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Subject: Evaluation of Pathologic Data from
Two-Year Feeding Study in Mice
Treated with Benomyl, DuPont Haskell Laboratory Study

OFFICE OF TOXIC SUBSTANCES

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SUMMARY

Male and female mice, 80/group, were fed with Benomyl for two years at dose levels of 0, 500, 1,500, and 5,000 ppm (parts per million). An increased incidence of liver (25/77, 35/80, 52/79 and 27/80) and lung (13/79, 24/80, 23/79 and 16/80) neoplasms were detected in male mice. In female mice, an increased incidence of liver neoplasms (4/77, 9/80, 13/79 and 21/77) were reported by DuPont, Haskell Laboratory. For the submitted data, we are in agreement with the Company's pathologist that oncogenesis was established in the livers of male mice at low and intermediate levels and in female mice livers at all dose levels. There was an increased incidence not only in benign but also in malignant hepatocellular and alveolar cell neoplasms. A proportionally higher incidence in decrease of latency, both in female liver neoplasms (0, 5, 4, 5,) and male lung neoplasms (1, 13, 10, 9) occurred. The presence of malignant tumors, the increased incidence in lung tumors in male mice, the decrease in latency in test animals, and the earlier occurrence of female liver tumors in test animals compared with controls are supporting data for oncogenicity. A compound-related effect on male gonads was observed at the high dose level.

INTRODUCTION

Male and female mice were divided into 8 groups of 80 animals per group. Male (I, III, V, VII) and female (II, IV, VI, VIII) groups were fed with Benomyl at 0, 500, 1,500, 5,000 ppm (the dose level of 5,000 ppm was lowered from 7,500 ppm after 38 weeks of testing), respectively. The animals were on test material for 740 - 744 days and then sacrificed. Gross pathologic observation was made, and all major tissues and organs were collected for histopathologic observation.

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The results are presented in 8 tables. Table I (page 5 - 137) shows the gross findings. Table II (page 139 - 1050) illustrates the individual histopathologic diagnoses. Table III (page 1051 - 1079) describes the major causes of death or moribund and killed mice. Table IV (page 1080 - 1135) summarizes the histopathologic findings in different groups. Table V (page 1142 - 1143) is the summary incidence table of compound-related histopathologic changes. Table VII (page 1144) shows the statistical analysis of hepatocellular neoplasms, and Table VIII (page 1145) describes the times of hepatocellular tumor discovery. The code used in Table II is presented on page 133.

The objective of this report is the review of pathologic data in the report and to make comments about the adequacy of the presented data.

MATERIALS AND METHODS

In the summary incidence table, the distribution of histopathologic changes was observed. Those organs and tissues in which major differences were seen are listed in this report. For comparative reasons, the neoplasms in the livers and lungs were tabulated according to whether the neoplasms were observed before the final sacrifice or at the time of terminal sacrifice. Also the dates of the first observations of liver and lung tumors were also reported. With several organs and tissues, the lesions were counted in the individual histopathologic tabulation and their numbers were compared to the numbers shown in the summary incidence tables. Benign and malignant neoplasms of the same cellular origin were listed separately and also counted together in our tabulation.

In the male control and high dose groups, liver gross and histopathologic findings were compared in 24 randomly selected animals.

To establish oncogenicity the following three criteria were primarily considered:

- 1) Increases in neoplasm incidence.
- 2) Decrease in the latency of tumor appearance.
- 3) Presence of rare tumors.

RESULTS

The submitted pathologic data in the report were presented in a well-organized fashion. The tissues and organs are listed in twelve groups (I - XII) based on body systems (e.g., the endocrine system). Under the individual organs, the diagnoses are listed and the lesions graded. There is good correlation between the individual histopathologic table (II) and summary incidence table (VI). Some of our major findings are illustrated in the following tables:

2

General Information

003726

	Groups							
	I	II	III	IV	V	VI	VII	VIII
	M	F	M	F	M	F	M	F
No. Animals/Group	80	80	80	80	80	80	80	80
Dose	0	0	500	500	1,500	1,500	5,000	5,000
Survival	43	33	36	31	40	23	40	23

There are no significant differences in the survival rates in either male or female groups.

Selected Histopathologic Findings in Male Mice

	Groups			
	I	III	V	VII
<u>SKIN</u>				
Ulcerative Dermatitis (Body & Ear)	6/79	15/80	19/79	18/80
<u>LUNG</u>				
Alveolar Cell Carcinoma	13/79	24/80	23/79	16/80
<u>LIVER</u>				
Adenoma	9/77	9/80	11/79	10/80
Carcinoma	16/77	26/80	41/79	17/80
Total Neoplasms	25/77	35/80	52/79	27/80
<u>TESTES</u>				
Atrophy	12/78	12/79	8/79	31/79
Interstitial Cell Hyperplasia	4/78	4/79	7/79	18/79
<u>EPIDIDYMS</u>				
Depletion Sperm	18/78	11/78	12/79	30/79
Distended Tubuli with Degenerated Sperms	9/78	5/78	11/79	17/79
<u>ADRENAL GLAND</u>				
Focal Cortical Hyperplasia	2/66	6/77	11/78	3/67
<u>THYROID</u>				
Distended Follicles	4/65	13/74	6/73	18/71
<u>HARDERIAN GLAND</u>				
Focal Hyperplasia	3/71	6/66	9/69	6/68

003726

Presence of Lesions Before and At the Time of Terminal Sacrifice

(in Male Mice)

	Groups							
	I		III		V		VII	
<u>LUNG</u>	I* ()#	T**	I	T	I	T	I	T
Alveolar Cell Carcinoma	1(659), 12		13(445), 11		10(380), 13		9(574), 7	
<u>LIVER</u>								
Adenoma	5(530), 4		4(445), 5		6(541), 5		3(627), 7	
Carcinoma	<u>8(545), 8</u>		<u>10(470), 16</u>		<u>14(590), 27</u>		<u>5(508), 12</u>	
Total	13(530), 12		14(445), 21		20(541), 32		8(508), 19	
Neoplasms								

* Lesions before terminal sacrifice.

First day on test when lesion was detected.

**Lesions at the time of terminal sacrifice.

Selected Histopathologic Findings in Female Mice

	<u>Groups</u>			
	II	IV	VI	VIII
<u>SPLEEN</u>				
Myeloid Metaplasia	10/76	22/79	24/78	16/74
<u>LIVER</u>				
Adenoma	2/77	2/80	7/79	7/77
Carcinoma	2/77	7/80	6/79	14/77
Total Neoplasms	4/77	9/80	13/79	21/77

4

Presence of Lesions Before and At the Time of Terminal Sacrifice

(in Female Mice)

	Groups							
	II		IV		IV		VIII	
	I*(#)	T**	I	T	I	T	I	T
<u>LIVER</u>								
Adenoma	0 (), 2		1(641), 1		4(650), 3		2(644), 5	
Carcinoma	0 , 2		4(640), 3		0 (), 6		3(426), 11	
Total	0	4	5	4	4	9	5	16
Neoplasms								

* Lesions before terminal sacrifice.

First day on test when lesion was detected.

** Lesions at the time of terminal sacrifice.

There is a higher incidence of lung and liver neoplasms in male low dose and middle dose groups in comparison with the controls. The increase is more remarkable in the malignant tumors than in the benign. The highest group has tumors, both benign and malignant, in similar incidence as the controls. -We accept the interpretations of the company pathologist in the original report, on page 2, "Significant increase in hepatocellular carcinomas and combined hepatocellular neoplasms (adenoma, carcinoma, and hepatocellular neoplasm -- NOS*) occurred at the low and intermediate treatment level". Also a compound-related effect on gonads was observed at the high dose level. Other than neoplasms and the effect on gonads, the pathologic changes which were found and were compound related are considered less important.

In female mice, there is an increased incidence of liver neoplasms at all dose levels. In addition to the benign neoplasms, the malignant tumor incidence also increased. We agree with the interpretations of these findings by the Company pathologist on page 2, "Significant increases in hepatocellular carcinoma occurred in female mice at the high (5,000 ppm) and low (500 ppm) feeding levels. Significant increases were also shown for combined hepatocellular neoplasms (by one or more statistical tests, Table VII) for intermediate and high dose females." Other than neoplasms, the pathologic changes which were reported and were compound related are considered less important.

When the times of tumor occurrences were checked, proportional decreases in latency were present in lung alveolar carcinoma at all dose levels in male mice and moderate decreases in latency were detected at all dose levels in female liver neoplasms.

There were only minor differences in times of the first observed neoplasms in the male mice. In the female groups, the liver tumors in controls all were detected at the time of terminal sacrifice. On the other hand, in test groups, liver tumors were observed both before and at the time of terminal sacrifice.

*Not otherwise specified.

5

When the gross and histopathologic findings were compared in 12 male controls (6531, 6467, 6515, 6513, 6468, 6516, 6537, 6535, 6538, 6468, 6463, 6520) and in 12 male high-dose animals (6719, 6732, 6773, 6755, 6771, 6752, 6741, 6711, 6723, 6726, 6733, 6735), a good correlation was found between the gross histopathologic observations and histopathologic description of the lesions. 003726

DISCUSSION AND CONCLUSION

When Benomyl was fed to mice in a chronic feeding study, an oncogenic effect was detected both in male and female animals. This effect was compound related in males where the tumor induction was limited to low and medium dose levels. In the female livers, the oncogenic effect was compound and dose related. There is no definite scientific explanation for the lack of oncogenic response in male mice at the high dose level; however, there are other oncogenic compounds too which produce neoplasms in a similar fashion. Other than oncogenic response, the pathologic changes have secondary importance related to the adverse effects of this compound.

Because of the lack of dose-related response in male mice and the presence of increased malignancy and decreased latency in test animals, it can be concluded that Benomyl is a moderately severe oncogenic compound in mice.

6