18-MONTH CHRONIC AND CARCINOGENIC INHALATION TOXICOLOGICAL STUDY OF METHANOL IN B6C3F1 MICE: REVIEW OF PATHOLOGY DATA

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18-MONTH CHRONIC AND CARCINOGENIC INHALATION
TOXICOLOGICAL STUDY OF METHANOL IN B6C3F1 MICE
(Test No.: 4A-223)

REVIEW OF PATHOLOGY DATA

1 Executive Summary

At the request of the Methanol Institute, Experimental Pathology Laboratories, Inc. (EPL®), conducted a review of the pathology data included in the final toxicology report. The purpose of this review was to provide an overall assessment of the accuracy, completeness and quality of the study data and interpretations. The review of the pathology data included a review of the individual animal pathology data from the control and high dose animals, review of the summary pathology data, and the pathology results, discussion and conclusion in the narrative portion of the report.

Review of the summary incidence tables indicated a few potential synonymous diagnoses for similar morphologic changes or terminology that was unclear. Except for the liver diagnosis of “neoplastic nodule”, these generally involved diagnoses that were of low incidence. All were considered to have minimal to no affect on the overall interpretation of the histopathologic data.

No obvious potential treatment-related changes were present in male or female B6C3F1 mice. There was a very slight increase in papillary adenoma in the lungs of male mice. These were thought to be unrelated to exposure to methanol gas because they were not accompanied by nonneoplastic proliferative lesions in the lung. Since these neoplasms are generally late occurring, even when induced in B6C3F1 mice, comparison to laboratory historical control results for 18 month carcinogenicity studies in this strain of mouse may support this conclusion.

Although there were several comments noted during this review, most of them were of little significance with respect to the overall interpretation of the histopathologic findings in this study. There was a very high correlation between the gross findings with corresponding histopathological findings noted in the individual animal results. Tissue accountability was generally considered excellent.

2 Introduction

A continuous inhalation test was performed on B6C3F1 mice using systemic exposure method to study 18-month (78-week) carcinogenicity of methanol by the Mitsubishi Chemical Safety Institute, Ltd. The purpose of the study was to investigate the carcinogenicity of long-term low level exposure to methanol gas in mice. The results of this study were included in the final study report dated March 30, 1985.

The test groups consisted of four groups including a control group kept inside an inhalation chamber, and each group contained 52 male and 53 female rats. Exposure levels are 0, 10, 100 and 1000 ppm by volume.

Toxic effects of methanol gas was investigated through observation of general conditions, body weight check, food consumption check, laboratory tests (urinalysis, hematological test, and serum biochemistry test), organ weight check and pathological tests (pathological autopsy and histopathological test). In the final toxicology report, the pathology findings were summarized as follows:

- All groups showed various types of nonneoplastic changes; however, the majority of the changes are nonspecific and naturally occurring changes that are often experienced by 18-month old B6C3F1 mice and no changes that may have been caused by the exposure to methanol gas were observed.
- Various neoplastic changes were observed in all groups; however, all the changes were naturally occurring changes experienced by 18-month old B6C3F1 mice.
- The neoplasms observed in the cases found dead as well as the cases killed *in extremis* were mainly age-associated naturally occurring tumors, and were not considered caused by the exposure to methanol gas.

The conclusion in the final study report stated that: “the result of chronic inhalation toxicological/carcinogenic study on B6C3F1 mice though 18-month consecutive and systemic exposure to methanol gas did not indicate toxicological or carcinogenic effect of exposure to methanol gas at levels lower than 1000 ppm in either male or female mice.”

3 Review of Pathology Data

At the request of the Methanol Institute, Experimental Pathology Laboratories, Inc. (EPL®), conducted a review of the pathology data included in the final toxicology report. The purpose of this review was to provide an overall assessment of the accuracy, completeness and quality of the study data and interpretations. The review of the pathology data included a review of the individual animal pathology data from the control and high-dose animals, review of the summary pathology data, review of histopathological sample photographs, and the pathology results, discussion and conclusion in the narrative portion of the report. During this review the following steps were performed:

- Review of gross observations for the control and high dose male and female mice with comparison to the histologic diagnoses or descriptions to confirm that each gross lesion was considered microscopically.
- The morphologic diagnoses were reviewed to determine that the nomenclature is acceptable and that each protocol-required tissue was addressed in the individual pathology tables.
- Summary tables were examined for acceptable nomenclature (topography or morphology) used to record diagnoses.
- Summary tables were reviewed for tumor rates in controls that are unusually low or high when compared to published historical control rates.
- Identification of all negative and positive trends in the data in the summary tables for consideration as possible test article related findings.
- The narrative portion of the report was reviewed to assure that treatment-related trends and variations in control incidence rates were discussed.

- Review of Histopathological Sample Photographs (Volume 8) for the diagnostic criteria and nomenclature used to characterize the lesion illustrated.

4 Results of the Review

4.1 Review of gross observations

Since complete histopathological examination was performed only on all cases in the control group and the high level exposure group (1000 ppm), a comparison of the gross findings to histopathological findings to assess the degree of correlation was only performed for the control and high dose groups for each sex. Since no changes suspected to be caused by the methanol gas inhalation were identified in the high level exposure group, organs in the low (10 ppm) as well as the medium level exposure groups (100 ppm) were not examined for histopathological findings. During this review only gross findings possibly indicating a neoplasm were considered (i.e., enlargement and nodule). One gross finding indicating a possible neoplasm was noted in the lung of one male mouse in the high exposure group that did not have correlating histopathological findings. Only three were noted in the control and high exposure groups of female mice. These gross findings are listed by group, sex and animal number below:

Group/Sex	Animal Number	Gross Finding	Histopathological Finding
High Male	0339	Lung – Nodule, slight	No Finding Reported
Control Female	1015	Ovaries – Enlargement, unilateral	No Finding Reported
	1039	Lung - Nodule	No Finding Reported
High Female	1329	Uterus - Enlargement	No Finding Reported

There was a very high degree of correlation between the gross findings noted at necropsy and histopathological findings reported during microscopic examination. Gross findings without corresponding histopathological findings noted in two control females

and one high dose female mouse. They did not affect the overall interpretation of the study.

4.2 Review of morphologic diagnoses for acceptable nomenclature

The morphologic diagnoses in the Summary Incidence Tables in Volume 2 were reviewed to determine if the nomenclature was clear and that the terminology was generally recognized as that generally used by toxicologic pathologists evaluating carcinogenicity bioassays in laboratory rodents.

Terminology (Synonymous Terms)

Organ	Histopathological Finding
Lymph Nodes (mesent.)	Lymphocytic Leukemia Malignant Lymphoma (Histiocytic sarcoma) Malignant Lymphoma (Lymphocytic sarcoma)
Spleen	Lymphocytic Leukemia Malignant Lymphoma (Lymphocytic sarcoma) Malignant Lymphoma (Reticulum-cell sarcoma)
Liver	Lymphocytic Leukemia Malignant Lymphoma (Histiocytic sarcoma) Malignant Lymphoma (Lymphocytic sarcoma)
Uterus	Malignant Lymphoma (Histiocytic sarcoma) Malignant Lymphoma (Reticulum-cell sarcoma)

Terminology (Unusual Morphology)

Organ	Histopathological Finding
Kidneys	Angitis
Kidneys	Urine Cast
Kidneys	Atrophy of urinary tubular epithelium
Kidneys	Hyperplasia of mucosal epithelium
Prostate Gland	Hyperplasia of mucosal epithelium
Uterus	Dilatation of duct
Thyroid Glands	Angitis
Liver	Neoplastic nodule
Adrenal Glands	Cortical tumor
Skin	Dermatofibrosarcoma

4.3 Tissue accountability

All protocol-required tissues were required to be examined in on the cases killed on schedule on Week 78 among the control group and the high level exposure group (1000 ppm). Since no changes suspected to be caused by the methanol gas inhalation were identified in the high level exposure group, organs in the low (10 ppm) as well as the medium level exposure groups (100 ppm) were not examined for histopathological findings. The protocol-required tissues were brain, pituitary gland, thyroid gland, heart, lung, bronchi, thymus gland, liver, kidneys, spleen, pancreas, adrenal glands, ovaries, uterus, vagina, testis, epididymis, prostate, vesicular gland, preputial glands, bladder, esophagus, stomach, duodenum, jejunum, ileum, colon, appendix, rectum, lymph nodes (submandibular, hilar, and mesenteric), salivary glands (sublinqual, subaural, and submandibular), perilacrimal gland, eyeballs (including optic nerves), harderian gland, ischiadic nerve, femoral muscles, femurs, bone marrow (femur), nasal cavities, laryngopharynx, spinal cords, thoracic aorta, skin, mammary gland, and other locations of visible lesion.

Tissue accountability can only be assessed in the control and high dose groups where all protocol-required tissues were required to be examined. Additionally, tissue accountability can be assessed in the low and medium dose groups for the liver in both sexes since it was examined for all animals in all groups. The overall tissue accountability was considered to be excellent with only a few protocol-tissues not examined microscopically. With the exception of the tissues listed below, 100% tissue accountability was present for all protocol required tissues in all the control and high dose groups for both sexes:

	Male (N=52 per Group)		**Female (N=53 per Group)**	
Tissue	**Control**	**High Dose**	**Control**	**High Dose**
Thymus	52	50	52	53
Lymph Nodes (Submaxi)	52	52	52	53
Lymph Nodes (Pulmo. Hilus)	49	45	51	49
Lymph Nodes (Mesent.)	52	51	51	53
Spleen	52	52	52	53

	Male (N=52 per Group)		Female (N=53 per Group)	
Tissue	**Control**	**High Dose**	**Control**	**High Dose**
Bone Marrow	52	52	52	53
Nasal Cavity	52	52	52	53
Laryngopharynx	50	52	51	52
Trachea	50	52	52	53
Stomach	52	52	52	53
Ileum	51	52	52	53
Colon	51	52	52	53
Submaxillary Glands	52	52	52	53
Sublingual Glands	52	52	52	53
Parotid Glands	51	52	52	53
Extraorbital Lacrimal Glands	51	52	52	53
Pancreas	52	52	52	53
Urinary Bladder	52	52	52	52
Epididymis	51	52	--	--
Preputial Gland	50	51	--	--
Ovaries	--	--	52	53
Vagina	--	--	52	52
Pituitary Gland	51	49	51	51
Thyroid Glands	49	51	51	52
Adrenal Glands	52	52	52	53
Bone (Femur)	52	52	52	53
Muscle (Q. femoris)	52	52	52	53
Skin	52	52	52	53
Mammary Gland	52	52	52	53
Eye Balls	52	52	52	53
Harderian Glands	52	52	50	51
Spinal Cord	52	52	52	53

4.4 Increasing and decreasing trends in the incidence of hyperplastic and neoplastic histopathological findings

Sex	Male				Female			
Dose (ppm)	0	10	100	1000	0	10	100	1000
No. Necropsied	52	52	52	52	53	52	53	53
Organ Findings								
Lung (No. Examined)	(52)	(3)	(3)	(52)	(53)	(0)	(5)	(53)
Adenomatosis	0	0	0	0	0	0	0	1
Papillary Adenoma	4	0	0	7	3	0	0	2

The only tumor incidence that was increased in the 1000 ppm dose group as compared to the 0 ppm dose group was papillary adenoma in the lung of male mice. All lung tumors in both sexes were observed in animals sacrificed following 18 months of treatment. In view of the lung changes reported in male mice, the incidence of lung neoplasms in male mice should be considered. Additionally, this study was terminated following only 18 months of exposure which is six months shorter that carcinogenesis bioassay studies routinely conducted by the National Toxicology Program in this same strain of mouse. Laboratory historical control data from this time interval should be compared to the current study results to help interpret the slight increase in lung tumors in the 1000 ppm dose group. The lack of adenomatosis in the control or treated male mice would help support the conclusion that the tumors observed in this study are likely not related to exposure to methanol gas.

4.5 Data with uncertain morphologic criteria and nomenclature for neoplastic diagnoses

Sex	Male				Female			
Dose (PPM)	0	10	100	1000	0	10	100	1000
No. Necropsied	52	52	52	52	53	52	53	53
Organ Findings								
Liver (No. Examined)	(52)	(52)	(52)	(52)	(53)	(52)	(53)	(53)
Hepatocellular Adenoma	3	2	2	4	1	1	1	4
Hepatocellular Carcinoma	2	4	0	1	3	0	3	2
Neoplastic Nodule	16	13	16	20	1	0	0	1

Although there is no apparent relationship to treatment involving any of the neoplastic findings in the liver, the use of the morphologic diagnosis of adenoma (presumably benign), carcinoma (presumably malignant) and neoplastic nodule (uncertain classification) has never been typically used to characterize neoplasms in the liver of mice. Therefore, it is not possible to know exactly what the nature of the neoplastic nodules are and whether they should be considered benign liver neoplasms or malignant liver neoplasms.

This study was conducted before pathology peer review was commonly performed on carcinogenicity studies. The only way to be able to determine the exact nature of the lesion diagnosed as neoplastic nodule would be reexamination of the tissue sections of liver. This would allow the lesions to be evaluated using recommended diagnostic criteria and nomenclature that has been published by the Society of Toxicologic Pathology and the International Agency on Research on Cancer (WHO). Unfortunately, the slides from the study are no longer available for examination.

4.6 Review of histopathological sample photographs

In volume 8 of the final study report, 64 sample photographs were included to illustrate some of the findings reported in the study. The purpose of the review of the sample photographs was to determine if appropriate diagnostic criteria and nomenclature were used to characterize the lesion illustrated in the photograph. The photograph details were listed in a table at the beginning of volume 8 on pages numbered (i) to (iii).

The majority of the photographs consisted of a single hematoxylin and eosin stained image of each lesion taken at 100X magnification. This magnification was sufficient to confirm most of the non-neoplastic changes being illustrated, but was too high of a magnification to confirm the specific diagnosis for a many of the neoplastic lesions being illustrated. Although photographs of the neoplasms may illustrate the lesion, at this high magnification it is not possible to assess the diagnostic criteria necessary to confirm the diagnosis. These include the following diagnoses:

Photo No.	Organ	Finding
40	Spleen	Hemangioendothelioma
41	Lung	Papillary Adenoma
42	Liver	Neoplastic Nodule
43	Liver	Hepatocellular Adenoma
45	Liver	Hemangioendothelioma
46	Epididymis	Fibroma
47	Scrotum	Lipoma
48	Ovaries	Granulosa Cell Tumor
49	Uterus	Endometrial Stromal Tumor
50	Uterus	Hemangioendothelioma
51	Vagina	Leiomyoma
52	Pituitary Gland	Chromophobe Adenoma

Photo No.	Organ	Finding
53	Thyroid Gland	Follicular Adenocarcinoma
54	Parathyroid Glands	Adenoma
55	Adrenal Glands	Cortical Tumor
56	Adrenal Glands	Pheochromocytoma
57	Skin	Sebaceous Adenoma
58	Skin	Dermatofibrosarcoma
60	Brain	Meningeal Sarcoma
61	Bone Marrow	Lymphocytic Leukemia
62	Lymph Nodes (Mesenteric)	Malignant Lymphoma (Histiocytic Sarcoma)
63	Spleen	Malignant Lymphoma (Lymphocytic Sarcoma)
64	Spleen	Malignant Lymphoma (Reticuloendothelial Sarcoma)

5 Conclusions

Although there were several comments noted during this review, most of them were of little significance with respect to the overall interpretation of the histopathologic findings in this study. There were very few gross findings without corresponding histopathological findings noted in the individual animal results.

There were a few potential diagnoses that were possible synonyms or duplications in terminology for the same morphologic changes. A few findings had morphologic diagnoses that were unusual or did not distinguish between benign and malignant tumors. Most of these involved findings of low incidence and even if combined would have no effect on interpretation of the incidence data.

In this study, the systemic neoplasms that affect multiple organs (i.e., malignant lymphoma and leukemia) was recorded under the tissue that it was observed in and was never tabulated as a systemic neoplasm with the number of animals affected rather than the incidence in separate organs. For these types of neoplasms, it is generally thought that the number of animals affected is the most appropriate way to assess a potential treatment-related affect. In this study the incidence of these neoplasms in quite low in all dose groups. This low incidence may be explained by the fact that this is a neoplasm that occurs late in the normal life span of the B6C3F1 mouse. The incidence is much

lower that reported in carcinogenicity bioassays conducted by the National Toxicology Program, where the studies are not terminated until 24 months on test.

When the incidence lymphoma and leukemia are tabulated by animal rather than by organ, there is no apparent difference between the incidence in the control and high dose groups for either sex. The incidence of Lymphocytic Leukemia and Malignant Lymphoma tabulated by number of animals affected are presented in the table below:

	Males				**Females**			
Level (ppm)	**0**	**10**	**100**	**1000**	**0**	**10**	**100**	**1000**
No. of Animals Necropsied	52	52	52	52	53	52	53	53
Lymphocytic Leukemia	0	0	1	0	1	0	0	0
Malignant Lymphoma (Reticulum-Cell Sarcoma)	1	0	0	0	1	0	1	3
Malignant Lymphoma (Lymphocytic Sarcoma)	0	1	0	1	6	0	1	4
Malignant Lymphoma (Histiocytic Sarcoma)	0	0	0	0	2	1	1	0

The neoplastic findings reported for the liver are difficult to interpret because of the unusual terminology used to classify the hepatocellular neoplasms. The Summary Incidence Tables report hepatocellular adenoma, hepatocellular carcinoma, and neoplastic nodule. The morphologic term “neoplastic nodule” has not been typically used by toxicologic pathologists to diagnosis hepatocellular neoplasms in the liver of mice so it is difficult to determine exactly the nature of the neoplasm being diagnosed. The only way to resolve this diagnostic question is to review the liver slides and apply recommended terminology to classify the hepatocellular tumors. Regardless of the nature of the lesion there is no apparent relationship of any of the tumors reported for the liver to exposure to methanol gas.

Tissue accountability was very high for all tissues.

Although the review of the Summary Incidence Tables confirmed that there were no obvious treatment-related proliferative changes that may be considered potentially related to exposure to the inhalation of methanol gas for up to 18-months, a slight increase in the incidence of papillary adenoma was present in the lungs of male mice. These neoplasms were all observed at the terminal sacrifice (18 months) and were not accompanied by proliferative nonneoplastic lesions (adenomatosis). This would suggest that the slight increase is not likely related to exposure to methanol gas. Comparison to historical incidence of lung neoplasms from the testing laboratory in carcinogenicity studies conducted in B6C3F1 mice for 18 months would be helpful in arriving at a definitive conclusion, especially in light of the findings observed in the lungs of male rats. Since these are generally considered to be late occurring tumors in the lung of mice, comparison of the tumor incidence from studies conducted by the National Toxicology Program in B6C3F1 mice would not be useful since they do not terminate studies until 24 months of exposure.

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