Burnam

5/4/82

MEMORANDUM

TO: Jeff Kempter, Chief
SPRD (TS-791)

SUBJECT: Statistical Evaluation and Oncogenicity Risk Assessment of Benomyl, Benlate and MBC 2-Year Feeding Studies in Mice

Summary

In the following report it is shown that Benomyl and its metabolite MBC are associated with a statistically significant dose-related increase in the incidence of hepatocellular adenomas and/or carcinomas in both male and female mice. The female mice fed 500 and 1500 ppm MBC and their randomized control group are found to be the most sensitive group for this tumor type and therefore the group most appropriate for quantitative risk assessment. Low-dose extrapolation was performed using five current models and both the point estimators and lower 95% confidence bounds on "Virtually Safe Doses" associated with selected risks are presented in Table 2. Using criteria developed by the Food Safety Council ("Proposed System for Food Safety Assessment", June 1980) the multi-stage model is used to extrapolate cancer risks in man by the potency estimate $Q^*_1 = 2.065 \times 10^{-3}$. Multiplying proposed human exposures by Q^*_1 will estimate the upper 95% bound on cancer risks to man from lifetime exposures.

Discussion

INT-1991, Benomyl Benlate in mice MRP #3194-001, Haskell Laboratory Report No. 20-82 of chronic feeding study conducted from 8/30/78 to 9/12/80 on CD-1 mice, (born 7/17/78 from Charles River Breeding Labs, Wilmington, Mass. received 8/17/78). Three hundred twenty mice were allocated by computer stratified randomization into 4 groups of 80 males and 4 groups of 80 females to Purina Laboratory Chow plus 0, 500, 1500 or 7,500 ppm Benomyl. Due to the delay in weight gain observed in high dose mice of both sexes this dose was reduced from 7,500 to

5,000 ppm after week 37 and survivors were maintained on 5,000 ppm for the remaining 69 weeks of study. Nevertheless the weight Tables and graphs (See Table I & III of Haskell Laboratory Report 20-82 pages 34-37) presented by the applicant demonstrate that there is a significant reduction in body weights of mid- and high-dosed compared to control animals. As these differences persist after the second week for high dose animals of both sexes and for mid-dosed males, the size of the effect was used to determine whether doses were excessive. The data in these tables demonstrate approximately a 10% reduction among the high dosed animals and 5% or less in the mid-dosed animals. This 10% weight reduction in body weight criterion has been routinely used by the National Toxicology Program as the basis for considering that the maximum tolerated dose has been exceeded. We have therefore omitted the high dose group of each sex from further consideration in the analysis except for the presentation of data in Table I below.

MBC,INT-965 in mice MRP #3207-001, Haskell Laboratory Report No. 70-82 of chronic feeding study conducted 10/3/78-10/16/80 on CD-1 mice, (born 8/29/78 from Charles River Breeding Labs, Wilmington, Massachusetts, 362 were received on 9/28/78.) Three hundred and twenty mice were allocated by computer to stratified randomization into 4 groups of 80 males and 4 groups of 80 females to Purina Laboratory Chow plus 0, 500, 1500, on 7,500 ppm MBC. High dose males had a higher mortality rate during weeks 52-64. The MBC in the diet was reduced to 3,750 ppm at week 66 (after 1 week of no chemical) -- however this group was sacrificed at week 73 due to continued excessive mortality. The reduction in survival and early curtailment of the high-dosed male mice effectively eliminates this group from analysis of tumor incidence. While survival and weight observed in other groups presented no statistically significant trends, the decreases in red cell blood count and hemoglobin in high-dose females and the increased hematocrit and other hematological markers in mid-dosed males may be evidence of competing toxicological manifestations which could bias the rate of tumor formation in these study groups.

Thus in the Benomyl mouse assay the high dosed animals appear to have been exposed to a dose that exceeds the maximum tolerated dose and in the metabolite (MBC) assay males in the high-dose and possible mid-dose have exceeded the MTD while females in the high-dose group may also have exceeded the MTD. Only liver and lung adenomas and/or carcinomas appear in significantly increased proportions. The attached Table I displays the number of liver adenoma and/or carcinoma bearing animals among those still surviving on the date that the first mouse died with a liver tumor. The proportion or rates for each sex and dose group is shown for those mice dying during study, interim findings, and for those animals diagnosed

at terminal kill. The company analysis, see Attachment 1, addresses only the total findings summarizing or combining the above without considering the additional information to be gained by analysis of these two data sub-sets. When the data in Table I are examined it is immediately evident that the response rates in the high-dose groups do not behave in a pattern consistent with low- and mid-dose responses. This phenomenon has already been anticipated by the weight gain, survival and hematological differences discussed above. There is however an increase in the dose-response slopes for females compared to males in both studies and this effect is most pronounced among the interim MBC females compared to the Benomyl interim or final findings. These findings for hepatocellular adenomas and/or carcinomas are largely due to the increase in carcinoma incidence of treated mice of both sexes. Although the findings for carcinoma alone and adenoma and/or carcinoma are essentially identical, the more general problem of oncogenesis is more inclusive and therefore a better basic model for estimating oncogenic potential.

Statistical analysis of hepatocellular adenoma and/or carcinoma in the MBC female sub-study using exact tests (Gart, Chu & Tarone, JNCI 66 pp 1175-1181) comparing the incidence in 500 ppm (125 mg/kg/d) treated females to randomized controls results in P=.03 for incidental diagnoses, P=.145 for the terminal kill, with a combined P=.0075. With respect to the 1500 ppm (380 mg/kg/d) dose, the increased incidence of proliferative liver neoplasms is P=.000,03 for incidental diagnoses, P<.001 for the final kill and $P<5x10^{-8}$ overall.

When utilizing mouse liver data as the information base for extrapolating cancer risks to man it is important to consider all relevant ancillary data available from the available information profile. With respect to Benomyl, no cancer data has been reported in rats, liver and to a lesser degree lung cancer findings have been reported in both sexes in 3 mouse-feeding studies of Benomyl and metabolites of Benomyl. The liver has been reported as a target organ in several species so that oncogenesis may be expected as an ultimate end-point. Other target tissue are, the reproduction system and blood including degeneration of germinal epithelium in males and females, Red Blood Cell count, hemaglobin and hematocrit (data on these 3 variables will be analyzed in a subsequent report). Mutagenicity data submitted have indicated that under selected conditions Benomyl metabolites have mutagenic potential e.g. Sister-Chromatid Exchange and Mouse Lymphoma Cell Point Mutation. From the preceeding it seems clear that parametric models for one-hit, multi-stage or multi-hit theories of carcinogenesis are to be preferred over tolerance based models (probit or Weibull); (see Rai & Van Ryzin pages 99-117 of Sims Conference Proceedings "Energy & Health" ed by Breslow & Whittemore, Siam 1979)

Goodness-of-fit is suggested by the Food and Safety Council (see reference in opening paragraph) as a basis for selecting among these models when biological data does not suggest that a particular model is the most appropriate choice. Therefore, the female MBC data from control low and mid-doses have been fit to several models and both point estimates and lower 95% confidence bounds on virtually safe dose levels associated with selected risks of cancer are shown in Table 2. The multi-stage model provides the best fit in that the lack-of-goodness-of-fit has a P>.999 indicating almost perfect fit of the observed data to the model.

Using the multi-stage model one can obtain a potency estimator Q^*_1, which is slope of the lower 95% confidence bound on the virtually safe dose. The MBC data have a Q^*_1 of 2.065×10^{-3}. When Q^*_1 is multiplied by the exposure estimated for a particular crop or commodity we obtain the upper 95% bound on the expected lifetime cancer risk from the estimated exposure. Note, that following the Food Safety Council procedures, no intraspecies dietary correction is used.

Bertram Litt, Statistician
Toxicology Branch, HED (TS-769)

Attachment

cc: O. Paynter
W. Burnam
M. Sochard
A. Barton

BLitt/ccw 5/4/82 #7

Table 1 Benomyl Mouse Bioassy Liver Adeour and/or Carcionma

(5)

Group	Benomyl (Surivors after first liver tumor) Interim Findings	Terminal Kill	Total
Males			
0 mg/kg/d	13/31	12/43	25/74
64 mg/kg/d	14/33	21/41	35/74
187 mg/kg/d	20/32	32/40	52/72
678 mg/kg/d	8/36	19/40	27/76
Females			
0 mg/kg/d	0/40	4/35	4/73
103 mg/kg/d	5/39	4/31	9/70
286 mg/kg/d	5/41	8/31	13/72
959 mg/kg/d	5/32	16/33	21/65

Males	MBC (Survivors after 1st liver tumor) Interim Findings	Terminal Kill	Total
0 mg/kg/d	7/53	6/18	13/71
81 mg/kg/d	12/51	8/14	20/65
259 mg/kg/d	22/53	1/9	23/62
(1560) mg/kg/d	2/23	1/23	3/46
Females			
0 mg/kg/d	0/29	1/21	1/50
125 mg/kg/d	6/41	3/13	9/54
380 mg/kg/d	13/30	8/14	21/44
1886 mg/kg/d	5/32	10/21	15/53

Table 2. Benomyl/MBC Quantitative Risk-Low Dose Extrapolation

Point Estimate of VSD				Added Risk Over Baseline	Lower 95% Confidence Bound on VSD				
One-Hit	Multi-Stage	Multi-Hit	Weibull		Mantel-Bryan	One Hit	Multi-Stage	Multi-Hit	Weibull
	(mg/kg/day)				(mg/kg/day)				
6.47×10^{-6}	8.91×10^{-6}	3.73×10^{-4}	1.54×10^{-4}	1×10^{-8}	2.05×10^{-3}	3.42×10^{-6}	5.34×10^{-6}	1.82×10^{-8}	1.74×10^{-5}
6.47×10^{-5}	8.91×10^{-5}	2.16×10^{-3}	1.02×10^{-3}	1×10^{-7}	5.29×10^{-3}	3.42×10^{-5}	5.34×10^{-5}	4.03×10^{-7}	1.30×10^{-4}
6.47×10^{-4}	8.91×10^{-4}	1.25×10^{-2}	6.72×10^{-3}	1×10^{-6}	1.48×10^{-2}	3.42×10^{-4}	5.34×10^{-4}	8.92×10^{-6}	9.87×10^{-4}
6.47×10^{-3}	8.91×10^{-3}	7.22×10^{-2}	4.46×10^{-2}	1×10^{-5}	9.54×10^{-2}	3.42×10^{-3}	5.34×10^{-3}	1.97×10^{-4}	7.69×10^{-3}
6.47×10^{-2}	8.91×10^{-2}	4.18×10^{-1}	2.45×10^{-1}	1×10^{-4}	1.60×10^{-1}	3.42×10^{-2}	5.34×10^{-2}	4.36×10^{-3}	6.18×10^{-2}
6.18×10^{-1}	8.91×10^{-1}	2.42	1.95	1×10^{-3}	6.80×10^{-1}	3.43×10^{-1}	5.34×10^{-1}	9.62×10^{-2}	5.22×10^{-1}

q_0 2.02×10^{-2}
q_1 1.12×10^{-3}
q_2 1.4×10^{-6}
q_3 ——

$$Q^*_1 = 2.065 \times 10^{-3}$$

Appendix Table 7

(7)

TABLE VII

SUMMARY OF STATISTICAL ANALYSES OF HEPATOCELLULAR NEOPLASMS IN
CD®-1 MICE FED CARBAMIC ACID, (1-[(BUTYLAMINO) CARBONYL]-1H-BENZIMIDAZOL-2-YL)-, METHYL ESTER (INT-1991, BENOM

Group Designation	III	V	VII*	IV	VI	VIII*
Sex	♂	♂	♂	♀	♀	♀
Dose (ppm)	500	1500	5000	500	1500	5000
Hepatocellular Adenomas						
Fisher's Exact (livers)	NS	NS	NS	NS	NS	NS
Mantel Haenszel X^2 (Life table)	NS	NS	NS	NS	NS	NS
X^2 Test for Trend (dose response)	NS			†		
Hepatocellular Carcinomas						
Fisher's Exact	NS	†††	NS	NS	NS	††
Mantel Haenszel X^2	†	†††	NS	†	NS	††
X^2 Test for Trend	NS			††		
Hepatocellular Adenomas, Carcinomas, and Autolyzed Neoplasm						
Fisher's Exact	NS	†††	NS	NS	†	†††
Mantel Haenszel X^2	†	†††	NS	NS	††	†††
X^2 Test for Trend	NS			†††		

† = $P < 0.05$
†† = $P < 0.01$
††† = $P < 0.001$
NS = $P > 0.05$
* Dose level lowered from 7500 ppm after 38 weeks.

SUMMARY OF STATISTICAL ANALYSES OF HEPATOCELLULAR NEOPLASMS IN
CD®-1 MICE FED CARBAMIC ACID, 1H-BENZIMIDAZOL-2-yl-, METHYL ESTER (INE-965, MBC)

Group Designation	III	V	VII*	IV	VI	VIII
Sex	♂	♂	♂	♀	♀	♀
Dose (ppm)	500	1500	3750	500	1500	7500
Hepatocellular Adenomas						
Fisher's Exact	NS	NS	**	†	†	NS
Mantel Haenzel X^2 (Life table)	NS	NS		†	NS	NS
X^2 Test for Trend (dose response)	NS			NS		
Hepatocellular Carcinomas						
Fisher's Exact	NS	†		NS	†††	††
Mantel Haenszel X^2	NS	†		NS	†††	†††
X^2 Test for Trend	†			†††		
Hepatocellular, Adenomas, Carcinomas and Hepatoblastoma						
Fisher's Exact	NS	†		††	†††	†††
Mantel Haenszel X^2	†	†		††	†††	†††
X^2 Test for Trend	†			†††		

†= P <0.05

††= P <0.01

†††= P <0.001

NS = P > 0.05

* = Group VII mice received 7500 ppm for first 15 months on study.

** = Terminal sacrifice of males receiving the highest treatment level occurred after 17 months on test. Terminal sacrifice for all other treatment groups occurred after 24 months on test.