

US EPA ARCHIVE DOCUMENT

02/27/90

PEER REVIEW FILES

CHEMICAL NAME: Benomyl (MBC)
CASWELL NO.: 075A
CAS NO.: 17804-35-2
REVIEWER: Copley

007710

TXR 007710

CURRENT AGENCY DECISION

C; 4.2 x 10⁻³

TUMOR TYPE / SPECIES

Liver tumors (hepatocellular adenomas and carcinomas) in two genetically related strains of mice (CD-1 & Swiss SPF) (M & F).

REVIEWER PEER REVIEW PACKAGE	PEER REVIEW MEETING DATE	PEER REVIEW DOCUMENTS	PEER REVIEW CLASSIFICATION
5. / /	5. / /	5. / /	5.
4. / /	4. / /	4. / /	4.
3. 01/20/89	3. 01/25/89	3. 04/07/89	3. C; 4.2 x 10 ⁻³
2. 03/11/86	2. 01/07/86	2. 03/31/86	2. C; 3.9 x 10 ⁻³
1. 10/03/85	1. 10/03/85	1. / /	1. Prelim. meeting

SAP MEETING	SAP CLASSIFICATION
2. 05/21/86	2. C
1. 11/29/79	1.

QUALITATIVE/QUANTITATIVE RISK ASSESSMENT DOCUMENT	GENETIC TOXICITY ASSESSMENT DOCUMENT
2. / /	1. / /
1. 05/15/89	

MISCELLANEOUS:

First Peer Review Document missing; First SAP 10/09-10/79 and 11/29/79. Stamped 2/2/90; #PR-007710; 140 p.; nha.

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Peer Review Documents
(Information supplied regarding PR Documents
by Dr. Marion Copley 12/12/89)

4/7/89 (3rd)
3/31/86 (follow-up)
1st. no document

4/7/89



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

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FILE COPY

APR 7 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Third Peer Review of Benomyl/MBC

FROM: John A. Quest, Ph.D., Head *JAQ 2/14/89*
Science Support Staff
Science Analysis and Coordination Branch
Health Effects Division (TS-769C)

TO: Jane Mitchell, PM Team 21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

The Health Effects Division Peer Review Committee met on January 25, 1989 to discuss whether or not the tumor data on Benomyl/MBC necessitated a quantification of oncogenic risk.

A. Individuals in Attendance

1. Peer Review Committee: (Signature indicates concurrence with peer review unless otherwise stated.)

Robert Beliles: *Robert Beliles*

William Burnam: *William Burnam*

Marion P. Copley: *Marion P. Copley*

Bernice Fisher: *Bernice Fisher*

Marcia van Gemert: *Marcia van Gemert*

Judith W. Hauswirth: *Judith W. Hauswirth*

John A. Quest: *John A. Quest*

William Sette: *William Sette*

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2. Peer Review Committee Members in Absentia:
(Committee members who were not able to attend the discussion; signature indicates concurrence with the overall conclusions of the committee).

Reto Engler: *Reto Engler*

Richard N. Hill: —

Diane Beal: —

Kerry Dearfield: *Kerry Dearfield*

Lynnard Slaughter: *L. Slaughter*

Esther Rinde: *Esther Rinde*

Richard Levy: *Richard A. Levy*

3. Interested Observers:

Albin Kocialski: *A. Kocialski*

Phil Hurdemann: —

B. Material Reviewed:

This material available for review by the Committee was a package prepared by Dr. Copley containing information on most of the major scientific and regulatory activities conducted by the OPP over the past several years.

C. Background

Background information on Benomyl/MBC is comprehensively provided in Dr. Copley's memorandum of January 20, 1989 (attached). In brief, at the Peer Review Committee meeting of January 7, 1986, it was determined that Benomyl/MBC met some of the criteria for both the B2 and C categories of carcinogen classification. In support of a B2 category classification, both Benomyl and MBC produced an increased incidence of malignant or combined malignant and benign tumors of the liver. In the case of MBC, tumors were produced in multiple strains of mice (closely related CD-1 and Swiss SPF strains) and in multiple experiments. Furthermore, MBC produced an unusual type of hepatocellular tumor (hepatoblastoma) in male Swiss SPF mice. In support of a C category classification, it was noted that: 1) the oncogenic responses observed with Benomyl and MBC were confined solely to the mouse liver, even with repeated experiments; 2) the liver tumors produced by Benomyl and MBC were observed in two related strains of mice (CD-1 and Swiss

SPF) known to have high background incidence rates of liver tumors whereas no liver tumors were produced by MBC in another strain of mice [HOE NMRKf (SPF 71)] known to have a low background incidence rate of liver tumors; and 3) Benomyl and MBC produced weak mutagenic effects consistent with spindle poison activity rather than gene mutation or DNA repair activity.

Based on the above information, the Peer Review Committee decided that there was insufficient evidence for the B2 category and classified Benomyl/MBC as a Category C oncogen. Although there was some discussion by the Committee of possible quantification of risk, a formal decision about whether or not to quantify was not made. A similar situation prevailed at an SAP meeting on Benomyl/MBC held May 21, 1986. It should be noted that at that time, HED had calculated interim estimates of cancer potency for both Benomyl ($Q1^* = 5.9 \times 10^{-3}$; human risk) and MBC ($Q1^* = 3.9 \times 10^{-3}$; human risk) using tumor information from the female mouse portion of an MBC study where the incidence of liver tumor bearing animals (adenomas, carcinomas, and hepatoblastomas) was 1/79 at 0 ppm, 9/78 at 500 ppm, 21/80 at 1500 ppm, and 15/78 at 7500 ppm. To resolve the outstanding issue of whether the group C categorization of Benomyl/MBC is appropriate for quantification of risk using the $Q1^*$, the Registration Division requested that the present Peer Review Committee be convened.

D. Conclusion of the Peer Review Committee on Risk Quantification

The Committee determined that quantification of risk was warranted for Benomyl/MBC in view of the above described biological data supportive of the category B2 classification. In particular, this data included the occurrence of a mostly malignant hepatocellular tumor response with MBC in two strains of mice (and with Benomyl in one strain of mouse), the fact that the malignant response was generally seen in both sexes of mice, and the presence of the unusually occurring and malignant hepatoblastomas with MBC in male SPF Swiss mice. In addition, mutagenicity information was provided by Dr. Dearfield indicating that the aneuploidy (i.e., loss of chromosome material) known to be produced by Benomyl could theoretically result in a loss of tumor suppressor genes and a potential oncogenic effect (see Cancer Research 48:1623-1632, 1988).

The assignment of a $Q1^*$ value for human risk to Benomyl/MBC was temporarily deferred until a brief review of the incidence data for MBC-induced liver tumors in female mice is conducted to check for numerical accuracy of numerator and denominator values. In all probability, the

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Q1* value cited above in this document for MBC will be employed for MBC and Benomyl.

Other Deliberations of the Committee

The Committee also briefly considered whether a quantitative risk assessment should be performed on Thiophanate Methyl, another pesticide that, like Benomyl, is metabolized to MBC in both animals and plants. It was decided that the Q1* value derived for MBC from Benomyl metabolism could now be used to characterize the Q1* for MBC derived from Thiophanate-Methyl metabolism, provided that the latter agent results in MBC residues on plants. This issue can be considered further in the future when Thiophanate Methyl per se is peer reviewed. At present, a chronic mouse study on Thiophanate methyl is outstanding and the Committee could not comment further on this parent compound.

In view of the Agency's issue paper on mouse liver tumors and the recent workshop held in Virginia Beach, Virginia, both of which discussed the relevance of these tumors to humans, the Committee considered that the need for quantitative risk assessment on Benomyl/MBC could be modified. Further information on Benomyl/MBC that could influence this decision would include data on comparative metabolism, peroxisome proliferation, hepatic microsomal drug metabolism, and hepatocytotoxicity in mice. The Committee will schedule a separate meeting to discuss these generic issues.

Attachment

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TOXICOLOGY SUMMARY FOR THE THIRD PEER REVIEW OF BENOMYL AND MBC

Data Evaluation Report for the
Third Meeting of the
Peer Review Committee for Benomyl and MBC

Submitted by Marion P. Copley, D.V.M., Sect.2, Tox. Br.1, HED
Through Judith Hauswirth, Ph.D., Branch Chief
Toxicology Branch 1 (IRS), Hazard Evaluation Division

completed January 19, 1989

TOXICOLOGY SUMMARY FOR THE THIRD PEER REVIEW OF BENOMYL AND MBC

Data Evaluation Report for the
Third Meeting of the
Peer Review Committee for Benomyl and MBC

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1. Preliminary Peer Review of Benomyl - DER (10/3/85)	
2. Second Peer Review of Benomyl - DER (12/20/85)	
3. Peer Review of Benomyl and MBC document (3/31/86)	
4. Statistics memos	
5. Appropriate Study DERs not included in previous packages.	
A. Mouse oncogenicity - Swiss Random	
b. Mouse oncogenicity - NMRKf(SPF71)	

Data Evaluation Report for the Third Meeting of the
Peer Review Committee for Benomyl and MBC

Submitted by Marion P. Copley, D.V.M., Sect.2, Tox. Br.1, HED
Through Judith Hauswirth, Ph.D., Branch Chief
Toxicology Branch 1 (IRS), Hazard Evaluation Division

1. Issues

The Hazard Evaluation Division (HED) Peer Review Committee (formerly the Toxicology Branch (TB) Peer Review Committee) is requested to:

a) reevaluate whether Benomyl and MBC should be evaluated using the multistage model of risk quantification. This should take into consideration that this Committee already classified Benomyl and MBC as C oncogens based on liver tumors.

b) If a Q_1^* is deemed appropriate, to determine whether the previous calculations are adequate or whether they should be redone.

2. Background

a) Benomyl produces liver tumors, both hepatocellular adenomas and hepatocellular carcinomas in two closely related strains of mice (males and females) but not in an unrelated strain of mice or in rats.

Benomyl and MBC were discussed by the Peer Review Committee first on 10/3/85. At that time additional information was requested from the reviewer. No Peer Review Document resulted from that preliminary meeting. On 12/19/85 the Committee reconvened and following review of tumor data, metabolism and structure-activity information, historical control information, mutagenicity data and a listing of one-liner material, classified both fungicides as Category C (possible human) carcinogens.

Although it was discussed at some length, the Committee did not establish whether this compound was suitable for risk quantification by the standard procedures.

b) Benomyl has undergone a complete Special Review cycle. The result of the PD4 (10/1/82) was to regulate exposure by requiring dust masks.

A risk quantification was conducted for the PD4 with the Q_1^* of 2.065×10^{-3} (mg/kg/day)⁻¹. This was based on a benomyl chronic/oncogenicity study that has since been core-graded as supplementary for oncogenicity. Since that time a new value for the human Q_1^* was calculated: 3.9×10^{-3} (mg/kg/day)⁻¹ (see appendix 4 for statistical memos). This used data from

Peer Review

Benomyl, MBC

an MBC study which was core-graded minimum for oncogenicity.

NOTE: As stated in the PD4, benomyl rapidly hydrolyses to MBC in an aqueous environment. MBC also appears to be the initial metabolite in mammalian systems. It has similar or increased toxicity, both acute and chronic, to benomyl. For these reasons MBC data has been used to confirm and supplement benomyl data where applicable.

c) Benomyl was presented to the Scientific Advisory Panel in 5/21/86. They agreed with the classification of Benomyl and MBC as class C (possible human) carcinogens. No comment was given to the question of when to quantify using the multistage model. However the panel stated that,

"... Benomyl and its major metabolite ... MBC produce tumors in livers of two genetically related strains of mice. It does not produce tumors in a genetically unrelated mouse strain nor does it produce tumors in a two-year rat study. Both benomyl and MBC produce weak mutagenic effects consistent with spindle poison activity rather than gene damage and DNA repair activity. In view of these species differences in oncogenic activity and lack of evidence of any direct action on DNA, there are reasonable grounds for doubt that benomyl and its major metabolite MBC are human oncogens. The Panel believes that the classification C seems appropriate."

d) There have been two MBC studies reviewed since the previous peer review. They were discussed and World Health Organization summaries of these studies were included with the previous peer review. Attached in Appendix 5 are completed DERs for:

1) Repeated-dose (24-month) feeding study for determination of the cancerogenic effect of HOE 17411 O F AT204 (Carbendazim) in mice. (NMRKf(SPF71) strain)

and

2) Carcinogenicity study with Carbendazim in mice. (Swiss random strain)

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Peer Review

Benomyl, MBC

3. Summary Weight-of-the-Evidence

Category C oncogen (possible human oncogen) for Benomyl and MBC

1. Tumors in one specie (mouse)
2. Tumors in two strains of mouse (CD-1 and Swiss random)
 - a. Tumors in two sexes (of above studies)
 - b. Both benign and malignant hepatocellular tumors
 - c. Genetically related - both are outbred derivitaves of the Swiss strain
 - d. Both strains have high historical control values for liver tumors in male mice
 - e. Tumors limited to one organ (liver)
 - f. Tumors only at end of study
 - g. Tumors primarily only at high doses
 - h. No evidence for metastases or invasion
 - i. No evidence for decreased time to occurrence of tumors.
3. Tumors not in one (genetically unrelated) strain
 - a. NMRKf strain;
 - b. Low historical control values for liver tumors.
 - c. Evidence for hepatotoxicity is present
4. Mutagenicity - weak
 - a. Genotoxicity - equivocal: DNA repair, gene mutation
 - b. Cytotoxicity - Spindle inhibition
5. Teratogenic (microphthalia in mice)

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

MAR 31 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Benomyl and MBC

FROM: *for* John A. Quest, Ph.D.
Team Leader, Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Henry Jacoby
Product Manager #21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

The Toxicology Branch Peer Review Committee met on January 7, 1986 to discuss and evaluate the data base on Benomyl and its primary metabolite, MBC, with particular reference to the oncogenic potential of the chemical. A preliminary meeting was held on October 3, 1985 on Benomyl to determine the information that would be required to hold an in-depth discussion on this compound.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with peer review unless otherwise stated).

Theodore M. Farber

William Burnam

Anne Barton

Reto Engler

R. Bruce Jaeger

Bertram Litt

in John A. Quest

2. Reviewers: (Non-panel members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Marion Copley

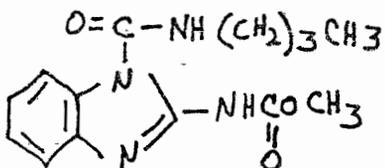
Jane E. Harris

B. Material Reviewed:

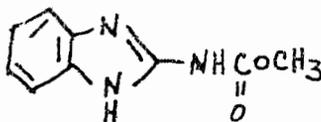
The material available for review consisted of a comprehensive summary of toxicology information on Benomyl (Copley/Harris memorandum dated 12/19/85), including tumor data on Benomyl and its major metabolite MBC, metabolism and structure-activity information, historical control information, mutagenicity data, and a listing of one-liner material on the Benomyl/MBC data base. A copy of the information reviewed is appended to this panel report.

C. Background Information:

Benomyl is a benzimidazole carbamate compound (methyl-1(Butylcarbamoyl)-2-benzimidazolecarbamate) that is metabolized under aqueous conditions both in vivo and in vitro to its major metabolite MBC (methyl 2-benzimidazole carbamate). Both compounds are systemic fungicides and both are associated with hepatocellular tumors in certain strains of mice but not in rats.



BENOMYL



MBC

The Review Committee evaluated oncogenicity data on these chemicals from 4 studies performed in mice and from 2 studies performed in rats. The oncogenicity data are summarized below:

D. Evaluation of Oncogenicity Evidence for Benomyl and MBC:

1. Mouse Oncogenicity Study of Benomyl:

Haskell Laboratory administered Benomyl in the diet to groups of 80 male and 80 female Charles River CD-1 mice at concentrations of 0, 500, 1500 or 7500/5000 ppm for 2 years. The high dose of 7500 ppm was reduced to 5000 ppm at 37 weeks in males and females due to weight loss. The following incidence patterns of tumors suggestive of a compound related effect were observed.

Tumor Site and Type	Sex	Dose (ppm)			
		0	500	1500	7500/5000
Lung:					
Alveologenic carcinoma	M	13/79(16%)	24/79(30%)*	23/79(29%)*	16/80(20%)
	F	16/77(21%)	7/79(9%)	4/78(5%)	6/74(8%)
Liver:					
Adenoma	M	9/77(12%)	9/80(11%)	11/79(14%)	10/80(12%)
Carcinoma	M	16/77(21%)	26/80(32%)*	41/79(52%)*	17/80(21%)
Combined	M	25/77(32%)	35/80(44%)*	52/79(66%)*	27/80(34%)
Adenoma	F	2/77(2.5%)	2/80(2%)	7/79(9%)	7/77(9%)
Carcinoma	F	2/77(2.5%)	7/80(9%)*	6/79(7%)	14/77(18%)*
Combined	F	4/77(5%)	9/80(11%)	13/79(16%)*	21/77(27%)*

*= p<0.05 compared to controls

Pulmonary carcinomas were significantly elevated in male mice (low and mid doses). The effect did not appear to be compound related however, since a dose-response effect was not observed in the Cochran-Armitage test for trend; the observed incidences were within the range of historical control rates for this tumor in other studies conducted at Haskell Labs (i.e., 16% to 36%); and the MBC metabolite did not produce an increase in pulmonary tumors in other studies performed in CD-1 mice.

Hepatocellular carcinomas were significantly elevated in male (low and mid doses) and female (low and high doses) mice. In addition, adenomas and carcinomas combined were significantly elevated in males (low and mid doses) and females (mid and high doses). The tumorigenic responses appeared to be compound related; e.g., they occurred with significant positive trends, and the elevated incidences exceeded historical rates for these tumor responses in 2 other studies (an "unnamed" study, and the MBC study cited below under No. 2) conducted at the registrant's laboratory (see Copley/Harris memorandum of 12/19/85, page 10 for data). Furthermore, similar liver tumorigenic responses were produced by the MBC metabolite in other studies performed in CD-1 mice (see below). The oncogenic responses that were produced by Benomyl in treated mice were not accompanied by increased incidences of hepatocellular adenomas or hyperplasia.

The highest dose of benomyl tested in male mice in this study probably exceeded a MTD level. This dose in males produced a decreased weight gain (approximately -9%), hepatocellular toxicity (e.g., foci of cellular alteration, cytomegaly, and foci of degeneration), and degenerative changes in the testes (e.g., atrophy, seminiferous tubule degeneration, and interstitial cell hyperplasia) and in the epididymis (aspermia). This dose did not produce liver tumors in males, possibly because of the hepatocellular toxic changes that were observed (e.g., the observed liver toxicity may have altered the ability of benomyl to be metabolized to MBC). The low and mid dose levels of benomyl did produce liver tumors in males, but these doses were not associated with any other toxic effects and thus did not approximate a MTD level.

The highest dose of benomyl tested in females probably approximated a MTD level as evidenced by findings of decreased weight gain (approximately -9%), elevated liver weights, reduced kidney weight, and spleen hemosiderosis. This dose in females did produce liver tumors, as did lower doses of the compound. Benomyl did not produce the exaggerated liver toxic changes in female mice that were observed in male mice.

2. Mouse Oncogenicity Study of MBC:

Haskell Laboratory administered MBC in the diet to groups of 80 male and 80 female Charles River CD-1 mice at concentrations of 0, 500, 1500, 7500 (females) or 7500/3750 (males) ppm for 2 years. The high dose of 7500 ppm was reduced to 3750 ppm at 66 weeks in males due to increased mortality, and all males were ultimately sacrificed at 73 weeks. The following incidence pattern of liver tumors was observed.

Liver Tumor Type	Sex	Dose (ppm)			
		0	500	1500	7500**
Adenoma	M	11/80(14%)	15/80(19%)	14/80(17%)	3/80(4%)
Carcinoma	M	2/80(2%)	5/80(6%)	9/80(11%)*	0/80(0%)
Combined	M	13/80(16%)	20/80(25%)	23/80(28%)*	3/80(4%)
Adenoma	F	0/79(0%)	5/78(6%)*	5/80(6%)*	3/78(4%)
Carcinoma	F	1/79(1%)	4/78(5%)	15/80(18%)*	12/78(15%)*
hepatoblastoma	F	0/79(0%)	0/78(0%)	1/80(1%)	0/79(0%)
Total	F	1/79(1%)	9/78(11%)*	21/80(26%)*	15/78(19%)*

* = p < 0.05 compared to controls

** = Reduced to 3750 ppm in males at 66 weeks.

Hepatocellular carcinomas, and adenomas and carcinomas combined, were significantly elevated in male mice (mid dose level); no increase in adenomas occurred in males. The lack of oncogenic response in high dose males is likely to be explained by their early deaths and sacrifice at 73 weeks. In female mice there were significant increases in adenomas (low and mid doses), carcinomas (mid and high doses), and adenomas and carcinomas (all 3 dose level tested). The Committee noted that this profile of liver tumors resembled that described above for benomyl in CD-1 mice. No increased incidence of liver hyperplasia occurred in treated mice. A comparison of the MBC liver tumor data with historical control data from 2 other studies conducted at Haskell Laboratory (the "unnamed" study and the benomyl mouse study in CD-1 mice; see Copley/Harris memorandum of 12/19/85, page 10) indicated that only the carcinomas (mid and high dose levels) and the adenomas/carcinomas combined (all 3 dose levels tested) in female mice exceeded the control response rates in the other studies.

The high dose level of MBC tested in male mice clearly exceeded a MTD level because of excessive mortality. The mid dose level appeared to approximate a MTD level. Both of these doses in males caused reduced weight gain, hepatocellular toxicity (e.g., pigmented macrophages, hypertrophy, and centrilobular necrosis), renal tubular pigmentation, thymic lymphoid depletion, and sperm stasis. The changes however were more severe at the high dose level.

The highest dose of benomyl tested in females appeared to approach but did not exceed the MTD level. This dose caused increased liver weight and foci of eosinophilic hepatocellular alteration, renal tubular pigmentation, and thymic lymphoid depletion.

3. Mouse Oncogenicity Study of Carbendazim (99% MBC):

In a study performed by the Central Institute for Nutrition and Food Research, TNO, and reviewed in summary form by the WHO (see Copley/Harris memorandum of 12/19/85, page 7), MBC was administered in the diet to groups of 100 male and 100 female SPF Swiss mice at concentrations of 0, 150, 300 or 1000/5000 ppm for 80 weeks. The 1000 ppm concentration was increased to 5000 ppm in males and females at week 8. Data were presented in summary form only. The following incidence pattern of liver tumors was observed (Note: In this study the term "neoplastic nodule" was used in place of the term "adenoma"; the term "hepatoblastoma" refers to a more uncommon and malignant type of liver tumor than hepatocellular carcinoma).

Liver Tumor Type	Sex	Dose (ppm)			
		0	150	300	1000/5000
Neoplastic Nodule	M	9/100(9%)	7/98(7%)	14/100(14%)	16/100(16%)
Carcinoma	M	1/100(1%)	1/98(1%)	9/100(2%)	3/100(3%)
Hepatoblastoma	M	0/100(0%)	1/98(1%)	1/100(1%)	7/100(7%)*
Total	M	10/100(10%)	8/98(8%)	16/100(16%)	17/100(17%)
Neoplastic Nodule	F	0/97(0%)	1/99(1%)	1/98(1%)	9/97(9%)*
Carcinoma	F	1/97(1%)	0/99(0%)	0/98(0%)	0/97(0%)
Hepatoblastoma	F	0/97(0%)	0/99(0%)	0/98(0%)	0/97(0%)
Total	F	1/97(1%)	1/99(1%)	1/98(1%)	9/97(9%)

*= P<0.01 compared to controls, Exact test.

Hepatoblastomas were significantly elevated in male mice (high dose level), and neoplastic nodules (i.e., adenomas) were significantly elevated in female mice (high dose level). The Committee noted that the Swiss SPF strain of mouse used in this study is similar to the CD-1 strain of mouse in which benomyl and MBC were tested; both strains are Swiss derived and tend to exhibit a high background incidence of liver tumors in male mice.

Based on the summary information available for this study, the highest dose level of MBC tested did not appear to exceed a MTD level. The HDT caused increased relative liver weights and clear cell and/or mixed hepatic cell foci in males and females.

4. Mouse Oncogenicity Study of Carbendazim (MBC):

In another study reviewed by the WHO (see Copley/Harris memorandum of 12/19/85, page 8), MBC was administered in the diet to groups of 100 male and 100 female HOE NMRKF (SPF 71) mice at concentrations of 0, 50, 150, 300 or 1000/5000 ppm for 22 months. The 1000 ppm concentration was increased to 5000 ppm at week 8. No evidence of an oncogenic response in the liver or at any other site was observed. The Committee noted that the NMRKF strain of mouse, in contrast to Charles River CD-1 and Swiss SPF mice, normally exhibits a low background incidence of liver tumors.

The highest dose of MBC tested in this study appeared to be close to a MTD level as indicated by findings of liver toxicity in both male and female mice (e.g., liver cell hypertrophy, clear cell foci, liver cells in mitosis, pigmented Kupffer cells, enlarged cell nuclei, and multiple cell necrosis).

5. Rat Oncogenicity Studies of Benomyl and MBC:

Benomyl was studied in a 2-year dietary study (0, 100, 500 or 2500 ppm) in Charles River CD rats; the highest concentration was a systemic NOEL and no oncogenic effects occurred. MBC was also studied in a 2 year dietary study (0, 100, 500, 2500/10,000 or 5000 ppm) in Charles River CD rats; on oncogenic effects occurred. In this study, the highest dose level was a MTD level as evidence by findings of weight loss in males and females (10%-20% less than controls) and hepatic pericholangitis. Both of the above studies were performed by Haskell Laboratory.

E. Additional Toxicology Data on Benomyl and MBC:

1. Metabolism:

Limited studies conducted in mice indicate that benomyl is primarily metabolized to MBC, which in turn is converted to 2-aminobenzamidole (2-AB) and also to 5-OH-MBC and 5-OH-2-AB. The latter 2 metabolites undergo sulfate and glucuronide conjugation. Elimination of metabolites occurs rapidly in urine and feces (e.g., 94% of an orally administered radiolabelled dose was excreted in 96 hours in mice as the metabolites, with no parent compound detected). No unusual localization of benomyl or its metabolites has been found in animal tissues.

2. Teratology:

Benomyl has been demonstrated to be teratogenic in several oral (gavage) studies conducted in both Wistar and Charles River CD rats at doses ranging from 62.5 to 125 mg/kg/day. The most common abnormality in these studies was microphthalmia. In most of these studies, fetotoxic and embryotoxic effects were also observed at similar or greater dose levels. Benomyl was also reported to be teratogenic in one study in Charles River CD-1 mice at oral (gavage) doses of 100 mg/kg or more. The major anomalies noted were cleft palate, supernumerary ribs, and subnormal vertebral centrum (no compound-related microphthalmia was reported).

3. Mutagenicity:

Data provided in the Position Document 4 on Benomyl and MBC indicated that both compounds are spindle poisons. For example, nondisjunction was reported in A. nidulans and many other test systems with both agents. The compounds

also produced positive effects in tests to assess structural chromosome aberrations which were consistent with a spindle effect; e.g., benomyl was weakly positive for sister chromatid exchange in vitro in Chinese hamster ovary cells with and without activation, and both benomyl and MBC caused increased incidences of micronuclei in polychromatic erythrocytes in mice bone marrow. In other studies performed to assess gene mutations, equivocal results were obtained. That is, MBC was weakly positive in one mouse lymphoma test (L5178Y TK⁺/-) but was negative in a second test, Benomyl and MBC produced both positive and negative results in different Ames tests, and both compounds produced negative results in Chinese hamster ovary cells (HGPRT). Finally, negative results were obtained for DNA repair with Benomyl and MBC in several studies in primary mouse and rat hepatocyte cultures. The Peer Review Committee was of the opinion that these results, when taken together, indicated that both Benomyl and MBC have weak mutagenic activity that is primarily attributable to adverse effects on the cellular spindle apparatus. The pattern of results observed did not appear to correlate with heritable disease or oncogenic effects, but may relate to the teratogenic effects observed with Benomyls.

4. Structure-Activity Correlations:

Both Benomyl and MBC bear a close structural resemblance to several other benzimidazole compounds that are suspect oncogens (e.g., fenbendazole and albendazole). The potential oncogenic effects of these compounds are currently under review by the Center for Veterinary Medicine, Food and Drug Administration and were recently discussed in a Congressional Subcommittee Hearing (reference: Human Food Safety and the Regulation of Animal Drugs; 27th Report by the Committee on Government Operations, December 31, 1985. Union Calendar, No. 274. Intergovernmental Relations and Human Resources Subcommittee. Ted Weiss, New York, Chairman; pp. 1-115). In the case of fenbendazole, a high incidence of liver nodular hyperplasia and low incidences of liver neoplastic nodules, adenomas and carcinomas were observed in rats. In the case of albendazole, histiocytic sarcomas were observed in rats and uterine polyps were observed in rats and mice. The Committee was aware that final decisions regarding the classification of these chemicals as oncogens had not yet been made by the FDA.

F. Weight of Evidence Considerations:

The committee considered the following facts regarding toxicology data on Benomyl and MBC to be of importance in a weight of the evidence determination of oncogenic potential.

1. Benomyl (methyl-1(butylcarbamoyl)-2-benzimidazole carbamate) and MBC (methyl-2-benzimidazole carbamate) are structurally related compounds. Pharmacokinetic studies in mice have demonstrated that Benomyl is rapidly metabolized to MBC in vivo, and that MBC is the primary metabolite of Benomyl. The toxicity of Benomyl may be primarily due to the formation of the MBC metabolite.
2. Both Benomyl and MBC produced significantly elevated incidences of liver tumors (e.g., carcinomas, and carcinomas and adenomas combined) in male and female Charles River CD-1 mice, a non-inbred strain of Swiss mouse known to exhibit a high background incidence of liver tumors in males. (see Sher; Toxicol. Appl. Pharmacol. 30: 337, 1974; and historical control data in Copley/Harris memo of 12/19/85, page 10). The tumors were observed at similar dose levels for Benomyl and MBC, and were also similar in both incidence and type. No hepatocellular hyperplasia was observed in Charles River CD-1 mice exposed to either chemical, but there were increases in foci of cellular alteration.
3. MBC also produced a significantly elevated incidence of liver tumors (i.e., hepatoblastoma - a more uncommon and malignant tumor than hepatocellular carcinoma) in male Swiss SPF mice and a significantly elevated incidence of liver neoplastic nodules (i.e., adenomas) in female mice of the same strain. The CD-1 strain of mouse is similar to the Swiss SPF strain of mice in that it is Swiss-derived and also exhibits a high background incidence of liver tumors in males (Sher, 1974).
4. The tumorigenic responses observed with both Benomyl and MBC in Charles River CD-1 mice (e.g., carcinomas, and carcinomas and adenomas combined) and those observed with MBC in Swiss SPF mice (i.e., hepatoblastomas and neoplastic nodules) generally occurred at doses which were either lower than or approximately near maximum tolerated dose (MTD) levels. (See discussions of MTD levels for each study in sections D.1., D.2., and D.3.).
5. Oncogenic responses to Benomyl and MBC in Charles River CD-1 mice and Swiss SPF Swiss mice occurred only in the liver; no other type of organ or tissue exhibited an oncogenic response.
6. MBC was not oncogenic in HOE NMRKE (SPF 71) mice. This strain of mouse differs from Charles River CD-1 and Swiss SPF mice in that it normally exhibits a low background incidence rate of liver tumors (Weisse et al., Z. Versuchstierk 17: 91, 1975). In addition, neither Benomyl nor MBC were oncogenic in studies in Charles River CD rats.

7. Benomyl and/or MBC produced positive mutagenic effects that were consistent with adverse effects on the cellular spindle apparatus. These included nondisjunction in A. nidulans, sister chromatid exchange in CHO cells, and micronuclei formation in mouse bone marrow cells. In contrast, equivocal results (both positive and negative findings) for gene mutation were found in Ames tests and mouse lymphoma tests, and negative results for DNA repair were found in primary rat and mouse hepatocyte cultures. The pattern of mutagenicity results appeared to correlate poorly with heritable spindle effects or point mutagenicity.
8. Benomyl was teratogenic following oral (gavage) administration in rats (e.g., microphthalmia) and mice (e.g., cleft palate, supernumary ribs, subnormal vertebral centrum), and also evoked embryotoxic effects in these species. The Committee noted a possible correlation between these effects and the ability of benomyl to act as a spindle poison.
9. Benomyl and MBC are structural congeners of other benzimidazole compounds (e.g., fenbendazole and albendazole) that are currently under review by the FDA Center for Veterinary Medicine; no final determination of oncogenicity has been made by the FDA at this time for these analogues.

G. Classification of Oncogenic Potential:

The Committee concluded that the data available for Benomyl and its primary metabolite, MBC, provides limited evidence of oncogenicity for both chemicals in male and female mice. Criteria contained in the proposed EPA Guidelines (CFR, November 23, 1984) for classifying a carcinogen in either Category B₂ or C were considered. Benomyl and MBC met some of the criteria specified for the B₂ classification. That is, both Benomyl and MBC produced an increased incidence of malignant or combined malignant and benign tumors of the liver. In the case of MBC, tumors were produced in multiple strains of mice (closely related CD-1 and Swiss SPF strains) and in multiple experiments. Furthermore, MBC did produce an unusual type of hepatocellular tumor (hepatoblastoma) but only in male Swiss SPF mice.

Alternatively, the panel considered the guideline criteria for Category C (limited evidence of carcinogenicity), and classified Benomyl in this category for the following reasons: (1) The oncogenic responses observed with Benomyl and MBC were confined solely to the mouse liver, even with repeated experiments; (2) the liver tumors produced by Benomyl and MBC were observed in 2 related strains of mice (CD-1 and Swiss SPF) known to have high background incidence rates of liver tumors whereas no liver tumors were produced by MBC in

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another strain of mice [HOE NMRKÉ (SPF 71)] known to have a low background incidence rate of liver tumors; (3) Benomyl and MBC produced weak mutagenic effects consistent with spindle poison activity rather than gene mutation or DNA repair activity; the committee considered this pattern of mutagenic activity to correlate better with the observed teratogenic effects of Benomyl than with the oncogenic responses to Benomyl and MBC. Because of these factors the Committee determined that there was insufficient evidence for the B₂ category and therefore, in conformity with the EPA Guidelines noted above, classified both Benomyl and its primary metabolite, MBC, as Category C (possible human) carcinogens.

#9 2/6/86
rew: 3/19/86

MEMORANDUM

SUBJECT: Definition and Use of the Term "MTD"
(Maximum Tolerated Dose)

FROM: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769) *RBJ 3/26/86*

My signature acknowledges concurrence with the peer review on Benomyl/MBC providing the use of the term "MTD" in this document is consistent with the definition and use as given in: (1) HED SEP: Oncogenicity Potential (Guidance for Analysis and Evaluation of Long Term Rodent Studies) (EPA-540/9-85-019, June 1985); (2) Report of the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation, DHHS (August 17, 1984); and (3) Chemical Carcinogens; A review of the Science and its Associated Principles, February 1985, Office of Science and Technology Policy (FR/Vol. 50, No. 50/March 14, 1985).

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SAP Executive Summary; Meeting Date(s)

5/21/86
10/9/79 & 11/29/79

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MAY 28 1986

MEMORANDUM

SUBJECT: Transmittal of the Final FIFRA Scientific Advisory Panel Reports on the May 21-22, 1986 Meeting

TO: Steven Schatzow, Director
Office of Pesticide Programs (TS-766)

The above mentioned meeting of the FIFRA Scientific Advisory Panel (SAP) was an open meeting held in Washington, D.C., to review the following topics:

- (1) Consideration by the Agency of a proposed testing battery for inerts.
- (2) A set of scientific issues being considered by the Agency in connection with the Special Review of Diazinon.
- (3) A set of scientific issues being considered by the Agency in connection with the Registration Standard for Benomyl.
- (4) A set of scientific issues being considered by the Agency in connection with the Registration Standard for Thiophanate Methyl.
- (5) A set of scientific issues being considered by the Agency in connection with the Registration Standard for Pronamide.
- (6) Presentation of paper on maximum tolerated dose.
- (7) Review of a draft paper entitled "Neoplasia Induced by Inhibition of Thyroid Gland Function (Guidance for Analysis and Evaluation)"
- (8) Review of twelve draft reporting guidelines.

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Please find attached the SAP's final reports on the eight issues discussed at the meeting.



Stephen L. Johnson, Executive Secretary
FIFRA Scientific Advisory Panel (TS-769)

Attachments

- cc: Panel Members
- John A. Moore
- James Lamb
- Al Heier
- Susan Sherman
- John Melone
- Douglas Campt
- EPA Participants

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

A Set of Scientific Issues Being Considered by the Agency in Connection with the Draft Registration Standard for Benomyl

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed review of the data base supporting the Environmental Protection Agency's (EPA) decision to classify benomyl as a Group C, or possible human oncogen. The review was conducted in an open meeting held in Washington, D.C., on May 21, 1986. All Panel members were present for the review.

Public notice of the meeting was published in the Federal Register on Friday, April 25, 1986.

Oral statements were received from staff of the Environmental Protection Agency.

In consideration of all matters brought out during the meeting and careful review of all documents presented by the Agency, the Panel unanimously submits the following report.

REPORT OF SAP RECOMMENDATIONS

Risk Assessment Procedures

Several of the EPA documents studied by the Panel in connection with the Registration Standards for pesticides include the following statement pertaining to the Agency's risk assessment procedures:

"The use of the upper 95% bound has been adopted principally to provide assurance that the estimated risks will not be materially affected by using the results of any particular positive study."

The Panel disagrees with this approach. While it may be mathematically satisfying, it is biologically irrational and insensitive to reality. The upper 95% confidence limit represents a linear extrapolation of high dose data, when in fact non-linearities are likely to be present at lower doses. Thus, it is not scientifically valid to only present the upper 95% bound.

The Panel reiterates its request that Agency risk assessment discussions should include not only upper, but also lower 95% confidence bound values, and the MLE (maximum likelihood estimate) in order to provide information on the degrees of uncertainty involved in any given risk assessment.

The Panel does not believe that it is justified to make quantitative risk assessments for any pesticides classified in Groups C or D in regards to oncogenicity, unless there are mitigating circumstances.

Benomyl

The Agency requested the Panel to focus its attention upon a set of issues relating to the pesticide benomyl. There follows a list of the issues and the Panel's response to the issues.

1. The Agency asks the Panel to consider its analysis of the weight-of-the-evidence in classifying benomyl as a Group C oncogen, or possible human oncogen.

Panel Response:

Benomyl and its major metabolite methyl-z-benzimidazole carbamate (MBC) produce tumors in the livers of two genetically related strains of mice. It does not produce tumors in a genetically unrelated mouse strain nor does it produce tumors in a two-year rat study. Both benomyl and MBC produce weak mutagenic effects consistent with spindle poison activity rather than gene damage and DNA repair activity. In view of these species differences in oncogenic activity and lack of evidence of any direct action on DNA, there are reasonable grounds for doubt that benomyl and its major metabolite MBC are human oncogens. The Panel believes that the classification C seems appropriate.

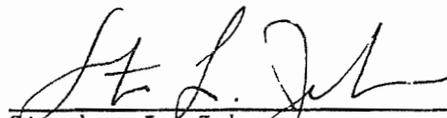
2. The Agency asks the Panel to consider what weight the Agency should place on a quantitative risk assessment for benomyl, as a class C oncogen, causing only liver tumors in certain strains of mice.

Panel Response:

The C classification indicates the possibility that a compound may be a human oncogen, but it also indicates that there is some degree of doubt about human oncogenicity. The doubt may reflect conflicting evidence or important data gaps. Since quantitative risk assessment is less than a precise tool, it seems inappropriate to proceed with a quantitative risk assessment for humans on such data.

FOR THE CHAIRMAN

Certified as an accurate report of Findings:



Stephen L. Johnson
Executive Secretary
FIFRA Scientific Advisory Panel

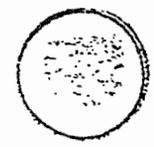
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BENOMYL

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FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT
SCIENTIFIC ADVISORY PANEL

A Set of Scientific Issues Being Considered by the Agency in
Connection with the Draft Registration Standard for
Thiophanate Methyl

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed review of the data base supporting the Environmental Protection Agency's (EPA) decision to classify thiophanate methyl as a Group C, or possible human oncogen. The review was conducted in an open meeting held in Washington, D.C., on May 21, 1986. All Panel members were present for the review.

Public notice of the meeting was published in the Federal Register on Friday, April 25, 1986.

Oral statements were received from staff of the Environmental Protection Agency and from Mr. Joseph Panetta and Dr. Bernalyn D. McGaughey, Pennwalt Corporation.

In consideration of all matters brought out during the meeting and careful review of all documents presented by the Agency, the Panel unanimously submits the following report.

REPORT OF SAP RECOMMENDATIONS

Risk Assessment Procedures

Several of the EPA documents studied by the Panel in connection with the Registration Standards for pesticides include the following statement pertaining to the Agency's risk assessment procedures:

"The use of the upper 95% bound has been adopted principally to provide assurance that the estimated risks will not be materially affected by using the results of any particular positive study."

The Panel disagrees with this approach. While it may be mathematically satisfying, it is biologically irrational and insensitive to reality. The upper 95% confidence limit represents a linear extrapolation of high dose data, when in fact non-linearities are likely

N-05313/Thiophanate Methyl

to be present at lower doses. Thus, it is not scientifically valid to only present the upper 95% bound.

The Panel reiterates its request that Agency risk assessment discussions should include not only upper, but also lower 95% confidence bound values, and the MLE (maximum likelihood estimate) in order to provide information on the degrees of uncertainty involved in any given risk assessment.

The Panel does not believe that it is justified to make quantitative risk assessments for any pesticides classified in Groups C or D in regards to oncogenicity, unless there are mitigating circumstances.

Thiophanate Methyl

The Agency requested the Panel to focus its attention upon a set of issues relating to the pesticide thiophanate methyl. There follows a list of the issues and the SAP's response to the issues.

1. The Agency asks the Panel to consider the analysis of the weight-of-the-evidence in classifying thiophanate methyl as a Group C oncogen, or possible human oncogen, and what weight the Agency should place on a quantitative risk assessment for thiophanate methyl as a class C oncogen based on results with the metabolite MBC. In addition, the Agency requests the Panel to consider the need for a quantitative risk analysis for applicators who are expected to be exposed primarily to thiophanate methyl, and as part of such an assessment, the need for a dermal absorption study.

Panel Response:

The Panel notes that thiophanate methyl (TPM) was not oncogenic in male and female mice fed 0, 10, 40, 160, and 640 ppm for 2 years. The MTD was not reached in this study and it was concluded that this study was inadequate. TPM was not oncogenic in male and female rats at doses of 0, 10, 40, 160, and 640 ppm. The MTD was reached. TPM was essentially not mutagenic.

Methyl-z-benzimidazole carbamate (MBC), a major metabolite, was oncogenic in both male and female mice at 500 - 1000 ppm in the diet and the MTD was reached. MBC was not oncogenic in rats at 2500 - 10,000 ppm and the MTD was reached. MBC was weakly mutagenic in 2 tests and negative in 3 tests.

Recommendation:

1. The Panel believes that the weight-of-the-evidence warrants classification of TPM in Class D.

2. The Panel recommends that the mouse study be redone achieving an MTD.
3. The Panel recommends that dose-related metabolite-pharmacokinetic studies be performed to further consider the role of the metabolite, MBC, in evaluating the oncogenicity of TPM.
4. The Panel recommends that a quantitative risk assessment should not be done.
5. The Panel believes that a dermal absorption study is unwarranted at the present time.

FOR THE CHAIRMAN

Certified as an accurate report of Findings:



Stephen L. Johnson
Executive Secretary
FIFRA Scientific Advisory Panel

Date: 5/28/86

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9/79 + 11/29/79



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

November 30, 1979

OFFICE OF TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of FIFRA Section 6(b)(1) Action on Benomyl and Thiophanate-methyl

FROM: Dr. H. Wade Fowler, Jr. *H. Wade Fowler Jr.*
Executive Secretary
FIFRA Scientific Advisory Panel (TS-766)

TO: Deputy Assistant Administrator
for Pesticide Products (TS-766)

The FIFRA Scientific Advisory Panel has completed review of the Notices of Determination concluding the Rebuttable Presumptions Against Registration (RPAR's) of pesticide products containing benomyl and thiophanate-methyl. The review was completed during open meetings held in Arlington, Virginia, during the periods October 9-10, 1979, and November 29, 1979.

Attachment

cc: Panel Members
Mr. Conlon
Esther Saito
OPP Division Directors

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT (FIFRA)
SCIENTIFIC ADVISORY PANEL

Review of Preliminary Notices to
conclude Rebuttable Presumptions Against Registration
(RPARS) of products containing benomyl and thiophanate-methyl

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel has completed review of plans by the Environmental Protection Agency for initiation of regulatory action on benomyl and thiophanate-methyl pesticide products under the Provisions of Section 6(b)(1) of FIFRA, as amended. The review was completed during open meetings in Arlington, Virginia, during the periods October 9-10, 1979, and November 29, 1979.

Maximum public participation was encouraged for the review. Federal Register Notices were published on September 21, 1979, November 14, 1979, and November 27, 1979. In addition telephonic calls and special mailings were sent to the general public who had previously expressed an interest in activities of the Panel.

Written and oral statements were received from representatives of E. I. DuPont Nemours and Company and Pennwalt Corporation in respect to proposed regulatory action on benomyl and thiophanate-methyl, respectively.

In consideration of all matters brought out during the meeting and careful review of all documents submitted by the Agency and other parties, the Panel unanimously submits the following report:

Panel Position on Thiophanate-methyl

The Scientific Advisory Panel concurs with the Agency's position to terminate the RPAR against thiophanate-methyl.

In regard to the option under consideration by the Agency for additional gene mutation tests for MBC, a metabolite of thiophanate-methyl, the Scientific Advisory Panel offers the following comment:

In May 1978, the FIFRA Scientific Advisory Panel issued the following statement relative to the mutagenesis subsection of subpart F, Hazard Evaluation: Human and Domestic Animals, of the Proposed Guidelines for Registering Pesticides in the United States:

"The Scientific Advisory Panel strongly endorses the need for mutagenic assays as a survey for potential environmental hazards and endorses the principles of the proposed guidelines. Moreover we feel that mutagenesis and oncogenesis should be considered together as related rather than isolated phenomena. To these ends, therefore, and in the interest of simplicity and practicality, the following core battery of tests selected from the proposed guidelines is recommended for mutagenicity screening of all pesticides.

1. A sophisticated microbiological test such as an enhanced Ames assay with appropriate dose response.
2. A mammalian cell point mutation assay such as the hamster embryo or mouse lymphoma.
3. An in vivo cytogenetics assay.

In addition the Panel recommends that a multiple generation test which is part of the reproductive study (162.83-4) be modified to provide a statistically significant assay for dominant lethal mutations. The Panel also recommends that oncogenicity studies (162.83-2) be used in the overall evaluation of mutagenicity. Finally the Panel recommends that both the core battery of tests outlined in this report and the dominant lethal test be performed on each pesticide. The results of these tests will provide the Agency with the information necessary to estimate the mutagenic potential of pesticides".

In evaluating the data available on the mutagenicity of MBC, the Scientific Advisory Panel has also evaluated mutagenicity studies with benomyl since metabolism studies have shown that at least 80% of benomyl is metabolized to MBC in vivo. On examination of the mutagenicity tests that have been conducted using MBC, benomyl, and thiophanate-methyl, the Panel is of the opinion that the requirement

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for a sophisticated microbiological test has been met. In addition, the Panel is of the opinion that a suitable in vivo cytogenetics assay has been completed. However, the Panel does not believe that an appropriate mammalian cell point-mutation assay has been performed. Therefore, we recommend that a mammalian cell point mutation assay be conducted using MBC and a mouse lymphoma or Chinese hamster embryo cell line or any other equally well validated test for point mutations in mammalian cells. If the results of this test are negative, the Scientific Advisory Panel is of the opinion that the mutagenic potential of thiophanate-methyl be considered to have been completely rebutted and no further tests should be required.

In regard to the potential for spindle effects from human exposure to thiophanate-methyl, the Scientific Advisory Panel offers the following conclusions: A negative result has been obtained in a multigeneration reproductive study and a dominant lethal study using thiophanate-methyl. The Scientific Advisory Panel is of the opinion that these two tests and an appropriate in vivo cytogenetics test constitute an adequate measure for adverse health effects in man from spindle poisons. Having met the proposed requirements the Scientific Advisory Panel is of the opinion that there is no significant risk from spindle inhibition in man as a result of exposure to thiophanate-methyl in its normal use in agriculture.

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PANEL POSITION ON BENOMYL

In regard to the specific issues and questions posed by the Agency to the Panel regarding the benomyl RPAR, the Scientific Advisory Panel offers the following responses:

Questions from EPA Concerning Its Regulatory Decision.

Question 1. Based on the data available at this time do you agree with the agency's conclusion of low risk from the spindle effects associated with use of benomyl?

Answer: Yes (see Appendix I).

Question 2. Would you suggest a method of estimating the mutagenic risk for spindle effects?

Answer: See Appendix I.

Question 3. We proposed to request gene mutation tests in (1) Drosophila, (2) mammalian somatic cells in culture, and (3) an appropriate eukaryotic microorganism. In your opinion are these tests adequate?

Answer: See Appendix 1.

Question 4. During the April 1979 Scientific Advisory Panel meeting we discussed the DuPont dermal absorption study. Would you comment on this study and the agency's conclusion that the dermal component of the total body dose is extremely low.

Answer: We have examined the data from the study conducted by DuPont on dermal absorption of 2-¹⁴C-benomyl through rat skin. We concur with the agency's position that it would not be possible to obtain a level of benomyl in the blood through dermal absorption which would pose a significant risk.

Panel Comments on Benomyl Regulatory Decision

The EPA has proposed the continued registration of benomyl for all uses, but with an amendment of the terms and conditions of registration.

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These amendments of terms and conditions of registration entail the requirement for (1) additional mutation studies, (2) the requirement for water soluble packaging for benomyl sold in units of 5 pounds or larger, (3) that a cloth mask be required during mixing and loading for aerial application if the product is not packaged in water soluble bags, (4) that a monitoring study of the fate of benomyl in the environment be required for continued registration for rice uses and, (5) that label changes will be required including a precautionary statement relative to reproductive hazards.

Mutation Studies (1)

Concerning the requirement for additional mutation studies see Appendix 1.

Water Soluble Packaging and Respiratory Protection (2) and (3)

The FIFRA Scientific Advisory Panel supports the requirement that a protective mask be worn during the mixing, loading and transfer operations for aerial application of benomyl. However, the Panel recommends the use of commercial disposable paper masks, in addition to the cloth mask specified in the decision document be allowed if, in the opinion of the agency, they provide sufficient protection against respiratory exposure to benomyl during these operations. With the recommendation for use of respiratory protection in mixing, loading and transfer operations involving benomyl, the Scientific Advisory Panel believes that the requirement for water soluble packaging for units of 5 pounds or larger should not be required.

Monitoring Studies (4)

The Panel supports the requirement for additional monitoring studies on benomyl for rice use. These studies will allow the EPA to make a more accurate determination of the potential risks to aquatic organisms of the use of benomyl on rice.

Label Changes (5)

The Scientific Advisory Panel does not concur in the suggested precautionary warning statement to be included on the label for benomyl. The basis for the Panel's non-concurrence is that if this type of labeling is required for benomyl it should also be required for all other pesticide products which cause adverse health effects in man. This, of course, would include almost all pesticide products currently in use. The Panel might favorably consider specific health effects warning statements on pesticide labels were this requirement uniformly applied to all pesticide products. However, we are of the opinion that the selective requirement for this kind of warning label on benomyl is not appropriate in view of past regulatory decisions that have been made relative to other pesticide products that have undergone an examination through the RPAR process.

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Additional Comments

The no effect level for teratogenesis from benomyl administered by gavage to rats has not yet been established. Two studies, one by the Environmental Protection Agency and another by DuPont are currently underway in which the Scientific Advisory Panel has an interest. Any conclusions by the Panel favoring continued use of benomyl are contingent upon a no effect level for reproductive toxicology being established in these studies. Furthermore, the level at which no effect is seen should provide a satisfactory margin of safety relative to the exposure levels which have been calculated from dietary intake and exposure during mixing, loading, transfer or application of benomyl. It should be noted that dietary studies of the teratogenic properties of both benomyl and the breakdown product MBC in rats have been carried out and the results of these studies fail to show an effect at dose levels of 400 mg/kg/day. Teratogenic studies in rabbits at approximately 125 mg/kg/day in the diet also showed no evidence of compound related teratogenicity. Therefore, it is the opinion of the Scientific Advisory Panel that any no effect level established through the gavage method of administration of benomyl should be qualified by the essentially negative results obtained in these dietary studies.

Except as noted above, the Panel concurs with the agency's position for continued registration of benomyl for all uses.

APPENDIX I

Mutation Studies

The Scientific Advisory Panel offers the following comment in regard to the agency's request that additional gene mutation tests using benomyl be carried out in Drosophila, mammalian somatic cells in culture, and in an appropriate eukaryotic microorganism.

On May 31, 1978, the FIFRA Scientific Advisory Panel issued the following statement relative to the mutagenesis subsection of subpart F, Hazard Evaluation: Human and Domestic Animals, of the Proposed Guidelines for Registering Pesticides in the United States:

"The Scientific Advisory Panel strongly endorses the need for mutagenic assays as a survey for potential environmental hazards and endorses the principles of the proposed guidelines. Moreover, we feel that mutagenesis and oncogenesis should be considered together as related rather than isolated phenomena. To these ends therefore, and in the interest of simplicity and practicality, the following core battery of tests selected from the proposed guidelines is recommended for mutagenicity screening of all pesticides.

1. A sophisticated microbiological test such as an enhanced Ames assay with appropriate dose response.
2. A mammalian cell point mutation assay such as the hamster embryo or mouse lymphoma.
3. An in vivo cytogenetic assay.

In addition the Panel recommends that a multiple generation test which is part of the reproduction study (162.83-4), be modified to provide a statistically significant assay for dominant lethal mutations. The Panel also recommends that oncogenicity studies (162.83-2) be used in the overall evaluation of mutagenicity. Finally the Panel recommends that both the core battery of tests outlined in this report and the dominant lethal tests be performed on each pesticide. The results of these tests will provide the agency with the information necessary to estimate the mutagenic potential of pesticides".

The Scientific Advisory Panel is of the opinion that the stated requirement for a sophisticated microbiological test using benomyl has been met. In addition, the Panel believes that sufficient tests for in vivo cytogenetics have also been completed. It appears, however, that an appropriate mammalian cell point mutation assay has not been carried out using benomyl. Therefore, we recommend that a mammalian cell point mutation assay be performed using either a mouse lymphoma or a Chinese hamster embryo cell line or another equally well validated mammalian cell test for point mutation. If the results of this assay is negative, as has been the case for the microbiological test using Salmonella typhimurium mutants and the in vivo cytogenetics assays, we believe that no further mutation testing should be required and the mutation trigger should be considered to have been completely rebutted.

Spindle Effects: Recommendations for Testing

The Agency has submitted to the Scientific Advisory Panel a request for suggestions for methods for estimating risks in man from compounds such as benomyl which cause spindle effects. It is our opinion that the best methods for predicting adverse health effects in man from spindle poisons are the multigeneration reproductive test and the dominant lethal test. As noted in our previous recommendation on the proposed mutagenesis guidelines, we recommend that the multigeneration reproductive test be modified to provide a statistically significant assay for dominant lethal mutations. We believe that a multigeneration study modified to provide for an examination for dominant lethal mutations should be the primary means by which the agency estimates the potential risk to man from spindle poisons. If the multigeneration and dominant lethal studies are negative we recommend that cytogenetics be conducted on the sperm and bone marrow cells of an adequate number of the animals used in these or other studies. Consideration should also be given to the extension of the technique of amniocentesis and chromosome banding to the rodent model. In addition to the in vivo tests described above the Panel believes that it is desirable to have a reproducible and practical in vitro assay for spindle effects of pesticides, their contaminants, and breakdown products. A starting point may be an examination of metaphase arrest and growth inhibition in a mammalian cell line in culture (e.g. Chinese hamster embryo cells). In this regard, we urge the Agency to fund research with the aim of developing the amniocentesis rodent model and the in vitro test in mammalian cells for spindle effects of pesticides and pesticide products. Research should also be encouraged to evaluate the predictability of in vitro tubulin binding of spindle poisons.

It is the opinion of the Scientific Advisory Panel that a threshold does exist for spindle effects from compounds such as colchicine and benomyl. This is based, in part, on the kinetics of the affinity of benomyl and colchicine for a tubulin binding site and the resulting inhibition of polymerization of tubulin to microtubules. This opinion is strengthened by the results of studies by Cox and Puck (1) in Chinese hamster cells in vitro using colcemid in which no appreciable changes in chromosomes were seen at 0.01 ug/ml. At 0.015 ug/ml nondisjunction appeared with tetraploidy occurring at 0.03 to 0.07 ug/ml. Similar results were obtained by Wilson et al using Vinca alkaloids in EHB cells in culture (2). In addition, studies by Seiler (3) with benomyl measuring micronucleated erythrocytes in mouse bone marrow following in vivo administration of benomyl also suggests there is a threshold for the spindle effects of this compound. The results of these studies (3) indicate the no effect level for benomyl for this measure of spindle effects in this mammalian species is between 400-500 mg/kg. Considering the level of exposure to benomyl of mixers and loaders and the exposure of the general population by way of the diet, the Scientific Advisory Panel is of the opinion that an adequate margin of safety exists for the potential spindle effects of benomyl in man.

In summary, the Panel believes that if negative results are obtained in an adequately conducted multigeneration reproductive test, in a dominant lethal test, and in the proposed in vivo cytogenetics assays, that this is adequate evidence that no adverse effects will occur in humans as a result of exposure to the spindle inhibitor in question. Since the results of a multigeneration reproductive test, the dominant lethal tests and the in vivo cytogenetics tests using benomyl have been negative the Panel is of the opinion that benomyl poses no significant risk as a spindle poison to human populations as a result of its current use in agriculture.

- (1) D.M. Cox and T. T. Puck, Cytogenetics 8: 158-169 (1969).
- (2) L. Wilson, K. Anderson and D. Chen, in Cell Motility, Cold Springs Harbor Symposium, D. Goldman, T. Pollard and J. Rosenbaum, eds., Cold Springs Harbor Press, New York, Vol. 3, 1051-1064 (1976).
- (3) J. D. Seiler, in Progress in Genetic Toxicology, D. Scott, B. A. Bridges and F. H. Sobels, eds., Elsevier, North Holland Biomedical Press, pp. 233-238 (1977).

FOR THE CHAIRMAN:

Certified as an accurate report of findings:



H. Wade Fowler, Jr., Ph.D.
Executive Secretary
FIFRA Scientific Advisory Panel

Date: November 29, 1979

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Qualitative/Quantitative Risk Assessment

7/15/89

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

BENOMYL

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MAY 15 1989

Subject: MRC(INF-965) - Qualitative and Quantitative Risk
Assessment, CD-1 Mouse Study (re-evaluation)
caswell no. 79C

From: Bernice Fisher, Biostatistician
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C) *Bernice Fisher 5/10/89*

To: Marion P. Copley, D.V.M., Section Head
Review Section II
Toxicology Branch I - Insecticides/Rodenticides
Health Effects Division (H7509C)

Thru: John A. Quest, Ph.D., Section Head
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C) *John A. Quest 5/15/89*

Summary

The estimated unit risk, Q_1^* of benomyl is $4.20 \times 10^{-3} (\text{mg/kg/day})^{-1}$ in human equivalents. This estimate of Q_1^* is based upon the outcome of the re-evaluation of hepatocellular (adenoma and/or carcinoma) tumors in CD-1 female mice with dose levels of 0, 500, 1500, and 7500 ppm.

This unit risk is essentially at the same level as the previously reported ($Q_1^* = 3.9 \times 10^{-3} (\text{mg/kg/day})^{-1}$ in human equivalents, - Benomyl Risk Assessment for $Q_1^* = 3.9 \times 10^{-3}$ for Carcinogenicity Potency, R.Litt - 3/86). The only difference in the two analysis is the modification of the denominators of tumor rates in female mice, used in the qualitative and quantitative risk assessment. Currently the denominators include only animals at risk (i.e. the total number of animals that were examined with the exclusion of those that died during the first year).

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Background

The Peer Review Committee on Renomv1/MRC, January 25, 1989 recommended a re-evaluation of the MRC study in CD-1 female mice for the qualitative and quantitative risk assessment. This current evaluation used the collection of individual animal data and then the application of the Stattox program to obtain statistical outcomes on survival, tumorigenicity and a unit risk analysis.

The 2 year CD-1 mouse study was conducted by Haskell Labs for F.I. duPont de Nemours and Company, Inc. and reported in January 26, 1982. The mice were assigned in a random manner to the following groups:

Table 1. MRC, CD-1 Mouse, Experimental Design of the Dietary Study

Dose (ppm)	Number of		weeks on Study
	Males	Females	
0	80	80	104
500	80	80	104
1500	80	80	104
7500	80 ^a	80	104 ^a

a due to the high mortality of males during weeks 52-64 in the high dose group, the dose was reduced to 3750 ppm at week 66 for males and the remaining animals were sacrificed at week 74 instead of 105.

Survival Analysis

In male mice there was a significant ($p < .001$) increasing trend in mortality with dose increments of MRC. There also was a significant ($p < .05$) difference between controls and the high (7500-3750 ppm) dose group as well as a significant ($p < .01$) difference between the mid (1500 ppm) dose group and controls (Table 2).

In the females, there was no statistical evidence of dose related mortality either in the trend analysis or in the the pair-wise comparison of control and each dose group (Table 3).

The statistical evaluation of mortality in the mouse was based upon the Thomas, Breslow and Gart computer program.

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Table 2. MRC - Male Mouse Study, Mortality Rates[†]
and Cox or Generalized K/W Test Results

Dose(ppm)	Week				Total
	1-26	27-52	53-73 ^a	74-104 ^b	
0	1/80	3/79	25/76	33/51	62/80 (78)**
500	0/80	8/80	33/72	24/39	66/80 (83)
1500	0/80	9/80	36/71	26/35	71/80 (89)**
7500- 3750 ^c	4/80	12/76	41/64	—	57/80 (71)*

[†] Number of animals that died during interval/ Number of animals alive at the beginning of the interval.

() percent

a Final Sacrifice at week 74 for highest (7500-3750 ppm) dose group.

b Final Sacrifice at week 105 for 0, 500, and 1500 ppm dose groups.

c Dose reduced from 7500 to 3750 ppm at week 66 in highest dose group.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then $p < .05$ and if ** then $p < .01$.

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Table 3. MBC - Female Mouse Study, Mortality Rates⁺ and Cox or Generalized K/W Test Results

Dose(ppm)	Week				Total
	1-26	27-52	53-78	79-104 ^a	
0	3/81	4/78	26/74	26/48	59/81 (73)
500	4/79	6/75	17/69	36/52	63/79 (80)
1500	2/80	3/78	27/75	34/43	66/80 (83)
7500	2/80	2/78	23/76	32/53	59/80 (74)

⁺ Number of animals that died during interval/ Number of animals alive at the beginning of the interval.

() percent

^a Final Sacrifice at week 105

Note: Time intervals were selected for display purposes only.
 Significance of trend denoted at Control.
 Significance of pair-wise comparison with control denoted at Dose level.

If * then $p < .05$ and if ** then $p < .01$.

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Tumor Analysis

In mice, both sexes had elevated tumors in the liver with dose increments of benomyl.

In the males, with dose related significant mortality, the Peto Prevalence method was used to evaluate tumor trends and the pair-wise comparison with controls and each dose group. In addition, tumorigenicity in the highest (7500-3750ppm) dose group was not analysed because of high mortality and thus the lack of sufficient animals for justifiable statistical evaluation. The results indicated that there was a significant ($p=.01$) increasing trend in hepatocellular carcinoma tumor rates and a significant ($p=.005$) increasing trend in the combined hepatocellular (adenoma and/or carcinoma) tumor rates with incremental doses of benomyl. In the pair-wise comparison of controls and the mid (1500 ppm) dose group, there was a significant ($p=.012$) difference in liver carcinoma tumor rates and also a significant ($p=.007$) difference in the combined liver (adenoma and/or carcinoma) tumors. In the pair-wise comparison of control and the low (500 ppm) dose group, there was a significant ($p=.009$) difference in the combined liver (adenoma and/or carcinoma) tumors (Table 4).

In this qualitative risk analysis of female mice, the denominators for liver tumor rates included only animals at risk. By definition it included all animals examined, less those that died during the first year of the study. While in the previous risk assessment - Statistical Evaluation and Oncogenicity Risk Assessment of Benomyl, Benlate, and MBC 2-Year Feeding Studies in Mice, R.Litt, 5/82 - all animals that were examined were included in the denominator without exception. In female mice, not having significant dose related mortality, the Cochran-Armitage trend test and the Fisher Exact test for pair-wise comparisons was used to evaluate liver tumor data. The outcome of these tests indicated a significant ($p=.010$) dose related trend in liver carcinoma tumor rates and also a significant ($p=.019$) dose related trend in the combined liver (adenoma and/or carcinoma) tumors. In the pair-wise comparison of controls and the highest (7500 ppm) dose group there was a significant ($p<.001$) difference in combined liver (adenoma and/or carcinoma) tumors and also a significant ($p=.001$) difference in liver carcinomas. In the pair-wise comparison of control and the mid (1500 ppm) dose group there was a significant difference in liver adenomas ($p=.030$) and in liver carcinomas ($p<.001$) and in the combined liver (adenoma and/or carcinoma) tumors ($p<.001$). In addition the pair-wise comparison of controls and the lowest (500 ppm) dose group resulted in a significant difference in the combined liver (adenoma and/or carcinoma) tumors ($p=.007$) and in liver adenoma tumors ($p=.025$) (Table 5).

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Table 4. MEC - Male Mice, Hepatocellular Tumor Rates[†]
and the Peto Prevalence Test Results

Liver Tumor	Dose (ppm)			
	0	500	1500	7500-3750 ^a
Adenoma	11/76 (14)	15/72 (21)	14/73 (19)	3 ^b /67 (4)
p=	0.155	0.072	0.131	— ^c
Carcinoma	2/76 (3)	5/72 (7)	9 ^d /73 (12)	0/67 (0)
p=	0.010*	0.080	0.012**	— ^c
Combined tumors	13/76 (17)	20/72 (28)	23/73 (32)	3/67 (4)
p=	0.005**	0.009**	0.007**	— ^c

Number of tumor bearing animals/ Number of animals at risk (excluding those that died before 52 weeks).

) percent

7500 ppm dose reduced to 3750 ppm at week 66.

first adenoma observed at week 62.

animals at high dose (7500-3750 ppm) were not evaluated because of early high mortality and subsequent final sacrifice at week 74.

first carcinoma observed at week 88.

Note: Significance of trend denoted at Control.

Significance of pair-wise comparison with control denoted at Dose level.

If * then $p < .05$ and if ** then $p < .01$.

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Table 5. MRC - Female Mice Hepatocellular Tumor Rates⁺
and Cochran-Armitage Trend Test and Fisher's Exact
Test Results

	<u>Dose (ppm)</u>			
<u>Liver Tumor</u>	0	500	1500	7500
Adenoma	0/74 (0)	5/70 (7)	5/75 (7)	3 ^a /75 (4)
p=	0.441	0.025*	0.030*	0.125
Carcinoma	1/74 (1)	4/70 (6)	15 ^b /75 (20)	12/75 (16)
p=	0.010*	0.166	0.000**	0.001**
Combined tumors	1/74 (1)	9/70 (13)	20/75 (27)	15/75 (20)
p=	0.019*	0.007**	.00**	0.000**

Number of tumor bearing animals/ Number of animals at
risk (excluding those that died before 52 weeks).

) percent

first adenoma observed at week 90.
first carcinoma observed at week 77.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with
control denoted at Dose level.

If * then $p < .05$ and if ** then $p < .01$.

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Dose-Response Analysis

The most sensitive measurable reaction to benomyl occurred in female mice in terms of significant dose related trends and pair-wise significant differences between controls and selected dose levels in liver tumors. Since there was no statistical evidence of significant dose related mortality in the females, the estimate of unit risk, Q_1^* of benomyl, based upon the liver tumor data, was calculated by the use of Global86 (Multi-Stage process) computer program of K.Crump.

The unit risk calculated from the female mouse liver tumor data in ppm doses was converted to mouse mg/kg/day by the use of Lehman's tables and then to human equivalents by the use of interspecies surface area adjustments as recommended by EPA Cancer Guidelines (1986).

The resultant estimate of Q_1^* is as follows:

Female liver tumors (adenomas &/or carcinomas)	Mouse, Q_1^* (mg/kg/day) ⁻¹	In Human Equivalents
	3.14×10^{-4}	4.20×10^{-3}

References

Armitage, P. (1955) Tests for Linear Trends in Proportions, Biometrics 11, 375-386.

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Howe, R.B., Crump, K.S., and Van Landingham, C. (1986) A Computer Program to Extrapolate Quantal Animal Toxicity Data to Low Doses (unpublished report), 25 pgs.

Peto, R., Pike, M., Day, P., Gray, P., Parish, S., Peto, J., Richard, S., and Wahrendorf, J. (1980) Guidelines for Simple, Sensitive, Significant Tests for Carcinogenic Effects in Long-term Animal Experiments. - Monograph on the Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal. International Agency on Research on Cancer Monograph - Supplement 7., 311-426. Lyons, France.

Thomas, D.G., Breslow, N., and Gart, J.J. (1977) Trend and Homogeneity Analysis of Proportions and Life Table Data, Computers and Biomedical Research 10, 373-381.

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viewer's Peer Review Package for 3rd Meeting 1/20/89

2/89

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

Benomyl, MBC

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Weight-of-the-evidence and oncogenic properties of Benomyl and MBC revisited : The third peer review.

Tox. Chem. No. 75A, 79C

TO: Peer Review Committee for Benomyl and MBC
Health Effects Division (TS-769C)

FROM: Marion P. Copley , D.V.M., Toxicologist
Section 2, Toxicology Branch 1 (IRS)
HED (TS-769C) *Marion P. Copley*
1/20/89

THRU: Judith W. Hauswirth, Branch Chief
Toxicology Branch 1 (IRS)
HED (TS-769C) *Judith W. Hauswirth*
1/20/89

Attached are the data evaluation reports and documents from the two previous peer reviews for benomyl and MBC including the following material:

1. Statement of issues, background and summary.
2. Initial Peer Review DER (10/3/85)
3. Follow-up Peer Review DER (12/19/85)
4. Peer Review Document (3/31/86)
5. Statistics memos
6. Appropriate Study DERs not included in previous packages.

Mouse oncogenicity - Swiss Random
Mouse oncogenicity - NMRkf(SPF71)

APPENDIX B

Reviewed by: Dynamac - P. Wennerberg, W. McLellan, I.C. Felkner
Secondary reviewers: Marion P. Copley, D.V.M. *M.P.C. 4/15*
Jane Harris, PhD, Section Head *J.H. 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: BenomyI

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, BenomyI,
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 femal
pulmonary alveologenic carcinomas in males,
Degenerative changes in the testes and epididymides
at 5000-7500 ppm (HDT)

Classification: Core-minimum

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

SEE ATTACHED REVIEW

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

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
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Program Manager
Dynamac Corporation

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APPROVED BY:

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

Signature: _____
Date: _____

Jane Harris, Ph.D.
EPA Section Head

Signature: _____
Date: _____

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BEST AVAILABLE COPY

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 methods¹. Comparisons of survival distributions and tumor incidences were analyzed by the Mantel-Haenszel method². 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)				
	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* 8.7%
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* 8.5%

* 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					
	Body Weight	Liver	Thymus	Testes	Brain ^c	Relative Liver
Control	44.35	2.58	0.07	0.43	1.14	5.86
500	42.30	2.64	0.05*	0.41	1.21*	6.26
1500	42.13	3.29*	0.05*	0.44	1.19	7.80*
5000-7500 ^b	40.34*	3.06	0.05*	0.38*	1.24*	7.54*
	FEMALES					
	Body Weight	Brain	Kidney	Brain	Relative 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 a

b 7500 ppm changed to 5000 ppm after week 37.

c Organ:body weight ratio.

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67

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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).

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

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

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

b 7500 ppm changed to 5000 ppm after week 37.

c No. of animals examined.

d No data entry signifies a non-significant finding.

e Significant trend ($p < 0.05$) using Cochran - Armitage trend test by our reviewers.

TABLE 4. Selected^a Incidences of Neoplasms in Nice 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.

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TABLE 5. Mean-Time-to, and Median-Day-of Death, When Lung Alveolar Cell Carcinomas were Present in mice 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

^aUpper value is the mean, the middle value is the standard deviation, the bottom value is the median-day-of-death.

^b7500 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.

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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.

Section VI, Tox. Branch (TS-769C)
Secondary reviewer: Jane Harris, Ph.D.
Section VI, Tox. Branch (TS-769C)

JCH 12/17/85

DATA EVALUATION REPORT

STUDY TYPE: Chronic onco - mouse

TOX. CHEM. NO.: 79C

ACCESSION NUMBER: 256028, 256029

TEST MATERIAL: MBC

SYNONYMS: Carbendazim
INE-965
2-Benzimidazolecarbamic acid, methyl ester

STUDY NUMBER(S): Med. Res. Proj. # 3207-001
Haskell Lab Report # 70-82

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

TESTING FACILITY: Haskell Lab. for Tox. and Industrial Med.,
Newark, Del.

TITLE OF REPORT: Long-term feeding study with 2-Benzimidazole-
carbamic acid, methyl ester (MBC, INE-965) in
mice

AUTHOR(S): CK Wood, PW Schneider, HJ Trochimowicz

REPORT ISSUED: Jan. 26, 1982

CONCLUSION:

Systemic NOEL = 500 ppm (75 mg/kg/day)
Systemic LEL = 1500 ppm (225 mg/kg/day) based on hepatotoxicity
(males) and lymphoid depletion (males and females).
Oncogenicity - Increased incidences of hepatocellular
carcinomas in males at 1500 ppm and hepatocellular
carcinomas and adenomas at all doses in females.

Classification: core-minimum

MATERIALS:

1. Test compound: MBC, (INE-965-212), off-white solid, 99.3 %
pure, Haskell Lab. #11201
2. Test animals: CD-1 mice were received from Charles
River Breeding Labs., Wil., Mass. at 30 days of age.
They were 6-7 weeks old at the start of the study.

B. STUDY DESIGN:

1. Rats were assigned randomly to the following test groups:

Test Group	Dose in diet (ppm)	male	female	weeks on test
control	0	80	80	104
low (LDT)	500	80	80	104
mid (MDT)	1500	80	80	104
high (HDT)	7500	--	80	104
high (HDT)	7500/3750*	80	--	73

* due to high mortality during weeks 52-64 in the HDT male rats, treatment was removed for 7 days then reincorporated at a lower dose (3750 ppm) at week 66 until termination of the group in week 73.

2. Diet preparation - Diet was prepared weekly and refrigerated until needed. The vehicle for the MBC, Mazola® Corn Oil, was present in the diet at 1% (w/w). Samples of fresh diet, refrigerated diet (7 days) and room t° diet (24 hr, 7 days) were frozen and analyzed.

Results - Material was stable for at least 7 days at both room temperature and under refrigeration.

3. Animals received food (Purina® Lab Chow) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

Weight - one-way analysis of variance

Hematology - crossed and nested analysis of variance

Least sig. diff. and/or Dunnett stat. - when the ratio of variance (f-ratio) indicated sig. diff. among groups.

Survival - Kaplan-Meier Methods

Survival distribution - Mantel-Haenszel Method

Absolute proportion of survival - Fisher Exact Test

Tumor incidence - Fisher Exact Test or Mantel-Haenszel Method

Dose response of tumor incidence - Chi square test for trend

(*) significance was set at $p \leq 0.05$ level of probability.

C. METHODS AND RESULTS:

1. Observations - Animals were inspected daily for signs of toxicity and examined individually (palpated) biweekly.

Results -

Toxicity - Lesions, including alopecia, dermatitis, ocular discharge, ruffled fur, wet and stained perineum, swollen eyelids, weakness and palpable masses occurred in most or all groups with no relation to treatment in either duration, incidence or severity.

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Survival - The following table indicates the median survival time and the numbers alive at termination (table from study report). The number of survivors (23) in the male high dose group at sacrifice was significantly less than controls (50) at 73 weeks. A dose-related decrease in survival time was evident in all treatment groups. Survival in the females was not affected by treatment.

<u>Treatment Group</u>	<u>Median Survival Time (weeks)</u>	<u># Mice Alive at Group's Termination</u>
<u>Male</u>		
Control	78.5	18
500 ppm MBC	72.0	14
1,500 ppm MBC	69.0	9*
7,500-3,750 ppm MBC ⁺	63.5	23
<u>Female</u>		
Control	91.0	22
500 ppm MBC	91.0	15
1,500 ppm MBC	91.0	13
7,500 ppm MBC	91.0	20

+ The male 7,500-3,750 group was terminated during test week 73. All other treatment groups were terminated during test week 104.

* Significantly different from control at $P < 0.05$ level of probability by Fisher's Exact Test.

2. Body weight - They were weighed weekly for the first 6 months, biweekly for the second 6 months, then monthly for the last year-of the study.

Results - Although there were no consistent statistical changes in body weight for MBC treated males, they often weighed slightly less than controls (5 %). At 104 weeks, mid dose treated males weighed significantly less (12 %) than controls. There was no treatment related change in weight or weight gain in any female group.

3. Food consumption and compound intake - Consumption was determined during each weighing period and mean daily diet consumption was calculated. Efficiency was calculated from the consumption and body weight gain data.

Results -

a. Food consumption and efficiency - They were not affected by MBC treatment in either males or females. Food consumption in all groups was reported to be correlated with age due to excessive spillage.

b. Compound intake - After diets were changed for the HDT and in both the low and mid dose groups, males received approximately 33 % less MBC/kg/day than females (see following table).

Mean Daily Intake (mg/kg/day) of MBC

Dose (ppm)	males				females			
	0	500	1500	7500/3750	0	500	1500	7500
week 72				1560*				2216
" 104	0	81	257	---	0	125	380	1886

*all surviving males were sacrificed by week 73.

4. Ophthalmological examinations - not performed.

5. Clinical chemistries - not done.

Hematology - Blood was collected at 1, 3, 6, 12, 17 and 24 months of the study (from the same 10 mice/sex/group when possible).

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

Results - Males given 1500 ppm (MDT) had slightly increased HCT, MCV, MCH, and TP. These values however, were within the range for other time periods. RCB and HGB were slightly (sig. at $p < .05$) decreased in the LDT and HDT females but not at the mid dose, suggesting no treatment related effect at 24 months. There were no other significant treatment related changes.

6. Urinalysis - not done

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the following tissues were collected for histological examination. Starred (*) organs were weighed.

Digestive system	Cardiovasc./hematopoietic	Neurologic
Salivary glands	Aorta	Brain*
Esophagus	Heart*	Sciatic nerve
Stomach	Bone marrow(sternum, femur)	Spinal cord
Small intestine	Lymph nodes	Pituitary
Cecum	Spleen*	Glandular
Colon	Thymus*	Eyes
Liver*	Urogenital	Thyroids
Pancreas	Kidneys (adrenals)*	(parathyroids)
Respiratory	Urinary bladder	Lacrimal gland
Trachea	Testes (epididymides)*	Miscellaneous
Lung*	Prostate	Skeletal muscle
	Ovaries	Skin
	Uterus	Mammary gland
		Femur
		All gross lesions and masses

Results -

a. Organ weight - Only thymus and liver weight changes appear to be treatment related. Absolute and relative thymus weight appeared to be decreased at all dose levels in both sexes but did not show a dose-response relationship. Relative liver weight was increased in the mid and high dose females with an absolute increase only in the HDT females. No other significant organ weight changes were observed.

Absolute (gm) and Relative (abs/body wt) Organ Weight

	control		500 ppm		1500 ppm		7500/3750 ³ ppm	
	abs	rel	abs	rel	abs	rel	abs	rel
le								
kidney	1.0067	2.3535	.8907 ¹	2.3438	.9233 ²	2.5361	.8267 ¹	2.0043 ¹
brain	.5117	1.1945	.5064	1.3376 ¹	.4789	1.3203 ²	.4756 ²	1.1520
thymus	.0644	.1479	.0500 ²	.1309	.0478 ¹	.1335	.0426 ¹	.1027 ¹
heart	.2483	.5778	.2521	.6726 ²	.2178	.5968	.2028 ¹	.4920
lung	.3989	.9305	.4393	1.1675 ¹	.4056	1.1127 ¹	.3602	.8764
male								
kidney	.7064	1.9267	.6287 ²	1.7749	.6767	1.9576	.7662	2.0192
brain	.5232	1.4389	.4693 ¹	1.3360	.4900	1.4296	.5086	1.3483
thymus	.0669	.1838	.0340 ¹	.0960 ¹	.0460 ¹	.1301 ¹	.0629	.1654
liver	2.1336	5.8131	1.9273	5.5197	2.6433	7.6034 ¹	3.0825 ¹	7.9995 ¹

p < 0.05 by Dunnett and LSD

p < 0.05 by LSD only

dose in HDT males was lowered due to toxicity; they were sacrificed at 17 months instead of 2 years making comparison to controls unreliable.

b. Gross pathology - Lesions observed at necropsy were difficult to analyze because no summary tables were presented, however a random comparison indicated good agreement between individual animal macroscopic and microscopic data.

c. Microscopic pathology

1) Non-neoplastic - A statistical increase in non-neoplastic lesions was present in the male liver, kidney, lacrimal gland, stomach, thymus, thyroid and testes and female liver, cecum, lung, thymus and kidneys (see following table). Both males (MDT and HDT) and females (HDT) had a dose-related increase in incidence in granular pigment in the renal tubules. Females (HDT) also had an increased incidence of pigmented macrophages in the renal interstitium as well. There was a marked increase in mice with thymic lymphoid depletion in both mid and high dose males and females. There was an increased incidence of centrolobular hepatocellular necrosis in the mid and high dose males and a dose related increase in hepatocellular hypertrophy⁷³

in all male groups receiving MBC. Females had areas of eosinophilic cellular alteration present only at the HDT. Pigmented interstitial macrophages in the liver were present in 36 % of the HDT males as compared to 18 % of the control males. There was a dose related increased incidence of sperm stasis, particularly in the mid and high dose males.

Incidence (%) of Non-neoplastic Lesions

dose (ppm)	males				females			
	0	500	1500	7500/3750	0	500	1500	7500
Kidney								
Pigment in tubules	9	4	24*	59*	0	0	4	54*
Pigment in Macs.	-- ¹	--	--	--	6	5	4	28*
Thymus								
Lymphoid depletion	26	33	55*	49*	8	11	27*	26*
Liver								
Pigment in Macs.	14	14	16	29*	--	--	--	--
Hepatocell. necr. centrolobular	0	1	11*	18*	--	--	--	--
Hepatocellular hypertrophy	0	11*	16*	21*	--	--	--	--
Cellular alteration eosinophilic	--	--	--	--	0	0	0	8*
basophilic	--	--	--	--	4	3	3	10
Testes								
Sperm stasis	10	16	20*	30*				

* Fisher's exact test, $p < .05$

¹ no significant increase

Other lesions in the males, with slight but significant increases ($p < .05$) were: single cell and focal/multi-focal hepatocellular necrosis, centrolobular hepatocellular swelling, hepatic peliosis, amyloidoses of the lamina propria of the glandular stomach, lymphocytic infiltration of the extraorbital lacrimal gland and follicular cysts of the thyroid. Other lesions increased in the females included cecal submucosal edema and pulmonary paravascular/para-alveolar amyloidosis.

2) Neoplastic - There was a treatment related increase of liver tumors in males and females.

Animals with Treatment Related Hepatic Tumors

dose (ppm)	males				females			
	0	500	1500	7500/3750 ¹	0	500	1500	7500
No. examined	80	80	80	80	79	78	80	78
adenoma	11	15	14	3	0	5*	5*	3
carcinoma	2	5	9*	0	1††	4	15***	12**
hepatoblastoma	0	0	0	0	0	0	-1	0
Total hepatic neopl.	13†	20	23*	3	1††	9**	21***	15***

¹ not included in stat. analysis, high dose males were sac. at 17 months

* Fisher's exact test, $p < .05$

** Fisher's exact test, $p < .01$

*** Fisher's exact test, $p < .001$

† Chi squared trend test, $p < .05$

†† Chi squared trend test, $p < .001$

There was a treatment related increase in hepatic tumors for both males and females. In the males, hepatocellular carcinomas and total hepatic neoplasms were increased statistically in the mid dose while there was a dose related trend¹ only with total hepatic neoplasms.

In the females however, there was a treatment related increase for adenomas (LDT, MDT), carcinomas (MDT, HDT) and total hepatic tumors (LDT, MDT, HDT). A dose related increase occurred for carcinomas and total hepatic tumors.

Metastasis from the liver was not treatment related. Histomorphologic appearance of the liver neoplasms was described as the same for all groups (treatment and control).

The time to tumor data did not indicate a decrease in latency in the males. The data suggested a slight, but somewhat equivocal decreased latency in the females with increasing dose (see table below).

Time to hepatic tumor (days)

	Males (ppm)				Females (ppm)			
	0	500	1500	7500/3750	0	500	1500	7500
hepatic tumor								
finding mice	13	20	23	3*	1**	9	21	15
first hepatic								
or detected	430	467	459	434	732**	648	536	551
time to tumor	628	671	628	*	732	706	689	653
time to tumor	633	697	651	*	732	724	697	733**

is not meaningful due to early sacrifice of this group at 17 months⁷⁵
 final sacrifice

due to early sacrifice, tumors in the high dose males were not 33
 included in the statistical calculations

Analysis of the mean and median time to tumor, exclusive of animals from the terminal sacrifice (as recommended by the Toxicology Branch statistics department (HED)) also only weakly suggests a decreased latency in the females with increasing dose.

Mean and median time to tumor (days) for mice necropsied prior to the terminal sacrifice

	Males (ppm)				Females (ppm)			
	0	500	1500	7500/3750	0	500	1500	7500
of mice sac. early	62	66	71	80*	0	9	19	17
of hepatic tumor bearing mice	7	12	22	3	0	6	13	10
mean time to tumor	539	630	623	*	-	693	662	632
median time to tumor	517	657	651	510*	-	699	670	640

Value not meaningful due to early sacrifice of entire group at 17 months

D. DISCUSSION

Although there were no treatment related clinical signs of toxicity (males and females), male mortality was markedly increased in a dose related fashion resulting in the decision to decrease the dose from 7500 ppm to 3750 ppm at week 66 and then to terminate the HDT males at 73 weeks (17 months). Other treatment related signs of toxicity included very slight decreases in weight gain (males only). Body weight was often slightly decreased in the HDT males until sacrifice at 17 months. By 2 years, the MDT males weighed 12 % less than the the controls. Food consumption and efficiency were not affected by MBC ingestion (males, females). Hematologic changes in the males and females were probably not treatment related.

MBC appears to be hepatotoxic as indicated by increased incidences of hepatocellular carcinoma, hepatocellular swelling, hypertrophy and necrosis in males, and hepatocellular carcinoma, adenoma, as well as eosinophilic areas of hepatocellular alteration in females. This was consistent with an increase in absolute and relative liver weights (females).

The background incidence of hepatocellular tumors for this strain of mouse (CD-1) in two year studies from the same laboratory is presented below.

Hepatocellular Tumors (% Incidence) in Controls from 3 Studies

	A(unnamed)	Benomyl	MBC*
Males			
# of livers examined	80	77	80
# of adenomas	9 (11%)	9 (12%)	11 (14%)
# of carcinomas	4 (5%)	16 (21%)	2 (3%)
combined	13 (16%)	25 (32%)	13 (16%)
Females			
# of livers examined	80	77	79
# of adenomas	5 (6%)	2 (3%)	0
# of carcinomas	1 (1%)	2 (3%)	1 (1%)
combined	6 (8%)	4 (5%)	1 (1%)

* the present study under review 34

As indicated in the above table, the male control incidence of carcinomas (3 %) and total hepatic tumors (16 %) in the MBC study are similar to those in study A but less than half of the values in the benomyl study. Female control values from the MBC study are similar to the other studies. When historical control values from both the benomyl and study A are taken into consideration, the increased incidence of hepatic tumors in females, due to MBC treatment, appears biologically significant while it is equivocal in the males. Clinical chemistries may have aided in further characterizing the hepatotoxicity of MBC.

There was also a treatment related decrease in female thymic weight (abs. and rel.) and a dose related decrease in male thymic weight. This was consistent with a treatment related lymphoid depletion observed in both males and females.

MBC, in this study, has a systemic NOEL of 500 ppm with a LEL of 1500 ppm based on hepatotoxicity (males), and lymphoid depletion in the thymus of both males and females. MBC appears to be a hepatocarcinogen associated with an increased incidence of hepatic tumors in males (carcinomas) and females (adenomas and carcinomas). Evidence for decreased latency however, is equivocal in the females and negative in the males.

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APPENDIX C

(23)

Pesticide residues in food - 1983

The monographs

Data and recommendations
of the joint meeting
of the
FAO Panel of Experts on Pesticide Residues
in Food and the Environment
and the
WHO Expert Group on Pesticide Residues
Geneva, 5 - 14 December 1983

BENOMYL P 7

CARBENDAZIM P 89

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FAO
PLANT
PRODUCTION
AND PROTECTION
PAPER

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FOODS
AND
AGRICULTURE
ORGANIZATION
OF THE
UNITED NATIONS

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APPENDIX D

21-day dermal - rabbit; Haskell lab.; 211-69; 7/20/69	52.5-53 % a.i. in sugar	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	IV	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 (HJT)(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 chronic 004679 -

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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 microphthalmia) Dosage = 62.5, 125, 250, 500 mg/kg (gavage) in Wistar strain	000722
Acu rabbit; #54-80, 50-1; 3/80	Benomy1 - 75% (Benlate DF)	Acc.# 243043 MRII 0006822	LD ₅₀ > 2000 mg/kg Severe skin irritation.	Guideline 000863 004679 -
Primary eye irritation - rabbit; Haskell; #497-80; 6/13/80	Benomy1 - 75% (Benlate DF)	Acc.# 243043 MRII 00064820	Corneal opacity at 8 days. For the irrigated eyes, irritation cleared by day 8. PIS day 1 = 28, day 11 = 0	Guideline 000863 004678 004679 -
Primary dermal irritation - rabbit; Haskell; #367-80;5/12/80	Benomy1 - 75% (Benlate DF)	Acc.# 243043 MRII 00064821	Slight edema and slight erythema at 24 hours; at 72 hours, only very slight erythema. PIS = 0.67 All scores were 0 by day 6.	Guideline 000863 004679 -

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Study/Lab/Study #/Date	Material	Accession No.	Results:	TOX Category	CORE Grad 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	LD50, LC50, PIS, NOEL, LEL 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 Minimum 003042
2 Year feeding - mouse; Dupont Haskell Lab; 20-80; 1/26/82	Benomy1 99-99.28 pure	MRID 00096514	Oncogenic NOEL < 500 ppm male and female, significant increase in hepatocellular neoplasms in male and female; 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	Benomy1	GS0119-018 MRID 00078620	Terata NOEL = 5000 ppm (HDT) (inconclusive result since ingested dose not measured accurately)Chr-CD		003728
Teratology - rat; Midwest Res. Inst.; #68-02-2982 1979	Benomy1	GS0119-016	doses 0, 100, 500, 2500, 5000 ppm 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	Benomy1	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	Benomy1	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

Study/Lab/Study #/Date	Material	Accession No.	Results: LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	TOX Category	CORE Grade/Doc. No.
Teratology - rat; Haskell Lab; #649-80; 1980	Benamyl	Acc.# 256575 CS0119-009	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 Supplementar 004689 Minimum when combined wit 003042
Neurotoxicity - chicken; IRDC; 125-039; 10/8/79	Benamyl in corn oil (99% Tech.)	Acc.# 241930 CS0119-007	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	Benamyl	CS0119-003	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	Benamyl (99% a.i.)	CS0119-002	Dose related increase in mutation frequency at TK locus of L5178Y cells, <u>in vitro</u> - weak mutagen with and without activation		003744 acceptable 004679 -
Mutagenic - SCE- chinese hamster ovary; SRI Int.; LSU-5778; Aug, 80	Benamyl (99% a.i.)	CS0119-004	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	Benamyl		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	Benamyl (99.6% a.i.)	CS0119-001	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	Benamyl (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	No.	LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	Category	Loc. No.
Mutagenic - mouse - DNA repair; Haskell; 741-81; 10/20/81 (Tong, 1981)	Benomy1	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)	Benomy1		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	Benomy1 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	Benomy1 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	Benomy1 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		supplement: 004679 -
Mutagenic micronucleus - mouse; J.P.Seiler; 1976	Benomy1 MBC	GS0119-008	increased micronucleus formation in mouse bone marrow NOEL = 500 mg/kg benomy1 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:	TOX Category	COKE Grade/Doc. No.
Acute oral - rats; Haskell Labs; 179-65; 12/15/65	Benomyl	MRID 00066779	LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL 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		supplemental 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 reduced spermatogenic activity at 14 d (not present 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, PCC; 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 DUC #
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 from .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 - <u>A. nidulans</u> ; 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 <u>A. nidulans</u> .		PD4 #
Primary eye irritation - rabbits; Haskell labs; 179-81; 4/6/81	Benlate DF 75 % a.i.	MRID 00084579	day 1 4 7 28 7.3 0	III	minimum 004679 -

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DATE OF LAST REVIEW

MRID

50% WP (53% tech) MBC

90-day feeding-dog Haskell Lab.; #283-70; 1970

2 Year feeding/onco-rat; Haskell Lab.; 195-72; 1972

2 Year feeding - dog; Haskell Lab.; 195-72; 1972

3 generation repro - rat; Haskell Lab.; 195-72; 1972

minimum 004679 -

minimum 004679 -

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.

Acc. # 232870-C 232871 MRID 00088333

50 or 70 % ai (53 or 72.2 % tech.) MBC

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.

Acc. # 232870-C 232871 MRID 00088333

50 or 70 % ai (53 or 72.2 % tech.) MBC

minimum 004679 -

minimum 004679 -

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.

Acc. # 232870-C 232871 MRID 00088333

50 or 70 % ai (53 or 72.2 % tech.) MBC

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.

Acc. # 232870-C 232871 MRID 00088333 CS0119-010

50 or 70 % ai (53 or 72.2 % tech.) MBC

minimum 004679 -

minimum 004679 -

WHO review

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96 Week onco - mice;
Kramer and Weigand et
al., 1982.

80 Week Onco - Mice
Beens, 1976

MBC

MBC

GS0119-011

GS0119-012

Not oncogenic at 5000 ppm

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 NWRKf(SPF 71)strain

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.

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BENOMYL

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Explanation

Benomyl was reviewed by the Joint Meetings of 1973, 1975 and 1978 (FAO/WHO 1974a,b, 1976a,b, 1979a).^{1/} An acceptable daily intake (ADI) was not allocated because of insufficient acute oral, short-term and long-term studies. Furthermore, a carcinogenic study, as well as observations in humans, were indicated as desirable information. These studies have been reviewed and are summarized in the toxicology section of the following monograph.

In the 1978 report it was recommended that residue guideline levels for benomyl and carbendazim should be replaced by a single list of guideline levels for carbendazim residues that occur as metabolic products of benomyl or thiophanate-methyl or from the direct use of carbendazim. That procedure is continued in the section on residues of this evaluation.

Extensive information was available on approved uses of benomyl in 22 countries, the fate of residues, international maximum residue limits (MRLs) and the results of a market-basket survey in the United States. Residue data from supervised trials were available from the United States, Africa, the Federal Republic of Germany, the United Kingdom, Australia and Japan.

TOXICOLOGY

EVALUATION FOR ACCEPTABLE DAILY INTAKE

BIOCHEMICAL ASPECTS

Absorption, Distribution and Excretion

Absorption, distribution and elimination in rats following dermal application (0.1, 1, 10 and 100 mg) was measured using ¹⁴C-labelled benomyl. Blood levels, mode of excretion, metabolic products and rate of penetration were also examined. A separate group of rats, dosed intravenously with ¹⁴C-benomyl for comparison of blood level concentrations, revealed that absorption into the bloodstream was non-linear with respect to dose. Blood values, which were low (0.004-0.07 ppm), first appeared 30 min. after treatment, peaked at all treatment levels after 4 h and were eliminated in urine after 30 min. linearly with time. The low blood concentrations resulted from limited absorption with rapid metabolism and elimination via the urine. The major urinary metabolite identified was 5-HBC and, to a lesser extent, MBC. Greater than 95 percent of the radioactivity was eliminated in 24 h in the urine following intravenous injection. No radioactivity was found in any body tissues sampled 24 h after injection (<0.1 percent) (Fisher et al., 1981).

In a similar evaluation, blood levels of benomyl and its metabolites were measured in rats following inhalation exposure to actual time-weighted averages of 0.32 and 3.3 mg/l. Exposure intervals of 0.5, 1, 2 and 6 h were utilized. Urinary residues consisted primarily of 5-HBC, with limited amounts of benomyl/MBC detected. The methodology did not distinguish between benomyl and MBC. At both exposure levels blood concentration of benomyl/MBC was greater than that of 5-HBC after 6 h, with levels ranging from 0.39-2.3 and 0.25-1.2 ppm, respectively. At 18 h post-exposure, 5-HBC was the only residue detected in the blood (1.1 ppm) and only at the high dose.

^{1/} See Annex 2 for FAO and WHO documentation.

Rapid elimination of benomyl was also demonstrated in mice and hamsters following intragastric intubation. 90 percent of the radioactivity was eliminated in the urine and faeces in 72 h. Little to no radioactivity was evident in tissues. Conjugates of 5-HBC and MBC appeared in the urine and faeces, respectively (Han 1971).

Lactating and non-lactating goats were given five consecutive daily doses of 2-¹⁴C benomyl by capsule at rates equivalent to 36 and 88 ppm in the total daily diet, respectively. Evaluation of radioactivity in various tissues, milk (where present), urine and faeces, as well as characterization of the bound and unbound residues, revealed a similar metabolism and elimination pattern identified for rats, dogs, chickens and dairy cows. Most of the radioactivity was eliminated in the urine and faeces and identified as 5-HBC and 4-HBC. Milk residues, principally of 5-HBC with minor amounts of 4-HBC and 5-hydroxy-2-aminobenzimidazole (5-HAB), accounted for approximately 2 percent of the total dose. Approximately 25 percent of the milk radioactivity was reincorporated into the natural milk components casein and whey protein. There were no detectable residues in muscle tissue and fat (<0.01 ppm). However, radioactivity detected in liver and kidney amounted to 3.8 and 0.09 ppm, respectively, with 5-HBC identified as the principal benomyl metabolite (ca. 6 percent). Much of the liver residues were reincorporated into glycogen, protein, fatty acids and cholesterol and accounted for approximately 35 percent of the liver residues. Further characterization of the bound liver tissue residues following enzymatic and trifluoroacetic anhydride hydrolysis identified 5-hydroxybenzimidazole moieties as the principal (at least 77 percent) ¹⁴C-residue found in goat liver. No free benomyl, MBC or 5-hydroxy-MBC were determined in the liver (Han 1980; Hardesty 1982).

In a series of metabolic studies, benomyl and/or Benlate were administered either by gavage or in the diet to pregnant ChR-CD rats to determine the concentrations of benomyl, MBC and two MBC metabolites (4-OH MBC and 5-OH MBC) in maternal blood and embryonic tissue. The design of these studies followed typical teratology schedules wherein dosing was administered on days 7 through 16 of gestation and included levels of 125 mg/kg/day via gavage or 5 000 - 10 000 ppm (ca. 400-800 mg/kg) in the diet. Blood samples from the dams and tissue samples from their embryos were examined at several time points during compound administration. Residue analyses revealed that benomyl was either much more efficiently metabolized or biologically unavailable following dietary administration. The half-life for benomyl/MBC in maternal blood, following gavage, was approximately 45 min. and was less than 45 min. in embryonic tissue. The metabolite 4-OH MBC was not detectable (< 0.03 ppm), while 5-OH MBC demonstrated a half-life for maternal blood of 2-3 h and 4-8 h in embryos. Similar analyses demonstrated that levels of benomyl/MBC in maternal blood varied from 0.23 to 0.61 ppm, regardless of dose, while 5-OH MBC averaged 0.44-4.4 ppm and 0.33-3.3 ppm in blood and embryo, respectively (Culik et al 1981).

Effects on Enzymes and Other Biochemical Parameters

A study conducted using acetylcholinesterase from bovine erythrocytes showed that benomyl did not inhibit the functioning of this enzyme. The acetylcholinesterase inhibition constant, K_i , for benomyl was greater than 1×10^{-3} M (Belasco undated).

TOXICOLOGICAL STUDIES

Acute Toxicity

The acute toxicity of benomyl in several animal species is summarized in Table 1.

Table 1 Acute Toxicity of Benomyl in Animals

Chemical	Species	Sex (Number)	Route	Vehicle	mg/kg b.w. ^{1/}	Reference
Benomyl	Rat	M (1/dose)	Oral	Peanut Oil	ALD > 1 000	Zwicker 1965
	Rat	M (1/dose)	Oral	Peanut Oil	ALD > 9 590	Sherman & Krauss 1966
	Rat	M/F (10/dose)	Oral	Peanut Oil	LD ₅₀ > 10 000	Sherman 1969a
	Rabbit	M (1/dose)	Oral	50% Wettable Powder	ALD > 3 400	Fritz 1969
	Dog	M (1)	Oral	Evaporated Milk and Water (1:1)	ALD > 1 000	Sherman 1969b
	Rabbit	M/F (4/dose)	Dermal	50% Wettable Powder	LD ₅₀ > 10 000	Busey 1968a
	Rat	M (5/dose)	Inhal. (4 hr)	50% Wettable Powder	LC ₅₀ > 0.82 mg/l	Hornberger 1969
	Rat	M (6/dose)	Inhal. (4 hr)	50% Wettable Powder	LC ₅₀ > 4.01 mg/l (analytical)	Busey 1968b
Dog	M (10/dose)	Inhal. (4 hr)	50% Wettable Powder	LC ₅₀ > 1.65 mg/l (analytical)	Littlefield 1969	
Benlate (53% Rat benomyl)	M/F (10 dose)	Oral	Aqueous suspension	LD ₅₀ > 10 000	Sherman 1969a	
2-Benzimidazole carbamic acid, methyl ester	M/F (10/dose)	Oral	Corn Oil	LD ₅₀ > 10 000	Goodman 1975	
5-Hydroxy-2-benzimidazole-carbamic acid, methyl ester	M (1 dose)	Oral	Corn Oil	ALD > 7 500	Snee 1969	
2-Aminobenzimidazole	M (1/dose)	Oral	Peanut Oil	ALD > 3 400	Sherman & Fritz 1969	

^{1/} Based on active ingredient.

Gross and histopathological changes were evaluated and selected organs in several species were examined with emphasis on the male gonads. Testicular degeneration, necrosis of germinal epithelium and aspermatogenesis were observed in male rats following some acute oral intubation and acute inhalation exposures. Dogs, exposed via inhalation for 4 h at 1.65 mg/l, presented evidence of focal aspermatogenesis and reduced spermiogenesis.

Special Study on Eye and Skin Irritation

The eye irritation properties of benomyl were examined in albino rabbits in several tests using technical grade benomyl, 50 percent wettable powder, and a suspension in mineral oil. Mild conjunctival irritation and minor transitory corneal opacity were reported in all tests (Reinke 1966; Frank 1986, 1972).

A 50 percent wettable powder applied to the clipped intact and abraded abdomen of albino rabbits produced moderate to marked erythema, slight oedema and slight desquamation. Exposure was for 24 h to occluded skin sites at doses greater than 0.5 g/animal. Albino guinea pigs similarly exposed to 10, 25 and 40 percent dilutions of technical grade benomyl in dimethyl phthalate presented only mild irritation of both intact and abraded skin sites (Busey 1968a; Majkut 1966; Colburn 1967; Frank 1967).

Special Study on Sensitization

Albino guinea pigs exposed to benomyl, either technical material or a 50 percent sucrose formulation, produced mild to moderate skin sensitization reactions following both intradermal injections or repeat applications to abraded skin (Majkut 1966; Colburn 1967; Frank 1969).

Short-term Studies

Rat

Benomyl, intubated into Chr-CD male rats at 200 and 3 400 mg/kg in peanut oil five times a week for two weeks produced mortality in 4/6 animals at the high dose. Animals dosed with 3 400 mg/kg demonstrated evidence of degeneration of germinal epithelium, multinucleated giant cells and reduction or absence of sperm. Very minor changes were observed in the testes of the animals dosed with 200 mg/kg (Sherman & Krauss 1966). Chr-CD male rats similarly dosed with 200 mg/kg of the metabolite 5-hydroxy-2-benzimidazole-carbamic acid (methyl ester) presented no toxic symptoms or evidence of effects on the testes (Snee 1969).

Groups of rats (16 of each sex, four-week-old Chr-CD rats/group) were fed benomyl (72 percent a.i.) in the diet at dosage levels of 0, 100, 500 and 2 500 ppm for 90 days. Animals were observed daily for behavioral changes and body weights and food consumption were recorded at weekly intervals. Haematological examinations were conducted on six male and six female rats in each group at 30, 60 and 90 days. Routine urinalyses were performed on the same animals as well as plasma alkaline phosphatase and glutamic pyruvic transaminase activity. After 76-103 days of continuous feeding 10 male and 10 female rats in each group were killed and selected organs weighed. Additional organs were preserved for microscopic examination. The six male and six female animals remaining in each group after the terminal sacrifice were subjected to a reproduction study.

There were no gross toxic signs of poisoning and no compound-related effects on weight gain, food consumption, food efficiency, haematology, biochemistry or urinalysis determinations. Liver-to-body weight ratio in females was slightly increased at 2 500 ppm, compared with control rats. Gross and microscopic examinations of tissues and organs showed no significant effects attributable to the presence of benomyl in the diet at levels up to and including 2 500 ppm (Sherman et al. 1967).

Rabbit

Groups of five male and five female New Zealand albino rabbits, weighing 2 to 2.4 kg, were exposed to 15 dermal applications of a 50 percent benomyl formulation (equivalent to 1 000 mg/kg b.w.) on both abraded and intact abdominal skin sites. Animals were exposed for six hours each day, five days/week for three weeks. After each daily application, the abdomen was washed with tap water. Observations were made daily for mortality and toxic effects and weekly for body weight changes. Gross necropsy and microscopic examinations were performed. Slight erythema, oedema and atonia were

observed for both abraded and intact skin sites. Slight to moderate desquamation was reported throughout the study. No apparent compound-related body weight or organ-to-body weight changes were reported. Microscopic examination of the males demonstrated that administration of 1 000 mg/kg of benomyl produced degeneration of the spermatogenic elements of the seminiferous tubules of the testes, including vacuolated and multinucleated spermatocytes. A slight increase in haematogenic activity in the bone marrow, as well as acanthosis and hyperkeratosis of the skin, were reported for treated animals (Busey 1968d).

In a separate repeated-dose dermal study, groups of five male and five female New Zealand albino rabbits, weighing 3 kg, were exposed to doses of benomyl equivalent to 0, 50, 250, 500, 1 000 and 5 000 mg/kg b.w. applied to non-occluded abraded dorsal skin sites six hours a day, five days a week for three weeks. Test material was removed by washing the skin site and drying with a towel. There were decreased body weight gains for both males and females at 1 000 mg/kg and greater. Mild to moderate skin irritation was reported for all groups but was most notable at the higher doses. Functional disturbances of the alimentary canal and kidney, including diarrhoea, oliguria and haematuria, were observed in males and females at 1 000 and 5 000 mg/kg. There was a decreased average haemoglobin concentration in the 1 000 mg/kg males, although this was not significant. Decreased average testicular weights and testes-to-body weight ratios were observed at the 1 000 mg/kg dose only. There were no histopathological changes reported and the lower testicular weights were not considered to be directly related to compound administration (Hood 1969).

Dog

Groups of beagle dogs (four males and four females per group) were administered benomyl 50 percent wettable powder in the diet at dosage levels of 0, 100, 500 and 2 500 ppm (based on active ingredient) for three months. Dogs were 7-9 months old. Food consumption and body weight data were recorded weekly. Clinical laboratory examinations including haematology, biochemistry and urinalysis, were performed pre-test and after 1, 2 and 3 months of feeding. At the conclusion of the study all animals were killed, selected organs weighed and additional organs subjected to gross and microscopic evaluations. There was no mortality or adverse clinical observation over the course of the study and growth and food consumption were normal. Urinalyses were unaffected by treatment. There were no dose-related effects on the haematological values; however, alkaline phosphatase and glutamic pyruvic transaminase activities were increased in high dose males and females. Furthermore, there were significant decreases in the albumin/globulin ratio in both males and females fed 2 500 ppm in the diet. Organ-to-body weight changes were observed in the high dose males and females for the thymus (decreased) and thyroid (increase). One female fed 2 500 ppm had an enlarged spleen, which was consistent with the decreased erythrocyte count, haemoglobin concentration and haematocrit values. Histopathological examination revealed myeloid hyperplasia of the spleen and bone marrow with erythroid hyperplasia for this same animal at the 3-month examination. Group mean values were not significantly different, however. Three out of four males fed 2 500 ppm had reduced relative prostate weights when compared with controls. Microscopic examination of tissues and organs did not indicate a consistent lesion effect in animals fed benomyl for 90 days. A no-effect level (NOEL) was demonstrated at 500 ppm (Sherman 1968).

Special Studies on Reproduction

The effects of exposure to benomyl on male reproductive development was evaluated in prepubertal Sprague-Dawley male rats (33 days old), which were gavaged daily for 10 days at doses of 0 and 200 mg/kg/day. Eight animals per group were killed at 3, 17, 31, 45 and 59 days after the last treatment. Selected tissues, including liver, kidney testes, seminal vesicles and epididymides, were removed, weighed and examined histologically. Samples of seminal fluid from the vas deferens were also examined. Observation intervals were pre-selected to coincide with stages of spermatogenesis. Data were presented on tissue weights, total epididymal sperm counts, vas deferens sperm concentrations, or testicular histology. There were no effects related to treatment (Carter 1982).

In a similar experiment, adult male Sprague-Dawley rats (65 days old) received 10 daily treatments of 0, 200 or 400 mg benomyl/kg/day by gavage. At 14 days after the last treatment, body weight, tissue weights, total epididymal sperm counts, sperm concentration in the vas deferens and testes histology were performed. Production of testosterone by the Leydig cells was artificially stimulated by subcutaneous injections of hypothalamic chorionic gonadotrophin (HCG) 2 h prior to sacrifice. There were no compound-related effects on body weight, liver, kidney, adrenal, testes or seminal vesicle weights. Caudate epididymal weights were, however, depressed by treatment with benomyl. There were also treatment-related reductions in epididymal sperm count (caput and caudate) as well as in the vas deferens sperm concentration. The study was designed to evaluate alterations in spermatozoa undergoing spermiogenesis in the seminiferous tubules of the testes during exposure to benomyl. Animals exposed to 400 mg/kg/day presented histologic evidence of hypospermatocytogenesis with generalized disruption of all stages of spermatogenesis, when compared with controls (Carter & Laakey 1972).

Special Studies on Teratogenicity

Mouse

Groups of pregnant CD-1 mice (20-25 mice/group) were administered benomyl via gavage at dose levels of 0, 50, 100 and 200 mg/kg/day on days 7 to 17 of gestation. Animals were killed on day 18, pups delivered by Caesarean section, the number of live, dead and resorbed fetuses determined, and fetuses examined for gross anomalies. Half of the fetuses were examined for visceral abnormalities and the other half for skeletal abnormalities. Maternal indices were unaffected by treatment. However, foetal mortality increased, foetal weight decreased and foetal development was adversely affected by treatment. The high dose caused an increased supraoccipital score, decreased numbers of caudal and sternal ossifications and increased incidences of enlarged lateral ventricles and enlarged renal pelvises. The latter, while not significant at the lower doses, did demonstrate dose-related increases at all other doses. The occurrence of supernumerary ribs and subnormal vertebral centrum was significant and increased in a dose-related manner at all dose levels. There was an increase in the number of abnormal litters and fetuses, which was significantly different from the control at 100 and 200 mg/kg/day. Major anomalies included: exencephaly, hydrocephaly, cleft palate, hydronephrosis, polydactyly, oligodactyly, umbilical hernia, fused ribs, fused vertebrae and short/kinky tail. Although benomyl demonstrated dose-related fetotoxicity at all levels, it was not teratogenic at 50 mg/kg/day in mice (Kavlock *et al.* 1982).

Rat

Groups of rats (ca. 26-28 pregnant CBR-CD rats/group) were administered benomyl (53.5 percent a.i.) in the diet at dosages of 0, 100, 500, 2 500 and 5 000 ppm from day 5 through day 15 of gestation. Average doses were equivalent to 0, 8.6, 43.5, 209.5 and 372.9 mg/kg/day. On day 20 of gestation, all pregnant animals were sacrificed and fetuses delivered by Caesarean section.

Determinations of the number and location of live fetuses, dead fetuses and resorption sites were made, as well as of body weights, crown-rump length, sex and external gross examination for visible abnormalities. Two thirds of the fetuses were prepared for examination of skeletal abnormalities, and the rest were examined for visceral and soft tissue anomalies.

There were no mortalities attributable to benomyl, no clinical signs of toxicity reported and no adverse effects on body weight of dams. Dams in the 5 000 ppm group had a reduced food intake during the period benomyl was administered in the diet, but it returned to comparable control levels for the remainder of the study. The data related to reproduction (implantation sites, resorption sites and live/dead fetuses) were not affected by benomyl up to and including the highest dose level. There were no external or internal abnormalities reported, except for three litters at the highest dose with incidences of hydronephrosis and retarded ossification (interparietal and occipital bones). There were no teratogenic effects noted with the administration of benomyl in the diet to pregnant rats during the critical period of organogenesis (Sherman *et al.* 1970).

Groups of pregnant Wistar rats (27-28 rats/group) were fed benomyl in the diet at dose levels of 0, 690, 3380 and 6760 ppm (time-weighted doses of 0, 169, 298 and 505 mg/kg/day, respectively) from days 7 to 16 of gestation. Foetuses were delivered by Caesarean section on day 21, weighed, examined grossly and fixed for evaluation of visceral and skeletal abnormalities. Food consumption decreased at 3380 and 6760 ppm with resultant decreases in body weight gain for dams, significantly so in the high dose group. Weight gain was significantly reduced in mid- and high-dose group foetuses and a significant decrease in the ossification of the supraoccipital bone in the latter. Furthermore, the percentage of enlarged renal pelvises was increased in the two highest dosage groups, compared with the control. No dose-related anomalies or major malformations were apparent from exposure to benomyl at any of the dose levels utilized (Kavlock et al. 1982).

Benomyl (99.2 percent a.i.) was administered by gavage to groups of pregnant rats (Chr-CD) at dose levels of 0, 3, 10, 30, 62.5 and 125 mg/kg/day from days 7 through 16 of gestation. There were 60 dams in the control group (corn oil) and 27 in each of the other test groups. Dams were observed daily for signs of toxicity and changes in behaviour.

No clinical signs of toxicity or mortality were observed among dams in any dose group. Body weight gain was comparable to controls, as was the incidence of pregnancy, corpora lutea, implantation sites and sex ratio. However, foetal body weight was significantly decreased in the 62.5 and 125 mg/kg/day dose groups. There was also an increased incidence of embryo-foetal mortality at 125 mg/kg/day.

Some foetuses exhibited external and visceral malformations, which were dose-related and significant at 10 mg/kg/day and greater. Malformations observed included microphthalmia, anophthalmia and hydrocephaly (distended lateral ventricles). These occurred predominantly at the higher doses. Histological examination of eyes from all groups revealed pathologic changes, consisting of irregular lenses, retro-bulbar glandular adnexa, distorted or compressed retinal layers and thickened nerve fibres in the 10, 62.5 and 125 mg/kg/day treatment groups. No alterations occurred in control or 30 mg/kg groups. Major skeletal malformations observed in the 125 mg/kg dose group included fused ribs, fused sternbrae and fused thoracic arches. Additional skeletal variations were also increased at 62.5 and 125 mg/kg/day, including misaligned and unossified sternbrae and bipartite vertebral centra.

Benomyl produced teratogenic responses in Chr-CD rats at doses of 10 mg/kg/day and greater. Microphthalmia at 10 mg/kg was reported to be compound related, on the basis of the severity of the pathologic changes and the finding that the increased incidence at 62.5 and 125 mg/kg/day was not a direct reflection of reduced foetal body weight (Staples & Culik 1980).

Groups of pregnant rats were administered benomyl (99.1 percent a.i.) via gavage at dosage levels of 0, 3, 6.25, 10, 20, 30 and 62.5 mg/kg/day from days 7 through 16 of gestation. Each group contained 50 animals, except the high dose group, which contained 20 female rats. Reproductive status was determined on a per litter basis following gross pathological evaluation. The number of implantation sites, resorptions, dead and live foetuses, stunted foetuses and mean weight of live foetuses per litter were determined. This study was performed to determine a no-effect level for microphthalmia and hydrocephaly; therefore, only foetal heads were fixed and examined. Microphthalmia was determined on the basis of the smallest eye in the control group (< 1.8 mm).

Mean foetal body weight was significantly lower in the high dose group. There were only two incidences of malformations, both in the 62.5 mg/kg/day group. One foetus had internal hydrocephaly and another, in a separate litter, had unilateral microphthalmia. These were not statistically different from controls. The only type of variation reported was subcutaneous haematoma, which was not dose related. There was no teratogenic response at 30 mg/kg (Staples 1982).

The teratogenic potential of benomyl was examined in groups of Wistar rats (12 to 30 pregnant rats/group) orally gavaged with dose levels of 0, 15.6, 31.2, 62.5 and 125 mg/kg/day administered on days 7 to 16 of gestation. Pups were delivered by Caesarean section on day 21 of gestation. Weight gain in high-dose dams was decreased from days 17 to 20 of gestation. Foetal resorption was increased in this group, with six litters completely resorbed. Foetal weight was also adversely affected, with significant decreases at 62.5 and 125 mg/kg/day and a significant increase in foetal mortality at 125 mg/kg/day. There were several skeletal and visceral variants observed among foetuses in the 62.5 and 125 mg/kg/day group, including: increased supraoccipital score, decreased number of sternal and caudal vertebrae, increased percentage of enlarged lateral ventricles and enlarged renal pelvis. Major anomalies observed primarily at 62.5 mg/kg/day and above included: encephaloceles, hydrocephaly, microphthalmia, fused vertebrae and fused ribs. A dose level of 31.2 mg/kg/day appeared to be without adverse effects on the developing rat foetus in this evaluation (Kavlock *et al.* 1982).

Benomyl was administered via gavage to groups of Wistar rats at dose levels of 0, 15.6 and 31.2 mg/kg/day from day 7 of gestation through day 15 of lactation (day 22 of gestation was considered day 0 of lactation). The litters were reduced to 8 pups/litter, equal in sex, on day 3 of lactation. The litters were weighed on days 8, 15, 22, 29 and 100, and locomotor activity was evaluated periodically throughout the study. At 100 days of age, several organs were weighed, including adrenals, liver, kidney, ovaries, testes and the ventral prostate plus seminal vesicles. There were no compound-related effects either on litter size at birth or weaning, or on body weights of foetuses (by sex). Growth, survival and locomotor activity were comparable with controls throughout the study. Organ weights were comparable with controls except for decreased testes and ventral prostate/seminal vesicle weights, which were significantly reduced at 31.2 mg/kg/day but not at 15.6 mg/kg (Kavlock *et al.* 1982).

Rabbit

Groups of rabbits (15 artificially inseminated albino rabbits/group) were administered benomyl (50 percent a.i.) in the diet at dosage levels of 0, 100 and 500 ppm from day 8 through day 16 of gestation. Mortality, clinical observations and food consumption were determined daily and body weights were measured weekly. On day 29 or 30 of gestation, selected pregnant animals were sacrificed and foetuses delivered by Caesarean section; the remaining does were allowed to hatch normally on day 35.

One low level doe and one high level doe aborted on days 3 and 6, respectively, and were sacrificed and excluded from the study. There were 12 pregnant does in the control group, 13 in the low dose group and 9 in the high dose group. Of these, 6, 7 and 5, respectively, were sacrificed and foetuses were delivered by Caesarean section; the remaining does hatched normally.

There was no mortality attributable to benomyl. General appearance, behaviour, body weight gain and food consumption were comparable among all groups. The data related to reproduction (implantation sites, resorption sites and live young) were not affected by benomyl. There were no external or internal abnormalities associated with benomyl treatment of pregnant rabbits. Internal development, including somatic and skeletal development, was normal, except for a marginal increase in rudimentary ribs at 500 ppm. Numbers of pregnant rabbits, of litters and of foetuses examined were less than adequate to assess the foetotoxic or teratogenic potential of benomyl to pregnant rabbits (Busey 1968c).

Special Studies on Neurotoxicity

Hen

Studies performed using hens gave no indication of neurotoxic potential at single oral doses up to and including 5 000 mg/kg (Goldenthal *et al.* 1978; Jessup & Dean 1979; Jessup 1979).

Long-Term Studies

Rat

Groups of weanling rats (36 male and 36 female Charles River albino rats/group) were administered benomyl (50-70 percent a.i.) in the diet for 104 weeks at dosage levels of 0, 100, 500 and 2 500 ppm. Growth, as observed by body weight changes and food consumption data, was recorded weekly for the first year and twice a month thereafter. Daily observations were made of behavioural changes and mortality. At periodic intervals over the course of the study, haematologic, urinalysis and selected clinical chemistry examinations were performed. After one year each group was reduced to 30 males and 30 females by interim sacrifice for gross and microscopic evaluations. At the conclusion of the study, all surviving animals were sacrificed and gross examinations of tissues and organs were made. Initially, microscopic examinations of tissues and organs from the control and 2 500 ppm groups were conducted, along with liver, kidney and testes examinations of animals in the 100 and 500 ppm dose groups. In a follow-up pathology evaluation, all of the tissues and organs of the control, low-, intermediate- and high-dose groups were examined microscopically.

There was no mortality in the study attributable to the presence of benomyl in the diet. Survival decreased to approximately 50 percent during the second year, but was comparable among all groups. Body weight, food consumption and food efficiency were unaffected by treatment. The average daily dose for the 2 500 ppm group was 330 mg/kg b.w./day (initially, M/F), 91-106 mg/kg (at one year) and 70-85 mg/kg (at two years). There were no compound-related clinical manifestations of toxicity. Haematologic, urinalysis and liver function examinations were unaffected by treatment. There were no observed differences in organ weights or organ-to-body weight changes. Histopathological examinations revealed no differences between treatment and control groups. The most frequently observed tumors were mammary, pituitary and adrenal ones, which were equally distributed among all groups. Hepatic toxicity was similar among all groups, including controls, with no demonstrated compound-related effects. A high incidence of testicular degeneration was observed in control males and, therefore, no conclusion could be made with regard to compound-related effects on male gonads.

Benomyl was without adverse effects in this study at levels up to and including 2 500 ppm (Sherman et al. 1969; Lee 1977).

Dog

Groups of beagle dogs (four males and females/group) one to two years old, were administered benomyl (50 percent a.i.) in the diet at dosage levels of 0, 100, 500 and 2 500 ppm for two years. Food consumption and body weight data were obtained weekly and animals were examined daily for clinical signs of toxicity. Haematological, biochemical and urinalysis examinations were performed periodically throughout the study. Interim sacrifice after one year was performed on one male and one female per group from the 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 one, which demonstrated both decreased food intake and body weight gain. The average daily dose was 55-58 mg/kg b.w. (initially, M&F), 74-79 mg/kg (at one year) and 45-55 mg/kg (at two years). One dog in the high-dose group lost its appetite and was replaced. No other clinical signs of toxicity were observed. Haematological evaluations and urinalyses were similar to the control. Males in the 2 500 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 marked, effects in high dose females. Cholesterol and total protein were similar to controls among the females examined.

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

Focal testicular degeneration was present in all treatment groups, with marked testicular degeneration (reduced testes weight, absence of spermatozoa and spermatid cells) in 1/3 dogs at 2 500 ppm. A complete histological evaluation of the testes and secondary sex organs of historical control dogs from the testing facility in conjunction with the findings from the present report demonstrated that the testicular lesions reported did not appear to be attributable to benomyl ingestion. However, an outbreak of inflammatory infection causing orchitis in beagle colonies at that time may have contributed to the unusually high level in control dogs; therefore, no clear evidence exists for the absence of testicular effects. Data from the carbendazim two-year dog study provide additional confirmatory results for the absence of these effects at dietary levels of 100 ppm carbendazim. Furthermore, as indicated, only testes and liver were examined microscopically in all dose groups in this benomyl two-year dog study. The two-year carbendazim study provides added assurance for the absence of adverse effects in organs or tissues in other dose groups. Considering the absence of such data for benomyl, a NOEL for benomyl of greater than or equal to 100 ppm can be assigned (Sherman 1970; Lee 1970, 1971a,b,c, 1977).

Special Studies for Carcinogenicity

Mouse

Male and female CD-1 mice (80 males and 80 females per group) were administered benomyl (99 percent a.i.) in the diet at dose levels of 0, 500, 1 500 and 5 000 ppm (5 000 ppm was lowered from 7 500 ppm after 37 weeks) for two years. Mice were 6-7 weeks old at the start of the study. Animals were examined daily for behaviour and clinical signs of toxicity, biweekly for palpable masses and regularly weighed for body weight changes. Food consumption was similarly determined on a routine basis. Mortality was noted and recorded. Peripheral blood was collected periodically throughout the study for haematological examinations. Urine and faecal samples were collected and evaluated prior to terminal sacrifice. Selected organs were weighed, including brain, heart, lungs, liver, spleen, kidney, testes and thymus. Microscopic examination was performed on tissues and organs.

Median survival time was unaffected by treatment. Male and female mice fed 1 500 and 5 000 ppm benomyl had dose-related body weight decreases. Food consumption was variable throughout the study, although high dose females appeared to consume less food. The average daily intake of benomyl for males was 1 079 mg/kg b.w./day (initially), 878 mg/kg (1 yr.) and 679 mg/kg (2 yr.); for females it was 1 442 mg/kg (initially), 1 192 mg/kg (1 yr.) and 957 mg/kg (2 yr.). Clinical signs of toxicity were not different between groups. Alopecia/dermatitis were observed in all groups with equal severity and occurrence. The aetiology of this symptom was unknown. There were no apparent differences between treatment and control groups for palpable masses, number of mice affected or latency period of discovery. Haematology examinations were unremarkable except for decreased erythrocyte counts for males at 1 500 and females at 5 000 ppm. Haemoglobin and haematocrit values were also depressed in males of the intermediate dose group.

Several significant organ weight changes occurred among treated groups. In male mice, mean absolute thymus weights were depressed at all levels and relative thymus weights decreased at 500 and 1 500 ppm (decrease at 5 000 was not significant). Relative thymus weights were increased among females at 5 000 and 1 500 ppm (not significant). Relative brain weights were increased in males and females at 5 000 ppm, in males at 500 and in females at 1 500 ppm. The most significant compound-related organ weight changes involved absolute and relative liver weights for males at 1 500 and 5 000 ppm

and for females at 5 000 ppm. Male mice also presented decreased absolute testes weights at the high dose. The liver and testes weight changes were accompanied by histomorphaic changes in these tissues.

The incidence of hepatocellular carcinomas and benign neoplasms in female mice was increased and dose dependent. Hepatocellular carcinomas were significant in females at 500 and 5 000 ppm, while hepatic neoplasms were significant at 1 500 and 5 000 ppm. In male mice, hepatocellular carcinomas and neoplasms were significantly increased at 500 and 1 500 ppm, but not at the high dose. Lung tumours (alveolozenic carcinomas) in 500 and 1 500 ppm dose groups were also significantly increased in males but not in females. Several non-neoplastic organ changes were increased in males (5 000 ppm), but were confined to liver (degeneration, pigment, cytomegaly), thymus (atrophy) and testes, epididymus, prostate (degeneration of seminiferous tubules, atrophy, aspermatogenesis, distended acini). Splenic haemosiderosis was significantly increased at 5 000 ppm, as was submucosal lymphocytic infiltration of the trachea at 1 500 ppm, in female mice.

The latency period for liver tumour induction (adenomas and carcinomas) was determined from palpation, gross necropsy and histopathology performed on all animals throughout the study. These findings demonstrated that there is no measurable difference in time elapsed from mean test day to tumour between control animals and treatment groups with regard to liver neoplasms.

Benomyl, when fed to CD-1 mice at dose levels of 500 and 5 000 ppm in the diet, is oncogenic in male and female animals. This effect was compound-related in males at the low and intermediate dose levels. In the liver of female mice, the oncogenic effect was compound- and dose-related at all levels. A no-effect level was not observed in male or female mice for hepatocellular carcinomas or combined hepatocellular neoplasms (Wiechman et al. 1982).

Human Exposure

Potential dermal and respiratory exposure to benomyl under actual use situations was determined for: mixing procedures for aerial application, reentry into treated fields and home use (garden, ornamental and greenhouse). Maximum exposure occurred in the loading and mixing operation for aerial application, where dermal exposure was 26 mg benomyl per mixing cycle, primarily to hands and forearms (70 percent). Respiratory exposure average 0.03 mg of benomyl. Reentry data demonstrated dermal and respiratory exposures of 5.9 mg/h and less than 0.002 mg/h, respectively. Home use situations produced exposures of 1 mg and 0.003 mg per application cycle for dermal and respiratory routes, respectively (Everhart & Holt 1982).

Field use conditions involved in spraying fruit orchards were examined for potential dermal and respiratory exposures of humans involved in mixing and applying 20 and 100 gallons of Benlate per acre (1 acre = 0.4 hectares). The application cycle was approximately 70 min. and resulted in total dermal and respiratory exposure of 11 or 15 mg benomyl/cycle. Essentially all exposure was dermal, resulting in 12.2 mg/cycle dermally, with less than 0.05 mg/cycle via the respiratory route (DuPont 1979a, undated).

Selected blood profiles from 50 factory workers involved in the manufacture of Benlate were compared to a control group of 48 workers who were not exposed to Benlate. White blood count, red blood count, haemoglobin and haematocrit values were comparable among the two groups. There were no quantitative estimates of exposure given for the factory workers. There were no female employees included in the control group (DuPont 1979b).

An epidemiology survey was performed to determine whether potential exposure to Benlate had an adverse effect on the fertility of 278 male workers exposed to benomyl between 1970 and 1977. The workers ranged from 19 to 64 years of age, with 73 percent between 20 and 39. Seventy-eight percent of the spouses were similarly between 20 and 39 years. Exposure duration ranged from less than one month to 95 months, with more than 51 percent of the workers potentially exposed from 1 to 5 months. The birth rates of exposed workers' spouses were compared with those of four comparison populations from

the same county, state, region and country (USA). There was no reduction in fertility as evidenced by the birth rates for the study population, which were generally higher than the comparison populations. Spermatogenesis among workers was not examined (Gooch 1978).

Special Studies on Mutagenicity

Results of mutagenicity assays of benomyl are summarized in Table 2.

Table 2 Results of Mutagenicity Assays of Benomyl/Benlate

Test Organism	Test Substance	Results	Reference	
Gene Mutation Studies				
BACTERIA				
<u>Salmonella typhimurium</u>	Benlate or similar formulation	Positive	Kappas <u>et al.</u> 1976	
		Negative	DuPont 1977	
	Benomyl	Series of tests: spot, liquid culture and host-mediated assays using strains <u>his</u> G46, TA1530, TA1535, TA1550 using benomyl. No mutagenic activity was noted in any of these tests		
		Bacterial assays with Benomyl. Strains TA98, TA100, TA1535, TA1537, and TA1538. Doubtful mutagenic activity was reported for benomyl both with and without metabolic activation.		Ercegovich & Rashid 1977; Rashid & Ercegovich 1976
		Positive	Kappas <u>et al.</u> 1976	
		Negative	Shirasu <u>et al.</u> 1978	
		Negative	Ficsor <u>et al.</u> 1978	
		Benomyl was not mutagenic at doses as high as 250 µg per plate in strains TA1535, 1537, 98 and 100		Donovan & Krahn 1981
		Positive/Negative	Russel 1978; Donovan & Krahn 1981	
		Spot tests on plates with <u>Salmonella</u> strains TA1535, 1536, 1537 and 1538 and with <u>Streptomyces</u> . No mutagenic activity was reported for benomyl in either organism.		Carere <u>et al.</u> 1978
<u>S. typhimurium</u> and <u>Streptomyces coelicolor</u>				

Test Organism	Test Substance	Results	Reference
<u>S. typhimurium</u> (host-mediated assay)	Benomyl	Negative	Shirasu <u>et al.</u> 1978
<u>Escherichia coli</u>	Benlate	Positive	Kappas <u>et al.</u> 1976
	Benomyl	Positive	Kappas <u>et al.</u> 1976
		Negative	Shirasu <u>et al.</u> 1978
YEAST AND FUNGI			
<u>Fusarium oxysporum</u>	Benomyl	Inconclusive	Dassenoy & Meyer 1973
<u>Aspergillus nidulans</u>	Benlate	Negative	Eastie 1970
<u>A. nidulans</u>	Benomyl	Benomyl tested in an excision repair deficient strain. A dose-dependent effect was reported.	Kappas & Bridges 1981
<u>Saccharomyces cerevisiae</u> and <u>A. nidulans</u>	Benomyl	Mitotic gene conversion study was negative on testing benomyl with metabolic activation.	De Bertoldi <u>et al.</u> 1980
<u>A. nidulans</u>	Benomyl	Negative for nondisjunction or crossing over. Increased frequency of segregants due to spindle inhibition reported.	De Bertoldi & Griselli 1980
INSECTS			
<u>Drosophila melanogaster</u>	Benomyl/Benlate	Genetic toxicity tested in <u>Drosophila</u> . Noted sterility in some broods. This was considered to be consistent with spindle effects of benomyl.	Lamb & Lilly 1980
Chromosomal Effects Cytogenetics - <u>in vitro</u>			
Chinese hamster ovary cells <u>in vitro</u>	Benomyl	Weakly positive results for sister chromatid exchange were reported, with and without metabolic activation.	Evans & Mitchell 1980
Chinese hamster ovary cells <u>in vitro</u>	Benomyl	Looked for mutations at the HGPRT locus. Benomyl was not mutagenic under these test conditions.	Fitzpatrick & Krahn 1980
Human leukocyte cell cultures	Benlate	Primary cultures were treated with benomyl. Cells examined in the high dose cultures did not show a statistically significant increase in incidence of chromosomal aberrations.	Gupta & Legator 1975

Test Organism	Test Substance	Results	Reference
<u>Cytogenetics - In Vivo</u>			
Rats	Benomyl/Fundazol 50WP	Bone marrow and cultures of embryo cells of pregnant rats were examined for chromosomal aberrations after treatment with benomyl on days 7 to 14 of gestation. No increase in frequency of cells with chromosomal aberrations were reported in bone marrow cells. Embryonic cells had 5 times more chromosomal damage at the two highest doses (200, 500 mg/kg) than controls.	Ruzicska <u>et al</u> 1976
Human	Benomyl/Fundazol 50WP	Peripheral blood cells from workers at a benomyl (Fundazol 50WP) plant were examined. No effects attributable to benomyl reported.	Ruzicska <u>et al</u> 1976
<u>Dominant Lethal</u>			
Rat	Benomyl	Negative	Sherman <u>et al</u> 1975
<u>Micromucleus Test</u>			
Mice	Benomyl	Dosed by gavage at 250, 500 or 1 000 mg benomyl/kg on two consecutive days. Reported a statistically significant increase in number of micromuclei in bone marrow from femur bones at 48 h in 250 and 500 mg/kg dose groups and 1 000 mg group at 24 and 48 h.	Kirchart 1980
<u>DNA Damage and Repair</u>			
B6C3F1 Mice and F344 Rats	Benomyl	Benomyl was tested for DNA repair using primary hepatocyte cultures. Benomyl did not induce DNA repair in either rat or mouse.	Tong 1981a,b
<u>Mitotic Gene Conversion</u>			
<u>S. cerevisiae</u>	Benlate Benomyl	Negative Negative	Siebert <u>et al.</u> 1970 De Bertoldi <u>et al.</u> 1980
<u>A. nidulans</u>	Benomyl	Negative	De Bertoldi <u>et al.</u> 1980
<u>Mitotic Crossing-Over</u>			

Test Organism	Test Substance	Results	Reference
Differential Toxicity (Bacteria)			
<u>Bacillus subtilis</u>	Benomyl	Negative	Shirasa <u>et al.</u> 1978
Plant Studies			
<u>Allium cepa</u>	Benomyl	Negative	Dassenay & Meyer 1973
		Positive	Richmond & Phillips 1975
Chromosome Nondisjunction Yeast and Fungi			
<u>A. nidulans</u>	Benlate	Positive	Hastie 1970
	Benomyl	Positive	Kappas <u>et al.</u> 1974
		Positive	De Bertoldi & Griselli 1980
Mammals			
<u>Microtus oeconomus</u>	Benlate	Inconclusive	Tates 1979
	Benomyl	Inconclusive	Tates 1979

Mouse, micronucleus

Groups of 24 male mice were given two daily doses by gavage of 250, 500 or 1 000 mg benomyl per kg b.w. on consecutive days. A vehicle control (DMSO) group was also included. 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 were found.

The statistical procedure used showed that four groups had significantly increased numbers of cells with micronuclei. These groups included the low- and mid-dose groups at 48 h (15/3 500 and 16/4 000, respectively as compared with 5/3 000 cells from vehicle controls) and in the high-dose group at 24 and 48 h after treatment (20/3 500 and 17/3 500, respectively; respective control values were 5/3 500 and 6/5 000) (Kirkhart 1980).

Ovary cells in vitro

Chinese hamster ovary cells in cultures were exposed to varying concentrations of benomyl without metabolic activation (0, 0.625, 1.25, 2.5, 5, or 10 µg/ml) and with metabolic activation (0, 9.375, 18.75, 37.5, 75 or 150 µg/ml). EMS and dimethylnitrosamine (DMN) were used as positive controls and a vehicle control (0.95 percent ethanol in culture medium) was included. Two samples of 25-cells each were scored for the number of sister chromatid exchanges (SCE) and number of chromosomes. A total of 50 cells were scored for each group. The 5 and 10 µg/ml dosages did not allow sufficient numbers of second division metaphases to occur for an evaluation.

The number of SCE in the ethyl methane-sulphonate group was triple that of the negative controls, while the three non-activated benomyl groups had one-third more SCE than controls. In the experiments with activated benomyl, the DMN positive controls had approximately twice the number of SCE found in negative controls. The benomyl groups had increased numbers of SCE (by approximately 25 to 100 percent above controls). The number of SCE per cell was increased by one sixth to one half above that for negative

controls for non-activated benomyl. The activated fungicide increased the number of SCE by approximately 15 to 100 percent over that seen in controls. Benomyl proved weakly positive in this study (Evans & Mitchell 1980).

A mutagenicity assay with a Chinese hamster ovary cell line, which can demonstrate mutations at the gene locus coding for hypoxanthine-guanine phosphoribosyl transferase (HGPRT) was conducted using benzo(a)pyrene and ethyl methane sulphonate as positive controls, as well as a vehicle control (DMSO). Benomyl was added to test cultures with or without metabolic activation. Resistance of cells to 6-thioguanine was used as the indicator of mutagenic effects.

A dose-related cytotoxic response 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 at concentrations ranging from 17 to 172 μ M. Positive controls demonstrated that the test system was sensitive and cell survival was greater than 10 percent at most concentrations used. Benomyl was not mutagenic under these test conditions (Fitzpatrick & Krahn 1980).

DNA repair

DNA repair assays in rat or mouse hepatocyte primary cultures (HPC) were evaluated for benomyl along with dimethylnitrosamine, dimethylformamide, fluorene and 2-amino-fluorene, which were used as positive controls. The liver was removed and primary cultures were initiated with hepatocytes from B6C3F1 mice or 344 rats. Benomyl and tritiated thymidine (10 μ Cl) were added to the culture medium. After 18 to 20 h incubation they were fixed and examined microscopically for morphological changes and absence of S-phase nuclei indicative of cytotoxicity. Autoradiographic techniques are used to determine the number of nuclear grains induced by test chemicals. Background counts were obtained by evaluating three nuclear-sized areas in the cytoplasm; these values were averaged and subtracted from the number counted in the nucleus to obtain a net value for each nucleus.

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

Bacterial tests

Benomyl and Benlate were tested for mutagenic activity according to the plate incorporation procedure, essentially as described by Ames et al. (1975). Salmonella typhimurium strains used included TA1535, TA1537, TA98 and TA100. DMSO was the solvent used for benomyl and water was the solvent for Benlate. One Benlate sample, tested at concentrations up to 1 200 μ g/plate, was not mutagenic under the test conditions (DuPont 1977). At concentrations up to 500 μ g/plate, benomyl (technical grade) exhibited slight mutagenic activity in TA1537, but only in the presence of the activation system. The induced reversion frequency was 3.8 times the control value and the average revertants/n mole value was 0.06 (Russel 1978). Benomyl was tested with and without activation by three different liver microsomal enzyme preparations (mouse liver S-9 mixes containing 0.8 or 2.5 mg of protein per plate, and rat liver S-9 mix containing 3.5 mg of protein/plate). Only one of two trials with strain TA1535 showed a statistically significant dose-related trend in the induction of mutations. Dose group differences were not significantly-greater than controls. Benomyl at dosages as high as 250 μ g/plate was not mutagenic under the test conditions (Donovan and Krahn 1981). An analytical grade sample of benomyl was not mutagenic at concentrations up to 500 μ g/plate (Russell 1978).

A series of spot tests was conducted with paper disks containing benomyl. The disks were placed on media, which were streaked with S. typhimurium (strains TA1535, TA1536, TA1537, or TA1538) or Streptomyces coelicolor. Benomyl was used alone or combined with liver microsomal enzymes for activation and methylnitrosnitroguanidine was used as a reference mutagen. Disks contained 20 μ g or 500 μ g benomyl for the S. typhimurium and S. coelicolor tests, respectively. No mutagenic activity was noted for benomyl in these tests (Carere et al. 1978).

The mutagenic activity of benomyl, Benlate^R and Fundazol 50 WP was investigated in a series of spot, liquid culture and host mediated assays in strains his G46, TA1530, TA1535 and TA195C of S. typhimurium. Doses of 0.25 to 10 000 $\mu\text{g/ml}$ in overlay spot test and liquid culture treatments were negative. Mice given subcutaneous injections of 500 mg/kg did not produce mutations in S. typhimurium strain his G46. No mutagenic activity was observed in S. typhimurium TA195C when rats and mice were orally dosed with 4 000 mg/kg benomyl (Ficsor et al. 1978).

In similar bacterial assays with benomyl in S. typhimurium TA28, TA100, TA1535, TA1537 and TA1538, there was doubtful mutagenic activity in the base substitution sensitive strains (TA100 or TA1535) for benomyl (1 to 325 $\mu\text{g/plate}$) with and without liver microsomal activation. Responses were defined in terms of the ratio of the number of revertants observed on treated plates to that found on untreated control plates and a doubtful response was defined as a ratio of 1.5 to 2 (Ercegovich & Rashid 1977; Rashid & Ercegovich 1976).

Benlate^R was tested for mutagenic activity in S. typhimurium and Escherichia coli, using a simplified fluctuation assay. Benlate^R was mutagenic in the base pair substitution specific strain, TA1535, but not in the frameshift specific strain, TA1538. It was also mutagenic for E. coli strain WP2 uvrA, which lacks excision repair, but not for E. coli strain WP2 (DNA repair proficient) and CM611 (misrepair and excision repair deficient). With both S. typhimurium and E. coli, the degree of mutagenic activity observed at concentrations between 0.125 to 1 $\mu\text{g/ml}$ was similar when tested at concentrations greater than 1 mg/ml Benlate^R was not mutagenic (Kappas et al. 1976).

Benomyl was non-mutagenic in S. typhimurium TA1535, TA1537, TA1538, TA28 and TA100, and E. coli WP2 hcr. Concentrations between 5 and 1 000 $\mu\text{g/plate}$ were tested in a plate incorporation assay with DMSO as the solvent both in the presence and absence of an activation system, which included a 9 000 x g supernatant fraction of homogenized livers from Aroclor 1254-treated Sprague Dawley rats (Shirasu et al. 1978).

Tests with yeast and fungi

Benomyl added to cultures of an Aspergillus nidulans strain, which is excision repair deficient, induced back mutations to biotin and pyridoxin-requiring strains. Mutagenic activity was observed at 0.25, 0.3 and 0.4 $\mu\text{g/ml}$ but there was a plateau of positive response rather than a linear dose-response through zero concentration. Benomyl dissolved in ethanol was not mutagenic in either of the repair-proficient strains (Kappas & Bridges 1981).

No mitotic gene conversion was noted in diploid strains of Saccharomyces cerevisiae or A. nidulans exposed to as much as 3 200 ppm or 200 ppm benomyl, respectively. Benomyl was activated with a mouse liver microsomal fraction; survival at the highest level tested was equal to 83 percent and 51 percent for S. cerevisiae and A. nidulans, respectively (de Bertoldi et al. 1980).

In another study with A. nidulans exposed to benomyl, no non-disjunction or crossing over were reported. An increased frequency of segregants occurred, probably due to benomyl's spindle-inhibiting effects (de Bertoldi et al. 1980).

Insect tests

Gamma radiation was used as the reference mutagen. Treated and untreated adult male Drosophila melanogaster were mated with virgin females so that at least nine broods of offspring were produced from each male. Each male was mated with two untreated females for two to three days following treatment. At that time, a subsequent mating was conducted. The offspring were classified by sex and phenotype (regular or exceptional). Some of the exceptional offspring could have resulted from exchanges between the X and Y chromosomes in the parent male rather than from whole or partial chromosome loss. Exceptional offspring resulting from chromosome loss, breakage or non-disjunction could, therefore, not be clearly distinguished from those resulting from the sex chromosome exchanges.

No deaths resulted from the treatments. However, there was an increased incidence of sterility in the later broods from treated Oregon-R males, but no effect in treated Y^+B/BSY^+ males. The first and second broods result from germ cells which were post-meiotic at the time of treatment, those in the third and fourth broods were from meiotic germ cells and those in the last broods were from germ cells that were premeiotic at the time of treatment. No compound-related effects were noted when chromosomes were examined for breakage in a second set of experiments and the overall incidence of recessive lethal mutations reported was 5/4 807 (0.1 percent). The sterility observed in broods from matings involving mitotic spermatosomal cells is probably consistent with the suspected spindle effects of the chemical (Lamb & Lilly 1980).

Cytogenetics

Primary cultures of human leukocytes were treated with Benlate^R in DMSO at 0, 200, 2 000 or 20 000 ppm. Each test solution (0.1 ml) was added to 5 ml cultures, which were examined for cells with chromosome breaks, deletions and small fragments after exposure. In cultures exposed to 0, 200 or 2 000 ppm benomyl, 1.3, 5.8, and 3.8 percent of the cells examined had chromosomal aberrations. Cultures exposed to the highest dose contained fewer dividing cells, owing to toxicity. Those cells examined in high-dosed cultures did not show a significantly increased incidence of chromosomal aberrations above that in control cultures. The mitotic index was not measured nor were replicates performed. Thus, the results are difficult to assess, particularly regarding toxicity (Gupta & Legator 1975).

Bone marrow and cultures of the embryo cells of pregnant rats were examined for chromosomal aberrations. The rats were given daily doses of 0, 25, 50, 200 or 500 mg Fundazol 50WP per kg b.w. by gavage on days 7 through 14 of gestation. No increases in frequency of cells with chromosomal aberrations were reported in bone marrow. However, embryonic cells from rats given 200 or 500 mg/kg/day had five times the frequency of cells with damaged chromosomes. The aberrations were described as rings, acentric chromatids and translocations (Ruzicka et al. 1976).

COMMENTS

Available information demonstrates that benomyl and carbendazim have a similar metabolism. Benomyl is rapidly metabolized to carbendazim in mammals and is eliminated preferentially in urine as methyl 5-hydroxy-2-benzimidazolecarbamate (5-hydroxycarbendazim, 5-HBC). In rats following intubation, inhalation and dietary exposures, benomyl and carbendazim were present in the blood within the first six hours, together with comparable levels of 5-HBC. Within 18 hours after exposure, only 5-HBC was identified in blood. Metabolism proceeds via hydroxylation and ester hydrolysis in the liver followed by elimination, primarily in the urine (41-71 percent) and to a lesser extent in faeces (21-46 percent). There is no retention of ¹⁴C activity in muscle tissue or fat; only liver and kidney demonstrate bound residues identified as 5-HBC.

Benomyl is not acutely toxic to mammals. It has an acute oral LD₅₀ in rats and an acute dermal LD₅₀ in rabbits above 10 000 mg/kg. Gross and histopathological examination of many of these animals demonstrated a measurable compound-related effect on the male gonads at high doses (testicular degeneration, necrosis of germinal epithelium and aspermatogenesis).

Spermatogenesis in pre-pubertal rats was not affected at dose levels affecting adult rats. Previous JMPR evaluations have identified variations in teratogenic response dependent on the mode of oral administration (diet or gavage). The present Meeting reviewed additional data, which confirmed the difference in sensitivity according to the route of administration in the rat, the dietary NOEL for teratogenicity being 6 760 ppm. This dose did, however, induce embryonic toxicity. In the mouse, gavage studies resulted in terata induction at levels above 500 mg/kg. In the rabbit, a limited study did not result in induction of terata at 500 mg/kg in the diet.

Short- and long-term dietary studies in rats did not demonstrate compound-related effects at doses up to and including 2 500 ppm. Ninety-day and two-year dietary studies in dogs demonstrated adverse effects on the liver at 2 500 ppm, but not at 500 ppm, evidenced by increased cholesterol, alkaline phosphatase and GPT levels, by decreased total protein and albumin/globulin ratio, and by bile duct proliferation and haemosiderosis (see also carbendazim).

A carcinogenicity study in CD-1 mice at 500, 1 500 and 5 000 ppm in the diet showed oncogenicity at all levels. Neoplastic changes included lung carcinomas in males, but not in females, at 500 and 5 000 ppm. There was an increased incidence of hepatocellular carcinomas in males at 500 and 1 500 ppm and in females at 500 and 5 000 ppm. There was no measurable effect on latency period. It was concluded that benomyl was hepatocarcinogenic to mice (see also carbendazim).

Mutagenicity studies with benomyl gave both positive and negative results. Benomyl was positive in the micronucleus, yeast, fungi and drosophila tests. Conflicting negative and positive results in other tests prevented evaluation of the mutagenic potential. The potential impact of these results on human health cannot be adequately assessed at this time.

The monographs on benomyl and carbendazim have stated that the metabolism of the two compounds is essentially the same, with benomyl being converted rapidly to carbendazim in mammals. Accordingly, the available data for benomyl and carbendazim should be considered collectively for the evaluation of specific studies such as teratology, reproduction, chronic toxicity and oncogenicity, taking into account the different molecular weights of the two compounds.

Previous Meetings in 1970, 1973 and 1976, have discussed the etiology and pathogenesis of liver tumours in certain strains of mice, with particular emphasis on organochlorine pesticides. It was recognized that liver tumours are known to develop spontaneously in many strains of mice, at relatively high incidence, without intentional exposure to chemicals. Evidence of such tumours in several strains of mice has been found in many of the oncogenicity studies performed with benomyl and carbendazim. Furthermore, one strain of mouse used (HOE NMR) is known to have a low background incidence of liver tumours (1-2 percent) and did not provide evidence of oncogenicity when exposed to carbendazim at doses up to and including 5 000 ppm. Two additional studies have been carried out in rats using both benomyl and carbendazim. Both studies were negative for oncogenicity at doses up to and including 2 500 and 10 000 ppm, respectively. The hepatic tumours produced in mice, therefore, appear to be a species-related phenomenon.

The Meeting expressed concern at the equivocal nature of the results of a wide range of mutagenicity studies. The possibility that conflicting results were due to variations in the type and amount of impurities was considered. However, the Meeting received information that current levels of the relevant impurities are very low in technical materials.

In view of established no-observed-effect levels determined in several studies, including teratology, reproduction and chronic feeding, an ADI for both benomyl and carbendazim could be estimated. However, a safety factor of 200 was used to reflect the Meeting's concern for the paucity of individual animal data for many studies on carbendazim, which reflect the toxicity of benomyl.

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TOXICOLOGICAL EVALUATIONLevel causing no toxicological effect

Rat: 2500 ppm in the diet, equivalent to 125 mg/kg b.w.
 Dog: (see carbendazim)
 Rat: 30 mg/kg b.w./day (teratology)

Estimate of acceptable daily intake for man

0-0.02 mg/kg b.w.

FURTHER WORK OR INFORMATIONDesirable

1. Data on individual animals in studies on carbendazim that have been identified in the monograph.
2. Additional data to elucidate the mechanism of degenerative testicular effects on mammals.
3. Elucidation of the variability of the mutagenicity data.

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Reviewer's Peer Review Package for 2nd Meeting 3/11/86

3/11/86

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

II. D.

007710

MAR 11 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Benomyl Risk Assessment Assumption for $Q_1^* = 3.9 \times 10^{-3}$
for Carcinogenicity Potency

FROM: Bertram Litt, Leader, Statistical Team
Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Marion Copley
Section VI
Toxicology Branch/HED (TS-769)

THRU: Reto Engler, Chief
Mission Support Staff
Toxicology Branch/HED (TS-769)

Based on the suggestions of the HED/TOX Peer Review Committee the liver tumor data for all females studied in the MBC mouse 2-year feeding study (CD-1 strain) were used as the source for estimating cancer potency of Benomyl.

The data were fitted to a polynomial of the 3rd degree by Crump's Global82 program using Abbott's correction.

The data fitted were:

Dose mg/kg/day	0	75	257	1125
Tumor Bearing Animals	1/	9/	21/	15/
No. Examined	/79	/78	/80	/78

and a Q_1^* of 3.1×10^{-4} was estimated for mice $(\text{mg/kg/day})^{-1}$.

The mouse estimate was then extrapolated to man by the surface area adjustment of $(\frac{60,000 \text{ g. human body wt.}}{30 \text{ g mouse wt.}})^{1/3} = 12.6$

i.e. $3.1 \times 10^{-4} \times 12.6 = 3.9 \times 10^{-3} (\text{mg/kg/day})^{-1} = \text{human } Q_1^*$.

2/14/84

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

FEB 14 1984

FEB 14 1984

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Interim Revised Estimate of Benomyl Cancer Potency.

FROM: Bertram Litt, Leader *Hebut assays for Baker field*
Biostatistics Team/MSS
Toxicology Branch/HED (TS-769)

TO: Peer Review Committee
Toxicology Branch

THRU: Reto Engler, Chief
Mission Support Staff
Toxicology Branch/HED (TS-769)

Following the recent peer review of Benomyl carcinogenicity it was agreed that two estimates of cancer potency were indicated. The first was a best estimate based on the Q_1^* for MBC female liver data and the geometric mean of the Q_1^* for female liver tumors in Benomyl MBC. Secondly an alternative was to be developed which would serve as an example of how to estimate risk for class C oncogens. The following Q_1^* estimates fulfill the first request, a subsequent report will be prepared from the alternative approach.

	<u>Animal Risk</u>	<u>Human Risk</u>
MBC	3.1×10^{-4}	3.9×10^{-3}
Benomyl	4.7×10^{-4}	5.9×10^{-3}
Geometric Mean	3.8×10^{-4}	4.8×10^{-3}

26/84

007710

MEMORANDUM

SUBJECT: Definition and Use of the Term "MTD"
(Maximum Tolerated Dose)

FROM: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769) *RBj 3/26/86*

My signature acknowledges concurrence with the peer review on Benomyl/MBC providing the use of the term "MTD" in this document is consistent with the definition and use as given in: (1) HED SEP: Oncogenicity Potential (Guidance for Analysis and Evaluation of Long Term Rodent Studies) (EPA-540/9-85-019, June 1985); (2) Report of the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation, DHHS (August 17, 1984); and (3) Chemical Carcinogens; A review of the Science and its Associated Principles, February 1985, Office of Science and Technology Policy (FR/Vol. 50, No. 50/March 14, 1985).

19/85

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

DEC 19 1985

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Follow-up to the Data Evaluation Report submitted to the Tox. Br. Peer Review Panel

FROM: Marion P. Copley, D.V.M., D.A.B.T. *MC 12/18/85*
Section 6
Toxicology Branch/HED

THROUGH: Jane Harris, PhD, Section Head *JCH 2/19/85*
Section 6
Toxicology Branch/HED

TO: The peer review panel for benomyl

Attached is the follow-up Data Evaluation Report (DER) to the Peer Review Committee for Benomyl. At the meeting of 11/8/85 the committee requested the following information: 1) evaluation of the 2 year mouse studies with MBC, 2) historical control data for the mouse liver tumors from the testing laboratory, 3) activity (toxicologic) of thiophanate methyl and thiophanate ethyl, and 4) tables comparing oncogenicity and other properties for use in the weight-of-the-evidence determination of oncogenic classification.

This document contains the requested information and should be used in conjunction with the original DER submitted to the Peer Review Committee.

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Follow-up Data Evaluation Report for the
Peer Review Committee for Benomyl

Submitted by Marion P. Copley, D.V.M., D.A.B.T.

Sect. 6, Tox. Br., HED

Through Jane Harris, PhD, Section Head, Sect. 6, Tox. Br., HED

1. Background

Benomyl (methyl-1(Butylcarbamoyl)-2-benzimidazolecarbamate) and its major metabolite MBC (methyl 2-benzimidazole carbamate) are systemic fungicides. MBC is also the primary metabolite for the fungicides thiophanate methyl and thiophanate ethyl.

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. The oncogenic lesions in question were hepatocellular adenomas and hepatocellular carcinomas 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. With respect to acute and chronic toxicity, MBC is either similar to or more potent 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).

2. Metabolism

Although valid metabolism studies have not been performed to adequately describe the metabolism of benomyl in animals, benomyl and its metabolites do not accumulate in animal tissue and are rapidly eliminated in the urine or feces as sulphate and glucuronide conjugates.

One postulated pathway is as follows:

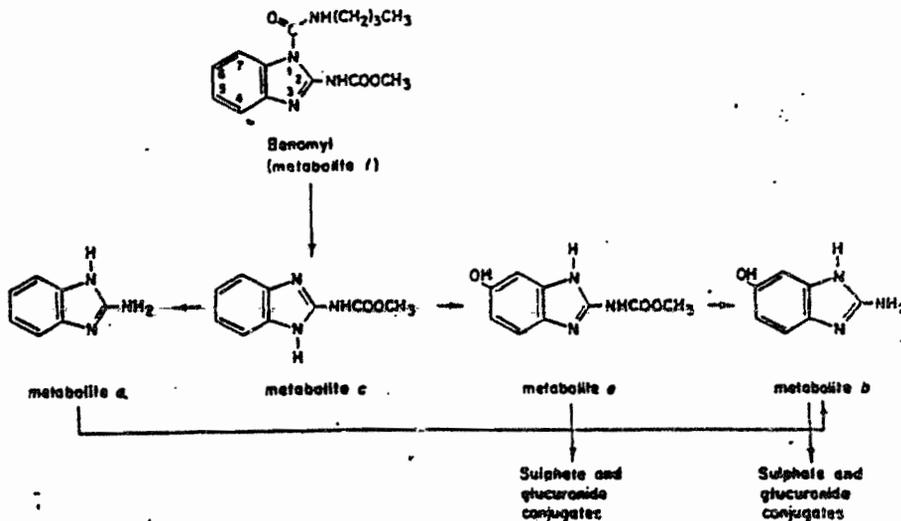


Fig. 3. Metabolic pathway of Benomyl in mice, rabbits and sheep.

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

The metabolite profile (a, b, c, and e) and urinary and fecal excretory pattern appeared similar after oral and intraperitoneal exposure to high doses of benomyl suggesting enterohepatic recirculation of the metabolites. The distribution of metabolites also appeared similar for the mouse, rabbit and sheep. 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 high exposures may not however, represent the pathway that would occur with lower dietary exposure levels.

After repeated exposure to a lower dose in a single male rat 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.

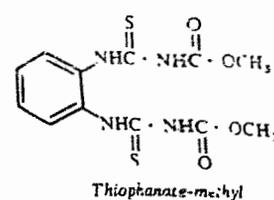
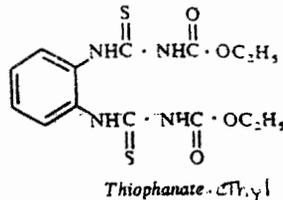
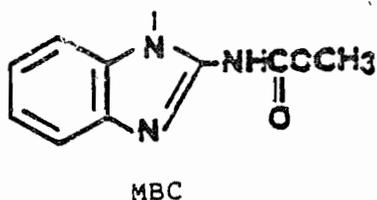
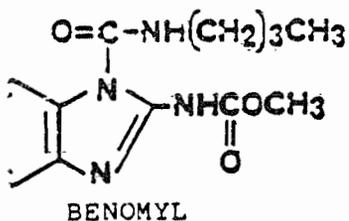
3. Structure Activity Relationship

Benomyl is primarily metabolized to MBC (see below). The acute and chronic toxicologic properties of MBC are similar to benomyl. They are both associated with hepatocellular tumors and are both spindle poisons.

Thiophanate methyl (TM) (see structure below) is also metabolized to MBC. Thiophanate ethyl (TE), a structural analogue of TM, may also be metabolized to ethyl 2-benzimidazole carbamate (EBC). Although the metabolism is not well established for either thiophanate fungicide, it appears that between 80 and 90 % of the TM label is excreted after 24 hours with about 46 % of the administered dose converted to MBC and 5-OH-MBC. There is no indication that either TM or TE is oncogenic or mutagenic.

The following table relates the similarities and differences among these four compounds.

	Benomyl	MBC	TM	TE
hepatic tumors, mice	X	X	-	-
mutagenic (spindle effects)	X	X	-	-



4. Relevant Chronic or Life Time Studies (see appendices A and B for combined tumor incidence tables and Data Evaluation Report)

- A. Long-term feeding study with Methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate (INT-1991), Benomyl (Benlate®) in mice (see DER appendix B-1).

Species/Strain: CD-1 strain mouse

Testing facility: Haskell Lab. for Toxicology and Industrial Medicine

Number of mice in study: 30/sex/group

Mice received in their diet either 0, 500, 1500 or 7500-5000 ppm of benomyl for 2 years. At week 37 the high dose of 7500 ppm was decreased to 5000 ppm for both males and females due to marked weight loss. Although the incidence of alveologenic carcinoma was increased (see

table below) in the mid and low dose male groups (30% and 29 %, respectively) there was no dose response in the Chi² test for trend and the incidences were within the range for this tumor in historical controls at the same laboratory (20 % to 36 %) (see section 5 for historical values). In the same mouse strain, MBC does not appear to be associated with an increased incidence of lung tumors.

Incidence¹ of Lung Alveologenic Carcinoma in CD-1 Mice Receiving Benomyl for 104 Weeks

Study Strain	dose in ppm			
	0	500	1500	7500/5000
Male	13/79†	24/79*	23/79*	16/80
Female	16/77	7/79	4/78	6/74

Benomyl, however was associated with an increased incidence of hepatocellular carcinomas in both males (LDT and MDT) and females (LDT and HDT) for both a pair-wise comparison and the trend test (see table below) in both males and females. No increased incidences of hepatic adenoma or hyperplasia were observed in treated mice as compared with controls.

Incidence¹ of Hepatic Neoplasms in CD-1 Mice Receiving Benomyl for 104 Weeks (Haskell Labs.)

Study Strain	liver neopl.	dose in ppm			
		0	500	1500	7500/5000
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*

The systemic no observable effect level (NOEL) was 1500 ppm (225 mg/kg/day) the lowest effect level (LEL) was 7500/5000 ppm (1125/750 mg/kg/day), based on decreased weight gain and liver and testicular pathology. There were no clinical chemistries tested. At 2 years the HDT males and females weighed about 9 % less than controls*. Non-neoplastic histopathologic lesions were observed in the HDT male liver* and testes* (see table). These included foci of hepatocellular alteration, hepatic karyo- and cytomegaly, and foci of hepatocellular

¹ number of animals with tumors/number of animals examined.

* significantly different from control (p < 0.05) analysis by study authors using the Fisher's exact test.

† significant trend (p < 0.05) using Cochran-Armitage test.

ballooning and degeneration. There there was an increased incidence of testicular atrophy and degenerated seminiferous tubules, with epididymal aspermia and distended tubules filled with degenerated sperm.

Incidence¹ of Selected Non-neoplastic Lesions
in the Liver and Testes
for Male Mice Treated for 104 weeks with Benomyl

Lesion in males	dose in ppm			
	0	500	1500	7500/ 5000
Liver				
hepatocell. alteration	1/77	3/80	2/79	8/80*
karyo- and cytomegaly	9/77	5/80	12/79	21/80*
hepatocell. ballooning and degen.	0/77	1/80	0/79	6/80*
Testic. tubular degen.	10/78	19/79	15/79	27/79*

¹ number of animals with tumors/number of animals examined.

* significantly different from control (p < 0.05) analysis by study authors using the Fisher's exact test.

† significant trend (p < 0.05) using Cochran-Armitage test.

- B. Long-term feeding study with 2-benzimidazole-carbamic acid, methyl ester (MBC, INE-965) in mice, Parts I and II. (DER appendix B-2)
Species, Strain: CD-1 mouse
Testing facility: Haskell Lab. for Toxicology and Industrial Medicine
Number of mice in study: 80/sex/group

Mice received MBC in their diet at either 0, 500, 1500, 7500 (females) or 7500/3750 (males) ppm for 2 years (HDT males were sac. at 73 weeks). At 66 weeks the male high dose (7500 ppm) was decreased to 3750 ppm due to increased mortality. There were increases in the incidence of hepatocellular carcinomas in males at 1500 ppm and the combined incidences of hepatocellular carcinomas and adenomas at all doses in females (see table). No increased incidence of liver hyperplasia was observed in treated vs. control mice.

The NOEL was 500 ppm (75 mg/kg/day). The LEL, based on hepatotoxicity (males) and lymphoid depletion of the thymus (males and females) was 1500 ppm (225 mg/kg/day). There was a dose related increase in mortality in the males only. The HDT males were sacrificed at 73 weeks since only 23 HDT males were alive at that time as compared to 50 control males. At 104 weeks (termination) the MDT males weighed about 12 % less than controls. Relative liver weight was increased in the MDT and HDT females. Hepatotoxicity was characterized in the males by an increased incidence of hepatocellular

Incidence¹ of Hepatic Neoplasms in Mice
Receiving MBC for 104 weeks

Study # Strain	liver neopl.	dose in ppm				7500
		0	500	1500	3750/ 7500 ³	
MBC						
Haskell)	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 ²	0/79	0/78	1/80		0/79
	total	1/79††	9/78**	21/80***		15/78***

¹ number of animals with tumors/number of animals examined.

² Hb - Hepatoblastoma

³ Dose in the HDT males was decreased to 3750 ppm at week 66.
This group was terminated during week 73.

* significantly different from control (p < 0.05) analysis by study authors using the Fisher's exact test.

** signif. diff. from control (p < 0.01), Fisher's exact test.

*** signif. diff. from control (p < 0.01), Fisher's exact test.

† significant trend (p < 0.05) using Cochran-Armitage test.

†† significant trend (p < 0.001) using Cochran-Armitage test.

necrosis (centrolobular) and hypertrophy. There was an increased incidence of foci of eosinophilic hepatocellular alterations in the female (see table). There was increased incidence of thymic lymphoid depletion and decreased thymic weight at the mid and high doses in both males and females. The testes had evidence of sperm stasis at the MDT and HDT as well.

Incidence (%) of Non-neoplastic Lesions
in the Liver of Mice Treated for 104 Weeks with MBC

dose (ppm)	males				females			
	0	500	1500	7500/3750	0	500	1500	7500
Hepatocellular necrosis ¹	0	1	11*	18*	--	--	--	--
Hepatocellular hypertrophy	0	11*	16*	21*	--	--	--	--
Cellular alteration eosinophilic	--	--	--	--	0	0	0	8*

* Fisher's exact test, p < .05

¹ centrolobular

-- no significant change

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- C. Carcinogenicity Study with Carbendazim (99 % MBC) in Mice -
Summary - Report No. R4936 of The Central Institute for
 Nutrition and Food Research. (Review of study by WHO
 in Appendix C, pages 100 - 101)
 Species/Strain: SPF Swiss mice
 Testing facility: Central Inst. for Nutr. and Food Res., TNO
 Number of mice in study: 100/sex/group

Mice were treated for 80 weeks with either 0, 150, 300 or 1000 ppm of MBC in their diet. The 1000 ppm dose was increased to 5000 ppm at week 8. Results were presented in summary form only. High dose females had an increased incidence of hepatic neoplastic nodules (adenomas) while high dose males had an increased incidence of hepatoblastomas.

It was determined after reexamination of the hepatic sections by Haskell Labs., that all the hepatoblastomas occurred within the borders of hepatocellular adenomas or carcinomas, and that these lesions were not counted separately in the original report. The hepatoblastoma is considered to be a more uncommon and malignant liver tumor than the hepatocellular carcinoma (reevaluation by R. Everett, Haskell Labs.).

Incidence of Liver Neoplasms¹ in SPF-Swiss Mice
 after 80 Weeks Treatment with MBC

ppm	0	150	300	5000	0	150	300	5000
	MALE				FEMALE			
NN ³	9/100	7/98	14/100	16/100	0/97	1/99	1/98	9/97*
malig ³	1/100	1/98	2/100	3/100	1/97	0/99	0/98	0/97
Hb	0/100	1/98	1/100	7/100*	0/97	0/99	0/98	0/97
total ²	10/100	8/98	16/100	17/100	1/97	1/99	1/98	9/97

¹ liver neopl.

malig. (adenocarcinomas)

N.N. (neoplastic nodules - benign)

² Animals with multiple tumors were counted once in the totals.

³ includes the NN and malig. that occurred in conjunction with Hb. They were observed by the Haskell pathologist but omitted in the original TNO report.

* P < 0.01 (chi-square test)

The NOEL was 300 ppm (45 mg/kg/day). The LEL based on hepatic alterations, was 5000 ppm (750 mg/kg/day). Body weight was not affected by the compound. Rel. liver weights were increased in the high dose male (30 %) and female (20 %) groups. Clear cell and mixed cell foci were present at increased incidences in livers of the high dose males and females.

Incidence of Non-neoplastic Liver Lesions after MBC Treatment

Lesions	Male (ppm)				Female (ppm)			
	0	150	300	5000 ¹	0	150	300	5000 ¹
cellular alt.								
clear	0/100	0/98	1/100	5/100*	0/97	1/99	0/98	8/97**
mixed	1/100	6/98	6/100	10/100**	0/97	1/99	3/98	0/97

* P < 0.05 (chi-square test)

** P < 0.01 (chi-square test)

¹ raised from 1000 at week 8.

- D. Repeated Dose Feeding Study for Determination of the Carcogenic Effect of HOE 17411 OFAT204 (carbendazim or MBC) in mice. (Review of study by WHO in Appendix C, page 101)
 Species/Strain: HOE NMRKf (SPF 71) mice
 Testing facility: BASF
 Number of mice in study: 100/sex/group (20 additional mice/sex/control and HDT were treated for an 18 month sacrifice.

Mice were treated for 97 weeks (22 months) with either 0, 50, 150, 300 or 1000 of MBC in their diet. The 1000 ppm dose was increased to 5000 ppm at week 8.

The systemic NOEL was 300 ppm (45 mg/kg/day). The LEL, based on liver toxicity (males and females) was 5000 ppm (750 mg/kg/day). The effects observed at the high dose consisted of a significant increase in liver cell hypertrophy, clear cell foci and hepatocellular necrosis. There was also an increase in relative liver weight at both 18 and 22 months.

The frequency and the distribution of possible preneoplastic changes and primary liver neoplasms are shown in the following table.

Table 3.	Controls	50 ppm	150 ppm	300 ppm	5 000 ppm
	97♂ 98♀	99♂ 98♀	99♂ 95♀	95♂ 95♀	99♂ 95♀
Clear cell foci.					3 4
Basophilic foci.		1	1		
Neoplastic nodules (adenomas)	3	2		1	1
Haemangiomas.		2	3	2	2 1

No significant treatment-related increases of preneoplastic or neoplastic hepatic lesions were apparent in the NMRKf mouse.

- E. There was no increase in neoplasia in the dog or albino Charles River CD rat. Although there was no maximum tolerated dose (MTD) established in the chronic benomyl rat study (high dose 2500 ppm), the MBC chronic rat study established an MTD at 5000 ppm without any significant treatment-related increase in oncogenicity. At 2 years the males and females receiving 5000 ppm weighed between 10 - 20 % less than controls. They also had an increased incidence of hepatic pericholangitis and altered hematologic parameters.

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5. Historical Control Information

a) Organ/lesion: Lung/pulmonary tumors
 Species/strain: Mice/control male
 Laboratory: Haskell Labs.

- The incidence rate of pulmonary tumors observed in the control male mice in the benomyl study was at the low extreme of our historical range for pulmonary tumors in control male mice. The studies reported below were conducted between September, 1977 and March, 1981.

<u>Compound</u>	<u>Number of Pulmonary Tumors in Male Control Group/ Number of Lungs Examined</u>	<u>Percentage of Male Mice With Pulmonary Tumors</u>
H-11086	13/80	16
H-11135	18/98	18
H-11201	16/80	20
H-12700	19/79	24
H-10963	17/67	25
H-10720	23/80	29
H-11265	29/80	36
Benomyl	13/79	16

Mean = 24

- The incidences of pulmonary tumors in male mice in the low and intermediate dose groups in the benomyl study were 24 of 80 (30 percent) and 25 of 79 (29 percent), respectively. While these incidence rates were statistically significant ($P < 0.05$, Fisher's Exact Test) when compared to the low incidence of pulmonary tumors in the benomyl controls, both incidence rates were within the range for our historical controls (16 to 36 percent).

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b) Organ/lesion: hepatic neoplasia (hepatocellular adenoma and hepatocellular adenocarcinoma)
 Species/strain: CD-1 mice
 Laboratory: Haskell Labs.
 Study type: 24 month feeding

Hepatocellular Tumors (% Incidence) in Controls from 3 Studies

	A (unnamed)	Benomyl	MBC
Males			
# of livers examined	80	77	80
# of adenomas	5 (11%)	9 (12%)	11 (14%)
# of carcinomas	4 (5%)	16 (21%)	2 (3%)
combined	13 (16%)	25 (32%)	13 (16%)
Females			
# of livers examined	80	77	79
# of adenomas	5 (6%)	2 (3%)	0
# of carcinomas	1 (1%)	2 (3%)	1 (1%)
combined	6 (8%)	4 (5%)	1 (1%)

range	male	female
adenomas	11 - 14 %	0 - 6 %
carcinomas	3 - 21 %	1 - 3 %
combined	16 - 32 %	1 - 8 %

c) Organ/lesion: hepatic neoplasia (hepatocellular adenoma and hepatocellular adenocarcinoma)
 Species/strain: CD-1 mice
 Laboratory: Chevron
 Study type: Chronic mouse feeding study (2 year)

Hepatocellular Tumors (% Incidence) in Controls from 3 Studies

	1	2	3
Males			
# of livers examined	51	80	52
# of adenomas	16 (31%)	17 (21%)	8 (15%)
# of carcinomas	1 (2%)	0	0
combined	17 (33%)	17 (21%)	8 (15%)
Females			
# of livers examined	52	80	50
# of adenomas	4 (8%)	2 (3%)	1 (2%)
# of carcinomas	0	0	0
combined	4 (8%)	2 (3%)	1 (2%)

1 Captafol controls - Chevron Labs.
 2 Captan controls - "
 3 Folpet controls - "

range	male	female
adenomas	15 - 31 %	2 - 8 %
carcinomas	0 - 2 %	0
combined	15 - 33 %	2 - 8 %

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6. Mutagenicity

The mutagenic activity of benomyl and MBC was discussed in detail in the first DER to the Benomyl Peer Review Committee. Both compounds are spindle poisons which could result in nondisjunction and aneuploidy. Nondisjunction has been reported in A. nidulans after exposure to benomyl and MBC. Benomyl was associated with gene mutation in strains of S. typhimurium with activation. Although MBC* was weakly mutagenic in a mouse lymphoma cell (L5178Y TK+/-) study (with and without activation), a second study was negative. In Chinese hamster ovary cells (HGPRT) however, benomyl, was not mutagenic with or without activation. Benomyl was not associated with increased DNA repair in primary mouse and rat hepatocyte cultures. There were no chromosome breaks in vivo with Chinese hamster bone marrow cells. 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. Benomyl was weakly positive for sister chromatid exchange (SCE) in vitro in Chinese hamster ovary cells with and without activation.

It was concluded in 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 test compound in this study was initially reported as benomyl but subsequently changed to MBC.

7. Summary

Benomyl, a benzimidazole carbamate, is initially metabolized to MBC. It is expected that the toxicity of Benomyl is either equal to, or less than MBC since benomyl rapidly hydrolyzes to MBC both in vitro and in vivo in an aqueous environment.

Both benomyl and MBC are hepatotoxic and are associated with an increased incidence of hepatocellular adenomas and/or carcinomas in the Charles River CD-1 (benomyl and MBC) mouse. MBC is also associated with an increased incidence of hepatoblastomas (a malignant liver tumor) (males) and neoplastic nodules (benign liver tumors) (females) in the SPF Swiss (MBC) strain of mouse. These two strains however, have a high background incidence of liver tumors, particularly in the male. MBC is not oncogenic in the NMRKf mouse, a strain with a low background incidence of liver tumors. Neither fungicide is associated with increased tumorigenicity in other species.

Both benomyl and MBC are spindle poisons which could result in nondisjunction and aneuploidy. They are also weakly mutagenic for the gene mutation end point in vitro.

Other related pesticides, thiophanate methyl and thiophanate ethyl, which are metabolized to MBC and a structural analogue of MBC, respectively, are not associated with oncogenicity or mutagenicity.

Appendix A

Incidence¹ of Liver Tumors²
in Mice from Positive Studies

compound/ strain/ sex	ppm mg/kg	0	150	300	500	1500	3750 ³ 562 ³	5000 750	7500 1125
Benamyl (Haskell) CD-1-male	benign	9/77			9/80	11/79		10/80	
	malig. total ⁴	16/77 25/77			26/80* 35/80*	41/79* 52/79*		17/80 27/80	
-female	benign	2/77			2/80	7/79		7/77	
	malig. total	2/77 4/77			7/80* 9/80	6/79 13/79*		14/77* 21/77*	
MBC (Haskell) CD-1-male	benign	11/80			15/80	14/80	3/80 ³		
	malig. total	2/80 13/80†			5/80 20/80	9/80* 23/80*	0/80 ³ 3/80 ³		
-female	benign	0/79			5/78*	5/80 *			3/78
	malig. Hb total	1/79†† 0/79 1/79††			4/78 0/78 9/78**	15/80*** 1/80 21/80***			12/78** 0/79 15/78***
MBC ⁵ (TNO) Swiss-male	NN	9/100	7/98	14/100				16/100	
	malig. Hb total	1/100 0/100 10/100	1/98 1/98 8/98	2/100 1/100 16/100				3/100 7/100** 17/100	
-female	NN	0/97	1/99	1/98				9/97**	
	malig. Hb total	1/97 0/97 1/97	0/99 0/99 1/99	0/98 0/98 1/98				0/97 0/97 9/97	

¹ number of animals with tumors/number of animals examined.

² liver neopl.

benign (adenomas)

malig. (adenocarcinomas)

N.N. (neoplastic nodules - benign)

Hb. (Hepatoblastoma - a malignant liver tumor)

³ Dose in the HDT males was decreased from 7500 ppm (1125 mg/kg/day) to 3750 ppm (562 mg/kg/day) at week 66, this group terminated during week 73.

⁴ Animals with multiple tumors were counted once in the totals.

⁵ includes the NN and malig. that occurred in conjunction with Hb. They were observed by the Haskell pathologist but omitted in the original TNO report.

* significantly different from control ($p < 0.05$) analysis by study authors using the Fisher's exact test.

** signif. diff. from control ($p < 0.01$), Fisher's exact test.

*** signif. diff. from control ($p < 0.01$), Fisher's exact test.

† significant trend ($p < 0.05$) using Cochran-Armitage test.

†† significant trend ($p < 0.001$) using Cochran-Armitage test.

7/5/82 + add. 1/5/86

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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Jeff Kempter, Chief
SPRD (TS-791)

SUBJECT: Statistical Evaluation and Oncogenicity Risk
Assessment of Benomyl, Benlate and MBC 2-Year
Feeding Studies in Mice

Summary

In the following report it is shown that Benomyl and its metabolite MBC are associated with a statistically significant dose-related increase in the incidence of hepatocellularomas and/or carcinomas in both male and female mice. The mice fed 500 and 1500 ppm MBC and their randomized control group are found to be the most sensitive group for this tumor type and therefore the group most appropriate for quantitative risk assessment. Low-dose extrapolation was performed using five current models and both the point estimators and lower 95% confidence bounds on "Virtually Safe Doses" associated with selected risks are presented in Table 2. Using criteria developed by the Food Safety Council ("Proposed System for Food Safety Assessment", June 1980) the multi-stage model is used to extrapolate cancer risks in man by the potency estimate $Q^* = 2.065 \times 10^{-5}$. Multiplying proposed human exposures by Q^* will estimate the upper 95% bound on cancer risks to man from lifetime exposures.

Discussion

INT-1991, Benomyl Benlate in rice MPP #3194-001, Haskell Laboratory Report No. 21-82 of chronic feeding study conducted from 8/30/78 to 9/12/80 on CD-1 mice, (born 7/17/78 from Charles River Breeding Labs, Wilmington, Mass. received 8/17/78). Three hundred twenty mice were allocated by computer stratified randomization into 4 groups of 60 males and 4 groups of 30 females to Purina Laboratory Chow plus 0, 500, 1500 or 7,500 ppm Benomyl. Due to the delay in weight gain observed in high dose mice of both sexes this dose was reduced from 7,500 to

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5,000 ppm after week 37 and survivors were maintained on 5,000 ppm for the remaining 69 weeks of study. Nevertheless the weight Tables and graphs (See Table I & III of Haskell Laboratory Report 20-82 pages 34-37) presented by the applicant demonstrate that there is a significant reduction in body weights of mid- and high-dosed compared to control animals. As these differences persist after the second week for high dose animals of both sexes and for mid-dosed males, the size of the effect was used to determine whether doses were excessive. The data in these tables demonstrate approximately a 10% reduction among the high dosed animals and 5% or less in the mid-dosed animals. This 10% weight reduction in body weight criterion has been routinely used by the National Toxicology Program as the basis for considering that the maximum tolerated dose has been exceeded. We have therefore omitted the high dose group of each sex from further consideration in the analysis except for the presentation of data in Table I below.

MBC, INT-965 in mice MRP #3207-001, Haskell Laboratory Report No. 70-82 of chronic feeding study conducted 10/3/78-10/16/80 on CD-1 mice, (born 8/29/78 from Charles River Breeding Labs, Wilmington, Massachusetts, 362 were received on 9/28/78.) Three hundred and twenty mice were allocated by computer to stratified randomization into 4 groups of 80 males and 4 groups of 80 females to Purina Laboratory Chow plus 0, 500, 1500, or 7,500 ppm MEC. High dose males had a higher mortality rate during weeks 52-64. The MEC in the diet was reduced to 3,750 ppm at week 66 (after 1 week of no chemical) -- however this group was sacrificed at week 73 due to continued excessive mortality. The reduction in survival and early curtailment of the high-dosed male mice effectively eliminates this group from analysis of tumor incidence. While survival and weight observed in other groups presented no statistically significant trends, the decreases in red cell blood count and hemoglobin in high-dose females and the increased hematocrit and other hematological markers in mid-dosed males may be evidence of competing toxicological manifestations which could bias the rate of tumor formation in these study groups.

Thus in the Benomyl mouse assay the high dosed animals appear to have been exposed to a dose that exceeds the maximum tolerated dose and in the metabolite (MBC) assay males in the high-dose and possible mid-dose have exceeded the MTD while females in the high-dose group may also have exceeded the MTD. Only liver and lung adenomas and/or carcinomas appear in significantly increased proportions. The attached Table I displays the number of liver adenoma and/or carcinoma bearing animals among those still surviving on the date that the first mouse died with a liver tumor. The proportion or rates for each sex and dose group is shown for those mice dying during study, interim findings, and for those animals diagnosed

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at terminal kill. The company analysis, see Attachment 1, addresses only the total findings summarizing or combining the above without considering the additional information to be gained by analysis of these two data sub-sets. When the data in Table I are examined it is immediately evident that the response rates in the high-dose groups do not behave in a pattern consistent with low- and mid-dose responses. This phenomenon has already been anticipated by the weight gain, survival and hematological differences discussed above. There is however an increase in the dose-response slopes for females compared to males in both studies and this effect is most pronounced among the interim MBC females compared to the Benomyl interim or final findings. These findings for hepatocellular adenomas and/or carcinomas are largely due to the increase in carcinoma incidence of treated mice of both sexes. Although the findings for carcinoma alone and adenoma and/or carcinoma are essentially identical, the more general problem of carcinogenesis is more inclusive and therefore a better basic model for estimating oncogenic potential.

Statistical analysis of hepatocellular adenoma and/or carcinoma in the MBC female sub-study using exact tests (Carr, Chu & Tarone, JNCI 66 pp 1175-1181) comparing the incidence in 500 ppm (125 mg/kg/d) treated females to randomized controls results in $P=.03$ for incidental diagnoses, $P=.145$ for the terminal kill, with a combined $P=.0075$. With respect to the 1500 ppm (380 mg/kg/d) dose, the increased incidence of proliferative liver neoplasms is $P=.000,03$ for incidental diagnoses, $P<.001$ for the final kill and $P<5 \times 10^{-6}$ overall.

When utilizing mouse liver data as the information base for extrapolating cancer risks to man it is important to consider all relevant ancillary data available from the available information profile. With respect to Benomyl, no cancer data has been reported in rats, liver and to a lesser degree lung cancer findings have been reported in both sexes in 3 mouse-feeding studies of Benomyl and metabolites of Benomyl. The liver has been reported as a target organ in several species so that oncogenesis may be expected as an ultimate end-point. Other target tissue are, the reproduction system and blood including degeneration of germinal epithelium in males and females, Red Blood Cell count, hemoglobin and hematocrit (data on these 3 variables will be analyzed in a subsequent report). Mutagenicity data submitted have indicated that under selected conditions Benomyl metabolites have mutagenic potential e.g. Sister-Chromatid Exchange and Mouse Lymphoma Cell Point Mutation. From the preceding it seems clear that parametric models for one-hit, multi-stage or multi-hit theories of carcinogenesis are to be preferred over tolerance based models (probit or Weibull); (see Rai & Van Poyen pages 99-117 of Sims Conference Proceedings "Energy & Health" ed. by Breslow & Whittetore, Star 1979)

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Goodness-of-fit is suggested by the Food and Safety Council (see reference in opening paragraph) as a basis for selecting among these models when biological data does not suggest that a particular model is the most appropriate choice. Therefore, the female MBC data from control low and mid-doses have been fit to several models and both point estimates and lower 95% confidence bounds on virtually safe dose levels associated with selected risks of cancer are shown in Table 2. The multi-stage model provides the best fit in that the lack-of-goodness-of-fit has a $P > .999$ indicating almost perfect fit of the observed data to the model.

Using the multi-stage model one can obtain a potency estimator Q^*_1 , which is slope of the lower 95% confidence bound on the virtually safe dose. The MBC data have a Q^*_1 of 2.065×10^{-3} . When Q^*_1 is multiplied by the exposure estimated for a particular crop or commodity we obtain the upper 95% bound on the expected lifetime cancer risk from the estimated exposure. Note, that following the Food Safety Council procedures, no intraspecies dietary correction is used.



Bertram Litt, Statistician
 Toxicology Branch, HED (TS-769)

Attachment

- cc: O. Paynter
- W. Burnam
- M. Sochard
- A. Barton

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Table 1. Benomyl Mouse Bioassay Liver Adenomas and/or Carcinoma

Group	Benomyl (Survivors after first liver tumor)		
	Interim Findings	Terminal Kill	Total
<u>Males</u>			
0 mg/kg/d	13/31	12/43	25/74
64 mg/kg/d	14/33	21/41	35/74
187 mg/kg/d	20/32	32/40	52/72
678 mg/kg/d	8/36	19/40	27/76
<u>Females</u>			
0 mg/kg/d	0/40	4/35	4/73
103 mg/kg/d	5/39	4/31	9/70
286 mg/kg/d	5/41	8/31	13/72
959 mg/kg/d	5/32	16/33	21/65

Males	MBC (Survivors after 1st liver tumor)		
	Interim Findings	Terminal Kill	Total
0 mg/kg/d	7/53	6/18	13/71
81 mg/kg/d	12/51	8/14	20/65
259 mg/kg/d	22/53	1/9	23/62
(1560) mg/kg/d	2/23	1/23	3/46
<u>Females</u>			
0 mg/kg/d	0/29	1/21	1/50
125 mg/kg/d	6/41	3/13	9/54
380 mg/kg/d	13/30	8/14	21/44
1886 mg/kg/d	5/32	10/21	15/53

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Table 2 Binomial M.B.C. Quantitative Risk-Low Dose Extrapolation

Point Estimate of VSD	Added Risk (over Baseline)	Lower Bound	95% Guidance Bound	Upper Bound
6.47 x 10 ⁻⁸	1 x 10 ⁻⁸	2.05 x 10 ⁻³	3.42 x 10 ⁻⁶	1.5 x 10 ⁻⁸
6.47 x 10 ⁻⁷	1 x 10 ⁻⁷	5.29 x 10 ⁻³	2.42 x 10 ⁻⁵	4.03 x 10 ⁻⁷
6.47 x 10 ⁻⁶	1 x 10 ⁻⁶	1.48 x 10 ⁻²	3.42 x 10 ⁻⁴	8.92 x 10 ⁻⁶
6.47 x 10 ⁻⁵	1 x 10 ⁻⁵	4.54 x 10 ⁻²	3.42 x 10 ⁻³	1.97 x 10 ⁻⁴
6.47 x 10 ⁻⁴	1 x 10 ⁻⁴	1.60 x 10 ⁻¹	3.42 x 10 ⁻²	4.26 x 10 ⁻³
6.47 x 10 ⁻³	1 x 10 ⁻³	6.80 x 10 ⁻¹	2.43 x 10 ⁻¹	9.62 x 10 ⁻²
6.47 x 10 ⁻²	1 x 10 ⁻²			5.22 x 10 ⁻¹
6.47 x 10 ⁻¹	1 x 10 ⁻¹			

$$Q_1^* = 2.065 \times 10^{-3}$$

TABLE VII

SUMMARY OF STATISTICAL ANALYSES OF HEPATOCELLULAR NEOPLASMS IN CD-1 MICE FED CARBANIC ACID, (1-[(BUTYLAMINO) CARBONYL]-1H-BENZIMIDAZOL-2-YL)- METHYL ESTER (INT-1991, BENIGN)

Group Designation	III	V	VII*	IV	VI	VIII*
Sex	♂	♂	♂	♀	♀	♀
Dose (ppm)	500	1500	5000	500	1500	5000
<u>Hepatocellular Adenomas</u>						
Fisher's Exact (Livers)	NS	NS	NS	NS	NS	NS
Mantel Haenszel X ² (Life table)	NS	NS	NS	†	NS	NS
X ² Test for Trend (dose response)	NS			†		
<u>Hepatocellular Carcinomas</u>						
Fisher's Exact	NS	+++	NS	NS	NS	++
Mantel Haenszel X ²	†	+++	NS	†	NS	++
X ² Test for Trend	NS			++		
<u>Hepatocellular Adenomas, Carcinomas, and Autolyzed Neoplasm</u>						
Fisher's Exact	NS	+++	NS	NS	†	+++
Mantel Haenszel X ²	†	+++	NS	NS	++	+++
X ² Test for Trend	NS			+++		

† = P < 0.05
 ++ = P < 0.01
 +++ = P < 0.001
 NS = P > 0.05
 * Dose level lowered from 7500 ppm after 30 weeks.

Appendix Table 1A

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SUMMARY OF STATISTICAL ANALYSES OF HEPATOCELLULAR NEOPLASMS IN CD⁰-1 MICE FED CARBAMIC ACID, III-BENZINIDAZOL-2-YL-, METHYL ESTER (INE-965, NBC)

Appendix Table LB

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Group Designation	III	V	VII*	IV	VI	VIII
Sex	♂	♂	♂	♀	♀	♀
Dose (ppm)	500	1500	3750	500	1500	7500
<u>Hepatocellular Adenomas</u>						
Fisher's Exact	NS	NS	**	†	†	NS
Mantel Haenszel X ² (Life table)	NS	NS		†	NS	NS
X ² Test for Trend (dose response)	NS			NS		
<u>Hepatocellular Carcinomas</u>						
Fisher's Exact	NS	†		NS	†††	††
Mantel Haenszel X ²	NS	†		NS	†††	†††
X ² Test for Trend	†			†††		
<u>Hepatocellular, Adenomas, Carcinomas and Hepatoblastoma</u>						
Fisher's Exact	NS	†		††	†††	†††
Mantel Haenszel X ²	†	†		††	†††	†††
X ² Test for Trend	†			†††		

† = P < 0.05 * = Group VII mice received 7500 ppm for first 15 months on study.

†† = P < 0.01 ** = Terminal sacrifice of males receiving the highest treatment level occurred after 17 months on test. Terminal sacrifice for all other treatment groups occurred after 24 months on test.

††† = P < 0.001

NS = P > 0.05

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Peer Review

Benomy1, MBC

5. Appropriate Study DERS not included in previous packages.
 - A. Mouse oncogenicity - Swiss Random
 - b. Mouse oncogenicity - NMRKf(SPF71)

Reviewed by: Marion Copley, D.V.M., D.A.B.T. *5/15/80*
Section VI, Tox. Branch (TS-769C)
Secondary reviewer: Jane Harris, Ph.D. *JH 9/15/80*
Section VI, Tox. Branch (TS-769C)

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005531

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity - mouse TOX. CHEM. NO.: 79C

ACCESSION NUMBER: 256029 (Vol 5: #2, 3, 4)

TEST MATERIAL: MBC

SYNONYMS: Carbendazim
INE-965
2-methoxycarbonyl-amino-benzimidazol

STUDY NUMBER(S): Report # R4936

SPONSOR: Hoechst AG

TESTING FACILITY: The Central Institute for Nutrition and
Food Research, Zeist, Holland (TNO)

TITLE OF REPORT: Carcinogenicity study with Carbendazim in mice

AUTHOR(S): RB Beems, HP Til, CA van der Heijden

REPORT ISSUED: Sept. 1976

CONCLUSIONS: NOEL = 300 ppm (45 mg/kg/day)
LEL = 5000 ppm (750 mg/kg/day) based on hepatic
alterations including increased relative liver weight
(males and females), increased number of foci of cellular
alteration in the livers, neoplastic nodules (adenomas) in
the females and hepatoblastomas in the males.

Classification: core-supplementary

A. MATERIALS:

1. Test compound: MBC, Description greyish-colored, finely granular material. Coded # Hoe 17411 OF AT001 (BAS 346 OF), Purity 99 %.
2. Test animals: Species: mice; Strain: SPF, Swiss random; Age: newly weaned; Weight: not specified; Source: Central Institute for the Breeding of Lab. Animals TNO, Zeist.

B. STUDY DESIGN:

1. Animal assignment: - Mice were received in two shipments, one week apart. Each shipment of 200 males and 200 females, were assigned equally to the following test groups:

Test Group	Dose in diet (ppm)	Main Study 80 weeks	
		male	female
1 Cont.	0	100	100
2 Low (LDT)	150	100	100
3 Mid (MDT)	300	100	100
4 High(HDT) ¹	5000	100	100

¹ Treatment was started at 1000 ppm, increased to 2000 ppm after 4 weeks and again to 5000 ppm after 8 weeks.

2. Diet preparation - The diet was mixed with the compound (frequency of preparation was unspecified), molasses added as a binder, pelleted and stored at -20°C. There is no mention of analysis for stability and concentration.
3. Animals received food and water ad libitum.
4. Statistics - The following procedures were utilized in analysing the numerical data: body weight - Student's t-test, mortality and tumor incidence - chi-square test.
5. A signed quality assurance statement was not included.

C. METHODS AND RESULTS:

1. Observations - Animals were inspected frequently for condition and behaviour.

Results - Toxicity - There is no report of toxicity observed during the study. However, there is no raw or summary data to support this observation.

Mortality (survival) - There is no treatment related effect on mortality which ranged from 30 to 36 % in males and 20 to 23 % in females.

2. Body weight - They were weighed at 0, 2, and 4 weeks then every four weeks for the remainder of the study.

Results - There were no apparent adverse effects on body weight. Summary tables were present, however individual animal data were not present.

3. Food consumption and compound intake - Consumption was not determined.

Results - Food consumption - not applicable

4. Ophthalmological examinations were not performed.
5. There was no hematology or clinical chemistry analysis.
6. There was no urinalysis.
7. Sacrific and Pathology -
 All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination, except where prevented by advanced autolysis. However, only organs with lesions suspected of being tumors and livers (2 sections) were examined histologically.
 In addition, the DOUBLE CHECKED (XX) organs were weighed (not on mice found dead).

<u>X</u>	<u>X</u>	<u>X</u>
Digestive system	Cardiovasc./Hemat.	Neurologic
X Tongue	X Aorta*	X Brain*
X Salivary glands*	X Heart*	Periph. nerve*
X Esophagus*	X Bone marrow*(sternum	X Spinal cord (3 levels)*
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	X Spleen*	Eyes (optic n.)*
X Jejunum*	Thymus*	Glandular
X Ileum*	Urogenital	X Adrenals*
X Cecum*	XX Kidneys*	X Lacrimal gland
X Colon*	X Urinary bladder*	Mammary gland*
Rectum*	Testes*	Parathyroids*
XX Liver*	Epididymides	X Thyroids*
Gall bladder*	Prostate	Other
X Pancreas*	X Seminal vesicle	Bone*
Respiratory	Ovaries	X Skeletal muscle*
X Trachea*	X Uterus*	X Skin
X Lung*	X Genitals (NOS)	X All gross lesions
	X coagulation gland	and masses

NOS - not otherwise specified

Results -

- a. Organ weight - Kidney weight was not affected by treatment. Relative (31% in males and 20% in females) and absolute (38% in males and 20% in females) liver weights in the 5000 ppm group were increased. Individual animal data was not presented.
- b. Gross pathology - There was a treatment related increase in visible liver nodules.

Liver nodules (number observed/number of animals)

Dose (ppm)	males	females
0	4/100	0/100
150	8/100	1/100
300	10/100	1/100
5000	12/100	5/100

These nodules were between a few mm to 1.5 cm in size, and were greyish-white, liver colored or hemorrhagic in appearance. A number of white liver tumors was also observed.

Liver tumors (number observed/number of animals)		
Dose (ppm)	males	females
0	8/100	2/100
150	6/100	0/100
300	5/100	0/100
5000	7/100	1/100

Without individual animal data, it could not be determined whether any mice had both types of liver lesions. These lesions were reported to correspond histologically to neoplastic nodules, hepatocellular carcinomas, fibrosarcomas, leukemias or metastases of mainly lymphoreticular origin. Other gross lesions in tissues other than the liver, did not appear to be treatment related.

c. Microscopic pathology

1) Non-neoplastic lesions, other than liver alterations were not discussed in the report. Clear cell and mixed cell foci were present at increased incidences in livers of the high dose males and females.

Incidence of Non-neoplastic Liver Lesions after MBC Treatment								
Lesions	Male (ppm)				Female (ppm)			
	0	150	300	5000	0	150	300	5000 ¹
cellular alt.								
clear	0/100	0/98	1/100	5/100*	0/97	1/99	0/98	8/97**
mixed	1/100	6/98	6/100	10/100**	0/97	1/99	3/98	0/97

* P < 0.05 (chi-square test)

** P < 0.01 (chi-square test)

2) Neoplastic -

High dose females had an increased incidence of hepatic neoplastic nodules (adenomas) while high dose males had an increased incidence of hepatoblastomas.

It was determined after reexamination of the hepatic sections by Haskell Labs., that all the hepatoblastomas occurred within the borders of hepatocellular adenomas or carcinomas, and that these lesions were not counted separately in the original report. The hepatoblastoma is considered to be a more uncommon and malignant liver tumor than the hepatocellular carcinoma (reevaluation by R. Everett, Haskell-Labs.).

Incidence of Liver Neoplasms¹ in SPF-Swiss Mice
after 80 Weeks Treatment with MBC

ppm	0	150	300	5000	0	150	300	5000
	MALE				FEMALE			
NN ³	9/100	7/98	14/100	16/100	0/97	1/99	1/98	9/97*
malig ³	1/100	1/98	2/100	3/100	1/97	0/99	0/98	0/97
Hb	0/100	1/98	1/100	7/100*	0/97	0/99	0/98	0/97
total ²	10/100	8/98	16/100	17/100	1/97	1/99	1/98	9/97

¹ liver neopl.

malig. (adenocarcinomas)

N.N. (neoplastic nodules - benign)

² Animals with multiple tumors were counted once in the totals.

³ includes the NN and malig. that occurred in conjunction with Hb. They were observed by the Haskell pathologist but omitted in the original TNO report.

* P < 0.01 (chi-square test)

Although there is no indication that treatment related adverse effects occurred in other organs, this could not be confirmed since tissues other than liver were not routinely sectioned unless they contained visible masses.

D. DISCUSSION: This study indicated that MBC is oncogenic for the Swiss random strain mouse causing neoplastic nodules (females) and hepatoblastomas (males) in the liver, particularly in the high dose. Absolute and relative liver weights are increased in the high dose males and females.

Sher (Toxicol. Appl. Pharmacol. 30: 337, 1974) has reported that the male Swiss mouse has a high background incidence of liver tumors. There is no historical control data from the testing facility.

This data can not be adequately examined due to lack of individual animal microscopic pathology and necropsy reports. It could not be readily determined when one mouse had multiple liver lesions.

The study design is limited to the question of the effect of chronic dietary exposure to MBC on the incidence of liver neoplasms in mice. It is only a summary report. The methods were brief and incomplete and diet was not analyzed for compound homogeneity and stability. This study can not be adequately analyzed since there were no individual animal data. Although a complete list of tissues were removed and fixed at necropsy, only all livers (2 sections) and organs with lesions suspected of being tumors were examined histologically. Tables comparing gross and microscopic observations for each animal as well as individual animal data should have been submitted. There was no signed quality assurance statement. Because of the above limitations, this mouse oncogenicity study has been classified core-supplementary.

COPLEY, Disc 17/12, MBC, # 79C, Ln 77, 9/2/86

* Recommended by Subdivision F (Oct. 1982) guidelines for chronic studies. 146

Reviewed by: Marion R. Copley, D.V.M., D.A.B.T.: *MC 4/17/86*
Section VI, Tox. Branch (TS-769C)
Secondary reviewer: Jane Harris, Ph.D. *JCH 4/9/86*
Section VI, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Chronic onco - mice TOX. CHEM. NO.: 79C

ACCESSION NUMBER: 256030-2,4 (Vol. 6-9)

TEST MATERIAL: MBC

SYNONYMS: Carbendazim
INE-965
Hoe 17411 O F AT204

STUDY NUMBER(S): Study # 606
Report No. 643/82

SPONSOR: Hoechst AG

TESTING FACILITY: Pharma Res. Tox., Hoechst AG, Frankfurt

TITLE OF REPORT: Repeated-dose (24-month) feeding study for
determination of the cancerogenic effect of HOE 17411 O F
AT204 (carbendazim) in mice.

AUTHOR(S): Donaubaueer, Schutz, Weigand, and Kramer

REPORT ISSUED: Oct. 13, 1982

CONCLUSIONS: Incidence of liver tumors was not increased in
MBC exposed mice.

NOEL = 300 ppm (35-40 mg/kg/day)
LEL = 5000 ppm (520-650 mg/kg/day) based on liver toxicity
(males and females) consisting of increase in liver
cell hypertrophy, clear cell foci and hepatocellular
necrosis.

Classification: Core-supplementary due to incomplete
examination of most recommended tissues.

A. MATERIALS:

1. Test compound: MBC, > 99 % active compound.
2. Test animals: Species: mouse; Strain: NMRKf(SPF71);
Age: approx. 4 weeks; Weight: mean 20 gm (male), 19 gm (female);
Source: Hoechst AG, Tierzucht Kastengrund

B. STUDY DESIGN:

1. Animal assignment - Animals were assigned randomly to five
test groups. One hundred animals per sex per group were
on test for 22 months and an additional 20 animals per
sex in the controls and high dose groups were interim
sacrificed at 18 months.

005531

Test Group	Dose in diet (ppm)	Total Mice on test		Interim Sac. 18 months	
		male	female	male	female
1 Cont.	0	120	120	20	20
2 Low (LDT)	50	100	100		
3 Mid1(MDT1)	150	100	100		
4 Mid2(MDT2)	300	100	100		
5 High(HDT)	1000/5000*	120	120	20	20

* increased from 1000 to 2000 ppm after 4 weeks and 5000 ppm after another 4 weeks.

2. Diet preparation - Diet was prepared weekly and pelleted. Samples of treated food were analyzed for stability and concentration at 1 to 3 month intervals with samples that were from 0 to 21 days old.

Results - The sample concentration varied irradically with age and from preparation to preparation. It could not be determined whether these variations were due to: 1) inconsistent diet preparation, from week to week or lack of homogeniety; 2) inconsistency in analytical technique and extraction or 3) diet instability. The latter is probably not a factor. The high dose concentrations obtained at analysis deviated most from the theoretical dose (5000 ppm), ranging from 3192 to 4843 ppm, and averaging about 82 % of the theoretical dose.

3. Animals received food (Altromin®) and water ad libitum.
4. Statistics - The following procedures were utilized in analyzing the numerical data: distributed-free method by Nemenyi/Sidak.
5. Signed quality assurance inspection records were submitted periodically to the sponsor.

C. METHODS AND RESULTS:

1. Observations - Animals were inspected daily for signs of toxicity and mortality. A more detailed examination was performed weekly.

Results - Toxicity - There were no treatment-related changes noted in the report.

Mortality (survival) - There was no treatment-related change in survival.

2. Body weight - They were weighed weekly.

Results - There was no treatment related change in body weight.

3. Food consumption and compound intake - Consumption was determined weekly and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.
Water consumption - It was measured monthly using 10 mice/sex/group during a 16 hr period.

- Results - There were no treatment related changes in food consumption, food efficiency and water consumption.
Compound intake - Mean Daily Compound (MBC) Intake

Treatment group (ppm)	for week #s	males mg/kg	females mg/kg
50*	1-96	5.8	7.1
150*	1-96	17.1	21.2
300*	1-96	34.4	41.9
5000	1-96	522.2	648.0
(1000)*	1-4	198.3	215.0
(2000)*	5-8	269.6	327.1
(5000)*	9-96	548.4	682.3

*actual concentration of MBC in diet

4. Ophthalmological examinations were not performed.

5. Blood and urine were not collected.

7. Sacrifice and Pathology -

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues, primarily lung, liver and gross lesions, were collected for histological examination. Lung and liver were also weighed.

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

- Results -

- a. Organ weight - Relative liver weight was increased ($p > 0.05$) at both 18 and 22 months in males and females at 5,000 ppm in comparison with controls.

dose (ppm)	22 month rel. liver wt. (% body wt.)	
	males	females
0	5.62	6.04
50	5.27	5.83
150	5.49	6.02
300	5.47	6.39
5000	7.10	7.95

Lung weight changes were probably not treatment related.

- b. Gross pathology - The only macroscopic change noted was a slight increased frequency of liver changes in the high dose at 18 and 22 months.

- c. Microscopic pathology -

1) Non-neoplastic - The only effects observed at the high dose were a significant increase in liver cell hypertrophy, clear cell foci and hepatocellular necrosis (see tables). Three high dose females had clear cell foci at 18 months. Animals at 10 months had centrilobular hypertrophy and single cell necrosis. Those sacrificed at term showed more marked hypertrophy (centrilobular extending to intermediate areas) along with single cell necrosis. There were also larger areas of necrosis with scarring and fibrosis in many of the high dose term animals. No treatment-related lung lesions were noted.

2) Neoplastic - The incidence of liver tumors was not increased due to treatment with MBC (see table). Lung tumors were also not treatment-related.

Incidence (%) of hepatocellular hypertrophy and/or necrosis

	controls		50 ppm		150 ppm		300 ppm		5,000 ppm	
	M	F	M	F	M	F	M	F	M	F
22 month sac. and early deaths*	(N)(82)	(85)	(88)	(93)	(84)	(78)	(80)	(82)	(80)	(75)
necrosis only	1	11	7	8	8	11	11	16	6	16
necr. & hypert.	0	2	0	0	0	0	0	0	56	60
hypertrophy only	0	2	0	0	0	0	0	0	21	16
18 month sac.	(N)(20)	(20)							(20)	(20)
necrosis only	0	0							0	5
necr. & hypert.	0	0							65	55
hypertrophy only	0	0							35	25

150

* not including early deaths occurring prior to 18 months or animals too autolyzed for adequate histologic examination.

The frequency and the distribution of possible preneoplastic changes and primary liver neoplasms are shown in the following table.

Table 3.	Number of animals								
	Controls	50 ppm		150 ppm		300 ppm		5 000 ppm	
	97♂ 98♀	99♂ 98♀	99♂ 95♀	95♂ 95♀	99♂ 95♀	99♂ 95♀			
Clear cell foci.						3	4		
Basophilic foci.		1	1						
Neoplastic nodules (adenomas)	3	2			1	1			
Hemangiomas.		2	3		2	2		1	

D. DISCUSSION:

There were too many inconsistencies in the diet analysis results to assure that the theoretical levels of the MBC test material were those achieved in the diet. The HDT appeared to average about 18 % lower than 5000 ppm. This study however, was designed to specifically answer the question of hepatic oncogenicity and therefore was classified as core-supplementary (recommended tissues were not routinely sampled, except for liver and lung). There was sufficient hepatotoxicity observed at the high dose to determine that an maximum tolerated dose (MTD) was obtained. No significant treatment-related increases of preneoplastic or neoplastic hepatic or lung lesions were apparent at any dose. This permitted the conclusion that an increased incidence of hepatic or lung tumors at the MTD (high dose) was not associated with MBC in the NMRKf mouse, even though the actual concentration in this group is in doubt.

The systemic NOEL was 300 ppm (35-40 mg/kg/day). The LEL, based on liver toxicity (males and females) was 5000 ppm (520-650 mg/kg/day).

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Reviewer's Peer Review Package for 1st Meeting

10/3/85

007710



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

FILE COPY

OCT 3 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review on Benomyl.
FROM: Reto Engler, Chief
Mission Support Staff
Toxicology Branch/HED (TS-769)
TO: Addressees

A peer review of the weight-of-the-evidence of Benomyls' oncogenic/toxic properties is scheduled for Tuesday, October 8, 1985 at 10:00 AM in Dr. Farber's office (room 816, CM-2).

Benomyl has undergone extensive review including Special Review in the Agency, now a Registration Standard is forthcoming. The committee is expected to assess the Benomyl data base with respect to the Agency's cancer/reproductive effects guidelines.

Attachment

Addressees

- Theodore Farber
- William Burnam
- John Quest
- Louis Kasza
- Bertram Litt
- Marion Copley
- Jane Harris
- Irving Mauer
- Roger Gardner
- Steve Dapson
- Richard Hill

MEMORANDUM

SUBJECT: Weight-of-the-evidence and oncogenic/toxic properties of benomyl.

FROM: Marion P. Copley, D.V.M. *M.P. Copley 10/4/85*
Section 6
Toxicology Branch/HED

THROUGH: Jane Harris, PhD, Section Head *J.H. 10/4/85*
Section 6
Toxicology Branch/HED

TO: The peer review panel for benomyl

Contents

1. Background metabolism and structure of benomyl and MBC (1)
2. Oncogenicity
 - a. overview of mouse oncogenicity (2)
 - b. study information from the PD4 (3)
 - c. risk discussion from the PD4 (6)
3. Mutagenicity
 - a. study information from the PD4 (7)
 - b. risk discussion from the PD4 (8)
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 - a. study information from the PD4 (9)
 - b. risk discussion from the PD4 (10)
5. Spermatogenic effects
 - a. study information from the PD4 (12)
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6. Tables 1, 2, 3, 4, 5, and 7 (there is no 6) (14)
7. Complete one liners (draft)
8. DER for mouse oncogenicity study in CD-1 mice; benomyl
9. Reviews by L. Kasza for oncogenicity CD-1 mice; benomyl and MBC

The following information (except as noted with a (†) is directly from the PD4 published in 1982. The additional information denoted by the (†) is data obtained subsequent to the PD 4 or data not discussed in that document.

1. Background

olism

In addition to the new toxicology data, other information has come to the Agency's attention since 1979 which necessitates changes in or amendments to the exposure estimates presented in the PD 2/3 on benomyl and the PD 2 on thiophanate-methyl.

The Agency indicated in PD 1 (US EPA, 1977a) that benomyl hydrolyzed to MBC in aqueous solution under laboratory conditions (Anonymous, 1974). Further study indicated that in aqueous solution benomyl rapidly breaks down to MBC with conversion being complete within one week (Peterson and Edington, 1969). However, benomyl is relatively stable in spray suspension. Benzimidazole-containing residues of benomyl can be found in soil although most parent benomyl rapidly disappears. Their half-life is three to six months on turf and six to twelve months on bare soil with the major portion of the metabolites being found in the top four inches of the soil (Bauze et al., 1974). Benomyl and its metabolites do not move significantly from the site of application (Rhodes and Lowen, 1974). Benomyl remains on foliar surfaces as a relatively stable residue (Bauze et al., 1973). Relatively little benomyl penetrates leaves. That which does is translocated as MBC and 2-aminobenzimidazole (2-AB) (Hammerschlag and Sissler, 1972 and 1973). MBC is taken up into plants when benomyl is applied to the root area. Only a small amount of benomyl was found in the flesh of oranges stored two months after benomyl dipping. The major portion remained in the peel (Lowen, 1974).

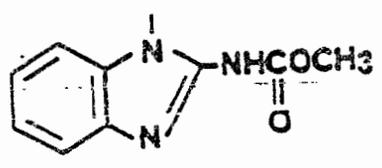
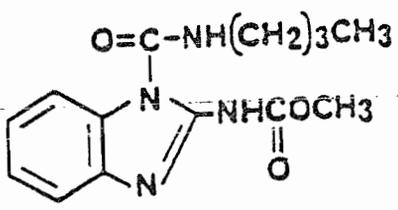
Benomyl is normally metabolized in animals to 5-hydroxy-2-benzimidazolecarbamate (5-HBC), 99 percent of which appears in the urine and feces of dogs or rats within 72 hours of administration of a single oral dose. Other possible metabolites include MBC and 4-hydroxy-2-benzimidazolecarbamate (4-HBC). Benomyl and its metabolites do not accumulate in animal tissues but are eliminated rather rapidly as sulphate or glucuronide conjugates in the urine or feces (Gardiner et al., 1974; Douch, 1973). The Agency indicated in PD 2/3 (U.S. E.P.A., 1979a) that benomyl or its metabolites can reach the mammalian gonads (Styles and Garner, 1974; Gardiner et al., 1974).

Benomyl

(Butylcarbamoyl)-2-benzimidazole-carbamic acid, methyl ester

MBC

2-methylbenzimidazole carbamate



† Q. Oncogenicity - The question of oncogenicity concerning benomyl arose because of the 2 year mouse feeding study (MRID 0096514). An initial review of this and a similar study with MBC (the major, and probably active, metabolite of benomyl) indicated that in CD⁰1 mice, benomyl was associated with an increased incidence of lung and liver tumors (the DERs for the benomyl study and reviews by Dr. Kasza for both compounds are attached. Table 1 has the tumor incidences for alveolar cell carcinoma, liver adenoma and liver carcinoma for males, as well as the day each type of lesion was first detected and how many were observed at the terminal sacrifice. Table 2 has the same information for liver adenomas and carcinomas for females. Table 3 and 4 contain the same data as tables 1 and 2, for MBC.

Benomyl - Chronic mouse: Mice were treated for 2 years with diets containing 0, 500, 1500 or 5000-7500 ppm benomyl. The 7,500 ppm level was reduced to 5000 ppm due to marked reductions in weight gain (male and female). Testicular changes were noted in the high dose group (see table 1). Weight gain also appeared to be decreased in many of the mid and high dose animals.

There was no increased incidence of neoplasms in the 2 year rat or dog feeding studies. The high dose in the rat study was 2500 ppm, no MTD was established.

MBC - Chronic mouse: This 2 year study also used CD⁰1 mice treated at 0, 500, 1500 and 7500 ppm. The HDT group was decreased to 3700 ppm due to excessive toxicity. They (HDT) were sacrificed early due to increased mortality (73 weeks - about 17 months).

A study recently submitted by DuPont, tested MBC in NMRKG(SPF71) mice for 22 months. Although this has not been reviewed thoroughly, preliminary evaluation indicates no increase incidence in neoplasia (no liver tumors at all), but an increased incidence in toxic liver damage consisting of hypertrophy of centrolobular and intermediary hepatocytes, liver necrosis and increased mitotic activity at the HDT, 5000 ppm.

An additional mouse study performed by Beems et al. published in 1976, used SPF, albino, Swiss random bred mice. There was an increased incidence of malignant liver tumors in females at 5000 ppm (duration 80 weeks).

Table 5 presents the historical control data submitted on-12/6/82 by the registrant (for the testing lab.) for pulmonary tumors.

b. Oncogenicity Studies

1 Benzyl Study

a. DuPont Chronic Feeding Study

On March 20, 1981, DuPont provided the Agency with preliminary information from their analysis of liver tissue samples collected during a two-year mouse feeding study with benzyl, which showed an increased incidence of hepatocellular neoplasms (Chen, 1981). On October 27, 1981, DuPont submitted the completed report on the review of all tissues from this study which concluded, "No new oncogenic effects were found during the complete histomorphologic evaluations" (Everett, 1981a). On January 27, 1982, DuPont submitted the final report of this study, which indicated that no tumorigenic effect was observed in any organ system other than the liver (Wiechman, 1982).

In this study, male and female CD-1 mice were given diets containing 0, 500, 1,500 or 5,000-7,500 ppm benzyl. The concentration of benzyl in the diet of the high-dose group was reduced from 7,500 ppm to 5,000 ppm after 37 weeks on test because marked reductions in mean body weights and weight gains were observed in both male and female mice. DuPont scientists concluded, "Benzyl administration in the diets of male and female mice under the conditions of this study resulted in a hepatic carcinogenic effect" (Wiechman, 1982). Survival and the incidence of and time to first observation of palpable tissue masses among mice were comparable in test and control groups. Decreased body weight gains in male and female mice at the 1,500 ppm and 5,000-7,500 ppm groups when compared to controls as well as hepatotoxic changes related to benzyl treatment in the 5,000-7,500 ppm groups were reported. No clinical observations or hematological changes attributed to benzyl were reported.

Although alveolar cell carcinomas occurred at significantly increased rates in the low and intermediate dosed males ($p < 0.05$, Fisher's Exact Test), DuPont scientists concluded that this increase was not an effect of compound administration based on the following information:

- (1) while statistically significant when compared to the pulmonary tumors in the benzyl control male mice (16%), the incidences of pulmonary tumors in the low and intermediate doses (30% and 29%, respectively), were within the range for incidence of such tumors in historical control animals from other such tests in their laboratory (20% to 36% in four other studies);
- (2) a dose response in the X^2 Test for Trend was not observed for pulmonary tumors in male mice receiving benzyl;
- (3) calculation of the median time-to-pulmonary tumor discovery in male mice did not provide evidence for a dose-related response for tumor latency;
- (4) the percent of total pulmonary tumors discovered in animals dying prior to terminal sacrifice did not demonstrate a dose response; and
- (5) a decrease in incidences of pulmonary tumors occurred in male mice treated with MBC compared to their concurrent control group (the metabolic and chemical behavior of benzyl and MBC are reported to be analogous by DuPont) (Everett, 1982).

The Agency has reviewed this study (Kasza, 1981a) and agrees with DuPont's pathologist that oncogenesis was established in the livers of male mice at low and intermediate dose levels and in female mouse livers at all dose levels. There was an increased incidence, not only in benign, but also in malignant hepatocellular and alveolar cell neoplasms in the male. A proportionally higher incidence in decrease of latency (the time interval between treatment and tumor appearance), both in female liver neoplasms and male lung neoplasms occurred. The presence of malignant liver tumors in males and females, the increased incidence in lung tumors in male mice, the decrease in latency in test animals, and the earlier occurrence of female liver tumors in test animals, compared with controls are supporting data for oncogenicity.

Regarding DuPont's conclusion on the incidence of pulmonary tumors in male mice, the Agency considered that it would be just as logical, when referring to the spontaneous incidence of pulmonary tumors in DuPont's historical controls, to remove the high value of 36% (Velpar® test) as an outlier as it would be to remove benomyl. The remaining four studies (Terbacil, MEC, DPX-4189, benomyl) would then have a mean for spontaneous pulmonary tumor incidence in controls of 20% with DPX-4198 being 4% higher and benomyl 4% lower than the mean (Litt, 1982a). As a consequence of its review of the DuPont study, the Agency noted that data exist to support the hypothesis of decreased latency for lung carcinoma in male mice only (Litt, 1982b).

2. MEC Studies

a. DuPont Chronic Feeding Study.

On March 20, 1981, DuPont provided the Agency with preliminary information from their analysis of liver tissue samples collected during a two year mouse feeding study with MEC which showed compound related neoplastic effects in the livers of male and female mice (Everett, 1981b). On October 27, 1981, DuPont submitted the completed report on the review of all tissues from this study which concluded that "no new oncogenic effects were found during the complete histomorphologic evaluations" (Everett, 1981a). On January 27, 1982, DuPont submitted their final report of this study which indicated that no carcinogenic effect was observed in any organ system other than the liver (Wood, 1982).

In this study, male and female CD-1 mice were given diets containing 0, 500, 1,500 or 7,500 ppm MEC. The concentration of MEC in the diet of the high dose male group was reduced from 7,500 to 3,750 during test week 66 and finally, the group was terminated during week 73. DuPont scientists concluded that "MEC administration in the diets of male and female mice under conditions of this study, resulted in a hepatic carcinogenic effect" (Wood, 1982). Male mice in the high dose group tended to consume more diet and had a lower food efficiency than male mice in the control group. Survival of male mice in the intermediate (1,500 ppm) and high (7,500-3,750 ppm) dose groups was significantly lower than that of male control mice. Male mice in the intermediate (1,500 ppm) and high (7,500-3,750 ppm) dose groups and female mice in the high (7,500 ppm) dose group exhibited compound-related histomorphological changes in the kidney. Degenerative or toxic changes were observed in the livers of male mice in the test groups. No effects on body weight, weight gain, clinical observations or hematological parameters that could be attributed to dietary administration of MEC were noted.

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The Agency has reviewed this study and is in agreement with DuPont's pathologist that compound-related hepatic carcinogenicity was established in the male intermediate dose group (1,500 ppm). However, the Agency does not agree that a valid conclusion can be made regarding the high dose male group (7500-3,750 ppm) since it was terminated before the majority of the tumors developed in other groups. In females, the hepatic oncogenic effect was compound and dose related at all dose levels. There was an increased incidence of both benign and malignant tumors in both sexes. Dose-related decreases in latency of tumor appearance were present in both sexes. The oncogenic effect of MEC was comparable to that of the parent chemical, benomy. (in both males and females, the incidence of hepatocellular neoplasms increased in test groups; there was no increased incidence in lung tumors with MEC) (Kasza, 1982b).

b. Beems, Til and van der Heijden

The Agency has reviewed an additional report on the oncogenicity of MEC (Beems, Til and van der Heijden, 1976) that is consistent with the results of DuPont's study on MEC. In this study, SPF, albino, Swiss random bred mice were fed diets containing 0, 150, 300 and 1,000 ppm MEC (high dose increased first to 2,000 ppm and then to 5,000 ppm at weeks 4 and 8, respectively) for 80 weeks. It was concluded that feeding MEC to mice in this study resulted in an increased incidence of malignant liver tumors in males and an increased occurrence of benign liver tumors in females at 5,000 ppm (Sochard, 1981a).

From PD 4

//

C. Oncogenic Risk

a. Derivation of the Potency Estimator

The Agency had no data indicating that benomyl was an oncogen prior to publication of P1 2/3. When the Agency received data which showed that benomyl and its metabolite, MBC, were hepatocarcinogenic in mice, it became necessary to calculate the oncogenic risk presented to humans by this compound.

In its assessment of benomyl's oncogenic risk, the Agency evaluated long term feeding studies in mice with benomyl (Wiechman, 1982) and MBC (Wood, 1982). The details of these studies are discussed in Section II.A. of this position document. The Agency's quantitative risk estimate was based upon data on hepatocellular adenomas and carcinomas in female mice fed 500 and 1500 ppm MBC, since these were found to be the most sensitive groups for this tumor type in either the Wood (1982) or the Wiechman (1982) studies.

When using mouse liver data as the basis for extrapolating cancer risks to man, it is important to consider all relevant ancillary data available. No positive cancer effects have been reported in benomyl tests with rats. In mice, liver tumors were reported in both sexes in one study with benomyl (Weickman, 1982) and two studies with its metabolite, MBC, (Wood, 1982; Beems, Til and van der Heijden, 1976). Evidence also exists to support the hypothesis of a decreased latency for lung carcinomas in male mice treated with benomyl (Weickman, 1982). Data also show that under selected conditions, benomyl metabolites have mutagenic potential (e.g., weakly positive effect in a sister-chromatid exchange study and mouse lymphoma cell point mutation). The liver has been reported to be a target organ for various non-oncogenic effects from benomyl in several species. Target sites for additional effects are the reproductive system and blood.

Since the biological data did not suggest that a particular model was more appropriate than any other for risk estimation, the female MBC data were fit to several models (multi-stage, multi-hit, one-hit, probit, Weibull) and the results analyzed for goodness of fit as suggested by the Food Safety Council (1980). The multi-stage model provided the best fit. Lack of goodness of fit had a $p < 0.999$ indicating almost perfect fit of the data to this model (Litt, 1982a).

The potency estimator, Q^* , derived by the Agency using the multi-stage model was 2.065×10^{-3} (mg/kg body weight/day⁻¹). This is the slope of the lower 95% confidence bound on the virtually safe dose. Q^* is multiplied by the estimated exposure to obtain the upper 95% bound on the expected lifetime cancer risk from that exposure (Litt, 1982a).

The Agency's risk assessment is based on exposure data generated for benomyl use and an oncogenic risk estimator derived from data on liver tumor incidence in a two-year chronic feeding study in mice with the benomyl/thiophanate-methyl metabolite, MBC. Though not estimated directly, the oncogenic risk from thiophanate-methyl is assumed to be about the same as that from benomyl. This assumption is based upon their similar use patterns and data showing that both benomyl and thiophanate-methyl degrade to MBC in aqueous solution, in the soil, and in plants which are ingested by humans or domestic animals.

3 a. Mutagenicity Studies

1. Benomyl

a. Summary of PD 1 Information

In PD 1, the Agency presumed against benomyl registrations based upon evidence that it and the metabolite, MBC, caused mutagenic effects (US EPA, 1977a). PD 1 indicated that both benomyl and MBC caused chromosomal effects in mammalian cell cultures (Styles and Garner, 1974; Debrander et al., 1976) and mammalian tests in vivo (Styles and Garner, 1974; Seiler, 1976). In addition, benomyl caused chromosomal effects in plants (both higher and lower plants) (Zutshi and Kaul, 1975; Boyle, 1973; Dassenoy and Meyer, 1973; Richmond and Phillips, 1975; Davidse, 1973; Hammerschlag and Sisler, 1973).

PD 1 also indicated that benomyl caused point mutations in Fusarium oxysporum (Dassenoy and Meyer, 1973), that Benomyl and MBC caused point mutations in Salmonella tychimurium strains G46, TAL530 and TAL535 (without metabolic activation), and Escherichia coli strain WP2 uvrA, and the MBC caused mutations in Salmonella strain LT-2 (Seiler, 1972; Kappas et al., 1976). PD 1 also included a statement from Seiler (1973, 1975) indicating that point mutations appeared to result from incorporation of benzimidazoles into DNA in place of purine bases. One negative point mutation study was reported in PD 1 (Ficsor and Bordas, unpublished).

b. Summary of PD 2/3 Information

In PD 2/3, the Agency responded to rebuttals regarding mutagenicity. The Agency accepted DuPont's rebuttal of Styles and Garner (1974) which stated that insufficient data were presented in the study to support the claim of chromosomal breakage or anaphase bridges. In addition, DuPont cited several other studies as evidence that benomyl or MBC do not cause chromosomal effects (Dassenoy and Meyer, 1973; Sherman, Culik and Jackson, 1975; Hoffman and Peh, 1974; Mollet, 1976; Siebert, Zimmerman and Lemperle, 1970; Seiler, 1977). The Agency agreed that these studies supported the hypothesis that benomyl does not produce direct chromosomal damage. However, the Agency did not accept them as evidence that chromosomal effects could not result from other mechanisms such as spindle interference or mitotic interruption.

In regard to point mutations, the Agency accepted the DuPont rebuttal argument that Dassency and Meyer (1973) did not unequivocally demonstrate such activity. In addition, the Agency accepted DuPont's argument that Seiler (1972) contained certain omissions that limited its utility for determining the potential for point mutagenic activity and that Kappas et al. (1976) alone was inadequate to determine if benomyl was a point mutagen. Further, the Agency agreed that the studies by Seiler (1972, 1975) did not unequivocally demonstrate that benzimidazole is incorporated into DNA. Data from a mouse spot test (Fabrig and Seiler, 1979) were not definitive enough to demonstrate point mutagenicity in vivo.

DuPont submitted several studies as evidence that benomyl and MBC do not cause point mutations (Haskell Laboratory, 1977). The Agency concluded that the positive results in some of these studies could have been due to a contaminant. Further, the Agency discussed negative results in studies with Bacillus subtilis, Salmonella tychimurium, and Escherichia coli (Shirasu, Mariya and Kato, 1978) and a negative sex-linked recessive lethal test in Drosophila (Lamb and Lilly 1973).

The Agency concluded in PD 2/3 that the available evidence was insufficient to conclude that either benomyl or MBC react directly with DNA to cause point mutations or direct chromosomal aberrations in mammalian systems. However, the Agency indicated that available evidence supported the presumption that benomyl or its metabolite, MBC, were spindle poisons capable of inducing non-disjunction, and that benomyl and its metabolites may reach the mammalian gonad. This evidence provided an adequate basis for a qualitative presumption against benomyl for potentially heritable spindle effects. However, the Agency indicated that evidence was lacking to demonstrate definitively that benomyl induced such effects in germ cells, although preliminary information (Tates, 1978) indicated that MBC might cause non-disjunction in germ cells. The Agency indicated that persons exposed to high levels of benomyl might be at risk from induction of spindle effects. However, a means to quantify such risk was not available and the existing data were not adequate to demonstrate the existence of a significant risk at estimated exposure levels (US EPA, 1979a).

c. Data Review

Additional data bearing on the mutagenicity of benomyl were received subsequent to publication of the PD 2/3.

Existing data on mutagenicity are summarized in this section.

1) Gene Mutations

Two studies provided evidence of no mutagenic activity in S. tychimurium (Fischer et al., 1978; Shirasu et al., 1978), however, the dosage levels used were below the 5,000 to 10,000 ug/plate range at which activity was noted in the other studies. Similar results in S. tychimurium were reported for technical and analytical grade MBC. Mutagenic activity was reported at a dose range from 4,000 to 10,000 ug/plate for MBC.

Several reports on the mutagenic activity of benomyl and MBC in fungi (Dassenoy and Meyer, 1973; Guerzoni et al., 1976; and Kappas and Bridges, 1981) suggest that benomyl can induce gene mutations in Aspergillus nidulans and such activity has also been reported in A. nidulans and C. cucumerinum when MBC was tested (Nirenberg and Speakman, 1981; and Speakman and Nirenberg, 1981). However, purity of the benomyl and MBC which was tested was not established, therefore, a comparison of these results cannot be made with those results obtained in bacterial tests. These results also need to be considered in light of the studies by Kumari et al. (1977) which suggest that benomyl and MBC do not directly interact with DNA in fungi. (See discussion under primary DNA damage).

In vitro mammalian cell assays present uncertain results for benomyl in that no genetic effects were noted in Chinese hamster ovary cells (Fitzpatrick and Krahn, 1980) while the fungicide was mutagenic in mouse lymphoma cells (Jotz et al., 1980). One study with MBC in Chinese hamster ovary cells has been reported but no gene mutation induction was noted (Waters and Krahn, 1980).

No sex-linked recessive lethal mutations were reported by Mollet (1976) or Lamb and Lilly (1980) in Drosophila melanogaster exposed to benomyl or MBC. However, these studies need confirmation from other test systems or repeated assay with Drosophila

2) Primary DNA Damage

No effect was observed in a Bacillus subtilis differential toxicity assay reported by Shirasu et al. (1978) or a mitotic gene conversion study in Saccharomyces cerevisiae (Siebert et al., 1978), however, dosages tested in these studies were not at or near cytotoxic levels established in other test systems. However, a study by de Bertoldi et al. (1980) showed that benomyl did not induce mitotic gene conversions in S. cerevisiae or A. nidulans.

Studies of the uptake of radiolabelled phosphorous into DNA, DNA melting profiles, and occurrence of ¹⁴C-label in DNA after exposure of fungi to labelled MBC suggested that the fungicide and its metabolite do not directly interact with DNA (Kumari et al., 1977). The study indicated that interference with the processes of DNA precursor biosynthesis without becoming part of the macromolecule itself occurred. These findings suggested that mutagenic activity of the fungicide and its metabolite noted in fungi is likely to result from a contaminant in the test material.

An increased frequency of sister chromatid exchanges in Chinese hamster ovary cells in vitro was reported by Evans and Mitchell (1980) after exposure of the cells to benomyl with and without metabolic activation. However, the response was described as weak.

In vitro studies were conducted with benomyl and MBC to determine the effect of the chemicals on DNA synthesis in primary rat and mouse hepatocyte cultures (Tong, 1981a, b, c, and d). Neither chemical induced DNA synthesis.

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3) Chromosome Damage

The Agency originally cited reports in which benomyl and MBC were shown to damage chromosomes. Rebuttal arguments were accepted by the Agency against some of the studies, but additional data in support of the presumption have been found. Richmond and Phillips (1975) noted the occurrence of chromosomal aberrations (bridges). Zutshi and Kaul (1975) reported observing metaphase aberrations in barley exposed to Benlate and isolated breaks and chromatid exchanges in treated broad beans have also been observed.

Cultured human leukocytes treated with benomyl had more chromosomal aberrations than untreated cultures (Gupta and Legator, 1975), while in a separate experiment, human leukocytes did not have increased frequencies of chromosomal aberrations when treated with MBC (Lamb and Lilly, 1980).

In vivo cytogenetics studies were reported in several animal systems. No effects on chromosomes of bone marrow cells were reported in rats treated with Fundazole 50 WP (Ruzicska et al., 1975). No effects were noted (Seiler, 1976) on chromosomes in bone marrow cells from Chinese hamsters and mice treated with MBC.

Doses as high as 2500 ppm in the diet of rats did not induce dominant lethal effects (Sherman et al., 1975). Similar studies were conducted with MBC in mice (BASF, 1973; Hoechst, 1974; and BASF, 1975) (see PD 2 for thiofanate-methyl). These studies were negative.

A micronucleus test reported by Kirkhart (1980) showed that benomyl increased the number of micronuclei in polychromatic erythrocytes in treated mice. However, the effect could result from spindle effects (see Spindle Effects section below).

4) Chromosomal Nondisjunction

Chromosomal nondisjunction was observed in A. nidulans exposed to Benlate® 50 WP and benomyl (Dassency and Meyer, 1973; Kappas et al., 1974). MBC was also found to induce chromosomal nondisjunction in A. nidulans.

Benlate, benomyl, and MBC were reported to induce lagging chromosomes at telophase in mitosis of onion root tip cells. Boyle (1973) reported that the metaphase chromosomes were more condensed than normal.

5) Spindle Effects

MBC has been found to interfere with polymerization of microtubulin which is needed for the formation of spindle fibers in cell division. Many of the results from tests evaluating chromosomal effects may arise from affected cell division processes. Therefore, those studies could be reflective of microtubule inactivation. Chromosomal nondisjunction, lagging chromosomes, multinucleation, micronuclei formation, and chromosomal breaks and aberrations could result from microtubule inhibition during cell division.

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conclusion of PD 4 b Mutagenic Risk

The Agency issued a presumption based on suspected mutagenicity against benomyl on December 6, 1977 (US EPA, 1977a) and on thiophanate-methyl on December 7, 1977 (US EPA, 1977b). Both fungicides are metabolized to MEC which was presumed to have a role in their mutagenicity.

Studies specific for detection of gene mutation suggest that benomyl and MEC may be active. However, comparison of test results in the bacterial systems strongly suggest that the activity reported was initiated by contaminants of the active ingredients. This consideration is also applicable to interpretation of the results with the mouse lymphoma cell system. Consideration of results in the fungal DNA tests and yeast gene conversion tests also strongly support a conclusion that benomyl and MEC do not directly interact with DNA, although their contaminants might do so.

Although benomyl has been shown to induce sister chromatid exchanges in Chinese ovary cells in vitro, studies with the same cell type showed no mutagenic activity at the HGPRT locus. Based on studies with the fungicide and its metabolite in cultures of rat and mouse liver cells, it is unlikely that the two chemicals induce DNA synthesis. In view of these findings and conditions of the SCE assay performed, the results cannot be considered as positive evidence that benomyl is capable of damaging DNA in Chinese hamster ovary cells. The other studies mentioned above suggest that the fungicide does not induce primary DNA damage.

Interference with cell division through binding to microtubulin potentially results in numerical chromosomal aberrations such as those detected in plants (tripolar anaphase nuclei, metaphase arrest, unbalanced chromosome number) and in animals (micronuclei, anaphase bridges, etc.). These effects are likely to result in teratogenicity in laboratory animals since morphological development of organs also depends on microtubules. Studies regarding these effects are discussed in the teratogenicity sections of this PD 4.

Studies cited by the Agency in PD 1 for benomyl (Busey, 1968; Littlefield, 1969; and Hoffman and Kirsch, 1974) suggest that it is capable of reaching the gonads of experimental animals. However, the genotoxic effects discussed herein are more likely to arise from the effects on microtubules in dividing cells. Because there is no validated method for quantitatively assessing the hazards which may result from these effects, the significance of the presence of residues of benomyl or MEC in the gonads cannot be quantified (Gardner, 1982).

The Agency previously concluded in its PD 2 that thiophanate-methyl is not mutagenic. However, its metabolite MEC was considered to be a spindle poison. Available data and the absence of validated test procedures do not provide a basis for changing the previous conclusions as stated by the Agency in its Thiophanate-methyl PD 2, which indicated that the Agency does not have the means to estimate risk from such exposures.

4 a. Teratogenicity Studies

The Agency considered three studies in concluding that the risk criterion for teratogenicity had been exceeded in its benomyl PD 1 (US EPA, 1977a). Schtenberg and Torchinski (1972) reported effects including brain hernias, hydrocephalia, microphthalmia and anophthalmia when benomyl was administered by gavage to Wistar rats (either on days 1 to 20 or days 7 to 15 of gestation). Doses of 125 mg/kg/day were teratogenic. The NOEL was 62.5 mg/kg/day. Torchinski (1973) also induced teratogenic effects in Wistar rats given a 145 mg/kg dose of benomyl by gavage on day 12 of gestation. PD 1 also discussed a study (Sherman, Culik and Jackson, 1975) which reported that a diet containing about 400 mg/kg/day benomyl was not teratogenic to Charles River-CD rats.

In PD 2/3 the Agency concluded that the risk criterion for teratogenicity had not been rebutted (US EPA, 1979a). The Agency considered the teratology studies discussed in PD 1 and additional studies received after its publication. These included Agency-sponsored studies showing teratogenic effects down to the lowest dose tested (gavage) 62.5 mg/kg/day (Short et al., 1979; Kavlock, 1979), a DuPont study showing no teratogenic effects in CHR-CD rats following dietary dosing of the benomyl metabolite MBC up to 500 mg/kg/day (Baskell Laboratory, 1978), and a study showing teratogenic effects in Sprague-Dawley rats following gavage administration of MBC (Delatour and Richard, 1976). None of these studies established a firm NOEL for benomyl's induction of teratogenic effects. Therefore, both EPA and DuPont committed themselves to perform additional teratology studies on benomyl. The Agency assumed in PD 2/3 that the NOEL for teratogenic effects would not be less than the NOEL for spermatogenic effects (the most sensitive effect for benomyl). Therefore, for its risk estimates in PD 2/3 the Agency used the level of 7.5 mg/kg/day (from a 1969 test by Hornberger showing spermatogenic effects in rats following inhalation exposure to benomyl). The margins of safety (MOS) for exposed populations was then estimated to range from 21 to 380.

Since publication of the PD 2/3 the Agency has received additional studies on the teratogenicity of benomyl. Kavlock, Chernoff, and Gray (1980) showed teratogenic effects in Wistar rats following gavage dosing at 0, 15.6, 31.2, 62.5, and 125 mg/kg/day (days 7-16 of gestation). The lowest effect level (LEL) was 62.5 mg/kg/day and the NOEL was 31.2 mg/kg/day. A dietary dosing support of this study did not show teratogenic effects when rats were fed diets containing up to 500 mg/kg/day of benomyl. A study by DuPont (Staples, 1980) showed teratogenic effects when CHR-CD rats were given benomyl by gavage. Dose levels were 0, 3.0, 10.0, 30.0, 62.5, and 125.0 mg/kg/day. The LEL was 62.5 mg/kg/day and the NOEL was 30 mg/kg/day. Unilateral microphthalmia was reported at 10 mg/kg/day in two animals in this study. Although the effect was biologically significant, its occurrence was not statistically significant at that level. This effect occurred at a higher dose level at a statistically significantly increased incidence. DuPont is currently performing a follow-up study (using doses of 30 mg/kg/day and below) to further examine occurrence of this eye effect following gavage dosing. The Agency has, therefore, provisionally set a NOEL of 30 mg/kg/day for use in its teratogenic risk assessment.

† A rat teratology study, performed specifically to determine the NOEL for the occurrence of microphthalmia, has been reviewed. The levels tested were 0, 3, 6.25, 10, 20, 30 and 62.5 mg/kg. Microphthalmia was only observed at 62.5 mg/kg, mid-maternal-toxicity also was observed at this level. The NOEL for teratogenicity was set at 30 mg/kg and the LEL was set at 62.5 mg/kg.

b. Teratogenic Risk

Table 7 presents the results of benomyl teratology testing. These results show that teratogenic effects have occurred following gavage dosing of rats at levels ranging from 62.5 mg/kg/day to 500 mg/kg/day, and in mice at 200 mg/kg/day. The lowest NOEL determined by gavage testing was 30 mg/kg/day (Staples, 1980). Dietary dosing did not result in teratogenic effects in three studies with rats with doses from 5 to 500 mg/kg/day of benomyl or 500 mg/kg/day of MBC.

The Agency calculated margins of safety (MOS) for both dietary and non-dietary exposure to benomyl based on the provisional NOEL of 30 mg/kg/day. This was taken from the DuPont teratogenicity study (Staples, 1980) in which CHR-CD rats receiving a dose of 0, 3, 10, 30, 62.5 or 125 mg/kg/day of benomyl by gavage developed teratogenic effects at doses of 62.5 mg/kg/day and above. This study is being repeated by DuPont to confirm this NOEL, since biologically relevant eye effects were seen in two animals at 10 mg/kg/day; however, the incidence of this effect was not statistically significant at this level (Sochard, 1981b). This risk will be reassessed in the future if the results from DuPont's new study show a need to do so.

It should be noted that the procedure of using results of animal tests employing gavage dosing (as was done by Staples, 1980 from which the 30 mg/kg/day NOEL was derived) to calculate human risk from dietary exposure is controversial. DuPont (Smith, 1981) considers such risk estimates to be inappropriate, preferring instead to use results from studies employing dietary dosing of animals. DuPont points out, that gavage studies with benzyl have induced teratogenic effects at doses of 62.5 mg/kg/day and greater. Dietary dosing on the other hand, has not induced teratogenic effects.

Further, DuPont maintains that benzyl and MBC can cross the placenta following gavage administration to rats (Haskell Laboratory, 1980a), but with dietary administration, benzyl does not cross the placenta in detectable quantities (Haskell Laboratory, 1980b). DuPont considers that a large "bolus" dose of the compound administered by gavage might overwhelm the metabolic capacity of the liver, resulting in high doses of benzyl to the embryo. Large dietary doses, however, are more slowly absorbed and can be rapidly metabolized by the liver, giving low maternal blood levels and no detectable embryonic levels of benzyl. They concluded that dietary administration was a more appropriate method for human hazard assessment.

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 benzyl should be qualified by the essentially negative results obtained in dietary studies (Fowler, 1979).

Kavlock et al. (1982) compared dietary and gavage administration in rats and mice. Oral administration was teratogenic above 31.2 mg/kg/day in the rat and above 50 mg/kg/day in the mouse. Dietary administration caused only fetal growth retardation in rats at the highest test dose (505 mg/kg/day). Kavlock et al. (1982) pointed out that rats consume most of their feed in short bouts throughout the night with some being consumed during the day. Assuming equal metabolic potential and absorption into the blood, dietary administration of chemicals like benzyl with short half-lives would result in low circulating levels in the blood since excretion per unit of time can equal or exceed ingestion per unit time. Since humans consume most of their food at two to three discrete daily time periods, higher peak plasma levels of benzyl would be expected from the human feeding pattern than would occur in the rat. Kavlock et al. (1982) concluded that for chemicals like benzyl which have short biological half-lives, gavage is the appropriate dosing regimen for basing human risk extrapolations from dietary exposure.

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 Kavlock et al. (1982) opinion, believing that gavage assures relevance of treatment to the human condition for chemicals like benzyl, 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 clearly parallels the human situation than continuous uptake of the chemical in the rat diet. Even so, the Agency considers that gavage may be a more conservative indicator of this risk and in interpreting MCS's the Agency has taken this into consideration.

5 a. Spermatogenic Effects Studies

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In PD 1 the Agency considered a number of studies which identified the testes as a primary target of benomyl. In an acute toxicity test, Sherman and Krauss (1966) reported the degeneration of germinal tissue and aspermatogenesis in

rats receiving gavage treatment of benomyl. The lowest effect level (LEL) was 3400 mg/kg, the lowest dose tested. Sherman and Krauss (1966) also reported a reduction of sperm in mature rats receiving ten doses of benomyl at 200 mg/kg/day. Sherman (1965) also reported reduction of sperm in rats receiving a single 670 mg/kg benomyl dose by gavage.

Sperm production was not affected in chronic feeding studies in dogs (Sherman et al., 1970) and rats (Sherman et al., 1969) receiving benomyl at doses of up to 2500 ppm in the diet. Further, the fertility index was not affected in a three-generation reproductive effects study in rats receiving up to 2500 ppm benomyl in the diet (Sherman et al., 1975).

PD 1 also reported on the results of inhalation studies with benomyl on rats (Hornberger, 1969) and dogs (Littlefield, 1969) which showed a reduction of spermatogenic activity. The lowest NOEL (7.5 mg/kg/day) occurred in the rat study (Hornberger, 1969). In PD 2/3 (US EPA, 1979a) the Agency concluded that the risk criterion had not been rebutted. The margins of safety (MOS) for this effect were based upon the NOEL of 7.5 mg/kg/day via the inhalation route of exposure (Hornberger, 1969). These ranged from 21 to 380.

In a study by Carter and Laskey (1982) adult Sprague-Dawley rats were given ten daily doses of 0, 200 or 400 mg benomyl/kg/day by gavage. Various parameters were measured 14 days after treatment ended. Carter and Laskey reported statistically significant depression in the total epididymal (caudal and caput) sperm counts and in the vas deferens sperm concentration in rats treated with 200 to 400 mg/kg/day. A slight to severe generalized hypospermatogenesis in two animals and a slight to moderately severe hypospermatocytogenesis in test animals was reported in the six animals given histological evaluation of the testes (dose level was 400 mg/kg/day). In addition, caudal epididymis weights were significantly depressed with benomyl treatment.

However, Carter, Hein and Laskey (1982) reported that ten daily gavage treatments of prepubertal Sprague-Dawley rats with 0 or 200 mg benomyl/kg/day did not cause damage to the reproductive system while the same exposure in adults caused significant reproductive effects (Carter and Laskey, 1982).

Kavlock et al. (1982) reported a decrease in testicular weights at 31.2 mg/kg/day in the offspring of Wistar rats receiving gavage treatments of benomyl during both gestation and lactation. No effects on any parameters were evident in rats receiving 15.6 mg/kg/day by gavage. The above studies all show effects at levels higher than those reported by Hornberger (1969). Therefore, the NOEL from that study is used in the spermatogenic effects risk assessment in this document.

† The MOS for dietary exposure would probably be more sound if based on a chronic dietary study rather than the inhalation study as discussed in the PD4. The dog NOEL would be the lowest for dietary exposure.

b Risk from Spermatogenic Effects in Males

Table 7 presents the results of benomyl/MBC spermatogenic effects testing. These results show that spermatogenic effects have occurred following both single and multiple gavage dosing of rats at dose levels from 62.5 mg/kg/day to 3400 mg/kg/day. The lowest NOEL determined by gavage dosing (both during gestation and lactation) was 15.6 mg/kg/day (Kavlock, Chernoff and Gray, 1982). Inhalation dosing in dogs and rats caused a reduction of spermatogenic activity in rats at 33 mg/kg/day (Hornberger, 1969) and in dogs at 82 mg/kg/day (Littlefield, 1969). The NOEL determined by inhalation testing was 7.5 mg/kg/day. The next highest dose level was 33 mg/kg/day (Hornberger, 1969). Dietary dosing of dogs and rats at levels greater than 2,500 ppm did not affect sperm production. Further, the fertility index of rats was not affected by dietary dosing in a three-generation reproductive effects study at levels up to 2,500 ppm.

The Agency calculated margins of safety (MOS) for both dietary and non-dietary exposure to benomyl based on the NOEL of 7.5 mg/kg/day developed in the inhalation study (Hornberger, 1969). Non-dietary MOS's are shown in Table 8. The MOS's range from 21 to dietary background levels for benomyl users without respiratory protection. Exposure for mixer/loaders could be reduced by up to 90 percent by use of a dust mask (EPA/OPP Protective Clothing Workgroup, 1982). This would cause the spermatogenic effects MOS's to range from 150 to dietary background levels if a dust mask were used during mixing/loading of benomyl.

† Although the rat and dog chronic feeding studies indicated no testicular lesions at the HDT, 2500 ppm (dog - 62.5 mg/kg/day, rat - 125 mg/kg/day) the benomyl CD-1 mouse study indicated degenerative testicular and epididimal changes at 5000 ppm (750 mg/kg/day). The resultant NOEL was 1500 ppm (225 mg/kg/day).

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Table 1 benomyl in male mice

Selected Histopathologic Findings in Male Mice

	Groups			
	I	III	V	VII
	control	75 mg/kg/day 500ppm	225 mg/kg/day 1500ppm	750-1125 mg/kg/day 5000-7500ppm
<u>LUNG</u>				
Alveolar Cell Carcinoma	13/79	24/80	23/79	16/80
<u>LIVER</u>				
Adenoma	9/77	9/80	11/79	10/80
Carcinoma	16/77	26/80	41/79	17/80
Total Neoplasms	25/77	35/80	52/79	27/80
<u>TESTES</u>				
Atrophy	12/78	12/79	8/79	31/79
<u>Interstitial Cell</u>				
Hyperplasia	4/78	4/79	7/79	18/79
<u>EPIDIDYMS</u>				
Atrophy Sperm	18/78	11/78	12/79	30/79
<u>Distended Tubuli with Degenerated Sperms</u>	9/78	5/78	11/79	17/79

(in Male Mice)

	Groups								
	I		III		V		VII		
	I*	()#	T**	I	T	I	T	I	T
<u>LUNG</u>									
Alveolar Cell Carcinoma	1	(659),	12	13	(445),	11	10	(380),	13
9	(574),	7							
<u>LIVER</u>									
Adenoma	5	(530),	4	4	(445),	5	6	(541),	5
Carcinoma	8	(545),	8	10	(470),	16	14	(590),	27
5	(508),	12							
Total Neoplasms	13	(530),	12	14	(445),	21	20	(541),	32
8	(508),	19							

* Lesions before terminal sacrifice.

First day on test when lesion was detected.

**Lesions at the time of terminal sacrifice.

Table 2 benomyl in female mice

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Selected Histopathologic Findings in Female Mice

	Groups			
	II control	IV 75mg/kg/day 500ppm	VI 225mg/kg/day 1500ppm	VIII 750-1125mg/kg/day 5000-7500ppm
<u>SPLEEN</u>				
Myeloid Metaplasia	10/76	22/79	24/78	16/74
<u>LIVER</u>				
Adenoma	2/77	2/80	7/79	7/77
Carcinoma	<u>2/77</u>	<u>7/80</u>	<u>6/79</u>	<u>14/77</u>
Total Neoplasms	4/77	9/80	13/79	21/77

Presence of Lesions Before and At the Time of Terminal Sacrifice

(in Female Mice)

	Groups							
	II		IV		IV		VIII	
	I*()#	T**	I	T	I	T	I	T
<u>LIVER</u>								
Adenoma	0 (), 2		1(641), 1		4(650), 3		2(644), 5	
Carcinoma	<u>0 , 2</u>		<u>4(640), 3</u>		<u>0 (), 6</u>		<u>3(426), 11</u>	
Total Neoplasms	0 4		5 4		4 9		5 16	

Lesions before terminal sacrifice.

First day on test when lesion was detected.

Lesions at the time of terminal sacrifice.

Table 3 MBC in male mice

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Selected Histopathologic Findings in Male Mice

	<u>Groups</u>			
	<u>I</u> control	<u>III</u> 75mg/kg/day 500 ppm	<u>V</u> 225mg/kg/day 1500 ppm	<u>VII</u> 555-1125mg/kg/day 3400-7500 ppm
<u>KIDNEY</u>				
Tubules contain yellow-brown granular material	7/30	3/79	19/78	47/79
<u>LIVER</u>				
Adenoma	11/80	15/80	14/80	3/80
Carcinoma	2/80	5/80	9/80	0/80
Total Neoplasms	13/80	20/80	23/80	3/80
Hepatocellular Necrosis Focal and Centrolobular	1/80	7/80	10/80	13/80
<u>TESTES</u>				
Sperm Stasis Bilateral, Unilateral	7/77	13/78	16/80	22/74

Presence of Liver Neoplasms Before and At the Time of Terminal Sacrifice
in Male Mice

	<u>Groups</u>						
	<u>I</u>	<u>III</u>		<u>V</u>		<u>VII</u>	
	I* ()#, T**	I	T	I	T	I	T (NA) ^c
<u>LIVER</u>							
Adenoma	6(430), 5	10(467), 5		14(459), 0		3(434),	c
Carcinoma	1(629), 1	2(649), 3		8(616), 1		0 (0),	c
Total Neoplasms	7(430), 6	12(467), 8		22(459), 1		3(434),	c

* Lesions before terminal sacrifice.

First day on test when lesion was detected.

** Lesions at the time of terminal sacrifice.

c Not applicable. mice were sacrificed at 73 weeks due to related mortality

Table 4 MBC in female mice

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Selected Histopathologic Findings In Female Mice

	<u>Groups</u>			
	II	IV	VI	VIII
	control	75mg/kg/day 500ppm	225mg/kg/day 1500 ppm	555-1125mg/kg/day 3400-7500ppm
<u>KIDNEY</u>				
Macrophages with yellow-brown pigment	5/80	4/78	3/80	21/76
<u>LIVER</u>				
Adenoma	0/79	5/78	5/80	3/78
Carcinoma	1/79	4/78	15/80	12/78
Hepatoblastoma	0/79	0/78	1/80	0/78
Total Neoplasms	1/79	9/78	21/80	15/78
<u>THYMUS</u>				
Lymphoid Depletion	3/38	4/25	12/44	10/38

Presence of Liver Neoplasms Before and At the Time of Terminal Sacrifice
in Female Mice

	<u>Groups</u>								
	II		IV		VI		VIII		
<u>LIVER</u>	I*	()#	T**	I	T	I	T	I	T
Adenoma	0	(0)	0	4(648), 1	4(636), 1	1(624),	2		
Carcinoma	0	(0)	1	3(704), 1	7(536), 8	6(551),	6		
Hepatoblastoma	0	(0)	0	0(--), 0	1(704), 0	0(--),	0		
Total Neoplasms	0	(0)	1	7(648), 2	12(536), 9	7(551),	8		

* Lesions before terminal sacrifice.
First day on test when lesion was detected.
** Lesions at the time of terminal sacrifice.
c Not applicable.

- The incidence rate of pulmonary tumors observed in the control male mice in the benomyl study was at the low extreme of our historical range for pulmonary tumors in control male mice. The studies reported below were conducted between September, 1977 and March, 1981.

<u>Compound</u>	<u>Number of Pulmonary Tumors in Male Control Group/ Number of Lungs Examined</u>	<u>Percentage of Male Mice With Pulmonary Tumors</u>
H-11086	13/80	16
H-11135	18/98	18
H-11201	16/80	20
H-12700	19/79	24
H-10963	17/67	25
H-10720	23/80	29
H-11265	29/80	36
Benomyl	13/79	16

} Mean = 24

- The incidences of pulmonary tumors in male mice in the low and intermediate dose groups in the benomyl study were 24 of 80 (30 percent) and 25 of 79 (29 percent), respectively. While these incidence rates were statistically significant ($P < 0.05$, Fisher's Exact Test) when compared to the low incidence of pulmonary tumors in the benomyl controls, both incidence rates were within the range for our historical controls (16 to 36 percent).

Table 7
Teratogenic and Reproductive Effects of Bromoxy

Species	Dosing	LEL/NREL (mg/kg/day)	Results	Reference
I. Teratogenic Effects				
A. Gavage Dosing				
Wistar Rats	1) 0, 62.5, 125, 250, 500 mg/kg/day during days 7-15 gestation 2) as above, days 1-20 gestation Gavage	NREL = 62.5 LEL = 125	Brain hernias, hydrocephalia microphthalmia, arnophthalmia	Richtenberg and Torchinski, 1972
Wistar Rats	145 mg/kg, day 12 gestation Gavage	NREL = none LEL = none Calculated insufficient data	Teratogenic	Torchinski, 1973
Wistar Rats	0-500 mg/kg/day; days 6-15 gestation Gavage	NREL = <62.5 (LPM) ¹ LEL = 62.5	Dose-related fetal weight decrease + increase in mal- formations-OES herniations & reduction defects of extremities, lack of eye bulges	Sixt et al., 1979
CD-1 Mice	200 or 400 mg/kg/day in corn oil, days 8-12 of gestation Gavage	NREL = <200 LPL = 200	Complete resorption of 63% at 400 mg/kg/day; litter size 59% of controls. At 200 mg/kg/day weights of pups diminished by 11% herniations of OES and reduction of extremities reduced above 11.2 mg/kg/day.	Kavlock, 1979
CHR-CD Rats	0, 3, 10, 30, 62.5, 6 125 mg/kg/day Gavage	NREL = 30 LEL = 62.5	Unilateral microphthalmia at 10 mg/kg/day in 2 animals. Embryotoxicity at 62.5 mg/kg/day above.	Staples, 1980
Sprague- Dawley Rat	Gavage-days 8-15 of gestation dose MFC=9.55 mg/kg dose bromoxy=116 mg/kg	Small sample size precipitates deter- mination.	Terata produced-encephaly (MFC treated) and other malformations	Delatour and Richard, 1976

Table 7 Continued

I. Teratogenic Effects Continued

A. Gavage Dosing Continued	
CD-1 Mice	
0, 50, 100, 200 mg/kg/day on days 7-17 of gestation Gavage	<p>NMEL = 50 LEL = 100</p> <p>All parameters affected at high dose. Weight, supra-occipital scars, supernumerary ribs, subaxial vertebral centrae affected at 100 mg/kg/day.</p> <p>Kavlock et al., 1982</p>
Mistar Rat	
0, 15.6, 31.2, 62.5, 125 mg/kg/day on days 7-16 of gestation Gavage	<p>NMEL = 31.2 LEL = 62.5</p> <p>Embryonic resorptions at 125 mg/kg/day. Fetal weight reduced at 31.2 mg/kg/day and above skeletal maturity affected at 62.5 mg/kg/day and above. Detectable abnormalities at 62.5 mg/kg/day and above.</p> <p>Kavlock et al., 1982</p>
Mistar Rat	
0, 15.5, 31.2 mg/kg/day from day 7 of gestation to day 15 of lactation Gavage	<p>NMEL = 15.6 LEL = 31.2</p> <p>58 reduction in weight of testes and a 13% reduction in the weight of the ventral prostate and seminal vesicles at 31.2 mg/kg/day..</p> <p>Kavlock et al., 1982</p>
B. Dietary Dosing	
Charles River, CD Rats	
5 mg/kg-400 mg/kg/day dietary through gestation day 15	<p>NMEL = 400 (HRT) LEL = >400 (change)</p> <p>Not teratogenic.</p> <p>Sherman, Cullk and Jackson, 1975</p>
CDR-CD Rats	
Dietary MEC days 6-15 gestation; 0-10,000 ppm (500 mg/kg/day)	<p>NMEL = 250 (5000 ppm) LEL = 375 (7500 ppm) (fetal weight)</p> <p>Not embryotoxic or teratogenic. Slightly lower, not statistically significant fetal weights at 7500 ppm.</p> <p>Haskell Lab., 1978</p>
Mistar Rat	
0, 125, 250, 500 mg/kg/day on days 7-16 of gestation-diet (calculated)	<p>NMEL = >500 LEL = >500</p> <p>No dose related incidence of anomalies/malformations in fetuses.</p> <p>Kavlock et al., 1982</p>

Table 7 Continued

II. Spermatogenic Effects						
A. Gavage Dosing						
Rats	>3400 mg/kg gavage (acute and subacute tests)	NOEL = <3400 LEL = 3400 (LJTT)	Deprecation of germinal tissue and spermatogenesis. Testes = 1 ^o target of toxicity (mature rats)	Sherman and Krauss, 1966		
Rats	670 mg/kg (single dose) gavage (acute test)		Reduction of sperm (mature rats)	Sherman, 1965		
Rats	10 doses, 200 mg/kg/dose gavage (subacute test)		Reduction of sperm (mature rats)	Sherman and Krauss, 1966		
Winter Rats	0, 15.6, 31.2 mg/kg/day on day 7 of gestation to day 15 of lactation gavage	NOEL = 15.6 LEL = 31.2	58 reduction in weight of testes and 13% reduction in weight of ventral prostate and seminal vesicles at 31.2 mg/kg/day	Kavlock et al., 1982		
Sprague-Dawley Rat-Adult	0, 200, 400 mg/kg/day for 10 days gavage	NOEL = not determined LEL = 200	15-48% depression in total epididymal sperm counts & vas deferens sperm concentration at 200 or 400 mg/kg/day. Caudal weights depressed.	Carter and Laskey, 1982		
Sprague-Dawley Rat-Adult	0, 400 mg/kg/day for 10 days gavage	NOEL = not determined LEL = 400	slight to moderately severe hypogonadotropinemia and slight to severe hypospERMATOGENESIS	Carter and Laskey, 1982		
Sprague-Dawley Rat-Prepubertal	0, 200 mg/kg/day for 10 days by gavage	NOEL = >200 LEL = >200	No damage to the reproductive system	Carter, Hein and Laskey, 1982		

Table 7 Continued

II. Spermatogenic Effects Continued

B. Dietary Dosing

Dogs	0, 100, 500, 1,500, and 2,500 ppm Dietary chronic 2 year feeding ^a	NOEL = >2,500 ppm LEL = >2,500 ppm	No effect on sperm production (mature animals)	Sherman, Barnes and Rittle, 1970
Rats	0, 100, 500 ppm Dietary chronic feeding	NOEL = >2,500 LEL = >2,500	No effect on sperm production (mature animals)	Sherman, Barnes and Rittle, 1969
Rats (3 generation reproductive studies)	Up to 2,500 ppm in diet ^a	NOEL = 500 ppm LEL = 2,500 ppm (fetal weight decrease)	No effect on fertility index - no pathological changes attributable to HBC in diets - weights slightly decreased at 2,500-10,000 ppm in fetuses	Sherman, Cullik and Jackson, 1975

C. Inhalation Dosing

Rats	Inhalation 33 mg/kg	NOEL = 7.5 LEL = 33	Reduction of spermatogenic activity (mature rats)	Horobeger, 1969
Dogs	Inhalation 82 mg/kg	NOEL = 32 LEL = 82	Reduction of spermatogenic activity (mature dogs)	Littlefield, 1969

HBC fed in diet

1/
LEL = lowest effect level
NOEL = no observed effect level
LIT = lowest dose tested
HIT = highest dose tested

Study/Lab/Study #/Date	Material	Accession No.	Results: LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	TOX Category	CORE Grade/Doc. No.
Acute oral LD ₅₀ - rat; M.B.A. Laboratories #K4028; 6/18/77	Benomy1 - 0.3% Dimethylformamide - 99.7%		LD ₅₀ = 5,300 mg/kg	IV	000719
Acute dermal LD ₅₀ - rabbit; M.B.A. Laboratories	Benomy1 - 0.3% Dimethylformamide - 99.7%		LD ₅₀ = 4,000 mg/kg	III	000719
Primary dermal irritation - rabbit; M.B.A. Labs.; #K4028; 6/18/77	Benomy1 - 0.3% Dimethylformamide - 99.7%	MRID 00079441	PIS = 0.5/8.0	IV	000719
Primary eye irritation - rabbit; M.B.A. Labs.; #K4028; 7/12/77	Benomy1 - 0.3% Dimethylformamide - 99.7%		Positive as an eye irritant. Dulling of cornea still present in 2 rabbits at 6 days.	I	000719
Acute oral LD ₅₀ - rat; WARF; #4123189 12/31/74	Pro Turf DSB Fungicide (1.1% Benomy1)		LD ₅₀ > 20 gm/kg	IV	000719 004678
Primary dermal irritation - rabbit; WARF; #4123189; 12/31/74	Pro Turf DSB Fungicide (1.1% Benomy1)		No irritation noted 24 hr exposure	IV	000719 004678
Acute dermal LD ₅₀ - rabbit; WARF; #4123189; 12/31/74	Pro Turf DSB Fungicide (1.1% Benomy1)		LD ₅₀ > 8 gm/kg 24 hr exposure	III	000719 004678
21-Day inhalation - rat; Haskell Lab.; 4/30/70	53.5% a.i. in sugar Benlate		LC ₅₀ > 0.2 mg/L, 4 hours/day dose levels - .02, .2 mg/L		000720 004678
21-day dermal - rabbit; Haskell lab.; 211-69; 7/20/69	52.5-53 % a.i. in sugar	MRID 00097287	NOEL not determined LEL < 50 mg/kg based on decreased rel. & abs. testes weight levels tested 50 to 5000 mg/kg		supplementary 004679 -
21-day dermal - rabbit; Haskell lab.; 211-69; 7/20/69	51.5 % a.i. in Ca propionate	MRID 00097287	NOEL = 1000 mg/kg (only dose tested)		minimal 004679 -

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Study/Lab/Study #/Date	Material	Accession No.	Results:		TOX Category	CORE Grade/ Doc. No.
			LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL			
Acute oral LD ₅₀ - rat Haskell labs; 17-69; 1/22/69	50% WP 53% tech	MRID 00097277	LD ₅₀ > 10,000 mg/kg		IV	000721 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 oral LD ₅₀ - rabbit Haskell labs; 109-69; 1969	50% WP	MRID 00097277	LD ₅₀ > 3,400 mg/kg		III	000721
Acute dermal LD ₅₀ - rabbit; Hazleton Labs; 201-216	50% WP	MRID 00097602	LD ₅₀ > 4,640 mg/kg (24 hr exposure) Levels tested: 464, 1000, 2150, 4640, 3430*, 10,000* mg/kg (*1/dose)		III	000721 004678 minimum 004679 -
Acute inhalation LC ₅₀ - rat	50% WP		LC ₅₀ > 2.0 mg/L		III	000721
Acute inhalation LC ₅₀ - rat, Haskell labs summary 7/16/68	50% WP	Acc.# 105912-A MRID 00097597	LC ₅₀ > 1.37 mg/L		II	000721 suppl ementary 004678
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 irrita- tion - guinea pig; Haskell labs; 85-69; 4/18/69	50% WP (52% tech)	Acc.# 050427-X MRID 00097290	Mild irritation		IV	000721
Dermal sensitization - guinea pig; Haskell labs; 85-69; 4/18/69	50% WP (52% tech)	Acc.# 050427-X MRID 00097290	Mild sensitization			000721

Study/Lab/Study #/Date	Material	Acc.#	Results: LD50, LC50, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary dermal irritat. - guinea pig; Haskell lab.; 174-66; 9/28/66	technical	Acc.# 105908-A MRID 00057244	no skin irritation Levels tested: 10, 25, and 40% paste	IV	004678
Dermal sensitization - guinea pig; 174-66; 9/28/66	technical	Acc.# 105908-A MRID 00057244	mild sensitization		004678
Primary dermal irritat. - 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	moderate sensitization		minimum 004679 -
Primary eye irritation - rabbit; Haskell labs; 233-72; 6/22/72	53% tech 50% WP		Mild irritation		000721 004678
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, 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 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		000721 004678 minimum 004679 -

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Study/Lab/Study #/Date	Material	MRID No.	Results: LD50, LC50, 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 adverse effects on testis. No effect on sperm production 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(al)		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; Haskell Lab.; #206-70; 1970	53.5% WP		Terata NOEL = 5,000 ppm (HDT)		000722
Teratology - rat; Schtenberg & Torchinsky; 1972	Technical		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 microphthalmia) Dosage = 62.5, 125, 250, 500 mg/kg (gavage)		000722
51-Week feeding - mice; Haskell Lab.; #539-78; 10/13/78	TMT-1991	236765	Study terminated after 51 weeks due to high morbidity and mortality in all groups (systemic bacterial infection) Levels tested = 0, 500, 1500, 5000 and 7000 ppm		Supplementary 000723

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COKE grade/

FOA

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Study/Lab/Study #/Date	Material	No. Acc.#	LD50, LC50, PIS, NOEL, LEL	Category	Guideline Doc. No.
Acute dermal LD50 - rabbit; Haskell; #554-80, 7/23/80	Benomy1 - 75% (Benlate DF)	243043 MRID 00064822	LD50 > 2000 mg/kg Severe skin irritation.	III	Guideline 000863 004679 -
Primary eye irritation - rabbit; Haskell; #497-80; 6/13/80	Benomy1 - 75% (Benlate DF)	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	Benomy1 - 75% (Benlate DF)	243043 MRID 00064821	Slight edema and slight erythema at 24 hours; at 72 hours, only very slight erythema. PIS = 0.67 ALL scores were 0 by day 6.	IV	Guideline 000863 004679 -
Teratology - rat; Haskell Labs.; report #587-82; E.I. DuPont de Nemours; 1982	Technical 99.1% Pure	248563 249749 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)		Supplementary 002578 Upgraded to Minimum 003042
2 Year feeding - mouse; Dupont Haskell Lab; 20-80; 1/26/82	Benomy1 99-99.2% pure	MRID 00096514	Onco-genic NOEL < 500 ppm male and female, significant increase in hepatocellular neoplasms in male and female, pulmin. alveol. carcin. males, degen. of testes and epid. at 5000 ppm. Dosage levels = 500, 1500, 5000 ppm (5000 lowered from 7500 ppm)		003726 minimum 004679 -
Teratology - rat; Sherman et al.; 1975	Benomy1		Terata NOEL = 400 mg/kg (HDF) (inconclusive result since ingested dose not measured accurately)		003728
Teratology - rat; Midwest Res. Inst.; 1979	Benomy1		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		003728

Study/Lab/Study #/Date	Material	No.	LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	Category	Doc. No.
Teratology - mice; Health Effects Res. Lab; US EPA; 1979	Benomy1		Terata NOEL < 200 mg/kg (LDT; increased resorptions, decreased litter size; herniations; reduction of extremities) Dosage levels = 200 and 400 mg/kg by gavage		003728
Teratology - rat; Health Effects Res. Lab; US. EPA; 1980	Benomy1		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		003728
Teratology - rat; Health Effects Res. Lab; US EPA; 1980	Benomy1		NOEL = 169 mg/kg LEL = 298 mg/kg (weight change) No dose related incidences of anomalies or malformations Dosage levels = 0 - 500 mg/kg in diet		003728
Post natal teratology - rat; health Effects Res. Lab; US EPA; 1980	Benomy1		Benomy1 was administered by gavage to dams from day 7 of gestation to day 15 of lactation Fetotoxic NOEL = 31.2 mg/kg Fetotoxic LEL = 62.5 mg/kg (decreased weight of testes, ventral prostate, and seminal vesicles)		003728
Teratology - rat; Haskell Lab; #649-80; 1980	Benomy1	Acc.# 25675 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		003728 Supplementary 004689 Minimum when combined with 003042

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Study/Lab/Study #/Date	Material	No.	LD50, LC50, PIS, NOEL, LEL	Category	Doc. No.
Neurotoxicity - hen; IRDC; 125-028; 1/5/79	Benomyl in corn oil (Tech.)	Acc.# 241930	Inconclusive results due to underlying disease in hens Dosage levels = 500, 2500, 5000 mg/kg (single dose)		003728 supplementary 004679 -
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 femur 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, <u>in vitro</u> - 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 mg)		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 17-192 uM		003744 acceptable 004679 -
Muta -rat - DNA repair, Haskell; 741-82; 10/20/81 (T'ong, 1981)	Benomyl	GS0119-006	Not a mutagen when tested for DNA repair using rat hepatocyte cultures		003744 acceptable 004679 -

Study/Author/Year	Material	No.	Category	Doc. No.
Mutagenic ovary cells - Chinese hamster; Waters and Kahn; 1980	Benomyl		Not mutagenic with or without metabolic activation at HGPRT locus	003744
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 004672 -
Mutagenic - microorganisms; Carere et al; 1978	Benomyl		Not a mutagen in salmonella and streptomycetes	003744
Mutagenic - microorganisms; Fiscor et al; 1978	Benomyl		no mutagenic activity noted	003744
Mutagenic - microorganisms; Ercegovich and Rashid; 1976	Benomyl and MBC		Doubtful mutagenic activity was reported for benomyl and MBC both with and without metabolic activation	003744
Mutagenic drosophyllia; Lamb and Lilly; 1980	Benomyl and MBC		Noted sterility in some broods	003744
Mutagenic - human; leukocyte culture; Gupta and Legator; 1975	Benomyl		No compound related chromosome aberrations observed	003744
Mutagenic micronucleus - rat; Ruzicka et al; 1976	Benomyl		Benomyl was given to pregnant rats at 200 and 500 mg/kg/day from days 7 to 14 of gestation. No increase in frequency of chromosomal aberrations in bone marrow cells. Increased incidence of chromosomal damages in embryonic cells	003744

Study/Lab/Study #/Date	Material	No.	Ld50, LC50, PIS, NOEL, LEL	Category	Doc. No.
1-Generation reproduction - rat; Kavlock et al; 1982	Benomyl		NOEL = 15.6 mg/kg LEL = 31.2 mg/kg (decrease testicular weights in offspring) Material given by gavage during gestation and lactation		003744
Teratology - mice; Kavlock et al; 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)		003744
Teratology - rat; Kavolck et al; 1982	Benomyl		Dosage levels = 0, 125 and 500 mg/kg/day (calculated dose in diet) Terata NOEL > 500 mg/kg (HDF)		003744
Teratology - rat; Torchinski; 1973	Benomyl		145 mg/kg given by gavage on day 12 of gestation (single dose tested)	Supplementary (insufficient data) 003728	003744
10-Day spermatogenic - rat, Carter and Laskey; 1982 Toxicology Letters, 11:87-94	Benomyl		Dose levels = 0, 200, 400 mg/kg/day for 10 days. Animals sacrificed 14 days later (gavage) Depression of sperm count and decreased caudal epididymis weights at 200 mg/kg; moderately severe hypospermatocytogenesis (400 mg/kg)		003744
10-Day spermatogenic - rat; Carter et al; 1982	Benomyl		Dosage levels = 0, 200 mg/kg/day by gavage to prepubertal rats NOEL > 200 mg/kg		003744
Spermatogenic - rat; Sherman and Krauss; 1966	Benomyl		Degeneration of germinal tissue and aspermatogenesis at 3400 mg/kg (LDF)		003744
Acute spermatogenic - rat; Sherman; 1965	Benomyl		Reduction of sperm at 670 mg/kg (single dose used)		003744

10-Day spermatogenic - rat; Sherman and Kraus; 1966	Benomy1		MRID 00097281	NOEL < 200 mg/kg (single dose tested)	003744
Acute inhalation - rat; Haskell Lab; #95-69; 4/24/69	Benomy1		MRID 00097601	NOEL = .82 mg/L (7.5 mg/kg) LEL = .20 mg/L (reduction of spermatogenic activity) (33 mg/kg)	003744 minimum 004679 -
14-day intubation - rats Haskell labs; 100-66; 7/15/66	Benomy1 1991 (% unspecified)		MRID 00097601	Systemic NOEL > 200 mg/kg/day LEL = 3400 mg/kg/day Levels tested; 200, 3400 mg/kg/day	000720
Acute oral - rats; Haskell labs; 100-66; 7/15/66	Benomy1 1991 (% unspecified)		MRID 00097601	LD50 > 9590 mg/kg Levels tested: 500, 2250, 3400, 3600, 7500, 9590 mg/kg in CHR-CD rats	supplementary 004679 -
Mutagenic micronucleus - mouse; J.P.Seiler; 1976	Benomy1 MBC	G50119-008		author's conclusions for micronucleus formation- mouse bone marrow NOEL = 500 mg/kg benomy1 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 "	incomplete 004679 -
Acute Oral - rats; Haskell labs; 421-80; 5/23/80	Benlate(75% a.i)		MRID 00064819	LD50 > 5000 mg/kg (only dose tested) testes- small, soft discolored	minimum 004678 004679 -
Acute Dermal - rabbits Haskell labs, 554-80; 6/23/80	Benlate DF 75 % a.i.		MRID 00064822	LD50 > 2000mg/kg	guideline 004678 004679 -

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Study/Lab/Study #/Date	Material	Accession No.	Results: LD50, LC50, PIS, NOEL, LEL	Tox Category	Core Grade/Doc. No.
Acute oral - rats; Maskell 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 degen and necrosis was present at 670 and 1000 mg/kg		supplementary 004679 -
Acute inhalation - dogs; Hazelton labs; HLR#192-69; 7/14/69	Benomyl WP 50%	MRID 00097275	LC50 > 1.65 mg/l (HDF no deaths) NOEL = .65 mg/l LEL 1.65 mg/l based on reduced spermatogenic activity at 14 d (not present at 28 days)	II	minimum 004679 -
Primary eye irritation - rabbits; Maskell labs; 179-81; 4/6/81	Benlate DF 75% a.i.	MRID 00084579	PIS day 1 4 7 28 7.3 0	III	minimum 004679 -
Registration Standard			Caswell 79C - 2 benzimidazole carbamic acid, methyl ester synonyms: MBC 2 methyl benzimidazole carbamate		
Methylolites					

Reviewed by: Dynamac - P. Wennerberg, W. McLellan, I.C. Felkner

Secondary reviewers: Marion P. Copley, D.V.M. *M. C. 4/1/85*
Jane Harris, PhD, Section Head *JAH 6/2/85*
Section 6, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

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

ACCESSION NUMBER: 246948A, 246949, 246950 MR ID 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,
Benlat[®]) in mice.

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

REPORT ISSUED: Jan. 26, 1982

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

Classification: Core-minimum

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

SEE ATTACHED REVIEW

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

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

BEST AVAILABLE COPY

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.)

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EPA Section Head

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Date: _____

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, Benomy1, 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 methods¹. Comparisons of survival distributions and tumor incidences were analyzed by the Mantel-Haenszel method². 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.

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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.

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

Group/Dose (ppm)	MALES					FEMALES					
	Body Weight	Liver	Thymus	Testes	Brain ^c	Relative Liver	Thymus	Kidney	Brain	Relative 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				
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.

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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 the treated groups were significantly lower than control (Table 5).

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

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

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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.

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

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

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

^b 7500 ppm changed to 5000 ppm after week 37.

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

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

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

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

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

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

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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.

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

Date: December 4, 1981

Subject: Evaluation of Pathologic Data from OFFICE OF TOXIC SUBSTANCES
Two-Year Feeding Study in Mice
Treated with INE-965, MBC (Benomyl Metabolite)

From: Louis Kasza, D.V.M., Ph.D. *Louis Kasza*
Pathologist, Toxicology Branch, TS-769
Hazard Evaluation Division

To: Jeff Kempter, Chief
Chemical Review Branch #3, TS-791
Special Pesticide Review Division

Through: Orville Paynter, Ph.D., Chief *OP*
Toxicology Branch, TS-769
Hazard Evaluation Division

No.: 75A
352-354

SUMMARY

Male and female mice, 80/group, were fed with MBC (Benomyl metabolite), for two years at dose levels of 0, 500, 1,500, and 3,705 (in males, it was reduced from 7,500 after 15 months), and 7,500 (females) ppm (parts per million), respectively. An increased incidence of liver neoplasms (13/80, 20/80, 23/80, 3/80) were observed in male mice. In female mice, an increased incidence of liver neoplasms (1/79, 9/78, 20/80, 15/78) was diagnosed by DuPont, Haskell Laboratory. From the submitted data, we are in agreement with the Company's pathologist that a compound-related oncogenesis was established in the male intermediate group; however, we do not consider that a valid conclusion can be made regarding the high-dose group since this group was terminated (at 516 days) before the majority of the tumors developed in the other groups. In the female group, the oncogenic effect was compound and dose related at all dose levels. There was an increased incidence not only in benign but also in malignant tumors in both sexes. Dose-related decreases in latency in the appearance of tumors were present in both sexes.

Regarding oncogenicity, the effect of MBC was comparable to the parent chemical, Benomyl. (Results of the pathologic evaluation of the Benomyl experiment were summarized in a memorandum of 11/18/81 by L. Kasza to J. Kempter).

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INTRODUCTION

Male and female mice were divided into 8 groups of 80 animals per group. Male (I, III, V, VII) and female (II, IV, VI, VIII) groups were fed with INE-965, MBC (Benomyl metabolite) at 0, 500, 1,500, 3,750* (male), and 7,500 (female) ppm, respectively. The animals were on test material 516 (Group VII) and 731 - 733 days, then sacrificed. Gross pathologic observation was made, and all major tissues and organs were collected for histopathologic observation. The results are presented in six tables. Table I (pages 5 - 121) shows the gross findings. Table II (pages 122 - 562) illustrates the individual histopathologic diagnoses. Table III (pages 563 - 592) presents the summary incidence table of histopathologic findings by group and sex. Table IV (pages 593 - 597) is the summary incidence table for neoplasms per group and sex. Table V (pages 598 - 600) illustrates the presence and statistical significance of compound-related histopathologic changes. Table VI (page 601) is the summary of statistical analyses of hepatocellular neoplasms. The code used in Table II is presented in a footnote on page 122.

The objective of this report is the review of pathologic data in the DuPont, Haskell Laboratory report, and to make comments about the adequacy of the presented data.

MATERIALS AND METHODS

The primary source of this report is the "Summary Incidences of Histopathologic Changes" (Table III). Selected organs and tissues in which major histopathologic changes were seen are listed here and the lesions tabulated. For comparative reasons, the neoplasms in the livers were tabulated according to whether the neoplasms were observed before or at the time of terminal sacrifice. Also the dates of the first observations of liver tumors were reported. With several organs and tissues, the lesions were counted in the individual histopathologic tabulation (Table II) and the accuracy of the numbers was compared to the numbers shown in the summary incidence table (Table III). Benign and malignant liver neoplasms of the same cellular origin were listed separately and also counted together as the basis of establishing oncogenecity.

In female intermediate and high-dose groups, liver gross and histopathologic findings were compared in 24 randomly selected mice.

* Group VII mice received 7,500 ppm for the first 15 months of the study. Because of compound-related increased mortality, Group VII mice were sacrificed after 17 months on test.

To establish oncogenicity, the following criteria were primarily considered:

1. Increase in neoplasm incidence;
2. Decrease in latency of neoplasms appearance;
3. Presence of rare tumors;
4. Consideration was given to:
 - a. Presence and increase in number of malignant neoplasms;
 - b. Only compound relationship;
 - c. Compound and dose relationship;
 - d. Presence of tumors in difference sexes.

RESULTS

The submitted pathologic data were presented in a well-organized system and in an easy-to-read format. The tissues and organs listed in Table II are in alphabetical order. Under individual tissues and organs, the diagnoses are listed and the lesions graded. There is a good correlation between the data of individual histopathological diagnoses table (II) and the summary incidence table (III). Some of our major findings are illustrated in the following tables:

General Information

	Groups							
	I	II	III	IV	V	VI	VII*	VI
	M	F	M	F	M	F	M	F
Number of Animals/Group	80	80	80	80	80	80	80	80
Dose (ppm)	0	0	500	500	1,500	1,500	3,750	7,500
Survival (731-733 days)	18	22	14	16	9	14	0	21

The survival rate is rather low, and it is a dose-related decrease in the male groups.

*The dose was reduced from 7,500 ppm after 15 months on test. This group was terminated after 17 months on study (516 days).

Selected Histopathologic Findings in Male Mice

	<u>Groups</u>			
	<u>I</u>	<u>III</u>	<u>V</u>	<u>VII</u>
<u>KIDNEY</u>				
Tubules contain yellow-brown granular material	7/30	3/79	19/78	47/79
<u>LIVER</u>				
Adenoma	11/80	15/80	14/80	3/80
Carcinoma	<u>2/80</u>	<u>5/80</u>	<u>9/80</u>	<u>0/80</u>
Total Neoplasms	13/80	20/80	23/80	3/80
Hepatocellular Necrosis Focal and Centrolobular	1/80	7/80	10/80	13/80
<u>TESTES</u>				
Sperm Stasis Bilateral, Unilateral	7/77	13/78	16/80	22/74

Presence of Liver Neoplasms Before and At the Time of Terminal Sacrifice
in Male Mice

	<u>Groups</u>							
	<u>I</u>		<u>III</u>		<u>V</u>		<u>VII</u>	
<u>LIVER</u>	I* ()#	T**	I	T	I	T	I	T (NA) ^c
Adenoma	6(430),	5	10(467),	5	14(459),	0	3(434),	c
Carcinoma	<u>1(629),</u>	<u>1</u>	<u>2(649),</u>	<u>3</u>	<u>8(616),</u>	<u>1</u>	<u>0 (0),</u>	c
Total Neoplasms	7(430),	6	12(467),	8	22(459),	1	3(434),	c

* Lesions before terminal sacrifice.
First day on test when lesion was detected.
** Lesions at the time of terminal sacrifice.
c Not applicable.

Selected Histopathologic Findings In Female Mice

	Groups			
	II	IV	VI	VIII
<u>KIDNEY</u>				
Macrophages with yellow-brown pigment	5/80	4/78	3/80	21/76
<u>LIVER</u>				
Adenoma	0/79	5/78	5/80	3/78
Carcinoma	1/79	4/78	15/80	12/78
Hepatoblastoma	0/79	0/78	1/80	0/78
Total Neoplasms	1/79	9/78	21/80	15/78
<u>THYMUS</u>				
Lymphoid Depletion	3/38	4/25	12/44	10/38

Presence of Liver Neoplasms Before and At the Time of Terminal Sacrifice
in Female Mice

	Groups							
	II		IV		VI		VIII	
<u>LIVER</u>	I* ()#	T**	I	T	I	T	I	T
Adenoma	0 (0)	0	4(648), 1	4(636), 1	1(624), 2			
Carcinoma	0 (0)	1	3(704), 1	7(536), 8	6(551), 6			
Hepatoblastoma	0 (0)	0	0(--), 0	1(704), 0	0(--), 0			
Total Neoplasms	0 (0)	1	7(648), 2	12(536), 9	7(551), 8			

* Lesions before terminal sacrifice.

First day on test when lesion was detected.

** Lesions at the time of terminal sacrifice.

‡ Not applicable.

There is an increased incidence of hepatocellular neoplasms in male mice at low and intermediate dose levels. The tumor incidence at high dose level (3) should be disregarded in comparison since this group was killed at 17 months on test and the majority of tumors in other groups were observed between 18 - 24 months. Considering this fact, it can be concluded that the oncogenic effect in livers of male mice was compound and dose related. The presence of malignant tumors and the decrease in latency of tumor appearance in test groups are supporting data for oncogenicity. There was an increased incidence of hepatocellular necrosis (focal plus centrolobular) in test groups compared with controls.

In comparing the Benomyl and MBC in male mice experiments, the effect of both materials was similar on liver oncogenesis. There were differences: Benomyl affected the male gonads more markedly than MBC, and induced increased incidence in lung tumors. On the other hand, MBC produced marked necrosis in the liver.

Basically, we are in agreement with the Company pathologist's opinion (page 2) regarding the oncogenicity, "In male mice, a significant increase in hepatocellular carcinomas occurred at the intermediate treatment level. A similar increase was observed for the combined incidences of all primary hepatocellular neoplasms in the intermediate dose group. The X² Test for Trend was significant (P < 0.05) for the combined incidences of primary hepatocellular tumors".

Other than neoplasms and hepatocellular necrosis, the diagnosed compound related lesions have less importance.

In female mice, there is increased incidence of liver neoplasms which is compound and dose related. Similar to the male test group, there was an increased number of malignant tumors and decrease in latency in test groups compared to the controls. We are in agreement with the Company pathologist's opinion (page 2), "Significant increase in hepatocellular carcinomas occurred in female mice at the high (7,500 ppm) and intermediate (1,500 ppm) feeding levels. Significant increases were also shown for hepatocellular adenomas (by one or more statistical tests, Table VI) for all compound-related groups of females. The X² Test for Trend (dose-response) was also significant (P < 0.001) for hepatocellular neoplasms (hepatocellular adenomas, carcinomas and hepatoblastomas). The effects of Benomyl and MBC were similar in female mice.

Other than neoplastic changes, the compound-related lesions are less important.

When the gross and histopathologic findings were compared in the livers of 12 female intermediate dose animals (8470, 8445, 8433, 8429, 8428, 8420, 8418, 8425, 8459, 8401, 8463, 8439) and in 12 female high-dose animals (8491, 8495, 8497, 8404, 8507, 8515, 8502, 8523, 8525, 8534, 8553, 8512), a good correlation was found between gross pathologic observation and histopathologic description of the lesions.

DISCUSSION AND CONCLUSION

When the oncogenic effects of Benomyl and its metabolite, MBC, were compared in different mouse experiments, comparable results were found to be induced by both materials. In both males and females, the incidence of hepatocellular neoplasms increased in test groups. In MBC-treated male animals, there was no increased incidence in lung tumors versus the Benomyl-treated male mice, where alveolar cell carcinomas increased at low and middle dose levels.

The MBC-induced oncogenic effect was compound related in male, and compound and dose related in female mice. The effects in the male high-dose group could not be compared to the other groups since this group was sacrificed at 17 months on test versus 24 months in other groups. The increased incidences in malignant hepatocellular neoplasms and the decrease in latency in appearance of tumors are supporting data for oncogenicity.

The increased incidence of liver necrosis in male mice indicates hepatotoxicity related to the treatment with MBC.

Based on increased incidence of neoplasms both in male and female mice, but only in one organ, and as supporting data, the presence of malignant tumors and the decrease of latency in tumor appearance, it can be concluded that MBC (a Benomyl metabolite) is a moderately severe oncogenic compound in mice.

cc: William Burnam, Deputy Chief
Toxicology Branch, TS-769
Hazard Evaluation Division

007710

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INFORMATION**



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

Date: November 18, 1981

Subject: Evaluation of Pathologic Data from
Two-Year Feeding Study in Mice
Treated with Benomyl, DuPont Haskell Laboratory Study

From: Louis Kasza, D.V.M., Ph.D. *Louis Kasza* **75A**
Pathologist, Toxicology Branch, TS-769
Hazard Evaluation Division

To: Jeff Kempter, Chief
Chemical Review Branch #3, TS-791
Special Pesticide Review Division

Through: William Burnam, Acting Chief *WAB*
Toxicology Branch, TS-769
Hazard Evaluation Division

No.: 75A
352-354

SUMMARY

Male and female mice, 80/group, were fed with Benomyl for two years at dose levels of 0, 500, 1,500, and 5,000 ppm (parts per million). An increased incidence of liver (25/77, 35/80, 52/79 and 27/80) and lung (13/79, 24/80, 23/79 and 16/80) neoplasms were detected in male mice. In female mice, an increased incidence of liver neoplasms (4/77, 9/80, 13/79 and 21/77) were reported by DuPont, Haskell Laboratory. For the submitted data, we are in agreement with the Company's pathologist that oncogenesis was established in the livers of male mice at low and intermediate levels and in female mice livers at all dose levels. There was an increased incidence not only in benign but also in malignant hepatocellular and alveolar cell neoplasms. A proportionally higher incidence in decrease of latency, both in female liver neoplasms (0, 5, 4, 5,) and male lung neoplasms (1, 13, 10, 9) occurred. The presence of malignant tumors, the increased incidence in lung tumors in male mice, the decrease in latency in test animals, and the earlier occurrence of female liver tumors in test animals compared with controls are supporting data for oncogenicity. A compound-related effect on male gonads was observed at the high dose level.

INTRODUCTION

Male and female mice were divided into 8 groups of 80 animals per group. Male (I, III, V, VII) and female (II, IV, VI, VIII) groups were fed with Benomyl at 0, 500, 1,500, 5,000 ppm (the dose level of 5,000 ppm was lowered from 7,500 ppm after 38 weeks of testing), respectively. The animals were on test material for 740 - 744 days and then sacrificed. Gross pathologic observation was made, and all major tissues and organs were collected for histopathologic observation.

037710

75A

Date Out: EFB: 28 OCT 1981

To: Product Manager 21 Jacoby
TS-767

From Dr. Willa Garner *lll*
Chief, Review Section No. 1
Environmental Fate Branch

Attached please find the environmental fate review of:

Reg./File No.: 46262 - EUP - 1

Chemical: Benomyl

Type Product: Fungicide

Product Name: Benlate

Company Name: Dupont

Submission Purpose: Basal & foliage treatment on caks

ZBB Code: Sec. 5

ACTION CODE: 720

Date in: 9/10/81

EFB # 940

Date Completed: 28 OCT 1981

TAIS (level II) Days

Deferrals To: 52 4

 Ecological Effects Branch

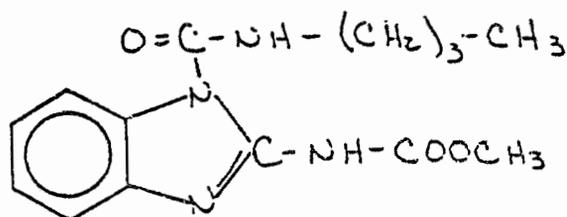
 X Residue Chemistry Branch

 X Toxicology Branch

1.0 INTRODUCTION

The applicant, Professional Tree Service, College Station, Texas, is applying for an experimental use permit for a Benlate/dimethylformamide basal treatment and a foliage treatment of Benlate in a 1 1/2 percent oil emulsion in water to be used as a fungicide system in the control of the persimmon wilt fungus on broadleaved trees (mostly oaks, elms, and sycamores).

2.0 Benlate: benconyl



methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate

3.0 DISCUSSION

- 3.1 No new environmental chemistry data submitted. All requirements for an EUP appear to have been satisfied.
- 3.2 This request seems to be a renewal of EPA Experimental Use Permit No. 35899-EUP-2.
- 3.3 This use is confined to the state of Texas in the vicinity of the cities of Austin, Benders, Bryan, Kerrville, Rockport, and Temple. Approximately 2000 trees will be treated with 400 lb Benlate and 4000 gal dimethylformamide. Tests are planned using Benlate/DMF basal treatment alone and in combination with the foliage spray, and with the foliage spray in a oil emulsion suspension alone to compare efficacy of treatments. Treatment period is to last one year. Dosage rate is determined by diameter of each tree at breast height.

4.0 RECOMMENDATIONS

- 4.1 EFB defers to Toxicology Branch to comment on the toxicity data presented for benconyl and DMF and to Residue Chemistry Branch to comment on benconyl residue data.
- 4.2 EFB concurs with the granting of this EUP.

Revised 11/1/68

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The results are presented in 8 tables. Table I (page 5 - 137) shows the gross findings. Table II (page 139 - 1050) illustrates the individual histopathologic diagnoses. Table III (page 1051 - 1079) describes the major causes of death or moribund and killed mice. Table IV (page 1080 - 1135) summarizes the histopathologic findings in different groups. Table V (page 1142 - 1143) is the summary incidence table of compound-related histopathologic changes. Table VII (page 1144) shows the statistical analysis of hepatocellular neoplasms, and Table VIII (page 1145) describes the times of hepatocellular tumor discovery. The code used in Table II is presented on page 138.

The objective of this report is the review of pathologic data in the report and to make comments about the adequacy of the presented data.

MATERIALS AND METHODS

In the summary incidence table, the distribution of histopathologic changes was observed. Those organs and tissues in which major differences were seen are listed in this report. For comparative reasons, the neoplasms in the livers and lungs were tabulated according to whether the neoplasms were observed before the final sacrifice or at the time of terminal sacrifice. Also the dates of the first observations of liver and lung tumors were also reported. With several organs and tissues, the lesions were counted in the individual histopathologic tabulation and their numbers were compared to the numbers shown in the summary incidence tables. Benign and malignant neoplasms of the same cellular origin were listed separately and also counted together in our tabulation.

In the male control and high dose groups, liver gross and histopathologic findings were compared in 24 randomly selected animals.

To establish oncogenicity the following three criteria were primarily considered:

- 1) Increases in neoplasm incidence.
- 2) Decrease in the latency of tumor appearance.
- 3) Presence of rare tumors.

RESULTS

The submitted pathologic data in the report were presented in a well-organized fashion. The tissues and organs are listed in twelve groups (I - XII) based on body systems (e.g., the endocrine system). Under the individual organs, the diagnoses are listed and the lesions graded. There is good correlation between the individual histopathologic table (II) and summary incidence table (VI). Some of our major findings are illustrated in the following tables:

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General Information

	Groups							
	I	II	III	IV	V	VI	VII	VIII
	M	F	M	F	M	F	M	F
No. Animals/Group	80	80	80	80	80	80	80	80
Dose	0	0	500	500	1,500	1,500	5,000	5,000
Survival	43	33	36	31	40	23	40	23

There are no significant differences in the survival rates in either male or female groups.

Selected Histopathologic Findings in Male Mice

	Groups			
	I	III	V	VII
<u>SKIN</u>				
Ulcerative Dermatitis (Body & Ear)	6/79	15/80	19/79	18/80
<u>LUNG</u>				
Alveolar Cell Carcinoma	13/79	24/80	23/79	16/80
<u>LIVER</u>				
Adenoma	9/77	9/80	11/79	10/80
Carcinoma	16/77	26/80	41/79	17/80
Total Neoplasms	25/77	35/80	52/79	27/80
<u>TESTES</u>				
Atrophy	12/78	12/79	8/79	31/79
Interstitial Cell Hyperplasia	4/78	4/79	7/79	18/79
<u>EPIDIDYMS</u>				
Depletion Sperm	18/78	11/78	12/79	30/79
Distended Tubuli with Degenerated Sperms	9/78	5/78	11/79	17/79
<u>ADRENAL GLAND</u>				
Focal Cortical Hyperplasia	2/66	6/77	11/78	3/67
<u>THYROID</u>				
Distended Follicles	4/65	13/74	6/73	18/71

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Presence of Lesions Before and At the Time of Terminal Sacrifice

(in Male Mice)

	Groups							
	I		III		V		VII	
	I*	()# T**	I	T	I	T	I	T
<u>LUNG</u>								
Alveolar Cell Carcinoma	1(659),	12	13(445),	11	10(380),	13	9(574),	7
<u>LIVER</u>								
Adenoma	5(530),	4	4(445),	5	6(541),	5	3(627),	7
Carcinoma	8(545),	8	10(470),	16	14(590),	27	5(508),	12
Total Neoplasms	13(530),	12	14(445),	21	20(541),	32	8(508),	19

* Lesions before terminal sacrifice.

First day on test when lesion was detected.

**Lesions at the time of terminal sacrifice.

Selected Histopathologic Findings in Female Mice

	Groups			
	II	IV	VI	VIII
<u>SPLEEN</u>				
Myeloid Metaplasia	10/76	22/79	24/78	16/74
<u>LIVER</u>				
Adenoma	2/77	2/80	7/79	7/77
Carcinoma	2/77	7/80	6/79	14/77

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Presence of Lesions Before and At the Time of Terminal Sacrifice
(in Female Mice)

	Groups							
	II		IV		IV		VIII	
	I*()	T**	I	T	I	T	I	T
<u>LIVER</u>								
Adenoma	0 (),	2	1(641),	1	4(650),	3	2(644),	5
Carcinoma	0,	2	4(640),	3	0 (),	6	3(426),	11
Total	0	4	5	4	4	9	5	16

Neoplasms

- * Lesions before terminal sacrifice.
First day on test when lesion was detected.
** Lesions at the time of terminal sacrifice.

There is a higher incidence of lung and liver neoplasms in male low dose and middle dose groups in comparison with the controls. The increase is more remarkable in the malignant tumors than in the benign. The highest group has tumors, both benign and malignant, in similar incidence as the controls. We accept the interpretations of the company pathologist in the original report, on page 2, "Significant increase in hepatocellular carcinomas and combined hepatocellular neoplasms (adenoma, carcinoma, and hepatocellular neoplasm -- NOS*) occurred at the low and intermediate treatment level". Also a compound-related effect on gonads was observed at the high dose level. Other than neoplasms and the effect on gonads, the pathologic changes which were found and were compound related are considered less important.

In female mice, there is an increased incidence of liver neoplasms at all dose levels. In addition to the benign neoplasms, the malignant tumor incidence also increased. We agree with the interpretations of these findings by the Company pathologist on page 2, "Significant increases in hepatocellular carcinoma occurred in female mice at the high (5,000 ppm) and low (500 ppm) feeding levels. Significant increases were also shown for combined hepatocellular neoplasms (by one or more statistical tests, Table VII) for intermediate and high dose females." Other than neoplasms, the pathologic changes which were reported and were compound related are considered less important.

When the times of tumor occurrences were checked, proportional decreases in latency were present in lung alveolar carcinoma at all dose levels in male mice and moderate decreases in latency were detected at all dose levels in female liver neoplasms.

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When the gross and histopathologic findings were compared in 12 male controls (6531, 6467, 6515, 6513, 6468, 6516, 6537, 6535, 6538, 6468, 6463, 6520) and in 12 male high-dose animals (6719, 6732, 6773, 6755, 6771, 6752, 6741, 6711, 6723, 6726, 6733, 6735), a good correlation was found between the gross histopathologic observations and histopathologic description of the lesions.

DISCUSSION AND CONCLUSION

When Benomyl was fed to mice in a chronic feeding study, an oncogenic effect was detected both in male and female animals. This effect was compound related in males where the tumor induction was limited to low and medium dose levels. In the female livers, the oncogenic effect was compound and dose related. There is no definite scientific explanation for the lack of oncogenic response in male mice at the high dose level; however, there are other oncogenic compounds too which produce neoplasms in a similar fashion. Other than oncogenic response, the pathologic changes have secondary importance related to the adverse effects of this compound.

Because of the lack of dose-related response in male mice and the presence of increased malignancy and decreased latency in test animals, it can be concluded that Benomyl is a moderately severe oncogenic compound in mice.

MBC

89

CARBENDAZIM

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Explanation

Carbendazim (methyl 2-benzimidazole carbamate) was evaluated by the Joint Meetings of 1973, 1976, 1977 and 1978 (FAO/WHO 1974, 1977, 1978, 1979).^{1/} The data available were not considered adequate for the estimation of an acceptable daily intake (ADI). Data necessary for estimating an ADI were identified in 1976 and 1978 and listed under "Further Work or Information". These data have been provided and reviewed in this monograph addendum.

New, additional, or updated information on use patterns, environmental chemistry and plant metabolism was also made available to the Meeting, along with new or additional data on crop residues from supervised trials.

TOXICOLOGY

EVALUATION FOR ACCEPTABLE DAILY INTAKE

BIOCHEMICAL ASPECTS

Absorption, Distribution and Excretion

NMRI mice and Wistar rats were given carbendazim, via intragastric intubation, as a single dose of 3 mg/kg b.w. and one of 300 mg/kg b.w. Urine was collected during the first 6 h, after which the animals were killed. Analyses revealed no sex differences. Almost all metabolites in urine were conjugated as sulphate esters. Cleavage of these conjugates by B-glucuronidase/arylsulphatase released 5-HBC as the only extractable metabolite from water. Urine of mice contained a higher portion of compounds that remained polar after enzyme treatment than the corresponding urine of rats. Polarity was caused by a functional group (e.g. phenolic hydroxyl group) that was introduced into the MBC molecule by a conjugation reaction. These water-soluble compounds were not identified. Essentially the same metabolites were found in both mouse and rat urine samples with only quantitative differences observed between species (Dorn *et al.* 1983).

Effects on Enzymes and Other Biochemical Parameters

Groups of Wistar-SPF male rats and Swiss-SPF male mice were administered carbendazim in the diet at dosage levels of 0 to 10 000 ppm for 60 days. The induction of liver enzyme activities by carbendazim was examined and compared with a positive control, which received phenobarbital sodium (administered via drinking water).

Growth and food consumption were decreased in rats at 10 000 ppm but not in mice administered up to 5 000 ppm in the diet. Relative liver weights were increased in rats fed 2 000 and 10 000 ppm and in mice receiving 1 000 and 5 000 ppm of carbendazim. Phenobarbital groups were similarly affected. Protein concentrations in the total liver homogenates and in post-mitochondrial fractions of rats were not affected by carbendazim, whereas in mice both fractions were increased at 5 000 ppm.

The feeding of carbendazim to rats at dose levels of 2 000 ppm and higher resulted in slight to moderate induction of several drug-metabolizing enzymes of phase 1 (7-ethoxycoumarin-O-deethylase, biphenyl-4-hydroxylase, aniline hydroxylase, 4-methoxybiphenyl-5-demethylase and cytochrome-o-reductase). Similar increased activities of phase 2 drug metabolizing enzymes (glucuronyl transferase I and II) and glutathione content were moderately to markedly increased at this dose.

The feeding of carbendazim to mice at dose levels of 1 000 ppm and higher resulted in moderate to marked increases in drug metabolizing enzymes of phase 1 (including cytochrome P-450 and aminopyrine-N-demethylase). Cytochrome-o-reductase activity was decreased. Glucuronyl transferase and glutathione-S-transferase activities, along with glutathione content, were slightly increased.

^{1/} See Annex 2 for FAO and WHO documentation.

There were no measureable differences noted between rats and mice in regard to the metabolism of the test substance, although exhaustion of the detoxification mechanism was more evident in the mouse at the higher dose levels. The detoxification and elimination of carbendazim and its metabolites proceed more rapidly in the rat than in the mouse. This is reinforced by the increased glutathione content in rat liver and increased activity of phase 2 enzymes (Falke *et al.* 1982a,b).

TOXICOLOGICAL STUDIES

Acute Toxicity

The acute toxicity of carbendazim in several animal species is summarized in Table 1.

Gross and histopathological changes were observed in the testes and epididymides of male rats orally dosed with carbendazim at doses of 1 000 mg/kg b.w. and greater. Testes were small, soft and discoloured with greater than 70 percent of the tubules showing degenerative changes. Sperm was reduced or absent in the epididymides examined.

Table 1 Acute Toxicity of Carbendazim in Animals

Chemical	Species	Sex (Number)	Route	Vehicle	ADL/LD ₅₀ ^{1/} (mg/kg b.w.)	Reference
Carbendazim (MBC)	Rat	M/F (10/dose)	Oral	Corn oil	LD ₅₀ > 10 000	Goodman 1975
	Rat	M (1/dose)	Oral	Peanut oil	ALD > 11 000	Sherman 1965
	Rat	M/F (10/dose)	Oral	Sesame oil	LD ₅₀ > 15 000	Kramer & Weigand 1971
	Rat	M (1/dose)	Oral	Peanut oil	ALD > 17 000	Sherman & Krauss 1966
	Mouse	M (10/dose)	Oral	Propylene glycol	LD ₅₀ > 15 000	Til & Beems 1981
	Dog	M/F (2/dose)	Oral	Sesame oil	ALD ₅₀ > 5 000	Scholz & Weigand 1972
	G. pig	M (10/dose)	Oral	Corn oil	LD ₅₀ > 5 000	Dashiell 1975a
	Mouse	M/F (10/dose)	I.P.	Sesame oil	LD ₅₀ > 15 000	Scholz & Weigand 1972
	Rat	M (10/dose)	I.P.	0.9% saline & Tween 80	LD ₅₀ > 2 000	Scholz & Weigand 1972
	Rat	M (6/dose)	Inhal. Dust (1 h.)		ALC > 5.9 mg/l (time weighted concentration)	Sarver 1975
	Rabbit	M (10/dose)	Dermal	50/50 Aqueous paste	LD ₅₀ > 10 000	Edwards 1974a
	Rat	F (5/dose)	Dermal	Sesame oil	ALD > 2 000	Kramer & Weigand 1971
75% Wettable Powder	Rat	M/F (5/dose)	Oral	Corn oil	LD ₅₀ > 5 000	Hinckle 1981
75% Wettable Powder	Rat	M/F (10/dose)	Inhal. Dust (4 h.)		LC ₅₀ > 5 mg/l	Nash 1982
75% Wettable Powder	Rabbit	M/F (5/dose)	Dermal	Physiologic saline	LD ₅₀ > 2 000	Pord 1982

^{1/} Based on mg/kg a.i.

Special Studies on Eye and Skin Irritation

The eye irritation evaluation of technical carbendazim was negative in albino rabbits. The 75 percent wettable powder formulation when tested in rabbits produced transient corneal opacity in 6/6 unwashed and 2/3 washed eyes. Biomicroscopic examination confirmed this finding as mild to moderate. Conjunctival irritation (redness, swelling, discharge) was also transient. All eyes were normal at day 4 of the observation period. Irritation response was probably related to the inert ingredients in the wettable powder formulation (Edwards 1974b; Henry 1982).

A 75 percent wettable powder formulation produced transient slight irritation when applied to the intact and abraded skin of albino rabbits (Ford 1981a). A 55 percent suspension of the 75 percent wettable powder formulation in dimethyl phthalate produced mild irritation to the shaved intact skin of albino guinea pigs. A 5.5 percent concentration produced no irritation (Ford 1981b).

Special Study on Sensitization

Albino guinea pigs (10 males) exposed to carbendazim, either technical material or a 75 percent wettable powder formulation, presented no evidence of dermal sensitization following both intradermal injections and repeat applications to shaved intact skin (Ford 1981b).

Short-Term Studies

Rat

Groups of ChR-CD male rats (6/dose) were intubated with 200, 3 400 or 5 000 mg carbendazim/kg/day, five times/week for two weeks. Mortality occurred in 2/6 rats at the 3 400 mg/kg dose only. Animals at all levels demonstrated gross and microscopic evidence of adverse effects on testes and reduction or absence of sperm in the epididymides. Testes were small and discoloured, with tubular degeneration and evidence of aspermatogenesis. Morphological changes were also reported at 3 400 mg/kg for the duodenum, bone marrow and liver (Sherman 1965; Sherman & Krauss 1966).

Groups of ChR-CD rats (16 males and 16 females per group) were fed carbendazim (72 percent a.i.) in the diet for 90 days at dosage levels of 0, 100, 500 and 2 500 ppm. Animals were observed daily for behavioural changes and body weight and food consumption were recorded at weekly intervals. Haematological examinations were conducted on 10 male and 10 female rats in each group at 30, 60 and 90 days. Routine urinalyses were performed on the same animals, as well as plasma alkaline phosphatase and glutamic pyruvic transaminase levels. After 90-98 days of continuous feeding, 10 male and 10 female rats in each group were killed and selected organs weighed. Additional organs were preserved for microscopic examination. The six male and six female rats remaining in each group after terminal sacrifice were subjected to a reproduction study.

There were no gross toxic signs of poisoning and no compound-related effects on weight gain, food consumption, food efficiency or haematology. There were no control data for biochemistry, urinalysis determinations or differential white blood counts. The average daily dose for the high dose group animals was 360 mg/kg b.w./day, initially, and 123-152 mg/kg b.w./day at sacrifice. Liver to body weight ratio in females at 2 500 ppm was slightly increased compared with control rats. There were no effects on testicular weights in any of the treatment groups. Microscopic examination of selected tissues and organs in the control and high dose groups demonstrated no adverse effects attributable to the presence of carbendazim in the diet (Sherman 1968).

Rabbit

New Zealand Albino rabbits (6 males/group) were treated with 0 and 2 000 mg/kg of carbendazim, applied as a 50 percent aqueous paste to the shaved intact dorsal skin. Material was applied repeatedly, six hours/day for ten consecutive days. There were 222

untoward effects on body weight, clinical symptoms, organ weights, gross or histopathology of selected organs. There was focal necrosis of the epidermis and polymorphonuclear cell infiltration of the dermis in 5/6 rabbits exposed to carbendazim. No other effects were observed (Dashiell 1975b).

Dog

Groups of one-year-old beagles (4 males and four females per group) were administered carbendazim (53 percent a.i.) in the diet for three months at dosage levels of 0, 100, 500 and 2 500 ppm. The 2 500 ppm level was reduced to 1 500 ppm because of loss of appetite and decreased body weight. However, compound administration was interrupted when animals were placed on a control diet for a few days and then restarted at the 1 500 ppm dietary level. Therefore, the data generated from the high dose group are not considered in the evaluation of this study.

Food consumption and body weight data were recorded weekly. Clinical laboratory examinations, including haematological, biochemistry and urinalysis measurements were performed pre-test and after 1, 2 and 3 months of feeding. At the conclusion of the study all animals were killed, selected organs weighed and additional organs subjected to gross and microscopic evaluations.

There was no mortality or adverse cageside observations over the course of the study and growth and food consumption were normal, except as noted at the high dose level (1 500-2 500 ppm). Urinalysis measurements were unaffected by treatment. There were no dose-related effects on the haematological measurements. Females at the mid-dose level showed a trend toward increased cholesterol levels at 1, 2 and 3 months compared with pre-test and control. High-dose females had similarly elevated cholesterol levels. Organ-to-body weight changes were observed for the thymus of low- and mid-dose males and for the prostate of mid-dose males. All weights for these organs were increased compared with control values. However, only liver, kidney and testes were examined histologically in low- and mid-dose group dogs. Limited histopathology data did not indicate compound-related effects (Sherman 1970).

Groups of beagles (four males and four females per group) were administered carbendazim in the diet at dosage levels of 0, 100, 300 and 1 000 ppm for 13 weeks. The 1 000 ppm level was increased to 2 000 ppm after six weeks of treatment. Body weights, haematological and blood chemistry measurements, urinalyses and liver/kidney function tests were examined periodically during the test. Gross and microscopic examinations of all animals were performed at the conclusion of the study.

There were no reported compound-related effects on clinical behaviour, body weight, food consumption, haematology, kidney function (phenol red excretion) or liver function (BSP retention) examinations. Blood chemistry measurements were normal, except for a slight decrease in albumin in mid- and high-dose males at 12 weeks. These values differed from week 0 measurements only in high-dose males. Urinalysis was normal except for a high bacteria count in high-dose females at week 13. Blood clotting time was slightly reduced in high-dose dogs at week 12. There were slight increases in relative liver and thyroid weights and a decrease in relative heart weights in the 2 000 ppm group compared with control. There were no microscopic changes in these organs or any other organs that could be associated with treatment. There was an increase in submucosal lymphocytic infiltrates in female dogs in all groups, which was significant at the high dose. Carbendazim appeared to be without adverse effects on beagles when incorporated in the diet for 13 weeks at dietary levels of 300 ppm or less (Til et al. 1972). (NOTE: All measurements for males and females were combined and averaged. This practice can complicate interpretation of the results when there are slight or marginal effects in one sex, such as in this study.)

Special Study on Reproduction

Rat

A one-generation reproduction study in rats was performed with 24 male and 24 female rats that had been removed from a 90-day dietary feeding study. Dietary levels of carbendazim administered were 0, 100, 500 and 2 500 ppm. There were six male and six

female rats per group. After each female had been exposed to three males from the same dosage group they were separated to produce the F_{1A} generation. Litters were reduced to 10 pups/litter on the fourth day after birth. The F₀ animals were mated again after approximately one week in order to produce the F_{1B} litter. Reproduction indices, litter data at birth and on days 4, 12 and 21 were recorded, along with body weights at weaning.

Data presented were extremely limited and submitted as group data only. There were no pregnancies at 100 ppm for either F_{1A} or F_{1B} matings. There were no apparent effects on the reproduction indices or weaning weights. However, the fertility index for all groups, which was 33-67 percent, prevents any meaningful interpretation of the data (Sherman 1968).

Groups of Chr-CD rats (three male and 16 female rats/group; high-dose group was 20 females) were fed carbendazim in the diet at dosage levels of 0, 100, 500, 5 000 and 10 000 ppm and subjected to a standard two-litter per generation, three-generation reproduction study. Animals were fed from 21 days of age until 100 days of age, when they were mated to initiate the study. Number of matings, pregnancies and young in each litter at birth, and on days 4, 12 and 21 were recorded, along with body weights of pups at weaning. Litters were culled to 10 pups/litter on day 4. After one week, F₀ parents were mated again to produce the F_{1B} litters, after the F_{1A} had been sacrificed. The F_{1B} litters were maintained on their respective diets for 110 days and then mated to produce the F_{2A} and F_{2B} litters. The F_{3A} and F_{3B} litters were similarly produced. Gross and histopathological examination of selected tissues and organs were performed on two males and two females in each of five litters from the control, 5 000 and 10 000 ppm dose group pups from the F_{3B} litter.

Reproduction indices, including mating, fecundity, fertility, gestation, viability and lactation, were calculated and compared with control values. There were no compound-related effects on any of the reproduction indices other than reduced average litter weights at 5 000 and 10 000 ppm in all generations at weaning. Histopathological examination of F_{3B} weanlings did not reveal any effects that were considered compound related. MBC is considered to be without adverse effects on reproduction in the rat when administered in the diet at dose levels up to and including 500 ppm (Sherman 1972).

Carbendazim was administered to groups of Wistar Rats (10 males and 20 females/group) at dietary levels of 0, 150, 300 and 2 000 ppm for three generations. Two successive litters were reared from each female. General condition and behaviour were routinely observed and individual body weights were recorded throughout the study. The number of pups in each litter were recorded and culled to eight on day 1. Total weight of each litter was measured at days 1, 10 and 20. The F_{1A} and F_{2A} litters were discarded at weaning and the F_{1B} and F_{2B} litters were used to produce succeeding generations. The F_{3A} offspring were selected for use in a teratology study, while F_{3B} offspring were used in a 4-week short-term toxicity evaluation.

Health and body weight gain were not affected by carbendazim. However, treatment groups weighed significantly more than controls in all generations. There were no compound-related effects on fertility or survival at birth, day 10 or day 20. Litter size was not affected by treatment, except for a marginal decrease in F_{2A} litters in all dose groups. There were no differences in the F_{2B} litters at 300 and 2 000 ppm; however, there was a decrease in litter size and increase in mortality at birth at 150 ppm. Birth weight during the lactation period was comparable among all groups. There were no gross abnormalities related to treatment.

Autopsy of rats in the four-week short-term feeding study demonstrated increased relative liver weights and decreased relative spleen weights in females fed 2 000 ppm. There were also significant decreases in relative ovarian weights for females in all dose groups. Histopathology of the livers did not indicate any compound-related changes. There was no histopathology of the other organs presented.

There was no maternal or fetotoxicity evident in the teratology portion of the study. There were similarly no differences in visceral anomalies at 0 or 2 000 ppm (only groups examined). Thoracic vertebral bodies were reduced at 2 000 ppm and a significant reduction of the cervical vertebral bodies at 2 000 ppm. However, controls presented more significant changes throughout with regard to absent or delayed ossification of skeletal structures.

Although there were no apparent adverse effects on reproduction and no teratogenic effects at dietary levels of carbendazim up to and including 2 000 ppm, there were no individual animal data presented. Histopathology of animals in the four-week study was incomplete and did not include evaluations of spleen or ovaries. Such additional data are needed to confirm the absence of adverse effects in this three-generation reproduction study in rats (Koeter *et al.* 1976).

Special Studies on Teratogenicity

Rat

Groups of ChR-CD rats (27-28 pregnant rats/group) were administered carbendazim (53 percent a.i.) in their diet at dosages of 0, 100, 500, 2 500, 5 000, 7 500 and 10 000 ppm, from day 6 through day 15 of gestation. Average doses were equivalent to 0, 8.9, 45.9, 218.4, 431.6, 625.5 and 746.9 mg/kg day, respectively. On day 20 of gestation all pregnant animals were sacrificed and fetuses delivered by Caesarean section.

Determination of the number and location of live/dead fetuses and resorption sites were performed, as well as body weights, crown-rump length, sex and external examination for visible abnormalities. Two thirds of the fetuses were prepared for examination of skeletal abnormalities and the remaining ones were examined for visceral and soft tissue anomalies.

There was no mortality, no adverse effect on body weight or clinical signs of toxicity. Food intake was reduced in the 10 000 ppm group during the period the test diet was administered, but returned to comparable control levels from days 16 to 20. The data related to reproduction (implantation sites, resorption sites and live/dead fetuses) were not adversely affected by carbendazim. There were no external or internal abnormalities reported that were considered compound related. There was no individual litter data presented. It was concluded that carbendazim was not teratogenic when administered to ChR-CD rats at dietary levels up to and including 10 000 ppm during the critical period of organogenesis (Sherman *et al.* 1970).

Groups of pregnant Wistar-SPF rats (18-22 females per group) were administered carbendazim in the diet at dosage levels of 0, 500, 2 000 and 6 000 ppm from days 6 through 15 of gestation. On day 21 of gestation all pregnant rats were sacrificed and pups delivered by Caesarean section.

Dams were weighed periodically during the test and food consumption measured for specific periods. The number of corpora lutea were determined, ovaries weighed and fetuses weighed and examined. The number of implantation and resorption sites were recorded and the empty uterine horns weighed. One third of the fetuses were fixed and stained for skeletal examination and the remaining two thirds were examined for soft tissue anomalies.

Although 23 females per group were mated, the pregnancy rate was variable, with 18, 22, 20 and 18 pregnant in the 0, 600, 2 000 and 6 000 ppm groups, respectively. The mean body weight gain and food consumption for the high-dose females were significantly decreased in comparison with controls. The number of live/dead fetuses, implantation sites, embryonal resorptions, foetal resorptions and corpora lutea/dam were comparable among all groups. Ovarian weights and weight of the empty uterus were not affected by treatment. The mean foetal weight/litter and sex ratio were comparable among all groups. Pre- and post-implantation losses were not affected by treatment.

No visceral anomalies were reported that were significantly different from the control response. Misshapen and fused bones were much more frequent occurrences in the high-dose groups than any of the other treatment or control groups. Supernumerary ribs were also significantly increased in high-dose females. Ossification was significantly delayed or absent in high-dose group pups, particularly for forelimb, hindlimb, sternbrae and skull bones. Ossification was significantly delayed or absent in cervical vertebral bodies in all treatment groups when compared with control pups.

There were no individual animal or litter data and variations in ossification and other skeletal abnormalities were presented as percentages. The teratogenic or fetotoxic potential of carbendazim to pregnant Wistar-SPF rats, therefore, cannot be determined from the results and data presented (Koeter 1975a).

Rabbit

Groups of pregnant New Zealand albino rabbits (3-11 females/group) were administered carbendazim in the diet at dosage levels of 0, 600, 2 000 and 6 000 ppm from day 6 through day 18 of gestation. On day 29 of gestation, all pregnant animals were sacrificed and fetuses delivered by Caesarean section.

Does were weighed periodically and food consumption was determined for specific periods. The number of corpora lutea were determined, ovaries weighed and fetuses weighed and examined. The number of implantation sites and resorption sites were recorded and the empty uterus weighed. One half of fetuses were stained and sectioned for skeletal anomalies and the other half examined for soft tissue abnormalities. Only the fetuses in the high-dose and control groups were examined for visceral anomalies.

Although 18 females per group were artificially inseminated the pregnancy rate was extremely variable among groups, with 9 in control, 3 in 600 ppm, 3 in 2 000 ppm, and 11 in the 6 000 ppm group. The mean body weight gain was significantly decreased in the high-dose group, although food consumption did not vary among groups. The number of live/dead fetuses, implantation sites, embryonal resorptions, foetal resorptions and corpora lutea/dam were comparable among all groups. The ovarian and uterine weights in the high-dose group were depressed in comparison with control females. Pre- and post-implantation losses were not affected by treatment.

There were apparent differences between high-dose and control groups for visceral anomalies. However, too few litters and fetuses were examined to enable making any conclusions.

There was a significant increase in the number of supernumerary ribs (bilateral) and skull bones in the high-dose group. Ossification was significantly delayed or absent in high-dose group fetuses, most notably in the forelimb metacarpals and phalanges. There was also incomplete ossification of the sternbrae and skull bones, which was significant at 600 ppm and 6 000 ppm. Misshapen sternbrae were also present in the 6 000 ppm group.

There were no individual animal or litter data, variations in ossification were presented as percentages and visceral anomalies were evaluated in only 2/4 of the groups. The teratogenic potential of carbendazim to pregnant New Zealand albino rabbits, therefore, cannot be ascertained from the results and data presented (Koeter 1975b).

Special Study on Neurotoxicity

A neurotoxicity study performed using chickens gave no indication of neurotoxic potential at single oral doses up to and including 5 000 mg/kg (Goldenthal et al. 1978).

Long-Term Studies

Rat

Groups of weanling rats (36 male and 36 female Chr-CD albino rats/group) were administered carbendazim (50-70 percent a.i.) in the diet for 104 weeks at dosage levels of 0, 100, 500, 2 500-10 000, and 5 000 ppm. Growth was observed by body weight changes and food consumption data, which were recorded weekly for the first year and twice a month thereafter. Daily observations were made with respect to behavioural changes and mortality. At periodic intervals throughout the study, haematologic, urinalysis and selected clinical chemistry examinations were performed. After one year each group was reduced to 30 male and 30 female rats by interim sacrifice for gross and microscopic evaluations. At the conclusion of the study all surviving animals were sacrificed and gross pathological examination of tissues and organs was made. Microscopic examination of all tissues and organs from the control and 2 500 ppm groups were conducted, along with liver only from the 100 and 500 ppm groups, and liver, kidney testes and bone marrow from the 5 000 ppm group animals.

The few mortalities observed in the first year were not attributable to the presence of carbendazim in the diet. Survival decreased during the second year to approximately 50 percent for males and 39 percent for females, but was not related to treatment. Body weight gain was depressed for males and females in the 2 500-10 000 ppm group and for females in the 5 000 ppm group when compared to control groups. Food consumption did not differ among groups. The average daily dose for the 500 ppm group was 65 mg/kg b.w./day (initially, M and F), 18 mg/kg (at one year) and 15 mg/kg (at two years). Haematologic examinations demonstrated reduced erythrocyte count, haemoglobin and haematocrit values for females at 3-24 months in the 2 500 and 5 000 ppm groups; and for males at 24 months in the 2 500 ppm group. There were no compound-related clinical manifestations of toxicity and no effects observed in urinalysis examination. Alkaline phosphatase and glutamic pyruvic transaminase activities varied throughout the test at 2 500 and 5 000 ppm but did not demonstrate a consistent dose response. There were no apparent differences in the organ weights or organ-to-body weight measurements, except for female livers in the 2 500 and 5 000 ppm group. This increase in the liver-to-body weight ratio was reflective of lower body weights for both groups and therefore, not compound related. Histopathologic evaluation of the livers did not demonstrate any compound-related effects. Histopathologic examinations demonstrated an increased incidence of pigment deposition in spleen and bone marrow for both males and females at the 5 000 ppm level. This is consistent with the haematology data for the same group. Males in the 2 500-10 000 ppm group presented marginal increases for diffuse testicular atrophy and prostatitis. Carbendazim is considered to be without adverse effects on Chr-CD rats when incorporated in the diet at levels up to and including 500 ppm (Sherman).

Groups of Wistar rats (60 males and 60 females/group) were administered carbendazim (99 percent pure) in the diet at dosage levels of 0, 150, 300 and 2 000 ppm for two years. The 2 000 ppm dose was increased to 5 000 ppm after one week and then to 10 000 ppm after two weeks for the remainder of the study. Clinical signs of toxicity and general health were determined daily. Body weight and food consumption were measured regularly throughout the study. Haematology (peripheral blood), blood chemistry (orbital sinus) and urinalysis evaluations were periodically conducted during the study. All animals were subjected to complete gross necropsy and selected organs weighed. A complete list of tissues and organs was prepared and examined microscopically in 20 male and 20 female rats of the control and high-dose groups. All tumours and gross abnormalities were also examined histologically.

There were no differences between test groups and control animals concerning clinical signs of toxicity or food consumption. Body weights were significantly reduced in low-dose males at week 88 to term and in high dose females at week 12 to term. Urinalyses and kidney function (specific gravity) were comparable among all groups. Of the haematological measurements examined, Hgb was depressed in high-dose females at week 26, 52 and 103 and PCV was depressed in high-dose females at week 103. There were no compound related effects in males. SGOT activity was decreased in high-dose males at term, but

not in females. High-dose females had increased SGPT activity and decreased total serum protein at term. There were no compound-related effects on organ weights except for increased relative liver weights in high-dose females. There were also no compound-related effects on mortality, with 50 percent mortality in control males at week 76, and at week 92 in treated group males. There was 50 percent mortality in control and low-dose females at week 88 and at 72-96 weeks in mid- and high-dose females. Survival at termination of the study was comparable among all groups.

There were no measurable histological differences between control and treated groups, except for an increased incidence of diffuse proliferation of parafollicular cells of the thyroid in the high-dose females. The number of tumour-bearing animals and total number of primary tumours were comparable among all groups, and there were no compound-related oncogenic effects reported. (NOTE: All data presented were group mean values with SD. There were no data on individual animals.)

The no observed effect level (NOEL) is 300 ppm, based on body weight changes, decreased Hgb and PCV values and increased relative liver weight in high-dose females. There was no tumorigenic effect in this strain of rat at doses up to and including 10 000 ppm for 104 weeks (Til et al. 1976).

Dog

Groups of beagles (four males and four females/group) were administered carbendazim (53 percent a.i.) in the diet at dosage levels of 0, 100, 500 and 2 500 ppm for two years. Dogs were one to two years of age at the start of the test. Some dogs in the high-dose group received only 1 500 ppm. Food consumption and body weight data were obtained weekly and animals were examined daily for clinical signs of toxicity. Haematological, biochemical and urinalysis examinations were performed periodically throughout the study. Interim sacrifice after one year was performed on one male and one female from the 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 the test diet. Organ weights, gross necropsy and histopathological evaluations were performed at the conclusion of the study. Only the livers and testes were examined histologically in the 100 and 500 ppm dose groups.

There was no mortality reported for the control or 100 and 500 ppm dose groups. However, three males in the high-dose group were sacrificed after 22 and 42 weeks because of poor nutrition. No females in the high-dose group died. Body weight and food consumption were all adversely affected 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 and F), 14-18 mg/kg (one year) and 10-16 mg/kg (two years). Dogs in the highest dose group developed anorexia, distended abdomens and overall poor nutritional condition. Haematological evaluations and urinalyses were not apparently affected by treatment. The dogs in the 500 ppm and 1 500-2 500-dose groups had increased cholesterol, BUN, total protein, GPT and APase levels and presented evidence of a decreased A/G ratio throughout the study. This biochemical evidence of liver effect was supported by liver pathology, with incidences of hepatic 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 the other dose group or in control males. Based on the lack of supporting data in the other dose group males, these findings are not considered as being compound-related.

The NOEL in this study appears to be 100 ppm, based on the liver effects noted at 500 ppm and greater (Sherman 1972).

Groups of beagles (four males and four females/group) were fed carbendazim in the diet at dosage levels of 0, 150, 300 and 2 000 ppm for 104 weeks. After 33 weeks the 2 000 ppm dose was increased to 5 000 ppm. Dogs were 22-27 weeks old at the start of the study. Daily examinations were made for clinical signs of poisoning and adverse behaviour. Growth, as evidenced by body weight, was recorded regularly throughout the study, as were food consumption data. At periodic intervals (weeks 13, 26, 52, 78 and 104), haematology, blood chemistry and urinalysis were performed. Liver function (BSP retention) and kidney function (phenol red excretion) tests were evaluated at weeks 26, 52 and 104. At the conclusion of 104 weeks of dietary administration, each dog was sacrificed and gross and microscopic examination of tissues and organs were performed.

There was no mortality in any group except for one female in the high-dose group which was killed in a moribund state after week 36. Growth, as measured by body weight, was decreased in mid-dose males and high-dose males and females. Food consumption was comparable among all groups. Blood clotting times were significantly reduced in high-dose males from week 13 to term, with slight decreases noticed in high-dose females. Serum alkaline phosphatase activity was increased in the high-dose group dogs throughout the study. There were no compound-related effects on SGPT or SGOT levels. All other haematological and blood chemistry measurements were comparable with control groups. There were no differences among groups for BSP retention, phenol red excretion or urine analyses.

Absolute liver and thyroid weights were significantly increased in high-dose group dogs. Relative liver, thyroid and pituitary weights were also significantly increased at the high dose. There were no reported microscopic changes in these organs related to treatment. There was an increased incidence of prostatitis (3/4 vs 1/4) in high-dose males compared with controls. Also noted in 1/4 high dose males was interstitial nuclear inflammatory cell infiltrates and atrophic tubules of the testes.

(Summary tables only were provided for the number of dogs with the indicated pathological response. Severity of response, identity of dog involved, gross and histopathology reports of individual animals were not provided. Data provided were generally not separated according to sex.)

The feeding of carbendazim in the diet to dogs for two years was without apparent adverse effects at levels up to and including 300 ppm (Reuzel *et al.* 1976).

Special Studies for Carcinogenicity

Groups of CD-1 mice (80 males and 80 females/group) were administered carbendazim (99 percent a.i.) in the diet at dose levels of 0, 500, 1 500 and 7 500 ppm for two years. The 7 500 ppm dose was reduced to 3 750 ppm after 66 weeks for the males because of increased mortality. Females received 7 500 ppm throughout the study period. Animals were 6-7 weeks old at the start of the study. Mice were examined daily for behaviour and clinical signs of toxicity, biweekly for palpable masses and regularly weighed for body weight changes. Food consumption was similarly determined on a routine basis. Mortality was noted and recorded. Peripheral blood was collected periodically throughout the study for haematological examinations. Selected organs were weighed, including brain, heart, lungs, liver, spleen, kidney, testes and thymus. Microscopic examination was performed on a complete list of tissues and organs. Urine and faecal samples were also analysed.

Mortality was compound related in male mice. The high-dose group males terminated at week 73 because of significant increase in mortality. Only nine males in the 1 500 ppm group survived to week 104, compared with 18 for control males. Females were unaffected by treatment in this respect.

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There were no dose-related effects on body weight or food consumption throughout the study, although terminal body weights for low- and mid-dose group males were less than control and high-dose group males. Clinical signs of toxicity were similar among all treatment and control groups. Haematological determinations in males were unaffected by treatment. Females in the 7 500 ppm group had reduced erythrocyte counts and marginal decreases in haemoglobin concentration.

Both absolute and relative thymus weights were significantly decreased in females in the 500 and 1 500 ppm groups, but not in the high-dose group. Absolute liver weight was increased in the 7 500 ppm females, with relative liver weight increased in the 1 500 and 7 500 ppm groups. Organ weights for the males were variable with only the kidney and thymus weights apparently decreased by treatment. Absolute kidney and thymus weights were depressed in all male treatment groups. However, relative kidney and thymus weights were significantly decreased in the high-dose males only. The lower absolute kidney and thymus weights in the low- and mid-dose group males were probably a reflection of reduced terminal body weights.

Histological examination revealed dose-related changes in the thymus (lymphoid depletion) and kidneys (bilateral/unilateral accumulation of yellow-brown pigment in the tubules) for mid- and high-dose group male mice. Examination of the testes demonstrated a marginal increase in the finding of sperm stases (bilateral/unilateral combined) in treated males, with a similar finding of increased germinal cell atrophy (bilateral only). There was an opposite trend, however, for unilateral germinal cell atrophy, where the incidence in controls was greater or equal to treated males. These effects are, therefore, not considered compound related.

Examination of livers of male mice revealed a significant hepatotoxic effect at 1 500 and 7 500 (3 750) ppm, demonstrated by centrilobular hypertrophy, necrosis and swelling. There was no increase in the finding of hepatocellular adenoma, which occurred with equal frequency in control and treatment groups. A significant increase occurred for hepatocellular carcinomas at the 1 500 ppm dose only. However, too few high-dose males survived to 17 months (510 days) to support the conclusion of no oncogenic effect at that dose level.

Histomorphologic evaluation of the female mice revealed an increased incidence of lymphoid depletion in the thymus in the mid- and high-dose groups. There was a significant accumulation of yellow-brown pigment in the macrophages and tubules in the kidneys, as well as an increase in cystic tubules for high-dose group females. The occurrence of hepatocellular carcinomas was significantly increased in the mid- and high-dose females. However, there was no apparent compound-related effect on the latency period for this finding. The finding of hepatocellular adenomas was marginally increased in the low- and mid-dose females, but not in the high-dose group, compared with control. Other findings indicative of hepatotoxicity were more prominent in the control females than in the treatment groups. Hepatocellular chromatin aggregation and necrotic (focal, multifocal, single cell) were increased in controls. There was also a significant increase for the incidence of macrophages containing yellow-brown pigment. These findings appear to indicate different metabolic or detoxification mechanisms, which are sex dependent. The carcinogenic response in the liver, although significant at 1 500 and 7 500 ppm for females and at 1 500 ppm for males, is considered a weak response in light of the histomorphologic changes in the livers in male and female control group mice (Wood 1982).

* Carbendazim was administered in the diet to groups of SPF Swiss mice (100 males and 100 females/group) at dosage levels of 0, 150, 300 and 1 000 ppm for 80 weeks. The 1 000 ppm dose was increased to 2 000 ppm at week 4 and to 5 000 ppm at week 8 for the remainder of the study. Animals were observed for behaviour and clinical signs of toxicity. Body weight measurements were determined throughout the study. Gross necropsies were performed on all animals, liver and kidney weights recorded, and a complete list of organs and tissues was examined microscopically.

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There were no compound-related effects on general condition, mortality or body weight. Survival at term was 70 percent for males and 80 percent for females. Relative liver weights in high-dose males and females were significantly different from controls. There were no changes in kidney weights. Gross and histopathology examinations demonstrated a compound-related effect on the livers of both male and female mice in the high-dose group. There was a significant increase in the number of mice with clear cell foci in high-dose males and females and in mixed cell foci for high-dose males. Neoplastic nodules were reportedly increased in high-dose females, while the incidence of hepatoblastoma was increased in high-dose males. There were no differences between control and treatment groups for the finding of hepatocellular carcinoma. It was concluded that carbendazim is oncogenic to this strain of mouse at dietary doses greater than or equal to 5 000 ppm (Beems *et al.* 1976). (NOTE: All data presented were group mean values. There were no data on individual animals.)

* Carbendazim was administered in the diet to groups of HOE NMRKf (SPF 71) mice (100-120 males and females/group) for 96 weeks at dosage levels of 0, 50, 150, 300 and 1 000 ppm. The 1 000 ppm dose was increased to 2 000 ppm at week 4 and to 5 000 ppm at week 8 for the remainder of the study. Animals were observed for behaviour and general condition, as well as body weight, food/water consumption and mortality. Gross necropsies were performed on all animals, liver and lung weights were recorded, and a complete list of organ and tissues was examined microscopically. An interim sacrifice was made at 18 months on 20 males and 20 females from the control group and the 5 000 ppm group.

There were no compound-related effects on behaviour, body weight gain, food/water consumption or mortality. At 22 months there was 24-31 percent mortality in male mice and 17-52 percent mortality in females, for all groups. Mean daily consumption of carbendazim in mg/kg was 5.8-7.1 at 50 ppm, 17.1-21.2 at 150 ppm, 34.4-41.9 at 300 ppm, and 548.4-682.3 at 5 000 ppm.

Examination of lung and liver weights at 18 and 22 months demonstrated an increase in absolute and relative liver weights in both male and female mice at 5 000 ppm.

Gross and microscopic examination of animals at 18 months revealed compound-related effects on the liver at 5 000 ppm. There were reported increases in centrilobular hypertrophy of liver cells, single cell necroses, liver cells in mitosis and pigment in Kupffer cells. Controls presented evidence of fatty change of liver cells only. Microscopic evaluation of tissues/organs at 22 months demonstrated a definite compound-related effect on liver at 5 000 ppm in both males and females. There was marked liver cell hypertrophy, clear cell foci, liver cells in mitosis, abundant inclusion bodies in enlarged cell nuclei, multiple cell necroses and greenish yellow pigment in Kupffer cells. Neoplastic nodules (adenomas), carcinomas, fibrosarcomas and other tumourigenic responses in the liver were equally distributed among all groups. The occurrence of hemangiomas, evident in treated groups with none in the control groups, was randomly distributed (both by dose and sex), not dose related, not significantly different from control and, therefore, not considered compound related. The finding of any neoplasms (such as adenomatosis) were equally distributed among all groups. There was no effect on incidence or time of onset of tumours by carbendazim and the total number of benign and malignant tumours were comparable among groups. There was a significant increase in liver toxicity in both males and females at 5 000 ppm. However, there was no evidence of a carcinogenic effect from carbendazim when administered in the diet to mice at doses up to and including 5 000 ppm for 22 months (Kramer & Weigand 1982).

Special Studies on Mutagenicity

Results of the various mutagenicity assays are summarized in Table 2.

Bacteria

MBC was examined for mutagenic activity in *Salmonella typhimurium* following the plate incorporation protocol of Ames *et al.* (1975), at concentrations between 1 and 125 μ /g plate, with and without activation. MBC increased the reversion frequency 3-5 times the control frequency, without activation. Doubtful activity was found with activation. Source and purity of test substance were not provided and there were no actual data presented (Rashid & Ercegovic 1976; Ercegovich & Rashid 1977).

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MBC was tested for mutagenic activity on *S. typhimurium*, strains TA1535, TA1537, TA98 and TA100, with and without activation. MBC, dissolved in DMSO, was not mutagenic at concentrations between 4 and 2 500 $\mu\text{g}/\text{plate}$ (Hoechst 1977).

MBC and some of its commercial preparations were examined for mutagenic potential in *S. typhimurium* following different treatment protocols. An overlay spot test was used to test MBC at concentrations of 50 and 100 $\mu\text{g}/\text{spot}$ in strains *his* G46, TA1530 and TA1950. Only one sample of MBC (Seiler) exhibited weak mutagenic activity at 100 $\mu\text{g}/\text{spot}$. In a plate incorporation assay using strain TA100, MBC was not mutagenic at concentrations between 50 and 200 $\mu\text{g}/\text{plate}$. Liquid culture assays with 1 000 $\mu\text{g}/\text{ml}$ of MBC showed no evidence of mutagenicity in *his* G46 and TA1950 (Ficsor *et al.* 1978).

MBC was non-mutagenic in *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100, and in *Escherichia coli* strain WP2 *hcr*⁻. Concentrations between 5 and 1 000 $\mu\text{g}/\text{plate}$ were tested in a plate incorporation assay with DMSO as the solvent both in the presence and absence of an activation system, which included a 9 000 x g supernatant fraction of homogenized livers from Aroclor 1254-treated Sprague-Dawley rats (Shirasu *et al.* 1977).

MBC and 5-hydroxy-MBC were tested for mutagenic activity in *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 according to the plate incorporation procedure of Ames *et al.* (1975). DMSO was the solvent. Each sample was evaluated in the presence and absence of an activation system. Five different samples of MBC were tested at concentrations up to 10 mg/plate . A sample of technical grade MBC was mutagenic in the presence of rat liver homogenate in strains TA1537, TA1538 and TA98. A 99.6 percent pure MBC sample, in the presence of rat and mouse activation systems, was mutagenic in TA1537, TA98, TA1535 and TA100. Rat and mouse liver activation systems gave essentially identical results in TA1537 and TA98. A third sample (97.6 percent, Czech-Polish) was mutagenic with rat liver activation in strains TA1537, TA98 and TA100. An MBC sample from Hoechst (95-100 percent pure) was not mutagenic in any of the *Salmonella* strains. Analytical grade MBC (99.5 percent pure) was weakly mutagenic in TA1537 at concentrations up to 15 mg/plate . A sample of 5-hydroxy MBC was not mutagenic at concentrations up to 20 mg/plate (Russell 1977 a, b, 1978; Donovan 1981 a, b).

In a host mediated assay in male ICR mice given total doses of either 1 000 or 4 000 mg/kg the mutation frequencies observed in the *S. typhimurium* strain *his* G46 from treated animals were identical to mutation frequencies in bacteria from control animals (Shirasu *et al.* 1977).

Yeast and fungi

MBC was evaluated for mutagenic activity in *Aspergillus nidulans* and *Cladosporium cucumerinum*. In *A. nidulans*, MBC at a concentration of 2.77 μM (0.53 $\mu\text{g}/\text{ml}$), caused an increase in the frequency of colonies resistant to MBC (2 $\mu\text{g}/\text{ml}$) but did not increase the frequency of colonies resistant to carboxin (20 $\mu\text{g}/\text{ml}$). In *C. cucumerinum*, MBC at 0.58 μM (0.11 $\mu\text{g}/\text{ml}$) caused an increase in carboxin (20 $\mu\text{g}/\text{ml}$) resistant colonies, but had no effect on the frequency of colonies resistant to MBC (0.8 $\mu\text{g}/\text{ml}$). An activation system was not used for any of these studies (Speakman & Nirenberg 1981).

Experiments were conducted in *A. nidulans* and *C. cucumerinum* to evaluate the effect of altering the pH of the agar medium, using pHs of 5, 5.2-5.3 and 6.8. The pH of the treatment medium had a significant effect on the activity of MBC, which was mutagenic in *A. nidulans* only at a pH of 5.2-5.3 (MBC resistance) and in *C. cucumerinum* only at pH 6.8 (carboxin resistance). The concentrations of MBC exhibiting mutagenic activities were 0.53 $\mu\text{g}/\text{ml}$ and 0.11 $\mu\text{g}/\text{ml}$ for *A. nidulans* and *C. cucumerinum*, respectively. The effect of an activation system was not studied. The authors concluded that MBC had weak mutagenic activity (Nirenberg & Speakman 1981).

Cultured mammalian cells

Chinese hamster ovary cells in culture were exposed to varying concentrations of MBC without activation (3 to 654 μM) and with activation (3 to 628 μM), to detect mutations at the gene locus coding for hypoxanthine guanine phosphoribosyl transferase (HGPRT). A dose-related cytotoxic response was evident in cultures exposed to MBC without activation, with a decreased survival at 16 μM . No statistically significant differences in mutation frequency were noted and MBC was not mutagenic under the test conditions used (Waterer & Krahn, 1980).

Insects

MBC dissolved in DMSO at 0.5 mg/ml did not cause a significant increase in the frequency of sex-linked recessive lethals when given to *Drosophila melanogaster*. The only indication that the substance may have some potential for damaging germ cells comes from the observation that it caused an increased incidence of sterility in the later broods from treated Oregon-R males. However, this effect was not observed in treated $y^w/B^s/Zy^+$ males. No compound-related effects were noted when chromosomes were examined for breakage in a second set of experiments and the overall incidence of recessive lethal mutations reported was 5/4807 (0.1 percent). The sterility observed in broods from matings involving mitotic spermatogonial cells is consistent with the suspected spindle effects of the chemical (Lamb & Lilly 1980).

Mouse embryo

Mouse embryos, heterozygous for four different recessive coat colour genes, were treated in utero by dosing the mother orally with MBC at dose levels of 100 to 300 mg/kg b.w. If mutations are induced in pigment precursor cells in a wild type allele of one of the genes under study, a spot of an altered colour may appear on the coats of the offspring. At 200 mg/kg the number of spots was significantly different from controls (Fabrig & Seiler 1979).

Cytogenetics

The ability of MBC to cause chromosomal aberrations was evaluated in human lymphocytes in culture. MBC, at a concentration of 0.5 mg/ml, did not increase the frequency of chromosome aberrations over the DMSO control in a system without activation. Cells treated with MBC did exhibit grossly contracted chromosomes, an effect induced by spindle poisons such as colchicine (Lamb & Lilly, 1980).

MBC, with and without metabolic activation, was evaluated for its ability to induce forward mutations at the thymidine kinase (TK) locus in mouse L5178Y lymphoma cells. Metabolic activation was accomplished by microsomal enzymes obtained from induced rat liver preparations (S-9 mix). Ethylmethane sulphonate (EMS) and 3-methylcholanthrene were used as positive controls.

MBC was mutagenic in this test system with activation, the mutation frequency being increased in a dose-related manner. There was no mutagenic response without metabolic activation at doses of 50 to 250 μM . The results indicated that metabolic activation enhanced MBC's mutagenic activity at 100 μM (Jotz *et al.* 1980).

The effects of MBC on the mouse lymphoma L5178Y cell line at the thymidine kinase ($TK^{+/-}$) locus were examined both with and without metabolic activation. Concentrations tested included 12.5 to 200 μM of MBC, DMSO as negative control, 2 000 μM of ethylmethane sulphonate (positive control without activation) and 15 μM of 3-methylcholanthrene (positive control with activation). The positive controls gave the expected response. However, MBC, both with and without activation tested in replicate trials, was not mutagenic at levels up to and including 200 μM (Krahn *et al.* 1983).

Technical grade MBC was tested for its ability to cause chromosome aberrations in rat bone marrow cells in vivo. Male and female Sprague-Dawley rats were given a single oral dose of 300 mg/kg. Metaphase cells from treated animals, sacrificed at 6, 24, and 48 hours after treatment, did not exhibit an increased frequency of chromosome aberrations (BASF 1975a).

MBC did not produce chromosome breakage in bone marrow cells from Chinese hamsters given oral doses of 1 000 mg/kg. Only one chromatid break was observed in a total of 500 metaphases from four animals. The mitotic figures were examined in bone marrow cells from ICR mice given two oral doses of MBC at 1 000 mg/kg each. Twelve of the 1 000 nucleated anaphase cells exhibited lagging chromosomes, bridge formation, tripolar spindle formation or unequal chromatin distribution. MBC does not break chromosomes but probably exerts its effect by interfering with spindle function (Seiler 1976).

Rodent dominant lethal test

Twenty NMRI mice were given intraperitoneal injections of MBC (500 mg/kg) on five successive days. A 0.5 percent solution of the vehicle CMC was given to 20 mice and served as controls. Animals receiving MBC did not exhibit any clinical signs of toxicity. The body weights of control and MBC-treated animal groups were identical after the first week of mating. No macroscopically observable pathological changes were seen in dissected mice from the MBC-treated group. MBC did not exhibit a dominant lethal effect (Hoechst 1974).

Twenty-two male NMRI mice were given MBC by stomach tube (300 mg/kg) on five successive days. The vehicle for delivering MBC was not given. The same number of untreated mice served as controls. MBC-treated mice did not exhibit clinical symptoms of toxicity, body weight changes or macroscopically recognizable pathological changes in the internal organs. MBC did not cause a change in the mutagenicity index over that of the control (BASF 1975b).

DNA damage and repair

DNA repair assays in rat (F344) or mouse (B6C3F1) hepatocyte primary cultures (HPC) were evaluated for MBC along with dimethylnitrosamine and 2-amino-fluorene, which were used as positive controls. MBC and tritiated thymidine (10 μ Cl) were added to the culture medium. After 18 to 20 hours of incubation they were fixed and examined microscopically for morphological changes and absence of S-phase nuclei indicative of cytotoxicity. Autoradiographic techniques were used to determine the number of nuclei grains induced. MBC did not induce DNA repair in rat or mouse hepatocytes. The positive controls gave the expected response (Tong 1981a,b).

Differential toxicity to bacterial strains with different repair capacities

MBC (typically 99 percent pure, sources unspecified) was tested for toxicity to recombination repair-proficient and repair-deficient strains of Bacillus subtilis. MBC was tested at concentrations between 20 and 1 000 μ g/disk. MBC did not cause a zone of killing in either strain and, thus, was negative in the assay. The absence of toxicity to either strain indicated that MBC was either non-toxic under the test conditions or the limited solubility prevented diffusion from the disk. An activation system was not used (Shirasu et al. 1977).

Table 2

Mutagenicity Assays

Test Organism	Test Substance	Result	Reference
Gene Mutation Studies			
Bacteria			
<u>Salmonella typhimurium</u>	MBC	Bacterial assays with MBC. Strains TA98, TA100, TA1535, TA1537, and TA1538. Doubtful mutagenic activity was reported for MBC both with and without metabolic activation.	Ercegovich & Rashid 1977 Rashid & Ercegovich 1976
		Negative	Shirasu <u>et al.</u> 1977
		Negative. Results dependent on sample source and purity.	Russell 1977a,b, 1978
		Negative.	Hoechst 1977
		Series of tests: spot and liquid culture assays using strains <u>his</u> G46 and TA1530, TA1535, TA1950. No mutagenic activity except one weak positive in <u>his</u> G46.	Ficsor <u>et al.</u> 1978
		Plates treated with 100 to 10 000 ug MBC, with activation. The number of revertants/plate increased from 4.2 to 8.95 times in the trials with positive responses in TA98 and from 3.7 to 6.4 times in TA1537.	Donovan 1981a
MBC (Hoechst)	Negative. Same as previous citation, with and without activation. Results dependent on sample source and purity.	Donovan 1981b	
<u>S. typhimurium</u> (host mediated assay)	MBC	Negative	Shirasu <u>et al.</u> 1977
Yeast and Fungi			
<u>Asp gillus nidulans</u>	MBC	Positive at pH 5.2 and 5.3	Speakman & Nirenberg 1981 Nirenberg & Speakman 1981
	MBC	Positive.	Kappas <u>et al.</u> 1974
	MBC	Positive.	Davidse 1973
<u>Cladosporium cucumerinum</u>	MBC	Positive at pH 6.8	Speakman & Nirenberg 1981 Nirenberg & Speakman 1981

Test Organism	Test Substance	Result	Reference
Cultured Mammalian Cells			
Chinese hamster ovary cells <u>in vitro</u>	MBC	Negative	Waterer & Krahn 1980
Insects			
<u>Drosophila melanogaster</u>	MBC	Noted sterility in some broods. This was considered to be consistent with spindle effects of MBC.	Lamb & Lilly 1980
Mammals			
Mouse, <u>in utero</u>	MBC	Positive.- coat colour changes	Fahrig & Seiler 1979
Chromosomal effects			
Cytogenetics-in vitro			
Human lymphocytes	MBC	Grown in culture medium containing 0.5 mg MBC. No compound related chromosome aberrations.	Lamb & Lilly 1980
Mouse lymphoma L5178Y cells	MBC	Dose-related increase in mutation frequency with metabolic activation at TK ⁺ /- locus at 100 µM.	Jotz <u>et al.</u> 1980
Mouse lymphoma L5178Y cells	MBC	MBC was not mutagenic at the TK ⁺ /- locus with or without activation at concentrations up to and including 200 µM.	Krahn <u>et al.</u> 1983
Cytogenetics - <u>in vitro</u>			
Rat bone marrow	MBC	Negative.	BASF 1975a
Chinese hamster bone marrow	MBC	Negative.	Seiler 1976
Mouse bone marrow	MBC	Negative.	Seiler 1976
Dominant lethal-rodents			
Rat	MBC	Negative.	Benes & Sram 1976
Mice	MBC	Negative.	BASF 1975b Hoechst 1974
Micronucleus Test			
Mouse bone marrow	MBC	Positive.	Seiler 1976
DNA Damage and Repair			
Mice B6C3F1 and F344	MBC	MBC was tested for DNA repair using primary hepatocyte cultures. MBC did not induce DNA repair in either rat or mouse.	Tong 1981a,b

Test Organisms	Test Substance	Result	Reference
<u>Differential Toxicity-Bacteria</u>			
<u>Bacillus subtilis</u>	MBC	Negative.	Shirasu <u>et al.</u> 1977
<u>Gene Mutation-Bacteria</u>			
<u>S. typhimurium</u>	5-hydroxy-MBC	Negative. Negative	Cannon Laboratories 1978 Russell 1977b
<u>Plant Studies</u>			
<u>Allium cepa</u>	MBC	Positive.	Richmond & Phillips 1975

COMMENTS

Carbendazim follows a similar metabolic pathway to benomyl in rats and mice, being excreted in urine as 5-hydroxy carbendazim (5-HBC). Enzyme induction studies demonstrate that the rat is more efficient than the mouse in metabolizing and eliminating carbendazim and its metabolites.

Carbendazim is not acutely toxic to mammals as demonstrated by acute oral and dermal LD₅₀s of >10 000 mg/kg in rat and rabbit, respectively. Gross and histopathological examinations performed in many of these acute studies indicated that doses ≥1 000 mg/kg produced adverse effects on the testes (small, soft, discoloured, degenerative changes of the tubules) and epididymides (reduced or absent sperm).

A three-generation reproduction study in rats demonstrated a NOEL of 500 ppm, with higher doses resulting in reduced average litter weights.

Teratology studies in which the test material was administered in the diet of rats indicated in one study the absence of induction of terata at 10 000 ppm and in the second study a low incidence of misshapen, fused or incompletely ossified bones at 6 000 ppm. A limited study in rabbits did not indicate the induction of terata following dietary administration at 6 000 ppm (see also benomyl).

A short-term dietary study in rats indicated increased liver to body weight ratios in females at 2 500 ppm, although no compound-related histomorphologic changes were evident. There were no effects on testicular weight and the NOEL was 500 ppm. Two short-term dietary studies in dogs demonstrated that 300 and 500 ppm, respectively, caused no adverse effects. However, at doses of 1 000 and 2 500 ppm animals lost their appetite, lost weight and had increased cholesterol levels and relative liver weight increases.

In two separate long-term feeding studies in rats, carbendazim produced relative liver weight increases, deposition of pigment in the spleen and bone marrow, and decreased haemoglobin, haematocrit and red blood cell counts at the higher doses. It was without adverse effects at 300 and 500 ppm, respectively, and there was no oncogenic response at doses up to 10 000 ppm.

Beagles appeared to be more sensitive than rats to dietary exposure to carbendazim. Hepatic cirrhosis, vacuolated hepatic cells and increased levels of cholesterol, BUN, total protein, GPT and alkaline phosphatase, with decreased A/C ratio, were evidence of liver toxicity at levels greater than 100 ppm for two years.

Oncogenicity studies were performed using three strains of mice (CD-1, Swiss SPF and HOE-NMR). In CD-1 mice there was a significant increase in hepatocellular carcinomas at 1 500 and 7 500 ppm in females and 1 500 ppm in males. However, there were also substantial histomorphic changes in the livers of male and female control animals. There was no oncogenic response at 500 ppm. Swiss mice, exposed for 80 weeks to 150, 300 and 1 000-5 000 ppm carbendazim, showed an oncogenic response at 5 000 ppm, which was evidenced by significant increases in the incidence of neoplastic nodules and hepatoblastomas. There were no compound-related effects in this study at 300 ppm. HOE-NMR mice exposed to 50-5 000 ppm carbendazim for 96 weeks presented no evidence of an oncogenic response. It was concluded that carbendazim was hepatocarcinogenic to mice at high dose levels.

Mutagenicity studies with carbendazim gave both positive and negative results. Carbendazim was positive in the micronucleus, yeast, fungi and Drosophila tests. Conflicting negative and positive results in other tests prevented evaluation of the mutagenic potential. The potential impact of these results on human health cannot be adequately assessed at this time.

The data for benomyl and carbendazim have indicated that the metabolism of the two compounds is essentially the same, with benomyl converted rapidly to carbendazim in mammals. Accordingly, the available data for benomyl and carbendazim should be considered collectively for the evaluation of specific studies such as teratology, reproduction, chronic toxicity and oncogenicity, taking into account the different molecular weights of the two compounds.

Previous Meetings have considered the aetiology and pathogenesis of liver tumours in certain strains of mice, with particular emphasis on organochlorine pesticides (FAO/WHO 1970, 1973, 1976). It was recognized that liver tumours are known to develop spontaneously in many strains of mice, at relatively high incidence and without intentional exposure to chemicals. Evidence of such tumours in several strains of mice has been found in many of the oncogenicity studies performed with benomyl and carbendazim. Furthermore, one strain of mouse used (HOE-NMR) is known to have a low background incidence of liver tumours (1-2 percent) and did not provide evidence for oncogenicity when exposed to carbendazim at doses up to and including 5 000 ppm. Two additional studies have been carried out in rats using both benomyl and carbendazim. Both studies were negative for oncogenicity at doses up to and including 2 500 and 10 000 ppm, respectively. The hepatic tumours produced in mice, therefore, appear to be a species-related phenomenon.

The Meeting expressed concern at the equivocal nature of the results of a wide range of mutagenicity studies. The possibility that conflicting results were due to variations in the type and amount of impurities was considered, but the Meeting was informed that current levels of the impurities in question are very low in technical material.

In view of established NOEL determined in several studies, including teratology, reproduction and chronic feeding, an ADI for both benomyl and carbendazim could be estimated. However, a safety factor of 200 was used to reflect the concern of the Meeting for the paucity of individual animal data for many studies on carbendazim.

TOXICOLOGICAL EVALUATION

Level Causing no Toxicological Effect

Rat: 500 ppm in the diet, equivalent to 25 mg/kg b.w.

Dog: 100 ppm in the diet, equivalent to 2.5 mg/kg b.w.

Rat: Teratology (see benomyl)

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Estimate of Acceptable Daily Intake for Man

0-0.01 mg/kg b.w.

FURTHER WORK OR INFORMATIONDesirable

1. Data on individual animals used in studies on carbendazim that have been identified in this evaluation addendum.
2. Additional data to elucidate the mechanism of degenerative testicular effects on mammals.
3. Elucidation of the variability of the mutagenicity data.

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