DATE: **December 3, 1997**

MEMORANDUM

SUBJECT: **BENOMYL - *FQPA REQUIREMENT*** - Report of the Hazard Identification Assessment Review Committee.

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THROUGH: K. Clark Swentzel, Chairman,
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BACKGROUND: On November 25, 1997, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) met to determine the Uncertainty Factors and the Margins of Exposure for dietary and non-dietary risk assessments as required by the Food Quality Protecting Act (FQPA) of 1996. The Committee's decisions are attached.

A. INTRODUCTION

On January 9, 1997, the Health Effects Division's RfD/Peer Review Committee evaluated the toxicology data base of Benomyl and reassessed the Reference Dose but deferred to a later date the need for an additional Uncertainty Factor for the enhanced sensitivity to infants and children (as required by FQPA) as well as the final decision regarding the need for a developmental neurotoxicity study (Memorandum: G. Ghali, HED to C. Welch, RD, dated 05/28/97).

On January 14, 1997, the Health Effects Division's Toxicology Endpoint Selection Committee selected the doses and endpoints for acute dietary as well as occupational and residential exposure risk assessments. but did not address the Margins of Exposure (MOEs) required for the various exposure scenarios.

On November 25, 1997, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) met to determine the Uncertainty Factors and the MOEs for dietary and non-dietary risk assessments as required by the Food Quality Protecting Act (FQPA) of 1996.

The reader is referred to the initial RfD and TES Committee reports for summaries of the studies as well as the rationale for the doses and endpoints selected for the various exposure scenarios. The decisions made by HIARC on the Uncertainty Factors and/or the Margins of Exposure for acute and chronic dietary as well as occupational/residential risk assessments are provided below.

B. DETERMINATION OF UNCERTAINTY FACTORS AND/OR MARGINS OF EXPOSURES

1. Acute Dietary Risk Assessment

For acute dietary risk assessment, the Toxicology Endpoint Selection Committee selected the developmental NOEL of 30 mg/kg/day based on increased incidence of microphthalmia at 62.5 mg/kg/day (LOEL) in pregnant rats given oral administrations of Benomyl at 0, 3, 10, 30, 62.5 or 125 mg/kg/day during gestation days 7 through 16.

For this risk assessment, HIARC determined that the **10 x** factor to account for enhanced sensitivity of infants and children **(as required by FQPA) should be retained.** Although no increased sensitivity was observed in young rabbits following *in utero* exposure or in pups as compared to adults in the two-generation reproduction study in rats, **A MOE of 1000 is required** because:

(i) There is increased sensitivity of rat fetuses as compared to maternal animals following *in utero* exposure in a prenatal developmental toxicity study in rats. Increased sensitivity manifested as developmental anomalies (decreased fetal body weight and ocular and/or cerebral malformations) at doses which were found to be not maternally toxic. For developmental toxicity the NOEL was 30 mg/kg/day whereas for maternal toxicity, the NOEL was $\geq$125 mg/kg/day (highest dose tested).

(ii) There is concern for the developmental neurotoxic potential of Benomyl.

This is based on the evidence of neurotoxic effects in the acute and subchronic neurotoxicity (Subdivision F Guideline) studies as well as evidence from published literature which indicates that Benomyl produces CNS anomalies in rats when administered during late gestation as well as neurotoxic effects (CNS and cholinesterase inhibition, presumably in humans).

(iii) Mutagenicity studies provide evidence of aneuploidy induction following oral dosing in mice. The genetic imbalances resulting from aneuploidy in germinal cells may contribute to birth defects. Mutagenicity data support the evidence of developmental anomalies in rats.

2. Chronic Dietary Risk Assessment

For chronic dietary risk assessment, the RfD/Peer review Committee selected a NOEL of 2.5 mg/kg/day based on histopathological lesions of the liver characterized as swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis at 12.5 mg/kg/day (LOEL) in both sexes of dogs given oral administrations of Carbendazim, a primary metabolite of Benomyl at 0, 2.5, 12.5 or 62.5 mg/kg/day for 52 weeks.

For this risk assessment, the HIRAC determined that the **10 x** factor to account for enhanced sensitivity of infants and children **(as required by FQPA) should be removed. The present UF of 100 is adequate** to ensure protection from chronic exposure to Benomyl. **Therefore, the RfD remains at 0.03 mg/kg/day.** A UF of 100 is adequate because: 1) the RfD provides adequate protection for potential *in utero* exposure to humans (i.e., the NOEL of 30 mg/kg/day in rat developmental study is 12 times greater than the NOEL of 2.5 mg/kg/day in the chronic dog study used for deriving the RfD), and 2) the endpoint of concern following chronic exposure is liver pathology in adult dogs while the developmental effects of concern are seen following short-term *in utero* exposure (i.e., gestation days 6-16).

The Committee also determined that the decisions made on Benomyl are also applicable to Carbendazim, the primary metabolite product of Benomyl, for acute and chronic dietary risk assessments.

3. Occupational/Residential Exposure Risk Assessments

For **Short-**and **Intermediate-Term Dermal risk assessments**, HIARC selected the developmental NOEL of 30 mg/kg/day based on increased incidence of microphthalmia at 62.5 mg/kg/day (LOEL) in pregnant rats given oral administrations of Benomyl at 0, 3, 10, 30, 62.5 or 125 mg/kg/day during gestation days 7 through 16. Since a dose from an oral study (i.e., oral NOEL) was selected, a dermal absorption rate of 3.5% should be used for these risk assessments.

For these risk assessments, HIARC determined that the **10 x** factor to account for enhanced sensitivity of infants and children **(as required by FQPA) should be retained.**

Although no increased sensitivity was observed in young rabbits following *in utero* exposure or in pups as compared to adults in the two-generation reproduction study in rats, **A MOE of 1000 is required** because:

(i) There is increased sensitivity of rat fetuses as compared to maternal animals following *in utero* exposure in a prenatal developmental toxicity study in rats. Increased sensitivity manifested as developmental anomalies (decreased fetal body weight and ocular and/or cerebral malformations) at doses which were found to be not maternally toxic. For developmental toxicity the NOEL was 30 mg/kg/day whereas for maternal toxicity, the NOEL was ≥125 mg/kg/day (highest dose tested).

(ii) There is concern for the developmental neurotoxic potential of Benomyl. This is based on the evidence of neurotoxic effects in the acute and subchronic neurotoxicity (Subdivision F Guidelines) studies as well as evidence from published literature which indicates that Benomyl produces CNS anomalies in rats when administered during late gestation as well as neurotoxic effects (CNS and cholinesterase inhibition, presumably in humans).

(iii) Mutagenicity studies provide evidence of aneuploidy induction following oral dosing in mice. The genetic imbalances resulting from aneuploidy in germinal cells may contribute to birth defects. Mutagenicity data support the evidence of developmental anomalies in rats.

For Long-Term Dermal risk assessment, HIARC selected a NOEL of 2.5 mg/kg/day based on histopathological lesions of the liver characterized as swollen, vacuolated hepatic cells, hepatic cirrhosis and chronic hepatitis at 12.5 mg/kg/day (LOEL) in both sexes of dogs given oral administrations of Carbendazim, a primary metabolite of Benomyl at 0, 2.5, 12.5 or 62.5 mg/kg/day for 52 weeks. This dose was also used in establishing the RfD. Since a dose from an oral study (i.e., oral NOEL) was selected, a dermal absorption rate of 3.5% should be used for these risk assessments.

For this risk assessment, HIRAC determined that the **10 x** factor to account for enhanced sensitivity of infants and children **(as required by FQPA) should be removed. A MOE of 100 is adequate** because: 1) the dose (2.5 mg/kg/day) selected provides adequate protection for potential *in utero* exposure to humans (i.e., the developmental NOEL of 30 mg/kg/day is 12 times greater), and 2) the endpoint of concern after chronic exposure is liver pathology in adult dogs while the developmental effects of concern are seen following short-term *in utero* exposure (i.e., gestation days 6-16).

For Inhalation risk assessments, HIARC selected the oral NOEL of 30 mg/kg/day for Short- and Intermediate Term exposures and a oral NOEL of 2.5 mg/kg/day for Long-Term exposure risk assessment, the same doses used in respective dermal risk assessments. Although a 90-day inhalation study (MRID No. 40399501) was available, it was not used for this risk assessment, because that study measured a non-specific endpoint (histopathology suggestive of olfactory degeneration) and did not evaluate the *in utero* effect of concern. Since the doses identified for inhalation risk assessments are from oral studies (i.e., use of oral NOEL) the risk assessment should be as follows:

(i) The inhalation exposure component (i.e., mg/L) using a 100 % absorption rate (default value) should be converted to an equivalent oral dose (mg/kg/day).

(ii) The dermal exposure component (i.e., mg/kg/day) using 3.5% dermal absorption should be combined with this converted oral dose (mg/kg/day).

(iii) This dose should then be compared to the oral NOEL of 30 mg/kg/day for Short- and Intermediate-Term exposure and 2.5 mg/kg/day for Long-Term exposures to calculate the Margins of Exposure.

For Short-and Intermediate-Term Inhalation risk assessments, HIARC determined that the **10 x** factor to account for enhanced sensitivity of infants and children **(as required by FQPA) should be retained.** Although no increased sensitivity were observed in young rabbits following *in utero* exposure or in pups as compared to adults in the two-generation reproduction study in rats, **A MOE of 1000 is required** for the same reasons stated under the Acute and Chronic dietary risk assessments.

For Long-Term Inhalation risk assessment, HIRAC determined that the **10 x** factor to account for enhanced sensitivity of infants and children **(as required by FQPA) should be removed. A MOE of 100 is adequate** because: 1) the dose (2.5 mg/kg/day) provides adequate protection for potential *in utero* exposure to humans (i.e., the developmental NOEL of 30 mg/kg/day is 12 times greater), and 2) the endpoint of concern after chronic exposure is liver pathology in adult dogs while the developmental effects of concern are seen following short-term *in utero* exposure (i.e., gestation days 6-16).

C. RECOMMENDATION FOR A DEVELOPMENTAL NEUROTOXICITY STUDY

The RfD/Peer Review Committee recommended that the decision for the need for a developmental neurotoxicity study be made by the Developmental/Reproductive Peer Review Committee. This recommendation was re-evaluated by HIARC. Based on the following weight-of-the-evidence, HIARC determined that a developmental neurotoxicity study *is required.*

- The prenatal developmental toxicity study in rats demonstrated central nervous system (CNS) anomalies in the fetuses following maternal exposure during gestation. The CNS anomalies included anophthalmia, microphthalmia, and hydrocephaly (MRID No. 00148393 and 0015764).

- A number of other studies available in the literature have also demonstrated similar observations (Zeman et al., 1986; Ellis et al., 1987, 1988; Hess et al., 1987; Hoogenboom et al., 1991 and Lu et al., 1994).

- There is a suggestion that administration of Benomyl in late gestation, as opposed to administration only during the period of major organogenesis, enhances the incidences of CNS anomalies in rats (Zeman et al., 1987 and Ellis et al., 1988).

- In mutagenicity studies, there is evidence of aneuploidy induction following oral dosing in mice (MRID No. 42911601, 42911602). The genetic imbalances resulting from aneuploidy in germinal cells may contribute to birth defects. Mutagenicity data support the evidence of developmental anomalies in rats. Hoogenboom et al. (1991) postulated that the known antitubulin action of Benomyl may impair microtubule formation and produce brain and ocular malformations by disruption of neuronal proliferation and migration.

- In an acute neurotoxicity study a single dose of Benomyl at 2000 mg/kg caused a decrease in motor activity in females along with a decrease in body weight gain. Therefore, the former effect was not considered to be evidence of neurotoxicity. On the other hand, the decrease (6%) in absolute brain weight in males at 500 or 2000 mg/kg was considered to be a possible indicator of neurotoxicity (MRID No. 42817003).

- In a subchronic neurotoxicity study in rats, the increased motor activity observed in females given repeated oral administration of Benomyl at 578 mg/kg/day was considered to be indicative of a possible neurotoxic effect. The Committee noted that functional effects were not measured in this study (MRID No. 43277901).

- A summary of a monograph prepared for NIOSH indicated that Benomyl produces neurotoxic effects (CNS and cholinesterase inhibition), presumably in humans. The data upon which these conclusions were based was not specified in the summary (NIOSH, 1992).

The Committee recommended that highest dose level tested in this study should be sufficiently high to demonstrate the CNS defects observed in other studies. A clear differential in fetal response to gavage versus dietary exposure to Benomyl has been demonstrated, with gavage dosing producing anomalies at approximately one-tenth of the dietary level (Kavlock et al., 1982; Chernoff, 1985). This would need to be considered when the protocol is designed and dose levels are selected for the developmental neurotoxicity study.

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