] F # 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 # 258027)

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

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

MR-3501-001
MLR-6-9-80

00467

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[g,j]
-bipartite	1/1				1/1	2/2
-misaligned (2°)[e]	7/6	4/3	1/1		4/4	11/9[f,*]
-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[g]
-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	40/17	39/17
-rudimentary[i]	19/12	6/6	6/4	18/12[g]	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] $100 \times \bar{I}\left[\frac{\text{No. malformed fetuses in the litter}}{\text{Total no. of fetuses in the litter}}\right]$ /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

[†] 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

MRID 00081913

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

122

* 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, urobilinog· acetone, bilirubin, occult blood and microscopic sediment examin· Tissues from the control and 2500 ppm groups were fixed in Bouin' and stained with hematoxylin and eosin for histologic examinatio· included:

†brain	†adrenal	mammary gland	tonsil
†heart	prostate	esophagus	trachea
†lung	†pitultary	cecum	gall bladder
†liver	pancreas	colon	spinal cord
†spleen	urinary bladder	trachea	salivary gland
†kidney	epididymis	skeletal m.	
††estis	Fallopian tubes	peripheral n.	
thymus	uterus	bone marrow	
†stomach	ovary	eye	
††hyroid	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 relat·			
Prot.(2 yr)	6.32	6.36	6.27	5.75	change			

†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.

004679

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

69

Reviewed by: Marion P. Copley, D.V.M.
Section 6 , Tox. Branch (TS-769C)
Secondary reviewer: Jane Harris, Ph.D.
Section 6 , Tox. Branch (TS-769C)

JH 10/1.85

004679

DATA EVALUATION REPORT

STUDY TYPE: Mutagenesis - micronucleus TOX. CHEM. NO.: 75 79

ACCESSION NUMBER: (GS0189-008)

TEST MATERIAL: MBC, Benomyl

JOURNAL ARTICLE: Mutation Research, 40 (1976) 339-348

TITLE OF REPORT: The mutagenicity of benzimidazole and benzimidazole derivatives. VI. Cytogenetic effects of benzimidazole derivatives in the bone marrow of the mouse and the Chinese hamster.

AUTHOR(S): J.P.Seiler

REPORT ISSUED: 1976

CONCLUSION: author's conclusions for micronucleus formation:
MBC (mice) NOEL = 50 mg/kg
LEL = 100 mg/kg
Benomyl (mice) NOEL = 500 mg/kg
LEL = 1000 mg/kg
serum concentration of MBC NOEL = 8 ug/l (as long as 24 hr)
LEL = 11.5 ug/l (for less than 6 hr)

Classification: incomplete because the above mentioned conclusions could not be confirmed or rejected with the available data.

MATERIALS: Benzimidazole, MBC, 2-benzimidazolylurea, benzimadozolecarbamonitril, 2-aminobenzimidazole, benomyl

Animals: ICR mice, 8 wk old, 25-30 gm

METHODS: Micronucleus test: Material was administered by i.p. (intraperitoneal) or be gavage (in 2% gum arabic) twice, 24 hr apart. The mice were sacrificed 30 hr after the first treatment. Bone marrow smears were prepared for micronucleus and metaphase examination. See table I from the article for treatment summary and results. Serum levels of [2-^{14}C]MBC were measured at various times after single treatment with the labelled compound. See table IV from the article for treatment summary and results.

RESULTS and DISCUSSION: MBC, administered i.p. appeared to be insoluble as evidenced by a mass of MBC in the peritoneal cavity and constant serum levels regardless of dose or time (see table III). There was an increase in micronuclei both polychromatic and normochromatic. The author examined Chinese hamster bone marrow smears after gavage administration of

126

1000 mg/kg MBC for chromosome breakage. Due to negative results, he concluded that the micronucleus formation was due to interference with the mitotic process rather than chromosome breaks. He felt this was supported by an earlier onset of action with MBC (micronucleus formation as early as 6-8 hr) than with the known alkylator, trenimon (16 hr).

The author suggested there may be a threshold limit (of 11.5 ug/l MBC in the serum) for spindle inhibition and that MBC is 10 times more potent for this effect than benomyl. However, there is no mention of number of replicates or animals/dose, no indication as to sample variation or whether gum arabic was given to the control group in table 1. The methods and results were presented in abbreviated and vague terms. For the above reasons the authors conclusions could not be confirmed or rejected with the available data.

ABLE I

ICRO NUCLEATED ERYTHROCYTES (PER 1000 POLYCHROMATIC ERYTHROCYTES) FROM OUSE BONE MARROW AFTER TREATMENT WITH SEVERAL BENZIMIDAZOLE COMPOUNDS

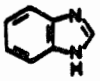
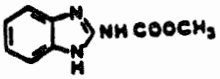
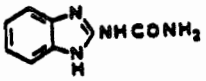
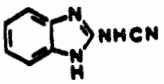
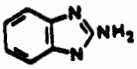
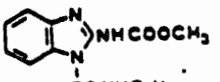
mpound	Formula	Appli-cation	Amount mg/kg	Micronucleated polychromatic erythrocytes	Micronucleated normochromatic erythrocytes
enzimi-azole		i.p.	100	4.3	2.8
			300	3.1	2.6
BC		i.p.	500	4.5	1.8
		p.o.	50	4.2	2.9
			100	13.4	6.5
			500	19.7	12.3
			1000	26.8	18.2
2-Benzimi-dazolylurea		p.o.	500	7.2	3.7
			1000	16.3	5.0
Benzimida-zolecarba-monitril		p.o.	500	3.5	2.5
2-Amino-benzimi-dazole		p.o.	100	3.2	3.0
			500	4.1	1.9
			1000	2.8	2.5
Benomyl		p.o.	500	4.2	3.0
			1000	12.6	5.6
None (control)		—	—	3.6	2.5

TABLE IV

[2-^{14}C]MBC IN BLOOD SERUM OF MICE TREATED ORALLY OR INTRAPERITONEALLY (RADIOACTIVITY COUNTS PER ML SERUM)

The specific activity of the MBC suspension was 1.15 μCi/mg.

Treatment	Amount (mg/kg)	Time after treatment (h)	cpm · 10^{-3}	MBC conc. μg/ml serum
p.o.	100	4	29	11.5
		6	20	8
	500	2	42	17
		4	61	24.5
		6	53	21
		8	34	13.5
		16	24	9.5
		24	27	11
i.p.	100	4	20	8
		6	19	7.5
i.p.	500	2	20	8
		4	19	7.5
		6	20	8
		8	21	8.5
		16	22	9
		24	21	8.5

004679

Reviewed by: Marion P. Copley, D.V.M.
Section 6 , Tox. Branch (TS-769C)
Secondary reviewer: Jane Harris, Ph.D. JH 10/10/85
Section 6 , Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Metabolism TOX. CHEM. NO.: 75A

ACCESSION NUMBER: 091561-F MRID NO.: 00066776

TEST MATERIAL: Benomyl

STUDY NUMBER(S): none given

SPONSOR: E.I. DuPont De Nemours and Co., Inc.

TESTING FACILITY: not specified

TITLE OF REPORT: Metabolism of Methyl 1-(Butylcarbamoyl)-2-C-14*Benzimidazolecarbamate

AUTHOR(S): JA Gariner, H. Sherman, RW Reiser

REPORT ISSUED: not specified (1968?)

CONCLUSION: The major urinary metabolites appear to be conjugates of 5-OH-MBC.

Classification: unacceptable , only 1 male rat was tested

MATERIALS: compound - Benomyl, Methyl 1-(Butylcarbamoyl)-Benzimidazolecarbamate with and without label
animals - rat, male, ChR-CD, 172 gm at start

METHODS: A single rat was treated with 2500 ppm unlabeled benomyl in the food (1 % corn oil was added). After 12 days (weight of the rat was 264 gm) the rat was given 7.7 mg of benomyl-$2^{14}C$ by gavage and placed in a glass metabolism cage. The system contained a trap for converting organic volatiles to CO_2 and a CO_2 trap. Urine and feces were collected daily for 72 hours. After 3 days, blood was drawn by cardiac puncture, the animal sacrificed and the following tissues removed for radiolabel analysis: brain, lungs, heart, liver, spleen, kidney, testes, gastrointestinal tract muscle, fat and the carcass.

RESULTS: The rat weighed 268 grams at sacrifice. Table 1 (from the report) indicates the percent of recovered radioactivity in the various organs and excreta. At 72 hours less than 1% remained in the carcass,with 86 % of recovered radioactivity found in the urine and 13 % in the feces. TLC analysis of the urine initially indicated 2 spots (1 major and 2 minor). After enzyme hydrolysis however only 1 spot accounting for 80 % of the radioactivity on the plate, remained. Its R_f value differered from the other three. This indicated that glucuronide

and/or sulfate conjugates were excreted in the urine. Very little, if any parent or MBC was present in the urine. The spot had the same R_f as 5-hydroxy-methyl-benzimidazolecarbamate (5-OH-MBC). Its identity was confirmed by mass spectroscopic analysis.

DISCUSSION: This study is limited because only one rat (male) was tested using pretreatment. Females may have a different metabolic pattern. Pretreatment may have resulted in more rapid metabolism of the parent. This study would need to be compared to single dose studies in order to develop a clearer understanding of the metabolism of benomyl.

74

004679

[illegible] - [illegible] carbamate

[illegible]	Percent of Recovered Radio[illegible][1]		
	[illegible]	[illegible] hrs.	[illegible]
carbon dioxide	N.D.[2]	N.D.	N.D.
urine	78.9	85.3	85.[illegible]
feces	8.7	12.2	13.1
acid wash of cage			0.[illegible]
fur			[illegible].2
[illegible]			
blood			< [illegible]
brain			< [illegible]
fat			< 0.[illegible]
g.i. tract			0.2
heart			< [illegible].01
kidneys			< 0.[illegible]
liver			0.2
lungs			< 0.[illegible]
muscle			< [illegible]
spleen			< 0.01
testes			< 0.[illegible]1
carcass (less fur)			[illegible]
	87.6	97.5	100

[1]Over-all recovery was 91.8% of administered C^{14}-activity.

[2]N.D. - None detected.

BEST AVAILABLE COPY

Data Evaluation Report

004679

Compound Benomyl (Benolate® 50WP)

Citation
2-^{14}C-Benomyl (50% WP) absorption through rat skin Part II: Effect of time and dose, I.J. Belsco, Biochemicals Dept, Research Div, Experimental Sta, E. I. du Pont de Nemours & Co Inc, Wlimington, Delaware, 19898, March 9. 1979.

Reviewed by Robert P. ~~Zendzian~~ PhD 11/9/81
Pharmacologist

Core Classification Acceptable

Conclusion
Benomyl was absorbed through the rat skin from Benolate 50WP in small amounts in a nonlinear dose and duration related manner. Percent absorption ranged from 0.031 (high dose) to 3.518 (low dose) for the maximum exposure of 10 hours.

Materials

[2-^{14}C] Benomyl as 50% wettable powder (Benolate® Fungicide)
Formulation #1 1.642 uCi/mg
Formulation #2 0.162 uCi/mg

Eighty male ChR-CD rats, 224 - 250 gm.

Methods

"Treatment Rates

(1) 0.2 mg ^{14}C-Benolate®/rat - Formulation 1

(2) 2 mg ^{14}C-Benolate®/rat - Formulation 1

(3) 20 mg ^{14}C-Benolate®/rat - Formulation 2

(4) 200 mg ^{14}C-Benolate®/rat - Formulation 2

Exposure time for each treatment was 0.5, 1, 2, 4 and 10 hours. Four animals were used at each treatment level and time interval."

The back of each rat was clipped and the clipped area washed with acetone 24 hours before treatment. Doses 1 and 2 were applied by syringe as water suspensions and doses 3 and 4 by spatula as a thin paste. Exposure area was 4 in^2 (approximately 16% of the rat's surface area). Applied material was spread with a Teflon® rod. 'Application' devices were counted for loss of dose. Rats were placed in individual collection containers and urine and feces collected separately. Blood samples were taken from all

animals at termination. All samples were assayed for total benomyl.

In addition urine samples were assayed qualititively for benomyl metabolites.

Results

Table A. Benomyl in the Blood. a) Mean equivalent concentrations of benomyl (ppm or ug/ml). b) Mean quantity (ug) assuming 6.4% blood volume. Data from tables 1, 2, 3, and 4 of the report.

Dose* mg/rat		Duration of exposure (hours) 0.5	1.0	2.0	4.0	10
0.2	a	0.001	0.004	0.004	0.004	0.003
	b	0.016	0.064	0.064	0.041	0.048
2.0	a	0.006	0.009	0.008	0.008	0.004
	b	0.096	0.144	0.128	0.128	0.064
20	a	0.026	0.028	0.034	0.036	0.024
	b	0.416	0.448	0.544	0.576	0.384
200	a	0.033	0.054	0.048	0.070	0.064
	b	0.528	0.864	0.768	1.120	1.020

* Benolate 50% WP

Table B Mean equivalent total amounts of benomyl in the urine (ug). Data from tables 1, 2, 3, and 4 of the report.

Dose* mg/rat	Duration of exposure (hours) 0.5	1.0	2.0	4.0	10
0.2	0.03 (.030)	0.11 (.110)	0.65 (.650)	1.67 (1.60)	3.47 (3.470)
2.0	0.07 (.008)	0.31 (.032)	1.05 (.106)	3.27 (.328)	4.85 (.486)
20	0.23 (.002)	0.67 (.006)	3.29 (.032)	4.03 (.040)	9.27 (.092)
200	0.40 (.001)	3.62 (.004)	8.79 (.008)	28.55 (.028)	30.33 (.030)

* Benolate 50% WP
(percent of dose of active ingredient)

Table C Mean equivalent total amounts (ug) and percent of dose of benomyl in urine and blood. Sum of data from tables A and B

Dose* mg/rat	Duration of exposure (hours) 0.5	1.0	2.0	4.0	10
0.2	0.046 (0.046)	0.174 (0.174)	0.714 (0.714)	1.734 (1.734)	3.518 (3.518)
2.0	0.166 (0.016)	0.454 (0.045)	1.178 (0.118)	3.398 (0.340)	4.914 (0.491)
20	0.645 (0.006)	1.108 (0.011)	3.924 (0.039)	4.606 (0.046)	9.624 (0.096)
200	0.928 (0.001)	4.484 (0.004)	9.549 ~~(0.095)~~ .010	28.670 (0.029)	31.359 (0.031)

* Benolate 50% WP
(percent of dose of active ingredient)

"Thin-layer chromatographic analysis of an extract of the composite urine from the 2 and 200 mg treatments for 10 hours revealed the presence of 5-HBC as the major benomyl metabolite with a lesser amount of MBC as shown in radio scan and autoradiogram. ---- No 4-HBC was detected in these samples."

Discussion

This study does not provide data on the amount of compound which may have been retained in the rat at termination of a particular exposure. However, the Gardiner et. al (1968) study of benomyl metabolism in the rat provides information indicating that any retained material would be insignificant. Following a single oral dose of 29 mg/kg (7.7 mg/rat) the post absorption phase equilibrium $t_{1/2}$ has been graphically determined to be 10.5. Under the circumstances of this dermal absorption study such a half-time should not lead to significant bioaccumulation of the compound. The dermal absorption generated in this study may be used for evaluating dermal exposure/dose of benomyl.

Quantity and percent of each dose that was absorbed increased with duration of exposure but the increase was not linear with time. For example, a ten fold increase in duration of exposure, from 1 to 10 hours, resulted in 20.6, 11.0, 8.8 and 7.0 fold increases in the quantities absorbed per respective dose. A five fold increase in duration of exposure, from 2 to 10 hours, resulted in 4.6, 4.2, 2.5 and 3.2 fold increases in the quantities absorbed per respective dose.

For each unit time of exposure the quantity of material absorbed increased with increasing dose but the percent of the dose absorbed decreased with increasing dose. This relationship was also not linear.

These dose/time relationships are expected with the majority of compounds which are absorbed through the skin.

Reference

Gardiner, J.A., Sherman, H. & Reiser, R.W. (1968?) Metabolism of Methyl 1-(Butylcarbamoyl)-2-C-14* Benzimidazolecarbamate in the Rat. E.I. du Pont.

d by: Marion P. Copley, [redacted] V.M. 79
6 , Tox. Branch (TS-76[redacted])
ry reviewer: Jane Harris, Ph.D.
6 , Tox. Branch (TS-769C)

00437

DATA EVALUATION REPORT

STUDY TYPE: TOX. CHEM. NO.: 75A
1) Teratology - mouse - gavage
2) Teratology - rat - gavage
3) Teratology - rat - dietary
4) Postnatal - rat - gavage (dams)

ACCESSION NUMBER: (GS0119-017)

TEST MATERIAL: Benomyl

JOURNAL ARTICLE: Toxicology and Applied Pharmacology 62,44-54(1982)
(HERL - USEPA: 1/11/80)

TITLE OF REPORT: Teratogenic effects of benomyl in the Wistar rat and CD-1 mouse, with emphasis on the route af administration

AUTHOR(S): RJ Kavlock, N Chernoff, LE Gray,Jr., JA Gray, and D Whitehouse

REPORT ISSUED: 1982

CONCLUSION:
1) Teratology - mouse - gavage
teratogenic NOEL = 50 mg/kg
LEL = 100 mg/kg (supra occipital scars, subnormal vertebral centrum, supernumerary ribs and cleft palate)

CORE-CLASSIFICATION: minimal

2) Teratology - rat - gavage
teratogenic NOEL = 31.2/mg/kg
LEL = 62.5 mg/kg (microphthalmia and increased fetal mortality, reduced fetal weight)

CORE-CLASSIFICATION: minimal

3) Teratology - rat - dietary
teratogenic NOEL > 500 mg/kg (HDT) (approx. 6760 ppm*)
toxic. NOEL = 169 mg/kg (approx. 1690 ppm*)
LEL = 298 mg/kg (approx. 3380 ppm*)(weight decrease in fetuses)

CORE-CLASSIFICATION: minimal

4) Postnatal - rat - gavage (dams)
fetotoxic NOEL = ~~31.2~~ 15.6 mg/kg
fetotoxic LEL = ~~62.5~~ 31.2 mg/kg (decreased weight of testes, ventral prostate, and seminal vesicles)

CORE-CLASSIFICATION: supplementary

SEE ATTACHED ARTICLE

136

*time weighted averages for dietary concentration

Emphasis on the Route of Administration[1]

Robert J. Kavlock,[2] Neil Chernoff, L. Earl Gray, Jr., Jacqueline A. Gray, and Douglas Whitehouse

Experimental Biology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

Received March 27, 1981; accepted September 1, 1981

Teratogenic Effects of Benomyl in the Wistar Rat and CD-1 Mouse, with Emphasis on the Route of Administration. Kavlock, R. J., Chernoff, N., Gray, L. E., Jr., Gray, J. A., and Whitehouse, D. (1982). *Toxicol. Appl. Pharmacol.* 62, 44–54. Benomyl, a systemic fungicide whose molecular basis of action is inhibition of tubulin polymerization, was administered during organogenesis via the dietary and gavage routes to pregnant Wistar rats, and via the gavage route to pregnant CD-1 mice. Benomyl was fetotoxic and teratogenic in both species via the po route of administration, producing a broad spectrum of malformations at a dose of 62.5 mg/kg/day in the rat and 100 mg/kg/day in the mouse. Via the dietary route of administration, benomyl produced fetotoxicity, but no teratogenic effects. The fetotoxic potential of benomyl from dietary exposure was approximately an order of magnitude less effective than from gavage exposure. Benomyl did not affect postnatal growth, viability, or locomotor activity at subteratogenic doses. The most sensitive indicator of perinatal exposure to benomyl via the po route of administration was a permanent reduction in testes and accessory sex gland weight noted in male offspring of dams receiving 31.2 mg/kg/day benomyl during gestation and lactation. [illegible] effects on any parameters were evident in rats receiving 15.6 mg/kg/day by po gavage. The relevance of the two routes of administration for risk extrapolation is discussed.

Benomyl ((methyl 1-butylcarbamoyl)-2-benzimidazole carbamate) is a fungicide registered for fruits, nuts, vegetables, rice, bulbs, flowers, ornamental crops, shade trees, grasses, and roses (*Federal Register*, December 6, 1977). It was developed in the late 1960's as one of the first systemic fungicides. Since that time, it has become widely used in both the field and the home (Beat, 1979). Its effectiveness against fungal growth is attributed to its ability to interfere with mitosis (Hammerschlag and Sisler, 1973), specifically by binding to tubulin (Davidse, 1973; Davidse and Flach, 1977), and thus preventing tubulin polymerization. While benomyl and its breakdown product in aqueous medium (methyl-2-benzimidazol carbamate, or MBC) (Kilgore and White, 1970) do effectively bind to fungal tubulin and prevent mitosis, both have relatively low affinity for mammalian tubulin (Davidse and Flach, 1977; Friedman and Platzer, 1978; Ireland *et al.*, 1979) and do not readily [illegible] toxicity of these compounds despite their effective fungicidal action. The oral LD50 in adult rats is in excess of 10,000 mg/kg (Seiler, 1975). Approximately 90% of a po administered dose is eliminated from the rat within 24 hr. The major metabolite is methyl 5-hydroxy-2-benzimidazole-carbamate (5-HBC) (Gardiner *et al.*, 1974). While the accepted method of residue analysis does not distinguish between benomyl and MBC (Kirkland, 1973), both compounds have identical metabolism in the rat (Gardiner *et al.*, 1974).

Despite low toxicity to adult mammals, several authors have cautioned that due to the biochemical basis of action, benzimidazole compounds may pose a genetic risk to man (Seiler, 1975; Bignami *et al.*, 1977; Davidse and Flach, 1977), and exposure of rats to both benomyl and MBC has been linked with damage to the reproductive system. Administration of benomyl to Wistar rats on gestation Days 1 to 20 or 7 to 15 by gavage resulted in increased incidences of resorbed embryos and malformed fetuses at doses of 125 mg/kg/day and greater (Shtenberg and Torchinsky, 1972). Anomalies included exencephalies, hydrocephaly, and microphthalmia; the incidences were dose related. The authors reported no effect on fetuses exposed to 62.5 mg/kg/day. Torchinsky *et al.* (1976) observed changes in testicular biochemistry and histology, sperm motility, and cerebellar acetylcholinesterase activity in male Wistar rats receiving peroral administration of benomyl at 500 mg/kg/day for up to 11 months. No histological changes were observed at the next lowest dose (31.5 mg/kg/day), but testicular succinate dehydrogenase and cerebellar acetylcholinesterase activities were depressed. No effects were observed in the lowest dose tested (6.25 mg/kg/day). Delatour and Richard (1976), in a study of the embryo-[illegible] Dawley rats on Days 6 to 15 of gestation did not affect embryonic development, administration of MBC at doses of 19.1 mg/kg and greater increased the incidence of resorption, decreased fetal weight, and produced terata (anomalies were not detailed, but included exencephaly, hydrocephaly, microphthalmia, hypodysplasia of the limbs, fusion of vertebral bodies, cleft palate, and hydronephrosis). Contrary to these reproductive effects of benomyl and MBC following po administration, Sherman *et al.* (1975) found no effect of dietary administration of 2500 ppm benomyl to Sprague–Dawley rats in a three-generation reproduction study or of 5000 ppm in a teratology study. This latter dietary level was approximately 400 mg/kg/day, or more than three times the effective dose noted by Shtenberg and Torchinsky (1972) in po-dosed Wistar rats. The present studies were initiated to further evaluate the effect of route of administration of benomyl on embryonic development in the rat. We also examined the effect of oral administration of benomyl in a second species, the mouse.

METHODS

Compound

Technical grade benomyl supplied by E. I. DuPont deNemours and Company (Wilmington, Dela.) was used.

Animals

Sixty-day-old time pregnant CD-1 mice and Wistar rats were obtained from Charles River Breeding Laboratory (Wilmington, Mass.). Day 1 of pregnancy was defined as the day on which a sperm plug (mice) or sperm in a vaginal smear (rats) was detected. Animals were maintained in constant temperature (20 to 24°C), humidity rooms with controlled lighting (6 AM to 6 PM). Upon arrival, mice were identified with an ear punch and rats with a metal ear tag (see 5, National

---

[1] This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation.

[2] Send requests for reprints to: Dr. Robert J. Kavlock, Developmental Biology Branch, Experimental Biology Division (MD-72), Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, N.C. 27711.

Band and Tag Co., Newport, Ky.). Mice were housed five per cage and rats were housed individually on pine-shaving bedding in polypropylene cages. They received tap water and Purina Lab Chow *ad libitum*.

Mouse Prenatal Study

Benomyl was administered via gavage as a suspension in stripped corn oil (Lot D-4-25, Eastman Kodak Co., Rochester, N.Y.) at a rate of 0.1 ml/mouse/day. The suspensions were prepared daily with the aid of a magnetic stirrer. Doses were 200, 100, 50, and 0 mg/kg/day, based on the Day 6 maternal weight. Control animals received the vehicle alone. Animals were randomly assigned to treatment groups and treated on Days 7 to 17 of gestation.

Animals were killed by decapitation on Day 18 of gestation. The uteri were removed, weighed, and examined to determine the number of live, dead, and resorbed fetuses. The maternal weight gain was calculated as the overall weight gain of pregnancy less the weight of the gravid uterus. The maternal liver was removed and weighed. Live fetuses were examined for gross anomalies, blotted, and weighed as a litter. Half of each litter was fixed in a solution of 5% formaldehyde, 5% glacial acetic acid, and 70% ethanol while the other half was preserved in 65% ethanol. The former were necropsied, while the latter were stained with Alizarin Red S and examined for skeletal abnormalities and maturity. Scoring systems to describe the ossification of the supraoccipital and the vertebral centras and the maturation of the lateral cerebral ventricles and renal pelves have been described (Chernoff *et al.*, 1979). In the tables, enlarged lateral ventricles and enlarged renal pelves refer to occurrence of those organs which have a score of greater than 2; while hydrocephaly and hydronephrosis refer to those cases with a score of 4, the "percentage with subnormal centrums" refers to the average litter percentage of fetuses with at least one centrum with a score of 2 or greater. Three classes of supernumerary lumbar ribs were distinguished in the skeletal examination: "extra" refers to lumbar ribs with a length of at least one-half of the last thoracic rib; "small" refers to ribs larger than a small ossification spot, but less than one-half the length of the last thoracic rib, and "very small" refers to small ossification spots adjacent to the first lumbar vertebral arches. In the tables, all three classes of supernumerary ribs were combined and presented as a single statistic.

Rat Prenatal Studies

Benomyl was administered to the dams either in the diet or by gavage. After blocking on the basis of Day 4 maternal weights, rats were randomly assigned to treatment groups such that the mean and variance of Day 4 weight were the same for each treatment group. In the gavage experiment, benomyl was administered as a suspension in stripped corn oil at a rate of 1 ml/rat/day. The suspensions were prepared fresh daily with the aid of a magnetic stirrer. Doses were 125, 62.5, 31.2, 15.6, and 0 mg/kg/day based on the Day 4 maternal weight. Control animals received vehicle alone. Animals were treated on Days 7 to 16 of gestation. In the dietary experiment, benomyl was administered at dose levels calculated to result in daily intakes of approximately 500, 250, 125, and 0 mg/kg/day on the basis of the Day 4 maternal weight. The high dose of benomyl was mixed into the ground chow in a planetary mixer for 30 min. Lower doses were prepared as dilutions of the high dose. Control animals received regular chow. Animals were treated on Days 7 to 16 of gestation. To assess the effects of administration of benomyl on food consumption for the two routes of administration, 24-hr food consumption and weight of females were measured on Days 7, 10, 14, and 17 of gestation. On the days when these measurements were taken, fresh diet preparations were supplied and the concentration of the compound in the diet adjusted to reflect increasing food consumption during pregnancy and to maintain dosage levels comparable to that in the oral teratology study. Females which were determined at necropsy to have no live fetuses were excluded from the analysis of food consumption and weight gain during pregnancy. Time-weighted averages for the dietary concentrations in this study were 1690, 3380, and 6760 ppm. These concentrations resulted in time-weighted dosage levels of 169, 298, and 505 mg/kg/day, respectively.

Rats were killed by decapitation on Day 21 of gestation. Fetuses were processed as described above.

Samples of the dietary formulations were taken at the time of their preparation and also when they were replaced by fresh formulations. These samples were preserved at −60°C. Benomyl in random feed samples was analyzed as methyl-2-benzimidazole carbamate (MBC) by HPLC to determine the uniformity of the preparation and the stability of benomyl. Five-gram samples were hydrolytically extracted four times with 25 ml of a 50/50 acetonitrile/phosphate buffer, pH 2.65 solution. The combined extract was filtered through a 45-μm filter and analyzed on an ion-exchange column under the following conditions: column, partisil 10 SCX, 25 cm × 4.6 mm; mobile phase, 20/80 acetonitrile/phosphate buffer, pH 2.65; flow rate, 1.0 ml/min; UV detector wavelength 283 nm. The compound was found to be present in the diet at the desired concentration, to be uniformly distributed, and to be stable over the 3 to 4-day period of utilization.

Rat Postnatal Study

Benomyl was administered via gavage as described above. The dose levels were 31.2, 15.6, and 0 mg/kg/day. The dams were treated from Day 7 of gestation through Day 15 of lactation (Day 22 of gestation was considered Day 0 of lactation). At birth, the pups were counted, examined for external defects, separated by sex and weighed. On Day 3 of lactation, the litters were reduced to no more than eight pups, with as equal a sex ratio as possible. The litters were then weighed on Days 8, 15, 22, 29 (weaning), and 100. Locomotor activity (Reiter, *et al.*, 1977) was determined at 15, 28, and 100 days of age in a figure-eight maze. At the conclusion of the last activity measurement, the animals were killed and the paired adrenals, liver, right kidney, and paired ovaries or paired testes, and the ventral prostate plus seminal vesicles were weighed.

Statistical Analyses

Data are presented as the mean plus or minus the standard error of the mean. The litter was regarded as the experimental unit of comparison for all analyses, except data recorded for postweanling animals where the individual was the unit of comparisons. Treatment effects were determined by Fisher's exact test for quantal data and by ANOVA for nonquantal data. When a significant treatment effect was determined by ANOVA, Duncan's multiple-range test was applied for pooled comparisons. All effects are reported significant at the $p < 0.05$ level.

RESULTS

Mouse

Oral teratology study. Administration of up to 200 mg/kg/day of benomyl during Days 7 to 17 of gestation did not affect maternal viability of growth (Table 1). Benomyl did, however, exert adverse influence upon fetal development. Embryonic viability, fetal weight, skeletal maturity (supraoccipital ossification), decreased numbers of sternal and caudal ossifications, delayed development of the vertebral centrums, visceral maturity (incidence of enlarged cerebral ventricles and enlarged renal pelves), and the occurrence of supernumerary ribs were all adversely affected by benomyl exposure. The responses were dose related. All these features for fetuses in the high-dose group were significantly different from control values. Only a few parameters for fetuses in the 100 mg/kg/day group were different from control values. While values for fetuses in the lowest dose group were generally in line with the dose response, no paired comparisons with control values were significantly different. It is noteworthy that all classes of supernumerary lumbar ribs were affected by treatment. The percentage of lumbar ribs in increasing order of dose were: extra: 2.4, 8.4, 15.5, and 17.5; small: 1.7, 6.9, 16.7, and 13.8; and very small: 6.4, 12.6, 9.6, and 22.7.

Major anomalies observed in mice fetuses exposed to benomyl during organogenesis are presented in Table 5. The overall incidence was 1.3, 1.0, 16.8, and 47.3% at 0, 50, 100, and 200 mg/kg/day, respectively.

Rats

Oral teratology study. Administration of up to 125 mg/kg/day of benomyl during organogenesis did not affect food consumption but did affect maternal weight change after cessation of treatment (Table 2). This effect was limited to a decrease in weight change in the highest-dose group and probably resulted from reduced litter size due to increased fetal resorption in that group.

The effects of benomyl on the maternal organism and fetal development are presented in Table 3. No treatment effects were evident either on maternal viability or on maternal weight gain. There was a significant treatment-related effect on the incidence of embryonic resorptions, although it was individually significant from the control value only at the highest-dose level. Six litters in the high-dosage group were completely resorbed. Fetal weight was significantly affected by the administration of benomyl, and fetuses from the dosage groups above 31.2 mg/kg/day weighed significantly less than control fetuses. Indicators of skeletal maturity (the developmental score for

TABLE 1

EFFECTS OF ORAL ADMINISTRATION OF BENOMYL ON FETAL DEVELOPMENT IN MICE

Observation	Dose (mg/kg/day) 0	50	100	200
Maternal				
No. treated	25	25	20	25
No. died	0	0	0	1
No. pregnant	20	19	18	17
No. resorbed (term)	1	0	0	1
Weight gain (g)	5.4 ± 0.3	6.5 ± 0.4	6.0 ± 0.2	6.5 ± 0.4
No. implantations	12.1 ± 0.5	11.9 ± 0.6	10.3 ± 0.7	11.8 ± 0.8
Fetal				
Mortality (%)	8.8 ± 4.5^{a}	10.7 ± 2.8^{a}	3.3 ± 1.2^{a}	21.7 ± 6.8^{a}
Weight (g)	1.00 ± 0.02^{a}	0.96 ± 0.03^{a}	0.86 ± 0.04^{b}	0.72 ± 0.03^{c}
Supraoccipital score	1.3 ± 0.1^{a}	1.4 ± 0.1^{a}	1.8 ± 0.2^{a}	2.6 ± 0.2^{b}
No. sternebrae	5.9 ± 0.1^{a}	5.7 ± 0.1^{a}	5.6 ± 0.1^{a}	5.0 ± 0.2^{b}
No. caudal vertebrae	4.0 ± 0.2^{a}	4.1 ± 0.2^{a}	3.9 ± 0.3^{a}	2.7 ± 0.3^{b}
Subnormal vertebral centrums (%)	1.0 ± 1.0^{a}	6.1 ± 4.5^{a}	36.9 ± 10.2^{b}	75.1 ± 9.9^{c}
Supernumerary ribs (%)	10.5 ± 5.3^{a}	27.9 ± 6.6^{a,b}	41.9 ± 8.1^{b,c}	54.0 ± 7.8^{c}
Enlarged lateral ventricles (%)	5.4 ± 2.0^{a}	10.8 ± 4.0^{a,b}	6.7 ± 2.9^{a}	23.2 ± 8.7^{b}
Enlarged renal pelves (%)	6.8 ± 2.8^{a}	11.4 ± 5.9^{a,b}	22.2 ± 6.6^{a,b}	25.7 ± 6.6^{b}

Note. $\bar{X}$ ± SE, means with same superscript are not significantly different, $p < 0.05$.

the ossification of the supraoccipital, the number of sternal and caudal ossification centers, and the occurence of subnormally ossified vertebral centrums) and visceral maturity (the occurrence of enlarged cerebral ventricles) were adversely affected by benomyl treatment and reflect the effect upon fetal weight. When individual dosage groups were compared with control values, it was evident that these effects were not observed at dosages at or below 31.2 mg/kg/day.

Major anomalies present in fetuses exposed to benomyl by po administration are presented in Table 5. The malformation rate in the control group was approximately 1.4%. In the lowest two dosage groups (15.6 and 31.2 mg/kg/day), the incidence of malformations was 3.1 and 5.2% of the total fetuses, respectively. Approximately 19% of the fetuses from the 62.5 mg/kg/day group had a detectable abnormality. At 125 mg/kg/day, 35% of the fetuses had a detectable abnormality, the most prominent being encephaloceles, hydrocephaly, clefts of the lip or jaw, gastroschisis, and fused vertebrae.

Dietary teratology study. Dietary administration of benomyl during organogenesis resulted in significantly reduced food consumption at all monitoring periods (Table 2). Pair-wise comparisons between control and treated groups indicated that initially both the 3380 and 6760 ppm diets were aversive to the dams, but only the 6760 ppm diet was aversive during the latter two sampling periods. As a result of this reduction in food consumption, maternal weight change during organogenesis was adversely affected during Days 7 to 10 of gestation. The reduction in weight gain continued for dams in the high-dosage group during Days 10 to 14. During the last measurement period (between the time of return to control diet and sacrifice of the dams), dams in the high-

TABLE 2

EFFECTS OF ADMINISTRATION OF BENOMYL TO WISTAR RATS ON FOOD CONSUMPTION AND WEIGHT CHANGE DURING PREGNANCY

Route/dose (mg/kg/day)	No. animals	Food consumption (g/pregnant rat) Days 7-8	Days 10-11	Days 14-15	Days 17-18	Weight change (g/pregnant rat) Days 7-10	Days 10-14	Days 14-17	Days 17-20
Gavage									
0	20	18.7 ± 1.0	19.8 ± 1.2	24.3 ± 1.7	28.3 ± 1.4	16.8 ± 1.4	20.2 ± 1.4	23.7 ± 1.5	52.0 ± 2.0^{a}
15.6	17	18.7 ± 1.2	18.0 ± 0.9	23.2 ± 2.1	28.2 ± 1.6	14.6 ± 1.0	17.7 ± 2.1	21.7 ± 2.5	48.4 ± 3.0^{a}
31.2	24	18.2 ± 0.8	19.3 ± 0.9	24.2 ± 1.8	32.8 ± 1.2	14.0 ± 1.0	19.8 ± 1.2	22.7 ± 1.3	51.9 ± 1.8^{a}
62.5	24	16.8 ± 1.3	19.5 ± 0.8	25.6 ± 2.5	29.0 ± 1.5	12.6 ± 1.4	18.9 ± 1.1	19.7 ± 1.6	47.4 ± 1.5^{a}
125	5	16.7 ± 2.0	22.8 ± 3.0	30.1 ± 2.6	31.3 ± 1.7	16.2 ± 2.2	17.5 ± 3.3	20.2 ± 2.1	34.2 ± 4.2^{b}
Dietary									
0	24	21.1 ± 1.1^{a}	24.6 ± 1.0^{a}	25.8 ± 2.1^{a,b}	32.4 ± 1.6	15.5 ± 0.8^{a}	22.7 ± 0.8^{a}	19.3 ± 1.5	55.7 ± 2.0^{a}
169	22	19.6 ± 1.0^{b}	23.6 ± 1.3^{a}	28.7 ± 1.3^{a}	34.6 ± 1.4	14.7 ± 0.7^{a}	26.5 ± 1.1^{b}	21.1 ± 1.5	54.0 ± 2.7^{a}
298	19	14.6 ± 1.0^{b,c}	23.4 ± 0.9^{a}	23.4 ± 1.4^{b,c}	29.5 ± 1.2	10.7 ± 1.2^{b}	24.1 ± 1.1^{a,b}	16.8 ± 1.3	53.6 ± 2.2^{a}
505	22	12.6 ± 1.2^{c}	19.6 ± 1.3^{b}	19.2 ± 1.6^{c}	32.6 ± 1.6	0.7 ± 1.2^{c}	15.3 ± 1.9^{c}	18.8 ± 1.3	65.4 ± 2.6^{b}

Note. $\bar{X}$ ± SE, [illegible] with same superscript are not significantly different.

dosage group reversed the previous weight gain and increased their body weight significantly more than did controls. Time-weighted averages of actual dietary concentration and food consumption indicated that animals received approximately 169, 298, and 505 mg/kg/day in the low-, middle-, and high-dietary concentration groups, respectively.

The effects of dietary exposure to benomyl on fetal development are presented in Table 4. A significant effect was observed on maternal weight gain during gestation. Only dams in the highest dosage group had weight changes individually different from the control value. No dose-related change in the incidence of fetal mortality was observed. Fetal weight was significantly affected by treatment, with the effect individually significant from the control value for fetuses in both the 3380 and 6760-ppm dosage groups. The only indicators of skeletal or visceral maturity affected by treatment were the ossification of the supraoccipital bone (with a significant difference between high and control dosage groups) and the presence of enlarged renal pelves (with the effect most prominent in the two highest dosage groups).

No dose-related incidences of anomalies and/or malformations were found in fetuses from dams in the dietary regimen (Table 5).

Postnatal teratology study. The results of po administration of 0, 15.6, or 31.2 mg/kg/day of benomyl to rats during gestation and lactation are presented in Table 6. Benomyl did not affect litter size or weight at birth, nor growth and survivability of the pups to weaning. At birth, two pups with anomalies were found in the 15.6 mg/kg/day dosage groups (one with a short tail, one with anasarca). No activity changes due to treatment were noted when animals were tested in a figure-eight maze at 15, 28, or 100 days of age. A permanent treatment-related reduction in the weight of the testes and the ventral prostate and seminal vesicles was observed. This effect was a 5% reduction in the weight of the testes and a 13% reduction

82

004679

TABLE 3

EFFECTS OF ORAL ADMINISTRATION OF BENOMYL ON FETAL DEVELOPMENT IN RATS

Observation	Dose (mg/kg/day) 0	15.6	31.2	62.5	125
Maternal					
No. treated	30	30	30	30	12
No. dead	0	0	1	1	1
No. pregnant	28	18	26	24	5
No. resorbed (at term)	0	1	1	1	6
Weight gain (g)	51.1 ± 2.2	49.4 ± 2.2	51.5 ± 2.0	48.9 ± 2.4	47.8 ± 3.1
No. implantations	11.2 ± 0.6	10.1 ± 0.6	10.8 ± 0.5	10.9 ± 0.6	12.3 ± 0.5
Fetal					
Mortality (%)	7.4 ± 1.9	20.3 ± 6.7	13.3 ± 4.7	15.0 ± 4.7	75.8 ± 10.6
Weight (g)	3.65 ± 0.10	3.44 ± 0.12	3.72 ± 0.08	3.10 ± 0.10	2.96 ± 0.19
Supraoccipital ossification (score)	1.20 ± 0.09	1.08 ± 0.09	1.04 ± 0.08	1.31 ± 0.10	1.57 ± 0.10
No. sternebrae	4.9 ± 0.2	4.9 ± 0.4	5.2 ± 0.1	4.2 ± 0.1	3.2 ± 1.4
No. caudal ossifications	3.7 ± 0.2	3.4 ± 0.5	4.8 ± 0.1	3.2 ± 0.2	2.9 ± 1.0
Subnormal centrums (%)	11.7 ± 3.4	11.3 ± 3.8	12.6 ± 3.7	48.4 ± 7.9	70.2 ± 27.0
Supernumerary ribs (%)	18.9 ± 5.5	3.3 ± 1.9	8.0 ± 3.7	19.6 ± 4.6	66.5 ± 25.5
Wavy ribs (%)	14.6 ± 6.0	5.5 ± 2.8	12.0 ± 5.2	14.0 ± 5.5	8.3 ± 9.6
Enlarged lateral ventricles (%)	9.3 ± 3.0	7.7 ± 2.8	2.8 ± 1.4	9.1 ± 5.7	51.5 ± 27.2
Enlarged renal pelves (%)	16.5 ± 10.2	5.2 ± 2.9	9.0 ± 4.4	9.2 ± 4.4	48.0 ± 20.3

Note. $\bar{X} \pm SE$, means with same superscript are not significantly different, $p < 0.05$.

in the weight of the ventral prostate and seminal vesicles for animals in the 31.2 mg/kg/day dosage group. No other treatment-related differences in organ weights were evident.

DISCUSSION

The studies reported are in agreement with both the work of Shtenberg and Torchinsky (1972), who reported on the teratogenic activity of benomyl following administration via oral gavage, and the work of Sherman *et al.* (1975), who reported on the lack of teratogenic activity following dietary administration. In the present study, oral administration of benomyl produced gross terata at doses above 31.2 mg/kg/day in the rat and 50 mg/kg/day in the mouse. The defects observed involved a broad range of tissues and were consistent with the action of microtubule inhibitors (Tamaki *et al.*, 1966). Furthermore, oral administration of 31.2 mg/kg/day benomyl to rats throughout gestation resulted in a permanent reduction in the size of the testes and male accessory sex glands. Only fetal growth retardation was observed in rat fetuses from dams receiving 6760 ppm (approximately 505 mg/kg/day) benomyl in the diet.

Since fetal growth retardation was observed in all three experimental groups (gavage rat, dietary rat, and gavage mouse), a comparison of the potency of benomyl in each experiment can be accomplished by calculating the regression of fetal weight on dose for the three cases and then determining the dosage necessary to effect a 10% decrease in fetal weight. The calculated regressions are: gavage rat, fetal weight = −(0.00680) (dose) + 3.7399; dietary rat; fetal weight = −(0.000753) (dose) + 3.8058, gavage mouse, fetal weight = −(0.00141) (dose) + 1.0089. Therefore, 55, 512, and 78 mg/kg/day are necessary to produce a 10% fetal weight reduction when benomyl is administered by gavage to the rat, in the diet to the rat, and by gavage in the mouse, respectively. By using a similar approach and

TABLE 4

EFFECTS OF DIETARY ADMINISTRATION OF BENOMYL ON FETAL DEVELOPMENT IN RATS

Observation	Dose (ppm in diet) 0	1690	3380	6760
Maternal				
No. treated	28	27	27	30
No. dead	0	0	0	0
No. pregnant	26	22	19	22
No. resorbed (at term)	0	0	0	0
Weight gain (g)	46.9 ± 1.9	51.8 ± 2.2	44.8 ± 2.1	30.4 ± 2.2
No. implantations	12.3 ± 0.4	12.1 ± 0.5	11.9 ± 0.6	12.1 ± 0.5
Fetal				
Mortality (%)	7.0 ± 1.4	7.3 ± 2.5	10.6 ± 2.7	6.8 ± 2.0
Weight (g)	3.80 ± 0.07	3.60 ± 0.09	3.57 ± 0.09	3.41 ± 0.09
Supraoccipital ossification (score)	1.02 ± 0.06	1.07 ± 0.07	1.07 ± 0.07	1.17 ± 0.09
No. sternebrae	5.2 ± 0.2	5.1 ± 0.1	5.3 ± 0.1	4.9 ± 0.1
No. caudal ossifications	4.1 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	3.9 ± 0.1
Subnormal centrums (%)	11.8 ± 4.6	10.3 ± 4.3	13.9 ± 5.7	22.2 ± 6.6
Supernumerary ribs (%)	17.4 ± 4.4	13.5 ± 4.0	5.0 ± 3.1	6.0 ± 6.7
Wavy ribs (%)	5.8 ± 3.0	4.6 ± 3.7	5.2 ± 2.1	8.6 ± 2.9
Enlarged lateral ventricles (%)	4.9 ± 2.1	5.7 ± 1.9	6.1 ± 3.5	3.2 ± 3.3
Enlarged renal pelves (%)	3.9 ± 1.9	6.2 ± 3.1	16.9 ± 6.1	11.7 ± 3.7

Note. $\bar{X} \pm SE$, means with same superscript are not significantly different, $p < 0.05$.

TABLE 5

MAJOR ABNORMALITIES IN RODENT FETUSES EXPOSED TO BENOMYL DURING ORGANOGENESIS[a]

	Dose (mg/kg/day): Rat—oral gavage 0	15.6	31.2	62.5	125	Rat—dietary 0	[illegible]	[illegible]	[illegible]	Mouse—oral gavage 0	50	100	200
No. litters	30	16	26	26	5	26	22	19	22	30	19	16	17
No. fetuses (necropsy)	101	79	111	110	10	136	123	91	120	112	97	89	83
No. fetuses (Alizarin)	100	82	122	120	16	140	110	107	125	123	107	96	86
No. abnormal litters	2	2	5	5	4	3	2	1	2	3	2	12	15
No. abnormal fetuses	3	5	7	27	12	4	2	2	2	3	2	30	79
Encephalocele		2/2	2/1	1/1	6/2	–	–	1/1	–	–	–	1/1	3/1
Exencephaly		–		–		1/1	–	–	–	–	–	3/3	9/1
Hydrocephaly				9/3	2/2	–	1/1	–	–	–	–	–	5/6
Microphthalmia		1/1		3/3	1/1	1/1	–	–	–	–	–	–	–
Micrognathia				–	–		–	–	–	–	–	1/1	5/6
Cleft palate					–	–	–	–	–	–	–	7/2	14/8
Polydactyly	–				–	–	–	–	–	1/1	–	–	6/6
Oligodactyly	–	–			–			–	–	–	–	–	10/3
Umbilical hernia							–	–	–	–	–	1/1	5/6
Ectopic gonads	1/1	1/1	1/6	–	1/1	2/2	–	1/1	1/1	–	2/2	–	3/3
Hydronephrosis	–	1/1	1/1	–	–	–	1/1	–	–	–	–	3/3	4/4
Fused ribs				11/0	–	–	–	–	–	–	–	8/6	10/7
Fused vertebrae		–	–	8/4	1/2	–	–	–	1/1	–	–	5/4	20/10
Short and/or kinky tail	2/2	1/1	1/1	–	2/2	–	–	–	–	–	–	7/3	77/10

Note. Data presented as number of fetuses affected/number of litters affected.
[a] Infrequently observed abnormalities are not presented in the table. [illegible] 15 and 31 mg/kg/day [illegible] 62 mg/kg/day [illegible] 125 mg/kg/day. In the dietary rat experiment, [illegible] 200 mg/kg/day. In the mouse experiment, [illegible] 200 mg/kg/day. Two fetuses from the control litters and three fetuses from two 200 mg/kg/day litters had small [illegible].
[b] Significantly different from control response, $p < 0.05$ [illegible].
[c] Significantly different from control response, $p < 0.001$ [illegible].

83

004679

TABLE 6

EFFECTS OF ORAL ADMINISTRATION OF BENOMYL DURING THE PERINATAL PERIOD ON POSTNATAL DEVELOPMENT IN RATS ($\bar{X}$ ± SE)[a]

	Dose (mg/kg/day)					
	0		116		[illegible]	
	Males	Females	Males	Females	Males	Females
No. litters	8		9		7	
Litter size at birth	10.0 ± 0.4		10.1 ± 0.6		10.4 ± 0.5	
Litter size at weaning[a]	7.8 ± 0.3		7.0 ± 0.5		6.9 ± 0.8	
Body weight (g)						
Day 1	6.1 ± 0.1	6.1 ± 0.1	6.6 ± 0.1	6.5 ± 0.3	6.2 ± 0.1	5.9 ± 0.2
Day 29	94 ± 4	87 ± 3	96 ± 2	85 ± 7	93 ± 2	88 ± 7
Day 100	473 ± 15	280 ± 6	491 ± 7	283 ± 5	474 ± 14	271 ± 7
Organ weights, Day 100 (N)						
Adrenals (mg)	71 ± 5 (8)	86 ± 4 (8)	—	—	70 ± 7 (8)	97 ± 1 (8)
Liver (g)	24.2 ± 1.0 (8)	12.8 ± 0.4 (8)	—	—	21.4 ± 1.0 (8)	[illegible]
Kidney (g)	1.94 ± 0.09 (8)	1.14 ± 0.05 (8)	—	—	1.85 ± 0.08 (8)	[illegible]
Ovaries (mg)		103 ± 4 (8)		—		94 ± 12 (8)
Testes (mg)	3634 ± 99 (22)		3576 ± 60 (30)		3030 ± 60 (16)[b]	
Seminal vesicle and prostate (mg)	1616 ± 53 (22)		1512 ± 43 (30)		1405 ± 50 (16)[b]	
Locomotor activity (N)[c]						
Day 15	337 ± 29 (13)		300 ± 27 (15)		289 ± 34 (19)	
Day 20	648 ± 38 (13)		486 ± 44 (17)		480 ± 47 (19)	
Day 100	5440 ± 282 (16)	6072 ± 375 (16)	—	—	5265 ± 315 (16)	5138 ± 368 (16)

[a] Litters were reduced to no more than eight pups on postnatal Day 3.
[b] Significantly different from control value, $p < 0.05$.
[c] Values are the number of photocell interruptions during the test period. Testing period was 60 min on Days 15 and 20 and 16 hr on Day 100. No sex differences in locomotor activity existed prior to puberty and data for the two sexes were pooled for Days 15 and 20.

assuming the slopes of the dose-response curves are similar for fetal growth retardation and for the induction of terata, it can be seen that since malformations occur in the rat after gavage at a dose that produces a 15% reduction in fetal weight (62.5 mg/kg/day) a similar weight reduction (and therefore malformations) would, theoretically, be expected to occur at a dietary exposure level of 663 mg/kg/day. Thus, in the rat, the dietary route of administration appears to be an order of magnitude less potent than the gavage route of administration.

Other examples of alterations in teratogenic potency subsequent to alterations in route of administration exist in the literature. Dipterex is teratogenic in the rat when administered in the diet, but not when given once per day by gavage (Staples *et al.*, 1976). However, administration of the compound three times per day by gavage did produce malformations in the rat (Staples and Goulding, 1979). Similarly, EDTA is a more effective teratogen when administered in the diet as compared with once per day sc injection or twice per day by gavage (Kimmel, 1977).

In view of the factors that regulate the amount of substance reaching the fetus, it is not surprising that large differences in teratogenic response would result from differing routes of administration. The important question to ask is which route of exposure, if any, is the more appropriate one from which to extrapolate results to the human situation. Fundamental differences in consummatory behavior exist between the species commonly used in teratology bioassays and humans. Feeding behavior in the laboratory rat does not occur in a distinct temporally concentrated pattern. While some food is consumed during the diurnal phase of the light-dark cycle, most food consumption occurs in short bouts throughout the nocturnal phase (Borbely and Huston, 1974). Thus, given equal potential for metabolism of a chemical by gut microflora and equal potential for absorption into the blood, dietary administration of chemicals with short biological half-lives such as benomyl would be expected to result in low circulating levels in the bloodstream, as excretion per unit time can equal or exceed ingestion per unit time. Humans, on the other hand, consume the bulk of their food at two or three discrete time periods during the day. Such temporally concentrated feeding behavior would be expected to result in higher peak plasma levels of short-lived chemicals administered in the diet than would occur in the rat. To the authors, this difference in feeding behaviors between humans and rodents indicates that for this compound and others with similar biological half lives, gavage is the appropriate dosing regimen when expected human exposure is through indirect contamination of the food, and risk extrapolations should be based on this regimen.

ACKNOWLEDGMENTS

We thank Drs. D. Brunel and C. Tate of the Analytical Chemistry Branch, Environmental Toxicology Division, for analyses of benomyl levels.

REFERENCES

BENT, K. J. (1979). Fungicides in perspective: 1979. *Endeavour* 3(1), 7-14.
BORBELY, A. A., AND HUSTON, J. P. (1974). Effects of two-hour light-dark cycles on feeding, drinking, and motor activity of the rat. *Physiol. Behavior* 13, 795-802.
BIGNAMI, M., AULICINO, F., VELCICH, A., CARERE, A., AND MORPURGO, G. (1977). Mutagenic and recombinogenic action of pesticides in *Aspergillus nidulans*. *Mutat. Res.* 46, 395-402.
CHERNOFF, N., KAVLOCK, R. J., HANISCH, R. C., WHITEHOUSE, D. A., GRAY, J. A., GRAY, L. E., JR., AND SOVOCOOL, G. W. (1979). Perinatal toxicity of endrin in rodents. I. Fetotoxic effects of prenatal exposure in hamsters. *Toxicology* 13, 155-165.
DAVIDSE, L. C. (1973). Antimitotic activity of methyl benzimidazol-2-yl carbamate (MBC) in *Aspergillus nidulans*. *Pestic. Biochem. Physiol.* 3, 317-325.
DAVIDSE, L. C., AND FLACH, W. (1977). Differential binding of methyl benzimidazol-2-yl carbamate to fungal tubulin as a mechanism of resistance to this antimitotic agent in mutant strains of *Aspergillus nidulans*. *J. Cell Biol.* 72, 174-193.
DELATOUR, P., AND RICHARD, Y. (1976). Embryotoxic and antimitotic properties of benzimidazole compounds. *Therapie* 31, 505-515.
FRIEDMAN, P. A., AND PLATZER, E. G. (1978). Interaction of anthelmintic benzimidazoles and benzimidazole derivatives with bovine brain tubulin. *Biochim. Biophys. Acta* 544, 605-614.
GARDINER, J. A., KIRKLAND, J. J., KLOPPING, H. L., AND SHERMAN, H. (1974). Fate of benomyl in animals. *J. Agr. Food Chem.* 22, 419-427.
HAMMERSCHLAG, R. S., AND SISLER, H. D. (1973). Benomyl and methyl-2-benzimidazolecarbamate (MBC): Biochemical, cytological, and chemical aspects of toxicity to *Ustilago maydis* and *Saccharomyces cerevisiae*. *Pesticide Biochemistry and Physiology* 3, 42-54.
IRELAND, C. M., GULL, K., GUTTERIDGE, W. E., AND POGSON, C. I. (1979). The interaction of benzimidazole carbamates with mammalian microtubule protein. *Biochem. Pharmacol.* 28, 2680-2682.
KILGORE, W. K., AND WHITE, E. R. (1970). Decomposition of the systemic fungicide 1991 (Benlate). *Bull. Environ. Contam. Toxicol.* 5, 67-69.
KIMMEL, C. A. (1977). Effect of route of administration on the toxicity and teratogenicity of EDTA in the rat. *Toxicol. Appl. Pharmacol.* 40, 299-306.
KIRKLAND, J. J. (1973). Method for high-speed liquid chromatographic analysis of benomyl and/or metabolite residues in cow milk, urine, feces, and tissues. *Agr. Food Chem.* 21, 171-177.
REITER, L., ANDERSON, G., ASH, M., AND GRAY, L. E. (1977). Locomotor activity measurements in behavioral toxicology: Effects of lead administration on residential maze behavior. In *Behavioral Toxicology: An Emerging Discipline* (J. Zenick and L. Reiter, eds.), EPA-600/9-77-042. U.S. Environmental Protection Agency, Washington, D.C.
SEILER, J. P. (1975). Toxicology and genetic effects of benzimidazole compounds. *Mutat. Res.* 32, 151-168.
SHERMAN, H., CULIK, R., AND JACKSON, R. A. (1975)

Reproduction, teratogenic, and mutagenic studies with benomyl. *Toxicol. Appl. Pharmacol.* 32, 305-315

SHTENBERG, A. I., AND TORCHINSKY, A. M. (1972) On the interrelationship of general toxic, embryotoxic and teratogenic action of chemicals extraneous for the agronom and the possibilities for prognosticating their influence on the antenatal period of ontogenesis. *Vestnik Akad. Nauk SSR* 3, 39-46.

STAPLES, R. E., KELLAM, R. G., AND HASEMAN, J. K. (1976) Developmental toxicity in the rat after ingestion or gavage of organophosphate pesticides (Dipterex, Imidan) during pregnancy. *Environ. Health Perspect.* 13, 133-140

STAPLES, R. E., AND GOULDING, E. H. (1979) Dipterex teratogenicity in the rat, hamster, and mouse when given by gavage. *Environ. Health Perspect.* 30, 105-113

TAMARI, M., SUGAWARA, T., KAMIYAMA, Y., AND MURAKAMI, Y. (1966) Vinclozine induced malformations in the rat embryo. *Annual Report of the Research Institute of Environmental Medicine, Nagoya University*, Vol. 15

TORCHINSKY, A. M., OBLOVA, N. V., SHMIDOVA, Y. B., AND MAKAROVA, L. F. (1976) Data on the toxicological and public health characteristics of the carbamate-group pesticide Bendiocarb. *Gig. Sanit.* 2, 35-39

Reviewed by: Dynamac [redacted]. Wennerberg, W. McLellan, J.C. Felkner 004679
Secondary reviewers: Marion P. Copley, D.V.M.
Jane Harris, PhD, Section Head 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 fem
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-1991-4
grey crystalline material.

SEE ATTACHED REVIEW

004679

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

William L. McLellan, Ph.D.
Senior Scientist
Dynamac Corporation

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

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

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

APPROVED BY:

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

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

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 $p < 0.05$.

---

[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.

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 0	500	1500	5000-7500[b]	Female 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)				
-spermia	18	11	12	30*				
-distended tubules/ tubules filled with degenerated sperm	9	5	11	17*				
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 Neoplasms 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.

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] 23.4 743	674.0 101.9 740
500	665.4* 94.1 728	674.4 84.6 715
1500	688.9* 96.6 741	719.5 33.0 736
5000-7500[b]	702.2* 54.9 739	730.7 11.3 737

[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.

00467?

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

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

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) rati

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.

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

STUDY TYPE: Acute Neurotoxicity-Hens TOX. CHEM. NO.: 79C

HASKELL LAB. REPORT NUMBER: HLO 28-79 ACCESSION NO: 241931
IRDC No.: 125-029 (study 1)
125-028 (study 2)

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

STUDIES PERFORMED AT: International Research and Development Corporation, Mattawan, Michigan

AUTHORS: E.I. Goldenthal

DATE REPORT SUBMITTED: 4/13/78 (original), 6/5/78 (addendum)

TEST MATERIAL: 2-benzimidazolecarbamic acid, methyl ester (99.3 % Tech., % a.i. not given) (metabolite of Benomyl)

SYNONYMS: MBC
INE-965

MATERIAL AND METHODS: Fasted White Leghorn hens (1108-2254 gm, 6-14 months old) were given by gavage, single doses of the following test materials in 20 ml corn oil/kg:

Compound	Dose (mg/kg)	# treated*		Mortality
		Study 1	Study 2	
0(vehical cont.)	0	10	10	0
TOTP(pos. cont.)	750	10	10	0
MBC	500		10	1
"	2500		10	0
"	5000	10		2(day 3)

TOTP - tri-o-tolyl phosphate
*Study 1 was initiated 3 weeks prior to study 2.

They were individually housed in environmentally controlled rooms and given water and food ad libitum. After treatment they were observed daily for pharmacotoxic signs including neurotoxicity and weighed pretest and weeks 1, 2, 3 and 4. All hens were necropsied an examined grossly. Microscopic examination was performed on selected nerve tissue from spinal cord (3 levels) and the sciatic nerve.

RESULTS AND DISCUSSION: Two of the 10 high dose MBC treated (5000 mg/kg) hens died on day 3. The deaths were considered to be the consequence of acute toxicity of MBC. The 1 LDT death was due to accidental injury. Body weight in the TOTP and 5000 mg/kg groups decreased 8-11% from pretest values while there was no significant change for the other groups.

004679

Signs of toxicity are as follows:

Dose (mg/kg)	Sign(s)	Study 1	study 2
0 (cont.)	sl. ataxia	1/10 (days 15-16)	
TOTP	salivation	5/10 (days 2-3)	4/10 (days 2-3)
	leg weakness, ataxia and/or goose step.	10/10 (days 7-28)	9/10 (days 7-28)
500 MBC	salivation		1/10 (day 2)
2500 MBC	salivation		1/10 (day 2)
5000 MBC	salivation, sl ataxia	*2/10 (day 2)	
	leg weakness	8/10 (1-8 days)	

* both of these died

Although MBC treated hens showed some neurotoxicity at 5000 mg/kg, only the TOTP treated hens showed symptoms of delayed neurotoxicity. The neurotoxic behavioral symptoms displayed early in the 5000 mg/kg MBC treated hens were attributed to acute toxic effects of the chemical. Hens treated with mid and low doses of MBC (2500 mg/kg and 500 mg/kg, respectively) appeared normal except for some salivation. No treatment related gross pathological effects were seen at sacrifice in the MBC treated hens. There were perivascular lymphoid infiltrates (cervical, thoracic and lumbar cord segments) present in all groups. Axonal degeneration and demyelination were not present in either the vehicle controls or MBC treated hens. Microscopic examination of spinal cord and sciatic nerves showed a spectrum of (expected) positive findings characteristic of TOTP treatment in the TOTP treated hens.

CONCLUSION: MBC does not appear to have delayed neurotoxic potential

NOEL for other neurotoxic signs: 2500 mg/kg

CORE-CLASSIFICATION: guideline

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

MRID
D00 88333

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.: 232870-232871

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.R.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 I	36	36	0 (0)
group Ia	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

161

† 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 contr 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 aort
†heart	†pituitary	thymus
†kidney	epididymis	bone marrow
†adrenal	lymph node	lumber spinal
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 an abnormal tis

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

RESULTS: Clinical Signs: There were no treatment related signs o 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 treatmen related.

Body Weight Gain: There was a decrease in weight gain evident fro 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 consumpt 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.

00467

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 IN 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	8.5	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. Prostatit 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 "pericholan and cholangiohepatitis" although not serious, is a toxic effect of the compound. The significance of increased prostati 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 parameter (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) increase cholangiohepatitis and pericholangitis in males and females.

CORE-CLASSIFICATION:

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

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

164

STUDY TYPE: Two-year Feeding study-Dog TOX. CHEM. NO.: 79C

HASKELL LAB. REPORT NO: 195-72 FICHE/MASTER: 000883
MR NO.: 1149 ACCESSION NO.: 23287
23287

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.Wasilecki

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 consumpt 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 affect by treatment. The dogs in 500 ppm and 1500/2500 ppm dose groups ha increased cholesterol, BUN, total protein, GPT and APase levels while similarly presenting evidence of a decreased A/G ratio throug

* see addendum for clarification and additional information.

00467

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			

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

004675

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 I	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)

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