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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: July 21, 2023

SUBJECT: **Carbendazim (MBC):** Report of the Cancer Assessment Review Committee

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Health Effects Division (7509T)

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On June 7 and 15, 2023, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) reevaluated the carcinogenic potential of carbendazim (MBC) in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). Attached please find the Cancer Assessment Document.

The conclusions conveyed in this assessment were developed in full compliance with *EPA Scientific Integrity Policy for Transparent and Objective Science*, and EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions.* The full text of *EPA Scientific Integrity Policy for Transparent and Objective Science*, as updated and approved by the Scientific Integrity Committee and EPA Science Advisor can be found here: https://www.epa.gov/sites/default/files/2014-02/documents/scientific_integrity_policy_2012.pdf. The full text of the EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions* can be found here: https://www.epa.gov/scientific-integrity/approaches-expressing-and-resolving-differing-scientific-opinions.

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF ***CARBENDAZIM (MBC)***

Date of the Report: July 21, 2023

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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EXECUTIVE SUMMARY

On June 7 and 15, 2023, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) in the Office of Pesticide Programs (OPP) met to reevaluate the carcinogenic potential of carbendazim (MBC). MBC, a benzimidazole fungicide, was classified under EPA's 1986 *Guidelines for Carcinogen Risk Assessment* (September, 1986) as "Group C-possible human carcinogen" (TXRs 0005576 and 0050625, J. Quest, 03/31/1985 and 04/07/1989, respectively). The classification was based on increased incidences of hepatocellular tumors in male and female CD-1 mice and hepatoblastoma tumors in male SPF Swiss mice, with evidence for genotoxicity (aneugenicity). The current Q_1* of 2.39 x 10^{-3} for MBC is based on combined hepatocellular adenomas and carcinomas in female CD-1 mice (TXR 0013859, L. Brunsman, 11/18/1999).

The Registrant (Troy Corporation) submitted a Pesticide Registration Improvement Act (PRIA) action for a cancer reassessment of MBC under the Agency's updated 2005 *Guidelines for Carcinogen Risk Assessment* (March, 2005). The submission included a proposed tumorigenic mode of action (MOA) for liver tumors in mice. The proposed MOA involves the non-linear (i.e., threshold) induction of aneugenicity and hepatotoxicity resulting in hepatocellular tumors (MRID 51768701). In support of this MOA, the proposal discussed reviews of the genotoxicity and carcinogenicity of MBC from other regulatory and health organizations and a published comprehensive review of genotoxicity and carcinogenicity data on MBC and benomyl (McCarroll *et* al., 2002). Prior to cancellation of its registration in 2002, the carcinogenicity of benomyl, also a benzimidazole compound, had been reviewed together with MBC because it rapidly converts to MBC *in vivo.* The latter review, prepared by EPA scientists with expertise in genotoxicity and chemical carcinogenesis, included an evaluation of aneugenicity as a potential MOA for liver tumor induction by MBC (and benomyl) using the International Programme for Chemical Safety (IPCS) Mode of Action Framework Analysis.

Mice

The CARC re-reviewed the carcinogenicity study in CD-1 mice (Wood *et al.*, 1982; MRID 00154676) under the current cancer guidelines. MBC (99.3% a.i.) was administered in the diet to 80 mice/sex/dose at dose levels of 0, 500, 1500, or 7500 ppm for up to 104 weeks (equivalent to 75, 225 or 563/1125 (males/females) mg/kg/day). Due to increased mortality between weeks 52-64, the high dose males were removed from treatment for one week (65 to 66), followed by resumption of treatment at 3750 ppm at week 66, until early termination during week 73 due to continued increased mortality and morbidity (not associated with liver tumors). The remaining dose groups showed no effects of treatment on survival.

Liver Tumors: The hepatocellular tumor incidence data in both the original study pathology report and a subsequent pathology reevaluation of liver tumors (Frame and VanPelt, 1990; MRID 41607904) were considered by the CARC. The reevaluation was conducted prior to the publication of the current standards for a Pathology Working Group review (Pesticide Regulation Notice 94-5, August 24, 1994). The data from the pathology reevaluation were not previously evaluated by the full committee.

In males, the high dose group was excluded from the tumor statistical analyses due to excessive toxicity (non-tumor related mortality) at this dose resulting in the early sacrifice of the remaining animals in this group during week 73. Statistically significant increases by pair-wise comparison to controls were observed for combined hepatocellular adenomas and carcinomas ($p<0.01$) and a significant positive trend ($p<0.01$) was observed at the low and mid dose groups in the original pathology report. Carcinomas were statistically significantly increased by pair-wise comparison to controls at the mid dose with a statistically significant positive trend ($p<0.01$). In the pathology reevaluation, statistical significance of pair-wise comparison ($p<0.01$) of tumor incidence (adenoma, carcinoma and combined adenomas and carcinomas) in males was achieved only at the mid dose though a statistically significant positive trend was observed ($p<0.01$) for adenomas and combined tumor incidence. The incidence of combined tumors in all dose groups (17-32%, original report and 15-31%, reevaluation report) was similar or less than the incidence observed in control males (32%) from the concurrently conducted study on benomyl, but similar or higher than the incidence observed in control males from a second historical control data set (16%). Non-neoplastic lesions of hepatocellular hypertrophy and necrosis were increased in males at mid and high dose.

In females, there was little change in combined hepatocellular tumor incidence or the pattern of statistical significance between the original and reevaluated reports. A statistically significant increase by pair-wise comparison to controls in combined hepatocellular adenomas and carcinomas was observed at all dose levels (all $p<0.01$ except low dose in the reevaluation, $p<0.05$), with a statistically significant positive trend ($p<0.05$ or 0.01, original and reevaluation, respectively). In the pathology reevaluation, the incidence of adenomas was statistically significantly increased by pair-wise comparison to controls (all dose groups, $p<0.01$) compared to the original report ($p<0.05$, low and mid dose) due to reclassification of carcinomas to adenomas. The incidence of carcinomas in the original evaluation was statistically significantly increased by pair-wise comparison to controls at mid and high dose ($p<0.01$) with a statistically significant trend ($p<0.05$) but in the reevaluation was only increased at mid dose with a significant positive trend (both $p<0.01$). The incidence of combined tumors in all dose groups (13-27%, original report and 13-28%, reevaluation report) was greater than the available historical control incidence observed in control females from the study on benomyl (5%) and from a second historical control data set (8%). Pre-neoplastic lesions of eosinophilic foci (mid and high dose) and basophilic foci (high dose) were increased in females.

Adequacy of Dosing: Dosing in the high dose males was considered excessive based on increased moribundity during the second year of treatment that resulted in early termination of this dose group during Week 73. The CARC considered the **dosing to be adequate in the low and mid dose males and in females at all dose levels** based on hepatocellular necrosis and hypertrophy in males at the mid dose and eosinophilic foci in females at the mid and high dose. The incidence of hepatocellular basophilic foci was also increased in females at the high dose

The CARC concluded that the combined hepatocellular tumors (adenomas + carcinomas) in male and female CD-1 mice were treatment-related at all dose levels tested (≥500 ppm; ≥75 mg/kg/day). Although the pathology reevaluation in males no longer showed statistical significance at the low dose by pair-wise comparison to controls for combined adenomas and carcinomas, the increases in combined tumors at both the low and mid doses were considered

treatment-related in males. This was based on the limited available historical control data, lack of interim sacrifice or subchronic data, statistically significant trends in both the original and reevaluated pathology reports and the statistically significant pair-wise increases compared to controls at both low and mid dose, observed in the original report. The lack of tumor incidence data at a third dose level (due to early sacrifice of the high dose males) to better evaluate the tumor dose-response, was also taken into consideration. The CARC also noted that CD-1 mice may be susceptible to liver tumor induction. Non-neoplastic toxicity included an increased incidence in hepatocellular hypertrophy and necrosis in males at mid and high dose. In females, the incidence of combined adenomas and carcinomas was statistically significantly increased by pair-wise comparison to controls in both evaluations at all dose levels along with a statistically significant positive trend. Pre-neoplastic lesions in females included increases in eosinophilic foci (mid and high dose) and basophilic foci (high dose).

Previous cancer assessments of MBC (TXRs 0005576 and 0050625, J. Quest, 03/31/1985 and 04/07/1989, respectively) included two additional mouse studies. A study in SPF Swiss mice (Beams *et al.*, 1976; MRID 00153420) identified an increased incidence of hepatoblastoma in males at the highest dose tested of 750 mg/kg/day. A second study in NMRKf mice (Donaubauer *et al.,* 1982; MRID 00154679) did not identify treatment-related tumors at doses up to 522/648 mg/kg/day (males/females). However, both studies were classified as unacceptable due to significant deficiencies in study conduct and reporting, such as uncertainties regarding dietary dose concentrations, lack of individual animal data, and/or an incomplete histopathological evaluation. **The CARC concluded that the data from the two additional mouse studies in SPF Swiss and NMRKf mice were not sufficiently reliable and should not be considered in the weight-of-evidence (WOE) determination, based on their study deficiencies and unacceptable classification.**

Rats

Two rat chronic toxicity/carcinogenicity studies were considered in the previous cancer assessments of MBC and in this reevaluation. In a two-year rat chronic toxicity/carcinogenicity study (MRID 00088333) MBC (INE-195, 53-72% a.i.) was administered to 36 CRL:CD1 rats/sex/dose for two years in the diet at concentrations of 0 (two control groups of 36/sex), 100, 500, 5000, or 2500/7500/10,000 ppm (time-weighted average 8557 ppm). The compound intakes were 0, 5, 25, 250 or 125/500 (time-weighted average 430) mg/kg/day.

Tumors: No treatment-related tumors were observed in this study.

Adequacy of Dosing: **Dosing at the high dose was considered adequate and not excessive in males and females**, based on decreased body weight and slightly increased severity of cholangiohepatitis and pericholangitis (both sexes) and decreases in red blood cell parameters (females).

A second rat chronic toxicity/carcinogenicity study was reviewed by the World Health Organization (WHO, 1993) and Australia Pesticides and Veterinary Medicines Authority (APVMA, 2009) but was not available to the Agency for review. Only summaries of the study were available for consideration. Sixty Wistar rats/sex/dose were administered MBC (99% a.i.)

for two years at dietary concentrations of 0, 150, 300, or 2000 ppm (week 1)/5000 ppm (week 2)/10,000 ppm (week 3 through study termination) (0, 7.5, 15 or 100/250/500 mg/kg/day; 500 mg/kg/day was administered except during the initial two weeks of the study).

Tumors: No treatment-related tumors were observed in this study.

Adequacy of Dosing: The CARC concluded that adequacy of dosing could not be definitively established due to the limited data presented in the study summary. However, dose levels tested were comparable to the CRL:CD1 rat study.

The CARC concluded that there were no treatment-related tumors after MBC exposure in the rat. Although details of the second study in Wistar rats were unavailable, the CARC considered the study to be supportive of the conclusions of the study in the CRL:CD1 rat.

Mutagenicity

The CARC concluded that the available genotoxicity data supports that MBC induces aneugenicity, a threshold genotoxic effect resulting from its disruption of mitotic spindle formation during cell division. Aneugenicity, an indirect (non-DNA targeted) genotoxic effect, requires exposures sufficient to disrupt normal assembly of mitotic spindle components (tubulin, centrosomes, associated proteins) during mitosis, leading to inaccurate chromosomal segregation. The database indicates that the potential for direct damage to DNA (i.e., mutagenicity) by MBC is low. Similar conclusions were made in other scientific reviews of MBC (WHO, 1993; ECHA, 2019; JMPR, 2005). The review by McCarroll *et al.* (2002) also concluded that MBC displays positive results for aneuploidy but is largely negative for gene mutations.

Structure Activity Relationship

The CARC concluded that other compounds in the benzimidazole fungicide chemical class (thiophanate-methyl [TM], thiabendazole, benomyl) demonstrate aneugenicity and cause liver toxicity. TM and benomyl induced liver tumors in CD-1 mice. TM and thiabendazole were classified as "*likely to be carcinogenic to humans."* Cancer risk was quantified for TM; the reference dose (RfD) approach was used for thiabendazole. Prior to cancellation, benomyl cancer risk was based on MBC.

Mode of Action

The Registrant submitted a proposal for aneugenicity, in concert with hepatotoxicity, as the carcinogenic MOA of MBC for liver tumors in the mouse (MRID 51768701). Under the proposed MOA, a threshold (i.e., non-linear) dose level of MBC is required to disrupt assembly of mitotic spindle apparatus components during cell division, leading to aneuploidy and, together with hepatotoxicity, eventually to induction of mouse liver tumors. Since cells have an excess of microtubules and other mitotic spindle components, MBC can reduce the number of functioning spindles without causing perceptible effects until a threshold dose is reached: therefore the dose-response would not be linear. The Registrant based their proposal on data submitted to the

Agency and conclusions regarding aneugenicity and carcinogenicity of MBC from the scientific reviews described above, including the comprehensive review of open literature and Office of Pesticide Programs data on MBC and benomyl (McCarroll *et al.*, 2002).

The IPCS MOA framework analysis of McCarroll *et al.* (2002) was used as the basis for this evaluation of the proposed cancer MOA. No new mechanistic or cancer studies were provided by the Registrant in support of the current proposal. A search of the open literature was also conducted to identify additional studies that could inform the MOA analysis. Relevant studies meeting the Agency's criteria for full review were selected for detailed review and the findings are discussed in the MOA analysis.

The CARC concluded that the molecular initiating event/ Key Event #1 (interaction of MBC with components of the mitotic spindle, resulting in inhibition and/or disruption of spindle formation), Key Event #2 (inaccurate chromosomal segregation during cell division, chromosomal non-disjunction and mal-segregation), and Key Event #3 (lagging chromosomes, micronucleus formation) were adequately supported by the available data. Key Event #4 (hepatotoxicity) was determined by the CARC to be partially supported. Although liver tumors were observed in both males and female mice, the liver histopathology supported a more robust liver toxicity response in males (necrosis, hypertrophy) than in females (low incidence of eosinophilic and basophilic foci) in the two-year CD-1 mouse study. There was also a lack of adequate data in shorter-term studies in CD-1 mice to evaluate liver toxicity at earlier time points and progression of hepatotoxicity over time.

The CARC concluded that the dose-response concordance of the Key Events and tumorigenicity was not fully supported for MBC due to the lack of data supporting Key Event effects at the tumorigenic dose level of 75 mg/kg/day. The CARC concluded that temporal concordance of the key events was supported. The CARC concluded that, although the proposed MOA was plausible and relevant to humans, the available data were insufficient to eliminate conclusively other potential non-genotoxic MOAs (for example, oxidative stress and damage, signal transduction alterations) that could lead to liver tumor formation. While the data were not considered sufficient under the IPCS MOA framework analysis to support the proposed MOA for induction of liver tumors in the mouse, the data were considered adequate to support aspects of the MOA; in particular, a threshold dose level of MBC being required to cause aneugenicity. These conclusions are consistent with those reported by McCarroll *et al.* (2002).

The CARC concluded that the proposed carcinogenic MOA for hepatocellular tumors in male and female CD-1 mice was not fully supported by the available data under the IPCS MOA framework. Although aneugenicity was considered a plausible MOA, the CARC did not consider the data adequate to fully support the registrant's proposal under the IPCS MOA framework, due to lack of adequate evidence of dose concordance of the Key Events at the lowest tumorigenic dose, absence of causative evidence of aneuploidy for liver tumor formation in mice, and lack of sufficient data to exclude alternative non-genotoxic MOAs. However, the CARC determined that aspects of the MOA, namely aneugenicity and a threshold dose level of MBC required to cause aneugenicity, and the low potential for mutagenicity (direct DNA interaction), were supported by the data, are relevant to humans, and should be included in the weight-of-evidence considerations.

Cancer Classification

In accordance with EPA's *Final Guidelines for Carcinogen Risk Assessment* (March, 2005), the CARC classified MBC as **"Suggestive Evidence of Carcinogenic Potential."** This classification is based on the following weight-of-the-evidence considerations:

(1) A treatment-related increased incidence of combined hepatocellular adenomas and carcinomas was observed in male and female CD-1 mice;
(2) The CD-1 mouse shows increased susceptibility to liver tumors, indicating it may be a particularly sensitive strain;
(3) Tumors were late-onset (in males, Week 66, adenoma and Week 88, carcinoma; in females, Week 90, adenoma and Week 77, carcinoma), were not associated with increased mortality, and the available non-neoplastic liver histopathology did not demonstrate robust evidence of pre-neoplastic lesions;
(4) No treatment-related tumors were identified in CRL:CD1 or Wistar rats;
(5) Genotoxicity studies demonstrated that MBC shows low potential for direct DNA damage (mutagenicity or clastogenicity) but does demonstrate aneugenicity and the requirement for a threshold dose to induce this effect. Disruption of spindle assembly by MBC results in chromosomal mal-segregation and abnormal chromosomal distribution (micronucleus formation, aneuploidy). The MOA is considered relevant to humans since aneuploidy is often associated with tumors in humans;
(6) The proposed cancer MOA of aneugenicity for induction of mouse liver tumors, while considered plausible and relevant to humans, was not fully supported by the available data under the IPCS framework analysis due to lack of data adequately demonstrating dose-concordance, causality of aneugenicity to induction of mouse liver tumors, and lack of data excluding alternative non-genotoxic MOAs.
(7) However, aspects of the MOA are supported. Data supporting MOA Key Events #s 1-3 (aneugenicity), and the genotoxicity database for MBC as a whole, provide robust support for induction of aneuploidy (a threshold effect) and lack of direct DNA damage by MBC.

Quantification of Human Cancer Risk

Based on this classification and the threshold effect of MBC- induced aneugenicity, the quantification of cancer risk using a Q1* approach is not required. A non-linear approach (i.e., RfD) would adequately account for all chronic toxicity, including carcinogenicity, which could potentially result from exposure to MBC. The RfD is also protective for potential aneugenicity resulting from MBC exposure.

I. INTRODUCTION AND OVERVIEW

Purpose of Meeting: On June 7 and 15, 2023, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED), Office of Pesticide Programs (OPP), met to reevaluate the cancer classification of the fungicide carbendazim (MBC). Currently, MBC is classified as "Group C- possible human carcinogen" under the Agency's 1986 *Guidelines for Carcinogen Risk Assessment* (September 24, 1986), based on an increase in liver tumors in two strains of mice in both sexes and evidence of aneugenicity, with a Q_1* of 2.39×10^{-3} $(mg/kg/day)^{-1}$.

Registrant's Reclassification Proposal (MRID 51768701): The Registrant (Troy Chemical Corporation) submitted a PRIA action for (1) CARC reevaluation of MBC under the Agency's *Guidelines for Carcinogen Risk Assessment* (March 2005) and (2) review a proposed a cancer mode of action (MOA) of aneugenicity, generally considered to be a threshold genotoxic effect, for induction of liver tumors in the mouse. Aneuploidogens (or aneugens) may be defined as chemical "agents that induce the loss or gain of chromosomes resulting from improper segregation at mitosis or meiosis" (McCarroll *et al.*, 2002, p. 322).

The proposed MOA is based on assessments of MBC's genotoxicity and carcinogenicity that have been conducted by various health organizations, regulatory entities and in published literature reviews. In particular, the comprehensive survey of genotoxicity and carcinogenicity data on MBC and benomyl, a related (now cancelled) fungicide, of McCarroll *et al.* (2002; MRID 51768701), was referenced in the Registrant's proposal. This article was authored by Agency toxicologists, including several experts on genotoxicity and carcinogenicity. It included an International Programme on Chemical Safety (IPCS) MOA framework analysis (for both compounds) of aneugenicity as (1) a threshold genotoxic effect and (2) potential tumorigenic MOA for the induction of mouse liver tumors. No new mechanistic studies were submitted with the Registrant's proposal. However, in order to better characterize the aneugenic response and genotoxicity of MBC and evaluate the proposed MOA, HED has reviewed several studies from the open literature that relate to key events of the MOA.

The Registrant's cancer reclassification proposal included a WOE determination in support of classifying MBC as "Suggestive Evidence for Carcinogenic Potential" with no quantification of cancer risk.

Scope of the Cancer Reevaluation: This reevaluation of MBC evaluates the appropriate cancer classification under the criteria of the Agency's 2005 *Guidelines for Carcinogen Risk Assessment* and the need for quantification when assessing cancer risk. In this reevaluation: (1) The tumorigenic dose in the CD-1 mouse carcinogenicity study used to quantify cancer risk (Wood *et al.*, MRID 00154676) is reassessed, taking into consideration the tumor incidence data from the original pathology report vs. the pathology reevaluation (Frame *et al.*, MRID 41607904). A new qualitative assessment of tumor incidence or detailed reevaluation of the study findings was not performed; (2) Two other mouse carcinogenicity studies with significant study deficiencies (Beems *et al.*, MRID 00153420; Donaubauer *et al.*, MRID 00154679) that were considered in the previous cancer reviews of MBC are summarized and reevaluated by CARC as to whether it is appropriate to include them in the WOE consideration, due to the study deficiencies; (3) The

Registrant's proposed MOA (aneugenicity) and WOE assessment are considered. This proposal did not itself provide a detailed framework analysis for the MOA but rather referred to the conclusions of other reviews (summarized in Table 1). The mouse and rat carcinogenicity data on MBC are presented in the Background Information section of this document since they were reviewed in the previous cancer assessments.

II. BACKGROUND INFORMATION AND SUMMARY OF CARCINOGENICITY STUDIES

A. Chemical description and use pattern:

MBC (methyl 1H-benzimidazol-2-ylcarbamate; PC Code 128872; CAS No. 10605-21-7; also known as carbendazim), a systemic benzimidazole fungicide, is registered as an active ingredient (a.i.) for nonfood/nonfeed uses as an antimicrobial pesticide in paint and treated vinyl flooring. There is currently one conventional use for tree injection, an application that is not expected to result in human exposure to MBC or its metabolites. In addition to its registered uses as an a.i., MBC is a metabolite of thiophanate-methyl (TM; PC Code 102001; CAS No. 23564-05-8), a systemic benzimidazole fungicide registered for use on numerous agricultural crops and turf. Therefore, dietary exposure to MBC may occur from residues on food crops and in drinking water as a result of TM agricultural uses and exposure to MBC, as a metabolite of TM, is assessed along with TM in human health risk assessments of TM. Due to differences in their toxicological profiles, both non-cancer and cancer risk are assessed separately for TM and MBC, using chemical-specific endpoints.

The chemical structure of MBC is shown below (Figure 1):

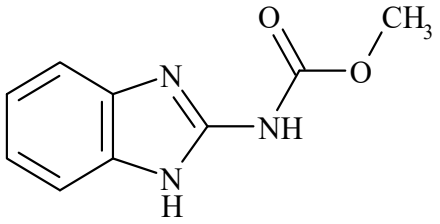


Figure 1. Structure of MBC

B. Previous Agency cancer assessments of MBC:

The Agency has previously reviewed the carcinogenicity of MBC, including a review by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Science Advisory Panel (SAP). Dates and summaries for each meeting are provided below. Previous assessments were conducted together with benomyl, since benomyl rapidly hydrolyzes to MBC *in vivo* and in the environment. Both compounds share similar profiles for hepatotoxicity, genotoxicity and hepatocellular carcinogenicity, and the cancer classification and quantification for benomyl was based on the mouse carcinogenicity data for MBC. Benomyl has since been voluntarily cancelled

for all uses (2001[1]; 2002[2]). Therefore, for the current cancer reevaluation, only MBC is being reclassified.

October 3, 1985 (preliminary meeting) and January 7, 1986 meeting (TXR 0005576, J. Quest, 03/31/1986): MBC and benomyl were classified under the 1986 EPA Guidelines for Carcinogenic Risk Assessment as Group C (possibly carcinogenic to humans), based on an increased incidence of hepatocellular tumors in male and female CD-1 and SPF Swiss mice and evidence of aneugenicity. The weight of the evidence considerations included: (1) increases in hepatocellular tumors in male and female CD-1 mice (MBC; MRID 00154676 and benomyl). An increase in foci of cellular alteration, but not hepatocellular hyperplasia, was observed for both compounds, along with foci of cellular degeneration and cytomegaly for benomyl (the report noted the lack of evidence of hyperplasia in the WOE considerations); (2) increases in hepatoblastoma in male SPF Swiss mice (MBC; MRID 00153420); (3) lack of tumor response in HOE NMRKf (SPF 71) mice, a strain with lower spontaneous hepatocellular tumor incidence than CD-1 mice (MBC; MRID 00154679); (4) the elevated liver tumor responses in CD-1 and SPF Swiss mice were seen in outbred Swiss mice strains that were stated to have a historically higher background incidence of liver tumors, suggesting a strain-specific response (in-house historical control data were not mentioned in the cancer assessment, however); (5) lack of tumor response in other tissues in all tested strains of mice; (6) lack of tumor response in Charles River CD rats; (7) positive genotoxicity results consistent with adverse effects on the cellular spindle apparatus and; (8) weak genotoxicity evidence for gene mutation or DNA damage. The WOE also noted late tumor onset, lack of significant preneoplastic (hyperplastic) liver changes, no effect on time to tumor or survival, and no evidence of tumor invasion or metastases. The Committee noted a structural relationship to fenbendazole and albendazole, veterinary compounds under review at that time by the Food and Drug Administration (FDA) for carcinogenicity. Classification as a Group B2 (probably carcinogenic to humans) was considered due to the rare tumor type (hepatoblastoma) in SPF Swiss mice, but the Committee determined that the preponderance of evidence supported the Group C category. A formal recommendation for quantification of cancer risk was not made at this meeting.

FIFRA Science Advisory Panel (SAP) meeting (May 21, 1986; May 26, 1986 memorandum from S. Johnson): The SAP recommended classification of MBC and benomyl in the Group C category, based on the considerations listed above. Quantification of cancer risk was not recommended due to the Group C classification, which reflects some uncertainties regarding the potential for carcinogenicity in humans.

January 25, 1989 meeting (TXR 0050625, J. Quest, 04/07/1989): Benomyl and MBC were reevaluated at the Registration Division's request to determine the need for quantification of cancer risk. The Committee also revisited the cancer classification discussion (Group C vs. B2), taking into consideration the 1986 FIFRA SAP conclusions. The Group C classification was reaffirmed. However, quantification of cancer risk was recommended, based on the increase in malignant liver tumors in two strains of mice and in both sexes, and increase in a rare tumor type (hepatoblastoma) in male SPF Swiss mice administered MBC.

[1] https://www federalregister.gov/documents/2001/05/23/01-12905/benomyl-receipt-of-request-for-registration-cancellations

[2] https://www federalregister.gov/documents/2002/01/15/02-958/benomyl-cancellation-order

Quantification of MBC Cancer Risk (TXR 0013859, L. Brunsman, 11/18/1999): The current cancer potency factor (Q_1^*) of 2.39 x 10^{-3} $(mg/kg/day)^{-1}$ is based on the incidence of combined hepatocellular adenoma and/or carcinoma in female CD-1 mice in the two-year dietary study on MBC (Wood, 1972; MRID 00088333). The value was updated from an earlier quantification to include the ¾ interspecies scaling factor (TXR 0007608, B. Fisher, 05/15/1989), as a 2/3 scaling factor was used previously.

Pathology Peer Review Group reevaluation of mouse liver tumors (MRID 41607904, reviewed in TXR 0010723, M. Morrow, 01/05/1994) and *Ad Hoc* (preliminary) CARC meeting, September 6, 2000 (TXR 0014346, D. Smegal, 09/21/2000): The Registrant (DuPont) submitted a request for cancer reclassification of MBC and benomyl based on a pathology peer review reevaluation of the mouse liver tumor data from the Wood (1972) study (MRID 41607904). The peer review group consisted of two pathologists from Haskell Laboratories (not including the original study pathologist) who reevaluated the liver tissue from the original study, followed by review from an outside consulting pathologist. A consensus was reached on the diagnoses between the two Haskell pathologists and the consulting pathologist. A copy of the original MRID submission is not available for review at this time.

This pathology reevaluation was conducted in 1990, prior to the Agency's development of criteria for conduct of a pathology working group (PWG) reevaluation. The PWG protocol is based on the PWG reevaluation quality assurance protocol of the National Toxicology Program (NTP), as set forth in Pesticide Regulation (PR) Notice 94-5 (August 24, 1994). This protocol provides for a PWG consensus review consisting of a group of experienced pathologists (including the study pathologist(s)), a PWG chair pathologist, and a peer review pathologist. The peer review pathologist conducts a reevaluation of all relevant tissue slides from the original study; the original study diagnoses and peer review diagnoses are discussed by the PWG and, where differences are identified, a consensus is reached.

An *ad hoc* (pre-) CARC meeting was held on September 6, 2000 to determine whether the reevaluation warranted a full Committee review. Although the pathology reevaluation identified a higher percentage of hepatocellular adenomas relative to carcinomas when compared to the original pathology report, the combined tumor incidence at each dose level was comparable to the combined tumor incidence in the original study and would not result in a significant change to the Q_1^* or the cancer classification. The *ad hoc* review also concluded that there were no data provided to support a cancer MOA for MBC or benomyl. Therefore, a full CARC meeting was not recommended, and no changes to the cancer classification or quantitation of carcinogenicity of MBC were made.

C. Reviews of the genotoxicity and carcinogenicity of MBC by other entities:

The genotoxicity and carcinogenicity of MBC have been reviewed by numerous entities (Table 1). The Registrant's submission included reviews prepared by the World Health Organization (WHO, 1993; Appendix I, p. 33 of MRID 51768701), the Joint Meeting of Pesticide Residues (JMPR, 2010 and update, 2021; Appendix VII, p. 357 of MRID 51768701) and the Australia Pesticides and Veterinary Medicines Authority (APVMA, 2009; Appendix III, p. 133 of MRID 51768701). The proposal also provided a WOE review of the genotoxicity and mutagenicity of

MBC in accordance with the European Commission (EC) Regulation No. 1272/2008 on Classification, Labelling and Packaging (CLP) of substances and mixtures (Gollapudi *et al.*, 2014, prepared by ExPonent for Troy Corporation; Appendix V, p. 285 of MRID 51768701) and an open literature survey of the genotoxicity and carcinogenicity data on MBC and benomyl (McCarroll *et al.*, 2002; Appendix VI, p. 318 of MRID 51768701). As previously noted, the review by McCarroll *et al.* (2002) was prepared by OPP and Office of Research and Development (ORD) scientists. A review by the European Chemical Agency (ECHA, 2019) was cited but not appended to the submitted proposal.

All reviews concluded that MBC is aneugenic in mammalian and lower eukaryotic cells. Chromosomal mal-segregation results from disruption of the normal spindle microtubule assembly and consequently, spindle formation during cell division. Genotoxicity from exposure to MBC is not considered the result of direct DNA interaction/damage, and a threshold dose is required for aneugenicity to occur. Based on a WOE determination, the review of Gollapudi *et al*. (2014) concluded that MBC did not meet the CLP criteria for a germ cell mutagen[3]. In addition, the review concluded that MBC is aneugenic, a genotoxic effect requiring a threshold dose. The extensive literature demonstrating aneugenicity of MBC and overall lack of direct DNA damage was cited by these reviews in support of this conclusion.

The reviews that evaluated the carcinogenicity of MBC concluded that there were treatment-related increases in hepatocellular tumors in the mouse and no evidence of carcinogenicity in the rat. APVMA and ECHA considered the mouse liver tumors to be a species-specific response in a strain of mouse (CD-1) sensitive to liver tumor induction and therefore not relevant to humans, whereas McCarroll *et al.* (2002) considered them relevant to humans, since the review included a summary of the Agency's cancer assessment. McCarroll noted that an aneugenic MOA was relevant to humans, stating that:

> " No data are available to exclude the possibility that benomyl/MBC can pose a carcinogenic risk to humans. On the basis of the positive *in vitro* data with human cell lines and human hybrid cell lines, there is a reasonable concern that a potential human health hazard is feasible. Additionally, the background incidence of aneuploidy is higher in humans than in other species that have been studied and an increased risk of tumor development in humans is seen in conjunction with certain congenital constitutional aneuploidies such as Down's, Turner's and Klinefelter's syndromes… " .

MBC has also been reviewed by the European Food Safety Authority (EFSA, 2010 and 2021), Canada's Pesticide Regulatory Management Agency (PMRA) and the California Department of Pesticide Regulation (CDPR). CDPR's review was prepared for benomyl and included evaluation of MBC as the active pesticidal moiety (CDPR; October, 1999). Aneugenicity was

[3] From Gollapudi *et al.*: "CLP Category 2 germ cell mutagens are "substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans". In order to be classified as a Category 2 mutagen, substances have to meet at least one of the following two criteria: (a) Positive results in somatic cell mutagenicity tests *in vivo* in mammals, or (b) Positive results in other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. CLP guidance further states that a substance shall be considered as Category 2 mutagen based solely on positive *in vitro* mammalian mutagenicity assays it also shows chemical structure activity relationship to known germ cell mutagens."

concluded to be the genotoxic MOA for MBC in each of these reviews. EFSA determined that MBC is not carcinogenic to humans: the liver tumors in the mouse were considered a species and strain-specific sensitivity and the tumor response not relevant to humans. However, PMRA used linear low-dose extrapolation to quantify cancer risk, based on lack of sufficient mechanistic data to support a non-linear response, noting the lack of *in vivo* evidence for MBC of aneuploidy in the target tissue (liver) or definitive data on liver cell proliferation relative to dose and time. PMRA therefore conducted a quantitative cancer risk assessment based on lack of sufficient mechanistic data to demonstrate a nonlinear mode of action, using a Q_1^* of 1.6×10^{-2} $(mg/kg/day)^{-1}$. CDPR also considered MBC and benomyl to be aneugenic and conducted a quantitative cancer risk assessment using a Q_1^* of 2.8×10^{-3} $(mg/kg/day)^{-1}$.

Table 1. Summary of Reviews of the Genotoxicity and Carcinogenicity of MBC Included in the Registrant Proposal Submission and Additional Reviews from other Entities.

REVIEWING ENTITY	GENOTOXICITY CONCLUSIONS/MOA	CANCER FINDINGS	CANCER CONCLUSIONS
Reviews included with proposal submission			
World Health Organization (WHO) Environmental Health Criteria 1993	Aneugenic, secondary to interaction with spindle microtubule assembly No evidence of direct genotoxicity or interaction with DNA resulting in gene mutation	Rat – no treatment-related tumors Mouse – increased incidence of liver tumors in two of three mouse carcinogenicity studies	Not considered carcinogenic in humans. Mouse liver tumors are considered a species-specific event and are not considered relevant to humans. Increases were observed in strains having higher spontaneous rates of liver tumors.
Australia Pesticides and Veterinary Medicines Authority (APVMA) 2009 Human health risk assessment	Aneugenic, threshold effect dependent on disruption of spindle tubulin assembly	Rat – no treatment-related tumors Mouse – increased incidence of liver tumors in two of three mouse carcinogenicity studies	Not considered carcinogenic in humans. Mouse liver tumors are considered a species-specific event and not relevant to humans. Variable tumor response among three studies on different mouse strains may relate to strain-specific liver metabolic enzyme differences.
Joint FAO/WHO Meeting on Pesticide Residues (JMPR) 2005 Assessment of genotoxicity	Aneugenic, threshold effect, Genotoxicity NOAEL for aneugenicity = 50 mg/kg/day based on spermatogenic effects in the male rat at 100 mg/kg.	Not evaluated	Not evaluated
Gollapudi *et al.* (ExPonent) 2014 Germ cell mutagenicity evaluation prepared for Troy Corporation. Review of MBC genotoxicity in accordance with EC Regulation No. 1272/2008 (CLP).	Aneugenic, not mutagenic	Not evaluated	Not evaluated

REVIEWING ENTITY	GENOTOXICITY CONCLUSIONS/MOA	CANCER FINDINGS	CANCER CONCLUSIONS
McCarroll *et al.* 2002, Mutation Research Survey of genotoxicity and carcinogenicity of MBC and benomyl with a mode of action framework analysis for aneugenicity and mouse liver tumor induction.	Aneugenic, not mutagenic.	Rat – no treatment-related tumors Mouse – increased incidence of liver tumors in two of three mouse carcinogenicity studies	Aneugenicity as the cancer MOA for mouse liver tumors is plausible, but available data are insufficient to support it definitively.
European Chemical Agency (ECHA) 2019/2013 Evaluation of active substances assessment report [for] carbendazim	Aneugenic, not mutagenic, threshold effect, Genotoxicity NOAEL = 50 mg/kg/day based on spermatogenic effects in the male rat at 100 mg/kg.	Rat – no treatment-related tumors Mouse – increased incidence of liver tumors in two of three mouse carcinogenicity studies.	Not considered carcinogenic in humans. Mouse liver tumors are considered a species-specific event and are not considered relevant to humans. Increases were observed in strains having higher spontaneous rates of liver tumors.
Additional reviews of genotoxicity and carcinogenicity of MBC (not referenced in proposal submission)			
European Food Safety Authority (EFSA) 2010	Aneugenic	Rat – no treatment-related tumors Mouse – increased incidence of liver tumors	Not considered carcinogenic in humans. Mouse liver tumors are considered a species-specific event and are not considered relevant to humans. Increases were observed in strains having higher spontaneous rates of liver tumors.
European Food Safety Authority (EFSA) 2021	Genotoxicity updated; aneugenic but not clastogenic	Not reevaluated. No change from 2010	Not reevaluated. No change from 2010.
Canada Pesticide Management Regulatory Agency (PMRA) 2011 Human health risk assessment	Aneugenic	Rat – no treatment-related tumors Mouse – increased incidence of liver tumors in two of three mouse carcinogenicity studies	Carcinogenic in the mouse. Aneugenic MOA for mouse liver tumors is plausible but conclusive mechanistic data are inadequate to support the MOA. Linear low-dose extrapolation was selected for quantification of cancer risk to humans. $Q1^* = 1.6 \times 10^{-2}$ $(mg/kg/day)^{-1}$ based on liver tumors in female CD-1 mice.
California Department of Pesticide Regulation (CDPR) 1999 MBC was evaluated as part of the benomyl human health risk assessment. Not updated since benomyl was cancelled (2002).	Aneugenic, evidence of other genotoxicity	Rat – no treatment-related tumors Mouse – increased incidence of liver tumors	Carcinogenic to humans based on liver tumors in mice and evidence of genotoxicity. $Q_1^* = 2.8 \times 10^{-3}$ based on liver tumors in CD-1 mice.

D. Summary of available mouse carcinogenicity studies:

Three oral carcinogenicity studies in the mouse are available for MBC. The study in CD-1 mice conducted by Wood *et al.* (MRID 00154676), demonstrated the most pronounced response for hepatocellular tumors and was used by EPA as well as CDPR and PMRA to calculate the Q_1* for MBC (and benomyl). A reevaluation of the liver tumor incidence was also considered in this reevaluation and the data are presented below together with the original pathology report incidence.

Two additional carcinogenicity studies in SPF Swiss mice (Beems *et al.*, MRID 00153420) and NMRKf mice (Donaubauer *et al.*, MRID 00154679), both conducted on MBC, were considered in the previous Cancer Peer Reviews of MBC and benomyl. The Registrant's proposal excluded consideration of the latter two studies due to limitations in study conduct and data reporting previously noted by the Agency. Both of these studies are summarized below and discussed by the CARC since they were considered in the previous cancer assessments.

Available copies of the original carcinogenicity study reports for MBC, all of which are older studies, are poorly legible and, where noted, lacked individual animal data. In-laboratory historical control values for mouse liver tumor incidence are available for two studies in the CD-1 mouse but unavailable for the other studies in SPF Swiss and NMRKf mice. Data are presented for this reevaluation to the best extent possible.

1. References: *Wood, C.K., Schneider, P.W. and Trochinowicz, N.J. (1982) Long-term feeding study with 2-benzimidazole-carbamic acid, methyl ester (MBC, INR-965). Study No. 70-82, January 26, 1982, Haskell Labs, Newark, DE. Unpublished report. MRID 00154676.*

   Frame, S.R. and VanPelt, C.S. (1990) Oncogenicity Studies with Benomyl and MBC in Mice (Supplemental Peer Review) Study Nos. 3194 and 3207 (based on original studies 20-82 and 70-82), June 28, 1990, Haskell Labs, Newark, DE. Unpublished report. MRID 41607904.

   a. Experimental Design

MBC (99.3% a.i.; Haskell Lab lot no. 11201) was administered in the diet to CD-1 mice (80/sex/dose) at dose levels of 0, 500, 1500, or 7500 ppm for up to 104 weeks. Due to increased mortality between weeks 5264, the high dose males were removed from treatment for one week (65 to 66), followed by resumption of treatment at 3750 ppm at week 66 until termination at 73 weeks. Treatment duration was 104 weeks for all remaining groups. Dietary levels (control to high dose, respectively) provided average daily doses (time-weighted averages) of 0, 75, 225 or 563 mg/kg/day (males) and 0, 75, 225 or 1125 mg/kg/day (females).

   b. Survival Analysis

Survival analyses of male and female CD-1 mice, prepared for previous cancer assessments of MBC and benomyl, are shown below (TXR 0007608, B. Fisher, 05/15/1989). At study

termination, mortality rates (control to high dose, respectively) were 78, 83, 89 and 71% in males (Table 2) and 73, 80, 83 and 74% in females (Table 3). The high dose males were sacrificed during week 73 due to an increase in mortality/moribund animals during the 53-73 week interval; all other dosing groups were continued until week 104. Survival at terminal sacrifice was <25% for the low- and mid-dose animals of both sexes (11 to 20%) and control males (22%), but >25% for control females and high dose males (at the time of early sacrifice) and females. Survival rates in all treatment groups were high (84-97%) for the first 52 weeks of the study and began to decline during the second year of dosing for both males and females, with high dose males showing the most pronounced change. These results were considered acceptable since adequate survival was maintained during the initial 18 months of the study.

Table 2. MBC - Male CD-1 Mouse Study Mortality[+] Rates and Cox or Generalized K/W Test Results (MRID 00154676)

	Week				
Dose in ppm (mg/kg/day)	**1-26**	**27-52**	**53-73[a]**	**74-104[b]**	**Total**
0	1/80	3/79	25/76	33/51	62/80 (78)**
500 (75)	0/80	8/80	33/72	24/39	66/80 (83)
1500 (225)	0/80	9/80	36/71	26/35	71/80 (89)**
7500/3500[c] (563)	4/80	12/76	41/64	--	57/80 (71)*

+ Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
() percent of sample size
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<0.5 and if ** then p<0.1.

Table 3. MBC - Female CD-1 Mouse Study Mortality[+] Rates and Cox or Generalized K/W Test Results (MRID 00154676)

	Week				
Dose in ppm (mg/kg/day)	**1-26**	**27-52**	**53-78**	**79-104[a]**	**Total**
0	3/81	4/78	26/74	26/48	59/81 (73)
500 (75)	4/79	6/75	17/69	36/52	63/79 (80)
1500 (225)	2/80	3/78	27/75	34/43	66/80 (83)
7500 (1125)	2/80	2/78	23/76	32/53	59/80 (74)

+ Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
() percent of sample size
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<0.5 and if ** then p<0.1.

c. Discussion of Tumor Data

The incidence and statistical analyses of liver tumors in male and female CD-1 mice, prepared for previous cancer assessments of MBC and benomyl from tumor data in the original study report (TXR 0007608, B. Fisher, 05/15/1989), are provided below in Tables 4 (males) and 5 (females). Limited historical control data from the study laboratory were available (discussed below). In male mice, the high dose group was excluded from the statistical analysis due to excessive toxicity (high mortality) resulting in an early sacrifice during week 73 (noted as week 74 in Table 4). A statistically significant increase in combined adenomas + carcinomas was observed at the low and mid doses. Carcinomas were increased at the mid dose. Significant positive trends were observed for both the carcinomas and combined tumor incidence. No significant differences were observed for the incidence of adenomas.

Table 4. MBC – CD-1 Mouse Study (MRID 00154676)-Original Pathology Report

Male Mice Hepatocellular Tumor rates[+] and the Peto Prevalence Test Results

	Dose (ppm)				
Liver Tumor	**0**	**500 (75 mg/kg/day)**	**1500 (225 mg/kg/day)**	**7500-3750[a] (563 mg/kg/day)**	**Historical Controls (% incidence) [e]**
Adenoma (%)	11/76 (14)	15/72 (21)	14/73 (19)	3[b]/67 (4)	(11, 12)
p=	0.155	0.072	0.131	---[c]	
Carcinoma (%)	2/76 (3)	5/72 (7)	9[d]/73 (12)	0/67 (0)	(5, 21)
p=	0.010*	0.080	0.012**	---[c]	
Combined tumors (%)	13/76 (17)	20/72 (28)	23/73 (32)	3/67 (4)	(16, 32)
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 of sample size
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.
e Historical controls from 2 studies, see details in Table 6. Each value represents the percentage incidence observed in the control group for that study.
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 $p<0.5$ and if ** then $p<0.1$.

In female mice, the incidence of combined adenomas + carcinomas was significantly increased by pair-wise comparison to controls in all treatment groups, and a significant positive trend was also observed. A significant pair-wise increase compared to controls was observed for adenomas at the low and mid doses, but not at the high dose, and a positive trend was not observed. The incidence of carcinomas was increased in mid and high dose groups by pair-wise comparison to controls, along with a positive trend. The tumor incidence did not show a proportional increase at the high dose when compared to the mid dose.

Table 5. MBC – CD-1 Mouse Study (MRID 00154676), Original Pathology Report

Female Mice Hepatocellular Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results

	Dose (ppm)				
Liver Tumor	**0**	**500 (75 mg/kg/day)**	**1500 (225 mg/kg/day)**	**7500[a] (1125 mg/kg/day)**	**Historical Controls (% incidence) [c]**
Adenoma (%)	0/74 (0)	5/70 (7)	5/75 (7)	3[a]/75 (4)	(6, 3)
p=	0.441	0.025*	0.030*	0.125	
Carcinoma (%)	1/74 (1)	4/70 (6)	15[b]/75 (20)	12/75 (16)	(1, 3)
p=	0.010*	0.166	0.000**	0.001**	
Combined tumors (%)	1/74 (1)	9/70 (13)	20/75 (27)	15/75 (20)	(8, 5)
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 of sample size
a first adenoma observed at week 90.
b first carcinoma observed at week 77.
c Historical controls from 2 studies, see details in Table 6. Each value represents the percentage incidence observed in the control group for that study.
Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.
If * then $p<0.5$ and if ** then $p<0.1$.

The study pathology report provided a table comparing control group hepatocellular tumor incidence of this study to the incidence in two other studies in CD-1 mice that were conducted by the laboratory (Table 6). The data indicated variability in tumor incidence in both sexes but particularly in males. The combined tumor incidence (28% and 32%) observed in low- and mid-dose males, respectively (Table 4), is comparable to the incidence (32%) observed for the male controls in the benomyl study (1982). Control females showed a slightly lower incidence in the MBC study than the other two studies. However, tumor incidence in females of all MBC-treated groups exceeded the values observed in controls from the other two studies.

Table 6. Hepatocellular Tumor Incidence (# Tumors (%)) in Control Groups from Three 2-Year Carcinogenicity Studies in CD-1 Mice: Comparison of Available Historical Control Studies to MBC CD-1 Mouse Study.

Hepatocellular tumor type	Historical Control Studies from Haskell Laboratory		(Wood *et al.* study)
	Compound A (unnamed; (study date not provided)	Benomyl (MRID 00096514) (1982)	MBC (MRID 00154676) (1982)
Males N =	80	77	80
Adenoma	9 (11)	9 (12)	11 (14)
Carcinoma	4 (5)	16 (21)	2 (3)
Combined	13 (16)	25 (32)	13 (17)
Females N =	80	77	79
Adenoma	5 (6)	2 (3)	0
Carcinoma	1 (1)	2 (3)	1 (1)
Combined	6 (8)	4 (5)	1 (1)

Data extracted from text table on p. 86, pathology report, MRID 00154676. Control tumor incidence shown for the MBC study is from the original pathology report.

As previously noted, the CARC did not conduct a full cancer assessment of the revised liver tumor incidence data from the 1990 pathology reevaluation (MRID 41607904; reviewed in TXR 0014346, D. Smegal, 09/21/2000), concluding that the reevaluation did not change the overall conclusions for carcinogenicity of MBC. Tumor incidence and a statistical analysis from both the original report and the pathology reevaluation were presented in McCarroll *et al.* (2002), and tumor incidence data from the pathology reevaluation were used for the liver tumor incidence in the Registrant's proposal. The pathology reevaluation reported a slightly lower combined tumor incidence and incidence of carcinomas for most treated groups than the original report. The reevaluation stated that many of the differences between the original and reevaluation findings were in the diagnosis of borderline lesions that were difficult to classify. The reevaluation was conducted prior to the Agency's 1994 guidance for a pathology peer review. The results of the reevaluation in males and females are presented below (Table 7) for comparison with the original pathology report.

In males, the incidence of adenomas and combined adenomas + carcinomas was increased at the mid dose by pair-wise comparison to controls, and a significant positive trend was observed. The incidence of carcinomas showed a significant pair-wise increase at the mid dose with no positive trend. In females, the incidence of adenomas and combined adenomas + carcinomas was increased at all dose levels by pair-wise comparison with controls, with a positive trend also observed. The incidence of carcinomas did not show pair-wise increases to controls except at the mid dose; a positive trend was also observed. As in the original pathology report, a dose-proportional increase in tumor incidence was not observed between the mid and high dose groups.

Table 7. Liver Tumors in CD-1 Mice – Peer Review Re-evaluation and Statistical Analysis of Tumor Incidence Data[a]

Males				
Liver Tumor Type	**0 ppm**	**500 ppm (75 mg/kg/day)**	**1500 ppm (225 mg/kg/day)**	**3750 ppm (563 mg/kg/day)**
Adenoma (%)	10/71 (14)	12/65 (18)	19/62 (31)	3[b]/45 (7)
P=	0.004**	0.110	0.005**	0.401
Carcinoma (%)	1[c]/33 (3)	2/25 (8)	3/25 (12)	0/0 (0)
p=	0.216	0.228	0.000**	--
Combined tumors (%)	11/71 (15)	13/65 (20)	19/62 (31)	3/45 (7)
p=	0.007**	0.091	0.007**	0.401
Females				
Liver Tumor	**0 ppm**	**500 ppm (75 mg/kg/day)**	**1500 ppm (225 mg/kg/day)**	**7500 (1125 mg/kg/day)**
Adenoma (%)	0/74 (0)	8/70 (11)	20/75 (27)	15[d]/75 (20)
p=	0.013*	0.003**	0.000**	0.000**
Carcinoma (%)	1/74 (1)	1/70 (1)	6[e]/75 (8)	3/75 (4)
p=	0.006**	0.60	0.000**	0.315
Combined tumors (%)	1/74 (1)	9/70 (13)	21/75 (28)	17/75 (23)
p=	0.006**	0.013*	0.000**	0.000**

a = Data table and statistical analysis extracted from McCarroll *et al.* (2002), Tables 3 and 4, journal citation page 330. Tumor incidence is based on the peer review reevaluation (MRID 41607904).
b = First adenoma in males seen at week 62.
c = First carcinoma in males seen at week 90.
d = First adenoma in females seen at week 90.
e = First carcinoma in females seen at week 77.
Number of tumor bearing animals/number of animals examined, excluding those that died before the first tumor.
Males: Peto's Prevalence Test - ** Significant (P<0.01) difference from control.
Females: Cochran–Armitage Trend and Fisher's Exact tests.* Significant (P<0.05); ** Significant (P<0.01) difference from control.

d. Non-Neoplastic Lesions

Treatment-related non-neoplastic histopathology findings were observed in the liver at the mid and high doses (Table 8). In males, increases in hepatocellular hypertrophy and centrilobular necrosis (mid and high dose), and a slight increase in pigment in macrophages (high dose only), were observed. An increase in hepatocellular hypertrophy was also observed at the low dose in males. In females, an increase in hepatocellular eosinophilic foci at the mid and high doses and a slight increase in centrilobular necrosis (3 vs. 1 control that included one animal each with minimal, moderate, and marked change) was seen at the mid dose. However, necrosis was not observed at the high dose. A slight increase in hepatocellular hypertrophy (minimal-mild) and basophilic foci was observed only at the high dose. Females did not show a treatment-related increase in pigment in liver macrophages.

Table 8. Incidence of Selected Non-neoplastic Histopathological Changes in CD-1 Mice Exposed to MBC for up to Two Years.

Tissue/lesion/severity		Dietary dose in ppm (mg/kg/day)			
		0	**500 (75)**	**1500 (225)**	**7500/3750 (563 M/1125 F)**
Males					
Liver	N=	(80)	(80)	(80)	(80)
Hepatocellular necrosis	total	0	1	9*	14*
	minimal	0	0	2	5
	mild	0	1	4	8
	moderate	0	0	1	1
	marked	0	0	1	0
Hepatocellular hypertrophy	total	0	9*	13*	21*
	minimal	0	3	0	2
	mild	0	4	10	13
	moderate	0	2	3	6
Pigment, macrophages	total	11	11	13	23*
	minimal	8	8	9	17
	mild	3	2	3	5
	moderate	0	1	1	1
Cellular alteration, eosinophilic foci		0	2	1	2
Cellular alteration, basophilic foci		2	2	2	1
Females					
Liver	N=	(79)	(78)	(80)	(78)
Hepatocellular necrosis	total	1	1	3	0
	minimal	0	0	1	0
	mild	1	0	0	0
	moderate	0	1	1	0
	marked	0	0	1	0
Hepatocellular hypertrophy	total	0	0	1	4
	minimal	0	0	0	3
	mild	0	0	1	1
	moderate	0	0	0	0
Pigment, macrophages	total	24[1]	13	10	13
	minimal	9	8	7	8
	mild	6	4	3	5
	moderate	1	1	0	0
Cellular alteration, eosinophilic foci		0	0	10	6
Cellular alteration, basophilic foci		3	2	2	8

Data extracted from MRID 00154676 pathology report, individual animal data tables and histopathology summary tables, pp. 285-884. N = total samples examined.

[1] Total examined with lesion (24) vs. number of animals with individual severity scores (15) differ for control group due to missing page for individual animal data in available study copy. Available severity scores are summarized.

- $p < 0.05$

e. Other Non-neoplastic Effects

Absolute liver weights were increased in females at the mid and high doses (absolute/relative 24%/31% and 44%/38% above controls, respectively). Although thymus weights were noted to be lower in treated groups of both sexes, a dose-response was not observed. Selected non-liver microscopic lesions are shown below in Table 9. Both sexes showed increases in thymus

lymphoid depletion at the mid and high dose. In the kidney, increases in pigment in tubules at the mid and high doses in males and in pigment in tubules and macrophages at the high dose in females were observed. Sperm stasis was also observed in males at mid and high dose.

Table 9. Incidence of Selected Non-liver Histopathological Changes in CD-1 Mice Exposed to MBC for up to Two Years.

Tissue/lesion/severity		Dietary dose in ppm (mg/kg/day)			
		0	500 (75)	1500 (225)	7500/3750 (563 M/1125 F)
Males					
Thymus	N=	(42)	(39)	(31)	(51)
Lymphoid depletion	total	11	13	17*	25*
	minimal	0	0	0	2
	mild	8	11	16	21
	moderate	3	2	1	2
Kidney	N=	(80)	(79)	(78)	(79)
Pigment in tubules	total	7	3	19*	47*
	minimal	0	2	17	35
	mild	7	1	2	10
	moderate	0	0	0	2
Pigment in macrophages	total	0	3	1	0
	minimal	0	0	0	0
	mild	0	2	2	0
	moderate	0	1	0	0
Testes	N=	(77)	(78)	(80)	(74)
Sperm stasis	total	7	13	16*	22*
	minimal	3	5	6	13
	mild	3	6	8	9
	moderate	0	2	2	0
	marked	1	0	0	0
Females					
Thymus	N=	(38)	(35)	(44)	(38)
Lymphoid depletion	total	3	4	12*	10*
	minimal	0	1	0	0
	mild	3	2	10	9
	moderate	0	1	2	3
Kidney	N =	(80)	(78)	(80)	(76)
Pigment, tubules	total	0	0	3	41*
	minimal	0	0	3	28
	mild	0	0	0	10
	moderate	0	0	0	3
Pigment, macrophages	total	5	4	3	21*
	minimal	3	3	3	15
	mild	0	0	0	5
	moderate	2	0	0	0
	marked	0	1	0	1

Data extracted from MRID 00154676 pathology report, individual animal data tables and histopathology summary tables, pp. 285-884.
N = total samples examined.
* $p<0.05$

f. Adequacy of Dosing

In males, dosing was considered adequate at the low and mid dose, based on increases in hepatocellular hypertrophy and necrosis, and sperm stasis at mid dose. The high dose was

considered excessive in males, based on increased moribundity during the second year of treatment, leading to early sacrifice (during Week 73) of all surviving males in this group. In females, dosing was considered adequate at all dose levels, based on increased liver eosinophilic foci at mid and high dose and basophilic foci at high dose.

The CARC considered the hepatocellular tumors in males and females to be treatment-related and reviewed the data to determine the tumorigenic dose. Both the original and reevaluated liver tumor incidence data were considered in the WOE determination. CARC noted the reevaluation was conducted prior to publication of the 1994 PR Notice 94-5 which provided guidance on conducting pathology reevaluations. In males, the incidence of combined adenomas and carcinomas was statistically significantly increased by pair-wise comparison to controls at the mid dose in both evaluations, but in the original review, was also statistically significant at the low dose by pair-wise comparison to controls. A statistically significant positive trend for incidence of combined adenomas and carcinomas was seen in both data sets. The CARC concluded that the combined adenomas and carcinomas in males were increased and treatment-related at the low dose and mid dose (≥500 ppm [75 mg/kg/day]). This determination took into consideration the limited available historical control data, lack of interim sacrifice or subchronic data (for evidence of early hepatotoxicity, pre-neoplastic lesions and/or aneuploidy in liver cells), significant hepatotoxicity at the mid and high dose (hypertrophy and necrosis), availability of only two dose levels for analysis of tumor incidence (due to late tumor onset and early sacrifice of the high dose males), the significant pair-wise increase in combined tumors at low dose in the original pathology evaluation, and the positive significant trend for combined tumor incidence observed in both pathology evaluations.

In females, both pathology evaluations identified statistically significant increases in combined adenomas and carcinomas by pair-wise comparison to controls at all dose levels tested, along with a statistically significant trend. Tumor incidence at all dose levels was also well above the limited available historical control values. Based on these considerations, the combined adenomas and carcinomas were considered treatment-related at ≥500 ppm (≥75 mg/kg/day) in females.

The CARC considered the high dose in males excessive based on poor condition/moribundity during the second year of dosing. Dosing was considered adequate in males at the low and mid-dose and in females at all doses, based on liver histopathology at the mid and high dose in both sexes. In males, hepatocellular necrosis and hypertrophy were observed. Sperm stasis was also observed in males at the mid and high doses. In females, an increased incidence of eosinophilic foci was observed at mid and high doses and of basophilic foci at the high dose.

2. *Reference: Beems, R., Til, H., Van der Heijden, C. (1976) Carcinogenicity Study with Carbendazim in Mice. Study No. R4930, September 1976, Central Institute for Nutrition and Food Research, Hoechst Aktiengesellschaft, Frankfurt, FRG. Unpublished report. MRID 00153420.*

In an oral carcinogenicity study (MRID 00153420), MBC (99% a.i.) was administered to 100 SPF Swiss mice/sex/dose at dietary levels of 0, 150, 300, or 5000 ppm (0, 23, 45, or 750 mg MBC/kg/day) for 80 weeks. An interim sacrifice group was not included in the study design.

There were no treatment-related clinical signs of toxicity or effects on mortality or body weight. Survival at Week 80 (control to high dose, respectively) was 64, 65, 70, and 70% (males) and 79, 78, 77, and 80% (females). The incidence of liver neoplastic findings is shown in Table 10. Hepatoblastoma, considered a rare liver tumor type, was increased in males at the high dose of 5000 ppm (750 mg/kg/day). The incidence of neoplastic nodule (adenoma) was significantly increased at the high dose in females.

Table 10. Incidence of Liver Tumors in SPF Swiss Mice Administered MBC in the Diet for 80 Weeks.

	Dietary dose in ppm (mg/kg/day)			
Liver tumor type	**0**	**150 (23)**	**300 (45)**	**1000/5000 (750)**
Males				
Neoplastic nodule	9/100	7/98	14/100	16/100
(%)	(9)	(7)	(14)	(16)
Carcinoma	1/100	1/98	9/100	3/100
(%)	(1)	(1)	(9)	(3)
Hepatoblastoma	0/100	1/98	1/100	7/100*
(%)	(0)	(1)	(1)	(7)
Total	10/100	8/98	16/100	17/100
(%)	(10)	(8)	(16)	(17)
Females				
Neoplastic nodule	0/97	1/99	1/98	9/97*
(%)	(0)	(1)	(1)	(9)
Carcinoma	1/97	0/99	0/98	0/97
(%)	(1)	(0)	(0)	(0)
Hepatoblastoma	0/97	0/99	0/98	0/97
(%)	(0)	(0)	(0)	(0)
Total	1/97	1/99	1/98	9/97
(%)	(1)	(1)	(1)	(9)

Table extracted from Third Cancer Peer Review (TXR 0050625, J. Quest, 04/07/1989).
* $p<0.01$ using Exact Test. [note: tumor nomenclature and statistical notation copied exactly from TXR 0050625, J. Quest, 04/07/1989).
Neoplastic nodule = adenoma.

The non-neoplastic liver microscopic findings are summarized below in Table 11 based on data presented in the study data evaluation record (DER):

Table 11. Hepatocellular Non-Neoplastic Lesions in SPF Swiss Mice Administered MBC in the Diet for 80 Weeks.

	Dietary dose in ppm (mg/kg/day)			
Liver/microscopic lesion	**0**	**150 (23)**	**300 (45)**	**1000/5000 (750)**
Males				
Hepatocellular foci				
Clear	0/98	0/98	1/100	5/100*
Mixed	1/100	6/98	6/100	10/100**
Females				
Hepatocellular foci				
Clear	0/97	1/99	0/98	8/97**
Mixed	0/97	1/99	0/98	0/97

Data extracted from Table 4, p. 20, MRID 00153420. * $p<0.05$ ** $p<0.01$

The study is Unacceptable because descriptions of the methods were incomplete, there were no non-neoplastic microscopic or gross pathology data for individual animals, and no assurance that

the diets were analyzed for compound homogeneity and stability. In addition, hematology, clinical chemistry, and urinalysis were not conducted. Only tissues with gross lesions suspected of being tumors and livers (2 sections per liver) were examined histologically.

3. *Reference: Donaubauer (1982) Repeated-dose (24-month) Feeding Study for Determination of the Cancerogenic Effect of HOE 17411 OF AT204 (Carbendazim) in Mice. Report no. 643/82, study no. 606, Hoechst Aktiengesellschaft. Unpublished Report. MRID 00154679.*

In an oral carcinogenicity study (MRID 00154679), MBC (99% a.i.) was administered to 100 NMRKf mice/sex/dose at dietary concentration levels of 0, 50, 150, 300, or 1000/2000/5000 ppm for 96 weeks. 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, continuing until study termination. An additional 20 mice/sex at 0 and 1000/2000/5000 ppm were treated for 18 months (interim sacrifice groups). Dietary intake was reported to be equivalent to 0, 5.8, 17.1, 34.4, or 522 mg/kg/day for males and 0, 7.1, 21.2, 41.9, or 648 mg/kg/day for females, respectively. This study was designed specifically to evaluate the finding of liver carcinogenicity, and other tissues were not subjected to a gross or microscopic examination.

There were no treatment-related clinical signs of toxicity or effects on survival. In the main groups, survival (control to high dose, respectively) was 69, 76, 71, 71, and 69% (males) and 48, 50, 63, 58, and 54% (females).

At the high dose (522/648 mg/kg/day male/females), liver toxicity was observed in both sexes which consisted of increased incidences of hepatocellular hypertrophy, clear cell foci, and hepatocellular necrosis (Table 12). The DER noted that the severity (but not incidence) of the necrosis/hypertrophy showed an increase with duration of exposure: "Animals at 10 months had centrilobular hypertrophy and single cell necrosis. Those sacrificed at term showed more marked hypertrophy (centrilobular extending to intermediate areas) along with single cell necrosis. There were also areas of necrosis with scarring and fibrosis in many of the high dose term animals."

Table 12. Percent (%) Incidence of Non-Neoplastic Hepatocellular Lesions in NMRKf Mice Treated with MBC in the Diet

Hepatocellular microscopic lesion	**Dietary Dose in ppm (mg/kg/day M/F)**				
	0	**50 (6/7)**	**150 (17/21)**	**300 (34/42)**	**1000/5000 (522/648)**
Males					
22 month (96 weeks) sacrifice group					
N =	**82**	**88**	**84**	**80**	**80**
Necrosis	1	7	8	11	6
Hypertrophy + necrosis	0	0	0	0	56
Hypertrophy alone	0	0	0	0	21
N =	**97**	**99**	**99**	**95**	**97**
Clear cell foci	0	0	0	0	3
18-month sacrifice					
N =	**20**	**-**	**-**	**-**	**20**
Necrosis	0	-	-	-	5
Hypertrophy + necrosis	0	-	-	-	65
Hypertrophy alone	0	-	-	-	35
Clear cell foci	0	-	-	-	0

Hepatocellular microscopic lesion	Dietary Dose in ppm (mg/kg/day M/F)				
	0	**50 (6/7)**	**150 (17/21)**	**300 (34/42)**	**1000/5000 (522/648)**
Females					
96 week (22 month) sacrifice					
N =	**85**	**93**	**78**	**82**	**75**
Necrosis	11	8	11	16	16
Hypertrophy + necrosis	2	0	0	0	60
Hypertrophy alone	2	0	0	0	16
N =	**98**	**98**	**95**	**95**	**95**
Clear cell foci	0	0	0	0	4
18-month sacrifice					
N =	**20**	**-**	**-**	**-**	**20**
Necrosis	0	-	-	-	5
Hypertrophy + necrosis	0	-	-	-	55
Hypertrophy alone	0	-	-	-	25
Clear cell foci	0	-	-	-	15

Summary data obtained from DER, TXR 0005531 (extracted from MRID 00154679). Not analyzed statistically.

Because of reported limitations in the analyses of MBC test diets and an incomplete gross and microscopic assessment, this study is Unacceptable. In addition, blood and urine were not collected.

The CARC concluded that the mouse carcinogenicity studies of Beems et al. (1976) and Donaubauer et al. (1982) were <u>not</u> sufficiently reliable to include in the cancer WOE considerations for MBC due to their study limitations and classification as unacceptable studies. The studies were taken into consideration in the previous cancer assessments, despite their shortcomings, to provide additional data on the mouse liver tumor response. The CARC noted the significant deficiencies in study design and conduct, which included uncertainties regarding dietary analyses and actual doses received, lack of individual animal data, and/or incomplete histopathological examination. These deficiencies were considered sufficient to exclude the studies from the WOE evaluation.

E. <u>Summary of Available Rat Carcinogenicity Studies</u>:

1. *Reference: Sherman, H., et al. (1972) Long-Term Feeding Studies in Rats with 2-Benzimidazole Carbamic Acid Methyl Ester [INE-195]. Study Report 195-72. Haskell Laboratories, Unpublished report. MRID 00088333.*

In an oral chronic toxicity/carcinogenicity study (MRID 00088333), 36 CRL:CD1 rats/sex/dose were administered carbendazim (INE-195 or MBC; 53% a.i., lot nos. Haskell 5793-2 and -3; to 72% a.i.; lot nos. Haskell 5061, 5793) in the diet at concentrations of 0 (two control groups of 36/sex), 100, 500, 5000, or 2500/7500/10,000 ppm (time-weighted average 8557 ppm). The high dose group was increased to 7500 ppm at 18 weeks and to 10,000 ppm from week 20 to study termination. Six rats/sex/dose were assigned for a one-year interim sacrifice. An additional group of 20 rats/sex/dose administered 5000 ppm MBC was added at 3 weeks into the study and continuing to the 24-month termination, with no pretreatment measurement of clinical parameters. The dietary concentrations of 0, 100, 500, 5000, and 2500/7500/10,000 ppm were equivalent to average daily intakes of 0, 5, 25, 250, or 125/500 (430) mg/kg/day, respectively.

There were no observed treatment-related clinical signs of toxicity, nor effects on mortality. At study termination, survival (control to high dose, respectively) was 47, 53, 43, 50, and 60%

(males) and 67, 47, 70, 53, 60, and 67% (females).

There was no evidence of treatment-related carcinogenicity (including the liver) reported in this study.

In males and females at high dose, mean body weight was decreased (not statistically significant) from about month 19-20 to termination compared to controls (10-16%). Females at the mid dose showed a more pronounced decrease (14-21%) than the high dose group during that time. At the mid- and high-dose, statistically significant decreases in red blood cell counts, hemoglobin and hematocrit values (14-20% below controls) were reported in females at 24 months. In males, non-significant decreases (10-21%) were observed at 24 months. There were no treatment-related effects on clinical chemistry, urinalysis, organ weight or gross pathology. In the histopathological evaluation, a slight increase in the severity of cholangiohepatitis and pericholangitis was observed in both sexes at the highest two dose levels (1.5-1.7 vs 1.0-1.2, control to mid dose, males; 1.9 vs 1.1-1.6, control to mid dose, females) (Table 14). The incidence, but not severity, of prostatitis was slightly higher at the high dose (21%, grade 1.3) than in the two control groups (11-12%, grade 1-2.3). Dosing was considered adequate based on decreased body weight in both sexes, hematological changes in females and slight liver/bile duct histopathology in both sexes.

The study is classified as acceptable/nonguideline due to the reduced number of animals tested per dose group and lack of complete individual animal data for several of the evaluations conducted in this study. Individual animal histopathology tables were provided.

2. *Reference: Til HP, Koellen C, & van der Heijden CA (1976) Combined chronic toxicity and carcinogenicity study with carbendazim in rats. The Hague, Central Institute for Nutrition and Food Research (TNO) (Unpublished report prepared for BASF AG, Ludwigshafen and Hoechst AG, Frankfurt) (summarized in WHO EHC, 1993 and in APVMA, 2008/09). Not submitted to the Agency-only study summaries are available.*

In an oral chronic toxicity/carcinogenicity study (not submitted to the Agency; available only as study summaries in WHO EHC, 1993, and APVMA, 2008/2009), 60 Wistar rats/sex/dose were administered MBC (99% a.i.) for two years at dietary concentrations of 0, 150, 300, or 2000 ppm (week 1)/5000 ppm (week 2)/10,000 ppm (week 3 through study termination). The administered dose levels were stated to be equivalent to average daily intakes of 0, 7.5, 15, or 100/250/500 mg/kg/day, respectively). The high dose was administered at 500 mg/kg/day for all but the initial two weeks of the study. Parameters evaluated throughout the study included daily clinical evaluations, regular measurement of body weight and food consumption (exact frequency was not specified), hematology, clinical chemistry, and urinalysis (26, 52, 103 weeks). All animals were necropsied, and weights were measured for unspecified organs (except for liver). Twenty rats/sex/dose were selected at termination for histopathological examination; a list of tissues was not provided. In addition, all tumors and gross abnormalities were examined. Information regarding the magnitude of effects as reported in the available summaries for this study is very limited.

Survival was reportedly unaffected by treatment. The summary noted that in males, 50%

mortality was observed at week 76 for controls and at week 92 for treatment groups. In females, 50% mortality was observed at week 88 in the control and low dose groups and at weeks 92 and 96 for the mid and high dose groups.

There were no reported increases in tumor incidence in males or females.

Clinical signs of toxicity, food consumption and urinalysis showed no effect of treatment. Decreased body weight was reported in males at the low dose (week 88 to termination) and females at the high dose (week 12 to termination), but the magnitude of the change was not provided. Other effects noted at the high dose included a slight decrease in hemoglobin in females (5%, 5%, and 12% below controls at 26, 52, and 103 weeks, respectively) and at termination, decreased hemoglobin in females, a reduction in serum AST activity in males (-25%), increased ALT and decreased serum total protein in females, and increased relative liver weights in females (10-15%). The only histopathological change reported was an increase in the incidence and severity of diffuse proliferation of thyroid parafollicular cells in females at 500 mg/kg/day.

Based on the limited information in the two available study summaries, adequacy of dosing cannot reliably be determined.

The CARC concluded that there were no treatment-related tumors following MBC exposure in the rat. Dosing in CD-1 rats was considered adequate based on decreased body weight and cholangiohepatitis/pericholangitis in both sexes and mild hematological effects in females. Although adequacy of dosing could not be established conclusively in the Wistar rat due to lack of study details, the CARC considered the second study, which tested up to 500 mg/kg/day, supportive of the lack of carcinogenicity in the CRL:CD1 rat.

III. TOXICOLOGY

A. Metabolism

MBC is well absorbed and extensively metabolized in the rat following a single low (50 mg/kg) or high (1000 mg/kg) oral dose or repeated low (50 mg/kg/day) oral dosing. Biliary excretion was not measured, but at 50 mg/kg, urinary excretion, together with the metabolite profile of the feces, indicated that about 80% of the administered dose (AD) was absorbed. Pharmacokinetic (PK) data were not collected in this study; therefore, a T_{max} was not determined, but absorption appeared to be rapid, based on the estimated excretion half-life of 12 hrs. Tissue bioaccumulation was not observed: at 72 hr after dosing, about 1% AD remained in tissues and carcass at the low dose, and ≤0.7% AD remained at the high dose. The highest tissue levels of MBC at 72 hr post-dosing were found in the liver, followed by the kidney, skin, GI tract, blood, and lungs. At the single or repeated low dose, excretion was primarily via the urine (54-66% AD) with the remainder excreted in the feces (24-38% AD). At the high dose, the percentage of excretion in the urine was lower (41% AD), and fecal excretion was greater (62-69%), indicating saturation of absorption or urinary excretion with increased dose. For all dosing groups, by 72 hr post-dosing, 87-100% AD was excreted, with the majority of this radioactivity (60-90% AD) excreted by 24 hr. The primary route of metabolism was oxidation of the phenyl ring, followed by sulfate

or glucuronide conjugation. The major urinary metabolite in males at all dose levels and females at low dose was 5-hydroxy benzimidazole carbamate sulfate (5-OH-BC-S), while the major metabolite in the feces, at all dose levels and both sexes, was 5-OH-BC. Subsequent phenyl ring oxidation and N-oxidation at the imidazole nitrogen yielded the metabolite 5,6-hydroxy-oxocarbendazim N-oxide glucuronide, which was more prevalent in females than in males. In females at the high dose and repeated low doses, this metabolite was present in the urine at higher amounts than 5-OH-BC-S. In the feces, unmetabolized MBC represented 1-2.8% AD at the low dose and 10-15% AD at the high dose, consistent with saturation of absorption or metabolism.

B. Mutagenicity:

Overview: In addition to studies submitted by the Registrant to the Agency, the genotoxicity of MBC has been extensively evaluated in the open scientific literature. Aneugenicity due to inhibition of tubulin polymerization and disruption of mitotic spindle formation during cell division is generally accepted to be the genotoxic MOA for MBC and other benzimidazole fungicides in both fungi, as the fungicidal MOA, and in mammalian cells.

Current Agency conclusions on MBC genotoxicity: Similar genotoxicity profiles are observed for MBC and benomyl. The previous cancer assessments reviewed both compounds together and concluded, based on the submitted genotoxicity data (see table below), that both MBC and benomyl alter mitotic spindle structure and chromosome segregation and are unlikely to cause direct DNA damage (quote extracted from first Cancer Peer Review memorandum, p. 10, TXR 0005576, J. Quest, 03/31/1986):

> "Benomyl and/or MBC produced positive mutagenic effects that were consistent with adverse effects on the cellular spindle apparatus. These included nondisjunction in *A. nidulans,* sister chromatid exchange in CHO cells, and micronuclei formation in mouse bone marrow cells. In contrast, equivocal results (both positive and negative findings) for gene mutation were found in Ames tests and mouse lymphoma tests, and negative results for DNA repair were found in primary rat and mouse hepatocyte cultures. The pattern of mutagenicity results appeared to correlate poorly with heritable spindle effects or point mutagenicity."

Genotoxicity studies submitted to the Agency. The submitted genotoxicity studies for MBC (summarized in Table 13) were considered in previous cancer assessments. The majority of bacterial reverse gene mutation assays and a forward gene mutation assay in CHO cells were negative. Some positive results were reported in a bacterial gene mutation assay and mouse lymphoma cell gene mutation assays conducted in the late 1970's - early 1980's. However, the registrant provided information indicating that low levels of impurities were present in some batches of MBC due to the manufacturing process used at the time. The impurities, 2,3-diaminophenazine (DAP) and 2-amino-3-hydroxyphenazine (AHP), are both mutagenic, but purified MBC is not mutagenic. Changes to the manufacturing process have since eliminated the presence of these compounds. MBC did not induce unscheduled DNA synthesis in rodent hepatocytes or human primary lung tumor cells, or increase DNA repair in *Bacillus subtilis.* Clastogenicity was not observed in CHO cells.

MBC inhibits fungal growth by disruption of microtubule polymerization and spindle assembly during cell division, thereby affecting spindle formation and resulting in inaccurate chromosomal segregation and distribution in daughter cells. This genotoxic MOA is applicable to mammalian cells as observed in rodent *in vivo* assays. Micronucleus assays in mouse bone marrow (*in vivo)* were positive for MBC. Of note, the studies identified doses of 50 to 66 mg/kg that were identified in *in vivo* micronucleus assays where no numerical chromosome effects were observed.

Table 13. Summary of Genotoxicity Studies Submitted in Support of MBC Registration

ASSAY TYPE	STUDY DETAILS	RESULTS
OPPTS §870.5100 Bacterial reverse gene mutation *Salmonella typhimurium*	MRID 00154668 (1983) TXR 0005531 Acceptable/guideline 100-5000 µg/plate +/- mammalian liver S9 metabolic activation MBC tech., 99% a.i.	Negative. Not mutagenic with or without S9 activation in strains TA1535, TA97, TA100 or TA98.
OPPTS §870.5100 Bacterial reverse gene mutation *Salmonella typhimurium*	MRID 00154669 (1983, 1986) TXR 0005531 Acceptable/guideline 100-5000 µg/plate +/- mammalian liver S9 metabolic activation MBC tech., 99% a.i.	Negative. Not mutagenic with or without S9 activation in strains TA1535, TA97, TA100 or TA98.
OPPTS §870.5100 Bacterial reverse gene mutation *Salmonella typhimurium* *Escherichia coli*	MRID 00154670 (1977) TXR 0005531 Acceptable/guideline 100-5000 µg/plate +/- mammalian liver S9 metabolic activation MBC tech., 99% a.i.	Negative. Not mutagenic with or without S9 activation in strains TA1535, TA97, TA100 or TA98 and *E. coli* strain WP2hcr-.
OPPTS §870.5100 Bacterial reverse gene mutation *Salmonella typhimurium*	MRID 00151825 (1977) TXR 0005531 Acceptable/guideline 200 – 10,000 µg/plate +/- mammalian liver S9 metabolic activation MBC, no purity information	**Positive without S9 activation in strains TA1537, TA1538 and TA98.** Negative in strains TA1535 and TA100 with or without S9 activation.
OPPTS §870.5100 Bacterial reverse gene mutation *Salmonella typhimurium*	MRID 00154753 (1983) TXR 0005531 Acceptable/guideline 100-10,000 µg/plate +/- mammalian liver S9 metabolic activation MBC, no purity information	Negative. Not mutagenic with or without S9 activation in strains TA1535, TA1537, TA97, TA100 or TA98.

ASSAY TYPE	STUDY DETAILS	RESULTS
OPPTS §870.5100 Bacterial reverse gene mutation *Salmonella typhimurium*	MRID 43205504 (1992) TXR 0011116 Acceptable/guideline 5000 µg/plate +/- mammalian liver S9 metabolic activation MBC tech., 99.7% a.i.	Negative. Not mutagenic with or without S9 activation in strains TA1535, TA97, TA100 or TA98 and *E. coli* strain WP2 uvrA +/-.
OPPTS §870.5100 Bacterial host-mediated reverse gene mutation *Salmonella typhimurium* Bacterial host-mediated assay of *Salmonella typhimurium* with ICR male mouse as host	MRID 00154670 (1977) TXR 0005531 Acceptable/guideline 500 or 2000 mg/kg once daily for two days via gavage MBC tech. 99% a.i.	Negative. Not mutagenic in the host-mediated assay with ICR male mice administered MBC and *Salmonella typhimurium* strain G46 (his-) as the indicator organism.
OPPTS §870.5300 Mammalian *in vitro* cultured cell forward gene mutation assay Chinese hamster ovary cell cultures, forward gene mutation at HGPRT locus	MRID 00154671 (1980) TXRs 0003744, 0005531 Acceptable/guideline 3-628 µM (120 µg/mL) - S9 activation 3-654 µM (125 µg/mL) with S9 activation MBC tech., 100% a.i.	Negative for forward gene mutation at all doses tested with or without S9 activation. Precipitation observed at ≥262 µM with and without S9 activation.
OPPTS §870.5300 Mouse lymphoma L5178y $TK^{+/-}$ forward gene mutation assay	MRID 00159370 (1983) TXR 0005531 Acceptable/guideline 50-250 µM (about 10-50 µg/mL) without S9 25-250 µM (about 5-25 µg/mL) with S9 MBC tech., >98% a.i.	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.** Additional information from the registrant indicated that positive results were from the presence of mutagenic contaminants from the manufacturing process at the time and not to MBC.
OPPTS §870.5300 Mouse lymphoma L5178y $TK^{+/-}$ forward gene mutation assay	MRID 00154674 (1983) TXR 0005531 Unacceptable/guideline 25-200 µM with and without S9 MBC tech., 100% a.i.	Negative at 25-200 µM with and without S9. Study classified as unacceptable due to lack of evidence of cytotoxicity or test material precipitation. Noted that (1) cytotoxicity was observed at 180 µM in one preliminary study but not a second and (2) reported MBC purity is higher than studies with positive results.

ASSAY TYPE	STUDY DETAILS	RESULTS
OPPTS §870.5300 Mouse lymphoma L5178y $TK^{+/-}$ forward gene mutation assay	MRID 00154673 (1980) TXRs 0005531, 0004679 Acceptable/guideline 5-50 µg/ml without S9 activation; 2-25 µg/ml with S9 activation MBC tech., 98% a.i.	**Positive for increases in mutation frequency at 50 µg/ml without S9; dose-dependent increases in mutation frequency at 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. MBC was weakly mutagenic.** Additional information from the registrant indicated that positive results were from the presence of mutagenic contaminants from the manufacturing process at the time, and not to MBC.
OPPTS §870.5375 Mammalian *in vitro* chromosomal aberration assay Chinese hamster ovary cells, cytogenetic assay	MRID 43205505 (1990) TXR 0011116 Acceptable/guideline 38 - 300 µg/mL in culture medium +/- S9 activation MBC, no purity information	Negative. No induction of chromosomal aberrations up to a precipitating and cytotoxic dose (300 µg/mL) with or without S9 activation.
OPPTS §870.5395 Mammalian *in vivo* chromosomal aberration assay Chinese hamster micronucleus assay	MRID 41051510 (1976) TXR 0004679 Unacceptable/guideline 500 mg/kg (intraperitoneal injection) or 50, 100, 500 or 1000 mg/kg once daily for two consecutive days (oral gavage) MBC, no purity information	Negative via intraperitoneal injection. **Positive via oral gavage. Dose-related increases in micronucleated polychromatic erythrocytes (MPEs) and micronucleated normochromatic erythrocytes (MNEs) were observed at 100 – 1000 mg/kg.** Study classified as unacceptable due to inadequate reporting of methodology and results. No effects at 50 mg/kg at 24 hr.
OPPTS §870.5395 Mammalian *in vivo* chromosomal aberration assay Mouse bone marrow micronucleus assay with immunohistochemistry using anti-kinetochore antibodies.	MRID 42911602 (1992) TXR 0010723 Acceptable/guideline 66, 1646, 3293 mg/kg single oral dose with sacrifice at 48 hr post-dose MBC tech., 99.3% a.i.	**Positive for total micronuclei and kinetochore-positive micronuclei at mid and high dose in females and high dose in males.** Cytotoxicity in the bone marrow was observed at mid dose in females and high dose in both sexes. No effects were observed at 66 mg/kg/day.

ASSAY TYPE	STUDY DETAILS	RESULTS
OPPTS §870.5500 Bacterial DNA damage/repair *B. subtilis* DNA damage/repair assay	MRID 00154670 (1977) TXR 0005531 Acceptable/guideline MBC tech., 99% a.i.	Negative. No increase in DNA damage or repair in *B. subtilis* strains H17 or M45.
OPPTS §870.5550 Unscheduled DNA synthesis *In vitro* unscheduled DNA synthesis assay in mouse primary hepatocyte cultures	MRID 00154754 (1981) TXR 0005531 Acceptable/guideline 0.0125 – 12.5 µg/mL MBC, no purity information	Negative. No treatment-related increase in unscheduled DNA synthesis in primary mouse hepatocyte cultures was observed.
OSPPTS §870.5550 Unscheduled DNA synthesis *In vitro* unscheduled DNA synthesis assay in rat primary hepatocyte cultures	MRID 00154672 (1981) TXR 0005531 Acceptable/guideline 0.0125 – 12.5 µg/mL MBC, no purity information	Negative. No treatment-related increase in unscheduled DNA synthesis in primary rat hepatocyte cultures was observed.
OPPTS §870.5550 Unscheduled DNA synthesis *In vitro* unscheduled DNA synthesis assay in human primary lung carcinoma cell cultures	MRID 43205506 (1992) TXR 0011116 Acceptable/guideline 0.3 – 300 µg/mL culture medium, +/- S9 activation MBC tech., 99.7% a.i.	Negative. No treatment-related increase in unscheduled DNA synthesis in primary human lung carcinoma cell cultures was observed up to precipitating (30 µg/mL) and cytotoxic (100 µg/mL) concentrations.

McCarroll *et al.* (2002) comprehensive survey of genotoxicity data: The results of genotoxicity studies on MBC and benomyl (up to the year 2001) were reviewed by Agency toxicologists in this survey article, including studies submitted to the Agency as well as reports published in the open scientific literature. The findings were analyzed to generate a genetic activity profile (GAP) using methodology jointly developed by the Agency and the International Agency for Research on Cancer (IARC). The review concluded that:

> "Both agents [MBC and benomyl] displayed consistent, positive result for aneuploidy induction but mostly negative results for gene mutations. Non-linear dose responses were seen both *in vitro* and *in vivo* for aneuploidy endpoints. No evidence was found suggesting that an alternative [genotoxicity] MOA other than aneuploidy may be operative." (p. 321 of the report)

Open literature studies: The Registrant did not provide a detailed review of data supporting the proposed aneugenic MOA or exploring alternative MOAs, relying instead on the IPCS framework analysis of McCarroll *et al.* (2002) and several reviews of MBC prepared by other entities (Table 1). McCarroll *et al.* (2002) provided a comprehensive bibliography of the data available at that time on the genotoxicity and carcinogenicity of MBC, which was reviewed by Agency scientists with expertise in those areas. For the current cancer reevaluation, an updated

search of the open literature for MBC was conducted[4] to identify newer or additional studies relevant to the key events of the proposed aneugenic MOA as outlined in McCarroll *et al.* (2002).

OPP's Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment[5] was used to screen and identify acceptable studies for detailed review. Studies were screened to identify reports summarizing the results of well-conducted independent research with information on test material purity, adequate descriptions of methodology and data presentation, and findings relevant to the proposed aneugenic cancer MOA. Abstracts of the 366 studies identified in the search were first screened for relevance to the proposed aneugenic MOA and acceptability for full-text screening. Of the 47 studies selected for full-text screening, six were selected for detailed review (Table 14). The remaining studies either did not fully meet the OPP Guidance criteria, were acceptable for review but did not directly bear on the aneugenic MOA or were acceptable for review but presented findings consistent with other studies selected for review. Three of these selected studies were cited in McCarroll *et al.* (2002), and two of the remaining three were published after 2002. These studies supplemented the findings of the guideline genotoxicity studies submitted to the Agency and informed the proposed aneugenic MOA framework analysis. Data from these studies are discussed in greater detail in the Mode of Action section, below (Section IV).

Comet assays (single cell gel electrophoresis to detect DNA strand breakage) did not show evidence of damage to DNA in liver cells from rats exposed *in vivo* to MBC (Bowen *et al.,* 2011) or cultured Chinese hamster ovary (CHO) cells (Vigreux *et al.,*1998). The study by Bowen *et al.* demonstrated positive responses for micronucleus formation (bone marrow) and aneuploidy (peripheral blood) at or above the limit dose, while Vigreux *et al.* demonstrated numerical chromosome abnormalities, but not gaps or breaks (clastogenicity) in treated CHO cells.

Barale *et al.* (1993) demonstrated a time-dependent increase in micronucleus formation and aneuploidy (hyperdiploidy and polyploidy) without significant increases in chromosomal breaks or gaps in an *in vivo* mouse bone marrow micronucleus assay. Formation of micronuclei occurs rapidly: peak effects were recorded between 36-48 hr, but induction of micronuclei and aneuploidy was seen as early as 6-12 hr. Bentley *et al.* (2000) evaluated aneuploidy in cultured human lymphocytes and identified a dose response and threshold doses of MBC for chromosomal non-disjunction and loss below which effects were not observed, consistent with an indirect, non-linear genotoxic response. The study also demonstrated induction of aneuploidy in human cells.

The study by Can and Albertini (1997) evaluated spindle and chromosomal organization of a single test concentration of MBC (30 µM) in cultured human granulosa cells by immunohistofluorescent staining of nucleic acid, tubulin, centromeres, and associated spindle components after a 3 to 15 hr exposure. The study visually demonstrated abnormal spindle

[4] Date and Time of Search: 06/08/2022; 02:45 pm. Search Details: ((*Carbendazim*) OR ("Methyl 2-benzimidazolecarbamate")) AND (rat OR mouse OR dog OR rabbit OR monkey OR mammal). PubMed hits: 366. Number of Swift Articles: 236 for Animal. Number of Swift Articles: 227 for Human. Number of Swift Articles: 0 for No Tag.

[5] https://www.epa.gov/sites/default/files/2015-07/documents/lit-studies.pdf

microtubule dynamics and chromosomal disorganization during cell division in mammalian cells, leading to a time-dependent increase in tri- to multipolar spindles.

Igarashi *et al.* (2007) examined micronucleus formation in liver following exposure of mice to MBC. Mice were treated with MBC by gavage at single doses between 125 to 1000 mg/kg/day following a partial hepatectomy, performed to stimulate hepatocellular division. The assay detected an increase in micronucleus formation and multinucleated cells in the liver in animals sacrificed six days post-dosing.

Table 14. Summary of Open Literature Reviews Reviewed for this Reevaluation

ASSAY TYPE	STUDY DETAILS	RESULTS
Open literature study Barale *et al.* (1993) Cytogenetic effects of benzimidazoles in mouse bone marrow. *Mutation Research* 300:15-28. Male Swiss albino mice, bone marrow micronucleus assay and cytogenetic assay	MRID 51975401 (1993) TXR 0058605 Acceptable/nonguideline MBC – 0.5 g/kg bw single gavage dose in DMSO vehicle Benomyl – 1.0 g/kg bw single gavage dose in DMSO vehicle Colchicine injected 2 hr before sacrifice. Both evaluated for micronuclei (MN) induction at 0, 6, 12, 18, 24, 30, 36, 42 and 48 hr post-dosing MBC tech., 95% a.i. (benomyl 99% a.i.)	Positive for aneugenicity. Both MBC and benomyl induced an increase in micronucleus formation and aneuploidy (hyperdiploidy and polyploidy), showing a fairly constant time-dependent increase in micronuclei, without significant effects on chromosome breaks or gaps. Maximum induction of micronuclei was observed at 36-42 hr and decreased at 48 hr; maximum induction of hyperdiploid and polyploid cells was observed at 42-48 hr. Slight effects were observed as early as 6-12 hours following dosing. **Demonstrated a time-dependent increase in micronuclei and aneuploidy with no significant effect on chromosomal breaks or gaps.**
Open literature study Bentley *et al.* (2000) Evaluation of thresholds for benomyl- and carbendazim-induced aneuploidy in cultured human lymphocytes using fluorescence *in situ* hybridization. *Mutation Research 464:41-51.* Human peripheral blood lymphocytes stimulated with phytohemagglutinin, micronucleus assay and fluorescence *in situ* hybridization assay	MRID 51975402 (2000) TXR 0058605 Acceptable/nonguideline MBC - 0 (control) and 20 concentrations between 21 to 5000 ng/mL Benomyl – 0 (control) and 18 concentrations between 25 to 3293 ng/mL Cells treated for 24 hr, then cytochalasin B added, then cells harvested at 48 hr (total 48 hr exposure to test material, 24 hr to cytochalasin B) Fluorescence *in situ* hybridization evaluated organization of microtubules, centrosomes (chromosome-specific), spindle-associated proteins and DNA using fluorescent centromeric probes MBC tech., 97% a.i. (benomyl 95% a.i.)	Positive for aneugenicity as measured in individual chromosomes. For MBC and benomyl, a threshold concentration for chromosomal non-disjunction was 600 mg/mL (1100 ng/mL for benomyl). Threshold concentrations for chromosome loss (chromosomes 1, 8, 11, 17,18, X) were equal to or greater than the non-disjunction thresholds. Centromeric positive micronuclei were assessed in benomyl only but demonstrated a similar threshold response as the other parameters. **Demonstrated a threshold concentration *in vitro* in human cells for centromeric positive micronuclei, chromosomal non-disjunction and chromosomal loss.**

ASSAY TYPE	STUDY DETAILS	RESULTS
Open literature study Bowen *et al.* (2011) Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the comet assay and the flow-cytometric peripheral blood micronucleus test. *Mutation Research* 722:7-19. Han Wistar or Sprague Dawley male rats	MRID 51975403 (2011) TXR 0058605 Acceptable/nonguideline 0, 1000, 1500, 2000 mg/kg bw by gavage in 1% aqueous methylcellulose vehicle, administered at 0, 24 and 45 hr with sacrifice at 3 hr post-dosing for the comet assay and bone marrow micronucleus formation by and 3, 24 and 44 hr post-dosing for the flow-cytometric micronucleus assay. Comet assay evaluated liver, stomach and blood. Flow-cytometric micronucleus assay evaluated peripheral blood. Analytical grade MBC; exact purity not given but >99% based on Sigma Aldrich analytical grade criteria.	Negative for DNA damage in the comet assay in the liver, stomach and blood at all doses tested. Positive for bone marrow micronucleus formation at 3 hr post-dosing at all doses tested. Positive for aneugenicity in the flow-cytometric peripheral blood micronucleus assay. Observed at all dose levels at 3 hr and at 1000 mg/kg at 24 and 44 hr post-dosing due to cytotoxicity at 1500 and 2000 mg/kg. **Demonstrated lack of DNA damage in liver and other tissues along with positive results for *in vivo* micronucleus formation in bone marrow and aneugenicity in peripheral blood cells by 3 hr post-dosing.**
Open literature study Can and Albertini (1997) M-phase specific centrosome-microtubule alterations induced by the fungicide MBC in human granulosa cells. *Mutation Research* 373:139-151. Human ovarian granulosa cells	MRID 51975404 (1997) TXR 0058605 Acceptable/nonguideline 30 µM (6 µg/mL culture medium) in DMSO vehicle, 12 hr treatment of 3-day cultures followed by a 9-day culture (mitotic index evaluation). Based on this assay, 3-day cultures were selected for fuse in immunofluorescence studies because they had the highest mitotic index; or 3, 6, 9, 12 or 15 hr treatment of 3-day cultures (immunofluorescent staining). Immunohistofluorescent staining of nucleic acid, tubulin, centromeres and centromere-associated proteins conducted to visualize spindle organization and chromosomal distribution. Tech., 99.7% a.i. MBC	Immunohistofluorescent staining of α-tubulin, acetylated α-tubulin, and nuclear proteins associated with the mitotic spindle (NuMA, centrophilin) demonstrated disruption of spindle formation with tri- to multi-polar spindles, chromosomal mal-segregation, and interference with centrosome organization during mitosis in human granulosa cells treated with MBC. Abnormal chromosomal organization due to disruption of microtubule and centrosome organization were observed during mitosis. An increased number of spindle poles associated with chromosomes displaced from the metaphase plate and increase from tri- to multi-polar spindles was observed. No effect on non-dividing cells. **Visually demonstrated disruption of normal spindle organization and resulting chromosomal mal-segregation in cells treated with MBC.**

ASSAY TYPE	STUDY DETAILS	RESULTS
Open literature study Igarashi *et al.* (2007) Optimum conditions for detecting hepatic micronuclei caused by numerical chromosome aberration inducers in mice. *Mutation Research* 632:89-98. Measurement of micronucleus formation in liver of Slc:ddY male mice following partial hepatectomy (PH) and dosing with MBC.	MRID 51975405 (2007) TXR 0058605 Acceptable/nonguideline 0, 125, 250, 500 or 1000 mg/kg bw MBC by gavage (0.5% aqueous methylcellulose vehicle), single dose on the day before or the day after PH; sampling on day 6. Tech., 99.9% a.i. MBC	Positive for increased induction of micronuclei in hepatocytes following PH at 500 mg/kg MBC. Increased binucleated cells were observed at 250 mg/kg; multinucleated cells were increased at 500 mg/kg. The study demonstrated aneuploidy in the liver of mice following oral exposure to MBC. **Demonstrated potential of MBC to cause aneuploidy in mouse liver following *in vivo* exposure and stimulation of hepatocellular proliferation.**
Open literature study Vigreux *et al.* (1998) DNA damaging effects of pesticides measured by the single cell gel electrophoresis assay (comet assay) and the chromosomal aberration test, in cultured CHOK1 cells. *Mutation Research* 419:79-90. Chinese hamster ovary K-1 cells evaluated in the comet assay and chromosomal aberration assay.	MRID 51975406 (1998) TXR 0058605 Acceptable/nonguideline Comet assay: 0, 25, 50 or 250 μM in cell culture medium, evaluated after 1 hr exposure Chromosomal aberration assay: 0, 25, 50 or 100 μM in cell culture medium, evaluated 18 hr following a 4 hr exposure Tech., >99% a.i. MBC	Negative for DNA strand breaks in the comet (single cell gel electrophoresis) assay, in the absence of cytotoxicity. Positive for numeric chromosomal aberrations at all concentrations tested, in the absence of a significant increase in structural (breaks or exchanges) aberrations, in the absence of cytotoxicity. **Demonstrated lack of DNA damage and supported numeric chromosomal aberrations induced by MBC.**

Conclusions: The genotoxicity database supports the conclusion that MBC is aneugenic and does not cause direct DNA damage. Overall, assays for gene mutations in bacterial and mammalian cells and for DNA damage/repair, including comet assays, were negative. In contrast, micronucleus and other assays evaluating chromosomal segregation and ploidy consistently demonstrated abnormalities. As evaluated in detail below in the MOA section of this memorandum, the aneugenicity of MBC results from disruption of spindle assembly during cell division, although the precise mechanism of the interaction has not been characterized for MBC. This conclusion is consistent with that of the earlier cancer peer reviews of MBC and reviews by outside entities.

Disruption of spindle assembly is considered a threshold event requiring dose levels sufficient to disrupt the function of mitotic spindle components. The protein components of the mitotic spindle are present in excess in the cell nucleus; therefore, disruption requires a dose adequate to affect their assembly. Studies evaluating micronuclei induction identified threshold dose levels below which no induction was observed. When observed, induction of aneuploidy occurs rapidly, within hours to days following exposure to MBC. There is evidence that MBC can induce micronucleus formation in mouse liver, based on results from an *in vivo* micronucleus

assay.

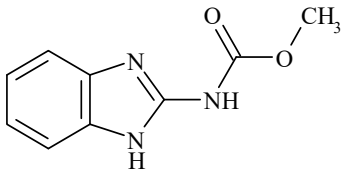
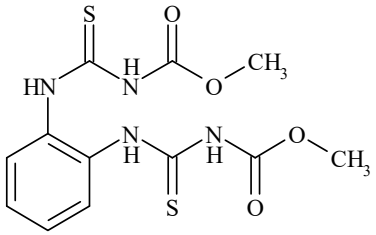
In accordance with the Agency's previous assessments of MBC, the review by McCarroll *et al.* (2002) and reviews by other regulatory agencies and health organizations (JMPR, WHO, PMRA, CDPR; refer to Table 1, Background section) have also consistently concluded that MBC is aneugenic and not likely to be mutagenic. A review of MBC for germ cell mutagenicity under the criteria of EC Regulation (No. 1272/2008) on CLP concluded MBC is an aneugen but not a mutagen or clastogen (ExPonent; Gollapudi *et al.,* 2014).

C. Structure-Activity Relationship

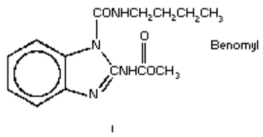
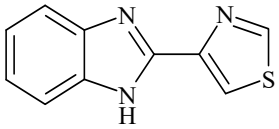
The Compendium of Common Pesticide Names website[6] lists several benzimidazole fungicides. Of these, only MBC, TM, and thiabendazole are currently registered for use in the United States; the remainder have either not been reviewed by the Agency or all registrations (e.g., benomyl) were cancelled. TM and benomyl (all uses cancelled in 2002) are rapidly hydrolyzed *in vivo* and in the environment to MBC, the fungicidally active moiety.

Table 15 summarizes the structure, toxicity (target organ and genotoxicity), and cancer classification and quantification for MBC and the related benzimidazole fungicides TM, benomyl, and thiabendazole. Like MBC, the cancer classification of TM has not been reevaluated under the 2005 cancer guidelines.

Table 15. Structure-Activity Comparison of Related Benzimidazole Fungicide Compounds

Chemical	Structure	Toxicity and Cancer Classification
Carbendazim (MBC) PC Code 128882 CAS No. 10605-21-7		Target organs: liver, testes Genotoxicity profile: aneugenic (aneuploidy) Tumor profile: mouse liver (male and female) Cancer classification from previous evaluations (TXRs 0005576, 0050625): **Group C, possibly carcinogenic to humans (1986 Guidelines)** Quantification of cancer risk (Q_1^*): 2.39 x 10^{-3} $(mg/kg/bw)^{-1}$ based on combined liver adenomas/carcinomas in female mice
Thiophanate-methyl (TM) PC Code 102001 CAS No. 23564-05-8		Target organs: liver, thyroid follicular cell Genotoxicity profile: aneugenic (aneuploidy) Tumor profile: rat thyroid and mouse liver (males and females) Current cancer classification: Likely to be **carcinogenic to humans (1996 Proposed Guidelines)** Quantification of cancer risk (Q_1^*): 1.16 x 10^{-2} $(mg/kg/bw)^{-1}$ based on combined liver adenoma/carcinoma/hepatoblastoma incidence in male mice.

[6] http://www.bcpcpesticidecompendium.org/

Chemical	Structure	Toxicity and Cancer Classification
Benomyl PC Code 099101 CAS No. 17804-35-2		Target organs: liver Genotoxicity profile: aneugenic (aneuploidy) Tumor profile: mouse liver (males and females) Cancer classification (at time of cancellation): **Group C, possibly carcinogenic to humans (1986 Guidelines)** Quantification of cancer risk (Q_1^*): 2.39×10^{-3} $(mg/kg/bw)^{-1}$ based on combined liver adenoma/carcinoma incidence in female mice in MBC study
Thiabendazole PC Code 060101 CAS No. 148-79-8		Target organs: liver, thyroid follicular cell Genotoxicity profile: weakly aneugenic (aneuploidy) Tumor profile: rat thyroid follicular cell tumors (males and females) Current cancer classification: "**Likely to be carcinogenic at doses high enough to cause a disturbance of the thyroid hormonal balance (2005 Guidelines).** It is not likely to be carcinogenic at doses lower that those which could cause a disturbance of this hormonal balance." Quantification of cancer risk: not required. A non-linear approach (*i.e.*, cRfD) will adequately account for all toxicity, including potential carcinogenicity.

MBC, TM, and benomyl all show consistent evidence of aneugenicity in genotoxicity studies. TM also showed a weak positive response (2x above controls) in the presence of rat liver S9 in *S. typhimurium* strains TA98 and TA100 at precipitating doses, a finding that was not observed in a repeat assay or a second Ames assay study. TM was positive in the Balb C3T3 cell transformation assay, which does not distinguish between DNA damage and other mechanisms of genotoxicity. The aneugenicity of thiabendazole, a less closely related structure, was characterized as weak.

MBC, benomyl, and TM were associated with an increase in the incidence of mouse liver tumors. Benomyl and MBC were classified under the Agency's 1986 cancer guidelines as Group C, or "possibly carcinogenic to humans". TM was classified as "likely to be carcinogenic to humans" under EPA's proposed 1996 cancer guidelines, based on an increased incidence of liver tumors in mice and thyroid tumors in rats. Quantification of cancer risk was based on mouse liver tumors for all three compounds. Thiabendazole was associated with an increase in the incidence of rat thyroid follicular cell tumors; the cRfD was considered protective of carcinogenic effects. Based on the available comparisons, benzimidazole fungicides demonstrate liver toxicity and aneugenicity.

D. Other Subchronic and Chronic Toxicity Studies

1. Subchronic Toxicity

Acceptable guideline subchronic oral studies in the mouse and rat were not submitted for MBC; the data requirement was satisfied by the rat and mouse carcinogenicity studies. No systemic toxicity was observed in the rat or mouse long-term dietary studies during the initial 3-6 months of exposure. A 15-week dietary study in the rat from the open literature was reviewed during registration review of MBC. An extended one-generation reproductive toxicity study (EOGRTS) in the rat that included a systemic toxicity cohort and a subchronic oral study in the dog are also available.

15-week dietary study in the rat: In a nonguideline subchronic oral study (MRID 50707603; published report), male Wistar Swiss albino rats were administered daily doses of carbendazim (MBC; 98% ai) at 0, 150, 300, or 600 mg/kg/day by gavage in corn oil vehicle (in a dosing volume of 0.275 mL/rat) for 15 weeks. Body weights, blood chemistry and hematology, and liver and kidney weights and histopathology were recorded at study termination.

At ≥150 mg/kg/day, statistically significant decreases in white blood cell (WBC) count (27, 28, and 43%, low to high dose, respectively) and lymphocyte count (29, 31, and 35%, low to high dose, respectively) were observed compared to controls. Histopathological effects in the liver and kidney were observed; however, severity was not reported. All liver and kidney findings at 150 mg/kg/day were of low incidence. At 150 mg/kg/day, histopathological effects in the liver (portal vein congestion, mononuclear cell infiltration; 2-3% incidence) and kidney (tubular degeneration, congestion, mononuclear cell infiltration; 1-2% incidence) were observed. In contrast, a dose-related increase in these lesions was seen at 300 and 600 mg/kg/day with incidence ranging from 50-80% and 40-100% in the liver and kidney, respectively. Hydropic degeneration of the liver was observed at 300 and 600 mg/kg/day, and fibrosis of the kidney was also seen at 600 mg/kg/day. Also, at 600 mg/kg/day, increased serum cholesterol (52%), albumin (27%), glucose (43%), creatinine (33%) and decreased very low-density lipoprotein (VLDL) (-47%) relative to controls were observed. Relative liver weights were slightly reduced (-16%) at 600 mg/kg/day. Body weights were unaffected by treatment.

EOGRTS: An EOGRTS in the rat was submitted that included cohorts for evaluation of systemic toxicity and thyroid effects.

In a modified EOGRTS (MRID 49547201), carbendazim (MBC technical, 99.5% a.i.; Batch no. 20080347) was administered to 30 Wistar Hanover Crl:WI(HAN) rats/sex/dose in the diet at dose levels of 0, 250, 1000, or 2000 ppm (equivalent to P generation premating average daily doses of 0, 13.9, 53.2, or 106.7 mg/kg/day in males and 0, 16.2, 67.6, and 136.8 mg/kg/day in females) for at least 4 weeks prior to mating, during the 14-day mating period, throughout gestation, and through lactation until weaning. Dietary adjustments of dose were not made during the course of any in-life phase of the study. As a result, test compound intake in females during lactation was up to 2-fold greater than other dose groups. The study protocol included evaluation of circulating thyroid hormone levels and a statistical semi-quantitative microscopic evaluation of thyroid colloid area and follicular cell height in parental animals and some

offspring cohorts, but did not include an immunotoxicity cohort as recommended in the Organization for Economic Cooperation and Development (OECD) guidelines. Study design and cohort assignment are summarized below:

P Generation: P males were exposed through postnatal day (PND) 85, and P females were exposed until lactation day (LD) 22. Parameters examined included body weight, food consumption, hematology, clinical chemistry, hormone analysis (thyroid and testosterone), organ weights, estrous cycle, ovarian follicle counts, sperm count, motility, and morphology, reproductive performance, gross pathology, histopathology, and a semi-quantitative microscopic analysis of the thyroid follicular cell height and colloid area.

F_1 Generation: Each set of F_1 offspring was maintained on the test diet from the time of weaning until termination. Litter parameters and pup weights were measured until animals were assigned to cohorts at weaning. F_1 offspring were evaluated for potential effects on the nervous system, reproductive and endocrine systems, thyroid function, and other systemic toxicity parameters. In-life parameters evaluated in all F_1 offspring included clinical observations, body weights, food consumption, anogenital distance, nipple retention and puberty onset. Selected F_1 offspring were divided into different groups (Cohorts 1a, 1b, 2a, 2b, and 3) at weaning on PND 21.

Cohort 1a (22/sex/group) and their F_2 offspring: assessment of reproductive and systemic toxicity which included estrous cycle evaluation and post-mortem evaluations that focused on thyroid hormones, reproductive organs, sperm assessment, and ovarian follicle counts. Males and females were mated on PND 90, and males were sacrificed on PND 148. Females were sacrificed on gestation day 20 and cesarean parameters evaluated. The F_2 fetuses from this mating were examined for external, visceral, and skeletal anomalies.

Cohort 1b (20/sex/group) and their F_2 offspring: assessment of reproductive toxicity which included estrous cycle evaluation and post-mortem evaluations that focused on clinical chemistry, hormones (testosterone and thyroid), hematology, reproductive organs, sperm assessment, ovarian follicle counts, and histopathology. Males and females were mated on PND 90, and males were sacrificed on PND 175. Females were sacrificed on LD 20. The F_2 offspring were sacrificed on PND 21 (n=12/sex/group for necropsy, perfusion of the nervous system, and gross brain measurements), PND 23 (n=12/sex/group for thyroid hormone analysis, necropsy and target organ pathology), and PND 45 (20 females/group for vaginal patency and thyroid hormone analysis).

Cohort 2a (10/sex/group): developmental neurotoxicity assessment, which included clinical signs, body weight, ophthalmology (~PND 45) functional observational battery (FOB, PND 52-55), motor activity (PND 63-66), and acoustic startle response (PND 58-61). On PND 70, animals were perfused for central nervous system and peripheral nerve neuropathology evaluation and brain morphometry.

Cohort 2b (12/sex/group): developmental neurotoxicity assessment, which included clinical signs, terminal body weight and brain measurements, perfusion for central nervous system and peripheral nerve neuropathology evaluation, and brain morphometry on PND 21.

Cohort 3 (12/sex/group): assessment of systemic toxicity which included thyroid hormone analysis, necropsy, and target organ pathology on PND 23.

Parental toxicity (P and F_1 adult animals): There were no treatment-related effects on P or F_1 parental mortality, clinical signs, body weight, body weight gain, food consumption, hematology or clinical chemistry parameters, urinalysis, and macroscopic findings. At 2000 ppm, P males showed decreases in monocytes (counts -29% and percent -33%), but values were within historical control range. In F_1 Cohort 1b, significantly increased white blood cell counts (40%) and absolute lymphocytes (46%) and segmented neutrophils (35%) were observed in parental females. These changes were within historical control range or, for segmented neutrophils, most or all groups (including concurrent controls) exhibited counts greater than the historical controls. Significantly decreased triglyceride levels in P females at 2000 ppm and F_1 parental females at 1000 and 2000 ppm were observed but no gross or histological correlates were observed.

Plasma testosterone levels in P males were minimally reduced at 1000 and 2000 ppm (25% and 24%, respectively; not statistically significant) but there was significant variation among individual animals and there were no effects on male sperm or reproductive parameters. In P males at 2000 ppm, very slight decreases in testes weight (4% below controls) and minimal or slight testicular atrophy (3/30) were not associated with lack of sperm or reproductive effects.

Changes in thyroid parameters were seen in P and adult F_1 animals but were most pronounced in P females, with alterations in both plasma hormone levels and thyroid histopathology observed at 1000 and 2000 ppm. In P females, plasma T4 levels were increased in all treatment groups (27, 34, and 18%, $p>0.05$ at 250 and 1000 ppm), as were TSH levels (64, 164, and 92%, $p<0.05$ at 1000 ppm). Minimal to slight microscopic follicular cell hypertrophy was observed at 1000 (2/29) and 2000 (3/23) ppm in P females only. A statistically significant decrease in colloid area and increase in follicular cell height was observed in the semi-quantitative microscopic analysis of the thyroid at 1000 and 2000 ppm. No thyroid effects were observed in P males except for a nonsignificant decrease (19%) in T4 at 2000 ppm.

In adult F_1 offspring, no thyroid effects were observed in Cohort 1a males (PND 148) or females (GD 20). F_1 Cohort 1b males (PND 175) showed a significant decrease in T3 (19% and 18%) at 1000 and 2000 ppm, but no other effects on thyroid hormones or histopathology. Testosterone levels were also unaffected by treatment. F_1 Cohort 1b females (LD 22) showed no effects on thyroid hormones, but at 2000 ppm, showed significant increases in absolute/relative thyroid weight (18%/25%). Decreased colloid area/increased follicular cell height were also observed. Analysis of follicular cell height and T4 or TSH levels did not show a consistent positive correlation among the different cohorts/generations of treated animals. However, in P females, the combined findings of changes in T4 and TSH, along with slight thyroid hypertrophy and increased follicular cell height/decreased follicular colloid area, were considered indicative of perturbation of thyroid homeostasis.

Reproductive toxicity: There were no treatment-related effects on reproductive indices, precoital intervals, gestation length, sexual development of F_1 parental animals, estrous cycle, ovarian follicle count, sperm parameters [number, motility, and morphology], parturition, lactation, or tissues and organs of the reproductive system.

Developmental toxicity (prenatal only): Fetuses from the F_1 Cohort 1a group mating were evaluated on GD 20. There were no treatment-related effects on live litter size, fetal anomalies (external, visceral or skeletal), fetal weight, or fetal sex ratio.

Offspring toxicity (systemic toxicity and developmental landmarks): There were no treatment-related effects on live litter size or pup viability, anogenital distance, developmental landmarks, food consumption, organ weights, or histopathology of the F_1 and F_2 offspring. The number of litters with pups found dead was increased in the P high-dose group (6/27) compared with controls (1/30), and the number of F_1 pups found dead during the lactation period was 15 and 1 for the high-dose and control group, respectively. However, the finding was considered incidental since the majority (10) of the pups were from one litter with complete loss during the lactation period, and an increase in dead pups was not seen in treated F_2 offspring. Body weight gain of F_1 male pups at 2000 ppm was significantly decreased from PND 4-18 by 10%, correlating with absolute mean body weights decreases of 6-7% (n.s.) from PNDs 14-21. After weaning, body weights of F_1 male offspring in both Cohorts 1a and 1b remained decreased, compared with controls, from PNDs 24-28 by 7-10% (not statistically significant), but were not significantly decreased at later time points. Mean body weights of F_2 females were significantly decreased by 8-11% on PNDs 24-28.

Changes in thyroid hormone levels, colloid area and follicular cell height were seen in offspring but were not consistent across cohorts or life stages. In F_1 PND 4 offspring, decreased T4 was observed at 1000 and 2000 ppm (24% and 35%; significant at high dose), but TSH was unaffected. Histopathology was not evaluated. At PND 23, male and female F_1 pups (Cohort 3) at 2000 ppm showed statistically significant increases in T4 (53%/22%), significant increases in TSH in females (41%), and significantly decreased colloid area/increased follicular cell height in both sexes (males -15%/+55%; females -22%/+85%). PND 23 males at 1000 ppm also showed significant increases in T4 and TSH (42%/21%) and significant colloid area/follicular cell height changes (-12%/+43%); in females, only TSH was increased (28%, n.s.). In contrast, F_2 pups at PND 23 showed only nonsignificant increases in T4 in males (20%) and TSH in females (26%). PND 45 females at 2000 ppm showed increased T4 (52%, $p<0.05$), TSH (41%, n.s.) and significantly altered colloid area and follicular cell height (-19%/+60%). Analysis of the correlation between follicular cell height and T4 or TSH levels did not show a consistent positive correlation in treated animals. No changes in thyroid parameters were observed in any offspring at 250 ppm.

Offspring developmental neurotoxicity: There was no effect of treatment on clinical signs, ophthalmology, FOB parameters, motor and locomotor activity, auditory startle parameters, brain weights, gross brain measurements, microscopic brain measurements and brain neuropathology, or other neuropathological findings. Statistically significant differences in brain morphometric measurements were of small magnitude, did not show a dose-response and were therefore not considered treatment-related.

Subchronic oral toxicity in the dog: In a subchronic oral toxicity study in the dogs (MRID 00091130), carbendazim (53% a.i.) was administered in the diet to 4 beagle dogs/sex/dose at concentrations of 0, 100, 500, or 2500/1500 ppm for 13 weeks. The high dose group initially received graduated doses starting with 500 ppm (3 days), 1000 ppm (2 days), 1500 ppm (2 days)

and 2500 ppm ("a few days"; no further details available) before the high dose was lowered back to 1500 ppm during week 3 due to weight loss and decreased food consumption. The exposures are equivalent to time-weighted average carbendazim consumption of 0, 2.7 (both sexes), 14.4/11.3 (M/F) or 40.7/35 (M/F) mg a.i./kg/day, respectively.

There were no treatment-related effects on mortality, clinical signs, body weight, hematologic or urinalysis parameters. At the high dose, effects on the liver were observed, particularly in males. Elevated alkaline phosphatase (AP, +350%) and glutamic-pyruvic transaminase (GPT, +560%) were observed in males. Liver histopathological effects included hepatic cirrhosis with hepatic cell necrosis (moderate in one male), marked bile duct proliferation in one male, tubular collapse and increased fibrous connective tissue around the triads (marked in one male and slight in one female). The mean testes weight was reduced (-17%), and diffuse testicular degeneration of moderate severity was noted in one male.

2. Chronic Toxicity

Chronic oral toxicity in the dog: In a chronic oral toxicity study (MRID 00164304), carbendazim (technical MBC, 98% a.i.) was administered to 5 beagle dogs/sex/dose at dietary concentrations of 0, 100, 200, or 500 ppm for one year (equivalent to average daily doses of 0, 2.93, 6.43, or 16.54 mg/kg/day in males and 0, 3.20, 7.19, or 17.07 mg/kg/day for females (average for both sexes 0, 3.06, 6.81, or 16.80 mg/kg/day). Clinical chemistry and hematology values were examined at pretest, and at 1, 3, 6, 9, and 12 months.

At 500 ppm, mean body weights of males were sporadically reduced by up to 10-12% below controls at week 32 or later, but the decreases were marginal and variable during this time, did not show a clear dose-response (similar decreases were sporadically observed in other dose groups), and were not statistically significant. Females did not show a treatment-related effect on mean body weight. Serum cholesterol was slightly increased (13-22% in males, $p<0.05$ at 9 months only; 20-48% in females, $p<0.05$ at 3, 6 and 9 months). The report stated that the values were within the laboratory historical control range but did not provide the data. There were also no changes observed in other clinical chemistry parameters or in liver histopathology. Increased relative kidney weight in males at 200 ppm (33%) and 500 ppm (21%) was observed but did not show a dose-response, and no associated clinical pathology or histopathology was observed. One high dose female had a thyroid follicular adenoma that is considered rare in dogs of this age. However, the tumor was considered incidental because there were no corresponding changes in thyroid histology and organ weight, or changes in clinical chemistries other than the cholesterol increase. There were no treatment-related effects on clinical observations, body weight, food consumption, hematology or urinalysis, organ weights, or pathology.

E. Mode of Action Studies

Mechanistic studies were not submitted for MBC. Open literature studies on the aneugenicity of MBC that were reviewed by the Agency are summarized in the MOA section of this document.

A nonguideline 28-day dietary mechanistic study in mice evaluating liver effects of benomyl was discussed in McCarroll *et al.* (2002). The study is summarized here to provide limited

information pertinent to liver effects of MBC, since benomyl converts rapidly to MBC:

In a nonguideline 28-day oral toxicity study in the mouse (MRID 41607903), benomyl (96.1% a.i.) was administered in the diet to 20 male CD-1 mice/dose at concentrations of 0, 100, 500, 3750, or 7500 ppm (equivalent to average daily intake of 0, 15.7, 85.4, 586, or 1180 mg benomyl/kg/day). Since benomyl is rapidly converted to MBC, the study was included as a bridging study to evaluate effects of MBC on mouse liver. When converted to molar equivalents of MBC, using a molecular weight adjustment factor of 0.66, benomyl doses are equivalent to average daily MBC intakes of 0, 10, 55, 382, or 777 mg/kg/day. The study evaluated clinical signs of toxicity, body weight and food consumption, and liver weight (14- and 28-day sacrifices) for all animals tested. On Days 14 and 28, 5 animals/dose group were injected with bromodeoxyuridine (BrdU) and sacrificed 2 hr later for histopathological examination and immunohistofluorescent staining for evaluation of hepatocellular proliferation (BrdU incorporation). An additional 5 animals/dose group were sacrificed on Days 14 and 28 for biochemical evaluations. Livers of these animals were homogenized, separated into microsomal and peroxisomal fractions, and assayed for cytochrome p450 and peroxisomal β-oxidation activity levels, respectively. Electron microscopy was also performed on samples from the liver to evaluate induction of microsomes and peroxisomal proliferation.

No effects were reported on survival, clinical signs of toxicity, body weight, or food consumption. Increases in mean absolute/relative liver weights were observed at 3750 and 7500 ppm at 28 days (25/22% and 26/28% above control, respectively; $p<0.05$). Hepatocellular hypertrophy was observed at 3750 and 7500 ppm at 14 days (5/5 for both dose levels) and 28 days (4/5 and 5/5, respectively). The BrdU labelling indices showed apparent increases relative to controls (not statistically significant) at 28-days at 3750 ppm (67%) and 14 and 28 days at 7500 ppm (55% and 91%, respectively). Cytochrome p450 levels were increased relative to controls at 7500 ppm at 14 and 28 days (35% and 37%, respectively, $p<0.05$). Levels of peroxisomal β-oxidation showed no treatment-related change with dose or time.

Based on these results, slight liver changes (increased weight, centrilobular hypertrophy, and p450 activity) along with a non-statistically significantly increased hepatocellular proliferation, were observed within 14-28 days of exposure at 3750 and 7500 ppm benomyl, dietary concentrations that are molar equivalents to 384 and 777 mg/kg/day MBC. No effects were observed at 500 ppm, molar equivalent to 55 mg/kg/day MBC. However, evidence of microscopic lesions demonstrating overt hepatotoxicity, such as hepatocellular necrosis or foci of cellular alteration, or evidence of aneugenicity in hepatocytes, was not observed under the conditions of this study.

IV. PROPOSED MODE OF ACTION FOR LIVER TUMORS IN THE MOUSE

A. Postulated Mode of Action for Mouse Liver Tumors

The Registrant proposed aneugenicity as the sole genotoxic MOA for MBC and the likely MOA for induction of liver tumors in the mouse, stating in their proposal that: “MBC-induced liver tumors in mice are likely to be induced through aneuploidy, a non-mutagenic mode of action” (p. 28). A detailed MOA framework analysis was not prepared for this proposal. Instead, the

Registrant's proposed MOA is based on the comprehensive literature survey and cancer MOA framework analysis of MBC and benomyl presented in McCarroll *et al.* (2002) and reviews of MBC conducted by WHO (1993), JMPR (2005), ECHA (2019/2013), APVMA (2009) and Gollapudi (2014) (refer to summaries in Table 1). All of these reviews concluded that MBC is aneugenic, not mutagenic, and supported a threshold for the genotoxic effects of MBC. WHO, ECHA, and APVMA also evaluated carcinogenicity and concluded that MBC is not a human carcinogen. The liver tumors in mice were considered to be an effect likely associated with increased susceptibility of specific mouse strains to liver tumors. All reviews concurred that MBC was not tumorigenic in the rat.

The MOA framework analysis of McCarroll *et al.* (2002) evaluated the plausibility of aneugenicity as a potential MOA for induction of mouse liver tumors under the IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for Humans (Boobis *et al.*, 2006). The cancer MOA framework analysis from McCarroll *et al.* (2002), which as noted in the publication was not an official statement of Agency views or policies, has not been formally reviewed by the Agency. Under the proposed MOA, disruption by MBC of mitotic spindle assembly during cell division, considered a threshold effect, occurs only at exposures high enough to interfere with assembly of the components of the spindle apparatus. MBC is considered to be an indirect genotoxicant since it does not directly interact with or damage DNA. The proposal noted the general scientific consensus regarding aneugenicity of MBC and benzimidazoles as a chemical class, which is based on (1) the large body of data demonstrating aneugenicity and (2) the overall lack of positive results in genotoxicity studies evaluating direct effects of MBC on DNA.

The Registrant's proposal was based on the liver tumor incidence from the pathology peer review (MRID 41607904) of the two-year study on CD-1 mice in their WOE for the MOA analysis. The proposal also noted the lack of microscopic evidence of preneoplastic liver lesions (e.g., hyperplasia) and late tumor onset. The WOE narrative from the Registrant's proposal, which proposed a cancer classification of "Suggestive evidence of carcinogenic potential" with no cancer quantification, is provided below.

No new mechanistic studies were submitted by the Registrant, as their proposal considered the available information adequate to establish the MOA. However, for the purpose of characterizing key events and aspects in the MOA framework analysis, the Agency conducted a search of the open literature, as previously described, to identify studies relevant to the MOA. Detailed reviews of several open literature studies were prepared by the Agency, in addition to the studies submitted for MBC, and are considered for the MOA.

1. Key Events for the Postulated MOA

The key events identified for the proposed mode of action for liver tumor formation in the mouse as outlined in McCarroll *et al.* (2002) are:

(1) Interaction of MBC with the target site (inhibition of microtubule assembly and mitotic spindle formation), the molecular initiating event;
(2) Inaccurate segregation of chromosomes during cell division (chromosomal non-disjunction and mal-segregation);

(3) Lagging of chromosomes (micronucleus and kinetochore-positive micronucleus formation);
(4) Hepatocellular toxicity.

Data supporting molecular initiating event and Key Event #1 (interaction with the target site; inhibition of microtubule assembly and mitotic spindle formation):

McCarroll *et al.* (2002) stated:

> "Because agents that induce aneuploidy generally act on components or structures associated with cell division, rather than DNA, the potential targets for aneuploidy inducing agents are many and not well defined…The multiple events required to impair processes leading to abnormal chromosome segregation are not fully understood." (p. 335)

Interference with spindle tubulin polymerization and microtubule assembly during cell division is the fungicidal MOA for MBC. No *in vivo* or *in vitro* data were identified that evaluated inhibition by MBC of tubulin polymerization or spindle microtubule assembly in somatic cells of the mouse. Several published reports evaluating the direct effects of MBC on mitotic spindle tubulin assembly and microtubule-associated proteins (MAPS) in various *in vitro* systems, including mammalian cells, were reviewed and summarized by McCarroll *et al.* (2002). These studies were not given detailed reviews for this reevaluation based on current Agency guidance for detailed review of open literature studies (e.g., test material purity not provided), but the available scientific literature, considered as a whole, provides consistent *in vitro* evidence of disruption of mitotic spindle microtubule assembly. The disrupting effects of the benzimidazole fungicides on microtubule polymerization and/or mitotic spindle assembly are well-established and generally accepted (Table 1), although the precise mechanism has not been established. MBC was shown to inhibit microtubule assembly in *Saccharomyces evarum* and in preparations of tubulin isolated from porcine, bovine, and rat brain and rat testes. Mammalian cells were shown to be less sensitive than fungal cells to these alterations. Effects of MBC on MAPS may also play a role in affecting spindle microtubule assembly, and its role may vary with species. The inhibition of tubulin polymerization/microtubule assembly in dividing cells by MBC and other benzimidazole fungicides in the *in vitro* assays using isolated tubulin occurs rapidly (within one hour of treatment).

***M-phase specific centrosome-microtubule alterations induced by the fungicide MBC in human granulosa cells.* MRID 51975404; open literature report; Can and Alp (1997).**

The disruption of mitotic spindle assembly, chromosomal segregation, and mitostatic action of MBC was evaluated using immunohistofluorescent staining with antibodies to mitotic spindle components and chromosomes of primary cultures of human ovarian granulosa cells (Can and Albertini, 1997). Cell cultures were treated with 0 or 30 µM of MBC (the dose was selected based on previous studies identifying an effective culture concentration for MBC). A separate positive control group was not included in the study. Mitotic index was evaluated for 2-9 days in control and treated cells; based on the results, 3-day cultures were selected as optimal for treatment at intervals between 3-15 hr. Following treatment, primary cultures of the enriched

granulosa cell suspension were mounted on glass coverslips. Spindles were visualized by incubation with antibodies against α-tubulin or acetylated α-tubulin. Centrosomes were visualized using human autoimmune sera against centrosomes (SPJ and 5051), considered "stable" markers that do not change with passage through the cell cycle. Nuclear proteins associated with mitotic spindles during cell division were visualized using antibodies against protein components of the nuclear mitotic apparatus, anti-NuMA-Ab-1 and anti-centrophilin. These two components are considered "transient" because they associate with the mitotic spindle during cell division but not during interphase. Nucleic acids were visualized using Hoechst 33258 stain. Multiple fluorescence labeling was performed to allow visualization of the relationship of the different components of the spindle and chromosomes within the same cell during the cell cycle. Images of spindles stained with anti-α tubulin and anti-acetylated α-tubulin antibodies were recorded on videotape, digitized, and quantified using commercially available image processing routines.

MBC caused metaphase arrest and abnormal spindle and chromosome organization following exposures of 3-15 hours. Alterations in spindle shape and microtubule composition were observed in treated cells. MBC exposure resulted in an increased number of spindle poles associated with chromosomes displaced from the metaphase plate. A gradual increase from tri- to multipolar spindles was noted with prolonged treatment, although a relatively constant fraction of bipolar spindles was maintained. MBC had no effect on microtubule organization in non-dividing cells. Analysis of mitotic figures by immunofluorescence microscopy showed a reduction in interpolar and astral microtubules in treated cells, while acetylated kinetochore microtubules were retained and their plus-ends were attached to metaphase chromosomes. In multipolar spindles, analysis of microtubule organizing centers (MTOC) with antisera to the stable centrosomal markers SPJ and 5051 revealed that only poles associated with displaced chromosomes retained these markers. In contrast, the transient centrosome markers NuMA and centrophilin were localized to all poles of multipolar spindles. The results of this study suggest that one mechanism of action of MBC may be impairment of spindle microtubule dynamics at the centrosome. The results of this study are consistent with disruption by MBC of the cell spindle apparatus during cell division and induction of aneuploidy during cell division.

MBC altered the normal process of spindle assembly and segregation of chromosomes during mitotic cell division. Immunofluorescent staining of mitotic spindles with anti-α-tubulin and anti-acetylated α-tubulin showed differences in staining patterns between treated and control cells (Figure 2). The micrographs and intensity plots demonstrated an increase in the number of spindle poles in cells exposed to MBC (Figures 2D and 2E, bipolar spindles; 2G and 2H, tripolar spindles) compared to untreated controls (Figures 2A and 2B). The interpolar intensity plots for control cells (Figure 2C) vs. bipolar (Figure 2F) and tripolar (Figure 2I) spindles show asymmetric spindle halves and an increase in the percentage of acetylated α-tubulin (dotted line) compared to α-tubulin (solid line).

Figure 2. (Figure 1 from MRID 51975404). Effects of MBC on Mitotic Spindle Patterns in Cultured Human Granulosa Cells

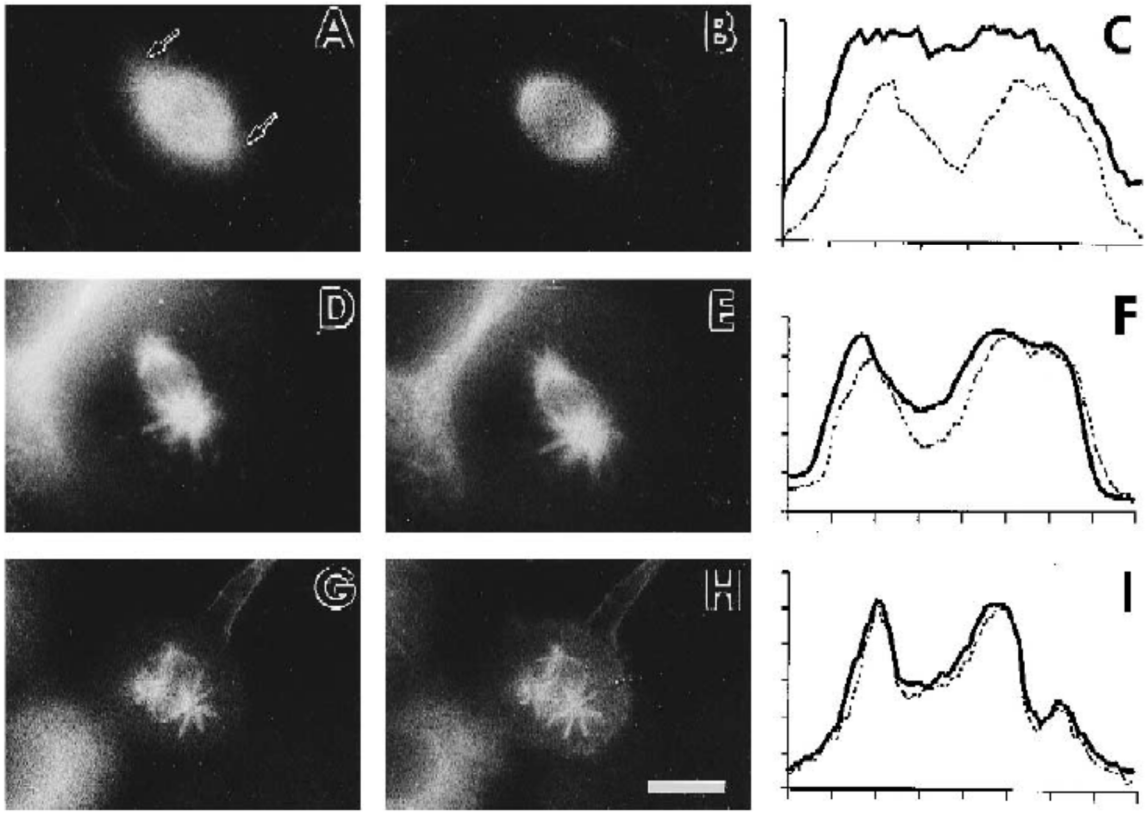


Fig. 3. α-Tubulin (A, D and G) and acetylated α-tubulin (B, E and H) staining patterns in control (A, B) and MBC-treated (D, E, G and H) cells. (A) A control cell in metaphase exhibiting astral (arrows), kinetochore and interpolar microtubules; acetylated α-tubulin staining (B) is restricted to kinetochore microtubules. Bipolar (D, E) and tripolar (G, H) spindles in MBC-treated cells show similar staining patterns with α-tubulin (D, G) and acetylated α-tubulin (E, H) antibodies. (C, F and I) Interpolar fluorescence intensity plots for α-tubulin (continuous line) and acetylated α-tubulin (broken line) along spindle axis. Pixel intensity (0–256) and spindle length (2 μm divisions) correspond to the y-axis and x-axis, respectively. Note continuous α-tubulin and two symmetric peaks of acetylated α-tubulin patterns in the control cell (C). In MBC-treated cells, α-tubulin and acetylated α-tubulin staining patterns are similar in both bipolar (F) and tripolar (I) cells. Note all α-tubulin is acetylated as expressed by the total absence of any area in between α-tubulin and acetylated α-tubulin patterns. MBC-treated cells also display asymmetric spindle halves for both labels (F and I). Bar 10 μm.

Figure extracted from p. 144 (journal), MRID 51975404.

The percentage of multipolar spindles vs. bipolar spindles over time was also evaluated for up to 15 hours in treated cells (Figure 3). A time-dependent increase in the percentage of multipolar spindles following exposure to MBC was observed in cells stained with antibodies to tubulin. The percentage of bipolar spindles remained relatively constant during this time.

Figure 3. (Figure 4 from MRID 51975404). Effects of MBC on Spindle Type with Time in Cultured Human Granulosa Cells Exposed for up to 15 Hours.

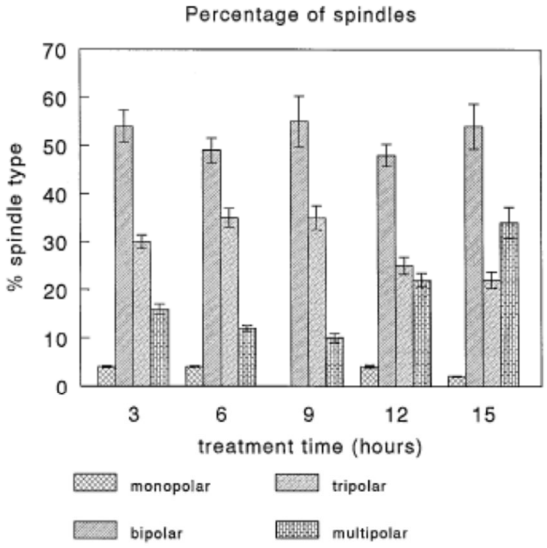


Fig. 4. Percentage of different spindle types observed in 3-day cultured human granulosa cells exposed to 30 μM MBC from 3 to 15 h. At each time point examined, bars represent, from left to right, monopolar, bipolar, tripolar, and multipolar (> 3) fraction of total number of mitotic cells measured. Note that the percentage of bipolar cells remains relatively constant at all time points whereas multipolar fractions increase with prolonged MBC exposure.

Figure extracted from p. 145 (journal), MRID 51975404.

Changes in the organization of centrosomes and their association with spindles were also observed (Figure 4). Correlating staining of nucleic acids (DNA) is shown for reference in Figures 4A and 4I (controls) and 4E and 4M (MBC-treated). In control cells at metaphase, stable centrosome components (SPJ and 5051) showed staining at the two mitotic spindle poles (Figures 4B and 4D). For cells exposed to MBC, although bipolar cells also showed two mitotic spindle poles (data not presented in report), cells showing tripolar and multipolar spindle formation were labeled with SPJ and 5051 only at one or two spindle poles; the remainder were therefore not associated with these centrosome components (Figures 4F and 4H).

Transient centrosome markers (NuMA and centrophilin) also showed abnormal patterns in MBC-treated cells. In control cells, the staining pattern of NuMA and centrophilin was associated with the spindle from prophase through anaphase stages of cell division (Figures 4J and 4L), but not during interphase (Figure 4L). Staining was observed in a normal pattern during the cell cycle: associated with chromosomes during prophase and at spindle poles at prometaphase through anaphase. In contrast, MBC-treated cells showed alterations in dividing cells, with staining observed as condensed spots at all spindle poles, with larger poles containing more intense staining (Figures 4N and 4P). Poles that lacked associated chromosomes and did not stain with stable centromere components did contain NuMA. From these data, it was concluded that in MBC-treated cells, the altered distribution of centrosome components was related to their association with chromosomes that were displaced from the metaphase plate.

Figure 4. (Figure 5 from MRID 51975404). Effects of MBC on Centromere Components in Cultured Human Granulosa Cells

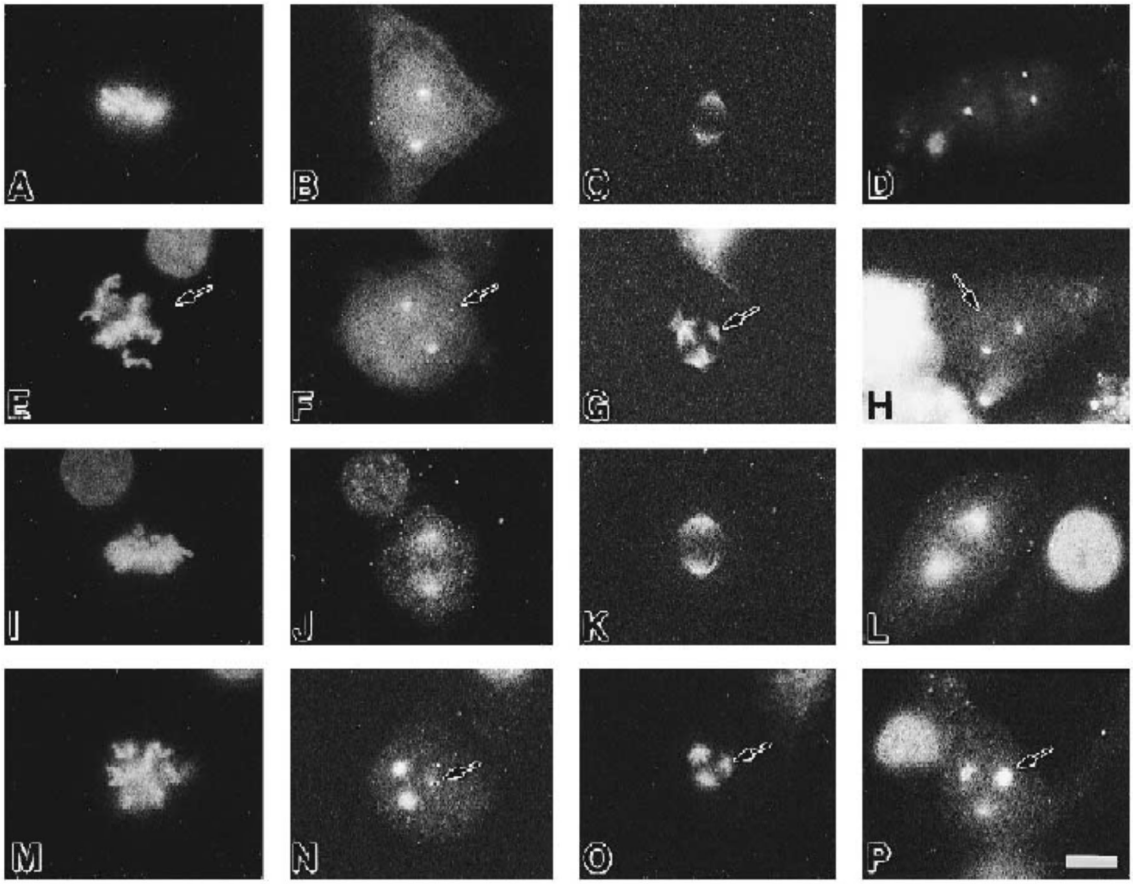


Fig. 5. Staining patterns of different centrosomal markers in control (A–D, I–L) and MBC-treated (E–H, M–P) cells. Correlative DNA patterns are shown (A, E, I and M). In control cells in metaphase (A–D), SPJ (B) and 5051 (D) is confined to centrosomes at spindle poles. In MBC-treated tripolar cells, SPJ (F) and 5051 (H) staining is restricted to two poles; displaced chromosomes are usually oriented to stained poles, whereas chromosomes are lacking at unstained spindle poles (arrows, E–H). NuMA and centrophilin staining show similar patterns in both control interphase and mitotic cells. Staining is punctate and intranuclear in control interphase cells (L) and in mitotic cells staining is confined to spindle halves including centrosomes (J and L). In MBC-treated cells, NuMA and centrophilin staining is concentrated at each pole although some poles exhibit variable staining intensity (arrows in N, O and P). Bar 10 μm.

Figure extracted from p. 146 (journal), MRID 51975404

The CARC concluded that the data adequately support the molecular initiating event/ Key Event #1. The interaction of MBC with tubulin and other mitotic spindle components is well-established. The open literature contains numerous studies in fungal and mammalian cells demonstrating interaction of MBC with components of the mitotic spindle (tubulin, centrosomal protein components), although data are not available in the mouse. Immunofluorescent staining of tubulin and centrosome components further demonstrate alteration of spindle formation by MBC.

Data supporting Key Event #2 (inaccurate segregation of chromosomes during cell division; chromosomal non-disjunction and mal-segregation):

McCarroll *et al.* (2002) discussed *in vitro* studies showing evidence of inaccurate segregation of

chromosomes and chromosomal non-disjunction in yeast and human cells. Some studies demonstrated evidence of a threshold dose requirement. Data in mammalian (human) cells is discussed further, below.

***Evaluation of thresholds for benomyl- and carbendazim-induced aneuploidy in cultured human lymphocytes using fluorescence in situ hybridization.* MRID 51975402; open literature report; Bentley *et al.* (2000).**

The study by Bentley *et al.* (2000) evaluated the effect of MBC and benomyl on chromosomal non-disjunction, chromosomal loss, and kinetochore-positive micronuclei for specific chromosomes in cultured peripheral blood human lymphocytes exposed *in vitro* to increasing concentrations of MBC. Peripheral blood samples were cultured in the presence of phytohemagglutinin to stimulate lymphocyte division. Twenty-four hours after culture initiation, separate cultures were treated with solvent (DMSO), various concentration ranges of MBC (tech., 97% a.i.; tested between 300 to 1500 ng/mL) or benomyl (tech., 95% a.i.; 600 to 1900 ng/mL), selected based on a range-finding cytotoxicity assay, or with the positive controls colchicine (9 or 12 ng/mL) or vinblastine (6 ng/mL). The positive controls demonstrated aneugenicity (chromosomal non-disjunction) at the concentrations tested in these assays. Twenty hours after these additions, cytochalasin B was added (final concentration 3 µg/mL). The cultures were harvested 72 hours after initiation, yielding 48 hours of test chemical exposure and 28 hours of cytochalasin B exposure. The lymphocytes were harvested, processed and fixed on slides for examination of chromosomes by fluorescence *in situ* hybridization (FISH). Chromosome pairs 1, 8, 11, 17, 18, and X were evaluated for aneuploidy in binucleated cells. The chromosomes were selected for evaluation due to their association with trisomy in various tumor types (1 and 8), Wilms tumor (11), p53/predisposition to breast cancer (17) or aneuploidy (18 and X). Abnormalities observed in the study were classified as chromosomal loss, gain, non-disjunction, polyploidy, and presence of centromere-positive micronuclei in binucleated cells. All types of abnormalities were evaluated for threshold effect levels.

MBC and benomyl showed a similar pattern of effects. Both demonstrated a dose-response curve with a threshold effect level for aneuploidy in human peripheral blood cells. Chromosomal non-disjunction data (Table 16) show that the threshold concentrations for chromosomal non-disjunction following treatment with benomyl were 1100 ng/mL for chromosomes 8, 11, 18, and X and 1200 ng/mL for chromosome 17; a threshold concentration was not determined for chromosome 1. For MBC, the non-disjunction threshold concentrations were 600 ng/mL for chromosomes 17 and X, 700 ng/mL for chromosome 1, and 800 ng/mL for chromosomes 8, 11, and 18 (Table 16). For both compounds, the threshold concentrations for chromosome X were similar to those of the other chromosomes examined, with increased frequency at the higher concentrations. Additionally, the concentrations of both compounds at which significant increases in non-disjunction were noted were approximately equimolar (3.8-4.1 µM for benomyl, 3.2-4.3 µM for MBC (Figure 5). Threshold concentrations for chromosome loss (benomyl and MBC), and centromeric positive micronuclei (benomyl only) also were determined and found to be equal or greater than those seen for non-disjunction (Table 17).

Table 16. (Table 1 from MRID 51975402). Mean Frequency of Non-Disjunction (Events/1000 cells) for Benomyl and Carbendazim-Induced Aneuploidy.

Table 1
Mean frequency of non-disjunction (events/1000 cells) for benomyl and carbendazim-induced aneuploidy

Test material (ng/ml)	Chromosome					
	1	8	11	17	18	X
Benomyl						
0	6	6.5	3.5	5	5	6.5
600	4	3	5	6	4.5	7.5
700	3.5	3	2.5	6	6	7.5
800	6.5	7	3.5	4.5	5	11.5
900	6	7.5	6	4	3.5	10
1000	5	6.5	5	8.5	9.5	11.5
1100	7	12*	7*	13.5	13.5*	23*
1200	8	14*	5*	14*	9*	26*
1300	11	17.5*	12*	15*	16.5*	30*
1400	7	18*	15.5*	21*	13.5*	34*
1500	14	–[a]	–	–	–	–
1700	12.5	–	–	–	–	–
1800	12	–	–	–	–	–
1900	24	–	–	–	–	–
Colchicine 9 or 12 ng/ml	46[b]	91[b]	–	70[b], 149[c]	–	64[b], 130[c]
Carbendazim						
0	3	8	1.5	3	3.5	4
300	4	10	2.5	2.5	6	6
400	5	12	3.5	2	4	9
500	4	4.5	5.5	3.5	7.5	8
600	4.5	9.5	3	6*	7	12.5*
700	10.5*	17	4	6*	12.5	15*
800	9.5*	22*	9*	9*	11*	22*
900	15*	27*	13.5*	8*	12*	32.5*
1000	20.5*	41*	21*	21.5*	20*	35.5*
1100	23.5*	38.5*	15.5*	21*	30*	47.5*
1200	11.5*	27*	6*	14.5*	11.5*	22*
1400	38*	54*	–	–	–	
1500	22*	55*	–	–	–	
Colchicine 12 ng/ml	24[b]	22[b]	–	46[d]	–	52[d]
Vinblastine 6 ng/ml	2[b]	11[b]	–	6[b]	–	11[b]

*Statistically significant increase relative to control; $p < 0.05$.
[a]Not analyzed.
[b]Data from the first experiment; 12 ng/ml colchicine or 6 ng/ml vinblastine.
[c]Mean frequency from two cultures evaluated in the second experiment.
[d]Mean frequency from one culture evaluated in the first experiment and from two cultures evaluated in the second experiment; 9 ng/ml colchicine.

Table extracted from p. 47 (journal), MRID 51975402.

Figure 5. (Figure 3 from MRID 51975402). Frequencies of Non-Disjunction of Select Chromosomes Following Treatment with Cytochalasin-B in Human Lymphocytes Treated with MBC and Benomyl.

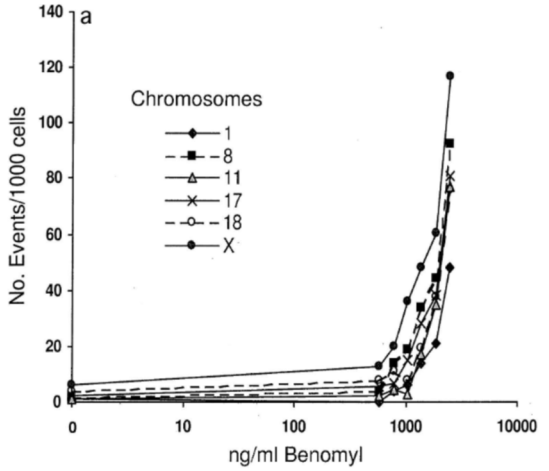


Fig. 3. Frequencies of non-disjunction for chromosomes 1, 8, 11, 17, 18, and X following treatment of cytochalasin B-blocked human lymphocytes with benomyl (a) and carbendazim (b). Results represent the mean response when 1000 binucleates per culture from two independently treated cultures (2000 cells total) were evaluated.

Figure extracted from p. 46 (journal), MRID 51975402.

Table 17. (Table 2 from MRID 51975402). Threshold for Benomyl- and MBC-Induced Aneuploidy.

Table 2
Thresholds for benomyl- and carbendazim-induced aneuploidy

Chromosome	Benomyl (ng/ml)			Carbendazim (ng/ml)		
	ND[a]	Loss	Cen + MN	ND	Loss	Cen + MN
1	> 1200*	> 1300	> 1200	700	> 1400	–[b]
8	1100	> 1500	> 1400	800	1000	–
11	1100	> 1500	> 1400	800	> 1200	–
17	1200	> 1500	1200	600	600	–
18	1100	1400	1400	800	900	–
X	1100	1400	1400	600	800	–

*Dose response tests for 1200 ng/ml and less showed no significance.
[a]ND = non-disjunction, Loss = chromosome loss, Cen + MN = centromeric positive micronuclei.
[b]Not determined.

Table extracted from p. 48 (journal), MRID 51947502.

***M-phase specific centrosome-microtubule alterations induced by the fungicide MBC in human granulosa cells.* MRID 51975404; open literature report; Can and Alp (1997).**

The previously discussed study by Can and Albertini also evaluated chromosomal distribution and spindle organization during mitosis in cultured cells exposed to MBC at a concentration of 30 µM. In addition to immunofluorescent staining by anti-tubulin antibodies to examine spindle formation, immunohistofluorescent staining using antibodies to nuclear proteins associated with the centrosome and Hoechst 33258 staining of nucleic acid to visualize chromosomal structure was performed. In contrast to normal chromosomal segregation shown in control cells (Figure 6, A and B), cells treated with MBC showed abnormal spindle organization as demonstrated using anti-tubulin immunohistofluorescent staining (Figure 6, C and E). Cells showed chromosomal mal-segregation as evidenced by displacement of chromosomes to spindle poles using Hoechst 33258 immunofluorescent stain (Figure 6, D and F).

Figure 6. (Figure 2 from MRID 51975404). Effects of MBC on Chromosome Distribution and Spindle Distribution in Cultured Human Granulosa Cells.

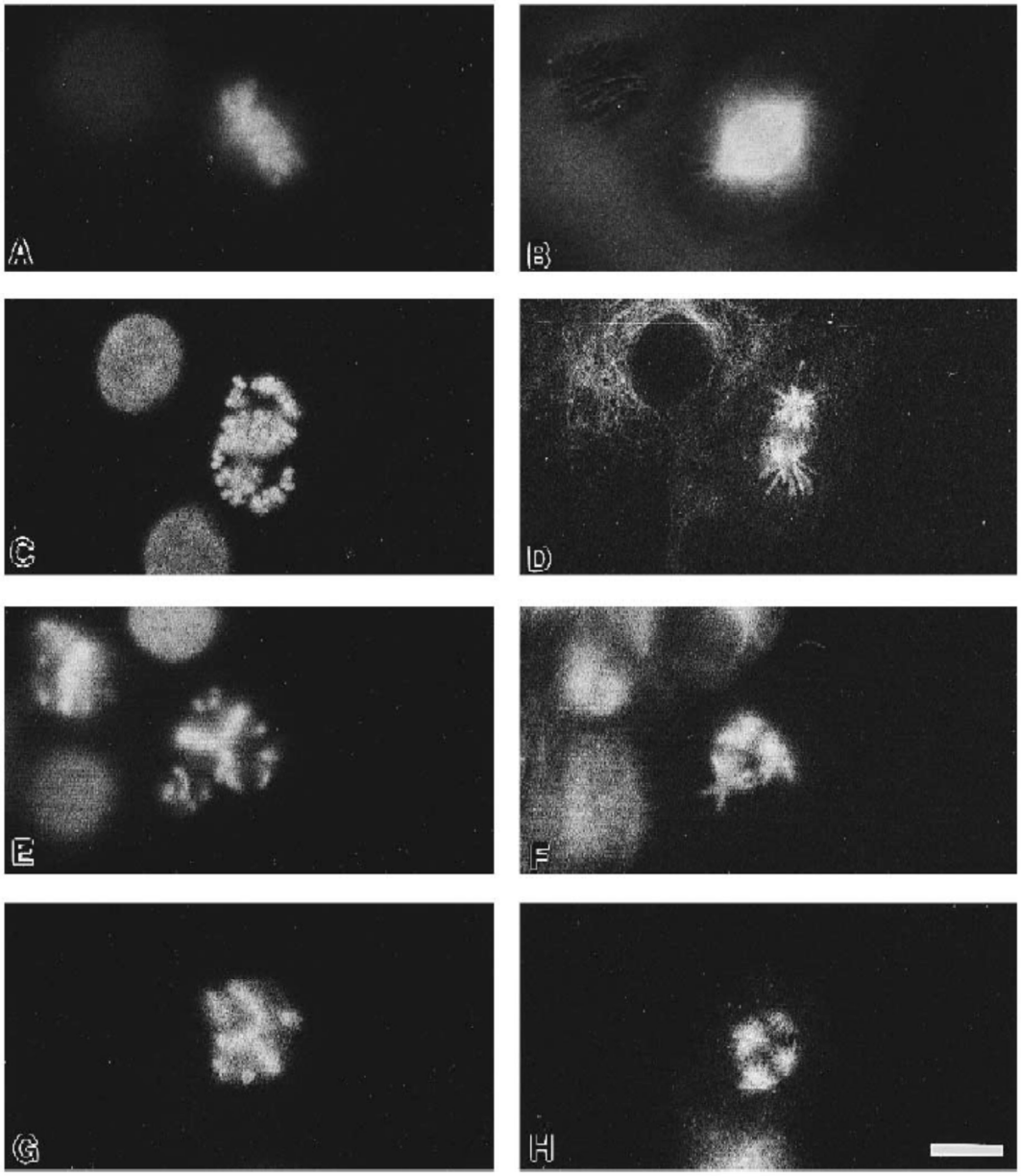


Fig. 2. Effects of MBC on chromosome distribution and spindle organization. (A, C, E and G) Stained with Hoechst 33258 showing a metaphase cell in control (A) and *type I* (C), *type II* (E) and *type III* (G) chromosome distribution patterns noted in MBC-treated cells. Corresponding cells stained with anti-α-tubulin antibody (B, D, F, and H) displaying normal bipolar mitotic spindle in control (B) and bipolar (D), tripolar (F) and quadripolar (H) spindles in MBC-treated cells. Note the displacement of chromosomes to spindle poles in mitotically active MBC-treated cells (C, E and G). Bar 10 μm.

Figure extracted from p. 143 (journal), MRID 51975404.

The CARC concluded that Key Event #2 is adequately supported by the available data. Effects occur within hours of exposure and chromosomal loss does not occur until nondisjunction occurs. The data indicate a threshold response for these effects. Immunohistofluorescent staining of spindle components demonstrates alterations in chromosomal segregation in cells

exposed to MBC.

Data supporting Key Event #3 (lagging of chromosomes):

Bone marrow micronucleus assays conducted in mice and rats have demonstrated induction of micronuclei following exposure to MBC (McCarroll *et al.,* 2002). Data on the mouse are presented, below, since mouse is the relevant species for the tumor MOA.

Cytogenetic effects of benzimidazoles in mouse bone marrow. **MRID 51975401; open literature report; Barale *et al.* (1993)**

In a study by Barale *et al.* (1993), the induction of micronuclei, structural chromosomal changes and ploidy in mouse bone marrow cells was evaluated over a 48-hour time window. Groups of 4-8 male Swiss Albino mice/dose/sampling time were administered single gavage doses in 5% aqueous DMSO of benomyl (99% a.i.) at 1 g/kg body weight or carbendazim (MBC; 95% a.i.) at 0.5 g/kg/body weight. Vehicle controls received only the DMSO vehicle. Bone marrow cells were harvested at several time points after dosing to establish a time course for chromosomal effects: vehicle controls at 0, 24, and 48 hr; MBC and benomyl at 6 hr intervals between 0 and 48 hr, inclusive (except that benomyl was evaluated at 38 instead of 36 hr).

MBC induced micronuclei and aneugenic (hyperdiploidy or polyploidy) effects (Table 18), with less evidence for induction of chromosome gaps and breaks (Table 19). Similar results were obtained for benomyl (data not presented here). The observation of aneugenicity is also consistent with chromosomal non-disjunction and mal-segregation discussed for Key Event #2 in human cell lines. The findings were observed as early as 6-12 hr following dosing, indicating that aneuploidy events can occur rapidly upon exposure to MBC or benomyl. Although the assay did not include a separate positive control group, both compounds are considered aneugens based on extensive genotoxicity testing in the scientific literature and Agency submissions. The results of this study are consistent with that determination and demonstrate induction of aneugenic changes in human cells.

Table 18. (Table 7 from MRID 51975401).

TIME COURSE OF MICRONUCLEUS INDUCTION IN MOUSE BONE MARROW CELLS AFTER A SINGLE i.p. ADMINISTRATION OF MBC (0.5 mg/kg)

Time (h)	MN/PCE	‰	MN/NCE	‰	NCE/PCE
Control [a]		1.68 ± 1.05		1.83 ± 0.91	1.11 ± 0.32
6	8/3000	2.66	1/1818	0.55	0.61
	7/3000	2.33	1/3222	0.31	1.07
	10/3000	3.33	11/5498	2.00	1.83
	8/4000	2.00	5/1608	3.11	0.40
mean ± SD		2.58 ± 0.57		1.49 ± 1.31	0.98 ± 0.63
12	12/4000	3.00	8/2288	3.49	0.57
	6/3000	2.00	4/2920	1.37	0.97
	17/5000	3.40	7/5308	1.32	1.06
	7/3030	2.12	9/3667	2.45	1.21
mean ± SD		2.63 ± 0.68		2.16 ± 1 ± b103	0.95 ± 0.27
18	17/3000	5.67	5/2188	2.28	0.73
	8/3000	2.66	4/2552	1.57	0.85
	4/3025	1.32	6/1844	3.25	0.61
	5/3000	1.66	7/2776	2.52	0.92
mean ± SD		2.83 ± 1.98		2.40 ± 0.69	0.78 ± 0.14
24	16/4000	4.00	4/4134	0.97	1.03
	16/4514	3.54	5/3565	1.40	0.79
	14/4000	3.50	11/3822	2.88	0.96
	12/3000	4.00	6/2821	2.17	1.06
mean ± SD		3.76 ± 0.28		1.85 ± 0.84	0.96 ± 0.12
30	15/2151	6.97	2/2243	0.89	1.04
	12/3000	4.00	1/1818	0.55	0.61
	8/3000	2.66	10/2345	4.26	0.78
	5/3000	1.66	4/3044	1.31	1.01
mean ± SD		3.82 ± 2.31		1.75 ± 1.70	0.86 ± 0.20
36	11/2600	4.23	6/4437	1.35	1.70
	39/4000	9.75	11/3878	2.84	0.97
	30/5000	6.00	17/6540	2.60	1.31
	18/3200	5.62	6/3327	1.80	1.04
mean ± SD		6.40 ± 2.36		2.14 ± 0.69	1.26 ± 0.33
42	13/3000	4.30	1/2225	0.45	0.74
	23/3558	6.46	18/4374	4.11	0.81
	26/3000	8.66	22/5723	3.84	0.52
	10/3115	3.21	12/4335	2.77	0.71
mean ± SD		5.66 ± 2.41		2.79 ± 1.66	0.69 ± 0.12
48	9/3000	3.00	6/3146	1.91	0.95
	11/3000	3.66	7/2873	2.44	1.04
	12/3000	4.00	26/5283	4.92	0.57
	11/3312	3.32	8/4016	1.99	1.21
mean ± SD		3.49 ± 0.43		2.81 ± 1.42	0.94 ± 0.27

[a] Control at 0 h (see Table 1).

Table extracted from p. 22 (journal), MRID 51975401.
Note: dosing in this study was peroral and not intraperitoneal injection (IP) as indicated in the Table title.

Table 19. (Table 8 from MRID 51975401).

TIME COURSE OF STRUCTURAL AND NUMERICAL CHROMOSOME ABERRATION INDUCTION IN MOUSE BONE MARROW CELLS AFTER p.o. ADMINISTRATION OF MBC (0.5 g/kg)

Time (h)	Meta-phases scored	Aberrations		% aber. cells		Hyperdiploid and polyploid cells		
		+gaps	−gaps	+gaps	−gaps	(2n+1, 2n+2)		4n
Control [a]	600	13	4	2.2±1.3	1.0±0.8	0	0	0
6	200	7	1	3.5	0.5	0	0	0
	200	6	3	3.0	1.5	0	0	0
	200	9	3	4.5	1.5	0	0	0
	200	12	6	6.0	3.0	0	0	0
mean±SD				4.2±1.3	1.6±1.0			
12	200	17	8	8.5	4.0	1	0	0
	200	12	3	6.0	1.5	0	0	0
	200	9	7	4.5	3.5	1	1	0
	200	23	8	11.5	4.0	0	0	0
mean±SD				7.6±3.1	3.2±1.2			
18	200	17	0	8.5	0.0	1	0	0
	200	10	7	5.0	3.5	1	0	0
	200	8	3	4.0	1.5	0	0	0
	200	10	1	5.0	0.5	0	0	1
mean±SD				5.6±1.9	1.4±1.5			
24	200	4	1	2.0	0.5	0	0	2
	200	7	3	3.5	1.5	1	0	0
	200	2	0	1.0	0.0	0	0	1
	200	4	2	2.0	1.0	0	0	1
mean±SD				2.1±1.0	0.7±0.6			
30	200	27	0	13.5	0.0	0	0	2
	200	9	0	4.5	0.0	0	0	1
	200	14	0	7.0	0.0	0	0	0
	200	7	3	3.5	1.5	1	0	0
mean±SD				7.1±4.5	0.4±0.7			
36	200	7	7	3.5	3.5	0	0	1
	200	12	0	6.0	0.0	0	0	2
	200	5	2	2.5	1.0	0	0	1
	200	6	1	3.0	0.5	1	0	0
mean±SD				3.7±1.6	1.2±1.5			
42	200	7	2	3.5	1.0	0	0	2
	200	7	0	3.5	0.0	0	0	3
	200	6	3	3.0	1.5	1	0	3
	200	4	1	2.0	0.5	1	0	0
mean±SD				3.0±0.7	0.7±0.6			
48	200	12	3	6.0	1.5	4	0	1
	200	7	6	3.5	3.0	1	0	2
	200	6	3	3.0	1.5	0	0	5
	200	6	1	3.0	0.5	0	1	1
mean±SD				3.9±1.4	2.0±0.9			

[a] Control at 0 h (see Table 2).

Table extracted from p. 23 (journal), MRID 51975401.

***Classification of DPX-E965-299, (Carbendazim, MBC)-Induced Micronuclei in Mouse Bone Marrow Erythrocytes Using Immunofluorescent Antikinetochore Antibodies.* MRID 42911602; submitted study; Bentley *et al.* (1992)**

A mouse bone marrow micronucleus assay (Bentley *et al.,* 1992) evaluated the effects of MBC on the proportion of treatment-induced kinetochore-positive and -negative micronuclei. Five B6D2-F1/CR-1BR mice/sex/dose were administered single gavage doses of MBC in 0.5% aqueous carboxymethyl cellulose vehicle at 0, 66, 1646, or 3293 mg/kg. Positive controls cyclophosphamide (40 mg/kg) and vincristine (0.125 mg/kg) were administered in normal saline by intraperitoneal injection. Animals were sacrificed at 48 hr post-dosing (24 hr for positive controls and their vehicle controls). Bone marrow samples were processed for detection of kinetochores in induced micronuclei by immunofluorescent staining with anti-kinetochore-specific antibodies.

Statistically significant increases in total micronucleus formation were observed at 1646 mg/kg in females and in both sexes at 3293 mg/kg (Table 20).

Table 20. Total Micronucleated Polychromatic Erythrocyte (MN-PCE) Frequencies for Mice Treated with MBC.

DPX-E965-299 (mg/kg)	Samp. Time (hrs)	Sex	N	Total %MN-PCEs Mean (SE)	Median (IQR)
0	24	M	5	0.12 (0.04)	0.10 (0.15)
0	24	F	5	0.08 (0.04)	0.10 (0.15)
PI(CP), 40	24	M	4[a]	4.08 (0.43)	3.85 (1.53)**
PI(CP), 40	24	F	4[a]	3.50 (0.21)	3.45 (0.80)**
PI(VCR), 0.125	24	M	5	5.76 (1.00)	5.10 (4.25)**
PI(VCR), 0.125	24	F	5	6.54 (1.50)	7.70 (6.40)**
0	48	M	5	0.34 (0.05)	0.30 (0.20)
0	48	F	5	0.28 (0.07)	0.30 (0.25)
66	48	M	5	0.40 (0.09)	0.40 (0.40)
66	48	F	4[a]	0.13 (0.03)	0.10 (0.08)
1646	48	M	5	1.02 (0.46)	0.80 (1.45)
1646	48	F	5	1.26 (0.26)	1.20 (1.15)* +
3293	48	M	5	1.74 (0.60)	1.00 (2.05)* ++
3293	48	F	5	3.24 (0.64)	2.90 (2.15)** ++

* P<0.05 using the Kruskal-Wallis test for equal medians.
** P<0.01 using the Kruskal-Wallis test for equal medians.
\+ P<0.01 using the Jonckheere-Terpstra test for increasing trend.
++ P<0.001 using the Jonckheere-Terpstra test for increasing trend.
[a] An animal was excluded from statistical analysis due to excessive weight loss.

PI – positive indicator; CP – cyclophosphamide; VCR – vincristine; SE – standard error; IQR – Interquartile range;
Data excerpted from Table 2, p. 20 (MRID 42911602)

Similar results were observed for kinetochore-positive micronuclei; a statistically significant increase in kinetochore-negative micronuclei was observed only in females at the high dose of 3293 mg/kg (Table 21). No increase in micronuclei was observed in males or females at 66 mg/kg. The induction of kinetochore-positive micronuclei reflects the presence of lagging chromosomes and is consistent with an aneugenic mode of genotoxicity.

Table 21. Frequencies of Kinetochore+ (KC+) and Kinetochore – (KC-) Micronucleated Polychromatic Erythrocytes (MN-PCEs)

DPX-E965-299 (mg/kg)	Samp. Time (hrs)	Sex	N	% KC+ MN-PCEs Mean (SE)	% KC+ MN-PCEs Median (IQR)	% KC- MN-PCEs Mean (SE)	% KC- MN-PCEs Median (IQR)
0	24	M	5	0.06 (0.04)	0.00 (0.15)	0.06 (0.02)	0.10 (0.10)
0	24	F	5	0.02 (0.02)	0.00 (0.05)	0.06 (0.02)	0.10 (0.10)
PI(CP), 40	24	M	4ª	0.35 (0.06)	0.35 (0.25)*	3.73 (0.38)	3.55 (1.38)**
PI(CP), 40	24	F	4ª	0.43 (0.13)	0.35 (0.48)**	3.08 (0.11)	3.10 (0.43)**
PI(VCR), 0.125	24	M	5	5.32 (1.02)	4.80 (4.30)**	0.44 (0.05)	0.40 (0.20)**
PI(VCR), 0.125	24	F	5	6.00 (1.43)	7.00 (6.00)**	0.54 (0.12)	0.60 (0.45)*
0	48	M	5	0.24 (0.06)	0.30 (0.25)	0.10 (0.03)	0.10 (0.10)
0	48	F	5	0.20 (0.05)	0.20 (0.20)	0.08 (0.04)	0.10 (0.15)
66	48	M	5	0.26 (0.08)	0.20 (0.35)	0.14 (0.02)	0.10 (0.10)
66	48	F	4ª	0.03 (0.03)	0.00 (0.08)	0.10 (0.04)	0.10 (0.15)
1646	48	M	5	0.90 (0.41)	0.60 (1.25)	0.12 (0.06)	0.10 (0.25)
1646	48	F	5	1.12 (0.25)	1.00 (1.10)* +	0.14 (0.04)	0.20 (0.15)
3293	48	M	5	1.62 (0.60)	0.90 (2.10)* ++	0.12 (0.02)	0.10 (0.05)
3293	48	F	5	2.68 (0.57)	2.40 (1.90)* ++	0.56 (0.07)	0.50 (0.25)* ++

* P<0.05 using the Kruskal-Wallis test for equal medians.
** P<0.01 using the Kruskal-Wallis test for equal medians.
\+ P<0.01 using the Jonckheere-Terpstra test for increasing trend.
++ P<0.001 using the Jonckheere-Terpstra test for increasing trend.
ª An animal was excluded from statistical analysis due to excessive weight loss.

PI – positive indicator; CP – cyclophosphamide; VCR – vincristine; SE – standard error; IQR – Interquartile range
Data excerpted from Table 3, p. 21 (MRID 42911602)

Optimum conditions for detecting hepatic micronuclei caused by numerical chromosome aberration inducers in mice. MRID 51975405; open literature report; Igarashi *et al.* (2007)

At the time of the McCarroll *et al.* (2002) paper, no studies were available evaluating induction of micronuclei by MBC in mouse liver. Since then, induction of hepatocyte micronuclei by MBC was demonstrated by Igarashi *et al.* (2007). Groups of 3-5 Slc:ddY male mice/dose group were administered MBC (99.9% a.i.; Wako Pure Chemical Industries) in aqueous methylcellulose vehicle via gavage at dose levels of 0, 125, 250, 500, or 1000 mg/kg on the day prior to or the day after partial hepatectomy (PH). All mice were euthanized on Day 6 post-PH, and hepatocytes were isolated. Isolated hepatocyte smears were prepared, stained, and evaluated for cell classification (the number of mono-, bi-, or multinucleated cells), as well as micronucleated hepatocytes (MNH). Additionally, relative liver weights and hepatocyte proliferation indices (HPIs) were evaluated. Although a designated positive control group *per se* was not included, the study tested six additional compounds known to induce numerical chromosomal aberrations, which were shown to induce MNH following PH in this assay (data not shown here).

Treatment with MBC yielded an increased induction of MNH after PH and increased nuclear fragmentation at 500 mg/kg, which induced the maximum incidence of MNH and multinucleated cells (Figures 7 and 8). This finding supports a relationship among parameters such as MNH, nuclear fragmentation, and multinucleated cells. This investigation determined optimum conditions for micronuclei detection in the livers of mice and demonstrated the potential for MN

formation and bi-/multi-nucleated/fragmented cells by MBC in mouse liver. Based on the results of this study, MBC caused an increase in hepatocellular proliferation and numerical chromosomal aberrations in mice following stimulus for cell division (PH). Positive results for MNH were also observed for other compounds tested in this study (data not shown).

Figure 7. (Figure 3 from MRID 51975405). Day 6 Incidence of Micronucleated Hepatocytes in CD-1 Mice Exposed to MBC.

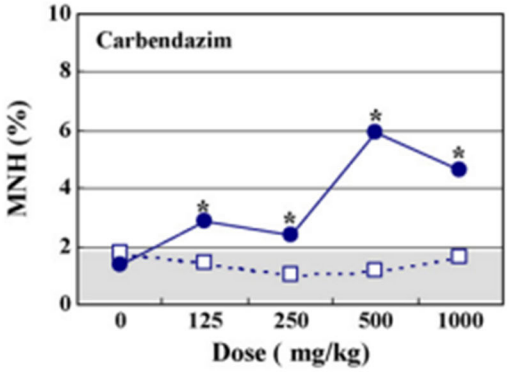


Fig. 3. *Experiment 2*. Incidence of micronucleated hepatocytes (MNH) on Day 6 in mice ($n = 3–5$) administered numerical aberration inducers before (open square) or after (closed circle) PH. The shadow area represents the vehicle control ranges. The asterisks show the values that exceeded these ranges and were significantly different from the concurrent control ($P < 0.05$, two-tailed Fisher's exact test).

Figure extracted from p. 94 (journal), MRID 51975405.

Figure 8. (Figure 4 from MRID 51975405). Day 6 Incidence of Bi- and Multi-Nucleated Hepatocytes on Day 6 in CD-1 Mice Exposed to MBC.

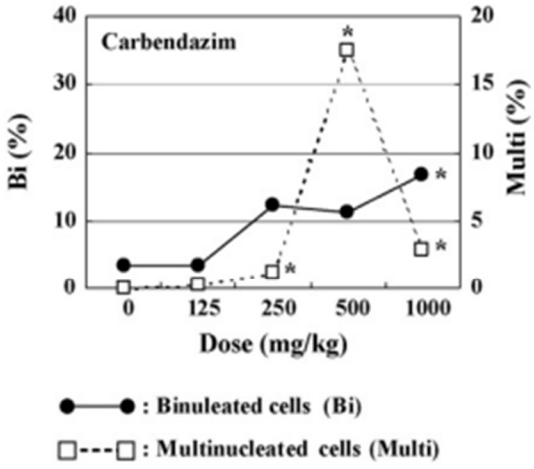


Fig. 4. *Experiment 2*. Incidence of binucleated (Bi) and multinucleated (Multi) hepatocytes on Day 6 in mice ($n = 3–5$) administered numerical aberration inducers after PH. The asterisks represent the values that exceeded the vehicle control ranges and were significantly different from the concurrent control ($P < 0.05$, two-tailed chi-square test).

Figure extracted from p. 95 (journal), MRID 51975405.

The CARC concluded that Key Event #3 (lagging chromosomes and micronucleus formation) is adequately supported by the available data. Data in mouse bone marrow and liver from

experimental in vivo studies demonstrate impaired chromosomal segregation and formation of micronucleus and kinetochore-positive micronucleus formation following MBC exposure. Data are supportive of a threshold dose for these effects. However, it is unclear whether the dose-response for the cytogenetic effects in bone marrow and liver cells observed in these studies would correlate to the dose-response observed in the mouse liver following in vivo exposure in dietary studies.

Data supporting Key Event #4 (hepatocellular toxicity):

Refer to Section II.D., Background Information, above, for study details of mouse carcinogenicity study. MRIDs 00154676, 41607904; Wood *et al.* (1982) and VanPelt *et al.* (1990).

The liver is a known target organ for MBC in the mouse. Histopathological non-neoplastic effects in the liver were observed in CD-1 mice (Wood *et al.*, MRIDs 00154676, 41607904). The incidence of hepatocellular hypertrophy, necrosis, and pigment in hepatic interstitial macrophages was increased in males at mid and high dose (≥225 mg/kg/day). Hypertrophy alone was observed at low dose (75 mg/kg/day) and was not considered evidence of hepatotoxicity in the absence of other histopathological findings. The incidence of combined hepatocellular adenomas and carcinomas in males was increased at low and mid dose (75 and 225 mg/kg/day; high dose was excessive). In females, an increase in the incidence of cellular alterations of eosinophilic foci was observed at mid and high dose (≥225 mg/kg/day) and basophilic foci at high dose (1125 mg/kg/day). An increase in hepatocellular hypertrophy and necrosis was not observed, but the findings of cellular alterations at mid and high dose were considered supportive of hepatotoxicity to females. The incidence of combined hepatocellular adenomas and carcinomas was increased in females at all doses tested in this study (≥75 mg/kg/day). Therefore, in both sexes, hepatocellular tumor incidence was increased at a dose (75 mg/kg/day) below that causing increased hepatocellular toxicity (225 mg/kg/day).

A 28-day mechanistic study on benomyl administered to male CD-1 mice in the diet identified adaptive changes in the liver at doses equivalent to ≥382 mg/kg/day MBC (MRID 41607903). These included increases in liver weight, hepatocellular hypertrophy, cytochrome P450 activity, and BrdU incorporation. No changes were observed at doses equivalent to 55 mg/kg/day MBC. While the data do not demonstrate overt toxicity to the liver and MBC was not assessed directly, they indicate some potential for liver toxicity and induction of liver enzyme activity.

The CARC concluded that Key Event #4 is partially supported by the available data, due to the data in males for hepatotoxicity being more robust than in females. In the CD-1 mouse carcinogenicity study at the mid and high dose, hepatocellular necrosis and hypertrophy were observed in males whereas a low incidence of cellular alterations (eosinophilic and basophilic foci) were observed in females. While hypertrophy and necrosis were not observed in females, the cellular alterations were considered supportive of hepatocellular toxicity. Slight increases in BrdU incorporation and cytochrome P450 activity in a 28-day mechanistic study on benomyl (at or above 382 mg/kg/day MBC-equivalent doses) were also observed in male CD-1 mice: while not adverse or tested directly with MBC, the findings indicate the potential for liver toxicity following enzyme induction by MBC.

2. Dose-Response Concordance of Key Events

A comparison of the dose response for the proposed key events as observed in mouse *in vivo* studies is summarized below in Table 22 (prepared by the reviewer). Studies evaluating liver effects from shorter-term studies on MBC are not available in the mouse. The results of the mouse 28-day mechanistic study on benomyl (with molar equivalent doses adjusted for the lower molecular weight of MBC) are included in the comparison due to rapid transformation of benomyl to MBC and the similarity of effects from the two compounds. There are no studies that provide *in vivo* dose-response data for the effects of MBC on spindle tubulin polymerization/microtubule assembly (Key Event #1) or chromosomal mal-segregation (Key Event #2) in the mouse. However, lagging of chromosomes and aneuploidy (Key Event #3) have been demonstrated *in vivo* in mice following exposure to MBC. Therefore, dose levels from mouse bone marrow and liver micronucleus assays can be compared to dose levels in mouse dietary studies.

Overall, there is not a full dose-response concordance for key events of the proposed MOA, where *in vivo* data in the mouse are available. Genotoxicity studies evaluating aneugenic effects are identified at higher dose levels than those inducing liver tumors in CD-1 mice. For example, the available data show a no observed effect level of 66 mg/kg/day for micronuclei induction in the mouse bone marrow (250 mg/kg/day in the liver) with effects observed at ≥1646 mg/kg/day in the bone marrow (≥500 mg/kg/day in the liver) (Key Event #3). Liver non-neoplastic histopathology in CD-1 mice was observed at doses ≥225 mg/kg/day with a no observed effect level of 75 mg/kg/day (Key Event #4). However, in CD-1 mice, statistical increases in combined liver tumor incidence were seen at the low dose of 75 mg/kg/day for both sexes in the original pathology report in the absence of hepatocellular toxicity (Tables 4-6). It is also unclear how the doses causing aneugenic effects in mouse bone marrow and mouse liver in partially hepatectomized animals relate to the dose levels that might cause aneuploidy in livers of mice with chronic dietary exposure to MBC.

Table 22. Dose-Response Concordance for the Key Events of the Proposed MOA in the Mouse Studies (*In Vivo* Data)

Endpoint	No Observed Effect Level mg/kg/day	Lowest Observed Effect Level mg/kg/day
Key Event #1. Interaction with the target site (spindle tubulin/microtubules)		
Inhibition of tubulin polymerization/ assembly	*In vivo* data unavailable for identification of a NOAEL	*In vivo* data unavailable for identification of a NOAEL
Key Event #2. Inaccurate chromosomal segregation		
Hyperdiploid and polyploid cells	*In vivo* data unavailable for identification of a NOAEL	*In vivo* data unavailable for identification of a NOAEL
Key Event #3. Lagging chromosomes (micronucleus and kinetochore-positive micronucleus formation)		
Kinetochore-positive micronuclei in mouse bone marrow (MRID 51975401)	66	1646
Micronucleus in mouse bone marrow (MRIDs 42911602, 51975401)	na	500 (only dose tested)
Micronucleus in mouse liver (MRID 51975405)	250	500

Endpoint	No Observed Effect Level mg/kg/day	Lowest Observed Effect Level mg/kg/day
Key Event #4. Hepatocellular toxicity and cell proliferation		
Male CD-1 mouse 28-day dietary study on benomyl (doses expressed in molar equivalents of MBC) (MRID 41607903)		
Hepatocellular BrdU incorporation	55	382
Increased liver weight	55	382
Hepatocellular hypertrophy	55	382
Increased cytochrome P450 activity	55	382
CD-1 mouse 2-year study (MRIDs 000154676, 41607904)		
Increased liver weight	75 (M/F)	225 (M/F)
Hepatocellular hypertrophy	<75 (M)	75 (M)
Hepatocellular necrosis	75 (M)	225 (M)
Pigment in hepatic macrophages	75 (M)	225 (M)
Eosinophilic foci	75 (F)	225 (F)
Basophilic foci	225 (F)	1125 (F)
Hepatocellular adenoma + carcinoma[1]	<75 (M/F)	≤75 (M/F)

[1] tumor incidence data from original study and pathology reevaluation identified different dose levels for significant tumor increases.
na - not available M – males; F – females

The CARC concluded that the available data do not fully support dose-concordance of the key events. There are no data in the mouse to establish dose-concordance for Key Events #1 and #2. While Key Events #3 and #4 identified threshold doses for their effects, no effects (except hepatocellular hypertrophy in males) were observed at or near the lowest dose at which hepatocellular tumors were observed in male and female mice (75 mg/kg/day).

3. Temporal Concordance of Key Events

The temporal relationship of key events is presented in Table 23 (prepared by the reviewer). Disruption of chromosomal segregation and formation of micronuclei occur rapidly in mice following *in vivo* exposure to MBC. As discussed above, formation of kinetochore-positive micronuclei has been demonstrated to occur within hours to days following exposure to MBC (Bentley *et al.*, MRID 51975402). Barale *et al.* (MRID 51975401) assessed micronucleus formation and aneuploidy in an *in vivo* mouse bone marrow assay over a time course up to 48 hrs following treatment. Micronucleus formation was observed at time points throughout the assay following oral dosing at 0.5 mg/kg (Table 18).

There are no studies in mice evaluating a time course for hepatotoxicity over short-term or subchronic exposure to MBC *per se*. The 28-day mechanistic study on benomyl in CD-1 mice (MRID 41607903) identified mild increases in hepatocellular proliferation, liver weight, hepatocellular hypertrophy, and increased cytochrome P450 activity at 2 and/or 4 weeks but did not identify other hepatocellular histopathological lesions. There are also no shorter-term studies directly evaluating the temporal association of aneugenicity, hepatocellular adaptive changes and proliferation, and overt hepatocellular toxicity in mice, such as necrosis or foci of cellular alteration. However, based on the rapid induction of aneuploidy as observed in rodent micronucleus and chromosomal segregation assays, it is plausible that aneuploidy would occur prior to, or in association with, overt hepatocellular toxicity and stimulation of hepatocellular proliferation, leading to eventual tumor induction. In the mouse CD-1 carcinogenicity study (MRID 00154676), liver adenomas or carcinomas were first observed between week 62-90 (a one-year interim sacrifice was not performed). However, the relationship between precursor

events and induction of liver tumors in the CD-1 mouse has not been established.

Table 23. Temporal Concordance for the Key Events of the Proposed MOA (*In Vitro and Vivo* Data)

Endpoint	Species tested/*in vivo* or *in vitro*	Exposure Duration
Key Event #1. Interaction with the target site (spindle tubulin/microtubules)		
Inhibition of tubulin/mitotic spindle assembly	Human ovarian granulosa cells (*in vitro*)	≤15 hours
Key Event #2. Inaccurate chromosomal segregation		
Chromosomal non-disjunction	Human lymphocyte cells (*in vitro*)	2-3 days
Hyperdiploid and polyploid cells	Swiss albino mouse (*in vivo*)	12-48 hours
Abnormal chromosomal distribution	Human ovarian granulosa cells (*in vitro*)	≤15 hours
Key Event #3. Lagging chromosomes (micronucleus and kinetochore-positive micronucleus formation)		
Lagging chromosomes	Human lymphocyte cells (*in vitro*)	6-12 hours
Micronuclei and/or kinetochore-positive micronuclei in bone marrow	CD-1 mouse (*in vivo*)	6-12 hours 2 days
Micronucleus in mouse liver	CD-1 mouse (*in vivo*)	5 days
Key Event #4. Hepatocellular toxicity and cell proliferation		
Hepatocellular BrdU incorporation	CD-1 mouse (*in vivo*)	2-4 weeks (M)
Increased liver cytochrome P450 activity	CD-1 mouse (*in vivo*)	4 weeks (M)
Increased liver weight (absolute)	CD-1 mouse (*in vivo*)	4 weeks (M) 2 years (M/F)
Hepatocellular hypertrophy	CD-1 mouse (*in vivo*)	2-4 weeks (M) 2 years (M/F)
Hepatocellular necrosis	CD-1 mouse (*in vivo*)	2 years (M)
Eosinophilic foci	CD-1 mouse (*in vivo*)	2 years (F)
Hepatocellular adenoma + carcinoma	CD-1 mouse (*in vivo*)	2 years (M/F)

M – males F – females

The CARC concluded that temporal concordance was demonstrated for the Key Events. Effects in available studies that relate to disruption of mitotic spindle formation and chromosomal segregation are observed within hours to days (depending on study design) and lagging chromosomes show temporal concordance with micronuclei formation and aneuploidy. Hepatotoxicity may occur as early as two weeks and continue throughout the lifespan based on studies in the CD-1 mouse. The Key Events may occur concurrently and/or sequentially.

4. Plausibility and Coherence of Proposed MOA

Aneuploidy is considered a plausible MOA for induction of liver tumors in the mouse by MBC. As discussed in McCarroll *et al.* (2002), aneuploid cells are frequently present in tumor cell populations and represent an abnormal distribution of genetic material during cell division that may affect regulation of cell metabolism and growth in the altered cells. The study of Igarashi *et al.* (MRID 51975405) suggests MBC may induce micronuclei formation in dividing hepatocytes in the mouse. The temporal responses for effects of MBC on disruption of mitotic spindle formation and hepatocellular toxicity suggest that they may coexist or that induction of aneuploidy may result in tumorigenesis through hepatocellular proliferation induced by hepatotoxicity. However, the relationship between aneuploidy, hepatocellular toxicity, and induction of hepatocellular carcinogenesis in mice has not been demonstrated for MBC. Evaluation of liver tumors in mouse studies on MBC did not include a cytogenetic analysis to identify the presence of aneuploid cells, and there are inadequate mouse data to evaluate

progression or a direct correlation between disruption of mitotic spindle assembly, aneuploidy, and liver tumorigenesis in the mouse.

The available studies suggest species differences in susceptibility to the induction of liver tumors by MBC. In the rat, increases in liver or other tumors were not observed following long-term exposure. In mice, Swiss-derived strains, CD-1 and SPF Swiss, showed treatment-related increases in liver tumors. Control liver tumor incidence in two other studies conducted in CD-1 mice by Haskell Laboratories showed high variability (Table 6).

The CARC concluded that the proposed MOA is plausible; however, shortcomings were identified. Aneuploid cells could result in abnormal gene expression and are frequently observed in tumor cell populations, although the role in tumorigenesis is uncertain. Experimental data in the mouse demonstrate susceptibility of the liver to micronucleus formation following in vivo exposure to MBC. However, at this time, the relationship between aneuploidy and liver tumors following MBC exposure is considered associative and not causative.

5. Alternative Plausible MOAs

Direct or indirect DNA interaction (mutagenicity/DNA damage): Overall, the available genotoxicity data on MBC show a low potential for direct interaction with DNA (DNA damage/mutation). As previously discussed, the majority of studies evaluating gene mutation, clastogenicity, or unscheduled DNA synthesis are negative, with some positive studies having tested with batches containing reported impurities. Published studies evaluating DNA integrity in comet assays (single-cell gel electrophoresis assays or SCGE) following exposure to MBC did not show evidence of DNA strand fragmentation. The general consensus of scientific reviews of MBC at this time supports aneugenicity and not direct DNA interaction (Section II.E, Background Information), and a threshold for genotoxicity based on the indirect effect of MBC on chromosomal segregation. This MOA is also consistent with the fungicidal MOA.

In a published study (below), *in vivo* exposure of male rats to MBC (up to 2000 mg/kg) by gavage did not cause DNA damage in the liver, stomach, or blood as assessed by the comet assay. In contrast, an increase in micronucleus induction in bone marrow and peripheral blood cells was observed.

Evaluation of a multi-endpoint assay in rats, combining the bone-marrow micronucleus test, the comet assay and the flow-cytometric peripheral blood micronucleus test. **MRID 51975403, open literature report; Bowen *et al.* (2011)**

In a multi-endpoint genotoxicity assay conducted by Bowen *et al.* (2011), groups of six Han-Wistar male rats/dose group were administered three consecutive doses of carbendazim (MBC, analytical grade from Sigma Aldrich, UK) in 1% aqueous (v/v) methylcellulose vehicle via oral gavage at dose levels of 0, 1000, 1500, or 2000 mg/kg/day at 0, 24, and 45 hours. The rats were euthanized three hours after the final dose administration (48 hours total exposure) and necropsied; the stomach, liver, and femurs were excised; and blood samples were obtained by cardiac puncture. Additional groups of five male rats/dose group were treated as above, and

blood samples were obtained from the tail vein at 24 hours and via cardiac puncture at 44 hours after the final dose administration. The tissues and blood were analyzed by the comet assay for DNA damage (blood, liver, stomach), flow cytometry for micronucleus formation (peripheral blood), and/or fluorescence microscopy for micronucleus formation (bone marrow). A positive control *per se* was not included in the study, but seven other compounds showing varying types of genotoxicity (clastogen, aneugen, DNA crosslinker, gene mutagen) were examined in the same manner in the study and showed appropriate positive responses (data not presented here).

As assessed by the comet assay, treatment with MBC did not cause DNA damage in blood, liver, or stomach (Table 24). In the liver, a small decrease ($p<0.05$) in tail intensity (TI; 0.8-fold) was observed at 2000 mg/kg/day; however, it was stated that all values fell within historical control ranges. In the stomach, a small increase ($p<0.05$) in TI (2.7-fold) was noted at 2000 mg/kg/day; however, this increase was associated with increased presence of clouds, i.e., comets lacking nucleic acid "heads" following electrophoresis, that were considered evidence of cytotoxicity (33.33% clouds treated vs. 19.70% control; 34.33% diffused cells treated vs. 24.00% control) and therefore was considered equivocal.

TABLE 24. DNA Damage in Tissues from Male Rats 3 hours after Administration of 3 Doses of MBC. [a]

Tissue	Dose (mg/kg/day)			
	0	1000	1500	2000
Liver				
%TI (fold increase) [b]	1.53 ± 0.15	1.46 ± 0.14 (1.0)	1.63 ± 0.26 (1.1)	1.17 ± 0.29* (0.8)
Cytotoxicity				
% clouds	2.42	3.25	4.42	4.92
% diffused cells	0.00	8.25	18.00	15.00
Stomach				
%TI (fold increase) [b]	3.12 ± 2.74	4.26 ± 1.36 (1.4)	4.44 ± 2.76 (1.4)	8.31 ± 3.68* (2.7)
Cytotoxicity				
% clouds	19.70	24.60	20.60	33.33
% diffused cells	24.00	23.80	25.50	34.33
Blood				
%TI (fold increase) [b]	2.08 ± 031	2.06 ± 0.29 (1.0)	1.87 ± 0.19 (0.9)	2.14 ± 0.20 (1.0)
Cytotoxicity				
% clouds	1.33	0.67	0.58	1.00
% diffused cells	0.17	0.00	0.67	0.67

a Data obtained from Table 3 on page 12-13 (journal citation page), MRID 51975603.
b Mean ± SD
* Significantly different from control; $p<0.05$.

Fluorescence microscopy revealed bone marrow toxicity that was indicated by a decrease in the percentage of polychromatic erythrocytes (PCE) noted at 1500 and 2000 mg/kg/day, and increased frequencies of micronucleated (MN)-PCE were noted at all dose levels (Table 25). Flow cytometry of peripheral blood (data not presented here) indicated cytotoxicity by a decrease in the percentage of reticulocytes at 1500 and 2000 mg/kg/day at the 3-hour sampling time point. Additionally, a dose-dependent 2.6- to 3.6-fold increase in the percentage of MN-reticulocytes (RET) that exceeded the 95% confidence interval of the laboratory's historical control range was observed at all dose levels tested. Based on the findings of the assays conducted in this study, MBC demonstrated genotoxic effects consistent with aneuploidy but did not show evidence of direct damage to DNA.

TABLE 25. Bone Marrow Micronucleus Formation in Male Rats 3 hours after Administration of 3 Doses of MBC. [a]

Index	Dose (mg/kg/day)			
	0	1000	1500	2000
%PCE	72.27	55.68	37.10	35.75
MN PCE/2000 PCE	1.3	7.5	11.0	9.3
%MN PCE [b]	0.047 ± 0.05	0.38 ± 0.29**	0.55 ± 0.36**	0.47 ± 0.28**

a Data obtained from Table 4 on page 14 (journal citation page), MRID 51975403.
b Mean ± SD
** Significantly different from control; p<0.01

***DNA damaging effects of pesticides measured by the single cell gel electrophoresis assay and the chromosomal aberration test.* MRID 51975406; open literature report; Vigreaux *et al.* (1998)**

Similar results were obtained in comet and chromosomal aberration assays conducted by Vigreux *et al*. (1998) using Chinese hamster ovary (CHOK1) cells exposed to MBC. Cultured CHOK1 cells were evaluated in the SCGE (comet) assay for evidence of DNA strand breaks and for chromosomal aberrations by cytogenetic examination. Cells were treated with MBC at concentrations of 0, 25, 50, or 250 µM for the comet assay and 0, 25, 50, or 100 µM for the chromosomal aberration assay. A positive control, etoposide, was tested at concentrations of 0, 0.2, 1, or 2 µM. The SCGE assay was performed immediately after the 1-hour treatment. Twenty-five randomly selected cells per slide were visually analyzed and submitted to image analysis. Cells were eye-graded into three categories depending on DNA migration level: intact cells (IC) or slightly-damaged cells (SDC), damaged cells (DC), and highly-damaged cells (HDC). For the chromosomal aberration assay, on the day after culture initiation, the cells were exposed to the test chemical and negative and positive controls for 4 hours, and then again at 18 hrs post-exposure. At the end of the post-incubation period, cells were treated with 0.6 mg/mL colcemid for 2 hours, and slides were scored (100 metaphase cells/dose) for chromosomal aberrations, which included chromosome and chromatid breaks, exchanges and gaps, pulverization, and numerical chromosome aberrations. The positive control, etoposide, an inhibitor of topoisomerase II known to induce DNA strand breaks, demonstrated dose-related increases in DNA migration in the SCGE assay and increased the percentage of aberrant cells in the chromosomal aberration assay.

Exposure to MBC demonstrated no treatment-related increases in cytotoxicity or DNA migration at all MBC treatment concentrations (Figure 9).

Figure 9. Comet Assay (Figure 6 from MRID 51975406). DNA Migration in CHOK1 Cells Exposed to Increasing Concentrations of MBC.

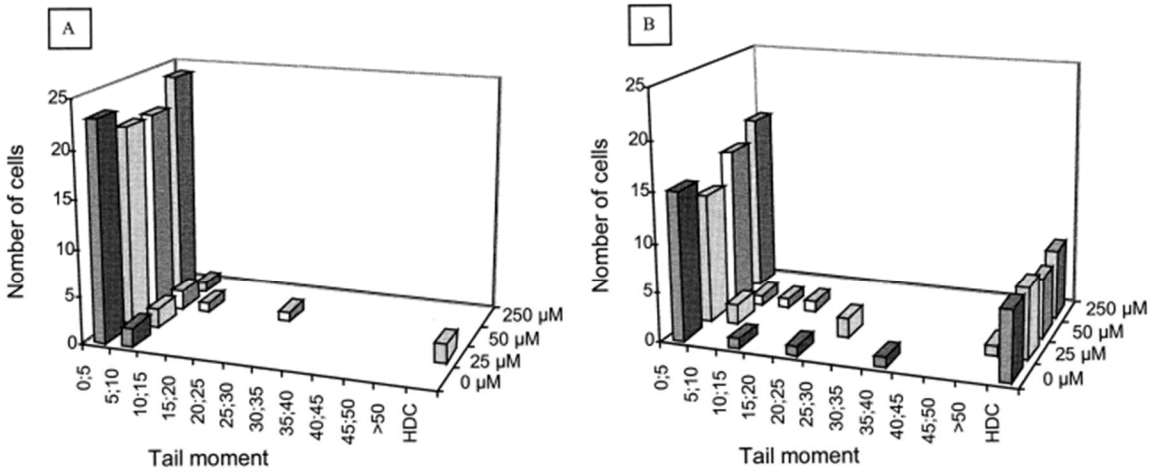


Fig. 6. Effects of increasing concentration of carbendazim on DNA migration measured by SCGE assay. Each histogram is a single representative experiment. In each experiment (A, B), no statistically significant dose–response relationship was observed. HDC: highly damaged cells.

Figure extracted from p. 85 (journal), MRID 51975406.

In contrast, positive results were obtained for numeric chromosomal aberrations at all concentrations tested (Table 26). Structural chromosomal aberrations such as gaps, breaks or exchanges were not observed. The findings indicate aneugenic potential for MBC in the absence of direct damage to DNA.

Table 26. (Table 4 from MRID 51975406). Chromosome Aberrations of Chinese Hamster Ovary Cells Induced by MBC

(µM)	Aberrations types							% Aberrant cells excluding gap		
	Gap	Chromosome		Chromatid		MA[a]	P[b]	Structural	Numeric[c]	Total
		Breaks	Exchanges	Breaks	Exchanges					
0	0	1	0	0	0	0	0	1	2	3
25	0	0	0	0	0	0	0	0	12 *	12 *
50	0	0	0	1	0	0	0	1	23 *	24 *
100	0	1	0	0	0	0	0	1	19 *	20 *

Total of 100 metaphase cells were counted for each treatment.
[a] Multiple aberrations
[b] Pulverization.
[c] Polyploidy, endoreduplication, hyperdiploidy
*: Indicates statistically significant difference from the concurrent control ($p<0.05$).
Table extracted from p. 86 (journal), MRID 51975406.

Non-genotoxic MOAs of liver tumor induction: Other plausible non-genotoxic (epigenetic) MOAs for induction/ of liver tumors could include peroxisomal proliferation, stimulation of hepatocellular proliferation, alterations to cell signaling pathways, or glutathione depletion.

No conclusive studies on alternative epigenetic MOAs for mouse liver tumors are available for MBC. The mouse 28-day study on benomyl (MRID 41607903) evaluated some liver parameters

relevant to alternative MOAs (Table 27). In this study, benomyl did not induce an increase in peroxisomal β-oxidation activity or evidence of peroxisome proliferation in electron micrographs. Mild adaptive changes in the liver were reported, which included increased cytochrome P450 activity, minimal to mild hepatocellular hypertrophy, and hepatocellular proliferation (BrdU incorporation). Other microscopic hepatocellular lesions were not observed.

Table 27. Summary of Liver Effects Following a 28-Day Exposure to Mice in the Diet (MRID 41607903; table extracted from DER, p. 43 of TXR 0010723, M. Morrow, 01/05/1994)

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

* p = 0.05

a = % BrdU positive hepatocytes per 100 hepatocytes evaluated

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

The CARC concluded that while direct DNA interaction (gene mutation and chromosomal damage) may be eliminated as an alternative MOA, there are other, plausible alternative MOAs that cannot be eliminated from consideration. The genotoxicity database for MBC indicates a lack of direct DNA interaction, based on negative comet assays in CHOK1 and rat cells, gene mutation, and clastogenicity assays. A mouse 28-day dietary study on benomyl suggests peroxisomal proliferation is not likely. However, robust mechanistic data for MBC evaluating other alternative MOAs (for example, cell signaling alterations, oxidative damage) were not provided.

6. Data Gaps and Uncertainties/Inconsistencies

The submitted MOA proposal did not directly address in detail data gaps or uncertainties/inconsistencies for the proposed MOA. While there is a robust database demonstrating aneugenicity of MBC based on *in vivo* assays conducted in the rodent (rat and mouse bone marrow micronucleus) and *in vitro* assays in mammalian (including human) cells, an association of aneuploidy with the induction of liver tumors in the mouse has not been demonstrated for MBC. The presence of aneuploid cells in mouse liver tumors was not examined in the available carcinogenicity studies. Although aneuploidy and liver toxicity-induced cell proliferation are a plausible MOA, there are insufficient data to demonstrate that the key events identified in the MOA are causative of the tumors. No MOA experiments were available that directly demonstrated the ability of aneuploid liver cells induced by MBC to induce liver tumors. In CD-1 mice, statistically significant increases in liver tumors were seen at the lowest dose tested (75 mg/kg/day), at which non-neoplastic hepatocellular toxicity was not reported in the two-year study.

Data directly demonstrating the nature of the interaction of MBC with spindle tubulin and associated spindle components and associated proteins in the mouse are not available. In addition, although aneugenicity presumably is likely to occur in other mammalian species and was demonstrated in micronucleus assays conducted in the rat (Bowen *et al.*, MRID 51975403), an increase in treatment-related tumors in the rat was not observed. There are no data characterizing inter-species differences or differing susceptibility of mouse strains to liver tumor induction via the proposed aneugenic MOA. Data are insufficient for MBC evaluating potential epigenetic MOAs for induction of liver tumors in the mouse, such as peroxisomal proliferation, alterations in signal transduction, glutathione depletion in the liver, or other cytotoxic MOAs, although results of the 28-day CD-1 mouse study on benomyl (and lack of tumor response in the rat) suggests that peroxisomal proliferation is not a likely MOA.

It is noted that while McCarroll *et al.* (2002) concluded that aneuploidy is a plausible MOA for mouse liver tumors induced by MBC and benomyl, the available data were not considered sufficient to establish the MOA conclusively:

> "At this time, a threshold response is demonstrated and provides clear evidence for a non-linear dose response [for genotoxicity of MBC]. However, direct evidence linking benomyl/MBC inhibition of tubulin polymerization to mouse liver carcinogenicity is not currently available." (p. 343)

The CARC concluded that data gaps exist in the MOA proposal. No data were available to directly demonstrate causality (including dose-concordance) of aneugenicity from MBC exposure and the induction of hepatocellular tumors in the mouse. The available genotoxicity data were considered adequate to exclude direct DNA interaction/damage as a potential MOA. However, data were inadequate to exclude definitively other alternative MOAs. A 28-day dietary mechanistic study in CD-1 mice conducted on benomyl provided only limited data on alternative MOAs (such as peroxisomal proliferation) and similar data on MBC itself were not submitted.

7. Relevance of MOA to Humans

There are no data excluding the relevance of the proposed MOA to humans. *In vitro* evidence of aneuploidy in cultured human lymphocytes and disruption of mitotic spindle assembly and chromosomal segregation in cultured human granulosa cells have been demonstrated following exposure to MBC (MRIDs 51975402 and 51975404, respectively), supporting susceptibility of human cells to induction of aneuploidy by MBC. McCarroll *et al.* (2002) noted a correlation between aneuploidy and certain tumor types in humans; numerous review articles discuss this subject (Aardema *et al*., 1998; Fang and Zhang, 2011; Lynch *et al.*, 2019).

The CARC concluded that the registrant's proposed MOA for mouse liver tumors is relevant to humans, based on susceptibility of all mammalian cells to aneugenic damage and potential association with cancer.

CARC conclusions on the proposed MOA for liver tumors in CD-1 mice:

Overall, the CARC concluded that the Registrant's proposed MOA for liver tumors in male and female CD-1 mice was not fully supported under the IPCS MOA framework. Key Events #1-3 were adequately supported, and Key Event #4 was partially supported (hepatocellular toxicity was more pronounced in males than females). Temporal concordance was observed. However, there was a lack of dose-concordance for the Key Events, with a lack of in vivo data for Key Events #s 1 and 2, and the tumor incidence was increased at a lower dose than dose levels showing evidence of hepatotoxicity. Although CARC considered the MOA plausible and relevant to humans, data directly demonstrating an association of disruption of mitotic spindle formation and aneuploidy with induction of liver tumors in mice were not provided. Alternative non-genotoxic MOAs could not be ruled out and represent data gaps and uncertainties in the proposal. However, the CARC concluded that aspects of the MOA (demonstration of aneugenicity and a threshold dose level of MBC required to cause aneugenicity) were adequately supported by the available data.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

A. Carcinogenicity

Mice

- The CARC concluded that MBC induced hepatocellular tumors in male and female CD-1 mice (MRID 00154676). The CARC considered the liver tumor incidence data from the

original study pathology report together with the incidence data from the pathology reevaluation (MRID 41607901). The high dose males were not included in the statistical analyses of tumor incidence due the excessive toxicity observed in that dose group.

- The CARC concluded that the hepatocellular tumors (combined adenomas and carcinomas) were treatment-related at ≥500 ppm (≥75 mg/kg/day) for males and females. In males, a statistically significant increase ($p<0.01$) by pair-wise comparison to controls in the incidence of combined hepatocellular adenomas and carcinomas was observed at both the low and mid doses in the original pathology report, but in the re-evaluation, significance was only observed at mid dose ($p<0.01$). However, a statistically significant positive trend ($p<0.01$) was observed in both evaluations. Tumor incidence in treated males was comparable to historical controls in a concurrent study on benomyl (32%) but higher than controls in a second undated study (16%) in the historical control data set. In females, statistically significant increases by pair-wise comparison to controls in combined hepatocellular adenomas and carcinomas were observed in both evaluations at all doses tested ($p<0.01$ except $p<0.05$ for low dose females in the reevaluation), along with a statistically significant positive trend ($p<0.01$), in both evaluations. Tumor incidence in treated females exceeded the incidence in the limited available historical control data set at all dose levels (8%, concurrent study in benomyl and 5%, undated study). Non-neoplastic liver lesions included hepatocellular hypertrophy and necrosis in males at 1500 ppm (and the discontinued high dose of 7500/3750 ppm) and cellular alterations of eosinophilic foci (1500 ppm) and basophilic foci (7500 ppm) in females.
- The CARC concluded dosing was adequate in males at the low and mid dose and at all dose levels in females.
- The CARC noted the late onset of tumors in both sexes, lack of robust evidence of pre-neoplastic liver lesions in the study, limited availability of historical control data, lack of a third dose group in males for evaluating tumor dose-response, and lack of interim or subchronic data on liver effects to evaluate progression of liver histopathology and presence of aneuploid liver cells.

Rats

- The CARC concluded that MBC is not carcinogenic in the rat.
- A two-year study in CRL:CD1 rats submitted to the Agency (MRID 00088333) showed no evidence of carcinogenicity up to 430 mg/kg/day. Dosing was considered adequate in males and females.
- A two year study in Wistar rats summarized in WHO (1993) and APVMA (2009) showed no evidence of carcinogenicity up to 500 mg/kg/day. The study is considered supportive of lack of carcinogenicity in the rat.

B. Mutagenicity

- The CARC concluded that the available data (submitted and open literature studies) demonstrate aneugenicity, an indirect mode of genotoxicity, for MBC (see MOA, below). Submitted and open literature studies support that MBC induces aneuploidy, tri/multi-polar mitotic spindles and micronuclei formation in dividing cells.
- The CARC concluded that the genotoxicity database indicates the potential for direct interaction of MBC with DNA, resulting in mutagenicity or clastogenicity, is low.

- The CARC noted that aneugenicity is widely accepted as the genotoxic MOA for MBC as summarized in other scientific reviews and open literature studies (WHO, ECHA, JMPR, McCarroll *et al., etc*.).

C. Structure Activity Relationship

- Structurally-related benzimidazole fungicides that are metabolized to MBC, benomyl (cancelled; same classification as MBC) and TM, are aneugenic and induce liver toxicity and liver tumors in CD-1 mice. TM was classified as "*Likely to be Carcinogenic to Humans"* based on an increase in mouse liver tumors and rat thyroid tumors.
- Thiabendazole demonstrated weak aneugenic potential and was classified as "*Suggestive Evidence of Carcinogenic Potential to Humans*" based on rat thyroid tumors.

D. Mode of Action

The Registrant proposed aneugenicity as the likely MOA of MBC for induction of hepatocellular tumors in CD-1 mice. The CARC evaluated the evidence supporting their proposal in an IPCS Framework MOA Analysis that was based on the framework analysis presented in McCarroll *et al.* (2002). No new data were submitted by the Registrant, but open literature studies relevant to the Key Events were identified and reviewed for this reevaluation. In assessing the proposed MOA, the CARC considered both whether the available data supported (1) aneugenicity (indirect genotoxicity, a threshold effect) as the genotoxic MOA of MBC and (2) the Registrant's proposal of aneugenicity as the MOA for induction of liver tumors in the mouse.

- Molecular Initiating Event/Key Event #1 (Interaction of MBC with Tubulin and/or Other Mitotic Spindle Components and Disruption of Spindle Assembly): The CARC concluded that the available data are adequate to support the molecular initiating event and Key Event #1. Binding to tubulin and/or other protein components of the mitotic spindle in mammalian and fungal cells, and subsequent disruption of spindle formation, is well-characterized in the open literature and widely accepted for MBC and other benzimidazole fungicides. Immunohistofluorescent staining of tubulin and centromere components in dividing human granulosa cells exposed to MBC demonstrated disruption of spindle assembly. However, data are not available in the mouse.

- Key Event #2 (Chromosomal Non-disjunction/Mal-segregation): The CARC concluded that the available data are adequate to support Key Event #2. Studies in cultured human lymphocytes exposed to MBC demonstrated that chromosomal loss does not occur until non-disjunction occurs, supporting a relationship between these two events, and these effects show a dose-dependent increase, occuring at/above a threshold dose. Immunohistofluorescent staining of α-tubulin and staining of DNA in dividing human granulosa cells exposed to MBC visually demonstrated inaccurate chromosomal segregation associated with altered spindle formation.

- Key Event #3 (Lagging Chromosomes/Micronucleus Formation): The CARC concluded that the available data are adequate to support Key Event #3. Micronucleus formation and/or aneuploidy demonstrate a threshold response in mouse bone marrow and mouse

liver following *in vivo* MBC exposure. An increase in numerical chromosome aberrations that increases over time was observed; a small change in structural chromosome aberrations (small increase in gaps early on that is not consistent over time was noted). Micronuclei formation occurs temporally with the numerical chromosome aberrations and the latter progress to polyploidy over time. Predominant genotoxic effects observed relate to abnormal chromosomal segregation. The observed effects in bone marrow cells were identified within 6-12 hours following exposure; mouse liver was not examined until several days after a partial hepatectomy and dosing with MBC, but data support aneugenicity occurring in CD-1 mouse liver.

- Key Event #4 (Hepatotoxicity): The CARC concluded that Key Event 4 is partially supported by the available data, based on a more robust toxic response in the liver of male CD-1 mice than females in the chronic toxicity/carcinogenicity study. Non-neoplastic lesions were observed at ≥225 mg/kg/day in both sexes. In males, hepatocellular necrosis, hypertrophy and pigment in hepatic interstitial macrophages were observed. In females, an increase in cellular alterations of eosinophilic and basophilic foci (the latter only at 1125 mg/kg/day) was noted; hepatocellular hypertrophy and necrosis were not observed. Interim sacrifice data were not available to assess effects present at shorter exposure durations or the presence of aneuploid hepatocytes. Liver is a target organ in the MBC database. A treatment related increase in hepatocellular combined adenomas and carcinomas was observed in both sexes at ≥75 mg/kg/day.

- The CARC concluded that the available data do not adequately support dose-concordance, due to lack of *in vivo* dose-concordance data in the mouse for Key Events #1 and #2, and lack of evidence of liver toxicity or aneuploidy at the tumorigenic dose of 75 mg/kg/day. However, temporal concordance was supported based on rapid onset of mitotic spindle disruption in treated cells and occurrence of hepatocellular toxicity within weeks to months.

- The CARC concluded that the proposed MOA is plausible. Aneuploid cells are frequently observed in tumor cell populations. Tumor induction may result from proliferation of cells with aberrant chromosomal distribution or through cell death and stimulation of proliferation. For similar reasons, and because the Key Events were demonstrated experimentally in human cells, the MOA is considered relevant to humans.

- The CARC concluded that the available data did not conclusively rule out all plausible alternative non-genotoxic MOAs. Studies assessing alternative MOAs (e.g., oxidative stress, *etc.* in the mouse liver) were not available. The mouse 28-day study on benomyl provided limited data on potential alternative MOAs (lack of peroxisomal proliferation) and did not directly test MBC.

- The CARC concluded that data gaps exist in the characterization of the proposed MOA. There is no evidence linking MBC inhibition of mitotic spindle assembly and aneuploidy/micronucleus formation to mouse liver carcinogenicity and no data demonstrating the presence of aneuploid cells in the liver tumors or their causality with

liver tumor induction. Liver toxicity, a key event of the MOA, was not observed at the tumorigenic dose of 75 mg/kg/day in the available study.

- Based on the above considerations, the CARC concluded that the Registrant's proposed MOA of aneuploidy for induction of mouse liver tumors was not fully supported under the IPCS MOA framework by the available data because it did not adequately demonstrate dose-concordance for the Key Events, evidence for aneuploidy in the mouse carcinogenicity study, causality of aneuploid cells to liver tumor induction, or conclusively eliminate the possibility of alternative non-genotoxic MOAs. The available studies demonstrating threshold effects tested at higher dose levels relative to the tumorigenic dose of 75 mg/kg/day in the mouse carcinogenicity study.

- The CARC concluded that aneugenicity is adequately supported by the available data. The data also support a threshold response for the aneugenic effect of MBC and low potential for direct interaction with DNA.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's *Final Guidelines for Carcinogen Risk Assessment* (March, 2005), the CARC classified MBC as "*Suggestive Evidence of Carcinogenic Potential."* This classification was based on the following weight-of-the-evidence considerations:

(1) A treatment-related increased incidence of combined hepatocellular adenomas and carcinomas was observed in male and female CD-1 mice;
(2) The CD-1 mouse shows increased susceptibility to liver tumors, indicating it may be a particularly sensitive strain, i.e., common tumor type in this strain of mouse;
(3) Tumors were late-onset (in males, Week 66, adenoma and Week 88, carcinoma; in females, Week 90, adenoma and Week 77, carcinoma), were not associated with increased mortality, and the available non-neoplastic liver histopathology did not demonstrate robust evidence of pre-neoplastic lesions;
(4) No treatment-related tumors were identified in the CRL:CD1 or Wistar rat;
(5) Genotoxicity studies demonstrated that MBC shows low potential for direct DNA damage (mutagenicity or clastogenicity) but does demonstrate aneugenicity and the requirement for a threshold dose to induce this effect. Disruption of spindle assembly by MBC results in chromosomal mal-segregation and abnormal chromosomal distribution (micronucleus formation, aneuploidy). The MOA is considered relevant to humans since aneuploidy is often associated with tumors in humans;
(6) The proposed cancer MOA of aneugenicity for induction of mouse liver tumors, while considered plausible and relevant to humans, was not fully supported by the available data under the IPCS framework analysis due to lack of data adequately demonstrating dose-concordance, causality of aneugenicity to induction of mouse liver tumors, and lack of data excluding alternative non-genotoxic MOAs.
(7) However, aspects of the MOA are supported. Data supporting Key Events #s 1-3 (aneugenicity), and the genotoxicity database for MBC as a whole, provide robust

support for induction of aneuploidy (a threshold effect) and lack of direct DNA damage by MBC.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Quantification of human cancer risk is not required for MBC. This determination took into consideration the genotoxicity data on MBC supporting aneugenicity, a non-linear threshold effect, as the genotoxic MOA.

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