

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

BENOMYL AND MBC (PRINCIPAL BENOMYL METABOLITE)

SB 950-201, Tolerance # 294
Chemical Code 1552

August 14, 1986

Revised 11/6/86, 9/15/87, 5/16/89, 9/21/89,
10/9/90, 3/14/91, 12/18/91, 9/24/93, 2/15/95, 9/3/96, 10/01/97

I. DATA GAP STATUS

Chronic rat:	No data gap, possible adverse effect.
Chronic dog:	No data gap, possible adverse effect.
Oncogenicity, rat:	No data gap, no adverse effect.
Oncogenicity mouse:	No data gap, possible adverse effect.
Reproduction rat:	No data gap, possible adverse effect.
Teratology rat:	No data gap, possible adverse effect.
Teratology rabbit:	No data gap, possible adverse effect.
Gene mutation:	No data gap, possible adverse effect.
Chromo. aberration:	No data gap, possible adverse effect.
DNA damage:	No data gap, possible adverse effect.
Neurotoxicity:	Not required at this time.

NOTE: Toxicology one-liners are attached. ** Before the one-liner indicates an acceptable study.
Bold face of volume and record numbers indicates a possible adverse effect.

Previous versions of Summary by F. Martz, and J. Gee. Rectified with Library printout of 2/15/95 including record #'s up to 131147 (Document No. 294-161) and 900000+. 10/9/90 update by Aldous, 3/14/91 and 12/18/91 by Gee, 9/24/93 and 2/15/95 by Kellner, 9/3/96 by Gee [volumes 140 and 146 were overlooked in previous reviews]. P. Iyer, 10/1/97.

MBC is methyl 2-benzimidazolecarbamate, a breakdown product of several fungicides including benomyl, thiophanate-methyl, and other thiophanates.

These pages contain summaries only. Each individual worksheet may contain additional effects.

II. TOXICOLOGY ONE-LINERS

294-140 123817 "Assessment of the Mammalian Toxicity and Potential Human Health Effects of Benomyl" (Hurt, M. E., Reynolds, V. L. and Stadler, J. C., Haskell Laboratory for Toxicology and Industrial Medicine, Du Pont, 1/93) This document reviews studies in many areas of toxicology including genotoxicity, acute toxicity, subchronic and chronic toxicity and effects on development and reproduction. It contains approximately 15 pages of citations. No worksheet. (Gee, 8/30/96)

RAT COMBINED TOXICITY/ONCOGENICITY STUDIES

NOTE: DPR considered the collective data on chronic rat feeding studies to serve the purpose of a "combined" rodent (chronic/oncogenicity) study as of 5/16/89, and no further rat chronic or oncogenicity study is required at this time. No individual study was classified as individually "acceptable", however studies 059:036267 and 079:044582, as supplemented by information requested by DPR, are considered to have addressed the basic purposes of a "combined" study.

Some major concerns which remained prior to 5/16/89 related to the test article: (1) the recognized instability of the parent compound, Benomyl, in diet; (2) the effects that formulation excipients might have on toxicity; and (3) lack of periodic analyses of test article in feed. These concerns were effectively addressed by Dr. O'Neal, in a meeting with DPR toxicologists on 4/21/88. Dr. O'Neal presented information showing that the "instability" of Benomyl was due to its hydrolysis to MBC. Modern methods of analysis, which quantitate both Benomyl and MBC, indicate that MBC is relatively stable. The excipients were examined, and none were considered likely to impact Benomyl or MBC stability. DPR noted that adequate stability of Benomyl/MBC had been shown in a more recent mouse oncogenicity study (060:036269). Thus, issues relating to test article were effectively resolved as of 4/21/89.

The other primary concern which DPR had about the rat chronic studies was lack of ophthalmology. DPR indicated in the meeting of 4/21/88 that the overall evaluation of chronic effects on the eye would be resolved by a combination of (1) multiple sections of eyes from dog chronic study 059:036268 and (2) the normal evaluations (single section per eye) of the two rat studies, 059:036267 and 079:044582. On receipt of the multiple section evaluations of eyes from dog study, 059:036268, DPR considered that the overall consideration of ophthalmology was complete for both species. At this time the rat chronic/oncogenicity data gap was considered filled (see review by J. Gee on the dog study, dated 5/16/89. (The above overview by Aldous, 12/22/89).

BENOMYL

294-059 036267 (with rebuttal in -076:043797): "Long-Term Feeding Study in Rats with 1-Butylcarbamoyl-2-Benzimidazolecarbamic Acid, Methyl Ester [INT-1991; Benlate; Benomyl];" Haskell Laboratory, 8/15/69; benomyl (INT-1991, 50% or 70% AI) at 2500, 500, 100, or 0 ppm AI in the feed. Deficiencies noted: no MTD, no ophthalmoscopic exams, and instability of Benomyl in the feed. As indicated above, this study was not considered independently acceptable, however this study is considered by DPR to contribute to filling the "combined" study data requirements. (Apostolou, 11/18/85; Martz, 6/4/86; Aldous, 12/22/89; the latter review did not involve a worksheet, but this Summary was updated for clarification).

EPA One-liner: "Systemic NOEL > 2500 ppm."

NOTE: The memo from EPA to DPR addressing differences in data gap status for this chemical (dated 2/07/89) notes EPA classification as "Core Minimum" as a chronic study, and "Supplementary" as an oncogenicity study..

294-076 043797: Rebuttal and supplemental information to # 036267. Contains narrative comments regarding dose level selection, data about feed analysis and stability and a supplemental pathology report. Stability data indicate 50% loss in 2 days at room temperature, with only 19% of activity remaining after 5 days. Refrigerated samples retained activity for 7 days. This stability issue was considered a major problem for this study until clarified as indicated in introductory paragraphs, above. One-liner added 9/4/87, Martz; modified on 12/22/89, Aldous.

MBC

294-079 044582 (with rebuttal in -095, Tabs 3 and 4): "Long-Term Feeding Studies in Rats and Dogs With 2-Benzimidazolecarbamic Acid, Methyl Ester [INE-965] in Rats"; Haskell Laboratory, 5/25/72; MBC formulated as wettable powder, at 5000, 500, 250, 100, or 0 ppm in the feed with a fifth group starting at 2500 ppm and increased to 10,000 ppm by week 20, to CD rats; slight increase in liver weight; **Possible adverse effect** in liver: increased incidence and severity of pericholangitis/cholangiohepatitis, mainly females, with NOEL = 100 ppm. First review: UNACCEPTABLE: no MTD, no feed analysis, inadequate group sizes and no ophthalmoscopic exams. Rebuttal partially answered deficiencies (9/4/87), but report was still classified as unacceptable due to the absence of feed analysis and ophthalmoscopic exams. This study was considered to contribute to filling the "combined" rat data requirement on 5/16/89, as indicated in the introductory paragraphs, above.

REVIEW: 7/15/86 by Martz, rebuttal response and second review 9/4/87 by Martz, with NOEL change (see "COMMENT" below). Updates by Aldous, 12/22/89.

NOTE: The memo from EPA to DPR addressing differences in data gap status for this chemical (dated 2/07/89) notes EPA classification as "Core Minimum" for oncogenicity and chronic study data requirements.

294-095 TABS 3 and 4 (no record#): Narrative rebuttal to # 044582. Provides comments about dose level selection, frequency of clinical observations, clarification of urinalysis and information about feed analysis. Analysis of single batch of blends indicated acceptable AI content, ranging from 87%-108% of intent. Reference is made to AI/feed stability analysis data generated in a mouse oncogenicity study with MBC which DPR accepted (see #44585 in -080). This could satisfy our concern retrospectively, except that stability data were generated with technical material whereas the combined rat study utilized formulated material containing approximately 20% to 50% excipients whose effect on stability is unknown. The 9/4/87 DPR review indicated that based on this as well as on the absence of ophthalmoscopic exams, this study could not be upgraded. As indicated in the note at the beginning of this section, the collective rat chronic data now are considered to fill the "combined" study data gap. Martz, 9/4/87 (no separate Worksheet for rebuttal itself); Aldous (no worksheet), 12/22/89.

COMMENT: The MBC rat feeding study (079:044582) was originally reviewed as demonstrating an adverse effect based on an increased incidence and severity of "spontaneously-occurring cholangiohepatitis/pericholangitis" in the 10,000 and 5000 ppm groups with 500 ppm being the NOEL. Re-review of this report as part of the rebuttal process led to a reduction of the NOEL to 100 ppm, based on an increased overall lesion incidence in 500 ppm females as well as an increase in lesion severity in that group. The tabulation of lesion and severity as well as the basis for the NOEL change is covered in a separate "Supplemental Information or Peer Review Worksheet" dated 9/4/87, by F. Martz.

CHRONIC DOG STUDIES

COMMENT ON CHRONIC DOG STUDIES: The hepatotoxic potential of benomyl/MBC is well documented when all 3 studies are considered together, with the newest study demonstrating a clear NOEL of 200 ppm. The new study [1986 Haskell Labs study on MBC] also demonstrated the absence

of testicular atrophy under guideline test and husbandry conditions, so that adverse effect noted in the earlier study [1970 Haskell Labs study on Benomyl] can be discounted. 9/3/87, Martz. With submission of record # 072845, reexamination of eyes from record # 036268 [1970 Haskell Labs study on Benomyl], the collective data on rodent/non-rodents are upgraded to adequate. Thus, **although no single dog chronic study is independently classified as "acceptable", the data requirement for a dog chronic study is filled.** Gee, 5/16/89.

BENOMYL

294-059 036268 (with rebuttals in -076, 43800, and -095, TAB 2): "Long-Term Feeding Study in Dogs with 1-Butylcarbamoyl-2-Benzimidazolecarbamic Acid, Methyl Ester [INT-1991; Benlate; Benomyl];" 2 year study with 1 year interim sacrifice; Haskell Laboratory, 3/17/70; INT-1991, 50% pure with remainder as formulation excipients, at 2500, 500, 100, or 0 ppm AI in the diet to 4/sex/level with interim sacrifice of 1/sex/level at 1 year. **Possible adverse effect** in liver: increased alkaline phosphatase, SGPT and cholesterol (males mainly) with "cirrhosis" at 2500 ppm; testicular atrophy with no clear NOEL due to intercurrent disease; rebuttals partially satisfy major deficiencies, but study still UNACCEPTABLE in absence of ophthalmoscopic exams.

REVIEW: original 11/20/85 by Apostolou, rebuttal reviews 6/9/86 and 9/2/87 by Martz. See comment under 107 # 072845 below. Gee, 5/16/89.

EPA One-liner: "systemic NOEL = 500 ppm,...LEL = 2500 ppm (HDT, cirrhosis and adverse effects on testis. No effect on sperm production." NOTE: The memo from EPA to DPR addressing differences in data gap status for this chemical (dated 2/07/89) notes EPA classification as "Core Minimum".

294-076 043800: Rebuttal and supplemental information to # 036268. Contains comments on number of animals, study duration, feed analysis and stability as well as a supplemental pathology report discussing liver and testicular findings. Does not upgrade study. One-liner added 9/3/87, Martz.

294-095 TAB 2 (no record#): Second rebuttal to # 036268. Provides clarification of feed preparation and feeding procedures, which generally ameliorate DPR concerns about compound/feed stability. Does not upgrade study in absence of ophthalmoscopic exams. 9/2/87, Martz (no separate Worksheet).

294-107 072845 Supplement to 036268. Results of additional sections of eyes from the study as discussed at the April 21, 1988, meeting with the registrant for upgrading the total data. Supplement dated 11/14/88. A total of seven sections, about 100 microns apart, including the original section, were evaluated histologically. With this submission, with negative results, the collective data for chronic feeding studies in rodents and non-rodents is considered acceptable. Gee, 5/16/89.

MBC

294-079 044584 "Long-Term Feeding Studies in Rats and Dogs with 2-Benzimidazolecarbamic Acid, Methyl Ester [INE-965]," Haskell Laboratory, 5/25/72; 2-year study with 1 year interim sacrifice; INE-965 formulated as a wettable powder, at 2500, 1500, 500, 100, or 0 ppm AI in the feed to 4/sex/level initially; anorexia and weight loss at 2500 ppm, several dogs reduced to 1500 ppm, no effect at 500 ppm; 2 high dose males sacrificed in extremis (and replaced) in week 22, another moribund in week 42; **ADVERSE EFFECT** in liver: elevated alkaline phosphatase, SGPT, and cholesterol, decreased albumin, as well as "hepatic cirrhosis, inflammation, and fatty liver," NOEL = 100 ppm, equivalent to about 3 mg/kg/day. UNACCEPTABLE AND NOT UPGRADEABLE, no feed analysis or ophthalmoscopic eye examinations.

REVIEW: 6/14/86 with second review 11/6/86, both by Martz.

NOTE: The memo from EPA to DPR addressing differences in data gap status for this chemical

(dated 2/07/89) notes EPA classification as "Core Minimum".

294-093 049262: "One-year Feeding Study in Dogs with INE-965;" Haskell Laboratory, 6/27/86; MBC, 98.8% pure, at 500, 200, 100, or 0 ppm in the feed for 1 year to 5/sex/level; no histopathologic changes; slight increase in serum cholesterol and decrease in serum albumin at 500 ppm, not considered adverse effects by themselves; NOEL = 200 ppm, equivalent to about 7 mg/kg/day; UNACCEPTABLE - no ophthalmoscopic exams (otherwise OK); possibly upgradeable with additional information.

REVIEW: 11/4/86 by Martz.

EPA One-liner: none in Branch Library.

MOUSE ONCOGENICITY STUDIES

NOTE: Additional information provided in Document 294-117 affects interpretation of Benomyl and MBC mouse oncogenicity studies in three important ways: (1) Record 088852 contains a peer review of all available liver slides from the two studies below, which are accepted by DPR. That review team concluded that hepatocellular adenoma incidence and multiplicity was increased by Benomyl and MBC without definitive NOELs. In addition, non-neoplastic foci of cellular alteration were observed in some instances. This is a major change from original reports, which had indicated increases in hepatocellular carcinoma incidence for both Benomyl and MBC. (2) Record 088852 also contains three publications. The chief importance of these articles for this Summary is that the comparatively uncommon tumors, hepatoblastomas, were almost always found within or adjacent to hepatocellular adenomas or carcinomas. Thus hepatoblastomas should not be considered as an independent tumor type. (3) Record 088853 presents several lines of evidence that Benomyl elicits significant liver toxicity at high doses, which would be expected to predispose these animals to hepatocellular tumors. Aldous, 9/21/90.

BENOMYL

****294-060 to -062, 036269-71** Wiechman, B.E., "Long-Term Feeding Study with Methyl-1-(Butylcarbamoyl)-2-Benzimidazolecarbamate, (INT-1991, Benomyl, Benlate) in Mice". Haskell Laboratory, Report No. 20-82, 1/26/82. Benomyl (INT-1991), 99% pure, at 0, 500, 1500, or 5000 ppm in the feed to CD-1 mice for 2 years [the latter group received 7500 ppm for 37 weeks before dose was reduced to 5000 ppm due to excessive toxicity]. **Possible adverse effect:** increased incidence and/or multiplicity of hepatocellular adenomas in both sexes without an apparent NOEL. High dose males and females also had increased incidence of foci of hepatocellular alteration. [See 117:088852 and associated DPR review for data on hepatocellular tumors and altered foci.] Additional non-neoplastic lesions in livers of high dose males considered to be treatment-related were noted in a 8/13/86 review by Martz. Other lesions in various tissues, possibly related to treatment, were also generally restricted to high dose males, and are noted in the same review. The rebuttal in Document 294-076 (below) was considered in the 8/13/86 review by Martz. DPR review history: Study **accepted** in 11/25/85 by Apostolou, with indication of "possible adverse effect" (liver neoplasia); re-examination by Martz on 8/13/86 confirmed acceptability and "possible adverse effects" status; re-examination by Aldous on 9/24/90 involved worksheets for supplementary data in Document 294-117 (see note above and individual 1-liners, below) noted that hepatocellular adenomas, not carcinomas, were increased in both sexes. Study status; **Acceptable, possible adverse effect**. Aldous, 9/24/90.

EPA One-liner: "Oncogenic NOEL < 500 ppm male and female significant increase in hepatocellular neoplasms in male and female." No grade given.

294-076 043798, 043799: Rebuttal asserting that the mouse hepatocellular tumors are not biologically significant [to human health]. Assertion is supported by an article, "The relevance of mouse liver hepatoma to human carcinogenic risk" [A report of The International Expert Advisory Committee to the

Nutrition Foundation, Sept. 1983]. The DPR review of 8/13/86 considered this article, but considered that the "weight of evidence" of a treatment effect of potential relevance to human health was sufficiently strong that DPR should continue to classify findings as "possible adverse effects". The elevated tumor incidence in high dose females, which do not have high background incidence of such tumors, was specifically mentioned by Martz in the 8/13/86 review.

294-117 088852 [supplementary to 294-060:036269 (mouse oncogenicity study with Benomyl) and 294-080:044585 (mouse oncogenicity study with MBC)]. Frame, S. R., and Van Pelt, C. S., "Oncogenicity studies with benomyl and MBC in mice: Supplemental peer review". Re-evaluation of liver slides for the above two studies by two Haskell Laboratory pathologists (presumably the two authors, above), together with EPL pathologist, Jerry F. Hardisty, D.V.M. Report date: 6/28/90. The re-evaluation employed NTP criteria for classifying lesions. The re-evaluation found the incidence and/or multiplicity of adenomas to be increased at one or more dose levels for both sexes following treatment with Benomyl or MBC. There was no definitive NOEL for adenomas. Unlike the original pathologist's evaluation, re-examination of slides did not confirm a treatment effect on hepatocellular carcinoma incidence. High dose males treated with Benomyl had increased incidence of foci of cellular alteration. New data are tabulated in the present review. Aldous, 9/19/90.

294-117 088852 [The first of 3 related published articles within this record, supplementary to lifetime Benomyl and MBC studies]. Nonoyama, T., Fullerton, F., Reznik, G., Bucci, T.J., and Ward, J.M. "Mouse hepatoblastomas: A histologic, ultrastructural, and immunohistochemical study". Vet. Pathol. 25:286-296 (1988). Hepatoblastomas were studied in mice with the following variables: strain (B6C3F1 and BALB/c), sex, and amount of dietary 2-acetylaminofluorene (2-AAF). There were 96/sex/group; males received 0, 20, 40, 60, or 80 ppm 2-AAF, and females received 0, 100, 125, 150, 200, or 250 ppm 2-AAF. Study duration: 2 yr. Twenty-two hepatoblastomas were found: 20 of these in the B6C3F1 mice. Hepatoblastomas were more common in males than in females, and appeared to be dose-related to 2-AAF. All but 3 of the 22 hepatoblastomas were located within or adjacent to hepatocellular adenomas or carcinomas. Morphological characteristics indicated that the hepatoblastomas were less differentiated than hepatocellular adenomas or carcinomas. Histogenesis of the hepatoblastomas could not be established, however investigators did not find evidences of transition between hepatoblastomas and other hepatocellular tumors. Aldous, 9/20/90.

294-117 088852 [The second of 3 related published articles within this record, supplementary to lifetime Benomyl and MBC studies]. Diwan, B.A., Ward, J.M., and Rice, J.M., "SHORT COMMUNICATION: Promotion of malignant 'embryonal' liver tumors by phenobarbital: increased incidence and shortened latency of hepatoblastomas in (DBA/2 X C57BL/6)F1 mice initiated with N-nitrosodiethylamine", Carcinogenesis 10:1345-1348 (1989). Male mice were used in a study in which variables were (a) strain, (b) presence or absence of initiator [N-nitrosodiethylamine (NDEA)], (c) presence or absence of promoter [phenobarbital (PB)], and (d) age to sacrifice (33 wk or 47 wk). Test animals were all hybrids of the above two strains, but groups were either offspring of DBA/2 males and C57BL/6 females (B6D2F1 mice), or of DBA/2 females and C57BL/6 males (D2B6F1 mice). Each combination of a, b, c, and d above involved 10 mice. As expected, the hepatocellular tumor yield was increased by NDEA, particularly in PB-promoted mice. The NDEA/PB mice of either strain had relatively high incidence of hepatocellular adenomas at wk 33, and of adenomas and carcinomas at wk 47. Ten mice had one or more hepatoblastomas: all of these from NDEA/PB groups, and 9 of these were D2B6F1 mice. Hepatoblastomas were generally found in or adjacent to hepatocellular adenomas or carcinomas. Investigators concluded that susceptibility to hepatoblastomas is based on an autosomal dominant trait. Aldous, 9/20/90.

294-117 088852 [The third of 3 related published articles within this record, supplementary to lifetime Benomyl and MBC studies]. Diwan, B.A., Rice, J.M., and Ward, J.M. "Strain-dependent effects of phenobarbital on liver tumor promotion in inbred mice". Prog. Clin. Biol. Res. 331:69-83 (1990). Note

from the 1-liner above that these investigators found strain differences in male mice to hepatoblastoma development, and found that initiation and promotion was required to elicit hepatoblastomas under conditions of the study. The present article summarizes a subsequent study, which confirmed the development of hepatoblastomas in D2B6F1 males, but which found no hepatoblastomas in D2B6F1 females under identical treatment. No DPR worksheet. Aldous, 9/20/90.

294-039 965471 Partial duplicate of 060:036269, above.

294-117 088853 [supplementary to 294-060:036269 (mouse oncogenicity study with Benomyl)]. Van Pelt, C. S., "28-Day feeding study with Benomyl in mice", Haskell Laboratory, 8/15/90. Benomyl (Belle Plant Lot #F60317K, 96.1%) was fed to CD-1*(ICR)BR mice. Doses of 0, 100, 500, 3750, or 7500 ppm Benomyl were administered to groups of 20 male mice: half were sacrificed at 14 days, half at 28 days. Emphasis was placed on liver toxicity: livers were evaluated for pathology, cell proliferation (BrdU incorporation), b-oxidation activity of peroxisomal fraction, and cytochrome P-450 content. Liver relative weights of 3750 and 7500 ppm groups were statistically significantly elevated at both sacrifice times, and absolute weights of both groups were statistically significantly elevated on day 14 also. Minimal to mild hypertrophy (generally centrilobular) was observed in both time periods in the two higher dose groups. Cell proliferation was suggested by non-significant elevations in BrdU uptake at 28 days in the 3750 and 7500 ppm groups. Statistically significant increases in cytochrome P-450 content were found at days 14 and 28 in 7500 ppm groups. Peroxisomal activity was apparently not affected in any groups. A provisional NOEL of 500 ppm is suggested, however additional electron micrographs will be examined for analysis of SER proliferation in liver. Data support, but do not prove, the idea that non-genotoxic mechanisms are causes of mouse liver parenchymal cell tumors. Aldous, 9/21/90.

MBC

****294-080 & 81, 044585 & 86;** "Long-Term Feeding Study with 2-Benzimidazole-carbamic Acid, Methyl Ester (MBC, INE-965) in Mice;" Haskell Laboratory, 1/26/82; MBC, 99.3% pure, at 7500, 1500, 500, or 0 ppm in the feed to CD-1 mice for 2 years; ONCOGENICITY EFFECT in liver: hepatocellular adenomas and carcinomas in females with NOEL<500 ppm (LDT); hepatotoxicity in males only with NOEL<500 ppm; report complete and study ACCEPTABLE.

REVIEW: 7/1/86 by Martz.

EPA One-liner: none in Branch Library.

294-039 965472 Summary of 080:044585, above.

294-081 044587-92: "Carcinogenicity Study with Carbendazim in Mice;" Central Institute for Nutrition and Food Research (Netherlands), 9/76; MBC, 99% pure, at 5000, 300, 100, or 0 ppm in the feed to Swiss mice for 18 months; ONCOGENICITY EFFECT in liver: at 5000 ppm, hepatocellular adenomas in females and hepatoblastomas in males; liver weight elevation in both sexes at 500 ppm; overall NOEL = 300 ppm; incomplete - summary only with additional review comments.

REVIEW: 7/16/86 by Martz.

EPA One-liner: none in Branch Library.

294-082 to -085 044593-96: "Repeated-dose (24 month) Feeding Study for Determination of the Carcinogenic Effect of HOE 17411 0 F AT204 (Carbendazim) in Mice;" Hoechst AG (Frankfurt), 10/13/82; MBC, >99% AI, at 5000, 300, 150, 50, or 0 ppm in the feed to Hoe:NMRKf(SPF71) mice for 22 months; **ADVERSE EFFECTS:** hepatotoxicity and ovarian granulosa cell tumors and/or luteomas with NOEL = 150 ppm for both effects. Acceptable, as supplement to #44587-92, with additional information.

REVIEW: 8/5/86 by Martz.

EPA One-liner: none in Branch Library.

REPRODUCTION AND FERTILITY STUDIES

BENOMYL

**** 294-124 096358** "Reproductive and Fertility Effects with DPX-T1991-529 (Benomyl) Multigeneration Reproduction Study in Rats." (Mebus, C. A., Haskell Laboratory, Report No. 765-90, 2/21/91) Benomyl, Lot # F60317K, 99%, was fed in the diet at 0, 100, 500, 3000 or 10,000 ppm to 30/sex/group CrI:CD BR rats. There were two generations with one litter in the first and two in the second. Pups were culled on day four. Parental males and females of the control and high dose groups were subjected to microscopic examination of the reproductive organs; in addition the testes and epididymides of all males were examined. Body weights were significantly lower at 10,000 ppm (nominal) for adult males and females of both the P1 and F1 generations. Pup weights were lower in the 3000 ppm in the F2A and B litters and in all litters in the 10,000 ppm groups with decreased live pups at culling at 10,000 ppm. **Possible adverse effect:** Lower sperm counts in the 3000 and 10,000 ppm males, testicular atrophy and degeneration (4/30 and 29/30 in P1 and 9/30 and 21/25 in F1 3000 and 10,000 ppm groups respectively), oligospermia in the epididymides (unilateral and bilateral with 1/30 at 3000 ppm and 26/30 at 10,000 ppm in P1, 9/30 and 20/25 in F1 respectively). NOEL = 500 ppm in males, 3000 ppm in females (decreased body weights). **Acceptable.** (Gee, 3/14/91)

294-111 73672 Protocol for Multigeneration reproduction study in rats (294-124:096358). No worksheet.

294-065 036315: "Three-Generation Reproduction Study in Rats with 1-Butylcarbamoyl-2-Benzimidazolecarbamic Acid, Methyl Ester (INT-1991);" Haskell Laboratory, 11/18/68; benomyl in wettable powder, 50% or 70% AI, at 2500, 500, 100, or 0 ppm AI in the feed to CD rats; no compound-related effects. Study UNACCEPTABLE AND NOT UPGRADEABLE, no MTD, no feed analysis (instability shown in rebuttal to benomyl combined rat study, see #43797, 076 above), inadequate group size.

REVIEW: 12/26/85 by Apostolou, second opinion 6/4/86 by Martz.

EPA One-liner: "Systemic NOEL = 100 ppm." NOTE: The memo from EPA to DPR addressing differences in data gap status for this chemical (dated 2/07/89) notes EPA classification as "Core Minimum".

MBC

294-077 044559 (with rebuttal in -095, TAB 4): "Long-Term Feeding Studies in Rats and Dogs with 2-Benzimidazolecarbamic Acid, Methyl Ester [INE-965];" Haskell Laboratory, 5/25/72; MBC formulated in wettable powder; 5000, 500, 100, or 0 ppm AI in the feed to CD rats with a fifth group receiving 2500 ppm increased to 10,000 ppm after 20 weeks; 3 generation, 2 litter study with F₀ rats "borrowed" from combined study; **ADVERSE EFFECT:** neonatal growth retardation @ 5000 and 10,000 ppm, NOEL = 500 ppm; no parental MTD. UNACCEPTABLE AND NOT UPGRADEABLE, no MTD.

REVIEW: 7/10/86 by Martz, rebuttal response 9/2/87 by Martz.

NOTE: The memo from EPA to DPR addressing differences in data gap status for this chemical (dated 2/07/89) notes EPA classification as "Core Minimum".

294-095, TAB 4: Rebuttal to # 044559, similar to that given for combined rat study for MTD and feed analysis. Does not upgrade study. 9/2/87, Martz (no separate Worksheet).

294-079 044583 Exact duplicate of 077:044559, above.

EXPLORATORY FERTILITY STUDIES

294-065 036317 Carter, S.D., and Laskey, J.W., "Effect of Benomyl on Reproduction in the Male Rat;" Health Effects Research Laboratory, US EPA, in Toxicology Letters **11**:87-94, 1972. Benomyl (technical grade) administered for 5 consecutive days/week for 2 weeks via gavage at 0, 200, and 400 mg/kg/day in block one and at 0 and 400 mg/kg/day in block two of male Sprague-Dawley rats (4-6 animals/treatment group). **Possible adverse effects indicated:** there was a 35-48% depression in total epididymal sperm count and in the vas deferens sperm concentrations at both treatment levels 14 days after termination of treatment with benomyl. Not applicable for reproduction data requirement purposes but has useful mechanistic information. First review 11/26/85 by Apostolou, second review and Worksheet by Margolis, 8/10/87; 9/2/87, Martz.

294-065 036316 Carter, S.D., "Effect of Benomyl on the reproductive development in the prepubertal male rat". [manuscript to be submitted to J. Toxicol. Environ. Health] [from 1982 Thesis]. 33-day old Sprague-Dawley rats were given 10 daily gavage treatments with 0 or 200 mg/kg/day Benomyl, and then killed at intervals of 3 to 59 days after cessation of dosing. There were no changes in sperm concentration in the vas deferens, in total epididymal sperm, nor were there changes in testicular histology. **No adverse effects indicated. Unacceptable.** Aldous (no worksheet), 9/27/90.

294-104 067403 Carter, S.D., Hess, R.A., and Laskey, J.W., "The Fungicide Methyl 2 Benzimidazole Carbamate Causes Infertility in Male Sprague-Dawley Rats." Biology of Reproduction **37**:709-717 (1987). (Health Effects Research Laboratory, U.S. EPA, Research Triangle Park). MBC (carbendazim), 98.1% and 1.9% inerts; given by oral gavage in corn oil (2 ml/kg) at 0 or 400 mg/kg/day to male Charles River rats for 10 days. These proven males (23 - 24 per group) were 90 days of age at treatment initiation. Each male was placed with 1 female for 1 week on day 3 of treatment. Females were replaced weekly with nulliparous females for 32 weeks after termination of treatment. All males were killed at week 35 post exposure, and testicular tissues were examined. Females were killed 12 days after breeding period, and uterine contents were examined. At termination, testicular weights in treated males were 39% lower than controls. In totally infertile males, this value was a 58% reduction. 10/24 males in the treated group failed to produce a pregnant female in the first week post treatment. By the 5th week, 16 males were infertile, and 12 remained infertile throughout the study. Pathology showed atrophic seminiferous tubules lined by Sertoli cells, but displaying very limited spermatogenesis, with 7/24 showing 100% tubule atrophy. **Not acceptable, due to study design**, but useful supplemental data demonstrating "**possible adverse effects**". Gee, 6/13/88.

294-104 067404 Carter, S.D., Hein, J.F., Rehnberg, G.L., and Laskey, J.W. "Effect of Benomyl on the Reproductive Development of Male Rats." J. Toxicol. Environ. Health **13**:53-68 (1984). (Health Effects Research Laboratory, U. S. EPA, Research Triangle Park). Benomyl, technical grade, no purity stated. Experiment 1: 33-day old males were given 10 daily doses by gavage at 0 or 200 mg/kg/day; after 3, 17, 31, 45 and 59 days post treatment, 8 males per group were sacrificed for gonadal tissue examinations. Experiment 2: 33, 54 and 75- day old rats [representing prepubertal, pubertal, and postpubertal ages, respectively] were given 0, 125, 250, 500 or 1000 mg/kg/day (5/interval/dose) for 5 days divided into two dosings/day. Blood samples were taken at 29 days after treatment and animals were sacrificed 31 days after treatment. Results of Expt. 1: no effect on weights of testes, seminal vesicles, or epididymides (caput or cauda), on sperm counts or on time of appearance of spermatozoa in treated group. Expt. 2: no significant effects were seen in prepubertal animals; epididymal sperm counts were depressed in pubertal animals; and postpubertal animals showed a wide variation in susceptibility of sperm counts. Histological exams of testicular tissue showed an increased incidence of diffuse hypospermatocytogenesis in pubertal and postpubertal males. **Unacceptable, not upgradeable** due to study design, with useful supplementary data showing a **possible adverse effect**. Gee, 6/13/88.

294-104 067405 Dashiell, O.L., "Ten-Dose Oral Subacute Test with Reproduction Study." (Haskell Laboratory, 3/10/78, Report 121-78). Benomyl, 50% with 50% inerts; given by gavage in corn oil at 0 or 200 mg/kg/day for 10 doses to young adult male ChR-CD rats, 30 per group. 5/group were each mated with 2 females 3 days after the last dose. The same males were mated a second time 59 days after the last dose. The number of pregnancies in the first mating was 9/10 for controls and 3/10 for treated group. In the second mating, the numbers were 9/10 and 10/10 respectively - no other reported parameters were affected. In addition, control and treated males, 5 per group, were sacrificed at 4 hours, 14, 28, 42, 70 and 90 days after the last dose and the testes and epididymides were examined. Testicular weights were reduced, and microscopic lesions were focal to diffuse degeneration of germinal epithelium, accompanied by giant cells, occasional sperm granulomas, and reduction or absence of sperm. Some effects were still seen at 90 days recovery. **Unacceptable, not upgradeable** due to study design, with useful supplementary data showing a **possible adverse effect**. Gee, 6/16/88.

RAT TERATOLOGY STUDIES

BENOMYL

****294-065 036320:** Staples, R.E., "Benomyl: Teratogenicity in the Rat After Administration by Gavage;" Haskell Laboratory, 9/18/80. Benomyl, 99.2% pure; 125, 62.5, 30, 10, 3, or 0 mg/kg/day by oral gavage to CD rats. **Possible adverse effects:** decreased litter size and fetal weights, microphthalmia or anophthalmia, and hydrocephaly. NOEL = 3 mg/kg/day (microphthalmia); maternal NOEL = 125 mg/kg/day. Report complete and study acceptable in conjunction with # 036323 below. Martz, 6/11/86.

EPA One-liner: "Unilateral microphthalmia at 10 mg/kg/day (2 animals), NOEL = 30 mg/kg/day, LEL = 62.5 mg/kg (embryotoxicity)." No grade given.

****294-065 036323:** Staples, R.E., "Benomyl Gavage: Teratogenicity in the Rat", Haskell Laboratory, 10/1/82. Benomyl, 99.1% pure; 62.5, 30, 20, 10, 6.25, 3, or 0 mg/kg/day by oral gavage to CD rats. **Possible adverse effects:** decreased fetal weights, microphthalmia and hydrocephaly at 62.5 mg/kg only. Study is valid and results support change of previous NOEL from 3 to 30 mg/kg/day. Report complete and acceptable as supplement to # 036320 above. Martz, 7/7/86.

EPA One-liner: "NOEL = 30 mg/kg, LEL = 62.5 mg/kg (microphthalmia)." Graded as "supplementary; upgraded to minimum."

COMMENT: Neither # 036320 nor # 036323 is independently acceptable. The former lacked dosing solution analysis, but this deficiency was corrected by analytical results in the latter study. The latter was unacceptable by itself because only heads were examined, in order to clarify craniofacial malformations noted in the former. However, both were excellent studies and complemented each other. They are both acceptable when taken together, and fill the data requirement. Note also that the latter study supports a change of the NOEL from 3 mg/kg to 30 mg/kg. Comment added 9/16/87, Martz.

294-065 036319 (with rebuttal in -076, 43804): "Teratogenic Study in Rats With 1-Butylcarbamoyl-2-Benzimidazolecarbamic Acid, Methyl Ester (INT-1991; Benlate; Benomyl);" Haskell Laboratory, 7/9/70; benomyl formulated as wettable powder, 50% AI at 5000, 2500, 500, 100, or 0 ppm in the feed to CD rats. No maternal or fetal effects. Original status was unacceptable but possibly upgradeable. Rebuttal cannot upgrade study, still UNACCEPTABLE but not upgradeable.

REVIEW: original 12/2/85 by Apostolou, rebuttal 6/15/86 by Martz.

EPA One-liner: "Terato NOEL = 5000 ppm (HDT)," with no grade given.

294-076 043804: Rebuttal to # 036319. Narrative explanation of randomization, dam necropsy observations, absence of corpora lutea counts, absence of soft tissue examination at lower dose levels,

and dose level justification. While the latter could satisfy the MTD criticism, documented AI instability in the feed (see # 043797, -076 above) renders study not upgradeable. One-liner added 9/8/87, Martz.

294-065 036324 Kavlock, R.J., Chernoff, N., Gray, L.E. Jr., Gray J.A., Whitehouse, D., "Teratogenic effects of Benomyl in the Wistar rat and CD-1 mouse, with emphasis on the route of administration", Toxicol. Appl. Pharmacol. 62:44-54 (1982). A rat and mouse study, with rat exposure via gavage or in diet. Only gavage portion of rat study is relevant, due to the high NOEL of the dietary segment. Rats were treated with 0, 15.6, 31.2, 62.5, or 125 mg/kg/day Benomyl (tech., in 1 ml corn oil/rat/day) days 6-15. Maternal toxicity was not apparent. NOEL for developmental toxicity = 31.2 mg/kg/day (dose-related reduction in fetal weight, hydrocephaly, microphthalmia, fused ribs, fused vertebrae, and decreased ossification in tail and in vertebral centra). Findings at the HDT of 125 mg/kg/day included: full litter resorptions in 6 of 11 surviving pregnant dams, enlarged lateral ventricles, enlarged renal pelves, and delayed ossification (more widespread than at 62.5 mg/kg/day). Fetotoxicity and teratogenicity findings in absence of obvious maternal toxicity indicate **possible adverse effects**. Note that similar findings were noted in records 036320 and 036323 in this volume. Since the latter studies identified a NOEL for developmental toxicity, it is unlikely that this study will be required for risk assessment. **Unacceptable, upgrade unlikely**. Apostolou, 12/3/85 (brief review): one-liner by Aldous, 9/25/90 (no written review).

294-039 965482 (duplicate of records 036324 (above) and 036325 (below)).

MBC

294-077 044558 (With rebuttal and supplemental information in -095, TAB 5 and 051508): "Teratogenicity Study in Rats with 2-Benzimidazolecarbamic Acid, Methyl Ester (INE-965);" Haskell Laboratory, 11/3/70; MBC with excipients, 53% AI as formulation; administered to CD rats at 10000, 7500, 5000, 2500, 500, 100, or 0 ppm AI in the feed. No effects on any parameters. **UNACCEPTABLE** and not upgradeable - inadequate dose levels, no maternal toxicity, no feed analysis, effects of formulation excipients on a.i. absorption unknown.

REVIEW: Martz, 7/8/86, with second review and rebuttal response, Margolis, 7/31/87.

EPA One-liner: none in Branch Library.

294-095 051508 [TAB 5]: Narrative rebuttal and supplemental information to # 044558 above regarding MTD. Argument doesn't fully answer concerns and can not upgrade study. 9/8/87, Martz (no separate Worksheet).

294-065 036326 Delatour, P., and Richard, Y. "Embryotoxic and antimitotic properties of benzimidazole compounds". Therapie 31:505-515 (1976). Several benzimidazole-related compounds were tested for developmental toxicity, and for in vitro and in vivo antimitotic activity. A few SD rats were treated orally (a total of 13 dams divided between 3 dosages between 9.6 and 38 mg/kg/day for MBC, 7 dams at 116 mg/kg/day for benomyl) for a limited teratology study. Benomyl was apparently inactive, but MBC was inactive at 9.6 mg/kg/day, caused 72% embryoletality and 100% "external anomalies" among survivors at 19 mg/kg/day, and 100% embryoletality at 38 mg/kg/day. The anomalies were not quantified, however exencephaly was noted as "common" for MBC, and malformations noted as common for the more active of the series of compounds included "exencephaly, meningocele, hydrocephaly, hare lip, micro-anophthalmia, hypodysplasia of the limbs, ectrodactylia, micro-anurous". **Unacceptable, not upgradeable** (due to limitations of study design), useful for general perspective of developmental toxicity of a chemical series. Aldous, (no worksheet) 9/26/90.

294-039 965483 Comments by L.W. Smith, who suggested that teratology studies for Benomyl should be by dietary rather than gavage exposure, to be relevant to human exposure situation. Gavage administration of Benomyl or MBC can be expected to elicit developmental effects by overwhelming the

organism, which is capable of tolerating comparably large doses if administered in the diet. No DPR review worksheet. "One-liner" by Aldous, 9/26/90.

RABBIT TERATOLOGY STUDIES

BENOMYL

294-065 036318: "Segment II - Teratology Study -Rabbits. Fungicide 1991 (MRO-1079);" Hazleton, 7/15/68; "Fungicide 1991", 50% benomyl, at 500, 100, or 0 ppm AI in the feed to NZW rabbits, days 8-16 (insemination=day 0); rib defects at 500 ppm, probably incidental; no other fetal or maternal effects; UNACCEPTABLE AND NOT UPGRADEABLE, no MTD, no feed analysis (AI instability in feed?), inadequate group size, insufficient skeletal exams.

REVIEW: original 12/3/85 by Apostolou, second opinion 7/7/86 by Martz.

EPA One-liner: "Terata NOEL = 500 ppm (HDT)." No grade given.

294-076 043805 Rebuttal comments to 065:036318, above. Comments were considered in 7/7/86 re-review by Martz.

178; 143105; "Developmental Toxicity Study of DPX-TI991-529 (Benomyl) in Rabbits" (Susan M. Munley; Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE; Lab Project No. 10126-001; 8/31/95; DPX-TI991-529 (Benomyl, technical grade, purity = 97.4%), dosed as a suspension in aqueous 0.5% methyl cellulose at a dosing volume of 2 ml dosing suspension/kg to groups of 20 mated NZW rabbits at dose levels of 0 (vehicle), 15, 30, 90, or 180 mg/kg/day on days 7-28 of gestation; seven animals died or were killed as a result of dosing errors; decreased food consumption was observed in high-dose animals on days 7-13 and 25-27; slight increases in the number of early resorptions and the number of litters with fetuses with small renal papilla were observed; **no adverse effects; maternal NOEL = 90 mg/kg/day (decreased food consumption); developmental NOEL = 90 mg/kg/day (fetuses with small renal papilla); **Acceptable**. (Duncan, 9/29/97)

MBC

****294-086 045741:** "Developmental Toxicity Study of H-15647 Administered via Gavage to New Zealand White Rabbits;" Argus Research Laboratories, 7/3/85; MBC ("H-15647"), 98.7% pure; 125, 20, 10, or 0 mg/kg once daily by oral gavage to 16-18 pregnant/level, days 7-19 (insemination day = 0); **ADVERSE EFFECTS:** maternal - weight loss, decreased feed consumption, and abortion at 125 mg/kg; malformations - rib and vertebral at 125 mg/kg; embryotoxicity - total litter resorption at 125 and 20 mg/kg, reduced litter size and increased postimplantation loss, 125, 20, and 10 mg/kg. **MATERNAL AND TERATOGENIC NOEL = 20 MG/KG, EMBRYOTOXIC NOEL < 10 mg/kg (LDT);** Report complete and study ACCEPTABLE.

REVIEW: 7/9/86 by Martz (one-liner revision 9/3/87).

The data were re-reviewed and evaluation of the developmental effects of MBC was conducted using the litter (not fetus) as the unit. MBC appears to cause significant effects (postimplantation loss) at the mid and high dose level. The study remains acceptable. **Developmental NOEL = 10 mg/kg/day.** P. Iyer, 10/1/97.

EPA One-liner: none in Branch Library.

MOUSE TERATOLOGY STUDIES

294-065 036325 Kavlock, R.J., Chernoff, N., Gray, L.E. Jr., Gray J.A., Whitehouse, D., "Teratogenic effects of Benomyl in the Wistar rat and CD-1 mouse, with emphasis on the route of administration", Toxicol. Appl. Pharmacol. 62:44-54 (1982). [The same study is under Record No. 036324 for rat data]. Benomyl (tech., in 0.1 ml corn oil/mouse/day) days 6-16 to mice at 0, 50, 100, or 200 mg/kg/day. There was no apparent maternal toxicity. Developmental effects NOEL = 50 mg/kg/day (based on fetal weight decrements, delayed ossification in vertebral centra, increased supernumerary ribs, enlarged renal pelvises; and the anomalies: cleft palate, hydronephrosis, fused ribs, fused vertebrae, and short and/or kinky tail). The above findings, at 100 mg/kg/day, were generally more markedly manifest at 200 mg/kg/day. Additional findings at 200 mg/kg/day were increased fetal mortality, enlarged lateral ventricles, hydrocephaly, micrognathia, polydactyly, oligodactyly, and umbilical hernia. Developmental findings are **possible adverse effects**, however the apparent NOEL for developmental toxicity is higher than that for rats or rabbits, therefore usefulness of these data for risk assessment is doubtful. Study is **unacceptable, and unlikely upgradeable**. Brief review by Apostolou, 12/3/85; one-liner (no worksheet) by Aldous, 9/25/90.

GENETIC TOXICITY

294-129 111296 "Assessment of the Genetic Toxicological Studies on Benomyl and Carbendazim: A Review." (Sarrif, A. M., Haskell Laboratory for Toxicology and Industrial Medicine, du Pont, 1/31/91) The document reviews numerous reports on a number of endpoints for somatic cell and germ cell genetic toxicity. Most are given a one paragraph summary and a brief assessment of the significance of the findings. No worksheet. Gee, 12/18/91.

294-140 123816 "A Review of the Genetic Toxicity Studies on Benomyl and Carbendazim" (Reynolds, V. L. and A. M. Sarrif, Haskell Laboratory for Toxicology and Industrial Medicine, Du Pont, HLR 1-93, 1/93) The document reviews numerous studies in different species using a tier approach. Tier I consists of studies with somatic cells and Tier II, studies with germ cells. The authors concluded that benomyl and its major metabolite, carbendazim (MBC), cause specific effects resulting in aneuploidy. This was thought not to be the result of direct interaction with DNA but with other targets, for example, tubulin. Positive results for gene mutation and structural aberrations were attributed to cytotoxicity at high concentrations. The authors considered benomyl to be negative for the induction of DNA damage and repair. They proposed that the causal event of aneuploidy has a threshold. The review contains about 15 pages of citations. No worksheet. (Gee, 8/30/96)

GENE MUTATION

BENOMYL

**294-039 965485: "Mutagenicity Evaluation in Salmonella Typhimurium;" Haskell Laboratory, 8/26/81; benomyl, 99.2% pure; strains TA1535, TA1537, TA98, and TA100 (with or without rat or mouse liver S9 activation); 1000, 500, 375, 250, 100, 50, 10, 5, or 0 ug/plate. Cytotoxicity above 250, no mutagenic activity. Study ACCEPTABLE.

REVIEW: 5/9/85 by Wong.

EPA One-liner: "Not mutagenic in TA1537, 1538, 98, or 100 up to dosage levels of 250 µg/plate."

**294-076 043811 & -12 (revised report of -063, 36279): "Mutagenicity Evaluation in Salmonella

Typhimurium;" Haskell Laboratory, 3/18/83 with revision 4/3/86; benomyl, 99% pure; strains TA1535, TA1537, TA98 and TA100 with or without rat liver S9 activation; 0, 10, 25, 50, 100, or 200 µg plate without S9; 0, 25, 50, 100, 250, or 500 µg/plate with S9; in duplicate, 2 trials. No evidence of increased reversion rate. Revised report complete and ACCEPTABLE.

REVIEW: original 12/5/85, rebuttal 6/17/86, both by (Remsen) Gee.

EPA One-liner: none in Branch Library.

****294-076 043809 & -10** (revised report of -063, 036278): "Mutagenicity Evaluation in Salmonella Typhimurium;" Haskell Laboratory, 3/18/83 with revision 4/7/86; benomyl, 99% pure; strains TA1535, TA1537, TA98 AND TA100 ± rat liver S9 activation; 0, 25, 50, 100, 250, or 500 µg plate, duplicate plates, 2 trials; cytotoxicity with TA1535 at 500 µg without S9 and at 1000 µg with S9. No increase in reversion rate reported. Revised report ACCEPTABLE with variances.

REVIEW: original 12/5/85, rebuttal 6/17/86, both by (Remsen) Gee.

EPA One-liner: none in Branch Library.

MBC

294-078 044572: "Mutagenicity Testing on Fungicide 1991 Metabolite (MBC) in Microbial Systems;" Institute of Environmental Toxicology (Japan), 10/17/77; MBC, 99% pure; TA1535, TA1537, TA1538, TA98, and TA100 ± rat liver activation, 1 trial in duplicate; 0, 10, 50, 100, 500, 1000, or 3000 µg/plate; results negative, study UNACCEPTABLE.

REVIEW: 6/18/86 by (Remsen) Gee.

EPA One-liner: none in Branch Library.

****294-095 051507, TAB 6 (revised version of 44567 in -078)**: "Mutagenicity Evaluation of 2-Benzimidazolecarbamic Acid, Methyl Ester in Salmonella typhimurium." Haskell Laboratory, 10/14/77, revised 11/3/86. MBC, 99.1% or 99.3% (two analyses). 5 tester strains ± rat liver activation; 0, 200, 400, 600, 800, 1000, 4000, 8000, or 10000 µg/plate. **POSITIVE**, concentration dependent response with S9 in TA1537, TA1538, and TA98 (frame shift) with revertant frequency > 2X background at ≥ 4000 µg/plate for all 3 strains. TA100 significant at 4000-8000 µg/plate, but frequencies < 2X background. Originally unacceptable but upgraded to **ACCEPTABLE** by revised report.

REVIEW: First 6/17/86 by (Remsen) Gee, re-issued version by Margolis, 7/30/87 and Martz, 9/2/87.

EPA One-liner: none in Branch Library.

****294-039 965486**: "Mutagenicity Evaluation in Salmonella Typhimurium;" Haskell Laboratory, 7/31/81; MBC, 99.6% pure; strains TA1535, TA1537, TA98, and TA100, with or without mouse or rat liver S9; 10000, 5000, 1000, 500, 100, or 0 µg/plate. **POSITIVE** response (significant and >2X background) in TA1537 and TA98 with either rat or mouse S9 ≥ 5000 µg/plate with trend at 1000 µg/plate; equivocal effect (concentration dependent but < 2X background) in TA100 with S9 and TA1537 without S9. Study ACCEPTABLE.

REVIEW: 5/9/85 by Wong and 9/16/87 by Martz.

EPA One-liner: none in Branch Library.

****294-078 044568**: "Mutagenicity Evaluation in Salmonella Typhimurium;" Haskell Laboratory, 6/1/83; MBC (INE-965), 99% pure; TA1537, TA1537, TA98, and TA100 ± rat liver S9; 0, 100, 500, 1000, 5000, or 10000 µg/plate; no increased reversion rate; no cytotoxicity at 10000 µg/plate; study ACCEPTABLE with minor variances.

REVIEW: 6/18/86 by (Remsen) Gee.

EPA One-liner: none in Branch Library.

****294-078 044569**: "Mutagenicity Evaluation in Salmonella;" Haskell Laboratory, 9/22/83; MBC, 99%

pure (Z08844); TA1535, TA97, TA98, and TA100 \pm rat liver activation; 0, 100, 500, 1000, 2500, or 5000 μ g/plate; no evidence of increased reversion rate in two trials; study ACCEPTABLE.

REVIEW: 6/18/86 by (Remsen) Gee.

EPA One-liner: none in Branch Library.

****294-078 044570:** "Mutagenicity Evaluation in Salmonella Typhimurium;" Haskell Laboratory, 9/22/83; MBC, 99% pure (Z08652); TA1535, TA97, TA98, and TA100 \pm rat liver activation; 0, 100, 500, 1000, 2500, or 5000 μ g/plate; no evidence of cytotoxicity at 10000 μ g/plate; no increase in reversion rate; study ACCEPTABLE.

REVIEW: 6/18/86 by (Remsen) Gee.

EPA One-liner: none in Branch Library.

****294-078 044574:** "Chinese Hamster Ovary Cell Assay for Mutagenicity;" Haskell Laboratory, 9/5/80; MBC, >99% pure; \pm rat liver activation, 0 to 628 μ M concentration duplicate cultures, 4 trials without S9, 3 trials with S9; concentrations changed with trials; precipitation at > 262 μ M which caused toxicity problems; no evidence of mutagenicity; report refers to MBC as a spindle poison; study ACCEPTABLE.

REVIEW: 6/18/86 by (Remsen) Gee.

EPA One-liner: "Not mutagenic with or without metabolic activation at HGPRT locus."

****294-078 044577 (With rebuttal in -095, TAB 9):** "An Evaluation of Mutagenic Potential of MBC Employing the L5178Y TK \pm Mouse Lymphoma Assay;" SRI International, 12/80; MBC, 99% pure; L51784 TK \pm \pm rat liver S9 (F344); trial 1 @ 0-1000 μ g/ml \pm S9 with precipitation at 80 μ g/ml, trial 2 @ 0-25 μ g/ml with S9, 0-100 μ g/ml without S9; **POSITIVE** mutagenic effect (frequency >2X background) at 50 μ g/ml without S9, at 12 μ g/ml with S9; originally unacceptable (AI not identified) but upgraded to ACCEPTABLE by rebuttal in -095 TAB 9.

REVIEW: Original 6/18/86 by (Remsen) Gee, rebuttal 9/9/87 by Martz.

EPA One-liner: "Dose related increase in mutation frequency at TK locus of L5178Y cells, in vitro." (Listed with benomyl one-liners)

294-095 TAB 9: Narrative rebuttal to #44577 identifying MBC as test article, upgrades study to acceptable. 9/9/87, Martz.

****294-078, 044578:** "L5178Y Mouse Lymphoma Cell Assay for Mutagenicity;" Haskell Laboratory, 7/12/83; MBC, >99% pure; L51784 TK \pm rat liver S9 (CD); 0, 25, 50, 100, 150, or 200 μ M (plus other intermediate concentrations); 2 trials; no increase in mutation frequency reported; study ACCEPTABLE.

REVIEW: 6/18/86 by (Remsen) Gee.

EPA One-liner: none in Branch Library.

5-hydroxy MBC

****294-076 043813 & -14 (revised report of -063, 36280):** "Mutagenic Activity of 2-Benzimidazolecarbamic Acid, 5-Hydroxy-, Methyl in the Salmonella/Microsome Assay;" Haskell Laboratory, 10/14/77, revised 4/7/86; 5-hydroxy MBC, purity 95%; strains TA1535, TA1537, TA1538, TA98, and TA100 \pm rat liver S9 activation; 0-16000 μ g/plate without S9, 0-20000 μ g/plate with S9, duplicate plates, 5 trials with increasing concentrations in each. No increase in reversion rate reported. Revised report complete and ACCEPTABLE.

REVIEW: original 12/5/85, rebuttal 6/17/86, both by (Remsen) Gee.

EPA One-liner: none in Branch Library.

COMMENT: In view of the conflicting results in numerous valid studies, the test article must be assumed to have mutagenic activity. In bacterial tests from the same laboratory, MBC caused increased

reversion frequencies in strains TA1537 and TA98 with rat liver activation in 2 of 5 studies, whereas negative results were obtained in the same tester strains in 3 other studies at similar MBC concentrations. To further confuse interpretation, similar methods as well as identical rat strains for activation systems were used in all 5 studies. Due to the differences in report dates, it can be assumed that different lots of test material were used. Therefore, the participation of impurities in the mutagenic process is open to question.

Opposite results also were noted in 2 mammalian cell tests from different laboratories using overlapping test article concentrations. In the mouse lymphoma assay, SRI reported positive results with activation at 12 µg/ml, which is equivalent to about 63uM. In contrast, the same test at Haskell Laboratory was negative with activation at concentrations up to 200uM, equivalent to about 38 µg/ml. Differing activation sources were used in each study, however. SRI used S9 liver preparations from F344 male rats whereas the S9 fractions used at Haskell Laboratory were obtained from CD (Sprague-Dawley descended) males. Although the mechanism is speculative, qualitative or quantitative metabolic pathway differences between the 2 rat strains could account for the opposite mutagenicity results. Alternatively, the use of different batches of test material (containing differing impurities) could possibly account for this discrepancy.

The registrant has advocated the latter but provided no analytical data or side-by-side assays to support that contention (see rebuttal to #44577 in TAB 9 of -095). Although that speculation is probable, it does not ameliorate concern about mutagenic activity of benomyl/MBC. Assuming that technical grade material contains similar mutagenic impurities, the manufactured pesticidal products would present mutagenic hazards regardless of the etiologic agent(s) involved. Consequently, an adverse effect status must be assigned regardless of the underlying cause. Gee, 1987.

GENE MUTATION: ADDITIONAL INFORMATION (ESPECIALLY PUBLICATIONS):

NOTE: The following studies were not included in previous Summaries of Toxicology Data, and these reports generally received only brief DPR reviews (in a few cases, no DPR worksheets had been generated). Aldous, 10/4/90.

294-064 036298 Kappas, A., Green, M.H.L., Bridges, B.A., Rogers, A.M., and Muriel, W.J., "Benomyl - A novel type of base analogue mutagen?". Mutation Research 40:379-382 (1976). Benlate (50% Benomyl a.i.) was administered at Benomyl concentrations of 0.125 to 5.0 µg/ml (slightly higher upper range for some test systems) for systems: E. coli strains WP2uvrA, WP2, CM611, Salmonella typhimurium TA1535 and TA1538. A simplified fluctuation test was used. Two assays were positive, WP2uvrA, and TA1535, both in the range of 0.125 to 1.0 µg/ml Benomyl. **Possible adverse effect indicated. Unacceptable** due to limitations in study design. de Vlaming/Apostolou, 12/3/85.

294-064 036299 Kappas, A., and Bridges, B.A. "Induction of point mutations by Benomyl in DNA-repair-deficient Aspergillus nidulans". Mutation Res. 91:115-118 (1981). Benomyl (0.25 to 0.40 µg/ml) induced reverse mutations from both biotin and pyridoxine requirement in the excision-deficient UT517 strain of Aspergillus nidulans, whereas there was no detectable mutagenic effect in the repair-proficient UT439. Benomyl (0.25 to 0.40 µg/ml) induced reverse mutations from adenine requirement in a UV-sensitive strain (UT540) of Aspergillus nidulans. **Possible adverse effect indicated. Unacceptable** due to limitations in study design. de Vlaming/Apostolou, 12/3/85.

294-064 036300 Dassenoy, B., and Meyer, J.A. "Mutagenic effect of Benomyl on Fusarium oxysporum". Mutation Res. 21:119-120 (1973). (Text without tables). Benomyl caused forward mutations, creating monoauxotrophs with several amino acid requirements. **Possible adverse effect indicated. Unacceptable** due to limitations in study design. de Vlaming/Apostolou, 12/3/85.

294-064 036301 Hastie, A.C. "Benlate-induced instability of Aspergillus diploids". Nature 226:771 (1970). Heterozygous diploid strains were distinguishable by colors (white and yellow) of the colonies. Benomyl, 0.25 or 0.5 ppm, caused increased segregation of colonies, and many of the colonies were haploid. **Possible adverse effect indicated. Unacceptable** due to limitations in study design. de Vlaming/Apostolou, 12/3/85.

294-064 036302 Bignami, M., Aulicino, F., Velcich, A., Carere, A., and Morpurgo, G. "Mutagenic and recombinogenic action of pesticides in Aspergillus nidulans". Mutation Res. 46:395-402 (1977). Benomyl (500 µg per 3 cm x 5 cm filter paper triangle) was tested for ability to induce point mutations to 8-azaguanine resistance in Aspergillus nidulans. This compound did not increase mutation frequency. **Unacceptable** due to limitations in study design. de Vlaming/Apostolou, 12/3/85.

294-064 036304 Carere, A., Ortali, V.A., Cardamone, G., Torracca, A.M., Raschetti, R. "Microbiological mutagenicity studies of pesticides in vitro". Mutation Res. 57:277-286 (1978). Benomyl (at 20 or 500 µg/3 cm x 2 cm triangular absorbent paper) was not mutagenic in the TA1535, TA1536, TA1537, or TA1538 strains of S. typhimurium in a reverse mutation spot test with and without activation with phenobarbital-induced male rat liver. **Unacceptable** due to limitations in study design. de Vlaming/Apostolou, 12/3/85; updated by Gee, 10/1/90.

294-064 036310 Fiscor, G., Bordas, S., and Stewart, S.J. "Mutagenicity testing of Benomyl, methyl-2-benzimidazole carbamate [MBC], streptozotocin and N-methyl-N'-nitro-N-nitrosoguanidine in Salmonella typhimurium in vitro and in rodent host-mediated assays". Mutation Res. 51:151-164 (1978). Benomyl and two of its commercial preparations were tested in various gene mutation assays. These compounds were negative in in vitro spot tests, in microsomal plate assay, in liquid-culture treatments, and in the rodent host-mediated assay. The base-pair substitution S. typhimurium mutant hisG46 and the hisG46-bearing uvrB excision-repair deficient mutants TA100, TA1530, TA1535, or TA1950 were used as test organisms. At the dose levels used, benomyl was not mutagenic. MBC was tested in some of the above systems, and was also negative for mutagenicity. **Unacceptable** due to limitations in study design. de Vlaming/Apostolou, 12/4/85.

294-041 and -063 965489 Russell, J.F., Jr. "Mutagenic activity of 2-benzimidazolecarbamic acid, 1-(butylcarbamoil)-, methyl ester in the Salmonella/microsome assay". Haskell Lab Report No. 819-77, dated 10/14/77. Test article was Benlate* (50% wettable powder). Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100 were tested at concentrations of the formulation up to 1200 µg/plate in the absence of S9 or up to 750 µg/plate in presence of S9. There were two replicates per dose level in two separate experiments. Report indicates that treatments included a "slightly toxic" range (>50% of control survival: data not provided). **No adverse effect indicated**: all strains were negative with and without S9. **Unacceptable** due to choice of test article, but useful information. Aldous, 10/1/90 (no DPR worksheet).

294-041 and -063 024912 Russell, J.F., Jr. "Mutagenic activity of 2-benzimidazolecarbamic acid, 1-(butylcarbamoil)-, methyl ester in the Salmonella/microsome assay". Haskell Lab Report No. 18-78, dated 1/20/78. Test article was Benomyl, 99.05 to 99.4% purity. Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100 were tested at concentrations of the formulation up to 500 µg/plate in the presence or absence of S9. There were two replicates per dose level in up to 8 separate trials per strain/treatment/S9 combination. Evidence of toxicity was extremely variable between strains and between trials. In most cases, some toxicity was observed in several dose levels in tests without S9 activation. Strain 1537 without S9 had elevated numbers of revertants in several trials and at several dose levels. For this reason, test article was considered to be mutagenic in that strain in the absence of an activation system. This is a **"possible adverse effect"**. **Unacceptable**: Excessive variability in toxicity suggests problems in execution of study. (Upgradeability is not an issue, since later studies have been accepted.) A brief DPR review was written by J. Wong on 5/10/85. This 1-liner is by Aldous,

10/1/90 (no new DPR worksheet).

294-041 and -063 965490 Shirasu, Y., Moriya, M., and Kato, K., "Mutagenicity testing on Fungicide 1991 in microbial systems". Institute of Environmental Toxicology, Tokyo, 1/23/78. Benomyl, 99%, was tested in several systems, primarily to detect gene mutations. Tests included "Ames" test with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100; also reverse mutation test in Escherichia coli WP2 hcr. A host-mediated assay was done using Salmonella typhimurium strain G46 in mice. In addition, a DNA damage/repair study was performed: a rec-assay in Bacillus subtilis strains M45 and H-17. All results were negative. These studies were classified as **unacceptable** by J. Wong on 5/10/85, based on the following deficiencies: dosage ranges were not justified, there were no analyses of dosing solutions, there was no QA/GLP statement, and there were insufficient individual data for independent analyses. One liner by Aldous, 10/2/90.

294-041 965491, 038196, and 038197 (for host-mediated assay, rec-assay, and "Ames"-style plate tests, respectively). Shirasu, Y., Moriya, M., and Kato, K.; "Mutagenicity testing on Fungicide 1991 metabolite (MBC) in microbial systems". Institute of Environmental Toxicology, Tokyo, 10/17/77. MBC, 99%, was tested in the same systems as reported for Benomyl, above (294-041 and -063 965490). All results were negative. These studies were classified as **unacceptable** by J. Wong on 5/10/85, based on the same deficiencies listed in the cited 1-liner (above). One-liner by Aldous, 10/2/90.

294-063 036287 Lamb, M.J., and Lilly, L.J. "An investigation of some genetic toxicological effects on the fungicide Benomyl". Toxicology 17:83-95 (1980). [see also DPR review of the chromosomal effects portions of this report, under Record Nos. 036288 and 036289]. Either 1 mg/ml Benlate* (powdered formulation which is 50% Benomyl) or 0.5 mg/ml MBC was dissolved in 0.5% DMSO. Benomyl and MBC were fine suspensions at these concentrations. Food and water were retained from adult male D. melanogaster [Oregon-R strain] flies for 16 hr, then flies were placed in the presence of a drop of Benomyl, MBC, or DMSO vehicle. Differences in weights of groups of 5 flies were taken as estimates of consumption. Neither Benomyl nor MBC increased the numbers of recessive lethals significantly (even though an unusually low zero incidence in controls was obtained). Significant increases in numbers of sterile males were noted at a time period corresponding to exposure to predominantly pre-meiotic spermatocytes and spermatogonial cells for both Benomyl and MBC. The increase in sterile males was considered a **possible adverse effect** in the 12/6/85 review. It should be noted that no increases in male sterility were noted in yw⁺B/B^SYy⁺ males used in chromosome loss and breakage tests in the same report. Original DPR review by Remsen (Gee) 12/6/85. One-liner by Aldous, 10/2/90.

294-039 and -063 965487 Summers, J.C., "Chinese hamster ovary cell assay for mutagenicity". Haskell Lab. Report No. 438-80, 5/16/80. The BH4 clone of the CHO-K1 cell line was used by method of A.W. Hsie at Oak Ridge National Laboratory. 99.9 to 100% purity benomyl was tested in 4 trials with activation [from Aroclor 1254-induced CD rat livers] at concentrations up to 172 μ M and in 5 trials without activation at concentrations up to 805 mM (typically two reps at each dose level in each trial). No treatment effect on chromosomal aberrations. **Unacceptable**: large variability between trials suggested technical problems; lack of QA/GLP. Original CDFA review by Wong, 5/10/85; one-liner by Aldous, 10/3/90.

294-140 123816 Reynolds, V. and Sarrif, A. "A Review of the Genetic Toxicity Studies on Benomyl and Carbendazim" (Haskell Laboratory, Du Pont, 1/93). In a review of genetic toxicity studies on benomyl and carbendazim, occasional positive findings were noted in many study types, including the Salmonella/Ames test (frameshift mutations), yeast and fungal reversion assays, mouse lymphoma assay, structural chromosomal aberration assay in human leukocytes, human/rodent hybrid cell assay (to quantitate chemically-induced aneuploidy), and the Sister Chromatid Exchange (SCE) assay in CHO cells. Although numerous genetic toxicity studies were cited which had positive responses, the authors concluded that impurities such as 2,3-diaminophenazine (DAP) that may arise during benomyl or

carbendazim synthesis were responsible for the positive responses. The use of test compound with no more than 5 ppm DAP resulted in negative findings in the Ames test. Conflicting gene toxicity findings were also attributed to inadequate protocols or test systems, lack of supportive data, and the sensitivity of the test system (particularly in vitro mammalian systems) to cytotoxicity. According to the author, the only genotoxic endpoint showing a specific benomyl-related effect was numerical chromosome aberrations (aneuploidy).

Adverse effects from animal studies were also discussed, including acute dermal sensitization in guinea pigs, benign liver tumors in mouse chronic studies (possibly through induction of liver enzymes, modulating growth of spontaneous neoplasms), reproductive effects (eg. decreased sperm counts, decreased testicular weights and histopathologic changes) and developmental effects in rats and rabbits (anomalies of the eyes, skull and head). The liver was reported as the primary target organ as evidenced from serum enzyme changes, elevated organ weight and histopathic changes. No worksheet. Kellner, 9/24/93.

CHROMOSOMAL ABERRATION

BENOMYL

** **294-117 088850** "Benomyl: Micronucleus Test in Mice." (Sasaki, Y. F. X., The Institute of Environmental Toxicology, Tokyo, IET 89-0046, 3/2/90) Benomyl, lot AG 0079-37, 95% pure, was given in a single dose by oral gavage at 0 (0.5% aqueous sodium carboxymethylcellulose), 1250, 2500 or 5000 mg/kg. Each group contained 6/sex with sacrifice times of 24, 48 and 72 hours. No mortalities were reported. No effect on the PCE/(PCE + NCE) was noted. Formation of micronuclei per 1000 polychromatic erythrocytes was evaluated. An increase in micronuclei formation in PCE's was reported at 24 and 48 hours with statistical significance at all three doses and at 72 hours, for 2500 and 5000 mg/kg. A statistically significant trend was also seen at 24 and 48 hours. **Possible adverse effect. Acceptable.** (Gee, 9/27/90)

294-117 088851 "In vivo Evaluation of IN T1991-259 for Chromosome Aberrations in Mouse Bone Marrow." (Stahl, R. G., Jr., Haskell Laboratory for Toxicology and Industrial Medicine, Report No. 401-90, 7/23/90) Benomyl technical, 96.1%, was given in a single dose by oral gavage to 5/sex/group of B6D2F1/Cr-1BR mice. Doses were 0 (corn oil), 625, 1250, 2500 or 5000 mg/kg. Cyclophosphamide was the positive control. Animals were sacrificed 24 hours after dosing and at least 2 slides/animal were prepared. When possible, 50 cells/animal were scored [by Hazleton Laboratory Americas] for chromosome and chromatid aberrations and the mitotic index recorded. **No increase in aberrations** at the 24-hour harvest time. **Unacceptable** (single sacrifice time without justification). (Gee, 9/28/90)

294-063 036286 (With rebuttal in -076, 043808): "Micronucleus Test on Benomyl," in mice; SRI, 2/12/80; benomyl, 1000, 500, 250, or 0 (DMSO) mg/kg once, kill at 48, 72, and 96 hours; 1000 mg/kg once with kill at 24 and 72 hours. Tested males only, 500 PCE's/animal. **Micronuclei** increased at 1000 mg/kg. Reviewer questioned solubility of AI at high dose, and whether mice received full dose. Rebuttal below cannot answer deficiencies, still **Unacceptable** and not **upgradeable**.

REVIEW: original 12/6/85, rebuttal 6/17/86, by (Remsen) Gee.

EPA One-liner: "Dose related significant increase in micronuclei in bone marrow from femur bones."

294-076 043808: Narrative rebuttal of #36286, does not address deficiencies: no females used, failure to sample at 12-24 hours in lower dose level groups, insufficient cells/animal scored, no positive control, and solubility problems with vehicle. One-liner added 9/8/87, Martz.

294-063 036282 (With rebuttal in -076, 43806): "Benlate - Dominant Lethal Study in Male Rats;" Haskell Laboratory, 3/28/74; benlate (54% AI) at 5000, 2500, 500 or 0 ppm (AI or bulk?) in the feed for 7 days;

mated weekly for 6 weeks. No effects noted, but no positive control, too few animals, and other deficiencies [AI instability in feed] confounds interpretation. Contains no useful information. Rebuttal below does not upgrade status. **Unacceptable** and not **upgradeable**.

REVIEW: original 12/5/85, rebuttal 6/17/86, by (Remsen) Gee.
EPA One-liner: none in Branch Library.

294-076 043806: Narrative rebuttal to #36282 above attempting to justify dose level selection, dietary route of administration, and 7 day exposure period. Uncorrectable deficiencies cannot be addressed: mating performance of controls, lack of positive controls, and no feed analysis. One-liner added 9/8/87, Martz.

****294-112 075752** "Benomyl: In vitro Cytogenetics Test." (Institute of Environmental Toxicology, Tokyo, Japan, IET 88-0043, 11/28/88) Benomyl, Lot # F90707, 98.7%, tested with Chinese hamster lung (CHL) cells with and without male rat liver activation (Aroclor-induced); without activation, incubated for 24 or 48 hours, 2 cultures per concentration, two trials at 0 (non-treated and solvent - DMSO), 1.416, 2.832, 5.664, 11.33 or 22.66 µg/ml. With activation, treated for 6 hours, washed and incubated a further 12 or 18 hours, duplicate cultures, two trials. In trial 1, concentrations were 0, 3.119, 6.238, 12.48, 24.95 or 49.9 µg/ml. In trial 2, at 0, 5.664, 11.33, 22.66, 45.31, or 90.63. One hundred metaphases per culture were scored for polyploidy (37 or more chromosomes from a modal number of 25) and for chromatid/chromosome aberrations. Results were positive for **ADVERSE EFFECTS** on structural and numerical chromosomal aberrations with and without activation. Results without activation were greater than with activation. **ACCEPTABLE** study.

REVIEW: 9/21/89, by Gee.
EPA One-liner: None in Branch Library.

**** 146 126870** "Classification of DPX-T1991-529 (Benomyl)-Induced Micronuclei in Mouse Bone Marrow Erythrocytes Using Immunofluorescent Antikinetochores Antibodies", (K.S. Bentley, E.I. DuPont De Nemours & Co., Haskell Laboratory for Toxicology and Industrial Medicine, HLR Report No. 568-92, 10/12/92). Benomyl technical [[1-[(butylamino)carbonyl]-1H-benzimidazol-2-yl]-carbamic acid, methyl ester], purity 96.1-97.4%, was administered in a single oral intubation at concentrations of 0 (0.5% methyl cellulose), 100, 2500, or 5000 mg/kg to 5 B6D2F1/Cr-1BR mice/sex/group. Test animals were sacrificed 48 hours after treatment. **Possible adverse effect: Increase in micronucleated polychromatic erythrocytes**; NOEL = 100 mg/kg. Micronuclei increases were primarily kinetochores positive, and, as a result, DPX-T1991-259 was classified as an aneugen. **Acceptable**. (Kishiyama and Gee, 8/30/96).

MBC

294-078 044579 (With rebuttal in -095, TAB 10): "The Specific Arrest of HeLa Cells in Mitosis by Methyl 2-Benzimidazolecarbamate;" du Pont, no date given; MBC, purity not given; cells exposed to 10^{-7} to 10^{-4} M or 20 µg/ml for various time periods; **ADVERSE EFFECT:** cell cycle progression was halted in mitosis, cells entered mitosis but could not divide to G1 stage; status not applicable for data requirement due to nonstandard design but otherwise well done and useful for genotoxic evaluation [referenced to be a spindle poison in mammalian toxicology reports and summaries].

REVIEW: 6/18/86 by (Remsen) Gee and 9/9/87 by Martz.
EPA One-liner: none in Branch Library.

294-095 TAB 10: Narrative rebuttal arguing for acceptability of #44579 based on study's scientific value and merit. The latter are agreed with, but study design is not applicable for specific data requirements in spite of good scientific merit. 9/9/87, Martz (no separate Worksheet).

*** COMMENT:-063, 036285 (With rebuttal in -076, 43807) was originally classified as 844 but can also fulfill 843 requirement. This report, whose one-liner is located below in "DNA CHANGES (BENOMYL), in combination with # 044579 and # 036286 provide sufficient information to fill the chromosomal aberration data requirement. Added 9/9/87, Martz.

CONCLUSION: A "possible adverse effect" status is being assigned to this test category on the basis of 3/4 positive studies, one of which was acceptable. 9/16/87, Martz. Note: Additional positive studies have been reviewed. Gee, 9/3/96.

146 126869 "Classification of DPX-E965-299 (Carbendazim, MBC)-Induced Micronuclei in Mouse Bone Marrow Erythrocytes Using Immunofluorescent Antikinetochore Antibodies", (K.S. Bentley, E.I. DuPont De Nemours & Co., Haskell Laboratory for Toxicology and Industrial Medicine, HLR Report No. 569-92, 9/3/92). Carbendazim technical (1H-benzimidazol-2-yl-carbamic acid, methyl ester), purity 99.3%, was administered in a single oral intubation at concentrations of 0 (0.5% methyl cellulose), 66, 1646, or 3293 mg/kg to 5 B6D2F1/Cr-1BR mice/sex/group. Test animals were sacrificed 48 hours after treatment. **Possible adverse effect: Increase in micronucleated polychromatic erythrocytes**; NOEL = 66 mg/kg. Micronuclei increases were primarily kinetochore positive. As a result, DPX-E965-299 was classified as an aneugen. **Supplemental data.** (Kishiyama and Gee, 8/29/96)

CHROMOSOMAL EFFECTS: ADDITIONAL INFORMATION (ESPECIALLY PUBLICATIONS)

294-064 036303 Bignami, M., Aulicino, F., Velcich, A., Carere, A., and Morpurgo, G. "Mutagenic and recombinogenic action of pesticides in Aspergillus nidulans". Mutation Res. 46:395-402 (1977). Benomyl (2 mg per 3 cm x 5 cm filter paper triangle) induced a high frequency of mitotic non-disjunction in Aspergillus nidulans in the spot test. Benomyl at 0.4 µg/ml also promoted non-disjunction in Aspergillus in a "non-selective" test. **Unacceptable** due to limitations in study design. **Possible adverse effect indicated.** de Vlaming/Apostolou, 12/3/85.

294-064 036306 Seiler, J.P. "The mutagenicity of benzimidazole and benzimidazole derivatives. VI. Cytogenetic effects of benzimidazole derivatives in the bone marrow of the mouse and the Chinese hamster." Mutation Res. 40:339-348 (1976). MBC at doses of 100 mg/kg or above was mutagenic in mice in the micronucleus test. Benomyl at 1000 mg/kg was positive in the same test. Doses were given twice and the mice sacrificed 6 hours after the second dosing. **Unacceptable** due to limitations in study design. **Possible adverse effect indicated.** de Vlaming/Apostolou, 12/4/85; updated by Gee, 10/1/90.

294-064 036307 Richmond, D.V., and Phillips, A. "The effect of Benomyl and Carbendazim [MBC] on mitosis in hyphae of Botrytis cinerea Pers. ex Fr. and roots of Allium cepa L." Pesticide Biochem. Physiol. 5:367-379 (1975). Benomyl and MBC at 100 µg/ml induced chromosome aberrations in hyphae of Botrytis cinerea and root tips of onion Allium cepa. **Unacceptable** due to limitations in study design. **Possible adverse effect indicated.** de Vlaming/Apostolou, 12/4/85.

294-063 036288, 036289 Lamb, M.J., and Lilly, L.J. "An investigation of some genetic toxicological effects on the fungicide Benomyl". Toxicology 17:83-95 (1980). [see also CDFA review of the recessive lethal portion of this report, under Record No. 036287]. To evaluate chromosome loss or breakage in Drosophila, either 1 mg/ml Benlate* (powdered formulation which is 50% Benomyl) or 0.5 mg/ml MBC was dissolved in 0.5% DMSO. Benomyl and MBC were fine suspensions at these concentrations. Food and water were withheld from adult male D. melanogaster [yw^+B/B^SYy^+ strain] flies for 16 hr, then flies were placed in the presence of a drop of Benomyl, MBC, or DMSO vehicle. Differences in weights of groups of 5 flies were taken as estimates of consumption. Neither Benomyl nor MBC increased the numbers of offspring resulting from paternal chromosome losses, exchanges, or breaks. The human

lymphocyte study involved blood samples cultured with MBC for 44 hr. Chromosomes from cells cultured with MBC were more contracted than controls, however MBC did not increase the numbers of cells with chromosome aberrations. Thus both studies were negative. Original brief CDFA reviews by Remsen (Gee) 12/6/85. One-liner by Aldous, 10/2/90.

294-063 036291 (also 036290 and 036292) Ruzicska, P., Pe'ter, S., Laczi, J., and Czeizel, E. "Study on the chromosome mutagenicity of Fundazol 50WP". Ege'sze'gtudoma'ny (Budapest) 20:74-83 (1976). Fundazol 50WP is similar to Benlate* formulation. Rats ("of R Amsterdam and Long Evans type") were used to derive (1) bone marrow cell suspension samples of 21-day pregnant rats for chromosomal aberration analyses, and to derive (2) embryonic tissues (of unspecified gestational age) for culturing in Parker 199 solution with 20% calf serum, for chromosomal aberration analyses, as above. At least the first of these rat studies involved gavage of dams on days 7 through 14 with 25, 50, 200, or 500 mg/kg/day Fundazol WP. In addition, peripheral blood samples of 20 male workers in a Fundazol WP plant were compared with those of 15 controls for chromosomal aberration analyses, as above. The bone marrow samples, and the worker epidemiological studies were negative. The investigators concluded that "in the rat-embryonic tissue the ratio of chromosome aberrations considerably increased" (chromosome aberration incidence was reported to be statistically significant in 200 and 500 mg/kg/day groups). This is technically a "**possible adverse effect**", however the report is not sufficiently complete nor are the methods sufficiently validated to be useful for quantitative risk analysis. **Unacceptable**. Review by Remsen (Gee), 12/6/85; one liner updated by Aldous, 10/3/90.

DNA DAMAGE/REPAIR

BENOMYL

****294-063 036285** (With rebuttal in -076, 43807): "An Evaluation of the Effect of Benomyl on Sister Chromatid Exchange Frequencies in Cultured Chinese Hamster Ovary Cells;" SRI, 8/80, benomyl, 99% pure; CHO cells with S9 (F344) activation at 150, 75, 37.5, 18.8, 9.4, or 0 µg/ml for 2 hours; or without activation at 10, 5, 2.5, 1.25, 0.63, or 0 µg/ml for 22 hours. SCE increase at all concentrations tested both with and without activation, but high background rate confounds interpretation. Originally unacceptable but upgraded to **ACCEPTABLE** by rebuttal, with **ADVERSE EFFECT** noted.

REVIEW: original 12/5/85, rebuttal 6/17/86, both by (Remsen) Gee.

EPA One-liner: "Weakly positive for sister chromatid exchange."

COMMENT: This report can satisfy an 843 requirement also.

294-076 043807: Narrative rebuttal to # 036285 addressing inconsistency between cytogeneticists, cell cycle delay evaluation and test article stability. To quote: "...these deficiencies are irrelevant since the test material was shown to induce SCE's in CHO cells with and without metabolic activation." The rebuttal upgraded study to acceptable. One-liner added 9/9/87, Martz.

MBC

****294-078 044575** (With rebuttal in -095, TAB 7): "The Hepatocyte Primary Culture/DNA Repair Assay on Compound 11,201-01 Using Mouse Hepatocytes in Culture;" Naylor Dana Institute, 10/20/81; MBC, purity not given; B6C3F₁ male hepatocytes with 18-20 hour exposure to 0, 0.0125, 0.125, 1.25, 12.5, or 125 µg/ml; no effect reported; originally unacceptable but upgraded to **acceptable** with rebuttal in -095, TAB 7 (see below).

REVIEW: Original 6/18/86 by (Remsen) Gee, rebuttal response 9/9/87 by Martz.

EPA One-liner: "Not a mutagen when tested for DNA repair using mouse and rat hepatocyte cultures."

294-095 TAB 7: Narrative rebuttal to # 044575 adequately answering concerns of hepatocyte viability, number of cells scored, and test article purity. Upgraded study to acceptable. 9/9/87, Martz.

294-078 044576 (With rebuttal in -095, TAB 8): "The Hepatocyte Primary Culture/DNA Repair Assay on Compound 11,201-01 Using Rat Hepatocytes in Culture;" Naylor Dana Institute, 10/20/81; MBC, purity not given; male F344 rat hepatocytes with MBC at 0, 0.0125, 0.125, 1.25, or 12.5 µg/ml; 125 µg/ml was toxic; triplicate cover slips, 2 trials, autoradiography; no evidence of unscheduled DNA synthesis; originally unacceptable but upgraded to **acceptable with rebuttal in -095, TAB 8 (see below).

REVIEW: Original 6/18/86 by (Remsen) Gee, rebuttal response 9/9/87 by Martz.

EPA One-liner: "Not a mutagen when tested for DNA repair using mouse and rat hepatocyte cultures."

294-095 TAB 8: Narrative rebuttal to # 044576 adequately answering concerns of hepatocyte viability, number of cells scored, and test article purity. Upgraded study to acceptable. 9/9/87, Martz.

294-078 044571: "Mutagenicity Testing on Fungicide 1991 Metabolite (MBC) in Microbial Systems," Bacillus subtilis; Institute of Environmental Toxicology (Japan), 10/17/77; MBC, 99% pure; M45 and H17 without activation with 0, 20, 100, 200, 500, or 1000 µg/10 mm disk in 20 µl; no evidence of differential growth inhibition or cytotoxicity; **unacceptable** and not **upgradeable** (no activation).

REVIEW: 6/16/86 by (Remsen) Gee.

EPA One-liner: none in Branch Library.

CONCLUSION: A "possible adverse effect" status is being assigned to this test category on the basis of 1/3 acceptable studies being positive. 9/16/87, Martz.

DNA DAMAGE/REPAIR: ADDITIONAL INFORMATION (ESPECIALLY PUBLICATIONS)

294-064 036309 Kappas, A., Georgopoulos, S.G., and Hastie, A.C. "On the genetic activity of benzimidazole and thiophanate fungicides on diploid Aspergillus nidulans". Mutation Res. 26:17-27 (1974). Benomyl induced genetic segregation (haploidization) in a diploid strain of Aspergillus nidulans. **Unacceptable** due to limitations in study design. **Possible adverse effect indicated.** de Vlaming/Apostolou, 12/4/85.

294-064 036311 Morpurgo, G., Bellincampi, D., Gualandi, G., Galdinelli, L., and Serlupi Crescenzi, O. "Analysis of mitotic nondisjunction with Aspergillus nidulans". Environ. Health Perspect. 31:81-95 (1979). A plate test and a liquid test were performed with Benomyl and MBC. Both were positive for nondisjunction, apparently due to interference with spindle fiber formation. **Unacceptable** due to limitations in study design. **Possible adverse effect indicated.** de Vlaming/Apostolou, 12/4/85.

294-064 036312 Bates, A.D. "Microtus oeconomus (Rodentia), a useful mammal for studying the induction of sex-chromosome nondisjunction and diploid gametes in male germ cells." Environ. Health Perspect. 31:151-159 (1979). Contents of seminiferous tubules of field voles were examined at 1-16 days following MBC (2 gavage treatments, 24 hr apart, of 250 mg/kg MBC in gum arabic) or 1-14 days following Benomyl treatment (single ip injection of 50, 250, or 500 mg/kg Benomyl, with sacrifice for examinations of seminiferous tubules at varying times, generally 10 to 14 days). Frequencies of non-disjunction spermatids or of diploid spermatids were measured. Data for Benomyl were reportedly negative, but investigators concluded that "MBC was effective in inducing nondisjunction in young primary spermatocytes (day 10 after treatment)". The investigator noted that these results were "preliminary". **Unacceptable** due to limitations in study design. **Possible adverse effect indicated.** de Vlaming/Apostolou, 12/4/85: one-liner updated by Aldous, 9/28/90.

294-041 and -063 965490 Shirasu, Y., Moriya, M., and Kato, K., "Mutagenicity testing on Fungicide 1991 in microbial systems". Institute of Environmental Toxicology, Tokyo, 1/23/78. A (negative) rec assay as part of a series of studies on Benomyl. These studies were classified as **unacceptable**. One-liner for this report is near the end of the "Gene Mutation" section of this Summary. Aldous, 10/2/90.

294-041 038196 (the rec-assay portion of a series of studies). Shirasu, Y., Moriya, M., and Kato, K.; "Mutagenicity testing on Fungicide 1991 metabolite (MBC) in microbial systems". Institute of Environmental Toxicology, Tokyo, 10/17/77. MBC, 99%, was tested in the same systems as reported for Benomyl, above (294-041 and -063 965490). All results were negative. These studies were classified as **unacceptable**. One-liner for this report is near the end of the "Gene Mutation" section of this Summary. Aldous, 10/2/90.

294-039 965492 Tong, C., "The hepatocyte primary culture/DNA repair assay on Compound 10,962-02 [Benomyl] using rat hepatocytes in culture." Study apparently was performed at Naylor Dana Institute, Valhalla, NY; report issued 10/20/81. Seeded hepatocytes were treated with tritiated thymidine in the presence of 0.00005, 0.0005, 0.005, 0.05, or 0.5 mg/ml Benomyl. Nuclear grain counts above background [minimum of 5 counts/nucleus to be "positive"] were recorded. Results were negative, by those criteria. Report is classified as **unacceptable**: inadequate description of test article, no analysis of dosing solution, insufficient individual data, no justification for the wide range of dosages selected, no QA/GLP. Original review by Wong, 5/9/85. One-liner by Aldous, 10/3/90.

294-039 965493, 965494, and 965495 Studies identical to 965492, above, except that -492 and -493 tested Benomyl, whereas the latter two tested MBC; and the even-numbered reports used F344 rat hepatocytes, whereas the odd-numbered reports used B6C3F1 mouse hepatocytes. All tests were negative, and all are classified **unacceptable** for the reasons given in the 1-liner above. Original reviews by Wong, 5/9/85. One-liner by Aldous, 10/4/90.

Note that one of these reports, 965494, was submitted separately and given document:record number 294-078:044576. The latter study was eventually upgraded to acceptable status following submission of additional information (see 1-liner, about 3 pages toward the front of this Summary).

294-039 965488 A summary of journal articles and studies concerning the oncogenic, mutagenic and reproductive toxicity potential of Benomyl. Duplicate of record -063:36297. No worksheet. Kellner, 9/24/93.

NEUROTOXICITY STUDIES

BENOMYL

161 131147 Foss, J. "Subchronic Neurotoxicity Study of DPX-T1991-529 (Benomyl) Administered Orally Via The Diet To CrI:CD*BR VAF/Plus® Rats" (Argus Research Laboratories, Inc., Report No. 104-019, DuPont Study #HLO 551-93, 6/13/94). Benomyl (Lot No. F60317K, 97.4% purity) was administered orally via the feed to 11 CrI:CD® BR VAF/Plus® (Sprague-Dawley) rats/sex/dose at levels of 0, 100, 2500 or 7500 ppm for 92 to 95 consecutive days. Feed consumption and body weight gain was reduced in high-dose rats. Motor activity (total movements) for high-dose males was increased 40%, 56% and 48% in weeks 4, 8 and 13, respectively; similar increases were seen in high-dose females. No compound-related FOB findings, gross or microscopic neuronal lesions were reported. NOEL (for body weight and motor activity effects) = 2500 ppm. **No Adverse Neurotoxic Effects. ACCEPTABLE. Kellner, 1/25/95.

-143 125046 Foss, J. "Acute Neurotoxicity Study of DPX-T1991-529 (Benomyl) Administered Orally Via

Gavage to Crl:CD® BR VAF/Plus® Rats" (Argus Research Laboratories, Inc., Report No. HLO 825-92, 6/14/93). Benomyl (Lot No. F60317K, 97.4% purity) was administered orally via gavage to 10 Crl:CD® BR VAF/Plus® (Sprague-Dawley) rats/sex/dose at levels of 0, 500, 1000 and 2000 mg/kg. By day 2, most of the high-dose males and one female showed soft or liquid feces during the Functional Observation Battery (FOB). Between day 1 and 3, 1 female each in the mid- and high-dose groups showed urine-stained fur during clinical observations and FOB. Number of movements and time spent in movement was reduced in high-dose females on the day of dosage. Body weight gain was reduced in all dose groups on day 1 and 2 (statistically significant in the mid- and high-dose males only) accompanied by significantly reduced feed consumption values (all males and mid-, high-dose females). Systemic NOEL < 500 mg/kg. **No Adverse Neurotoxic Effects.** Acceptable as supplementary data. Kellner and Aldous, 11/10/93.

-141 124974 Interim report (2 pages) for acute neurotoxicity study -143: 125046. No Worksheet. Kellner, 2/15/95.

294-065 036332 (With rebuttal in -076, 043801 & -02): "Neurotoxicity Study in Hens;" IRDC, 6/5/78; benomyl (H-10962, purity not given), 5000, 2500, 500, or 0 mg/kg by oral gavage with TOCP at 750 mg/kg as positive control; results equivocal because study compromised due to intercurrent disease, has no useful data. Not acceptable, but not a required test.

REVIEW: original 11/25/85 by Apostolou, rebuttal 6/11/85 by Martz.

EPA One-liner: "Inconclusive results due to underlying disease in hens." No grade given.

294-076 043801 & -02: Rebuttal to # 036332 above. Clarifies nerve selection 29for histopathology and absence for forced activity assessment as well as a revised pathology report consisting of the opinions of 5 pathologists about the equivocal findings. One-liner added 9/8/87, Martz.

BENOMYL

294-065 036331 (With rebuttal in -076, 043803): "Acute Delayed Neurotoxicity Study in Chickens;" IRDC, 12/7/79; benomyl (H-10962, purity not given), 5000, 2500, 500, or 0 mg/kg by oral gavage with TOCP at 1200 mg/kg as positive control; death, ataxia, low carriage, and wing drop at 5000 mg/kg, but 2500 and 500 mg/kg groups asymptomatic; no histological evidence of delayed neurotoxicity. Rebuttal (below) clarified major criticisms. INCOMPLETE but upgradeable, however not a required test.

REVIEW: original 11/25/85 by Apostolou, rebuttal 6/11/86 by Martz.

EPA One-liner: "No evidence of delayed neurotoxicity was found." No grade given.

294-076 043803: Narrative rebuttal to # 036331 above. Clarifies nerve selection for histopathology, not having used atropine protection, no repeat dosing on day 21, and the consistency of results with the previous equivocal study (#36332). One-liner added 9/8/87, Martz.

MBC

294-077 044556 & -7: "Neurotoxicity Study in Hens;" IRDC, 6/5/78; MBC (H-11,201, purity not given), 5000, 2500, 500, or 0 mg/kg by oral gavage with TOCP at 750 mg/kg as positive control; clinical signs of neurotoxicity at 5000 mg/kg; no histopathologic evidence of neurotoxicity in any MBC treated hens; INCOMPLETE BUT UPGRADEABLE with additional information, however, not a required test.

REVIEW: 6/13/86 by Martz. (No EPA 1-liner on file with CDFA).