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For Counsel file

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

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APR 22 1986

MEMORANDUM:

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Executive summary concerning benomyl for the
Science Advisory Panel

Tox. Chem. No. 75A

TO: S. L. Johnson
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Attached is the "Information Concerning Benomyl for the Science
Advisory Panel". It consists of:

- I. The set of scientific issues considered by the Agency in connection with the Registration Standard of benomyl and
- II. The supporting background documents.

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INFORMATION CONCERNING BENOMYL
FOR THE
SCIENTIFIC ADVISORY PANEL

April 23, 1986

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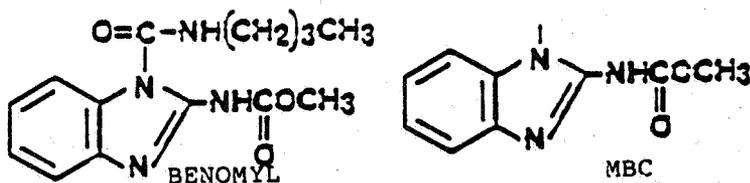
SUMMARY

The Panel should address what weight they feel should be placed on this risk assessment for benomyl (and MBC) as a class C oncogen demonstrating mouse liver tumors. Currently the Agency does risk assessment on class C oncogens on a case by case basis. The Panel is requested to consider what factors and weight-of-the-evidence should go into the determination of whether a risk assessment should be performed on class C oncogens.

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

Introduction

Benomyl, a systemic fungicide and MBC, its primary metabolite are benzimidazole carbamates. Their structures are as follows:



The original Special Review on benomyl was concluded in the 47 Federal Register 46747, October 20, 1982 and supported in the Position Document 4 (PD-4). The Agency determined that the potential oncogenic, mutagenic, teratogenic, and spermatotoxic risks of benomyl were exceeded by the benefits associated with its use provided that dust masks were worn by mixer/applicators for aerial application. The Agency has recently reassessed its regulatory decision on benomyl in the registration standard process.

The relevant oncogenicity data base for benomyl consists of one rat and one chronic mouse (Charles River CD-1 strain) feeding/oncogenicity study. The data base for the metabolite MBC consists of one chronic rat and three chronic mouse (CD-1, SPF Swiss and NMRKf-SPF 71 strains) feeding/oncogenic studies. The rat studies for benomyl and MBC are negative. The mouse study for benomyl and two of the MBC mouse studies (using genetically related CD-1 and Swiss strains) have shown oncogenic effects consisting solely of mouse liver tumors in both males and females. However, MBC has shown no oncogenic effect in the NMRKf mouse (a genetically unrelated strain). The Agency believes that the weight of the evidence does not support doing a quantitative oncogenic risk assessment for benomyl. However, since a quantitative risk assessment was already performed in the benomyl PD-4, the Agency recently completed a revised worst case risk assessment for the Registration Standard (see memorandum from B. Litt for Q1* calculations). This was based on tolerances rather than actual residues resulting in a very conservative estimate of risk.

The Agency asks the Panel to consider: 1) its analysis of the weight-of-the-evidence in classifying benomyl as a Group C oncogen, or possible human oncogen, and 2) what weight the Agency should place on a quantitative risk assessment for benomyl, as a class C oncogen causing only liver tumors in certain strains of mice.

Weight-of-the-Evidence

A. Evaluation of Oncogenicity Evidence for Benomyl and MBC:

1. Mouse Oncogenicity Study of Benomyl:

Haskell Laboratory administered benomyl in the diet to groups of 80 male and 80 female CD-1 mice at concentrations of 0, 500, 1500 or 7500/5000 ppm for 2 years. The high dose of 7500 ppm was reduced to 5000 ppm at 37 weeks in males and females due to weight loss. There were no significant differences in survival rates in either males or females. The following incidence patterns of tumors suggest a compound-related effect.

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

*= p<0.05 compared to controls

Pulmonary carcinomas were significantly elevated in male mice at low and mid but not high doses. The effect did not appear to be compound related for the following reasons: 1) A dose-response effect was not observed in the Cochran-Armitage test for trend. 2) Low tumor incidences in high doses could not be attributed to early death (see attached table with relation of tumor incidence to survival). 3) All tumor incidences were within the range of historical controls (16% to 36%). The mean \pm S.D. for pulmonary tumors for seven studies, not including benomyl, conducted at Haskell Labs for the two years preceding and subsequent to the benomyl study was $24 \pm 17\%$ (total of 564 animals). 4) The tumor incidence in the benomyl control group was equal to the lowest incidence level observed in the historical control group. 5) Pulmonary tumor incidences in the low and mid dose groups are not statistically different from the historical controls and only marginally significant ($p = 0.05$) from the concurrent controls. 6) In addition, nearly all benomyl administered is rapidly

converted to MBC and MBC did not produce an increase in pulmonary tumors in other studies performed in CD-1 and SPF Swiss mice. Therefore, the increases in lung carcinomas in male mice at the low and mid dose groups are not considered to be biologically significant or compound-related.

Hepatocellular carcinomas were significantly elevated in male (low and mid doses) and female (low and high doses) mice. In addition, adenomas and carcinomas combined were significantly elevated in males (low and mid doses) and females (mid and high doses). The tumorigenic responses appeared to be compound-related; e.g., they occurred with significant positive trends, and the elevated incidences exceeded historical rates for these tumor responses in two other studies conducted at the registrant's laboratory (see Copley/Harris memorandum of 12/19/85, page 10 for data). Furthermore, similar liver tumorigenic responses were produced by the MBC metabolite in other studies performed in CD-1 mice (see below). The oncogenic responses that were produced by benomyl in treated mice were not accompanied by significant increased incidences of hepatocellular adenomas or hyperplasia.

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

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

2. Mouse Oncogenicity Study of MBC:

Haskell Laboratory administered MBC in the diet to groups of 80 male and 80 female CD-1 mice at concentrations of 0, 500, 1500, 7500 (females) or 7500/3750 (males) ppm for 2 years. The high dose of 7500 ppm was reduced to

3750 ppm at 66 weeks in males due to increased mortality, and all males were ultimately sacrificed at 73 weeks. The following incidence pattern of liver tumors was observed.

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

* = p < 0.05 compared to controls

= Reduced to 3750 ppm in males at 66 weeks.

Hepatocellular carcinomas were significantly elevated in male mice at the mid dose level; no significant increases in adenomas occurred in males at any dose level. The lack of oncogenic response in high dose males may be attributed to the early deaths (possibly due to hepatotoxicity) and sacrifice at 73 weeks. In female mice, there were significant increases in adenomas (low and mid doses), carcinomas (mid and high doses), and adenomas and carcinomas (all 3 dose levels tested). The Toxicology Branch Peer Review Committee of the Office of Pesticide Programs noted that this profile of liver tumors resembled that described above for benomyl in CD-1 mice. No increased incidence of liver hyperplasia occurred in treated mice. A comparison of the MBC liver tumor data with historical control data from two other studies conducted at Haskell Laboratory (see Copley/Harris memorandum of 12/19/85, page 10) indicated that only the carcinomas (mid and high dose levels) and the adenomas/carcinomas combined (all 3 dose levels tested) in female mice exceeded the control response rates in the other studies.

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

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

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

Limited information is available on a study performed by the Central Institute for Nutrition and Food Research (TNO), and reviewed in summary form by the World Health Organization (WHO) (see Copley/Harris memorandum of 12/19/85, page 7), MBC was administered in the diet to groups of 100 male and 100 female SPF Swiss mice at concentrations of 0, 150, 300 or 1000/5000 ppm for 80 weeks. The 1000 ppm concentration was increased to 5000 ppm in males and females at week 8. Data were presented in summary form only. The following incidence pattern of liver tumors was observed.

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

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

Hepatoblastomas (a less common and more malignant liver tumor than hepatocellular carcinoma) were significantly elevated in male mice (high dose level); neoplastic nodules (i.e., adenomas) were significantly elevated in female mice (high dose level). The Toxicology Branch Peer Review Committee noted that the SPF Swiss strain of mouse used in this study is genetically similar to the CD-1 strain of mouse in which benomyl and MBC were tested. The CD-1 strain is an outbred strain of the SPF Swiss mouse. Both strains tend to exhibit a high background incidence of liver adenomas in male mice.

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

4. Mouse Oncogenicity Study of Carbendazim (99.3% MBC):

In another study reviewed by the WHO (see Copley/Harris memorandum of 12/19/85, page 8 and Copley/Harris review), MBC was administered in the diet to groups of 100 male and 100 female HOE NMRKf (SPF 71) mice at concentrations of 0, 50, 150, 300 or 1000/5000 ppm for 22 months. The 1000 ppm concentration was increased to 5000 ppm at week

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1000 ppm concentration was increased to 5000 ppm at week eight. No evidence of an oncogenic response in the liver or at any other site was observed. The Toxicology Branch Peer Review Committee noted that the NMRKf strain of mouse, in contrast to CD-1 and SPF Swiss mice, normally exhibits a low background incidence of liver tumors.

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

5. Rat Oncogenicity Studies of Benomyl and MBC:

Benomyl was studied in a 2-year dietary study (0, 100, 500 or 2500 ppm) in Chr CD rats; the highest concentration was a systemic NOEL and no oncogenic effects occurred. A MTD was not established for benomyl.

MBC was also studied in a 2 year dietary study (0, 100, 500, 2500/10,000, 5000 ppm) in Chr CD rats; no oncogenic effects occurred. The 2500 ppm dose was increased to 10,000 ppm (HDT) during week 20 of the study. The MTD was established at the highest dose demonstrated by weight loss in males and females (10%-20% less than controls) and hepatic pericholangitis. Both of the above studies were performed by Haskell Laboratory.

B. Additional Toxicology Data on Benomyl and MBC:

1. Metabolism:

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

2. Teratology:

Benomyl has been demonstrated to be teratogenic in several oral (gavage) studies conducted in both Wistar and Chr CD rats at a dose of 62.5 mg/kg/day and higher. The most common abnormality in these studies was microphthalmia. In most of these studies, fetotoxic and embryotoxic effects were also observed at similar or greater dose levels. Benomyl was also reported to be teratogenic in one study in CD-1 mice at oral (gavage) doses of 100 mg/kg or more. The major anomalies noted

were cleft palate, supernumerary ribs, and subnormal vertebral centrum (no compound-related microphthalmia was reported).

3. Mutagenicity:

Data provided in the Position Document 4 on benomyl and MBC indicated that both compounds are spindle poisons often associated with aneuploidy and nondisjunction. For example, nondisjunction was reported in *A. nidulans* with both agents. The compounds also produced positive effects in tests to assess structural chromosome aberrations which were consistent with a spindle effect; e.g., benomyl was weakly positive for sister chromatid exchange in vitro in Chinese hamster ovary cells with and without activation, and both benomyl and MBC caused increased incidences of micronuclei in polychromatic erythrocytes in mice bone marrow. In other studies performed to assess gene mutations, equivocal results were obtained. That is, MBC was weakly positive in one mouse lymphoma test (L5178Y TK⁺/-) but was negative in a second test, benomyl and MBC produced both positive and negative results in different Ames tests, and both compounds produced negative results in Chinese hamster ovary cells (HGPRT). Finally, negative results were obtained for DNA repair with benomyl and MBC in several studies in primary mouse and rat hepatocyte cultures. The Toxicology Branch Peer Review Committee concluded that these results, when taken together, indicated that both benomyl and MBC have weak mutagenic activity that is primarily attributable to adverse effects on the cellular spindle apparatus. Inhibition of the cellular spindle apparatus may explain the observed teratogenic and/or spermatotoxic effects of benomyl, and other benzimidazole compounds such as MBC, cambendazole and parbendazole. Correlation, or the lack thereof, with oncogenicity has not been demonstrated conclusively.

4. Structure-Activity Correlations:

Both benomyl and MBC bear a close structural resemblance to several other benzimidazole compounds that are suspect oncogens (e.g., fenbendazole and albendazole). The potential oncogenic effects of these compounds are currently under review by the Center for Veterinary Medicine, Food and Drug Administration and were recently discussed in a Congressional Subcommittee Hearing (reference: Human Food Safety and the Regulation of Animal Drugs; 27th Report by the Committee on Government Operations, December 31, 1985. Union Calendar, No. 274. Intergovernmental Relations and Human Resources Subcommittee. Ted Weiss, New York, Chairman; pp. 1-115). In the case of albendazole, histiocytic sarcomas were observed in rats and uterine polyps were observed in rats and mice. Fenbendazole may be associated with rat liver tumor. The Toxicology Branch Peer Review

Committee was aware that FDA has deferred making final decisions regarding the classification of these chemicals as oncogens until the slides from these studies have been reread.

C. Classification of Oncogenic Potential:

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

Despite these considerations, the Toxicology Branch Peer Review Committee decreased the classification to Category C (limited evidence of carcinogenicity), for the following reasons: 1) Neither benomyl nor MBC produced tumors in the rat. 2) The oncogenic responses observed with benomyl and MBC were confined solely to the mouse liver in two genetically related strains of mice (CD-1 and SPF Swiss). 3) No liver tumors were produced by MBC in a genetically unrelated strain of mouse [HOE NMRkf (SPF 71)]. 4) The genetic toxicity of benomyl and MBC is minimal, that is, they produced weak mutagenic effects consistent with spindle poison activity rather than gene mutation or DNA repair activity. Because of these factors, the Toxicology Branch Peer Review Committee determined that there was insufficient evidence for the B₂ category and therefore, in conformity with the EPA Guidelines noted above, classified both Benomyl and its primary metabolite, MBC, as Category C (possible human) carcinogens.

Conclusions

The Agency has reviewed oncogenicity studies for benomyl and its metabolite MBC, and concluded that these data provide limited evidence of oncogenicity for these chemicals in male and female mice. According to EPA Proposed Guidelines for Carcinogen Risk Assessment (November 23, 1984, 49 FR 46294), benomyl has been classified as a Group C oncogen, that is, a possible human oncogen.

The Toxicology Branch Peer Review Committee chose to classify benomyl and MBC in Group C (limited evidence of carcinogenicity) for the following reasons:

- a) The oncogenic responses observed with benomyl and its metabolite were confined solely to the mouse liver.
- b) Neither benomyl nor MBC were oncogenic in Chr-CD rats.
- c) The liver tumors produced by benomyl and MBC were observed in two genetically related strains of mice (CD-1 and SPF Swiss), whereas no liver tumors were produced by MBC in a genetically unrelated strain of mouse [HOE NMRKf (SPF-71)].
- d) Benomyl and MBC produced weak mutagenic effects consistent with spindle poison activity rather than gene mutation or DNA repair activity. The Toxicology Branch Peer Review Committee noted that this pattern of mutagenic activity correlates well with teratogenic and spermatotoxic effects. Correlation, or lack thereof, with oncogenicity has not been demonstrated.

Because of these factors it was determined that there was insufficient evidence for the B₂ category and therefore, in conformity with the EPA Guidelines noted above, classified both Benomyl and its primary metabolite, MBC, as Category C (possible human) oncogens.

Oncogenic Risk Assessment

The 95 % upper confidence level potency estimator, Q_1^* , for oncogenicity is 3.9×10^{-3} (mg/kg/day)⁻¹ (see attached memorandum from B. Litt to Marion Copley dated March 11, 1986). This estimate is based on liver tumors observed in female CD-1 mice from a 2 year MBC feeding study (HLR# 70-82) (see memorandum by M. Copley dated Dec. 19, 1985, page 27).

- A. Dietary Risk: The exposure, based on tolerances (a worst case estimate) is 0.0337 mg/kg/day. It is reduced to 0.0074 mg/kg/day when corrected for percent of crop treated. The resultant risks are 10^{-4} and 10^{-5} for uncorrected and corrected dietary exposures, respectively (see the Registr. Standard for Benomyl). When actual residue data are available the risks may be several order of magnitudes lower.
- B. Applicator Risk: Inhalation exposure is the primary route of applicator exposure. The worst case job related exposure, 0.35 mg/kg/day, occurs with mixer/loaders using aerial applications for grapes/fruit crops. Dust masks reduce this by approximately 90 %. The resultant risks for this worst case exposure are 10^{-4} - 10^{-5} and 10^{-5} with and without dust masks, respectively. Dermal exposure to benomyl is considered minimal when compared to inhalation exposure, due to poor dermal absorption.

Summary

Based on the weight-of-the-evidence assessment with emphasis on the occurrence of liver tumors limited to two genetically related mouse strains, but not in a genetically unrelated mouse strain, or in rats, the Agency has classified benomyl and MBC as class C (possible human) oncogens. The Agency specifically requests any comments that the Panel may wish to present with regard to our assessment of the weight-of-the-evidence and subsequent determination of oncogenicity according to the Agency's Cancer Guidelines.

Since a quantitative oncogenic risk assessment for benomyl was presented in the PD-4, the Agency has presented an updated risk assessment in the Registration Standard. The Panel should address what weight they feel should be placed on this risk assessment for benomyl (and MBC) as a class C oncogen demonstrating mouse liver tumors. Currently the Agency does risk assessment on class C oncogens on a case by case basis. The Panel is requested to consider what factors and weight-of-the-evidence should go into the determination of whether a risk assessment should be performed on class C oncogens.