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Memorandum

SUBJECT: Toxicology Chapter for Benomyl and Carbendazim. DP Barcode D264602, Case 819338, Benomyl PC Code 099101, Carbendazim PC Code 128872.

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This memorandum summarizes the guideline studies submitted by the registrant, and other relevant toxicity studies considered by HED in developing the acute and chronic reference doses (RfDs) and toxicity endpoints for use in risk assessment for benomyl and its primary metabolite, carbendazim (Methyl 2-Benzimidazole Carbamate or MBC).

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I. HAZARD ASSESSMENT OVERVIEW

The hazard assessment addresses issues and data related to benomyl, which is currently registered with EPA and has been supported for reregistration. The toxicology data in support of benomyl reregistration are complete in accordance with the Subdivision F Test Guidelines for a food use chemical. The Hazard Identification Assessment Review Committee (HIARC) has requested a developmental neurotoxicity (DNT) study in rats for benomyl. Toxicology data for carbendazim (Methyl 2-Benzimidazole Carbamate) or MBC, the primary environmental breakdown product of benomyl, are also considered in this memorandum, and are incomplete. In foods and the environment, benomyl rapidly transforms to MBC, hence residues in foods are primarily MBC. MBC is also registered for use as a fungicide, but has no registered food uses in the US. The HIARC requested two toxicity studies with MBC, a 21 day dermal toxicity study in rats and a developmental neurotoxicity study in rats. In addition, the 2-generation rat reproduction and subchronic studies for MBC fail to meet the Subdivision F Guidelines.

Acute Toxicity. Both benomyl and MBC are of low toxicity following acute exposures. Guideline studies for acute toxicity indicate that both chemicals are classified as category IV for acute oral toxicity, category III for acute dermal toxicity, and category IV for primary skin irritation. Benomyl is in category II, while MBC is in category III for inhalation toxicity and primary eye irritation. Benomyl is classified as a mild to moderate skin sensitizer, while MBC is not a skin sensitizer.

Subchronic Toxicity. Several subchronic studies are available for benomyl including one oral study in rats, mice and dogs, a 21 day dermal toxicity study in rabbits, and an inhalation study in rats. All three oral studies (in rats, mice and dogs) fail to meet the test guidelines, however, chronic mouse and dog studies are available to satisfy these guidelines. Benomyl is most toxic following subchronic inhalation exposures and causes respiratory effects characterized by cell necrosis, chronic and acute inflammation and loss of olfactory epithelium with foci of repair in rats exposed to doses as low as 4.8 mg/kg/day (50 mg/m^3). In all animal species the most sensitive toxicological endpoint following subchronic oral exposure is liver toxicity manifested as induction of liver enzymes accompanied by liver cell hypertrophy and proliferation and increased liver weight at doses as low as 62.5 mg/kg/day. Dogs appear to be the most sensitive species following subchronic oral exposure to benomyl. Rabbits dermally exposed to benomyl dose levels at and above 1000 mg/kg exhibited diarrhea, oliguria and hematuria. Biologically significant effects on testicular weight were also noted following dermal exposure to 1000 mg/kg.

Only one subchronic oral study in dogs was available for MBC. Although classified as unacceptable, both liver and testicular effects were noted at MBC doses as low as 35-40 mg/kg/day.

Chronic Toxicity and Carcinogenicity. Benomyl and MBC were evaluated for carcinogenic potential in both rats, and mice. In addition, benomyl and MBC were evaluated for chronic

toxicity in dogs. In all species (except rats treated with benomyl), the most sensitive toxicological endpoint is liver toxicity that occurred at levels as low as 62.5 mg/kg/day for benomyl and 12.5 mg/kg/day for MBC, indicating that MBC may be more toxic than benomyl following chronic exposure. Dogs appear to be the most sensitive species for liver toxicity following chronic oral exposure to both fungicides. For benomyl, liver effects were characterized by hepatic cirrhosis, bile duct proliferation with corresponding biochemical changes indicative of liver injury. Testicular degeneration was noted in dogs at benomyl doses as low as 62.5 mg/kg/day, and in mice at much higher doses of 1125/750 mg/kg/day.

Both benomyl and MBC are classified in group C (possible human carcinogens) because they induced liver tumors (hepatocellular adenoma and/or carcinomas) in mice. There is no evidence of carcinogenicity in rats for either fungicide. HED calculated a Q_1* of 2.39×10^{-3} $(mg/kg/day)^{-1}$ for both benomyl and MBC based on a mouse carcinogenicity study with MBC that observed statistically significant increases in liver adenomas and carcinomas in females (Wood et al. 1982). The Q_1* was calculated using the $(mg/kg/day)^{3/4}$ cross species scaling factor. It is noted that the benomyl and MBC rat studies only tested 36 rats/sex/dose (and only 20/sex/dose in the 250 mg/kg/day MBC dose group), when current guidelines require 50 rats/sex/dose.

Developmental Toxicity. Both benomyl and MBC induce developmental toxicity in the absence of maternal toxicity. Benomyl was evaluated for developmental toxicity in rats and rabbits in registrant-submitted studies. In rats, developmental effects were noted at doses ranging from 62.5 to 125 mg/kg/day in the absence of maternal toxicity, indicating increased fetal sensitivity. At 62.5 mg/kg/day developmental effects included increased incidence of ocular malformations (microphthalmia and anophthalmia), increased fetal mortality and reduced fetal weight. Effects at 125 mg/kg/day included increased incidence of malformations of the brain, characterized by distended lateral ventricles and hydrocephaly. Fetuses of rabbit does exposed to 180 mg/kg/day developed a significant increase incidence in visceral variations (small renal papillae) that were not readily attributed to exposure and were not considered to be malformations because they may have occurred as a result of incomplete maturation. Nevertheless, the visceral variations occurred at maternally toxic doses as indicated by stained tails and reduced feed consumption at 180 mg/kg/day.

Literature studies have also demonstrated that benomyl induces developmental effects in rats and mice following gavage administration to pregnant animals at doses as low as 62.5 mg/kg/day and 100 mg/kg/day, respectively (Kavlock et al. 1982, Chernoff 1985). Developmental effects in rats include small eyes (microphthalmia), decreased fetal weight, increased fetal mortality and delayed skeletal and visceral maturation, while effects in mice include cleft palate, supernumerary ribs and subnormal vertebral centrum (no compound-related microphthalmia was reported). Literature studies have also demonstrated a difference in fetal response to gavage versus dietary exposure to benomyl, with gavage dosing producing anomalies at approximately one-tenth of the dietary dose (Kavlock et al. 1982, Chernoff 1985). In addition, literature studies suggest that the incidence and severity of the developmental effects appear to be increased when the dams are nutritionally comprised (protein deficient) and by late gestation dosing.

Benomyl has also been associated with sustained adverse effects on the male reproductive system (decreased weight of testes, prostate, and seminal vesicles) in a postnatal rat study at doses as low as 31.2 mg/kg/day (Kavlock et al. 1982).

There is increased sensitivity of rat and rabbit fetuses as compared to maternal animals following *in utero* exposure to MBC, in prenatal developmental toxicity studies. In the MBC rat study, increased sensitivity manifested as developmental anomalies (decreased fetal body weight and increases in skeletal variations and a threshold for malformations) at doses of 20 mg/kg/day which were not maternally toxic. At higher doses of 90 mg/kg/day, treatment-related malformations of the CNS were observed which included exencephaly, domed head, anophthalmia, microphthalmia and bulged eyes. For developmental toxicity the NOAEL was 10 mg/kg/day, whereas for maternal toxicity, the NOAEL was 20 mg/kg/day (based on a slight increase in liver weight at 90 mg/kg/day).

In the rabbit developmental study with MBC, increased sensitivity manifested as decreased implantations and litter size, and increased resorptions at 20 mg/kg/day; the NOAEL is 10 mg/kg/day. Maternal toxicity was not observed until higher doses of 125 mg/kg/day, based on abortions and decreased maternal body weight; the maternal NOAEL is 20 mg/kg/day.

Reproductive Effects. Both benomyl and MBC are associated with adverse reproductive effects, including effects on the male reproductive system. Benomyl induced reproductive toxicity in rats, but only at dose levels that induced parental toxicity. Reproductive effects included reduced pup weights and testicular pathology, while parental effects included decreased sperm counts as well as histological lesions of the testes (atrophy and degeneration of the seminiferous tubules) at doses as low as 168 mg/kg/day.

Adverse testicular effects have been observed in rats, dogs, mice and rabbits exposed to benomyl by oral (gavage and dietary), dermal and inhalation routes in registrant-submitted and literature studies. Testicular effects (decreased spermatogenesis) were observed in acute LC_{50} studies in both rats and dogs 14 days following a single 4-hour inhalation exposure at doses of 33 mg/kg (0.82 mg/L) and 82 mg/kg (1.65 mg/L), respectively. The acute inhalation NOAELs were 7.5 mg/kg (0.2 mg/L) and 32 mg/kg (0.65 mg/L) for rats and dogs, respectively. Testicular effects (decreased size of the testes, lesions, degeneration) were also noted in the 1992 acute neurotoxicity study in rats, and in the 1982 mouse oncogenicity study. Literature studies have also reported testicular effects 2 days or 70 days post exposure in adult male rats given single gavage doses of benomyl as low as 50 or 100 mg/kg, respectively. Effects include dose-dependent increases in the premature release of germ cells (sloughing), seminiferous tubular atrophy and occluded efferent ductules (Hess et al. 1991). Male offspring in a postnatal rat study (dams dosed from gestation day 7 to lactation day 15) exhibited testicular effects, including permanent reductions in testes weight, and ventral prostate and seminal vesicles at 31.2 mg/kg.

MBC was associated with adverse reproductive effects (decreased birth weight at weaning) in an unacceptable reproductive toxicity study in rats. MBC also caused adverse testicular effects

characterized by premature release of immature germ cells, atrophy of a few seminiferous tubules and significant decrease in seminiferous tubule diameter following a single gavage dose with 50 mg/kg (Nakai et al. 1992). In addition, evidence of testicular effects has been demonstrated in the unacceptable 90-day subchronic dog study with MBC.

Mutagenicity. Both benomyl and MBC have marginal mutagenic activity in standard in vitro studies. In contrast, there is clear and reproducible evidence of aneuploidy (i.e., abnormal number of chromosomes) both in vitro and in vivo. There is also convincing evidence that the induction of aneuploidy by benomyl and MBC is primarily attributed to adverse effects on cellular spindle apparatus. Both benomyl and MBC are established spindle poisons that induce aneuploidy effects in both in vitro and in vivo test systems. For example, nondisjunction was reported in A. nidulans and many other test systems with both agents. Both fungicides also produced positive effects in bone marrow antikinetochore micronucleus assays, which were consistent with a spindle effect. However, neither compound is clastogenic. Since the genotoxic activity of benomyl and MBC is well known, these pesticides are frequently used as test chemicals (i.e., positive controls) for the assessment of new assay systems for the detection of aneuploidy induction.

In mutagenicity studies with benomyl and MBC, there is compelling evidence of aneuploidy induction following oral dosing in mice. Mutagenicity data support the evidence of developmental anomalies in rats and hepatocellular tumors in several strains of male and female mice.

Neurotoxicity. No treatment-related neurotoxicity was observed in the acute or subchronic rat studies with benomyl. Although increased motor activity was noted at the highest dose tested (456-578 mg/kg/day) in the benomyl subchronic rat study, this observation was discounted due to the presence of systemic toxicity. Benomyl and MBC do not appear to cause delayed neurotoxicity in hens.

Metabolism/Pharmacokinetic Studies. In the rat, benomyl and MBC are excreted primarily in the urine with lesser amounts excreted in the feces, and MBC is poorly distributed to the tissues. MBC was rapidly absorbed and extensively metabolized in CD/BR rats following single oral doses up to 1000 mg/kg. The half-life of MBC was approximately 12 hours, and 98% of carbendazim was excreted by 72 hours post- administration. The primary reactions involved in the metabolism of MBC were oxidation of the phenyl ring, followed by conjugation to yield sulfate and glucoronide conjugates of 5-hydroxycarbendazim and 5,6-dihydroxycarbendazim. Subsequent phenyl ring oxidation and N-oxidation at the imidazole nitrogen led to significant levels of 5,6-hydroxy-oxo-carbendazim N-oxide glucuronide conjugate, especially in female rats.

Dermal Absorption. The dermal absorption of benomyl is low and ranges from 0.031 to 3.5 percent in rats.

Mechanism of Action. In 1997, the HED RfD/Peer Review Committee summarized a

mechanism of action for benomyl. The following summary is an excerpt from the 5/28/97 report. "Benomyl has been reported to inhibit the in vitro polymerization of the rat neurotubulin at approximately 7.5 μg/mL (Albertine et al. 1995). This finding is consistent with the known mechanism of aneuploidy induction by the benzimidazole class of compounds (i.e., in vitro inhibition of yeast and/or mammalian tubulin polymerization with impairment of the spindle apparatus and resulting aneuploidy in the daughter cells).

Since it is generally acknowledged that somatic cell aneuploidy may be in involved in carcinogenesis and that the genetic imbalances resulting from aneuploidy in germinal cells may contribute to birth defects, it is not surprising that the results from genetic toxicology testing with benomyl correlate with the data from chronic feeding studies demonstrating hepatocellular carcinomas in male and female mice. Similarly, the genetic toxicology data support the evidence of developmental effects in rats. Hoogenboom et al. (1991) postulated that the known antitubulin action of benomyl may impair microtubule formation and produce brain and ocular malformations by disruption of neuronal proliferation and migration.

Other metabolites. The primary metabolites of MBC are 5-hydroxy-2-benzimidazolecarbamic acid, methyl ester (5-HBC) and 2-aminobenzimidazole (2-AB). The acute toxicity of 5-HBC and 2-AB could not be compared to MBC since they were not tested at levels higher than 3400 and 7500 mg/kg, respectively. MBC did not cause death in rats following single oral doses of 5000 mg/kg. Deaths (6/6) occurred with 2-AB following 10 doses at 670 mg/kg/day (2/6 occurred with MBC at 3400 mg/kg/day). 5-HBC was not tested higher than 200 mg/kg/day for 10 doses over 2 weeks. Testicular degeneration was observed with 5-HBC at 3400 mg/kg but not with 2-AB up to 7500 mg/kg.

II BENOMYL

a. Acute Toxicity

Benomyl is of low toxicity following acute exposures. Guideline studies for acute toxicity indicate that benomyl is classified as category IV for acute oral toxicity, category III for acute dermal toxicity, category II for inhalation toxicity and primary eye irritation, and category IV for primary skin irritation. Benomyl is classified as a mild to moderate skin sensitizer. Acute toxicity values and categories for benomyl are summarized in the following table.

Table 1 Acute Toxicity of Benomyl					
Guideline No.	**Study Type**	**% a.i.**	**MRID or Accession No.**	**Results**	**Toxicity Category**
870.1100 (81-1)	Acute Oral, Rat	75	00064819	LD_{50} = >5000 mg/kg,	IV
870.1200 (81-2)	Acute Dermal, Rat	75	243043	LD_{50} = >2000 mg/kg,	III
870.1300 (81-3)	Acute Inhalation, Rat	50	00097281	LC_{50} >0.82 mg/L	II
870.2400 (81-4)	Primary Eye Irritation, Rabbit	75	00064820 243043 (Acc. No)	irritant	II
870.2500 (81-5)	Primary Skin Irritation, Rabbit	75	243043 (Acc. No)	Non-irritant	IV
870.2600 (81-6)	Dermal Sensitization, Guinea Pig	not given	050427 (Acc. No)	mild to moderate dermal sensitizer	N/A
870.6100a (81-7)	Delayed neurotoxicity, hen	not given	241930	NOAEL = 2500 mg/kg	N/A
870.6200a (81-8)	Acute Neurotoxicity, Rat	97.4	42817003	NOAEL >2000 mg/kg	N/A

N/A Not applicable

b. Subchronic Toxicity

Several subchronic studies are available for benomyl including one oral study in rats, mice and dogs, a 21 day dermal toxicity study in rabbits, and an inhalation study in rats. All three oral studies (in rats, mice and dogs) fail to meet the test guidelines, however, acceptable chronic mouse and dog studies are available to satisfy these guidelines. Benomyl is most toxic following subchronic inhalation exposures and causes respiratory effects characterized by cell necrosis, chronic and acute inflammation and loss of olfactory epithelium with foci of repair in rats exposed to doses as low as 4.8 mg/kg/day (50 mg/m^3). In all animal species the most sensitive toxicological endpoint following subchronic oral exposure is liver toxicity manifested as induction of liver enzymes accompanied by liver cell hypertrophy and proliferation and increased liver weight at doses as low as 62.5 mg/kg. Dogs appear to be the most sensitive species following subchronic oral exposure to benomyl. Rabbits dermally exposed to benomyl dose levels at and above 1000 mg/kg exhibited diarrhea, oliguria and hematuria. Non-significant

effects on testicular weight were also noted following dermal exposure to 1000 mg/kg, however, these findings were not dose related (most likely due to only 2 animals tested at the high dose of 5000 mg/kg). The following table summarizes the subchronic toxicity studies for benomyl:

Table 2. Subchronic Toxicity of Benomyl

GDLN	STUDY	DOSE (mg/kg/day) (1)	RESULTS (mg/kg/day) (1)
870.3100 (82-1(a))	Subchronic Feeding in Rats (90 days) MRID #: 00066771 1967 Core Grade: unacceptable guideline	M: 0, 9, 45, or 214 F: 0, 9, 46, or 234 (0, 100, 500 or 2500 ppm)	**72% a.i. benomyl** NOAEL: 45 (M), 46 (F) LOAEL: 214 (M), 234 (F) Effects: increases in SGPT in males and increased relative and absolute liver weights in females
not applicable	Subchronic (28 day) Oral in Male CD-1 Mice MRID #: 41607903 1990 Core Grade: supplementary, not conducted to satisfy guidelines	0, 15.7, 85.4, 586 or 1180 (0, 100, 500, 3750 or 7500 ppm) (males only)	**96.1% a.i. benomyl** NOAEL: 85.4 LOAEL: 586 Effects: At 586 and 1180 mg/kg/day there was an increase in relative and absolute liver weights, an increase in the incidence of cellular hypertrophy and increased cell proliferation. At 1180 mg/kg/day there was evidence of nuclear hypertrophy and proliferation of the smooth endoplasmic reticulum, and an increase in cytochrome P_{450} activity after 14 and 28 days.
870.3150 (82-1(b))	90 Day Oral (Diet) in Dogs MRID #: 00066785 1968 Core Grade: unacceptable guideline. Two chronic studies exist that would supercede this data requirement	0, 2.5, 12.5 or 62.5 (0, 100, 500 or 2500 ppm)	**51% a.i. benomyl** NOAEL:12.5 LOAEL: 62.5 Effects: increased SGPT, alkaline phosphatase and albumin:globulin ratio in males

Table 2. Subchronic Toxicity of Benomyl			
GDLN	**STUDY**	**DOSE (mg/kg/day) (1)**	**RESULTS (mg/kg/day) (1)**
870.3200 (82-2)	21-Day Dermal Toxicity Study in Rabbits MRID #: 00097287 (Hood et al. 1969) Core Grade: acceptable guideline	0, 50, 250, 500, 1000 and 5000 (Doses already adjusted for % a.i. in study)	**53% a.i benomyl** NOAEL: 500 LOAEL: 1000 Effects: Males of the 1000 mg/kg/day group exhibited 30% and 24% decreases in testicular weight and testes-to-body weight ratios, respectively (both not statistically significant), which was not apparent at 5000 mg/kg/day. However, lack of testicular effects at 5000 mg/kg/day may be due to a low number of animals (2/sex) evaluated at this dose. Females exposed to 1000 and 5000 mg/kg/day exhibited diarrhea, oliguria and hematuria. Moderate skin irritation was reported for all dose groups.
870.3465 (82-4)	Subchronic Inhalation in Sprague-Dawley Rats (90 days) MRID #: 40399501 (Warheit 1987) Core Grade: acceptable guideline	Males: 0.96, 4.8 or 19.2 mg/kg/day Females: 1.4, 7.0 or 28.8 mg/kg/day (0, 10, 50 or 200 mg/m^3, or 0, 0.01, 0.05 or 0.2 mg/L) 4 hr/day	**95% a.i. benomyl** NOAEL: 0.96 (males) LOAEL: 4.8 (males) Effects: At 4.8 mg/kg/day olfactory degeneration was characterized by necrosis, chronic and acute inflammation and loss of olfactory epithelium with foci of repair. Males exposed to 19.2 mg/kg/day had decreased body weights (10.8%) and body weight gains (13.6%).

(1) Unless specified, mg ai benomyl/kg/day.
NOAEL = No Observable Adverse Effect Level
LOAEL = Lowest Observable Adverse Effect Level
SGPT = Serum Glutamic Pyruvic Transaminase

c. Chronic Toxicity and Carcinogenicity

Benomyl was evaluated for carcinogenic potential in both rats, and mice. In addition, benomyl was evaluated for chronic toxicity in dogs. In dogs and mice, the most sensitive toxicological endpoint is liver toxicity that occurred at levels as low as 62.5 mg/kg/day. Dogs appear to be the most sensitive species for liver toxicity following chronic oral exposure. Liver effects were characterized by hepatic cirrhosis, bile duct proliferation with corresponding biochemical changes indicative of liver injury. Benomyl induced liver tumors (hepatocellular carcinomas) in mice. There is no evidence of carcinogenicity in rats. Testicular effects in mice were characterized as degenerative changes in the testes and epididymides at very high doses of 1125/750 mg/kg/day. Benomyl is classified in group C (possible human carcinogen). HED

calculated a Q1* of 2.39×10^{-3} $(mg/kg/day)^{-1}$ for both benomyl and MBC based on a mouse carcinogenicity study with MBC.

The following tables summarize the chronic toxicity/carcinogenicity studies for benomyl:

Table 3. Chronic Toxicity/Carcinogenicity of Benomyl

GDLN	STUDY	DOSE (mg/kg/day)	RESULTS (mg/kg/day)
870.4100a 870.4200 (83-1(a) 83-2)	Chronic feeding study in CD rats Accession # 051427 Sherman et al. 1969 Core Grade: minimum	0, 5, 25, or 125 (0, 100, 500 or 2500 ppm) (Doses adjusted for % a.i.)	**51 or 72.2% a.i. benomyl** NOAEL: >125 (HDT) LOAEL: none established Effects: None observed. No evidence of carcinogenicity. Deficiencies: Limited clinical chemistry analysis, and only 36 rats/sex/dose were evaluated.
870.4100b (83-1b)	Chronic feeding study in beagle dogs (2 yrs) MRID # 00061618, 00081913, 0097305, 00097318, 00097326 Sherman et al. 1970 Core Grade: acceptable guideline	0, 2.5, 12.5, or 62.5 (0, 100, 500 and 2500 ppm) (Doses adjusted for % a.i.)	**50% a.i. benomyl** NOAEL: 12.5 LOAEL: 62.5 (HDT) Effects: At 62.5 mg/kg/day effects include hepatic cirrhosis, bile duct proliferation, testicular degeneration, as well as decreased weight gain and food consumption. Males had increased in cholesterol, alkaline phosphatase and SGPT and decreased total protein and albumin/globulin (A/G) ratio, which were correlated with chemically-induced hepatic injury. Focal testicular degeneration was present in all treatment groups, with marked testicular degeneration (reduced testes weight, absence of spermatozoa and spermatic giant cells) in 1/3 dogs at 62.5 mg/kg/day.

Table 3. Chronic Toxicity/Carcinogenicity of Benomyl			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
870.4200b (83-2b)	Chronic feeding study in CD-1 mice (2 yrs) MRID # 00096514 Schneider et al. 1982 Core Grade: acceptable guideline	0, 75, 225 or 1125 (750) (0, 500, 1500 and 7500 ppm) (the 7500 ppm dose level was reduced to 5000 ppm after week 37).	**99, 99.2% a.i. benomyl** NOAEL: none LOAEL: 75 Effects: significant increase in hepatocellular carcinomas in both males and females at 75 mg/kg/day. There was also an increase in the combined incidence of hepatocellular adenomas and carcinomas in mid and high dose females. At the highest dose tested, the testes and epididymides showed degenerative changes, which were characterized by degeneration of the seminiferous tubules, atrophy and tubular degeneration.

HDT = Highest Dose Tested
SGPT = Serum Glutamic Pyruvic Transaminase

Chronic/Carcinogenicity Study in Rats

In a study conducted in 1969, benomyl (51.5 or 72.2%) was administered in the diets of CD rats (36/sex/dose) at levels of 0, 100, 500 or 2500 ppm for two years (Accession # 051427, Sherman et al. 1969). This is approximately equivalent to 0, 5, 25 or 125 mg ai benomyl/kg/day. Six rats/sex/dose were sacrificed and examined for gross and microscopic pathology at 1 year. After two years, the surviving rats were also sacrificed. All tissues from the brain, heart, kidney, adrenal, stomach, liver, spleen, testes and lung were examined microscopically in the control and high dose group at both sacrifices, while all tissues were examined only at the 2 year sacrifice in the low and mid dose groups. In addition, histopathology was conducted on a more comprehensive list of organs in the control and high-dose groups at 1 and 2 years, but only for the liver, kidney and testes of the low and mid-dose groups at 2 years.

There were no treatment-related effects on mortality, body weight, food consumption, organ weights, clinical chemistry, urinalysis, gross pathology or histopathology. Only two clinical chemistry parameters, alkaline phosphatase (AP) and serum glutamic-pyruvic transaminase (SGPT), were evaluated, which makes it difficult to confirm that there were no adverse effects. There were no treatment-related organ weights or histopathologic changes in any of the groups tested at either the 1 (6 rats/sex/dose) or 2 year (30 rats/sex/dose) sacrifices. Therefore, the toxicological significance of any clinical chemistry alterations is questionable in the absence of corroborative changes in organ weight and histopathology in the liver. Liver changes and testicular degeneration were a frequent occurrence, but were equally spread between control and test groups. There was no evidence of carcinogenicity.

This study was conducted in 1969, prior to the 1984 Subdivision F guidelines for a chronic toxicity study (83-1) and chronic feeding/oncogenicity study (83-2), and therefore is classified as minimum (i.e., does not meet current evaluation standards, however, is adequate for risk assessment). Deficiencies include limited clinical chemistry analysis, failure to identify a LOAEL, the maximum tolerated dose (MTD) was not established and only 36 rats/sex/dose were evaluated (when 50/sex/dose are currently required for a carcinogenicity study).

Chronic/Carcinogenicity Study in Mice

Benomyl (99%, 99.2%) was administered in the diets of CD-1 mice (80/sex/dose) at levels of 0, 500, 1500 and 7500/5000 ppm (the 7500 ppm dose level was reduced to 5000 ppm after week 37) for two years. This is equivalent to 0, 75, 225 or 1125/750 mg/kg/day. Hepatocellular carcinomas were significantly elevated in male (500 and 1500 ppm) and female (500 and 7500/5000 ppm) mice. In addition, the combined incidence of hepatocellular adenomas and carcinomas were significantly elevated in males (500 and 1500 ppm) and females (500 and 7500/5000 ppm). The tumorigenic response appeared to be compound-related (e.g., they occurred with significant positive trends, and the incidence exceeded historical rates). Pulmonary alveologenic carcinomas were significantly elevated in the low and mid dose males, but not at the high dose. Therefore, the pulmonary tumors did not appear to be compound-related since there was no dose-response and the observed incidences were within the range for historical control rates at the laboratory. At the highest dose tested, the testes and epididymides showed degenerative changes (aspermia), which were characterized by degeneration of the seminiferous tubules, atrophy and tubular degeneration.

There was a statistically significant decrease in body weight reported for both males and females in the highest dose tested. The body weights were approximately 10% lower than controls in both sexes from weeks 13 to study termination at week 104. Sporadic decreases in body weights were also reported at one or two weighing intervals in mice receiving 1500 ppm but did not appear to be statistically or biologically significant. The highest dose of benomyl tested in male mice appeared to exceed the maximum tolerated dose (MTD). High dose males exhibited an approximate 10% decreased body weight gain, hepatocellular toxicity (e.g., foci of cellular alteration, cytomegaly, and foci of degeneration) and degenerative changes in the testes. The high dose did not produce 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 the be metabolized to MBC).

This study is **acceptable** and satisfies the requirement for a carcinogenicity study in mice. Based on the reported decreases in body weight and the increase in incidence of liver tumors, the dose selection in this study appears to be adequate (MRID 00096514).

Chronic Toxicity Study in Dogs

Groups of 4/sex/dose beagle dogs were administered a formulated product containing benomyl in

the diet at dosage levels of 0, 100, 500 and 2500 ppm for 2 years (MRID 00061618). The dietary concentrations are equivalent to 0, 2.5, 12.5 and 62.5 mg/kg/day ai benomyl. After one year, one dog/sex from control and high dose groups were sacrificed. Organ weights, gross necropsy and histopathological evaluations were conducted after two years. Only the livers and testes were examined histologically in the 100 and 500 ppm groups.

There were no treatment-related effects on mortality, hematology, urinalysis, or clinical signs. Body weight gain and food consumption were decreased in the high dose group. Males in the high dose group had increased cholesterol, alkaline phosphatase and glutamic-pyruvic transaminase (GPT) values, as well as decreased total protein and albumin/globulin (A/G) ratio. Similar effects, other than cholesterol and total protein, were noted in the high dose females. The clinical chemistry observations support the adverse liver effects in the high dose group, characterized as cirrhosis (one male at 1 year and 2 males and 1 female at 2 year sacrifice) and slight to marked bile duct proliferation in 4/6 dogs of the 2500 ppm (62.5 mg/kg/day ai) group. Focal testicular degeneration was present in all treatment groups, with marked testicular degeneration (reduced testes weight, absence of spermatozoa and spermatic giant cells) in 1/3 dogs at 2500 ppm.

The NOAEL is 500 ppm (12.5 mg/kg/day ai) based on hepatic cirrhosis, clinical chemistry alterations, testicular degeneration as well as decreased weight gain and food consumption noted at 2500 ppm. This study is **acceptable** and satisfies the guideline requirements for a chronic dog study.

Classification of Carcinogenic Potential

Both benomyl and MBC are classified as group C chemicals (possible human carcinogens) by the Cancer Peer Review Committee. On 5/21/86, the Scientific Advisory Panel (SAP) concurred with the classification of benomyl. The rationale for this classification is as follows: (1) the carcinogenic response for both benomyl and MBC are 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) know to have high background incidence rates of liver tumors, whereas no liver tumors were produced by MBC in another strain of mice [NMRKf (SPF 71)] known to have a low background incidence rate of liver tumors (see discussion under Section III Carbendazim Carcinogenicity Discussion); (3) benomyl and MBC produced weak mutagenic effects consistent with spindle poison activity rather than gene mutation or DNA repair activity.

The Peer Review Committee noted the occurrence of mostly malignant hepatocellular tumor response with MBC in two stains of mice, and the presence of unusually occurring and malignant hepatoblastomas with MBC in male SPF Swiss mice. In addition, the mutagenicity information indicates that the aneuploidy known to be produced by benomyl could theoretically result in a loss of tumor suppressor genes and a potential oncogenic effect.

HED estimated a unit risk Q_1* of 2.39×10^{-3} $(mg/kg/day)^{-1}$ for both benomyl and MBC. This estimate is based on the outcome of the re-evaluation of the hepatocellular (adenoma and/or carcinoma) tumors in CD-1 female mice with dose levels of 0, 500, 1500 or 7500 ppm MBC (Wood et al. 1982). The Q_1* was calculated using the $(mg/kg/day)^{3/4}$ species scaling factor. Details of the quantitative estimate are presented in Attachment 1 of this memorandum.

d. Developmental Toxicity

Benomyl was evaluated for developmental toxicity in rats and rabbits in registrant-submitted studies. In rats, developmental effects were noted at doses ranging from 62.5 to 125 mg/kg/day in the absence of maternal toxicity. At 62.5 mg/kg/day effects included increased incidence of ocular malformations (microphthalmia and anophthalmia), increased fetal mortality and reduced fetal weight. Effects at 125 mg/kg/day included increased incidence of malformations of the brain, characterized by distended lateral ventricles and hydrocephaly. Fetuses of rabbit does exposed to 180 mg/kg/day developed a significant increase incidence in visceral variations (small renal papillae) that were not readily attributed to exposure and were not considered to be malformations because they may have occurred as a result of incomplete maturation. Nevertheless, the visceral variations occurred at maternally toxic doses as indicated by stained tails and reduced feed consumption at 180 mg/kg/day.

Literature studies have also demonstrated that benomyl induces developmental effects in rats and mice following gavage administration to pregnant animals at doses as low as 62.5 mg/kg/day and 100 mg/kg/day, respectively (Kavlock et al. 1982, Chernoff 1985). Developmental effects in rats include microphthalmia, decreased fetal weight, increased fetal mortality and delayed skeletal and visceral maturation, while effects in mice include cleft palate, supernumerary ribs and subnormal vertebral centrum. Literature studies have also demonstrated a differential in fetal response to gavage versus dietary exposure to benomyl, with gavage dosing producing anomalies at approximately one-tenth of the dietary dose (Kavlock et al. 1982, Chernoff 1985). In addition, benomyl caused sustained adverse effects on the male reproductive system in a postnatal rat study at doses as low as 31.2 mg/kg/day (Kavlock et al. 1982).

The following table summarizes the developmental studies for benomyl:

Table 4. Developmental Toxicity of Benomyl			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
870.3700a (83-3a)	Developmental Study in CHR:CR rats (gavage) MRID# 00148393 (Accession # 256575) (Staples and Aftosmis 1980) Core Grade: acceptable guideline when considered with MRID # 00115674, 00126522 (below)	0, 15.6, 31.2, 62.5 or 125 (gestation day 7-16)	**99% a.i. benomyl** Maternal NOAEL: 125 Maternal LOAEL: None Developmental NOAEL:31.2 Developmental LOAEL: 62.5 based on a significant increase in fetal and litter incidence of ocular malformations, specifically, microphthalmia and anophthalmia and decreased fetal weight. At 125 mg/kg, benomyl was associated with an increased incidence of malformations of the brain, characterized by distended lateral ventricles and hydrocephaly.
870.3700a (83-3a)	Developmental Study in CHR:CR rats (gavage) MRID# 00115674, 00126522 (Staples 1982) Core Grade: acceptable when considered with MRID 00148393 (above)	0, 3, 6.25, 10, 20, 30 or 62.5 mg/kg (gestation day 7-16)	**99.1% a.i. benomyl** Maternal NOAEL: 62.5 Maternal LOAEL: None Developmental NOAEL: 30 Developmental LOAEL: 62.5 based on microphthalmia Note: This study was conducted to evaluate a specific effect on the development of the eyes in fetal rats.
870.3700a (83-3a)	Developmental Study in Wistar rats (gavage) Accession # GS0119017 Kavlock et al. 1982 Literature Study	0, 15.6, 31.2, 62.5 or 125 mg/kg (gestation day 7-16)	**% a.i. benomyl not given (technical)** Maternal NOAEL: 125 (HDT) Maternal LOAEL: none Developmental NOAEL: 31.2 Developmental LOAEL: 62.5 based on the increased incidence of microphthalmia, increased fetal mortality reduced fetal weight, and delayed skeletal and visceral maturation.

Table 4. Developmental Toxicity of Benomyl			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
870.3700a (83-3a)	Developmental Study in Wistar rats (diet) Accession # GS0119017 Kavlock et al. 1982 Literature Study	0, 169, 298, 505 (approximately 0, 1690, 3380, and 6760 ppm) (gestation day 7-16)	**% a.i. benomyl not given (technical)** Maternal NOAEL: 298 Maternal LOAEL: 505 (reduced weight gain) Developmental NOAEL: 169 Developmental LOAEL: 298 based on weight decreases in fetuses and enlarged renal pelves.
NA	Postnatal Study in Wistar rats (gavage) Accession # GS0119017 Kavlock et al. 1982 Literature Study	0, 15.6, or 31.2 mg/kg (gestation day 7 through lactation day 15)	**% a.i. benomyl not given (technical)** Maternal NOAEL: 31.2 (HDT) Maternal LOAEL: none Developmental NOAEL: 15.6 Developmental LOAEL: 31.2 based on decreased weight of testes, ventral prostate and seminal vesicles.
870.3700a (83-3a)	Developmental Study in CD-1 Mice (gavage) Accession # GS0119017 Kavlock et al. 1982 Literature Study	0, 50, 100, or 200 mg/kg (gestation day 7-17)	**% a.i. benomyl not given (technical)** Maternal NOAEL: 200 (HDT) Maternal LOAEL: none Developmental NOAEL: 50 Developmental LOAEL: 100 based on supra occipital scars, subnormal vertebral centrum, supernumerary ribs and cleft palate.

Table 4. Developmental Toxicity of Benomyl			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
870.3700b (83-3 (b))	Developmental Study in Hra (New Zealand White) SPF rabbits (gavage) MRID# 43788301 1995 Core Grade: acceptable guideline	0, 15, 30, 90 or 180 mg/kg (gestation day 7-28)	**97.4% a.i. benomyl** (DPXT-1991-529) Maternal NOAEL: 90 Maternal LOAEL: 180 clinical signs of toxicity (stained tails and reduced feed consumption) Developmental NOAEL (systemic): 180 Developmental LOAEL(systemic): none established Comments: At 180 mg/kg/day, there was a significant increase in the incidence of small renal papillae in the fetuses that was not readily attributable to the administration of benomyl. Small papillae were present in 4 control fetuses and involved one litter. Only the litter incidence was increased by one when doses of 0 and 180 mg/kg are compared. The biological significance of small renal papillae in the absence of other renal developments is unclear, in addition there was no dose response relationship. The small renal papillae are considered visceral variations rather than malformations and may have occurred as a result of incomplete maturation.

NA= Not applicable

Scientific Literature

Literature studies have also demonstrated that benomyl induces developmental effects in rats and mice and causes permanent effects on the male reproductive system (Kavlock et al. 1982). In this study, pregnant Wistar rats were administered either 0, 15.6, 32.2, 62.5 or 125 mg/kg/day benomyl via gavage or time-weighted average dietary concentrations of 0, 169, 298 or 505 mg/kg/day (0, 1690, 3380, 6760 ppm) on gestation days 7 to 16. Gavage exposures to rats resulted in both teratogenic and fetotoxic effects, while dietary exposure only resulted in fetotoxicity, but not teratogenicity. Benomyl was approximately an order of magnitude more fetotoxic following gavage administration than dietary exposure. However, the authors concluded that gavage exposure regimen is more relevant to assess human exposure for chemicals such as benomyl with short-half lives. This is because humans consume the bulk of their food in two or three meals, which is more similar to a gavage exposure, resulting in higher peak plasma levels of benomyl compared to the rat dietary pattern where food is consumed in short bouts throughout the nocturnal phase. In Position Document 4 (PD 4) the Agency concurred with the Kavlock et al. (1982) opinion. The Office of Pesticides (OPP) considers gavage (bolus) dosing of test material to be more acceptable than dietary administration for determining a NOAEL for developmental effects because it eliminates problems with

palatability, drug stability, nutrient integrity, and calculations of accurate dose levels.

In the gavage study, fetuses of the 62.5 and 125 mg/kg/day groups weighed significantly less than controls, and exhibited delayed skeletal maturation (the developmental score for ossification of the supraoccipital, the number of sternal and caudal ossification centers and the occurrence of subnormally ossified vertebral centrum) and visceral maturation (the occurrence of enlarged cerebral ventricles). In addition, there was an increased incidence of microphthalmia, increased fetal mortality and reduced fetal weight. In the dietary study, initial reduced food consumption was noted in the mid and high dose groups, resulting in reduced maternal weight gain during organogenesis during days 7 to 10, which persisted for the high dose group during days 10 to 14. However, dams in the high dose group significantly increased their body weight upon return to the control diet. Only dams in the high dose group had weight changes individually different from the control values. Fetal weight was significantly reduced in the mid and high dose groups. There were no dose-related incidences of anomalies and/or malformation in the fetuses. Effects on skeletal or visceral maturity were noted on the ossification of the supraoccipital bone (high dose group) and the presence of enlarged renal pelves (in mid and high dose groups).

Kavlock et al. (1982) also evaluated the developmental effects of benomyl in CD-1 mice. Pregnant mice were administered 0, 50, 100 or 200 mg/kg/day benomyl via gavage on gestational days 7 through 17. There were dose-related effects on embryonic viability, fetal weight, skeletal maturity (supraoccipital ossification), decreased numbers of sternal and caudal ossifications, delayed developmental of the vertebral centrums, visceral maturity (incidence of enlarged cerebral ventricles and enlarged renal pelves) and the occurrence of supernumerary ribs. Effects in the fetuses of the 100 mg/kg/day group included supra occipital scars, subnormal vertebral centrum, supernumerary ribs and cleft palate.

In a postnatal study, Kavlock et al. (1982) dosed pregnant rats with 0, 15.6 or 31.2 mg/kg/day benomyl via gavage from gestation day 7 to lactational day 15. There were no treatment-related effects on litter size, weight at birth, growth, survivability of the pups to weaning or locomotor activity. Male offspring of the 31.2 mg/kg/day group exhibited permanent treatment-related reductions in the weight of the testes (5%) and ventral prostate and seminal vesicles (13%).

Scientific Literature Considered by the RfD Peer Review Committee

The HED RfD/Peer Review Committee (the predecessor to the Hazard Identification Assessment Review Committee, HIARC) reviewed several literature studies in 1997 that pertained to the developmental toxicity of benomyl and MBC. The following summary are excerpts from the 5/28/97 report.

Literature studies suggest that administration of benomyl in late gestation, as opposed to administration only during the period of major organogenesis enhances the incidence of CNS anomalies in rats (Zeman et al. 1986, Ellis et al. 1988). It was hypothesized that the known anti-tubulin action of benomyl may impair microtubule formation and produce brain and ocular

malformations by disruption of neuronal proliferation and migration (Hoogenboom et al. 1991).

Malformations of the brain of Sprague-Dawley rats, following gavage dosing of the dams with 62.5 mg/kg/day of benomyl on GD 7-21 were noted in the open literature. These included observations of hydrocephaly and exencephaly in a study by Zeman and Hoogenbom (1986); other findings such as meningocoele, anencephaly, periventricular necrosis, periventricular overgrowth, and corpus callosum agenesis were observed by Ellis et al. (1987). The incidence and severity of the developmental effects appear to be increased when the dams are nutritionally comprised (protein deficient) and by late gestation dosing (Ellis et al. 1987, Hess et al. 1987, Hoogenboom et al. 1988, 1991), although Zeman et al. (1986) demonstrated a decreased incidence of abnormalities of the brain in the fetuses of protein deficient dams at a gavage dose of 31.2 mg/kg/day. The periventricular overgrowth observed in histopathological examination of fetal brains and described by Ellis et al. (1988) is hypothesized to cause cerebral aqueductal stenosis and subsequent hydrocephalus. In Wistar rats, dose-related incidences of exencephaly were reported by Lu et al. (1994) at doses of 200-500 mg/kg/day (GD 7-16); it cannot be determined whether the reduced response can be attributed to the strain of rat used, to the lack of dosing in late gestation, or to some other unknown factor.

e. Reproductive Toxicity

Benomyl is associated with adverse reproductive effects, including effects on the male reproductive system. Benomyl induced reproductive toxicity in rats, but only at dose levels that induced parental toxicity. Reproductive effects included reduced pup weights and testicular pathology, while parental effects included decreased sperm counts as well as histological lesions of the testes (atrophy and degeneration of the seminiferous tubules) at doses as low as 168 mg/kg/day.

Adverse testicular effects have been observed in rats, dogs, mice and rabbits exposed to benomyl by oral (gavage and dietary), dermal and inhalation routes in registrant-submitted and literature studies. Testicular effects (decreased spermatogenesis) were observed in acute LC_{50} studies in both rats and dogs 14 days following a single 4-hour inhalation exposure at doses of 33 mg/kg (0.82 mg/L) and 82 mg/kg (1.65 mg/L), respectively. The acute inhalation NOAELs were 7.5 mg/kg (0.2 mg/L) and 32 mg/kg (0.65 mg/L) for rats and dogs, respectively. Testicular effects (decreased size of the testes, lesions, degeneration) were also noted in the 1992 acute neurotoxicity study in rats, and in the 1982 mouse oncogenicity study. Literature studies have also reported testicular effects 2 days or 70 days post exposure in adult male rats given single gavage doses of benomyl as low as 50 or 100 mg/kg, respectively. Effects include dose-dependent increases in the premature release of germ cells (sloughing), seminiferous tubular atrophy and occluded efferent ductules (Hess et al. 1991). Male offspring in a postnatal rat study (dams dosed from gestation day 7 to lactation day 15) exhibited testicular effects, including permanent reductions in testes weight, and ventral prostate and seminal vesicles at 31.2 mg/kg.

The following table summarizes the reproduction study for benomyl, in addition to several studies that evaluated and/or reported adverse effects on the male reproductive system:

Table 5. Reproductive Toxicity of Benomyl

GDLN	STUDY	DOSE (mg/kg/day)	RESULTS (mg/kg/day)
870.3800 (83-4)	2-Generation Reproduction Toxicity in Crl:CDBR Rats (diet) MRID No: 41887901 1991 Core Grade: acceptable guideline	P1 Males: 0, 5, 28, 168 and 553 mg/kg P1 Females: 0, 7, 35, 210 and 712 mg/kg F1 Males: 0, 8, 38, 234 and 954 mg/kg F1 Females: 0, 9, 47, 280 and 1,168 mg/kg (0, 100, 500, 3000 and 10,000 ppm)	**99% a.i. benomyl** (DPX-T1991-529) Parental NOAEL: 28 (M), 35 (F) (500 ppm) Parental LOAEL: 168 (M), 210 (F) (3000 ppm) Reproductive NOAEL: 28-47 (500 ppm) Reproductive LOAEL: 168-280 (3000 ppm) Effects: At 3000 ppm there were decreases in the body weights of F2a and F2b offspring on days 14 and 21 of lactation, decreases in sperm counts reported for F1 parental males, and testicular pathology (atrophy, degeneration of the seminiferous tubules) in both generations. At 10,000 ppm oligospermia in addition to statistically significant decreases in the birth and lactation weights of F1, F2a and F2b pups, decreased testicular weights in P1 and F1 males and decreases in body weights, body weight gains and food consumption in parental animals. In the F2b offspring, there was an increase in the number of pups with partially opened or unopened eyes and a nonsignificant decrease in pup survival.
NA	Single oral dose (gavage) study Hess et al. 1991	0, 25, 50, 100, 200, 400 or 800 mg/kg	NOAEL: none observed LOAEL: 25 Effects: biologically significant premature release of germ cells and occlusions of the efferent ductules of the testes.
870.1300 (81-3)	Acute rat inhalation study (nose-only) MRID #: 00097281 Hornberger 1969	0, 7.5, 33 mg/kg (0, 0.2, 0.82 mg/L)	NOAEL: 7.5 LOAEL: 33 Effects: decreased spermatogenesis.
NA	Acute dog inhalation study MRID #: 00097275 Littlefield 1969	0, 32, or 82 mg/kg (0, 0.65 or 1.65 mg/L)	NOAEL: 32 LOAEL: 82 Effects: decreased spermatogenesis.

Not applicable

Scientific Literature: Effects on the Male Reproductive System

Adult male Sprague-Dawley rats (approximately100 days of age, 20 rats/dose) were given a single gavage dose of 0, 25, 50, 100, 200, 400 or 800 mg/kg body weight benomyl (95% a.i.) in corn oil (Hess et al. 1991). Eight animals/group were sacrificed at 2 days and 12 animals/group at 70 days (except for the 800 mg/kg group) after treatment. The testis and excurrent ducts were examined each time to determine benomyl effects on spermatogenesis and on the epididymis. The primary effects seen at day 2 were testicular swelling and occlusions of the efferent ductules. Premature release of germ cells (sloughing) was the most sensitive short-term response to benomyl and was not observed in the controls. At 25 and 50 mg/kg biologically significant sloughing occurred in 1% and 2.8% of the tubules, respectively. Sloughing was statistically significant ($p<0.05$) at doses of 100 mg/kg to 800 mg/kg (approximately 25 to 55% of the tubules). Occlusions of the efferent ductules of the testis were dose dependent at 0, 0, 10, 60, 83, 93 and 92% for the 0, 25, 50, 100, 200, 400 and 800 mg/kg groups, respectively and correlated with the increase in testis weight on day 2. Occluded efferent ductules were identified by compacted luminal contents, swollen ductules and the presence of granulomas. Testes weight was significantly increased in the 200 to 800 mg/kg groups (approximately 29 to 44%).

Long-term effects (70 days) were seen in the 100, 200 and 400 mg/kg groups, e.g., decreased testis weight (400 mg/kg), dose-dependent increases in seminiferous tubular atrophy, and increases in the number of reproductive tracts containing occluded efferent ductules. No long-term effects were seen in the 0, 25 or 50 mg/kg groups. The NOAEL is 25 mg/kg/day, and the LOAEL is 50 mg/kg/day based on biologically significant sloughing and occlusions of the efferent ductules of the testes.

f. Mutagenicity Studies

Both benomyl and MBC have marginal mutagenic activity in standard in vitro studies. In contrast, there is clear and reproducible evidence of aneuploidy (i.e., abnormal number of chromosomes) both in vitro and in vivo. There is also convincing evidence that the induction of aneuploidy by benomyl and MBC is primarily attributed to adverse effects on cellular spindle apparatus. Both benomyl and MBC are established spindle poisons that induce aneuploidy effects in both in vitro and in vivo test systems. For example, nondisjunction was reported in *A. nidulans* and many other test systems with both agents. Both fungicides also produced positive effects in bone marrow antikinetochore micronucleus assays, which were consistent with a spindle effect. However, neither compound is clastogenic. Since the genotoxic activity of benomyl and MBC is well known, these pesticides are frequently used as test chemicals (i.e., positive controls) for the assessment of new assay systems for the detection of aneuploidy induction.

In mutagenicity studies with benomyl and MBC, there is compelling evidence of aneuploidy induction following oral dosing in mice. Mutagenicity data support the evidence of developmental anomalies in rats and hepatocellular tumors in several strains of male and female mice.

Table 6 summarizes the genotoxicity studies for benomyl.

Table 6. Genotoxicity of Benomyl

STUDY	DOSE	RESULTS
GENE MUTATION		
Ames Assay *S.* typhimurium assay Horst A.L. and Krahn, D.F. 1980; Doc No. 003744, 004679 Acceptable guideline	up to 10,000 μg/plate	Positive: TA1537, TA98: Doses: 5000 and 10,000 μg/plate with S9; Negative TA1535, TA1538, TA100 at 100-10,000 μg/plate -/+S9 and all nonactivated doses with TA1537, TA98.
Ames Assay *S.* typhimurium assay MRID # 00103119 Doc No. 003744 Acceptable guideline	5 to 1,000 μg/plate	Not mutagenic with or without S-9 activation in strains TA-1535 (100-1000 μg/plate with S-9 only), TA-1537, TA-100, or TA-98 at 5-250 μg/plate
S. typhimurium/ Streprotomyces coelicolor spot tests MRID # 00123282 Doc No. 003744 Acceptable guideline	20 μg/plate (*S. typhimurium*); 500 μg/plate (Streprotomyces coelicolor)	Negative: TA1535, T1536, TA1537 & TA1538 at 20 μg/plate +/-S9; Negative: *S. coelicolor* at 500 μg/plate--only doses tested.
Chinese hamster ovary (CHO)/HGPRT forward Gene Mutation Assay MRID No. 00038808 Doc No 04679 Acceptable guideline	17-172 μM (≈5-50 μg/mL) HID:172 μM +/-S9	Negative up to cytotoxic concentrations of 120 μM (≈35 μg/mL) without S9 or 17 μM (≈50 μg/mL) with S9
CHROMOSOMAL ABERRATION		
In vitro CHL chromosome aberrations MRID No. 41184601 Doc No. 007649 Acceptable guideline	12-90 μg/mL	**98% a.i.** Positive for structural aberrations at 12-22 μg/mL without S9; 45-90 μg/mL with S9 and Positive for numerical aberrations at 12-22 μg/mL without S9.

Table 6. Genotoxicity of Benomyl		
STUDY	**DOSE**	**RESULTS**
MICRONUCLEUS INDUCTION		
In vivo Bone marrow micronucleus Swiss Webster mice (Kirkhart, 1980) Doc. No. 004679 Acceptable guideline	250, 500 & 1000 mg/kg/day once daily for 2 days by oral gavage.	Positive all doses at 48 hrs post exposure. Lowest Effective Dose (LED) was 250 mg/kg. The high dose also caused significant effects at 72 hrs post exposure.
In vivo Bone marrow micronucleus BD-F1 mice MRID No. 41607901 Doc. No. 010723 Acceptable guideline	1250, 2500 & 5000 mg/kg once by oral gavage	Positive dose-related and significant increases in micronucleated polychromatic erythrocytes (MPEs) in bone marrow at all dose & all sample times (24,48 & 72 hrs post exposure); LED:1250 mg/kg.
In vivo Bone marrow micronucleus ICR mice MRID No. 41051510 Doc. No. 004679 Acceptable guideline	500 & 1000 mg/kg/day once daily by oral gavage	Positive (≈3.5-fold) increase in micronucleated polychromatic erythrocytes (MPEs) at 1000 mg/kg; no effect was observed at 500 mg/kg. An ≈2.2-fold increase in micronucleated normochromatic erythrocytes (MNEs) was also seen at 1000 mg/kg, but only sampled at 24 hr.
ANEUPLOIDY INDUCTION		
In vivo Bone marrow erythrocyte immunofluorescent antikinetochore micronucleus assay BD-F1 mice MRID No. 42911601 Doc. No. 010723 Acceptable non guideline	100, 2500 & 5000 mg/kg/day once by oral gavage	Positive (significant increases in total MPEs) at ≥2500 mg/kg; 82% of micronuclei were kinetochore-positive based on immunofluorescent antikinetochore-specific antibody staining; no effects at 100 mg/kg. The finding of increased KC+ micronuclei suggests an aneuploidy effect since micronuclei staining positive for kinetochores are presumed to contain intact chromosomes while those staining negative for kinetochores contain chromosome fragments resulting from structural damage to the chromosomes (i.e., a clastogenic effect).
In vivo Bone marrow erythrocyte immunofluorescent antikinetochore micronucleus assay B6D2-F1 mice MRID No. 42911602 Acceptable non guideline	1646, and 3293 mg/kg once by oral gavage	Significant increases in total MPEs and the frequency of kinetochore-positive (83-93% KC+) micronuclei in both sexes of mice administered 3293 mg/kg. Positive effects were also seen at 1646 mg/kg in the females.

Table 6. Genotoxicity of Benomyl		
STUDY	**DOSE**	**RESULTS**
STRUCTURAL CHROMOSOMAL ABERRATION INDUCTION		
Bone marrow cytogenetics BD-F1 mice MRID NO. 41607902 Doc. No. 010723 Acceptable guideline	1250, 2500 & 5000 mg/kg/day once by oral gavage.	**96% a.i.** Negative, no cytotoxicity (based on mitotic indices) or clastogenic activity. No evidence of overt toxicity (no effect on body weight, mortality or clinical signs)
SISTER CHROMATID EXCHANGE		
In Vitro Sister Chromatid Exchange in CHO (Evans, E.L. & Mitchell, A.D. 1980) (Doc. No. 004679) Acceptable guideline	0.625 to 150 μg/mL	Positive at 0.625 μg/mL (LDT) to 2.5 μg/mL without S9; Positive at 0.375 μg/mL (LDT) to 150 μg/mL with S9; response only dose-dependent with S9.
UNSCHEDULED DNA SYNTHESIS		
In Vitro Unscheduled DNA synthesis in B6C3F1 mice MRID No. 00163411; Doc. No. 004679 Acceptable guideline	HID: 50 μg/mL	Not mutagenic in primary mouse hepatocyte cultures up to a cytotoxic concentration of 50 μg/mL.
Unscheduled DNA synthesis in F344 rats MRID No. 00103118; Doc. No. 004679 Acceptable guideline	HID: 5 μg/mL	Not mutagenic in primary rat hepatocyte cultures.

HID= Highest Ineffective Dose
LED = Lowest Effective Dose

Mutagenicity Data Considered by the RfD Peer Review Committee

In 1997, the HED RfD/Peer Review Committee reviewed a summary of the mutagenicity data for benomyl and MBC (N. McCarrol, 5/28/97). The majority of the studies considered by the Committee are summarized on Tables 6 and 7. In addition, the Committee considered literature data. The following summary is an excerpt from the 1997 report.

Several mutagenicity reviews exist for benomyl (Georgieva et al. ,1990; WHO, 1993) including one prepared by HED (see MRID No. NR 41051523). In general, the genotoxicity assessments in these reviews compare favorably with the results of the registrant-submitted acceptable studies. Overall, the findings indicate that benomyl is at best weakly mutagenic in bacteria and

cultured mammalian cells and produces conflicting results in in vitro chromosome assays (i.e., generally causing cell cycle delay but no clear effects on chromosome structure). There is also convincing and supporting evidence that while both benomyl and the MBC metabolite induce micronuclei in vivo, neither compound is clastogenic. In addition, results from a dominant lethal assay indicated that 70 days of oral exposure to 50 mg/kg benomyl significantly reduced the percentage of females with implants at mating weeks 1, 2 and 3 but there was no evidence of a dominant lethal effect (Georgieva et al., 1990).

In contrast to the largely negative database for gene mutations and structural chromosome aberrations, a sizable database exists from various assay systems indicating that benomyl and MBC are aneugenic following oral dosing in mice. A summary of the more recent work with benomyl in assays designed to detect aneuploidy is presented in Table 7. As shown, benomyl was positive for mitotic chromosomal malsegregation in Saccharomyces cerevisiae causing dose-dependent increases in the frequency of malsegregation at 30-60 µg/mL (Albertini 1991; Albertini et al. 1993). It is noteworthy that in both series of experiments, MBC served as the positive control, producing positive effects at 2.5 µg/mL (≈10-fold less than the lowest effective dose of benomyl). In a series of investigations with cultured human lymphocytes, Georgieva et al. (1990) found that benomyl concentrations ranging from 0.5 to 2.0 µg/mL significantly decreased the number of human lymphocytes undergoing third cell division and that 0.25-4.0 µg/mL induced significant and dose-related aneuploidy. There was, however, no adverse effect on chromosome structure. Gibson at al. (1995) also reported that 2.0 µg/mL benomyl caused a significant increase in morphological transformation of Syrian hamster embryo cell; higher doses (≥2.5 µg/mL) were cytotoxic. Similarly, results from the study of Zelesco et al. (1990) provided compelling evidence of dose-dependent aneuploidy and polyploidy in a human-Chinese hamster hybrid cell line at 4 and 8 µg/mL benomyl. In vivo, benomyl has been shown to induce significant increases in total MPEs as well as kinetochore-positive micronuclei in the bone marrow cells of male and female mice (see MRID No. 42911601); comparable results were found with MBC (see MRID No. 42911602). Finally, Mailhes and Aardema (1992) demonstrated aneuploidy induction in germinal cells. In this study, superovulated female ICR mice receiving single oral administrations of 500 to 2000 mg/kg benomyl had significantly increased percentages of hyperploid oocytes but no increases in structural chromosome damage. The response was dose-related, and peak activity was detected at 1500 mg/kg.

Table 7. Summary of Recent Aneuploidy Studies with Benomyl

ASSAY	CELL TYPE	RESULT	REFERENCE
IN VITRO TEST SYSTEMS			
Mitotic chromosomal malsegregation	Saccharomyces cerevisiae D61.M	positive	Albertini (1991)

Table 7. Summary of Recent Aneuploidy Studies with Benomyl			
ASSAY	CELL TYPE	RESULT	REFERENCE
Mitotic chromosomal malsegregation	Saccharomyces cerevisiae D61.M	positive	Albertini et al. (1993)
Morphological transformation	Syrian hamster embyro cells	positive	Gibson et al. (1995)
Aneuploidy/polyploidy	Chinese hamster-human hybrid EUBI cells	positive	Zelesco et al. (1990)
Aneuploidy	Human lymphocytes	positive	Georgieva et al. (1990)
IN VIVO TEST SYSTEMS			
Antikinetochore antibody formation	Mouse bone marrow cells	positive	Bentley (1992) (MRID No. 42911601)
Mouse oocytes aneuploidy	Mouse MI and MII oocytes	positive	Mailhes and Aardema (1992)

Since it is generally acknowledged that somatic cell aneuploidy may be involved in carcinogenesis and that the genetic imbalances resulting from aneuploidy in germinal cells may contribute to birth defects, it is not surprising that the results from genetic toxicology testing with benomyl correlate favorably with the data from chronic feeding studies demonstrating hepatocellular carcinomas in male and female mice. Similarly, the genetic toxicology data support the evidence of developmental effects in rats.

In light of the sizable body of evidence indicating that benomyl is an aneugen, it is concluded that no additional genetic toxicology testing is warranted. The acceptable studies satisfy both the Pre-1991 and the New mutagenicity initial testing battery guidelines.

g. Neurotoxicity

No treatment-related neurotoxicity was observed in the acute or subchronic rat studies with benomyl. Although increased motor activity was noted at the highest dose tested (456-578 mg/kg/day) in the subchronic rat study, this observation was discounted due to the presence of systemic toxicity. Benomyl does not appear to cause delayed neurotoxicity in hens. The following Table summarizes the registrant-submitted neurotoxicity studies for benomyl:

Table 8. Neurotoxicity of Benomyl			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
870.6100b (81-7)	Delayed Neurotoxicity Study in Hens MRID No: 241930 1979 Core Grade: not acceptable guideline	0, 500, 2500 or 5000	**% a.i. benomyl not given** NOAEL: 2500 LOAEL: 5000 based on decreased activity; No delayed neurotoxicity Note: 5/10 hens exposed to 5000 mg/kg died between 6-9 days of treatment.
870.6200 (81-8)	Acute Neurotoxicity Study in Crl:CDBR Rats MRID # 42817003 1993 Core Grade: acceptable guideline	0, 500, 1000 or 2000	**97.4% a.i. benomyl** (DPX-T1991-529) NOAEL (systemic): 2000 LOAEL (systemic): none established Effects: No signs of neurotoxicity observed, Testicular lesions were observed at 500 and 1000 mg/kg.
870.6200a (82-8)	13 Week Rat (Sprague-Dawley) Neurotoxicity Study MRID 43277901 1994 Core Grade: acceptable guideline	M: 0, 6, 158, or 456 F: 0, 8, 199 or 578 (0, 100, 2500 or 7500 ppm)	**97.4% a.i. benomyl** (DPX-T1991-529) NOAEL (systemic): 158 (M), 199(F) LOAEL (systemic): 456 (M), 578 (F) Effects: Increased motor activity and decreased terminal body weight (15% males and 12% females) and decreased body weight gain (approximately 25% for both sexes). Benomyl was not considered neurotoxic because the increased motor activity occurred in the presence of systemic toxicity.

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

compared to controls and body weight gain was approximately 25% lower than controls for both sexes. Motor activity was also increased at 7500 ppm for both males and females. No neuropathological lesions attributable to the administration of benomyl were observed when the tissues were subjected to histological examination.

The NOAEL was 2500 ppm and the LOAEL was 7500 ppm based on decreased terminal body weight (15% for males and 12% for females), decreased body weight gain (approximately 25% for both sexes) and increased motor activity. Based on the results, the compound is not considered a neurotoxicant because the increase in motor activity was observed at a dose level where systemic toxicity occurred (MRID 43277901).

h. Metabolism/Pharmacokinetic Studies

In the rat, benomyl is excreted primarily in the urine with lesser amounts excreted in the feces. In ~~an~~ unacceptable rat metabolism study with benomyl, 86% and 13% of recovered radioactivity was detected in the urine and feces, respectively within 72 hours of dosing (MRID 00066776). Very little MBC was present in the urine. This study is limited because only one male rat was evaluated, and pretreatment with 2500 ppm unlabeled benomyl in the diet 1 day prior to the gavage dosing of 7.7 mg/kg radiolabelled benomyl may have enhanced metabolism of the parent compound.

This guideline is fulfilled by the rat metabolism study with carbendazim discussed later in this memorandum.

i. Dermal Absorption

A dermal absorption study was conducted in rats (4 rats/time point/dose) using benomyl in the form of Benlate (50% wettable powder formulation). The test material was applied at dose levels of 0.2, 2, 20 or 200 mg of Benlate (equivalent to 0.1, 1, 10 or 100 mg a.i. of benomyl). The exposure durations were 0.5, 1, 2, 4 and 10 hours. The amount of benomyl absorbed ranged from 0.031 to 3.5 percent from the highest to the lowest dose, respectively, following the maximum exposure period of 10 hours. The majority of the absorbed benomyl (96-99%) was excreted in the urine after 10 hours (MRID 00097287). This study is acceptable.

j. Mechanism of Action

In 1997, the HED RfD/Peer Review Committee summarized a mechanism of action for benomyl. The following summary is an excerpt from the 5/28/97 report. "Benomyl has been reported to inhibit the in vitro polymerization of the rat neurotubulin at approximately 7.5 μg/mL (Albertine et al. 1993). This finding is consistent with the known mechanism of aneuploidy induction by the benzimidazole class of compounds (i.e., in vitro inhibition of yeast and/or mammalian tubulin polymerization with impairment of the spindle apparatus and resulting aneuploidy in the daughter cells)(Albertini et al. 1988)."

Since it is generally acknowledged that somatic cell aneuploidy may be in involved in carcinogenesis and that the genetic imbalances resulting from aneuploidy in germinal cells may contribute to birth defects, it is not surprising that the results from genetic toxicology testing with benomyl correlate with the data from chronic feeding studies demonstrating hepatocellular carcinomas in male and female mice. Similarly, the genetic toxicology data support the evidence of adverse reproductive effects in rats (i.e., degeneration of seminiferous tubules, reduced sperm counts, decrease in F2b offspring and decreased survival in days). Hoogenboom et al. (1991) postulated that the known antitubulin action of benomyl may impair microtubule formation and produce brain and ocular malformations by disruption of neuronal proliferation and migration.

III. CARBENDAZIM (MBC)

a. Acute Toxicity

Carbendazim is of low toxicity following acute exposures. Guideline studies for acute toxicity indicate that the carbendazim is classified as category IV for acute oral toxicity, category III for acute dermal and inhalation toxicity and primary eye irritation, and category IV for primary skin irritation. Carbendazim is not a skin sensitizer, and there is no evidence of delayed neurotoxicity in hens. Acute toxicity values and categories for carbendazim are summarized in the following table.

Table 9
Acute Toxicity of Carbendazim

Guideline No.	Study Type	% a.i.	MRID or Accession No.	Results	Toxicity Category
870.1100 (81-1)	Acute Oral, Rat	75	256025 (Acc No)	LD_{50} = >5000 mg/kg,	IV
870.1200 (81-2)	Acute Dermal, Rabbits	75	256025 (Acc No)	LD_{50} = >2,000 mg/kg formulation	III
870.1300 (81-3)	Acute Inhalation, Rat	75	256025 (Acc No)	LC_{50} >5 mg/L	III
870.2400 (81-4)	Primary Eye Irritation, Rabbit	>98	256025 (Acc No)	minimal to no irritation	III
870.2500 (81-5)	Primary Skin Irritation, Rabbit	75	256025 (Acc No)	slight irritation at 24 hr, normal by 72 hr	IV
870.2600 (81-6)	Dermal Sensitization, Guinea Pig	98	256025 (Acc No)	not a dermal sensitizer	N/A

Table 9 Acute Toxicity of Carbendazim					
Guideline No.	**Study Type**	**% a.i.**	**MRID or Accession No.**	**Results**	**Toxicity Category**
870.6100a (81-7)	Delayed neurotoxicity, hen	Not given	241931 (Acc No)	NOAEL = 2500 mg/kg	N/A

N/A Not applicable

b. Subchronic Toxicity

Both liver and testicular effects were noted in dogs following subchronic oral exposure to carbendazim as doses as low as 35-40 mg/kg/day. The following table summarizes the subchronic toxicity study for carbendazim.

Table 10. Subchronic Toxicity of Carbendazim			
GDLN	**STUDY**	**DOSE (mg/kg/day) (1)**	**RESULTS (mg/kg/day) (1)**
870.3150 (82-1(b))	Subchronic Feeding in Dogs (90 days) MRID #: 00099130 Sherman et al. 1970 Core Grade: unacceptable guideline	M: 0, 2.7, 14.4, or 40.7 F: 0, 2.7, 11.3, or 35 (0, 100, 500 or 1500/2500 ppm)	**53% a.i. carbendazim** NOAEL: 11.3 (F), 14.4 (M) LOAEL: 35 (F), 40.7 (M) Effects: Histopathology changes in liver (1/4 males and 1/4 females) and testes (1/4 males) and increased alkaline phosphatase, cholesterol and SGPT. Liver effects included hepatic cirrhosis (hepatic cell necrosis, tubular collapse, and increased fibrous connective tissue around triads). Decreased testes weight in 3/4 males in the high dose. Note: The HDT group was gradually given increasing amounts of MBC using the following schedule: 500 ppm (3 days); 1000 ppm (2 days); 1500 ppm (2 days); 2500 ppm for a short time before the dose was lowered to 1500 ppm (week 3) due to decreased food consumption and weight loss.

SGPT = Serum Glutamic Pyruvic Transaminase

c. Chronic Toxicity and Carcinogenicity

Carbendazim was evaluated for carcinogenic potential in both rats, and mice. In addition, carbendazim was evaluated for chronic toxicity in dogs. In all animal species, the most sensitive toxicological endpoint is liver toxicity that occurred at levels as low as 12.5 mg/kg/day. Dogs appear to be the most sensitive species for liver toxicity following chronic oral exposure. Carbendazim induced liver tumors (hepatocellular carcinomas) in mice. There is no evidence of carcinogenicity in rats, however, the rat study only tested 36 rats/sex/dose (and only 20/sex/dose in the 250 mg/kg/day dose group) (when current guidelines require 50 rats/sex/dose). The following table summarizes the chronic toxicity/carcinogenicity studies for carbendazim:

Table 11.
Chronic Toxicity/Carcinogenicity of Carbendazim

GDLN	STUDY	DOSE (mg/kg/day)	RESULTS (mg/kg/day)
870.4100 870.4200 (83-1& 2)	Chronic feeding/ carcinogenicity study in CD rats (2 yrs) MRID # 00088333 Accession #: 232870-0, 232871 Sherman et al. 1972 Core Grade: minimum	0, 5, 25, 250 or 125/500 (430) [0, 100, 500, 5000 or 2500/10000 (8557) ppm]	**53% a.i. carbendazim** NOAEL:25 LOAEL: 250 Effects: Statistically significant decreases in red blood cell parameters (hematocrit, hemoglobin an red blood cells) in females and histological lesions in the liver (cholangiohepatitis and pericholangitis) in males and females. No evidence of carcinogenicity. Note: Dietary levels in 2,500 ppm were increased to 7,500 ppm at 18 weeks and to 10,000 ppm from weeks 20-104 for a time-weighted average of approximately 8557 ppm (430 mg/kg/day). Deficiencies: Only 36 rats/sex/dose tested (only 20 rats/sex were in 250 mg/kg/day dose group). Lack of complete clinical chemistry data and histopathology examination. At 24 months, only liver evaluated in 5 and 25 mg/kg/day groups and only liver, kidney and testes evaluated in 250 mg/kg/day group.

Table 11. Chronic Toxicity/Carcinogenicity of Carbendazim			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
870.4100b (83-1b)	Chronic feeding study in beagle dogs (2 yrs) MRID # 00088333 Accession #: 232870-0, 232871 (Sherman et al. 1972) Core Grade: acceptable guideline	0, 2.5, 12.5, or 37.5/62.5 (0, 100, 500 and 1500/2500 ppm) (Doses adjusted for % a.i.)	**53% a.i. carbendazim** NOAEL: 2.5 LOAEL: 12.5 Effects: At 12.5 mg/kg/day swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis and biochemical alterations indicative of liver damage (i.e., increased cholesterol, total protein, SGPT and alkaline phosphatase levels, and decreased A/G ratio). At 37.5/62.5 mg/kg/day, anorexia, distended abdomens and poor nutritional condition were reported.
870.4100b (83-1b)	Chronic feeding study in beagle dogs (1 yr) Accession # 265664 (Stadler et al. 1986) Core Grade: acceptable guideline	F:0, 2.93, 6.43 or 16.54 mg/kg M: 0, 3.2, 7.19, 17.07 (0, 100, 200, or 500 ppm)	**98.8% a.i. carbendazim** NOAEL: 6.43 (200 ppm) LOAEL: 16.54 (500 ppm) Effects: Possible transient increase in cholesterol (males and females) consistent with previous dog feeding studies.
870.4200b (83-2b)	Chronic feeding study in CD-1 mice (2 yrs) MRID # 256028, and 256029 Wood et al. 1982 Core Grade: acceptable guideline. The study was designed to specifically evaluate the liver carcinogenicity potential of MBC	0, 75, 225, 1125 (females) or 1125/563 (males) (0, 500, 1500 or 7500 (females) or 7500/3750 (males) ppm)	**99.3% a.i. carbendazim** NOAEL (non-cancer systemic): 75 LOAEL (non-cancer systemic): 225 Effects: liver toxicity (hepatocellular necrosis and swelling), body weight decrease and lymphoid depletion. In both sexes, there was an increased incidence of liver tumors. In males, hepatocellular carcinomas were noted at 225 mg/kg/day, while females exhibited carcinomas and adenomas at all dose levels. Note: The 7500 ppm was reduced to 3750 ppm at 66 weeks in males due to increased mortality.

Table 11. Chronic Toxicity/Carcinogenicity of Carbendazim			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
870.4200b (83-2b)	Chronic feeding/ carcinogenicity study in NMRKf mice (2 years) MRID # 2560302 (Donaubauer et al. 1982) Core Grade: unacceptable guideline	0; 5.8-7.1; 17.1 - 21.2; 34.4 - 41.9 or 522 - 648 (0, 50, 150, 300 or 1000/5000 ppm)	**99% a.i. carbendazim** NOAEL (non-cancer systemic): 34.4 - 41.9 LOAEL (non-cancer systemic): 522 - 648 Effects: increases the incidences of hepatic cell hypertrophy, clear cell foci and hepatocellular necrosis. No increased incidence of carcinogenicity was noted. Note: The 1000 ppm dose was increased to 2000 ppm after 4 weeks and to 5000 ppm after an additional 4 weeks. Deficiencies: incomplete examination of most recommended tissues, blood and urine were not collected for analysis.
870.4200b (83-2)	Chronic feeding/ carcinogenicity study in Swiss mice (80 weeks) MRID # 256029 (Beems et al. 1976) Core Grade: unacceptable guideline	0, 22.5, 45 or 750 (0, 150, 300 or 5000 ppm)	**99% a.i. carbendazim** NOAEL:45 LOAEL:750 Effects: hepatic alterations which included increased relative liver weights in both sexes, increased number of foci of cellular alterations in the liver in females, neoplastic nodules in females and hepatoblastomas in males Deficiencies: Brief methods, there were no historical data or microscopic or gross pathology reports for individual animals, and there was no assurance that the diets were analyzed for compound homogeneity and stability. In addition, there were no hematology or clinical chemistry analysis, nor urinalysis. Only organs or lesions suspected of being tumors and livers (2 sections) were examined histologically.

Chronic/Carcinogenicity Study in Rats

MBC (methyl ester, 53%) was administered in the diets of CRL: CD1 rats (36/sex/dose, except for 20/sex at 250 mg/kg/day group) at dietary levels of 0, 100, 500, 5,000 or 2,500/10,000 ppm (8557 ppm) (equivalent to 0, 5, 25, 250 or 125/500 (430) mg/kg/day)(MRID 00088333, Sherman et al. 1972). The dietary levels were increased in the 2,500 ppm group to a level of 7,500 ppm at 18 weeks and to 10,000 ppm from weeks 20 to study termination at week 104, yielding an approximate time-weighted average (TWA) daily dose of 8557 ppm (430 mg/kg/day) for the 104 week study duration. Treatment was initiated for the 5,000 ppm group 3 weeks late (age 33

weeks of age) without preliminary hematology. There were no apparent treatment related signs of toxicity nor were there any effects on mortality, food consumption or feed efficiency. In females in the highest dose group, there was a decrease in body weight gain when compared to controls at both 15 (14% lower) and 24 months (24% lower).

At 5000 and 2500/10,000 ppm (250 and TWA 430 mg/kg/day), statistically significant decreases in red blood cell counts, hemoglobin and hematocrit values were reported in females at 24 months. Transient, non-significant elevations in SGPT were reported in males and females in the high dose group (TWA 430 mg/kg/day) at 12 months, but these findings were not apparent at 24 months and therefore, were not considered to be related to the administration of benomyl. In males and females receiving 5000 ppm and 2500/10,000 ppm (TWA 8557 ppm) benomyl, there was an increase in the incidence and severity of cholangiohepatitis and pericholangitis. There was no evidence of carcinogenicity reported in this study, however, this study is classified as minimum, and does not meet the Subdivision F chronic toxicity or oncogenicity guidelines (see below). Thus, the NOAEL in this study was 500 ppm (25 mg/kg/day), based on statistically significant decreases in red blood cell parameters and histological lesions in the liver (cholangiohepatitis and pericholangitis) at both 5000 and 2500/10,000 ppm (8557 ppm) (250 and TWA 430 mg/kg/day.

This study was conducted in 1972, prior to the 1984 Subdivision F guidelines for a chronic toxicity study (83-1) and chronic feeding/oncogenicity study (83-2), and therefore is classified as minimum (i.e., does not meet current evaluation standards, however, is adequate for risk assessment). Deficiencies include small sample size (36/sex/dose except 20/sex in 5000 ppm group, when 50/sex/dose are required for 83-2 and current oncogenicity guidelines, limited histopathology (the target organ testes was not evaluated in the two lowest dose groups) and limited clinical chemistry evaluation [i.e., only plasma alkaline phosphatase (AP) and Serum Glutamic Pyruvic Transaminase (SGPT)] only in the two highest dose groups. Based on the observed decreases in body weight at the highest doses tested and the observations of liver lesions and decreases in hematology measurements, the study appears to have been conducted at adequate dose levels. The adequacy of the doses tested is further supported by the results of the 90 day neurotoxicity study, where terminal body weights and body weight gains were decreased when compared to controls at the highest dose tested of 7500 ppm (MRID 00088333).

Chronic/Carcinogenicity Study in Mice

In a carcinogenicity study conducted with MBC (99.3%), the test material was administered in the diets of CD-1 mice (80/sex/dose) at levels of 0, 500, 1500, 7500 (females) or 7500/3750 (males) ppm (equivalent to 0, 75, 225, 1125 (females) or 1125/563 (males) mg/kg/day) (MRID # 256028, and 256029, Wood et al. 1982). In males receiving 7500 ppm, the dose was reduced to 3750 at week 66 and fed until this group was terminated at week 73. All other groups were fed at the designated dietary levels up to week 104. Survival of the male mice in the intermediate (1,500 ppm) and high (7,500-3750 ppm) dose groups was significantly lower than that of male control mice. A 12% decrease in body weight was reported in males receiving 1500 ppm when

compared to controls at week 104. Hepatoxicity, characterized by hepatocellular necrosis and swelling was also reported in males at 1500 ppm. In both sexes, an increased incidence of liver tumors was reported. Heptatocelluar carcinomas, and adenomas and carcinomas combined, were significantly elevated in male mice (mid dose level); no increase in adenomas (alone) occurred in males. The lack of carcinogenic response in high dose males is likely to be explained by either their early deaths or 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 levels tested) of the liver. No increased incidence of liver hyperplasia occurred in treated mice. Only the carcinomas (mid and high dose levels) and the adenomas/carcinomas combined (all 3 dose levels) in female mice exceeded the historical control response rates. There was a treatment-related decrease in female thymic weight (absolute and relative) and a dose-related decrease in male thymic weight. This was consistent with the treatment-related lymphoid depletion observed in both sexes of the mid and high dose groups.

The high dose level of MBC clearly exceeded the MTD level in male mice (but not females) because of excessive mortality. The mid dose level appeared to approximate the MTD level for males. The non-cancer systemic NOAEL was 500 ppm based on liver toxicity, body weight decrease and lymphoid depletion reported at 1500 ppm (MRID 256028, 256029). This study is **acceptable** and satisfies the 83-2 guidelines for an oncogenicity study in mice.

In another carcinogenicity study in NMRKf mice (100/sex/dose), MBC (99%) was administered at dietary levels of 0, 50, 150, 300 or 1000/5000 ppm for 2 years. The 1000 ppm dose was increased to 2000 ppm after 4 weeks and from 2000 ppm to 5000 ppm after an additional 4 weeks on the study. Dietary concentrations were reported to be equal to 0, 5.8, 17.1, 34.4 or 522 mg/kg for males and 0, 7.1, 21.2, 41.9 or 648 mg/kg for females, respectively. This study was designed to specifically address the finding of liver carcinogenicity and all tissues were not subjected to a gross or microscopic examination. The systemic NOAEL was 300 ppm (34.4 - 41.9 mg/kg) and the systemic LOAEL was 5000 ppm (520 - 650 mg/kg) based on liver toxicity in both sexes which consisted of increases the incidences of hepatic cell hypertrophy, clear cell foci and hepatocellular necrosis. The incidence of carcinogenicity was not increased in this study. The NMRKf strain of mouse, in contrast with the CD-1 and Swiss SPF mice, normally exhibits a low background incidence of liver tumors. Because of the reported inconsistencies in the analysis of MBC and because an incomplete gross and microscopic assessment, this study was classified as **not acceptable** guideline. In addition, blood and urine were not collected for evaluation (MRID 2560302).

Carbendazim was also associated with an increase in the incidence of hepatoblastomas in Swiss mice. MBC (99%) was administered in the diets of SPF Swiss mice (100/sex/dose) at dietary levels of 0, 150, 300 or 5000 ppm (equivalent to 22.5, 45 or 750 mg/kg/day) for 80 weeks (Accession No. 256029, Beems et al. 1976). The systemic NOAEL was 300 ppm (45 mg/kg) and the systemic LOAEL was 5000 ppm (750 mg/kg) based on hepatic alterations which included increased relative liver weights in both sexes, increased number of foci of cellular alterations in the liver in females, neoplastic nodules in females and hepatoblastomas in males.

This study was actually a report and was classified as **unacceptable** guideline because the methods were brief, there were no historical data provided, there were no microscopic or gross pathology reports for individual animals, and there was no assurance that the diets were analyzed for compound homogeneity and stability. In addition, there were no hematology or clinical chemistry analysis, nor urinalysis. Only organs or lesions suspected of being tumors and livers (2 sections) were examined histologically (MRID 256029).

Chronic Toxicity Study in Dogs

Beagle dogs (4/sex/dose) were administered a product formulation containing 53% a.i. carbendazim, a primary metabolite of benomyl at dietary doses levels of 0, 100, 500 or 1500/2500 ppm for two years (MRID 00088333, Accession #: 232870-0,232871, Sherman et al. 1972). Due to weight loss and decreased appetite, the dose to some dogs in the 2500 ppm group was reduced to 1500 ppm. This is equivalent to 0, 2.5, 12.5 or 37.5/62.5 mg/kg/day ai MBC. One dog/sex from control and 500 ppm group, as well as one female from the high dose group was sacrificed at 1 year. One male from the high dose group was sacrificed in extremis after 42 weeks on the test diet. Only the livers and testes were examined histologically in the 100 an 500 ppm dose groups.

There was no mortality reported for the control or 100 and 500 ppm groups. However, three of the males in the high dose group were sacrificed after 22 and 24 weeks because of poor nutrition. No females in the high dose group died. Body weight and food consumption were adversely affected in all high dose group animals. No treatment-related effects were noted in dogs fed 100 ppm (2.5 ai mg/kg/day). Diffuse and marked testes atrophy and aspermatogenesis were observed in 2/4 males of the 100 ppm group, which were not considered treatment-related because these observations were not present in the other dose groups. Dogs of both sexes in the mid and high dose groups (12.5 ai or 37.5/62.5 mg/kg/day) exhibited liver pathology characterized as swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis. There were no apparent effects on the organ weights or organ to body weight ratios. At 500 ppm and 1500/2500 ppm, there were also reported increases in cholesterol, total protein, SGPT and alkaline phosphatase, in addition to a decrease in the albumin/globulin (A/G) ratio throughout the study. Dogs in the 1500/2500 ppm (37.5/62.5 mg/kg/day ai) groups exhibited anorexia, distended abdomens and poor nutritional condition.

The NOAEL is 100 ppm (2.5 mg/kg/day ai). The LOAEL is 500 ppm (12.5 mg/kg/day ai) based on biochemical and histological alterations indicating liver damage. Histopathological lesions of the liver were characterized as swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis in both sexes of dogs. This study is **acceptable** and satisfies the guideline for a chronic feeding study in dogs (83-1b).

In a more recent study (Accession # 265664, Stadler et al. 1986) beagle dogs (5/sex/dose) were administered carbendazim (98.8% ai) at dietary doses levels of 0, 100, 200 or 500 ppm for one year. Based on compound intake, these doses were equivalent to 0, 2.93, 6.43 or 16.54

mg/kg/day in females and 0, 3.2, 7.19 or 17.07 mg/kg/day in males (average for both sexes were 0, 3.06, 6.81 or 16.8 mg/kg/day).

There were no treatment-related effects on clinical observations, body weight, food consumption, hematology, and urinalysis. The only possible treatment-related observation was an increase in cholesterol. Although these values were noted as within the historical control range for the laboratory, actual historical control ranges were not given in the report. In several other dog studies with carbendazim, there were definite dose-related cholesterol increases at higher doses and a borderline increase at 500 ppm. Therefore, it is possible that this change, although minimal and transient, is treatment-related. There was a statistical increase in relative renal weight in the mid and high dose males, however, there were no corresponding effects in clinical chemistries or histopathology. Renal weights were not affect in other carbendazim dog studies. There were slight brain weight changes only in the mid dose group. Therefore, renal and brain weight changes are probably due to individual animal variation. One high dose female had a thyroid follicular adenoma that is considered rare in dogs of this age. However, this tumor was not considered to be treatment-related because there were no corresponding changes in thyroid histology and organ weight or changes in clinical chemistries other than the possible cholesterol increase. This study is **acceptable** and satisfies the guideline for a chronic feeding study in dogs (83-1b).

Classification of Carcinogenic Potential

See previous discussion under benomyl carcinogenic potential classification.

d. Developmental Toxicity

There is increased sensitivity of rat and rabbit fetuses as compared to maternal animals following *in utero* exposure to MBC, in prenatal developmental toxicity studies. In the MBC rat study, increased sensitivity manifested as developmental anomalies (decreased fetal body weight and increases in skeletal variations and a threshold for malformations) at doses which were not maternally toxic. At higher doses, treatment-related malformations of the CNS were observed which included exencephaly, domed head, anophthalmia, microphthalmia and bulged eyes. For developmental toxicity the NOAEL was 10 mg/kg/day, whereas for maternal toxicity, the NOAEL was 20 mg/kg/day (based on a slight increase in liver weight at 90 mg/kg/day).

In the rabbit developmental study with MBC, increased sensitivity manifested as decreased implantations and litter size, and increased resorptions at 20 mg/kg/day; the developmental NOAEL is 10 mg/kg/day. Maternal toxicity was not observed until higher doses of 125 mg/kg/day, based on abortions and decreased maternal body weight; the maternal NOAEL is 20 mg/kg/day.

Table 12. Developmental Toxicity of Carbendazim			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
870.3700a (83-3a)	Developmental Study in Crl:CE BR rats (gavage) MRID# 40438001 Alvarez 1987 Core Grade: acceptable guideline	0, 10, 20, 90 gestation day 7-16	**98.8% a.i. carbendazim** Maternal NOAEL: 20 Maternal LOAEL:90 (increased absolute liver weight) Developmental NOAEL:10 Developmental LOAEL: 20 based on decreased fetal body weight and increases in skeletal variations and a threshold for malformations.
870.3700b (83-3b)	Developmental Study in New Zealand White Rabbits (gavage) Accession # 260571 (Christian et al. 1985) Core Grade: acceptable guideline	0, 10, 20 or 125 gestation day 7-19	**98.7% a.i. carbendazim** Maternal NOAEL: 20 Maternal LOAEL:125 (abortions and decreased body weight) Developmental NOAEL:10 Developmental LOAEL: 20 mg/kg/day based on decreased implantations and litter size, and increased resorptions. Malformations (fused ribs, and malformed cervical vertebrae) were noted at 125 mg/kg/day Deficiencies: Terminal maternal body weights were not corrected by using empty uterine weight instead of gravid weights.

Rat Developmental Toxicity Study

In a developmental toxicity study (MRID No.: 40438001), 25 Crl:CE BR strain presumed pregnant rats per dose group were dosed with 0, 5, 10, 20 or 90 mg/kg/day of carbendazim (MBC, 98.8% a.i. in 0.5% methyl cellulose) by gavage on days 7 through 16 of gestation. The rats were sacrificed on day 22 of gestation. There was no effect on maternal body weight or other extrauterine parameters but there was a 10% increase ($p < 0.05$) in absolute liver weight at 90 mg/kg/day. Lower mean body weight on days 17-22 of gestation in the high dose group prior to sacrifice was attributed to intrauterine effects such as resorptions and not maternal toxicity. There were 24, 23, 24, 22 and 15 dams that delivered pups for the control to high dose groups, respectively. The low number of dams delivering pups in the high dose group was attributed to only 19 dams being pregnant in this group as well as one death (by mechanical dosing trauma) and three dams with total resorptions. The maternal LOAEL is 90 mg/kg/day based on absolute liver weight increase. The maternal NOAEL is 20 mg/kg/day.

There were a total of 312, 310, 281, 288 and 149 fetuses for the control to high dose groups, respectively available for examination. At 20 mg/kg/day there was a decrease in fetal weight

(-5% $p < 0.05$ for combined sexes). The mean percent of fetuses with variations due to retarded development was 22.9%, 25.0%, 19.5%, 41.6% ($p < 0.5$) and 52.5% ($p < 0.05$) for the control to high dose groups, respectively. At 20 mg/kg/day the vertebrae showed increases in bipartite ossification (21 incidents in 8 litters) and dumbbelled centrum (44 incidents in 13 litters) due to retarded development as well as misaligned sternebrae and extra ossification of the ribs that were not described as being related to retarded growth. At 90 mg/kg/day, there were a variety of developmental effects including decreases in mean live fetuses per litter (-24%, $p<0.05$), early and late resorptions, and decreased fetal weight (-26%, $p < 0.05$, both sexes combined). There were 3/2, 1/1, 1/1, 3/3, and 91/15 ($p < 0.01$) fetuses/litters affected with malformations in the control to high dose groups, respectively. These malformations included a variety of conditions mainly in the head (exencephaly, domed head), eyes (none, small or bulge), paws (clubbed) and skeleton (fused vertebrae, ribs and sternum or malformed scapula). Some of these malformations were seen in the three fetuses affected in the 20 mg/kg/day dose group and not in the lower doses or controls. Thus, 20 mg/kg/day is considered a threshold for malformations. The developmental LOAEL is 20 mg/kg/day based on decreased fetal body weight and increases in skeletal variations and a threshold for malformations. The developmental NOAEL is 10 mg/kg/day.

Rabbit Developmental Toxicity Study

In a developmental toxicity study (Accession No.: 260571), 20 Hra:(NZW)SPF strain presumed pregnant rabbits per dose group were dosed with 0, 10, 20 or 125 mg/kg/day of carbendazim (MBC, 98.7% a.i. in 0.5% methyl cellulose) by gavage on days 7 through 19 of gestation. The rats were sacrificed on day 29 of gestation. The only signs of maternal toxicity were in the 125 mg/kg/day group and included a slight decrease in food consumption and a body weight loss noted only during the treatment period. A significant increased incidence of anorexia was noted in dams during treatment with 125 mg/kg/day group. In addition, two dams in the 125 mg/kg/day group aborted during gestation (on gestation days 22 and 25), which may have been treatment-related. There was a treatment-related increase in resorptions at the high dose ($p \leq 0.01$) and a non-significant increase in the mid-dose. There was a significant decrease in the number of live fetuses ($p \leq 0.05$) in the 20 and 125 mg/kg/day groups which may be attributed to a slight decrease in implantations, and the increase in resorptions in these groups. The maternal LOAEL is 125 mg/kg/day based on abortion and decreased maternal body weight. The maternal NOAEL is 20 mg/kg/day.

Signs of developmental toxicity included abortions (125 mg/kg/day), resorptions (20 and 125 mg/kg/day), decreased implantations (20 and 125 mg/kg/day), and an increase in total fetal malformations (primarily fused ribs and malformed cervical vertebrae) (125 mg/kg/day). Resorptions and decreased implantations resulted in a decrease in liver fetuses at term in the 20 and 125 mg/kg/day dose groups. Although fused ribs may be a major abnormality, it has also been related to maternal stress. It is increased only at a maternally toxic levels and in historical controls, the incidence is increased when there is maternal stress (such as sham injections, cornstarch in diet, etc.). The malformations of the cervical vertebrae, although not reversible,

were minor variations, possibly due to development. There were no treatment-related external, head or visceral malformations. There was an increase in total fetuses with skeletal malformations only at 125 mg/kg/day, consisting primarily as malformed cervical vertebrae and interrelated malformation of the ribs (fused) and proximate thoracic vertebrae. Other malformations occurred sporadically in all groups including: malformed thoracic vertebrae, angulated or shortened hyoid alae, fused thoracic arches, fused sternebrae, flattened cranium, spina bifida, adactyly of one digit, malformed eyes, fused caudal vertebrae, domed head with hydrocephalus, shortened tail, ectoic kidneys. The developmental LOAEL is 20 mg/kg/day based on decreased implantation and liver litter size, and increased resorption. The developmental NOAEL is 10 mg/kg/day.

Scientific Literature Considered by the RfD Peer Review Committee

The HED RfD Peer Review Committee reviewed several literature studies in 1997 that pertained to the developmental toxicity of benomyl and MBC. The following summary is an excerpt from the 5/28/97 report.

"Cummings et al. (1990) showed that dosing during early gestation (GD 4-8) with MBC induced embryonic death, growth retardation, and developmental abnormalities at 200-600 mg/kg/day; fetal findings at GD 20 included fore- and hindlimb anomalies, and reductions in ossification of the sternebrae, caudal vertebrae, and phalanges; malformations of the eye were not mentioned and are apparently induced by dosing later in gestation."

e. Reproductive Toxicity

Carbendazim was associated with adverse reproductive effects (decreased birth weight at weaning) in an unacceptable reproduction study in rats. Carbendazim also caused adverse testicular effects characterized by premature release of immature germ cells, atrophy of a few seminiferous tubules and significant decrease in seminiferous tubule diameter following a single gavage dose with 50 mg/kg. In addition, evidence of testicular effects has been demonstrated in the 1970 90-day subchronic dog study with MBC.

Table 13. Reproductive Toxicity of Carbendazim			
GDLN	**STUDY**	**DOSE (mg/kg/day)**	**RESULTS (mg/kg/day)**
870.3800 (83-4)	Reproductive Study in ChR-CD rats (diet) MRID# 40438001 Sherman et al. 1972 Core Grade: unacceptable guideline	0, 5, 25, 250 or 125/500 (0, 100, 500, 5000 or 2500/10,000 ppm)	**50 or 70% a.i. carbendazim** Reproductive NOAEL:25 Reproductive LOAEL: 250 based on toxic signs of decreased pup weight noted at weaning. Deficiencies: Litter (or fetal) weights were not measured at birth, therefore it is impossible to attribute weight decrease in 5000 and 2500/10000 ppm groups to prenatal or lactaction period. Only 16 dams (20 dams for 5000 ppm), resulting in 10-16 litters per group were available, rather than the 20 litters recommended in the guideline. There was no special attention for the testes, a known target organ, including organ weights measurements. Note: 2500 ppm group increased to 7,500 ppm at week 18 and to 10,000 ppm at week 20 to end of study.
NA	Single dose (gavage) rat study Nakai et al. (1982)	0, 50, 100, 200, 400 or 800 mg/kg	NOAEL: none observed LOAEL: 50 Effects: premature release of immature germ cells 2 days post exposure, and atrophy of a few seminiferous tubules and significant decrease in seminiferous tubule diameter 70 days post exposure.

NA = Not Applicable

Scientific Literature: Effects on the Male Reproductive System

Nakai et al. (1992) conducted two experiments to investigate the effects of MBC on the male reproductive system. In experiment one, 16 groups of 8 male rats (86 days of age) were treated with a single oral dose of 0 or 400 mg/kg Carbendazim (methyl 2-benzimidazole carbamate, MBC, purity not specified), and effects on the testis and sperm were evaluated between 2 hours and 32 days after exposure. Significantly increased testis weight, significantly decreased testicular spermatid numbers and significantly decreased percentage of morphologically normal cauda sperm were noted beginning 8 hours post-exposure.

In experiment 2, groups of male rats (n=20/dose between 97 and 105 days of age) were treated with a single oral dose of 0, 50, 100, 200, 400 or 800 mg/kg MBC and killed on day 2 or 70 post-treatment. On day 2, at 50 mg/kg, round spermatids were sloughed (prematurely released) from stage I and II epithelium and elongated spermatids were sloughed from stage VII epithelium. In addition a dose-dependent increase in testicular weight was seen at dose levels of 100 mg/kg and higher that was accompanied by significant increases in mean seminiferous

tubular diameter at 400 and 800 mg/kg. At 100 mg/kg, the disappearance of germ cells was more severe and statistically significant and sloughing of elongated spermatids extended into stages XII and XIV. In animals treated with 100 mg/kg or more, there was a dose-dependent increased incidence of occlusions in the efferent ductules of the testes. The rete testis was swollen with sloughed germ cells indicating that ductal blockage had occurred further down the tract. At doses of 200 mg/kg and above, missing germ cells extended into all stages except stages IX-XI, while, at doses of 400-800 mg/kg, some of the seminiferous epithelia were damaged so severely that it was difficult to identify the stage.

On day 70, tubule diameter was significantly decreased at all doses in a dose-dependent relationship. Histologically, these decreases were associated with a dose-dependent increase in seminiferous tubular atrophy (significant at 100 mg/kg and higher). No atrophic tubules were seen in the control rats, however, atrophy of a few seminiferous tubules in one testicle was noted at 50 mg/kg. The atrophied tubules contained primarily Sertoli cells and occasional spermatogonia and were surrounded by a thickened basement membrane. Pathological alterations were also noted in the efferent ductules of the treated animals, 50% or more of the ducts being occluded in rats dosed with 100 mg/kg or more. Minimal effects were seen at 50 mg/kg, where slight abnormal growth of the efferent ductules was seen in only one specimen. The occlusions were characterized as compacted luminal contents, spermatic granulomas, mineralizations and obliterations of the original lumen by fibrotic connective tissue. In addition, mean testis weight showed a dose-dependent decrease that was statistically significant at doses of 100 mg/kg and greater.

This study identifies a LOAEL of 50 mg/kg/day for MBC; no NOAEL was identified based on sloughing (premature release) of immature germ cells 2 days postexposure; and atrophy of a few seminiferous tubules in one testicle, significant decrease in seminiferous tubule diameter, and slight abnormal growth of the efferent ductules at 70 days postexposure. The subtle effects detected in the epididymal sperm at 50 mg/kg may be attributed to the direct effect of MBC on the seminiferous epithelium.

f. Mutagenicity Studies

Both benomyl and MBC have marginal mutagenic activity in standard in vitro studies. In contrast, there is clear and reproducible evidence of aneuploidy (i.e., abnormal number of chromosomes) both in vitro and in vivo. There is also convincing evidence that the induction of aneuploidy by benomyl and MBC is primarily attributed to adverse effects on cellular spindle apparatus. Both benomyl and MBC are established spindle poisons that induce aneuploidy effects in both in vitro and in vivo test systems. For example, nondisjunction was reported in A. nidulans and many other test systems with both agents. Both fungicides also produced positive effects in bone marrow antikinetochore micronucleus assays, which were consistent with a spindle effect. However, neither compound is clastogenic. Since the genotoxic activity of benomyl and MBC is well known, these pesticides are frequently used as test chemicals (i.e.,

positive controls) for the assessment of new assay systems for the detection of aneuploidy induction.

In mutagenicity studies with benomyl and MBC, there is compelling evidence of aneuploidy induction following oral dosing in mice. Mutagenicity data support the evidence of developmental anomalies in rats and hepatocellular tumors in several strains of male and female mice. Table 14 summarizes the genotoxicity studies for MBC.

Table 14. Genotoxicity of Carbendazim

STUDY	DOSE	RESULTS
GENE MUTATION		
Ames Assay MRID No. 00154668; Doc No. 005531 Acceptable guideline	100-5,000 μg/plate	Not mutagenic with or without S-9 activation in strains TA-1535, TA-1537, TA-1538, TA-100, or TA-98
Ames Assay MRID No. 00154669; Doc No. 005531 Acceptable guideline	100-5,000 μg/plate	Not mutagenic with or without S-9 activation in strains TA-1535, TA-1537, TA-1538, TA-100, or TA-98
Ames Assay *S. typhimurium* assay Russell J.F. & Krahn, D.F. 1977; Doc No. 005531 Acceptable guideline	200-16,000 μg/plate without S9; 200-20,000 μg/plate with S9	Not mutagenic with or without S-9 activation in strains TA-1535, TA-1537, TA-1538, TA-100, or TA-98
Ames Assay *S. typhimurium* assay MRID No. 00151825; Doc No. 005531 Acceptable guideline	200-10,000 μg/plate	Negative at 200-10,000 μg/plate with and without S9. Positive in TA1537, TA1538 & TA98 at 4000, 8000 & 10,000 μg/plate +S9. Negative TA1535& TA100 +/-S9.
Ames Assay *S. typhimurium* assay Russell, J.F., Arce, G.T. & Sarrif, A.M., 1983; Doc No. 005531 Acceptable guideline	100-10,000 μg/plate	Not mutagenic with or without S-9 activation in strains TA-1535, TA-1537, TA-1538, TA-100, or TA-98

Table 14. Genotoxicity of Carbendazim		
STUDY	**DOSE**	**RESULTS**
S. typhimurium/E. coli assay MRID No. 43205504; Doc No. 011116 Acceptable guideline	HID: 5000 μg/plate	Not mutagenic with or without S-9 activation in strains TA-1535, TA-1537, TA-100, or TA-98; Negative for E. coli WP2 uvrA +/-S9
S. typhimurium host mediated assay MRID No. 00154670; Doc No. 005531 Acceptable guideline	500 or 2000 mg/kg once daily for 2 days by oral gavage	Negative in ICR male mice administered 500 or 2000 mg/kg once daily for 2 days by oral gavage using strain G46 as the indicator organism.
S. typhimurium/ E.coli assay MRID No. 00154670; Doc No. 005531 Acceptable guideline	10-3000 μg/plate without S9; 10-1000 μg/plate with S9; HID: 1000 μg/plate without S9; 3000 μg/plate with S9.	Negative TA1535, TA1537, TA1538, TA98, TA100; E. coli WP2 uvrA +/-S9
CHO/HGPRT assay MRID No. 00154671; Doc No 003744, 005531 Acceptable guideline	3-628 μM (120 μg/mL) without S9; 3-654 μM (125 μg/mL) with S9	Negative: Dose Range: 3-628 μM without S9 HID = 628 μM (≈ 120 μg/mL) without S9; Dose range with S9: 3-654 μM with S9 HID = 654 μM (≈ 125 μg/mL) with S9; ppt at ≥262 μM (≈ 50 μg/mL)+/-S9.
Mouse lymphoma l5178y $TK^{+/-}$ Forward Gene Mutation Assay MRID No. 00154673; Doc. No. 004679, 005531 Acceptable guideline	5-50 μg/ml without S9; 2-25 μg/ml with S9	**98% a.i.** Positive: (LED:50 μg/ml without S9); dose-dependent increases in mutation frequency over 8 concentrations of 12-25 μg/ml with S9. The response peaked at 25 μg/ml with S9, with a 7-fold increase in mutation frequency and 10% total growth. Colony sizing not performed.
Mouse lymphoma $TK^{+/-}$ assay MRID No. 00159370; Doc. No. 005531 Acceptable guideline	50-250 μM (≈ 10-50 μg/mL) without S9 25-250 μM (≈ 5-25 μg/mL) with S9	Negative at 50-250 μM (≈ 10-50 μg/mL) without S9. Positive at 75, 87.5, 100, 112.5, 125, 150, 200 and 250 μM with S9 but additional information sent by the registrant indicated that the mutagenicity was due to contaminants not to MBC.

Table 14. Genotoxicity of Carbendazim		
STUDY	**DOSE**	**RESULTS**
CHROMOSOMAL ABERRATIONS		
CHO chromosome aberrations MRID No. 43205505; Doc No. 011116 Acceptable guideline	38-300 μg/mL	Negative with and without S9 activation up to a ppt and cytotoxic dose (≥300 μg/mL).
MICRONUCLEUS INDUCTION		
In vivo Bone marrow micronucleus ICR mice MRID No. 41051510 Doc. No. 004679 Acceptable guideline	500 mg/kg (ip) or 50, 100, 500 & 100 mg/kg/day once daily for 2 days by oral gavage.	Negative via ip; positive via oral gavage; dose-related increase in micronucleated polychromatic erythrocytes (MPEs) and micronucleated normochromatic erythrocytes (MNEs) at 100-1000 mg/kg-- no effect at 50 mg/kg but only sampled at 24 hours. MBC was a ≈10 times more potent inducer of micronuclei than benomyl.
ANEUPLOIDY		
Bone marrow antikinetochore micronucleus assay BD-F1 mice MRID No. 42911602 Doc. No. 010723 Acceptable guideline	66, 1646, & 3293 mg/kg once by oral gavage.	Positive at 1646 & 3293 mg/kg in females (48 hrs.) and 3293 mg/kg in males (48 hrs); 83-93% of micronuclei were kinetochore-positive at 3293 mg/kg; no effects at 1646 mg/kg (males) or 66 mg/kg (females).
UNSCHEDULED DNA SYNTHESIS		
In vitro Unscheduled DNA synthesis in mice (1981) MRID No. 00154754 Doc. No. 005531 Acceptable guideline	0.0125-12.5 μg/mL	Not mutagenic in primary mouse hepatocyte cultures.
In vitro Unscheduled DNA synthesis in rats MRID No. 00154672 Doc. No. 005531 Acceptable guideline	0.0125-12.5 μg/mL	Not mutagenic in primary rat hepatocyte cultures.

Table 14. Genotoxicity of Carbendazim		
STUDY	**DOSE**	**RESULTS**
In vitro Unscheduled DNA synthesis assay in Human cells (explant from lung carcinomas) MRID No. 43205506 Doc No. 011116 Acceptable guideline	0.3-300 μg/mL +/- S9.	Negative up to a ppt ($\geq$30 μg/mL) and cytotoxic doses($\geq$100 μg/mL).
DNA DAMAGE/REPAIR		
Bacillus subtilis DNA damage/repair rec assay MRID No. 00154670 Doc No. 005531 Acceptable guideline	up to 1000 μg/plate	Negative up to 1000 μg/plate without S9 *B. subtilis* H17, M45

HID= Highest Ineffective Dose
LED = Lowest Effective Dose

Mutagenicity Data Considered by the RfD Peer Review Committee

In 1997, the HED RfD/Peer Review Committee reviewed a summary of the mutagenicity data for benomyl and MBC that was prepared by N. McCarrol (5/28/97). The majority of the studies considered by the Committee are summarized on Tables 6, 7 and 14. In addition, the Committee considered literature data. A summary from this report was previously presented in the benomyl mutagenicity section.

g. Neurotoxicity

MBC (% a.i. not given) was not associated with delayed neurotoxicity up to 5000 mg/kg (Accession No. 241931). At 5000 mg/kg, 2/10 hens had slight ataxia and salivation as a result of acute toxicity of MBC, while 8/10 hens developed leg weakness. The NOAEL for neurotoxicity is 2500 mg/kg. This study satisfies the guideline (81-7) for an acute neurotoxicity study in the hen.

h. Metabolism/Pharmacokinetic Studies

In the rat, carbendazim is excreted primarily in the urine, and is poorly distributed to the tissues. Carbendazim (94% ai) was rapidly absorbed and extensively metabolized in CD/BR rats following single oral doses of 50 and 1000 mg/kg and at repeated oral doses of 50 mg/kg (MRID 41419201). Urinary excretion accounted for 62-66% in males and 54-62% of radiolabeled carbendazim in males and females, respectively. Less than 1% of the total administered dose was detected as residues in the liver and carcass, indicating that carbendazim was poorly

distributed. At 1000 mg/kg, the urinary route of excretion became saturated, allowing for only 41% excretion. The secondary route of excretion was through the feces. The half-life of carbendazim was approximately 12 hours, and 98% of carbendazim was excreted by 72 hours post- administration. The primary reactions involved in the metabolism of carbendazim were oxidation of the phenyl ring, followed by conjugation to yield sulfate and glucoronide conjugates of 5-hydroxycarbendazim and 5,6-dihydroxycarbendazim. Subsequent phenyl ring oxidation and N-oxidation at the imidazole nitrogen led to significant levels of 5,6-hydroxy-oxo-carbendazim N-oxide glucuronide conjugate, especially in female rats. This study is acceptable and satisfies the guideline requirement for a metabolism study (85-1).

i. Dermal Absorption

No dermal absorption studies were located for MBC.

IV. OTHER METABOLITES

The primary metabolites of MBC are 5-hydroxy-2-benzimidazolecarbamic acid, methyl ester (5-HBC) and 2-aminobenzimidazole (2-AB). The acute toxicity of 5-HBC and 2-AB could not be compared to MBC since they were not tested at levels higher than 3400 and 7500 mg/kg, respectively. MBC did not cause death following single oral doses of 5000 mg/kg. Deaths (6/6) occurred with 2-AB following 10 doses at 670 mg/kg/day (2/6 occurred with MBC at 3400 mg/kg/day). 5-HBC was not tested higher than 200 mg/kg/day for 10 doses over 2 weeks. Testicular degeneration was observed with 5-HBC at 3400 mg/kg but not with 2-AB up to 7500 mg/kg.

Table 15. Toxicity Data for 5-HBC

GDLN	STUDY	% a.i.	RESULTS (mg/kg)	Classification
870.1100 (81-1)	Acute Oral-rat	99	LD50 ≥3400 NOAEL = 1500 LOAEL = 3400 (testicular changes)	unacceptable
870.1100 (81-1)	Acute Oral-rat	99	LD50 ≥7500 NOAEL ≥ 7500	unacceptable
	14 Day subacute oral, rat	99	No deaths > 200 NOAEL ≥ 200	unacceptable
870.5100 (84-2)	Gene mutation (Ames)		No evidence for mutagenic activity	unacceptable

Table 16. Toxicity Data for 2-AB				
GDLN	STUDY	% a.i.	RESULTS (mg/kg)	Classification
870.1100 (81-1)	Acute Oral-rat	100	deaths > 3400 (males only) NOAEL = 1500 LOAEL = 3400 (testicular changes)	unacceptable
	14 Day subacute oral, rat	100	deaths ≥ 680 (no testicular changes)	unacceptable

V. REFERENCES

Albertini, S. (1991). Reevaluation of the 9 compounds reported conclusive positive in yeast Saccharomyces cerevisiae aneuploidy test systems by the Gene-Tox Program using strain D61.M of Saccharomyces cerevisiae. Mutat Res 260: 165-180.

Albertini, S, Brunner, M, and Wurgler, F E (1993). Analysis of the six additional chemicals for in vitro assays of the European Economic Communities' EEC Aneuploidy Programme using Saccharomyces cerevisiae D61.M and the in vitro porcine brain tubulin assembly assay. Environ Mol Mutagen 21: 180-192.

Chernoff, N. 1985. The science of teratology in a regulatory setting. Basic Life Science 34:285-301.

Cummings, A.M.., Harris, S.T., and Rehnberg G.L. 1990. Effects of methyl benzimidazolecarbamate during early pregnancy in the rat. Fundamental and Applied Toxicology 15(3): 528-535.

Ellis W.G., Semple, J.L., Hoogenboom E.R., Kavlock R.J., and Zeman, F.J. 1987. Benomyl-induced craniocerebral anomalies in fetuses of adequately nourished and protein-deprived rats. Teratogenesis, Carcinogenesis, and Mutagenesis. 7(4):357-375.

Ellis, WG, DeRoss, F, Kavlock, RJ and Zeman, FJ. (1988). Relationship of Periventricular Overgrowth to Hydrocephalus in Brains of Fetal Rats Exposed to Benomyl. T*eratogenesis, Carcinogenesis and Mutagenesis* 8(6):377-392.

Georgieva, V, Vachkova, R, Tzoneva, M, and Kappas, A (1990). Genotoxic activity of benomyl in different test systems. Environ Mol Mutagen 16: 32-36

Gibson, D P, Aardema, M J, Kerckaert, G A, Carr, G J, Brauninger, R M, LeBoeuf, R A (1995). Detection of aneuploidy-inducing carcinogens in the Syrian hamster embryo (SHE) cell transformation assay. Mutat Res 343: 7-24.

Hess N, Ellis, W., Kavlock, R.J., and Zeman F. 1987. Fetal brain development in the malnourished benomyl-exposed rat. Federal Proceedings 46(3):956.

Hess, R.A., Moore, B.J., Forrer, J., Linder, R.E., Abuel-Atta, A.A. 1991 "The Fungicide Benomyl (Methyl 1-(Butylcarbamoyl)-2-benzimidazolecarbamate) Causes Testicular Dysfunction by Inducing the Sloughing of Germ Cells and Occlusion of Efferent Ductules." Fundamentals and Applied Toxicology: 17, 733-745.

Hoogenboom, E.R., Ransdell, J.F., Ellis, W.G., Taubeneck, M.W., Kavlock, R.J., and Zeman, F.J. 1988. Effects of benomyl exposure and protein deficiency on the fetal rat eye. Teratology 37(5):465.

Hoogenboom, ER, Ransdell, JF, Ellis, WG, Kavlock, RJ and Zeman, FJ. (1991). Effects on the Fetal Rat Eye of Maternal Benomyl Exposure and Protein Malnutrition. *Current Eye Research* 10(7):601-612.

Kavlock, R.J., Chernoff N., Gray Jr. LE, Gray JA, and Whitehouse D. 1982. Teratogenic effects of benomyl in the Wistar rat and CD-1 mouse, with emphasis on the route of administration. Toxicology and Applied Pharmacology, 62:44-54

Lu S.Y., Lin, H.W., and Wang, S.C. 1994. Studies on teratogenicity of benomyl in rats. Journal of Chinese Society of Veterinary Science. 20(4):248-356.

Mailhes, J B and Aardema, M J (1992). Benomyl-induced aneuploidy in mouse oocytes. Mutagenesis 7: 303-309.

Nakai, M.,. Hess, R.A., Moore, B.J., Guttroff, R.F., Strader, L.F., and, Linder, R.E. 1992. "Acute and Long-term Effects of a Single Dose of the Fungicide Carbendazim (Methyl 2-Benzimidazole Carbamate) on the Male Reproductive System in the Rat." Journal of Andrology. 13(6):507-517.

WHO 1993.

Zeman, FJ, Hoogenboom, ER, Kavlock, RJ and Semple, JL. (1986). Effects of the Fetus of Maternal Benomyl in the Protein-Deprived Rat. *J. Toxicol. Environ. Health* 17(4):405-417.

Zelesco, P A, Barbieri, I, Marshall Graves, J A (1990). Use of a cell hybrid test system to demonstrate that benomyl induces aneuploidy and polyploidy. Mutat Res 242: 329-335.

MEMORANDUM November 18, 1999

SUBJECT: REVISED Benomyl/MBC Quantitative Risk Assessment (Q_1^*) Based On CD-1 Mouse Dietary Study Using mg/kg b.w.^$^3/_4$'s/day Cross Species Scaling Factor

P.C. Code 099101

TO: Deborah Smegal, Toxicologist
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FROM: Lori L. Brunsman, Statistician
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THROUGH: William L. Burnam, Branch Chief
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The upper bound estimate of unit risk, Q_1^*(mg/kg/day)$^{-1}$, of Benomyl/MBC based upon female mouse liver adenoma and/or carcinoma combined tumor rates is 2.39 x 10^{-3} in human equivalents. The dose levels used from the 105-week dietary study were 0, 500, 1500, and 7500 ppm of MBC. The corresponding tumor rates were 1/74, 9/70, 20/75, and 15/75, respectively.

Background

On January 25, 1989, the Cancer Peer Review Committee classified Benomyl/MBC as a Group C - possible human carcinogen, and recommended that, for the purpose of risk characterization, a low dose extrapolation model be applied to the experimental animal tumor data for quantification of human risk (Q_1^*). A Q1* was generated using mg/kg b.w.^$^2/_3$'s/day cross species scaling factor (MBC(INE-965) - Qualitative and Quantitative Risk Assessment, CD-1 Mouse Study, B. Fisher, 5/10/89). This revised memo has been generated to reflect the Agency policy change from use of the $^2/_3$'s to the $^3/_4$'s scaling factor

in 1994[1].

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All unit risks have been converted from animals to humans by use of the $^3/_4$'s scaling factor (Tox_Risk program, Version 3.5, K. Crump, 1994)[1]. For the conversion to human equivalents, weights of 0.03 kg for the mouse and 70 kg for humans were used.

It is to be noted that the Q_1^* $(mg/kg/day)^{-1}$ is an estimate of the upper bound on risk and that, as stated in the EPA Risk Assessment Guidelines, "the true value of the risk is unknown, and may be as low as zero."

Dose-Response Analysis

The statistical evaluation of mortality (MBC(INE-965) - Qualitative and Quantitative Risk Assessment, CD-1 Mouse Study, B. Fisher, 5/10/89) indicated no significant incremental changes with increasing doses of MBC in female mice. The unit risk, Q_1^*, was obtained by the application of the Multi-Stage model (Tox_Risk program, Version 3.5, K. Crump, 1994).

Female mice had a significant increasing trend at $p < 0.05$, and significant differences in the pair-wise comparisons of all dosed groups (500, 1500 and 7500 ppm) with the controls at $p < 0.01$, for liver adenoma and/or carcinoma tumors combined.

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[1]See memo - Deriving Q_1^*s Using the Unified Interspecies Scaling Factor, P.A. Fenner-Crisp, Director, HED, 7/1/94.

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

MEMORANDUM November 18, 1999

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: REVISED Benomyl/MBC Quantitative Risk Assessment (Q_1^*) Based On CD-1 Mouse Dietary Study Using mg/kg b.w.^$^3/_4$'s/day Cross Species Scaling Factor

P.C. Code 099101

TO: Deborah Smegal, Toxicologist
Reregistration Branch 3
Health Effects Division (7509C)

FROM: Lori L. Brunsman, Statistician
Science Analysis Branch
Health Effects Division (7509C)

THROUGH: William L. Burnam, Branch Chief
Science Analysis Branch
Health Effects Division (7509C)

The upper bound estimate of unit risk, $Q_1^*(mg/kg/day)^{-1}$, of Benomyl/MBC based upon female mouse liver adenoma and/or carcinoma combined tumor rates is 2.39×10^{-3} in human equivalents. The dose levels used from the 105-week dietary study were 0, 500, 1500, and 7500 ppm of MBC. The corresponding tumor rates were 1/74, 9/70, 20/75, and 15/75, respectively.

Background

On January 25, 1989, the Cancer Peer Review Committee classified Benomyl/MBC as a Group C - possible human carcinogen, and recommended that, for the purpose of risk characterization, a low dose extrapolation model be applied to the experimental animal tumor data for quantification of human risk (Q_1^*). A Q1* was generated using mg/kg b.w.^$^2/_3$'s/day cross species scaling factor (MBC(INE-965) - Qualitative and Quantitative Risk Assessment, CD-1 Mouse Study, B. Fisher, 5/10/89). This revised memo has been generated to reflect the Agency policy change from use of the $^2/_3$'s to the $^3/_4$'s scaling factor in 1994[1].

All unit risks have been converted from animals to humans by

[1]See memo - Deriving Q_1^*s Using the Unified Interspecies Scaling Factor, P.A. Fenner-Crisp, Director, HED, 7/1/94.

use of the $^3/_4$'s scaling factor (Tox_Risk program, Version 3.5, K. Crump, 1994)[1]. For the conversion to human equivalents, weights of 0.03 kg for the mouse and 70 kg for humans were used.

It is to be noted that the Q_1^* $(mg/kg/day)^{-1}$ is an estimate of the upper bound on risk and that, as stated in the EPA Risk Assessment Guidelines, "the true value of the risk is unknown, and may be as low as zero."

Dose-Response Analysis

The statistical evaluation of mortality (MBC(INE-965) - Qualitative and Quantitative Risk Assessment, CD-1 Mouse Study, B. Fisher, 5/10/89) indicated no significant incremental changes with increasing doses of MBC in female mice. The unit risk, Q_1^*, was obtained by the application of the Multi-Stage model (Tox_Risk program, Version 3.5, K. Crump, 1994).

Female mice had a significant increasing trend at $p < 0.05$, and significant differences in the pair-wise comparisons of all dosed groups (500, 1500 and 7500 ppm) with the controls at $p < 0.01$, for liver adenoma and/or carcinoma tumors combined.

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

OFFICE OF PESTICIDES AND TOXIC SUBS

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

From: Bernice Fisher, Biostatistician
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Bernice Fisher 5/10/89

To: Marion P. Coplev, D.V.M., Section Head
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Thru: John A. Quest, Ph.D., Section Head
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John A. Quest 5/15/89

Summary

The estimated unit risk, Q_1^* of benomyl is 4.20×10^{-3} (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 ($Q_1^* = 3.9 \times 10^{-3}$ (mg/kg/day)$^{-1}$ in human equivialents, - Benomyl Risk Assessment for $Q_1^* = 3.9 \times 10^{-3}$ for Carcinogenicity Potency, B.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 Benomyl/MBC, January 25,1989 recommended a re-evaluation of the MBC 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 Statox program to obtain statistical outcomes on survival, tumorgenicity and a unit risk analysis.

The 2 year CD-1 mouse study was conducted by Haskell Labs for E.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. MBC, CD-1 Mouse, Experimental Design of the Dietary Study

Dose (ppm)	Number of Males	Number of Females	weeks on Study
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 MBC. There also was a significant ($p<.05$) diference 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.

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

Dose(ppm)	Week 1-26	27-52	53-73[a]	74-104[b]	Total
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 $p<.01$.

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

Dose(ppm)	Week 1-26	27-52	53-78	79-104a	Total
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 $p<.01$.

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, tumorgenicity 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 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, B.Litt, 5/82 - all animals that were examined were included in the denomiator 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).

Table 4. MBC - 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

a 7500 ppm dose reduced to 3750 ppm at week 66.
b first adenoma observed at week 62.
c animals at high dose (7500-3750 ppm) were not evaluated because of early high mortality and subsequent final sacrifice at week 74.
d 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 $p<.01$.

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

	Dose(ppm)			
Liver Tumor	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**	0.000**	0.000**

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

( ) percent

a first adenoma observed at week 90.
b 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 $p<.01$.

Dose-Responce 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:

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

References

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

Cochran, W.G. (1954) Some Methods for Strengthening the Common X^2 Test, Biometrics 10, 417-451.

Cox, D.R., (1972) Regression Models and Life Tables (with discussion) J. Royal Stat. Soc. Ser. B. 34, 187-220.

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 2., 311-426. Lyons, France.

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