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Memorandum

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SUBJECT: Metalaxyl Mouse Oncogenicity Study. Registration No. 100-607, Acc. No. 247660. TOX Chem. No. 375AA.

Attached is a review and discussion of the 104-week mouse oncogenicity study cited above.

Conclusions and Recommendations

The results of the study suggested a treatment related trend in the increased incidence of hepatocellular adenomas and carcinomas in male mice. However, statistical analysis (trend analysis and Chi-square tests) suggest that the results could be an artifact or that they could possibly be a low dose effect.

Additional specific historical control data could be useful in characterizing the significance of the reported results, and it should be requested. Such data should include information on the variation encountered in the background incidence of each tumor type from the control groups which were apparently pooled to get the reported figures cited in the report.

No final conclusion regarding the study can be made until the specific historical control data are available for review.

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Citation:

McSheehy, T.W., S.M. MaCrae and J.C. Whitney. October 31, 1980. CGA-48-988: Oncogenicity in dietary administration to mice for two years. Unpublished report submitted by Ciba-Geigy Corporation. EPA Accession No. 247660.

Materials and Methods:

Test Substance: Metalaxyl Fungicide (CGA-48-988) was used in three batches as provided by the study's sponsor. The authors stated that the sponsor determined the purity and composition of the test material, and this description was not included in the report.

Test Species: Swiss mice of the ICI Alderley Park Strain were used.

Experimental Procedures: Mice were assigned to one of four test groups which contained 60 of each sex. Assignments were made so that the group mean body weights did not differ by more than 1 g.

The mice were given diets containing 0, 50, 250 or 1250 ppm Metalaxyl for 104 weeks. Test diets were prepared fresh each week and samples were analyzed for concentration of test substance and homogeneity of the dietary mixtures. The stability of Metalaxyl in test diet was also evaluated prior to the start of the study.

Mice were observed twice daily for signs of toxicity during the first two weeks and from week 28 until the end of the study. These observations were made once a day during weeks 3 through 27 of the study. Each animal was palpated once a week during the study. Body weights were measured weekly during the first 14 weeks and from week 40 until the end of the study. The mice were weighed at monthly intervals from the 14th through 40th weeks of the experiment.

Animals described as "severely debilitated" were isolated to prevent loss to the study, and moribund animals were sacrificed.

The food consumption was estimated weekly for each cage (four mice). These data were used with body weights to estimate efficiency of food conversion and with chemical analysis data to estimate the achieved daily dosage (mg test substance per kg body weight).

Mice sacrificed in extremis during the study or survivors sacrificed at termination were necropsied. These animals were subjected to gross internal examination and blood and femoral marrows mears were made when possible immediately after sacrifice. Organs or tissues which were found with gross lesions were preserved for microscopic examination. The tissues collected and preserved from every animal for histological examination included endocrine glands, gastrointestinal tract, heart, liver, kidney, lungs, gonads (including accessory tissues), bone, lymphatic system, nervous system, skin, skeletal muscle, urinary bladder, uterus, and tissue masses. Not all tissues from all animals were examined, and a tabulated summary and individual animal reports described the losses.

Statistical analysis of body weight, food consumption, and food conversion efficiencies was conducted by "T" tests, and incidence of tumors or non-neoplastic lesions was analyzed with the Chi-square tests.

Reported Results

The authors reported that no compound-related signs of toxicity were observed in treated mice during the study. Signs of a mild infection were noted during weeks 54 through 57 and 64 through 67, and they were described as sneezing, nasal and occular discharges, minor body weight losses, (1 or 2 g) and fluctuations in feed consumption.

Palpuble masses reported in approximately 17% of the animals were associated with swollen lymph nodes in both sexes, mammary glands, and preputial glands in male mice. Reported times of occurrance and distribution of these observations in the four test groups was not dose related according to the authors.

The high dose group was reported to have reduced body weight gains in comparison with that of the control group. The male mice in the group had reduced weight grains during weeks 11 through 30 while the female mice receiving the high concentration showed reduced weight gain during weeks 31 through 56. The group mean body weights for each sex were decreased by less than 10% below the appropriate mean body weights for the control group. No statistically significant differences for this parameter in the females were found according to the authors.

Mortality at week 52 was reported to be 17, 13, 15, and 12% in control, low, mid and high dose groups of male mice, respectively. The reported mortality in female mice from the control, low, mid, and high dose groups at 52 weeks was 12, 17, 12, and 25%, respectively. By week 78, mortality was reported to be 47, 45, 43, and 50% in the control, low, mid, and high dose groups, respectively and 33, 47, and 48% in female mice from the control, low, mid, and high dose groups respectively.

The reported achieved daily intake of the test substance (mg/kg/day) determined from body weight, food consumption, and dietary analysis data ranged from 10.0 to 3.3 mg/kg/day for the low dose group males, and from 10.2 to 3.6 mg/kg/day for females given the 50 ppm diet. Respective ranges for males and females receiving the 250 ppm diet were 49.0 to 15.9 and 51.0 to 17.9 mg/kg/day. The ranges for male and female mice given the 1250 ppm diet were 254.7 to 86.4 and 250.2 to 95.2 mg/kg/day, respectively. It should be noted that the higher intakes were reported during the first weeks of the study, while the lower values were observed late in the study.

The most frequently observed histopathology according to the authors was nephropathy including hydronephrosis and dystrophic mineralization in the kidneys; hepatocytic fatty vaculation, Tyzzer's disease, liver telangiectasis; extramedullary hematopoiesis in the spleen; cataracts ovarian follicular cysts; abscesses and chronic inflamation of preputial gland in males; abscesses, chronic inflamation and ulceration of the skin and subcutis; degeneration of germinal epithelium in the testes; and cystic areas, endometrial glandular hyperplasia, hydrometra, and polyps in the uterus. These findings were described as being consistent with those found normally in aging mice of the same strain (no historical data were reported), and no dose-related incidences were noted.

Neoplastic lesions most frequently observed according to the report included malignant lymphoma, hepatocellular adenoma, alveolar adenoma in the lungs, hardarian gland adenomas, pituitary adenomas (female mice), and lipomas in the skin and subcutis.

The number of tumor bearing animals was determined from individual animal reports is summarized as follows:

Dietary Concentration (ppm)	Tumor bearing animals in each group of 60 examined	
	Males	Females
0	44	49
50	33	37
250	43	39
1250	48	37

The first tumor (malignant lymphoma) was diagnosed in a male mouse receiving the highest dietary concentration. The animal died during the fifth week of feeding. Generally, early tumor diagnoses were made during the 24th through 27th weeks, and the time to diagnosis did not appear to be dose-related according to the authors.

The incidence of hepatocellular adenomas and carcinomas in male mice was the only parameter affected by treatment. The incidence of these tumors (number with tumor/number examined) in animals sacrificed in extremis or at termination of the sutdy is summarized as follows:

Dietary Concentration (ppm)	Time of sacrifice (weeks) 0-52	53-104	Termination	Total
0	1/10	11/46	2/4	14/60
50	0/8	20/46	2/6	22/60
250	0/9	15/45	2/6	17/60
1250	1/7	16/43	6/10	23/60

The first liver tumors were diagnosed during the 45th week of feeding (male from the control group) and week 55 (male receiving the 1250 ppm diet). These tumors first occurred during that time period (weeks 45 through 55) in the low and mid dose groups.

Discussion

Trend analysis of the results for the incidence of hepatocellular adenomas and carcinomas combined in groups of male mice examined during the study (weeks 53 through 104) and all male animals from each group (n = 60) showed no significant trends. However, paired comparisons between a treated group and the control group showed a statistically significant difference between the combined incidence of liver tumors in the low dose and control groups ($P = 0.047$, $X^2 = 3.941$).

A similar analysis of hepatocellular adenomas or carcinomas showed no significant trends, and paired comparisons for adenomas indicated that incidence in the low dose group males was significantly different from control ($X^2 = 4.079$, $P = 0.043$). No significant differences were found when the incidence of hepatocellular carcinomas was considered.

The analyses indicate that the suggested trend which is apparent from the results could be an artifact or a low dose effect. The results are therefore equivocal.

Although no specific historical data were presented to describe the background incidence of tumors in the variety of swiss mouse used, a comparison of the reported tumor data with control data from another variety of swiss mouse (Homburger, et al., 1975) can be conducted. Some example comparisons are

summarized as follows:

Tumor Type	Test Data (%)	Control Data (%) (Homburger et al., 1976)
Tumor bearing animals		
Males	72.5%	54%
Females	67.5%	75%
Hepatocellular tumors		
Males	21.7%	7.1%

It should be noted that the incidences reported in the study described above were generally similar as indicated in the above examples. However, Homburger, et al (1975) reported observed incidences for hepatocellular tumors in male mice which varied from 0 to 17% The highest incidence was reported for one of six control groups. The results for the Metalaxyl study indicate that a generally higher incidence of liver tumors could be characteristic for the mice used.

There are no data reported in the study to indicate the variation likely in control groups from several studies with the variety of swiss mouse used in the Metalaxyl experiment.

This type of data would provide a basis on which to determine whether the 37% incidence noted in the high and low dose groups (hepatocellular tumors in all high and low dose males in the experiment) is within the limits of the background observed for the strain of mouse tested. Such data could provide the basis for determination of the significance of the apparently treatment-related increase in liver tumors observed in the male mice tested.

References

Homburger, F.A., B. Russfield, J.H. Weisburger, S. Lim, S.P. Chak, and E.K. Weisburger. 1975. Aging changes in CD™ 1 HaM/ICR mice reared under standard laboratory conditions. J. Nat. Cancer Inst. 55:37-45.

Core Classification: Miniumum

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