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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 Dose Feed study on CITRAL (5392-40-5) 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.

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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.



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Sincerely,



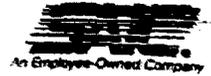
William Eastin, Ph.D.
Head, Information Systems & Central Files
Environmental Toxicology Program

Encls: PWG Report and Pathology Summary Tables for Rats & Mice
cc: Central Data Management
Dr. Bucher
Dr. Chhabra

A-05



Pathology Associates International
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CAS No. 5392-40-5

PATHOLOGY WORKING GROUP

**Chairperson's Report
of the**

**Two year Chronic Study of Citral (C56348A/56348-03)
in
Male and Female F344/N Rats**

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Prepared by:

Peter B. Little

**Pathology Associates International (PAI)
4915 Prospectus Drive
Durham, NC 27713**

Submitted to:

**National Toxicology Program/NIEHS
Research Triangle Park, NC**

March 29, 2000

The pathologist performing this review, Dr. Peter B. Little has had no involvement with any laboratory or organization concerned with this study other than the NTP, and has not been involved in the origination or any previous review of data from this study.

PATHOLOGY WORKING GROUP CHAIRPERSON'S REPORT

Two year Chronic Study of Citral in F344 Rats

I. Introduction:

Date of PWG Review: March 1, 2000
 Site: NIEHS, Research Triangle Park, NC.

Participants:

Robert Maronpot, D.V.M.; NIEHS; Ronald Herbert, D.V.M., Ph.D.; NIEHS; Robert Sills, D.V.M., Ph.D.; NIEHS; Cynthia Shackelford, D.V.M., M.S., Ph.D.; EPL; John Seely, D.V.M.; EPL; Gordon Flake, M.D.; NIEHS (observer); Jeffrey Wolf, D.V.M., EPL (observer); and Peter L. Little D.V.M., M.S., Ph.D.; PAI (PWG Chairperson)

This NTP PWG was conducted to evaluate selected slides from a two year study of male and female F344 rats exposed to Citral ad libitum dosed feed at concentrations of 0% (untreated control), 0% (vehicle control), 0.312% (low dose), 0.625% (mid dose), and 1.25% (high dose). The study consisted of 50 rats per sex per dose group.

The study was conducted at Batelle Columbus Ohio. The study pathologist (SP) for the rat study was Dr. Allen W. Singer, and the Quality Assessment Pathologist (QAP) was Dr. Cynthia Shackelford of Experimental Pathology Laboratories, Inc.

II. SUMMARY OF THE PWG FINDINGS

The average severity of Kidney - Mineralization was increased in the mid and high dose groups of males as compared with controls.

III. CONDUCT OF THE PWG REVIEW

The PWG Chairperson examined the pathology tables, the SP's narrative, the Quality Assessment Report, and microslides of tissues selected for QA review. Examples of potential treatment related lesions and discrepancies in diagnosis among the SP, the QAP and the PWG Chairperson were selected for examination by the PWG.

The QAP had examined selected organs for all tumor and nontumor diagnoses for all animals in all groups. These organs were as follows:

Male Rats

Adrenal Medulla
 Prostate
 Thyroid Gland, C-Cell

Female Rats

Adrenal Cortex
 Adrenal Medulla
 Clitoral Gland
 Mammary Gland
 Uterus

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

Male Rats

Adrenal cortex - Angiectasis
Kidney - Mineralization
Liver - Eosinophilic Focus
Liver - Mixed Cell Focus
Pituitary Gland - Angiectasis

Female Rats

Lymph Node, Mandibular - Ectasia
Lymph Node, Mandibular - Hyperplasia, Plasma Cell
Pituitary Gland - Angiectasis
Spleen - Hematopoietic Cell proliferation

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

Male Rats

Liver - Hyperplasia, Adenomatous
Stomach, Forestomach - Hyperplasia
Stomach, Forestomach - Hyperplasia , Basal Cell
Skin, Subcutaneous Tissue - Trichoepithelioma
Skin, Dermis - Schwannoma, Malignant
Lung, Bronchiole - Carcinoma

Female Rats

Liver - Hyperplasia, Adenomatous
Spleen - Hyperplasia, Adenomatous
Pituitary Gland - Carcinoma

All tumor diagnoses (except Testes- Interstitial Cell Tumor) in all organs from all animals in all groups were reviewed.

The PWG chair reviewed all slides and target organs examined by the QAP.

IV. RESULTS OF THE PWG REVIEW

KIDNEY

The SP had diagnosed greater incidences of Kidney - Mineralization of the renal pelvis in the mid and high dose male groups as compared with controls and similar, very low incidences in females. The QAP diagnosed minimal mineralization in most of the remaining kidneys in males and females such that the incidence of this lesion was similar in all groups of male and females. However, the increased severity of

mineralization was confirmed by the QAP. The PWG Chair confirmed the QAP's findings. Microscopically, the lesion was characterized by the presence of minute to focally extensive mineralization of the stromal tissue between the collecting ducts which was most severe in the two highest dosed groups of male animals.

The PWG examined examples of renal pelvic mineralization of minimal and moderate severity, and agreed on the presence of the lesion and the severity grades diagnosed. The PWG confirmed the presence of minimal mineralization in some kidneys, as had been diagnosed by the QAP. The PWG decided, as a result of this discussion, to require as a post PWG action item that the kidneys of rats from the thirteen week subchronic Citral study be examined for the presence or absence of minimal renal pelvic mineralization.

FORESTOMACH

The SP had diagnosed similar incidences of **Forestomach - Hyperplasia** in the control and treated male and female groups. The QAP changed the SP's diagnosis of **Stomach, Forestomach - Hyperplasia** to the more specific **Stomach, Forestomach, Epithelium - Hyperplasia, Basal Cell or Stomach, Forestomach, Epithelium - Hyperplasia**. The PWG chairperson identified 17 further examples of epithelial hyperplasia in males and females, as well as one ulcer. The PWG examined these lesions and confirmed 11 of the 17. The forestomach ulcer in the vehicle control group was also confirmed by the PWG. While the QAP had diagnosed forestomach epithelial basal cell hyperplasia, the PWG elected to include that diagnosis under the inclusive term of forestomach epithelial hyperplasia rather than diagnosing it as a separate entity. The conclusion was that this lesion was not treatment related, and the additional cases of forestomach hyperplasia had no effect on that conclusion.

PROSTATE

The SP did not observe any treatment related effects in the prostate. This finding was confirmed by the QAP and PWG Chair. The QAP diagnosed **Prostate, Ventral - Hyperplasia** in seven males from various dose groups; the incidences were similar across dose groups. The PWG chairperson identified two additional ventral prostatic hyperplasias, one in the vehicle control group and one in the mid dose group. The PWG confirmed both additional prostatic hyperplasias. The addition of these examples did not change the conclusion that this lesion was not treatment related.

ADRENAL GLAND

The SP had diagnosed **Adrenal Cortex - Angiectasis** in a few more males in the mid and high dose groups than in the control and low dose group. The QAP considered this a normal feature, and the PWG chair concurred with this opinion. The PWG was shown two examples of this change in the high dose group, one of which was confirmed by the PWG, while the second was not confirmed. The PWG considered that no treatment related effect was present.

The SP's findings showed a similar incidence of Adrenal Medulla -- Hyperplasia across all groups. The QAP added more examples in most groups. The PWG chair diagnosed adrenal medullary hyperplasia in four additional males, but none of these four were confirmed by the PWG. The PWG concluded that there was no treatment related effect on the incidences of adrenal medullary hyperplasia in males or females.

PITUITARY GLAND

The SP had diagnosed higher incidences of Pituitary Gland -- Angiectasis, characterized microscopically by dilated vascular spaces, in untreated control males as compared with other male groups, and in vehicle control and treated groups of females as compared with the untreated control females. The QAP rediagnosed these as occurring either in the pars distalis or pars intermedia. This change was not considered to represent a treatment related effect in males. In females however, both the SP's and QAP's incidences did suggest a treatment related effect. The PWG Chair, in his review, considered that angiectasis had been inappropriately diagnosed in most cases by both the SP and QAP, since in most cases dilated vascular spaces were associated with the occurrence of focal hyperplasia or adenoma. The PWG Chair considered this vascular enlargement to be secondary to the underlying lesion. The PWG was shown six examples of angiectasis diagnosed by the SP and QAP, and none were confirmed by the PWG. The PWG consensus was that this diagnosis was inappropriate and should be deleted from all animals in this study.

The PWG Chair found what he considered to be many examples of previously undiagnosed pituitary adenomas. The PWG was shown all of the adenomas diagnosed by the PWG Chair, plus three examples of pituitary adenoma that the SP, QAP and PWG chair agreed on. The PWG concurred with the diagnosis of pituitary adenoma for the three animals on which the SP, QAP, and PWG Chair agreed. Of the pituitary adenomas diagnosed by the PWG chair in female rats five of ten were confirmed, while in males five of fifteen were confirmed. The PWG examined the data on pituitary adenomas and concluded that there was no evidence of a treatment related effect for this lesion.

CLITORAL GLAND

There were three discrepancies among the SP, QAP and PWG Chair in diagnoses of proliferative clitoral gland lesions which were shown to the PWG. Clitoral gland adenoma, carcinoma, and duct cysts were not considered to be treatment related.

MANDIBULAR LYMPH NODE

The SP had diagnosed Hyperplasia, Plasma Cell in a number of animals in each female group; however the QAP considered the lesion not to be present except in two cases in high dose animals. Tabulated data from the SP's diagnosis of this lesion indicated no treatment related effect. The PWG was shown four examples of this lesion diagnosed by the SP, and in all cases the PWG unanimously considered

that hyperplasia was not present. Therefore, the PWG consensus was that all diagnoses of **Mandibular Lymph Node - Hyperplasia, Plasma Cell** be deleted for all animals in this study.

THYROID GLAND

The SP had diagnosed somewhat higher incidences of **C-Cell - Hyperplasia** in vehicle control and treated groups of females as compared with the untreated control females. The QAP disagreed with some of the SP's findings such that the incidences of C-cell hyperplasia diagnosed by the QAP were more similar across female groups. Five discrepancies in diagnosis of thyroid follicular or C-cell lesions were shown to the PWG. The PWG examined the SP's and QAP's findings and concluded that there was no treatment related effect for thyroid gland C-cell proliferative lesions.

MAMMARY GLAND

The SP had diagnosed **Mammary Gland - Galactocoele** and **Mammary Gland - Hyperplasia** in a number of animals in each female group. In most cases the QAP considered the change diagnosed as galactocoele to be part of a fibroadenoma, and either disagreed that hyperplasia was present or rediagnosed it as focal or lobular hyperplasia. The PWG Chair concurred with the QAP in most cases. The incidences as diagnosed by the QAP did not indicate a treatment related effect. Since there was good agreement between the QAP and PWG Chair concerning these lesions, no slides were reviewed by the PWG.

UTERUS

The SP had diagnosed similar incidences of **Hyperplasia, Cystic** in control and treated groups of females. The QAP added a few diagnoses of hyperplasia to each group and suggested use of the terminology **Uterus, Endometrium - Hyperplasia, Cystic** to diagnose this change. The PWG Chair confirmed the QAP's findings. Neither the SP's nor the QAP's findings were indicative of any treatment related effect. Since there was good agreement between the QAP and PWG Chair and since there was no treatment related effects in the uterus, no slides were reviewed by the PWG.

SPLEEN

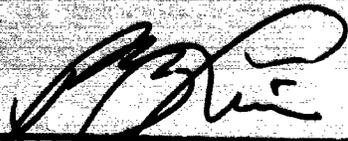
The SP diagnosed higher incidences of **Hematopoietic Cell Proliferation** in untreated and vehicle control females as compared with the treated female groups. The SP's findings were confirmed by the QAP and PWG Chair. Since there was good agreement on these lesions, no slides were reviewed by the PWG.

MISCELLANEOUS

There were a number of lesions in which there was discrepancy among the SP, QAP and PWG Chairperson concerning the nature of various neoplastic lesions. All examples were shown to the PWG, and the resulting diagnoses are listed in the accompanying tables. None were considered to be treatment related.

V. Post PWG Review Actions:

Kidneys of rats from the 13 week subchronic study are to be reviewed and assessed for the presence of renal pelvic mineralization.



Peter B. Ellis, DVM, M.S., Ph.D.
Diplomate AQVP
PWG Chairperson

March 29/2000
Date



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**PATHOLOGY WORKING GROUP
CHAIRPERSON'S REPORT**

5892-40-5

06348 A

**2-YEAR CHRONIC STUDY OF
CITRAL (C56348A)
ADMINISTERED BY DOSED FEED
TO B6C3F1 MICE**

PWG:CHM

Prepared by:

Micheal P. Jokinen, DVM
Pathology Working Group Chairperson

Pathology Associates International
4915D Prospectus Drive
Durham, NC 27713

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Submitted to:

National Toxicology Program/NIEHS
Research Triangle Park, NC

March 20, 2000

The pathologist performing this review, Dr. Micheal P. Jokinen, has had no involvement with any laboratory or organization concerned with this study other than NTP, and has not been involved in the origination or any previous review of data from this study.

PATHOLOGY WORKING GROUP CHAIRPERSON'S REPORT

2-Year Chronic Study of Citral in B6C3F1 Mice

Participants: Drs. M. Jokinen (PAI - PWG Chairperson), A. Brix (EPL- QAP), R. Herbert (NIEHS), G. Flake (NIEHS - observer), R. Maronpot (NIEHS), A. Nyska (NIEHS), and H. Wall (GlaxoWellcome)

Date: February 29, 2000

Site: NIEHS, Research Triangle Park, NC

The PWG was convened to evaluate selected slides from B6C3F1 mice administered citral by dosed feed for two years. The citral was placed in microcapsules mixed in the feed. Consequently, two control groups were used for this study: an untreated control group which received normal feed, and a vehicle control group which received feed mixed with empty microcapsules. The doses and numbers of animals examined microscopically per group were as follows:

Dose	M	F
Untreated Control	50	50
Vehicle Control	50	50
0.156%	50	50
0.312%	50	50
0.625%	50	50

The study was conducted at Battelle Columbus Laboratories. The Study Pathologist (SP) was Dr.M. Ryan 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

- Oral Mucosa
- Prostate
- Stomach, Forestomach
- Bone Marrow
- Spleen
- Lung

Female Mice

- Oral Mucosa
- Ovary
- Stomach, Forestomach
- Bone Marrow
- Spleen
- Lung
- Lymph Node, Mesenteric
- Thymus
- Islets, Pancreatic

In addition, adrenal cortex was reviewed from all males or all females when diagnoses of Zona Glomerulosa - Hyperplasia, Focal; Hyperplasia, Focal; Hypertrophy, Focal; or Vacuolization, Cytoplasmic, Focal were present, and the glandular stomach of females was reviewed when the diagnosis of Epithelium - Hyperplasia, Atypical, Focal was present. All diagnosed neoplasms in all tissues in all animals were also reviewed.

A few slides containing minor lesions not considered to be of sufficient significance to warrant review by the entire PWG were examined immediately following the PWG by Drs. Jokinen, Maronpot, and Brix. The slides reviewed concerned disagreements as to whether inflammation and/or ulcer were present in the oral mucosa, whether hematopoietic cell proliferation was present in the spleen, whether some proliferative lung lesions were alveolar epithelial hyperplasias, or alveolar/bronchiolar adenomas or carcinomas, and whether or not hyperplasia of the pancreatic islets was present. The findings of this review were included with the other PWG findings.

SUMMARY OF REVIEW FINDINGS

The incidences of Lymphoma, Malignant in the mid and high dose (0.312% and 0.625%) groups of females were increased as compared with the control groups.

Incidences of Oral Mucosa - Inflammation, Chronic Active were increased in some treated groups of males and females as compared with controls. The incidences of Oral Mucosa - Ulcer were increased in treated groups of females as compared with controls, while the incidence of Oral Mucosa - Ulcer in the untreated male group was lower than in the vehicle control and treated male groups.

The combined incidences of Lung - Alveolar/Bronchiolar Adenoma and Lung - Alveolar/Bronchiolar Carcinoma were lower in the high dose (0.625%) male group and the mid and high dose (0.312% and 0.625%) groups of females as compared with respective controls.

The incidences of Bone Marrow - Hyperplasia in untreated control males and females were lower than in the respective vehicle control and treated 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**

Incidences of Lymphoma, Malignant were increased in the mid and high dose (0.312 and 0.625%) female groups as compared with the respective control and low dose female groups. Tissues most commonly affected with lymphoma were the **Spleen; Lymph Node, Mesenteric; Thymus; and to a lesser extent Ovary.** These tissues were reviewed from all females.

Overall, there was very good agreement among the SP, QAP, and PWG Chair concerning the presence of lymphoma. The PWG reviewed tissues of a number of diagnosed lymphomas that were considered to be early by the PWG Chair and the PWG confirmed the diagnosis. The PWG also reviewed a few disagreements, and a few cases diagnosed as lymphoid hyperplasia by the SP that the PWG Chair thought potentially may have been early lymphomas.

Spleen

The most important finding, as mentioned above, was an increased incidence of lymphoma in the mid and high dose female groups. The SP had diagnosed a somewhat lower incidence of **Hematopoietic Cell Proliferation** in the high dose male group as compared with the control groups and other treated male groups. The QAP added or deleted diagnoses of **Hematopoietic Cell Proliferation** in each of the male groups such that the difference in incidence was somewhat less, although the incidence in the high dose male group was still somewhat lower than in untreated male group but not the vehicle control group. With few exceptions, the PWG Chair agreed with the QAP's diagnoses. The SP had diagnosed similar incidences of **Hematopoietic Cell Proliferation** in control and treated groups of females; this was confirmed by the QA/PWG review. The SP also diagnosed similar incidences of **Lymphoid Follicle - Atrophy** and **Lymphoid Follicle - Hyperplasia** in treated and control groups of males and females; these findings were also confirmed by the QA/PWG review. Since there had been good agreement among the SP, QAP, and PWG Chair, and since the only potential treatment related effect seen in the spleen was an increase in malignant lymphoma in females, the PWG only reviewed a few spleens in which lymphoma, or lymphoid hyperplasia that may have been early lymphoma, had been diagnosed. A few spleens in which there was a disagreement as to whether or not hematopoietic cell proliferation was present were reviewed immediately after the PWG by Drs. Jokinen, Maronpot, and Brix.

Ovary

The incidences of **Lymphoma, Malignant** were increased in the mid and high dose female groups, as mentioned above. The SP had diagnosed similar

incidences of **Cyst** in the control and treated groups of females, and this was confirmed by the QA/PWG review. The PWG reviewed one ovary diagnosed by the SP as containing a cystadenocarcinoma. The PWG considered the ovarian lesion to be a squamous cell carcinoma metastatic from the forestomach.

Mesenteric Lymph Node

The incidences of **Lymphoma, Malignant** were increased in the mid and high dose female groups, as mentioned earlier. Otherwise, there were no significant findings in the mesenteric lymph node.

Thymus

The incidence of **Lymphoma, Malignant** was increased in the high dose female group, as mentioned earlier. The SP had diagnosed a somewhat lower incidence of **Thymus - Atrophy** in the high dose female group as compared with the control and other treated female groups. This was confirmed by the QA/PWG review. Atrophy was characterized microscopically by reduction in the number of cortical lymphocytes and consequent reduction in the size of the thymus. In most cases the atrophy appeared to be normal involution that occurs with age, although in a few early death animals there appeared to be loss of lymphocytes secondary to the moribund condition of the animal prior to death. The lower incidence of atrophy in the high dose group may have been due in part to the increased incidence of lymphoma in that group, as the presence of lymphoma would have masked the presence of atrophy. The SP had diagnosed a somewhat greater incidence of **Thymus - Hyperplasia, Lymphoid** in the mid and high dose female groups. The QAP added some additional diagnoses to the untreated control group so that the incidences in the untreated control group and the mid and high dose female groups were similar; the incidence in the vehicle control group as diagnosed by the QAP was still somewhat lower than in the mid and high dose groups. The PWG Chair confirmed the QAP's findings.

Oral Mucosa

The SP had diagnosed greater incidences of **Oral Mucosa - Inflammation, Chronic Active** in the mid and high dose male groups than in the two control groups, and greater incidences of **Oral Mucosa - Ulcer** in the vehicle control and treated groups of males as compared with the untreated male control group. The SP also diagnosed greater incidences of **Ulcer** in the treated groups of females as compared with the control female groups. A number of changes to the SP's findings were made as a result of the QA/PWG review. The SP's findings concerning the trends in the incidences of inflammation and ulcer in males and of ulcer in females were confirmed. However, the QAP deleted a number of diagnoses of inflammation from the control groups of females such that the QAP's findings indicate greater incidences of inflammation in the treated female groups as compared with the female control groups. The PWG Chair

agreed with most, but not all of the QAP's findings, and final conclusions regarding the incidences of these two changes must await preparation of the final pathology tables. However, it appeared in general that the QAP's findings were confirmed. The PWG examined some representative examples of inflammation and ulcer and confirmed the diagnoses.

Microscopically, the areas of inflammation and ulcer were, with rare exception, directly medial to the molar teeth in nose section III. Chronic active inflammation was, generally, a minimal to moderate change characterized by accumulation of mixed inflammatory cells within and beneath the oral mucosal epithelium directly adjacent to the medial aspect of the tooth. In a majority of the cases hair shafts were present in the inflamed area, and appeared to have been driven into the tooth socket where they penetrated the oral mucosa into the underlying connective tissue. Ulcers were, generally, of minimal to mild severity and consisted of focal areas of loss of the oral mucosal epithelium that were almost always located at the points at which hair shafts penetrated the oral mucosa.

The opinion of the PWG was that the inflammation and ulcer were secondary to the presence of the hair, and that in those sections with inflammation in which hair shafts were not seen, a hair shaft was most likely present but not in the plane of section. The PWG was also of the opinion that the inflammation and ulcer were probably not a direct toxic effect of the test chemical but were more likely a secondary effect, possibly due to some type of behavioral change induced by the test chemical or by the presence of the microcapsules in the feed.

Forestomach

The SP diagnosed higher incidences of **Stomach, Forestomach, Epithelium - Hyperplasia, Diffuse** in mid and high dose males as compared with control and low dose males, and similar incidences of diffuse epithelial hyperplasia of the forestomach in control and treated groups of females. The QAP either deleted all of these diagnoses, in most cases considering the apparent change to be due to incomplete inflation of the stomach or, in a few animals, generally early death animals, considered the change to be **Stomach, Forestomach, Epithelium - Hyperkeratosis, Diffuse**. The PWG Chair confirmed the QAP's findings.

The PWG reviewed representative examples in which the QAP considered diffuse hyperplasia to be absent or diffuse hyperkeratosis to be present, and confirmed the QAP's findings. The PWG was of the opinion that the forestomachs in which the SP had diagnosed diffuse hyperplasia were within the range of normal and that the apparent thicker epithelium in some forestomachs was due to the fact that these stomachs were not as fully inflated as were other stomachs.

Lung

The SP had diagnosed lower combined incidences of Lung – A/B Adenoma and Lung – A/B Carcinoma in the high dose males, and mid and high dose females as compared with the respective control and other treated groups. Incidences of Lung. Alveolar Epithelium – Hyperplasia, Focal were low and similar across control and treated groups of both males and females. These findings were confirmed by the QA/PWG review. The PWG reviewed a few slides in which the PWG Chair felt a lesion was present that had not been diagnosed previously. Also, a few slides of disagreements concerning whether a lung lesion represented a hyperplasia, adenoma, or carcinoma were reviewed by Drs. Jokinen, Maronpot, and Brix immediately after the conclusion of the PWG.

Bone Marrow

The SP diagnosed higher incidences of Bone Marrow – Hyperplasia in the vehicle control and treated groups of males and females than in the untreated control groups of males and females. These findings were confirmed by the QA/PWG review. The QAP disagreed with a number of the SP's diagnoses of bone marrow hyperplasia in each of the male groups, but primarily in the vehicle control and treated groups of males; the PWG Chair generally concurred with the QAP's findings. The QAP's findings confirmed the lower incidence of hyperplasia in the untreated controls as compared with the vehicle control and treated groups, but the difference in incidence as diagnosed by the QAP was less than that diagnosed by the SP. The PWG reviewed a few examples of bone marrow hyperplasia in which there was agreement among the SP, QAP, and PWG Chair, and confirmed the diagnoses. The PWG also reviewed a few slides in which the PWG Chair felt hyperplasia was present that had not been diagnosed previously, and confirmed the PWG Chair's findings. The PWG Chair noted that in many cases the bone marrow hyperplasia appeared to have occurred secondary to a neoplastic or inflammatory process.

Adrenal Cortex

No potential treatment related effects were observed in the adrenal cortex. The SP had diagnosed similar incidences of Adrenal Cortex – Hyperplasia, Focal and Adrenal Cortex – Hypertrophy, Focal in control and treated groups of males and females. The QAP disagreed with some of the SP's findings, but confirmed the SP's findings of similar incidences in control and treated groups. In addition, the SP had diagnosed similar incidences of Adrenal Cortex – Vacuolization, Cytoplasmic, Focal in groups of control and treated males; the SP had also diagnosed focal cytoplasmic vacuolization of the adrenal cortex in one female. The QAP noted that the cytoplasmic vacuolization occurred within foci of hypertrophy and therefore considered the vacuolization to be part of the hypertrophy. Consequently, the QAP considered the cytoplasmic vacuolization not to warrant a separate diagnosis, and recommended deleting the diagnoses

of vacuolization. The PWG Chair concurred with the QAP's finding, noting that, with rare exception, the cytoplasmic vacuolization was present within the foci of hypertrophic cells. In those uncommon cases in which the SP diagnosed vacuolization that was not present within hypertrophic cells, the PWG Chair concurred with the SP. The PWG reviewed representative examples and concurred with the QAP's recommendation.

The SP had also diagnosed **Adrenal Cortex, Zona Glomerulosa – Hyperplasia, Focal** in a few males, primarily in the control groups. The PWG Chair considered these lesions to represent **Adrenal Cortex, Subcapsular – Hyperplasia**. The PWG reviewed all of these lesions and in some cases considered them to be **Adrenal Cortex, Subcapsular – Hyperplasia** or **Adrenal Cortex, Subcapsular – Adenoma**. These hyperplasias and adenomas consisted of small, basophilic, spindle-like A-Cells and/or large, polygonal, foamy B-Cells, and thus differed from the typical subcapsular hyperplasias which consist solely of A-Cells. The PWG was also of the opinion that lesions of the adrenal cortex need not be specified as to which zone (glomerulosa, fasciculata, or reticularis) is affected.

Pancreatic Islets

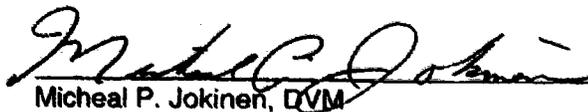
No potential treatment related effects were observed in the pancreatic islets. The SP had diagnosed similar incidences of **Islets, Pancreatic – Hyperplasia** in the untreated control and treated groups of females, and a lower incidence of this change in the vehicle control females. The QAP considered the pancreatic islets in all of these cases to be normal and recommended deleting the SP's diagnosis. With a few exceptions, the PWG Chair concurred with the QAP's recommendations. Two examples in which the QAP recommended deleting the SP's diagnoses were examined by Drs. Jokinen, Maronpot, and Brix immediately after the PWG and all were in agreement that hyperplasia was not present.

Prostate

The SP reported no significant findings in the prostate in any of the control or treated groups of males. This was confirmed by the QAP/PWG review. Since there were no significant findings, the PWG did not review any slides.

POST-PWG ACTION ITEMS

There were no post-PWG action items.



Micheal P. Jokinen, DVM
Diplomate, ACVP
PWG Chairperson

3/20/00
Date

NATIONAL TOXICOLOGY PROGRAM

TR-505 Citral

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 - untreated control vs vehicle control

B 07

NTP Experiment-Test: 56348-03
Study Type: CHRONIC
Route: DOSED FEED

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
CITRAL

FINAL #1/RATS

Report: PRHPT03
Date: 06/09/00
Time: 10:51:48

Facility: Battelle Columbus Laboratory
Chemical CAS #: 5392-40-5
Lock Date: 03/25/99
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

Page 1

NTP Experiment-Test: 56348-03
 Study Type: CHRONIC
 Route: Dosed Feed

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
 CTRLAL

Report: P2RPR03
 Date: 06/09/00
 Time: 10:51:48

DISPOSITION SUMMARY	FISCHER 344 RATS FEMALE		VEHICLE CONTROL		.312%		.625%		1.25%	
	UNTREATED CONTROL	VEHICLE CONTROL								
Animals Initially in Study	50	50	50	50	50	50	50	50	50	50
Early Deaths	12	10	11	11	11	11	11	11	12	12
Morbund Sacrifice	3		3	3	3	3	3	3	2	2
Natural Death										
Surgical Sacrifice	34	40	36	36	36	36	36	36	36	36
Natural Death	1									
Animals Examined Microscopically	50	50	50	50	50	50	50	50	50	50
ALIMENTARY SYSTEM										
Intestine Large, Colon	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Inflammation	2 (4%)	2 (4%)					1 (2%)	1 (2%)	2 (4%)	2 (4%)
Parasite Metaston										
Ulcer										
Intestine Large, Sigmoid	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Parasite Metaston	7 (14%)	4 (8%)	5 (10%)	6 (12%)	5 (10%)	6 (12%)	5 (10%)	6 (12%)	4 (8%)	4 (8%)
Intestine Large, Cecum	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Inflammation										
Intestine Small, Duodenum	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Inflammation										
Parasite Metaston										
Intestine Small, Jejunum	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(49)	1 (2%)	1 (2%)
Dysplasia										
Inflammation							1 (2%)	1 (2%)		
Polyp Inflammatory							1 (2%)	1 (2%)		
Ulcer										
Lymphatic, Cyst										
Intestine Small, Ileum	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Inflammation										
Liver	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Angiectasis										
Basophilic Focus	42 (84%)	3 (6%)	44 (88%)	45 (90%)	44 (88%)	45 (90%)	44 (88%)	45 (90%)	42 (84%)	42 (84%)
Clear Cell Focus	1 (2%)	2 (4%)	2 (4%)	2 (4%)	2 (4%)	2 (4%)	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Degeneration, Cystic	8 (16%)	8 (16%)	7 (14%)	7 (14%)	7 (14%)	7 (14%)	7 (14%)	7 (14%)	9 (18%)	9 (18%)
Basophilic Focus										
Fatty Change										
Fibrosis										
Hematopoietic Cell Proliferation	1 (2%)	3 (6%)	1 (2%)	3 (6%)	1 (2%)	3 (6%)	1 (2%)	3 (6%)	4 (8%)	4 (8%)
Hepatodysplastic Nodule	10 (20%)	8 (16%)	8 (16%)	8 (16%)	8 (16%)	8 (16%)	8 (16%)	8 (16%)	5 (10%)	5 (10%)

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

NTP Experiment-Test: 56348-03
 Study Type: CHRONIC
 Route: DOSED FEED

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
 CITRUS

Report: PRRPT03
 Date: 06/09/00
 Time: 10:51:48

FISCHER 344 RATS FEMALE	UNTREATED CONTROL		VEHICLE CONTROL		.312%		.625%		1.25%	
ALIMENTARY SYSTEM - CONT										
Inflammation	40 (80%)	44 (88%)	44 (88%)	41 (82%)	43 (86%)					
Mixed Cell Focus	16 (32%)	18 (36%)	11 (22%)	16 (32%)	15 (30%)					
Necrosis	5 (10%)	12 (24%)	7 (14%)	4 (8%)	6 (12%)					
Vacuolization Cytoplasmic	25 (50%)	15 (30%)	17 (34%)	13 (26%)	13 (26%)					
Bile Duct, Hyperplasia	5 (10%)	6 (12%)	9 (18%)	7 (14%)	7 (14%)					
Centrilobular, Degeneration	(10)	(5)	(1)	(5)	(7)					
Mesentery	1 (10%)	2 (40%)								
Hemorrhage										
Inflammation										
Thrombosis										
Fat, Necrosis										
Pancreas	8 (80%)	3 (60%)	1 (100%)	4 (80%)	1 (14%)					
Atrophy	(50)	(50)	(50)	(50)	5 (71%)					
Cyst	8 (16%)	12 (24%)	20 (40%)	8 (16%)	10 (20%)					
Cytoplasmic Alteration	2 (4%)	2 (4%)	1 (2%)		2 (4%)					
Hyperplasia	1 (2%)	2 (4%)	2 (4%)							
Inflammation	1 (2%)		1 (2%)							
Metaplasia, Hepatocyte	(50)	(50)	(50)	(50)	1 (2%)					
Salivary Glands	2 (4%)		(50)	(50)	1 (2%)					
Atrophy										
Inflammation	(50)	2 (4%)	(50)	(50)	(50)					
Stomach, Fore stomach	1 (2%)	(49)								
Edema										
Hyperplasia										
Inflammation	2 (4%)	1 (2%)	1 (2%)		1 (2%)					
Ulcer	6 (12%)	2 (4%)	4 (8%)	3 (6%)	1 (2%)					
Stomach, Glandular	(50)	(50)	(50)	(50)	1 (2%)					
Erosion	2 (4%)	1 (2%)	1 (2%)		2 (4%)					
Fibrosis	1 (2%)									
Inflammation										
Mineralization		1 (2%)	1 (2%)							
Ulcer										
Epithelium, Mineralization	1 (2%)									
Tooth										
Inflammation			(1)	(2)	(2)					
Malformation				2 (100%)	1 (50%)					
Periodontal tissue, Inflammation			1 (100%)	1 (50%)	1 (50%)					
CARDIOVASCULAR SYSTEM										
Blood Vessel	(50)	(50)	(50)	(50)	(50)					

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

B 09

NTP Experiment-Test: 56348-03
 Study Type: CHRONIC
 Route: DOSED FEED

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
 CITRAL

Report: PEIRPT03
 Date: 06/08/00
 Time: 10:51:48

FISCHER 344 RATS FEMALE UNTREAT CONTROL VEHICLE CONTROL .312% .625% 1.25%

GENITAL SYSTEM

Clitoral Gland	(49)	(49)	(79)	(50)	(49)
Hyperplasia	7 (14%)	17 (35%)	8 (16%)	15 (30%)	4 (8%)
Inflammation	13 (27%)	22 (45%)	20 (41%)	19 (38%)	24 (49%)
Metaplasia			1 (2%)		
Bilateral, Cyst	5 (10%)	1 (2%)	3 (6%)	6 (12%)	4 (8%)
Duct, Cyst	(50)	(50)	(50)	(50)	(50)
Ovary					
Atrophy	3 (6%)	11 (22%)	6 (12%)	1 (2%)	5 (10%)
Cyst					
Infiltration Cellular, Lymphocyte	(50)	(50)	(50)	(50)	(50)
Uterus					
Fibrosis	1 (2%)	1 (2%)		1 (2%)	1 (2%)
Hemorrhage					
Hyperplasia					
Hyperplasia, Cystic	1 (2%)	1 (2%)			1 (2%)
Inflammation					
Cervix, Prolapse					
Endometrium, Hyperplasia, Cystic	9 (18%)	12 (24%)	15 (30%)	1 (2%)	14 (28%)
Vagina					
Dilatation		(2)	(1)	(2)	
Hyperplasia		1 (50%)			
Hypertrophy					
Inflammation, Acute			1 (100%)		

HEMATOPOIETIC SYSTEM

Bone Marrow	(50)	(50)	(50)	(50)	(50)
Angiogenesis	1 (2%)	1 (2%)		2 (4%)	1 (2%)
Atrophy	1 (2%)	9 (18%)	12 (24%)	10 (20%)	10 (20%)
Hyperplasia	11 (22%)		1 (2%)		1 (2%)
Myelofibrosis	1 (2%)				
Necrosis	(7)	(4)	(7)	(10)	(5)
Lymph Node					
Lumber, Hyperplasia, Histiocytic		1 (25%)			
Mediastinal, Congestion		1 (25%)			
Mediastinal, Hyperplasia, Plasma Cell	(50)	(50)	(50)	1 (10%)	(50)
Lymph Node, Mediastinal					
Ecstasia		10 (20%)	3 (6%)	5 (10%)	7 (14%)
Hyperplasia, Plasma Cell	6 (12%)	17 (34%)	17 (34%)	7 (14%)	17 (34%)
Necrosis					
Lymph Node, Neutrotic	(50)	(50)	(50)	(50)	(50)

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

NTP Experiment-Test: 56348-03
 Study Type: CHRONIC
 Route: DOSED FEED

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
 CITRAL

Report: PEIRPT03
 Date: 06/09/00
 Time: 10:51:48

FISCHER 344 RATS FEMALE	UNTREATED CONTROL		VEHICLE CONTROL		.312%		.625%		1.25%	
HEMATOPOIETIC SYSTEM - CONT										
Atrophy			1 (2%)							
Ectasia					1 (2%)					
Hyperplasia, Lymphoid					1 (2%)					
Spleen	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Accessory Spleen										
Endothelial Tissue			1 (2%)	1 (2%)						
Fibrosis			1 (2%)	1 (2%)						
Granuloma										
Hematopoietic Cell Proliferation			11 (22%)	11 (22%)			8 (16%)	5 (10%)	5 (10%)	
Hemorrhage										
Hyperplasia, Histiocytic			1 (2%)							
Hyperplasia, Macromatous			1 (2%)							
Infect										
Infiltration Cellular, Lipocyte										
Neerosis										
Pigmentation			1 (2%)		1 (2%)					
Red Pulp Degeneration Cellular			1 (2%)		1 (2%)			3 (6%)		
Thymus			1 (2%)		1 (2%)					
Atrophy			45 (92%)	46 (98%)	45 (92%)	45 (92%)	44 (92%)	44 (92%)	45 (92%)	45 (92%)
INTRODUCTORY SYSTEM										
Mammary Gland										
Ductalocle										
Hyperplasia										
Hyperplasia, Focal										
Hyperplasia, Lobular			4 (8%)	5 (10%)	10 (20%)	6 (12%)	3 (6%)	1 (2%)	3 (6%)	
Skin										
Cyst Epithelial Inclusion			(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Fibrosis										
Hyperkeratosis			1 (2%)	1 (2%)						
Hyperplasia			1 (2%)	1 (2%)						
Inflam. ation			1 (2%)	1 (2%)						
Der. at. Fibrosis			1 (2%)							
Subcutaneous Tissue, Inflammation				1 (2%)						
MUSCULOSKELETAL SYSTEM										
Bone										
Osteopetrosis			(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
			3 (6%)	3 (6%)	12 (24%)	5 (10%)	8 (16%)			

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

NTP Experiment-Test: 56348-03
 Study Type: CHRONIC
 Route: DOSED FEED

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (*)
 CITRAL

Report: PEHF03
 Date: 06/09/00
 Time: 10:51:48

FISCHER 344 RATS FEMALE UNDEAF CONTROL VEHICLE CONTROL .312% .625% 1.25%

NERVOUS SYSTEM

Brain	(50)	(50)	(50)	(50)	(50)	(50)
Hemorrhage	3 (6%)	2 (4%)	4 (8%)	4 (8%)	4 (8%)	1 (2%)
Necrosis		1 (2%)		4 (8%)	1 (2%)	
Spinal Cord				1 (2%)	1 (2%)	
Hemorrhage				1 (2%)	1 (2%)	

RESPIRATORY SYSTEM

Lung	(50)	(50)	(50)	(50)	(50)	(50)
Fibrosis				1 (2%)	1 (2%)	1 (2%)
Hemorrhage	2 (4%)		1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, Lymphoid			1 (2%)	1 (2%)	1 (2%)	1 (2%)
Inflammation	9 (18%)	7 (14%)	17 (34%)	5 (10%)	9 (18%)	9 (18%)
Alveolar Epithelium, Hyperplasia	9 (18%)	10 (20%)	9 (18%)	10 (20%)	11 (22%)	11 (22%)
Nose	(50)	(50)	(50)	(50)	(50)	(50)
Foreign Body	3 (6%)	1 (2%)	4 (8%)	1 (2%)	3 (6%)	3 (6%)
Inflammation	4 (8%)	5 (10%)	5 (10%)	2 (4%)	2 (4%)	2 (4%)
Trachea	(50)	(50)	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	1 (2%)	3 (6%)	1 (2%)	1 (2%)	1 (2%)

SPECIAL SENSES SYSTEM

Eye	(2)	(3)	(1)	(1)	(2)	(2)
Cataract	2 (100%)	2 (67%)	1 (100%)	1 (100%)	1 (50%)	1 (50%)
Cornea, Inflammation		1 (33%)			1 (50%)	
Rotina, Degeneration	2 (100%)	2 (67%)	1 (100%)		1 (50%)	1 (50%)
Harderian Gland						
Atrophy						1 (100%)

URINARY SYSTEM

Kidney	(50)	(50)	(50)	(50)	(50)	(50)
Accumulation, Nyaline Droplet		1 (2%)			1 (2%)	1 (2%)
Cyst		1 (2%)			2 (4%)	1 (2%)
Hydrophrosis	1 (2%)	1 (2%)			1 (2%)	1 (2%)
Inflammation	41 (82%)	43 (86%)	43 (86%)	30 (75%)	41 (82%)	41 (82%)
Mineralization						2 (4%)
Necrosis						2 (4%)

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

NTP Experiment-Test: 56348-03 INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
 Study Type: CHRONIC CYTAL
 Route: DOSED FEED Report: PETRPRO3
 Date: 06/09/00
 Time: 10:51:48

URINARY SYSTEM - CONT	FISCHER 344 RATS FEMALE		INCIDENCE RATES	
	URINARY CONTROL	VEHICLE CONTROL	.312%	.625%
Nephropathy	41 (82%)	44 (88%)	45 (90%)	48 (96%)
Pigmentation	2 (4%)	1 (2%)	4 (8%)	44 (88%)
Renal tubule, Hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Transitional Epithelium, Hyperplasia	(50)	(50)	(50)	(50)
Urinary bladder	1 (2%)		1 (2%)	1 (2%)
Calculus Gross obstruction				1 (2%)
Hemorrhage				1 (2%)
Inflammation				1 (2%)
Ulcer				1 (2%)
Transitional Epithelium, Hyperplasia				1 (2%)

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