

**EPL®**

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.  
P.O. BOX 12766, RESEARCH TRIANGLE PARK, NC 27709 (919)544-8061 Fax: (919)544-7289

May 4, 2000

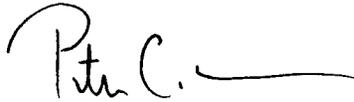
Dr. Doug Wolf  
Environmental Protection Agency  
ERC Building, MD68  
Research Triangle Park, NC 27711

Dear Dr. Wolf:

Enclosed please find an original and a copy of the report from the Pathology Working Group of the Effects of Ammonium Perchlorate on Thyroids, Task 0029, 0030 and 0031, EPL Project Nos. 480-025 - 480-034.

If there are any questions regarding this report, please do not hesitate to contact me.

Sincerely,

A handwritten signature in black ink, appearing to read "Peter C. Mann", followed by a horizontal flourish line.

PETER C. MANN, D.V.M.  
Pathologist

PCM:asc  
Encl.

THE EFFECTS OF  
AMMONIUM PERCHLORATE ON THYROIDS  
PATHOLOGY WORKING GROUP REPORT

Author

Peter C. Mann, D.V.M.

Performing Laboratory

Experimental Pathology Laboratories, Inc.  
P.O. Box 12766  
Research Triangle Park, NC 27709  
919-998-9407

Sponsor

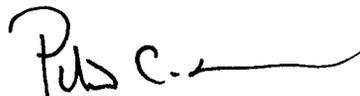
Environmental Protection Agency  
ERC Building, MD68  
Research Triangle Park, NC 27711  
919-541-4137

May 4, 2000

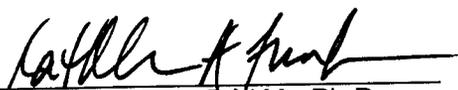
**TABLE OF CONTENTS**

	<u>Page</u>
PWG Participants' Page	
Quality Assurance Statement	
Narrative Summary .....	1
APPENDIX A	
PWG Consensus Diagnoses for Individual Animals Reviewed in Each Group	
APPENDIX B	
Curricula Vitae	

## PWG PARTICIPANTS:



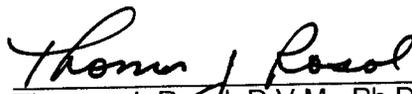
Peter C. Mann, D.V.M.  
Diplomate A.C.V.P.  
Experimental Pathology  
Laboratories, Inc.  
(Chairperson)



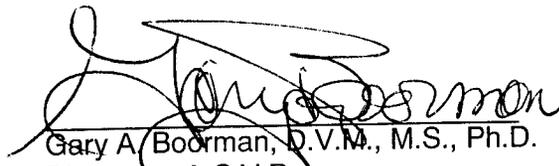
Kathleen A. Funk, D.V.M., Ph.D.  
Diplomate A.C.V.P.  
Experimental Pathology  
Laboratories, Inc.



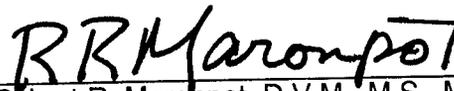
Steven R. Frame, D.V.M., Ph.D.  
Diplomate A.C.V.P.  
Consultant Pathologist



Thomas J. Rosol, D.V.M., Ph.D.  
Diplomate A.C.V.P.  
Consultant Pathologist



Gary A. Boorman, D.V.M., M.S., Ph.D.  
Diplomate A.C.V.P.  
Diplomate ABT  
National Toxicology Program



Robert R. Maronpot, D.V.M., M.S., MPH  
Diplomate A.C.V.P.  
Diplomate ABT  
National Toxicology Program

**EXPERIMENTAL PATHOLOGY LABORATORIES, INC.  
QUALITY ASSURANCE FINAL CERTIFICATION**

**Study Title:** The Effects of Ammonium Perchlorate on Thyroids Pathology Working Group Report

**Client Study:** Task 0029, 0030, & 0031/  
Protocol 1416-002, 1416-002P,  
Mouse MUSC, 1613-002, SLS  
Study No. 3455.1, A-10, 25F, and  
1416-001

**EPL Project Coordinator:** Dr. Mann

**EPL Project Number:** 480-025, 480-027-480-034

**EPL Pathologist:** Dr. Mann

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Project Coordinator and Management are indicated below.

<u>Area Inspected</u>	<u>Dates</u>	
	<u>Inspection</u>	<u>Reporting</u>
EPL Project Sheets	February 23, 2000;	February 23, 2000;
	February 24, 2000;	February 24, 2000;
	March 1, 2000	March 1, 2000
Peer Review	February 24, 2000;	February 24, 2000;
	March 1, 2000;	March 1, 2000;
	March 10, 2000	March 10, 2000
Draft Report	April 25, 2000	April 25, 2000
Final Report	May 4, 2000	May 4, 2000
=====		
Date of last quarterly facility inspection	_____	
=====		

Jane J. Hollingsworth  
EPL Quality Assurance Unit

May 4, 2000  
Date

**THE EFFECTS OF  
AMMONIUM PERCHLORATE ON THYROIDS  
PATHOLOGY WORKING GROUP REPORT**

Ammonium perchlorate is an oxidant utilized in solid fuel for rockets. Due to its potential environmental impact, a number of studies have recently been conducted on behalf of the Environmental Protection Agency (EPA) to evaluate the effects of ammonium perchlorate on the thyroid gland in laboratory animals. The studies were conducted in several different species at several contract laboratories. The studies included the following:

- Oral (Drinking Water) Developmental Toxicity Study of Ammonium Perchlorate in Rabbits
- Effects of Ammonium Perchlorate on Immunological, Hematological, and Thyroid Parameters
- A Neurobehavioral Development Study of Ammonium Perchlorate Administered Orally in Drinking Water to Rats
- A 90-Day Drinking Water Toxicity Study in Rats with Ammonium Perchlorate
- A Fourteen-Day Oral Dosing Toxicity Evaluation of Ammonium Perchlorate Administered in the Drinking Water of Sprague-Dawley Rats
- Neurobehavioral Development Study of Ammonium Perchlorate Administered in Drinking Water to Rats
- Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats
- Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats: F1 Generation
- Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats: F1 and F2 Weanlings

These studies were originally evaluated by several pathologists, who used slightly different diagnostic terminology and criteria to describe lesions in the thyroid. In order to improve the consistency and accuracy of the diagnoses as an aid to risk assessment, Dr. Douglas Wolf of the EPA re-evaluated all slides from all the studies, without knowledge of sex

or dose group, using the following criteria, which had been developed jointly by pathologists from the EPA, the NTP and EPL (manuscript in preparation):

**Colloid**

Coded as decreased colloid based on either:

- a. Reduction or absence of colloid, or
- b. Pale, lacy, or granular colloid

**Follicular Cell Hypertrophy**

Diagnosed ONLY when not associated with follicular cell hyperplasia in the same gland

Coded as AREA AFFECTED

Grade	% of Follicles Affected
1	1-10
2	11-50
3	51-100

**Follicular Cell Hyperplasia**

Diagnosed only when there was either stratification (multiple layers) or papillary infolding of single or multiple layers of follicular cells. A capillary may be seen in the core of the papillary infolding. Hypertrophic cells may be present, but were not diagnosed separately when associated with follicular cell hyperplasia. In such instances, only follicular cell hyperplasia was diagnosed. Most of the cells in the affected follicle were smaller than normal.

Coded as AREA AFFECTED

Grade	% of Follicles Affected
1	1-10
2	11-50
3	51-100

Coded as AVERAGE SEVERITY

The average severity of all the affected follicles with follicular cell hyperplasia, using the severity grades described below:

Coded as GREATEST SEVERITY

The severity grade given to the follicle with the greatest degree of follicular hyperplasia. The severity grading of the follicular hyperplasia was based on the complexity of the pattern of hyperplasia and/or multiplicity within the affected thyroid gland follicle

Grade	Description
1	Simple papillary infolding of follicular epithelium, usually with a capillary at its core
2	Multiple papillary infolding with or without stratification of the follicular epithelium in other parts of the same thyroid follicle
3	Hyperplasia of the follicular epithelium having a complex pattern (e.g. microfollicular pattern or extensive branching). The follicular cells may appear more basophilic. There is no compression due to the hyperplasia. Areas of hyperplasia are generally smaller and less complex than with follicular cell adenomas

**Example:** If follicular cell hyperplasia is seen in 8 follicles, with 4 follicles of grade 1 severity, 2 of grade 2 severity, and 2 of grade 3 severity:

Area affected = 1% = Severity Grade 1

Overall Severity = Severity Grade 2 (rounded up from 1.75)

Greatest Severity = Severity Grade 3

Following the reevaluation by Dr. Wolf, a peer review was conducted by Dr. Peter Mann of Experimental Pathology Laboratories, Inc. (EPL®), Research Triangle Park, NC. Dr. Mann examined all sections of thyroid from all the studies. For the purposes of the peer review, Dr. Mann utilized Dr. Wolf's criteria for thyroid lesions. Following the initial peer review, Dr. Mann and Dr. Wolf examined all thyroids where there was a difference of opinion as to the presence or severity of a particular lesion. For most of these differences, it was possible to reach a consensus opinion. For those lesions where it was not possible to reach a consensus, the thyroids were submitted to the Pathology Working Group.

A Pathology Working Group (PWG) was convened in Research Triangle Park, North Carolina, on March 15-17, 2000 and involved a panel of experienced toxicologic pathologists who examined a subset of slides from all studies. Curricula vitae for the PWG chairperson and each of the PWG participants are presented in Appendix B. Individuals participating in the PWG or attending as an observer are listed below:

Dr. Peter C. Mann, Diplomate, A.C.V.P	(PWG Chairperson)
Dr. Robert R. Maronpot, Diplomate, A.C.V.P Diplomate, ABT	(PWG Participant)
Dr. Gary A. Boorman, Diplomate, A.C.V.P Diplomate, ABT	(PWG Participant)
Dr. Kathleen A. Funk, Diplomate, A.C.V.P	(PWG Participant)
Dr. Steven R. Frame, Diplomate, A.C.V.P	(PWG Participant)
Dr. Thomas Rosol, Diplomate, A.C.V.P	(PWG Participant)
Dr. Doug Wolf	(Observer)
Ms. Annie Jarabek	(Observer)
Dr. William Farland	(Observer)
Dr. Dorothy Kantor	(Observer)
Dr. Steve Lamm	(Observer)
Dr. Jerry Hardisty	(Observer)

The PWG examined coded slides without knowledge of treatment group. The PWG examined approximately 15% of the thyroids, chosen at random, from each of the treated groups from each study. In addition, the PWG examined all slides where differences between the study pathologist and the reviewing pathologist could not be resolved. The total number of slides examined was approximately 18% of all treated dose groups. The PWG was informed of the sex, but did not know the dose group. For each study, the PWG was provided with control animals from each sex to aid in its deliberations. Each PWG participant recorded his/her

diagnoses and comments on worksheets, which were prepared by the PWG chairperson. The PWG examined the slides for colloid depletion, hypertrophy, hyperplasia, and neoplasia. Each lesion was discussed by the group, reexamined if necessary, and the final opinions were recorded on the chairperson's worksheets. The consensus diagnoses of the PWG were reached when a majority of the PWG participants were in agreement. PWG consensus diagnoses for individual animals are presented in Appendix A.

During the course of its deliberations, the PWG determined that although Dr. Wolf had conducted a consistent and careful evaluation of the thyroids from the nine studies, it was not reasonable or feasible in three days for the Pathology Working Group to apply the same criteria. The PWG decided that the complexity of the classification scheme used by Dr. Wolf, while useful as a study-specific classification system, was more appropriate in a research setting and would be difficult to apply generally to toxicology studies. The PWG concurred that the original goal of elucidating a subtle dose response was appropriate, but felt that they could not consistently reproduce the results in the time available to the PWG.

The PWG developed a set of criteria, which they applied to the thyroids they examined. These criteria follow:

### **Colloid Depletion**

A reduction in the amount of colloid was graded as 0 (no reduction), 1, or 2. The grade assigned was based primarily on reduction in area of colloid in thyroid follicles, and secondarily on the tinctoral quality or staining character of colloid. The PWG felt that the amount of colloid normally present varied due to species, sex and age of the animal, and so they compared the amount of colloid depletion to concurrent sex-matched controls for each study.

### **Hypertrophy**

The degree of hypertrophy was graded as 0 (no hypertrophy), 1, or 2. The PWG felt that hypertrophy in the ammonium perchlorate studies represented a minimal physiologic response of the thyroid gland.

Grade 1. The follicular epithelium was taller than the normal cuboidal, often approaching columnar. Individual follicular epithelial cells exhibited increased height and width. In addition, the cytoplasm:nucleus ratio was also increased. The nucleus generally appeared to be somewhat wider and the cell higher. Hypertrophy was generally more intense in the center of the gland. A grade 1 hypertrophy was considered just above the limits of detection.

Grade 2. The follicular epithelial cells were distinctly larger than normal. The follicular lumen was consistently obliterated by the hypertrophied cells. Columnar cells were easily found in the affected gland.

The PWG felt that it was inappropriate to regulate compounds on the basis of hypertrophy alone. Follicular cells in rats are typically large (tall cuboidal to columnar) compared to follicular cells of other animals, due to the high level of follicular cell activity in normal rats. Thyroid hormone in rats have a short half life in blood compared to other animals and humans, which necessitates greater synthesis of hormones to maintain normal blood levels. Follicular cells in rats have a limited ability to increase further in size. For these reasons, hypertrophy should be considered a relatively insensitive indicator of cellular activity in rat thyroid glands. The PWG felt that it might be useful to evaluate Ammonium Perchlorate induced hypertrophy in a higher mammal, such as a primate. The PWG felt that hypertrophy should be diagnosed in all cases where it was present, whether hyperplasia was present or not.

## **Hyperplasia**

The degree of hyperplasia was graded as 0 (no hyperplasia), 1, or 2. The PWG evaluated the mean severity of hyperplasia, which in a sense was a combination of Dr. Wolf's diagnoses of area affected and average severity of hyperplasia.

The PWG differentiated between physiologic hyperplasia, which tends to be more diffuse and involve more cells and treatment-related hyperplasia which is more focal. The PWG did not grade physiologic hyperplasia in their deliberations of the perchlorate studies.

The PWG felt that the change diagnosed as grade 1 hyperplasia was quite subtle, and there was a tremendous overlap of normal thyroids and grade 1 lesions. The grade 1 developed by this PWG is specific to these studies and might be considered within normal limits in other studies.

Grade 1 - included stratification of cells, a microfollicular appearance to affected follicles, papillary infolding, and tinctoral (hyperchromatic) changes. Not all affected follicles exhibited all of these changes. Lesions were either focal or multifocal. A section of thyroid might have either individual follicles affected or nodules, consisting of several affected follicles.

The PWG felt that generally there had to be more than one follicle per section affected before a diagnosis of grade 1 hyperplasia was made. Follicles on the periphery of a section of thyroid often had more papillary infoldings and microfollicular appearance. These changes were regarded as within normal limits on the periphery.

Grade 2 – the hyperplasia was well-demarcated and involved multiple contiguous follicles in a given thyroid. The affected areas were larger than in grade 1.

Diagnoses of follicular cell adenoma were made by both Dr. Wolf and the PWG. In both instances, the criteria published by the NTP (Hardisty, et al, 1990) were used.

#### **Follicular Cell Adenoma**

- Discrete and well-demarcated mass; generally not encapsulated
- Compression of adjacent tissue
- Growth pattern that varies from normal (complex papillary or follicular)
- Cells well differentiated but abnormal in size and staining quality
- Cell nuclei abnormal in size and chromatin content (e.g., small and hyperchromatic or large with prominent nucleoli)
- No invasion of capsule, adjacent tissue, or metastases

After the PWG completed the slide review and the diagnoses were recorded by the PWG chairperson, the slides were decoded and the microscopic findings were decoded by treatment group. The PWG discussed its set of criteria in comparison to the criteria used by Dr. Wolf at some length. The PWG felt that they had been consistent in applying their criteria to the thyroids examined, and that they would utilize the same criteria again. They also felt that their criteria were more appropriate and would be easier to use in routine toxicology studies. They felt that Dr. Wolf had been consistent in his evaluation, but that the complex grading scheme was too study specific and would be best utilized in a research setting. The PWG then discussed their findings for each study in comparison to those of Dr. Wolf. Since the criteria were different, it was not possible to conduct an exact comparison for each diagnosis for

individual animals. However, after the PWG examined incidence tables for each study using their criteria and compared them to incidence tables generated using Dr. Wolf's criteria, the PWG agreed that the effects of ammonium perchlorate on the thyroid were similar as in regards to dose, regardless of the criteria used to diagnose the changes. The PWG made a number of suggestions to Dr. Wolf for further evaluation of the thyroids, based on their conclusions and discussions to enable him to finalize his report.

The PWG felt that the degree of colloid normally present was highly age-dependent. They felt that since Dr. Wolf did not have knowledge of the appearance of controls in the studies involving young animals (since he evaluated the slides without knowledge of dose or sex), it would be appropriate for him to re-evaluate the studies involving PND5 pups for colloid depletion, with knowledge of the appearance of the controls from these studies.

The PWG felt that to determine if the processes of hypertrophy and hyperplasia were distinct and represented different responses to physiologic or toxic changes, Dr. Wolf should re-evaluate all thyroids for which he had made a diagnosis of hyperplasia (and thus would not have diagnosed hypertrophy) to determine if there was also hypertrophy present in those animals.

Finally, the PWG was concerned that the very subtle nature of the Grade 1 lesions, both with their criteria and with Dr. Wolf's criteria, might present potential difficulties. The PWG's concern was that the criteria were highly study-specific, and that the Grade 1 lesions would probably be considered to be within normal limits by pathologists who had not had the opportunity to review all of the ammonium perchlorate studies. The PWG recommended that Dr. Wolf review all of the lesions that he diagnosed as Grade 1 to determine whether or not they were actually within normal limits.

Dr. Wolf was present during the PWG's deliberations and discussions and he agreed to comply with the panels' requests. Following his reevaluation, he will finalize his tables from the nine studies and issue reports as appropriate.

---

PETER C. MANN, D.V.M.  
Diplomate, A.C.V.P.  
Chairperson

---

DATE

PCM:asc

**Reference:**

Hardisty JF and Boorman GA (1990). Thyroid. In: Pathology of the Fischer Rat. Eds. Boorman GA, Eustis SL, Elwell MR, Montgomery CA, and MacKenzie WF. Academic Press, San Diego, pp 519-536.