UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

003727

Date: December 4, 1981

OFFICE OF TOXIC SUBSTANCES

Subject: Evaluation of Pathologic Data from Two-Year Feeding Study in Mice Treated with INE-965, MBC (Benomyl Metabolite)

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No.: 75A
352-354

SUMMARY

Male and female mice, 80/group, were fed with MBC (Benomyl metabolite), for two years at dose levels of 0, 500, 1,500, and 3,705 (in males, it was reduced from 7,500 after 15 months), and 7,500 (females) ppm (parts per million), respectively. An increased incidence of liver neoplasms (13/80, 20/80, 23/80, 3/80) were observed in male mice. In female mice, an increased incidence of liver neoplasms (1/79, 9/78, 20/80, 15/78) was diagnosed by DuPont, Haskell Laboratory. From the submitted data, we are in agreement with the Company's pathologist that a compound-related oncogenesis was established in the male intermediate group; however, we do not consider that a valid conclusion can be made regarding the high-dose group since this group was terminated (at 516 days) before the majority of the tumors developed in the other groups. In the female group, the oncogenic effect was compound and dose related at all dose levels. There was an increased incidence not only in benign but also in malignant tumors in both sexes. Dose-related decreases in latency in the appearance of tumors were present in both sexes.

Regarding oncogenecity, the effect of MBC was comparable to the parent chemical, Benomyl. (Results of the pathologic evaluation of the Benomyl experiment were summarized in a memorandum of 11/18/81 by L. Kasza to J. Kempter).

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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 INE-965, MBC (Benomyl metabolite) at 0, 500, 1,500, 3,750* (male), and 7,500 (female) ppm, respectively. The animals were on test material 516 (Group VII) and 731 - 733 days, then sacrificed. Gross pathologic observation was made, and all major tissues and organs were collected for histopathologic observation. The results are presented in six tables. Table I (pages 5 - 121) shows the gross findings. Table II (pages 122 - 562) illustrates the individual histopathologic diagnoses. Table III (pages 563 - 592) presents the summary incidence table of histopathologic findings by group and sex. Table IV (pages 593 - 597) is the summary incidence table for neoplasms per group and sex. Table V (pages 598 - 600) illustrates the presence and statistical significance of compound-related histopathologic changes. Table VI (page 601) is the summary of statistical analyses of hepatocellular neoplasms. The code used in Table II is presented in a footnote on page 122.

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

MATERIALS AND METHODS

The primary source of this report is the "Summary Incidences of Histopathologic Changes" (Table III). Selected organs and tissues in which major histopathologic changes were seen are listed here and the lesions tabulated. For comparative reasons, the neoplasms in the livers were tabulated according to whether the neoplasms were observed before or at the time of terminal sacrifice. Also the dates of the first observations of liver tumors were reported. With several organs and tissues, the lesions were counted in the individual histopathologic tabulation (Table II) and the accuracy of the numbers was compared to the numbers shown in the summary incidence table (Table III). Benign and malignant liver neoplasms of the same cellular origin were listed separately and also counted together as the basis of establishing oncogenecity.

In female intermediate and high-dose groups, liver gross and histopathologic findings were compared in 24 randomly selected mice.

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* Group VII mice received 7,500 ppm for the first 15 months of the study. Because of compound-related increased mortality, Group VII mice were sacrificed after 17 months on test.

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To establish oncogenecity, the following criteria were primarily considered:

1. Increase in neoplasm incidence;
2. Decrease in latency of neoplasms appearance;
3. Presence of rare tumors;
4. Consideration was given to:
   a. Presence and increase in number of malignant neoplasms;
   b. Only compound relationship;
   c. Compound and dose relationship;
   d. Presence of tumors in difference sexes.

RESULTS

The submitted pathologic data were presented in a well-organized system and in an easy-to-read format. The tissues and organs listed in Table II are in alphabetical order. Under individual tissues and organs, the diagnoses are listed and the lesions graded. There is a good correlation between the data of individual histopathological diagnoses table (II) and the summary incidence table (III). Some of our major findings are illustrated in the following tables:

General Information

	Groups							
	I	II	III	IV	V	VI	VII*	VIII
	M	F	M	F	M	F	M	F
Number of Animals/Group	80	80	80	80	80	80	80	80
Dose (ppm)	0	0	500	500	1,500	1,500	3,750	7,500
Survival (731-733 days)	18	22	14	16	9	14	0	21

The survival rate is rather low, and it is a dose-related decrease in the male groups.

*The dose was reduced from 7,500 ppm after 15 months on test. This group was terminated after 17 months on study (516 days).

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Selected Histopathologic Findings in Male Mice

	Groups			
	I	III	V	VII
KIDNEY				
Tubules contain yellow-brown granular material	7/30	3/79	19/78	47/79
LIVER				
Adenoma	11/80	15/80	14/80	3/80
Carcinoma	2/80	5/80	9/80	0/80
Total Neoplasms	13/80	20/80	23/80	3/80
Hepatocellular Necrosis Focal and Centrolobular	1/80	7/80	10/80	13/80
TESTES				
Sperm Stasis Bilateral, Unilateral	7/77	13/78	16/80	22/74

Presence of Liver Neoplasms Before and At the Time of Terminal Sacrifice in Male Mice

	Groups								
	I		III		V		VII		
LIVER	I* ( )#,	T**	I	T	I	T	I	T	(NA)ç
Adenoma	6(430),	5	10(467),	5	14(459),	0	3(434),		ç
Carcinoma	1(629),	1	2(649),	3	8(616),	1	0 (0),		ç
Total Neoplasms	7(430),	6	12(467),	8	22(459),	1	3(434),		ç

* Lesions before terminal sacrifice.
\# First day on test when lesion was detected.
** Lesions at the time of terminal sacrifice.
ç Not applicable.

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Selected Histopathologic Findings In Female Mice

	Groups			
	II	IV	VI	VIII
KIDNEY				
Macrophages with yellow-brown pigment	5/80	4/78	3/80	21/76
LIVER				
Adenoma	0/79	5/78	5/80	3/78
Carcinoma	1/79	4/78	15/80	12/78
Hepatoblastoma	0/79	0/78	1/80	0/78
Total Neoplasms	1/79	9/78	21/80	15/78
THYMUS				
Lymphoid Depletion	3/38	4/25	12/44	10/38

Presence of Liver Neoplasms Before and At the Time of Terminal Sacrifice in Female Mice

	Groups							
	II		IV		VI		VIII	
LIVER	I* ( )#,	T**	I	T	I	T	I	T
Adenoma	0 (0)	0	4(648),	1	4(636),	1	1(624),	2
Carcinoma	0 (0)	1	3(704),	1	7(536),	8	6(551),	6
Hepatoblastoma	0 (0)	0	0(--),	0	1(704),	0	0(--),	0
Total Neoplasms	0 (0)	1	7(648),	2	12(536),	9	7(551),	8

* Lesions before terminal sacrifice.
\# First day on test when lesion was detected.
** Lesions at the time of terminal sacrifice.
ç Not applicable.

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There is an increased incidence of hepatocellular neoplasms in male mice at low and intermediate dose levels. The tumor incidence at high dose level (3) should be disregarded in comparison since this group was killed at 17 months on test and the majority of tumors in other groups were observed between 18 - 24 months. Considering this fact, it can be concluded that the oncogenic effect in livers of male mice was compound and dose related. The presence of malginant tumors and the decrease in latency of tumor appearance in test groups are supporting data for oncogenecity. There was an increased incidence of hepatocellular necrosis (focal plus centrolobular) in test groups compared with controls.

In comparing the Benomyl and MBC in male mice experiments, the effect of both materials was similar on liver oncogenesis. There were differences: Benomyl affected the male gonads more markedly than MBC, and induced increased incidence in lung tumors. On the other hand, MBC produced marked necrosis in the liver.

Basically, we are in agreement with the Company pathologist's opinion (page 2) regarding the oncogenecity, "In male mice, a significant increase in hepatocellular carcinomas occurred at the intermediate treatement level. A similar increase was observed for the combined incidences of all primary hepatocellular neoplasms in the intermediate dose group. The X^2 Test for Trend was significant ($P < 0.05$) for the combined incidences of primary hepatocellular tumors".

Other than neoplasms and hepatocellular necrosis, the diagnosed compound related lesions have less importance.

In female mice, there is increased incidence of liver neoplasms which is compound and dose related. Similar to the male test group, there was an increased number of malignant tumors and decrease in latency in test groups compared to the controls. We are in agreement with the Company pathologist's opinion (page 2), "Significant increase in hepatocellular carcinomas occurred in female mice at the high (7,500 ppm) and intermediate (1,500 ppm) feeding levels. Significant increases were also shown for hepatocellular adenomas (by one or more statistical tests, Table VI) for all compound-related groups of females. The X^2 Test for Trend (dose-response) was also significant ($P < 0.001$) for hepatocellular neoplasms (hepatocellular adenomas, carcinomas and hepatoblastomas)." The effects of Benomyl and MBC were similar in female mice.

Other than neoplastic changes, the compound-related lesions are less important.

When the gross and histopathologic findings were compared in the livers of 12 female intermediate dose animals (8470, 8445, 8433, 8429, 8428, 8420, 8418, 8425, 8459, 8401, 8463, 8439) and in 12 female high-dose animals (8491, 8495, 8497, 8404, 8507, 8515, 8502, 8523, 8525, 8534, 8553, 8512), a good correlation was found between gross pathologic observation and histopathologic description of the lesions.

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DISCUSSION AND CONCLUSION

When the oncogenic effects of Benomyl and its metabolite, MBC, were compared in different mouse experiements, comparable results were found to be induced by both materials. In both males and females, the incidence of hepatocellular neoplasms increased in test groups. In MBC-treated male animals, there was no increased incidence in lung tumors versus the Benomyl-treated male mice, where alveolar cell carcinomas increased at low and middle dose levels.

The MBC-induced oncogenic effect was compound related in male, and compound and dose related in female mice. The effects in the male high-dose group could not be compared to the other groups since this group was sacrificed at 17 months on test versus 24 months in other groups. The increased incidences in malignant hepatocellular neoplasms and the decrease in latency in appearance of tumors are supporting data for oncogenecity.

The increased incidence of liver necrosis in male mice indicates hepatotoxicity related to the treatement with MBC.

Based on increased incidence of neoplasms both in male and female mice, but only in one organ, and as supporting data, the presence of malignant tumors and the decrease of latency in tumor appearance, it can be concluded that MBC (a Benomyl metabolite) is a moderately severe oncogenic compound in mice.

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