FYI-1200-1397 MRH 42194

A

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

December 11, 2000

Document Control Office (7407)
Attn: TSCA Section FYI
East Tower Room G99
Office of Pollution Prevention & Toxics
401 M St. SW
Washington, DC 20460-0001

Contain NO CBI

RECEIVED
OPPT CBIC
2000 DEC 18 AM 10: 49

Dear Document Control Office:

In compliance with the National Toxicology Program's (NTP) mission to keep our colleagues informed of the current NTP findings during ongoing studies, a copy of the Pathology Working Group (PWG) report and the Summary Pathology Tables for the chronic Dosed Feed; Gavage; Inhalation study on O-NITROTOLUENE (88-72-2), ACRYLONITRILE (107-13-1), VANADIUM PENTOXIDE (1314-62-1) are enclosed for your review.

The NTP assembles a Pathology Working Group to review every study and to resolve any differences between the study laboratory and quality assessment pathology evaluations. Please note that the PWG conclusion of the study results is based solely on the pathology for this study and may not reflect final NTP conclusions. In determining final conclusions, the NTP assesses a broad array of information that includes other results from this study and historical control data.

The Summary Pathology Tables contain the Incidence Rates of Neoplastic and Non-neoplastic Lesion data and the Statistical Analysis of Primary Tumors data pertaining to the laboratory animals. All study data are subject to an NTP retrospective audit and the interpretation may be modified based on the findings.

A wide variety of NTP information is also available in electronic format on the world-wide web, for example, the NTP Annual Plan, abstracts of NTP Reports, study data, and the status of all NTP studies. To view this information requires access to the internet and a Web browser such as Netscape Navigator or Internet Explorer. To access the NTP home page, use the URL http://ntp-server.niehs.nih.gov/. Comments on the usefulness of this site and suggestions for improvement are encouraged.

Please contact Central Data Management (CDM) at (919)541-3419 if you have any questions. You may also fax your requests for information to CDM at (919)541-3687 or send them via e-mail to cdm@niehs.nih.gov.

2000 DEC 26 AM 10: 44

04

Hard copies of documents such as NTP Technical Reports, short-term Toxicity Reports, and the Report on Carcinogens are available from the Environmental Health Information Service (EHIS). You can contact EHIS by phone at (919) 541-3841, by fax at (919)541-0273, or by e-mail at ehis@niehs.nih.gov.

Sincerely,

William Eastin

William Eastin, Ph.D.
Head, Information Systems & Central Files
Environmental Toxicology Program

Encls: PWG Report and Pathology Summary Tables for Mice & Rats; Mice; Mice & Rats
cc: Central Data Management

RECEIVED

05

88-72-2
PWG CHR
C62340D

CHAIRPERSON'S REPORT
PATHOLOGY WORKING GROUP (PWG) REVIEW
o-NITROTOLUENE IN F344/N RATS
(C62340D/05203-08) A 14-WEEK INTERIM SACRIFICE AND TWO-YEAR CORE AND STOP STUDIES ADMINISTERED BY DOSED FEED AT SOUTHERN RESEARCH INSTITUTE

RECEIVED
OPPT CBIC
2000 DEC 18 AM 10:

DATE OF PWG: August 31, 2000

LOCATION OF PWG: NIEHS, Research Triangle Park, NC

PARTICIPANTS:

Drs. R. Hailey (NIEHS), R. Herbert (NIEHS), R. Sills (NIEHS), A. Nyska (NIEHS), G. Flake (NIEHS), C. Shackelford (EPL-Reviewing Pathologist), J. Everitt (CIIT), J. Heath (Souther Research Institute-Study Pathologist), T. Suwa (NIEHS-observer) and P. Hildebrandt (PATHCO-PWG Chairperson).

SUMMARY AND CONCLUSION

The feeding of o-nitrotoluene to rats for 14-weeks resulted in numerous non-neoplstic lesions. The feeding of o-nitrotoluene to rats for up to two years resulted in numerous non-neoplastic and neoplastic lesions.

A. 14-Week Interim Sacrifice (male rats)

1. No neoplasms were diagnosed in the 14-week interim sacrifice study.

2. Many non-neoplastic lesions were observed in the 5000 ppm dose group. These changes consisted of:

   a. Germinal epithelial degeneration/atrophy and mineralization of the testes

   b. Cellular atypia and atrophy (hypospermia) of luminal contents of the epididymis These were secondary changes due to the testicular lesions.

   c. Cytoplasmic vacuolization in the liver

   d. Hyaline droplet formation in tubular epithelial cell of the kidney

   e. Olfactory epithelial degeneration in the nose

   f. Hyperplasia of pancreatic islets

   g. Preputial and salivary gland atrophy

   h. Hematopoietic cell proliferation and congestion of the spleen

RECEIVED
OPPT NCIC
2000 DEC 26 AM 10:14

These lesions were all significantly increased in the 5000 ppm dose group. The salivary gland atrophy was also significantly increased in the 2000 ppm.

B. Two-Year Core Study and Special Studies (male stop dose groups)

1. Numerous neoplastic lesions occurred in both sexes

a. Mesotheliomas of the peritoneum occurred at a very high incidence in male rats. The mesotheliomas occurred in all dose groups in a dose related fashion.

b. Skin, subcutaneous tissue

The incidence of fibromas, fibrosarcomas, and lipomas noted grossly as nodules and large masses on the torso, head, and appendages were greatly increased in a dose related fashion in treated rats. In most treated rats there were multiple such lesions.

c. Mammary gland

Treated rats of both sexes had a marked increase in mammary tumors. The majority of these were fibroadenomas and were multiple in many females.

d. There was an increase in liver tumors in all dose groups of males and in high dose females. Several liver tumors in males had biliary components.

e. Pulmonary tumors were increased in the high dose of both sexes but only considered significant in high dose males.

2. Non-neoplastic lesions

The same non-neoplastic lesions in the 14-week study were present in the two-year studies with the following exceptions.

a. Olfactory degeneration of the nose was not seen in the two-year studies. This is believed to be the result of healing and "adaption" of the epithelium to the insult.

b. The F344 rat has a very high incidence of interstitial cell tumors in the testes. These tumors, with associated degeneration/atrophy in older rats, made it difficult to detect a compound effect.

c. The hematopoietic cell proliferation observed in the spleen and bone marrow may have been partially due to subcutaneous masses that were infected/ulcerated.

d. A high incidence of cytoplasmic alteration in the pituitary occurred in high dose males.

TUDY DESIGN

Sixty male and female rats were administered o-Nitrotoluene at the following doses and designated core udy.

Core Study

	Control	625 mg/kg	1250 mg/kg	2000 mg/kg
Male	70*	60	60	60
Female	60	60	60	60

*10 control males sacrificed at 14 weeks

Special Study Group

	2000 mg/kg	5000 mg/kg
Male	70	70
	10 rats sacrificed at 14 weeks {The remaining 60 rats fed control diet after 14 weeks (Stop Study)}	10 rats sacrificed at 14 weeks {The remaining 60 rats fed control diet after 14 weeks (Stop Study)}

ANIMAL DISPOSITION SUMMARY

Males	0 ppm	625 ppm	1250 ppm	2000 ppm	2000 ppm (stop study)	5000 ppm (stop study)
Animals Initially in Study	70	60	60	60	70	70
Moribund	18	35	48	53	39	57
Natural Deaths	4	7	6	7	10	3
Animals surviving to study termination	38[a]	18	3	0	11[a]	0[a]
Females						
Animals Initially in Study	60	60	60	60	NA	NA
Moribund	11	12	19	22	NA	NA
Natural Deaths	4	2	2	5	NA	NA
Animals surviving to study termination	45	46	39	33	NA	NA

[a] After 3 months of treatment, 10 animals were necropsied at the interim sacrifice
NA = Not applicable

CONDUCT OF THE PWG

Prior to the PWG the chairperson reviewed the pathology incidence tables, the study pathologist's narrative, the report prepared by the reviewing pathologist, and microscopic slides of potential target organs and lesions with discrepancies in diagnoses between the study pathologist (SP) and reviewing pathologist (RP). All tumor diagnoses in all tissues were also reviewed by the chairperson except the testes where only limited numbers were re-examined.

RESULTS

Potential target organs in the 14-week study were considered to be:

Skin, subcutaneous tissue	Nose, olfactory epithelium
Mammary gland	Preputial gland
Peritoneum	Salivary gland
Liver	Pituitary gland
Lung	Spleen
Testes	Bone marrow
Epididymis	Kidney
Prostate	Islet, pancreatic

Potential target organs for the two-year chronic core study and the chronic special study (stop study) were considered to be:

Male Rats	Femal. Rats
Skin	Skin
Mammary gland	Mammary gland
Peritoneum	Liver
Liver	Lung
Lung	Clitoral gland
Testes	Salivary gland
Epididymis	Bone marrow
Prostate	Spleen
Preputial gland	Pituitary gland, Pars Distalis
Salivary gland	Kidney
Pituitary gland	Mandibular lymph node
Spleen	
Bone marrow	
Islet, pancreatic	
Kidney	

The kidneys from all rats were examined for the diagnosis of cytoplasmic alteration, pigmentation and nephropathy. The mandibular lymph node in female rats was evaluated for hyperplasia.

At the PWG, introductory remarks presented by Dr. Sills included background information on the chemistry of o-nitrotoluene and human exposure potential with products containing o-nitrotoluene.

A 09

The chairperson led a short discussion in the form of introductory remarks which included the fo

1. Survival

Male Rats	Female Rats
0 ppm - 65%	0 ppm - 78%
625 ppm - 30%	625 ppm - 78%
1250 ppm - 5%	1250 ppm - 65%
2000 ppm - 0 %	2000 ppm - 55%
2000 ppm (stop) - 18%	
5000 ppm (stop) - 0%	

2. Body Weights

Males: There was reduced weight gain in the 1250 and 2000 (stop) dose group but was less than different from controls. The 5000 (stop) and 2000 core had reduced weight gains that ranged from 10-30 than controls. The 625 ppm dose group weight gain was similar to controls.

Females: Both 1250 ppm and [illegible] ppm groups gained weight similar to controls. The 2000 ppm group showed a mild to moderate weight gain deficit compared to controls. The maximum deficit occurr week 84 (-12%).

3. Clinical Signs

The primary clinical signs consisted of large subcutaneous masses in the torso area and were seen both sexes. There was also an increase in masses of the head and appendages for treated rats of both sexe compared to controls.

4. Gross Lesions

Treatment related gross lesions recorded at necropsy consisted of large subcutaneous masses, perit masses and small preputial and clitoral glands. There was good correlation of gross lesions and microsco observations. There was a rather aggressive moribund sacrifice in this study due to large subcutaneous m

5. Findings in 13-week subchronic study were discussed.

The PWG chairperson projected 34 (2x2) photomicrographs of lesions that were photographed du preparation for the PWG. These lesions were representative of lesions occurring in the respective target o and were presented to acquaint the PWG members with the lesions to be examined.

There were few discrepancies between the diagnoses of the study pathologist and the reviewing pathologist in this study.

A. 14-Week Study

The PWG examined lesions in the salivary gland, preputial gland, kidney, testes, epididymis, islets pancreas and olfactory epithelium of the nose.

A 10

1. Salivary Gland

The PWG agreed that atrophy of the salivary gland was present. The lesion was characterized by a reduction in size and number of eosinophilic cytoplasmic granules in the epithelial cells of secretory ducts in the submandibular salivary gland. In some salivary glands the eosinophilic granules were essentially absent and the epithelial cells were reduced in size.

2. Preputial Glands

The PWG agreed that preputial glands of the 5000 ppm dose group had moderate to marked atrophy. The glandular epithelial cells had fewer and smaller cytoplasmic granules than normal. Interstitial tissue was more prominent than normal, most likely a relative increase due to decreased size of glandular epithelial cells. A number of preputial glands were recorded to be grossly smaller than normal at necropsy.

3. Testes

The PWG examined a series of testes and agreed that degeneration of the germinal epithelium of seminiferous tubules existed. The majority of affected seminiferous tubules were lined only be Sertoli cells with few or no spermatogenic cells remaining. There was variation in numbers of seminiferous tubules affected within testicles. Some testicles only had a few seminiferous tubules involved whereas, in some testicles, almost all tubules were involved. These were usually diagnosed as atrophy and degree of severity was graded on numbers of tubules involved. Grading was based on the following criteria.

Minimal - approximately 5% or less of seminiferous tubules atrophic;
Mild - approximately 6-20% of seminiferous tubules atrophic;
Moderate - approximately 21-50% of seminiferous tubules atrophic;
Marked - approximately 51% or greater of seminiferous tubules atrophic.

Cellular debris and multinucleated giant cells were observed in some tubules but was not a prominent feature. Mineralization of degenerating seminiferous tubules was present in some testicles at a minimal to moderate degree of severity.

4. Epididymis

The diagnosis of cellular atypia and atrophy of the epididymis was recorded for a number of male r The study pathologist utilized these diagnostic terms to diagnose changes in the luminal contents of the epididymis. The epididymis proper was within normal limits. The TDMS system is limited in diagnostic terms regarding contents of the epididymis.

The PWG examined a series of slides with the epididymis and agreed luminal contents of 5000 ppm dose rats were different than controls. Cellular atypia was characterized by the presence of rounded and enlarged or multinucleated degenerated spermatozoa while atrophy was characterized by the presence of a reduction in number (hypospermia) of normal-appearing spermatozoa. The PWG agreed that changes recorded for the epididymis were the result of testicular changes and not a direct compound effect on the epididymis.

5. Pancreas

The PWG examined a series of slides with the pancreas and agreed that islet hyperplasia was present in the 5000 ppm dose group. The hyperplasia was characterized by increased numbers and size of islets compared to the pancreas of controls. Some hyperplastic islets were over twice the size of controls. Larger islets had slightly enlarged islet cells which were less orderly arranged than the normal glandular pattern of controls. Also, in some hyperplastic islets there was a slight increase in connective tissue stroma.

6. Kidney

A series of kidney slides from control and 5000 ppm dose group rats were presented to the PWG for evaluation.

The PWG agreed that hyaline degeneration was present in the 5000 ppm dose group and minimal nephropathy was also present in a few of these kidneys.

The hyaline degeneration was characterized by a slight increase in the presence of hyaline droplets within renal tubular epithelial cells over that which was present in control animals. These cytoplasmic droplets were homogeneous, eosinophilic and rounded (3-5 microns in diameter). Larger droplets appeared to be extruded into the tubular lumina.

The PWG agreed that the term "hyaline degeneration" was the more appropriate term for this lesion rather than "cytoplasmic alteration".

Although all kidneys were not examined, the PWG accepted the diagnosis of the reviewing pathologist that very minimal nephropathy was present in a number of these kidneys.

7. Nose - olfactory epithelium

The PWG examined a series of slides containing the nose and agreed that degeneration of the olfactory epithelium existed in sections II and III. The degeneration was characterized by disruption, vacuolization or atrophy of the olfactory epithelium. In some areas the epithelial cells were somewhat flattened, suggesting very early attempts toward squamous cell metaplasia. The degenerative changes primarily involved the most dorsal aspect of the ethmoid turbinates, nasal septum and dorsal meatus. Interestingly, many of the changes were unilateral. All 5000 ppm dose rats and approximately ½ of the 2000 ppm were diagnosed with olfactory epithelial degeneration.

B. Two-Year Study

1. Mesothelioma

A very high incidence of mesotheliomas occurred in treated male rats both in the core study and in the special study (stop study) and there was a dose relationship.

The PWG examined a series of slides with mesotheliomas that were diagnosed by the SP and RP. These lesions were mostly large masses of neoplastic mesothelial cells associated with the reproductive organs (testes, epididymis) and their tunics. In some cases mesotheliomas were observed in the abdominal cavity, both on the abdominal wall and on the surface of abdominal organs.

12

The reviewing pathologist had diagnosed a few more male rats with mesothelioma than the study pathologist. A series of these discrepancies were presented to the PWG for evaluation. The PWG agreed that most of these lesions were small mesotheliomas; however, several were considered to be focal mesothelial hyperplasia.

The majority of the mesotheliomas in this study were large and consisted of rather solid areas or sheets of pleomorphic mesothelial cells. In some areas of the tumor, attempts of tubular formation were present. Invasion of adjacent organs was limited to the serosa or occasionally the capsule of the organ. There was the impression that distant foci of neoplastic cells occurred by extension or implantation.

The PWG noted the overwhelming incidence of mesotheliomas in treated male rats in this study and recognized that the rats in the stop study had almost the same incidence as rats in the core study.

2. Mammary Gland

There was an increase in mammary gland tumors in treated male and female rats over that of controls. In male rats mammary fibroadenomas were diagnosed only in treated groups and occurred in essentially a dose related fashion. In females mammary gland tumors consisted primarily of fibroadenomas although an occasional adenoma and carcinoma were diagnosed. There was a dramatic increase in fibroadenomas and fibroadenoma, multiple in treated females and a dose response was present.

The PWG examined a small series of mammary fibroadenomas and agreed that these were characteristic of typical fibroadenomas.

There was a large number of mammary glands diagnosed with hyperplasia. The PWG examined a series of these mammary glands and agreed with the RP that many of these glands had a few dilated ducts containing eosinophilic desiccated material. There was no proliferating ductal or glandular epithelium and the diagnosis of dilatation was more appropriate.

3. Skin, subcutaneous tissue

A large number of masses and nodules in the skin of the torso, head, and appendages had been recorded clinically and at necropsy. The majority of these lesions in male rats were fibromas. Many rats had multiple fibromas. Fibrosarcomas and multiple fibrosarcomas were also present at high incidence. Lipomas were also diagnosed in the subcutaneous tissue of treated male rats. No lipomas were observed in male controls or in any females.

The PWG examined a small series of these subcutaneous tumors for verification and agreed that these were typical fibromas and fibrosarcomas. The lipomas were focal nodules of variable-sized fat cells often infiltrated with streaks of fibrous tissue and occasionally a few chronic inflammatory cells were present.

In females many of these skin masses were mammary fibroadenomas. There were a large number of fibromas and a few fibrosarcomas in the 1250 ppm and 2000 ppm female dose groups. The SP had only diagnosed a single tumor when there may have been more than one present. The RP utilized the term "multiple" when more than one tumor was present.

The PWG agreed that a very dramatic increase in subcutaneous tumors occurred in treated rats of both sexes and occurred in a dose related fashion.

4. Liver

A higher incidence of hepatic tumors were reported to be present in treated rats of both sexes over t of controls. Also, a higher incidence of non-tumor lesions, i.e. basophilic foci, eosinophilic foci, clear cell a mixed cell foci, and mixed cell infiltrates occurred in treated rats over that of controls.

The hepatic tumors of male rats had a rather high incidence of bile duct associated tumors (cholangiocarcinoma, hepatocholangioma and hepatocholangiocarcinoma). The PWG examined all such hepatic tumors and all hepatocellular adenomas and carcinomas diagnosed in high dose and control male and female rats. The PWG agreed with the diagnosis of the cholangiocarcinomas, hepatocholangiocarcinoma, an hepatocholangioma. The PWG also noted that several of the hepatocellular adenomas had more biliary proliferation than is usually seen in such tumors. It was noted that bile duct hyperplasia was diagnosed less frequently in the liver of treated rats in the study compared to controls. The PWG agreed that an increase in liver tumors occurred in all groups of treated male rats and at the 2000 ppm female dose group.

5. Lung

A higher incidence of pulmonary tumors (Alveolar/Bronchiolar adenoma and Alveolar/Bronchiolar carcinoma) had been diagnosed in the 5000 ppm (stop) males dose group and 2000 ppm female dose group o that of controls.

The PWG examined all control high dose male and female lungs in which a tumor had been diagnos There was quite good agreement in the diagnoses of tumors although an occasional A/B adenoma was considered by the PWG to be A/B hyperplasia.

It was the PWG opinion that there was a higher incidence of pulmonary tumors in the male 5000 ppm (stop) dose group over that of controls. Although a slightly higher incidence of pulmonary tumors were prese in the female 2000 ppm dose group, a statistically significant increase is questioned. The PWG noted that alveolar epithelial hyperplasia was diagnosed at a higher incidence in treated rats of both sexes over that of controls.

6. Urinary Bladder

The PWG examined several lesions in the bladder that had been diagnosed as tumors. Three mesenchymal tumors that had been diagnosed were considered by the PWG to be inflammation and/or fibrosis Two squamous cell carcinomas were considered to be transitional cell papillomas with areas of squamous cell differentiation. No treatment related lesions were considered to be present in the urinary bladder of either sex.

7. Kidney

A very high incidence of minimal nephropathy had been diagnosed in the kidney of both sexes. The PWG reviewed two examples of minimal nephropathy and agreed that these examples were indeed minimal. It was noted that the threshold for the diagnosis of minimal nephropathy versus no remarkable lesion can be quite subjective. The degree of nephropathy in many of the kidneys in this study consisted of only 2-3 epithelial cell within a single tubule that were slightly larger and slightly more basophilic than normal. The RP diagnosed these as nephropathy, minimal whereas the SP considered these to be within normal limits. The PWG agreed that no compound effect appeared to be present in the kidney of the two-year studies.

8. Pituitary Gland

A change in the pituitary gland diagnosed as cytoplasmic alteration was observed in most male 5000 ppm (stop) dose group. Only a few pituitaries in lower male dose groups had this change and none in any females were involved.

The cytoplasmic alteration was characterized by individual cells in the pars distalis that were hypertrophied and had increased lightly eosinophilic cytoplasm that often contained a vacuole. These cells have been termed "castration cells" and have been observed in rats following orchidectomy. These cells have also been seen in controls and in rats of other studies. There were increased numbers of these cells in the 5000 ppm (stop) dose group in this study compared to concurrent controls.

9. Testes and Epididymis

There were interstitial cell tumors in most testes of all male dose groups. There was a slight decrease in tumors in dose groups with a high incidence of early deaths. Atrophy of the testes was also seen in association with many testes having interstitial cell tumors. The cellular atypia and atrophy of the epididymal luminal contents as seen in the 14-week study were also present in the two-year studies.

The PWG agreed that very prominent changes were present in the testes of the 14-week interim sacrifice discussed earlier. Because of the very high incidence of interstitial cell tumors in older F344 rats, it is difficult to differentiate direct compound related lesions from those secondary to the interstitial cell tumors.

10. Salivary Gland, Preputial Gland and Clitoral Gland

The PWG accepted the diagnosis of the SP, RP and chairperson regarding atrophy of these organs. The change was similar to those examined by the PWG in the 14-week study.

11. Pancreas - Islet Cell

Hyperplasia of pancreatic islets occurred at a higher incidence in the male 5000 ppm (stop) dose group over that of controls. The character of the lesion was similar to that observed in the 14-week interim sacrifice study. There was no increase in adenomas/carcinomas in any dose group over that of controls.

12. Bone Marrow and Spleen

The PWG noted that an increased incidence of hematopoietic cell proliferation was reported in the bone marrow and spleen of treated male and female rats. It was recognized that many of the rats had subcutaneous masses that were ulcerated and had varying degrees of inflammation. These inflammatory lesions would result in increased hematopoietic cell proliferation and clouded a direct treatment related change if present.

B 01

13. Mononuclear Cell Leukemia

The PWG noted that a reduced incidence of mononuclear cell leukemia existed in treated rats of both sexes compared to controls. The PWG was of the opinion that this lower incidence could not be explained by reduced longevity or a reduction in weight gain and represented a compound effect.

Paul K. Hildebrandt, D.V.M.
Diplomate, American College of
Veterinary Pathologists

10/11/00
Date

PATHOLOGY WORKING GROUP CHAIRPERSON'S REPORT

2-Year Chronic Study of o-Nitrotoluene in B6C3F1 Mice

Participants: Drs. M. Jokinen (PAI - PWG Chairperson), D. Farnell (Southern Research - SP), G. Flake (NIEHS), J. Hailey (NIEHS), R. Herbert (NIEHS), A. Nyska (NIEHS), C. Okerberg (EPL – QAP), K. Ozaki (NIEHS – Observer), J. Pletcher (PAI), C. Qualls (Glaxo Wellcome), and R. Sills (NIEHS).

Date: August 24, 2000
Site: NIEHS, Research Triangle Park, NC

The PWG was convened to evaluate selected slides from the two year dosed feed study of o-nitrotoluene in B6C3F1 mice. Dose levels and numbers of animals examined microscopically per group were as follows:

Dose	M	F
0 ppm	60	60
1250 ppm	60	60
2500 ppm	60	60
5000 ppm	60	60

The study was conducted at Southern Research Institute. The Study Pathologist (SP) was Dr. D. Farnell and the Quality Assessment Pathologist (QAP) was Dr. C. Okerberg of EPL.

A number of organs were reviewed by the QAP for potential treatment-related effects: the QAP reviewed these organs in all animals for all diagnoses, neoplastic and nonneoplastic. Organs reviewed for all diagnoses were as follows:

Male Mice	Female Mice
Intestine Large, Cecum	Intestine Large, Cecum
Liver	Liver
Stomach, Forestomach	Stomach, Forestomach
Stomach, Glandular	Stomach, Glandular
Kidney	Kidney

In addition, all neoplasms from all organs from all animals were reviewed, prostate from all males was reviewed for chronic inflammation, spleen from all males and females was reviewed for hematopoietic cell proliferation, and skin subcutaneous tissue was reviewed from all males and females for edema.

SUMMARY OF PWG FINDINGS

Terminal survival was low in all treated male groups and in the 5000 ppm female group. All 5000 ppm males were dead by Week 64 of the study. None of the 2500 ppm males lived until study termination at 104 weeks, and only one male from this

group was still alive at Week 100. Thirty-six 1250 ppm males lived until the termination of the study, while 52 control males lived until study termination. Six 5000 ppm females lived until study termination. The survival until study termination in the two remaining treated female groups and control females was similar, although the survival in the treated groups was marginally lower than in the controls. Low survival in treated groups of males and females was considered to be secondary to neoplasms.

Incidences of **Hemangiosarcoma** and **Hemangiosarcoma, Multiple** were increased in all dosed groups of males and females as compared with controls. Incidences of hemangiosarcoma in 2500 and 5000 ppm males and 5000 ppm females were markedly increased as compared with controls. Organs primarily involved were the subcutaneous tissue of the skin, the mesentery, and the skeletal muscle.

Incidences of **Intestine Large, Cecum – Carcinoma** were increased in the 1250 and 2500 ppm male groups and in all treated female groups as compared with respective controls. No cecal carcinomas were observed in the 5000 ppm males, presumably due to low survival.

Combined incidences of **Liver – Hepatocellular Adenoma; Hepatocellular Adenoma, Multiple; Hepatocellular Carcinoma; and Hepatocellular Carcinoma, Multiple** were increased in 2500 and 5000 ppm females as compared with 1250 ppm and control females. Combined incidences of hepatocellular neoplasms in 2500 and 5000 ppm males were decreased as compared with 1250 ppm and control males, presumably as a result of low survival in the two top male groups. Incidences of **Liver – Eosinophilic Focus** were increased in treated groups of females and in the 1250 ppm group of males. Incidences of **Liver – Necrosis** were increased in all treated groups of males as compared with control males, and in the 5000 ppm female group as compared with other treated and control female groups. In addition, incidences of **Liver, Hepatocyte – Syncitial Alteration, Focal** were increased in all treated groups of males as compared with control males.

Incidences of **Kidney, Renal Tubule – Pigment** were increased in all treated groups of males and females as compared with controls. The incidences were markedly elevated in the 2500 and 5000 ppm male groups, and in the 5000 ppm female group. The incidence of **Kidney, Renal Tubule – Accumulation, Hyaline Droplet** was increased in the 5000 ppm female group as compared with the other female groups. The incidences of **Kidney – Nephropathy** were decreased in the 2500 and 5000 ppm male groups as compared with the 1250 ppm and control male groups, presumably due to the low survival in the two highest dose male groups.

Incidences and average severities of **Spleen – Hematopoietic Cell Proliferation** were increased in all treated groups of males and females as compared with respective controls. This was presumably secondary to the increased incidences of neoplasms in these dose groups.

and thus were primary at these sites. In all of the other animals, hemangiosarcomas were present in one or more other sites, and thus the possibility that the hemangiosarcomas in the heart, prostate, or uterus of these animals may have been metastases from another site.

Large Intestine, Cecum

The SP diagnosed increased incidences of **Large Intestine, Cecum – Carcinoma** in each of the treated groups of males and females, except the 5000 ppm males. The SP's findings were confirmed by the QA/PWG review. Incidences were highest in the 1250 ppm male group. No cecal carcinomas were observed in control males and females or in 5000 ppm males. The absence of cecal carcinomas in the 5000 ppm male group was considered due to the low survival in that group. The PWG examined a number of representative examples and in each case confirmed the diagnosis of carcinoma.

Microscopically, the carcinomas generally were characterized by proliferation of glandular structures, composed of moderately pleomorphic mucosal epithelial cells, that had invaded the cecal wall. In some carcinomas, presumably earlier in development, only a small amount of invasion was seen but in large, apparently more developed, carcinomas, extensive invasion was sometimes seen with neoplastic cells extending through the cecal wall into the attached mesentery. A few of the lesions were unusual in that they consisted of a diffuse thickening of the submucosa by solid masses of large cells with abundant clear cytoplasm and a peripherally located nucleus, resembling the so-called "signet ring cells", mixed with smaller numbers of epithelial cells with large nuclei and small amounts of cytoplasm. Invasion of the cecal wall was not apparent in these lesions. It was noted that areas of similar appearing cells were seen in other carcinomas with extensive invasion. After considerable discussion, the PWG came to the conclusion these lesions were all carcinomas which represented a variant of the other carcinomas. However, the PWG did recommend that immunostaining be conducted on these unusual carcinomas for additional verification.

Liver

The SP diagnosed greater combined incidences of hepatocellular neoplasms **(Hepatocellular Adenoma; Hepatocellular Adenoma, Multiple; Hepatocellular Carcinoma; Hepatocellular Carcinoma, Multiple)** in the 2500 and 5000 ppm female groups, and lower incidences in the 2500 and 5000 ppm males, as compared with respective controls. Quite a number of the SP's diagnoses were changed as a result of the QA/PWG review; specifically, many of the SP's diagnoses of hepatocellular carcinoma were changed to hepatocellular adenoma or eosinophilic focus. Despite that, however, the SP's overall findings were confirmed. The low incidences of hepatocellular neoplasms in the 2500 and 5000 ppm male groups was considered to be secondary to the low survival in these groups. The PWG Chair disagreed with a number of the SP's and QAP's diagnoses concerning hepatocellular neoplasms; all of these disagreements were reviewed by the PWG.

Skin, Subcutaneous Tissue – Edema occurred in numerous males in the 2500 and 5000 ppm groups and in a few animals in the 1250 ppm male groups and in all the treated female groups.

CONDUCT OF THE PWG

Prior to the PWG, the PWG Chairperson reviewed the pathology tables, the SP's narrative, the Pathology Data Review, the Quality Assessment Report, and microslides of tissues selected for QA review. The PWG Chair then selected slides for review by the PWG, including representative examples of lesions, and lesions for which there was a difference in diagnosis among the SP, QAP, and PWG Chair.

RESULTS OF THE PWG REVIEW

Multiple Organs

The SP had diagnosed increased incidences of **Hemangiosarcoma** in several organs in both treated males and females. The SP's findings were confirmed by the QA/PWG review. Incidences were highest in the 2500 and 5000 ppm males and 5000 ppm females, and probably was the cause of the low survival in those groups. The organs primarily affected with hemangiosarcoma were the **Skin, Subcutaneous Tissue, Skeletal Muscle,** and **Mesentery,** with a scattering in a few other tissues. Many of the animals, particularly the high dose males, had multiple hemangiosarcomas. There was excellent agreement among the SP, QAP, and PWG Chair concerning the diagnoses of hemangiosarcoma, so the PWG reviewed only representative examples and in each case unanimously agreed that hemangiosarcoma was present.

Hemangiosarcomas ranged from small neoplasms, approximately 1-2 mm in diameter, to large masses up to 1 cm or more in diameter. Microscopically, they consisted of numerous, irregular, variably sized, blood-filled vascular channels, lined by large, pleomorphic endothelial cells, and separated by variable amounts of fibrous stroma. The central portions of most of the hemangiosarcomas had undergone necrosis leaving a large blood filled cavity surrounded by a rim of neoplastic vascular tissue. Many of the animals, particularly those with multiple and/or large hemangiosarcomas, had small hemangiosarcomas in other tissues, primarily the lung, that were considered to be metastases.

It was noted during the PWG that, although hemangiosarcomas appeared primarily in the skin, mesentery, and skeletal muscle of treated animals, a few hemangiosarcomas were diagnosed in other sites including heart and prostate in males and the uterus in females. The question was raised as to whether the hemangiosarcomas in these sites in these animals represented primary neoplasms or were possibly metastastic lesions. Examination of the pathology findings for these animals indicated that the hemangiosarcoma in the heart of one 1250 ppm male, in the prostate of one 2500 ppm male, and in the uterus of one 1250 ppm female and one 5000 ppm female were the only hemangiosarcomas diagnosed in these animals,

and thus were primary at these sites. In all of the other animals, hemangiosarcomas were present in one or more other sites, and thus the possibility that the hemangiosarcomas in the heart, prostate, or uterus of these animals may have been metastases from another site.

Large Intestine, Cecum

The SP diagnosed increased incidences of **Large Intestine, Cecum – Carcinoma** in each of the treated groups of males and females, except the 5000 ppm males. The SP's findings were confirmed by the QA/PWG review. Incidences were highest in the 1250 ppm male group. No cecal carcinomas were observed in control males and females or in 5000 ppm males. The absence of cecal carcinomas in the 5000 ppm male group was considered due to the low survival in that group. The PWG examined a number of representative examples and in each case confirmed the diagnosis of carcinoma.

Microscopically, the carcinomas generally were characterized by proliferation of glandular structures, composed of moderately pleomorphic mucosal epithelial cells, that had invaded the cecal wall. In some carcinomas, presumably earlier in development, only a small amount of invasion was seen but in large, apparently more developed, carcinomas, extensive invasion was sometimes seen with neoplastic cells extending through the cecal wall into the attached mesentery. A few of the lesions were unusual in that they consisted of a diffuse thickening of the submucosa by solid masses of large cells with abundant clear cytoplasm and a peripherally located nucleus, resembling the so-called "signet ring cells", mixed with smaller numbers of epithelial cells with large nuclei and small amounts of cytoplasm. Invasion of the cecal wall was not apparent in these lesions. It was noted that areas of similar appearing cells were seen in other carcinomas with extensive invasion. After considerable discussion, the PWG came to the conclusion these lesions were all carcinomas which represented a variant of the other carcinomas. However, the PWG did recommend that immunostaining be conducted on these unusual carcinomas for additional verification.

Liver

The SP diagnosed greater combined incidences of hepatocellular neoplasms (**Hepatocellular Adenoma; Hepatocellular Adenoma, Multiple; Hepatocellular Carcinoma; Hepatocellular Carcinoma, Multiple**) in the 2500 and 5000 ppm female groups, and lower incidences in the 2500 and 5000 ppm males, as compared with respective controls. Quite a number of the SP's diagnoses were changed as a result of the QA/PWG review; specifically, many of the SP's diagnoses of hepatocellular carcinoma were changed to hepatocellular adenoma or eosinophilic focus. Despite that, however, the SP's overall findings were confirmed. The low incidences of hepatocellular neoplasms in the 2500 and 5000 ppm male groups was considered to be secondary to the low survival in these groups. The PWG Chair disagreed with a number of the SP's and QAP's diagnoses concerning hepatocellular neoplasms; all of these disagreements were reviewed by the PWG.

As a result of the QA review the QAP diagnosed greater incidences of **Eosinophilic Focus** in the 1250 ppm male and 5000 ppm female groups as compared with respective controls. In addition, the PWG Chair noted a number of altered hepatocellular foci, primarily eosinophilic foci, that had not been diagnosed previously and showed of all those to the PWG as well. Thus, it appeared that greater incidences of eosinophilic focus may have occurred in some treated groups. However, any conclusions regarding this will have to wait until final pathology tables, which take into account all changes made during the review, are available.

Microscopically, eosinophilic focus, hepatocelluar adenoma, and hepatocellular carcinoma had the typical appearance of those lesions in B6C3F1 mice. Eosinophilic foci were small to moderately large lesions composed of hepatocytes with eosinophilic cytoplasm that generally were somewhat enlarged. The hepatocytes were arranged in normal hepatic cords that merged imperceptibly with the surrounding normal hepatocytes. Foci usually produced little or no compression of the surrounding normal hepatocytes, although some degree of compression was present in some larger foci. Adenomas were discrete masses with distinct borders that caused definite compression of the surrounding normal parenchyma. Adenomas usually were composed of hepatocytes that appeared similar to those seen in eosinophilic foci except that in adenomas the normal lobular architecture was not apparent, and plates of neoplastic hepatocytes intersected the surrounding normal hepatoctyes at sharp angles, rather than merging with them, as in foci. Carcinomas were discrete masses that generally had irregular borders due to localized areas of growth of neoplastic hepatocytes into the surrounding normal parenchyma. The neoplastic hepatocytes often were somewhat atypical, but the major distinguishing features of carcinomas was the presence of abnormal patterns of growth. The most common abnormal growth pattern was formation of trabeculae of neoplastic hepatocytes that were three or more cell layers thick, while less commonly the neoplastic cells formed glandular structures or solid masses.

The SP had diagnosed greater incidences of **Liver, Hepatocyte – Syncytial Alteration, Focal** in all treated groups of males. This was confirmed by the QA/PWG review. The PWG examined some representative examples and agreed that the change was present. Microscopically, syncytial alteration was generally a minimal change consisting of one to several scattered hepatocytes which contained multiple (usually 4 to 6) small nuclei. The significance of this change was uncertain, but some PWG members noted that this change has been seen as a treatment effect in other NTP studies.

The SP had diagnosed **Liver – Infiltrate Cellular, Mixed Cell** in most livers in control and treated males and females. Microscopically, mixed cell infiltrate was a minor change, usually of minimal severity, consisting of one to several small to focal aggregates of mixed inflammatory cells, usually macrophages and lymphocytes, sometimes admixed with a few neutrophils. These aggregates of inflammatory cells usually had a few hepatocytes with brightly eosinophilic cytoplasm, that appeared to be necrotic, around the periphery or mixed with the inflammatory cells. This change

had been diagnosed by the SP as **Liver, Hepatocyte – Necrosis, Focal**. The QAP agreed with the SP's diagnoses in nearly every case.

The PWG Chair was of the opinion that the focal necrosis diagnosed by the SP was actually part of the mixed cell infiltrate and did not warrant a separate diagnosis. Also, the PWG Chair noted that mixed cell infiltrate and focal hepatocyte necrosis had been diagnosed in a number of control and treated animals of both sexes in which the PWG Chair considered the change(s) not to be present. The PWG Chair also noted that one or more discrete foci of coagulative necrosis of hepatocytes, consisting of hepatocytes with uniformly eosinophilic cytoplasm and pyknotic or karrhyorectic nuclei, were seen more commonly in treated livers than in controls, and this necrosis appeared to represent a treatment related effect. In these livers, the PWG Chair had concurred with the diagnosis of hepatocyte necrosis.

The incidences of mixed cell infiltrate, as diagnosed by the PWG Chair, were lower in the 2500 and 5000 ppm dose groups, as compared with the control and 1250 ppm groups, and were similar in control and 1250 ppm males. The lower incidences of mixed cell infiltrate in the 2500 and 5000 ppm dose groups were presumed to be related to the low survival in those groups. The incidences of mixed cell infiltrate in control and treated groups of females were similar. The incidences of hepatocyte necrosis diagnosed by the PWG Chair were greater in all treated male groups than in control males, and were greater in the 5000 ppm female group than in the other treated and control female groups.

The PWG examined representative examples of control livers in which mixed cell infiltrate and focal hepatocyte necrosis had been diagnosed by the SP and concurred with the PWG Chair's opinion. The PWG members noted that mixed cell infiltrate is a common aging finding in the livers of B6C3F1 mice and that a few necrotic hepatocytes are commonly seen as part of the lesion. The PWG was of the opinion that the inflammatory infiltrate and necrotic hepatocytes were all part of one, presumably inflammatory, process and did not warrant separate diagnoses.

The PWG then examined some livers from treated animals in which mixed cell infiltrate and/or focal hepatocyte necrosis had been diagnosed and in which the PWG Chair either considered the changes to be present or not present. The PWG also examined livers from treated animals in which the focal areas of coagulative necrosis were present. The PWG agreed with the PWG Chair that some of the livers in which mixed cell infiltrate and/or focal hepatocyte necrosis had been diagnosed did not contain these changes. The PWG Chair noted that many of these livers were somewhat autolyzed and that the changes diagnosed as mixed cell infiltrate of focal hepatocyte necrosis may have represented autolytic changes. The PWG also agreed that some of the livers had discrete foci of coagulative hepatocyte necrosis that warranted diagnosis. One of the foci of coagulative necrosis contained a minimal mixed cell infiltrate. The PWG was of the opinion that the infiltrate was a reaction to the necrosis, and as such, part of the process of necrosis, and thus did not warrant separate diagnosis.

Following this review the PWG recommended that (1) the diagnoses of mixed cell infiltrate and focal hepatocyte necrosis be deleted whenever recommended by the PWG Chair, and (2) all remaining diagnoses of hepatocyte necrosis be changed simply to **Liver – Necrosis.**

Kidney

The SP diagnosed greater incidences of **Kidney, Renal Tubule – Pigmentation** in all treated groups of males and in the 2500 and 5000 ppm groups of females than in respective controls. This was confirmed by the QA/PWG review. The QAP agreed with the SP's diagnoses of pigmentation in each case. The PWG Chair agreed in most cases, but in a few instances did not see any pigmentation. The PWG examined representative examples of the pigmentation and confirmed its presence. It also examined representative examples of the kidneys in which the PWG Chair did not see any pigmentation, and concurred with the PWG Chair's findings. Based upon that, the PWG recommended deleting the diagnosis of pigmentation in those instances in which the PWG Chair did not see any pigmentation. The incidences of pigmentation diagnosed by the PWG Chair are similar to those diagnosed by the SP but slightly lower, and the treatment related increase in the incidences of pigmentation was still present. Microscopically, pigmentation was generally a minimal to moderate change consisting of fine dark brown granules within the cytoplasm of cortical renal tubular epithelial cells. Minimal pigmentation was a slight change consisting of small numbers of granules either diffusely scattered or within scattered clusters of epithelial cells, and often required careful examination at high magnification to detect. Moderate pigmentation consisted of numerous, diffusely scattered granules and was easily detectable at low to moderate magnification.

The SP also diagnosed greater incidences of **Kidney, Renal Tubule – Accumulation, Hyaline Droplet** in all treated groups of males and females, as compared with respective controls. The incidences were marginally increased in all treated groups except the 5000 ppm females, in which the incidence was moderately increased. Microscopically, hyaline droplet accumulation consisted of small homogeneous brightly eosinophilic droplets within the epithelium of renal cortical tubules. The PWG examined some representative examples and confirmed the presence of the change. Hyaline droplet accumulation such as seen in this study is sometimes seen in animals with neoplasms, and the increased incidences in the treated animals in this study was considered to be secondary to the presence of neoplasms.

The SP had diagnosed slightly higher incidences of **Kidney – Nephropathy** in treated groups of females as compared with control females. The PWG Chair noted the presence of nephropathy in a few kidneys in which it had not been diagnosed previously and showed all of these kidneys to the PWG, which concurred with the PWG Chair's findings. The incidences of nephropathy as diagnosed by the PWG Chair were similar among the control and 2500 and 5000 ppm treated groups, and were slightly higher in the 1250 ppm group. Final conclusions regarding the significance of these findings must await preparation of final pathology tables.

The SP had also diagnosed **Kidney, Renal Tubule – Casts Protein**, one to few prominently dilated tubules filled with homogeneous eosinophilic proteinaceous material, in a few animals in the control and 1250 and 2500 ppm female groups, but not in the 5000 ppm female group. Protein casts are a common spontaneously occurring change. The PWG Chair noted the same change in a few animals in each of the female groups in which it had not been diagnosed previously. The PWG examined representative examples of protein casts diagnosed by the SP and by the PWG Chair and concurred with the diagnosis in each case, with the exception of one kidney that also had nephropathy. The PWG was of the opinion that if nephropathy was present then the protein casts should be considered part of the nephropathy. If protein casts were present and nephropathy was not present, then protein casts should be diagnosed. Based upon this, the PWG recommended adding the additional diagnoses of protein casts made by the PWG Chair be added to the pathology findings, unless the animal also had a diagnosis of nephropathy, in which case the additional diagnosis of protein casts should not be added. The incidences of protein casts as diagnosed by the PWG Chair were similar across groups with no indication of a treatment effect.

Spleen

The SP had diagnosed greater incidences and severities of **Hematopoietic Cell Proliferation** in treated groups of males and females as compared with respective controls. This was confirmed by the QA/PWG review. The PWG reviewed a few representative examples and confirmed the presence of the change. The increased hematopoietic cell proliferation in treated groups was presumed to be secondary to the greater incidences of neoplasms in these groups.

Skin

The SP had diagnosed **Skin, Subcutaneous Tissue – Edema** in a number of animals in each of the treated male and female groups, but not in any of the controls. The greatest incidences were in the 2500 and 5000 ppm groups. The PWG reviewed a few representative examples and confirmed its presence. Microscopically the edema appeared as thickening of the subcutaneous tissue with the subcutaneous connective tissues separated by abundant clear space caused by the accumulation of fluid. The exact cause of the edema was unclear. However, the PWG noted the presence of dilated lymphatics in the edematous areas suggesting the edema may have resulted from lymphatic obstruction caused by the presence of neoplasms.

Stomach

The SP had diagnosed slightly greater incidences of **Stomach Glandular, Glands – Degeneration, Cystic, Focal** in the 2500 ppm female group. The PWG Chair noted the same change was present in many stomachs in control and treated males and females in which it had not been previously diagnosed. The incidences in males as diagnosed by the PWG Chair were similar in the control and 1250 ppm and lower in the 2500 and 5000 ppm groups. In females, the incidences diagnosed by the PWG

Chair were similar across groups. The PWG examined representative examples of this change as diagnosed by the SP and by the PWG Chair and in all cases agreed it was there. However, the PWG further noted this change occurs commonly in aging mice and generally is considered a normal background change that does not warrant diagnosis. The lower incidences of this change diagnosed by the PWG Chair in the 2500 and 5000 ppm male groups are consistent with its being an aging change, as there was low survival in these male groups, and most animals died before they could develop the change. Since there was no indication that the incidence of this change increased with treatment, and at the suggestion of the SP, the PWG recommended that the diagnosis be deleted from the study.

The PWG also reviewed a number of additional slides of glandular and forestomach lesions, mainly epithelial hyperplasias and ulcers/erosions observed by the PWG Chair and which had not been diagnosed previously.

Miscellaneous

In a number of instances the SP diagnosed a single neoplasm when two or more of the same neoplasms were present. The preferred NTP terminology in these cases is use to the qualifier Multiple to indicate more than one neoplasm of the same type was present in that tissue. In these instances, the PWG Chair changed the diagnosis to the preferred terminology without showing the slides to the PWG, since there was no disagreement on diagnoses. For example, in 5000 ppm female number 432 Liver – Hepatocellular Carcinoma was diagnosed when two separate carcinomas were present and both had been diagnosed by the SP as carcinomas, as indicated by the fact that the SP correlated the diagnosis of carcinoma with two separate Trace Gross Lesions. The PWG Chair changed the diagnosis to Liver – Hepatocellular Carcinoma, Multiple.

POST-PWG ACTION ITEMS

The PWG recommended that immunostains be performed on the unusual cecal carcinomas to confirm the diagnosis. The results of the immunostains will be included in addendum to the report.

Micheal P. Jokinen, DVM — 9/19/00
Diplomate, ACVP — Date
VG Chairperson

National Toxicology Program

TR-504 o-Nitrotoluene

Pathology Tables - Rats

P03 - Incidence Rates of Non-Neoplastic Lesions - 2 year study

P05 - Incidence Rates of Neoplasms by Anatomic Site (systemic lesions abridged) - 2 year study

P08 - Statistical Analysis of Primary Tumors

P08 - Statistical Analysis of Primary Tumors - control vs stop

Pathology Tables - Mice

P03 - Incidence Rates of Non-Neoplastic Lesions

P05 - Incidence Rates of Neoplasms by Anatomic Site (systemic lesions abridged)

P08 - Statistical Analysis of Primary Tumors

F 03

107-13-1
PWG CHM
C 50215B

PATHOLOGY WORKING GROUP
CHAIRPERSON'S REPORT

Two-Year Chronic Study of Acrylonitrile (C50215B/50215-03) Administered by Gavage, Water to B6C3F1 Mice

DATE OF PWG: August 29, 2000

LOCATION OF PWG: NIEHS, Research Triangle Park, NC

PARTICIPANTS:

Drs. B. Davis (NIEHS), J. C. Seely (EPL-representing the QA pathologist), J. Toft (Battelle Columbus-Study Pathologist), T. Rosol (Ohio State University), B. Gottschling (Indiana University-observer), D. Thake (Monsanto/Searle-observer), J. Quast (AN Group-observer), G. Flake (NIEHS), R. Herbert (NIEHS), A. Nyska (NIEHS), S. Ching (ILS-PWG Chairperson).

RECEIVED
OPPT CBIC
2000 DEC 26 AM 10:47

INTRODUCTION/STUDY DESIGN:

The PWG was convened to evaluate selected H & E slides from B6C3F1 mice administered acrylonitrile by gavage for up to 105 weeks. The doses and numbers of animals examined microscopically per group were as follows:

Dose (mg/kg)	Male	Female
0 (water)	50	50
2.5	50	50
10	50	50
20	50	50

Survival to termination in this study is summarized in the table below:

Control		2.5 mg/kg		10 mg/kg		20 mg/kg	
M	F	M	F	M	F	M	F
76%	78%	88%	64%	78%	78%	28%	47%

The study was conducted at Battelle Columbus. The Study Pathologist (SP) was Dr. J. Toft and the Quality Assessment Pathologist (QAP) was Dr. J Wolf of EPL.

2000 DEC 26 AM 10:45

F 04

SUMMARY OF FINDINGS FROM THE PWG OR CHAIRPERSON REVIEW:

The following is a summary of review findings.

- There was an increase in the incidence of age-related ovarian atrophy and cysts in female treated mice. The incidence of granulosa cell tumor and ovarian hemangioma was slightly increased in the mid-dose group only. There was agreement on the terminology of uterine cystic endometrial hyperplasia. In the uterus, the incidence of angiectasis was increased in the low and mid-dose groups. There was no treatment-related increase in uterine tumors.

- There was an increased incidence of squamous cell carcinomas, squamous cell papillomas, focal epithelial hyperplasia, and focal and diffuse hyperkeratosis in the mid and high-dose male mice. In the female mice, the incidence of squamous cell carcinomas was increased in the high-dose, and the incidences of squamous cell papillomas and focal epithelial hyperplasia were increased in the mid and high-dose groups.

- Increased incidences of Harderian gland adenomas and hyperplasia were confirmed in the low, mid and high-dose male mice, and the incidence of carcinomas was increased in the mid and high-dose groups. There were slightly increased incidences of hyperplasia in the mid and high-dose males. In the female mice, the incidence of Harderian gland adenomas and carcinomas was increased in the mid and high-dose groups. In both sexes, eye atrophy, corneal inflammation, cataract and/or iris synechia were observed and were considered to be secondary changes associated with presence of Harderian gland tumors.

- In the lung, there were higher incidences of alveolar/bronchiolar carcinomas in the low, mid and high-dose female mice compared to the control group. The incidence of alveolar/bronchiolar adenomas was increased in the mid-dose group.

- The higher incidences of combined primary liver tumors (hepatocellular adenomas, hepatocellular carcinomas) were confirmed in the low and mid-dose male mice. Increased incidences of eosinophilic, basophilic and mixed cell foci, chronic active inflammation, cytoplasmic vacuolization, angiectasis, and pigmentation were observed in low and mid-dose male mice. Lesions in the liver with increased incidences in low and mid-dose female mice included basophilic and mixed cell foci, cytoplasmic vacuolization, angiectasis, hematopoietic cell proliferation, necrosis and eosinophilic foci.

- There was no increased incidence of subcapsular hyperplasia in the adrenals of either sex nor an increase in the incidence of subcapsular adenomas in the high-dose female mice.

- There was a higher incidence of lymphoid hyperplasia in the mandibular lymph nodes in the mid-dose female mice but not in the mediastinal lymph nodes of treated female mice. There was a decrease in the incidence of splenic lymphoid hyperplasia in high-dose female mice.

95

CONDUCT OF THE PWG:

In preparation for the PWG, the PWG Chairperson (CP) reviewed the study pathology tables (summary and individual animal data), the study Final Report that included the SP's narrative, the Quality Assessment Report, any relevant literature/publications and H & E slides of tissues selected for the QA review. The CP then selected slides for review by the PWG, including representative examples of target organ or treatment-related lesions, lesions for which there was a difference in diagnosis among the SP, QAP and/or CP, and non-neoplastic and neoplastic lesions requiring confirmation of terminology. PWG participants reviewed all selected slides, voted on diagnoses where the final diagnoses for the lesions presented were determined by the consensus of the participants, and discussed issues.

The following potential target organs for all tumor and nontumor diagnoses for all animals in all groups were reviewed by the QAP.

Male Mice	Female Mice
Liver	Liver
Stomach, Forestomach	Stomach, Forestomach
Harderian Gland	Ovary
Eye*	Uterus
	Harderian Gland
	Eye*
	Lung

*Only previously processed eyes were reviewed

In addition, the following organs/tissues were examined for all animals in all groups for the following lesions.

Female Mice
Lymph Node, Mandibular-hyperplasia, lymphoid
Lymph Node, Mediastinal-hyperplasia, lymphoid
Lymph Node, Mediastinal-hyperplasia
Lymph Node, Mediastinal-hematopoietic cell proliferation
Spleen-hyperplasia, lymphoid

Sections of the following organs from the sex indicated were reviewed when the specific diagnosis listed was present.

Male Mice
Stomach, Glandular-hyperplasia
Adrenal Cortex, Capsule-hyperplasia
Adrenal Cortex, Subcapsular-hyperplasia

Female Mice
Liver-hyperplasia
Adrenal Cortex-hyperplasia
Adrenal Cortex-hypertrophy
Adrenal Cortex, Capsule-hyperplasia
Adrenal Cortex, Subcapsular-hyperplasia
Adrenal Cortex, Zona Fasciculata-hyperplasia
Stomach, Glandular-hyperplasia
Lung-Alveolar/Bronchiolar Carcinoma, Metastatic, Lung
Mesentery-Inflammation, Chronic Active
Mesentery, Fat-Inflammation, Chronic Active

All tumor diagnoses from all animals in all groups were also reviewed.

RESULTS OF THE PWG REVIEW:

Ovaries and Uterus

There was good agreement between the SP and QAP for the increased incidence of certain age-related ovarian lesions in female treated mice. The PWG confirmed the diagnoses of ovarian atrophy and ovarian cyst. Ovarian atrophy was characterized by the almost complete lack of histologically evident follicular and corpus luteum development and a predominance of interstitial tissue. The diagnosis of ovarian cyst was non-specific and included several different types of cysts such as follicular, epithelial inclusion, rete and paraovarian. All ovaries were also evaluated independently by Dr. B. Davis who agreed with the ovarian changes (see attached memo).

The incidence of granulosa cell tumors (benign and malignant) was slightly increased (4/50=8%) in the mid-dose group only. All of the granulosa cell tumors diagnosed in this study were reviewed by the PWG and the benign tumor in the one high-dose female, the 3 benign tumors in the mid-dose mice and the 1 malignant tumor in the mid-dose were confirmed. In the malignant tumor (mid-dose female #306), neoplastic cells were present in the surrounding ovarian fat. The criteria for benign and malignant granulosa cell tumors is summarized below (Pathology of the Mouse, ed. Maronpot, 1999).

- Varying-sized follicles that compress adjacent tissue or completely efface the ovary
- Round to cuboidal cells arranged on a delicate basement membrane
- Cell nuclei centrally-located, round, oval or indented w/coarsely stippled chromatin
- Mitotic figures few-many
- Pattern may be tubular
- Malignant GCT invade surrounding fat and bursae, may metastasize; pleomorphic cells often arranged in sheets occasionally forming discrete follicles; mitotic rate is often high.

The incidence of ovarian hemangioma as diagnosed by the SP was slightly increased (3/50=6%) in the mid-dose group. The QAP considered that the term "angiectasis" was more appropriate for the multiple, dilated, thin-walled blood vessels observed in the ovaries of some of the treated mice. The PWG reviewed representative examples of these lesions and concluded that these vascular changes were hemangiomas. PWG participants agreed that criteria for hemangiomas included presence of a focal, well demarcated, well-differentiated endothelial proliferation composed

of multiple, dilated, thin-walled cavernous spaces filled with erythrocytes. Some participants commented they included increased stromal supporting tissue, more endothelial cells per linear area and the presence of a mass rather than simply dilatation in the criteria.

Since there was generally good agreement between the SP, QAP and CP for the incidence and diagnosis of ovarian cystadenomas, only one example was reviewed by the PWG. The PWG agreed that the term ovarian cyst should be applied to simple walled structures, whereas cystadenomas contained fronds and branching papillary projections of cuboidal to columnar epithelial cells arranged on a thin fibrovascular stroma.

The PWG reviewed representative examples of uterus and confirmed that the SP diagnosis of "Uterus-hydrometra" should be changed to "Cystic Endometrial Hyperplasia" grade 3 or 4 in order to be consistent with the recent recommendation from the NTP (News Notes Vol 1 Issue 1). The PWG agreed that the term hydrometra is not considered to be a microscopic diagnosis but rather a gross finding and should be added into the lexicon for gross necropsy terminology. There was good agreement between the SP, QAP and CP for the incidence of cystic endometrial hyperplasia.

In the uterus, the incidence of mild to moderate angiectasis was slightly increased in the low and mid-dose groups and there was good agreement between the SP, QAP and CP. In mid-dose female 336 and low-dose female 268, the PWG agreed with the QAP diagnosis of angiectasis rather than the SP diagnosis of uterine hemangioma. Angiectasis was characterized by a sinusoidal-like dilation of multiple, thin-walled blood vessels, predominantly in the myometrium. Uterine hemangiomas were characterized by a focal, usually well-demarcated capillary or sinusoidal proliferation consisting of a mass of variably sized cavernous spaces lined by a single layer of well-differentiated endothelium. There was no treatment-related increase in the incidence of uterine hemangioma since the incidences were 1/50, 2/50, 0/50 and 2/50 in the control, low, mid, and high-dose groups, respectively.

Since the incidence of uterine hemangiosarcomas was increased in the high-dose group (3/50=6%), all of these were reviewed by the PWG. The PWG concluded that the lesion in high-dose females 363 and 367 was a hemangioma rather than hemangiosarcoma, and in high-dose female 352, the lesion was angiectasis rather than hemangiosarcoma. Therefore, it was considered that there was no increase of uterine hemangiosarcomas in the high-dose female mice in this study and no treatment-related increase in uterine tumors.

In low-dose female 276, the SP diagnosed an uterine leiomyoma and the QAP diagnosed "not present in section". The PWG agreed with the QAP and considered the change to be possibly myometrial hypertrophy.

Adrenal Cortex

The SP and QAP diagnosed an increased incidence (3/50=6%) of subcapsular adenomas in the adrenals of high-dose female mice. This was slightly higher than the NTP historical control range (2/50). The PWG reviewed all 3 cases and confirmed a subcapsular adenoma in high-dose female #380 with all others as subcapsular hyperplasia (high-dose females # 371, #389). Therefore, this revised incidence was within the historical control range and was not considered to be treatment-related. In addition, the PWG reviewed all cases considered by the CP to be borderline between subcapsular hyperplasia and subcapsular adenoma and concluded that all were subcapsular hyperplasia. Subcapsular adenomas were distinguished by a discrete nodular appearance and the

F 08

presence of at least slight compression of the surrounding cortex. Other criteria for subcapsular adenomas were (Pathology of the Mouse, ed. Maronpot, 1999):

- composed of varying combinations of type A and type B cells
- range from 6-15 mm diameter
- usually composed of fusiform cells arranged in cords and packets
- mild cytomegaly may be observed
- larger than hyperplasia
- may destroy the normal architecture of capsule and/or the gland

In order to maintain diagnostic consistency in terminology, representative examples of subcapsular hyperplasia were reviewed by the PWG and it was agreed that this term should be used for those lesions with the following criteria (Pathology of the Mouse, ed. Maronpot, 1999).

- Composed of type A (spindle) cells, type B (polygonal) cells, or a mix
- Can be focal, multifocal, diffuse or circumferential
- Can have wedge-shaped larger foci, spherical nests or glandular structures
- Usually no compression of surrounding tissue or bulging above surface

Liver

Since there was generally good agreement between the SP, QAP and CP for the significantly higher incidences of certain degenerative and proliferative lesions in the livers of male and female-treated mice, only selected controversial lesions were reviewed by the PWG. In the male mice only, the total incidence of primary liver tumors (hepatocellular adenomas, hepatocellular carcinomas) was increased in low and mid-dose groups compared to the control group. Other lesions with significantly increased incidences in the livers of male mice in the low and mid-dose groups included eosinophilic, basophilic and mixed cell foci, minimal to mild chronic active inflammation, minimal to moderate cytoplasmic vacuolization, mild to moderate angiectasis, and minimal to moderate pigmentation. Lesions with significantly increased incidences in the livers of low and mid-dose female mice included basophilic and mixed cell foci, minimal to mild cytoplasmic vacuolization, mild to marked angiectasis, minimal to moderate hematopoietic cell proliferation, minimal to moderate necrosis and eosinophilic foci.

None of the changes made by the PWG for the slides reviewed impacted the overall incidences of any of the diagnoses or changed the overall conclusions for changes observed in the liver.

Stomach, Forestomach

The SP did not originally classify inflammatory and hyperplastic lesions of the forestomach as focal or diffuse; during the review by the QAP and CP and in agreement with the NTP pathologist, it was decided to further qualify the distribution of the lesions as "focal" or "diffuse". Lesions affected by this change included epithelial hyperplasia, hyperkeratosis, ulcer, and chronic active inflammation.

There was good agreement in the incidences and diagnoses for squamous cell carcinomas and squamous cell papillomas by the SP, QAP and CP. These tumors were increased in mid and high-dose male mice and in female mice, the incidence of squamous cell carcinomas was increased in the high-dose group especially, and the incidences of squamous cell papillomas were increased in the mid and high-dose group. The histologic appearance of these lesions was generally consistent with descriptions found in Pathology of the Mouse, ed. Maronpot, 1999. Squamous cell papillomas consisted of a solitary stalk of lamina propria protruding into the stomach lumen, with multiple finger-like projections arising from the stalk. The squamous epithelium is usually hyperplastic. Squamous cell carcinomas appeared in both the well-differentiated squamous cell and poorly-differentiated spindle cell types, and were highly malignant and appeared to have metastasized to a number of organs (primarily the liver). Other organs that had metastases included pancreatic lymph node, pancreas, glandular stomach, gallbladder, spleen, kidney, lung, mesenteric lymph node, bronchial lymph node, mesenteric fat, skeletal muscle, mediastinal lymph node, prostate, and adrenal gland.

Due to the consensus of reviewing pathologists, these lesions were discussed with the PWG but not reviewed. Only a few controversial cases were reviewed by the PWG, however none of the changes had an impact in the overall conclusion of the study.

Lung

The review by the QAP and CP confirmed the presence of a significantly higher incidence of primary lung tumors in female treated mice. The incidences of alveolar/bronchiolar carcinomas were increased in the low, mid and high-dose groups (10%, 12%, 8%) compared to the control group and at or above the current NTP historical control range for female mice (oral feed studies: 0-8%). The incidence of alveolar/bronchiolar adenomas was increased in the mid-dose group compared to control. The histologic appearance of the lung tumors was generally consistent with descriptions in Pathology of the Mouse, ed. Maronpot, 1999. Primary alveolar/bronchiolar carcinomas were characterized by formation of nodular sheets, heterogenic growth patterns, nuclear atypia, localized invasiveness, and intrapulmonary and distant metastasis.

Since there was good agreement between the reviewing pathologists for the treatment-related lesions, it was agreed with the NTP pathologist to have the PWG review only a few controversial slides. The PWG also confirmed the bronchial adenoma in control female #223.

Eye and Harderian Glands

The review by the QAP and CP confirmed the increased incidence of Harderian gland adenomas and hyperplasia in the low, mid and high-dose male mice. The incidences of carcinomas were increased in the mid and high-dose male mice. In the female mice, the incidences of Harderian gland adenomas and carcinomas were significantly increased in the mid and high-dose groups. Harderian gland hyperplasia was generally focal, alveolar architecture was maintained, there were generally increased number of cells that were often larger than normal and tended to be tinctorially distinct. Harderian gland adenomas were characterized by a loss of alveolar structure and at least minimal compression of the surrounding tissue. This lesion was usually unilateral but was occasionally bilateral. Harderian gland carcinomas generally resembled adenomas, but featured localized invasion of structures adjacent to the gland and/or distant metastasis. Since there was good agreement between all the reviewing pathologists, only one example each of a Harderian gland adenoma and a carcinoma was shown to the PWG to confirm the diagnoses.

The only eyes examined histologically by the SP and QAP were those with the associated gross finding of "eye abnormality". Eye lesions were most commonly associated with the presence of Harderian gland tumors and were consistent with changes that would occur secondary to ocular compression by these neoplasms. Thus, the increase in eye lesions was not considered to be a direct effect of treatment with acrylonitrile. The SP diagnosed eye lesions as atrophy, synechia, lens cataract, corneal and scleral inflammation and retinal degeneration. The QAP consolidated these lesions into the primary diagnosis of "atrophy" in some animals when it was considered that the lesions were a sequelae to bulbous atrophy. In the few abnormal eyes where atrophy was not diagnosed, the original diagnoses of corneal or scleral inflammation and/or synechia were retained. The CP considered that there were inconsistencies in when the diagnosis of "atrophy" was made despite using the criteria that eye atrophy was characterized by a non-artifactual collapse of the aqueous and vitreous chambers, with or without lens luxation. During the review by the CP and in agreement with the NTP pathologist, it was decided that to be accurate, the diagnoses would not be consolidated and all eyes that met the criteria for "atrophy" would have the diagnosis in addition to all other ocular changes observed (cataract, inflammation, etc.), and those that did not have atrophy would still have the other changes diagnosed.

Mandibular and Mediastinal Lymph Nodes, and Spleen

Examples of lymph nodes and spleen were not reviewed by the PWG, but the review by the CP confirmed the findings of the SP and QAP that there was a higher incidence of minimal to marked lymphoid hyperplasia in the mandibular lymph nodes in the mid-dose group of female mice. The CP review also confirmed the QAP conclusion that the incidence of lymphoid hyperplasia was not significantly increased in the mediastinal lymph nodes of treated female mice. The CP also confirmed the conclusion of the QAP after reviewing all spleens from female mice that there was a slight decrease in the incidence of splenic lymphoid hyperplasia in high-dose animals compared to controls.

In the lymph nodes, lymphoid hyperplasia was characterized by increased numbers of small to medium-sized lymphocytes in follicular and/or parafollicular locations, inevitably resulting in some lymphadenomegaly. Lymphoid hyperplasia in the spleen included expansion and increased pallor of white pulp areas, coalescence of lymphoid follicles, and in some animals, splenomegaly.

Miscellaneous Lesions

A number of lesions were examined by the PWG to either confirm their diagnosis or because a discrepancy existed. In most instances, these lesions represented unusual or diagnostically challenging lesions/neoplasms that were either diagnosed once or only in a few instances. None were considered to be treatment-related. Two cases (control male #29; high-dose female #374) of liposarcoma with metastases that were diagnosed by the SP but considered to be sarcomas by the QAP were reviewed by the PWG. The PWG confirmed that these tumors should be designated sarcomas.

The PWG changed several diagnoses that were made by the SP and QAP. The final diagnoses were hemangioma (small intestine, ileum) in high-dose male #192, mucinous metaplasia (prostate) in high-dose male #194, adenoma (small intestine, duodenum) in low-dose male #58, and meningioma (brain) in control female #246.

POST-PWG ACTION ITEMS:

- CP to review the ovaries from the acrylonitrile subchronic study in mice for potential treatment-related lesions and confirmation of terminology

Shelley V. Ching

Shelley V. Ching, D.V.M., Ph.D.
Diplomate, ACVP
PWG Chairperson

9/25/00
Date

PATHOLOGY WORKING GROUP CHAIRPERSON'S REPORT

TWO-YEAR CHRONIC INHALATION TOXICITY AND CARCINOGENICITY STUDY OF VANADIUM PENTOXIDE (CAS# 1314-62-1; C61427) IN RATS

RECEIVED
OPPT CBIC
2000 DEC 18 AM 10:45

Participants: Mark Butt, DVM (PAI, Chairperson), Rick Hailey, DVM (NIEHS), Ronald Herbert, DVM, PhD (NIEHS), Abraham Nyska, DVM (NIEHS), Gordon Flake, MD (NIEHS), Robert Maronpot, DVM (NIEHS), Amy Brix, DVM, PhD (EPL), Michael Jokinen, DVM (PAI), Jeffrey Everitt, DVM (CIIT), Roger Renne, DVM (Battelle), Kristen Nikula, DVM, PhD (Monsanto), Donald Dungworth, BV Sc., DVM (UC Davis)

Date: May 31 and June 1, 2000
Site: NIEHS, Research Triangle Park, NC

The PWG was convened to evaluate selected slides from F344 rats administered vanadium pentoxide via whole body inhalation for two years. The doses and numbers of animals examined microscopically per group were as follows:

Dose	M	F
0	50	50
0.5 mg/m^3	50	50
1.0 mg/m^3	50	50
2.0 mg/m^3	50	50

This study was conducted at Battelle Toxicology Northwest, Richland, Washington. The study pathologist (SP) was Dr. Roger Renne. The Quality Assessment Pathologist (QAP) was Dr. Amy Brix.

A number of organs were reviewed by the QAP for potential treatment-related effects; the QAP reviewed these organs in all animals for all diagnoses, neoplastic and nonneoplastic. Organs reviewed for all diagnoses were as follows:

Male Rats	Female Rats
Larynx	Larynx
Lung	Lung
Nose	Nose
	Uterus

RECEIVED
OPPT NCIC
2000 DEC 26 AM 10:45

The following organs/tissues were examined for all animals in all groups for the below listed lesions:

Male Rats
Lymph Node, Bronchial – Pigmentation
Lymph Node, Mediastinal – Pigmentation

Female Rats
Lymph Node, Bronchial – Pigmentation
Lymph Node, Mediastinal – Pigmentation
Ovary – Cyst

The following organs from the sex indicated were reviewed when the specific diagnoses listed as follows were present:

Male Rats
Lymph Node, Renal, Iliac – Leukemia Mononuclear
Bone, Cranium, Mandible – Sarcoma, Metastatic, Skin
Pancreas – Infiltration Cellular, Lipocyte
Penis – Hyperplasia, Basal Cell
Eye, Lens – Cataract
All Tumors in All Organs (Except for Testes – Interstitial Cell Tumor)

Female Rats
Skin – Schwannoma Malignant
Skin, Pinna – Schwannoma Benign
Skin – Inflammation, Acute
Skin – Inflammation, Suppurative
Mammary Gland, Epithelium – Hyperplasia
Mammary Gland, Duct – Hyperplasia
Tooth, Periodontal Tissue – Hyperplasia
Eye – Cataract
Eye, Lens – Cataract
All Tumors in All Organs

SUMMARY OF REVIEW FINDINGS

The following were considered to be related to exposure to the test material.

Lungs
--Increased combined incidences of **Alveolar/bronchiolar adenoma and Alveolar/bronchiolar carcinoma** in males and females

--Increased incidence of **Alveolar epithelium, squamous metaplasia, atypical** in males
-- Increased incidence of **Alveolar epithelium, squamous metaplasia,** in males and females
-- Increased incidence of **Bronchiolar epithelium, squamous metaplasia,** in males and females
-- Increased incidence of **Alveolar epithelium, hyperplasia** in males and females
-- Increased incidence of **Bronchiolar epithelium, hyperplasia** in males and females
-- Increased incidence of **Alveoli, cellular infiltration, histiocytic** in males and females
-- Increased incidence of **Alveoli, pigment** in males and females
-- Increased incidence of **Interstituim, fibrosis** in males and females
-- Increased incidence of **Chronic/active inflammation** in males and females

Nose
-- Increased incidence of **Respiratory epithelium, goblet cell hyperplasia** in males and females

Larynx
-- Increased incidence of **Respiratory epithelium, degeneration** in males and females
-- Increased incidence of **Respiratory epithelium, squamous metaplasia** in males and females
-- Increased incidence of **Respiratory epithelium, hyperplasia** in males and females
-- Increased incidence of **Chronic inflammation** in males and females

CONDUCT OF THE PWG

Prior to the PWG, the PWG Chairperson reviewed all of the pathology data tables, the SP's narrative, the Pathology Data Review, the Quality Assessment Report, and microslides of tissues selected for QA review. The PWG Chair then selected slides for review by the PWG, including representative examples of lesions, and lesions for which there was a difference in diagnosis among the SP, QAP, and PWG Chair.

RESULTS OF THE PWG REVIEW

Lung-Neoplastic Lesions

There was, in general, very good agreement between the SP, QAP, PWG Chair and the PWG members regarding the diagnosis of Alveolar/Bronchiolar (A/B) adenoma and carcinoma. Minor differences and changes in diagnoses (from the original determination of the SP) did not affect the overall decision that the increased incidence of combined A/B adenomas and carcinomas was related to exposure to the test material in males and females.

The PWG confirmed the diagnoses of metastatic neoplasms in the lungs of various animals. These lesions were reviewed to verify that these neoplasms were not primary lung tumors.

The PWG did not confirm the occurrence of squamous cell carcinoma in the high dose males. Each of the six squamous cell carcinomas diagnosed by the SP in the high dose male rats was reviewed by the PWG. In each case, these lesions were diagnosed as "Alveolar epithelium,

metaplasia, squamous, atypical" by a consensus of the PWG. This issue is fully discussed in the section below on Lung-Non-Neoplastic Lesions.

Lung - Non-Neoplastic Lesions

The most striking pulmonary change encountered in this study was the multiple foci of "Alveolar epithelium – hyperplasia" and "Bronchiolar epithelium – hyperplasia". This change affected all male and female treatment groups. These foci were characterized by a proliferation of epithelium in alveoli, alveolar ducts and distal terminal bronchioles. The change was most pronounced in the high dose groups of both sexes. Multiple participants remarked that the alveolar and terminal bronchiolar epithelial hyperplasia was consistent with bronchiolization, a process where bronchiolar epithelium proliferates and migrates down into alveolar ducts and adjacent alveoli.

Various changes were associated with the hyperplasia of the bronchiolar and alveolar epithelium. In some animals, cells with altered phenotypes resembling goblet cells or respiratory epithelial (ciliated, columnar) cells were present within the foci of hyperplasia. In the initial reviews, the SP had diagnosed goblet cell metaplasia in 1 of 50 high dose females and 7 of 50 high dose males. The QAP diagnosed respiratory metaplasia in 41 of 50 high dose males and 38 of 50 high dose females. The PWG participants noted these phenotypic changes. However, it was the consensus of the PWG participants that these changes represented characteristics of the bronchiolar and alveolar hyperplasia and should not be diagnosed separately. Thus, "Alveolar epithelium, goblet cell metaplasia" was globally deleted from the pathology data in this study and the QAP diagnosis of "Alveolar epithelium, metaplasia, respiratory" was not added to the data.

Two other changes associated with the alveolar and bronchiolar hyperplasia were "Alveolar epithelium, squamous metaplasia" and "Bronchiolar epithelium, squamous metaplasia". The squamous metaplasia was considered to be a striking feature of test material exposure in the high dose males and females. As discussed above in the section on neoplastic changes in the lungs, some high dose males had foci of "Alveolar epithelium, squamous cell metaplasia, atypical" that resembled squamous cell carcinoma. Other high dose males and several high dose females had smaller, more typical foci of squamous metaplasia.

In association with the foci of hyperplasia were variable, but generally minimal, areas of inflammation. The inflammation was characterized by infiltrates of mononuclear cells and variable but fewer neutrophils, interstitial fibrosis, intraalveolar pigment and intraalveolar infiltrates of histiocytes. The PWG determined that, as determined by the SP, "Alveoli, cellular infiltration, histiocytic" should remain as a separate diagnosis since the incidence of these cells was so common in the treated groups and because the cells did occur without other features of inflammation. Similarly, the PWG determined that "Interstitium, fibrosis", although generally minimal in the animals in this study, should remain as a separate diagnosis since the degree of fibrosis varies widely in association with the inhalation of various particulate materials and

because of the importance of fibrosis in determining subsequent recovery from pulmonary injury and lung remodeling. The PWG determined that the diagnosis of "Interstitium, inflammation" should be changed globally in the pathology data to a diagnosis of "Inflammation, Chronic/active" since the inflammation was not always limited to the interstitium and because a more general term was deemed to be more appropriate. "Alveoli, pigment" was confirmed by the QAP and PWG Chair's review. The diagnosis of "Alveoli, proteinosis" was considered by the PWG participants to be an unnecessary diagnosis in this study. The SP recorded that in many animals, there were intraalveolar accumulations of a material consistent with surfactant and also accumulations of mucin. The SP choose Alveoli, proteinosis as being the best possible diagnostic term for these accumulations. The PWG participants agreed with the study pathologist that some animals had intraalveolar accumulations of probable surfactant and mucin but that these accumulations were consistent with the inflammation and hyperplastic changes already present and were not significant enough to warrant a separate diagnosis. Thus, "Alveoli, proteinosis" was globally deleted from the pathology data.

As previously noted, the PWG did not confirm the occurrence of squamous cell carcinoma in the high dose males. In each case, these lesions were diagnosed as "Alveolar epithelium, metaplasia, squamous, atypical". Because of this PWG decision, "Squamous Cell Carcinoma" in the lung was determined to not be related to the test material exposure. There was considerable discussion among the participants regarding this issue. It was agreed by the participants that the biology, and thus the pathogenesis and subsequent behavior of these squamous cell lesions, could not be precisely determined. In general, these lesions were not diagnosed as squamous cell carcinomas because of the lack of evidence of tissue invasion. "Alveolar epithelium, metaplasia, squamous, atypical" was decided to be an appropriate diagnosis because the lesions, in general, were not locally invasive but were larger than other foci of squamous metaplasia noted in this study, the cells exhibited more pleomorphism than expected in a focus of squamous metaplasia, cells often lacked polarity and orderly differentiation in areas of the lesion, and the lesion tended to distort the adjacent lung parenchyma but did not appear to exhibit local tissue invasion. This lack of local tissue invasion was the basis from separating "Alveolar epithelium, metaplasia, squamous, atypical" from squamous cell carcinoma.

As a result of the PWG consensus on the issue of "Squamous cell carcinoma" versus "Alveolar epithelium, metaplasia, squamous, atypical", the PWG chairperson was tasked with the review of all lesions diagnosed as "Alveolar epithelium, metaplasia squamous" and "Bronchiolar epithelium, metaplasia squamous" to decide if any of those lesions qualified as a diagnosis of "Squamous metaplasia, atypical".

As part of this review, the PWG chairperson reviewed the following reference:

Boorman GA et. al. 1996. Classification of cystic keratinizing squamous lesions of the rat lung: report of a workshop. Toxicologic Pathology 24 (5) 564-572.

This reference was also available and discussed at the PWG.

The workshop reported in that reference was convened to review and discuss the morphology and biologic significance of keratinizing lesions of the lung in rats associated with the inhalation of a variety of particulates. The workshop defined four specific keratinizing lesions: pulmonary squamous cell metaplasia, pulmonary keratinizing cyst, pulmonary cystic keratinizing epithelioma and squamous cell carcinoma. Pulmonary squamous cell metaplasia was characterized by a transition of alveolar cells (in a gradual manner) to squamous metaplastic cells with distortion but not obliteration of alveolar structure. Pulmonary keratinizing cyst (PKC) was characterized by being a discrete lesion lined by flattened squamous epithelium forming a thin cyst wall surrounding a central mass of keratin. Orderly maturation and few mitotic figures characterized the cyst wall. Pulmonary cystic keratinizing epithelioma (PCKE) was characterized by a more complex, disorganized and thicker cyst wall (when compared to the keratinizing cyst) filling continuous alveoli in an irregular "cobblestone" appearance. Additionally, there is a lack of orderly maturation, increased mitotic figures, and areas of basal cell metaplasia. Generally, but not in all cases, the epithelial component surrounds a prominent, central area of keratin. Squamous cell carcinoma was defined by cellular atypia, loss of polarity, increased mitotic activity and invasive growth into adjacent tissue. Some squamous cell carcinomas were observed to arise within the wall of PCKE; others arose *de novo*.

It was the decision of the PWG chairperson that no lesions other than those originally diagnosed by the study pathologist as "Squamous cell carcinoma" (and subsequently reviewed by the PWG) should be diagnosed as "Metaplasia squamous, atypical". In some animals (705, 726, 729, 608, 615), the squamous metaplasia lesions possessed some characteristics of PCKE. However, these lesions were not discrete but tended to affect locally extensive alveolar areas. Thus, a diagnosis of "Alveolar epithelium, metaplasia, squamous" was deemed to be appropriate since the epithelium did not exhibit the aforementioned features of "Metaplasia, squamous, atypical". Additionally, in two animals (630, 634) the squamous metaplasia lesions had focal areas that had features of PKC. However, because the lesions were locally extensive and not discrete, it was interpreted that the areas resembling PKC were areas of squamous metaplasia with excessive keratin accumulation. Thus, a diagnosis of "Alveolar epithelium, metaplasia, squamous" was deemed most appropriate.

Nose

Selected sections of the nose were reviewed by the PWG for the presence of Respiratory epithelium, goblet cell hyperplasia. The PWG agreed unanimously with the findings of the SP and QAP. A review of the data indicated that Respiratory epithelium, goblet cell hyperplasia was related to test material exposure in all male treatment groups and the 2.0 mg/m^3 females

Larynx

Selected sections of the larynx were reviewed by the PWG for the presence of the following lesions: Respiratory epithelium, degeneration; Respiratory epithelium, squamous metaplasia; Respiratory epithelium, hyperplasia; and Chronic inflammation. The PWG agreed with the findings of the SP, QAP and PWG chairperson (who were in general agreement regarding the larynx changes) that these lesions were related to exposure to the test material in all treated male and female groups.

Miscellaneous

Various selected tissue sections (containing changes not associated with exposure to the test material) were reviewed because of their uniqueness or because of differences between the SP, QAP and/or the PWG. For this set of lesions, some PWG members abstained from voting because of a lack of experience with lesions not related to the respiratory system.

Four brain neoplasms diagnosed as Glioma benign, Granular cell tumor malignant, Astrocytoma malignant or Glioma malignant were all diagnosed as Astrocytoma malignant by the PWG.

Three neoplasms on the pinna or skin diagnosed as Melanoma benign or Schwannoma benign were diagnosed by the PWG as Neural Crest neoplasm benign.

A few examples of Uterus – Polyp stromal diagnosed by the SP were reviewed by the PWG. These were selected for review because there was a slight increase of stromal polyps in the high dose females. There was general agreement between the SP, QAP and PWG chairperson on the overall incidence of stromal polyps in all groups. (The PWG agreed with the QAP that in one animal (514), a diagnosis of Uterus, endometrium – Hyperplasia was more appropriate than Stromal polyp.) However, all three pathologists, as well as the PWG, interpreted this increase to be incidental and unrelated to exposure to the test material.

POST-PWG ACTION ITEMS

Specific diagnoses made by a vote of the PWG members were changed in the pathology data; these changes are noted in the tables included in the PWG Chairperson's report.

The PWG chairperson was directed to review all squamous cell metaplasia lesions in the lungs of all males and females. (The results of this review are thoroughly discussed in the section on Lung-Nonneoplastic Lesions.) As a result of this review, only those lesions previously diagnosed by the SP as Squamous cell carcinoma were determined to be appropriately termed Alveolar epithelium, squamous metaplasia, atypical. All other changes were deemed by the PWG chairperson to be appropriately diagnosed in accordance with the decisions of the PWG. Because the PWG reviewed all diagnosis of Squamous cell carcinoma in the lung, these changes are already included in the tables accompanying the PWG Chairperson's report.

In accordance with the PWG's findings, all diagnoses of Lung, Interstitium – Inflammation were globally changed to Lung – Inflammation chronic active.

In accordance with the PWG's findings, Lung, Alveolar Epithelium – Goblet cell metaplasia was globally deleted as a diagnosis.

In accordance with the PWG's findings, Lung, Alveolus – Proteinosis was globally deleted.

Mark T. Butt

Mark T. Butt, DVM
Diplomate, American College of Veterinary Pathologists
PWG Chairperson

7.27.00

Date

D. 08.

PWGCHM
13141-62-1
C61427B

ADDENDUM

TO THE
PATHOLOGY WORKING GROUP CHAIRPERSON'S REPORT
FOR THE
2-YEAR CHRONIC INHALATION STUDY OF VANADIUM PENTOXIDE
IN B6C3F1 MICE

It was noted during the PWG for this chemical that no lymphomas had been diagnosed in the control females. Examination of NTP historical control data indicated this was a unique occurrence. Consequently, the PWG Chairperson was directed to review the lymphoid tissues (spleen, thymus, lymph nodes) as well as the liver from all control and exposed females from this study to determine the presence or absence of lymphoma. During the review proliferative lymphoid lesions that were potentially lymphomas were observed by the PWG Chairperson in a few animals in the control and exposed groups. Generally, the SP had diagnosed these proliferative lymphoid lesions as lymphoid hyperplasia. In addition, high dose female number 726, which had a lymphoma diagnosed in the mediastinal lymph node, appeared to have lymphoma in other tissues in which hyperplasia been diagnosed.

On August 30, 2000 a special Pathology Working Group was convened at NIEHS to review the slides which the PWG Chairperson felt potentially contained lymphomas. The participants were Drs. M. Jokinen (PAI-Chairperson), G. Boorman (NIEHS), J Hailey (NIEHS), R. Herbert (NIEHS), J. Mahler (NIEHS). R. Maronpot (NIEHS), A. Nyska (NIEHS), and J. Peckham (EPL). The PWG members were of the opinion that each of the animals examined but one had a lymphoma, and the remaining animal had a lymphoid hyperplasia. Findings in individual animals and tissues are listed in the attached table.

In addition, two animals (male number 641 and female number 736) had been diagnosed as having parathyroid gland carcinomas. These slides were examined by the original PWG which concurred with the diagnosis of parathyroid gland carcinoma for each neoplasm, but which also recommended the neoplasms be stained immunohistochemically to determine with certainty whether the neoplasms were of parathyroid gland origin or were of thyroid gland follicular cell or C-Cell origin. Immunohistochemical staining was performed and was strongly positive for calcitonin in each of the two neoplasms indicating that both neoplasms were C-Cell carcinomas. Consequently, the original diagnoses of parathyroid gland carcinomas in both animals were changed to C-Cell carcinoma in the pathology findings.

Micheal P. Jokinen, DVM
Diplomate, ACVP
PWG Chairperson

10/4/00
Date

D 09

PATHOLOGY WORKING GROUP CHAIRPERSON'S REPORT

2-Year Chronic Study of **Vanadium Pentoxide** in B6C3F1 Mice

Participants: Drs. M. Jokinen (PAI - PWG Chairperson), D. Dixon (NIEHS), J. Everitt (CIIT), G. Flake (NIEHS), J. Hailey (NIEHS), R. Herbert (NIEHS), M. Jayo (PAI – Observer), R. Miller (Battelle Northwest – SP), A. Nyska (NIEHS), K. Ozaki (NIEHS – Observer), and J. Seely (EPL).

Date: July 25, 2000
Site: NIEHS, Research Triangle Park, NC

The PWG was convened to evaluate selected slides from the two year inhalation study of vanadium pentoxide in B6C3F1 mice. Exposure levels and numbers of animals examined microscopically per group were as follows:

Dose	M	F
0 mg/m3	50	50
1.0 mg/m3	50	50
2.0 mg/m3	50	50
4.0 mg/m3	50	50

The study was conducted at Battelle Northwest Laboratories. The Study Pathologist (SP) was Dr. R. Miller and the Quality Assessment Pathologist (QAP) was Dr. A. Brix of EPL.

A number of organs were reviewed by the QAP for potential treatment-related effects: the QAP reviewed these organs in all animals for all diagnoses, neoplastic and nonneoplastic. Organs reviewed for all diagnoses were as follows:

Male Mice	Female Mice
Larynx	Larynx
Lung	Lung
Nose	Nose

In addition, all neoplasms from all organs from all animals were reviewed, bronchial lymph nodes were reviewed from all males and all females for Lymph Node, Bronchial – Hyperplasia, and uterus was reviewed from all females for Uterus – Hydrometra and Uterus – Hyperplasia, Cystic. Moreover, a variety of diagnoses were reviewed only in those animals in which they had been diagnosed.

SUMMARY OF REVIEW FINDINGS

Incidences of **Lung – Alveolar/Bronchiolar Adenoma, Multiple; Alveolar/Bronchiolar Carcinoma;** and **Alveolar/Bronchiolar Carcinoma, Multiple** were increased in all exposed groups of males as compared with control males.

Incidences of **Lung – Alveolar/Bronchiolar Adenoma; Alveolar/Bronchiolar Adenoma, Multiple; Alveolar/Bronchiolar Carcinoma;** and **Alveolar/Bronchiolar Carcinoma, Multiple** were increased in all exposed groups of females as compared with control females.

Incidences of **Lung, Alveolus – Infiltration, Cellular, Histiocyte; Lung, Alveolar Epithelium – Hyperplasia; Lung, Bronchiole, Epithelium – Hyperplasia;** and **Lung – Inflammation, Chronic** were increased in each of the exposed groups of males and females as compared with respective controls.

Incidences of **Larynx, Respiratory Epithelium, Epiglottis – Metaplasia, Squamous** were increased in all exposed groups of males and females, and incidences of **Larynx, Squamous Epithelium – Hyperplasia** were increased in exposed groups of males as compared with respective controls.

Incidences of **Nose – Inflammation, Suppurative** were increased in mid- (2.0 mg/m3) and high (4.0 mg/m3) exposure groups of males and females as compared with respective controls.

Incidences of **Nose, Olfactory Epithelium – Atrophy** were increased in high exposure groups of males and females as compared with controls.

Incidences of **Nose, Olfactory Epithelium – Degeneration, Hyaline; Nose, Respiratory Epithelium – Degeneration, Hyaline;** and **Nose, Respiratory Epithelium – Metaplasia, Squamous** were increased in all exposed groups of males and females as compared with controls.

Incidences of **Lymph Node, Bronchial – Hyperplasia** were increased in mid- and high exposure groups of males and in all exposed groups of females as compared with controls.

CONDUCT OF THE PWG

Prior to the PWG, the PWG Chairperson reviewed the pathology tables, the SP's narrative, the Pathology Data Review, the Quality Assessment Report, and microslides of tissues selected for QA review. The PWG Chair then selected slides for review by the PWG, including representative examples of lesions, and lesions for which there was a difference in diagnosis among the SP, QAP, and PWG Chair.

RESULTS OF THE PWG REVIEW

Lung

The SP had diagnosed increased incidences of **Lung – Alveolar/Bronchiolar Adenoma, Multiple; Alveolar/Bronchiolar Carcinoma;** and **Alveolar/Bronchiolar Carcinoma, Multiple** in all exposed groups of males as

compared with control males. In addition, the SP had diagnosed increased incidences of **Lung – Alveolar/Bronchiolar Adenoma; Alveolar/Bronchiolar Adenoma, Multiple; Alveolar/Bronchiolar Carcinoma;** and **Alveolar/Bronchiolar Carcinoma, Multiple** in all exposed groups of females as compared with control females. These findings were confirmed by the QA/PWG review. In general, there was good agreement among the SP, QAP, and PWG Chair concerning the diagnoses of lung neoplasms. The PWG reviewed several examples of lung neoplasms in which there had been agreement among the SP, QAP, and PWG Chair and concurred with the diagnoses. The PWG then reviewed a number of slides of disagreements concerning the diagnosis of lung neoplasms. Most of these concerned whether or not multiple neoplasms were present. Neoplasms examined by the PWG included examples of metastasis of alveolar/bronchiolar carcinoma into the mediastinum; the PWG confirmed the presence of the metastasis.

The SP had also diagnosed increased incidences of **Lung, Alveolar Epithelium – Hyperplasia; Lung, Bronchiole – Hypertrophy; Lung, Alveolus – Infiltration Cellular, Histiocyte; Lung, Interstitium – Inflammation;** and **Lung, Interstitium – Fibrosis** in exposed groups of males and females. The QAP concurred with the SP's findings of alveolar epithelial hyperplasia and bronchiole hypertrophy but recommended combining the diagnoses of histiocyte infiltrate, interstitial inflammation, and interstitial fibrosis under a single diagnosis of **Lung – Inflammation, Chronic Active.**

The PWG reviewed representative examples and concurred with the SP's diagnoses of **Lung, Alveolar Epithelium – Hyperplasia; Lung, Alveolus – Infiltration Cellular, Histiocyte;** and **Lung, Interstitium – Fibrosis**, and recommended all of these diagnoses be retained. In examination of the bronchiolar changes, the PWG members did agree that some hypertrophy of the bronchiolar epithelium was present but noted that a small degree of hyperplasia of the bronchiolar epithelium was also present. The PWG members were of the opinion that hyperplasia was the primary change in the bronchiolar epithelium and that the hypertrophy was a part of this change. Consequently, the PWG recommended the SP's diagnoses of **Lung, Bronchiole – Hypertrophy** be changed to **Lung, Bronchiole, Epithelium – Hyperplasia**, and that diagnosis was entered into the Action Taken column of the Slide Review Worksheets. It was later found that the site **Lung, Bronchiole, Epithelium** was unavailable on TDMS and the site **Lung, Bronchiole** had to be used, as this was the available TDMS term. Consequently, the PWG finding for this change was listed on the Chairperson's Report sheets in this report as **Lung, Bronchiole – Hyperplasia**, and the SP's original diagnoses of **Lung, Bronchiole – Hypertrophy** should be changed to **Lung, Bronchiole – Hyperplasia** in the final pathology tables.

In addition, the PWG members agreed that inflammatory changes were present in the lungs but were of the opinion that the diagnosis of **Lung – Inflammation, Chronic** was more appropriate than the SP's diagnosis of interstitial inflammation.

Accordingly, the PWG recommended that all the SP's diagnoses of interstitial inflammation be changed to chronic inflammation.

Microscopically, the alveolar/bronchiolar neoplasms had the typical appearance of these lesions seen in B6C3F1 mice. Adenomas were discrete nodular lesions which generally displaced adjacent normal parenchyma. Adenomas were composed of cuboidal to round or polygonal cells, with moderate amounts of eosinophilic cytoplasm, that formed papillary or alveolar structures, or solid clusters that filled alveolar spaces, or a combination of these. Carcinomas were nodular, generally large, masses, many of which contained areas of infiltration of the surrounding normal parenchyma and/or areas of metastasis within the lung. Several of the carcinomas invaded the adjacent mediastinum and a few were present in the bronchial or mediastinal lymph nodes. A few of the carcinomas metastasized to more distant sites. Carcinomas consisted of pleomorphic, round to cuboidal to tall columnar cells, which formed papillary or tubular structures, or solid sheets of cells, or a combination of these. Carcinomas were distinguished by cellular atypia, infiltrative growth, and the tendency to form multiple cell layers or larger solid cellular areas. The PWG members noted that the carcinomas generally tended to be quite large and it was not unusual to find a carcinoma well over 1cm in diameter.

The alveolar epithelial hyperplasia was seen in alveoli surrounding terminal bronchioles and alveolar ducts, and consisted of areas of alveoli lined by cuboidal epithelial cells, instead of the normal squamous epithelium. Normal alveolar architecture was maintained in hyperplasias. Occasionally, foci of alveolar epithelial hyperplasia were seen in alveoli not adjacent to terminal bronchioles. The severity ranged from minimal to marked, and the overall severity appeared to increase with increasing exposure level.

Hyperplasia of the bronchiolar epithelium was generally a subtle, minimal to mild change seen in the terminal bronchioles and extending into adjacent alveolar ducts. It was characterized microscopically by slightly increased numbers of bronchiolar epithelial cells that appeared to be somewhat more densely packed than normal. Infrequently, the epithelial cells formed small clusters two to three cell layers thick. Admixed with these were scattered larger, somewhat hypertrophic appearing cells with large, prominent nuclei.

Alveolar histiocyte infiltrate consisted of small to large numbers of large histiocytes within alveolar lumens. This change was most marked in lungs containing alveolar/bronchiolar neoplasms, and was especially prominent in lungs containing large alveolar/bronchiolar carcinomas in which large areas of alveoli surrounding the neoplasm were diffusely filled with histiocytes. Small numbers of histiocytes were observed within alveolar lumens in the areas of alveolar epithelial hyperplasia adjacent to terminal bronchioles in numerous lungs, and histiocytes were observed in these areas of alveolar epithelial hyperplasia even in lungs which contained no neoplasms. The PWG examined representative examples and confirmed the above observations concerning the alveolar histiocyte infiltrate. The PWG members noted that alveolar histiocyte infiltrate is a relatively common finding

13

secondary to the presence of alveolar/bronchiolar neoplasms and that the increased incidence of histiocyte infiltrate in exposed males and females was a reflection of the increased incidences of alveolar/bronchiolar neoplasms in these animals. However, the PWG further noted that the presence of this infiltrate in lungs that did not contain neoplasms indicated it occurred as a primary treatment effect independent of the presence of neoplasms. The PWG members also noted it would have been desirable to be able to separate alveolar histiocyte infiltrate secondary to the presence of a neoplasm from that occurring as a primary effect. However, it appeared this could not be accomplished easily or accurately, so the PWG recommended the alveolar histiocyte infiltration be diagnosed whether or not a neoplasm was present. It was further recommended that the incidence and severity of alveolar histiocyte infiltrate be examined in only those animals which did not have alveolar/bronchiolar neoplasms to determine if this change occurred as a primary treatment effect.

The change the SP had diagnosed as interstitial inflammation principally consisted of multifocal aggregates of mononuclear cells, primarily lymphocytes, within the interstitium around airways and vessels, and occasionally within interalveolar septa. Occasionally, foci of inflammation consisting of clusters of alveoli containing alveolar histiocytes and, sometimes, a few neutrophils and/or lymphocytes, often with slight fibrous thickening of the intervening interalveolar interstitium, were seen and were included under the diagnosis of interstitial inflammation. Some lungs, primarily those with marked alveolar histiocyte infiltrate around large alveolar/bronchiolar neoplasms, also contained scattered foci of neutrophilic infiltrate. The PWG examined representative examples and came to the conclusion that this change was most appropriately diagnosed as **Lung -- Inflammation, Chronic**. This inflammatory change was commonly seen in lungs containing alveolar/bronchiolar neoplasms. However, it was also observed in a few lungs without neoplasms.

Interstitial fibrosis was a minor change consisting, generally, of multiple small foci of thickening of the interalveolar interstitium with proliferating loose fibrous tissue. It tended to be most common in lungs containing marked alveolar histiocyte infiltrate secondary to large neoplasms that was accompanied by chronic inflammation.

Larynx

The SP had diagnosed increased incidences of **Larynx, Respiratory Epithelium, Epiglottis -- Metaplasia, Squamous** in all exposed groups of males and females, and increased incidences of **Larynx, Squamous Epithelium -- Hyperplasia** in exposed groups of males as compared with respective controls. These findings were confirmed by the QA/PWG review. The PWG examined representative examples and confirmed the presence of the changes.

Microscopically, squamous metaplasia was generally a minimal change seen at the base of the epiglottis and was characterized by replacement of the normal

respiratory epithelium by one to three layers of flattened squamous epithelial cells. Squamous epithelial hyperplasia was generally a minimal, focal change of the stratified squamous epithelium of the epiglottis. It was characterized by slight thickening of the stratified squamous epithelium due to replacement of the normal two flattened layers of squamous epithelial cells by three or four layers of larger, somewhat more polygonal squamous epithelial cells.

Nose

The SP had diagnosed similar incidences of **Nose – Inflammation, Suppurative** in control and exposed groups of males and females. The QAP disagreed with a number of the SP's diagnoses primarily in the control and low exposure level groups, such that the incidences as diagnosed by the QAP indicate an increase in incidence of suppurative inflammation of the nose in the mid- and high exposure level male and female groups. The PWG Chair generally concurred with the QAP's findings. The PWG Chair noted that the change diagnosed as suppurative inflammation in many of the noses from control and low exposure level animals consisted of slight suppurative infiltrate within the vomeronasal organ; these were the diagnoses of suppurative inflammation with which the QAP disagreed. In contrast, the change diagnosed as suppurative inflammation by the SP in most of the exposed animals, particularly in the mid- and high exposure levels, consisted of modest amounts of suppurative infiltrate within Nasal Section I; the QAP agreed with these diagnoses.

The SP also diagnosed slightly increased incidences of **Nose, Lateral Wall – Hyperplasia; Nose, Lateral Wall – Metaplasia, Squamous;** and **Nose, Respiratory Epithelium – Necrosis;** and moderate to pronounced increases in **Nose, Olfactory Epithelium – Atrophy; Nose, Olfactory Epithelium – Degeneration, Hyaline; Nose, Respiratory Epithelium – Degeneration, Hyaline;** and **Nose, Respiratory Epithelium – Metaplasia, Squamous.** All of these findings were confirmed by the QA/PWG review.

The PWG examined representative examples and concurred with the QAP's opinion that suppurative inflammation should not be diagnosed in those noses in which the only change was slight suppurative infiltrate within the vomeronasal organ. The PWG also confirmed the presence of suppurative inflammation in Nasal Section I in exposed animals. The PWG noted that slight suppurative infiltrate was present within the vomeronasal organ of animals which had suppurative inflammation in Nasal Section I. The PWG was not in strict disagreement with the SP, and agreed that slight suppurative inflammation was present in the noses which had only infiltrate in the vomeronasal organ. However, the PWG was of the opinion that the suppurative inflammation in the vomeronasal organ was a slight background lesion and that diagnosing inflammation in those cases would obscure the fact that suppurative inflammation did occur in Nasal Section I of exposed animals as a treatment related effect. Thus, the PWG recommended the QAP's diagnoses concerning suppurative inflammation in the nose.

The PWG also confirmed the presence of the other lesions, mentioned above, in the noses of exposed animals.

Microscopically, suppurative inflammation was generally a minimal to mild, generally focal change seen within the turbinates, septum, or lateral wall in Nasal Section I, consisting of aggregation of small to moderate number of neutrophils beneath the respiratory or transitional epithelium. In more severe cases it was accompanied by squamous metaplasia, in which the normal respiratory epithelium was replaced by one to three layers of flattened squamous epithelial cells. In a few animals hyperplasia of the respiratory epithelium, consisting of slight focal thickening of the epithelium from one layer up to two or three layers. If the squamous metaplasia or hyperplasia of the respiratory epithelium occurred on the lateral wall of the nasal cavity, it was diagnosed by the SP under the separate topographical site of lateral wall. In a few noses, primarily in the high exposure level groups, the suppurative inflammation was accompanied by focal ulceration of the epithelium, which the SP termed necrosis of the respiratory epithelium. Hyaline degeneration of the respiratory and olfactory epithelium had the typical appearance of this change in B6C3F1 mice and consisted of epithelial cells with eosinophilic hyaline epithelium. Atrophy of the olfactory epithelium was generally a slight, focal change seen in Nasal Section III consisting of thinning of the normal olfactory epithelium due to loss of olfactory cells. In some noses the olfactory epithelium was replaced by respiratory epithelium, but all these degenerative changes of the olfactory epithelium were included under the diagnosis of olfactory epithelial atrophy.

Bronchial Lymph Node

The SP diagnosed increased incidences of **Lymph Node, Bronchial – Hyperplasia** in mid- and high dose males and in all exposed groups of females. These findings were confirmed by the QA/PWG review. The PWG reviewed some representative examples and confirmed the presence of the change. Microscopically, hyperplasia of the bronchial lymph node was characterized by diffuse enlargement, up to approximately three times normal size, due to a diffuse increase in the number of lymphocytes.

Miscellaneous

The SP had diagnosed **Uterus – Hydrometra** in a few animals in each of the female groups, including controls, and had diagnosed a slightly higher incidence in controls than in high exposure level females. The SP had also diagnosed similar incidences of **Uterus – Hyperplasia, Cystic** in control and exposed groups of females. In some cases the SP had diagnosed both changes in the same uterus. The change diagnosed as hydrometra consisted of dilatation of the uterine lumen. According to NTP diagnostic criteria, this uterine dilatation is considered to be part of the spontaneously occurring cystic uterine hyperplasia in B6C3F1 mice and should be included under the diagnosis of cystic hyperplasia. Accordingly, the QAP changed all of the SP's diagnoses of hydrometra to cystic hyperplasia. The

PWG Chair concurred with the QAP. The incidences of cystic uterine hyperplasia as diagnosed by the QAP were similar across dose groups. Since there was agreement between the QAP and PWG Chair, and since there was no indication of a treatment related effect on the incidence of uterine cystic hyperplasia, there was no necessity to show any slides to the PWG.

The QAP had examined a few additional slides representing a number of minor changes occurring in a variety of tissues. None of these was considered to be potential treatment related lesions. There was good agreement among the SP, QAP, and PWG Chair on these lesions, thus there was no necessity to show any of them to the PWG.

POST-PWG ACTION ITEMS

Since malignant lymphoma was diagnosed in a few animals in each of the exposed female groups but not in any of the control females, and since lymphoid organs were not reviewed from all animals for the presence of lymphoma, the PWG directed the PWG Chair to review the spleen, thymus, and mesenteric lymph node from all emales for the presence of lymphoid neoplasia.

The SP had diagnosed two neoplasms arising in the thyroid gland as parathyroid gland carcinomas. The PWG recommended performing immunostains on these two neoplasms to confirm their identity prior to their being included in the pathology data base.

The results of the review for lymphomas and immunostains will be included in an addendum to this report.

Micheal P. Jokinen 8/21/00

Micheal P. Jokinen, DVM
Diplomate, ACVP
PWG Chairperson

Date