



FYI-0997-1297

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Environmental Health Sciences
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FYI-97-001297

September 12, 1997

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RE: Chronic Pathology Working Group Reports and Summary Tables

In compliance with the National Toxicology Program's (NTP) mission to keep our colleagues informed of the current NTP findings during on-going studies, a copy of the Pathology Working Group (PWG) report and the Summary Tables for the chronic dosed water studies on 1-Chloro-2-propanol (CAS No. 127-00-4), the dermal studies on Coconut Oil Acid Diethanolamine (CAS No. 68603-42-9), and the dermal studies on Lauric Acid Diethanolamine (CAS No. 120-40-1) 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 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.

In addition to contacting the NIEHS directly, the Institute makes available a wide variety of NIEHS and NTP information in electronic format world-wide. For example, the NTP Annual Plan, The 7th Annual Report on Carcinogens (Summary), Abstracts of NTP Reports, study data and the status of all NTP studies are all available via the internet. On a monthly basis, files accessed are now greater than 150,000 from the NTP Web server with unique users exceeding 17,000. To view the NTP information requires access to the Internet and a Web browser such as Netscape Navigator or Internet Explorer. To access the NTP WorldWide Web Homepage, use the URL <http://ntp-server.niehs.nih.gov/>. Comments on the usefulness of this site and suggestions for improvement are encouraged.



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Please contact Central Data Management at (919) 541-3419 if you have any questions. You may also fax your requests to CDM at (919) 541-3687 or send them via Internet e-mail to CDM@NIEHS.NIH.GOV.

Sincerely,



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

Encls: 1-Chloro-2-Propanol (CAS No.127-00-4)
Rats & Mice, PWG, Pathology Summary Tables

Coconut Oil Acid Diethanolamine Condensate (CAS No. 68603-42-9)
Rats, PWG, Pathology Summary Tables

Lauric Acid Diethanolamine Condensate (CAS No. 120-40-1)
Rats & Mice, PWG, Pathology Summary Tables

cc: Dr. J. Bucher
Dr. J. Dunnick
Dr. J. Leininger
Central Data Management



**CHAIRPERSON'S REPORT
PATHOLOGY WORKING GROUP REVIEW
1-CHLORO-2-PROPANOL (C61983C)
CHRONIC DOSED WATER STUDY
IN F344 RATS
CONDUCTED AT TSI MASON LABORATORIES**

Date of the Pathology Working Group Review: April 24, 1997

Participants: James Hailey, D.V.M.; NIEHS
Ronald Herbert, D.V.M., Ph.D.; NIEHS
Joel Leininger, D.V.M., Ph.D.; NIEHS
Ann Radovsky, D.V.M., Ph.D.; NIEHS
Roger Brown, D.V.M.; GLAXO
Volker Geiss, D.V.M., Ph.D.; NIEHS
(Observer)
John Peckham, D.V.M., M.S.; EPL
(QAP for M. Elwell, D.V.M.)
John Curtis Seely, D.V.M.; PATHCO
(PWG Chairperson)

STUDY DESIGN

Male and female F344 rats were exposed to 1-Chloro-2-Propanol by dosed water (7 days/week) at dose levels of 0, 150, 325 and 650 mg/kg for up to 104 weeks. Table 1 summarizes the animal disposition for the study.

**TABLE 1
Male Rats**

<u>Dose (mg/kg)</u>	<u>0</u>	<u>150</u>	<u>325</u>	<u>650</u>
Animals in Study	50	50	50	50
Moribund Sacrifice	12	13	7	10
Natural Deaths	18	14	19	17
Survivors	20	23	24	23
Anim. Exam. Micro.	50	50	50	50



Dose (mg/kg)	Female Rats			
	0	150	325	650
Animals in Study	50	50	50	50
Moribund Sacrifice	12	7	7	5
Natural Deaths	13	10	13	14
Survivors	25	33	30	31
Anim. Exam. Micro.	50	50	50	50

SUMMARY

Administration of 1-chloro-2-propanol by dosed water, under the conditions of this study, was not associated with any treatment-related histopathologic lesions indicative of any toxicity or carcinogenicity.

Evaluation of the strength and significance of the pathology findings must await generation of the final tables and statistical analyses of the data.

CONDUCT OF THE PATHOLOGY WORKING GROUP

Prior to the PWG, the chairperson reviewed the pathology tables, the original study pathologist's (SP) narrative, the quality assessment (QA) report prepared by the quality assessment pathologist (QAP) and microscopic slides of potential target organs and selected lesions with discrepancies in diagnoses between the SP and QAP.



The following potential target organs and/or specific organ diagnoses were reviewed by the QAP from all control and treated rats.

Male Rats

Mammary Gland
Pituitary Gland
Pancreas - Acinus Atrophy
All Tumor Diagnoses*

Female Rats

Mammary Gland
Pituitary Gland
Pancreas - Acinus Atrophy
All Tumor Diagnoses*

*(except for testis - interstitial cell adenoma)

The PWG chairperson selected a set of 36 slides for review by the PWG. These slides included representative examples of potential treatment-related lesions, lesions for which there was a difference of opinion between the SP and QAP and lesions selected because of general interest (see Chairperson's PWG Worksheets). All slides were examined by each participant without knowledge of the dose group or diagnoses rendered by the SP and QAP. Final diagnoses for the lesions presented were determined by the consensus of the PWG participants.

PWG RESULTS

Mammary Gland

Representative examples of non-neoplastic and neoplastic lesions of the mammary gland in male and female rats were shown to the PWG. These lesions were examined because of the



reported decreased incidence of fibroadenomas in the high-dose females compared to the controls and the small increase in hyperplasia in the treated male animals compared to the controls. In general, there was good agreement among the PWG participants in the diagnosis of these lesions. All of the unknown cases shown to the PWG represented discrepancies between non-tumor and tumor or in the type of tumor present.

Dilatation of the mammary gland was diagnosed more often in females than in males. Dilatation was characterized by the presence of usually several ducts which were dilated and contained an eosinophilic proteinaceous material. Dilatation was not diagnosed when it was a component of a neoplasm.

Hyperplasia was evident as a multifocal to diffuse change associated with increased numbers of acini around ducts consistent with lobular hyperplasia. Although epithelial cells were variable in size, they were well-differentiated and consisted of only a single layer of cells.

The following criteria were used to diagnose adenoma and fibroadenoma during the PWG review.

Adenoma

1. General lobular pattern with slightly thicker connective tissue septa between lobules.
2. Alveoli are lined by single layer of cuboidal to columnar epithelium which occasionally forms branching papillary structures.



3. Epithelium is generally uniform with little cellular atypia.
4. Epithelial stratification is usually not present but may be two or three cell layers thick in some areas.
5. Connective tissue stroma is generally scant and uniformly distributed.

Fibroadenoma

1. Composed of glandular epithelium (ducts, ductules, and/or alveoli) which are surrounded by fibrous connective tissue.
2. Lobular alveolar patterns and some dilated and cystic ducts may be present.
3. Epithelium is generally uniform and single-layered although some atypia or stratification may occur.
4. In some neoplasms, the fibrous connective tissue component may be the most prominent portion of the neoplasm.

Following the PWG review, the reported incidence trends, in general, remained as reported. The decrease in fibroadenomas in the high-dose females did not appear to be either toxicologic or biologically meaningful. Overall, the incidence of fibroadenomas in control females was slightly higher than normally reported in the NIEHS/NTP historical control data base for dosed water studies.

Pituitary Gland

The PWG reviewed several slides containing, for the most part, discrepancies involving proliferative lesions of the pars distalis. These lesions were examined because of the



non-statistically significant increase of adenomas in all three male treatment groups compared to the controls. The incidence of hyperplasia in males did not show any similar trends. Proliferative lesions from several female animals were also examined.

The following criteria were used to diagnose the proliferative lesions.

Hyperplasia

1. An absolute or relative increase of a cell type within a fixed area.
2. The area of hyperplasia blends with the surrounding parenchyma.
3. Compression is minimal unless angiectasis, hemorrhage or cyst formation accompanies the hyperplasia.

Adenoma

1. A discrete aggregation of a single cell type which compresses surrounding parenchyma.
2. Histologically, adenoma cells vary from an almost normal appearance to cells with considerable pleomorphism characterized by cytomegaly and karyomegaly.
3. Adenomas may become quite large.



Carcinoma

1. Invasion of the brain or surrounding bone and/or metastasis are conclusive evidence of malignancy regardless of cellular morphology.
2. In the absence of these indicators, cellular morphology including atypia and mitotic activity should be considered as relative criteria of malignancy.

Following the PWG review, all of the discrepancies which were noted by the QA examination were resolved by the PWG and the trend reported by the SP confirmed. However, the increased incidence of adenomas of the pituitary gland did not appear statistically significant in the mid and high dose males and was not accompanied by an increase in hyperplastic lesions. In addition, the incidence was within the historical control range. Therefore, these lesions were not considered to be treatment-related.

MISCELLANEOUS

A number of lesions were examined by the PWG to either confirm their incidence or because a discrepancy existed. In most instances, these lesions represented unusual or diagnostically challenging neoplasms which were diagnosed either once or only in a few instances from different dose groups, and were not considered to be related to chemical exposure.



HISTOTECHNIQUE

The overall quality of the slides as determined by the
Histotechnique Quality Assessment was good.

John Curtis Seely, D.V.M.
John Curtis Seely, D.V.M.
Diplomate, American College
of Veterinary Pathologists

June 20, 1997
Date



**CHAIRPERSON'S REPORT
PATHOLOGY WORKING GROUP (PWG) OF THE CHRONIC STUDY OF 1-CHLORO-2-
PROPANOL (C61983C) ADMINISTERED VIA DOSED WATER TO B6C3F1 MICE
PERFORMED BY TSI MASON RESEARCH INSTITUTE**

Date of PWG: April 24, 1997

Site of PWG: National Toxicology Program
Research Triangle Institute, NC

PWG Participants:	Paul K. Lundebrandt, D.V.M., Chairperson,	PATHCO
	James Hailey, D.V.M.	NIEHS
	Ronald Herbert, D.V.M., Ph.D.	NIEHS
	Joel Leininger, D.V.M., Ph.D.	NIEHS
	Ann Radovsky, D.V.M., Ph.D.	NIEHS
	John Peckham, D.V.M., M.S., Ph.D.	EPL
	Roger Brown, D.V.M.	Glaxo-Wellcome
Volker Geiss, D.V.M., Ph.D., (observer)	NIEHS	

Study Pathologist: Dr. Michael Stedham

Reviewing Pathologist: Dr. Margarita Grubbel

I. Summary and Conclusion

1-Chloro-2-Propanol administration via dosed water for two years did not result in any compound related neoplastic or non-neoplastic lesion. A number of spontaneous lesions were present and occurred with similar incidence in all dose groups including controls. Liver tumors, including hepatoblastomas were present at high but equal incidence in all dose groups including controls.

II. Study Design

Fifty mice/sex/group were administered 1-Chloro-2-Propanol in deionized water for up to two years at the following doses: 0 ppm (control), 250 ppm, 500 ppm and 1000 ppm. Feed was available *ad libitum*.



III. Animal Disposition Summary

A. Chronic Toxicity/Carcinogenicity Study

FEMALE MICE

Dosage in PPM	<u>0</u>	<u>250</u>	<u>500</u>	<u>1000</u>
Animals Initially in Study	50	50	50	50
Early Deaths				
Natural Death	7	11	10	11
Moribund Sacrifice	8	7	3	5
Accidentally Killed	3		1	2
Survivors				
Terminal Sacrifice	32	32	36	32
Animals Examined Microscopically	50	50	50	50

A. Chronic Toxicity/Carcinogenicity Study

MALE MICE

Dosage in PPM	<u>0</u>	<u>250</u>	<u>500</u>	<u>1000</u>
Animals Initially in Study	50	50	50	50
Early Deaths				
Moribund Sacrifice	8		7	4
Natural Death	2	6	9	4
Accidentally Killed			5	3
Survivors				
Terminal Sacrifice	40	44	29	39
Animals Examined Microscopically	50	50	50	50



IV. 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 lesions with discrepancies in diagnoses between the study pathologist (SP) and reviewing pathologist (RP). All tumors were also reviewed by the Chairperson.

At the PWG, the Chairperson led a short discussion in the form of introductory remarks which included the following:

- I. Survival was essentially equal in all groups including respective controls.
- II. No compound related clinical signs were recorded.
- III. Body weights and weight gains were essentially equal in all groups.
- IV. In the 90 day subchronic study, slight pancreatic acinar cell degeneration was present in mice that were dosed at higher levels than administered in this chronic study.

Although no compound related lesions were identified in this study, a few liver lesions and malignant lymphomas and/or lymphoid hyperplasia were presented to the PWG for interest.

Liver

Liver neoplasms occurred at a rather high incidence in all dose groups including controls in this study. These hepatic neoplasms consisted of hepatocellular adenomas, hepatocellular carcinomas and hepatoblastomas. There were few discrepancies between the SP and RP regarding the presence of liver tumors in individual mice although there was some discrepancy in the diagnosis of hepatoblastoma.

A series of livers with hepatic tumors were presented to the PWG for evaluation. The hepatoblastomas consisted of poorly differentiated, small, slightly elongated basophilic cells, often aligned radially around numerous blood vessels. Several of these hepatoblastomas had been diagnosed as hemangiosarcomas. The PWG agreed with the diagnosis of hepatoblastomas in livers where discrepancies had existed (see attached PWG worksheets). The PWG noted that in several livers, the cells of hepatoblastomas were slightly larger than generally observed in hepatoblastomas. The PWG also evaluated these livers for any morphologic evidence of helicobacter infection. It was agreed the enlarged hepatocytes with smudged and desiccated appearing cytoplasm, and containing enlarged variable shaped nuclei characteristic of helicobacter infection were not present in these livers.

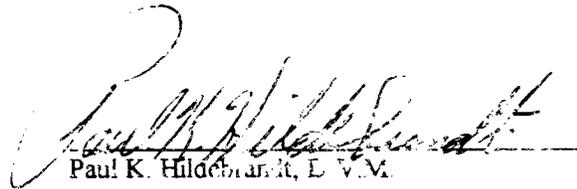


Malignant Lymphoma

There were a number of mice in each dose group with a diagnosis of lymphoid hyperplasia or malignant lymphoma. These were all within the incidence of historical controls. A few discrepancies in diagnosis between these two lesions existed and a sample of these were presented to the PWC. Results are presented in the attached PWG worksheets. No compound effect was present.

Several interesting and unusual lesions were presented to the PWG for review (see attached PWG worksheets).

The PWG agreed that no compound related lesions were observed in this study. There was no explanation for the rather high incidence of liver tumors in all dose groups including controls.



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

Report: PEIRPT03
Date: 07/30/97
Time: 09:11:09

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
1-CELORO-2-PROPANOL

NTP Experiment-Test: 05160-06
Study Type: CHRONIC
Route: DOSED WATER

2 YEAR CHRONIC

Facility: TSI Mason Research

Chemical CAS #: 127-00-4

Lock Date: 01/24/94

Cage Range: All

Reasons For Removal: All

Removal Date Range: All

Treatment Groups: Include All

a Number of animals examined microscopically at site and number of animals with lesion

Report: PEIRPT03
Date: 07/30/97
Time: 09:11:09

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
1-CHLORO-2-PROPANOL

NTP Experiment-Test: 05160-06
Study Type: CHRONIC
Route: DOSED WATER

B6C3F1 MICE FEMALE	0 PPM	250 PPM	500 PPM	1000 PPM
DISPOSITION SUMMARY				
Animals Initially In Study	50	50	50	50
Early Deaths	7	11	10	11
Natural Death	8	7	3	5
Moribund Sacrifice	3		1	2
Accidentally Killed				
Survivors	32	32	36	32
Terminal Sacrifice				
Animals Examined Microscopically	50	50	50	50

ALIMENTARY SYSTEM

Gallbladder	(42)	(44)	(45)	(40)
Inflammation, Acute	1 (2%)			1 (3%)
Necrosis	(44)	(46)	(48)	(49)
Intestine Large, Cecum	1 (2%)		1 (2%)	
Lymphoid Tissue, Hyperplasia			1 (2%)	
Serosa, Inflammation, Chronic Active	(46)	(45)	(47)	(46)
Intestine Small, Jejunum				1 (2%)
Hyperplasia, Lymphoid	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Peyer's Patch, Hyperplasia, Lymphoid				1 (2%)
Peyer's Patch, Inflammation, Acute	1 (2%)			1 (2%)
Serosa, Inflammation, Chronic Active	(46)	(44)	(45)	(45)
Intestine Small, Ileum			1 (2%)	
Serosa, Inflammation, Chronic Active	(50)	(50)	(50)	(50)
Liver				
Amyloid Deposition	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Angiectasis	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Clear Cell Focus	2 (4%)	1 (2%)	1 (2%)	31 (62%)
Cyst	29 (58%)	24 (48%)	29 (58%)	
Eosinophilic Focus		1 (2%)		
Fibrosis		1 (2%)	2 (4%)	
Hematopoietic Cell Proliferation	1 (2%)	1 (2%)		
Infiltration Cellular, Polymorphonuclear	1 (2%)		1 (2%)	
Inflammation, Chronic			1 (2%)	
Inflammation, Suppurative	1 (2%)			
Leukocytosis		2 (4%)		1 (2%)
Mixed Cell Focus	1 (2%)	3 (6%)	1 (2%)	
Necrosis				
Bile Duct, Hyperplasia	1 (2%)	1 (2%)		1 (2%)
Hepatocyte, Vacuolization Cytoplasmic				
Hepatocyte, Portal, Vacuolization Cytoplasmic				
Hepatocyte, Centrilobular, Vacuolization				
Cytoplasmic				
Serosa, Inflammation, Chronic Active	1 (2%)		1 (2%)	1 (2%)

a Number of animals examined microscopically at site and number of animals with lesions

**CHAIRPERSON'S REPORT
PATHOLOGY WORKING GROUP (PWG) REVIEW**

**THE CHRONIC DERMAL TOXICITY/CARCINOGENICITY
STUDY OF COCONUT OIL ACID DIETHANOLAMINE CONDENSATE
IN FISCHER 344 RATS (C55312B)**

Participants: Drs. M. Butt (PWG Chairperson), J. Hailey (NIEHS), J. Leininger (NIEHS), P. Mann (NIEHS), R. Miller (NCSU), A. Radovsky (NIEHS)

Date: March 27, 1997

Site: NIEHS, Research Triangle Park

INTRODUCTION

Coconut oil diethanolamine condensate (COD) was selected for testing by the National Cancer Institute as a representative of the carboxylic acid-diethanolamine condensates class. Because of its high production level and great potential for human exposure, COD was selected as a representative of the 2:1 (fatty acid:diethanolamine) condensate class. The purpose of this study was to determine the chronic toxicity and carcinogenicity of coconut oil diethanolamine (COD) when administered by dermal application to Fischer 344 rats for up to 104 consecutive weeks.

The PWG met on March 27, 1997 to review the results of the chronic dermal toxicity/carcinogenicity study of coconut oil acid diethanolamine condensate in F344 rats. This study was conducted at Battelle-Columbus. The study pathologist (SP) was Dr. J. Toft. The quality assessment pathologist (QAP) was Dr. Deborah A. Banas at Experimental Pathology Laboratories.

STUDY DESIGN

Male and female F344 rats were randomized into 50 rats/sex/dose group and exposed to COD by dermal application (5 times per week on weekdays excluding holidays) until their death, early sacrifice or scheduled sacrifice at study termination after approximately 104 weeks of treatment. Rats were treated at concentrations of 0, 50 or 100 mg/kg. Ethanol (95%) was the vehicle. The 0 mg/kg dose groups received 95% ethanol, only, and served as vehicle controls.

SURVIVAL

Survival until the start of the terminal sacrifice was 16, 24 and 22 percent for male rats and 56, 48 and 42 percent female rats for the vehicle control, 50 and 100 mg/kg dose groups, respectively. The lower survival percentages for males was attributed to a greater incidence of spontaneous disease complexes. Dermal application of COD had no apparent effect on survival in either sex.

CLINICAL OBSERVATIONS

Irritation at the site of vehicle or COD application was noted in the 100 mg/kg males (1/50), 0 mg/kg females (4/50), 50 mg/kg females (1/50) and 100 mg/kg females (24/50). These incidences indicated that clinically, irritation was treatment related in only the 100 mg/kg females although microscopically, the male and female 50 and 100 mg/kg dose groups had consistent evidence of treatment related skin changes. Other skin changes at the site of application (including papillomas) were found to be unrelated to treatment with COD.

BODY WEIGHTS

At study week 101 and at study termination, there was a statistically significantly decreased group mean body weight value (approximately 10% less than controls) in the 100 mg/kg females.

GROSS PATHOLOGY

There was a slight increase of crusts at the site of application in the 100 mg/kg males and females (5/50 and 6/50, respectively); this increase was considered to be related to treatment with COD. Lesions described as a "nodule" were observed in 3/50 vehicle control males. Lesions described as "mass" were observed in 3/50 50 mg/kg males, 1/50 100 mg/kg males, 1/50 50 mg/kg females and 1/50 100 mg/kg females. Microscopic examination indicated that the gross lesions of "nodule" and "mass" did not correlate to a treatment related increased incidence of neoplastic lesions.

MICROSCOPIC PATHOLOGY

All data generated by the PWG Chairperson was entered directly into the PWG Quality Assessment data program. This included data generated for preparation of the PWG Worksheets and data derived from the PWG review. For occasional animals, the SP diagnosis for the thyroid gland and/or pancreas was not present either on the Slide Review Worksheets in Volumes III or IV of the Quality Assessment Report or in the electronic data. In these animals, the PWG Chairperson reviewed the SP diagnoses in the individual animal data tables (reports PEIQAPTO9 and PEIQAPTO4).

The SP identified the following microscopic changes at the site of application to be related to treatment with COD: epidermal hyperplasia, sebaceous gland hyperplasia, hyperkeratosis and/or parakeratosis. Non-dermal changes stated by the SP to be possibly related to treatment with COD were forestomach lesions (epithelial hyperplasia, epithelial ulceration and chronic active inflammation) in females and pancreatic acinar cell atrophy in males. The study pathologist found no evidence of a carcinogenic effect related to dermal application of COD.

The QAP confirmed the SP findings for treatment-related changes in the skin at the site of application of COD. The QAP was in agreement with the SP regarding the incidence and character of forestomach lesions in both sexes but felt that the apparent increase of forestomach changes in the 50 and 100 mg/kg females may have been due to the increased severity of chronic nephropathy in the COD treated females. While noting the increased incidence of pancreatic acinar cell atrophy in males, the QAP felt that this lesion may not be treatment-related but rather due to biologic variation.

Tissues examined by the PWG chairperson were those selected and examined by the QAP as prescribed in Volume I of IV of the Quality Assessment Report. Briefly, the slides selected for review by the QAP included: eyes from males with cataracts; kidney (all, both sexes); pancreas (all, both sexes); skin site of application (all, both sexes); stomach, forestomach (all, both sexes); thyroid gland C cell (males); all clitoral glands and preputial glands with a diagnosis of hyperplasia or neoplasia by the study pathologist; all tumors in all organs from all animals in all groups (except for testicular interstitial cell tumors); and any tissues with potential duplication of diagnoses or an organ note discrepancy.

Diagnoses made by the PWG Chairperson were entered directly into the PWG slide review software and are recorded on the Slide Review Sheets in Volumes III and IV of the Quality Assessment Report. Diagnoses and comments on slides selected for review by the PWG are recorded on the PWG Chairperson's Report tables in Appendix 1 of this report.

Review of the toxicology data, the study pathologist's report, all pathology data (individual and summary), the Quality Assessment Report and all slides reviewed by the QAP disclosed nine issues requiring resolution by the PWG Chairperson and/or the PWG group. (A separate issue, the increased incidence of cataracts in the 100 mg/kg male rats, was examined by the QAP and PWG Chairperson and determined to be unrelated to treatment. No eyes with cataracts were selected for presentation at the PWG.)

Treatment related skin changes

A total of 12 slides from the COD treatment groups (in addition to controls) with representative lesions of the treatment-related skin lesions were presented for review by the PWG. The PWG members were in consistent agreement with the SP and QAP that epidermal hyperplasia, epidermal hyperkeratosis, epidermal parakeratosis and sebaceous gland hyperplasia were treatment-related microscopic changes in the 50 and 100 mg/kg males and females.

Pancreatic Acinar Cell Atrophy in Males

A total of 12 slides of the pancreas representing all male dose groups (including vehicle controls) were presented to the PWG for review. The PWG members agreed with the SP and QAP that acinar cell atrophy did exist. It was the general belief of the PWG members that the pancreatic acinar cell atrophy most likely represented biologic

variation and no members could relate previous experience with a study in which acinar cell atrophy was a treatment related effect. However, given the incidence of acinar cell atrophy in the COD treated males as compared to the controls (9, 21 and 23 by the SF and 11, 23 and 25 by the QAP in the 0, 50 and 100 mg/kg dose groups, respectively), the PWG members could not rule out that acinar cell atrophy might be related to treatment in the male rats.

Chronic Nephropathy in Females

The SP and QAP diagnosed a similar incidence of chronic nephropathy among males and female rats. The overall severity of nephropathy in males was similar. In female rats, however, the SP and QAP diagnosed an increased number of 50 and 100 mg/kg dose groups females with nephropathy of a moderate or marked severity. The QAP diagnosed a greater number of rats with moderate or marked nephropathy than did the SP. The SP did not describe this apparent increased severity of nephropathy among COD treated females to be related to treatment. The QAP did diagnose the increased severity of nephropathy among females to be treatment related in a dose dependent manner.

A total of 14 slides of the kidneys representing all female dose groups (including vehicle controls) were presented to the PWG for review. The PWG members agreed with the SP regarding the severity of the nephropathy diagnosed in the vehicle and 50 mg/kg female dose groups. The PWG tended to agree with the QAP regarding the severity of nephropathy in the 100 mg/kg dose group. It was the consensus of the PWG that the severity of nephropathy in the 50 and 100 mg/kg female dose groups was greater than that generally encountered in carcinogenicity studies with F344 rats. The PWG members agreed with the QAP that the severity of chronic nephropathy was a treatment related change in the 50 and 100 mg/kg females.

Forestomach Lesions in Females

The SP diagnosed a triad of forestomach lesions (epithelial hyperplasia, epithelial ulceration and chronic active inflammation) in female rats to be possibly related to treatment with COD. In the study pathologist's report, it was not specified whether both COD treated female dose groups or only the 100 mg/kg dose group were considered to be possibly affected. The study pathologist's report stated that the forestomach effects could be "true effects or secondary to general incidental debility, or simply spurious trends due to chance variation." The QAP confirmed and was in general agreement with the incidence and severity of the forestomach changes in both males and females. It was the opinion of the QAP that the increased severity of chronic nephropathy in the 100 mg/kg females was possibly responsible for the forestomach lesions in the high dose females.

The PWG members were in agreement with the SP that the forestomach lesions in the 100 mg/kg female rats were possibly related to treatment with COD.

Twenty six slides of the forestomach (representing all male dose groups and the 100 mg/kg female dose group) were selected by the PWG Chairperson for review by the PWG. Representative slides from the male groups were included so as to demonstrate to the PWG members that the severity of forestomach lesions was relatively similar across all male dose groups, including the vehicle control group. The PWG was in general agreement with the SP and QAP regarding the distribution and severity of the forestomach lesions. The PWG members commented that the forestomach lesions in the males and 100 mg/kg females were, in general, more severe than generally encountered in rats of this age. The PWG members could not, in their collective experience, support the QAP's association relating the forestomach lesions to the chronic nephropathy.

In order to investigate the possible relationship between the increased severity of nephropathy in high dose females and the incidence and severity of forestomach lesions, the PWG tasked the PWG Chairperson with examining the association in individual animals in all dose groups. For each dose group, the incidence of the various grades of chronic nephropathy (0=none, 1=minimal, 2=mild, 3=moderate, 4=marked) were tabulated and the number of animals with one or more of the forestomach lesions in question (epithelial hyperplasia, epithelial ulceration, chronic active inflammation) was tabulated. The results are in Tables 1A (females) and 1B (males). For the females (Table 1A), the SP and QAP chronic nephropathy diagnoses are presented since the QAP tended to grade the chronic nephropathy more severe than the SP in the 100 mg/kg dose group and the PWG tended to agree with the QAP's nephropathy grades. For the males (Table 1B), only the SP diagnoses are presented since there was general agreement between the SP, QAP and PWG. Since there was general agreement between the SP and QAP with respect to the forestomach lesions, the incidence of forestomach lesions in the individual animals were tabulated from the SP's data tables.

Tables 1A and 1B indicate that in the 100 mg/kg females and in all male dose groups, there was a correlation between the incidence of the specified forestomach lesions and the severity of chronic nephropathy. The correlation was most pronounced in the 100 mg/kg females and less pronounced in the male dose groups. It could not be determined whether this was an actual correlation or whether it was a circumstantial correlation.

Although there was an apparent correlation between the severity of forestomach lesions and the severity of chronic nephropathy in this study, in the collective experience of the PWG members, forestomach lesions of epithelial hyperplasia, epithelial ulceration and chronic active inflammation are not generally directly proportional to the severity of nephropathy. This indicates that other factors are present in this study which might explain this correlation.

One possible explanation for the forestomach lesions was the ingestion of vehicle from the site of application. The occurrence of forestomach lesions in the vehicle control males indicated that the forestomach changes were not strictly related to COD treatment. The vehicle for all groups in this study was 95% ethanol. A review of the

literature indicates that gastric lesions in rats are classically related to oral administration of ethanol.(1, 2) Given the occurrence of forestomach lesions in the vehicle control males, it is likely that the forestomach lesions encountered in this study were due to ingestion of the vehicle by the rats. While this explanation is consistent with the gastric changes occurring in the male rats, it does not readily explain the apparent increase of forestomach lesions in the 100 mg/kg females. Clinical observations did indicate a marked increase of irritation at the site of application in the 100 mg/kg females. This increased irritation may have led to or been a result of increased grooming activity at the site of application in the high dose females.

Table 1A Females
Severity of Nephropathy Compared to the Incidence of Forestomach Lesions

Group	Pathologist	Severity of Nephropathy				
		0	1	2	3	4
		Incidence (# with forestomach lesions/# with nephropathy)				
0 mg/kg	SP	0/3	0/18	2/29	--	--
	QAP	0/4	0/17	2/29	0/1	--
50 mg/kg	SP	0/4	2/9	3/29	1/6	0/2
	QAP	0/4	2/11	3/23	0/9	1/3
100 mg/kg	SP	0/4	0/5	2/22	10/18	1/1
	QAP	0/4	0/6	1/2	2/17	10/11

Table 1B Males
Severity of Nephropathy Compared to the Incidence of Forestomach Lesions

Group	Pathologist	Severity of Nephropathy				
		0	1	2	3	4
		Incidence (# with forestomach lesions/# with nephropathy)				
0	SP	0/1	2/4	1/15	6/14	10/16
50	SP	0/0	0/5	5/20	4/12	6/13
100	SP	1/1	2/2	4/22	7/16	4/9

The results of the microscopic evaluations of the SP and QAP, the PWG review of representative gastric lesions in males and females and chronic nephropathy in the females, and the further investigations of the PWG Chairperson allows several conclusions to be made. As stated in the previous section on chronic nephropathy, an increased severity of chronic nephropathy in females was related to COD treatment in both the 50 and 100 mg/kg females. Forestomach changes of epithelial hyperplasia, epithelial ulceration and chronic active inflammation may have been associated with ingestion of the vehicle 95% ethanol. These vehicle induced forestomach changes were exacerbated in animals of both sexes with moderate to marked chronic nephropathy.

Forestomach Squamous Neoplasms

The SP diagnosed a number of squamous neoplasms (generally squamous papillomas) in the forestomach which were considered to be non-neoplastic lesions by the QAP. Neither pathologist considered squamous neoplasia of the forestomach to be related to treatment.

A total of 16 slides from 10 animals with possible squamous cell neoplasia of the forestomach were presented to the PWG for review. In 7 of 10 of these animals, the PWG consensus was in agreement with the QAP that squamous neoplasia was not apparent. The PWG members agreed with the QAP that squamous cell carcinoma was not present in the only animal in which this neoplasm was diagnosed by the SP.

Several PWG members stressed that the microscopic diagnosis of squamous papilloma can be markedly affected by sectioning.

Prostatic Carcinomas Diagnosed by the SP

The SP diagnosed prostatic carcinomas in numerous males in all dose groups. Except for one male in the vehicle control group, the QAP disagreed with the diagnosis of carcinoma in the prostate.

Eleven slides of SP diagnosed prostatic carcinomas were presented to the PWG for review. In all cases except one, the PWG agreed with the QAP that a carcinoma was not present. The PWG agreed with the SP and QAP on the diagnosis of prostatic carcinoma in a single vehicle control male. The SP was apparently diagnosing prostatic carcinoma for lesions characterized by distended, mucus filled glands surrounded by a variable increase of fibrous connective tissue. The QAP consistently diagnosed this change as "Cyst, mucinous" and the PWG agreed with this diagnosis.

Thyroid Neoplasms

There was occasional disagreement between the SP and QAP regarding the diagnosis of thyroid C-cell neoplasms. Neither pathologist described C-cell neoplasia as a treatment related change.

Eight slides with thyroid neoplasms diagnosed by the SP and/or QAP were presented for review by the PWG. Seven of the eight neoplasms were C-cell lesions. For the C-cell lesions, the PWG consensus agreed with the SP in 2/7 cases and the QAP in 5/7 cases. For the single follicle (cell) lesion submitted for review, the SP diagnosed "Follicle, cyst" and the QAP diagnosed "Follicular Cell - Adenoma." The PWG members agreed with the SP pathologist that no neoplasm was present and agreed to accept the diagnosis of the SP.

Clitoral Gland and Preputial Gland Neoplasms

The QAP diagnosed an adenoma or carcinoma for multiple clitoral glands and preputial glands for which the SP had diagnosed hyperplasia or normal. Neither pathologist described clitoral gland or preputial glands to be related to treatment.

A total of 13 slides of suspected clitoral gland and preputial gland neoplasms were submitted to the PWG for review. In all cases, the QAP had diagnosed a neoplasm which the SP had not recorded. In one case, the QAP diagnosed a carcinoma where the SP had diagnosed an adenoma. The PWG consensus agreed with the SP in one case, agreed with the QAP in 4 cases and offered an alternate diagnosis in 7 cases. In most cases where the PWG consensus offered an alternate diagnosis, it was to diagnose an adenoma where the QAP had diagnosed a carcinoma.

Miscellaneous Neoplasms and Other Lesions

There were occasional differences regarding the diagnosis of neoplasia between the SP and QAP. None of these neoplasms were considered to be related to treatment.

A total of 36 slides were submitted to the PWG for a review of miscellaneous neoplasms and other lesions. Included in these slides were all renal neoplasms diagnosed by the SP and/or QAP.

A summary of the incidence of renal neoplasms diagnosed by the SP is provided in Table 2.

In general, there was agreement between the SP, QAP and PWG with regard to the renal neoplasms of tubule origin. The QAP diagnosed "Carcinoma, NOS" instead of "Renal Tubule - Carcinoma" for the 50 mg/kg female but the PWG consensus agreed with the SP.

Table 2 - Renal Neoplasms

Neoplasm	Dose Group					
	VF	LF	HF	VM	LM	HM
Renal Tubule, Adenoma		1		1	4	
Renal Tubule, Adenoma, Multiple					1	
Renal Tubule, Carcinoma		1				

Because of the unusual incidence of renal tubule neoplasms in this study and because renal tubule regeneration was a treatment related lesion in the 13-week rat study with COD, the PWG members agreed to recommend that an additional review of all renal sections from the chronic dermal toxicity/carcinogenicity study be reviewed by NTP pathologists. In addition to this review, step sectioning of the kidneys for the detection of previously undiagnosed proliferative lesions may be performed.

The PWG consensus opinion for the miscellaneous neoplasms selected for review by the PWG Chairperson is recorded on the PWG Chairperson's Report tables in Appendix 1.

Post-PWG Action Items

Brain slides from control male #26 (blocks 6 and 113) were recut from the paraffin blocks and were immunohistochemically stained for Glial Fibrillary Acidic Protein (GFAP) to help determine the origin of the neoplastic cells. The neoplastic cells did not show the presence of GFAP, which supports the PWG consensus diagnosis of meningeal sarcoma.

The adrenal cortex from control female #152 was reviewed by NTP pathologists as there was no agreement among the SP, QAP and PWG Chairperson. The consensus diagnosis was "adrenal cortex - hyperplasia, marked". Similarly, a change in the pancreatic islets was reviewed by NTP pathologists after the PWG. The consensus diagnosis was "hyperplasia, mild", in agreement with the PWG Chairperson.

PWG Conclusions

The following lesions were associated with the administration of coconut oil diethanolamine (COD) by dermal application under the conditions of this study: epidermal hyperplasia, sebaceous gland hyperplasia, hyperkeratosis and parakeratosis at the site of application in males and females treated with 50 and 100 mg/kg; and moderate to marked nephropathy in the 50 and 100 mg/kg females.

Pancreatic acinar atrophy in the 50 and 100 mg/kg males was considered to be possibly related to the application of COD. An increased incidence/severity of forestomach lesions (epithelial hyperplasia, epithelial ulceration, chronic active inflammation) in the 100 mg/kg females was possibly related to application of COD. Alternatively, these forestomach lesions (which occurred at approximately equal incidence and severity in all male dose groups) may have been related to ingestion of the vehicle (95% ethanol).

An unusual incidence of renal tubule neoplasms, while not interpreted to be related to COD treatment, justified a recommendation by the PWG that all kidney sections be reexamined by an NTP pathologist and that step sections of the kidneys, if deemed appropriate by the NTP pathologist, be performed in order to detect any undiagnosed proliferative lesions.

Mark T. Butt

Mark T. Butt, D.V.M.
Diplomate, American College
of Veterinary pathologists

5/22/97

Date

References

- 1) Hernandez-Munoz R, Montiel-Ruiz F. (1996) Reversion by histamine H2-receptor antagonists of plasma membrane alterations in ethanol-induced gastritis. *Dig Dis Sci* 41 (11) 2156-2165.
Toxline Abstract
- 2) al-Harbi MM, Qureshi S, Raza M, Ahmed MiM, Arzal M, Shah AH (1994) Evaluation of *Caralluma tuberculata* pretreatment for the protection of rat gastric mucosa against toxic damage. *Toxicol Appl Pharmacol* 128 (1) 1-8
Toxline Abstract



Pathology Associates International

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QUALITY ASSURANCE STATEMENT

The Path Working Group report has been audited by the Quality Assurance Unit of Pathology Associates International. PAI has a functioning and responsive Quality Assurance Unit which reports directly to management. The following is a record of audits and their resulting reports:

<u>Date of Inspection</u>	<u>Phase Inspected</u>	<u>Date Findings Reported to Management and Study Director</u>
5-29-97	Audit of Chairperson's Report	5-29-97

Deanna Croghan, B.S.
Quality Assurance Specialist

6-9-97

Date

Sponsor: National Toxicology Program
National Institute of Environmental Health Sciences

Title: The Chronic Dermal Study of Coconut Oil Acid Diethanolamine Condensate in Fischer 344 Rats (C55312B)

01124

NTP Experiment-Test: 55312-03
Study Type: CHRONIC
Route: SKIN APPLICATION

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
COCONUT OIL ACID DIETHANOLAMINE CONDENSATE

Report: PEIRPT03
Date: 08/21/97
Time: 14:54:32

CORE STUDY

FINAL #1

Facility: Battelle Columbus Laboratory

Chemical CAS #: 69603-42-9

Lock Date: 09/06/95

Cage Range: All

Reasons For Removal: All

Removal Date Range: All

Treatment Groups: Include All

a Number of animals examined microscopically at site and number of animals with lesion

NTP Experiment-Test: 55312-03
 Study Type: CHRONIC
 Route: SKIN APPLICATION

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
 COCONUT OIL ACID DIETHANOLAMINE CONSENSATE

Report: PEIRPT03
 Date: 06/21/97
 Time: 14:54:32

FISCHER 344 RATS FEMALE

	0 MG/KG	50 MG/KG	100 MG/KG
Animals Initially In Study	50	50	50
Early Deaths	15	11	18
Natural Death	7	15	10
Moribund Sacrifice	28	24	21
Terminal Sacrifice			1
Moribund Sacrifice			
Animals Examined Microscopically	50	50	50

DISPOSITION SUMMARY

Animals Initially In Study
 Early Deaths
 Natural Death
 Moribund Sacrifice
 Survivors
 Terminal Sacrifice
 Moribund Sacrifice
 Animals Examined Microscopically

ALIMENTARY SYSTEM

Zoephaagus	(50)	(50)	(50)
Perforation			1 (2%)
Intestine Large, Colon	(50)	(50)	(50)
Parasite Metazoan	2 (4%)	5 (10%)	1 (2%)
Intestine Large, Rectum	(50)	(50)	(50)
Parasite Metazoan	6 (12%)	4 (8%)	6 (12%)
Intestine Large, Cecum	(50)	(50)	(50)
Parasite Metazoan	2 (4%)		
Intestine Small, Duodenum	(50)	(50)	(50)
Epithelium, Ulcer			1 (2%)
Intestine Small, Jejunum	(50)	(50)	(50)
Inflammation, Chronic Active	(50)	(49)	(49)
Intestine Small, Ileum	(50)	(50)	(50)
Inflammation, Chronic Active	31 (62%)	17 (34%)	4 (8%)
Liver	5 (10%)	4 (8%)	1 (2%)
Basophilic Focus	1 (2%)		
Clear Cell Focus			
Fibrosis			
Hematopoietic Cell Proliferation	10 (20%)	1 (2%)	14 (28%)
Hepatodysplastic Nodule	29 (58%)	26 (52%)	13 (26%)
Inflammation, Chronic	1 (2%)	2 (4%)	
Mixed Cell Focus	1 (2%)		
Pigmentation, Hemosiderin	23 (46%)	20 (40%)	22 (44%)
Vacuolization Cytoplasmic	26 (52%)	21 (42%)	16 (32%)
Bile Duct, Hyperplasia			1 (2%)
Hepatocyte, Degeneration, Cystic	1 (2%)	1 (2%)	
Hepatocyte, Hyperplasia, Adenomatous	1 (2%)	2 (4%)	
Hepatocyte, Necrosis	2 (4%)	1 (2%)	2 (4%)
Hepatocyte, Centrilobular, Degeneration	1 (2%)		
Hepatocyte, Centrilobular, Necrosis	1 (2%)		
Serosa, Necrosis, Fibrinoid	(4)	(5)	(2)
Mesentery			1 (50%)
Fat, Inflammation, Chronic Active			

a Number of animals examined microscopically at site and number of animals with lesion

NTP Experiment-Test: 55312 03
 Study Type: CHRONIC
 Route: SKIN APPLICATION

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
 COCONUT OIL ACID DIETHANOLAMINE CONDENSATE

Report: PEIRPT03
 Date: 08/21/97
 Time: 14:54:32

FISCHER 344 RATS FEMALE

0 MG/KG 50 MG/KG 100 MG/KG

ALIMENTARY SYSTEM - CONT

Lesion	0 MG/KG	50 MG/KG	100 MG/KG
Oral Mucosa	3 (75%)	4 (80%)	1 (50%)
Gingival, Hyperplasia, Squamous	1 (100%)	(1)	(3)
Gingival, Inflammation, Chronic Act. vs.	(50)	(50)	1 (33%)
Pancreas			(50)
Inflammation, Granulomatous	1 (2%)	13 (26%)	11 (22%)
Acinus, Atrophy	19 (38%)	(50)	(50)
Stomach, Forestomach	1 (2%)	1 (2%)	2 (4%)
Edema	1 (2%)	1 (2%)	10 (20%)
Inflammation, Chronic Active	1 (2%)	1 (2%)	13 (26%)
Mineralization	2 (4%)	5 (10%)	11 (22%)
Epithelium, Hyperplasia	1 (2%)	3 (6%)	(50)
Epithelium, Ulcer	(50)	(50)	1 (2%)
Stomach, Glandular		1 (2%)	1 (2%)
Mineralization		1 (2%)	1 (2%)
Epithelium, Erosion		1 (2%)	1 (2%)
Epithelium, Hyperplasia	1 (2%)	1 (2%)	1 (2%)
Epithelium, Ulcer			
Glands, Hyperplasia			
Tooth			
Inflammation, Chronic Active	1 (1)	1 (2%)	1 (2%)
	1 (100%)		

CARDIOVASCULAR SYSTEM

Lesion	0 MG/KG	50 MG/KG	100 MG/KG
Blood Vessel	(50)	(50)	(50)
Artery, Mineralization		1 (2%)	1 (2%)
Pulmonary Artery, Mineralization		(50)	(50)
Heart	(50)	(50)	(50)
Cardiomyopathy, Chronic	22 (44%)	16 (32%)	14 (28%)
Inflammation, Chronic Active	1 (2%)		
Mineralization	1 (2%)	1 (2%)	1 (2%)
Atrio- Thrombosis		1 (2%)	3 (6%)

ENDOCRINE SYSTEM

Lesion	0 MG/KG	50 MG/KG	100 MG/KG
Adrenal Cortex	(50)	(50)	(50)
Accessory Adrenal Cortical Nodule		1 (2%)	
Atrophy		1 (2%)	
Degeneration, Cystic	8 (16%)	6 (12%)	8 (16%)
Degeneration, Fatty	24 (48%)	14 (28%)	16 (32%)
Fibrosis	1 (2%)		
Hyperplasia	22 (44%)	14 (28%)	11 (22%)
Hypertrophy	10 (20%)	3 (6%)	7 (14%)
Necrosis	1 (2%)		
Adrenal Medulla	(50)	(50)	(50)

a Number of animals examined microscopically at site and number of animals with lesion

DATE: 08/21/97 EXPERIMENT: 55312 TEST: 03
 STATISTICAL ANALYSIS OF PRIMARY TUMORS IN RATS (FISCHER344) -- 9 COCONUT OIL ACID DITETRAMOLAMINE CONDE

MALE TERMINAL SACRIFICE AT 105 WEEKS
 FEMALE TERMINAL SACRIFICE AT 104 WEEKS

- (a) Number of tumor-bearing animals / number of animals examined at site.
- (b) Kaplan-Meier estimated lifetime tumor incidence after adjustment for intercurrent mortality.
- (d) Observed incidence at terminal kill.
- (f) Beneath the control incidence are the p-values associated with the trend test. Beneath the dosed group incidence are the p-values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. Logistic regression is an alternative method for analyzing the incidence of non-fatal tumors. The Cochran-Armitage and Fisher's exact tests compare directly the overall incidence rates. For all tests, a negative trend is indicated by "N".
- (e) Value of Statistic cannot be computed.
- (1) Interim sacrifice
- (T) Terminal sacrifice
- ‡ Tumor rates based on number of animals necropsied.

* To the right of any statistical result, indicates significance at (P<0.05).
 ** To the right of any statistical result, indicates significance at (P<0.01).

PATHOLOGY WORKING GROUP CHAIRPERSON'S REPORT

The Chronic Dermal Toxicity/Carcinogenicity Study of Lauric Acid Diethanolamine Condensate (CAS NO. 120-40-1) in Fischer 344 Rats

PWG Participants: Drs. L. Lanning (PAI-Chairperson), J. Hailey (NIHES), R. Herbert (NIHES), A. Radovsky (NIHES), J. Leininger (NIHES), C. Shackelford (EPL), R. Cattley (CIIT)

Date: January 28, 1997

Site: NIHES, Research Triangle Park

The PWG convened to review selected slides from Fischer 344 rats administered lauric acid diethanolamine condensate (LAD) by dermal exposure for two years. Rats were exposed to 0, 50, or 100 mg/kg using dosing solutions (95% ethanol vehicle) at concentrations of 0, 85, and 170 mg/mL, respectively, 5 days per week for 104 weeks, exclusive of weekends and holidays. The experimental design is described below.

<u>Group Number</u>	<u>Dose Level (mg/kg)</u>	<u>Total Number of Animals</u>	<u>Animal ID Numbers</u>
Male	0	50	1 - 50
	50	50	51 - 100
	100	50	101 - 150
Female	0	50	151 - 200
	50	50	201 - 250
	100	50	251 - 300

The study was conducted at Battelle, Columbus Operations. The Study Pathologist (SP) was Dr. J. Yarrington and the Quality Assessment Pathologist (QAP) was Dr. C. Shackelford of Experimental Pathology Laboratories Inc. (EPL).

Potential target organs were examined by the QAP and PWG Chair for all tumor and nontumor diagnoses for all animals in all groups. Organs reviewed for all diagnoses were as follows:

Male Rats

Mammary gland
Skin, site of application(SOA)

Female Rats

Mammary gland
Skin, SOA

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

Male Rats

Preputial gland - Inflammation

Female Rats

Clitoral gland - Inflammation
Kidney - Nephropathy

In addition, all tumor diagnoses from all animals in all groups were reviewed (except for Testis - Interstitial Cell Adenoma).

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

Male Rats

Pituitary gland - Adenoma (no site)*

Female Rats

Kidney - Adenoma (no site)*

Pituitary gland - Adenoma (no site)*

Thyroid gland - Adenoma (no site)*

*The reviewing pathologist was asked to identify the site of the lesion. The PWG Chair agreed with the QAP on the sites reported for above lesions.

SUMMARY OF PWG FINDINGS

Mammary gland

No treatment effects were noted in the mammary gland.

Preputial gland

No treatment effects were noted in the preputial gland.

Clitoral gland

No treatment effects were noted in the clitoral gland.

Kidney (female rats only)

No treatment effects were noted in the kidney of female rats.

Skin, SOA

The incidence of lesions (minimal to moderate severity) of the skin at the site of application was increased in male and female rats exposed to 50 and 100 mg/kg of LAD as compared with those from the 0 mg/kg exposure group. The lesions included one or more of the following: epidermal hyperplasia, sebaceous gland hyperplasia, ulcer, parakeratosis, hyperkeratosis, and chronic active inflammation. One male rat exposed to 100 mg/kg had a basal cell adenoma.

01178

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 were differences in diagnoses among the SP, QAP, and PWG Chair.

RESULTS OF THE PWG REVIEW

Skin, SOA

The SP, QAP and PWG Chair concurred as to the presence of increased incidences of lesions in the skin, site of application, in rats exposed to 50 or 100 mg/kg LAD. The lesions included one or more of the following; epidermal hyperplasia (increased thickness of the epidermis), sebaceous gland hyperplasia (increased number of cells in each sebaceous gland), ulcer, parakeratosis (retention of the nuclei in the cells of the stratum corneum of the epidermis), hyperkeratosis (increased thickness of the keratinized layer of the epidermis), and chronic active inflammation. One male rat exposed to 100 mg/kg had a basal cell adenoma. The PWG examined some representative examples of lesions diagnosed by the SP as well as those diagnosed by the QAP and concurred with the QAP and PWG Chair. These findings are all associated with the processes involved with dermal irritation and the resolution of this irritation. The PWG maintained that the majority of these diagnoses may be consolidated into epidermis, hyperplasia and sebaceous gland hyperplasia, with more detailed descriptions of all findings associated with these changes in the narrative discussion. However, because the SP, QAP and PWG Chair were consistent in the use of the detailed terminology, the diagnoses should remain as listed in the Slide Review Worksheets.

Mammary gland

The SP, QAP and PWG Chair generally agreed on the incidences for neoplastic and nonneoplastic lesions in the mammary gland and that no LAD treatment-related trends were present. The SP did, however, diagnose higher incidences of mammary gland dilatation in the LAD treated groups of male rats and the low dose group of female rats as compared to their controls. The QA pathologist and PWG Chair diagnosed this change in many more animals resulting in a comparable incidence of this finding in all groups. In addition, the SP used the term 'galactoceles' more frequently than did the QAP or PWG Chair. This term was utilized by the QAP or PWG Chair only when markedly dilated ducts and/or alveoli filled with secretion were observed alone and completely separate from a hyperplastic lesion or neoplasm. If this change was noted associated with a hyperplastic lesion or a neoplasm, the term 'galactocyst' was replaced by the QAP and PWG Chair with the more appropriate term (ie. hyperplasia) and the qualifier 'cystic' was added to the diagnosis. No slides were shown to the PWG because no treatment related effect was found by the SP, QAP or PWG Chair and there was good agreement between the QAP and PWG Chair.

Preputial gland/clitoral gland

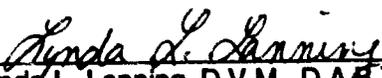
The SP, QAP and PWG Chair generally agreed on the presence of inflammatory lesions in the preputial glands from male rats and the clitoral glands from female rats and that no treatment-related trends were present. The SP did, however, diagnose higher incidences of chronic active inflammation in the LAD treated groups of male and female rats as compared to their controls. The QA pathologist and PWG Chair both found that the SP was inconsistent in diagnosing inflammation in these organs and diagnosed this change in many more animals resulting in a comparable incidence of this finding in all groups. No slides were shown to the PWG because no treatment related effect was found by the SP, QAP or PWG Chair and there was good agreement between the QAP and PWG Chair.

Kidney (female rats only)

The SP, QAP, and PWG Chair generally agreed on the presence of nephropathy of minimal severity in the kidneys from female rats and that no treatment-related trends were present. The SP did, however, diagnose higher incidences of nephropathy in the LAD treated groups of female rats as compared to their controls. The QA pathologist and PWG Chair both found that the SP was inconsistent in diagnosing nephropathy and diagnosed this change in many more animals resulting in a comparable incidence of this finding in all groups. No slides were shown to the PWG because no treatment related effect was found by the SP, QAP or PWG Chair and there was good agreement between the QAP and PWG Chair.

POST-PWG ACTION ITEMS

One post-PWG action item involved the review of slide #5 from animal number 135. The PWG Chair reviewed this slide and compared the forestomach to a piece of tissue on slide #12 which was reviewed by the PWG. Based upon this comparison, the piece of tissue in which edema was diagnosed was actually forestomach. Therefore, Skin - Edema, which was previously diagnosed by the SP and QAP on Slide #12, was changed to Forestomach - Edema. In addition, the skin tumor from animal #110 was reviewed by NTP pathologists post-PWG and the consensus diagnosis was basal cell adenoma. Therefore, the diagnosis was changed from sebaceous gland adenoma to basal cell adenoma.


Lynda L. Lanning, D.V.M., D.A.B.T.
PWG Chairperson

4/1/97
Date

PATHOLOGY WORKING GROUP CHAIRPERSON'S REPORT

The Chronic Dermal Toxicity/Carcinogenicity Study of Lauric Acid Diethanolamine Condensate (CAS NO. 120-40-1) in B6C3F₁ Mice

PWG Participants: Drs. L. Lanning (PAI-Chairperson), R. Herbert (NIEHS), A. Radovsky (NIEHS), J. Leininger (NIEHS), C. Shackelford (EPL), E. McConnell, J. Noid (PAI-Observer)

Date: March 21, 1997

Site: NIEHS, Research Triangle Park

The PWG convened to review selected slides from B6C3F₁ mice administered lauric acid diethanolamine condensate (LAD) by dermal exposure for two years. Mice were exposed to 0, 100, or 200 mg/kg using dosing solutions (95% ethanol vehicle), 5 days per week for 104 weeks, exclusive of weekends and holidays. The experimental design is described below.

Group Number	Dose Level (mg/kg)	Total Number of Animals	Animal ID Numbers
Male	0	50	1 - 50
	100	50	51 - 100
	200	50	101 - 150
Female	0	50	151 - 200
	100	50	201 - 250
	200	50	251 - 300

The study was conducted at Battelle, Columbus Operations. The Study Pathologist (SP) was Dr. M. Ryan and the Quality Assessment Pathologist (QAP) was Dr. C. Shackelford of Experimental Pathology Laboratories Inc. (EPL).

Potential target organs were examined by the QAP and PWG Chair for all tumor and nontumor diagnoses for all animals in all groups. Organs reviewed for all diagnoses were as follows:

Male Mice

Liver
Skin, site of application(SOA)

Female Mice

Liver
Skin, SOA

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

Male Mice

Islets, pancreatic - Hyperplasia
Thyroid gland, follicle - Hyperplasia

Female Mice

Islets, pancreatic - Hyperplasia
Kidney - Nephropathy
Thyroid gland, follicle - Hyperplasia

In addition, all tumor diagnoses from all animals in all groups were reviewed

Sections of the following organs from the sex indicated were reviewed by the QAP when the specific diagnoses listed were present. These sections were not provided to the PWG Chair for review.

Male Mice

None

Female Mice

Bone - Fibrosis

Bone - Fibrous osteodystrophy

SUMMARY OF PWG FINDINGS

Thyroid gland

The incidence of follicular hyperplasia was increased in male mice exposed to 100 or 200 mg/kg LAD as compared with those from the 0 mg/kg dose group.

Liver

The incidence of hepatocellular neoplasia (adenoma/carcinoma) was increased in female mice exposed to 100 or 200 mg/kg LAD as compared with those from the 0 mg/kg dose group.

Kidney (female mice only)

No treatment effects were noted in the kidney of female mice.

Islets, Pancreatic

No treatment effects were noted in the islets of the pancreas.

Skin, SOA

The incidence of lesions of the skin at the site of application was increased in male and female mice exposed to 100 and 200 mg/kg of LAD as compared with those from the 0 mg/kg exposure group. The lesions included one or more of the following; epidermal hyperplasia, sebaceous gland hyperplasia, ulcer, parakeratosis, hyperkeratosis, and chronic active inflammation. One female mouse exposed to 200 mg/kg had a fibrosarcoma in the subcutis at the site of application.

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 were differences in diagnoses among the SP, QAP, and PWG Chair.

RESULTS OF THE PWG REVIEW

Skin, SOA

The SP, QAP and PWG Chair concurred as to the presence of increased incidences of lesions in the skin, site of application, in mice exposed to 100 or 200 mg/kg LAD. The lesions included one or more of the following; epidermal hyperplasia (increased thickness of the epidermis), sebaceous gland hyperplasia (increased number of cells in each sebaceous gland), ulcer, parakeratosis (retention of the nuclei in the cells of the stratum corneum of the epidermis), hyperkeratosis (increased thickness of the keratinized layer of the epidermis), and chronic active inflammation. One female mouse exposed to 200 mg/kg had a fibrosarcoma in the subcutis at the site of application. The PWG examined some representative examples of lesions diagnosed by the SP as well as those diagnosed by the QAP and concurred with the QAP and PWG Chair. These findings are all associated with the processes involved with dermal irritation and the resolution of this irritation. The QAP and PWG Chair diagnosed these changes in several more animals in all groups, however the test material-related increase was confirmed. The PWG maintained that the lesion diagnosed as inflammation, chronic active (microscopically observed as an increased thickness of the dermis with scattered inflammatory cells including neutrophils, lymphocytes and macrophages) would more appropriately be described as dermal thickening, however because the SP, QAP and PWG Chair were consistent in the use of the inflammation terminology, the diagnoses should remain as listed in the Slide Review Worksheets.

Thyroid gland

The SP, QAP and PWG Chair generally agreed on the incidences for neoplastic and nonneoplastic lesions in the thyroid gland and that there was a dose-related increase in the incidence of follicular hyperplasia in male mice from the 100 and 200 mg/kg dose groups. The QAP and PWG Chair diagnosed this change in many more animals than did the SP, however the dose-related increase was confirmed. Corresponding increases in the incidences of neoplastic lesions involving the thyroid glands were not present. The PWG examined some representative examples of lesions diagnosed by the SP as well as those diagnosed by the QAP and concurred with the QAP and PWG Chair.

Liver

The SP, QAP and PWG Chair generally agreed on the incidences for neoplastic and nonneoplastic lesions in the liver. The incidences of hepatic neoplasms

(adenoma/carcinoma) were increased in female mice exposed to 100 and 200 mg/kg LAD as compared with those from the 0 mg/kg dose group. There was also a slight increase in the incidence of eosinophilic foci in the female treated mice. The QAP and PWG Chair diagnosed eosinophilic foci in several more animals in all groups, however the test material-related increase was confirmed. The PWG examined some representative examples of lesions diagnosed by the SP as well as those diagnosed by the QAP and concurred with the QAP and PWG Chair.

Kidney (female mice only)

The SP, QAP, and PWG Chair generally agreed on the presence of nephropathy of minimal severity in the kidneys from female mice and that no treatment-related trends were present. The SP did, however, diagnose a lower incidence of nephropathy in the high dose LAD treated groups of female mice as compared to their controls. The QA pathologist and PWG Chair both found that the SP was inconsistent in diagnosing nephropathy and diagnosed this change in many more animals resulting in a comparable incidence of this finding in all groups. No slides were shown to the PWG because no treatment related effect was found by the SP, QAP or PWG Chair and there was good agreement between the QAP and PWG Chair.

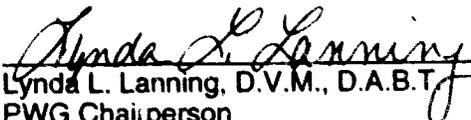
Islets, Pancreatic

The SP, QAP, and PWG Chair generally agreed on the presence of hyperplasia of minimal severity in the pancreatic islets from female mice and that no treatment-related trends were present. The SP did, however, diagnose a lower incidence of hyperplasia in the high dose LAD treated groups of male mice and higher incidences of hyperplasia in both LAD treated groups of female mice as compared to their controls. The QA pathologist and PWG Chair both found that the SP was inconsistent in diagnosing this lesion and diagnosed this change in fewer animals than did the SP, resulting in a similar incidence of the lesion in all groups. No slides were shown to the PWG because no treatment related effect was found by the SP, QAP or PWG Chair and there was good agreement between the QAP and PWG Chair.

POST-PWG ACTION ITEMS

The lungs from LM#72 and VF#178 were examined after the PWG by NTP Pathologists. Diagnoses for lung lesions on these slides were not agreed upon by the Study Pathologist, Reviewing Pathologist or PWG Chairperson during the pre-PWG slide review and were not shown to the PWG. The consensus diagnoses are:

LM#72--lung, alveolar epithelium-hyperplasia, marked
VF#178--lung, alveolar epithelium-hyperplaisa, marked


Lynda L. Lanning, D.V.M., D.A.B.T.
PWG Chairperson

5/19/97
Date

NTP Experiment - Test: 55323-03
Study Type: CHRONIC
Route: SKIN APPLICATION

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
LAURIC ACID DIETHANOLAMINE CONDENSATE

Report: PEIRPT03
Date: 07/17/97
Time: 12:22:52

CORE STUDY

Facility: Battelle Columbus Laboratory

Chemical CAS #: 120-40-1

Lock Date: 07/13/95

Cage Range: All

Reasons For Removal: All

Removal Date Range: All

Treatment Groups: Include All

* Number of animals examined microscopically at site and number of animals with lesion

Report: PEIRP103
Date: 07/17/97
Time: 12:22:52

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
LAURIC ACID DIETHANOLAMINE CONDENSATE

NTP Experiment - Test: 55323-03
Study Type: CHRONIC
Route: SKIN APPLICATION

FISCHER 344 RATS FEMALE	0 MG/KG	50 MG/KG	100 MG/KG
Animals Initially In Study	50	50	50
Early Deaths	11	12	22
Natural Death	10	12	5
Morbund Sacrifice	1		1
Accidentally Killed			
Survivors	28	26	22
Terminal Sacrifice			
Animals Examined Microscopically	50	50	50

DISPOSITION SUMMARY

Animals Initially In Study
Early Deaths
Natural Death
Morbund Sacrifice
Accidentally Killed
Survivors
Terminal Sacrifice
Animals Examined Microscopically

ALIMENTARY SYSTEM

Intestine Large, Colon	(45)	(46)	(43)
Parasite Metazoan	2 (4%)	4 (9%)	3 (7%)
Intestine Large, Rectum	(42)	(45)	(43)
Inflammation, Chronic Active	2 (5%)	2 (4%)	1 (2%)
Parasite Metazoan	(40)	(40)	1 (2%)
Intestine Large, Cecum	(49)	1 (3%)	(31)
Inflammation, Chronic Active	(49)	(49)	(49)
Intestine Small, Duodenum	1 (2%)	1 (2%)	1 (2%)
Inflammation, Chronic Active	(42)	(43)	(38)
Ulcer	1 (2%)	1 (2%)	1 (2%)
Intestine Small, Jejunum	(48)	(42)	(37)
Mineralization	(50)	(50)	(50)
Intestine Small, Ileum	1 (2%)	3 (6%)	1 (3%)
Parasite Metazoan	1 (2%)	3 (6%)	1 (2%)
Liver	12 (24%)	17 (34%)	2 (4%)
Basophilic Focus	13 (26%)	15 (30%)	2 (4%)
Clear Cell Focus	1 (2%)	1 (2%)	4 (8%)
Zoophilic Focus	3 (6%)	4 (8%)	2 (4%)
Hepatodiphragmatic Nodule	5 (6%)	6 (12%)	2 (4%)
Inflammation, Chronic Active	1 (2%)	1 (2%)	1 (2%)
Mixed Cell Focus	(c)	(2)	(4)
Necrosis	4 (8%)	1 (50%)	3 (75%)
Vacuolization Cytoplasmic	6 (12%)	1 (50%)	2 (50%)
Bile Duct, Hyperplasia	1 (2%)	1 (2%)	
Central Vein, Dilatation	1 (2%)	1 (2%)	
Hepatocyte, Hyperplasia	4 (80%)	1 (50%)	
Mesentery	1 (100%)	1 (50%)	
Necrosis	1 (100%)	1 (50%)	
Fat, Inflammation, Chronic Active	(50)	(50)	(49)
Fat, Mineralization			
Oral Mucosa			
Hyperplasia			
Pancreas			

a Number of animals examined microscopically at site and number of animals with lesion

Report: PEIRPT03
Date: 07/17/97
Time: 12:22:52

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
LAURIC ACID DIETHANOLAMINE CONDENSATE

NTP Experiment-Test: 55223-03
Study Type: CHRONIC
Route: SKIN APPLICATION

FISCHER 344 RATS FEMALE	0 MG/KG	50 MG/KG	100 MG/KG
ALIMENTARY SYSTEM - CONT			
Inflammation, Chronic Active		1 (2%)	1 (2%)
Necrosis		5 (10%)	1 (2%)
Acinus, Atrophy	3 (6%)		1 (2%)
Arteriole, Thrombosis	(50)	(50)	(50)
Stomach, Forestomach		1 (2%)	2 (4%)
Hemorrhage		2 (4%)	1 (2%)
Inflammation, Chronic Active	2 (4%)		1 (2%)
Ulcer	(50)	(50)	(49)
Stomach, Glandular			
Erosion	3 (6%)		
CARDIOVASCULAR SYSTEM			
Blood Vessel	(50)	(50)	(50)
Inflammation, Chronic Active	(50)	(50)	1 (2%)
Heart			(49)
Hemorrhage			1 (2%)
Mineralization		1 (2%)	
Myocardium, Inflammation, Chronic Active	27 (54%)	31 (62%)	35 (71%)
ENDOCRINE SYSTEM			
Adrenal Cortex	(50)	(50)	(50)
Accessory Adrenal Cortical Nodule	2 (4%)	2 (4%)	3 (6%)
Angiectasis	2 (4%)		
Degeneration	1 (2%)		
Hematopoietic Cell Proliferation		1 (2%)	
Hemorrhage	2 (4%)	2 (4%)	1 (2%)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)
Hypertrophy			
Mineralization		1 (2%)	
Necrosis	1 (2%)	4 (8%)	1 (2%)
Vacuolization Cytoplasmic	5 (10%)	(50)	7 (14%)
Adrenal Medulla			(50)
Hyperplasia	(47)	(40)	(45)
Parathyroid Gland	1 (2%)	2 (4%)	2 (4%)
Hyperplasia	(50)	(50)	(47)
Pituitary Gland	8 (16%)	5 (10%)	3 (6%)
Cyst			1 (2%)
Hemorrhage			2 (4%)
Hyperplasia	4 (8%)		1 (2%)
Thrombosis	1 (2%)	2 (4%)	2 (4%)
Pars Distalis, Angiectasis	1 (2%)		2 (4%)
Pars Distalis, Hyperplasia	(50)	(50)	(50)
Thyroid Gland			

a Number of animals examined microscopically at site and number of animals with lesion