Summary Report of the National Toxicology Program and Environmental Protection Agency-Sponsored Review of Pathology Materials from Selected Ramazzini Institute Rodent Cancer Bioassays

November 29, 2011

1. EXECUTIVE SUMMARY

A preliminary National Toxicology Program (NTP) review of pathology data was conducted for the Ramazzini Institute (RI) chronic rat bioassay for methanol in the spring of 2010. The review identified some differences in pathology diagnoses of lymphoid neoplasms and inner ear and cranium neoplasms. Based on these preliminary findings, the NTP and US Environmental Protection Agency (EPA) proposed that five RI chronic rodent cancer studies be subjected to an independent pathology review. The five studies selected were methanol, methyl-*t*-butyl-ether (MTBE), ethyl-*t*-butyl-ether (ETBE), acrylonitrile, and vinyl chloride (VC).

A pathology review, based on NTP procedures including a pathology data review (PDR), quality assessment (QA), and Pathology Working Group (PWG), was conducted on selected treatment-related findings from the five studies. In general, there was good agreement between the RI study diagnosis (SD) and PWG opinions for neoplasms in the acrylonitrile, VC, and ETBE studies. The PWG and SD differed on some diagnoses in the methanol and MTBE studies. The PWG diagnosed fewer lymphoid neoplasms, mainly of the respiratory tract, and fewer neoplasms of the inner ear and cranium. In both of these studies, there was chronic inflammation of the nasal cavity, ear canal, trachea, and lung, indicating infection by one or more respiratory pathogens. Many of the malignant neoplasms and the lymphoid dysplasias diagnosed by the RI were considered by the PWG as cases of hyperplasia related to chronic infection. The PWG did agree with some cases of unusual findings in the rat such as primary lung lymphoma, squamous cell carcinoma of the inner ear, and osteosarcoma, fibrosarcoma, or sarcoma of the cranium. It is not unusual in the setting of chronic inflammation that regenerative or lymphocytic proliferations take on some neoplastic features. In conclusion, it was the opinion of the PWG that fewer malignant neoplasms occurred in the methanol and MTBE studies than were diagnosed by the RI.

2. BACKGROUND

In late April 2010, a team of three pathologists and two technicians representing the NTP visited the RI in Bentivoglio, Italy to carry out a review of RI pathology procedures and selected pathology data including histopathology diagnoses. The review was performed under a general agreement between the RI and the National Institute of Environmental Health Sciences (NIEHS) related to the two institutes' shared interest in harmonization of methods for performance and reporting of rodent cancer bioassays.

The purpose of the visit was to conduct a limited assessment of pathology materials from the RI methanol study, as suggested by the RI. The review entailed an audit of pathology specimens (APS) and QA review of selected rat tissues in the RI methanol study but did not constitute a complete NTP pathology QA review or NTP PWG evaluation. The findings were submitted as a report (Appendix), considered preliminary, and intended as a basis for recommendations, rather than as final conclusions about any possible or reported effect of the chemical under study.

During the April 2010 visit, the RI was found to be a well-organized, clean facility including animal rooms, necropsy room, histology laboratory, and archives. The RI staff appeared to apply meticulous detail to the necropsy and to the recording, collecting, and archiving of materials and tissues from their studies. The same methods have been consistently applied over the decades, thus providing an ability to compare studies over time. The audit of data and pathology specimens revealed only minor discrepancies at a frequency typical for laboratories carrying out studies of this type. The histopathologic diagnoses by the NTP pathologists for a number of lesions in the methanol study differed from those of the RI. In general, the NTP pathologists diagnosed fewer neoplasms and more inflammatory lesions. The NTP pathologists also reviewed histologic diagnoses in a set of mouse and rat neoplasms and selected tissues from a more recent chronic study and generally agreed with the RI diagnoses. In the two chronic studies reviewed, including the methanol study, there was evidence of chronic airway (nasal, tracheal, and lung) and inner ear inflammation in many animals examined.

Recommendations for follow-up evaluations were prompted by the initial pathology review. In NTP studies, pathology diagnoses go through a several-step process of verification, including independent pathology peer reviews, namely a QA review (which includes a pathology data review or PDR, an audit of pathology specimens or APS, and QA of histopathology), and then a PWG to resolve discrepancies, achieve consensus, and confirm histopathology of treatment-related findings. Differences between a study pathologist's and a reviewing pathologist's diagnoses are not uncommon occurrences and at the NTP are typically resolved through the formal pathology peer-review process. It was the recommendation of the three visiting NTP pathologists that a more formal and rigorous pathology peer review should be performed on the methanol and some other selected RI studies. Also, it was recommended that going forward the RI consider applying an equivalent pathology review process to all studies.

3. OBJECTIVES

Conduct independent pathology peer reviews based on NTP procedures, including a QA review and PWG of reported treatment-related findings for five rodent chronic bioassays (Table 1) conducted at the RI.

Table 1. RI Chronic Bioassay Studies Selected for Pathology Peer Review

Chemical	RI Study Number	Year Study Started
Methyl-*t*-butyl-ether	BT 958	1988
Methanol	BT 960	1990
Ethyl-*t*-butyl ether	BT 959	1993
Vinyl chloride	BT 10	1974
Acrylonitrile	BT 201	1975

The RI accepted these recommendations, and plans were made to carry out a formal, NTP-based pathology peer review on the selected RI studies. These studies were of mutual interest to the RI, the NTP, and the EPA. The interests were based on the nature of the lesions originally reported by the RI, the differences in some lesion diagnoses noted by the NTP pathology review team in its partial review of the RI methanol study, and the potential for utilization of the findings from these studies in risk assessments carried out by the EPA.

4. GENERAL OVERVIEW OF METHODS

On February 7-18, 2011, a QA review team from Experimental Pathology Laboratories, Inc. (EPL), under contract to the NTP, visited the RI for the purpose of reviewing the pathology materials for the desired lesions to enable the design of the subsequent PWG. On April 4-8, 2011, a PWG was convened at the RI to assess the quality of the pathology data, address any discrepancies, and confirm the diagnoses from the selected studies. The PWG was coordinated by Dr. Robert Maronpot, EPL. Following the PWG, reports on the studies were compiled, reviewed by NTP pathologists, and used to prepare this summary report.

PWG Panel[1]	
PWG Coordinator	Robert R. Maronpot, DVM, MPH (EPL, Durham, NC)
QA Pathologists	Jerry F. Hardisty, DVM (EPL, Durham, NC)
	Peter C. Mann, DVM (EPL, Durham, NC)
PWG Members	Charles Clifford, DVM, PhD (Charles River Labs)
	Sabine Francke-Carroll, DVM, PhD (U.S. Food and Drug Administration)
	Peter Greaves, MBChB (Leicester University, United Kingdom)
	James B. Nold, DVM, PhD (Biotechnics, Hillsborough, NC)
Outside Observer	Steven Mog, DVM (U.S. Food and Drug Administration)
RI Observers[2]	Fiorella Belpoggi, PhD (RI, Bentivoglio, Italy)
	Morando Soffritti, MD (RI, Bentivoglio, Italy)
	Eva Tibaldi, PhD (RI, Bentivoglio, Italy)
	Laura Falcioni, DVM (RI, Bentivoglio, Italy)

[1] Curricula vitae for the PWG Panel are on file at the NIEHS

[2] Did not participate in the discussions

5. FINDINGS

a. Methyl-*t*-butyl ether (MTBE)

The MTBE study was carried out at the RI by gavage administration of the chemical in olive oil. The study started in 1988. Groups of 60 male and 60 female Sprague-Dawley rats received olive oil containing 0, 250, or 1000 mg/kg body wt per day. Results of the study and more complete materials and methods have been reported by the RI (see references below).

The pathology peer review consisted of analysis of the RI data and QA review of selected tissues and organs. The tumor types of interest were hemolymphoreticular tumors in male and female rats and testicular tumors in male rats. The QA and PWG reviews focused on the following:

- Thymus, liver, lung, spleen, and/or lymph nodes in all rats for hemolymphoreticular tumors
- Nose, ear, and lung for inflammation in all rats
- Testes from all male rats in all groups

All original study pathologist's (RI) or QA pathologist's diagnoses of lymphoma or leukemia and proliferative lesions in the testes were included in the set of slides examined by the PWG.

All materials necessary for this study review and all others were made readily available by the RI and were well organized. All slides requested by the QA pathologist were present. The histologic quality of the sections for all studies was considered to be very good by the QA pathologist with no deficiencies that interfered with the examination of the tissues present or the interpretation of histopathologic changes.

During the QA review, some terminology issues were noted by the QA pathologists that were not consistent with currently accepted nomenclature and diagnostic criteria. The issues identified and applied in the review were as follows:

- Lymphoreticular neoplasms diagnosed or confirmed during the QA review were categorized as either malignant lymphoma or mononuclear cell leukemia according to NTP practices and current literature recommendations.
- Dysplasia in lymph nodes diagnosed by the RI pathologist was considered to be normal or lymphoid hyperplasia by the QA and PWG pathologists.
- Testicular atrophy was diagnosed as testicular degeneration by the QA pathologist. Both are indicative of a similar process and may or may not be mutually exclusive processes.

The results of the pathology QA review generally confirmed the RI pathologist's findings for proliferative lesions of the testes. Some of the original diagnoses of lymphoma or leukemia were not confirmed by the QA pathologist. The PWG reviewed 179 slides representing 74 rats without knowledge of treatment group, sex, or previous diagnoses by the study pathologist or the QA pathologist. Following examination of a small number of specific tissues from 10 to 20 rats, the PWG Coordinator recorded consensus diagnoses for the relevant tissues for each rat. The consensus diagnosis represented the majority opinion of the PWG participants. Occasional differences of opinion among the PWG members were discussed and, if a consensus diagnosis was not initially achieved, the relevant slides were re-examined by each member followed by further discussion until a consensus diagnosis was achieved. The most significant lesions of concern involved diagnoses of inflammatory lesions in the airways, testicular interstitial cell hyperplasia and adenoma, and malignant lymphoma and/or mononuclear cell leukemia.

Inflammatory lesions in the lung consisted of infiltration by lymphocytes, plasma cells, and other mononuclear cells (i.e. macrophages) associated with the presence of neutrophils and accumulation of necrotic cellular debris in dilated airways (bronchiectasis). Inflammation in the nose consisted of neutrophils, mononuclear cells, necrotic cellular debris in the mucosa, and abundant exudate in the nasal air spaces. Acute and chronic cellular infiltrates accompanied by fibroplasia and hyperostosis of the surrounding soft tissue were characterized as inflammation of the middle and inner ear. The incidences of inflammation (including abscesses) in the lung, nose, and ear reported by the RI pathologist, QA pathologist, or PWG findings are summarized in Table 2.

Table 2. Summary Incidences of Inflammation of the Lung, Nose, and Ear of Male and Female Rats in the MTBE Study

	0 mg/kg		250 mg/kg		1000 mg/kg	
MALES						
Number of Animals per Group	60	60	60	60	60	60
	SD	PWG	SD	PWG	SD	PWG
Lung Inflammation	44	45	44	50	44	48
Nose Inflammation	10	34	10	31	13	30
Ear Inflammation	14	19	20	22	16	17
FEMALES						
Number of Animals per Group	60	60	59	60	60	60
	SD	PWG	SD	PWG	SD	PWG
Lung Inflammation	57	58	46	52	46	56
Nose Inflammation	13	34	13	24	14	28
Ear Inflammation	15	20	12	17	15	17

SD = RI study diagnosis, PWG = PWG Panel consensus

Diagnostic criteria applied for the diagnosis of testicular interstitial cell hyperplasia and adenoma were as described in Pathology of the Fischer Rat (Ed. Boorman *et al*., 1990. Academic Press, New York). The PWG examined slides from 31 male rats with a RI

pathologist and/or QA pathologist diagnosis of interstitial cell hyperplasia or adenoma. During tabulation of the PWG consensus for interstitial cell hyperplasia and adenoma, unilateral and bilateral lesions were combined. Results of the PWG consensus diagnoses for testicular lesions are shown in Table 3.

Table 3. Testicular Interstitial Cell Adenoma (Unilateral and Bilateral Combined) and Interstitial Cell Hyperplasia (Unilateral and Bilateral Combined) in the MTBE Study

	0 mg/kg		250 mg/kg		1000 mg/kg	
Number of Animals per Group	60		60		60	
	SD	PWG	SD	PWG	SD	PWG
Adenoma, Interstitial Cell	3	3	5	5	11	11
Hyperplasia, Interstitial Cell	4	3	7	4	8	3

SD = RI study diagnosis, PWG = PWG Panel consensus

Diagnostic criteria for malignant lymphoma and mononuclear cell leukemia used by the PWG followed internationally accepted guidelines (International Classification of Rodent Tumours. Part I: The Rat. 4. Hematopoietic System. IARC Scientific Publication No. 122. 1993; http://goreni.org/). The diagnostic criteria for malignant lymphoma included:

- Disruption of architecture of involved organs by neoplastic lymphocytes
- Aggressive growth with focally extensive or diffuse infiltration of affected organs
- Uniform infiltrating lymphocytes and non-cohesive; some larger lymphocytes may be present
- Densely clumped nuclear chromatin

To be consistent with NTP guidelines, lymphoid neoplasms were classified as malignant lymphomas and were not sub-classified. Evidence of leukemic infiltration and cell morphology characteristic of large granular lymphocytic leukemia was diagnosed as mononuclear cell leukemia. There were rare instances of myelogenous leukemia. A diagnosis of malignant lymphoma was typically based upon examination of lung in addition to other tissues such as thymus, spleen, liver, and lymph nodes.

The PWG rendered opinions on the presence or absence of malignant lymphoma from all rats with a study diagnosis of lymphoma/leukemia by the RI pathologist and/or the QA pathologist. The diagnostic terminology agreed upon by the PWG for positive cases was malignant lymphoma or mononuclear cell leukemia. Diagnosis of malignant lymphoma in the lungs was usually confirmed by finding involvement in other tissues such as thymus, liver, spleen, and/or lymph nodes. There was agreement for a diagnosis of malignant lymphoma confined just to the lungs by the RI, QA, and PWG for one control male and one high-dose female. Results for combined malignant lymphoma and leukemia are presented in Table 4.

Table 4. Summary Incidences of Malignant Lymphoma[1] or Leukemia[2] in Male and Female Rats in the MTBE Study

	0 mg/kg		250 mg/kg		1000 mg/kg	
MALES						
Number of Animals per Group	60	60	60	60	60	60
	SD	PWG	SD	PWG	SD	PWG
Lymphoma/Leukemia	8	8	7	1	6	3
FEMALES						
Number of Animals per Group	60	60	59	60	60	60
	SD	PWG	SD	PWG	SD	PWG
Lymphoma/Leukemia	2	0	7	1	12	4

SD = RI study diagnosis, PWG = PWG Panel consensus

[1] "Lymphoma" includes lymphoblastic lymphoma, lymphocytic lymphoma, lymphoimmunoblastic lymphoma for SD diagnoses. For QA pathologist and PWG, all lymphoma sub types were diagnosed as malignant lymphoma.

[2] "Leukemia" includes lymphoblastic leukemia and myeloid leukemia for SD diagnoses, and for QA pathologist and PWG, includes myeloid leukemia and mononuclear cell leukemia.

References

Belpoggi, F., Soffritti, M., Filippini, F., and Maltoni, C. (1997). Results of long-term experimental studies on the carcinogenicity of methyl tert-butyl ether. Ann N Y Acad Sci **837**, 77-95.

Belpoggi, F., Soffritti, M., and Maltoni, C. (1995). Methyl-tertiary-butyl ether (MTBE)--a gasoline additive--causes testicular and lymphohaematopoietic cancers in rats. Toxicol Ind Health **11**, 119-149.

Belpoggi, F., Soffritti, M., and Maltoni, C. (1998). Pathological characterization of testicular tumours and lymphomas-leukemias, and of their precursors observed in Sprague-Dawley rats exposed to methyl-tertiary-butyl ether (MTBE). Eur J Oncol **3**: 201-206.

Belpoggi, F., Soffritti, M., and Maltoni, C. (1999). Immunoblastic lymphomas in Sprague-Dawley rats following exposure to the gasoline oxygenated additives methyl-tertiary-butyl ether (MTBE) and ethyl-tertiary-butyl ether (ETBE): Early observations on their natural history. Eur J Oncol **4**: 563-572.

b. Methanol

The methanol study was conducted by the RI as a dosed-water study. The study started in 1990. Groups of 100 male and 100 female rats received drinking water containing 0, 500, 5000, or 20,000 ppm methanol for their lifetime. Experimental details and study findings have been published (see reference below).

The QA review included examination of the following tissues from all male and female rats in all groups. (These lesions and tissues were identified as tissues of interest during the initial pathology review by 3 NTP pathologists in April 2010).

- Thymus, liver, lung, spleen and lymph nodes for malignant lymphoma
- Lung for proliferative and inflammatory lesions
- Nose for inflammation
- Ear (inner) for neoplastic changes and inflammation
- Zymbal's gland for neoplasia and inflammation
- Cranium (near inner ear) for osteoma and osteosarcoma

The PWG Panel examined a total of 744 slides from 367 rats in the methanol study. The PWG examined slides from all animals with a previous diagnosis of lymphoma or leukemia reported by the RI pathologist or QA pathologist, all animals with a neoplasm diagnosed by the RI pathologist in the ear or bone (cranium) and six control and six high dose males without a previous diagnosis of lymphoma or leukemia by the SD or QA pathologist. Results for combined malignant lymphoma and leukemia are presented in Table 5.

Table 5. Summary Incidences of Malignant Lymphoma[1] or Leukemia[2] in Male and Female Rats in the Methanol Study

	0 ppm		500 ppm		5000 ppm		20000 ppm	
MALES								
Number of Animals per Group	100		100		100		98-100	
	SD	PWG	SD	PWG	SD	SD	SD	PWG
Lung Only	7	0	10	0	13	1	13	0
Multiple Tissues	19	13	21	11	22	12	26	8
Total Rats with Lymphoma/Leukemia	26	13	31	11	35	13	39	8
FEMALES								
Number of Animals per Group	100		100		100		99-100	
	SD	PWG	SD	PWG	SD	PWG	SD	PWG
Lung Only	3	3	5	0	6	0	7	0
Multiple Tissues	9	5	16	4	16	7	18	6
Total Rats with Lymphoma/Leukemia	12	8	21	4	22	7	25	6

SD = RI study diagnosis, PWG = PWG Panel consensus

[1] "Lymphoma" includes lymphoblastic lymphoma, lymphocytic lymphoma, lymphoimmunoblastic lymphoma for SD diagnoses. For QA pathologist and PWG, all lymphoma sub types were diagnosed as malignant lymphoma.

[2] "Leukemia" includes lymphoblastic leukemia and myeloid leukemia for SD diagnoses and for QA pathologist and PWG, includes myeloid leukemia and mononuclear cell leukemia.

The PWG diagnosed fewer cases of lymphoma or leukemia than did the SD (Table 5). Diagnosis of malignant lymphoma in the lungs was usually associated with involvement

in other tissues such as thymus, liver, spleen, and/or lymph nodes. Malignant lymphoma was confined only to the lungs for one male exposed to 5000 ppm methanol and in three females in the 0 ppm group.

The PWG examined slides with neoplasms diagnosed by the RI study pathologist or QA pathologist involving the inner ear, bone (cranium), and Zymbal's gland from all rats in all groups. The incidences of neoplastic lesions reported by the RI study pathologist or the PWG are summarized in Table 6a (males) and Table 6b (females).

The PWG diagnosed fewer squamous cell carcinomas of the inner ear in both treated and untreated male and female rats. Squamous cell hyperplasia was observed in many rats and was considered related to chronic otitis interna. A smaller number of osteosarcomas of the cranium were confirmed by the PWG in male and female treated and control rats; however, a few lesions originally called osteosarcoma by RI were diagnosed as sarcoma or fibrosarcoma. There was general agreement regarding neoplasms of the Zymbal's glands.

The PWG diagnosed inflammation of the airway (nose, trachea, and lung) more frequently than RI, including in control rats. The findings suggest that the male and female rats in this lifetime drinking water study had a respiratory infection.

Table 6a. Summary Incidences of Neoplasms of Inner Ear, Bone (Cranium), and Zymbal's Gland in Males in the Methanol Study

	0 ppm		500 ppm		5000 ppm		20000 ppm	
Number of Animals per Group	100		100		100		99	
	SD	PWG	SD	PWG	SD	PWG	SD	PWG
EAR								
Papilloma	0	0	0	0	0	0	0	1
Squamous Cell Carcinoma	9	5	12	6	16	2	24	7
Carcinoma	0	0	1	0	1	1	0	0
Sarcoma	0	1	0	0	0	1	0	0
Lymphoma, Malignant	0	1	0	0	0	1	0	0
Lymphoma, Lymphoblastic	0	0	0	0	1	0	0	0
Leukemia, Mononuclear Cell	0	1	0	0	0	2	0	0
Leukemia, Myelogenous	1	0	0	0	0	0	0	0
BONE, CRANIUM								
Sarcoma	0	3	0	1	0	3	0	1
Osteoma	0	0	0	0	1	0	0	0
Osteosarcoma	6	0	6	1	13	2	11	3
Fibrosarcoma	0	1	0	1	0	1	0	1
Squamous Cell Carcinoma	0	0	0	0	0	0	0	1
ZYMBAL's GLAND								
Squamous Cell Carcinoma	1	1	2	1	3	3	3	1
Leukemia, Mononuclear Cell	0	1	0	1	0	1	0	0
Leukemia, Myelogenous	1	0	1	0	1	0	0	0

SD = RI study diagnosis, PWG = PWG Panel consensus

Table 6b. Summary Incidences of Neoplasms of Ear, Bone (Cranium), and Zymbal's Gland in Females in the Methanol Study

	0 mg/kg		500 ppm		5000 ppm		20000 ppm	
Number of Animals per Group	100		100		100		99-100	
	SD	PWG	SD	PWG	SD	PWG	SD	PWG
EAR								
Squamous Cell Carcinoma	9	3	8	6	16	6	19	7
Fibrosarcoma	0	0	0	0	0	0	0	1
BONE, CRANIUM								
Sarcoma	0	1	0	0	0	0	0	0
Osteoma	0	0	0	0	0	0	1	0
Osteosarcoma	1	0	4	0	3	0	5	1
Fibrosarcoma	0	0	0	1	0	1	0	2
Squamous Cell Carcinoma	0	0	0	1	0	0	0	1
ZYMBAL'S GLAND								
Adenoma	0	0	0	0	0	0	2	1
Squamous Cell Carcinoma	1	1	4	1	3	2	4	4

SD = RI study diagnosis, PWG = PWG Panel consensus

The incidence of inflammation (including abscesses) in the lung, nose, and ear reported by either the RI study pathologist (SD), QA pathologist, or the PWG is summarized in Table 7.

Table 7. Summary Incidences of Inflammation in the Lung, Nose, and Ear of Male and Female Rats in the Methanol Study

	0 ppm		500 ppm		5000 ppm		20000 ppm	
MALES								
Number of Animals per Group	100		100		100		98-100	
	SD	PWG	SD	PWG	SD	PWG	SD	PWG
Lung	54	91	48	84	47	84	32	89
Ear	70	95	71	94	61	92	60	94
Nose	37	95	25	90	16	80	24	84
FEMALES								
Number of Animals per Group	100		100		100		99-100	
	SD	PWG	SD	PWG	SD	PWG	SD	PWG
Lung	69	85	60	84	49	83	52	83
Ear	79	99	80	100	72	95	57	84
Nose	26	96	26	99	9	64	8	42

SD = RI study diagnosis, PWG = PWG Panel consensus

Reference

Soffritti, M. et al., (2002). Results of long-term experimental studies on the carcinogenicity of methyl alcohol and ethyl alcohol in rats. Ann. NY Acad. Sci. 982: 46-69.

c. Ethyl-*t*-butyl ether (ETBE)

The ETBE study was conducted by the RI by gavage administration. The study started in 1993. Groups of 60 male and 60 female Sprague-Dawley rats received olive oil containing 0, 0.25, or 1 g ETBE/kg body weight/day. Experimental details and study findings have been published (see references below).

The QA review included examination of the following:

- All sections of the oral cavity from all male and female rats for hyperplastic and neoplastic changes
- All sections of uterus and vagina for hyperplastic and neoplastic lesions for all female rats

The PWG initially examined 36 slides from 12 rats across dose groups to determine the spectrum of proliferative changes in the oral cavity. These tissues were examined and the changes were discussed without voting on any diagnoses. The PWG then examined a total of 139 slides from 80 rats in the ETBE study without knowledge of treatment group, sex (except for uterus & vagina slides), or previous diagnoses by the RI or QA pathologists. The PWG examined slides from the following:

- All rats with a previous diagnosis of proliferative lesions including dysplasias, hyperplasias, and benign and malignant neoplasms affecting the oral mucosa and/or tongue.
- All proliferative, metaplastic, and neoplastic lesions in the uterus and vagina when there was a disagreement in diagnosis between the RI and the QA pathologist.

During the QA review, the original terminology used for oral cavity lesions was noted by the QA pathologist not to be consistent with NTP nomenclature. Specifically, RI diagnoses of dysplasia in the oral cavity were diagnosed by the QA pathologist as minimal squamous hyperplasia and RI diagnoses of proliferation in the oral cavity were diagnosed as minimal hyperplasia or considered within normal limits by the QA pathologist. The QA review consisted of oral cavity and tongue for identification of non-neoplastic and neoplastic proliferative lesions from all male and female rats in all groups. Uterus and vagina from all females in all groups were examined to document the presence of proliferative non-neoplastic and neoplastic lesions. The QA pathologist agreed with all study pathologist diagnoses of inflammation, atrophy, cysts, fibrosis, and ulceration in the oral cavity. Small areas of reactive hyperplasia of periodontal epithelium associated with inflammation were seen adjacent to broken teeth in some rats. Mixed inflammatory cell infiltrates present in the uterine and vaginal mucosa were confirmed by the QA pathologist.

The lesions of concern for this study involved diagnoses of epithelial tumors in the oral cavity and epithelial and mesenchymal neoplasms in the uterus and vagina. Diagnostic criteria for oral cavity and uterine/vaginal lesions used by the PWG followed internationally accepted guidelines (International Classification of Rodent Tumours. Part I: The Rat. 9. Female Genital System. IARC Scientific Publication No. 122. 1997; International Classification of Rodent Tumours. Part I: The Rat. 10. Digestive System. IARC Scientific Publication No. 122.1997; and Pathology of the Fischer Rat, Edited by Boorman et al., 1990. Academic Press, New York).

Oral cavity and uterine/vaginal lesion incidences are summarized in Tables 8 and 9 respectively.

Table 8. Incidence of Lesions in the Oral Mucosa/Tongue in the ETBE Study

	0 g/kg/day		0.25 g/kg/day		1.0 g/kg/day	
MALES						
Number of Animals per Group	60		60		60	
	SD	PWG	SD	PWG	SD	PWG
Hyperplasia, Squamous	0	14	0	28	0	18
Papilloma	1	0	0	0	2	0
Squamous Cell Carcinoma	0	0	0	0	1	0
FEMALES						
Number of Animals per Group	60		60		60	
	SD	PWG	SD	PWG	SD	PWG
Hyperplasia, Squamous	0	9	0	29	0	28
Papilloma	1	0	1	0	1	0
Squamous Cell Carcinoma	0	0	0	0	1	0

SD = RI study diagnosis, PWG = PWG Panel consensus

Table 9: Proliferative Lesions in the Uterus/Vagina in the ETBE Study

	0 g/kg/day		0.25 g/kg/day		1.0 g/kg/day	
Number of Animals per Group	60		60		60	
	SD	PWG	SD	PWG	SD	PWG
Metaplasia, Squamous	3	3	3	6	4	5
Stromal Polyp	21	21	11	12	14	14
Carcinoma	1	0	1	1	0	0
Leiomyoma	0	0	0	0	2	3
Leiomyosarcoma	1	1	2	0	0	0
Schwannoma, Malignant	0	0	6	7	2	2
Histiocytic Sarcoma	1	1	0	0	1	1

SD = RI study diagnosis, PWG = Panel consensus

References

Belpoggi, F., Soffritti, M., and Maltoni, C. (1999). Immunoblastic lymphomas in Sprague-

Dawley rats following exposure to the gasoline oxygenated additives methyl-tertiary-butyl ether (MTBE) and ethyl-tertiary-butyl ether (ETBE): Early observations on their natural history. Eur J Oncol **4**, 563-572.

Maltoni, C., Belpoggi, F., Soffritti, M., and Minardi, F. (1999). Comprehensive long-term experimental project of carcinogenicity bioassays on gasoline oxygenated additives: Plan and first report of results from the study on ethyl-tertiary butyl ether (ETBE). Eur J Oncol **4**, 493-508.

d. Vinyl chloride (VC)

The VC study was conducted by the RI by inhalation administration. The study started in 1974. One-day old Sprague-Dawley rats were exposed to VC by inhalation for 4 hours per day, 5 days per week, for a total of 5 weeks and then held without further treatment for their lifetime. Controls were comprised of groups of 109 males and 120 females; groups of 18 males and 25 females were exposed to 6000 ppm, and groups of 25 males and 21 females to 10,000 ppm. Results of VC study findings have been published previously (see references below).

The QA review resulted in several instances of changes in diagnostic nomenclature. Since the time this study was started in 1974, nomenclature for hepatoproliferative lesions has changed. RI study pathologist's liver diagnoses of neoplastic nodules and nodular hyperplasia are either no longer used in the case of neoplastic nodule or only rarely used in the case of nodular hyperplasia. What was diagnosed as neoplastic nodule is now considered either focus of cellular alteration or hepatocellular adenoma. The terms hepatocellular adenoma and carcinoma were recorded in the original study (Table 10). Contemporary use of nodular hyperplasia is uncommon and such lesions are typically diagnosed as foci of cellular alteration or regenerative hyperplasia when there is evidence of antecedent or contemporary hepatotoxicity. Furthermore, liver tumors consisting of hepatocellular and cholangiocellular elements are now diagnosed as hepatocholangiomas or hepatocholangiocarcinomas based on changes in nomenclature occurring since this study was completed.

The QA review consisted of examination of all livers for the presence of preneoplastic and neoplastic lesions. When different preneoplastic and neoplastic lesions were present in the same liver, each was diagnosed during the QA review. The PWG examined 144 liver sections from 82 rats. The primary focus during the PWG review was to confirm the presence of preneoplastic and neoplastic liver lesions for rats where there was a difference in diagnosis between the study and QA pathologist. Diagnostic criteria for proliferative liver lesions used during the QA and PWG reviews are provided in the references cited below.

The PWG found that the liver lesions were complex often with multiple distinct and merging tumors in the same liver and in the same tissue section. The hepatocellular

carcinomas tended to have a more glandular component than what is typically seen. When the glandular pattern was sufficiently prominent, the PWG preferred a diagnosis of hepatocholangiocarcinoma, or more rarely hepatocholangioma. There were a number of borderline lesions, particularly involving the lesions ranging from eosinophilic focus of cellular alteration to hepatocellular adenoma to hepatocellular carcinoma. Basophilic foci were hard to identify since they were not tinctorially distinct. Consensus diagnoses for each type of lesion were recorded by the PWG Coordinator. The neoplastic lesions are presented in Tables 10 and 11 for males and females, respectively. Although there are minor differences in the numbers of various types of liver neoplasms, the conclusions remain the same; that is, that VC caused an increase in benign and malignant hepatocellular, biliary, and endothelial (hemangioma and hemangiosarcoma) neoplasms.

Table 10. Liver Tumors in Male Sprague-Dawley Rats Exposed by Inhalation to VC for a Total of Five Weeks (Four Hours Per Day, Five Days per Week)

	0 ppm		6000 ppm		10000 ppm	
Number of Animals per Group	104		18		25	
	SD	PWG	SD	PWG	SD	PWG
Liver Lesion						
Hepatocellular Adenoma	0	0	4	2	7	3
Hepatocellular Carcinoma	0	0	5	8	6	11
Hepatocellular Adenoma and Carcinoma	0	0	0	0	0	1
Hepatocellular Adenoma or Carcinoma	0	0	9	10	13	13
Cholangioma	0	0	9	1	9	2
Cholangiocarcinoma	0	0	0	0	1	1
Cholangioma and Cholangiocarcinoma	0	0	0	0	0	1
Cholangioma or Cholangiocarcinoma	0	0	9	1	10	2
Hepatocholangioma	0	0	0	0	0	1
Hepatocholangiocarcinoma	0	0	0	3	0	5
Hemangioma	0	0	1	1	0	0
Hemangiosarcoma	0	0	5	4	6	4
Hemangioma or Hemangiosarcoma	0	0	6	5	6	4
Neoplastic Nodule	0	0	2	0	7	0

SD = RI study diagnosis, PWG = PWG Panel consensus

Table 11. Liver Tumors in Female Sprague-Dawley Rats Exposed by Inhalation to VC for a Total of Five Weeks (Four Hours Per Day, Five Days per Week)

	0 ppm		6000 ppm		10000 ppm	
Number of Animals per Group	117		25		20	
	SD	PWG	SD	PWG	SD	PWG
Liver Lesion						
Hepatocellular Adenoma	0	0	5	5	2	5
Hepatocellular Carcinoma	0	0	6	7	5	9
Hepatocellular Adenoma and Carcinoma	0	0	0	3	0	1
Hepatocellular Adenoma or Carcinoma	0	0	11	9	7	13
Cholangioma	0	0	7	2	3	0
Cholangiocarcinoma	0	0	1	0	2	2
Hepatocholangioma	0	0	0	1	0	0
Hepatocholangiocarcinoma	0	0	0	3	0	5
Hemangioma	0	0	0	0	0	1
Hemangiosarcoma	0	0	12	7	9	7
Hemangioma or Hemangiosarcoma	0	0	12	7	9	8
Neoplastic Nodule	0	0	4	0	4	0

SD = RI study diagnosis, PWG = PWG Panel consensus

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e. Acrylonitrile

Twelve-week old Sprague-Dawley rats were exposed to acrylonitrile by inhalation for 4 hours per day, 5 days per week, for a total of 52 weeks and then held without further treatment for their lifetime. The numbers of animals used and their allocation to dose groups are shown in Table 12. This study began in 1975. The results of the acrylonitrile studies have been published previously by the RI (see references below).

Table 12 – Study Design for Bioassay on Acrylonitrile Administered by Inhalation to Male and Female Sprague-Dawley Rats

Group No.	Concentration	Number of Animals and Sex
I	40 ppm	30 Males and 30 Females
II	20 ppm	30 Males and 30 Females
III	10 ppm	30 Males and 30 Females
IV	5 ppm	30 Males and 30 Females
V	0 ppm	30 Males and 30 Females

The peer review consisted of analysis of proliferative and neoplastic lesions of (1) brain/central nervous system, (2) extrahepatic angiomatous lesions, and (3) Zymbal's gland, liver, and mammary gland in exposed and control rats. The QA review included examination of all sections for hyperplastic and neoplastic changes from all male and female rats in all groups.

All slides with differences of opinion relating to the presence or absence of neoplastic lesions in the requested tissues between the SD and QA pathologist were identified for review by the PWG. The number of slides examined during the QA review of the acrylonitrile study is listed in Table 13.

Table 13. Number of Slides Reviewed during the QA Review

	Males	Females
Group	Slide Count	Slide Count
40 ppm	127	47
20 ppm	141	56
10 ppm	120	59
5 ppm	109	51
0 ppm	113	43
TOTAL	610	256

The PWG examined 73 tissue slides from 63 rats.

During the QA review, the original terminology for neoplastic lesions in the central nervous system, subcutis, Zymbal's gland, mammary gland, and liver were made consistent with current NTP nomenclature and practice. Diagnostic criteria for brain, Zymbal's gland, mammary gland, liver, and non-hepatic angiomatous lesions used by the PWG followed internationally accepted guidelines (see references below).

The PWG Panel noted that there was good diagnostic agreement between the QA and study pathologist for mammary gland neoplasms. The incidence of neoplastic lesions in male and female Sprague-Dawley rats is provided in Table 14.

Table 14. Neoplastic Lesions in Male and Female Sprague-Dawley Rats Exposed to Acrylonitrile by Inhalation for a Total of 52 Weeks

	0 ppm		5 ppm		10 ppm		20 ppm		40 ppm	
Number Animals per Group	30		30		30		30		30	
	SD	PWG	SD	PWG	SD	PWG	SD	PWG	SD	PWG
Mammary Gland – Males										
Fibroadenoma	0	1	0	0	0	1	0	2	0	2
Adenocarcinoma	1	0	0	0	0	0	0	0	1	1
Sarcoma	0	0	0	0	0	0	3	3	2	2
Fibroma*	0	0	0	0	1	0	2	0	3	1
Mammary Gland – Females										
Fibroadenoma	0	5	0	6	0	7	0	9	0	5
Adenocarcinoma	1	1	3	1	0	0	0	0	3	1
Sarcoma	0	0	1	1	0	0	1	1	2	2
Fibroma*	5	0	5	0	7	0	9	1	5	0
Squamous Cell Carcinoma	0	0	0	1	0	0	0	0	0	0
Brain – Males										
Microglioma	0	0	0	0	0	0	0	1	0	1
Oligodendroglioma	0	0	0	0	0	1	0	0	2	1
Brain – Females										
Malignant Reticulosis	0	0	0	0	0	0	0	1	0	1
Zymbal's Gland – Males										
Carcinoma	0	0	0	0	2	2	0	0	0	0
Zymbal's Gland – Females										
Carcinoma	0	0	0	0	1	0	1	1	0	0
Extrahepatic Vascular Tissue – Males										

	0 ppm		5 ppm		10 ppm		20 ppm		40 ppm	
Number Animals per Group	30		30		30		30		30	
	SD	PWG	SD	PWG	SD	PWG	SD	PWG	SD	PWG
Hemangioma**	0	1	0	0	0	0	2	2	0	0
Hemangiosarcoma	1	0	0	0	1	1	0	0	0	0
Extrahepatic Vascular Tissue – Females										
Hemangioma**	1	1	0	0	1	1	1	0	0	0
Hemangiosarcoma	0	0	0	0	0	0	0	1	0	0

SD = RI study diagnosis, PWG = PWG Panel consensus
*SD diagnosed fibroma and fibroadenoma as one type without distinction. Tabulated here as fibroma only for convenience. PWG classified them per NTP criteria.
** The RI pathologist considered some of these as synonymous to fibroangioma, a benign epithelial neoplasm with abundant collagen.

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6. SUMMARY/CONCLUSIONS

A preliminary NTP review of pathology data was conducted for the RI chronic rat bioassay for methanol in the spring of 2010. The review identified some differences in pathology diagnoses of lymphoid neoplasms and inner ear and cranial neoplasms. Based on these preliminary findings, the NTP and EPA proposed that five RI chronic rodent cancer studies be subjected to an independent pathology review. The five studies selected were methanol, MTBE, ETBE, acrylonitrile, and VC.

A pathology review, based on NTP procedures including pathology data review, QA, and PWG, was conducted on selected treatment-related findings in the five chronic rodent cancer studies carried out by RI. In general, there was good agreement between the RI diagnosis and PWG opinions with some minor issues in terminology for neoplasms in the acrylonitrile, VC, and ETBE studies. A number of tissues with discrepant diagnoses were

found in the methanol and MTBE studies and these were primarily of lymphoid neoplasms, mainly of the respiratory tract, and neoplasms of the inner ear and cranium. In both of these studies, there was chronic inflammation of the nasal cavity, ear canal, trachea, and lung indicating infection by one or more respiratory pathogens. Chronic airway inflammation, probably due to *Mycoplasma pulmonis* and perhaps other pathogens, and otitis media may have led to many of the differences of opinion concerning diagnoses of malignant lymphoma, squamous cell carcinoma, and/or osteosarcoma. The higher numbers of neoplasms diagnosed by the RI were considered by the PWG as cases of marked to severe hyperplasia (lymphoid related to chronic lung infection) and/or regenerative hyperplasia (squamous cells of the inner ear or bone remodeling and osteoid formation also related to chronic infection). It is not unusual in the setting of chronic inflammation that regenerative or lymphocytic proliferations take on some neoplastic features.

The PWG did agree with some cases of unusual findings in the rat such as primary lung lymphoma, squamous cell carcinoma of the inner ear, and osteosarcoma, fibrosarcoma, or sarcoma of the cranium. Despite terminology changes for liver neoplasms over the decades, there was good agreement between the SD and QA/PWG in the VC study, including for vascular neoplasms. In conclusion, while overall the frequency and nature of the differences in diagnostic opinions between the PWG and the RI SD were not considered unusual for studies of this type, it was the opinion of the PWG that fewer neoplasms than were originally diagnosed by the RI occurred in the methanol and MTBE studies.

7. COMMENTS AND ACKNOWLEDGEMENTS

Reviews of this type provide the prevailing opinions of the toxicologic pathology community concerning the lesions seen in a given study, and may reflect an evolution in thinking about the pathogenesis of a neoplastic or nonneoplastic disease process. The NTP appreciates the gracious and accommodating cooperation of the RI during the performance of these pathology reviews, recognizes that the RI may not agree with the PWG conclusions, and respects the rights of the RI in that regard.

The NTP appreciates the efforts and contributions of Drs. Maronpot, Hardisty, Mann, the PWG participants, and the EPL staff to this project.

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Appendix

NATIONAL TOXICOLOGY PROGRAM
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DATE: June 11, 2010

TO: John R. Bucher, Ph.D., DABT, Associate Director, National Toxicology Program

FROM:
NTP TEAM Dr. David Malarkey, Head, NTP Pathology Group; Dr. Ron Herbert, Head, NTP Pathology Support; Dr. Abraham Nyska, NTP Contractor Pathologist; Ms. Mary Ellen Sutphin, NTP Contractor Technical Staff; Ms. Kim Pernicka, NTP Contractor Technical Staff

SUBJECT: Report on visit (4/25/2010 - 4/30/2010) and assessment of the pathology procedures performed at the Ramazzini Institute (RI), Bentivoglio, Italy

OBJECTIVE: Conduct a limited assessment of the RI pathology procedures and histopathology for rodent chronic bioassays. This partial review focuses on the RI methanol study, but does not constitute an NTP Pathology Quality Assessment Review or an NTP Pathology Working Group (PWG). The findings are preliminary and intended as a basis for recommendations and are not intended to reach conclusions about any possible or reported effect of the chemical under study.

FINDINGS:

1. General:

The NTP team arrived at the RI on Monday, April 26, 2010, and was given a tour of the facilities including animal rooms, necropsy, histology laboratory, and archives. The RI recently received certification by the Organization for Economic Cooperation and Development (OECD), to conduct Good Laboratory Practice (GLP) studies. During our tour, we noted very organized and clean facilities. The animal housing did not appear to be a barrier facility and there were no ongoing studies. The necropsy area was fully equipped with four stations, instruments, and necessary necropsy records and guidance documents. Ethanol was the fixative of choice except for skulls, which are routinely fixed in 10% neutral buffered formalin prior to decalcification. The histology lab was orderly, clean, free from any solvent fumes, and had up-to-date automated processors (5) and microtomes. Technical staff wore lab coats and protective gloves. In general, the archives were very well organized and complete, and it was easy to retrieve documents or specimens from past studies. The wet tissues awaiting trimming, blocks, slides, and records were kept in secure areas, were extremely well organized and were easily retrievable. A fire detection and suppression system was in place for most of the archives, with further coverage planned for the near future.

Overall, the RI staff appears to apply meticulous detail to the necropsy, recording, collecting, and archiving of materials/tissues from their studies. The same methods have been consistently applied over the decades to provide an ability to compare studies over time. The standard operating procedures (SOPs), GLP documents, and necropsy records were within GLP expectations. Each animal had a necropsy record for gross observations, tissues collected, and histopathology findings.

The RI staff provided the following materials for review:

a. Original and revised SOPs from 1980-2010

b. GLP-related documents (from a recent GLP study) including protocols, lab reports, organizational chart, personnel information, and representative curriculum vitae

c. Documents (necropsy records, protocols, and publications), slides, and blocks for male high-dose group (n=100) and controls (n=100) from a past chronic study (methyl alcohol/methanol study)

d. Documents (necropsy records), slides, and blocks from control male and female mice and rats from a more recent study (n=40, 10 from each sex/species)

e. Histopathology sample set with a collection of about 150 H&E slides of various rat and mouse neoplasms from various past RI studies sorted by organ system and species

2. Assessment of pathology data from methyl alcohol study:

Partial audit of pathology data. The NTP team conducted an audit of in-life, necropsy, and pathology data on a sampling of 20% of the control and high-dose male Sprague-Dawley (SD) rats from the methyl alcohol study. For the audit of in-life data, the TDMSE report #EISRT12 (Animal History Observations or E12) and the original data logs were reviewed. The gross and microscopic entries were compared to the observations listed in the TDMSE summary report of individual animal findings (PEIRPT14 or P14). Minor discrepancies were identified, but believed not to affect the outcome of the study. The slide-block inventory, evaluation for slide quality, and data entry found minimal discrepancies or flaws.

Histopathology review. The three NTP pathologists reviewed the histologic sections of lung, liver, spleen, lymph nodes, thymus, ear canal, Zymbal's gland, and cranium from high dose (n=100) and control (n=100) male SD rats in the methyl alcohol study. For each animal the following were reviewed: 1) lymphoma, leukemia, or dysplasia in the thymus, lung, liver, spleen, and lymph nodes; 2) inflammation in the lung, nose, ear, and Zymbal's gland; 3) carcinoma, squamous cell carcinoma, or dysplasia of the ear canal or Zymbal's gland; and 4) osteoma or osteosarcoma of the cranium. Lymphomas of any subtype were combined with leukemias of any subtype, for the purpose of comparing lymphohematopoietic malignancies

identified by the RI and NTP pathologists. The NTP pathologists did not evaluate the consistency in RI diagnoses within or between studies.

Initially, the NTP pathologists each reviewed sections from the same 10 control and 10 high dose male rats before meeting together to discuss their findings. After discussion of the findings and achieving consensus for diagnostic criteria, each NTP pathologist reviewed histologic sections from 60 rats (30 controls and 30 high dose) for a total of 200 rats (100 control and 100 high-dose). Pathologic diagnoses from the NTP pathologists were compared with original findings of the RI.

Lung. The NTP pathologists were in general agreement with the occurrence of inflammation of the lung, but diagnosed it more frequently than was recorded in the original evaluation by the RI. The NTP pathologists preferred to diagnose the findings as "lung inflammation, chronic" whereas the RI usually recorded it as "lung, bronchus, inflammation." The lesions were generally characterized by chronic, moderate to severe, bronchopneumonia with marked lymphoplasmacytic infiltrates and proliferation (peribronchial and peribronchiolar), bronchiectasis, consolidation, and multifocal suppurative inflammation.

The NTP pathologists occasionally diagnosed "leukemia" or "lymphoma" of the lung, but at a lower frequency than the original findings. The NTP pathologists considered that the diagnosis of leukemia or lymphoma was sometimes difficult to distinguish from the intense, marked lymphocytic infiltrates related to the chronic inflammation of the lung. The leukemia and lymphoma cases for which there was consensus with the RI diagnosis were generally those in which monomorphic sheets of malignant cells effaced lung parenchyma and neoplastic infiltrates involved multiple organs.

Nose. The NTP pathologists diagnosed "inflammation, suppurative" in the nose of almost all animals. The inflammation was characterized by chronic, moderate to severe, suppurative rhinitis with turbinate atrophy and exudate in the nasal cavity. Inflammation within the nose was diagnosed at a lower incidence by the RI.

Inner ear. The NTP pathologists generally agreed with the occurrence and frequency of inflammation in the inner ear as reported in the original evaluation, but diagnosed about half of the squamous cell carcinomas reported in the original findings. The inflammation was characterized by chronic, severe, suppurative otitis interna with occasional associated osteomyelitis, bone destruction, and bone sequestra. Rafts of hyperplastic squamous cells were often admixed amongst the inflammation.

Cranium. The NTP pathologists diagnosed fewer osteoscarcomas than were reported by the RI. Many of the previously diagnosed osteoscarcomas were considered to be reactive tissue by the NTP pathologists.

Lymph node. The NTP pathologists diagnosed fewer "leukemias" or "lymphomas." In some animals advanced autolysis of lymph nodes precluded the ability to make a histologic diagnosis.

Dysplasia. The NTP pathologists did not apply this term during their review for several organs; such lesions are generally considered hyperplastic lesions that may or may not have been part of an immunologic response.

Liver, thymus, and spleen. There were occasional discrepancies between the NTP pathologists' diagnoses and the original findings; however, the differences did not appear to significantly affect the overall incidences of lymphomas and leukemias in these organs.

Zymbal's glands. No significant discrepancies were identified.

3. Assessment of pathology data from a more recent chronic study:

The NTP pathologists reviewed slides from 40 control male and female mice and rats (10 animals per sex and species) from a more recent study. They used slide review worksheets provided by RI staff that listed all neoplasms for selected individual animals. Animals were selected that presented a variety of neoplasms in the study. In addition to reviewing neoplasms, the NTP pathologists commented on noteworthy non-neoplastic changes. There was general agreement with RI diagnoses for most neoplasms, including malignant lymphomas. Lymphoma was not reported in the lung among these 40 animals. Chronic inflammation was noted in the nose, larynx, trachea, lung, and ear canal of many mice and rats, and a number of cutaneous ectoparasites (mites) were observed.

4. Assessment of RI training collection of sample rodent neoplasms:

The NTP pathologists reviewed about half of the RI slide set of rodent neoplasms commonly observed in their studies. There was general agreement with almost all of the diagnoses provided by RI staff.

5. Summary:

The OECD-certified RI is a well-organized, clean facility including animal rooms, necropsy room, histology laboratory and archives. The audit of data and pathology specimens revealed only minor discrepancies at a frequency typical for laboratories carrying out studies of this type. The histopathologic diagnoses by the NTP pathologists for a number of lesions in the methyl alcohol study differed from those of the RI. In general, the NTP pathologists diagnosed fewer neoplasms and more inflammatory lesions in the rats from the methyl alcohol study. The NTP pathologists generally agreed with the RI diagnoses reviewed from the more recent chronic study and the training collection of sample rodent neoplasms. In both chronic studies reviewed, there was a high incidence of chronic airway (nasal, tracheal,

and lung) and inner ear inflammation. There was also a high degree of autolysis in some tissues that occasionally precluded histological diagnosis by the NTP pathologists.

The pathology review conducted by the NTP pathologists was a partial review, and the findings are not sufficient to support or refute the overall conclusions of the studies as reported by the RI.

6. Recommendations:

In NTP studies, pathology diagnoses go through a several-step process of verification, including an independent pathology review and a quality review of the pathology data and specimens. Discrepancies between a study pathologist and a reviewing pathologist are typically resolved through use of a formal pathology peer review to verify the accuracy of the pathology data. It is recommended that the RI carry out an equivalent process to address the discrepancies identified in the methyl alcohol study.

The presence of inflammatory lesions in several tissues is consistent with chronic infection, which we understand is commonly observed in aging rats in the lifespan studies carried out by the RI. In addition, the practice of allowing animals to die spontaneously can lead to significant autolysis of some tissues. It is recommended that the RI take steps to minimize these factors in the conduct of future studies.