NTP
National Toxicology Program
U.S. Department of Health and Human Services

# NTP Technical Report on the Toxicology and Carcinogenesis Studies of 3,3′,4,4′-Tetrachloroazobenzene (TCAB) (CAS No. 14047-09-7) in Harlan Sprague-Dawley Rats and B6C3F1 Mice (Gavage Studies)

NTP TR 558

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NTP TECHNICAL REPORT

ON THE

# TOXICOLOGY AND CARCINOGENESIS STUDIES OF 3,3′,4,4′-TETRACHLOROAZOBENZENE (TCAB)

[CAS NO. 14047-09-7]

# IN HARLAN SPRAGUE-DAWLEY RATS AND B6C3F1 MICE

(GAVAGE STUDIES)

NATIONAL TOXICOLOGY PROGRAM
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## FOREWORD

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## SUMMARY

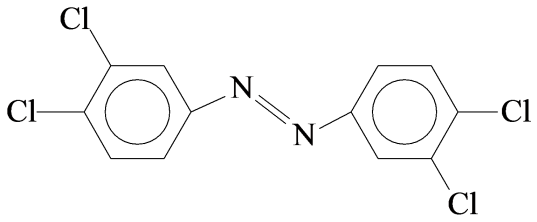
**Background**
3,3',4,4' Tetrachloroazobenzene (TCAB) is formed as a by-product in the manufacture of a variety of herbicides. TCAB is structurally similar to TCDD, one of a large family of hydrocarbons containing chlorine known as dioxins. We studied the effects of TCAB on male and female rats and mice to identify potential toxic or carcinogenic hazards.

**Methods**
We exposed groups of 50 male or female rats by depositing solutions of TCAB dissolved in corn oil through a tube directly into their stomachs five days a week for two years. Daily doses of TCDD were 10, 30, or 100 milligrams (mg) per kilogram of body weight. Similar groups of male or female mice received doses of 3, 10, or 30 mg/kg on the same schedule. Animals receiving corn oil alone served as the control group. Tissues from more than 40 sites were examined for every animal.

**Results**
Exposure to TCAB caused a variety of diseases in several organs. Cancers of the liver, lung, and mouth were seen in both male and female rats. Male rats also had increased rates of thyroid gland tumors and some female rats had tumors of the forestomach. Almost all exposed male mice developed carcinomas of the urethra. Both male and female mice also had a variety of tumors of the lung as well as cancers of the forestomach. Female mice also experienced cancers of the skin. A variety of other toxic effects were observed in exposed animals. In rats these included hypertrophy, hyperplasia, fibrosis, and necrosis of the liver; hyperplasia of the oral mucosa; metaplasia of the lung; inflammation and atrophy of the pancreas; and other nonneoplastic lesions of the forestomach, adrenal cortex, blood vessel, spleen, and mesenteric lymph node. In mice observed adverse outcomes included cardiomyopathy of the heart; atrophy of the thymus; hyperplasia of the skin, urinary bladder, forestomach and glandular stomach; and other nonneoplastic lesions of the spleen, liver, urethra, ureter, blood vessel, clitoral gland, ovary, and bone marrow.

**Conclusions**
We conclude that TCAB caused cancer and other toxic effects at several sites in male and female rats and mice.

# ABSTRACT




**3,3′,4,4′-TETRACHLOROAZOBENZENE**

**TCAB**

CAS No. 14047-09-7

Chemical Formula: $C_{12}H_6C_{l4}N_2$ Molecular Weight: 320.0

**Synonyms:** Azobenzene, 3,3′,4,4′-tetrachloro-(8CI); 3,3′,4,4′-tetrachloroazobenzene diazene, bis(3,4-dichlorophenyl)-(9CI); TCAB

3,3′,4,4′-Tetrachloroazobenzene (TCAB) is not commercially manufactured but is formed as an unwanted by-product in the manufacture of 3,4-dichloroaniline and its herbicidal derivatives Propanil®, Linuron®, and Diuron®. It occurs from the degradation of chloroanilide herbicides (acylanilides, phenylcarbamates, and phenylureas) in soil by peroxide-producing microorganisms and is formed by the photolysis and biolysis of 3,4-dichloroaniline. Humans may be exposed to TCAB during the manufacture as well as the application of herbicides containing TCAB as a contaminant. TCAB was nominated by the United States Environmental Protection Agency for toxicity and carcinogenicity testing based on its structural and biological similarity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the potential for human exposure from the consumption of crops contaminated with 3,4-dichloroaniline-derived herbicides. Male and female Harlan Sprague-Dawley rats and B6C3F1 mice were administered TCAB (at least 97.8% pure) in corn oil:acetone (99:1) by gavage for 3 months (rats only) or 2 years.

## 3-Month Study in Rats

Groups of 10 male and 10 female Harlan Sprague-Dawley rats were administered 0.1, 0.3, 1, 3, 10, 30, or 100 mg TCAB/kg body weight in corn oil:acetone (99:1) by gavage, 5 days a week, for 14 weeks; groups of 10 male and 10 female rats received the corn oil:acetone vehicle alone. Special study groups of 30 (dosed groups) or 6 (vehicle control group) female Harlan Sprague-Dawley rats were administered 0.1, 3, or 100 mg TCAB/kg body weight in corn oil:acetone (99:1) by gavage, 5 days a week, for 13 weeks; vehicle controls received the corn oil:acetone vehicle alone.

All male and female rats survived to the end of the study. Terminal mean body weights of males were not significantly different from vehicle controls in any group. Terminal mean body weights of females administered 10 mg/kg or greater were significantly less than those of the vehicle controls. Mean body weight gains of all dosed groups of females were significantly less than those of

the vehicle controls. The hematology results indicate that TCAB induced a microcytic normochromic responsive anemia in male Sprague-Dawley rats. Serum concentrations of total thyroxine ($T_4$) and free $T_4$ were significantly decreased in a dose-related manner in all dosed groups in both sexes compared to their respective vehicle controls; total triiodothyronine ($T_3$) and thyroid stimulating hormone (TSH) concentrations were generally unaffected. There were no statistically significant differences in the BrdU labeling indices in the liver of males or females exposed to TCAB compared to their respective vehicle controls. Significant induction of hepatic 7-ethoxyresorufin-*O*-deethylase (EROD) and 7-pentoxyresorufin-*O*-deethylase activities was observed in all dosed groups of males and females. Significant induction of hepatic acetanilide-4-hydroxylase activity was observed in males exposed to 3 mg/kg or greater and all treated groups of females. EROD activities in the lung generally increased with increasing dose and were significantly greater in all treated groups of males and females compared to their respective vehicle controls. The highest concentrations of TCAB were observed in fat tissue with lower concentrations in the liver and lung. TCAB concentrations were significantly increased in a dose-dependent manner in all tissues from dosed groups relative to vehicle controls.

At the end of the 3-month study, absolute and relative liver weights were significantly greater than those of the vehicle controls in all dosed groups of males and in females administered 10 mg/kg or greater. Absolute and relative lung weights were significantly greater in 100 mg/kg males and 3 mg/kg or greater females. Absolute and relative right kidney and spleen weights were generally significantly greater for all dosed groups of males. Absolute thymus weights of 10 mg/kg or greater males and absolute and relative thymus weights of 1 mg/kg or greater females were significantly less than those of the vehicle controls.

In the liver, the incidences of midzonal to diffuse hepatocytic hypertrophy in males administered 1 mg/kg or greater and in females administered 10 mg/kg or greater were significantly greater than the vehicle control incidences. Hematopoietic cell proliferation occurred in most males administered 3 mg/kg or greater and most females administered 10 mg/kg or greater. The incidences of midzonal hepatocytic cytoplasmic fatty vacuolization were significantly increased in males administered 3 mg/kg or greater. In the lung, significantly increased incidences of bronchiolar metaplasia of the alveolar epithelium and interstitial mononuclear cell infiltration occurred in 10, 30, and 100 mg/kg males. The incidence of interstitial mononuclear cell infiltration was also significantly increased in 100 mg/kg females. Significantly increased incidences of hematopoietic cell proliferation of the spleen occurred in males administered 10 mg/kg or greater. The incidences of hemosiderin pigment of the spleen were significantly increased in 10 mg/kg or greater females. Atrophy in the thymus was significantly increased in all dosed groups of females, except the 0.1 mg/kg group, and in males administered 10 mg/kg or greater.

## 2-Year Study in Rats

Groups of 50 male and 50 female Harlan Sprague-Dawley rats were administered 10, 30, or 100 mg TCAB/kg body weight in corn oil:acetone (99:1) by gavage, 5 days a week, for 2 years; groups of 50 male and 50 female rats received the corn oil:acetone vehicle alone. The survival of all dosed groups of males was significantly less than that of the vehicle controls. Mean body weights of 100 mg/kg males were less than those of the vehicle control group throughout the study. Mean body weights of 30 mg/kg males were 6% less than those of the vehicle control group after week 24, and those of 10 mg/kg males were 7% less than the vehicle control group after week 80. Mean body weights of 100 mg/kg females were less than those of the vehicle control group throughout the study, and those of 30 mg/kg females were 6% less than the vehicle control group after week 36.

In the lung, the incidences of multiple cystic keratinizing epithelioma and single or multiple cystic keratinizing epithelioma (combined) in males and females were significantly increased in all dosed groups (except multiple epithelioma in 10 mg/kg females). Significantly increased incidences of pigmentation, alveolar epithelium squamous metaplasia (except 10 mg/kg females), and alveolar epithelium bronchiolar metaplasia occurred in all dosed groups of males and females. The incidences of histiocytic cellular infiltration in all dosed groups of males were significantly increased.

In the liver, the incidences of cholangiocarcinoma (single or multiple) occurred in a positive trend in males and were significantly greater than that in the vehicle control group; the incidence in 100 mg/kg females was also increased. A significant dose-related increase in hepatic toxicity was observed in dosed rats and was characterized by increased incidences of numerous lesions includ-

ing hepatocyte hypertrophy, centrilobular degeneration, hepatocellular necrosis, pigmentation, fatty change, bile duct hyperplasia, oval cell hyperplasia, nodular hyperplasia, hematopoietic cell proliferation, eosinophilic focus, mixed cell focus, multinucleated hepatocytes, bile duct cyst, toxic hepatopathy, and cholangiofibrosis.

Significantly increased incidences of gingival squamous cell carcinoma within the oral mucosa occurred in 10 mg/kg males and 100 mg/kg males and females. The incidences of gingival squamous hyperplasia and cystic keratinizing hyperplasia in dosed groups of males and females were generally significantly increased.

The incidences of follicular cell adenoma (single or multiple) of the thyroid gland in 30 and 100 mg/kg males were significantly greater than that in the vehicle control group. The incidences of follicular cell hypertrophy, follicular cell hyperplasia, and inflammation were significantly increased in 30 and 100 mg/kg males.

Three incidences of single or multiple squamous cell papilloma of the forestomach occurred in 100 mg/kg females, and single incidences of squamous cell carcinoma of the forestomach occurred in 10 and 100 mg/kg females. Significantly increased incidences of epithelial hyperplasia occurred in all dosed groups of males and females.

There were three incidences of malignant schwannoma in the thoracic cavity in 100 mg/kg males and a single incidence in 30 mg/kg males.

In the adrenal cortex of 30 and 100 mg/kg females, there were slightly increased incidences of adenoma. In all dosed groups of males, the incidences of degeneration, cytoplasmic vacuolization, and hyperplasia of the zona fasciculata were significantly increased. Increased incidences and severities of necrosis occurred in 30 and 100 mg/kg males. Incidences of cytoplasmic vacuolization in 10 and 100 mg/kg females and hyperplasia of the zona fasciculata in 30 mg/kg females were significantly greater than those in the vehicle controls.

Numerous nonneoplastic effects were seen in other organs including atrophy, acinar cytoplasmic vacuolization, and inflammation of the pancreas; blood vessel inflammation; lymphoid follicle atrophy and pigmentation of the spleen; pigmentation and atrophy of the mesenteric lymph node; germinal epithelial degeneration of the testes; and inflammation of the nose.

## 2-Year Study in Mice

Groups of 50 male and 50 female mice were administered 3, 10, or 30 mg TCAB/kg body weight in corn oil:acetone (99:1) by gavage, 5 days a week, for 2 years; groups of 50 male and 50 female rats received the corn oil:acetone vehicle alone. Survival of 10 and 30 mg/kg males and 30 mg/kg females was significantly less than that of vehicle controls. All 30 mg/kg males died before the end of the study. Mean body weights of treated males were similar to or greater than those of the vehicle controls throughout most of the study. Mean body weights of 10 and 30 mg/kg males were 10% and 8% less than those of the vehicle controls at the last weighing at weeks 101 and 73, respectively. Mean body weights of 3 mg/kg females were 7% greater than those in the vehicle controls after week 64. The only chemical-related clinical finding was the appearance of ulcers or abscesses, primarily on the head and neck and exacerbated by scratching. These skin lesions appeared as areas of discoloration, ulceration, or thickening. In both sexes, the time when these lesions first appeared was dose related, with lesions appearing first in the 30 mg/kg groups.

The incidences of transitional epithelial carcinoma of the urethra were significantly increased in all dosed groups of males, and two of these neoplasms were observed in 30 mg/kg females. Transitional epithelial hyperplasia of the urethra and urinary bladder occurred in male and female mice. One 10 mg/kg male and one 30 mg/kg female had transitional epithelial carcinoma of the ureter. The incidences of dilatation and chronic active inflammation of the ureter were significantly increased in 10 and 30 mg/kg males.

In the lung, significantly increased incidences of alveolar/bronchiolar adenoma occurred in all dosed groups of males, and a significantly increased incidence of alveolar/bronchiolar carcinoma occurred in 30 mg/kg females. Significantly increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) occurred in 3 and 10 mg/kg males and 30 mg/kg females. A single incidence of cystic keratinizing epithelioma (CKE) and a single incidence of multiple CKE occurred in 30 mg/kg females. The incidences of chronic active inflammation were significantly increased in 10 and 30 mg/kg females.

In the forestomach, the incidences of squamous cell carcinoma in the 30 mg/kg groups were significantly greater than those in the vehicle control groups. Significantly

increased incidences of hyperplasia at the limiting ridge occurred in all dosed groups. In the glandular stomach, incidences of focal epithelial hyperplasia, epithelial cyst (except 10 mg/kg females), and subtle mucosal lymphoid cell infiltration were significantly increased in dosed groups of males and females; the incidences of mineralization were significantly increased in all dosed groups of males.

The incidences of subcutaneous fibrosarcoma and fibrosarcoma or malignant schwannoma (combined) of the skin were significantly increased in 30 mg/kg females. The skin lesions observed grossly were characterized histologically as chronic active inflammation, dermal fibrosis, and epidermal hyperplasia and ulcers. The incidences of these lesions in females were dose related, and all were significantly greater in the 30 mg/kg group than in the vehicle control group; the incidences of these lesions in all male dosed groups, except dermal fibrosis at 30 mg/kg, were significantly increased. The incidences of follicular dilatation were significantly increased in 10 and 30 mg/kg males and females. The incidences of sebaceous gland atrophy were significantly increased in 10 and 30 mg/kg males and 30 mg/kg females.

Statistically significant increased incidences of malignant lymphoma occurred in 10 and 30 mg/kg females. The lymphoma involved the spleen and various lymph nodes (i.e., mesenteric, mediastinal, and mandibular).

Numerous nonneoplastic effects were seen in other organs including urinary bladder transitional epithelial hyperplasia; atrophy, cystic ducts, and chronic inflammation of the clitoral gland; ovarian atrophy; bone marrow hyperplasia; atrophy and hematopoietic cell proliferation of the spleen; atrophy of the thymus; hematopoietic cell proliferation of the liver; cardiomyopathy; and aorta mineralization.

## CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity** of TCAB in male Harlan Sprague-Dawley rats based on increased incidences of cystic keratinizing epithelioma of the lung, cholangiocarcinoma of the liver, and gingival squamous cell carcinoma of the oral mucosa. The increased incidences of follicular cell adenoma of the thyroid gland were also considered to be related to TCAB administration. The marginally increased incidence of malignant schwannoma may have been related to TCAB administration. There was *clear evidence of carcinogenic activity* of TCAB in female Harlan Sprague-Dawley rats based on increased incidences of cystic keratinizing epithelioma of the lung and gingival squamous cell carcinoma of the oral mucosa. The increased incidences of cholangiocarcinoma of the liver and squamous cell papilloma or squamous cell carcinoma (combined) of the forestomach were also considered to be related to TCAB administration. The marginally increased incidences of adenoma of the adrenal cortex may have been related to TCAB administration. There was *clear evidence of carcinogenic activity* of TCAB in male B6C3F1 mice based on increased incidences of carcinoma of the urethra and alveolar/bronchiolar neoplasms of the lung. The increased incidences of squamous cell carcinoma of the forestomach were also considered to be related to TCAB administration. The marginally increased incidence of carcinoma of the ureter may have been related to TCAB administration. There was *clear evidence of carcinogenic activity* of TCAB in female B6C3F1 mice based on increased incidences of fibrosarcoma and fibrosarcoma or malignant schwannoma (combined) of the skin. The increased incidences of carcinoma of the urethra, alveolar/bronchiolar neoplasms and cystic keratinizing epithelioma of the lung, and squamous cell carcinoma of the forestomach were also considered to be related to TCAB administration. The marginally increased incidences of carcinoma of the ureter and malignant lymphoma may have been related to TCAB administration.

TCAB administration caused increased incidences of nonneoplastic lesions of the lung, liver, oral mucosa, forestomach, adrenal cortex, pancreas, blood vessel, spleen, and mesenteric lymph node in male and female rats; the thyroid gland and testis in male rats; the nose in female rats; the urinary bladder, forestomach, glandular stomach, skin, spleen, thymus, liver, and heart in male and female mice; the urethra, ureter, and blood vessel in male mice; and the lung, clitoral gland, ovary, and bone marrow in female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 14.

## Summary of the 2-Year Carcinogenesis Studies of TCAB

| | Male Harlan Sprague-Dawley Rats | Female Harlan Sprague-Dawley Rats | Male B6C3F1 Mice | Female B6C3F1 Mice |
|---|---|---|---|---|
| **Concentrations in corn oil/acetone by gavage** | 0, 10, 30, or 100 mg/kg | 0, 10, 30, or 100 mg/kg | 0, 3, 10, or 30 mg/kg | 0, 3, 10, or 30 mg/kg |
| **Body weights** | 100 mg/kg group 7% less than the vehicle control group after week 2; 30 mg/kg group 6% less than the vehicle control group after week 24; 10 mg/kg group 7% less than the vehicle control group after week 80 | 100 mg/kg group 6% less than the vehicle control group after week 3; 30 mg/kg group 6% less than the vehicle control group after week 36 | 10 mg/kg group 10% less than the vehicle control at week 101; 30 mg/kg group 8% less than the vehicle control group at week 73 | 3 mg/kg group 7% greater than the vehicle control group after week 64 |
| **Survival rates** | 28/50, 9/50, 4/50, 2/50 | 25/50, 30/50, 19/50, 17/50 | 35/50, 31/50, 5/49, 0/50 | 35/50, 30/50, 32/50, 20/50 |
| **Nonneoplastic effects** | Lung: pigmentation (3/50, 16/50, 12/50, 16/50); alveolar epithelial metaplasia, squamous (0/50, 14/50, 22/50, 22/50); alveolar epithelium, metaplasia, bronchiolar (1/50, 32/50, 32/50, 34/50); alveolus, infiltration cellular, histiocyte (23/50, 34/50, 35/50, 35/50)<br>Liver: hepatocyte hypertrophy (0/50, 6/50, 11/50, 22/50); centrilobular degeneration (0/50, 10/50, 23/50, 24/50); necrosis (1/50, 7/50, 18/50, 21/50); pigmentation (1/50, 4/50, 5/50, 6/50); diffuse fatty change (3/50, 9/50,18/50, 34/50); oval cell hyperplasia (0/50, 4/50, 8/50, 5/50); hematopoietic cell proliferation (5/50, 40/50, 37/50, 30/50); eosinophilic focus (3/50, 9/50, 4/49, 12/50); bile duct cyst (0/50, 0/50, 1/50, 4/50); toxic hepatopathy 0/50, 0/50, 5/50, 8/50) | Lung: pigmentation (1/50, 11/50, 21/49, 26/49); alveolar epithelium, metaplasia, squamous (2/50, 4/50, 18/49, 30/49); alveolar epithelium, metaplasia, bronchiolar (0/50, 21/50, 26/49, 35/49)<br>Liver: hepatocyte hypertrophy (4/50, 33/50, 38/49, 42/49); centrilobular degeneration (1/50, 2/50, 18/49, 17/49); necrosis (3/50, 6/50, 10/49, 10/49); pigmentation 1/50, 17/50, 32/49, 40/49); focal fatty change (2/50, 2/50, 2/49, 9/49); diffuse fatty change (0/50, 3/50, 10/49, 10/49); bile duct hyperplasia (12/50, 26/50, 29/49, 38/49); oval cell hyperplasia (0/50, 7/50, 24/49, 36/49); nodular hyperplasia (1/50, 3/50, 11/49, 22/49); hematopoietic cell proliferation (28/50, 42/50, 32/49, 37/49); eosinophilic focus (3/50, 27/50, 31/49, 38/49); mixed cell focus (6/50, 16/50, 14/49, 16/49); bile duct cyst (3/50, 4/50, 5/49, 12/49); | Urethra: transitional epithelial hyperplasia (0/50, 17/50, 2/49, 0/50)<br>Ureter: dilatation 0/43, 6/45, 22/47, 42/50); chronic active inflammation (0/43, 3/45, 24/47, 39/50)<br>Urinary Bladder: transitional epithelial hyperplasia (0/50, 1/50, 0/49, 4/50)<br>Forestomach: epithelial hyperplasia (8/50, 21/50, 33/49, 44/50)<br>Glandular Stomach: focal epithelial hyperplasia (0/50, 10/50, 20/49, 34/50); gland epithelium cyst (5/50, 18/50, 21/49, 26/50); lymphoid cellular infiltration (3/50, 20/50, 19/49, 15/50); mineralization (0/50, 4/50, 6/49, 13/50)<br>Skin: chronic active inflammation (3/50, 13/50, 12/49, 5/50); dermal fibrosis (3/50, 12/50, 12/49, 4/50); epidermal hyperplasia (3/50, 13/50, 12/49, 6/50); epidermal ulcer (3/50, 13/50, 11/49, 6/50); hair follicle dilatation (7/50, 10/50, 13/49, 28/50); sebaceous gland atrophy (13/50, 10/50, 20/49, 29/50) | Urinary Bladder: transitional epithelial hyperplasia (0/49, 0/50, 0/50, 4/50)<br>Lung: chronic active inflammation (0/49, 3/50, 5/50, 7/50)<br>Forestomach: epithelial hyperplasia (8/50, 27/50, 38/50, 43/50)<br>Glandular Stomach: focal epithelial hyperplasia (1/50, 19/50, 26/50, 28/50); gland epithelial cyst (8/50, 19/50, 13/50, 22/50); lymphoid cellular infiltration (1/50, 14/50, 30/50, 28/50)<br>Skin: chronic active inflammation (0/50, 2/50, 6/50, 11/50); dermal fibrosis (0/50, 1/50, 4/50, 11/50); epidermal hyperplasia (1/50, 2/50, 4/50, 11/50); epidermal ulcer (0/50, 1/50, 4/50, 11/50); hair follicle dilatation (2/50, 0/50, 11/50, 23/50); sebaceous gland atrophy (6/50, 10/50, 11/50, 15/50)<br>Clitoral Gland: atrophy (25/49, 44/50, 37/49, 40/50); cyst duct (5/49, 46/50, 43/49, 43/50); chronic active inflammation (3/49, 4/50, 17/49, 25/50) |

*(continued)*

**Summary of the 2-Year Carcinogenesis Studies of TCAB**

| | Male Harlan Sprague-Dawley Rats | Female Harlan Sprague-Dawley Rats | Male B6C3F1 Mice | Female B6C3F1 Mice |
|---|---|---|---|---|
| **Nonneoplastic effects (continued)** | Oral Mucosa: gingival squamous hyperplasia (2/50, 21/50, 24/50, 31/50); gingival cystic keratinizing hyperplasia (0/50, 4/50, 18/50, 11/50)<br>Thyroid Gland: follicular cell hypertrophy (2/50, 2/50, 6/50, 6/50); follicular cell hyperplasia (0/50, 2/50, 10/50, 12/50); inflammation (0/50, 3/50, 9/50, 14/50)<br>Forestomach: epithelium hyperplasia (8/50, 36/50, 44/50, 45/50)<br>Adrenal Cortex: degeneration (4/50, 14/50, 14/50, 9/50); vacuolization cytoplasmic (20/50, 29/50, 31/50, 25/50); zona fasciculata, hyperplasia (14/50, 22/50, 19/50, 21/50); necrosis (0/50, 2/50, 4/50, 13/50)<br>Pancreas: atrophy (4/50, 13/49, 10/50, 10/50); acinar vacuolization cytoplasmic (0/50, 16/49, 30/50, 13/50); inflammation (1/50, 7/49, 7/50, 3/50)<br>Blood Vessel: inflammation (21/50, 29/50, 30/50, 29/50)<br>Spleen: lymphoid follicle, atrophy (5/50, 4/50, 5/50, 12/50)<br>Lymph Node, Mesenteric: pigmentation (1/50, 24/48, 25/50, 20/50); atrophy (0/50, 1/48, 1/50, 5/50)<br>Testis: germinal epithelial degeneration (15/50, 16/50, 18/50, 21/50) | Liver: *(continued)* multinucleated hepatocyte (0/50, 2/50, 1/49, 28/49); toxic hepatopathy (0/50, 4/50, 14/49, 25/49); cholangiofibrosis (0/50, 1/50, 0/49, 11/49)<br>Oral Mucosa: gingival squamous hyperplasia (0/50, 8/50, 24/50, 24/50); gingival cystic keratinizing hyperplasia (0/50, 4/50, 9/50, 13/50)<br>Forestomach: epithelial hyperplasia (0/50, 32/50, 46/50, 46/50)<br>Adrenal Cortex: vacuolization cytoplasmic (7/50, 16/50, 13/50,15/49); zona fasciculata, hyperplasia (14/50, 20/50, 25/50, 21/49)<br>Pancreas: atrophy (0/50, 11/49, 12/49, 13/49); acinar vacuolization cytoplasmic (0/50, 27/49, 33/49, 40/49)<br>Blood Vessel: inflammation (1/50, 10/50, 14/50, 16/50)<br>Spleen: lymphoid follicle, atrophy (3/50, 4/50, 8/49, 10/49); pigmentation (31/50, 44/50, 42/49, 47/49)<br>Lymph Node, Mesenteric: pigmentation (14/50, 32/49, 32/50, 30/49); atrophy (0/50, 0/49, 4/50, 6/49)<br>Nose: inflammation (0/50, 7/50, 6/50, 8/50) | Spleen: atrophy (2/50, 9/50, 30/49, 39/49)<br>Thymus: atrophy (11/41, 15/42, 30/40, 45/49)<br>Liver: hematopoietic cell proliferation (2/50, 9/50, 9/49, 3/50)<br>Heart: cardiomyopathy (5/50, 5/50, 17/49, 9/50)<br>Blood Vessel: aorta mineralization (0/50, 0/50, 6/49, 8/50) | Ovary: atrophy (29/49, 44/50, 47/50, 45/50)<br>Bone Marrow: hyperplasia (2/50, 8/50, 9/50, 16/50)<br>Spleen: hematopoietic cell proliferation (10/49, 25/50, 21/50, 33/50)<br>Thymus: atrophy (7/48, 15/48, 12/45, 25/48)<br>Liver: hematopoietic cell proliferation (3/49, 9/50, 9/50, 21/50)<br>Heart: cardiomyopathy (3/49, 5/50, 4/50, 9/50) |

**Summary of the 2-Year Carcinogenesis Studies of TCAB**

| | Male Harlan Sprague-Dawley Rats | Female Harlan Sprague-Dawley Rats | Male B6C3F1 Mice | Female B6C3F1 Mice |
|---|---|---|---|---|
| **Neoplastic effect** | Lung: cystic keratinizing epithelioma (0/50, 14/50, 31/50, 37/50)<br>Liver: cholangiocarcinoma (0/50, 4/50, 4/50, 6/50)<br>Oral Mucosa: gingival squamous cell carcinoma (1/50, 5/50, 4/50, 5/50)<br>Thyroid Gland: follicular cell adenoma (0/50, 3/50, 4/50, 4/50) | Lung: cystic keratinizing epithelioma (0/50, 6/50, 26/49, 39/49)<br>Liver: cholangiocarcinoma (1/50, 1/50, 1/49, 3/49)<br>Oral Mucosa: gingival squamous cell carcinoma (0/50, 0/50, 4/50, 6/50)<br>Forestomach: squamous cell papilloma or squamous cell carcinoma (0/50, 1/50, 0/50, 4/50) | Urethra: transitional epithelial carcinoma (0/50, 32/50, 46/49, 49/50)<br>Lung: alveolar/bronchiolar adenoma (5/50, 16/50, 12/49, 6/50); alveolar/bronchiolar adenoma or carcinoma (7/50, 17/50, 15/49, 6/50)<br>Forestomach: squamous cell carcinoma 0/50, 1/50, 1/49, 3/50) | Skin: fibrosarcoma (1/50, 6/50, 5/50, 8/50); fibrosarcoma or malignant schwannoma (2/50, 8/50, 7/50, 12/50)<br>Urethra: transitional epithelial carcinoma (0/50, 0/50, 0/50, 2/50)<br>Lung: alveolar/bronchiolar carcinoma (0/49, 2/50, 1/50, 4/50); alveolar/bronchiolar adenoma or carcinoma (3/49, 8/50, 5/50, 10/50); cystic keratinizing epithelioma (0/49, 0/50, 0/50, 2/50)<br>Forestomach: squamous cell carcinoma (0/50, 1/50, 1/50, 4/50) |
| **Equivocal findings** | Malignant Schwannoma: (0/50, 0/50, 1/50, 3/50) | Adrenal Cortex: adenoma (1/50, 1/50, 3/50, 4/49) | Ureter: epithelial carcinoma (0/50, 1/50, 0/50, 0/50) | Ureter: epithelial carcinoma (0/50, 0/50, 1/50, 0/50)<br>Malignant Lymphoma: (2/50, 5/50, 8/50, 7/50) |
| **Level of evidence of carcinogenic activity** | Clear evidence | Clear evidence | Clear evidence | Clear evidence |

## EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that cannot be evaluated because of major flaws **(inadequate study)**. These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- Clear evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- Some evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- Equivocal evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- No evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- Inadequate study of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as "were also related" to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as "may have been" related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

## NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on TCAB on February 25, 2009, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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## SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On February 25, 2009, the draft Technical Report on the toxicology and carcinogenesis studies of 3,3′,4,4′-tetrachloroazobenzene (TCAB) received public review by the National Toxicology Program's Board of Scientific Counselors Technical Report Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. Michelle Hooth, NIEHS, introduced the toxicology and carcinogenesis studies of TCAB by describing its occurrence as a byproduct of herbicide production, its structural similarity to dioxins, the design of the short- and long-term studies, the toxic endpoints noted in the 3-month studies, and the body weight, survival, and neoplastic and nonneoplastic lesions observed in the 2-year studies. The proposed conclusions were:

> Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of TCAB in male Harlan Sprague- Dawley rats based on increased incidences of cystic keratinizing epithelioma of the lung, cholangiocarcinoma of the liver, and gingival squamous cell carcinoma of the oral mucosa. The increased incidences of follicular cell adenoma of the thyroid gland were also considered to be related to TCAB administration. The marginally increased incidence of malignant schwannoma may have been related to TCAB administration. There was *clear evidence of carcinogenic activity* of TCAB in female Harlan Sprague-Dawley rats based on increased incidences of cystic keratinizing epithelioma of the lung and gingival squamous cell carcinoma of the oral mucosa. The increased incidences of cholangiocarcinoma of the liver and squamous cell papilloma or squamous cell carcinoma (combined) of the forestomach were also considered to be related to TCAB administration. The marginally increased incidences of adenoma of the adrenal cortex may have been related to TCAB administration. There was *clear evidence of carcinogenic activity* of TCAB in male B6C3F1 mice based on increased incidences of transitional epithelial gland carcinoma of the urethra and alveolar/bronchiolar neoplasms of the lung. The increased incidences of squamous cell carcinoma of the forestomach were also considered to be related to TCAB administration. There was *clear evidence of carcinogenic activity* of TCAB in female B6C3F1 mice based on increased incidences of fibrosarcoma or malignant schwannoma (combined) of the skin. The increased incidences of transitional epithelial gland carcinoma of the urethra, alveolar/bronchiolar neoplasms and cystic keratinizing epithelioma of the lung, and squamous cell carcinoma of the forestomach were also considered to be related to TCAB administration. The marginally increased incidences of malignant lymphoma may have been related to TCAB administration.
>
> TCAB administration caused increased incidences of nonneoplastic lesions of the lung, liver, oral mucosa, forestomach, adrenal cortex, pancreas, blood vessel, spleen, and mesenteric lymph node in male and female rats; the thyroid gland and testis in male rats; the nose in female rats; the urinary bladder, forestomach, glandular stomach, skin, spleen, thymus, liver, and heart in male and female mice; the urethra, ureter, and blood vessel in male mice; and the lung, clitoral gland, ovary, and bone marrow in female mice.

Dr. Pino, the first primary reviewer, inquired why fibrosarcomas and malignant schwannomas of the skin in female mice were combined for analysis. He felt that fibrosarcomas alone might constitute clear evidence. Dr. Hooth agreed that the incidence of fibrosarcomas was sufficient for clear evidence and explained that the incidences were combined because both are cutaneous tumors of mesenchymal origin.

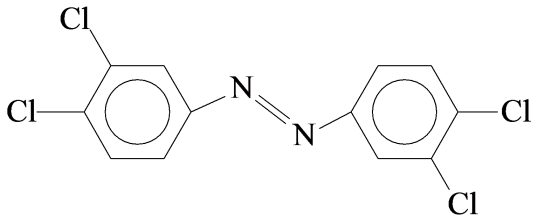
Dr. Nagarkatti, the second primary reviewer, felt the study was well designed and agreed with the conclusions. She felt it was worth noting in the discussion that TCAB had a lower potency than TCDD because it degraded more rapidly and thus did not bioaccumulate to the same extent. She offered suggestions for the possible design of future studies. She inquired about the possible interpretation of the lower incidence of pancreatic

tumors in dosed animals compared to controls. Dr. Hooth replied that the difference in pancreatic tumor incidence may reflect the higher mortality in the high dosed males, and thus more control animals may have been at risk for these late-developing tumors. She added that frozen tissues were saved to study gene expression and immunological studies are ongoing.

Dr. Eastmond, the third primary reviewer, felt the study was well conducted and presented. He suggested clarifying the distinction between lesions contributing to clear evidence versus some evidence in the same study and providing the rationale for each call. He also inquired about the historical incidence of the rare urethral tumors. Dr. Kissling replied that in a review of not only the current 5-year study window of historical controls but also of a larger examination of NTP studies, no such tumors had been observed in approximately 10,000 control mice.

Dr. Pino suggested that the clear evidence conclusion for female mice should be based on "fibrosarcoma and fibrosarcoma or malignant schwannoma (combined) of the skin." Dr. Eastmond moved and Dr. Nagarkatti seconded that the conclusions be accepted with the proposed amendment. The motion was accepted unanimously with 8 yes votes, 0 no votes, and 0 abstentions.

# INTRODUCTION



**3,3′,4,4′-TETRACHLOROAZOBENZENE**

**TCAB**

CAS No. 14047-09-7

Chemical Formula: $C_{12}H_6C_{l4}N_2$ Molecular Weight: 320.0

**Synonyms:** Azobenzene, 3,3′,4,4′-tetrachloro-(8CI); 3,3′,4,4′-tetrachloroazobenzene diazene, bis(3,4-dichlorophenyl)-(9CI); TCAB

## Chemical and Physical Properties

TCAB is not commercially manufactured but is formed as an unwanted by-product in the manufacture of 3,4-dichloroaniline and its herbicidal derivatives Propanil®, Linuron®, and Diuron® (Poland *et al.*, 1976; Sundström *et al.*, 1978; Bunce *et al.*, 1979; Hill *et al.*, 1981). In addition, environmental contamination by TCAB occurs from the degradation of chloroanilide herbicides (acylanilides, phenylcarbamates, and phenylureas) in soil by peroxide-producing microorganisms (Bartha *et al.*, 1968; Bartha and Pramer, 1969; Lay and Ilnicki, 1974). It is also formed by the photolysis and biolysis of 3,4-dichloroaniline (Miller *et al.*, 1980).

TCAB is a bright orange, crystalline solid and has a melting point of 158° C (Hsia and Burant, 1979) and a log octanol/water partition coefficient of 5.53 to 6.69 (USEPA, 1985; Hashimoto *et al.*, 1994). The solubility in water is calculated to be 1 µg/L (USEPA, 1985). In the *trans* configuration, TCAB can assume a planar conformation with a molecular shape similar to TCDD (Figure 1; Poland *et al.*, 1976).

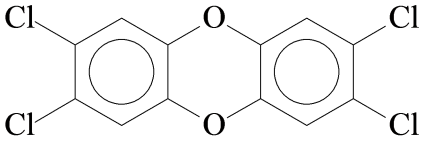


**Figure 1**
**Molecular Structure of**
**2,3,7,8-Tetrachlorodibenzo-*p*-dioxin**

## Production and Human Exposure

Propanil® has been reported to contain higher concentrations of TCAB than other herbicides (Sundström *et al.*, 1978; Bunce *et al.*, 1979; Hill *et al.*, 1981). The concentration of TCAB ranges from 1,000 to 2,700 µg/g in Propanil®, 6 to 28 µg/g in Diuron®, 8 to 9 µg/g in Linuron®, and 9 to 8,600 µg/g in 3,4-dichloroaniline. With a production volume of 10 million pounds of Propanil® per year, the resultant TCAB production amount could be as high as 12,000 kg per year (McMillan *et al.*, 1991). With a production volume of 100,000 to 1,000,000 pounds of 3,4-dichloroaniline per year, the resultant TCAB production amount could be as high as 3,900 kg per year (USEPA, 1985). Because 3,4-dichloroaniline is used as a precursor to dyes and, to a limited extent, as a heat transfer fluid in addition to its use in the manufacture of herbicides (USEPA, 1985), TCAB might be present in products other than herbicides.

The United States Environmental Protection Agency (USEPA) estimates the usage of various pesticides from a variety of published and proprietary sources available to the EPA and reports this information in Reregistration Eligibility Decision (RED) documents for individual pesticides. The estimate for the total annual domestic use of Diuron® in 2003 was approximately nine to 10 million pounds (USEPA, 2003), and the estimate for Linuron® in 1995 was approximately two million pounds (USEPA, 1995). Approximately 66% of the Diuron® was used agriculturally and 33% for other purposes; oranges and cotton accounted for the greatest agricultural use based on the number of pounds applied (USEPA, 2003). In 2006, the estimate for the total domestic use of Propanil® was approximately seven million pounds (USEPA, 2006).

Humans may be exposed to TCAB during the manufacture as well as the application of herbicides containing TCAB as a contaminant. Human exposure to TCAB has been reported in various manufacturing plants producing 3,4-dichloroaniline or herbicides derived from 3,4-dichloroaniline (Taylor *et al.*, 1977). Exposure of humans to TCAB may occur in rice fields; analyses of soil samples from a rice field plot treated with 6.7 kg Propanil®/hectare indicated a TCAB concentration of 0.09 ppm (Kearney et al., 1970). Six of 99 soil samples from the rice-growing states of Arkansas, California, Louisiana, Mississippi, and Texas contained 0.01 to 0.05 ppm TCAB, whereas no residual concentration of Propanil® was detected (Carey *et al.*, 1980). Rice plants grown in an artificial medium containing TCAB are able to absorb and translocate it to the aerial portions of the plants (Still, 1969). TCAB was detected in the roots and shoots of soybean plants grown experimentally in soil treated with 25 ppm TCAB (Worobey, 1984).

## Absorption, Distribution, Metabolism, and Excretion

### *Experimental Animals*

In male Sprague-Dawley rats administered a single gavage dose of 10 mg radiolabeled TCAB, 66% of the dose was excreted in urine and feces after 24 hours (Burant and Hsia, 1984). The pattern indicated a biphasic elimination consisting of an early rapid phase with a half-life of 18 hours and a slow terminal phase with a half-life greater than 20 days. The major route of excretion was via the feces with 55% of the total dose excreted over 48 hours. Total urinary excretion over 48 hours was approximately 27%. In tissues, the highest concentrations of TCAB were in the epididymal fat, adrenal gland, kidney, liver, lung, lymph nodes, pancreas, and urinary bladder. The lowest concentration was found in the brain. The liver-to-fat ratio of TCAB was 0.63.

Male F344 rats administered gavage doses of 3.2 or 32 mg TCAB/kg excreted 39% and 45%, respectively, of the doses in the urine in 96 hours (Pillai *et al.*, 1996). The major urinary metabolites were sulfate conjugates of mono-or dichloroaniline derivatives; some of these metabolites were *N*-acetylated. The excretion via feces within 96 hours was 53% and 56% of the doses, respectively. Less than 6% of the administered radioactivity remained in the tissues after 96 hours. Radiolabel accumulated in the adipose tissue and kidney, as shown by tissue-to-blood ratios greater than one. The liver-to-fat ratios were 0.22 and 0.09 at doses of 3.2 and 32 mg/kg, respectively. The lowest concentrations of TCAB were found in the brain. The oral bioavailability was 30% based on the ratio of the oral to intravenous total area under the blood concentration time curves. Extensive azo reduction was suggested as the reason for low systemic bioavailabilty of TCAB after oral administration. When 3.2 mg TCAB/kg was administered intravenously, 33% of the total dose was excreted in the bile 6 hours after administration, which is higher than the fecal elimination of 21% of the total dose after 24 hours. This finding suggests some enterohepatic recirculation of TCAB.

Inhalation by rats (strain not specified) and dermal application to male albino rabbits of TCAB resulted in vari-

ous systemic toxic effects, indicating that TCAB is absorbed after inhalation or dermal exposure (USEPA, 1983, 1985).

The *in vitro* metabolism of TCAB was studied in an NADPH-generating system and microsomes from male Sprague-Dawley rats exposed to TCAB by intraperitoneal injection (Hsia and Kreamer, 1981). The major metabolite was identified as a 3,3′,4,4′-tetrachloroazobenzene phenol. Two minor metabolites were identified as 3,3′,4,4′-tetrachlorohydrazobenzene and an N-hydroxylated derivative of 3,3′,4,4′-tetrachlorohydrazobenzene.

### *Humans*

No absorption, distribution, metabolism, or excretion studies of TCAB in humans were found in a review of the literature.

## TOXICITY

### *Experimental Animals*

In the *trans* configuration, TCAB can assume a planar conformation similar to that of TCDD. TCAB has been shown to bind to the aryl hydrocarbon receptor with a specific binding affinity of one-fifth that of TCDD (Poland *et al.*, 1976; Schneider *et al.*, 1995). Like TCDD, TCAB induces hepatic aryl hydrocarbon hydroxylase activity in mouse and chicken embryos (Poland *et al.*, 1976). Male Sprague-Dawley rats dosed with a single intraperitoneal injection of 100 mg/kg TCAB had a 120-fold increase in hepatic microsomal 7-ethoxyresorufin-*O*-deethylase activity and threefold increases in 7-pentoxyresorufin-*O*-deethylase and 7-benzyloxyresorufin-*O*-dealkylase activities (McMillan *et al.*, 1990). The porphyrinogenic effect of Propanil® in chick embryo liver cell cultures has been attributed to TCAB (Mensink and Strik, 1982).

The NTP performed 3-month studies of TCAB in male and female F344/N rats and B6C3F1 mice to aid in the design and dose selection for these 2-year carcinogenicity studies (Table 1; NTP, 1998a; Van Birgelen *et al.*, 1999a). Animals were exposed to TCAB by oral gavage at doses of 0, 0.1, 1.0, 3.0, 10, or 30 mg/kg 5 days a week for 3 months. In addition to histopathology, evaluations included clinical chemistry, hematology, thyroid hormone analyses, and reproductive parameters. TCAB caused typical dioxin-like effects. In the rat studies, terminal body weights were decreased in the 30 mg/kg males and females. Decreased thymus weights were accompanied by thymic atrophy in 10 and 30 mg/kg males and females, and increased spleen weights were observed in these groups. Liver weights were increased in 3 mg/kg or greater males in this rat study. Hepatic cytochrome P450 1A1 was induced in 30 mg/kg males and females. Circulating thyroxine ($T_4$) concentrations were significantly decreased in all dosed groups of rats, with hardly any $T_4$ detected at the highest doses. A decrease in triiodothyronine concentration also occurred in all dosed groups at the end of the study. However, thyroid-stimulating hormone concentrations were only marginally increased. Histopathologic lesions included hematopoietic cell proliferation in the spleen of 10 and 30 mg/kg males and females and hyperplasia of the forestomach in 3.0 mg/kg or greater males and 30 mg/kg females. A responsive anemia and decreased platelet counts were observed in 10 and 30 mg/kg rats. A no-observed-adverse-effect level (NOAEL) was not reached in rats. In the mouse studies, liver and spleen weights were increased in 10 and 30 mg/kg males and females and thymus weights were decreased in 30 mg/kg males. Increased incidences of hyperplasia of the forestomach were observed in male and female mice that received 1.0 mg/kg or greater. Additional histopathologic lesions observed in males included centrilobular hypertrophy of hepatocytes and an increase in hematopoietic cell proliferation in the spleen in groups receiving 3.0 mg/kg or greater. A significant decrease in epididymal spermatozoal concentration was observed in 3.0 and 30 mg/kg male mice. The NOAEL in mice was 0.1 mg/kg.

In several additional animal studies, exposure to TCAB resulted in typical dioxin-like effects including decreased body weight (Hsia and Kreamer, 1985), increased liver and spleen weights (Hsia *et al.,* 1982), thymic atrophy, and hepatotoxicity. A 120-day study of 100 ppm TCAB administered in the feed to male Sprague-Dawley rats resulted in a 9.4% decrease in mean body weight compared to the controls at the end of the study (Hsia *et al.*, 1980). The total consumption of TCAB per animal in this experiment was calculated to be 25.2 mg. Hematocrit and hemoglobin concentration were significantly decreased. TCAB exposure resulted in significant increases in relative liver weight, cytochrome P448 concentration, microsomal aryl hydrocarbon hydroxylase activity, and aspartate aminotransferase activity.

A 60-day study with male Sprague-Dawley rats administered 25 mg TCAB/kg per week by intraperitoneal injection resulted in a decrease in body weight, a decrease in

**TABLE 1**
**Summary of Selected Treatment-Related Effects in the 13-Week Gavage Studies of TCAB in F344/N Rats and B6C3F1 Mice[a]**

| Endpoint | Affected Dose Groups (mg/kg)[b] | | | |
|---|---|---|---|---|
| | Male Rats | Female Rats | Male Mice | Female Mice |
| Terminal body weight (decrease) | 30 | 30 | NS[c] | NS |
| Body weight gain (decrease) | NS | 30 | NS | NS |
| Liver | | | | |
| Weight (increase) | 3↑ | 10↑ | 10↑ | 10↑ |
| Centrilobular hypertrophy of hepatocytes (increased incidence) | NO[d] | NO | 3↑ | NO |
| Hepatic cytochrome P450 1A concentration (increase) | 30 | 10↑ | —[e] | — |
| Thymus | | | | |
| Weight (decrease) | 10↑ | 10↑ | 30 | NS |
| Atrophy (increased incidence) | 10↑ | 30 | NO | NO |
| Spleen | | | | |
| Weight (increase) | 10↑ | 30 | 30 | 10↑ |
| Hematopoietic cell proliferation (increased incidence) | 10↑ | 10↑ | 3↑ | NS |
| Responsive anemia | 10↑ | 10↑ | — | — |
| Platelet count (decrease) | 10↑ | 10↑ | — | — |
| Total T3 and T4 concentrations[f] (decrease) | 0.1↑[g] | 0.1↑[g] | — | — |
| Forestomach | | | | |
| Epithelial hyperplasia (increased incidence) | 3 and 30 | 30 | 1, 10, and 30 | 1↑ |
| Epididymal spermatozoal concentration[h] (decrease) | NS | — | 3[g] and 30 | — |

↑ All higher doses were affected
[a] NTP, 1998a
[b] Doses tested in rats and mice were 0, 0.1, 1.0, 3.0, 10, and 30 mg/kg
[c] NS = not significantly affected
[d] NO = not observed
[e] Not applicable or not analyzed
[f] $T_3$ = triiodothyronine; $T_4$ = thyroxine
[g] Lowest dose tested for this effect
[h] Doses tested for sperm motility evaluations were 3.0, 10, and 30 mg/kg

relative thymus weight, and an increase in relative liver weight (39% above controls) (Hsia *et al.*, 1981). Histological alterations were found in the liver, lung, lymph nodes, spleen, and thymus. In the liver of dosed animals, hepatocyte swelling and cytoplasmic vacuoles were observed. The cortex of the thymus, the outer cortical areas of the mesenteric lymph nodes, and the periarterial lymphatic sheaths of the spleen were atrophied. In addition, the lung of dosed rats contained thickened alveolar walls and foamy macrophages.

In studies of TCAB hepatotoxicity conducted by Schrankel *et al.* (1980), male Sprague-Dawley rats were given four daily intraperitoneal injections of 25 mg/kg and examined on day 5. Female ICR outbred Swiss albino mice were administered five daily 20 mg/kg intraperitoneal injections immediately following the lactation stage and were examined on day 6. In both studies, hepatocytes were enlarged and contained abundant cytoplasmic vacuoles. Proliferation of smooth endoplasmic reticulum was observed, and membranous arrays

occurred frequently. The hepatic mitotic index was increased in dosed animals compared to the controls. In addition, potential genotoxicity of TCAB was suggested by Schrankel *et al.* (1980) because of the occasional appearance of atypical mitotic figures.

Chloracne is a severe and persistent skin condition characterized by acne-like eruption of comedones and cysts on specific regions of the skin. It is associated with exposure to certain halogenated aromatic hydrocarbons; TCDD is the most potent chloracnegen. Although chloracne is the primary adverse effect observed in humans (see below), only one animal study has reported that TCAB induced chloracne-like lesions. The inner surface of the left ear of two adult female New Zealand White rabbits per group was painted daily for 5 days with 0.1 mL of a 0.001 to 0.08 mg/mL solution of TCAB in methyl isobutyl ketone (for a total dose of 0.5 to 32 µg TCAB) (Hill *et al.*, 1981). The right ear was used as an untreated control. The rabbits were killed between 14 and 22 days after their first treatment. A dose-dependent increase in the severity of hyperkeratosis was observed. Hyperkeratosis was characterized by epithelial proliferation, dilatation of the hair follicles, and sebaceous gland atrophy. The hyperkeratosis occurred at the lowest dose, 0.5 µg, suggesting that the chloracnegenic potency of TCAB is similar to that of TCDD.

### *Humans*

Three outbreaks of chloracne attributed to TCAB exposure have occurred among workers in chemical plants manufacturing 3,4-dichloroaniline or its derivatives. In manufacturing plants producing Diuron® and 3,4-dichloroaniline, 89 workers were exposed to TCAB and, to a lesser extent, 3,3′,4,4′-tetrachloroazoxybenzene, for 1 to 7 years (Scarisbrick and Martin, 1981). Thirty people (34%) in the exposed cohort had chloracne. Alanine aminotransferase activities and total cholesterol concentrations were greater in the exposed workers with chloracne than in exposed workers without chloracne or in unexposed workers. Triglyceride concentrations were elevated in exposed workers, with or without chloracne, in comparison to unexposed workers. Exposure resulted in chloracne in 30 people in one facility in 1989 (Dr. A. Smith, University of Leicester, United Kingdom, personal communication).

Morse *et al.* (1979) reported that 38% of 102 workers in an Arkansas Propanil® manufacturing plant had chloracne that was attributed to TCAB exposure. The highest incidence (61%) occurred in the production workers.

## Reproductive and Developmental Toxicity

### *Experimental Animals*

TCAB was tested for potential developmental and reproductive effects in Sprague-Dawley rats using the NTP Reproductive Assessment by Continuous Breeding (RACB) protocol (Hooth *et al.*, 2002; NTP, 2004). $F_0$ males and females (20/sex per group) were administered TCAB once daily by corn oil gavage at doses of 0, 1.0, 3.0, or 10 mg/kg per day. Mating pairs were bred continuously to produce three $F_1$ litters/pair ($F_{1a}$, $F_{1b}$, and $F_{1c}$), and the third litter was reared to adulthood. $F_{1c}$ weanlings were exposed to TCAB at the same concentrations as their $F_0$ parents, and on postnatal day 71 to 91, 20 pairs of $F_{1c}$ adults/dose were mated to produce three $F_2$ litters/pair ($F_{2a}$, $F_{2b}$, and $F_{2c}$). Parental observations included body weight, feed consumption, organ weights, and gross and microscopic pathology. Litter observations included number of live pups per litter and live pup weight. A dose-related decrease in mean body weights was observed in $F_0$ and $F_1$ males and females. The pregnancy index was not affected in the $F_0$ cohabitation, but by the $F_1$ cohabitation there were significant decreases in the pregnancy index in all treated groups. The $F_1$ pregnancy index was accompanied by an increase of 12% to 39% in the cumulative days to litter. There were significant reductions in the number of live $F_1$ pups per litter in all dosed groups except for 1.0 mg/kg males. Live pup weight was decreased at 10 mg/kg. The number of live $F_2$ pups per litter was significantly reduced in all dosed groups except for 1.0 mg/kg females, and live pup weight was decreased at 10 mg/kg. Sperm analysis of the $F_0$ males revealed no changes. Sperm analysis of 10 mg/kg $F_1$ males revealed decreases of total spermatids per testis by 26% and total sperm per cauda by 32%. Microscopic findings noted at 10 mg/kg included minimal retention of Step 19 spermatids in both $F_0$ and $F_1$ males.

As part of the NTP RACB study, a crossover mating was conducted with the $F_0$ parents to determine which sex was affected by TCAB treatment (Hooth *et al.*, 2002; NTP, 2004). Treated $F_0$ males and females from the

control and 10 mg/kg groups were paired with naive females and males for 7 days. The reproductive data for the treated males was comparable to the controls. When treated females were cohabited with naive males, the number of live males per litter was decreased by 53%. There was no effect on the number of primordial follicles in the ovaries of 10 mg/kg $F_0$ females relative to controls, suggesting that *in utero* developmental toxicity was responsible for the reductions in litter size.

The development of the $F_{1c}$ pups in the NTP RACB study suggested that TCAB produced some neurotoxic effects (Hooth *et al.*, 2002; NTP, 2004). There were decreases in landing foot splay and forelimb grip strength in 3.0 and 10 mg/kg males and females. Hind limb grip strength was decreased in 10 mg/kg males.

Based on the conditions of the NTP RACB study, TCAB resulted in reproductive toxicity at all doses as evidenced by decreased numbers of pups and pup body weights and increased incidences of microscopic findings in the testis; general toxicity was evidenced by decreased parental body weight and feed consumption at all doses and by increased incidences of microscopic findings in the liver, spleen, kidney, and thymus of 10 mg/kg animals (Hooth *et al.*, 2002; NTP, 2004). A NOAEL was not reached in this study.

The embryotoxicity and teratogenicity of TCAB in chick embryos was studied by Schrankel *et al.* (1982). Doses ranging from 0.0001 to 100 µg TCAB dissolved in corn oil were injected into the air cell of eggs on day 4 of incubation. In an additional group of eggs, 0.05 µg TCAB per egg was injected on days 11, 12, or 13 of incubation. The majority of embryo deaths occurred before day 13 of incubation for all groups treated with 0.005 to 100 µg. Eggs that were injected on days 11, 12, or 13 of incubation had a lower incidence of embryo mortality than those injected on day 4. The $LD_{50}$ was calculated to be 44 ng of TCAB. Numerous malformations were detected in hatched chicks and in embryos that died prior to hatching. Rump edema was the major abnormality observed in treated embryos. In addition, altered feather pattern, lack of down, hemorrhage, external viscera, reduced body size, failure to withdraw the yolk sac, beak malformation, dilation of blood vessels, and monomicrophthalmia were observed.

### *Humans*

No studies of the reproductive or developmental effects TCAB in humans were found in a review of the literature.

## Carcinogenicity

### *Experimental Animals*

The carcinogenic potential of TCAB has been investigated in *in vitro* studies. Hsia *et al.* (1977) reported on the ability of TCAB to induce focal morphologic changes, so-called type III foci, typical of transformed C3H/10T1/2 cells after a 10-day incubation. Transplantation of cells from type III foci into syngeneic C3H mice had been shown to result in the formation of neoplasms (Reznikoff *et al.*, 1973).

In a 60-week feed study by the National Cancer Institute, rats (strain not specified) were exposed to 4 mg TCAB per week for the first 3 weeks and 10 mg per week for the following 37 weeks (Bartha and Pramer, 1970). At the end of the 60-week period, no neoplasms were observed in exposed rats, although fatty degeneration in the liver was reported. Additional details of this study were not provided; no other *in vivo* studies of TCAB carcinogenicity were found in the literature.

### *Humans*

No epidemiology studies of TCAB in humans were found in a review of the literature.

## Genetic Toxicity

TCAB was not mutagenic in any of several strains of *Salmonella typhimurium*, with or without S9 metabolic activation enzymes, in standard plate incorporation assays (Gilbert *et al.*, 1980; McMillan *et al.*, 1988). Gilbert *et al.* (1980) reported weak, inconsistent mutagenicity for TCAB in an *S. typhimurium* fluctuation test in strains TA1532 and TA1538 (strains that detect frameshift mutations) in the presence of Aroclor 1254-induced Wistar rat liver S9. In NTP studies, TCAB (50, 75, or 100 µg/plate) was found to be weakly mutagenic in *S. typhimurium* strain TA97 (mutates via frameshift) when testing was carried out with a preincubation protocol in the presence of 30% induced rat liver S9 (NTP, 1998a); no mutagenic activity was detected in strain TA98, TA100, or TA1535 with or without S9 or in strain TA1537 with S9. TCAB did not induce unscheduled DNA synthesis (indicative of DNA damage and subsequent repair) in primary hepatocytes of rats in the absence of pretreatment with hepatic mixed-function oxidase inducers (McMillan *et al.*, 1988). However, TCAB was reported to induce dose-related increases in unscheduled DNA synthesis in primary hepatocytes

derived from rats pretreated with Aroclor 1254, phenobarbital, or TCAB (Shaddock *et al.*, 1989). Hsia and Kreamer (1979) found that a dose-dependent increase in unscheduled DNA synthesis was induced by TCAB in a freshly isolated suspension of rat hepatocytes. No increase in the frequency of hypoxanthine-guanine phosphoribosyl transferase mutations was seen in Chinese hamster ovary cells treated with TCAB in the presence or absence of S9 (McMillan *et al.*, 1988).

*In vivo*, no induction of micronuclei was noted in bone marrow reticulocytes of male mice treated with TCAB by intraperitoneal injection (three times at 24-hour intervals) (NTP, 1998a; Witt *et al.*, 2000). However, results of a peripheral blood erythrocyte micronucleus test, in which TCAB was administered once daily, 5 days per week, by gavage for 13 weeks, were positive in both male and female mice (NTP, 1998a; Witt *et al.*, 2000); the response observed in male mice in this test was stronger than that observed in females. For males and females, trend tests yielded significant P values, and 10 and 30 mg/kg male mice had frequencies of micronucleated erythrocytes that were significantly different from the frequencies seen in the vehicle controls. None of the treated groups of female mice had micronucleus frequencies that differed significantly from the vehicle controls. Although no significant increases in the percentage of polychromatic erythrocytes (reticulocytes) were seen in mice treated with TCAB for 13 weeks, erythropoiesis was observed in the spleen of these mice (Witt *et al.*, 2000). This observation is consistent with a compensatory response to treatment-related anemia, which may, by itself, result in increased frequencies of micronucleated erythrocytes in the absence of chemical exposure. Such a mechanism might have accounted for the different results in the short-term (72 hours) bone marrow study and the 13-week peripheral blood micronucleus test.

The close structural analogue 3,3′,4,4′-tetrachloroazoxybenzene was not mutagenic in *S. typhimurium* strains with or without rat liver S9 enzymes in a standard plate incorporation assay (Gilbert *et al.*, 1980); it also gave negative results in the *S. typhimurium* fluctuation test (Gilbert *et al.*, 1980). 3,3′,4,4′-Tetrachloroazoxybenzene (100 to 10,000 µg/plate) was not mutagenic in NTP *Salmonella* mutagenicity tests conducted in strains TA97, TA98, TA100, or TA1535 with or without S9 (NTP, 1998b). Similar to the results with TCAB *in vivo*, no significant increase in the frequency of micronuclei was noted in bone marrow erythrocytes of male mice treated with 3,3′,4,4′-tetrachloroazoxybenzene by intraperitoneal injection three times at 24-hour intervals (NTP, 1998b; Witt *et al.*, 2000). However, results of a mouse peripheral blood micronucleus test, in which 3,3′,4,4′-tetrachloroazoxybenzene (up to 30 mg/kg) was administered for 13 weeks by gavage, were positive in both males and females (NTP, 1998b; Witt *et al.*, 2000). Unlike the results with TCAB, the micronucleus frequencies observed in female mice treated for 13 weeks with 3,3′,4,4′-tetrachloroazoxybenzene increased with increasing dose and the two highest doses (10 and 30 mg/kg) produced significantly different responses from that of the vehicle controls. The responses seen in male mice were not well correlated with dose, with the peak response noted in the 10 mg/kg group. Erythropoiesis was also noted in the spleen of mice treated for 13 weeks with 3,3′,4,4′-tetrachloroazoxybenzene.

## Study Rationale

TCAB was nominated by the USEPA for toxicity and carcinogenicity testing based on its structural and biological similarity to TCDD and potential for human exposure from the consumption of crops contaminated with 3,4-dichloroaniline-derived herbicides. Workers may be exposed to TCAB during the manufacture as well as the application of herbicides containing TCAB as a contaminant. Prechronic studies conducted in F344/N rats and B6C3F1 mice were reported previously (NTP, 1998a, Van Birgelen *et al.,* 1999a). The goal of the 2-year studies in Harlan Sprague-Dawley rats was twofold: to characterize the chronic toxicity and carcinogenicity of TCAB and to compare the potential tumor outcome with that of TCDD and other dioxin-like compounds that were evaluated by the NTP in this species as part of the Toxic Equivalency Factor (TEF) evaluation studies. Currently, TCAB is not included in the TEF methodology used by the International Programme on Chemical Safety and the World Health Organization. However, based on results from NTP studies in female F344 rats, it was estimated that TCAB could account for more dioxin-like activity in the environment than polychlorinated dibenzo-*p*-dioxins and dibenzofurans together (NTP, 1998a; Van Birgelen *et al.*, 1999a). Oral gavage was used for these studies allowing direct comparison of the data to the other NTP dioxin toxic equivalency factor evaluation studies. Initial dose selection was based on the 13-week TCAB studies conducted in F344/N rats and B6C3F1 mice by the NTP (1998a).

# MATERIALS AND METHODS

## Procurement and Characterization

### *TCAB*

TCAB was obtained from AccuStandard (New Haven, CT) in two lots (110199MT-AC-1 and 10009-52-01 RTI). Lot 110199MT-AC-1 was used in the 3-month study and lot 10009-52-01 RTI was used in the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Research Triangle Institute (Research Triangle Park, NC) and the study laboratory at Battelle Columbus Operations (Columbus, OH); the analytical chemistry laboratory also conducted stability analyses (Appendix G). Melting point determination, elemental analyses, and Karl Fischer titration were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the TCAB studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

Both lots of the chemical, a fine, bright orange powder, were identified as TCAB by infrared (IR) and proton nuclear magnetic resonance spectroscopy; in addition, lot 10009-52-01 RTI was identified as TCAB by gas chromatography (GC) coupled with mass spectrometry and by melting point analysis. The purity of each lot was determined by GC, and elemental analysis and moisture content were determined for lot 10009-52-01 RTI.

For lot 110199MT-AC-1, GC by one system indicated one major peak and one minor impurity with an area accounting for approximately 0.1% of the total peak area; the overall purity of lot 110199MT-AC-1 was determined to be 99.7% or greater.

For lot 10009-52-01 RTI, Karl Fischer titration indicated a water content of 255 ppm. Elemental analyses for carbon, hydrogen, nitrogen, and chlorine were in agreement with the theoretical values for TCAB. GC by two systems indicated one major peak and 3 minor impurities, each with a peak area less than 0.1% of the total peak area. The overall purity of lot 10009-52-01 RTI was determined to be 99.8% or greater.

Stability studies of the bulk chemical were performed using GC. These studies indicated that TCAB was stable as a bulk chemical for 2 weeks when stored in sealed amber glass vials at temperatures up to 25° C. To ensure stability of the bulk chemical, lot 110199MT-AC-1 was stored at approximately 5° C in amber glass bottles. Lot 10009-52-01 RTI was stored in a –20° C freezer in clear glass bottles to decrease the possibility of reagglomeration of TCAB particles. Periodic reanalyses of the bulk chemical were performed during the 3-month and 2-year studies using GC, and no degradation of the bulk chemical was detected.

### *Formulation Materials*

Acetone was used with corn oil as the vehicle in all studies. For the 3-month study, one lot of USP-grade acetone was obtained from Spectrum Quality Products (Gardena, CA) and for the 2-year studies, one lot was obtained from Spectrum Quality Products and two lots from EM Science (Gibbstown, NJ). The identity of each lot was confirmed by the study laboratory using IR spectroscopy prior to its use. The purity of each lot was determined prior to the start of each study and at intervals of no more than 6 months thereafter using GC. All acetone lots had a purity greater than 99.9% except one lot that had a single impurity of 0.19% relative to the major peak. USP-grade corn oil for all studies was obtained from Spectrum Quality Products; periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than 3 mEq/kg.

## Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing TCAB with corn oil containing 1% acetone (Table G2).

Homogeneity studies of a 40 mg/mL dose formulation and stability studies of a 0.01 mg/mL dose formulation were conducted by the analytical chemistry laboratory, and homogeneity studies of 3, 4, and 40 mg/mL dose for-

mulations were conducted by the study laboratory; these formulations were all analyzed using GC. Homogeneity was confirmed and the stability of the dose formulations was confirmed for at least 43 days at 5° C and room temperature when stored in sealed amber glass containers, and for up to 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of TCAB were conducted by the study laboratory using GC. During the 3-month study, the dose formulations were analyzed at the beginning, midpoint, and end of the study; animal room samples of these dose formulations were also analyzed (Table G3). Of the dose formulations analyzed, all 21 were within 10% of the target concentrations; all 21 of the animal room samples were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed at least every 3 months; all 60 dose formulations analyzed and used for rats (Table G4) and all 36 for mice (Table G5) were within 10% of the target concentrations. Animal room samples were also analyzed; 11 of 12 for rats and all 12 for mice were within 10% of the target concentrations.

## 3-Month Study

The 3-month study was conducted to evaluate the cumulative toxic effects of repeated exposure to TCAB and to determine the appropriate dose concentrations to be used in the 2-year studies.

Male and female Harlan Sprague-Dawley rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). On receipt, the rats were approximately 5 weeks old. Animals were quarantined for 15 (males), 22 (core study females), or 19 (special study females) days and were 7 (males), or 8 (females) weeks old on the first day of the study. Before the study began, five males and five females were selected for parasite evaluation and gross observation for evidence of disease. At 4 weeks and at the end of the study, serologic analyses were performed on five male and five female sentinel rats using the protocols of the NTP Sentinel Animal Program (Appendix I).

Groups of 10 male and 10 female core study rats received TCAB in corn oil:acetone (99:1) by gavage at doses of 0.1, 0.3, 1, 3, 10, 30, or 100 mg/kg 5 days per week for 14 weeks; control animals received the corn oil:acetone (99:1) vehicle alone. For the special study, groups of 30 female rats received TCAB in corn oil:acetone by gavage at doses of 0.1, 3, or 100 mg/kg 5 days per week for 14 weeks, and a group of six females received the corn oil:acetone vehicle alone. To provide 2 consecutive dosing days prior to the start of sample collection, special study rats were dosed on Sunday of the last study week. A dosing volume of 2.5 mL/kg was used. Rats were housed five (core study) or three (special study) per cage. Feed and water were available *ad libitum*. Clinical findings for core study rats were recorded initially, weekly, and at the end of the study. All animals were weighed initially, then weekly during the study; core study animals were weighed at the end of the study. BrdU water consumption was recorded for 5 days prior to necropsy (core study only) for cell proliferation studies. Details of the study design and animal maintenance are summarized in Table 2.

The core study rats were bled on the first day of week 13, approximately 30 minutes after dosing for hematology and thyroid hormone analyses; blood was collected for clinical chemistry analyses at the end of the study. The parameters measured are listed in Table 2. At all time points, animals were anesthetized with a $CO_2/O_2$ mixture, and blood was drawn from the retroorbital sinus. Hematology samples were placed in tubes containing EDTA and stored at room temperature. Most of the hematology variables were measured using a Cell-Dyn® automated cell counter (Abbott Diagnostics, Santa Clara, CA). Leukocyte differentials, nucleated erythrocyte counts, and morphologic evaluation of blood cells were determined by light microscopic examination of blood films stained with a modified Wright-Giemsa stain using an Ames Hema-Tek® slide stainer (Miles Laboratory, Ames Division, Elkhart, IN). Smears made from preparations of equal volumes of new methylene blue (Sigma Chemical Company, St. Louis, MO) and whole blood were examined microscopically using the Miller disc method for the quantitative determination of reticulocytes. Samples for thyroid hormone and clinical chemistry analyses were collected into tubes without EDTA, allowed to clot at room temperature, and the serum was obtained by centrifugation. Radioimmunoassays were performed on serum for thyroid stimulating hormone, triiodothyronine, and free thyroxine ($T_4$) using a Packard Cobra II gamma counter (Packard Instrument Company, Meriden, CT). The assay for total $T_4$ was performed on a Hitachi 911® chemistry analyzer (Boehringer Mannheim®, Indianapolis, IN) using a Boehringer Mannheim immunoassay test system. Clinical chemistry analyses were also performed on a Hitachi 911® chemistry analyzer using reagents obtained from the manufacturer.

**TABLE 2**
**Experimental Design and Materials and Methods in the Gavage Studies of TCAB**

| | 3-Month Study | 2-Year Studies |
|---|---|---|
| **Study Laboratory** | Battelle Columbus Operations (Columbus, OH) | Battelle Columbus Operations (Columbus, OH) |
| **Strain and Species** | Harlan Sprague-Dawley rats | Harlan Sprague-Dawley rats<br>B6C3F1 mice |
| **Animal Source** | Harlan Sprague-Dawley, Inc. (Indianapolis, IN) | Rats: Harlan Sprague-Dawley, Inc. (Indianapolis, IN)<br>Mice: Taconic Farms, Inc. (Germantown, NY) |
| **Time Held Before Studies** | 15 (males), 22 (core study females), or 19 (special study females) days | Rats: 13 days (males) or 14 days (females)<br>Mice: 11 days (females) or 12 days (males) |
| **Average Age When Studies Began** | 7 (males) or 8 (females) weeks | Rats: 5 weeks<br>Mice: 5-6 weeks |
| **Date of First Dose** | December 14 (males), 21(core study females), or 18 (special study females), 2000 | Rats: January 29 (males) or 30 (females), 2003<br>Mice: February 3 (females) or 4 (males), 2003 |
| **Duration of Dosing** | 5 days/week for 14 weeks | Rats: 5 days/week for 104 weeks<br>Mice: 5 days/week for 104 (males) or 105 (females) weeks |
| **Date of Last Dose** | March 15 (males), 22 (core study females), or 19 (special study females), 2001 | Rats: January 21 (males) or 25 (females), 2005<br>Mice: January 31 (males) or February 3 (females), 2005 |
| **Necropsy Dates** | March 16 (males), 23 (core study females), or 19-24 (special study females), 2001 | Rats: January 24-26, 2005<br>Mice: January 31-February 4, 2005 |
| **Average Age at Necropsy** | 21 (males), 22 (core study females), or 21-22 (special study females) weeks | Rats: 109 weeks<br>Mice: 110 to 111 weeks |
| **Size of Study Groups** | Core study: 10 males and 10 females<br>Special study (clinical pathology and tissue concentration analysis): 30 (dosed groups) or 6 (vehicle control group) females | 50 males and 50 females |
| **Method of Distribution** | Animals were distributed randomly into groups of approximately equal initial mean body weights. | Animals were distributed randomly into groups of approximately equal initial mean body weights. |
| **Animals per Cage** | 5 (core study) or 3 (special study) | Rats: 3 (males) or 5 (females)<br>Mice: 1 (males) or 5 (females) |
| **Method of Animal Identification** | Tail tattoo | Tail tattoo |
| **Diet** | Irradiated NTP-2000 pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum,* changed weekly | Same as 3-month study |
| **Water** | Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), or Milli-Q water via amber glass bottles (last 10 days of core study), available *ad libitum* | Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI) |
| **Cages** | Polycarbonate (Lab Products, Inc., Seaford, DE), changed twice weekly | Polycarbonate (Lab Products, Inc., Seaford, DE), changed once (male mice) or twice weekly |

**TABLE 2**
**Experimental Design and Materials and Methods in the Gavage Studies of TCAB**

| | 3-Month Study | 2-Year Studies |
|---|---|---|
| **Bedding** | Irradiated Sani-Chips® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed twice weekly | Irradiated Sani-Chips® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed once (male mice) or twice weekly |
| **Cage Filters** | DuPont 2024 spun-bonded polyester (Snow Filtration Co., Cincinnati, OH); changed every 2 weeks | Same as 3-month study |
| **Racks** | Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks | Same as 3-month study |
| **Animal Room Environment** | Temperature: 72° ± 3° F<br>Relative humidity: 50% ± 15%<br>Room fluorescent light: 12 hours/day<br>Room air changes: at least 10/hour | Temperature: 72° ± 3° F<br>Relative humidity: 50% ± 15%<br>Room fluorescent light: 12 hours/day<br>Room air changes: at least 10/hour |
| **Dose Concentrations** | Core study: 0, 0.1, 0.3, 1, 3, 10, 30, or 100 mg/kg;<br>special study: 0, 0.1, 3, or 100 mg/kg;<br>dosing volume = 2.5 mL/kg body weight | Rats: 0, 10, 30, or 100 mg/kg (dosing volume = 2.5 mL/kg)<br>Mice: 0, 3, 10, or 30 mg/kg (dosing volume = 10 mL/kg) |
| **Type and Frequency of Observation** | Observed twice daily; all animals were weighed initially and then weekly, and core study animals were weighed at the end of the study; clinical findings were recorded initially, weekly, and at the end of the study. BrdU water consumption was recorded for 5 days prior to necropsy (core study only). | Observed twice daily; all animals were weighed on day 1, weekly for the first 13 weeks, every 4 weeks thereafter, and at the end of the study. Clinical findings were recorded on day 29, every 4 weeks thereafter, and at the end of the study. |
| **Method of Sacrifice** | Carbon dioxide asphyxiation | Carbon dioxide asphyxiation |
| **Necropsy** | Necropsies were performed on all animals. Organs weighed for the core study groups were the heart, right kidney, liver, lung, left ovary, left testis, thymus, thyroid gland, and spleen; organs weighed for the special study groups were liver and lung. | Necropsies were performed on all animals. |
| **Clinical Pathology** | Blood samples were collected from the retroorbital sinus of core study rats at approximately 30 minutes after dosing on the first day of week 13 for hematology and thyroid hormone analysis and at terminal sacrifice for clinical chemistry.<br>***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; leukocyte count and differentials; methemoglobin; and osmotic fragility<br>***Thyroid Hormone Analysis:*** thyroid stimulating hormone, total triiodothyronine, and total and free thyroxine determinations<br>***Clinical Chemistry:*** urea nitrogen, creatinine, total protein, albumin, cholesterol, triglyceride, alanine aminotransferase, alkaline phosphate, creatine kinase, sorbitol dehydrogenase, and bile acids | None |

**TABLE 2**
**Experimental Design and Materials and Methods in the Gavage Studies of TCAB**

| | 3-Month Study | 2-Year Studies |
|---|---|---|
| **Cell Proliferation** | Core study rats received BrdU in drinking water for 5 days before scheduled sacrifice. Samples from the liver and duodenum were measured for BrdU labeling. Vehicle control and 100 mg/kg groups were compared. | |
| **Cytochrome P450 Activities** | Tissue samples from the liver were taken from core study rats for 7-ethoxyresorufin-*O*-deethylase, 7-pentoxyresorufin-*O*-deethylase, and acetanilide-4-hydroxylase activities. Lung samples from these rats were analyzed for 7-ethoxyresorufin-*O*-deethylase activity. | |
| **Tissue Concentration Analysis** | Samples of fat, liver, and lung were taken from core study rats for analysis of tissue concentrations of TCAB at the end of the 3-month study. Samples of blood were taken from the retroorbital sinus of six special study rats per group for analysis of whole blood concentrations of TCAB at 0, 15, and 30 minutes and 1, 3, 6, 9, 12, 20, and 30 hours after the last dose. At 6, 30, 60, 91, and 120 hours after the last dose, the liver, lung, and fat tissue were harvested from six special study rats per dosed group for tissue concentration analyses; these tissues were harvested from vehicle controls at 6 hours only. | |
| **Histopathology** | Complete histopathology was performed on vehicle control and 100 mg/kg core study rats. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, heart, small intestine (duodenum), kidney, liver, lung, mammary gland, nose, ovary, pancreas, pituitary gland, prostate gland, skin, spleen, stomach (forestomach), testes with epididymis and seminal vesicles, thymus, thyroid gland, tongue, uterus, and vagina. In the remaining groups of core study rats, the liver, lung, and thymus were examined; the spleen was examined in the remaining groups of males and in 10 and 30 mg/kg females. | Complete histopathology was performed on all animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice only), harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testes with epididymis and seminal vesicles, thymus, thyroid gland, trachea, proximal ureter (male mice only), urethra, urinary bladder, and uterus. |

Ten days before scheduled sacrifice, all core study rats were switched to Milli-Q water; after 5 days, 5-bromo-2′-deoxyuridine (BrdU) was added (40 mg BrdU/100 mL of Milli-Q water). BrdU solutions were administered in amber glass water bottles (Allentown Caging Equipment Company, Inc., Allentown, NJ) equipped with Teflon®-lined lids and stainless steel sipper tubes; water consumption was measured daily during BrdU administration. Cell turnover rate in the liver of 100 mg/kg rats rats was compared to the turnover rate in the liver of vehicle control rats by determining the incorporation of BrdU into hepatocytes. Samples of duodenum (positive control) and liver were fixed for 18 to 24 hours in 10% neutral-buffered formalin then transferred to 70% ethanol. Representative sections of the liver and duodenum were trimmed and embedded, and two sections were cut. One of the sections was stained with hematoxylin and eosin and the other with anti-BrdU antibody complexed with avidin and biotin. Potential interlobular variation was determined by counting stained cells in the left lobe and right median lobe of the vehicle control and 100 mg/kg groups. Interlobular variation greater than 25% was considered significant. For quantitation, 2,000 labeled or unlabeled hepatocyte nuclei were counted using a 20× objective and ocular grid. The labeling index is expressed as the percentage of total nuclei that were labeled with BrdU.

For determination of cytochrome P450 activities, liver and lung tissue samples were collected from core study rats and stored frozen at –70° C. Microsomal suspensions were prepared using the Pearce method (Pearce *et al.,* 1996). The concentration of protein in each suspension was determined using the microtiter plate method of the Coomassie Plus Protein Assay (Pierce Chemical Co., Rockford, IL) with bovine serum albumin as the standard. Cytochrome P450 1A1 (CYP1A1)-associated 7-ethoxyresorufin-*O*-deethylase (EROD), CYP1A2-associated acetanilide-4-hydroxylase (A4H), and CYP2B-associated pentoxyresorufin-*O*-deethylase) (PROD) activities were determined in microsomal proteins, isolated from frozen liver or lung tissue. Data are shown as pmol/minute per mg (EROD and PROD) or nmol/minute per mg (A4H) microsomal protein.

Samples of fat, liver, and lung were taken from core study rats for analysis of tissue concentrations of TCAB at the end of the 3-month study. Six special study rats per dose group were bled at 0, 15, and 30 minutes and 1, 3, 6, 9, 12, 20, and 30 hours after the last dose; at the 6-hour timepoint, the six vehicle control rats were also bled. Blood was collected once from the control rats and twice from each dosed rat. Blood samples were placed in tubes containing EDTA and stored at approximately –20° C. At 6, 30, 60, 91, and 120 hours after the last dose, the liver, lung, and adipose tissue were harvested from six special study rats per dosed group; these tissues were harvested from vehicle controls at 6 hours only. Blood and tissue samples were shipped to Research Triangle Institute (Research Triangle Park, NC) for analysis of TCAB concentrations; details of these analyses are presented in Appendix J.

All animals were euthanized in a carbon dioxide chamber and necropsies were performed on all animals. The heart, right kidney, liver, lung, left ovary, left testis, thymus, thyroid gland, and spleen were weighed for the core animals; the liver and lung were weighed for the special study animals. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 µm, and stained with hematoxylin and eosin. For core study rats, histopathologic examinations were performed on vehicle control and 100 mg/kg groups. Table 2 lists the tissues and organs routinely examined.

## 2-Year Studies

### *Study Design*

Groups of 50 male and 50 female rats and mice received TCAB in corn oil:acetone (99:1) by gavage at doses of 10, 30, or 100 mg/kg (rats) or 3, 10, or 30 mg/kg (mice) 5 days per week for 2 years; groups of 50 male and 50 female rats and mice received the corn oil:acetone vehicle only. The dosing volume was 2.5 mL/kg for rats and 10 mL/kg for mice. The dose levels for the 2-year mouse studies were selected based on the results from NTP 13-week toxicity studies (NTP, 1998a and Van Birgelen *et al.,* 1999a). In the 13-week studies in mice (NTP, 1998a), there were no effects of TCAB exposure on mortality or body weights. Absolute and relative liver and spleen weights were significantly increased in males and females at 10 and 30 mg/kg. In male mice, absolute and relative thymus weights were significantly decreased at 30 mg/kg. Treatment-related effects included hematopoietic cell proliferation in the spleen of male mice and squamous hyperplasia in the forestomach epithelium of males and females. Centrilobular hypertrophy was present in the livers of male mice. Although the incidence of lesions increased with dose, the severity of the lesions was generally minimal and was not dose

limiting. Doses of 0, 3, 10, and 30 mg/kg per day were selected for the 2-year gavage study for male and female mice. A higher dose of 100 mg/kg was not selected because thymus weights were significantly decreased in male and female mice at this dose in the 16-day toxicity study (NTP, 1998a).

## *Source and Specification of Animals*

Male and female Harlan Sprague-Dawley rats and B6C3F1 mice were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN), and Taconic Farms, Inc. (Germantown, NY), respectively, for use in the 2-year studies. Rats were quarantined for 13 (males) or 14 (females) days and mice were quarantined for 11 (females) or 12 (males) days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats were approximately 5 weeks old and mice approximately 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix I).

## *Animal Maintenance*

Male rats were housed three per cage, male mice were housed individually, and female rats and mice were housed five per cage. Feed and water were available *ad libitum*. Cages and racks were rotated every two weeks. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix H.

## *Clinical Examinations and Pathology*

All animals were observed twice daily. Clinical findings were recorded at day 29, monthly thereafter, and at the end of the studies. Body weights were recorded on day 1, weekly for 13 weeks, monthly thereafter, and at the end of the studies.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 µm, and stained with hematoxylin and eosin (except eyes were first fixed in Davidson's solution) for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 2.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the liver, lung, stomach (forestomach and glandular), and thyroid gland of rats and the kidney, skin, and stomach (forestomach and glandular) of mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

## Statistical Methods

### *Survival Analyses*

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

### *Calculation of Incidence*

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A3, B1, B4, C1, C4, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g. mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g. leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

### *Analysis of Neoplasm and Nonneoplastic Lesion Incidences*

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k = 3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1– P with the letter N added (e.g., P = 0.99 is presented as P = 0.01N). For neoplasms and nonneoplastic lesions detected in the 3-month study, the Fisher exact test (Gart *et al.,* 1979), a procedure based on the overall proportion of affected animals, was used.

### *Analysis of Continuous Variables*

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, thyroid hormone, clinical chemistry, cell proliferation, cytochrome P450, and plasma concentra-

tion data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

## *Historical Control Data*

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year (mice) or 6-year (female Harlan Sprague-Dawley rats) period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration; for the current report the overall incidences of neoplasms for all routes of administration are included for the mouse study. There are several historical gavage studies using female Harlan Sprague-Dawley rats for comparison to the current study. However, because there are no other studies using male Harlan Sprague-Dawley rats, no comparisons to historical data will be made for male rats in this Technical Report.

## *Quality Assurance Methods*

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

# RESULTS

## RATS

### *3-Month Study*

All male and female rats survived to the end of the study (Table 3). Terminal mean body weights of males were not significantly different from vehicle controls in any group. Terminal mean body weights of females administered 10 mg/kg or greater were significantly less than those of the vehicle controls. Mean body weight gains of all dosed groups of females were significantly less than those of the vehicle controls. There were no treatment-related clinical finding.

The hematology and clinical chemistry data are listed in Table E1. At 3 months, a treatment-related anemia occurred in 10, 30, and 100 mg/kg males. The anemia was evidenced by decreases in hemoglobin concentrations and hematocrit values; erythrocyte counts were unaffected. The anemia was characterized as microcytic, normochromic, and responsive. Evidence of a microcytosis was demonstrated by decreases in mean cell volume values; normochromic erythrocytes were evidenced by no change in the mean cell hemoglobin concentrations. An erythropoietic response was demonstrated by increases in the reticulocyte counts. A minimal decrease in mean cell hemoglobin also occurred and would be consistent with the decreased mean cell volume values.

Increases in serum alkaline phosphatase and sorbitol dehydrogenase activities and bile acid concentration occurred in dosed male and female animals and were

**TABLE 3**
**Survival and Body Weights of Rats in the 3-Month Gavage Study of TCAB**

| Dose (mg/kg) | Survival[a] | Mean Body Weight[b] (g) | | | Terminal Weight Relative to Controls (%) |
|---|---|---|---|---|---|
| | | Initial | Terminal | Change | |
| **Male** | | | | | |
| 0 | 10/10 | 252 ± 2 | 421 ± 8 | 169 ± 7 | — |
| 0.1 | 10/10 | 252 ± 2 | 443 ± 4 | 191 ± 5 | 113 |
| 0.3 | 10/10 | 250 ± 3 | 413 ± 6 | 163 ± 6 | 96 |
| 1 | 10/10 | 249 ± 2 | 422 ± 5 | 173 ± 5 | 102 |
| 3 | 10/10 | 248 ± 2 | 416 ± 5 | 167 ± 4 | 99 |
| 10 | 10/10 | 250 ± 2 | 411 ± 8 | 161 ± 8 | 95 |
| 30 | 10/10 | 250 ± 3 | 404 ± 8 | 155 ± 7 | 92 |
| 100 | 10/10 | 254 ± 2 | 414 ± 7 | 161 ± 5 | 95 |
| **Female** | | | | | |
| 0 | 10/10 | 205 ± 4 | 285 ± 2 | 80 ± 5 | — |
| 0.1 | 10/10 | 207 ± 2 | 275 ± 4 | 67 ± 3* | 84 |
| 0.3 | 10/10 | 209 ± 3 | 276 ± 4 | 67 ± 3* | 84 |
| 1 | 10/10 | 205 ± 3 | 274 ± 4 | 69 ± 4* | 86 |
| 3 | 10/10 | 206 ± 4 | 274 ± 5 | 67 ± 4* | 84 |
| 10 | 10/10 | 203 ± 3 | 271 ± 5* | 68 ± 4* | 85 |
| 30 | 10/10 | 205 ± 2 | 263 ± 4** | 59 ± 4** | 74 |
| 100 | 10/10 | 207 ± 3 | 270 ± 3** | 63 ± 3** | 79 |

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

**TABLE 4**
**Thyroid Hormone Data for Rats in the 3-Month Gavage Study of TCAB[a]**

| | 0 mg/kg | 0.1 mg/kg | 0.3 mg/kg | 1 mg/kg | 3 mg/kg | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|---|---|---|---|
| Week 13 | | | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Male** | | | | | | | | |
| Total thyroxine ($T_4$) (µg/dL) | 4.86 ± 0.10 | 1.57 ± 0.15** | 0.72 ± 0.11** | 0.31 ± 0.07** | 0.19 ± 0.04** | 0.15 ± 0.00** | 0.15 ± 0.00** | 0.15 ± 0.00** |
| Free thyroxine ($T_4$) (ng/dL) | 2.59 ± 0.12 | 1.12 ± 0.09** | 0.77 ± 0.06** | 0.37 ± 0.03** | 0.42 ± 0.04** | 0.30 ± 0.04** | 0.18 ± 0.02** | 0.15 ± 0.00** |
| Total triiodothyronine ($T_3$) (ng/dL) | 109.8 ± 3.8 | 96.6 ± 7.5[b] | 98.2 ± 2.9 | 92.5 ± 5.5* | 87.7 ± 2.7**[b] | 104.3 ± 5.4 | 90.5 ± 4.2*[c] | 100.2 ± 3.6 |
| Thyroid stimulating hormone (TSH) (ng/mL) | 10.3 ± 0.7 | 12.9 ± 1.6 | 11.3 ± 1.1 | 10.9 ± 1.8 | 10.0 ± 1.4 | 11.6 ± 1.6 | 14.5 ± 2.8 | 10.1 ± 1.0 |
| **Female** | | | | | | | | |
| Total thyroxine ($T_4$) (µg/dL) | 5.25 ± 0.28 | 3.28 ± 0.24** | 1.68 ± 0.18** | 1.40 ± 0.12** | 1.23 ± 0.08** | 0.71 ± 0.06** | 0.51 ± 0.07** | 0.48 ± 0.08** |
| Free thyroxine ($T_4$) (ng/dL) | 2.18 ± 0.14 | 1.22 ± 0.10** | 0.65 ± 0.06** | 0.58 ± 0.03** | 0.39 ± 0.03** | 0.21 ± 0.03** | 0.17 ± 0.02** | 0.33 ± 0.03** |
| Total triiodothyronine ($T_3$) (ng/dL) | 95.7 ± 4.3 | 108.5 ± 6.3 | 63.1 ± 10.3* | 79.1 ± 5.5 | 64.0 ± 4.9** | 74.3 ± 6.2 | 89.3 ± 5.3 | 76.5 ± 7.6 |
| Thyroid-stimulating hormone (TSH) (ng/mL) | 9.4 ± 1.0 | 8.7 ± 0.6 | 7.7 ± 0.9 | 10.6 ± 1.4 | 10.6 ± 0.9 | 8.6 ± 0.9 | 8.3 ± 0.6 | 8.4 ± 0.7 |

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n = 9
[c] n = 8

consistent with the observed hepatic lesions. Additionally, serum cholesterol concentration demonstrated treatment-related increases; the mechanism was unknown.

Mean total thyroxine ($T_4$) and free $T_4$ serum concentrations were significantly decreased in a dose-related manner in all dosed groups of both sexes compared to their respective vehicle controls (Table 4). Mean triiodothyronine ($T_3$) serum concentrations were significantly decreased in 1, 3, and 30 mg/kg males and 0.3 and 3 mg/kg females. No significant differences were observed in mean thyroid stimulating hormone concentrations in any dosed group of either sex compared to their respective vehicle controls. There were no effects on absolute or relative thyroid gland weights (Table F1).

Hepatic cell proliferation was measured in the median and left liver lobes of vehicle control and 100 mg/kg male and female rats at the end of the 3-month study; no significant differences in the BrdU labeling index were observed between the vehicle control and 100 mg/kg groups [mean % of cells labeled: male left lobe (vehicle control, 0.723; 100 mg/kg, 0.532), male median lobe (0.495; 0.522), female left lobe (0.793; 0.861) female median lobe (0.830; 0.712)].

Hepatic P450 activity increased with dose in all treated groups of males and females compared to their respective vehicle controls (Table 5). Significant induction of hepatic 7-ethoxyresorufin-*O*-deethylase (EROD, CYP1A1) activity and 7-pentoxyresorufin-*O*-deethylase (PROD, CYP2B) activity was observed in all dosed groups of males and females. Significant induction of hepatic acetanilide-4-hydroxylase activity (CYP1A2) was observed in males exposed to 3 mg/kg or higher and all treated groups of females. The magnitude of the induction in the 100 mg/kg groups was approximately 4-fold for A4H, 7-fold for PROD, and 40- to 50-fold for EROD compared to vehicle controls.

EROD activities in the lung generally increased with dose and were significantly higher in all treated groups of males and females compared to their respective vehicle controls (Table 5). The magnitude of induction in the 100 mg/kg groups was approximately 29-fold in males and 11-fold in females compared to vehicle controls.

**TABLE 5**
**Liver and Lung Cytochrome P450 Data for Special Study Rats in the 3-Month Gavage Study of TCAB[a]**

| | 0 mg/kg | 0.1 mg/kg | 0.3 mg/kg | 1 mg/kg | 3 mg/kg | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|---|---|---|---|
| **Male** | | | | | | | | |
| n | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Liver Microsomes** | | | | | | | | |
| Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein) | 0.620±0.031 | 0.593±0.035 | 0.692±0.064 | 0.693±0.052 | 0.894±0.045** | 1.058±0.112** | 1.841±0.118** | 2.217±0.130** |
| 7-Ethoxyresorufin-O-deethylase (EROD) (pmol/minute per mg microsomal protein) | 33.41±2.04 | 175.2±8.01** | 320.4±11.20** | 483.1±36.33** | 657.6±38.66** | 764.2±37.94** | 1,204.1±69.06** | 1,629.0±40.34** |
| 7-Pentoxyresorufin-O-deethylase (PROD) (pmol/minute per mg microsomal protein) | 2.513±0.144 | 6.243±0.272** | 7.843±0.587** | 9.244±0.387** | 8.607±0.936** | 10.575±0.185** | 14.820±0.538** | 17.820±0.773** |
| **Lung Microsomes** | | | | | | | | |
| 7-Ethoxyresorufin-O-deethylase (EROD) (pmol/minute per mg microsomal protein) | 1.123±0.100[b] | 18.270±1.029** | 19.890±1.225** | 25.500±2.018** | 24.150±2.526** | 30.060±2.326** | 38.580±3.166** | 32.480±2.111** |
| **Female** | | | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Liver Microsomes** | | | | | | | | |
| Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein) | 0.503±0.030 | 0.679±0.024** | 0.748±0.051** | 0.926±0.036** | 0.844±0.047** | 1.088±0.069** | 1.626±0.074** | 1.946±0.101** |
| 7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein) | 55.22±5.07 | 325.80±16.81** | 563.40±46.39** | 920.80±51.53** | 965.80±51.12** | 1,344.1±91.09** | 1,686.0±101.47** | 2,134.0±97.58** |
| 7-Pentoxyresorufin-*O*-deethylase (PROD) (pmol/minute per mg microsomal protein) | 2.767±0.175 | 7.366±0.291** | 9.257±0.475** | 10.841±0.380** | 10.990±0.372** | 13.850±0.453** | 15.810±0.611** | 20.510±0.608** |
| **Lung Microsomes** | | | | | | | | |
| 7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein) | 2.537±0.548 | 14.301±2.302** | 19.700±1.120** | 18.450±1.122** | 16.939±1.652** | 17.790±0.878** | 26.930±2.356** | 28.560±1.942** |

** Significantly different (P≤0.01) from the control group by Shirley's test
[a] Data are presented as mean ± standard error.
[b] n = 10

**TABLE 6**
**Tissue Concentrations of TCAB in Rats in the 3-Month Gavage Study of TCAB[a]**

| 0 mg/kg | 0.1 mg/kg | 0.3 mg/kg | 1 mg/kg | 3 mg/kg | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|---|---|---|
| Week 13 | | | | | | | |
| n 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Male** | | | | | | | |
| Fat (ng/g) | | | | | | | |
| 3.06±1.18[b] | 160.3±16.7[**b] | 385.1±44.6[**] | 768.0±53.0[**b] | 3,524±259[**] | 10,908±541[**] | 19,078±1,101[**b] | 19,380±1,027[**] |
| Liver (ng/g) | | | | | | | |
| 0.025±0.006 | 0.579±0.071[**] | 1.230±0.190[**] | 4.373±1.539[**] | 8.348±0.591[**] | 34.62±12.55[**] | 69.71±5.91[**] | 135.6±23.6[**] |
| Lung (ng/g) | | | | | | | |
| 0.050±0.000 | 0.847±0.408[**] | 1.012±0.371[**] | 0.802±0.208[**] | 2.169±0.161[**] | 4.107±0.946[**] | 23.63±1.75[**b] | 40.88±6.98[**b] |
| **Female** | | | | | | | |
| Fat (ng/g) | | | | | | | |
| 3.09±1.25 | 136±12[**] | 390±38[**] | 633±28[**] | 3,699±292[**] | 8,698±650[**] | 14,140±550[**] | 10,936±822[**] |
| Liver (ng/g) | | | | | | | |
| 0.025±0.006 | 0.706±0.063[**] | 3.190±0.541[**] | 6.204±0.786[**] | 16.80±2.50[**] | 47.55±4.24[**] | 80.71±6.30[**] | 154.1±7.9[**] |
| Lung (ng/g) | | | | | | | |
| 0.056±0.006[b] | 0.692±0.389[*] | 0.792±0.311[**] | 0.350±0.115[**] | 3.551±0.532[**] | 20.45±6.91[**b] | 115.17±90.11[**b] | 48.46±13.52[**c] |

[**] Significantly different (P≤0.05) from the control group by Shirley's test
[*] P≤0.01
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n = 9
[c] n = 8

TCAB concentrations in core study male and female SD rats were determined in fat, liver, and lung 24 hours after the last oral TCAB dose at the end of the 13-week study. The highest concentrations of TCAB were observed in fat tissue with lower levels seen in the liver and lung (Table 6). TCAB concentrations were measurable in vehicle controls and increased in the dosed groups in a dose-dependent manner in all tissues. In fat tissue from dosed animals, mean TCAB concentrations increased from 160 to 19,380 ng/g in males and from 136 to 10,940 ng/g in females. Mean liver TCAB concentrations increased from 0.58 to 136 ng/g in dosed males and from 0.71 to 154 ng/g in dosed females. In lung, mean TCAB concentrations increased from 0.85 to 40.9 ng/g in males and from 0.69 to 115 ng/g in females. Tissue:fat ratios were calculated for liver and lung in both male and female rats based on the mean tissue concentrations for each dose group. Liver:fat ratios ranged from 0.00237 to 0.00699 in males and from 0.00454 to 0.0141 in females. Lung:adipose ratios ranged from 0.00038 to 0.00528 in males and from 0.00055 to 0.00814 in females.

At the end of the 3-month study, absolute and relative liver weights of all dosed groups of males and of females administered 10 mg/kg or greater were significantly greater than those of the vehicle controls (Tables 7 and F1). Absolute and relative lung weights were significantly greater in 100 mg/kg males and 3 mg/kg or greater females. Absolute and relative right kidney and spleen weights were generally significantly greater for all dosed groups of males. Absolute thymus weights of 10 mg/kg or greater males and absolute and relative thymus weights of 1 mg/kg or greater females were significantly less than those of the vehicle controls.

**TABLE 7**
**Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of TCAB[a]**

| | 0 mg/kg | 0.1 mg/kg | 0.3 mg/kg | 1 mg/kg | 3 mg/kg | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Male** | | | | | | | | |
| Necropsy body wt | 421±8 | 443±4 | 413±6 | 422±5 | 416±5 | 411±8 | 404±8 | 414±7 |
| R. Kidney | | | | | | | | |
| *Absolute* | 1.18±0.02 | 1.32±0.02* | 1.26±0.02* | 1.23±0.02* | 1.24±0.02* | 1.30±0.04** | 1.34±0.02** | 1.32±0.04** |
| *Relative* | 2.814±0.054 | 2.992±0.057* | 3.056±0.034* | 2.907±0.059 | 2.985±0.043 | 3.160±0.053** | 3.334±0.080** | 3.186±0.074** |
| Liver | | | | | | | | |
| *Absolute* | 13.44±0.38 | 15.59±0.34* | 14.17±0.28* | 14.57±0.29* | 14.90±0.31* | 16.79±0.57** | 17.87±0.48** | 18.97±0.70** |
| *Relative* | 31.898±0.689 | 35.232±0.739** | 34.264±0.458** | 34.502±0.386* | 35.811±0.537* | 40.742±0.731* | 44.202±0.684* | 45.687±1.250* |
| Lung | | | | | | | | |
| *Absolute* | 2.27±0.12 | 2.12±0.09 | 2.04±0.07 | 1.90±0.03 | 2.08±0.04 | 2.30±0.09 | 2.50±0.14 | 2.80±0.09** |
| *Relative* | 5.369±0.216 | 4.791±0.200 | 4.933±0.169 | 4.499±0.054 | 5.015±0.103 | 5.592±0.197 | 6.184±0.285** | 6.768±0.273** |
| Spleen | | | | | | | | |
| *Absolute* | 0.695±0.013 | 0.831±0.021* | 0.740±0.022* | 0.816±0.037** | 0.790±0.026** | 0.843±0.018** | 0.851±0.019** | 0.830±0.035** |
| *Relative* | 1.650±0.026 | 1.878±0.046* | 1.794±0.059* | 1.931±0.074** | 1.899±0.049** | 2.050±0.025** | 2.106±0.034** | 2.005±0.084** |
| Thymus | | | | | | | | |
| *Absolute* | 0.417±0.025 | 0.383±0.025 | 0.333±0.020 | 0.441±0.015 | 0.397±0.009 | 0.350±0.021* | 0.314±0.013** | 0.359±0.018** |
| *Relative* | 0.992±0.059 | 0.862±0.052 | 0.807±0.049* | 1.047±0.037 | 0.956±0.026 | 0.848±0.049 | 0.779±0.034** | 0.865±0.035 |
| **Female** | | | | | | | | |
| Necropsy body wt | 285±2 | 275±4 | 276±4 | 274±4 | 274±5 | 271±5* | 263±4** | 270±3** |
| Liver | | | | | | | | |
| *Absolute* | 9.01±0.25 | 8.72±0.28 | 9.95±0.36 | 9.40±0.21 | 9.73±0.32 | 10.60±0.24** | 11.17±0.27** | 11.93±0.22** |
| *Relative* | 31.596±0.805 | 31.756±0.879 | 35.974±1.038** | 34.331±0.528** | 35.500±0.650** | 39.158±0.617** | 42.395±0.713** | 44.262±0.879** |
| Lung | | | | | | | | |
| *Absolute* | 1.59±0.04 | 1.71±0.06 | 1.60±0.04 | 1.70±0.04 | 1.86±0.12* | 1.75±0.06* | 1.99±0.11** | 2.07±0.06** |
| *Relative* | 5.567±0.154 | 6.215±0.199 | 5.775±0.099 | 6.209±0.137 | 6.756±0.343** | 6.484±0.256** | 7.556±0.399** | 7.660±0.183** |
| Thymus | | | | | | | | |
| *Absolute* | 0.334±0.010 | 0.312±0.015 | 0.296±0.018 | 0.273±0.016* | 0.246±0.021** | 0.276±0.019** | 0.258±0.019** | 0.267±0.012** |
| *Relative* | 1.170±0.036 | 1.135±0.048 | 1.071±0.061 | 0.992±0.051* | 0.900±0.076* | 1.015±0.061* | 0.977±0.067* | 0.994±0.049* |

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

In the liver, the incidences of midzonal to diffuse hepatocytic hypertrophy in males administered 1 mg/kg or greater and in females administered 10 mg/kg or greater were significantly greater than the vehicle control incidences (Table 8). The hepatocytes were enlarged with increased cytoplasmic volume, clear cytoplasm, and perinuclear clumping of eosinophilic material presumed to be cellular organelles. Hematopoietic cell proliferation was significantly increased in 3 mg/kg or greater males and in 10 mg/kg or greater females. This change consisted of at least one cluster of hematopoietic cells, usually of erythrocytic series. Midzonal hepatocytic cytoplasmic fatty vacuolization occurred only in males; the incidences were significantly higher in the 3 mg/kg or greater males compared to the vehicle control incidence. The fatty change consisted of discrete clear vacuoles consistent with lipid in the cytoplasm of hepatocytes.

In the lung, significantly increased incidences of metaplasia of the alveolar epithelium occurred in 10, 30, and 100 mg/kg males (Table 8). This change was characterized by replacement of the normal alveolar epithelium with cuboidal to columnar, sometimes ciliated epithelium at the bronchiolar-alveolar junction. Increased incidences of interstitial mononuclear cell infiltration occurred in all dosed male groups, and the increases were significant at 10 mg/kg or greater. This lesion was also significantly increased in 100 mg/kg females. The lesion was characterized by the presence of interstitial, subpleural, and/or perivascular clusters of mononuclear cells, largely lymphocytes.

In the spleen of males, significantly increased incidences of hematopoietic cell proliferation, predominantly of the erythroid series, occurred at 10 mg/kg and greater (Table 8). In the spleen of females, pigmentation was significantly increased at 10 mg/kg and greater. This change was characterized by increased granular, golden, intrahistiocytic pigment presumed to be hemosiderin.

Atrophy in the thymus was significantly increased in all dosed groups of females, except the 0.1 mg/kg group, and in males administered 10 mg/kg or greater (Table 8). This change was characterized by decreased density of lymphocytes within the thymic cortex and/or decreased thickness of the thymic cortex relative to that of the medulla.

*Dose Selection Rationale:* There were no effects of TCAB exposure on mortality, and body weight effects were minimal. Histopathologic lesions consistent with the changes in organ weights were observed in the liver and thymus of males and females and the spleen and lung of males and were not considered life threatening. The average severity of these lesions was generally minimal. TCAB is approximately six to seven orders of magnitude less potent than TCDD using cytochrome P450 induction as the end point (Gerken *et al.,* 2002). A high dose of 100 mg/kg was selected for the 2-year study of TCAB in rats for a direct comparison to the 100 ng/kg dose of TCDD that produced hepatocellular carcinomas in female Harlan Sprague-Dawley rats (Kociba *et al.,* 1978). Doses of 0, 10, 30 and 100 mg/kg were selected for the 2-year study of TCAB in male and female Harlan Sprague-Dawley rats.

**TABLE 8**
**Incidences of Selected Nonneoplastic Lesions in Rats in the 3-Month Gavage Study of TCAB**

| | Vehicle Control | 0.1 mg/kg | 0.3 mg/kg | 1 mg/kg | 3 mg/kg | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|---|---|---|---|
| **Male** | | | | | | | | |
| Liver[a] | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Fatty Change[b] | 0 | 0 | 0 | 0 | 6** (1.2)[c] | 8** (1.0) | 10** (1.0) | 9** (1.3) |
| Hematopoietic Cell Proliferation | 2 (1.0) | 2 (1.0) | 2 (1.0) | 2 (1.0) | 9** (1.0) | 10** (1.0) | 10** (1.0) | 10** (1.0) |
| Hepatocyte, Hypertrophy | 0 | 0 | 2 (1.0) | 5* (1.2) | 7**(1.3) | 10** (1.5) | 10** (2.3) | 9** (2.4) |
| Lung | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 |
| Alveolar Epithelium, Metaplasia, Bronchiolar | 2 (1.0) | 0 | 3 (1.0) | 3 (1.0) | 3 (1.0) | 8* (1.0) | 8* (1.0) | 10** (1.0) |
| Interstitium, Infiltration Cellular, Mononuclear Cell | 1 (1.0) | 3 (1.0) | 3 (1.0) | 5 (1.0) | 5 (1.0) | 9** (1.0) | 10** (1.0) | 10** (1.0) |
| Spleen | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 |
| Hematopoietic Cell Proliferation | 2 (1.0) | 0 | 2 (1.5) | 3 (1.0) | 4 (1.0) | 9** (1.3) | 10** (1.5) | 8** (1.0) |
| Thymus | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 8 |
| Atrophy | 1 (1.0) | 1 (1.0) | 3 (1.0) | 1 (1.0) | 4 (1.0) | 7* (1.0) | 10** (1.2) | 6* (1.0) |
| **Female** | | | | | | | | |
| Liver | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematopoietic Cell Proliferation | 2 (1.0) | 3 (1.0) | 2 (1.0) | 3 (1.0) | 3 (1.3) | 9** (1.0) | 10** (1.0) | 10** (1.0) |
| Hepatocyte, Hypertrophy | 0 | 0 | 0 | 0 | 1 (1.0) | 6** (1.5) | 6** (1.3) | 5* (1.2) |
| Lung | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 10 |
| Interstitium, Infiltration Cellular, Mononuclear Cell | 0 | 1 (1.0) | 2 (1.0) | 2 (1.0) | 2 (1.0) | 0 | 2 (1.0) | 6** (1.2) |
| Spleen | 10 | 2 | 2 | 2 | 2 | 10 | 10 | 10 |
| Pigmentation, Hemosiderin | 0 | 0 | 1 (1.0) | 0 | 1 (1.0) | 4* (1.0) | 7** (1.0) | 7** (1.0) |
| Thymus | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Atrophy | 0 | 2 (1.0) | 8** (1.0) | 7** (1.0) | 6** (1.0) | 8** (1.0) | 9** (1.1) | 7** (1.3) |

* Significantly different ($P \leq 0.05$) from the vehicle controls by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

## *2-Year Study*

### Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 9 and in the Kaplan-Meier survival curves (Figure 2). The survival of all dosed groups of males was significantly less than that of the vehicle controls. However, survival of dosed female groups was similar to that of the vehicle controls. The primary cause of death in the 30 and 100 mg/kg groups was the presence of pulmonary cystic keratinizing epithelioma.

### Body Weights and Clinical Findings

Mean body weights of 100 mg/kg males were less than those of the vehicle control group throughout the study (Figure 3 and Table 10). Mean body weights of 30 mg/kg males were 6% less than those of the vehicle control group after week 24, and those of 10 mg/kg males were 7% less than the vehicle control group after week 80. Mean body weights of 100 mg/kg females were less than those of the vehicle control group throughout the study, and those of 30 mg/kg females were 6% less than the vehicle control group after week 36 (Figure 3 and Table 11).

**TABLE 9**
**Survival of Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Accidental deaths[a] | 1 | 0 | 1 | 1 |
| Moribund | 10 | 12 | 14 | 14 |
| Natural deaths | 11 | 29 | 31 | 33 |
| Animals surviving to study termination | 28 | 9 | 4 | 2 |
| Percent probability of survival at end of study[b] | 57 | 18 | 8 | 4 |
| Mean survival (days)[c] | 665 | 577 | 548 | 542 |
| Survival analysis[d] | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 |
| **Female** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Accidental death[a] | 1 | 0 | 2 | 0 |
| Moribund | 15 | 12 | 11 | 11 |
| Natural deaths | 9 | 8 | 19 | 22 |
| Animals surviving to study termination | 25 | 30 | 18 | 17 |
| Percent probability of survival at end of study | 51 | 60 | 40 | 34 |
| Mean survival (days) | 652 | 637 | 638 | 648 |
| Survival analysis | P = 0.085 | P = 0.687N | P = 0.390 | P = 0.196 |

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A lower mortality in a dosed group is indicated by N.

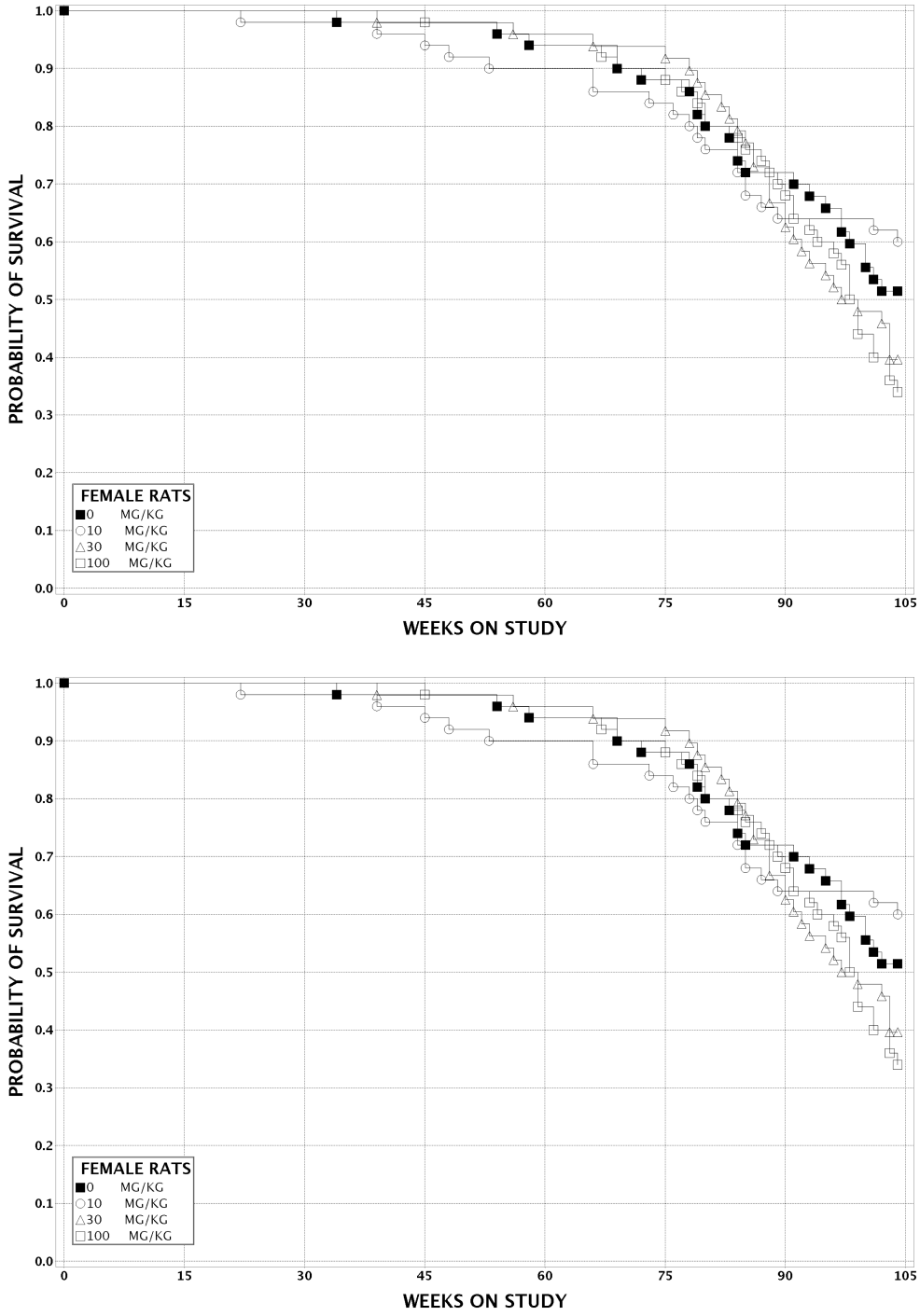
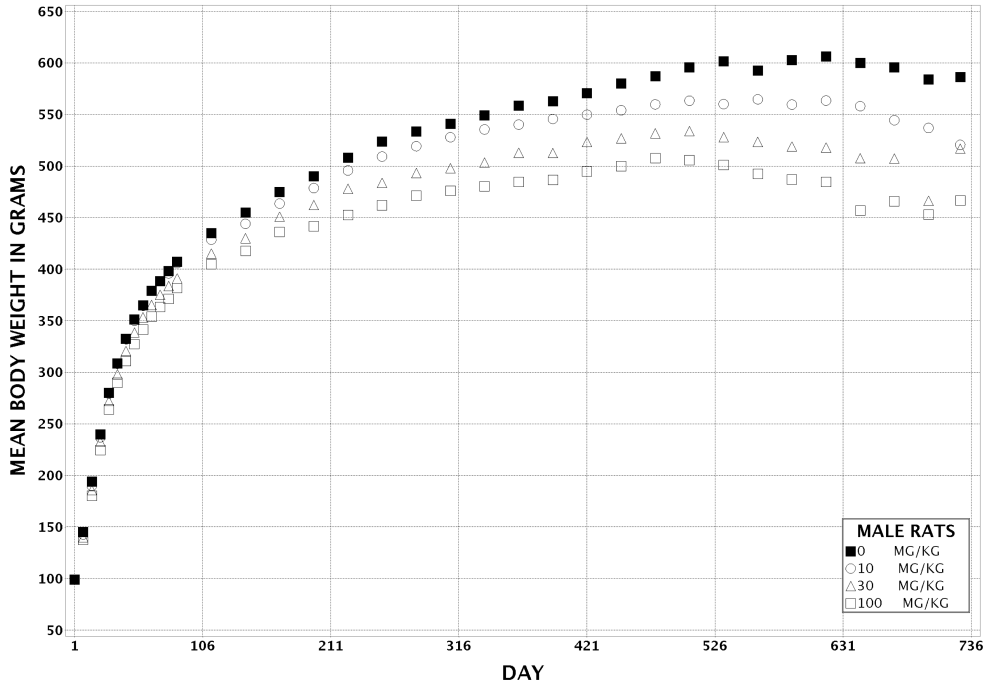



**FIGURE 2**
**Kaplan-Meier Survival Curves for Male and Female Rats Administered TCAB by Gavage for 2 Years**



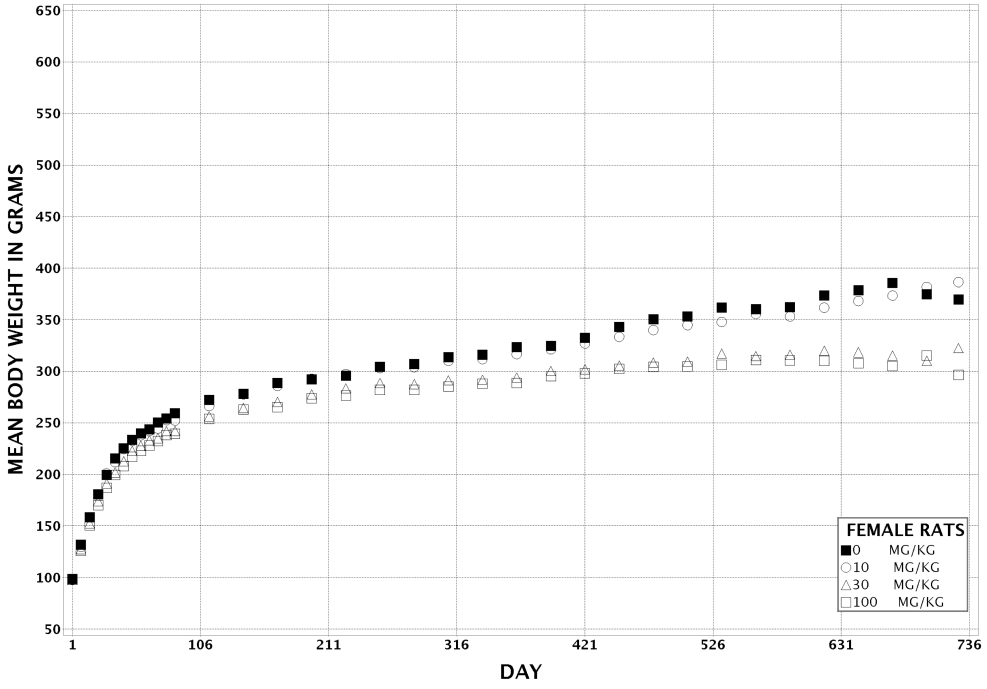




**FIGURE 3**
**Growth Curves for Male and Female Rats Administered TCAB by Gavage for 2 Years**

**TABLE 10**
**Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of TCAB**

| Days on Study | Vehicle Control | | 10 mg/kg | | | 30 mg/kg | | | 100 mg/kg | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Av. Wt. (g) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors |
| 1 | 99 | 50 | 99 | 100 | 50 | 99 | 100 | 50 | 99 | 100 | 50 |
| 8 | 145 | 50 | 143 | 98 | 50 | 140 | 96 | 50 | 138 | 95 | 50 |
| 15 | 194 | 50 | 190 | 98 | 50 | 186 | 96 | 50 | 180 | 93 | 50 |
| 22 | 240 | 50 | 237 | 99 | 50 | 233 | 97 | 50 | 225 | 94 | 50 |
| 29 | 280 | 50 | 279 | 100 | 50 | 272 | 97 | 50 | 264 | 94 | 50 |
| 36 | 309 | 50 | 308 | 100 | 50 | 298 | 97 | 50 | 290 | 94 | 50 |
| 43 | 333 | 50 | 332 | 100 | 50 | 321 | 96 | 49 | 311 | 93 | 50 |
| 50 | 351 | 50 | 350 | 100 | 50 | 339 | 96 | 49 | 327 | 93 | 50 |
| 57 | 365 | 50 | 365 | 100 | 50 | 353 | 97 | 49 | 342 | 94 | 50 |
| 64 | 379 | 50 | 379 | 100 | 49 | 366 | 96 | 49 | 354 | 93 | 50 |
| 71 | 389 | 50 | 388 | 100 | 49 | 375 | 97 | 49 | 364 | 94 | 50 |
| 78 | 398 | 50 | 396 | 99 | 49 | 384 | 96 | 49 | 371 | 93 | 50 |
| 85 | 407 | 50 | 406 | 100 | 49 | 391 | 96 | 49 | 382 | 94 | 50 |
| 113 | 435 | 50 | 429 | 99 | 49 | 415 | 95 | 49 | 405 | 93 | 50 |
| 141 | 455 | 50 | 444 | 98 | 49 | 430 | 95 | 48 | 418 | 92 | 50 |
| 169 | 475 | 50 | 464 | 98 | 49 | 451 | 95 | 48 | 436 | 92 | 50 |
| 197 | 490 | 50 | 479 | 98 | 48 | 462 | 94 | 48 | 442 | 90 | 48 |
| 225 | 508 | 50 | 496 | 98 | 48 | 478 | 94 | 48 | 453 | 89 | 47 |
| 253 | 524 | 50 | 509 | 97 | 48 | 484 | 92 | 48 | 462 | 88 | 47 |
| 281 | 534 | 49 | 519 | 97 | 48 | 493 | 92 | 46 | 471 | 88 | 45 |
| 309 | 541 | 49 | 528 | 98 | 48 | 498 | 92 | 44 | 476 | 88 | 45 |
| 337 | 549 | 49 | 536 | 98 | 47 | 504 | 92 | 44 | 480 | 87 | 45 |
| 365 | 559 | 49 | 540 | 97 | 47 | 513 | 92 | 44 | 485 | 87 | 44 |
| 393 | 563 | 49 | 546 | 97 | 46 | 513 | 91 | 43 | 487 | 86 | 43 |
| 421 | 571 | 47 | 550 | 96 | 46 | 523 | 92 | 43 | 495 | 87 | 42 |
| 449 | 580 | 47 | 554 | 96 | 45 | 527 | 91 | 41 | 500 | 86 | 40 |
| 477 | 587 | 47 | 560 | 95 | 42 | 532 | 91 | 37 | 508 | 87 | 39 |
| 505 | 596 | 45 | 564 | 95 | 38 | 534 | 90 | 34 | 506 | 85 | 34 |
| 533 | 602 | 44 | 560 | 93 | 33 | 528 | 88 | 33 | 501 | 83 | 31 |
| 561 | 593 | 43 | 565 | 95 | 28 | 524 | 88 | 31 | 492 | 83 | 27 |
| 589 | 603 | 41 | 560 | 93 | 26 | 519 | 86 | 22 | 487 | 81 | 20 |
| 617 | 606 | 39 | 564 | 93 | 22 | 518 | 85 | 16 | 485 | 80 | 16 |
| 645 | 600 | 36 | 558 | 93 | 17 | 508 | 85 | 16 | 457 | 76 | 11 |
| 673 | 596 | 33 | 545 | 91 | 15 | 508 | 85 | 9 | 466 | 78 | 5 |
| 701 | 584 | 31 | 537 | 92 | 10 | 467 | 80 | 7 | 453 | 78 | 4 |
| **Mean for weeks** | | | | | | | | | | | |
| 1–13 | 299 | | 298 | 100 | | 289 | 97 | | 281 | 94 | |
| 14–52 | 501 | | 489 | 98 | | 468 | 93 | | 449 | 90 | |
| 53–101 | 588 | | 554 | 94 | | 516 | 88 | | 486 | 83 | |

**TABLE 11**
**Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of TCAB**

| Days on Study | Vehicle Control | | 10 mg/kg | | | 30 mg/kg | | | 100 mg/kg | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Av. Wt. (g) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors |
| 1 | 99 | 50 | 98 | 100 | 50 | 98 | 99 | 50 | 98 | 99 | 50 |
| 8 | 132 | 50 | 130 | 99 | 50 | 128 | 97 | 50 | 126 | 96 | 50 |
| 15 | 159 | 50 | 158 | 100 | 50 | 153 | 96 | 50 | 151 | 95 | 50 |
| 22 | 181 | 50 | 181 | 100 | 50 | 174 | 96 | 50 | 170 | 94 | 50 |
| 29 | 200 | 50 | 201 | 101 | 50 | 191 | 96 | 50 | 187 | 94 | 50 |
| 36 | 216 | 50 | 212 | 98 | 50 | 202 | 94 | 50 | 200 | 93 | 50 |
| 43 | 225 | 50 | 225 | 100 | 50 | 213 | 95 | 50 | 208 | 92 | 50 |
| 50 | 234 | 50 | 233 | 100 | 50 | 223 | 96 | 50 | 217 | 93 | 50 |
| 57 | 240 | 50 | 238 | 99 | 50 | 228 | 95 | 50 | 223 | 93 | 50 |
| 64 | 244 | 50 | 242 | 99 | 50 | 233 | 96 | 50 | 228 | 94 | 50 |
| 71 | 250 | 50 | 245 | 98 | 50 | 235 | 94 | 50 | 232 | 93 | 50 |
| 78 | 255 | 50 | 253 | 99 | 50 | 242 | 95 | 50 | 238 | 94 | 50 |
| 85 | 259 | 50 | 252 | 97 | 50 | 242 | 93 | 50 | 239 | 92 | 50 |
| 113 | 272 | 50 | 266 | 98 | 50 | 256 | 94 | 50 | 254 | 93 | 50 |
| 141 | 278 | 50 | 278 | 100 | 50 | 265 | 95 | 50 | 263 | 95 | 50 |
| 169 | 289 | 50 | 286 | 99 | 49 | 270 | 94 | 50 | 265 | 92 | 50 |
| 197 | 292 | 50 | 293 | 100 | 49 | 278 | 95 | 50 | 274 | 94 | 50 |
| 225 | 296 | 50 | 297 | 101 | 49 | 284 | 96 | 50 | 277 | 94 | 50 |
| 253 | 304 | 49 | 303 | 100 | 49 | 289 | 95 | 49 | 282 | 93 | 50 |
| 281 | 307 | 49 | 304 | 99 | 48 | 288 | 94 | 48 | 282 | 92 | 50 |
| 309 | 314 | 49 | 310 | 99 | 47 | 291 | 93 | 48 | 285 | 91 | 50 |
| 337 | 316 | 49 | 312 | 99 | 46 | 292 | 92 | 48 | 288 | 91 | 49 |
| 365 | 323 | 49 | 317 | 98 | 46 | 294 | 91 | 48 | 289 | 89 | 49 |
| 393 | 325 | 48 | 321 | 99 | 45 | 300 | 92 | 47 | 296 | 91 | 48 |
| 421 | 333 | 47 | 327 | 98 | 45 | 302 | 91 | 47 | 298 | 90 | 47 |
| 449 | 343 | 47 | 334 | 97 | 45 | 306 | 89 | 46 | 302 | 88 | 47 |
| 477 | 351 | 47 | 340 | 97 | 43 | 309 | 88 | 45 | 304 | 87 | 46 |
| 505 | 353 | 44 | 345 | 98 | 43 | 310 | 88 | 45 | 305 | 86 | 45 |
| 533 | 362 | 44 | 348 | 96 | 41 | 317 | 88 | 44 | 306 | 85 | 44 |
| 561 | 360 | 40 | 356 | 99 | 38 | 315 | 87 | 41 | 311 | 86 | 40 |
| 589 | 362 | 37 | 353 | 98 | 34 | 316 | 87 | 38 | 311 | 86 | 39 |
| 617 | 374 | 35 | 362 | 97 | 33 | 320 | 86 | 32 | 310 | 83 | 35 |
| 645 | 379 | 34 | 368 | 97 | 32 | 319 | 84 | 28 | 308 | 81 | 32 |
| 673 | 386 | 32 | 374 | 97 | 32 | 316 | 82 | 24 | 305 | 79 | 29 |
| 701 | 375 | 27 | 382 | 102 | 32 | 310 | 83 | 23 | 315 | 84 | 22 |
| **Mean for weeks** | | | | | | | | | | | |
| 1–13 | 207 | | 205 | 99 | | 197 | 95 | | 194 | 94 | |
| 14–52 | 296 | | 294 | 99 | | 279 | 94 | | 274 | 93 | |
| 53–101 | 356 | | 348 | 98 | | 310 | 87 | | 305 | 86 | |

## Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of malignant schwannoma and neoplasms and/or nonneoplastic lesions of the lung, liver, oral mucosa, thyroid gland, forestomach, adrenal cortex, mammary gland, and nonneoplastic lesions of the tooth, pancreas, blood vessel, heart, bone marrow, spleen, lymph node (mesenteric), nose, testis, seminal vesicle, and ovary. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats. No historical data are available for male Harlan Sprague-Dawley rats; therefore, only historical data for females are presented in this Results section.

***Lung:*** Incidences of multiple cystic keratinizing epithelioma and single or multiple cystic keratinizing epithelioma (combined) in males and females occurred with positive trends and were significantly increased in all dosed groups (except multiple epithelioma in 10 mg/kg females) (Tables 12, A1, A2, B1, and B2). Significantly increased incidences of pigmentation, squamous metaplasia of the alveolar epithelium (except 10 mg/kg females), and bronchiolar metaplasia of the alveolar epithelium occurred in all dosed groups of males and females (Tables 12, A3, and B4). The incidences of histiocytic cellular infiltration in all dosed groups of males were significantly increased compared to the vehicle control incidence.

Cystic keratinizing epithelioma sometimes occurred singly but more often occurred as multiple lesions within the same lung, ranging from relatively small to very large lesions that replaced much of the normal lung parenchyma. Cystic structures consisted of a highly irregular wall of highly keratinized stratified squamous epithelium and a center filled with keratin. The outer portion of the lesion grew by expansion into the adjacent lung, but evidence of invasion was not observed (Plate 1); however, large lesions resulted in the death of male rats.

Pigmentation consisted of granular yellow-brown pigment within small, scattered clusters of alveolar histiocytes. Squamous metaplasia was generally a minor change consisting of one or more small, irregular foci of keratinizing stratified squamous epithelium that had replaced the normal alveolar epithelium (Plate 2). Bronchiolar metaplasia of the alveolar epithelium consisted of replacement of the normal alveolar epithelium by cuboidal to columnar, sometimes ciliated cells and was sometimes accompanied by mucus production in the affected area. The lesion generally diffusely affected the epithelium located at the bronchiolar-alveolar junction and adjacent alveoli. Alveolar histiocytes were present in lungs with bronchiolar metaplasia but were not associated with the areas of bronchiolar metaplasia, and alveolar histiocytes were present in lungs without bronchiolar metaplasia. Histiocytic cellular infiltration consisted of scattered individual alveolar histiocytes or clusters of alveolar histiocytes (Plate 3).

***Liver:*** The incidences of cholangiocarcinoma (single or multiple) occurred with a positive trend in males, and the incidences in all dosed groups were significantly greater than that in the vehicle control group; the incidence in 100 mg/kg females was also increased (Tables 13, A1, A2, B1, and B2). Only one carcinoma has been observed in 473 historical control female Harlan Sprague-Dawley rats from 2-year corn oil gavage studies. Cholangiocarcinoma consisted of an irregular, relatively large, noncircumscribed lesion that replaced normal liver parenchyma. The lesion consisted of fibrous connective tissue stroma containing numerous atypical bile ducts, which frequently contained mucinous material and cellular debris. The epithelium forming the atypical bile duct was often discontinuous, usually consisting of large atypical cells and displaying degenerative changes. Mitotic figures and localized invasion of adjacent liver parenchyma were also observed (Plates 4 and 5).

A single incidence of multiple hepatocholangiocarcinoma occurred in a 100 mg/kg male (Tables 13 and A1). Hepatocholangiocarcinoma was a distinct mass nearly one centimeter in diameter consisting primarily of irregularly arranged cords and broad trabeculae of densely packed, small to normal sized, slightly pleomorphic hepatocytes with vesicular nuclei, generally containing a prominent nucleolus, and small to moderate amounts of pale eosinophilic cytoplasm. In some areas at the periphery, the neoplastic hepatocytes infiltrated between and replaced the normal hepatocytes. A few nodular structures composed of broad trabeculae of moderate to extremely enlarged hepatocytes with abundant eosinophilic cytoplasm were scattered within the neoplasm. The center of the neoplasm contained an irregular area of dense fibrous connective tissue containing numerous scattered small biliary structures composed of large biliary epithelial cells with large vesicular nuclei and a small amount of clear cytoplasm. Numerous nar-

**TABLE 12**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Pigmentation[a] | 3 (1.0)[b] | 16** (1.3) | 12** (1.3) | 16** (1.1) |
| Alveolar Epithelium, Metaplasia, Squamous | 0 | 14** (2.0) | 22** (1.9) | 22** (3.0) |
| Alveolar Epithelium, Metaplasia, Bronchiolar | 1 (1.0) | 32** (2.0) | 32** (1.9) | 34** (2.1) |
| Alveolus, Infiltration Cellular, Histiocyte | 23 (1.3) | 34** (1.8) | 35** (1.8) | 35** (2.0) |
| Cystic Keratinizing Epithelioma, Multiple | 0 | 8** | 26** | 24** |
| Cystic Keratinizing Epithelioma (includes multiple) | | | | |
| Overall rate[c] | 0/50 (0%) | 14/50 (28%) | 31/50 (62%) | 37/50 (74%) |
| Adjusted rate[d] | 0.0% | 44.5% | 85.9% | 92.7% |
| Terminal rate[e] | 0/28 (0%) | 4/9 (44%) | 4/4 (100%) | 2/2 (100%) |
| First incidence (days) | —[f] | 576 | 367 | 192 |
| Poly-3 test[g] | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 |
| **Female** | | | | |
| Number Examined Microscopically | 50 | 50 | 49 | 49 |
| Pigmentation | 1 (1.0) | 11** (1.0) | 21** (1.4) | 26** (1.1) |
| Alveolar Epithelium, Metaplasia, Squamous | 2 (1.5) | 4 (2.5) | 18** (2.8) | 30** (2.6) |
| Alveolar Epithelium, Metaplasia, Bronchiolar | 0 | 21** (1.4) | 26** (1.5) | 35** (1.8) |
| Cystic Keratinizing Epithelioma, Multiple | 0 | 2 | 19** | 35** |
| Cystic Keratinizing Epithelioma (includes multiple)[h] | | | | |
| Overall rate | 0/50 (0%) | 6/50 (12%) | 26/49 (53%) | 39/49 (80%) |
| Adjusted rate | 0.0% | 15.5% | 62.6% | 89.5% |
| Terminal rate | 0/25 (0%) | 4/30 (13%) | 9/19 (47%) | 17/17 (100%) |
| First incidence (days) | — | 589 | 540 | 481 |
| Poly-3 test | P < 0.001 | P = 0.014 | P < 0.001 | P < 0.001 |

** Significantly different (P≤0.01) from the vehicle control group by the Poly-3 test
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Number of animals with neoplasm per number of animals with lung examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Not applicable; no neoplasms in animal group
[g] Beneath the vehicle 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 vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical control incidence for 2-year corn oil gavage studies in Harlan Sprague-Dawley rats: 0/471

row bands of dense fibrous tissue containing biliary structures extended outward from the fibrous center of the neoplasm among the neoplastic hepatocytes.

In males and females, there were generally dose-related increased incidences of hepatocellular hypertrophy, centrilobular degeneration, hepatocellular necrosis, and pigmentation; except for centrilobular degeneration and necrosis in 10 mg/kg females and pigmentation in 10 mg/kg males, the incidences in the dosed groups were significantly greater than the incidences in the vehicle control groups (Tables 13, A3, and B4). The incidences of focal fatty change in 30 mg/kg males and 100 mg/kg females were significantly greater than the incidences in the vehicle control groups. The incidences of diffuse fatty change in all treated males and 30 and 100 mg/kg females were significantly greater than those in the vehicle control groups. The severity of these lesions generally increased with increasing dose. The incidences of oval cell hyperplasia in all dosed groups were significantly increased, and there were dose-related increases in severity. The incidences of bile duct hyperplasia in all dosed female groups were significantly increased, and the severities increased with increasing dose in males and females. Incidences of hematopoietic cell proliferation were significantly increased in all dosed groups except 30 mg/kg females. The incidences of nodular hyperplasia were significantly increased in 30 and 100 mg/kg females. The incidences of toxic hepatopathy increased with increasing dose in males and females, and the severity increased with increasing dose in females. The incidences in 30 and 100 mg/kg males and females were significantly greater than the vehicle control incidences.

The incidences of eosinophilic focus increased with increasing dose in females and were significantly greater than the vehicle control incidences in all dosed groups of females and in 10 and 100 mg/kg males (Tables 13, A3, and B4). The incidences of mixed cell focus in all female dosed groups were also significantly increased. Dose-related decreases occurred in the incidences of clear cell focus in males.

Additionally, the incidences of cystic bile duct in 100 mg/kg males and females and multinucleated hepatocytes and cholangiofibrosis in 100 mg/kg females were significantly increased (Tables 13, A3, and B4).

Hepatocellular hypertrophy was characterized by enlarged hepatocytes with increased amounts of eosinophilic cytoplasm. As severity increased, greater proportions of the hepatic lobules were affected. Centrilobular degeneration consisted of the loss of hepatocytes within centrilobular areas with remaining hepatocytes appearing either atrophied (small with decreased cytoplasm), vacuolated, or undergoing individual cell necrosis and often caused centrilobular sinusoids to become enlarged and more prominent. Necrosis consisted of scattered necrotic areas of hepatic parenchyma that were often randomly distributed but, in more severe cases, were distributed more diffusely. Pigmentation consisted of light brown to golden pigment present within macrophages. The pigmented macrophages were often seen in portal areas but were also seen randomly scattered within the liver. Focal or diffuse fatty change were generally minor changes, consisting of discrete clear vacuoles (consistent with lipid) in the cytoplasm of hepatocytes that involved foci of hepatocytes (focal fatty change) or were scattered diffusely throughout the liver (diffuse fatty change). Bile duct hyperplasia consisted of increased numbers of portal bile ducts. Oval cell hyperplasia consisted of small ovoid cells, with basophilic cytoplasm and a round to ovoid nucleus, arranged in single or double rows and located predominantly in the portal areas.

Nodular hyperplasia was characterized by small to large nodular foci generally composed of hepatocytes that were considerably larger than normal hepatocytes (hepatocyte hypertrophy) sometimes mixed with areas of increased numbers of small hepatocytes (hepatocyte hyperplasia). Areas of nodular hyperplasia sometimes blended with the surrounding parenchyma, although they often had a distinct border. Large, focal to multifocal areas of nodular hyperplasia were sometimes seen that caused compression of surrounding tissue, and/or bulging of the capsular surface. The cells within nodular hyperplasia were generally very large, larger than cells seen within adenomas and usually larger than cells seen within foci, with abundant eosinophilic cytoplasm and often with variable degrees of cytoplasmic vacuolization. In a few areas of nodular hyperplasia, the cells were of more normal size or slightly smaller than normal. The cells appeared to be arranged in normal cords, but the cells were so large they obscured the sinusoids between the cords, giving the appearance of solid sheets of hepatocytes. Bile duct hyperplasia and portal areas were usually present within nodular hyperplasia. Blood vessels and/or central veins were sometimes seen within areas of nodular hyperplasia, usually when hepatocytes were not so hypertrophic as to completely obscure the normal architecture. The presence of hypertrophic, vacuolated hepatocytes together with proliferating bile ducts

**TABLE 13**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Hepatocyte, Hypertrophy[a] | 0 | 6** (2.3)[b] | 11** (2.7) | 22** (2.5) |
| Centrilobular, Degeneration | 0 | 10** (1.4) | 23** (1.4) | 24** (1.8) |
| Necrosis | 1 (1.0) | 7* (2.6) | 18** (1.9) | 21** (1.8) |
| Pigmentation | 1 (1.0) | 4 (1.5) | 5* (1.8) | 6* (1.2) |
| Fatty Change, Focal | 1 (1.0) | 2 (1.0) | 7** (1.1) | 3 (1.7) |
| Fatty Change, Diffuse | 3 (1.3) | 9* (1.7) | 18** (1.9) | 34** (1.6) |
| Bile Duct, Hyperplasia | 34 (1.4) | 38 (1.6) | 36 (1.7) | 29 (2.0) |
| Oval Cell, Hyperplasia | 0 | 4* (1.0) | 8** (1.6) | 5* (1.8) |
| Hematopoietic Cell Proliferation | 5 (1.4) | 40** (1.2) | 37** (1.3) | 30** (1.2) |
| Toxic Hepatopathy | 0 | 0 | 5** (1.6) | 8** (1.1) |
| Eosinophilic Focus | 3 | 9* | 4 | 12** |
| Clear Cell Focus | 32 | 12** | 8** | 4** |
| Bile Duct, Cyst | 0 | 0 | 1 (3.0) | 4* (1.5) |
| Cholangiofibrosis | 1 (2.0) | 3 (2.7) | 2 (2.5) | 0 |
| Hepatocholangiocarcinoma, Multiple | 0 | 0 | 0 | 1 |
| Cholangiocarcinoma, Multiple | 0 | 1 | 0 | 1 |
| Cholangiocarcinoma (includes multiple) | | | | |
| Overall rate[c] | 0/50 (0%) | 4/50 (8%) | 4/50 (8%) | 6/50 (12%) |
| Adjusted rate[d] | 0.0% | 13.3% | 14.3% | 22.6% |
| Terminal rate[e] | 0/28 (0%) | 1/9 (11%) | 0/4 (0%) | 1/2 (50%) |
| First incidence (days) | —[f] | 454 | 487 | 529 |
| Poly-3 test[g] | P = 0.007 | P = 0.030 | P = 0.026 | P = 0.003 |

**TABLE 13**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Female** | | | | |
| Number Examined Microscopically | 50 | 50 | 49 | 49 |
| Hepatocyte, Hypertrophy | 4 (1.5) | 33** (1.8) | 38** (2.3) | 42** (2.6) |
| Centrilobular, Degeneration | 1 (2.0) | 2 (1.0) | 18** (1.8) | 17** (1.5) |
| Necrosis | 3 (2.7) | 6 (1.5) | 10* (1.4) | 10* (2.2) |
| Pigmentation | 1 (3.0) | 17** (1.2) | 32** (1.6) | 40** (2.1) |
| Fatty Change, Focal | 2 (1.0) | 2 (1.0) | 2 (1.0) | 9* (1.3) |
| Fatty Change, Diffuse | 0 | 3 (1.0) | 10** (1.3) | 10** (1.6) |
| Bile Duct, Hyperplasia | 12 (1.1) | 26** (1.2) | 29** (1.6) | 38** (1.8) |
| Oval Cell, Hyperplasia | 0 | 7** (1.0) | 24** (1.3) | 36** (1.8) |
| Hyperplasia, Nodular | 1 | 3 | 11** | 22** |
| Hematopoietic Cell Proliferation | 28 (1.1) | 42** (1.1) | 32 (1.1) | 37* (1.2) |
| Toxic Hepatopathy | 0 | 4 (1.0) | 14** (1.5) | 25** (2.2) |
| Eosinophilic Focus | 3 | 27** | 31** | 38** |
| Mixed Cell Focus | 6 | 16** | 14* | 16** |
| Bile Duct, Cyst | 3 (1.3) | 4 (1.8) | 5 (1.4) | 12** (1.6) |
| Hepatocyte, Multinucleated | 0 | 2 (1.0) | 1 (1.0) | 28** (1.2) |
| Cholangiofibrosis | 0 | 1 (1.0) | 0 | 11** (1.6) |
| Cholangiocarcinoma[h] | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 1/49 (2%) | 3/49 (6%) |
| Adjusted rate | 2.6% | 2.6% | 2.7% | 7.9% |
| Terminal rate | 0/25 (0%) | 0/30 (0%) | 1/19 (5%) | 0/17 (0%) |
| First incidence (days) | 684 | 722 | 727 (T) | 658 |
| Poly-3 test | P = 0.153 | P = 0.756 | P = 0.747 | P = 0.295 |

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Number of animals with neoplasm per number of animals with liver examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Not applicable; no neoplasms in animal group
[g] Beneath the vehicle 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 vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical control incidence for 2-year corn oil gavage studies in Harlan Sprague-Dawley rats (mean ± standard deviation): 1/473 (0.2% ± 0.7%), range 0%-2%

were considered to be useful in differentiating from hepatocellular adenoma. In those incidences in which the hepatocytes were more normally sized, the presence of proliferating bile ducts served to differentiate nodular hyperplasia from focus or adenoma. In the current study, many of the foci of nodular hyperplasia were relatively small, particularly in the higher doses, but could be identified as nodular hyperplasia by the presence of small numbers of proliferating bile ducts. Nodular hyperplasia was seen most often in the higher dose groups where toxic changes were more prominent. However, a less severe degree of nodular hyperplasia was sometimes seen in lower dose animals in which toxic changes were minimal to unapparent; this change was not graded. Hematopoietic cell proliferation was characterized by variable numbers of scattered clusters of small, deeply basophilic hematopoietic cells that appeared to be mainly erythrocytic cells.

Toxic hepatopathy was diagnosed as a separate term and was used in addition to, not instead of, any of the individual nonneoplastic diagnoses already made for the liver to give an overall assessment of the degree of toxicity in the liver. This was to allow for easier comparison of both the incidence and severity of toxic changes among different dosed groups than would be possible if all the individual nonneoplastic changes were compared among the different groups. The changes evaluated under this diagnosis included foci of cellular alteration, multinucleated hepatocytes, fatty change, necrosis, pigmentation, nodular hyperplasia, bile duct cysts, bile duct hyperplasia, centrilobular hepatocyte degeneration, hepatocyte hypertrophy, and oval cell hyperplasia. Some dosed animals occasionally had a few of these changes present but this was not considered to be sufficient liver involvement to warrant a diagnosis of toxic hepatopathy. For example, when only findings of hepatocyte hypertrophy, pigmentation, and slight fatty change were present, no diagnosis was made. Minimal toxic hepatopathy was diagnosed when additional changes indicative of a toxic effect, usually a slight degree of bile duct and/or oval cell hyperplasia, sometimes a few large prominent altered hepatocellular foci, and occasionally a small focus of cholangiofibrosis, were present. Mild toxic hepatopathy was characterized by the presence of multiple toxic changes, all of which were of minimal to mild severity. In addition, multiple prominent altered hepatocellular foci and occasional foci of nodular hyperplasia were sometimes present. Moderate toxic hepatopathy was diagnosed when the entire or nearly the entire spectrum of toxic changes was present, generally with some degree of distortion of the normal liver structure caused by prominent altered hepatocellular foci, nodular hyperplasia, and possible cholangiofibrosis. Marked toxic hepatopathy was diagnosed when severe toxic changes were present with pronounced distortion of the liver architecture; livers with marked toxic hepatopathy often had a multinodular appearance due to the presence of numerous large foci of nodular hyperplasia that replaced much of the liver parenchyma.

Eosinophilic, mixed, and clear cell foci were characterized by a focus of hepatocytes with altered tinctorial properties. Eosinophilic focus was characterized by a focus of hepatocytes composed principally of cells with eosinophilic cytoplasm. Mixed cell focus was composed of a mixture of cells with different staining properties, generally a mixture of eosinophilic cells and clear cytoplasm (clear cells). To be classified as an eosinophilic focus, at least 80% of the cells within the focus had to be eosinophilic cells, otherwise the focus was classified as a mixed cell focus. Clear cell focus was composed of cells having clear cytoplasm. The margins of the focus were distinct, but the hepatic cords often merged imperceptibly with the surrounding hepatic cords. Some foci had a more definite border and the cords within the focus were not always smoothly continuous with those in the surrounding parenchyma. In addition, some larger foci caused variable degrees of compression of the surrounding hepatic parenchyma. Hepatocytes within foci were generally somewhat larger than normal but otherwise appeared normal. The cells were arranged in a relatively normal lobular pattern and foci sometimes contained blood vessels and/or portal areas. The presence of proliferating bile ducts or oval cells, however, was not considered characteristic of a focus. If two or more foci of a given type were present in a liver, it was diagnosed as multiple.

Bile duct cyst was characterized by either single or multiple dilated bile ducts that were lined by attenuated epithelium. Multinucleated hepatocytes were characterized by scattered hepatocytes that were enlarged and contained multiple nuclei (more than 2 and often 4 to 6). The presence of binucleated hepatocytes was not sufficient to make this diagnosis. Cholangiofibrosis was characterized by atypical ducts surrounded by abundant connective tissue. The ducts were often irregular,

dilated, and contained mucinous material and cellular debris.

***Oral Mucosa:*** Increased incidences of gingival squamous cell carcinoma occurred in dosed males and 30 and 100 mg/kg females; the increases in 10 mg/kg males and 100 mg/kg males and females were significant and increases in 30 mg/kg females exceeded the historical control range (Tables 14, A1, A2, B1, B2, and B3c). Incidences of gingival squamous cell hyperplasia and cystic keratinizing hyperplasia in male and female dosed groups were significantly increased compared to those of the vehicle control groups (except cystic keratinizing hyperplasia in 10 mg/kg females) (Tables 14, A3, and B4).

Squamous cell carcinoma within the oral mucosa adjacent to the molar tooth in nasal Section III was characterized by irregular cords and clusters of stratified squamous epithelial cells that invaded deep into the underlying connective tissue, usually accompanied by abundant fibrous tissue proliferation (Plate 6).

Squamous hyperplasia occurred in the stratified squamous epithelium of the gingival oral mucosa adjacent to the molar teeth in nasal Section III, and consisted of varying degrees of thickening of the epithelium, generally with the formation of epithelial rete pegs that extended into the underlying connective tissue. Minimal lesions located directly adjacent to the molar teeth consisted of a slight thickening of the epithelium. As severity increased, the thickening of the epithelium increased, usually with the formation of multiple papillary projections accompanied by abundant keratin accumulation and prominent rete pegs. In the more severe cases of hyperplasia, the hyperplastic epithelium formed prominent invaginations into the underlying palate, displacing normal tissue and somewhat resembling invasive lesions (Plate 6). In some sections containing the root of a molar tooth the hyperplastic epithelium tended to grow down along the root sending out rete pegs into the adjacent tissue. Some sections with hyperplasia had apparently isolated islands of epithelial cells beneath the hyperplasia epithelium on the surface; these islands were considered to be the ends of rete pegs extending from hyperplastic epithelium that ran along the root of the molar but out of the plane of the section. Sometimes, as the hyperplastic epithelium grew down along the root, it separated from the tooth leaving a narrow, elongated cavity between the tooth and the hyperplastic epithelium. Ends of hair shafts and/or some degree of inflammation were often present in the areas of squamous hyperplasia, commonly within the cavities seen with some hyperplastic lesions; in these cases the inflammation appeared to be secondary to the presence of the hair shafts.

Cystic keratinizing hyperplasia also occurred adjacent to the molar teeth in Section III and was characterized by variably sized cavities lined by stratified squamous epithelium and filled with keratin located deep in the lumenal surface. Minimal lesions consisted of one to few small, ovoid cavities lined by a thin layer of epithelium and containing small amounts of keratin, lying within the connective tissue of the palate a short distance below the oral lumenal surface. Some occurred in isolation while others occurred in association with diffuse hyperplastic lesions more typical of squamous hyperplasia and appeared to arise from the more diffuse hyperplasia. As severity increased, the size of the cavities and the depth of penetration into underlying tissues increased. In the case of moderate to severe lesions, the cystic structures penetrated deep into surrounding tissues, including the nasal cavity and the soft tissues attached to the maxilla (Plate 7). The lining of more severe lesions often tended to have areas of irregular thickening and formed multiple, adjacent cystic structures, giving the lesion a multilocular appearance. Generally, a connection could be seen between the cystic structure and the overlying gingival oral mucosa, suggesting the cystic keratinizing hyperplasias may have arisen from squamous hyperplasia. In some incidences of severe cystic keratinizing hyperplasias with penetration into the nasal cavity, variably sized islands and keratin pearls of stratified squamous epithelium, embedded within dense fibrous tissue, lay deep into the cystic structure and were indicative of invasion. These lesions were diagnosed as squamous cell carcinoma. Thus, it appears that squamous cell carcinoma may arise from cystic keratinizing hyperplasia.

***Tooth:*** Significantly increased incidences of inflammation of the periodontal tissue occurred in 30 and 100 mg/kg males and 30 mg/kg females (Tables 14, A3, and B4). Inflammation in the periodontal tissue around the incisor teeth in nasal Section III consisted of an infiltrate of small to moderate numbers of mixed inflammatory cells, mainly lymphocytes mixed with a few neutrophils. Hair shafts were often present between the periodontal tissue and tooth suggesting that the inflammation may have been related to the presence of hair shafts.

**TABLE 14**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Oral Cavity in Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Oral Mucosa[a] | 50 | 50 | 50 | 50 |
| Gingival, Hyperplasia, Squamous[b] | 2 (1.0)[c] | 21** (1.7) | 24** (1.8) | 31** (1.8) |
| Gingival, Hyperplasia, Cystic Keratinizing | 0 | 4* (1.1) | 18** (0.9) | 11** (1.4) |
| Gingival, Squamous Cell Carcinoma, Multiple | 0 | 1 | 0 | 0 |
| Gingival, Squamous Cell Carcinoma (includes multiple) | | | | |
| Overall rate[d] | 1/50 (2%) | 5/50 (10%) | 4/50 (8%) | 5/50 (10%) |
| Adjusted rate[e] | 2.5% | 16.7% | 14.5% | 18.8% |
| Terminal rate[f] | 0/28 (0%) | 2/9 (22%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | 694 | 504 | 474 | 524 |
| Poly-3 test[g] | P = 0.065 | P = 0.046 | P = 0.085 | P = 0.033 |
| Tooth | 50 | 50 | 50 | 50 |
| Periodontal Tissue, Inflammation | 11 (1.4) | 12 (1.3) | 19** (1.4) | 16** (1.4) |
| **Female** | | | | |
| Oral Mucosa | 50 | 50 | 50 | 50 |
| Gingival, Hyperplasia, Squamous | 0 | 8** (1.8) | 24** (1.4) | 24** (1.4) |
| Gingival, Hyperplasia, Cystic Keratinizing | 0 | 4 (1.0) | 9** (1.1) | 13** (1.3) |
| Gingival, Squamous Cell Carcinoma, Multiple | 0 | 0 | 0 | 1 |
| Gingival, Squamous Cell Carcinoma (includes multiple)[h] | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 4/50 (8%) | 6/50 (12%) |
| Adjusted rate | 0.0% | 0.0% | 10.6% | 15.4% |
| Terminal rate | 0/25 (0%) | 0/30 (0%) | 1/19 (5%) | 1/17 (6%) |
| First incidence (days) | —[i] | — | 574 | 551 |
| Poly-3 test | P = 0.002 | —[j] | P = 0.055 | P = 0.015 |
| Tooth | 50 | 50 | 50 | 50 |
| Periodontal Tissue, Inflammation | 5 (1.2) | 10 (1.2) | 14** (1.1) | 9 (1.4) |

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals necropsied
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[d] Number of animals with neoplasm per number of animals necropsied
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle 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 vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical control incidence for 2-year corn oil gavage studies in Harlan Sprague-Dawley rats (mean ± standard deviation): 4/473 (0.8% ± 1.0%), range 0%-2%
[i] Not applicable; no neoplasm in animal group
[j] Value of the statistic cannot be computed

***Thyroid Gland:*** The incidences of follicular cell adenoma (single or multiple) in 30 and 100 mg/kg males were significantly greater than that in the vehicle control group; one 100 mg/kg female also had this neoplasm (Tables 15, A1, A2, and B1). The incidences of follicular cell hypertrophy, follicular cell hyperplasia, and inflammation were significantly increased in 30 and 100 mg/kg males (Tables 15 and A3).

Adenoma was a discrete, well demarcated mass composed of abnormal, densely packed follicular structures containing follicular cells that ranged from small cells similar to those seen in hyperplasia to large cells similar to those seen in hypertrophy.

The glands affected with follicular cell hyperplasia or follicular cell hypertrophy were diffusely involved and usually enlarged, sometimes markedly. Follicular cell hyperplasia in most thyroid glands consisted of very large numbers of small to moderate sized, densely packed follicles lined by cuboidal to low columnar epithelium (Plates 8 and 9). Follicular cell hypertrophy consisted of follicles composed of larger than normal cells with abundant cytoplasm and enlarged nuclei. When affected glands had a mixture of hyperplastic and hypertrophic follicles, both lesions were diagnosed. Inflammation was characterized by diffusely scattered aggregates of moderate to large numbers of lymphocytes within the interstitium between follicles.

**TABLE 15**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Follicular Cell, Hypertrophy[a] | 2 (2.0)[b] | 2 (4.0) | 6* (2.7) | 6* (3.2) |
| Follicular Cell, Hyperplasia | 0 | 2 (4.0) | 10** (3.5) | 12** (3.6) |
| Inflammation | 0 | 3 (3.0) | 9** (2.8) | 14** (2.6) |
| Follicular Cell Adenoma, Multiple | 0 | 1 | 1 | 1 |
| Follicular Cell Adenoma (includes multiple) | | | | |
| Overall rate[c] | 0/50 (0%) | 3/50 (6%) | 4/50 (8%) | 4/50 (8%) |
| Adjusted rate[d] | 0.0% | 10.2% | 14.5% | 15.7% |
| Terminal rate[e] | 0/28 (0%) | 1/9 (11%) | 1/4 (25%) | 0/2 (0%) |
| First incidence (days) | —[f] | 635 | 491 | 476 |
| Poly-3 test[g] | P = 0.037 | P = 0.070 | P = 0.025 | P = 0.021 |
| **Female** | | | | |
| Number Examined Microscopically | 50 | 49 | 50 | 50 |
| Follicular Cell, Hypertrophy | 0 | 1 (2.0) | 2 (1.5) | 4 (2.0) |
| Follicular Cell, Hyperplasia | 0 | 1 (2.0) | 3 (3.0) | 3 (3.3) |
| Inflammation | 0 | 0 | 2 (3.0) | 4 (2.3) |
| Follicular Cell Adenoma[h] | 0 | 0 | 0 | 1 |

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Number of animals with neoplasm per number of animals with thyroid gland examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Not applicable; no neoplasms in animal group
[g] Beneath the vehicle 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 vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[h] Historical control incidence for 2-year corn oil gavage studies in Harlan Sprague-Dawley rats (mean ± standard deviation): 2/468 (0.4% ± 0.9%), range 0%-2%

***Forestomach:*** Three single or multiple squamous cell papillomas occurred in 100 mg/kg females, and single incidences of squamous cell carcinoma occurred in 10 mg/kg males and females and 100 mg/kg females (Tables 16, A1, B1, and B2). Dose-related significantly increased incidences and increased severities of epithelial hyperplasia occurred in males and females (Tables 16, A3, and B4). The incidence of inflammation was significantly increased in 100 mg/kg females.

Squamous cell papilloma was characterized by a polypoid or cauliflower-shaped mass on the mucosal surface; the attachment to the mucosa was generally a narrow stalk. There was generally an orderly maturation of the epithelium. Squamous cell carcinomas were characterized by areas of hyperplastic stratified squamous epithelium with small islands of epithelial cells scattered in the connective tissue deep into the neoplasm, indicative of invasion.

Epithelial hyperplasia was usually located at the limiting ridge and consisted of irregular, papillary thickening of the epithelium due to an increase in the number of cell layers, accompanied by an increase in the overlying keratin layer (Plates 10 and 11). In addition to epithelial hyperplasia at the limiting ridge, two animals had a cystic structure lined by a thin layer of stratified squamous epithelium and filled with keratin lying beneath the area of hyperplasia.

***Adrenal Cortex:*** There were slightly increased incidences of adenoma in 30 and 100 mg/kg females; these incidences exceeded the historical control range (Tables 17, B1, B2, and B3f). Incidences of degeneration, cytoplasmic vacuolization, and hyperplasia of the zona fasciculata in all dosed groups of males were significantly greater than those of the vehicle controls (Tables 17 and A3). Increased incidences of necrosis occurred in 30 and 100 mg/kg males, and the severity increased with increasing dose. Incidences of cytoplasmic vacuolation of the zona fasciculata in 10 and 100 mg/kg females and hyperplasia of the zona fasciculata and necrosis in 30 mg/kg females were significantly greater than those of the vehicle controls (Tables 17 and B4). While these lesions were generally increased in dosed females, the effect was not as strong as in males.

Adenoma was a large, discrete lesion with a well demarcated border that replaced glandular parenchyma and caused compression of the remaining normal tissue. Adenoma was distinguished from hypertrophy or hyperplasia by somewhat atypical cortical cells that were arranged in abnormal patterns, rather than normal appearing cells arranged in the normal cord pattern as with hypertrophy and hyperplasia.

Degeneration was a focal to multifocal, unilateral to bilateral lesion consisting of variably sized endothelial-lined spaces, usually containing blood and occasionally thrombi, located in the zona fasciculata and reticularis; larger lesions compressed or replaced adjacent parenchyma. Some lesions were very large, replaced much of the gland, and caused enlargement of the gland. Cytoplasmic vacuolization was a focal to multifocal to diffuse change consisting of small, discrete, clear intracytoplasmic vacuoles. The cytoplasm sometimes contained a large single vacuole that displaced the nucleus; the changes were morphologically consistent with the accumulation of lipid. Hyperplasia of the zona fasciculata was a focal to multifocal change consisting of a discrete area containing increased numbers of cortical cells. The hyperplastic cells were the same size or smaller than surrounding normal cortical cells and sometimes had slightly basophilic cytoplasm. With large lesions, sometimes there was compression of the surrounding tissue; these were distinguishable as hyperplasia because the cells still formed normal cords, particularly in the upper zona fasciculata. Necrosis was a focal to multifocal to locally diffuse change characterized by fragmentation and dissolution of cortical epithelial cells, suggestive of a change secondary to toxicity rather than a primary effect on the adrenal cortex.

***Malignant Schwannoma:*** There were three incidences of malignant schwannoma in the thoracic cavity in 100 mg/kg males and a single incidence in 30 mg/kg males (Tables 18, A1, and A2). Malignant schwannomas were large masses located within the mediastinum and all had a microscopic appearance typically seen with these neoplasms. The schwannomas were composed of sheets of loosely arranged cells with small, relatively uniform, moderately basophilic, round to ovoid nuclei, and pale inapparent cytoplasm. The cells often tended to form palisades and, within most of the neoplasm, were separated by abundant clear intracellular space; in some areas, the intercellular space contained eosinophilic fibrillar material that appeared to be collagen. In a few areas, the cells formed densely packed clusters and bundles with relatively little intercellular space. The neoplastic cells formed numerous small to very large irregular cavities lined by a layer of neoplastic cells producing cyst-like structures, some filled with blood. These cyst-like structures are very typical of malignant schwannoma.

**TABLE 16**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Forestomach in Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Number Necropsied | 50 | 50 | 50 | 50 |
| Epithelium, Hyperplasia[a] | 8 (1.4)[b] | 36** (1.8) | 44** (2.2) | 45** (2.5) |
| Squamous Cell Carcinoma | 0 | 1 | 0 | 0 |
| **Female** | | | | |
| Number Necropsied | 50 | 50 | 50 | 50 |
| Epithelium, Hyperplasia | 0 | 32** (1.7) | 46** (2.4) | 46** (2.5) |
| Inflammation | 1 (1.0) | 3 (2.0) | 5 (1.6) | 7* (2.3) |
| Squamous Cell Papilloma, Multiple | 0 | 0 | 0 | 1 |
| Squamous Cell Papilloma (includes multiple)[c] | | | | |
| Overall rate[d] | 0/50 (0%) | 0/50 (0%) | 0/50 (0%) | 3/50 (6%) |
| Adjusted rate[e] | 0.0% | 0.0% | 0.0% | 7.8% |
| Terminal rate[f] | 0/25 (0%) | 0/30 (0%) | 0/19 (0%) | 0/17 (0%) |
| First incidence (days) | —[g] | — | — | 590 |
| Poly-3 test[h] | P = 0.008 | —[i] | — | P = 0.116 |
| Squamous Cell Carcinoma[j] | 0 | 1 | 0 | 1 |
| Squamous Cell Papilloma or Squamous Cell Carcinoma[j] | | | | |
| Overall rate | 0/50 (0%) | 1/50 (2%) | 0/50 (0%) | 4/50 (8%) |
| Adjusted rate | 0.0% | 2.6% | 0.0% | 10.3% |
| Terminal rate | 0/25 (0%) | 1/30 (3%) | 0/19 (0%) | 0/17 (0%) |
| First incidence (days) | — | 727 (T) | — | 590 |
| Poly-3 test | P = 0.009 | P = 0.496 | — | P = 0.059 |

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Historical control incidence for 2-year corn oil gavage studies in Harlan Sprague-Dawley rats: 0/473
[d] Number of animals with neoplasm per number of animals necropsied
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Not applicable; no neoplasms in animal group
[h] Beneath the vehicle 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 vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[i] Value of the statistic cannot be computed
[j] Historical incidence (means ± standard deviation): 2/473 (0.4% ± 0.8%), range 0%-2%

**TABLE 17**
**Incidences of Selected Neoplasms and Nonneoplastic Lesions of the Adrenal Cortex in Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Degeneration[a] | 4 (1.0)[b] | 14** (1.4) | 14** (1.4) | 9* (1.8) |
| Vacuolization Cytoplasmic | 20 (1.2) | 29** (1.2) | 31** (1.5) | 25* (1.7) |
| Zona Fasciculata, Hyperplasia | 14 (1.6) | 22** (2.0) | 19* (1.9) | 21** (2.2) |
| Necrosis | 0 | 2 (1.0) | 4* (1.3) | 13** (1.8) |
| **Female** | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 49 |
| Degeneration | 13 (1.5) | 12 (1.7) | 17 (1.5) | 16 (1.5) |
| Vacuolization Cytoplasmic | 7 (1.3) | 16* (1.5) | 13 (1.5) | 15* (1.6) |
| Zona Fasciculata, Hyperplasia | 14 (1.6) | 20 (1.9) | 25* (1.9) | 21 (1.8) |
| Necrosis | 1 (2.0) | 0 | 7* (1.9) | 5 (1.8) |
| Adenoma[c] | | | | |
| Overall rate[d] | 1/50 (2%) | 1/50 (2%) | 3/50 (6%) | 4/49 (8%) |
| Adjusted rate[e] | 2.6% | 2.6% | 8.1% | 10.4% |
| Terminal rate[f] | 1/25 (4%) | 1/30 (3%) | 0/19 (0%) | 1/17 (6%) |
| First incidence (days) | 727 (T) | 727 (T) | 649 | 590 |
| Poly-3 test[g] | P = 0.093 | P = 0.757 | P = 0.289 | P = 0.175 |

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Historical control incidence for 2-year corn oil gavage studies in Harlan Sprague-Dawley rats (mean ± standard deviation): 5/471 (1.1 ± 1.4%), range 0%-4%
[d] Number of animals with neoplasm per number of animals with adrenal cortex examined microscopically
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle 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 vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

**TABLE 18**
**Incidences of Malignant Schwannoma in All Organs in Male Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Malignant Schwannoma** | | | | |
| Overall rate[a] | 0/50 (0%) | 0/50 (0%) | 1/50 (2%) | 3/50 (6%) |
| Adjusted rate[b] | 0.0% | 0.0% | 3.9% | 11.4% |
| Terminal rate[c] | 0/28 (0%) | 0/9 (0%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | —[d] | — | 712 | 398 |
| Poly-3 test[e] | P = 0.010 | —[f] | P = 0.413 | P = 0.060 |

[a] Number of animals with neoplasm per number of animals necropsied
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Not applicable; no neoplasms in animal group
[e] Beneath the vehicle 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 vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[f] Value of the statistic cannot be computed

***Pancreas:*** The incidences of atrophy and acinar cytoplasmic vacuolization in all dosed groups of rats were significantly greater than the vehicle control incidences (Tables 19, A3, and B4). The incidences of inflammation were significantly increased in 10 and 30 mg/kg males. The severities of these lesions increased with increasing dose in females.

Atrophy was a focal to multifocal change consisting of a reduction in the amount of acinar tissue and an associated increase in stromal fibrous connective tissue. Cytoplasmic vacuolization was generally a minimal to mild change consisting of small, clear, discrete intracytoplasmic vacuoles within pancreatic acinar cells. The severity of the change was determined by the degree of vacuolization per cell and the amount of tissue involved. Inflammation consisted of a diffuse infiltrate of small numbers of mononuclear inflammatory cells in the interstitial space between adjacent pancreatic acini; in some cases it appeared to extend from inflammation in adjacent tissues.

***Blood Vessel:*** There were significantly increased incidences of inflammation of the blood vessels in all dosed groups (Tables 19, A3, and B4). Sites commonly involved were the testes, mesentery, and pancreas, with occasional involvement of the epididymis and thymus. Inflammation was a focal to multifocal change characterized by a thick mantle of macrophages, lymphocytes, and plasma cells around the arteries, with infiltration into the muscular layers of the artery. There was often fibrinoid necrosis of the vessel, and the tunica intima was frequently thickened. Endothelial cells were swollen or decreased in number. This inflammatory reaction often extended into the surrounding parenchyma. In more chronic cases, the arterial wall was greatly thickened and appeared fibrotic. The affected vessels were sometimes greatly dilated (Plate 12).

***Heart:*** The incidence of cardiomyopathy in 100 mg/kg females was significantly greater than the vehicle control incidence (Tables 19 and B4). Cardiomyopathy had the microscopic appearance of this lesion typical of that seen in aging F344/N rats. It was a multifocal, generally minimal to mild lesion consisting of hypereosinophilic myofibers that lacked cross striations, infiltrates of mononuclear cells, and eventual replacement of myofibers by fibrous connective tissue. The severity was graded based upon the number and extent of foci of myocardial degeneration. Minimal cardiomyopathy

consisted of a few scattered foci while mild cardiomyopathy consisted of a greater number of lesions more diffusely scattered within the myocardium.

***Bone Marrow:*** Incidences of hyperplasia in 30 and 100 mg/kg males and 100 mg/kg females were significantly greater than those in the vehicle controls (Tables 19, A3, and B4). This change was characterized by an increase in the amount of hematopoietic tissue.

***Spleen:*** There were significantly increased incidences of atrophy of the lymphoid follicles in 100 mg/kg males and females and pigmentation in all dosed groups of females (Tables 19, A3, and B4). Increased severities of pigmentation, compared to vehicle controls, occurred in all dosed female groups. Lymphoid follicle atrophy was characterized microscopically by loss of lymphocytes from splenic lymphoid follicles with a subsequent decrease in the size of the lymphoid follicles. Pigmentation consisted of macrophages containing yellow-brown pigment.

***Lymph Node (Mesenteric):*** The incidences of pigmentation were significantly increased in all dosed groups, and the incidences of atrophy were significantly increased in the 100 mg/kg groups (Tables 19, A3, and B4). Pigmentation was characterized by macrophages containing yellow-brown pigment. Lymphoid atrophy was characterized by varying degrees of loss of lymphocytes.

***Nose:*** In all dosed female groups, incidences of inflammation were significantly greater than in the vehicle control group (Tables 19 and B4). This change consisted of infiltrates of small to moderate numbers of neutrophils mixed with debris located within the nasal cavity.

***Testis:*** There was a dose-related increase in the incidences of degeneration of the germinal epithelium, and the increase was significant at 100 mg/kg (Tables 19 and A3). Degeneration was characterized by varying numbers of seminiferous tubules lined by decreased numbers of spermatogenic cells, often lined by only a single layer of Sertoli cells. Necrosis consisted of complete fragmentation and disintegration of variably sized areas of seminiferous tubules.

***Seminal Vesicle:*** There were sporadic incidences of inflammation in all dosed groups and none in vehicle controls; at 100 mg/kg, the increase was significant (Tables 19 and A3). This change consisted of large aggregates of inflammatory cells, primarily neutrophils, mixed with cell debris.

***Ovary:*** Incidences of inflammation increased with increasing dose, and the incidence in 100 mg/kg females significantly exceeded the vehicle control incidence (Tables 19 and B4). This change was characterized by large aggregates of neutrophils mixed with cell debris and generally surrounded by a fibrous tissue (i.e., an abscess) that had replaced much or all of the normal ovary.

***Decreased Neoplasms:*** In males, decreased incidences of pituitary gland pars distalis adenoma were noted in all dosed groups when compared to vehicle controls (vehicle control, 13/50; 10 mg/kg, 6/50; 30 mg/kg, 7/50; 100 mg/kg, 0/49; Tables A1 and A2). Decreased incidences of pancreatic adenoma were noted in all dosed groups when compared to vehicle controls (9/50; 1/49; 0/50; 0/50). The decreased incidences of these neoplasms are suggested to be related to the decreased survival noted in all male dosed groups.

In females, decreased incidences of pituitary gland pars distalis adenoma were noted in groups administered 30 or 100 mg/kg when compared to vehicle controls (13/50; 11/50; 4/50; 6/50; Tables B1 and B2). Decreased incidences of mammary gland fibroadenoma were noted in groups administered 30 or 100 mg/kg when compared to vehicle controls (31/50; 30/50; 24/50; 20/50).

**TABLE 19**
**Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Pancreas[a] | 50 | 49 | 50 | 50 |
| Atrophy[b] | 4 (1.3)[c] | 13** (1.1) | 10* (1.1) | 10** (2.1) |
| Acinus, Vacuolization Cytoplasmic | 0 | 16** (1.3) | 30** (1.6) | 13** (1.4) |
| Inflammation | 1 (3.0) | 7** (1.9) | 7** (2.7) | 3 (3.3) |
| Blood Vessel | 50 | 50 | 50 | 50 |
| Inflammation | 21 (1.7) | 29** (2.7) | 30** (2.6) | 29** (2.6) |
| Bone Marrow | 50 | 50 | 49 | 50 |
| Hyperplasia | 12 (2.2) | 7 (2.7) | 21** (2.0) | 19** (2.2) |
| Spleen | 50 | 50 | 50 | 50 |
| Lymphoid Follicle, Atrophy | 5 (2.6) | 4 (2.0) | 5 (2.2) | 12** (2.5) |
| Lymph Node, Mesenteric | 50 | 48 | 50 | 50 |
| Pigmentation | 1 (1.0) | 24** (1.2) | 25** (1.1) | 20** (1.2) |
| Atrophy | 0 | 1 (2.0) | 1 (2.0) | 5** (2.6) |
| Testes | 50 | 50 | 50 | 50 |
| Germinal Epithelium, Degeneration | 15 (2.3) | 16 (2.7) | 18 (2.2) | 21** (2.7) |
| Seminal Vesicle | 50 | 50 | 50 | 50 |
| Inflammation | 0 | 2 (3.0) | 1 (2.0) | 4* (3.0) |
| **Female** | | | | |
| Pancreas | 50 | 49 | 49 | 49 |
| Atrophy | 0 | 11** (1.2) | 12** (1.6) | 13** (2.0) |
| Acinus, Vacuolization Cytoplasmic | 0 | 27** (1.7) | 33** (1.9) | 40** (2.1) |
| Inflammation | 1 (1.0) | 1 (1.0) | 4 (2.3) | 6 (2.5) |
| Blood Vessel | 50 | 50 | 50 | 50 |
| Inflammation | 1 (1.0) | 10** (1.9) | 14** (2.1) | 16** (2.4) |
| Heart | 50 | 50 | 49 | 49 |
| Cardiomyopathy | 12 (1.0) | 16 (1.1) | 13 (1.0) | 24* (1.1) |
| Bone Marrow | 50 | 50 | 50 | 50 |
| Hyperplasia | 36 (3.1) | 41 (2.6) | 40 (2.8) | 45** (2.8) |
| Spleen | 50 | 50 | 49 | 49 |
| Lymphoid Follicle, Atrophy | 3 (1.3) | 4 (2.3) | 8 (2.6) | 10* (2.3) |
| Pigmentation | 31 (1.2) | 44** (1.5) | 42** (1.6) | 47** (1.6) |
| Lymph Node, Mesenteric | 50 | 49 | 50 | 49 |
| Pigmentation | 14 (1.0) | 32** (1.3) | 32** (1.2) | 30** (1.3) |
| Atrophy | 0 | 0 | 4 (3.3) | 6* (3.0) |
| Nose | 50 | 50 | 50 | 50 |
| Inflammation | 0 | 7** (1.1) | 6* (1.2) | 8** (1.5) |
| Ovary | 50 | 50 | 50 | 50 |
| Inflammation | 0 | 1 (4.0) | 2 (4.0) | 5* (3.6) |

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
a Number of animals with tissue examined microscopically
b Number of animals with lesion
c Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

# MICE

## *2-Year Study*

Results of the NTP 3-month studies of TCAB in male and female B6C3F1 mice were published previously (NTP, 1998a, Van Birgelen, 1999a).

### Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 20 and in the Kaplan-Meier survival curves (Figure 4). Survival of 10 and 30 mg/kg males and 30 mg/kg females was significantly less than that of vehicle controls. All 30 mg/kg males died before the end of the study. Most of the deaths in the 10 and 30 mg/kg groups were due to transitional cell carcinomas of the urethra. Urethral carcinomas, malignant schwannomas, and fibrosarcomas contributed to decreased survival in 30 mg/kg females.

### Body Weights and Clinical Findings

Mean body weights of treated males were similar to or greater than those of the vehicle controls throughout most of the study. Mean body weights of 10 and 30 mg/kg males were 10% and 8% less than those of the vehicle controls at the last weighing at weeks 101 and 73, respectively (Figure 5 and Table 21). Mean body weights of 3 mg/kg females were 7% greater than those of the vehicle controls after week 64 (Figure 5 and Table 22).

**TABLE 20**
**Survival of Mice in the 2-Year Gavage Study of TCAB**

| | 0 mg/kg | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Animals initially in study | 50 | 50 | 49 | 50 |
| Accidental deaths[a] | 1 | 0 | 2 | 0 |
| Moribund | 10 | 11 | 16 | 19 |
| Natural deaths | 4 | 8 | 26 | 31 |
| Animals surviving to study termination | 35 | 31 | 5 | 0 |
| Percent probability of survival at end of study[b] | 72 | 62 | 11 | 0 |
| Mean survival (days)[c] | 702 | 667 | 575 | 396 |
| Survival analysis[d] | P < 0.001 | P = 0.283 | P < 0.001 | P < 0.001 |
| **Female** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Accidental death[a] | 1 | 0 | 1 | 1 |
| Moribund | 6 | 9 | 9 | 21 |
| Natural deaths | 8 | 11 | 8 | 8 |
| Animals surviving to study termination | 35 | 30 | 32 | 20[e] |
| Percent probability of survival at end of study | 71 | 60 | 65 | 39 |
| Mean survival (days) | 675 | 698 | 678 | 617 |
| Survival analysis | P < 0.001 | P = 0.438 | P = 0.715 | P = 0.002 |

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns.
[e] Includes one animal that died during the last week of the study

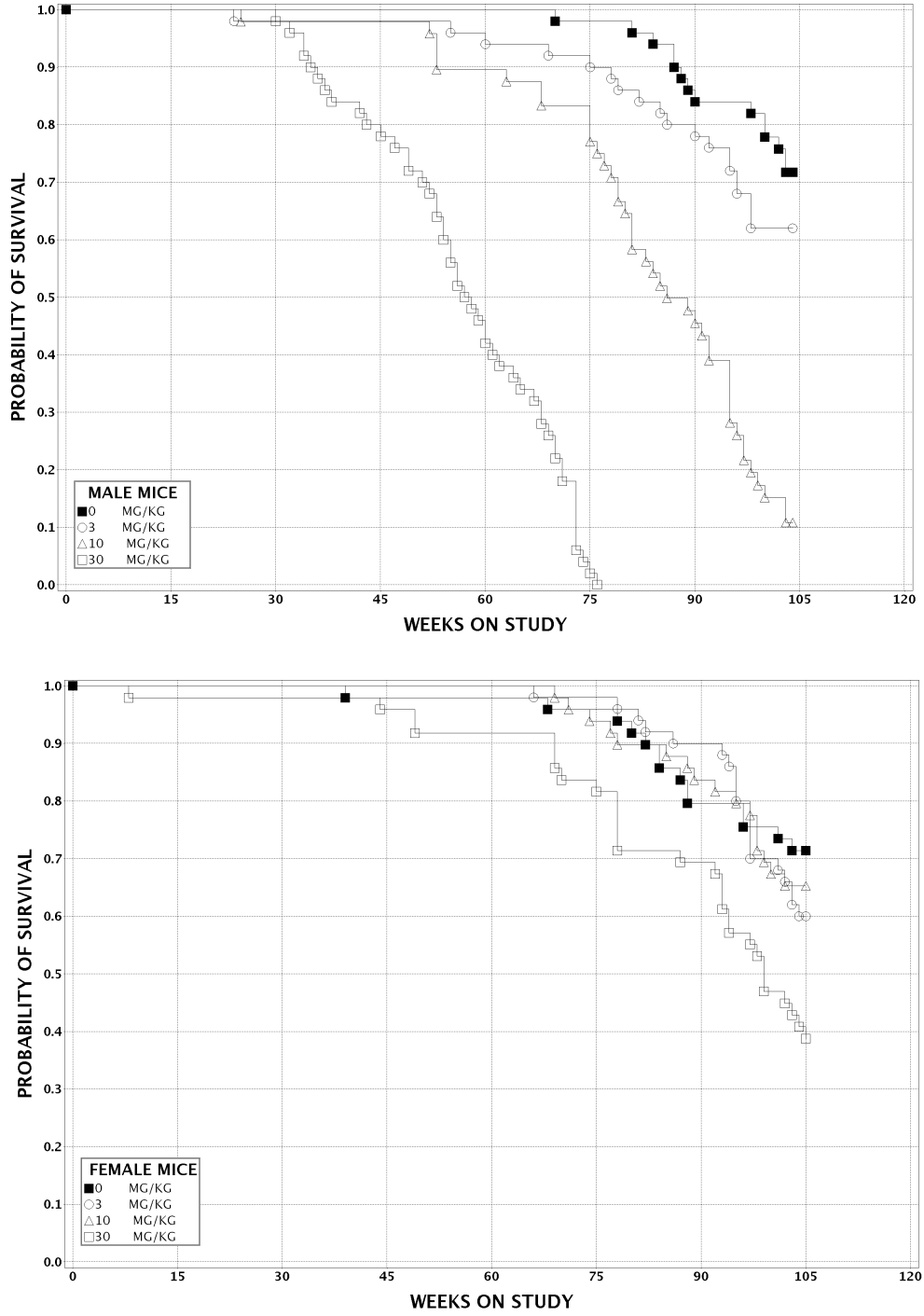



**FIGURE 4**
**Kaplan-Meier Survival Curves for Male and Female Mice Administered TCAB by Gavage for 2 Years**

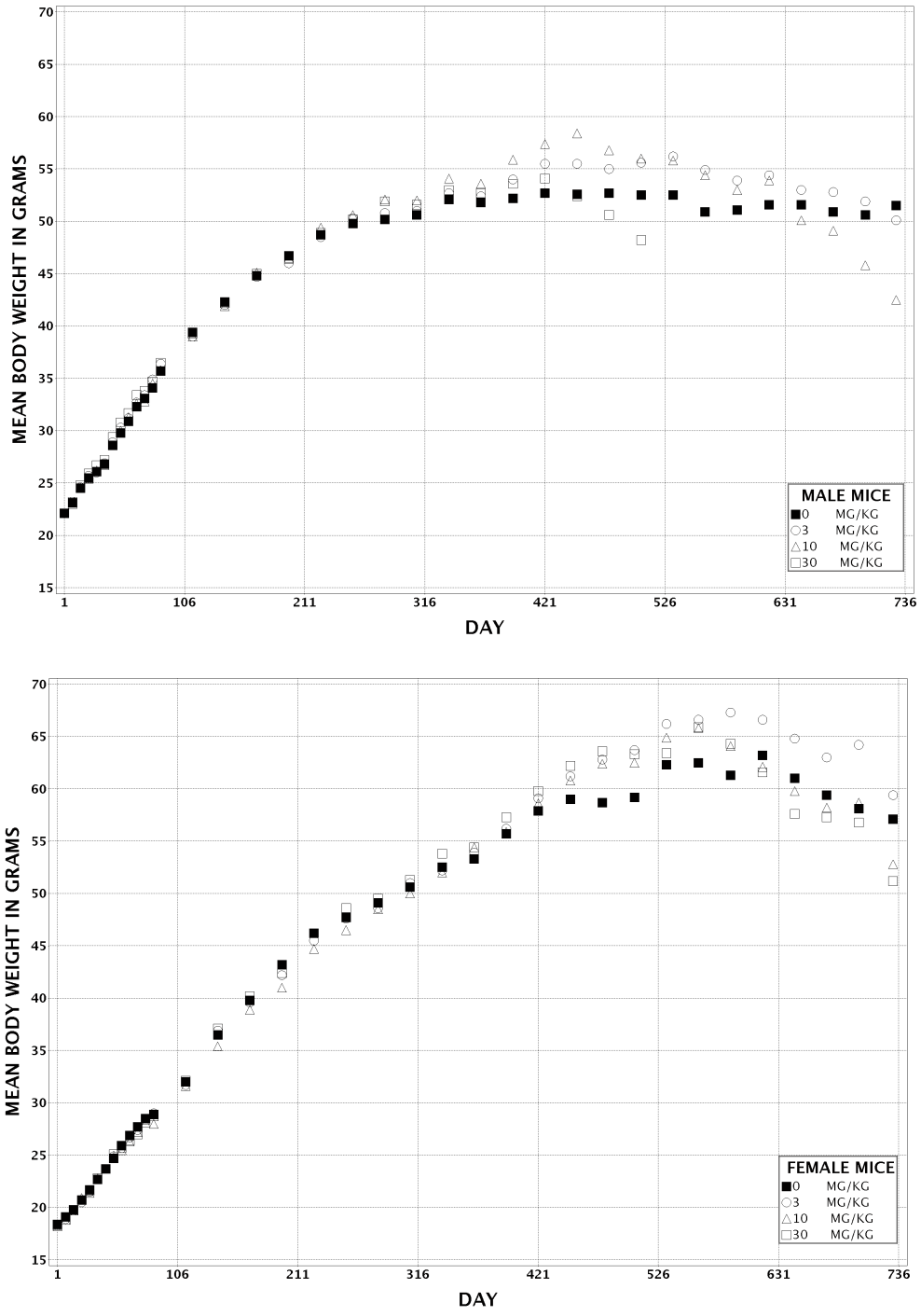



**FIGURE 5**
**Growth Curves for Male and Female Mice Administered TCAB by Gavage for 2 Years**

**TABLE 21**
**Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of TCAB**

| Days on Study | Vehicle Control | | 3 mg/kg | | | 10 mg/kg | | | 30 mg/kg | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Av. Wt. (g) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors |
| 1 | 22.1 | 50 | 22.1 | 100 | 50 | 22.1 | 100 | 50 | 22.1 | 100 | 50 |
| 8 | 23.1 | 50 | 23.2 | 100 | 50 | 23.0 | 99 | 49 | 23.2 | 101 | 50 |
| 15 | 24.5 | 50 | 24.6 | 101 | 50 | 24.6 | 100 | 49 | 24.8 | 102 | 50 |
| 22 | 25.5 | 50 | 25.7 | 101 | 50 | 25.4 | 100 | 49 | 25.9 | 102 | 50 |
| 29 | 26.1 | 50 | 26.0 | 100 | 50 | 26.2 | 101 | 49 | 26.7 | 103 | 50 |
| 36 | 26.8 | 50 | 26.9 | 100 | 50 | 26.7 | 100 | 49 | 27.2 | 101 | 50 |
| 43 | 28.6 | 50 | 28.9 | 101 | 50 | 28.6 | 100 | 49 | 29.4 | 103 | 50 |
| 50 | 29.8 | 50 | 30.3 | 102 | 50 | 30.0 | 101 | 49 | 30.8 | 104 | 50 |
| 57 | 30.9 | 50 | 31.2 | 101 | 50 | 31.3 | 101 | 49 | 31.7 | 103 | 50 |
| 64 | 32.3 | 50 | 32.7 | 101 | 50 | 32.6 | 101 | 48 | 33.4 | 103 | 50 |
| 71 | 33.1 | 50 | 33.4 | 101 | 50 | 32.8 | 99 | 48 | 33.8 | 102 | 50 |
| 78 | 34.1 | 50 | 34.9 | 102 | 50 | 34.5 | 101 | 48 | 34.7 | 102 | 50 |
| 85 | 35.7 | 50 | 36.4 | 102 | 50 | 35.8 | 101 | 48 | 36.5 | 102 | 50 |
| 113 | 39.4 | 50 | 39.0 | 99 | 50 | 39.0 | 99 | 48 | 39.3 | 100 | 50 |
| 141 | 42.3 | 50 | 42.0 | 99 | 50 | 41.9 | 99 | 48 | 42.2 | 100 | 50 |
| 169 | 44.8 | 50 | 44.7 | 100 | 49 | 45.1 | 101 | 47 | 45.0 | 101 | 50 |
| 197 | 46.7 | 50 | 46.0 | 99 | 49 | 46.5 | 100 | 47 | 46.4 | 100 | 50 |
| 225 | 48.7 | 50 | 48.5 | 100 | 49 | 49.4 | 101 | 47 | 48.9 | 100 | 48 |
| 253 | 49.8 | 50 | 50.3 | 101 | 49 | 50.6 | 102 | 47 | 50.2 | 101 | 44 |
| 281 | 50.2 | 50 | 50.8 | 101 | 49 | 52.1 | 104 | 47 | 51.9 | 103 | 42 |
| 309 | 50.6 | 50 | 51.0 | 101 | 49 | 52.0 | 103 | 47 | 51.6 | 102 | 40 |
| 337 | 52.1 | 50 | 52.7 | 101 | 49 | 54.1 | 104 | 47 | 53.0 | 102 | 37 |
| 365 | 51.8 | 50 | 52.4 | 101 | 49 | 53.6 | 104 | 46 | 52.7 | 102 | 34 |
| 393 | 52.2 | 50 | 54.0 | 103 | 48 | 55.9 | 107 | 43 | 53.6 | 103 | 26 |
| 421 | 52.7 | 50 | 55.5 | 105 | 47 | 57.4 | 109 | 43 | 54.1 | 103 | 21 |
| 449 | 52.6 | 50 | 55.5 | 106 | 47 | 58.4 | 111 | 42 | 52.4 | 100 | 18 |
| 477 | 52.7 | 50 | 55.0 | 104 | 47 | 56.8 | 108 | 40 | 50.6 | 96 | 14 |
| 505 | 52.5 | 49 | 55.6 | 106 | 46 | 56.0 | 107 | 40 | 48.2 | 92 | 7 |
| 533 | 52.5 | 49 | 56.2 | 107 | 45 | 55.8 | 106 | 35 | | | |
| 561 | 50.9 | 49 | 54.9 | 108 | 43 | 54.4 | 107 | 30 | | | |
| 589 | 51.1 | 47 | 53.9 | 106 | 42 | 53.0 | 104 | 26 | | | |
| 617 | 51.6 | 44 | 54.4 | 106 | 40 | 53.9 | 105 | 22 | | | |
| 645 | 51.6 | 42 | 53.0 | 103 | 38 | 50.1 | 97 | 18 | | | |
| 673 | 50.9 | 42 | 52.8 | 104 | 34 | 49.1 | 96 | 11 | | | |
| 701 | 50.6 | 38 | 51.9 | 102 | 31 | 45.8 | 90 | 7 | | | |
| **Mean for weeks** | | | | | | | | | | | |
| 1–13 | 28.7 | | 28.9 | 101 | | 28.7 | 100 | | 29.2 | 102 | |
| 14–52 | 47.2 | | 47.2 | 100 | | 47.9 | 101 | | 47.6 | 101 | |
| 53–101 | 51.8 | | 54.2 | 105 | | 53.9 | 104 | | 51.9 | 100 | |

**TABLE 22**
**Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of TCAB**

| Days on Study | Vehicle Control | | 3 mg/kg | | | 10 mg/kg | | | 30 mg/kg | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Av. Wt. (g) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors | Av. Wt. (g) | Wt. (% of controls) | No. of Survivors |
| 1 | 18.4 | 50 | 18.3 | 100 | 50 | 18.2 | 99 | 50 | 18.3 | 100 | 50 |
| 8 | 19.1 | 49 | 18.9 | 99 | 50 | 18.8 | 98 | 49 | 18.8 | 98 | 50 |
| 15 | 19.8 | 49 | 19.7 | 100 | 50 | 19.7 | 99 | 49 | 19.7 | 100 | 49 |
| 22 | 20.7 | 49 | 20.5 | 99 | 50 | 20.9 | 101 | 49 | 20.7 | 100 | 49 |
| 29 | 21.7 | 49 | 21.5 | 99 | 50 | 21.4 | 98 | 49 | 21.6 | 99 | 49 |
| 36 | 22.7 | 49 | 22.7 | 100 | 50 | 22.8 | 100 | 49 | 22.8 | 101 | 49 |
| 43 | 23.7 | 49 | 23.7 | 100 | 50 | 23.7 | 100 | 49 | 23.7 | 100 | 49 |
| 50 | 24.7 | 49 | 24.9 | 101 | 50 | 24.9 | 101 | 49 | 25.1 | 102 | 48 |
| 57 | 25.9 | 49 | 25.7 | 99 | 50 | 25.5 | 98 | 49 | 25.7 | 99 | 48 |
| 64 | 26.9 | 49 | 26.7 | 99 | 50 | 26.3 | 98 | 49 | 26.4 | 98 | 48 |
| 71 | 27.7 | 49 | 27.4 | 99 | 50 | 27.2 | 98 | 49 | 27.0 | 98 | 48 |
| 78 | 28.5 | 49 | 28.5 | 100 | 50 | 28.1 | 99 | 49 | 28.4 | 99 | 48 |
| 85 | 28.9 | 49 | 29.0 | 101 | 50 | 28.0 | 97 | 49 | 28.7 | 99 | 48 |
| 113 | 32.0 | 49 | 31.7 | 99 | 50 | 31.6 | 99 | 49 | 32.1 | 100 | 48 |
| 141 | 36.5 | 49 | 36.9 | 101 | 50 | 35.4 | 97 | 49 | 37.1 | 102 | 48 |
| 169 | 39.8 | 49 | 39.6 | 99 | 50 | 38.9 | 98 | 49 | 40.2 | 101 | 48 |
| 197 | 43.2 | 49 | 42.2 | 98 | 50 | 41.0 | 95 | 49 | 42.4 | 98 | 48 |
| 225 | 46.2 | 49 | 45.5 | 98 | 50 | 44.7 | 97 | 49 | 46.2 | 100 | 48 |
| 253 | 47.7 | 49 | 47.6 | 100 | 50 | 46.5 | 97 | 49 | 48.6 | 102 | 48 |
| 281 | 49.1 | 48 | 48.6 | 99 | 50 | 48.5 | 99 | 49 | 49.5 | 101 | 48 |
| 309 | 50.6 | 48 | 51.0 | 101 | 50 | 50.0 | 99 | 49 | 51.3 | 101 | 47 |
| 337 | 52.5 | 48 | 52.2 | 99 | 50 | 52.0 | 99 | 49 | 53.8 | 102 | 45 |
| 365 | 53.3 | 48 | 53.9 | 101 | 50 | 54.4 | 102 | 49 | 54.4 | 102 | 45 |
| 393 | 55.7 | 48 | 56.2 | 101 | 50 | 55.9 | 100 | 49 | 57.3 | 103 | 45 |
| 421 | 57.9 | 48 | 59.1 | 102 | 50 | 58.6 | 101 | 49 | 59.8 | 103 | 45 |
| 449 | 59.0 | 48 | 61.2 | 104 | 50 | 60.8 | 103 | 49 | 62.2 | 105 | 45 |
| 477 | 58.7 | 47 | 62.8 | 107 | 49 | 62.4 | 106 | 48 | 63.6 | 108 | 42 |
| 505 | 59.2 | 47 | 63.7 | 108 | 49 | 62.5 | 106 | 47 | 63.3 | 107 | 41 |
| 533 | 62.3 | 47 | 66.2 | 106 | 49 | 64.9 | 104 | 46 | 63.4 | 102 | 40 |
| 561 | 62.5 | 45 | 66.6 | 107 | 48 | 65.8 | 105 | 44 | 65.9 | 106 | 35 |
| 589 | 61.3 | 42 | 67.3 | 110 | 46 | 64.1 | 105 | 44 | 64.3 | 105 | 35 |
| 617 | 63.2 | 39 | 66.6 | 105 | 45 | 62.1 | 98 | 42 | 61.6 | 98 | 34 |
| 645 | 61.0 | 39 | 64.8 | 106 | 45 | 59.8 | 98 | 40 | 57.6 | 94 | 32 |
| 673 | 59.4 | 37 | 63.0 | 106 | 38 | 58.2 | 98 | 39 | 57.3 | 96 | 27 |
| 701 | 58.1 | 37 | 64.2 | 111 | 35 | 58.7 | 101 | 33 | 56.8 | 98 | 23 |
| **Mean for weeks** | | | | | | | | | | | |
| 1–13 | 23.7 | | 23.7 | 100 | | 23.5 | 99 | | 23.6 | 100 | |
| 14–52 | 44.2 | | 43.9 | 99 | | 43.2 | 98 | | 44.6 | 101 | |
| 53–101 | 59.4 | | 62.7 | 106 | | 60.6 | 102 | | 60.6 | 102 | |

The only chemical-related clinical finding was the appearance of ulcers or abscesses, primarily on the head and neck and exacerbated by scratching. These skin lesions appeared as areas of discoloration, ulceration, or thickening. The incidence of this finding ranged from 12% in vehicle controls to 34% in the 10 mg/kg males and 2% in vehicle controls to 26% in 30 mg/kg females. The lower incidence in 30 mg/kg males was due to the high mortality in this group. In both sexes, the time when these lesions first appeared was dose related, with lesions appearing first in the 30 mg/kg groups (Figure 6).

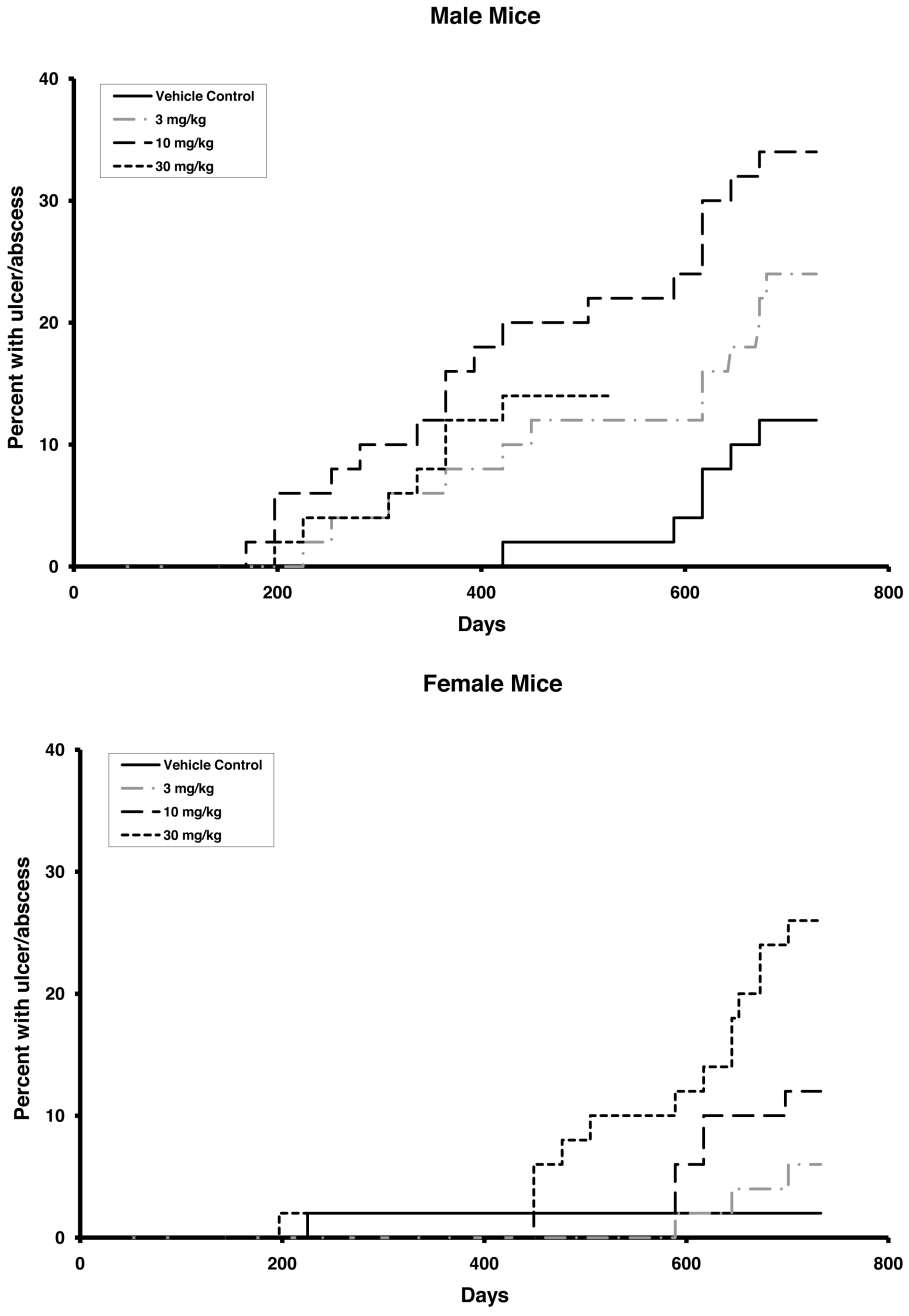



**FIGURE 6**
**Timeline of the Appearance of Gross Ulcers/Abscesses in Male and Female Mice**

## Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of malignant lymphoma and neoplasms and nonneoplastic lesions of the urethra, ureter, lung, stomach (forestomach and glandular), skin, and liver and nonneoplastic lesions of the urinary bladder, kidney, seminal vesicle, prostate gland, coagulating gland, epididymis, clitoral gland, ovary, bone marrow, spleen, thymus, heart and blood vessel. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

***Urethra:*** The incidences of transitional epithelial carcinoma were significantly increased in all dosed groups of males, and two of these neoplasms were observed in 30 mg/kg females (Tables 23, C1, C2, and D1). Transitional epithelial hyperplasia of the urethra occurred in 3 and 10 mg/kg males and 3 and 30 mg/kg females (Tables 23, C4, and D4). No spontaneous neoplasms of the urethra and ureter have been recorded in male or female mice in the NTP historical control reports.

Carcinomas were characterized by a poorly demarcated, hypercellular proliferation of glandular epithelium with a variably solid to glandular appearance surrounding the urethra with prominent cellular atypia and frequent mitotic figures. In some incidences, it was apparent that the neoplastic process was confined to the submucosal gland area, while in more invasive lesions, both the transitional epithelium lining and the submucosal compartment were involved (Plates 13 to 15). The urethral carcinomas were usually invasive into surrounding tissues and/or metastatic to tissues including the prostate gland, seminal vesicle, coagulating gland, ductus deferens, kidney, ureter, urinary bladder, lung, and skeletal muscle. Local invasion and/or metastases to other organs occurred in all dosed male groups and in 30 mg/kg females. It is considered that many of these urethral carcinomas most likely arose from the periurethral glands because of their overall glandular pattern, location, and the fact that in some incidences, the overlying transitional urothelium appeared to be uninvolved. Microscopically, transitional cell hyperplasia was characterized by a localized and well-demarcated increase in the number of transitional cells (Plates 16 and 17).

***Ureter:*** One 10 mg/kg male and one 30 mg/kg female had transitional epithelial carcinoma and one 30 mg/kg female had transitional epithelial hyperplasia (Tables 24, C1, C4, D1, and D4). The hyperplasia and carcinomas had the same morphological characteristics as those seen in the urethra (Plates 18 and 19). Some of the other findings present in the ureter were interpreted as secondary to the transitional epithelial lesions in the urethra, including chronic active inflammation and dilatation seen in all dosed male groups.

***Urinary Bladder:*** The incidences of transitional epithelial hyperplasia in 30 mg/kg males and females were significantly greater than those of the vehicle controls (Tables 25, C4, and D4). Microscopically, transitional cell hyperplasia was characterized by a localized and well demarcated increase in the number of urothelial cells.

***Kidney:*** In males, there were significant increases in the incidences of renal tubule dilatation (all dosed groups) and hydronephrosis (10 and 30 mg/kg) (Tables 25 and C4). In females, there were significant increases in the incidences of nephropathy and renal tubule dilatation (30 mg/kg) (Tables 25 and D4). Dilatation of tubules consisted of wider cortical and medullary tubules, while hydronephrosis consisted of dilation of the renal pelvic cavity. The dilation of the tubules and hydronephrosis are suggested to reflect retrograde pressure due to the presence of the transitional epithelial lesions of the urethra. Microscopically, nephropathy consisted of focal to multifocal regenerative renal tubules surrounded by a thickened basement membrane, glomerular thickening, tubular protein casts, and chronic inflammatory infiltrates with fibrosis. Increased incidences of other nonneoplastic lesions in the kidneys were interpreted as secondary to the neoplastic lesions of the urethra, including chronic active inflammation, thrombosis, glomerular amyloidosis, mineralization, and papillary necrosis (Tables C4 and D4).

***Lung:*** Significantly increased incidences of alveolar/bronchiolar adenoma occurred in all dosed groups of males, and a significantly increased incidence of alveolar/bronchiolar carcinoma occurred in 30 mg/kg females (Tables 26, C1, C2, D1, and D2). Significantly increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) occurred in 3 and 10 mg/kg males and 30 mg/kg females. Microscopically, alveolar/bronchiolar adenoma consisted of well-demarcated hypercellular masses distorting the normal septal architecture and was characterized by well-differentiated cuboidal to round cells forming papillary projections into the alveolar and bronchiolar lumens with slight compression of the surrounding parenchyma. Alveolar/bronchiolar carcinoma

**TABLE 23**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Urethra in Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Number Necropsied | 50 | 50 | 49 | 50 |
| Glands, Transitional Epithelium, Hyperplasia[a] | 0 | 17** (3.5)[b] | 2 (3.0) | 0 |
| Glands, Transitional Epithelium, Carcinoma | | | | |
| Overall rate[c] | 0/50 (0%) | 32/50 (64%) | 46/49 (94%) | 49/50 (98%) |
| Adjusted rate[d] | 0.0% | 70.7% | 99.7% | 99.9% |
| Terminal rate[e] | 0/35 (0%) | 21/31 (68%) | 5/5 (100%) | 0/0 (0%) |
| First incidence (days) | —[f] | 380 | 358 | 206 |
| Poly-3 test[g] | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 |
| **Female** | | | | |
| Number Necropsied | 50 | 50 | 50 | 50 |
| Glands, Transitional Epithelium, Hyperplasia | 0 | 4 (2.0) | 0 | 3 (2.7) |
| Glands, Transitional Epithelium, Carcinoma | 0 | 0 | 0 | 2 |

** Significantly different (P≤0.01) from the vehicle control group by the Poly-3 test
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Number of animals with neoplasm per number of animals necropsied
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Not applicable; no neoplasms in animal group
[g] Beneath the vehicle 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 vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

consisted of more irregular hypercellular masses distorting the normal septal architecture and was characterized by fairly pleomorphic, polygonal to columnar cells forming papillary and solid projections into alveolar or bronchiolar lumen with variable peripheral compression and invasion (Plate 20). One occurrence of squamous metaplasia of the alveolar epithelium occurred in a 30 mg/kg female (Tables 26 and D4).

A single incidence of cystic keratinizing epithelioma (CKE) and a single incidence of multiple CKE occurred in 30 mg/kg females (Tables 26 and D1). These two incidences did not appear exactly the same as the CKEs seen in Harlan Sprague-Dawley rats exposed to dioxin-like compounds in previous Toxic Equivalency Factor studies (NTP 2006a,b,c,d,e,f,g). The lesions seen in these mice were less organized and composed of clusters of squamous cells and keratin, but lacked the clear cyst-like component typical of these lesions (Plate 21). The incidences of chronic active inflammation were significantly increased in 10 and 30 mg/kg females. Chronic active inflammation was characterized by a mixture of lymphocytes, plasma cells, macrophages, and fewer neutrophils.

**TABLE 24**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Ureter in Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Number Examined Microscopically | 43 | 45 | 47 | 50 |
| Dilatation[a] | 0 | 6* (3.0)[b] | 22** (3.0) | 42** (2.8) |
| Inflammation, Chronic Active | 0 | 3 (3.3) | 24** (3.0) | 39** (3.0) |
| Transitional Epithelium, Carcinoma | 0 | 0 | 1 | 0 |
| **Female** | | | | |
| Number Examined Microscopically | 0 | 1 | 0 | 2 |
| Transitional Epithelium, Hyperplasia | | 0 | | 1 (2.0) |
| Transitional Epithelium, Carcinoma | | 0 | | 1 |

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

**TABLE 25**
**Incidences of Nonneoplastic Lesions of the Urinary Bladder and Kidney of Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Urinary Bladder[a] | 50 | 50 | 49 | 50 |
| Transitional Epithelium, Hyperplasia[b] | 0 | 1 (2.0)[c] | 0 | 4** (2.0) |
| Kidney | 50 | 50 | 49 | 50 |
| Renal Tubule, Dilatation | 1 (2.0) | 8* (2.6) | 31** (3.0) | 43** (2.9) |
| Hydronephrosis | 1 (2.0) | 5 (2.6) | 17** (2.4) | 29** (2.3) |
| **Female** | | | | |
| Urinary Bladder | 49 | 50 | 50 | 50 |
| Transitional Epithelium, Hyperplasia | 0 | 0 | 0 | 4* (2.5) |
| Kidney | 49 | 50 | 50 | 50 |
| Nephropathy | 29 (1.1) | 32 (1.3) | 38 (1.7) | 41* (1.5) |
| Renal Tubule, Dilatation | 1 (3.0) | 5 (2.4) | 6 (2.7) | 7* (3.0) |

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

**TABLE 26**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Number Examined Microscopically | 50 | 50 | 49 | 50 |
| Alveolar/bronchiolar Adenoma, Multiple[a] | 1 | 4 | 1 | 1 |
| Alveolar/bronchiolar Adenoma (includes multiple)[b] | | | | |
| Overall rate[c] | 5/50 (10%) | 16/50 (32%) | 12/49 (24%) | 6/50 (12%) |
| Adjusted rate[d] | 11.0% | 38.0% | 37.5% | 43.0% |
| Terminal rate[e] | 5/35 (14%) | 13/31 (42%) | 1/5 (20%) | 0/0 (0%) |
| First incidence (days) | 728 (T) | 574 | 470 | 357 |
| Poly-3 test[f] | P = 0.014 | P = 0.002 | P = 0.006 | P = 0.037 |
| Alveolar/bronchiolar Carcinoma, Multiple | 1 | 0 | 3 | 0 |
| Alveolar/bronchiolar Carcinoma (includes multiple)[g] | 3 | 1 | 4 | 0 |
| Alveolar/bronchiolar Adenoma or Carcinoma[h] | | | | |
| Overall rate | 7/50 (14%) | 17/50 (34%) | 15/49 (31%) | 6/50 (12%) |
| Adjusted rate | 15.3% | 40.2% | 45.8% | 43.0% |
| Terminal rate | 6/35 (17%) | 13/31 (42%) | 2/5 (40%) | 0/0 (0%) |
| First incidence (days) | 682 | 574 | 470 | 357 |
| Poly-3 test | P = 0.014 | P = 0.007 | P = 0.003 | P = 0.081 |
| **Female** | | | | |
| Number Examined Microscopically | 49 | 50 | 50 | 50 |
| Inflammation, Chronic Active | 0 | 3 (1.3)[i] | 5* (1.6) | 7** (2.6) |
| Alveolar Epithelium, Metaplasia, Squamous | 0 | 0 | 0 | 1 (2.0) |
| Cystic Keratinizing Epithelioma, Multiple | 0 | 0 | 0 | 1 |
| Cystic Keratinizing Epithelioma, (includes multiple)[j] | 0 | 0 | 0 | 2 |
| Alveolar/bronchiolar Adenoma, Multiple | 0 | 0 | 0 | 1 |
| Alveolar/bronchiolar Adenoma (includes multiple)[k] | 3 | 6 | 4 | 7 |
| Alveolar/bronchiolar Carcinoma[l] | | | | |
| Overall rate | 0/49 (0%) | 2/50 (4%) | 1/50 (2%) | 4/50 (8%) |
| Adjusted rate | 0.0% | 4.5% | 2.3% | 11.1% |
| Terminal rate | 0/35 (0%) | 2/30 (7%) | 1/32 (3%) | 1/19 (5%) |
| First incidence (days) | —[m] | 731 (T) | 731 (T) | 648 |
| Poly-3 test | P = 0.031 | P = 0.248 | P = 0.503 | P = 0.042 |

**TABLE 26**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Female** *(continued)* | | | | |
| Number Examined Microscopically | 49 | 50 | 50 | 50 |
| Alveolar/bronchiolar Adenoma or Carcinoma[n] | | | | |
| Overall rate | 3/49 (6%) | 8/50 (16%) | 5/50 (10%) | 10/50 (20%) |
| Adjusted rate | 7.1% | 18.0% | 11.5% | 27.3% |
| Terminal rate | 2/35 (6%) | 7/30 (23%) | 3/32 (9%) | 5/19 (26%) |
| First incidence (days) | 667 | 703 | 536 | 603 |
| Poly-3 test | P = 0.028 | P = 0.112 | P = 0.370 | P = 0.015 |

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 25/200 (12.5% ± 2.5%), range 10%-16%; all routes: 238/1,448 (16.4% ± 6.7%), range 2%-30%
[c] Number of animals with neoplasm per number of animals with lung examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Beneath the vehicle 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 vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[g] Historical incidence for corn oil gavage studies: 13/200 (6.5% ± 2.5%), range 4%-10%; all routes: 163/1,448 (11.3% ± 6.5%), range 2%-24%
[h] Historical incidence for corn oil gavage studies: 36/200 (18.0% ± 5.7%), range 14%-26%; all routes: 384/1,448 (26.5% ± 6.9%), range 14%-40%
[i] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[j] Historical incidence for corn oil gavage studies: 0/196; all routes: 0/1,496
[k] Historical incidence for corn oil gavage studies: 12/196 (6.1% ± 2.9%), range 2%-8%; all routes: 72/1,496 (4.8% ± 3.5%), range 0%-12%
[l] Historical incidence for corn oil gavage studies: 4/196 (2.0% ± 2.3%), range 0%-4%; all routes: 59/1,496 (3.9% ± 3.4%), range 0%-12%
[m] Not applicable; no neoplasms in animal group
[n] Historical incidence for corn oil gavage studies: 16/196 (8.1% ± 2.8%), range 6%-12%; all routes: 127/1,496 (8.5% ± 4.0%), range 2%-18%

***Stomach:*** In the forestomach, the incidences of squamous cell carcinoma in the 30 mg/kg groups were significantly greater than those in the vehicle control groups (Tables 27, C1, C2, D1, and D2). These were very aggressive tumors with invasion into the underlying musculature and metastases. Significantly increased incidences of hyperplasia at the limiting ridge occurred in all dosed groups (Tables 27, C4, and D4). Microscopically, forestomach hyperplasia was characterized by an increased thickness of the stratified squamous epithelium due to an increased number of epithelial cells. This change was usually accompanied by an increase in the thickness of the overlying keratin layer as well.

In the glandular stomach, incidences of focal epithelial hyperplasia, epithelial cyst (except 10 mg/kg females), and subtle mucosal lymphoid cell infiltration were significantly increased in dosed groups of males and females; the incidences of mineralization were significantly increased in dosed males and slightly increased in dosed females (Tables 27, C4, and D4). Microscopically, focal hyperplasia was characterized by disruption and disorganization of the normal glandular epithelium adjacent to the limiting ridge by the formation of small clusters of glandular epithelial cells (present in increased numbers), sometimes with the appearance of squamous metaplasia (Plate 22). The cysts were characterized by dilated glands.

**TABLE 27**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Stomach in Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Forestomach[a] | 50 | 50 | 49 | 50 |
| Epithelium, Hyperplasia[b] | 8 (2.5)[c] | 21** (1.4) | 33** (1.7) | 44** (2.0) |
| Squamous Cell Carcinoma[d] | | | | |
| Overall rate[e] | 0/50 (0%) | 1/50 (2%) | 1/49 (2%) | 3/50 (6%) |
| Adjusted rate[f] | 0.0% | 2.4% | 3.5% | 25.7% |
| Terminal rate[g] | 0/35 (0%) | 0/31 (0%) | 0/5 (0%) | 0/0 |
| First incidence (days) | —[h] | 416 | 584 | 376 |
| Poly-3 test[i] | P = 0.012 | P = 0.484 | P = 0.410 | P = 0.023 |
| Glandular Stomach | 50 | 50 | 49 | 50 |
| Epithelium, Hyperplasia, Focal | 0 | 10** (1.8) | 20** (1.9) | 34** (2.0) |
| Epithelium, Glands, Cyst | 5 (1.4) | 18** (1.4) | 21** (1.3) | 26** (1.3) |
| Infiltration Cellular, Lymphoid | 3 (1.0) | 20** (1.5) | 19** (1.6) | 15** (1.8) |
| Mineralization | 0 | 4* (1.0) | 6** (1.2) | 13** (1.4) |
| **Female** | | | | |
| Forestomach | 50 | 50 | 50 | 50 |
| Epithelium, Hyperplasia | 8 (1.9) | 27** (1.5) | 38** (1.7) | 43** (2.0) |
| Infiltration Cellular, Lymphoid | 2 (1.0) | 0 | 2 (1.5) | 10** (1.7) |
| Squamous Cell Carcinoma[j] | | | | |
| Overall rate | 0/50 (0%) | 1/50 (2%) | 1/50 (2%) | 4/50 (8%) |
| Adjusted rate | 0.0% | 2.3% | 2.3% | 11.2% |
| Terminal rate | 0/35 (0%) | 0/30 (0%) | 0/32 (0%) | 2/19 (11%) |
| First incidence (days) | — | 722 | 677 | 680 |
| Poly-3 test | P = 0.011 | P = 0.507 | P = 0.501 | P = 0.040 |
| Glandular Stomach | 50 | 50 | 50 | 50 |
| Epithelium, Hyperplasia, Focal | 1 (2.0) | 19** (1.5) | 26** (1.9) | 28** (2.1) |
| Epithelium, Glands, Cyst | 8 (1.6) | 19* (1.3) | 13 (1.2) | 22** (1.8) |
| Infiltration Cellular, Lymphoid | 1 (1.0) | 14** (1.4) | 30** (1.7) | 28** (1.7) |
| Mineralization | 1 (1.0) | 4 (1.0) | 3 (1.0) | 3 (1.7) |

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals necropsied
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[d] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 1/200 (0.5% ± 1.0%), range 0%-2%; all routes: 6/1,449 (0.4% ± 1.0%), range 0%-4%
[e] Number of animals with neoplasm per number of animals necropsied
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Not applicable; no neoplasms in animal group
[i] Beneath the vehicle 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 vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[j] Historical incidence for corn oil gavage studies: 0/198; all routes: 3/1,498 (0.2% ± 0.6%), range 0%-2%

***Skin:*** The incidences of subcutaneous fibrosarcoma and fibrosarcoma or malignant schwannoma (combined) were significantly increased in 30 mg/kg females (Tables 28, D1, and D2). Fibrosarcomas were characterized by a poorly demarcated, hypercellular mass of ovoid to spindle-shaped cells with a detectable intracellular collagenous matrix. Malignant schwannomas were characterized by a poorly demarcated, hypercellular mass of ovoid to spindle-shaped cells with a variable intracellular matrix ranging from scanty to prominent, consisting of a mucinous, amorphous material. For the statistical analysis, it is considered appropriate to combine the incidences of fibrosarcomas and schwannaomas because both are cutaneous tumors of mesenchymal origin.

The skin lesions observed grossly were characterized histologically as chronic active inflammation, dermal fibrosis, and epidermal hyperplasia and ulcers (Tables 28, C4, and D4) (Plate 23). The incidences of these lesions in females were dose related, and all were significantly greater in the 30 mg/kg group than in the vehicle control group. While not dose related in males due to high mortality, the incidences in all male dosed groups, except dermal fibrosis at 30 mg/kg, were significantly greater than the vehicle control group incidences.

Dorsal skin biopsies were taken routinely from all animals for histological evaluation; no clinical or macroscopic abnormalities were reported at this site. However, histopathologic examination of these sites revealed dose-related changes in males and females. The most obvious morphologic change was cystic dilatation of the infundibular segment of the hair follicle. The incidences of follicular dilatation were significantly increased in 10 and 30 mg/kg males and females (Tables 28, C4, and D4). The lumens of these dilated follicles contained variable quantities of delicate, desquamated keratin particles, and the lining follicular keratinocytes were generally more flattened squamous cells compared to the more normally present low cuboidal keratinocyte. The severity of follicular dilatation did not differ across dosed groups for males or females. Minimal severity was indicated when two to three follicles segmentally dilated to more than double the normal diameter, mild severity was four or five follicles more than double the normal diameter, and moderate was six or more. This dilatation was not accompanied by the presence of inflammatory cells (Plates 24 and 25). The associated sebaceous glands were either normal, atrophic due to reduced numbers of cells, or completely absent. The incidences of sebaceous gland atrophy were significantly increased in 10 and 30 mg/kg males and 30 mg/kg females, and severity increased with increasing dose in females. The severity grade was minimal when the total sebaceous gland area was reduced by approximately 10%, mild when reduced by approximately 33%, moderate when reduced by approximately 50%, and marked when reduced by more than 50%. No statistical correlation was found between the presence of follicular dilation and the presence of sebaceous gland atrophy.

***Malignant Lymphoma:*** Significantly increased incidences of malignant lymphoma occurred in 10 and 30 mg/kg females (Tables 29, D1, and D2). The lymphoma involved the spleen and various lymph nodes (i.e., mesenteric, mediastinal, and mandibular). Lymphoma was diagnosed when there was an effacement of the normal lymphoid architecture of the node due to increased numbers of lymphocytes. This effacement of the normal lymph node or splenic structure was the main feature differentiating hyperplasia from neoplasia; numerous mitoses could be present in hyperplastic nodes, as were blast-like cells.

***Genital System:*** In the coagulating gland, increased incidences of dilatation, chronic active inflammation, and fibrosis occurred in all dosed male groups (Tables 30 and C4). In the seminal vesicle, the incidences of these lesions were significantly increased in the 10 and 30 mg/kg groups. The incidences of chronic active inflammation were significantly increased in the prostate gland of all dosed groups of males. In the epididymis, increased incidences of sperm granuloma were observed in dosed male groups. Because these findings were present in mice with urethral neoplasms, they should be considered secondary effects.

In the clitoral gland, there were significant increases in atrophy and cystic ducts in all dosed females and chronic active inflammation in 10 and 30 mg/kg females (Tables 30 and D4). In the ovary, significantly increased incidences of atrophy occurred in all dosed females.

**TABLE 28**
**Incidences of Neoplasms and Nonneoplastic Lesions of the Skin in Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Number Necropsied | 50 | 50 | 49 | 50 |
| Inflammation, Chronic Active[a] | 3 (2.7)[b] | 13** (3.3) | 12** (3.3) | 5* (3.4) |
| Dermis, Fibrosis | 3 (3.0) | 12** (3.0) | 12** (3.1) | 4 (3.5) |
| Epidermis, Hyperplasia | 3 (3.0) | 13** (2.4) | 12** (3.3) | 6* (2.8) |
| Epidermis, Ulcer | 3 (4.0) | 13** (4.0) | 11** (4.0) | 6* (3.8) |
| Hair Follicle, Dilatation | 7 (1.3) | 10 (1.2) | 13* (1.2) | 28** (1.4) |
| Sebaceous Gland, Atrophy | 13 (2.8) | 10 (2.1) | 20** (2.6) | 29** (2.2) |
| **Female** | | | | |
| Number Necropsied | 50 | 50 | 50 | 50 |
| Inflammation, Chronic Active | 0 | 2 (3.0) | 6* (3.2) | 11** (3.6) |
| Dermis, Fibrosis | 0 | 1 (4.0) | 4 (3.8) | 11** (3.3) |
| Epidermis, Hyperplasia | 1 (2.0) | 2 (2.5) | 4 (3.8) | 11** (3.2) |
| Epidermis, Ulcer | 0 | 1 (4.0) | 4 (4.0) | 11** (4.0) |
| Hair Follicle, Dilatation | 2 (1.0) | 0 | 11** (1.3) | 23** (1.4) |
| Sebaceous Gland, Atrophy | 6 (2.2) | 10 (2.7) | 11 (2.9) | 15* (3.0) |
| Fibrosarcoma, Multiple | 0 | 0 | 0 | 1 |
| Fibrosarcoma (includes multiple)[c] | 1 | 6 | 5 | 8** |
| Malignant Schwannoma[d] | 1 | 2 | 2 | 4 |
| Fibrosarcoma or Malignant Schwannoma | | | | |
| Overall rate[e] | 2/50 (4%) | 8/50 (16%) | 7/50 (14%) | 12/50 (24%) |
| Adjusted rate[f] | 4.6% | 17.6% | 15.6% | 30.2% |
| Terminal rate[g] | 0/35 (0%) | 3/30 (10%) | 2/32 (6%) | 2/19 (11%) |
| First incidence (days) | 546 | 661 | 477 | 477 |
| Poly-3 test[h] | P = 0.004 | P = 0.051 | P = 0.084 | P = 0.001 |
| Fibrosarcoma Alone | | | | |
| Overall rate | 1/50 (2%) | 6/50 (12%) | 5/50 (10%) | 8/50 (16%) |
| Adjusted rate | 2.3% | 13.3% | 11.4% | 21.2% |
| Terminal rate | 0/35 (0%) | 3/30 (10%) | 2/32 (6%) | 2/19 (11%) |
| First incidence (days) | 606 | 661 | 477 | 477 |
| Poly-3 test | P = 0.023 | P = 0.062 | P = 0.105 | P = 0.008 |

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 3/198 (1.5% ± 1.0%), range 0%-2%; all routes: 30/1,498 (2.0% ± 2.6%), range 0%-8%
[d] Historical incidence for corn oil gavage studies: 3/198 (1.5% ± 2.0%), range 0%-4%: all routes, 10/1,498 (0.7% ± 1.3%), range 0%-4%
[e] Number of animals with neoplasm per number of animals necropsied
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle 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 vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

**TABLE 29**
**Incidences of Malignant Lymphoma in Female Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| Overall rate[a,b] | 2/50 (4%) | 5/50 (10%) | 8/50 (16%) | 7/50 (14%) |
| Adjusted rate[c] | 4.7% | 11.1% | 18.2% | 18.7% |
| Terminal rate[d] | 2/35 (6%) | 1/30 (3%) | 5/32 (16%) | 3/19 (16%) |
| First incidence (days) | 731 (T) | 664 | 541 | 50 |
| Poly-3 test[e] | P = 0.065 | P = 0.236 | P = 0.049 | P = 0.050 |

(T) Terminal sacrifice
[a] Number of animals with malignant lymphoma per number of animals necropsied
[b] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 26/198 (13.2% ± 7.6%), range 4%-22%; all routes: 307/1,498 (20.5% ± 9.7%), range 4%-54%
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle 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 vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

Chronic active inflammation in male and female mice consisted of a mixed population of lymphocytes, plasma cells, neutrophils, and macrophages. Atrophy of the clitoral gland was characterized by a decrease in the glandular acini, leaving only an enlarged duct lined by stratified squamous epithelium. Ovarian atrophy was characterized by small ovaries with decreased numbers of corpora lutea and increased numbers of interstitial cells.

***Bone Marrow:*** Significantly increased incidences of hyperplasia occurred in the 10 and 30 mg/kg male and female groups (Tables 31, C4, and D4). Because this finding was present in mice with urethral neoplasms, it is considered a secondary effect.

***Spleen:*** Increased incidences of atrophy (significant in dosed males), related to the depletion of lymphoid tissue, occurred in all dosed male and female groups (Tables 31, C4, and D4). Significantly increased incidences of hematopoietic cell proliferation occurred in all dosed female groups.

***Thymus:*** There was a dose-related increased incidence of atrophy, related to the depletion of cortical lymphoid tissue in dosed males, and the incidences in 10 and 30 mg/kg males and 30 mg/kg females were significantly greater than the vehicle control incidences (Tables 31, C4, and D4).

***Liver:*** Significantly increased incidences of hematopoietic cell proliferation occurred in 3 and 10 mg/kg males and 30 mg/kg females (Tables 31, C4, and D4). The decreased incidence of hepatocellular adenoma or carcinoma (combined) in 100 mg/kg males (vehicle control, 34/50; 3 mg/kg, 31/50, 10 mg/kg, 20/49; 30 mg/kg, 2/50; Tables C1 and C2) was attributed to the significant mortality in this group.

***Heart and Blood Vessel:*** Significantly increased incidences of cardiomyopathy occurred in 10 and 30 mg/kg males and 30 mg/kg females (Tables 31, C4, and D4). Three incidences of necrosis of the heart occurred in 30 mg/kg males. The necrosis was isolated and was not accompanied by any of the other characteristics of cardiomyopathy. Significantly increased incidences of mineralization occurred in the aorta of 10 and 30 mg/kg males. Cardiomyopathy was characterized by increased fibrosis and mononuclear cell infiltrates within the myocardium, usually in the ventricular wall.

**TABLE 30**
**Incidences of Nonneoplastic Lesions of the Genital System of Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Male** | | | | |
| Coagulating Gland[a] | 50 | 50 | 49 | 50 |
| Dilatation[b] | 9 (1.3)[c] | 16* (2.6) | 36** (2.6) | 47** (3.0) |
| Inflammation, Chronic Active | 0 | 6* (1.7) | 22** (2.2) | 33** (1.9) |
| Fibrosis | 0 | 6* (2.0) | 23** (2.5) | 30** (3.0) |
| Seminal Vesicle | 50 | 50 | 49 | 50 |
| Dilatation | 12 (2.3) | 14 (2.9) | 38** (2.9) | 41** (3.4) |
| Inflammation, Chronic Active | 0 | 4 (2.3) | 14** (2.6) | 22** (2.0) |
| Fibrosis | 0 | 3 (2.0) | 18** (2.7) | 29** (2.9) |
| Prostate Gland | 50 | 50 | 49 | 50 |
| Inflammation, Chronic Active | 3 (1.3) | 10* (2.8) | 27** (2.7) | 44** (2.2) |
| Epididymis | 50 | 50 | 49 | 50 |
| Granuloma Sperm | 1 (4.0) | 3 (2.3) | 6* (3.3) | 8** (3.0) |
| **Female** | | | | |
| Clitoral Gland | 49 | 50 | 49 | 50 |
| Atrophy | 25 (3.3) | 44** (3.3) | 37** (3.6) | 40** (3.6) |
| Duct, Cyst | 5 (3.0) | 46** (2.6) | 43** (3.1) | 43** (3.5) |
| Inflammation, Chronic Active | 3 (1.7) | 4 (1.5) | 17** (2.1) | 25** (2.3) |
| Ovary | 49 | 50 | 50 | 50 |
| Atrophy | 29 (3.3) | 44** (3.4) | 47** (3.4) | 45** (3.7) |

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
a Number of animals with tissue examined microscopically
b Number of animals with lesion
c Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

**TABLE 31**
**Incidences of Selected Nonneoplastic Lesions of Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | | 3 mg/kg | | 10 mg/kg | | 30 mg/kg | |
|---|---|---|---|---|---|---|---|---|
| **Male** | | | | | | | | |
| Bone Marrow[a] | 50 | | 50 | | 49 | | 50 | |
| Hyperplasia[b] | 13 | (4.0)[c] | 10 | (4.0) | 17* | (3.9) | 20** | (4.0) |
| Spleen | 50 | | 50 | | 49 | | 49 | |
| Atrophy | 2 | (2.5) | 9* | (2.9) | 30** | (3.2) | 39** | (3.0) |
| Hematopoietic Cell Proliferation | 18 | (2.9) | 16 | (3.0) | 12 | (3.5) | 7 | (2.7) |
| Thymus | 41 | | 42 | | 40 | | 49 | |
| Atrophy | 11 | (3.5) | 15 | (3.9) | 30** | (3.9) | 45** | (3.7) |
| Liver | 50 | | 50 | | 49 | | 50 | |
| Hematopoietic Cell Proliferation | 2 | (1.5) | 9* | (1.4) | 9** | (1.4) | 3 | (1.3) |
| Heart | 50 | | 50 | | 49 | | 50 | |
| Necrosis | 0 | | 0 | | 0 | | 3* | (2.0) |
| Cardiomyopathy | 5 | (1.4) | 5 | (2.2) | 17** | (1.7) | 9** | (1.4) |
| Blood Vessel | 50 | | 50 | | 49 | | 50 | |
| Aorta, Mineralization | 0 | | 0 | | 6** | (1.2) | 8** | (1.4) |
| **Female** | | | | | | | | |
| Bone Marrow | 50 | | 50 | | 50 | | 50 | |
| Hyperplasia | 2 | (4.0) | 8 | (4.0) | 9* | (4.0) | 16** | (4.0) |
| Spleen | 49 | | 50 | | 50 | | 50 | |
| Atrophy | 1 | (4.0) | 3 | (3.7) | 6 | (2.8) | 3 | (3.3) |
| Hematopoietic Cell Proliferation | 10 | (3.0) | 25** | (2.7) | 21* | (2.6) | 33** | (2.7) |
| Thymus | 48 | | 48 | | 45 | | 48 | |
| Atrophy | 7 | (3.3) | 15 | (3.5) | 12 | (3.9) | 25** | (3.8) |
| Liver | 49 | | 50 | | 50 | | 50 | |
| Hematopoietic Cell Proliferation | 3 | (1.3) | 9 | (1.3) | 9 | (1.6) | 21** | (1.7) |
| Heart | 49 | | 50 | | 50 | | 50 | |
| Cardiomyopathy | 3 | (1.3) | 5 | (1.2) | 4 | (1.3) | 9* | (1.7) |

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

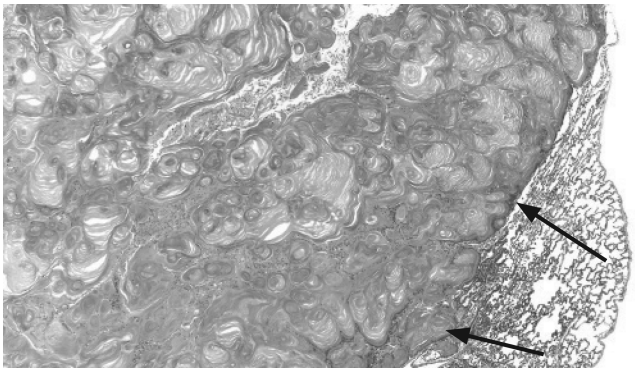


**Plate 1**
Cystic keratinizing epithelioma in the lung of a male Harlan Sprague-Dawley rat administered 100 mg/kg TCAB by gavage for 2 years. Note the cystic structure consisting of a highly irregular wall of highly keratinized stratified squamous epithelium and a center filled with keratin. The outer portion of the lesion grew by expansion into the adjacent lung but evidence of invasion was not observed (arrows). H&E

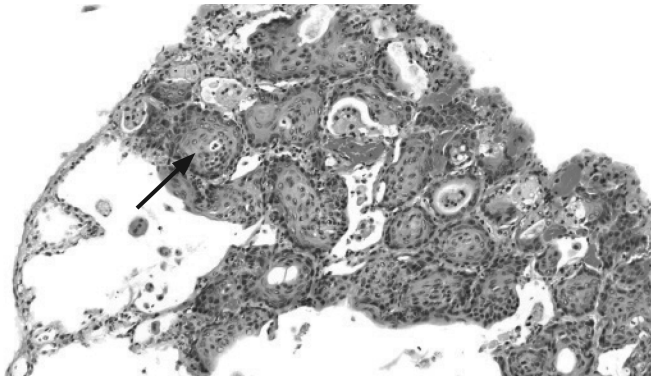


**Plate 2**
Alveolar epithelium squamous metaplasia in the lung of a male Harlan Sprague-Dawley rat administered 100 mg/kg TCAB by gavage for 2 years. Note the multiple, small, irregular foci (arrow) of keratinized stratified squamous epithelium that replace the normal alveolar epithelium. H&E

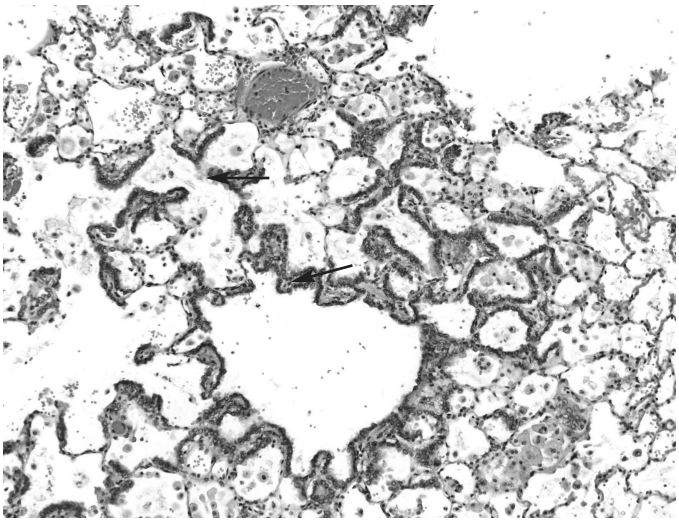


**Plate 3**
Histological section of pulmonary alveolar brochiolar metaplasia in a male rat treated for two years with 100 mg/kg of TCAB. The bronchiolar metaplasia of the alveolar epithelium consists of replacement of the normal alveolar epithelium by cuboidal to columnar, sometimes ciliated cells, and often accompanied by mucus production in the epithelium located at the bronchiolar-alveolar junction and adjacent alveoli (arrows). Aggregates of alveolar histiocytes are also present. H&E. x 16.

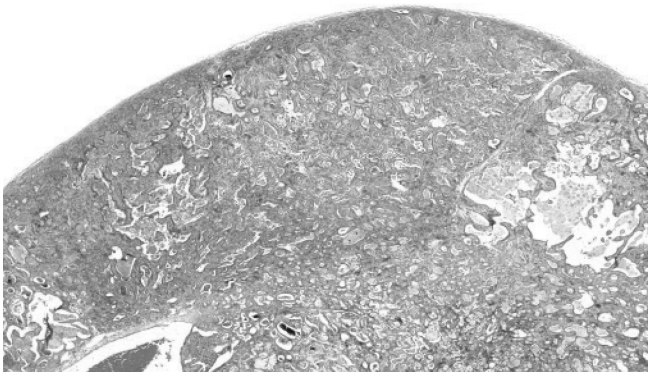


**Plate 4**
Cholangiocarcinoma in the liver of a male Harlan Sprague-Dawley rat administered 30 mg/kg TCAB by gavage for 2 years. Note the irregular, relatively large, noncircumscribed lesion that replaces normal liver parenchyma. The lesion consists of fibrous connective tissue stroma containing numerous atypical bile ducts, which frequently contain mucinous material and cellular debris. H&E

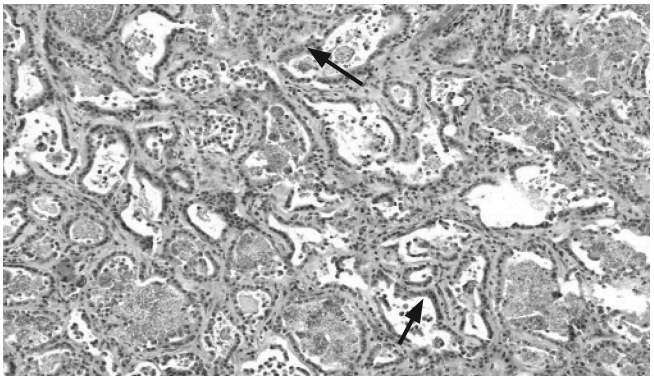


**Plate 5**
Higher magnification of the cholangiocarcinoma in Plate 4. The epithelium forming the atypical bile ducts is often discontinuous, usually consisting of large atypical cells and displaying degenerative changes (arrows). H&E

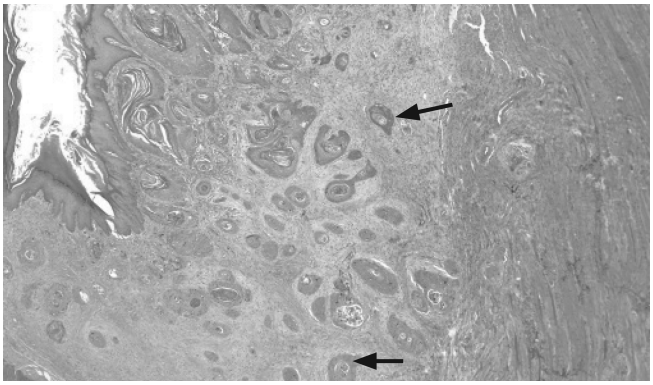


**Plate 6**
Squamous cell carcinoma in the oral cavity mucosa at nasal Section III (at the level of the molars) in a female Harlan Sprague-Dawley rat administered 100 mg/kg TCAB by gavage for 2 years. Note the irregular cords and clusters of stratified squamous epithelial cells that invade deeply into the underlying connective tissue (arrows). H&E

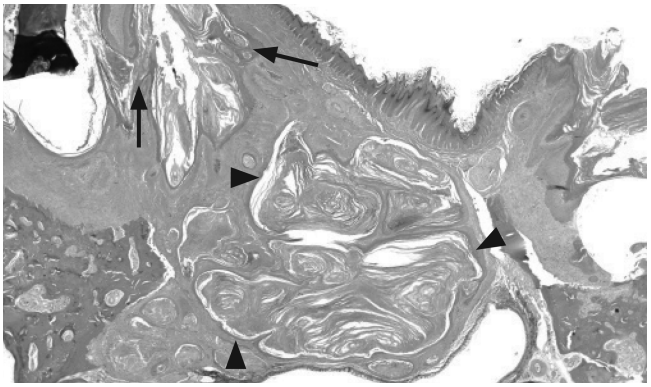


**Plate 7**
Oral cavity mucosa at nasal Section III (at the level of the molars) in a male Harlan Sprague-Dawley rat administered 100 mg/kg TCAB by gavage for 2 years. Note the presence of gingival squamous hyperplasia (arrows) and gingival cystic keratinizing hyperplasia (arrowheads). H&E

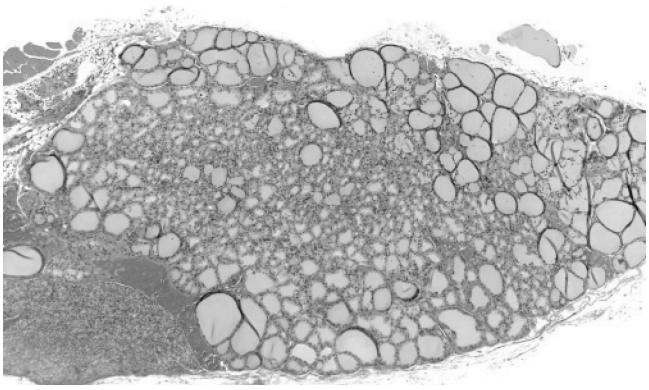


**PLATE 8**
Normal thyroid gland follicles in a female Harlan Sprague-Dawley vehicle control rat at 2 years in the gavage study of TCAB. H&E

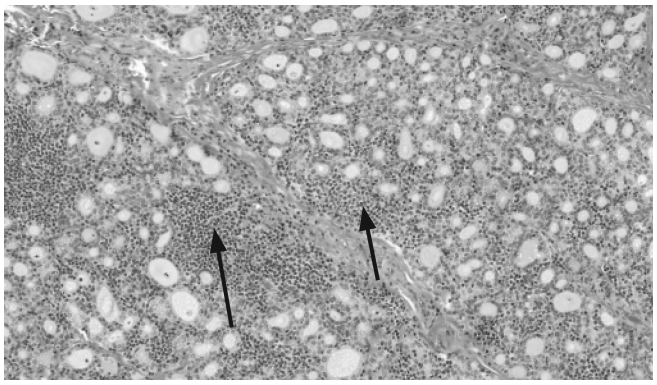


**PLATE 9**
Follicular cell hyperplasia in the thyroid gland of a female Harlan Sprague-Dawley rat administered 100 mg/kg TCAB by gavage for 2 years. The lesion consists of very large numbers of small follicles and densely packed follicles lined by cuboidal to low columnar epithelium. There is also inflammation, characterized by diffusely scattered aggregates of moderate to large numbers of lymphocytes within the interstitium between follicles (arrows). H&E

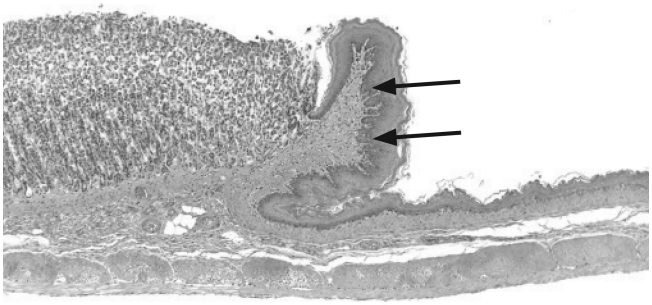


**PLATE 10**
Normal epithelium at the limiting ridge (arrows) of the forestomach in a male Harlan Sprague-Dawley vehicle control rat at 2 years in the gavage study of TCAB. H&E

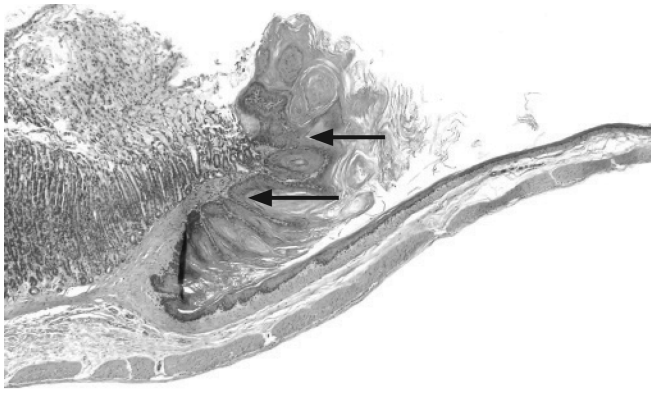


**PLATE 11**
Epithelial hyperplasia at the limiting ridge of the forestomach in a male Harlan Sprague-Dawley rat administered 100 mg/kg TCAB by gavage for 2 years. Note the presence of irregular papillary thickening of the epithelium due to an increase in the number of cell layers, accompanied by an increase in the overlying keratin layer (arrows). H&E

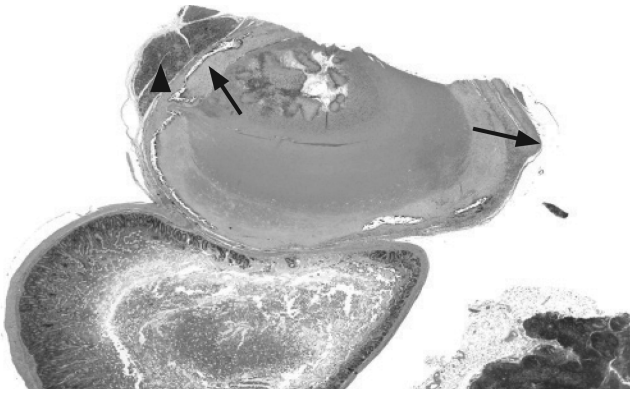


**Plate 12**
Inflammation of the blood vessels (arrows) associated with aneurism close to the pancreas (arrowhead) in a male Harlan Sprague-Dawley rat administered 100 mg/kg TCAB by gavage for 2 years. Note the great dilation of the vessel (aneurism). H&E

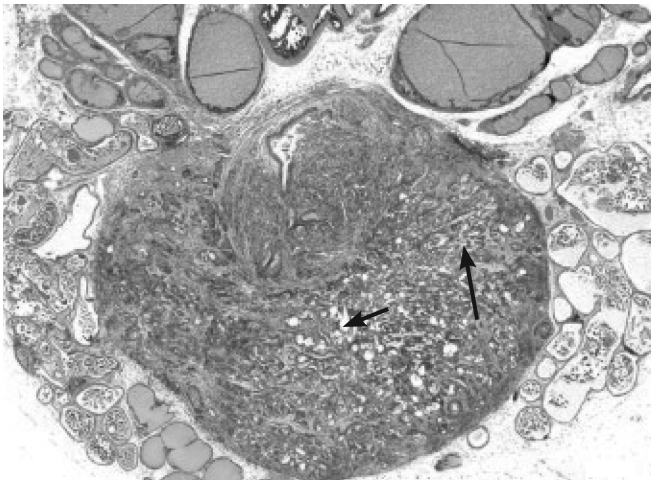


**Plate 13**
Carcinoma in the urethra of a male B6C3F1 mouse administered 10 mg/kg TCAB by gavage for 2 years. Note the poorly demarcated, hypercellular proliferation of neoplastic epithelium having a glandular appearance (arrows) with invasion into the submucosal compartment. H&E

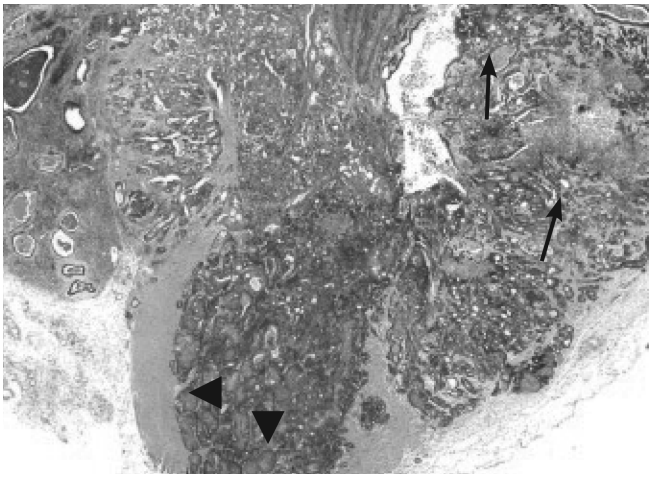


**Plate 14**
Carcinoma in the urethra of a male B6C3F1 mouse administered 10 mg/kg TCAB by gavage for 2 years. Note the poorly demarcated, hypercellular proliferation of neoplastic epithelium with mixed glandular (arrows) and solid (arrowheads) appearance and invasion into the submucosal compartment. H&E

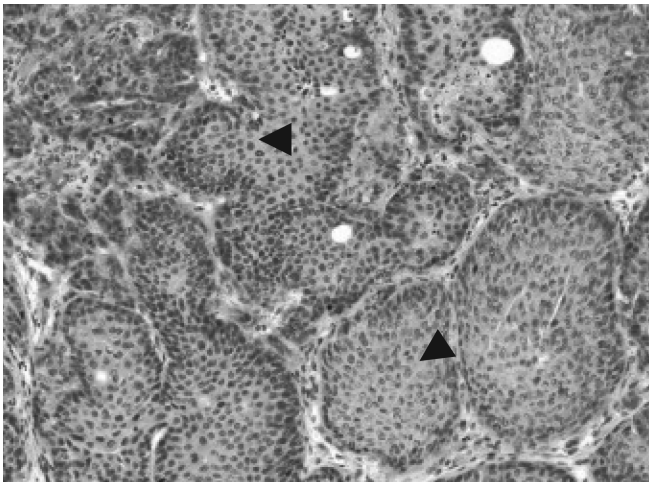


**Plate 15**
Higher magnification of Plate 14. Note the hypercellular proliferation of neoplastic epithelium with a solid appearance (arrowheads). H&E

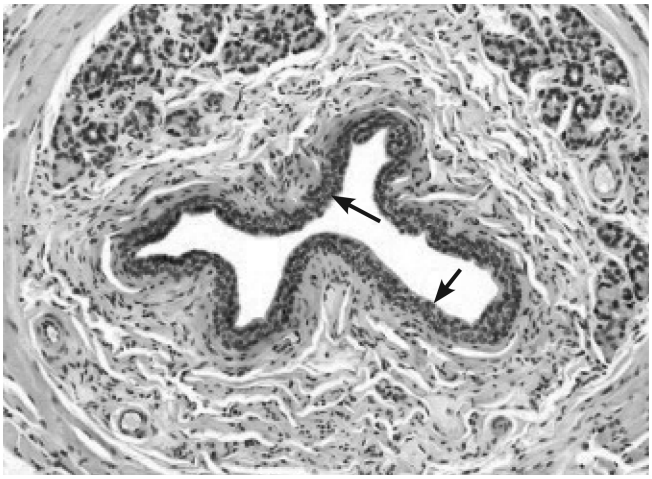


**Plate 16**
Normal epithelium (arrows) lining the urethra in a male B6C3F1 vehicle control mouse at 2 years in the gavage study of TCAB. H&E

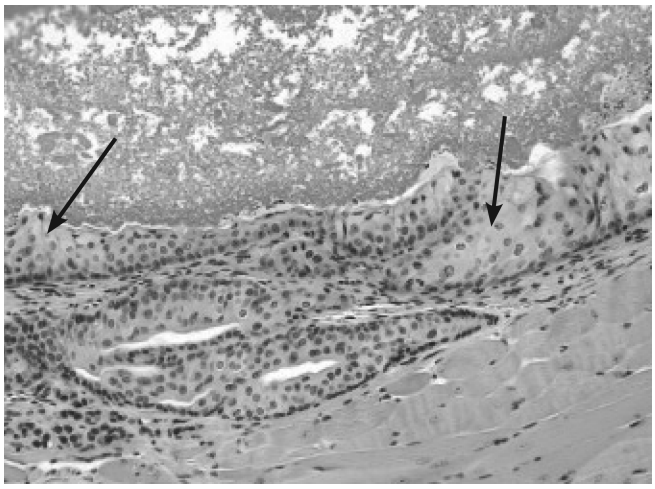


**Plate 17**
Transitional epithelium hyperplasia in the urethra of a male B6C3F1 mouse administered 10 mg/kg TCAB by gavage for 2 years. Note the localized and well-demarcated increase in the number of urothelial cells (arrows). H&E

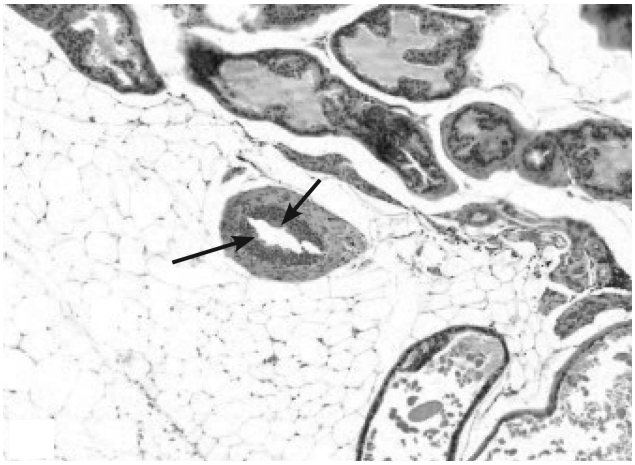


**Plate 18**
Normal epithelium (arrows) lining the ureter in a female B6C3F1 vehicle control mouse at 2 years in the gavage study of TCAB. H&E

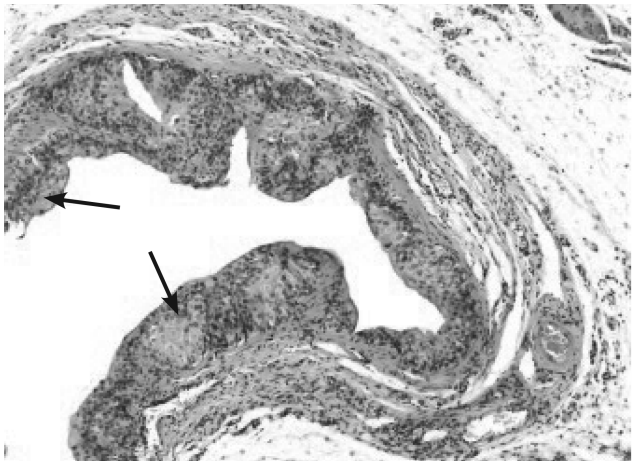


**Plate 19**
Transitional epithelium hyperplasia in the ureter of a female B6C3F1 mouse administered 30 mg/kg TCAB by gavage for 2 years. Note the localized and well-demarcated increase in the number of urothelial cells (arrows). H&E

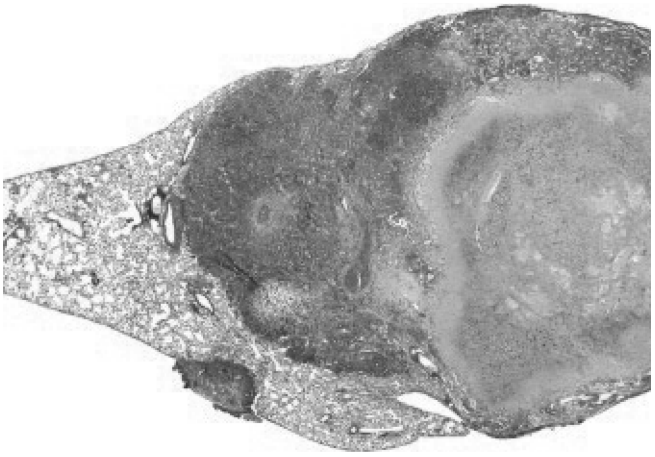


**PLATE 20**
Alveolar/bronchiolar carcinoma in the lung of a male B6C3F1 mouse administered 3 mg/kg TCAB by gavage for 2 years. Note that the mass, partially necrotic, is distorting the normal septal architecture with peripheral compression and invasion. H&E

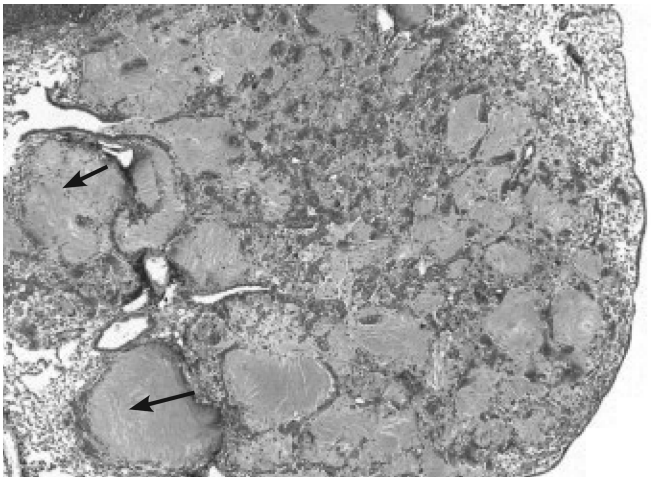


**PLATE 21**
Cystic keratinizing epithelioma in the lung of a female B6C3F1 mouse administered 30 mg/kg TCAB by gavage for 2 years. The lesion is composed of clusters of squamous cells and keratin (arrows) but lacks the clear cyst-like component typical of these lesions. H&E

**Plate 22**
Focal epithelium hyperplasia in the glandular stomach of a male B6C3F1 mouse administered 10 mg/kg TCAB by gavage for 2 years. Note the disruption and disorganization of the normal glandular epithelium adjacent to the limiting ridge by the formation of small clusters of glandular epithelial cells, sometimes with the appearance of squamous metaplasia (arrows). H&E

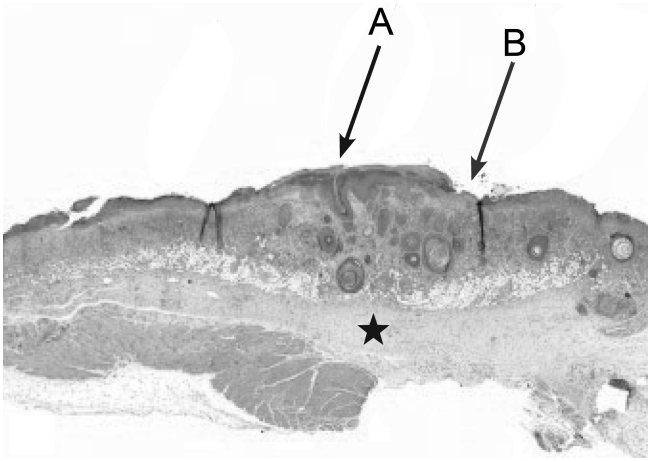



**Plate 23**
Histological section of the skin of a female B6C3F1 mouse administered 30 mg/kg TCAB by gavage for 2 years. Macroscopically, the mouse had ulceration on the torso, which histologically corresponds to chronic active inflammation accompanied by epithelial hyperplasia (A), ulceration (B), and dermal fibrosis (star). H&E

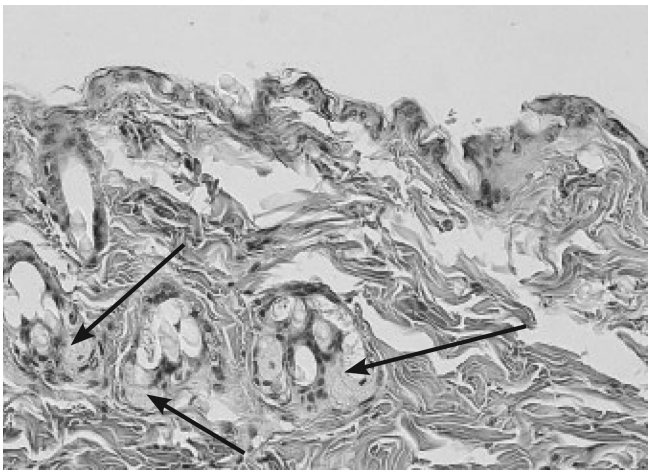


**Plate 24**
Normal hair follicles with adjacent sebaceous glands (arrows) in a male B6C3F1 vehicle control mouse at 2 years in the gavage study of TCAB. H&E

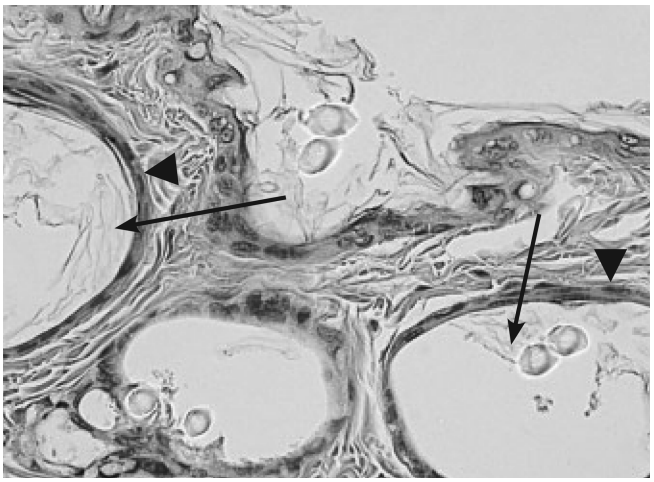


**Plate 25**
Dilatation of the hair follicles, atrophy of the lining epithelia (arrowheads) and of the adjacent sebaceous glands, and keratin debris (arrows) in the follicular lumena in the skin of a male B6C3F1 mouse administered 30 mg/kg TCAB by gavage for 2 years. Note that there is no inflammatory cell infiltration. Arrowheads indicate flattened epithelia lining the hair follicles. H&E

# DISCUSSION AND CONCLUSIONS

TCAB was nominated by the United States Environmental Protection Agency for toxicity and carcinogenicity testing based on its structural and biological similarity to TCDD and potential for human exposure from the consumption of crops contaminated with 3,4-dichloroaniline-derived herbicides including Propanil®, Linuron®, and Diuron®. In 2006, the estimate for total domestic use of Propanil® was approximately 7 million pounds of active ingredient; 50% to 70% of the United States rice crop is treated with Propanil® (USEPA, 2006). The concentration of TCAB in Propanil® ranges from 1,000 to 2,700 µg/g. With a production volume of approximately 10 million pounds of Propanil® per year, the resultant amount of TCAB produced as a contaminant could be as high as 12,000 kg per year (McMillan *et al.*, 1991; Van Birgelen *et al.,* 1999a).

In the *trans* configuration, TCAB can assume a planar conformation similar to that of TCDD. TCAB has been shown to bind to the aryl hydrocarbon receptor (AhR) with a specific binding affinity of one-fifth that of TCDD (Poland *et al.*, 1976; Schneider *et al.*, 1995). Previous subchronic studies conducted by the NTP demonstrated that administration of TCAB to rats and mice caused typical dioxin-like effects including body weight loss, thymic atrophy, induction of cytochrome P450 1A1, hepatotoxicity, anemia, and developmental toxicity (NTP, 1998a, Van Birgelen *et al.,* 1999a, Hooth *et al.*, 2002). Using thymic atrophy as the endpoint, Van Birgelen *et al.* (1999a) concluded that TCAB is about five to six orders of magnitude less potent than TCDD. These characteristics and the potential for human exposure via food warranted characterization of the chronic toxic and carcinogenic effects of TCAB and comparison of these effects with TCDD and other dioxin-like compounds. Currently, TCAB is not included in the Toxic Equivalency Factor (TEF) methodology used by the International Programme on Chemical Safety and the World Health Organization (Van den Berg, 2006). However, based on the calculated amount of TCAB produced and the relative potency of TCAB compared to TCDD, Van Birgelen *et al.* (1999a) estimated that TCAB could account for more dioxin-like activity in the environment than polychlorinated dibenzo-*p*-dioxins and dibenzofurans together.

The NTP performed 2-week and 3-month studies of TCAB in male and female F344/N rats and B6C3F1 mice that aided in the design and dose selection for the current 3-month and 2-year studies (NTP, 1998a; Van Birgelen *et al.*, 1999a). Initial dose selection for the 2-week studies was based on TCAB studies reported in the literature (NTP, 1998a). The current 3-month study was conducted in female Harlan Sprague-Dawley rats to compare the toxicity of TCAB with that of TCDD and other dioxin-like compounds that were evaluated by the NTP in this strain as part of the TEF evaluation studies. These studies also allowed comparison of the subchronic toxicity of TCAB in two different rat strains. In the current 3-month study in Harlan Sprague-Dawley rats, histopathologic lesions included hepatocellular hypertrophy and hematopoietic cell proliferation in the liver of males and females and increased incidences of metaplasia of the alveolar epithelium in males. These lesions were not observed in the 3-month study in F344/N rats (NTP, 1998a). However, increased incidences of hematopoietic cell proliferation in the spleen and thymic atrophy were observed in both rat strains (NTP, 1998a).

The hematology results indicated that TCAB induced a responsive anemia in male Harlan Sprague-Dawley rats similar to what was reported in the 3-month gavage study of TCAB and 3,3′,4,4′-tetrachloroazoxybenzene, a structurally related compound, in F344/N rats (NTP, 1998a,b; Van Birgelen *et al.,* 1999a,b). An erythropoietic response to the anemia was observed as evidenced by increased reticulocyte counts and hematopoietic cell proliferation observed microscopically in the liver and spleen. In the F344/N rat studies (NTP, 1998a,b), the mean cell volume was increased suggesting the erythrocytes were macrocytic. In contrast, the mean cell volume was decreased in the present study indicating microcytic erythrocytes and suggesting an effect on heme, hemoglobin, or erythrocyte production. Development of a normocytic, normochromic anemia consistent with early stage aplastic anemia was reported

for TCAB administered to rats by dosed feed (Hsia *et al.*, 1980). Reticulocyte counts to evaluate the erythropoietic capability of their test animals were not performed (Hsia *et al.*, 1980). TCDD and pentachlorodibenzofuran also caused a responsive anemia (Kociba *et al.*, 1976; Pluss *et al.*, 1988) and decreased platelet counts in male and female Sprague-Dawley rats (Weissberg and Zinkl, 1973; Kociba *et al.*, 1976; Pluss *et al.*, 1988). Decreased platelet counts were observed in the NTP F344/N rat TCAB and 3,3′,4,4′-tetrachloroazoxybenzene studies (NTP, 1998a,b), but thrombocytopenia did not occur in the present study.

The etiology of the responsive anemia is unknown. There was an increase of hemosiderin accumulation in the spleen (female rats only) which could suggest increased erythrocyte injury and turnover and consequent extravascular hemolytic anemia; the same change was also observed in the F344/N rat study (NTP, 1998a). Likewise, a number of structurally related compounds including 2,5-dichloroaniline, 2-chloroaniline, 3-chloroaniline, and 4-chloroaniline hydrochloride induce a dose-dependent hemolytic anemia and methemoglobinemia with compensatory responses in the erythropoietic system of bone marrow, spleen, liver, and kidney (ECB, 2006). However, in the current studies there were no changes in methemoglobin concentration and erythrocyte osmotic fragility evaluations. Thus, there was no evidence that TCAB increased erythrocyte injury.

Increased expression of CYP1A1 and CYP1A2 are characteristic responses to dioxin-like compounds in the liver and are directly linked to binding and activation of the aryl hydrocarbon receptor (AhR) by dioxin-like compounds (Whitlock, 1993). In many cases, the relative potency for induction of CYP1A1 *in vivo* is used as a surrogate for the dioxin-like activity of a given compound and is used in the assignment of TEFs (Van den Berg *et al.*, 1998, 2006). In this study, significant dose-dependent increases in hepatic and pulmonary CYP1A1 and hepatic CYP1A2 activities were observed in male and female rats as a result of TCAB administration. TCAB is approximately six to seven orders of magnitude less potent than TCDD using CYP1A1 induction as the end point (Gerken *et al.*, 2002).

Administration of TCAB to Harlan Sprague-Dawley rats for 3 months led to significant accumulation of TCAB in the fat, liver, and lung. The significant accumulation in fat is consistent with the lipophilic nature of TCAB. Previous studies of dioxin-like compounds indicate that the liver and fat are the main storage depots for dioxin-like compounds in rodents and together can comprise approximately 70% to 80% of the total body burden in rodents (DeVito *et al.*, 1995). The liver-to-fat ratio of dioxin-like compounds in rodents ranges from about 0.5 to 70 (DeVito *et al.*, 1995). "Hepatic sequestration" is a characteristic of some persistent dioxin-like compounds and is believed to be the result of binding of the compound to CYP1A2 whose expression is inducible by dioxin-like compounds in the liver (Diliberto *et al.*, 1997). By comparison, the liver-to-fat ratios in the current study ranged from 0.002 to 0.007 for male Harlan Sprague-Dawley rats and from 0.005 to 0.014 for female Harlan Sprague-Dawley rats indicating minimal CYP1A2-mediated sequestration of TCAB in the liver. These ratios were much lower than the liver-to-fat ratios of 0.1 to 0.2 reported in male Sprague-Dawley rats administered a single gavage dose of radiolabeled TCAB where the highest concentrations of TCAB were observed in the fat and kidney (Pillai *et al.*, 1996). One possible explanation for the diverse spectrum of neoplasms and nonneoplastic lesions observed in the 3-month and 2-year studies is that less sequestration in the liver and more distribution to fat in tissues resulted in greater systemic exposure to TCAB.

In the 2-year studies, significant mortality was observed in male rats and male and female mice. The mortality was unexpected and could not be predicted from the 3-month studies. Most of the deaths were attributed to the presence of neoplasms. Mean body weights of dosed rats and mice were less than those of the vehicle controls. Reduction in body weight gain is a characteristic toxic response to dioxin-like compounds and was also seen in other studies conducted as part of the NTP TEF evaluation studies (NTP, 2006a,b,c,d,e,f,g). Increased incidences of neoplasms and nonneoplastic lesions were observed in numerous tissues of Harlan Sprague-Dawley rats and B6C3F1 mice; nonneoplastic lesions were observed in a number of additional tissues. In contrast, lower incidences of mammary gland and pituitary gland neoplasms were observed following TCAB administration and may be related to the decrease in body weight gain seen in the higher dosed groups. A significant association between reduced body weight gain and lower incidences of mammary gland and pituitary gland neoplasms has been observed in many NTP studies (Seilkop, 1995).

The lung was a site of TCAB-induced neoplasms in rats and mice in the 2-year studies. There was clear evidence of carcinogenicity in male and female Harlan Sprague-

Dawley rats based on significantly increased incidences of cystic keratinizing epithelioma (CKE) of the lung that were dose-related. The incidences of multiple CKEs were also significantly increased in the females. CKEs were absent in the concurrent vehicle control animals and have not been observed in any female Harlan Sprague-Dawley vehicle controls in the NTP TEF evaluation studies (NTP, 2006a,b,c,d,e,f,g). The incidences of CKEs in the current study were greater than those observed following administration of TCDD or PCB 126 (NTP, 2006a,b). In female mice, two CKEs observed in the high dose group were considered to be related to TCAB exposure; no spontaneous CKEs have been observed in female mice in the current NTP historical control database. In mice, there were significantly increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined). The neoplastic response was stronger in the male mice leading to a conclusion of clear evidence of carcinogenicity in males; the increased incidences in females were also considered to be related to TCAB exposure.

There are at least two potential mechanisms involved in the increased incidences of neoplasms and nonneoplastic lesions in the lung. CYP1A1 is inducible in the lung by TCDD and dioxin-like compounds in several species (Beebe *et al.*, 1990; Walker *et al.*, 1995). The inducibility of CYP1A1 by TCDD is observable in Clara cells and bronchiolar cells and to a lesser degree in type II cells (Tritscher *et al.*, 2000). This indicates that the bronchiolar epithelium is clearly responsive to AhR ligands and suggests the potential for a direct effect on the lung. Induction of lung CYP1A1-associated 7-ethoxyresorufin-*O*-deethylase (EROD) activity was seen in the current 3-month study and in the other NTP studies of dioxin-like compounds (NTP, 2006a,b,c,d,e,f,g); however, there was significantly lower induction with TCAB compared to TCDD (NTP, 2006a). Another possible mechanism for the action of dioxin-like compounds on the lung may be an indirect effect due to the disruption of retinoid homeostasis in the liver. In rodents, mobilization of retinoid stores by TCDD and dioxin-like compounds leads to a disruption in retinoid homeostasis and vitamin A deficiency (Van Birgelen *et al.*, 1994, 1995a; Fiorella *et al.*, 1995; Fattore *et al.*, 2000; Schmidt *et al.*, 2003). A characteristic of retinoid deficiency is abnormal epithelial differentiation to a keratinized squamous phenotype (Lancillotti *et al.*, 1992; Lotan, 1994). The action of dioxin-like compounds may therefore be a disruption of retinoid action leading to altered growth and differentiation of the lung epithelium resulting in squamous metaplasia and ultimately neoplasia.

The forestomach was also a site of TCAB-induced neoplasms in Harlan Sprague-Dawley rats and B6C3F1 mice. In female rats, increased incidence of forestomach squamous cell papilloma or squamous cell carcinoma (combined) in the high dose group that exceeded the historical control range, was considered to be related to TCAB exposure. In mice, there were significant increases in the incidences of carcinoma in the high dose males and females. In addition, the incidences of hyperplasia were significantly increased in all dosed groups of male and female rats and mice. The background incidence of forestomach papilloma or carcinoma (combined) is low (less than 2%) in rats and mice. No intestinal tumors have been induced by TCDD and polychorinated biphenyls in the rodent models evaluated (Yoshizawa *et al.*, 2007).

In the 2-year mouse study, the incidence of a unique neoplasm, urethral transitional epithelial carcinoma, was significantly increased and compromised the survival of male mice. Two of these neoplasms were observed in the high dose females. Low incidences of nonneoplastic urethral lesions were observed in the 3-month mouse study. One 30 mg/kg male mouse had moderate hyperplasia of the urethra and one 0.1 mg/kg male mouse had mild hyperplasia of the urethra (data not shown). In the 2-year study, the neoplasms were often invasive in the surrounding tissues and organs (prostate gland, seminal vesicle, coagulating glands, ductus deferens, and skeletal muscle). TCAB administration was also associated with increased incidences of nonneoplastic lesions in the urinary tract and genital system of male mice. Most of the lesions in the urinary tract were considered to be secondary to obstruction and inflammation caused by urethral carcinomas. Many of these carcinomas appear to have arisen from the urethral glands because of their overall glandular pattern, location, and the fact that in some instances, the overlying transitional urothelium appeared to be unaffected. Some appear to be of transitional cell origin even when involving glands.

The urethra of the male mouse is divided into the membranous urethra and the penile urethra (Gaillard, 1999). The membranous urethra is located in the pelvic canal attached to the neck of the urinary bladder. The urethra forms a bulbous diverticulum near the posterior portion of the pelvic canal, which enters into the penis where it

is referred to as the penile urethra. The membranous urethra, urethral diverticulum and penile urethra are lined by transitional epithelium. In mice, the membranous urethra can be distinguished from the penile urethra by the presence of numerous glands called the urethral or periurethral glands (also called the urethral glands of Littré), which are located in the lamina propria of the membranous urethra. The urethral glands are lined by cuboidal epithelium and empty into the lumen of the membranous urethra. In rats, the urethral glands are located primarily surrounding the urethral diverticulum. In male rodents, several accessory sex organs are associated with the urethra including the prostate gland, seminal vesicles, coagulating glands, and ductus deferens. Studies in the literature suggest that the urethral glands in male mice are testosterone-dependent and may play a role in localized secretory immunity (Parr *et al.*, 1992, 1994). In male rats and mice, the glands contribute to the formation of semen and the copulation plug. The urethra of female rodents is relatively shorter than that in males. As in males, the lumen is lined by transitional epithelium and urethral glands are located in the lamina propria. In the female rat, occasional clusters of urethral glands are observed with ducts that empty into the lumen. The urethral glands are most developed at about 30 days of age and then undergo degeneration (Jokinen, 1990); this may partially explain the presence of fewer urethral tumors in female rats.

Spontaneous and chemically induced proliferative lesions are very rare in the lower urinary tract of rodents. No spontaneous neoplasms of the urethra and ureter have been recorded in male or female mice in the NTP historical control database. The exact incidences of the proliferative lesions in the urethra and ureter are not known since these tissues are not routinely examined microscopically in NTP studies. Only one other chemical tested by the NTP, nitrilotriacetic acid trisodium monohydrate, caused ureter transitional cell carcinoma in rodents (NCI, 1977). Chemically induced transitional epithelial tumors are rare and have been described mostly in the urinary bladder. In the urinary bladder, 24 chemicals induced tumors in one or more of the four gender-species experimental groups. Only three compounds induced urinary bladder tumors in male or female mice including *o*-anisidine hydrochloride (NCI, 1978a), *p*-cresidine (NCI, 1979), and 1,3-dichloropropene (Telone II®; NTP, 1985).

Structurally related chloroaniline compounds, such as 4-chloroaniline, are aromatic amines that were widely used in the dye, chemical, and pharmaceutical manufacturing industries. They are also persistent environmental degradation products of various herbicides. Several chloroaniline analogues were evaluated for carcinogenicity in experimental animals based on reports of high incidences of bladder cancer observed among dye manufacturing industry workers. However, IARC (1987) concluded that the evidence for carcinogenicity of aniline to humans was inadequate based on the conclusion that the excess of bladder cancer deaths observed in workers in the aniline-dye industry was attributed to exposure to chemicals other than aniline. 4-Chloroaniline hydrochloride induced fibrosarcomas in the spleen of male rats and hemangiosarcomas in the spleen and liver of male mice (NTP, 1989). Increased incidences of hepatocellular adenomas and carcinomas were also observed in male mice. Based on data in experimental animals, 4-chloroaniline was classified as possibly carcinogenic in humans (group 2B). 4-Chloro-*o*-phenylenediamine, another chemical containing a chloroaniline moiety, induced urinary bladder neoplasms in male and female rats (NCI, 1978b). No experimental animal data are available for 3,4-dichloroaniline and a number of other chemical analogues.

It is not clear why TCAB induced urethral and ureteral carcinomas only in mice and not in rats in the current studies. No neoplasms or nonneoplastic lesions were observed in the urinary tract of male rats. The mechanism of TCAB-induced urethral and ureteral carcinoma in mice is unclear and needs further investigation. It is also unclear whether the mechanism of these tumors is mediated through the AhR. Tumors of the urinary tract were not observed in a previous NTP 2-year gavage study of TCDD in B6C3F1 mice (NTP, 1982a). However, exposure to TCDD has been shown to cause proliferation of embryonic urothelium (Abbott *et al.*, 1987). The epithelial cells of the ureter express AhR and epithelial growth factor receptor (EGFR; Choi *et al.*, 2006). Expression of EGFR in ureter epithelium declines with age. TCDD inhibits the decrease in EGFR in ureter epithelium with subsequent proliferation of these cells (Abbott and Birnbaum, 1990). EGFR has also been shown to play an important role in *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine-induced bladder tumors in mice (El-Marjou *et al.*, 2001).

The NTP recently conducted 2-year bioassays in female Harlan Sprague-Dawley rats to evaluate the chronic toxicity and carcinogenicity of dioxin, dioxin-like compounds, structurally related polychorinated biphenyls

and mixtures of these compounds (NTP, 2006a,b,c,d,e,f,g). In these studies, increased incidences of neoplasms and nonneoplastic lesions occurred in several tissues including the liver, lung, oral mucosa, thyroid gland, and adrenal cortex. Overall, the spectrum of neoplasms and nonneoplastic lesions observed in the 2-year rat study of TCAB was comparable with that seen with TCDD and other dioxin-like compounds (Table 32; NTP, 2006a,b,c,d,e,f,g). However, much higher doses of TCAB were required to induce neoplasms confirming that the potency of TCAB is significantly less than that of TCDD and the other dioxin-like compounds (NTP, 2006a,b,c,d,e,f,g). Relative to TCDD, TCAB is rapidly metabolized and eliminated from the body resulting in a shorter elimination half-life and lower systemic bioavailability (Pillai *et al.,* 1996). Qualitative similarities and differences among TCAB and these compounds will be described here; quantitative analyses of tumor potency are beyond the scope of this Technical Report and will be reported in subsequent manuscripts.

Numerous studies have demonstrated that dioxin-like compounds induce hepatic neoplasms including cholangioma/cholangiocarcinoma, hepatocholangioma and hepatocellular adenoma and carcinoma (NTP, 2006a,b,c,d,e,f,g). Unlike the spectrum of hepatic neoplasms seen with the other dioxin-like compounds, only cholangiocarcinoma was observed in male and female rats administered TCAB for 2 years. The incidences of this malignant neoplasm were significantly increased in all dosed groups of males. In females, the incidence of cholangiocarcinoma in the 100 mg/kg group exceeded the historical control range. The incidences of cholangiocarcinoma in the current study are lower than those observed in the other NTP TEF evaluation studies (NTP 2006a,b,c). Spontaneous cholangioma and cholangiocarcinoma are rare in female Harlan Sprague-Dawley rats and were not observed in vehicle control animals from this group of studies. The incidence of cholangiofibrosis was significantly increased in 100 mg/kg females; cholangiofibrosis and cholangiocarcinoma appear to be a morphological continuum. Based on these observations, it was concluded that there was clear evidence of carcinogenicity in the liver of male rats; the increased incidences in females were also considered to be related to TCAB exposure.

The mechanism underlying the selectively increased incidences of biliary neoplasms is unknown. There has been a considerable amount of research examining the potential mode of action of dioxin-like compounds in the liver. There is a general scientific consensus that almost all responses require initial binding to the AhR. Given that dioxin-like compounds are not direct-acting genotoxic agents and are potent growth dysregulators, it is believed that their predominant mode of action is promoting the development of preneoplastic and neoplastic lesions. In short-term studies with TCDD there were significant increases in hepatocyte replication as demonstrated by BrdU labeling studies (Maronpot *et al.*, 1993; Walker *et al.*, 1998; Wyde *et al.*, 2001); however, increased hepatocyte replication was not observed following TCAB administration to Harlan Sprague-Dawley rats for 3 months. In the 2-year studies, TCAB administration increased the proliferation of hepatocytes and bile duct epithelial cells resulting in increased incidences of hepatic nonneoplastic lesions; however, hepatocellular neoplasms were not observed in this study. Cell damage and subsequent death of hepatocytes and/or bile duct cells may result in a repair process which involves the proliferation of these cell types and the formation of scar tissue (cholangiofibrosis). In addition, there may be a direct stimulatory effect on oval cells, the putative stem cells in the liver, as evidenced by the increased incidences of oval cell hyperplasia. Interestingly, neoplasms and nonneoplastic lesions were not observed in the liver of male or female mice. Increased incidences of hepatocellular carcinoma were observed in male and female mice administered TCDD by gavage for 2 years (NTP, 1982a). The factors contributing to this species difference in liver neoplasm induction are not known.

In the 2-year study, there was clear evidence of carcinogenicity in the oral mucosa of male and female rats administered TCAB. These neoplasms are rare in female Harlan Sprague-Dawley rats as evidenced by the mean historical control incidence of 1% for 2-year corn oil gavage studies. Increased incidences of gingival squamous cell hyperplasia and cystic keratinizing hyperplasia were increased in all dosed groups. In the current study, there was a morphological continuum from squamous hyperplasia to cystic keratinizing hyperplasia to squamous cell carcinoma. Cystic keratinizing hyperplasia was not diagnosed in the previous NTP TEF evaluation studies. It is not clear what the association is between gingival squamous cell hyperplasia and gingival squamous cell carcinoma. Based on the histopathologic similarity seen between these lesions during an examination of the H&E slides from the NTP TEF evaluation studies, Yoshizawa *et al.* (2005) suggested that gingival squamous cell hyperplasia may develop into gingival squamous cell carcinoma in animals treated with TCDD and

**TABLE 32**
**Histopathologic Neoplastic Effects of TCAB, TCDD, Pentachlorodibenzofuran, and Pentachlorobiphenyl 126 in 2-Year Gavage Studies in Harlan Sprague-Dawley Rats and B6C3F1 Mice[a]**

| | TCAB[b] | TCDD[c,d] | PeCDF[e] | PCB 126[f] |
|---|---|---|---|---|
| Adrenal Gland | | | | |
| Adenoma | +FR | — | — | +/– FR |
| Forestomach | | | | |
| Squamous cell papilloma or carcinoma | +FR, +MM, +FM | — | — | — |
| Liver | | | | |
| Cholangioma | — | +/–FR | — | +/–FR |
| Cholangiocarcinoma | +MR, +FR | +FR | +FR | +FR |
| Hepatocellular adenoma or carcinoma | — | +FR, +MM, +FM | +FR | +FR |
| Hepatocholangioma | — | +/–FR | — | +FR |
| Lung | | | | |
| Alveolar/bronchiolar adenoma or carcinoma | +MM, +FM | — | — | — |
| Cystic keratinizing epithelioma | +MR, +FR, +FM | +FR | +/–FR | +FR |
| Squamous cell carcinoma | — | — | — | +FR |
| Malignant lymphoma | +/–FM | — | — | — |
| Oral Mucosa | | | | |
| Gingival squamous cell carcinoma | +MR, +FR | +FR | +FR | +FR |
| Pancreas | | | | |
| Acinar adenoma or carcinoma | — | +/–FR | +/–FR | — |
| Skin | | | | |
| Fibrosarcoma or Malignant Schwannoma | +/–MR, +FM | — | — | — |
| Thyroid Gland | | | | |
| Follicular Cell Adenoma | +MR | +MR, +FM | — | — |
| Urethra/Ureter | | | | |
| Transitional epithelial gland carcinoma | +MM | — | — | — |
| Uterus | | | | |
| Squamous cell carcinoma | — | +FR | — | — |
| Carcinoma | — | – | +/–FR | — |

[a] + = clear or some evidence of carcinogenicity; +/– = equivocal evidence of carcinogenicity; — = no evidence of carcinogenicity; MR = male Harlan Sprague-Dawley or Osborne-Mendel rats; FR = female Harlan Sprague-Dawley rats; MM = male B6C3F1 mice; FM = female B6C3F1 mice

[b] TCAB: 0, 10, 30, or 100 mg/kg per day, 5 days a week for up to 104 weeks (present study)

[c] TCDD: 0, 3, 10, 22, 46, or 100 ng/kg, 5 days a week for up to 104 weeks in female Harlan Sprague-Dawley rats (NTP, 2006a)

[d] TCDD: 0, 0.01, 0.05, or 0.5 µg/kg, 2 days a week for up to 104 weeks in Osborne-Mendel rats and male B6C3F1 mice; 0, 0.04, 0.2, or 2.0 µg/kg, 2 days a week for up to 104 weeks in female B6C3F1 mice (NTP, 1982a)

[e] 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF): 0, 6, 20, 44, 92, or 200 ng/kg, 5 days a week for up to 104 weeks in female Harlan Sprague-Dawley rats (NTP, 2006c)

[f] 3,3′4,4′,5-Pentachlorobiphenyl 126 (PCB 126): 0, 30, 100, 175, 300, 550, or 1,000 ng/kg, 5 days a week for up to 104 weeks in female Harlan Sprague-Dawley rats (NTP, 2006b)

dioxin-like compounds. As noted above for effects of TCAB on the lung, the squamous lesions in the oral cavity may also be related to an alteration in retinoid homeostasis known to be induced by dioxin-like compounds.

There were increased incidences of thyroid gland follicular cell adenoma and follicular cell hypertrophy and hyperplasia in male Harlan Sprague-Dawley rats. Similarly, in a 2-year gavage study of TCDD, there were significantly increased incidences of follicular cell adenoma in male Osborne-Mendel rats and female B6C3F1 mice (NTP, 1982a). Based on these data, the increased incidences of thyroid gland neoplasms were considered related to TCAB exposure. Alteration in thyroid hormone homeostasis by dioxin-like compounds such as PCB 126, TCDD, TCAB and 3,3′,4,4′-tetrachloroazoxybenzene is well established (Van Birgelen *et al.*, 1994, 1995b, 1999a,b; Schmidt *et al.*, 2003; Tani *et al*., 2004). Significant reductions in thyroxine ($T_4$) concentrations were observed in the current 3-month study in Harlan Sprague-Dawley rats; however, there was little or no concomitant increase in circulating thyroid stimulating hormone (TSH) concentrations and no histopathologic changes were observed in the thyroid gland. Although a weak TSH response is unusual, the observed thyroid hormone changes are consistent with those described previously for TCAB and 3,3′,4,4′-tetrachloroazoxybenzene (NTP, 1998a,b). Based on these findings, an *in vitro* study evaluating the potential direct effects of TCAB on the $T_4$ immunoassay was performed and demonstrated that the addition of TCAB to rat serum did not interfere with the performance of the $T_4$ immunoassay (data not shown). The decrease in $T_4$ is likely due to an increase in $T_4$ glucuronidation as a result of uridine diphosphate glucuronyltransferase induction by TCAB, as seen with other dioxin-like compounds, resulting in increased $T_4$ metabolism. This is thought to result in decreased negative feedback inhibition of the pituitary gland leading to overexpression of TSH (Curran and DeGroot, 1991). It has been hypothesized that overstimulation of the thyroid gland by TSH and subsequent thyroid gland cell proliferation may be involved in the mechanism of follicular cell carcinogenesis (Hill *et al.*, 1989). Another hypothesis is that metabolites of TCAB might bind to transthyretin, a binding protein for $T_4$, resulting in destabilization of this complex and ultimately decreased $T_4$ concentrations (Van Birgelen *et al.*, 1999a). A possible explanation for the lack of TSH response is that TCAB or its metabolites mimic $T_4$, suppressing the feedback mechanism to stimulate TSH production and release (Van Birgelen *et al.*, 1999a).

Additional neoplasms that may have been related to TCAB administration in female Harlan Sprague-Dawley rats included a dose-related increase in the incidences of adrenal cortex adenoma that exceeded the historical control range for 2-year corn oil gavage studies. An increased incidence of hyperplasia in the zona fasciculata was observed in all dosed groups of males and in 30 mg/kg females. In the Kociba *et al.* (1978) feed study of TCDD, there was a significantly increased incidence of adrenal cortical adenoma in male but not female rats at the 100 ng/kg dose. In the current study, increased incidences of cytoplasmic vacuolization were observed in most dosed groups of male and female rats and the incidences of degeneration were significantly increased in males. These lesions may reflect continued stress in these animals leading to depletion of corticosteroid hormones or some other unknown mechanisms (Sapolsky *et al.*, 1987).

Administration of TCAB for 2 years also induced neoplasms and nonneoplastic lesions in the skin of B6C3F1 mice. There was clear evidence of carcinogenicity in the skin of female B6C3F1 mice based on a significant increase in the incidence of fibrosarcoma and fibrosarcoma or malignant schwannoma (combined) in the 30 mg/kg group. In male rats, the dose-related increase in the incidences of malignant schwannoma, although not statistically significant, may have been related to TCAB administration. TCDD induced fibrosarcoma in the integumentary system of female Swiss-Webster mice when applied dermally for 2 years (NTP, 1982b).

The incidences of nonneoplastic skin lesions characteristic of chloracne, including follicular dilatation and sebaceous gland atrophy, were significantly increased in male and female mice. Atrophy of sebaceous glands likely occurred secondary to follicular occlusion (Yamamoto and Tokura, 2003). These lesions are consistent with characteristics of chloracne-like lesions that have been described in hairless mice, rabbits, and monkeys after exposure to TCDD and dioxin-like compounds (Hill *et al.*, 1981). TCAB induced chloracne-like lesions following painting of the inner surface of the ears of female New Zealand rabbits for 5 days (Hill *et al.*, 1981). Typically chloracne-like skin lesions are not observed in haired mice, rats, or guinea pigs (Greene *et al.*, 2003). TCDD induces involution of sebaceous glands in haired

mice without other characteristics of chloracne (Puhvel and Sakamoto, 1988). Chloracne-like lesions were not observed in any of the NTP TEF evaluation studies, providing additional evidence that rat species are not sensitive to dioxin-induced chloracne (Greene *et al.*, 2003). The dose-dependent increase in chloracne-like lesions observed in the current study indicates that mouse strains other than hairless mice are susceptible to the development of these lesions. Chloracne-like lesions were observed in B6C3F1 mice in the 3-month study of 3,3′,4,4′-tetrachloroazoxybenzene (Van Birgelen *et al.*, 1999b). However, no lesions were observed in the skin of rats or mice in the 3-month TCAB studies (Van Birgelen *et al.*, 1999a) indicating that long-term studies may be needed to detect TCAB-induced chloracne-like skin lesions.

Chloracne is the primary adverse effect reported in humans exposed to TCAB. Three outbreaks of chloracne have occurred among workers following exposure to TCAB (Morse *et al.*, 1979; Scarisbrick and Martin, 1981) in chemical plants manufacturing 3,4-dichloroaniline or its derivatives. Chloracne developed in 34% to 61% of the exposed workers. The process by which TCDD and dioxin-like compounds, including TCAB, induce skin lesions such as chloracne requires further study (Loertscher *et al.*, 2001). One possible mechanism for the action of dioxin and dioxin-like compounds in the skin may be an indirect effect due to the disruption of retinoid homeostasis (described above) resulting in abnormal epithelial differentiation to a keratinized squamous phenotype. This leads to a stratified squamous cornifying epithelium at sites that normally contain secretory epithelia with atrophy of associated glands (Lancillotti *et al.*, 1992; Everts *et al.*, 2005). However, no changes in epidermal retinol have been observed in humans with chloracne (Coenraads *et al.*, 1977).

In addition to chloracne-like lesions, mice in this 2-year study developed treatment-related inflammatory skin lesions characterized as chronic active inflammation, dermal fibrosis, epidermal hyperplasia, and ulcer. Sporadic cases of these lesions are observed occasionally in the vehicle control B6C3F1 male mice in NTP studies, the cause of which is uncertain. These lesions are not part of the usual description for chloracne lesions that develop after dioxin exposure; chloracne is characterized by a non-inflammatory state even in the late stages of the disease (Coenraads *et al.*, 1994; Yamamoto and Tokura, 2003). Polycyclic aromatic hydrocarbons are potent inducers of AhR activity and elicit inflammatory skin responses such as irritant dermatitis and allergic contact dermatitis (Wu *et al.*, 2003; Yamamoto and Tokura, 2003). Using transgenic mice, investigators have recently demonstrated that constitutive activation of the AhR pathway alone can induce severe skin lesions with itching that mimic atopic dermatitis and contact hypersensitivity (Tauchi *et al.*, 2005). Notably, the inflammatory skin lesions were not seen in the NTP TEF evaluation studies with female Harlan Sprague-Dawley rats (NTP, 2006a,b,c,d,e,f,g) indicating that these effects might be species dependent, similar to the chloracne-like lesions.

Several nonneoplastic lesions related to TCAB administration were observed in the immune system tissues of rats and mice at 2 years and were consistent with those observed in the 3-month studies of TCAB (NTP, 1998a). These lesions included increased incidences of bone marrow hyerplasia, atrophy in the mesenteric lymph node, and hematopoietic cell proliferation in the spleen and liver. Thymic atrophy in mice is one of the hallmarks of immunotoxicity induced by dioxin-like compounds (DeWaal *et al.*, 1997) and lymphocytes are known to be one of the primary targets of these compounds. Increased incidences of malignant lymphoma in female mice may have been related to TCAB administration. Although the incidences in 10 and 30 mg/kg females were significantly increased, the incidence in the concurrent vehicle control group was unusually low (4%) compared to the mean historical control incidence for 2-year corn oil gavage studies (13%). Furthermore, the incidences in all dosed groups were within the range of historical controls (4% to 22%) for 2-year corn oil gavage studies. TCDD and other dioxin-like compounds were not carcinogenic in the lymphohematopoietic system of rats or mice (NTP, 2006a,b,c).

Nonneoplastic lesions were also consistently observed in the cardiovascular tissues of rats and mice administered TCAB and included inflammation of the blood vessels, mineralization in the aorta, and cardiomyopathy. Dioxin-like compounds have been found to produce effects on the cardiovascular system in rodent models (Yoshizawa *et al.*, 2007). The NTP TEF evaluation studies showed increased incidences of cardiomyopathy and chronic active arteritis mostly in the mesentery and pancreas of rats dosed with TCDD, PCB 126 and pentachlorodibenzofuran (Jokinen *et al.*, 2003; NTP, 2006a,b,c). The cardiomyopathy and arteritis observed

were similar to some lesions studied in humans (Jokinen *et al.*, 2003). A recent systematic review of the epidemiological studies in the literature suggests that dioxin exposure in humans is associated with increased deaths from cardiovascular disease (Humblet *et al.*, 2008).

Finally, the incidences of atrophy, acinar cytoplasmic vacuolization, and inflammation in the pancreas were significantly increased in the 2-year rat study. Similar treatment-related increased incidences of these lesions were observed in the NTP studies of TCDD, PCB 126, and pentachlorodibenzofuran (NTP, 2006a,b,c). Acinar atrophy of the pancreas may be related to the down-regulation of cholecystokinin (CCK), an important regulator of pancreatic growth and function (Baldwin, 1995; Varga *et al.*, 1998). Lee *et al.* (2000) have shown that the down-regulation of CCK is likely due to a general endocrine effect as a result of the reduction in body weight gain following exposure to PCB 126. The incidences of pancreatic adenoma were also significantly decreased in all dosed groups of male rats administered TCAB relative to the vehicle controls in the current study.

Taken together, the 3-month data in rats suggest that TCAB caused typical dioxin-like effects including decreased body weight gain, increased incidences of thymic atrophy, induction of cytochrome P450 enzymes, reduction of thyroid hormone concentrations, and development of regenerative anemia. The spectrum of neoplasms and nonneoplastic lesions observed in the 2-year rat study were similar between TCAB, TCDD, and other dioxin-like compounds (NTP, 2006a,b,c,d,e,f,g) including the induction of neoplasms in the liver, lung, oral mucosa, thyroid gland, and adrenal cortex. This pattern of responses indicates that TCAB is acting as a dioxin-like chemical and suggests that the effects of TCAB may be mediated through the AhR. In general, TCAB, TCDD, and other dioxin-like compounds are not directly genotoxic *in vitro* or *in vivo* (NTP, 2006a,b,c,d,e,f,g). Since many of the dioxin-like compounds were evaluated for carcinogenicity in female Harlan Sprague-Dawley rats, the potential carcinogenicity in an additional sex and species is not known. However, other data suggest that the toxicity and carcinogenicity of TCAB is mediated through a mechanism other than/in addition to the AhR pathway. TCAB is metabolized to mono- or dichloroaniline derivatives which are conjugated with sulfate or acetylated. Based on the TCAB liver-to-fat ratio, it appears that TCAB is not sequestered in the liver to any extent, allowing for greater systemic distribution and accumulation in fat tissue in a number of organs. Previous studies in the literature have demonstrated that the highest concentrations of TCAB in male rats were found in the adrenal gland, epididymal fat, kidney, liver, lung, lymph nodes, pancreas, and urinary bladder (Burant and Hsia, 1984). In the current study, all of these tissues were sites of TCAB-induced toxicity or carcinogenicity. The induction of urinary tract tumors in mice has not been described for TCDD or other dioxin-like compounds but appears to be consistent with neoplasms of the urinary bladder associated with 4-chloro-*o*-phenylenediamine, another chemical containing a chloroaniline moiety (NCI, 1978b). Whatever the mechanism, TCAB was clearly carcinogenic in a number of tissues of Harlan Sprague-Dawley rats and B6C3F1 mice.

## CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity** of TCAB in male Harlan Sprague-Dawley rats based on increased incidences of cystic keratinizing epithelioma of the lung, cholangiocarcinoma of the liver, and gingival squamous cell carcinoma of the oral mucosa. The increased incidences of follicular cell adenoma of the thyroid gland were also considered to be related to TCAB administration. The marginally increased incidence of malignant schwannoma may have been related to TCAB administration. There was *clear evidence of carcinogenic activity* of TCAB in female Harlan Sprague-Dawley rats based on increased incidences of cystic keratinizing epithelioma of the lung and gingival squamous cell carcinoma of the oral mucosa. The increased incidences of cholangiocarcinoma of the liver and squamous cell papilloma or squamous cell carcinoma (combined) of the forestomach were also considered to be related to TCAB administration. The marginally increased incidences of adenoma of the adrenal cortex may have been related to TCAB administration. There was *clear evidence of carcinogenic activity* of TCAB in male B6C3F1 mice based on increased incidences of carcinoma of the urethra and alveolar/bronchiolar neoplasms of the lung. The increased incidences of squamous cell carcinoma of the forestomach were also considered to be related to TCAB administration. The marginally increased incidence of carcinoma of the ureter may have been related to TCAB administration. There

was *clear evidence of carcinogenic activity* of TCAB in female B6C3F1 mice based on increased incidences of fibrosarcoma and fibrosarcoma or malignant schwannoma (combined) of the skin. The increased incidences of carcinoma of the urethra, alveolar/bronchiolar neoplasms and cystic keratinizing epithelioma of the lung, and squamous cell carcinoma of the forestomach were also considered to be related to TCAB administration. The marginally increased incidences of malignant lymphoma may have been related to TCAB administration.

TCAB administration caused increased incidences of nonneoplastic lesions of the lung, liver, oral mucosa, forestomach, adrenal cortex, pancreas, blood vessel, spleen, and mesenteric lymph node in male and female rats; the thyroid gland and testis in male rats; the nose in female rats; the urinary bladder, forestomach, glandular stomach, skin, spleen, thymus, liver, and heart in male and female mice; the urethra, ureter, and blood vessel in male mice; and the lung, clitoral gland, ovary, and bone marrow in female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 14.

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# APPENDIX A
# SUMMARY OF LESIONS IN MALE RATS
# IN THE 2-YEAR GAVAGE STUDYOF TCAB

**TABLE A1**
**Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of TCAB[a]**

| | Vehicle Control | 75 mg/kg | 150 mg/kg | 300 mg/kg |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Accidental deaths | 1 | | 1 | 1 |
| Moribund | 10 | 12 | 14 | 14 |
| Natural deaths | 11 | 29 | 31 | 33 |
| Survivors | | | | |
| Terminal sacrifice | 28 | 9 | 4 | 2 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Esophagus | (50) | (50) | (50) | (50) |
| Intestine large, cecum | (50) | (50) | (50) | (50) |
| Intestine large, colon | (50) | (50) | (50) | (50) |
| Intestine large, rectum | (50) | (50) | (50) | (50) |
| Intestine small, duodenum | (50) | (50) | (50) | (50) |
| Intestine small, ileum | (50) | (50) | (50) | (50) |
| Intestine small, jejunum | (50) | (50) | (50) | (50) |
| Liver | (50) | (50) | (50) | (50) |
| Cholangiocarcinoma | | 3 (6%) | 4 (8%) | 5 (10%) |
| Cholangiocarcinoma, multiple | | 1 (2%) | | 1 (2%) |
| Cholangioma | | | 1 (2%) | |
| Hepatocellular adenoma | | 1 (2%) | | |
| Hepatocholangiocarcinoma, multiple | | | | 1 (2%) |
| Mesentery | (0) | (0) | (0) | (1) |
| Transitional epithelial carcinoma, metastatic, kidney | | | | 1 (100%) |
| Oral mucosa | (50) | (50) | (50) | (50) |
| Gingival, squamous cell carcinoma | 1 (2%) | 4 (8%) | 4 (8%) | 5 (10%) |
| Gingival, squamous cell carcinoma, multiple | | 1 (2%) | | |
| Pancreas | (50) | (49) | (50) | (50) |
| Transitional epithelial carcinoma, metastatic, kidney | | | | 1 (2%) |
| Acinus, adenoma | 5 (10%) | 1 (2%) | | |
| Acinus, adenoma, multiple | 4 (8%) | | | |
| Salivary glands | (50) | (50) | (49) | (50) |
| Schwannoma malignant | 1 (2%) | 2 (4%) | | 1 (2%) |
| Stomach, forestomach | (50) | (50) | (50) | (50) |
| Squamous cell carcinoma | | 1 (2%) | | |
| Stomach, glandular | (50) | (50) | (50) | (50) |
| Tooth | (50) | (50) | (50) | (50) |
| **Cardiovascular System** | | | | |
| Blood vessel | (50) | (50) | (50) | (50) |
| Heart | (50) | (50) | (50) | (50) |
| Fibrosarcoma, metastatic, tissue NOS | | | 1 (2%) | |
| Schwannoma benign | | 3 (6%) | | |
| Schwannoma malignant, metastatic, tissue NOS | | | 1 (2%) | |
| Epicardium, sarcoma, metastatic, tissue NOS | | 1 (2%) | | |

**TABLE A1**
**Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (50) | (50) | (50) |
| Adenoma | 1 (2%) | 1 (2%) | | 2 (4%) |
| Adrenal medulla | (50) | (50) | (50) | (50) |
| Pheochromocytoma benign | 5 (10%) | 4 (8%) | 6 (12%) | 6 (12%) |
| Pheochromocytoma benign, multiple | | 1 (2%) | | |
| Pheochromocytoma malignant | | 1 (2%) | | |
| Bilateral, pheochromocytoma benign | | 1 (2%) | | 1 (2%) |
| Islets, pancreatic | (50) | (50) | (50) | (50) |
| Adenoma | 2 (4%) | | | |
| Parathyroid gland | (43) | (48) | (44) | (44) |
| Adenoma | 1 (2%) | | | |
| Pituitary gland | (50) | (50) | (50) | (49) |
| Schwannoma malignant, metastatic, eye | 1 (2%) | | | |
| Pars distalis, adenoma | 13 (26%) | 6 (12%) | 7 (14%) | |
| Thyroid gland | (50) | (50) | (50) | (50) |
| Bilateral, C-cell, adenoma | 1 (2%) | 1 (2%) | | |
| C-cell, adenoma | 12 (24%) | 2 (4%) | 1 (2%) | 4 (8%) |
| C-cell, carcinoma | 1 (2%) | 1 (2%) | | |
| Follicular cell, adenoma | | 2 (4%) | 3 (6%) | 3 (6%) |
| Follicular cell, adenoma, multiple | | 1 (2%) | 1 (2%) | 1 (2%) |
| **General Body System** | | | | |
| Tissue NOS | (0) | (1) | (2) | (3) |
| Mediastinum, fibrosarcoma | | | 1 (50%) | |
| Mediastinum, sarcoma | | 1 (100%) | | |
| Mediastinum, schwannoma malignant | | | 1 (50%) | 3 (100%) |
| **Genital System** | | | | |
| Coagulating gland | (50) | (50) | (50) | (49) |
| Epididymis | (50) | (50) | (50) | (50) |
| Preputial gland | (50) | (50) | (50) | (49) |
| Squamous cell papilloma | | 1 (2%) | 1 (2%) | 1 (2%) |
| Prostate | (50) | (50) | (50) | (50) |
| Adenoma | 3 (6%) | | | |
| Adenoma, multiple | 2 (4%) | | | |
| Carcinoma | | | | 1 (2%) |
| Seminal vesicle | (50) | (50) | (50) | (50) |
| Transitional epithelial carcinoma, multiple | | | | 1 (2%) |
| Testes | (50) | (50) | (50) | (50) |
| Interstitial cell, adenoma | 1 (2%) | 1 (2%) | 1 (2%) | |
| Interstitial cell, adenoma, multiple | 1 (2%) | | | |

**TABLE A1**
**Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Hematopoietic System** | | | | |
| Bone marrow | (50) | (50) | (49) | (50) |
| Lymph node | (38) | (28) | (29) | (41) |
| Mediastinal, fibrosarcoma, metastatic, tissue NOS | | | 1 (3%) | |
| Mediastinal, thymoma malignant, metastatic, thymus | | | | 1 (2%) |
| Lymph node, mandibular | (49) | (50) | (49) | (50) |
| Lymph node, mesenteric | (50) | (48) | (50) | (50) |
| Spleen | (50) | (50) | (50) | (50) |
| Thymus | (48) | (48) | (46) | (48) |
| Hemangioma | | 1 (2%) | | |
| Thymoma malignant | | | | 1 (2%) |
| **Integumentary System** | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Skin | (50) | (50) | (50) | (50) |
| Basal cell adenoma | 1 (2%) | 2 (4%) | | 1 (2%) |
| Keratoacanthoma | 2 (4%) | | | |
| Squamous cell papilloma | | 1 (2%) | | |
| Trichoepithelioma | | | 1 (2%) | |
| Pinna, neural crest tumor | 1 (2%) | | | |
| Subcutaneous tissue, fibroma | 5 (10%) | | | |
| Subcutaneous tissue, hemangiosarcoma | 1 (2%) | | | |
| Subcutaneous tissue, schwannoma malignant | | 1 (2%) | | |
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Skeletal muscle | (0) | (0) | (2) | (0) |
| **Nervous System** | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Astrocytoma benign | 1 (2%) | | | |
| **Respiratory System** | | | | |
| Lung | (50) | (50 | (50) | (50) |
| Alveolar/bronchiolar adenoma | 1 (2%) | 1 (2%) | 1 (2%) | |
| Alveolar/bronchiolar carcinoma | 1 (2%) | | | |
| Carcinoma, metastatic, thyroid gland | 1 (2%) | | | |
| Cystic keratinizing epithelioma | | 6 (12%) | 5 (10%) | 13 (26%) |
| Cystic keratinizing epithelioma, multiple | | 8 (16%) | 26 (52%) | 24 (48%) |
| Hemangiosarcoma, metastatic, skin | 1 (2%) | | | |
| Hepatocholangiocarcinoma, metastatic, liver | | | | 1 (2%) |
| Pheochromocytoma malignant, metastatic, adrenal medulla | | 1 (2%) | | |
| Transitional epithelial carcinoma, metastatic, kidney | | | | 1 (2%) |
| Nose | (50) | (50) | (50) | (50) |

**TABLE A1**
**Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Special Senses System** | | | | |
| Ear | (0) | (1) | (0) | (0) |
| Eye | (48) | (50) | (50) | (50) |
| Schwannoma malignant | 1 (2%) | | | |
| Harderian gland | (50) | (50) | (50) | (50) |
| Schwannoma malignant | | | 1 (2%) | |
| Schwannoma malignant, metastatic, eye | 1 (2%) | | | |
| Lacrimal gland | (0) | (1) | (0) | (0) |
| Zymbal's gland | (1) | (0) | (1) | (0) |
| Carcinoma | 1 (100%) | | 1 (100%) | |
| **Urinary System** | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Transitional epithelium, carcinoma | | | | 1 (2%) |
| Ureter | (41) | (31) | (42) | (49) |
| Urethra | (50) | (50) | (50) | (50) |
| Urinary bladder | (50) | (50) | (50) | (50) |
| **Systemic Lesions** | | | | |
| Multiple organs[b] | (50) | (50) | (50) | (50) |
| Leukemia mononuclear | 1 (2%) | | | |
| Lymphoma malignant | 1 (2%) | 1 (2%) | 1 (2%) | 4 (8%) |
| **Neoplasm Summary** | | | | |
| Total animals with primary neoplasms[c] | 44 | 39 | 39 | 46 |
| Total primary neoplasms | 71 | 62 | 67 | 80 |
| Total animals with benign neoplasms | 40 | 31 | 35 | 41 |
| Total benign neoplasms | 61 | 45 | 54 | 56 |
| Total animals with malignant neoplasms | 9 | 15 | 13 | 19 |
| Total malignant neoplasms | 9 | 17 | 13 | 24 |
| Total animals with metastatic neoplasms | 3 | 2 | 2 | 3 |
| Total metastatic neoplasms | 4 | 2 | 3 | 5 |
| Total animals with uncertain neoplasms, benign or malignant | 1 | | | |
| Total uncertain neoplasms | 1 | | | |

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

**TABLE A2**
**Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Adrenal Medulla: Benign Pheochromocytoma** | | | | |
| Overall rate[a] | 5/50 (10%) | 6/50 (12%) | 6/50 (12%) | 7/50 (14%) |
| Adjusted rate[b] | 12.2% | 19.3% | 21.8% | 26.4% |
| Terminal rate[c] | 4/28 (14%) | 2/9 (22%) | 0/4 (0%) | 1/2 (50%) |
| First incidence (days) | 650 | 500 | 554 | 544 |
| Poly-3 test[d] | P = 0.116 | P = 0.309 | P = 0.240 | P = 0.128 |
| **Adrenal Medulla: Benign or Malignant Pheochromocytoma** | | | | |
| Overall rate | 5/50 (10%) | 7/50 (14%) | 6/50 (12%) | 7/50 (14%) |
| Adjusted rate | 12.2% | 22.4% | 21.8% | 26.4% |
| Terminal rate | 4/28 (14%) | 2/9 (22%) | 0/4 (0%) | 1/2 (50%) |
| First incidence (days) | 650 | 500 | 554 | 544 |
| Poly-3 test | P = 0.139 | P = 0.203 | P = 0.240 | P = 0.128 |
| **Heart: Benign Schwannoma** | | | | |
| Overall rate | 0/50 (0%) | 3/50 (6%) | 0/50 (0%) | 0/50 (0%) |
| Adjusted rate | 0.0% | 10.0% | 0.0% | 0.0% |
| Terminal rate | 0/28 (0%) | 0/9 (0%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | —[e] | 521 | — | — |
| Poly-3 test | P = 0.427N | P = 0.073 | —[f] | — |
| **Liver: Cholangiocarcinoma** | | | | |
| Overall rate | 0/50 (0%) | 4/50 (8%) | 4/50 (8%) | 6/50 (12%) |
| Adjusted rate | 0.0% | 13.3% | 14.3% | 22.6% |
| Terminal rate | 0/28 (0%) | 1/9 (11%) | 0/4 (0%) | 1/2 (50%) |
| First incidence (days) | — | 454 | 487 | 529 |
| Poly-3 test | P = 0.007 | P = 0.030 | P = 0.026 | P = 0.003 |
| **Lung: Cystic Keratinizing Epithelioma** | | | | |
| Overall rate | 0/50 (0%) | 14/50 (28%) | 31/50 (62%) | 37/50 (74%) |
| Adjusted rate | 0.0% | 44.5% | 85.9% | 92.7% |
| Terminal rate | 0/28 (0%) | 4/9 (44%) | 4/4 (100%) | 2/2 (100%) |
| First incidence (days) | — | 576 | 367 | 192 |
| Poly-3 test | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 |
| **Oral Mucosa: Squamous Cell Carcinoma** | | | | |
| Overall rate | 1/50 (2%) | 5/50 (10%) | 4/50 (8%) | 5/50 (10%) |
| Adjusted rate | 2.5% | 16.7% | 14.5% | 18.8% |
| Terminal rate | 0/28 (0%) | 2/9 (22%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | 694 | 504 | 474 | 524 |
| Poly-3 test | P = 0.065 | P = 0.046 | P = 0.085 | P = 0.033 |

**TABLE A2**
**Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Pancreas: Adenoma** | | | | |
| Overall rate | 9/50 (18%) | 1/49 (2%) | 0/50 (0%) | 0/50 (0%) |
| Adjusted rate | 21.8% | 3.5% | 0.0% | 0.0% |
| Terminal rate | 7/28 (25%) | 0/9 (0%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | 592 | 541 | — | — |
| Poly-3 test | P = 0.005N | P = 0.035N | P = 0.016N | P = 0.021N |
| **Pituitary Gland (Pars Distalis): Adenoma** | | | | |
| Overall rate | 13/50 (26%) | 6/50 (12%) | 7/50 (14%) | 0/49 (0%) |
| Adjusted rate | 30.3% | 19.6% | 24.9% | 0.0% |
| Terminal rate | 5/28 (18%) | 1/9 (11%) | 2/4 (50%) | 0/2 (0%) |
| First incidence (days) | 420 | 535 | 533 | — |
| Poly-3 test | P = 0.006N | P = 0.227N | P = 0.413N | P = 0.005N |
| **Prostate Gland: Adenoma** | | | | |
| Overall rate | 5/50 (10%) | 0/50 (0%) | 0/50 (0%) | 0/50 (0%) |
| Adjusted rate | 12.2% | 0.0% | 0.0% | 0.0% |
| Terminal rate | 2/28 (7%) | 0/9 (0%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | 700 | — | — | — |
| Poly-3 test | P = 0.043N | P = 0.074N | P = 0.094N | P = 0.107N |
| **Prostate Gland: Adenoma or Carcinoma** | | | | |
| Overall rate | 5/50 (10%) | 0/50 (0%) | 0/50 (0%) | 1/50 (2%) |
| Adjusted rate | 12.2% | 0.0% | 0.0% | 4.1% |
| Terminal rate | 2/28 (7%) | 0/9 (0%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | 700 | — | — | 603 |
| Poly-3 test | P = 0.205N | P = 0.074N | P = 0.094N | P = 0.264N |
| **Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, or Basal Cell Adenoma** | | | | |
| Overall rate | 3/50 (6%) | 2/50 (4%) | 1/50 (2%) | 1/50 (2%) |
| Adjusted rate | 7.4% | 6.8% | 3.8% | 4.0% |
| Terminal rate | 3/28 (11%) | 0/9 (0%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | 727 (T) | 486 | 554 | 225 |
| Poly-3 test | P = 0.384N | P = 0.642N | P = 0.471N | P = 0.488N |
| **Skin (Subcutaneous Tissue): Fibroma** | | | | |
| Overall rate | 5/50 (10%) | 0/50 (0%) | 0/50 (0%) | 0/50 (0%) |
| Adjusted rate | 12.2% | 0.0% | 0.0% | 0.0% |
| Terminal rate | 3/28 (11%) | 0/9 (0%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | 650 | — | — | — |
| Poly-3 test | P = 0.043N | P = 0.074N | P = 0.094N | P = 0.108N |

**TABLE A2**
**Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Thyroid Gland (C-Cell): Adenoma** | | | | |
| Overall rate | 13/50 (26%) | 3/50 (6%) | 1/50 (2%) | 4/50 (8%) |
| Adjusted rate | 31.7% | 10.1% | 3.9% | 15.6% |
| Terminal rate | 11/28 (39%) | 0/9 (0%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | 671 | 576 | 712 | 586 |
| Poly-3 test | P = 0.110N | P = 0.031N | P = 0.008N | P = 0.126N |
| **Thyroid Gland (C-Cell): Adenoma or Carcinoma** | | | | |
| Overall rate | 13/50 (26%) | 4/50 (8%) | 1/50 (2%) | 4/50 (8%) |
| Adjusted rate | 31.7% | 13.5% | 3.9% | 15.6% |
| Terminal rate | 11/28 (39%) | 1/9 (11%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | 671 | 576 | 712 | 586 |
| Poly-3 test | P = 0.098N | P = 0.069N | P = 0.008N | P = 0.126N |
| **Thyroid Gland (Follicular Cell): Adenoma** | | | | |
| Overall rate | 0/50 (0%) | 3/50 (6%) | 4/50 (8%) | 4/50 (8%) |
| Adjusted rate | 0.0% | 10.2% | 14.5% | 15.7% |
| Terminal rate | 0/28 (0%) | 1/9 (11%) | 1/4 (25%) | 0/2 (0%) |
| First incidence (days) | — | 635 | 491 | 476 |
| Poly-3 test | P = 0.037 | P = 0.070 | P = 0.025 | P = 0.021 |
| **Tissue NOS: Malignant Schwannoma** | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 1/50 (2%) | 3/50 (6%) |
| Adjusted rate | 0.0% | 0.0% | 3.9% | 11.4% |
| Terminal rate | 0/28 (0%) | 0/9 (0%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | — | — | 712 | 398 |
| Poly-3 test | P = 0.010 | — | P = 0.413 | P = 0.060 |
| **Tissue NOS: Fibrosarcoma, Sarcoma, or Malignant Schwannoma** | | | | |
| Overall rate | 0/50 (0%) | 1/50 (2%) | 2/50 (4%) | 3/50 (6%) |
| Adjusted rate | 0.0% | 3.4% | 7.5% | 11.4% |
| Terminal rate | 0/28 (0%) | 0/9 (0%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | — | 617 | 438 | 398 |
| Poly-3 test | P = 0.037 | P = 0.434 | P = 0.158 | P = 0.060 |

**TABLE A2**
**Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **All Organs: Fibrosarcoma, Sarcoma, or Malignant Schwannoma** | | | | |
| Overall rate | 2/50 (4%) | 4/50 (8%) | 3/50 (6%) | 4/50 (8%) |
| Adjusted rate | 4.8% | 12.9% | 11.1% | 14.8% |
| Terminal rate | 0/28 (0%) | 0/9 (0%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | 549 | 380 | 438 | 398 |
| Poly-3 test | P = 0.184 | P = 0.213 | P = 0.316 | P = 0.169 |
| **All Organs: Fibrosarcoma, Sarcoma, or Benign or Malignant Schwannoma** | | | | |
| Overall rate | 2/50 (4%) | 7/50 (14%) | 3/50 (6%) | 4/50 (8%) |
| Adjusted rate | 4.8% | 21.7% | 11.1% | 14.8% |
| Terminal rate | 0/28 (0%) | 0/9 (0%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | 549 | 380 | 438 | 398 |
| Poly-3 test | P = 0.308 | P = 0.032 | P = 0.316 | P = 0.169 |
| **All Organs: Malignant Lymphoma** | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 1/50 (2%) | 4/50 (8%) |
| Adjusted rate | 2.5% | 3.4% | 3.8% | 15.3% |
| Terminal rate | 1/28 (4%) | 0/9 (0%) | 0/4 (0%) | 0/2 (0%) |
| First incidence (days) | 727 (T) | 62 | 573 | 503 |
| Poly-3 test | P = 0.026 | P = 0.686 | P = 0.655 | P = 0.077 |
| **All Organs: Benign Neoplasms** | | | | |
| Overall rate | 40/50 (80%) | 31/50 (62%) | 35/50 (70%) | 41/50 (82%) |
| Adjusted rate | 87.3% | 80.1% | 91.3% | 95.7% |
| Terminal rate | 25/28 (89%) | 7/9 (78%) | 4/4 (100%) | 2/2 (100%) |
| First incidence (days) | 398 | 486 | 367 | 192 |
| Poly-3 test | P = 0.022 | P = 0.241N | P = 0.389 | P = 0.104 |
| **All Organs: Malignant Neoplasms** | | | | |
| Overall rate | 9/50 (18%) | 15/50 (30%) | 13/50 (26%) | 19/50 (38%) |
| Adjusted rate | 20.7% | 43.7% | 40.8% | 56.4% |
| Terminal rate | 3/28 (11%) | 4/9 (44%) | 0/4 (0%) | 1/2 (50%) |
| First incidence (days) | 274 | 62 | 438 | 387 |
| Poly-3 test | P = 0.002 | P = 0.023 | P = 0.049 | P < 0.001 |
| **All Organs: Benign or Malignant Neoplasms** | | | | |
| Overall rate | 44/50 (88%) | 39/50 (78%) | 39/50 (78%) | 46/50 (92%) |
| Adjusted rate | 91.4% | 91.3% | 95.1% | 98.9% |
| Terminal rate | 25/28 (89%) | 8/9 (89%) | 4/4 (100%) | 2/2 (100%) |
| First incidence (days) | 274 | 62 | 367 | 192 |
| Poly-3 test | P = 0.029 | P = 0.662N | P = 0.370 | P = 0.085 |

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, heart, liver, lung, pancreas, pituitary gland, prostate gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of the statistic cannot be computed

**TABLE A3**
**Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of TCAB[a]**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Accidental deaths | 1 | | 1 | 1 |
| Moribund | 10 | 12 | 14 | 14 |
| Natural deaths | 11 | 29 | 31 | 33 |
| Survivors | | | | |
| Terminal sacrifice | 28 | 9 | 4 | 2 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Esophagus | (50) | (50) | (50) | (50) |
| Muscularis, inflammation | | 1 (2%) | | |
| Intestine large, cecum | (50) | (50) | (50) | (50) |
| Inflammation | | | 1 (2%) | |
| Necrosis | | | | 1 (2%) |
| Intestine large, colon | (50) | (50) | (50) | (50) |
| Inflammation | | | | 1 (2%) |
| Parasite metazoan | 6 (12%) | 6 (12%) | 3 (6%) | 5 (10%) |
| Thrombosis | | | | 1 (2%) |
| Intestine large, rectum | (50) | (50) | (50) | (50) |
| Inflammation | | | | 1 (2%) |
| Intestine small, duodenum | (50) | (50) | (50) | (50) |
| Fibrosis | | | | 1 (2%) |
| Necrosis | | | 1 (2%) | 1 (2%) |
| Intestine small, ileum | (50) | (50) | (50) | (50) |
| Dysplasia | | | 1 (2%) | |
| Fibrosis | | | 1 (2%) | |
| Inflammation | | | 1 (2%) | |
| Intestine small, jejunum | (50) | (50) | (50) | (50) |
| Necrosis | | | 1 (2%) | |
| Liver | (50) | (50) | (50) | (50) |
| Angiectasis | 2 (4%) | 1 (2%) | 1 (2%) | |
| Basophilic focus | 3 (6%) | 1 (2%) | 5 (10%) | 2 (4%) |
| Cholangiofibrosis | 1 (2%) | 3 (6%) | 2 (4%) | |
| Clear cell focus | 32 (64%) | 12 (24%) | 8 (16%) | 4 (8%) |
| Congestion | | 1 (2%) | | |
| Degeneration, cystic | 2 (4%) | | 1 (2%) | 1 (2%) |
| Eosinophilic focus | 3 (6%) | 9 (18%) | 4 (8%) | 12 (24%) |
| Fatty change, focal | 1 (2%) | 2 (4%) | 7 (14%) | 3 (6%) |
| Fatty change, diffuse | 3 (6%) | 9 (18%) | 18 (36%) | 34 (68%) |
| Fibrosis | | 1 (2%) | | 4 (8%) |
| Hematopoietic cell proliferation | 5 (10%) | 40 (80%) | 37 (74%) | 30 (60%) |
| Hepatodiaphragmatic nodule | | 2 (4%) | 1 (2%) | |
| Hyperplasia, nodular | | 1 (2%) | 1 (2%) | 3 (6%) |
| Inflammation, granulomatous | | | 1 (2%) | |
| Inflammation, chronic active | 40 (80%) | 37 (74%) | 30 (60%) | 28 (56%) |
| Mixed cell focus | 7 (14%) | 3 (6%) | 3 (6%) | 1 (2%) |
| Necrosis | 1 (2%) | 7 (14%) | 18 (36%) | 21 (42%) |
| Pigmentation | 1 (2%) | 4 (8%) | 5 (10%) | 6 (12%) |
| Toxic hepatopathy | | | 5 (10%) | 8 (16%) |

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

**TABLE A3**
**Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Alimentary System** *(continued)* | | | | |
| Liver *(continued)* | (50) | (50) | (50) | (50) |
| Bile duct, cyst | | | 1 (2%) | 4 (8%) |
| Bile duct, dilatation | | 1 (2%) | 3 (6%) | |
| Bile duct, fibrosis | 10 (20%) | 3 (6%) | 1 (2%) | 3 (6%) |
| Bile duct, hyperplasia | 34 (68%) | 38 (76%) | 36 (72%) | 29 (58%) |
| Bile duct, metaplasia | | | | 1 (2%) |
| Centrilobular, degeneration | | 10 (20%) | 23 (46%) | 24 (48%) |
| Centrilobular, fatty change | | | | 1 (2%) |
| Hepatocyte, hypertrophy | | 6 (12%) | 11 (22%) | 22 (44%) |
| Hepatocyte, multinucleated | | | 1 (2%) | 3 (6%) |
| Oval cell, hyperplasia | | 4 (8%) | 8 (16%) | 5 (10%) |
| Mesentery | (0) | (0) | (0) | (1) |
| Oral mucosa | (50) | (50) | (50) | (50) |
| Gingival, hyperplasia, cystic keratinizing | | 4 (8%) | 18 (36%) | 11 (22%) |
| Gingival, hyperplasia, squamous | 2 (4%) | 21 (42%) | 24 (48%) | 31 (62%) |
| Pancreas | (50) | (49) | (50) | (50) |
| Atrophy | 4 (8%) | 13 (27%) | 10 (20%) | 10 (20%) |
| Fibrosis | 1 (2%) | | | |
| Inflammation | 1 (2%) | 7 (14%) | 7 (14%) | 3 (6%) |
| Acinus, hyperplasia | 8 (16%) | 2 (4%) | 2 (4%) | |
| Acinus, necrosis | | 1 (2%) | | |
| Acinus, vacuolization cytoplasmic | | 16 (33%) | 30 (60%) | 13 (26%) |
| Salivary glands | (50) | (50) | (49) | (50) |
| Atrophy | | 1 (2%) | | 3 (6%) |
| Cyst | | | 1 (2%) | |
| Fibrosis | | | | 1 (2%) |
| Hyperplasia | 1 (2%) | | | |
| Inflammation | 1 (2%) | | | |
| Stomach, forestomach | (50) | (50) | (50) | (50) |
| Cyst | | | | 1 (2%) |
| Inflammation | 5 (10%) | 2 (4%) | 2 (4%) | 3 (6%) |
| Mineralization | 2 (4%) | | 1 (2%) | |
| Necrosis | | 3 (6%) | 1 (2%) | 1 (2%) |
| Epithelium, hyperplasia | 8 (16%) | 36 (72%) | 44 (88%) | 45 (90%) |
| Stomach, glandular | (50) | (50) | (50) | (50) |
| Inflammation | 2 (4%) | | 1 (2%) | |
| Mineralization | 3 (6%) | 2 (4%) | 2 (4%) | 1 (2%) |
| Necrosis | | | 1 (2%) | |
| Tooth | (50) | (50) | (50) | (50) |
| Peridontal tissue, inflammation | 11 (22%) | 12 (24%) | 19 (38%) | 16 (32%) |
| **Cardiovascular System** | | | | |
| Blood vessel | (50) | (50) | (50) | (50) |
| Inflammation | 21 (42%) | 29 (58%) | 30 (60%) | 29 (58%) |
| Mineralization | 5 (10%) | 1 (2%) | 4 (8%) | 3 (6%) |
| Aorta, intima, hyperplasia | | 1 (2%) | | |
| Heart | (50) | (50) | (50) | (50) |
| Cardiomyopathy | 47 (94%) | 48 (96%) | 45 (90%) | 40 (80%) |
| Inflammation | 1 (2%) | | 2 (4%) | 1 (2%) |
| Mineralization | 5 (10%) | | 1 (2%) | 1 (2%) |
| Necrosis | | | | 2 (4%) |
| Thrombosis | | 4 (8%) | 2 (4%) | 4 (8%) |
| Endocardium, hyperplasia | | 1 (2%) | | |

**TABLE A3**
**Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (50) | (50) | (50) |
| Angiectasis | 1 (2%) | | 1 (2%) | |
| Degeneration | 4 (8%) | 14 (28%) | 14 (28%) | 9 (18%) |
| Hypertrophy | 33 (66%) | 24 (48%) | 11 (22%) | 17 (34%) |
| Infiltration cellular, mononuclear cell | | 1 (2%) | | |
| Necrosis | | 2 (4%) | 4 (8%) | 13 (26%) |
| Vacuolization cytoplasmic | 20 (40%) | 29 (58%) | 31 (62%) | 25 (50%) |
| Zona fasciculata, hyperplasia | 14 (28%) | 22 (44%) | 19 (38%) | 21 (42%) |
| Adrenal medulla | (50) | (50) | (50) | (50) |
| Hyperplasia | 10 (20%) | 8 (16%) | 5 (10%) | 6 (12%) |
| Islets, pancreatic | (50) | (50) | (50) | (50) |
| Hyperplasia | 1 (2%) | | | 1 (2%) |
| Parathyroid gland | (43) | (48) | (44) | (44) |
| Hyperplasia | 8 (19%) | 9 (19%) | 6 (14%) | 2 (5%) |
| Pituitary gland | (50) | (50) | (50) | (49) |
| Angiectasis | | 1 (2%) | | |
| Hemorrhage | | | | 1 (2%) |
| Hyperplasia | 1 (2%) | | | |
| Necrosis | | | | 2 (4%) |
| Pars distalis, hyperplasia | 18 (36%) | 10 (20%) | 13 (26%) | 10 (20%) |
| Pars intermedia, necrosis | | 1 (2%) | | |
| Rathke's cleft, hyperplasia | 1 (2%) | | | |
| Thyroid gland | (50) | (50) | (50) | (50) |
| Angiectasis | | 1 (2%) | | |
| Atrophy | | | 1 (2%) | |
| Fibrosis | | | | 1 (2%) |
| Inflammation | | 3 (6%) | 9 (18%) | 14 (28%) |
| C-cell, hyperplasia | 7 (14%) | 1 (2%) | 2 (4%) | 1 (2%) |
| Follicle, cyst | 1 (2%) | | | |
| Follicular cell, hyperplasia | | 2 (4%) | 10 (20%) | 12 (24%) |
| Follicular cell, hypertrophy | 2 (4%) | 2 (4%) | 6 (12%) | 6 (12%) |
| **General Body System** | | | | |
| None | | | | |
| **Genital System** | | | | |
| Coagulating gland | (50) | (50) | (50) | (49) |
| Inflammation | 1 (2%) | 3 (6%) | 2 (4%) | 4 (8%) |
| Metaplasia, squamous | | | | 1 (2%) |
| Epididymis | (50) | (50) | (50) | (50) |
| Granuloma sperm | | 1 (2%) | 3 (6%) | 2 (4%) |
| Inflammation | 3 (6%) | | | |
| Preputial gland | (50) | (50) | (50) | (49) |
| Ectasia | 1 (2%) | | | |
| Hyperplasia, squamous | 2 (4%) | 3 (6%) | 1 (2%) | 4 (8%) |
| Inflammation | 5 (10%) | | | 1 (2%) |
| Prostate | (50) | (50) | (50) | (50) |
| Inflammation | 13 (26%) | 12 (24%) | 7 (14%) | 16 (32%) |
| Epithelium, hyperplasia | 12 (24%) | 4 (8%) | 3 (6%) | 2 (4%) |

**TABLE A3**
**Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Genital System** *(continued)* | | | | |
| Seminal vesicle | (50) | (50) | (50) | (50) |
| Hyperplasia | | | | 2 (4%) |
| Inflammation | | 2 (4%) | 1 (2%) | 4 (8%) |
| Metaplasia, squamous | | | | 3 (6%) |
| Mineralization | 1 (2%) | | | |
| Testes | (50) | (50) | (50) | (50) |
| Inflammation | 1 (2%) | 1 (2%) | | 1 (2%) |
| Necrosis | | 2 (4%) | 1 (2%) | 3 (6%) |
| Thrombosis | | 1 (2%) | | |
| Germinal epithelium, degeneration | 15 (30%) | 16 (32%) | 18 (36%) | 21 (42%) |
| Germinal epithelium, mineralization | 3 (6%) | 3 (6%) | 3 (6%) | 4 (8%) |
| Interstitial cell, hyperplasia | | 1 (2%) | | 1 (2%) |
| **Hematopoietic System** | | | | |
| Bone marrow | (50) | (50) | (49) | (50) |
| Atrophy | | 1 (2%) | 2 (4%) | 2 (4%) |
| Hyperplasia | 12 (24%) | 7 (14%) | 21 (43%) | 19 (38%) |
| Necrosis | | | | 1 (2%) |
| Lymph node | (38) | (28) | (29) | (41) |
| Pigmentation | | 1 (4%) | | |
| Deep cervical, atrophy | | | | 1 (2%) |
| Deep cervical, ectasia | | | | 1 (2%) |
| Deep cervical, hematopoietic cell proliferation | 1 (3%) | | | |
| Mediastinal, atrophy | 2 (5%) | | | 1 (2%) |
| Mediastinal, ectasia | | 1 (4%) | | 1 (2%) |
| Mediastinal, hemorrhage | | | 1 (3%) | 1 (2%) |
| Mediastinal, hyperplasia, lymphoid | | 1 (4%) | | 3 (7%) |
| Mediastinal, infiltration cellular, histiocyte | | 1 (4%) | | |
| Pancreatic, hyperplasia, lymphoid | | 1 (4%) | | |
| Lymph node, mandibular | (49) | (50) | (49) | (50) |
| Atrophy | 5 (10%) | 1 (2%) | | 7 (14%) |
| Congestion | | 1 (2%) | | |
| Ectasia | | 1 (2%) | | |
| Hyperplasia, lymphoid | 2 (4%) | 6 (12%) | 1 (2%) | 2 (4%) |
| Infiltration cellular, histiocyte | | | 1 (2%) | |
| Necrosis | | | 2 (4%) | |
| Pigmentation | 1 (2%) | | | 2 (4%) |
| Lymph node, mesenteric | (50) | (48) | (50) | (50) |
| Atrophy | | 1 (2%) | 1 (2%) | 5 (10%) |
| Hyperplasia, lymphoid | | 2 (4%) | 2 (4%) | |
| Inflammation, granulomatous | | | | 1 (2%) |
| Pigmentation | 1 (2%) | 24 (50%) | 25 (50%) | 20 (40%) |
| Spleen | (50) | (50) | (50) | (50) |
| Atrophy | 1 (2%) | | | |
| Congestion | | | 1 (2%) | |
| Hematopoietic cell proliferation | 38 (76%) | 48 (96%) | 44 (88%) | 41 (82%) |
| Pigmentation | 38 (76%) | 39 (78%) | 40 (80%) | 35 (70%) |
| Thrombosis | 1 (2%) | | | |
| Lymphoid follicle, atrophy | 5 (10%) | 4 (8%) | 5 (10%) | 12 (24%) |
| Thymus | (48) | (48) | (46) | (48) |
| Atrophy | 45 (94%) | 47 (98%) | 44 (96%) | 47 (98%) |
| Inflammation | | | | 1 (2%) |
| Thymocyte, hyperplasia | 1 (2%) | | | |

**TABLE A3**
**Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Integumentary System** | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Inflammation | | | | 1 (2%) |
| Mineralization | 1 (2%) | | | |
| Skin | (50) | (50) | (50) | (50) |
| Cyst epithelial inclusion | 2 (4%) | 3 (6%) | 2 (4%) | 4 (8%) |
| Hyperplasia | 2 (4%) | | 1 (2%) | |
| Inflammation | 1 (2%) | 1 (2%) | 2 (4%) | 2 (4%) |
| Ulcer | | 1 (2%) | 1 (2%) | 1 (2%) |
| Hair follicle, hyperplasia | | 1 (2%) | | |
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Hyperostosis | | | | 1 (2%) |
| Tendon, inflammation | | | 1 (2%) | 1 (2%) |
| Skeletal muscle | (0) | (0) | (2) | (0) |
| Hemorrhage | | | 1 (50%) | |
| **Nervous System** | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Edema | | 1 (2%) | | |
| Gliosis | | 2 (4%) | | |
| Hemorrhage | 1 (2%) | | | 2 (4%) |
| Necrosis | | 4 (8%) | 1 (2%) | 3 (6%) |
| **Respiratory System** | | | | |
| Lung | (50) | (50) | (50) | (50) |
| Congestion | 1 (2%) | 2 (4%) | | |
| Fibrosis | | 1 (2%) | | |
| Foreign body | 1 (2%) | | | |
| Hemorrhage | 1 (2%) | | | |
| Inflammation, granulomatous | 7 (14%) | 1 (2%) | 2 (4%) | 5 (10%) |
| Inflammation, chronic active | 6 (12%) | 6 (12%) | 6 (12%) | 5 (10%) |
| Mineralization | 1 (2%) | | | |
| Necrosis | 1 (2%) | 1 (2%) | | |
| Pigmentation | 3 (6%) | 16 (32%) | 12 (24%) | 16 (32%) |
| Alveolar epithelium, hyperplasia | 2 (4%) | 3 (6%) | | 2 (4%) |
| Alveolar epithelium, metaplasia, bronchiolar | 1 (2%) | 32 (66%) | 32 (64%) | 34 (68%) |
| Alveolar epithelium, metaplasia, squamous | | 14 (28%) | 22 (44%) | 22 (44%) |
| Alveolus, infiltration cellular, histiocyte | 23 (46%) | 34 (68%) | 35 (70%) | 35 (70%) |
| Smooth muscle, hyperplasia, focal | 1 (2%) | | | |
| Nose | (50) | (50) | (50) | (50) |
| Foreign body | 2 (4%) | | | |
| Inflammation | 10 (20%) | 5 (10%) | 9 (18%) | 9 (18%) |
| Nasolacrimal duct, hyperplasia | | | 1 (2%) | |

**TABLE A3**
**Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Special Senses System** | | | | |
| Ear | (0) | (1) | (0) | (0) |
| External ear, hyperplasia, squamous | | 1 (100%) | | |
| Eye | (48) | (50) | (50) | (50) |
| Anterior chamber, inflammation | 1 (2%) | 2 (4%) | 1 (2%) | 1 (2%) |
| Cornea, degeneration | | 1 (2%) | | |
| Cornea, hyperplasia, squamous | 1 (2%) | | | |
| Cornea, inflammation | 3 (6%) | 12 (24%) | 5 (10%) | 2 (4%) |
| Cornea, mineralization | | 1 (2%) | | |
| Lens, cataract | | | 1 (2%) | |
| Optic nerve, infiltration cellular, mononuclear cell | | | 1 (2%) | |
| Retina, atrophy | | | | 1 (2%) |
| Retina, degeneration | 1 (2%) | 1 (2%) | 6 (12%) | 2 (4%) |
| Harderian gland | (50) | (50) | (50) | (50) |
| Atrophy | 1 (2%) | | | 1 (2%) |
| Inflammation | | 1 (2%) | | |
| Lacrimal gland | (0) | (1) | (0) | (0) |
| Cytoplasmic alteration | | 1 (100%) | | |
| Zymbal's gland | (1) | (0) | (1) | (0) |
| **Urinary System** | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Mineralization | 22 (44%) | 20 (40%) | 24 (48%) | 24 (48%) |
| Nephropathy | 50 (100%) | 50 (100%) | 47 (94%) | 48 (96%) |
| Cortex, cyst | 2 (4%) | 3 (6%) | 1 (2%) | 2 (4%) |
| Pelvis, dilatation | | | | 1 (2%) |
| Pelvis, inflammation | 1 (2%) | 2 (4%) | | 2 (4%) |
| Pelvis, transitional epithelium, hyperplasia | 1 (2%) | 1 (2%) | 1 (2%) | 1 (2%) |
| Renal tubule, hyperplasia, atypical | | | 1 (2%) | |
| Transitional epithelium, hyperplasia | 1 (2%) | | | |
| Ureter | (41) | (31) | (42) | (49) |
| Transitional epithelium, hyperplasia | | | | 3 (6%) |
| Urethra | (50) | (50) | (50) | (50) |
| Transitional epithelium, hyperplasia | | | | 2 (4%) |
| Urinary bladder | (50) | (50) | (50) | (50) |
| Inflammation | 1 (2%) | 1 (2%) | 2 (4%) | |

# APPENDIX B
# SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR GAVAGE STUDY OF TCAB

**TABLE B1**
**Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of TCAB[a]**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Accidental deaths | 1 | | 2 | |
| Moribund | 15 | 12 | 11 | 11 |
| Natural deaths | 9 | 8 | 19 | 22 |
| Survivors | | | | |
| Terminal sacrifice | 25 | 30 | 18 | 17 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Intestine large, cecum | (50) | (48) | (50) | (49) |
| Intestine large, colon | (50) | (50) | (50) | 50) |
| Intestine small, duodenum | (50) | (50) | (50) | (49) |
| Carcinoma | 1 (2%) | | | |
| Intestine small, ileum | (50) | (49) | (49) | (49) |
| Intestine small, jejunum | (50) | (49) | (50) | (49) |
| Leiomyosarcoma | | 1 (2%) | | |
| Liver | (50) | (50) | (49) | (49) |
| Carcinoma, metastatic, pancreas | 1 (2%) | | 1 (2%) | |
| Cholangiocarcinoma | 1 (2%) | 1 (2%) | 1 (2%) | 3 (6%) |
| Hepatocellular adenoma | 2 (4%) | | 1 (2%) | 1 (2%) |
| Hepatocellular carcinoma | | 1 (2%) | | |
| Mesentery | (3) | (2) | (1) | (3) |
| Carcinoma, metastatic, pancreas | 1 (33%) | | | |
| Schwannoma malignant | | | | 1 (33%) |
| Oral mucosa | (50) | (50) | (50) | (50) |
| Gingival, squamous cell carcinoma | | | 4 (8%) | 5 (10%) |
| Gingival, squamous cell carcinoma, multiple | | | | 1 (2%) |
| Pancreas | (50) | (49) | (49) | (49) |
| Carcinoma, metastatic, intestine small, duodenum | 1 (2%) | | | |
| Duct, carcinoma | 1 (2%) | | 1 (2%) | |
| Salivary glands | (50) | (49) | (50) | (49) |
| Stomach, forestomach | (50) | (49) | (50) | (49) |
| Leiomyosarcoma | | | 1 (2%) | |
| Squamous cell carcinoma | | 1 (2%) | | 1 (2%) |
| Squamous cell papilloma | | | | 2 (4%) |
| Squamous cell papilloma, multiple | | | | 1 (2%) |
| Stomach, glandular | (50) | (49) | (49) | (49) |
| Carcinoma, metastatic, pancreas | 1 (2%) | | | |
| Tooth | (50) | (50) | (50) | (50) |
| **Cardiovascular System** | | | | |
| Blood vessel | (50) | (50) | (50) | (50) |
| Heart | (50) | (50) | (49) | (49) |
| Sarcoma | | | 1 (2%) | |
| Schwannoma benign | | 1 (2%) | | |
| Schwannoma malignant | | | | 1 (2%) |

**TABLE B1**
**Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (50) | (50) | (49) |
| Adenoma | 1 (2%) | 1 (2%) | 3 (6%) | 4 (8%) |
| Adrenal medulla | (50) | (50) | (50) | (49) |
| Pheochromocytoma benign | 3 (6%) | 2 (4%) | 2 (4%) | 1 (2%) |
| Pheochromocytoma benign, multiple | | | 1 (2%) | |
| Islets, pancreatic | (50) | (50) | (50) | (49) |
| Adenoma | | 1 (2%) | 1 (2%) | |
| Parathyroid gland | (46) | (46) | (46) | (45) |
| Adenoma | | | | 1 (2%) |
| Pituitary gland | (50) | (50) | (50) | (50) |
| Pars distalis, adenoma | 13 (26%) | 11 (22%) | 4 (8%) | 6 (12%) |
| Pars intermedia, adenoma | 1 (2%) | | | |
| Thyroid gland | (50) | (49) | (50) | (50) |
| Bilateral, C-cell, adenoma | 1 (2%) | | | |
| C-cell, adenoma | 9 (18%) | 9 (18%) | 11 (22%) | 1 (2%) |
| C-cell, carcinoma | 1 (2%) | 1 (2%) | | |
| Follicular cell, adenoma | | | | 1 (2%) |
| **General Body System** | | | | |
| Peritoneum | (0) | (1) | (1) | (0) |
| Carcinoma, metastatic, pancreas | | | 1 (100%) | |
| Sarcoma | | 1 (100%) | | |
| **Genital System** | | | | |
| Clitoral gland | (50) | (50) | (50) | (50) |
| Ovary | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, pancreas | 1 (2%) | | 1 (2%) | |
| Granulosa cell tumor malignant | 1 (2%) | | | |
| Schwannoma malignant | | | 1 (2%) | |
| Thecoma benign | | 1 (2%) | 2 (4%) | |
| Oviduct | (2) | (0) | (3) | (2) |
| Uterus | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, intestine small, duodenum | 1 (2%) | | | |
| Carcinoma, metastatic, pancreas | | | 1 (2%) | |
| Polyp stromal | 6 (12%) | 10 (20%) | 10 (20%) | 10 (20%) |
| Polyp stromal, multiple | | 1 (2%) | | |
| Sarcoma stromal | | | | 1 (2%) |
| Squamous cell carcinoma | 1 (2%) | 1 (2%) | | |
| Squamous cell papilloma | | | | 1 (2%) |
| Cervix, fibroma | | | 1 (2%) | |
| Endometrium, carcinoma | 1 (2%) | 1 (2%) | 2 (4%) | 2 (4%) |
| Endometrium, polyp stromal | 2 (4%) | | | |
| Vagina | (2) | (2) | (0) | (4) |
| Sarcoma stromal, metastatic, uterus | | | | 1 (25%) |

**TABLE B1**
**Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Hematopoietic System** | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Lymph node | (1) | (0) | (4) | (2) |
| Lymph node, mandibular | (50) | (49) | (49) | (49) |
| Lymph node, mesenteric | (50) | (49) | (50) | (49) |
| Carcinoma, metastatic, pancreas | | | 1 (2%) | |
| Spleen | (50) | (50) | (49) | (49) |
| Carcinoma, metastatic, pancreas | 1 (2%) | | | |
| Thymus | (50) | (50) | (47) | (50) |
| **Integumentary System** | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Adenoma | 1 (2%) | 1 (2%) | | |
| Carcinoma | 4 (8%) | 1 (2%) | | |
| Fibroadenoma | 23 (46%) | 21 (42%) | 20 (40%) | 12 (24%) |
| Fibroadenoma, multiple | 8 (16%) | 9 (18%) | 4 (8%) | 8 (16%) |
| Skin | (50) | (50) | (50) | (50) |
| Basal cell adenoma | | | | 1 (2%) |
| Subcutaneous tissue, fibrosarcoma | | | | 1 (2%) |
| **Musculoskeletal System** | | | | |
| Skeletal muscle | (2) | (1) | (0) | (0) |
| Rhabdomyosarcoma | 1 (50%) | | | |
| **Nervous System** | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Astrocytoma benign | | 1 (2%) | | |
| Peripheral nerve | (2) | (1) | (0) | (1) |
| Ganglioneuroma | | 1 (100%) | | |
| Schwannoma malignant | 1 (50%) | | | |
| Squamous cell carcinoma, metastatic, oral mucosa | | | | 1 (100%) |
| **Respiratory System** | | | | |
| Lung | (50) | (50) | (49) | (49) |
| Carcinoma, metastatic, mammary gland | 1 (2%) | | | |
| Cystic keratinizing epithelioma | | 4 (8%) | 7 (14%) | 4 (8%) |
| Cystic keratinizing epithelioma, multiple | | 2 (4%) | 19 (39%) | 35 (71%) |
| Sarcoma, metastatic, heart | | | 1 (2%) | |
| Schwannoma malignant, metastatic, heart | | | | 1 (2%) |
| Nose | (50) | (50) | (50) | (50) |
| Trachea | (50) | (50) | (50) | (50) |
| Schwannoma malignant, metastatic, heart | | | | 1 (2%) |
| **Special Senses System** | | | | |
| Ear | (0) | (0) | (1) | (0) |
| Eye | (50) | (50) | (50) | (50) |
| Harderian gland | (50) | (50) | (50) | (50) |
| Zymbal's gland | (0) | (1) | (0) | (0) |
| Carcinoma | | 1 (100%) | | |

**TABLE B1**
**Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Urinary System** | | | | |
| Kidney | (50) | (50) | (50) | (49) |
| Carcinoma, metastatic, intestine small, duodenum | 1 (2%) | | | |
| Lipoma | 1 (2%) | | | |
| Urethra | (50) | (50) | (50) | (49) |
| Urinary bladder | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, uterus | | | 1 (2%) | 1 (2%) |
| **Systemic Lesions** | | | | |
| Multiple organs[b] | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | 1 (2%) | 1 (2%) | | |
| Leukemia mononuclear | 1 (2%) | | | |
| Lymphoma malignant | | 1 (2%) | 2 (4%) | 2 (4%) |
| **Neoplasm Summary** | | | | |
| Total animals with primary neoplasms[c] | 44 | 44 | 42 | 44 |
| Total primary neoplasms | 86 | 88 | 99 | 108 |
| Total animals with benign neoplasms | 40 | 42 | 39 | 42 |
| Total benign neoplasms | 71 | 76 | 86 | 90 |
| Total animals with malignant neoplasms | 13 | 10 | 12 | 12 |
| Total malignant neoplasms | 15 | 12 | 13 | 18 |
| Total animals with metastatic neoplasms | 3 | | 3 | 3 |
| Total metastatic neoplasms | 9 | | 7 | 5 |

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

**TABLE B2**
**Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Adrenal Cortex: Adenoma** | | | | |
| Overall rate[a] | 1/50 (2%) | 1/50 (2%) | 3/50 (6%) | 4/49 (8%) |
| Adjusted rate[b] | 2.6% | 2.6% | 8.1% | 10.4% |
| Terminal rate[c] | 1/25 (4%) | 1/30 (3%) | 0/19 (0%) | 1/17 (6%) |
| First incidence (days) | 727 (T) | 727 (T) | 649 | 590 |
| Poly-3 test[d] | P = 0.093 | P = 0.757 | P = 0.289 | P = 0.175 |
| **Adrenal Medulla: Benign Pheochromocytoma** | | | | |
| Overall rate | 3/50 (6%) | 2/50 (4%) | 3/50 (6%) | 1/49 (2%) |
| Adjusted rate | 7.7% | 5.1% | 8.1% | 2.6% |
| Terminal rate | 2/25 (8%) | 1/30 (3%) | 2/19 (11%) | 0/17 (0%) |
| First incidence (days) | 684 | 366 | 668 | 684 |
| Poly-3 test | P = 0.280N | P = 0.500N | P = 0.638 | P = 0.315N |
| **Liver: Cholangiocarcinoma** | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 1/49 (2%) | 3/49 (6%) |
| Adjusted rate | 2.6% | 2.6% | 2.7% | 7.9% |
| Terminal rate | 0/25 (0%) | 0/30 (0%) | 1/19 (5%) | 0/17 (0%) |
| First incidence (days) | 684 | 722 | 727 (T) | 658 |
| Poly-3 test | P = 0.153 | P = 0.756 | P = 0.747 | P = 0.295 |
| **Lung: Cystic Keratinizing Epithelioma** | | | | |
| Overall rate | 0/50 (0%) | 6/50 (12%) | 26/49 (53%) | 39/49 (80%) |
| Adjusted rate | 0.0% | 15.5% | 62.6% | 89.5% |
| Terminal rate | 0/25 (0%) | 4/30 (13%) | 9/19 (47%) | 17/17 (100%) |
| First incidence (days) | —[e] | 589 | 540 | 481 |
| Poly-3 test | P < 0.001 | P = 0.014 | P < 0.001 | P < 0.001 |
| **Mammary Gland: Fibroadenoma** | | | | |
| Overall rate | 31/50 (62%) | 30/50 (60%) | 24/50 (48%) | 20/50 (40%) |
| Adjusted rate | 68.9% | 69.7% | 56.5% | 47.3% |
| Terminal rate | 14/25 (56%) | 20/30 (67%) | 11/19 (58%) | 6/17 (35%) |
| First incidence (days) | 478 | 335 | 273 | 467 |
| Poly-3 test | P = 0.010N | P = 0.558 | P = 0.155N | P = 0.028N |
| **Mammary Gland: Fibroadenoma or Adenoma** | | | | |
| Overall rate | 31/50 (62%) | 31/50 (62%) | 24/50 (48%) | 20/50 (40%) |
| Adjusted rate | 68.9% | 72.1% | 56.5% | 47.3% |
| Terminal rate | 14/25 (56%) | 21/30 (70%) | 11/19 (58%) | 6/17 (35%) |
| First incidence (days) | 478 | 335 | 273 | 467 |
| Poly-3 test | P = 0.008N | P = 0.460 | P = 0.155N | P = 0.028N |
| **Mammary Gland: Carcinoma** | | | | |
| Overall rate | 4/50 (8%) | 1/50 (2%) | 0/50 (0%) | 0/50 (0%) |
| Adjusted rate | 9.8% | 2.6% | 0.0% | 0.0% |
| Terminal rate | 0/25 (0%) | 1/30 (3%) | 0/19 (0%) | 0/17 (0%) |
| First incidence (days) | 372 | 727 (T) | — | — |
| Poly-3 test | P = 0.060N | P = 0.198N | P = 0.073N | P = 0.069N |
| **Mammary Gland: Adenoma or Carcinoma** | | | | |
| Overall rate | 5/50 (10%) | 2/50 (4%) | 0/50 (0%) | 0/50 (0%) |
| Adjusted rate | 12.2% | 5.3% | 0.0% | 0.0% |
| Terminal rate | 0/25 (0%) | 2/30 (7%) | 0/19 (0%) | 0/17 (0%) |
| First incidence (days) | 372 | 727 (T) | — | — |
| Poly-3 test | P = 0.027N | P = 0.244N | P = 0.040N | P = 0.037N |

**TABLE B2**
**Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma** | | | | |
| Overall rate | 32/50 (64%) | 31/50 (62%) | 24/50 (48%) | 20/50 (40%) |
| Adjusted rate | 69.7% | 72.1% | 56.5% | 47.3% |
| Terminal rate | 14/25 (56%) | 21/30 (70%) | 11/19 (58%) | 6/17 (35%) |
| First incidence (days) | 372 | 335 | 273 | 467 |
| Poly-3 test | P = 0.006N | P = 0.497 | P = 0.133N | P = 0.022N |
| **Oral Mucosa: Squamous Cell Carcinoma** | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 4/50 (8%) | 6/50 (12%) |
| Adjusted rate | 0.0% | 0.0% | 10.6% | 15.4% |
| Terminal rate | 0/25 (0%) | 0/30 (0%) | 1/19 (5%) | 1/17 (6%) |
| First incidence (days) | — | — | 574 | 551 |
| Poly-3 test | P = 0.002 | —[f] | P = 0.055 | P = 0.015 |
| **Pituitary Gland (Pars Distalis): Adenoma** | | | | |
| Overall rate | 13/50 (26%) | 11/50 (22%) | 4/50 (8%) | 6/50 (12%) |
| Adjusted rate | 32.9% | 28.8% | 10.7% | 15.6% |
| Terminal rate | 10/25 (40%) | 10/30 (33%) | 1/19 (5%) | 4/17 (24%) |
| First incidence (days) | 636 | 705 | 611 | 608 |
| Poly-3 test | P = 0.050N | P = 0.442N | P = 0.016N | P = 0.061N |
| **Stomach (Forestomach): Squamous Cell Papilloma** | | | | |
| Overall rate | 0/50 (0%) | 0/50 (0%) | 0/50 (0%) | 3/50 (6%) |
| Adjusted rate | 0.0% | 0.0% | 0.0% | 7.8% |
| Terminal rate | 0/25 (0%) | 0/30 (0%) | 0/19 (0%) | 0/17 (0%) |
| First incidence (days) | — | — | — | 590 |
| Poly-3 test | P = 0.008 | — | — | P = 0.116 |
| **Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma** | | | | |
| Overall rate | 0/50 (0%) | 1/50 (2%) | 0/50 (0%) | 4/50 (8%) |
| Adjusted rate | 0.0% | 2.6% | 0.0% | 10.3% |
| Terminal rate | 0/25 (0%) | 1/30 (3%) | 0/19 (0%) | 0/17 (0%) |
| First incidence (days) | — | 727 (T) | — | 590 |
| Poly-3 test | P = 0.009 | P = 0.496 | — | P = 0.059 |
| **Thyroid Gland (C-Cell): Adenoma** | | | | |
| Overall rate | 10/50 (20%) | 9/49 (18%) | 11/50 (22%) | 1/50 (2%) |
| Adjusted rate | 25.0% | 23.7% | 29.0% | 2.7% |
| Terminal rate | 7/25 (28%) | 9/30 (30%) | 5/19 (26%) | 1/17 (6%) |
| First incidence (days) | 587 | 727 (T) | 540 | 727 (T) |
| Poly-3 test | P = 0.003N | P = 0.553N | P = 0.442 | P = 0.005N |
| **Thyroid Gland (C-Cell): Adenoma or Carcinoma** | | | | |
| Overall rate | 11/50 (22%) | 9/49 (18%) | 11/50 (22%) | 1/50 (2%) |
| Adjusted rate | 27.5% | 23.7% | 29.0% | 2.7% |
| Terminal rate | 8/25 (32%) | 9/30 (30%) | 5/19 (26%) | 1/17 (6%) |
| First incidence (days) | 587 | 727 (T) | 540 | 727 (T) |
| Poly-3 test | P = 0.002N | P = 0.451N | P = 0.541 | P = 0.002N |
| **Uterus: Stromal Polyp** | | | | |
| Overall rate | 8/50 (16%) | 11/50 (22%) | 10/50 (20%) | 10/50 (20%) |
| Adjusted rate | 20.1% | 28.5% | 26.2% | 25.8% |
| Terminal rate | 6/25 (24%) | 10/30 (33%) | 5/19 (26%) | 7/17 (41%) |
| First incidence (days) | 504 | 560 | 560 | 608 |
| Poly-3 test | P = 0.460 | P = 0.272 | P = 0.353 | P = 0.366 |

**TABLE B2**
**Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Uterus: Stromal Polyp or Stromal Sarcoma** | | | | |
| Overall rate | 8/50 (16%) | 11/50 (22%) | 10/50 (20%) | 11/50 (22%) |
| Adjusted rate | 20.1% | 28.5% | 26.2% | 28.1% |
| Terminal rate | 6/25 (24%) | 10/30 (33%) | 5/19 (26%) | 7/17 (41%) |
| First incidence (days) | 504 | 560 | 560 | 590 |
| Poly-3 test | P = 0.356 | P = 0.272 | P = 0.353 | P = 0.283 |
| **All Organs: Fibrosarcoma, Sarcoma, or Malignant Schwannoma** | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 2/50 (4%) | 3/50 (6%) |
| Adjusted rate | 2.5% | 2.6% | 5.4% | 7.7% |
| Terminal rate | 0/25 (0%) | 1/30 (3%) | 1/19 (5%) | 0/17 (0%) |
| First incidence (days) | 575 | 727 (T) | 644 | 558 |
| Poly-3 test | P = 0.189 | P = 0.754 | P = 0.479 | P = 0.301 |
| **All Organs: Fibrosarcoma, Sarcoma, or Benign or Malignant Schwannoma** | | | | |
| Overall rate | 1/50 (2%) | 2/50 (4%) | 2/50 (4%) | 3/50 (6%) |
| Adjusted rate | 2.5% | 5.3% | 5.4% | 7.7% |
| Terminal rate | 0/25 (0%) | 2/30 (7%) | 1/19 (5%) | 0/17 (0%) |
| First incidence (days) | 575 | 727 (T) | 644 | 558 |
| Poly-3 test | P = 0.275 | P = 0.489 | P = 0.479 | P = 0.301 |
| **All Organs: Benign Neoplasms** | | | | |
| Overall rate | 40/50 (80%) | 42/50 (84%) | 39/50 (78%) | 42/50 (84%) |
| Adjusted rate | 88.3% | 93.5% | 87.3% | 93.1% |
| Terminal rate | 22/25 (88%) | 29/30 (97%) | 17/19 (90%) | 17/17 (100%) |
| First incidence (days) | 478 | 335 | 273 | 467 |
| Poly-3 test | P = 0.354 | P = 0.291 | P = 0.576N | P = 0.314 |
| **All Organs: Malignant Neoplasms** | | | | |
| Overall rate | 13/50 (26%) | 10/50 (20%) | 12/50 (24%) | 12/50 (24%) |
| Adjusted rate | 30.8% | 25.4% | 30.7% | 29.5% |
| Terminal rate | 5/25 (20%) | 7/30 (23%) | 5/19 (26%) | 3/17 (18%) |
| First incidence (days) | 372 | 527 | 574 | 551 |
| Poly-3 test | P = 0.532 | P = 0.386N | P = 0.592N | P = 0.545N |
| **All Organs: Benign or Malignant Neoplasms** | | | | |
| Overall rate | 44/50 (88%) | 44/50 (88%) | 42/50 (84%) | 44/50 (88%) |
| Adjusted rate | 92.7% | 96.7% | 92.0% | 95.5% |
| Terminal rate | 23/25 (92%) | 30/30 (100%) | 18/19 (95%) | 17/17 (100%) |
| First incidence (days) | 372 | 335 | 273 | 467 |
| Poly-3 test | P = 0.476 | P = 0.318 | P = 0.618N | P = 0.437 |

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of the statistic cannot be computed

**TABLE B3a**
**Historical Incidence of Cystic Keratinizing Epithelioma in the Lung of Vehicle Control Female Harlan Sprague-Dawley Rats[a]**

| Study (Study Start) | Incidence in Controls |
|---|---|
| PCB 126 (February, 1998) | 0/53 |
| TCDD (June, 1998) | 0/53 |
| PeCDF (April, 1999) | 0/53 |
| TEF Mixture (June, 1998) | 0/53 |
| PCB 153 (August, 1998) | 0/52 |
| Binary Mixture of PCB 126/PCB 153 (September, 1998) | 0/53 |
| Binary Mixture of PCB 126/PCB 118 (October, 1999) | 0/53 |
| PCB 118 (March, 2004) | 0/51 |
| TCAB (January, 2003) | 0/50 |
| **Overall Historical Incidence** | |
| Total | 0/471 |

[a] Data as of November 13, 2008

**TABLE B3b**
**Historical Incidence of Cholangiocarcinoma in the Liver of Vehicle Control Female Harlan Sprague-Dawley Rats[a]**

| Study (Study Start) | Incidence in Controls |
|---|---|
| PCB 126 (February, 1998) | 0/53 |
| TCDD (June, 1998) | 0/53 |
| PeCDF (April, 1999) | 0/53 |
| TEF Mixture (June, 1998) | 0/53 |
| PCB 153 (August, 1998) | 0/53 |
| Binary Mixture of PCB 126/PCB 153 (September, 1998) | 0/53 |
| Binary Mixture of PCB 126/PCB 118 (October, 1999) | 0/53 |
| PCB 118 (March, 2004) | 0/52 |
| TCAB (January, 2003) | 1/50 |
| **Overall Historical Incidence** | |
| Total (%) | 1 /473 (0.2%) |
| Mean ± standard deviation | 0.2% ± 0.7% |
| Range | 0% – 2% |

[a] Data as of November 13, 2008

**TABLE B3c**
**Historical Incidence of Squamous Cell Carcinoma in the Oral Mucosa of Vehicle Control Female Harlan Sprague-Dawley Rats[a]**

| Study (Study Start) | Incidence in Controls |
|---|---|
| PCB 126 (February, 1998) | 0/53 |
| TCDD (June, 1998) | 1/53 |
| PeCDF (April, 1999) | 1/53 |
| TEF Mixture (June, 1998) | 1/53 |
| PCB 153 (August, 1998) | 0/53 |
| Binary Mixture of PCB 126/PCB 153 (September, 1998) | 0/53 |
| Binary Mixture of PCB 126/PCB 118 (October, 1999) | 1/53 |
| PCB 118 (March, 2004) | 0/52 |
| TCAB (January, 2003) | 0/50 |
| **Overall Historical Incidence** | |
| Total (%) | 4/473 (0.9%) |
| Mean ± standard deviation | 0.8% ± 1.0% |
| Range | 0% – 2% |

[a] Data as of November 13, 2008

**TABLE B3d**
**Historical Incidence of Follicular Cell Adenoma in the Thyroid Gland of Vehicle Control Female Harlan Sprague-Dawley Rats[a]**

| Study (Study Start) | Incidence in Controls |
|---|---|
| PCB 126 (February, 1998) | 0/52 |
| TCDD (June, 1998) | 1/52 |
| PeCDF (April, 1999) | 0/53 |
| TEF Mixture (June, 1998) | 0/53 |
| PCB 153 (August, 1998) | 0/51 |
| Binary Mixture of PCB 126/PCB 153 (September, 1998) | 0/53 |
| Binary Mixture of PCB 126/PCB 118 (October, 1999) | 0/53 |
| PCB 118 (March, 2004) | 1/51 |
| TCAB (January, 2003) | 0/50 |
| **Overall Historical Incidence** | |
| Total (%) | 2/468 (0.4%) |
| Mean ± standard deviation | 0.4% ± 0.9% |
| Range | 0% – 2% |

[a] Data as of November 13, 2008

**TABLE B3e**
**Historical Incidence of Forestomach Neoplasms in Vehicle Control Female Harlan Sprague-Dawley Rats[a]**

| Study (Study Start) | Incidence in Controls | | |
|---|---|---|---|
| | Squamous Cell Papilloma | Squamous Cell Carcinoma | Squamous Cell Papilloma or Squamous Cell Carcinoma |
| PCB 126 (February, 1998) | 0/53 | 0/53 | 0/53 |
| TCDD (June, 1998) | 0/53 | 1/53 | 1/53 |
| PeCDF (April, 1999) | 0/53 | 0/53 | 0/53 |
| TEF Mixture (June, 1998) | 0/53 | 0/53 | 0/53 |
| PCB 153 (August, 1998) | 0/53 | 0/53 | 0/53 |
| Binary Mixture of PCB 126/PCB 153 (September, 1998) | 0/53 | 0/53 | 0/53 |
| Binary Mixture of PCB 126/PCB 118 (October, 1999) | 0/53 | 1/53 | 1/53 |
| PCB 118 (March, 2004) | 0/52 | 0/52 | 0/52 |
| TCAB (January, 2003) | 0/50 | 0/50 | 0/50 |
| **Overall Historical Incidence** | | | |
| Total (%) | 0/473 | 2/473 (0.4%) | 2/473 (0.4%) |
| Mean ± standard deviation | | 0.4% ± 0.8% | 0.4% ± 0.8% |
| Range | | 0% – 2% | 0% – 2% |

[a] Data as of November 13, 2008

**TABLE B3f**
**Historical Incidence of Adenoma in the Adrenal Cortex of Vehicle Control Female Harlan Sprague-Dawley Rats[a]**

| Study (Study Start) | Incidence in Controls |
|---|---|
| PCB 126 (February, 1998) | 0/52 |
| TCDD (June, 1998) | 1/53 |
| PeCDF (April, 1999) | 1/53 |
| TEF Mixture (June, 1998) | 0/52 |
| PCB 153 (August, 1998) | 0/53 |
| Binary Mixture of PCB 126/PCB 153 (September, 1998) | 0/53 |
| Binary Mixture of PCB 126/PCB 118 (October, 1999) | 0/53 |
| PCB 118 (March, 2004) | 2/52 |
| TCAB (January, 2003) | 1/50 |
| **Overall Historical Incidence** | |
| Total (%) | 5/471 (1.1%) |
| Mean ± standard deviation | 1.1% ± 1.4% |
| Range | 0% – 4% |

[a] Data as of November 13, 2008

**TABLE B4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of TCAB[a]**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Accidental deaths | 1 | | 2 | |
| Moribund | 15 | 12 | 11 | 11 |
| Natural deaths | 9 | 8 | 19 | 22 |
| Survivors | | | | |
| Terminal sacrifice | 25 | 30 | 18 | 17 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Intestine large, cecum | (50) | 48) | (50) | (49) |
| Necrosis | | | | 1 (2%) |
| Intestine large, colon | (50) | (50) | (50) | (50) |
| Parasite metazoan | 6 (12%) | 2 (4%) | | 3 (6%) |
| Intestine small, duodenum | (50) | (50) | (50) | (49) |
| Intestine small, ileum | (50) | (49) | (49) | (49) |
| Intestine small, jejunum | (50) | (49) | (50) | (49) |
| Liver | (50) | (50) | (49) | (49) |
| Angiectasis | | 4 (8%) | 3 (6%) | 5 (10%) |
| Basophilic focus | 14 (28%) | 10 (20%) | 9 (18%) | 7 (14%) |
| Cholangiofibrosis | | 1 (2%) | | 11 (22%) |
| Clear cell focus | 13 (26%) | 22 (44%) | 9 (18%) | 7 (14%) |
| Degeneration, cystic | | 2 (4%) | 3 (6%) | |
| Eosinophilic focus | 3 (6%) | 27 (54%) | 31 (63%) | 38 (78%) |
| Fatty change, focal | 2 (4%) | 2 (4%) | 2 (4%) | 9 (18%) |
| Fatty change, diffuse | | 3 (6%) | 10 (20%) | 10 (20%) |
| Fibrosis | 1 (2%) | | 2 (4%) | 6 (12%) |
| Hematopoietic cell proliferation | 28 (56%) | 42 (84%) | 32 (65%) | 37 (76%) |
| Hepatodiaphragmatic nodule | 1 (2%) | | 1 (2%) | 1 (2%) |
| Hyperplasia, nodular | 1 (2%) | 3 (6%) | 11 (22%) | 22 (45%) |
| Inflammation, chronic active | 29 (58%) | 29 (58%) | 32 (65%) | 30 (61%) |
| Mixed cell focus | 6 (12%) | 16 (32%) | 14 (29%) | 16 (33%) |
| Necrosis | 3 (6%) | 6 (12%) | 10 (20%) | 10 (20%) |
| Necrosis, focal | | | | 2 (4%) |
| Pigmentation | 1 (2%) | 17 (34%) | 32 (65%) | 40 (82%) |
| Thrombosis | 1 (2%) | | 1 (2%) | |
| Toxic hepatopathy | | 4 (8%) | 14 (29%) | 25 (51%) |
| Bile duct, cyst | 3 (6%) | 4 (8%) | 5 (10%) | 12 (24%) |
| Bile duct, fibrosis | 1 (2%) | 1 (2%) | 5 (10%) | 1 (2%) |
| Bile duct, hyperplasia | 12 (24%) | 26 (52%) | 29 (59%) | 38 (78%) |
| Centrilobular, degeneration | 1 (2%) | 2 (4%) | 18 (37%) | 17 (35%) |
| Hepatocyte, hypertrophy | 4 (8%) | 33 (66%) | 38 (78%) | 42 (86%) |
| Hepatocyte, multinucleated | | 2 (4%) | 1 (2%) | 28 (57%) |
| Oval cell, hyperplasia | | 7 (14%) | 24 (49%) | 36 (73%) |
| Serosa, fibrosis | | 1 (2%) | | |
| Mesentery | (3) | (2) | (1) | (3) |
| Thrombosis | | | 1 (100%) | |
| Fat, Necrosis | 1 (33%) | 1 (50%) | | 2 (67%) |
| Oral mucosa | (50) | (50) | (50) | (50) |
| Gingival, hyperplasia, cystic keratinizing | | 4 (8%) | 9 (18%) | 13 (26%) |
| Gingival, hyperplasia, squamous | | 8 (16%) | 24 (48%) | 24 (48%) |

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

**TABLE B4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Alimentary System** *(continued)* | | | | |
| Pancreas | (50) | (49) | (49) | (49) |
| Atrophy | | 11 (22%) | 12 (24%) | 13 (27%) |
| Inflammation | 1 (2%) | 1 (2%) | 4 (8%) | 6 (12%) |
| Acinus, hyperplasia | | | 1 (2%) | 1 (2%) |
| Acinus, vacuolization cytoplasmic | | 27 (55%) | 33 (67%) | 40 (82%) |
| Duct, atypia cellular | | | 1 (2%) | |
| Salivary glands | (50) | (49) | (50) | (49) |
| Atrophy | 1 (2%) | 1 (2%) | | 2 (4%) |
| Fibrosis | | 1 (2%) | | |
| Duct, cyst | | | | 1 (2%) |
| Stomach, forestomach | (50) | (49) | (50) | (49) |
| Cyst | | | | 2 (4%) |
| Inflammation | 1 (2%) | 3 (6%) | 5 (10%) | 7 (14%) |
| Mineralization | 2 (4%) | 2 (4%) | 1 (2%) | |
| Necrosis | | | 3 (6%) | 2 (4%) |
| Epithelium, hyperplasia | | 32 (65%) | 46 (92%) | 46 (94%) |
| Stomach, glandular | (50) | (49) | (49) | (49) |
| Inflammation | 1 (2%) | | | |
| Mineralization | | 1 (2%) | 1 (2%) | |
| Tooth | (50) | (50) | (50) | (50) |
| Peridontal tissue, inflammation | 5 (10%) | 10 (20%) | 14 (28%) | 9 (18%) |
| **Cardiovascular System** | | | | |
| Blood vessel | (50) | (50) | (50) | (50) |
| Inflammation | 1 (2%) | 10 (20%) | 14 (28%) | 16 (32%) |
| Mineralization | 2 (4%) | 1 (2%) | 2 (4%) | 1 (2%) |
| Thrombosis | | | | 1 (2%) |
| Heart | (50) | (50) | (49) | (49) |
| Cardiomyopathy | 12 (24%) | 16 (32%) | 13 (27%) | 24 (49%) |
| Dilatation | | | | 1 (2%) |
| Inflammation | | | 1 (2%) | 1 (2%) |
| Mineralization | | | 1 (2%) | |
| Necrosis | | | 1 (2%) | 1 (2%) |
| Thrombosis | | | 1 (2%) | 3 (6%) |
| Endocardium, hyperplasia | 2 (4%) | | | |
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (50) | (50) | (49) |
| Atrophy | | 1 (2%) | 1 (2%) | |
| Degeneration | 13 (26%) | 12 (24%) | 17 (34%) | 16 (33%) |
| Hypertrophy | 41 (82%) | 31 (62%) | 30 (60%) | 30 (61%) |
| Inflammation | | | | 1 (2%) |
| Necrosis | 1 (2%) | | 7 (14%) | 5 (10%) |
| Thrombosis | | 1 (2%) | 1 (2%) | 1 (2%) |
| Vacuolization cytoplasmic | 7 (14%) | 16 (32%) | 13 (26%) | 15 (31%) |
| Zona fasciculata, hyperplasia | 14 (28%) | 20 (40%) | 25 (50%) | 21 (43%) |
| Adrenal medulla | (50) | (50) | (50) | (49) |
| Hyperplasia | 11 (22%) | 5 (10%) | 3 (6%) | 6 (12%) |
| Islets, pancreatic | (50) | (50) | (50) | (49) |
| Hyperplasia | | | | 1 (2%) |
| Parathyroid gland | (46) | (46) | (46) | (45) |
| Hyperplasia | | | | 2 (4%) |

**TABLE B4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Endocrine System** *(continued)* | | | | |
| Pituitary gland | (50) | (50) | (50) | (50) |
| Necrosis | 1 (2%) | | 2 (4%) | |
| Pigmentation | 1 (2%) | | | |
| Pars distalis, hyperplasia | 20 (40%) | 13 (26%) | 12 (24%) | 19 (38%) |
| Pars intermedia, hyperplasia | | | 1 (2%) | |
| Thyroid gland | (50) | (49) | (50) | (50) |
| Inflammation | | | 2 (4%) | 4 (8%) |
| C-cell, hyperplasia | 12 (24%) | 5 (10%) | 3 (6%) | 4 (8%) |
| Follicle, cyst | | 1 (2%) | | 1 (2%) |
| Follicular cell, hyperplasia | | 1 (2%) | 3 (6%) | 3 (6%) |
| Follicular cell, hypertrophy | | 1 (2%) | 2 (4%) | 4 (8%) |
| **General Body System** | | | | |
| Peritoneum | (0) | (1) | (1) | (0) |
| **Genital System** | | | | |
| Clitoral gland | (50) | (50) | (50) | (50) |
| Cyst | 5 (10%) | 1 (2%) | 6 (12%) | |
| Hyperplasia, squamous | | 1 (2%) | 3 (6%) | |
| Inflammation | 3 (6%) | | | |
| Ovary | (50) | (50) | (50) | (50) |
| Cyst | 12 (24%) | 8 (16%) | 13 (26%) | 12 (24%) |
| Inflammation | | 1 (2%) | 2 (4%) | 5 (10%) |
| Necrosis | | | | 1 (2%) |
| Oviduct | (2) | (0) | (3) | (2) |
| Cyst | | | 2 (67%) | |
| Fibrosis | 1 (50%) | | | |
| Inflammation | 2 (100%) | | 1 (33%) | 2 (100%) |
| Metaplasia, squamous | 1 (50%) | | | |
| Uterus | (50) | (50) | (50) | (50) |
| Adenomyosis | | | | 1 (2%) |
| Angiectasis | | 1 (2%) | 1 (2%) | 2 (4%) |
| Cyst | | | | 2 (4%) |
| Hemorrhage | | | 1 (2%) | 1 (2%) |
| Hydrometra | | | | 1 (2%) |
| Inflammation | 4 (8%) | 5 (10%) | 3 (6%) | 3 (6%) |
| Endometrium, hyperplasia, adenomatous | 1 (2%) | | | |
| Endometrium, hyperplasia, cystic | 15 (30%) | 4 (8%) | 5 (10%) | 6 (12%) |
| Endometrium, metaplasia, squamous | 20 (40%) | 28 (56%) | 22 (44%) | 22 (44%) |
| Vagina | (2) | (2) | (0) | (4) |
| Fibrosis | | | | 1 (25%) |
| Hyperplasia, squamous | | | | 1 (25%) |
| Inflammation | | | | 1 (25%) |
| Epithelium, atypia cellular | 1 (50%) | | | |
| Epithelium, hyperplasia | 2 (100%) | 2 (100%) | | 1 (25%) |
| **Hematopoietic System** | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Atrophy | | | | 1 (2%) |
| Hyperplasia | 36 (72%) | 41 (82%) | 40 (80%) | 45 (90%) |
| Myelofibrosis | | | 1 (2%) | 1 (2%) |

**TABLE B4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Hematopoietic System** *(continued)* | | | | |
| Lymph node | (1) | (0) | (4) | (2) |
| Pancreatic, hemorrhage | | | 1 (25%) | |
| Popliteal, inflammation, chronic active | | | | 1 (50%) |
| Popliteal, necrosis | | | | 1 (50%) |
| Thoracic, hyperplasia, lymphoid | | | 1 (25%) | |
| Lymph node, mandibular | (50) | (49) | (49) | (49) |
| Atrophy | | | | 4 (8%) |
| Hyperplasia, lymphoid | | | 2 (4%) | |
| Infiltration cellular, plasma cell | 1 (2%) | | | |
| Lymph node, mesenteric | (50) | (49) | (50) | (49) |
| Atrophy | | | 4 (8%) | 6 (12%) |
| Atrophy, lymphoid | | | | 2 (4%) |
| Pigmentation | 14 (28%) | 32 (65%) | 32 (64%) | 30 (61%) |
| Spleen | (50) | (50) | (49) | (49) |
| Accessory spleen | 1 (2%) | | | |
| Hematopoietic cell proliferation | 41 (82%) | 47 (94%) | 45 (92%) | 45 (92%) |
| Necrosis, fibrinoid | | 1 (2%) | | |
| Pigmentation | 31 (62%) | 44 (88%) | 42 (86%) | 47 (96%) |
| Lymphoid follicle, atrophy | 3 (6%) | 4 (8%) | 8 (16%) | 10 (20%) |
| Thymus | (50) | (50) | (47) | (50) |
| Atrophy | 47 (94%) | 49 (98%) | 46 (98%) | 50 (100%) |
| Hyperplasia, lymphoid | 1 (2%) | | | |
| Inflammation, granulomatous | | | | 1 (2%) |
| Vein, thrombosis | | | | 1 (2%) |
| **Integumentary System** | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Dilatation | | 1 (2%) | | |
| Galactocele | 3 (6%) | | | |
| Hyperplasia | 4 (8%) | 11 (22%) | 11 (22%) | 4 (8%) |
| Inflammation | | | | 1 (2%) |
| Pigmentation | | 1 (2%) | | |
| Skin | (50) | (50) | (50) | (50) |
| Cyst epithelial inclusion | | | | 1 (2%) |
| Hyperplasia, squamous | | | | 1 (2%) |
| **Musculoskeletal System** | | | | |
| Skeletal muscle | (2) | (1) | (0) | (0) |
| Inflammation | | 1 (100%) | | |
| **Nervous System** | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Hemorrhage | | | 2 (4%) | |
| Necrosis | | | | 1 (2%) |
| Peripheral nerve | (2) | (1) | (0) | (1) |

**TABLE B4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|
| **Respiratory System** | | | | |
| Lung | (50) | (50) | (49) | (49) |
| Congestion | 1 (2%) | | | |
| Inflammation, suppurative | 1 (2%) | | | |
| Inflammation, chronic active | 4 (8%) | 5 (10%) | 1 (2%) | 4 (8%) |
| Metaplasia, cartilaginous | 1 (2%) | | | |
| Necrosis | 1 (2%) | 1 (2%) | | |
| Pigmentation | 1 (2%) | 11 (22%) | 21 (43%) | 26 (53%) |
| Thrombosis | | | 1 (2%) | 1 (2%) |
| Alveolar epithelium, hyperplasia | 5 (10%) | 1 (2%) | | |
| Alveolar epithelium, metaplasia, bronchiolar | | 21 (42%) | 26 (53%) | 35 (71%) |
| Alveolar epithelium, metaplasia, squamous | 2 (4%) | 4 (8%) | 18 (37%) | 30 (61%) |
| Alveolus, infiltration cellular, histiocyte | 36 (72%) | 23 (46%) | 29 (59%) | 33 (67%) |
| Artery, hypertrophy | | | | 1 (2%) |
| Serosa, inflammation, granulomatous | | | 1 (2%) | |
| Nose | (50) | (50) | (50) | (50) |
| Inflammation | | 7 (14%) | 6 (12%) | 8 (16%) |
| Trachea | (50) | (50) | (50) | (50) |
| **Special Senses System** | | | | |
| Ear | (0) | (0) | (1) | (0) |
| External ear, hyperplasia, squamous | | | 1 (100%) | |
| Eye | (50) | (50) | (50) | (50) |
| Synechia | | | | 1 (2%) |
| Anterior chamber, inflammation | | | 1 (2%) | |
| Cornea, inflammation | 2 (4%) | | | 2 (4%) |
| Lens, cataract | | | | 1 (2%) |
| Retina, degeneration | 1 (2%) | | 4 (8%) | 1 (2%) |
| Harderian gland | (50) | (50) | (50) | (50) |
| Inflammation | | | | 1 (2%) |
| Zymbal's gland | (0) | (1) | (0) | (0) |
| **Urinary System** | | | | |
| Kidney | (50) | (50) | (50) | (49) |
| Accumulation, hyaline droplet | | 2 (4%) | | |
| Casts | 1 (2%) | | | 2 (4%) |
| Infarct | | 1 (2%) | 4 (8%) | 2 (4%) |
| Inflammation, diffuse | | 1 (2%) | 3 (6%) | 2 (4%) |
| Mineralization | 36 (72%) | 32 (64%) | 28 (56%) | 29 (59%) |
| Nephropathy | 30 (60%) | 39 (78%) | 39 (78%) | 42 (86%) |
| Thrombosis | | | | 1 (2%) |
| Cortex, cyst | | 1 (2%) | | 1 (2%) |
| Papilla, necrosis | | | 1 (2%) | 2 (4%) |
| Pelvis, dilatation | | | 2 (4%) | 1 (2%) |
| Pelvis, inflammation | 1 (2%) | 2 (4%) | 1 (2%) | 2 (4%) |
| Pelvis, transitional epithelium, hyperplasia | 1 (2%) | 1 (2%) | 2 (4%) | 3 (6%) |
| Renal tubule, hyperplasia, atypical | 1 (2%) | | 1 (2%) | |
| Renal tubule, necrosis | | | 3 (6%) | 3 (6%) |
| Urethra | (50) | (50) | (50) | (49) |
| Inflammation | | 1 (2%) | | |
| Transitional epithelium, hyperplasia | | 1 (2%) | 1 (2%) | 1 (2%) |
| Transitional epithelium, metaplasia, squamous | | | | 1 (2%) |
| Urinary bladder | (50) | (50) | (50) | (50) |
| Inflammation | | 1 (2%) | | 1 (2%) |
| Metaplasia, squamous | | 1 (2%) | | |
| Transitional epithelium, hyperplasia | 1 (2%) | | | |

# APPENDIX C
# SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR GAVAGE STUDY OF TCAB

**TABLE C1**
**Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of TCAB[a]**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 49 | 50 |
| Early deaths | | | | |
| Accidental deaths | 1 | | 2 | |
| Moribund | 10 | 11 | 16 | 19 |
| Natural deaths | 4 | 8 | 26 | 31 |
| Survivors | | | | |
| Terminal sacrifice | 35 | 31 | 5 | |
| Animals examined microscopically | 50 | 50 | 49 | 50 |
| **Alimentary System** | | | | |
| Esophagus | (50) | (50) | (49) | (50) |
| Gallbladder | (49) | (50) | (49) | (50) |
| Intestine large, cecum | (50) | (50) | (49) | (50) |
| Intestine large, rectum | (50) | (50) | (49) | (50) |
| Carcinoma, metastatic, urethra | | | | 1 (2%) |
| Intestine small, duodenum | (50) | (50) | (49) | (50) |
| Adenoma | | 1 (2%) | | |
| Intestine small, ileum | (50) | (50) | (49) | (50) |
| Carcinoma | | | 1 (2%) | |
| Intestine small, jejunum | (50) | (50) | (49) | (50) |
| Carcinoma | 2 (4%) | | 1 (2%) | |
| Liver | (50) | (50) | (49) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (2%) | |
| Carcinoma, metastatic, islets, pancreatic | 1 (2%) | | | |
| Fibrosarcoma, metastatic, skin | 1 (2%) | | | |
| Hemangiosarcoma | | 2 (4%) | | |
| Hemangiosarcoma, multiple | 1 (2%) | | | |
| Hepatoblastoma | 2 (4%) | 1 (2%) | | |
| Hepatocellular adenoma | 12 (24%) | 14 (28%) | 10 (20%) | 2 (4%) |
| Hepatocellular adenoma, multiple | 10 (20%) | 13 (26%) | 7 (14%) | |
| Hepatocellular carcinoma | 14 (28%) | 10 (20%) | 7 (14%) | |
| Hepatocellular carcinoma, multiple | 3 (6%) | | 1 (2%) | |
| Hepatocholangiocarcinoma | 1 (2%) | | | |
| Mesentery | (5) | (5) | (4) | (2) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (25%) | |
| Carcinoma, metastatic, islets pancreatic | 1 (20%) | | | |
| Carcinoma, metastatic, uncertain primary site | | | | 1 (50%) |
| Carcinoma, metastatic, urethra | | | 1 (25%) | |
| Oral mucosa | (0) | (1) | (0) | (0) |
| Pancreas | (50) | (50) | (49) | (50) |
| Carcinoma, metastatic, islets, pancreatic | 1 (2%) | | | |
| Squamous cell carcinoma, metastatic, stomach, forestomach | | | 1 (2%) | |
| Salivary glands | (50) | (50) | (49) | (50) |
| Stomach, forestomach | (50) | (50) | (49) | (50) |
| Squamous cell carcinoma | | 1 (2%) | 1 (2%) | 3 (6%) |
| Squamous cell papilloma | 1 (2%) | | | 1 (2%) |
| Stomach, glandular | (50) | (50) | (49) | (50) |
| Squamous cell carcinoma, metastatic, stomach, forestomach | | 1 (2%) | 1 (2%) | 1 (2%) |
| Tongue | (0) | (1) | (0) | (0) |
| Tooth | (8) | (3) | (0) | (0) |
| Odontoma | 1 (13%) | | | |

**TABLE C1**
**Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Cardiovascular System** | | | | |
| Blood vessel | (50) | (50) | (49) | (50) |
| Aorta, alveolar/bronchiolar carcinoma, metastatic, lung | | 1 (2%) | | |
| Heart | (50) | (50) | (49) | (50) |
| Fibrosarcoma, metastatic, skin | 1 (2%) | | | |
| Hemangiosarcoma | | 1 (2%) | | |
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (50) | (49) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (2%) | |
| Carcinoma, metastatic, islets, pancreatic | 1 (2%) | | | |
| Hemangiosarcoma, metastatic, kidney | 1 (2%) | | | |
| Subcapsular, adenoma | 3 (6%) | 1 (2%) | | |
| Islets, pancreatic | (50) | (50) | (49) | (50) |
| Carcinoma | 2 (4%) | | | |
| Parathyroid gland | (45) | (47) | (46) | (44) |
| Pituitary gland | (49) | (50) | (48) | (50) |
| Pars intermedia, adenoma | | 1 (2%) | | |
| Thyroid gland | (50) | (50) | (49) | (50) |
| C-cell, carcinoma | 1 (2%) | | | |
| Follicular cell, carcinoma | | 1 (2%) | | |
| **General Body System** | | | | |
| None | | | | |
| **Genital System** | | | | |
| Coagulating gland | (50) | (50) | (49) | (50) |
| Carcinoma, metastatic, islets, pancreatic | 1 (2%) | | | |
| Carcinoma, metastatic, urethra | | 22 (44%) | 44 (90%) | 48 (96%) |
| Ductus deferens | (50) | (50) | (49) | (50) |
| Carcinoma, metastatic, islets, pancreatic | 1 (2%) | | | |
| Carcinoma, metastatic, urethra | | 23 (46%) | 45 (92%) | 33 (66%) |
| Epididymis | (50) | (50) | (49) | (50) |
| Carcinoma, metastatic, islets, pancreatic | 1 (2%) | | | |
| Preputial gland | (50) | (50) | (49) | (50) |
| Carcinoma | | | | 1 (2%) |
| Prostate | (50) | (50) | (49) | (50) |
| Carcinoma, metastatic, islets, pancreatic | 1 (2%) | | | |
| Carcinoma, metastatic, urethra | | 22 (44%) | 45 (92%) | 49 (98%) |
| Seminal vesicle | (50) | (50) | (49) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (2%) | |
| Carcinoma, metastatic, islets, pancreatic | 1 (2%) | | | |
| Carcinoma, metastatic, urethra | | 22 (44%) | 44 (90%) | 48 (96%) |
| Testes | (50) | (50) | (49) | (50) |
| Interstitial cell, adenoma | | | 1 (2%) | |

**TABLE C1**
**Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Hematopoietic System** | | | | |
| Bone marrow | (50) | (50) | (49) | (50) |
| Lymph node | (2) | (1) | (2) | (0) |
| Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (50%) | |
| Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (50%) | |
| Mediastinal, carcinoma, metastatic, islets, pancreatic | 1 (50%) | | | |
| Lymph node, mandibular | (49) | (50) | (48) | (49) |
| Lymph node, mesenteric | (50) | (49) | (48) | (50) |
| Spleen | (50) | (50) | (49) | (49) |
| Hemangiosarcoma | 2 (4%) | | | |
| Thymus | (41) | (42) | (40) | (49) |
| **Integumentary System** | | | | |
| Skin | (50) | (50) | (49) | (50) |
| Subcutaneous tissue, fibrosarcoma | 1 (2%) | | | |
| Subcutaneous tissue, hemangiosarcoma | 1 (2%) | 1 (2%) | | |
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (49) | (50) |
| Skeletal muscle | (50) | (50) | (49) | (50) |
| Carcinoma, metastatic, urethra | | 24 (48%) | 45 (92%) | 49 (98%) |
| Fibrosarcoma, metastatic, skin | 1 (2%) | | | |
| **Nervous System** | | | | |
| Brain | (50) | (50) | (49) | (50) |
| Meningioma malignant | 1 (2%) | | | |
| **Respiratory System** | | | | |
| Lung | (50) | (50) | (49) | (50) |
| Alveolar/bronchiolar adenoma | 4 (8%) | 12 (24%) | 11 (22%) | 5 (10%) |
| Alveolar/bronchiolar adenoma, multiple | 1 (2%) | 4 (8%) | 1 (2%) | 1 (2%) |
| Alveolar/bronchiolar carcinoma | 2 (4%) | 1 (2%) | 1 (2%) | |
| Alveolar/bronchiolar carcinoma, multiple | 1 (2%) | | 3 (6%) | |
| Carcinoma, metastatic, islets, pancreatic | 1 (2%) | | | |
| Carcinoma, metastatic, urethra | | | 1 (2%) | |
| Fibrosarcoma, metastatic, skin | 1 (2%) | | | |
| Hepatocellular carcinoma, metastatic, liver | 5 (10%) | 2 (4%) | 2 (4%) | |
| Squamous cell carcinoma, metastatic, stomach, forestomach | | 1 (2%) | | |
| Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung | 1 (2%) | 1 (2%) | 1 (2%) | |
| Mediastinum, fibrosarcoma, metastatic, skin | 1 (2%) | | | |
| Nose | (50) | (50) | (49) | (50) |
| Pleura | (1) | (1) | (1) | (0) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | 1 (100%) | 1 (100%) | |
| Fibrosarcoma, metastatic, skin | 1 (100%) | | | |
| Trachea | (50) | (50) | (49) | (50) |
| **Special Senses System** | | | | |
| Eye | (50) | (50) | (49) | (50) |
| Harderian gland | (50) | (50) | (49) | (50) |
| Adenoma | 6 (12%) | 3 (6%) | 1 (2%) | 1 (2%) |

**TABLE C1**
**Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Urinary System** | | | | |
| Kidney | (50) | (50) | (49) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | 1 (2%) | |
| Carcinoma, metastatic, urethra | | | | 1 (2%) |
| Hemangiosarcoma | 1 (2%) | | | |
| Ureter | (43) | (45) | (47) | (50) |
| Carcinoma, metastatic, urethra | | | | 6 (12%) |
| Transitional epithelium, carcinoma | | | 1 (2%) | |
| Urethra | (48) | (50) | (49) | (50) |
| Transitional epithelium, carcinoma | | 32 (64%) | 46 (94%) | 49 (98%) |
| Urinary bladder | (50) | (50) | (49) | (50) |
| Carcinoma, metastatic, urethra | | 1 (2%) | | 3 (6%) |
| **Systemic Lesions** | | | | |
| Multiple organs[b] | (50) | (50) | (49) | (50) |
| Histiocytic sarcoma | | 1 (2%) | | |
| Lymphoma malignant | 1 (2%) | 1 (2%) | | |
| **Neoplasm Summary** | | | | |
| Total animals with primary neoplasms[c] | 41 | 45 | 46 | 49 |
| Total primary neoplasms | 74 | 101 | 93 | 63 |
| Total animals with benign neoplasms | 30 | 32 | 24 | 10 |
| Total benign neoplasms | 38 | 49 | 31 | 10 |
| Total animals with malignant neoplasms | 28 | 42 | 46 | 49 |
| Total malignant neoplasms | 36 | 52 | 62 | 53 |
| Total animals with metastatic neoplasms | 9 | 27 | 45 | 49 |
| Total metastatic neoplasms | 24 | 121 | 238 | 240 |
| Total animals with malignant neoplasms of uncertain primary site | | | | 1 |

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

**TABLE C2**
**Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Adrenal Cortex: Adenoma** | | | | |
| Overall rate[a] | 3/50 (6%) | 1/50 (2%) | 0/49 (0%) | 0/50 (0%) |
| Adjusted rate[b] | 6.6% | 2.4% | 0.0% | 0.0% |
| Terminal rate[c] | 3/35 (9%) | 1/31 (3%) | 0/5 (0%) | 0/0 |
| First incidence (days) | 728 (T) | 728 (T) | —[d] | — |
| Poly-3 test[e] | P = 0.138N | P = 0.341N | P = 0.231N | P = 0.496N |
| **Harderian Gland: Adenoma** | | | | |
| Overall rate | 6/50 (12%) | 3/50 (6%) | 1/49 (2%) | 1/50 (2%) |
| Adjusted rate | 12.8% | 7.2% | 3.5% | 10.0% |
| Terminal rate | 2/35 (6%) | 2/31 (7%) | 0/5 (0%) | 0/0 |
| First incidence (days) | 486 | 680 | 567 | 505 |
| Poly-3 test | P = 0.224N | P = 0.308N | P = 0.189N | P = 0.573N |
| **Liver: Hepatocellular Adenoma** | | | | |
| Overall rate | 22/50 (44%) | 27/50 (54%) | 17/49 (35%) | 2/50 (4%) |
| Adjusted rate | 47.1% | 63.1% | 50.5% | 18.4% |
| Terminal rate | 17/35 (49%) | 22/31 (71%) | 3/5 (60%) | 0/0 |
| First incidence (days) | 609 | 552 | 437 | 381 |
| Poly-3 test | P = 0.231N | P = 0.089 | P = 0.471 | P = 0.164N |
| **Liver: Hepatocellular Carcinoma** | | | | |
| Overall rate | 17/50 (34%) | 10/50 (20%) | 8/49 (16%) | 0/50 (0%) |
| Adjusted rate | 35.5% | 23.4% | 26.7% | 0.0% |
| Terminal rate | 9/35 (26%) | 6/31 (19%) | 1/5 (20%) | 0/0 |
| First incidence (days) | 563 | 524 | 533 | — |
| Poly-3 test | P = 0.093N | P = 0.153N | P = 0.294N | P = 0.130N |
| **Liver: Hepatocellular Adenoma or Carcinoma** | | | | |
| Overall rate | 34/50 (68%) | 31/50 (62%) | 20/49 (41%) | 2/50 (4%) |
| Adjusted rate | 70.4% | 71.0% | 57.4% | 18.4% |
| Terminal rate | 24/35 (69%) | 24/31 (77%) | 4/5 (80%) | 0/0 |
| First incidence (days) | 563 | 524 | 437 | 381 |
| Poly-3 test | P = 0.008N | P = 0.568 | P = 0.150N | P = 0.016N |
| **Liver: Hepatocellular Carcinoma or Hepatoblastoma** | | | | |
| Overall rate | 19/50 (38%) | 10/50 (20%) | 8/49 (16%) | 0/50 (0%) |
| Adjusted rate | 39.6% | 23.4% | 26.7% | 0.0% |
| Terminal rate | 11/35 (31%) | 6/31(19%) | 1/5 (20%) | 0/0 |
| First incidence (days) | 563 | 524 | 533 | — |
| Poly-3 test | P = 0.047N | P = 0.075N | P = 0.183N | P = 0.104N |
| **Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma** | | | | |
| Overall rate | 34/50 (68%) | 31/50 (62%) | 20/49 (41%) | 2/50 (4%) |
| Adjusted rate | 70.4% | 71.0% | 57.4% | 18.4% |
| Terminal rate | 24/35 (69%) | 24/31 (77%) | 4/5 (80%) | 0/0 |
| First incidence (days) | 563 | 524 | 437 | 381 |
| Poly-3 test | P = 0.008N | P = 0.568 | P = 0.150N | P = 0.016N |
| **Lung: Alveolar/bronchiolar Adenoma** | | | | |
| Overall rate | 5/50 (10%) | 16/50 (32%) | 12/49 (24%) | 6/50 (12%) |
| Adjusted rate | 11.0% | 38.0% | 37.5% | 43.0% |
| Terminal rate | 5/35 (14%) | 13/31 (42%) | 1/5 (20%) | 0/0 |
| First incidence (days) | 728 (T) | 574 | 470 | 357 |
| Poly-3 test | P = 0.014 | P = 0.002 | P = 0.006 | P = 0.037 |

**TABLE C2**
**Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Lung: Alveolar/bronchiolar Carcinoma** | | | | |
| Overall rate | 3/50 (6%) | 1/50 (2%) | 4/49 (8%) | 0/50 (0%) |
| Adjusted rate | 6.6% | 2.4% | 14.0% | 0.0% |
| Terminal rate | 2/35 (6%) | 0/31 (0%) | 2/5 (40%) | 0/0 |
| First incidence (days) | 682 | 680 | 590 | — |
| Poly-3 test | P = 0.352 | P = 0.341N | P = 0.265 | P = 0.496N |
| **Lung: Alveolar/bronchiolar Adenoma or Carcinoma** | | | | |
| Overall rate | 7/50 (14%) | 17/50 (34%) | 15/49 (31%) | 6/50 (12%) |
| Adjusted rate | 15.3% | 40.2% | 45.8% | 43.0% |
| Terminal rate | 6/35 (17%) | 13/31 (42%) | 2/5 (40%) | 0/0 |
| First incidence (days) | 682 | 574 | 470 | 357 |
| Poly-3 test | P = 0.014 | P = 0.007 | P = 0.003 | P = 0.081 |
| **Stomach (Forestomach): Squamous Cell Carcinoma** | | | | |
| Overall rate | 0/50 (0%) | 1/50 (2%) | 1/49 (2%) | 3/50 (6%) |
| Adjusted rate | 0.0% | 2.4% | 3.5% | 25.7% |
| Terminal rate | 0/35 (0%) | 0/31 (0%) | 0/5 (0%) | 0/0 |
| First incidence (days) | — | 416 | 584 | 376 |
| Poly-3 test | P = 0.012 | P = 0.484 | P = 0.410 | P = 0.023 |
| **Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma** | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 1/49 (2%) | 3/50 (6%) |
| Adjusted rate | 2.2% | 2.4% | 3.5% | 25.7% |
| Terminal rate | 1/35 (3%) | 0/31 (0%) | 0/5 (0%) | 0/0 |
| First incidence (days) | 728 (T) | 416 | 584 | 376 |
| Poly-3 test | P = 0.043 | P = 0.743 | P = 0.645 | P = 0.062 |
| **Urethra: Carcinoma** | | | | |
| Overall rate | 0/50 (0%) | 32/50 (64%) | 46/49 (94%) | 49/50 (98%) |
| Adjusted rate | 0.0% | 70.7% | 99.7% | 99.9% |
| Terminal rate | 0/35 (0%) | 21/31 (68%) | 5/5 (100%) | 0/0 |
| First incidence (days) | — | 380 | 358 | 206 |
| Poly-3 test | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 |
| **All Organs: Hemangiosarcoma** | | | | |
| Overall rate | 3/50 (6%) | 2/50 (4%) | 0/49 (0%) | 0/50 (0%) |
| Adjusted rate | 6.5% | 4.8% | 0.0% | 0.0% |
| Terminal rate | 2/35 (6%) | 1/31 (3%) | 0/5 (0%) | 0/0 |
| First incidence (days) | 612 | 659 | — | — |
| Poly-3 test | P = 0.157N | P = 0.546N | P = 0.234N | P = 0.497N |
| **All Organs: Benign Neoplasms** | | | | |
| Overall rate | 30/50 (60%) | 32/50 (64%) | 24/49 (49%) | 10/50 (20%) |
| Adjusted rate | 62.4% | 73.3% | 64.8% | 58.6% |
| Terminal rate | 22/35 (63%) | 24/31 (77%) | 3/5 (60%) | 0/0 |
| First incidence (days) | 486 | 552 | 437 | 357 |
| Poly-3 test | P = 0.402N | P = 0.181 | P = 0.503 | P = 0.504N |
| **All Organs: Malignant Neoplasms** | | | | |
| Overall rate | 28/50 (56%) | 42/50 (84%) | 46/49 (94%) | 49/50 (98%) |
| Adjusted rate | 57.1% | 89.1% | 99.7% | 99.9% |
| Terminal rate | 16/35 (46%) | 27/31 (87%) | 5/5 (100%) | 0/0 |
| First incidence (days) | 563 | 380 | 358 | 206 |
| Poly-3 test | P < 0.001 | P < 0.001 | P < 0.001 | P < 0.001 |

**TABLE C2**
**Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **All Organs: Benign or Malignant Neoplasms** | | | | |
| Overall rate | 41/50 (82%) | 45/50 (90%) | 46/49 (94%) | 49/50 (98%) |
| Adjusted rate | 82.1% | 94.3% | 99.7% | 99.9% |
| Terminal rate | 27/35 (77%) | 29/31 (94%) | 5/5 (100%) | 0/0 (0%) |
| First incidence (days) | 486 | 380 | 358 | 206 |
| Poly-3 test | P = 0.002 | P = 0.055 | P = 0.003 | P = 0.002 |

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, and lung; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Not applicable; no neoplasms in animal group
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.

**TABLE C3a**
**Historical Incidence of Lung Neoplasms in Control Male B6C3F1 Mice[a]**

| Study (Study Start) | Incidence in Controls | | |
|---|---|---|---|
| | Alveolar/bronchiolar Adenoma | Alveolar/bronchiolar Carcinoma | Alveolar/bronchiolar Adenoma or Carcinoma |
| **Historical Incidence: Corn Oil Gavage Studies** | | | |
| Isoeugenol (May, 2002) | 6/50 | 2/50 | 7/50 |
| β-Myrcene (April, 2002) | 8/50 | 5/50 | 13/50 |
| Pulegone (April, 2003) | 6/50 | 3/50 | 9/50 |
| TCAB (February, 2003 | 5/50 | 3/50 | 7/50 |
| Total (%) | 25/200 (12.5%) | 13/200 (6.5%) | 36/200 (18.0%) |
| Mean ± standard deviation | 12.5% ± 2.5% | 6.5% ± 2.5% | 18.0% ± 5.7% |
| Range | 10% – 16% | 4% – 10% | 14% – 26% |
| **Overall Historical Incidence: All Routes** | | | |
| Total (%) | 238/1,448 (16.4%) | 163/1,448 (11.3%) | 384/1,448 (26.5%) |
| Mean ± standard deviation | 16.4% ± 6.7% | 11.3% ± 6.5% | 26.5% ± 6.9% |
| Range | 2% – 30% | 2% – 24% | 14% – 40% |

[a] Data as of November 19, 2008

**TABLE C3b**
**Historical Incidence of Squamous Cell Carcinoma in the Forestomach of Control Male B6C3F1 Mice[a]**

| Study (Study Start) | Incidence in Controls |
|---|---|
| **Historical Incidence: Corn Oil Gavage Studies** | |
| Isoeugenol (May, 2002) | 0/50 |
| β-Myrcene (April, 2002) | 0/50 |
| Pulegone (April, 2003) | 1/50 |
| TCAB (February, 2003) | 0/50 |
| Total (%) | 1/200 (0.5%) |
| Mean ± standard deviation | 0.5% ± 1.0% |
| Range | 0% – 2% |
| **Overall Historical Incidence: All Routes** | |
| Total (%) | 6/1,449 (0.4%) |
| Mean ± standard deviation | 0.4% ± 1.0% |
| Range | 0% – 4% |

[a] Data as of November 19, 2008

**TABLE C4**
**Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of TCAB[a]**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 507 |
| Early deaths | | | | |
| Accidental deaths | 1 | | 2 | |
| Moribund | 10 | 11 | 16 | 19 |
| Natural deaths | 4 | 8 | 26 | 31 |
| Survivors | | | | |
| Terminal sacrifice | 35 | 31 | 5 | |
| Animals examined microscopically | 50 | 50 | 49 | 50 |
| **Alimentary System** | | | | |
| Esophagus | (50) | (50) | (49) | (50) |
| Perforation | 1 (2%) | | | |
| Epithelium, necrosis | 1 (2%) | | | |
| Periesophageal tissue, inflammation, chronic active | 1 (2%) | | | |
| Gallbladder | (49) | (50) | (49) | (50) |
| Infiltration cellular, lymphoid | 2 (4%) | | | |
| Inflammation, chronic active | 1 (2%) | | | |
| Intestine large, cecum | (50) | (50) | (49) | (50) |
| Cyst | | | 1 (2%) | |
| Intestine large, rectum | (50) | (50) | (49) | (50) |
| Intestine small, duodenum | (50) | (50) | (49) | (50) |
| Intussusception | | 1 (2%) | | |
| Intestine small, ileum | (50) | (50) | (49) | (50) |
| Intestine small, jejunum | (50) | (50) | (49) | (50) |
| Liver | (50) | (50) | (49) | (50) |
| Amyloid deposition | | 3 (6%) | 2 (4%) | |
| Atypia cellular | | 1 (2%) | | |
| Basophilic focus | | 4 (8%) | 2 (4%) | 2 (4%) |
| Clear cell focus | 12 (24%) | 6 (12%) | 1 (2%) | |
| Congestion | | | 1 (2%) | 1 (2%) |
| Eosinophilic focus | 5 (10%) | 1 (2%) | 3 (6%) | |
| Hematopoietic cell proliferation | 2 (4%) | 9 (18%) | 9 (18%) | 3 (6%) |
| Hemorrhage | 1 (2%) | | 1 (2%) | |
| Infarct | 1 (2%) | 1 (2%) | | |
| Infiltration cellular, lymphoid | 7 (14%) | 16 (32%) | 11 (22%) | 2 (4%) |
| Inflammation, chronic active | 35 (70%) | 32 (64%) | 17 (35%) | 14 (28%) |
| Mineralization | 1 (2%) | 1 (2%) | | |
| Mixed cell focus | 4 (8%) | 6 (12%) | | 2 (4%) |
| Pigmentation | 2 (4%) | 1 (2%) | | |
| Tension lipidosis | 2 (4%) | 5 (10%) | 12 (24%) | 15 (30%) |
| Thrombosis | 1 (2%) | | | |
| Bile duct, hyperplasia | 1 (2%) | | | |
| Hepatocyte, necrosis | 5 (10%) | 4 (8%) | 2 (4%) | |
| Hepatocyte, vacuolization cytoplasmic | 31 (62%) | 31 (62%) | 23 (47%) | 11 (22%) |
| Mesentery | (5) | (5) | (4) | (2) |
| Fat, fibrosis | 4 (80%) | 2 (40%) | | |
| Fat, inflammation, chronic active | 3 (60%) | 5 (100%) | 3 (75%) | 1 (50%) |
| Fat, mineralization | 1 (20%) | 2 (40%) | | |
| Fat, necrosis | 4 (80%) | 3 (60%) | 1 (25%) | |
| Oral mucosa | (0) | (1) | (0) | (0) |
| Gingival, inflammation, chronic active | | 1 (100%) | | |

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

**TABLE C4**
**Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Alimentary System** *(continued)* | | | | |
| Pancreas | (50) | (50) | (49) | (50) |
| Infiltration cellular, lymphoid | 6 (12%) | 5 (10%) | 1 (2%) | |
| Inflammation, chronic active | | 2 (4%) | 1 (2%) | 1 (2%) |
| Duct, cyst | 1 (2%) | | | |
| Salivary glands | (50) | (50) | (49) | (50) |
| Degeneration | 1 (2%) | | | |
| Infiltration cellular, lymphoid | 43 (86%) | 34 (68%) | 22 (45%) | 4 (8%) |
| Mineralization | | | 1 (2%) | |
| Stomach, forestomach | (50) | (50) | (49) | (50) |
| Infiltration cellular, lymphoid | | | 1 (2%) | |
| Inflammation, chronic active | 6 (12%) | 7 (14%) | 6 (12%) | 3 (6%) |
| Epithelium, cyst | 1 (2%) | | | |
| Epithelium, hyperplasia | 8 (16%) | 21 (42%) | 33 (67%) | 44 (88%) |
| Epithelium, ulcer | 5 (10%) | 3 (6%) | 3 (6%) | 2 (4%) |
| Stomach, glandular | (50) | (50) | (49) | (50) |
| Infiltration cellular, lymphoid | 3 (6%) | 20 (40%) | 19 (39%) | 15 (30%) |
| Infiltration cellular, mast cell | | 1 (2%) | | |
| Inflammation, chronic active | | 1 (2%) | 1 (2%) | 1 (2%) |
| Mineralization | | 4 (8%) | 6 (12%) | 13 (26%) |
| Epithelium, hyperplasia | 1 (2%) | | | |
| Epithelium, hyperplasia, focal | | 10 (20%) | 20 (41%) | 34 (68%) |
| Epithelium, ulcer | | | 1 (2%) | |
| Epithelium, glands, cyst | 5 (10%) | 18 (36%) | 21 (43%) | 26 (52%) |
| Glands, cyst | | | 1 (2%) | 1 (2%) |
| Tongue | (0) | (1) | (0) | (0) |
| Cyst, multiple | | 1 (100%) | | |
| Artery, inflammation, chronic active | | 1 (100%) | | |
| Tooth | (8) | (3) | (0) | (0) |
| Dysplasia | 3 (38%) | | | |
| Malformation | 4 (50%) | 3 (100%) | | |
| **Cardiovascular System** | | | | |
| Blood vessel | (50) | (50) | (49) | (50) |
| Hyperplasia | 1 (2%) | | | |
| Aorta, inflammation, chronic active | | 2 (4%) | | |
| Aorta, mineralization | | | 6 (12%) | 8 (16%) |
| Heart | (50) | (50) | (49) | (50) |
| Cardiomyopathy | 5 (10%) | 5 (10%) | 17 (35%) | 9 (18%) |
| Hyperplasia, atypical | | 2 (4%) | | |
| Infiltration cellular, lymphoid | 1 (2%) | | | |
| Inflammation, chronic active | 1 (2%) | 1 (2%) | 2 (4%) | 1 (2%) |
| Mineralization | | 2 (4%) | | 1 (2%) |
| Necrosis | | | | 3 (6%) |
| Artery, hyperplasia | 1 (2%) | | | |
| Artery, inflammation, chronic active | 2 (4%) | 2 (4%) | | |
| Myocardium, necrosis | | 1 (2%) | | |

**TABLE C4**
**Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Endocrine System** | | | | |
| Adrenal cortex | (50) | (50) | (49) | (50) |
| Accessory adrenal cortical nodule | | 2 (4%) | 4 (8%) | |
| Amyloid deposition | | | 1 (2%) | |
| Hematopoietic cell proliferation | | | 1 (2%) | |
| Hyperplasia | 1 (2%) | | | |
| Hypertrophy | 9 (18%) | 5 (10%) | 1 (2%) | |
| Bilateral, accessory adrenal cortical nodule | | | | 1 (2%) |
| Subcapsular, hyperplasia | 45 (90%) | 38 (76%) | 37 (76%) | 27 (54%) |
| Islets, pancreatic | (50) | (50) | (49) | (50) |
| Hyperplasia | 5 (10%) | 3 (6%) | | |
| Parathyroid gland | (45) | (47) | (46) | (44) |
| Cyst | 1 (2%) | | | |
| Cyst, multiple | | 1 (2%) | | |
| Pituitary gland | (49) | (50) | (48) | (50) |
| Pars distalis, cyst | 2 (4%) | 4 (8%) | 1 (2%) | 3 (6%) |
| Pars distalis, cyst, multiple | 1 (2%) | | | 1 (2%) |
| Thyroid gland | (50) | (50) | (49) | (50) |
| Inflammation, chronic active | 2 (4%) | | | |
| Follicle, cyst | | 1 (2%) | | |
| Follicle, degeneration | 1 (2%) | | | |
| **General Body System** | | | | |
| None | | | | |
| **Genital System** | | | | |
| Coagulating gland | (50) | (50) | (49) | (50) |
| Atrophy | 1 (2%) | | | |
| Dilatation | 9 (18%) | 16 (32%) | 36 (73%) | 47 (94%) |
| Fibrosis | | 6 (12%) | 23 (47%) | 30 (60%) |
| Infiltration cellular, lymphoid | 3 (6%) | 6 (12%) | 2 (4%) | |
| Inflammation, chronic active | | 6 (12%) | 22 (45%) | 33 (66%) |
| Epithelium, hyperplasia | 1 (2%) | 1 (2%) | 5 (10%) | 1 (2%) |
| Ductus deferens | (50) | (50) | (49) | (50) |
| Inflammation, chronic active | | | 3 (6%) | |
| Epididymis | (50) | (50) | (49) | (50) |
| Atrophy | 1 (2%) | | | |
| Cyst | | 1 (2%) | 1 (2%) | |
| Granuloma sperm | 1 (2%) | 3 (6%) | 6 (12%) | 8 (16%) |
| Infiltration cellular, lymphoid | 40 (80%) | 30 (60%) | 21 (43%) | 15 (30%) |
| Inflammation, chronic active | | 3 (6%) | 2 (4%) | |
| Preputial gland | (50) | (50) | (49) | (50) |
| Atrophy | 20 (40%) | 23 (46%) | 24 (49%) | 9 (18%) |
| Infiltration cellular, lymphoid | 18 (36%) | 3 (6%) | | |
| Inflammation, chronic active | | 7 (14%) | 3 (6%) | 2 (4%) |
| Duct, ectasia | 19 (38%) | 15 (30%) | 23 (47%) | 10 (20%) |
| Prostate | (50) | (50) | (49) | (50) |
| Fibrosis | | 1 (2%) | 3 (6%) | 1 (2%) |
| Infiltration cellular, lymphoid | 42 (84%) | 43 (86%) | 26 (53%) | 9 (18%) |
| Inflammation, chronic active | 3 (6%) | 10 (20%) | 27 (55%) | 44 (88%) |
| Mineralization | 1 (2%) | | | |
| Artery, hyperplasia | 1 (2%) | | | |
| Artery, inflammation, chronic active | 1 (2%) | | | |
| Epithelium, hyperplasia | 4 (8%) | | 1 (2%) | |

**TABLE C4**
**Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Genital System** *(continued)* | | | | |
| Seminal vesicle | (50) | (50) | (49) | (50) |
| Atrophy | 1 (2%) | | | |
| Dilatation | 12 (24%) | 14 (28%) | 38 (78%) | 41 (82%) |
| Fibrosis | | 3 (6%) | 18 (37%) | 29 (58%) |
| Inflammation, chronic active | | 4 (8%) | 14 (29%) | 22 (44%) |
| Epithelium, hyperplasia | | 1 (2%) | 5 (10%) | 1 (2%) |
| Testes | (50) | (50) | (49) | (50) |
| Mineralization | 1 (2%) | 1 (2%) | 3 (6%) | 2 (4%) |
| Germinal epithelium, degeneration | 8 (16%) | 1 (2%) | 3 (6%) | 3 (6%) |
| **Hematopoietic System** | | | | |
| Bone marrow | (50) | (50) | (49) | (50) |
| Hyperplasia | 13 (26%) | 10 (20%) | 17 (35%) | 20 (40%) |
| Myelofibrosis | | | 1 (2%) | |
| Erythroid cell, hyperplasia | 1 (2%) | | | |
| Lymph node | (2) | (1) | (2) | (0) |
| Mediastinal, hyperplasia, lymphoid | | | 1 (50%) | |
| Lymph node, mandibular | (49) | (50) | (48) | (49) |
| Hematopoietic cell proliferation | | | 1 (2%) | |
| Hyperplasia, lymphoid | 3 (6%) | 7 (14%) | 7 (15%) | |
| Hyperplasia, plasma cell | | 2 (4%) | 1 (2%) | |
| Necrosis, lymphoid | | | 1 (2%) | |
| Lymph node, mesenteric | (50) | (49) | (48) | (50) |
| Hyperplasia, lymphoid | 1 (2%) | | 1 (2%) | |
| Spleen | (50) | (50) | (49) | (49) |
| Amyloid deposition | | 4 (8%) | 2 (4%) | |
| Atrophy | 2 (4%) | 9 (18%) | 30 (61%) | 39 (80%) |
| Hematopoietic cell proliferation | 18 (36%) | 16 (32%) | 12 (24%) | 7 (14%) |
| Artery, hyperplasia | 1 (2%) | | | |
| Artery, inflammation, chronic active | 1 (2%) | | | |
| Lymphoid follicle, necrosis, lymphoid | | | 1 (2%) | |
| Thymus | (41) | (42) | (40) | (49) |
| Atrophy | 11 (27%) | 15 (36%) | 30 (75%) | 45 (92%) |
| Cyst | 5 (12%) | 2 (5%) | 5 (13%) | 5 (10%) |
| Cyst, multiple | 19 (46%) | 28 (67%) | 27 (68%) | 35 (71%) |
| Ectopic parathyroid gland | 8 (20%) | 8 (19%) | 3 (8%) | 9 (18%) |
| Inflammation, chronic active | | | 1 (3%) | |
| Thymocyte, necrosis | | | 2 (5%) | 3 (6%) |
| **Integumentary System** | | | | |
| Skin | (50) | (50) | (49) | (50) |
| Cyst epithelial inclusion | | 1 (2%) | | |
| Hemorrhage | | | 1 (2%) | |
| Inflammation, acute | | | | 1 (2%) |
| Inflammation, chronic active | 3 (6%) | 13 (26%) | 12 (24%) | 5 (10%) |
| Dermis, fibrosis | 3 (6%) | 12 (24%) | 12 (24%) | 4 (8%) |
| Dermis, metaplasia, osseous | | 1 (2%) | | |
| Epidermis, hyperkeratosis | | 1 (2%) | | |
| Epidermis, hyperplasia | 3 (6%) | 13 (26%) | 12 (24%) | 6 (12%) |
| Epidermis, ulcer | 3 (6%) | 13 (26%) | 11 (22%) | 6 (12%) |
| Hair follicle, dilatation | 7 (14%) | 10 (20%) | 13 (27%) | 28 (56%) |
| Sebaceous gland, atrophy | 13 (26%) | 10 (20%) | 20 (41%) | 29 (58%) |
| Subcutaneous tissue, inflammation, chronic active | | | | 1 (2%) |

**TABLE C4**
**Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (49) | (50) |
| Osteopetrosis | | 2 (4%) | 1 (2%) | |
| Skeletal muscle | (50) | (50) | (49) | (50) |
| Infiltration cellular, lymphoid | 2 (4%) | | | |
| Inflammation, chronic active | | 1 (2%) | | |
| **Nervous System** | | | | |
| Brain | (50) | (50) | (49) | (50) |
| Degeneration | | 1 (2%) | | |
| Inflammation, chronic active | 1 (2%) | | | |
| Neuron, necrosis | | | 1 (2%) | |
| **Respiratory System** | | | | |
| Lung | (50) | (50) | (49) | (50) |
| Congestion | | | 1 (2%) | |
| Fibrosis | | 1 (2%) | 1 (2%) | |
| Infiltration cellular, lymphoid | | 4 (8%) | | |
| Inflammation, chronic active | | 2 (4%) | 1 (2%) | 1 (2%) |
| Alveolar epithelium, hyperplasia | 5 (10%) | 6 (12%) | 2 (4%) | 2 (4%) |
| Alveolus, infiltration cellular, histiocyte | 4 (8%) | 6 (12%) | 1 (2%) | |
| Mediastinum, inflammation, chronic active | | 1 (2%) | | |
| Nose | (50) | (50) | (49) | (50) |
| Hemorrhage | | | 1 (2%) | |
| Inflammation, chronic active | | | 1 (2%) | |
| Nasolacrimal duct, inflammation, chronic active | 1 (2%) | | | |
| Nasolacrimal duct, squamous epithelium, hyperplasia | 2 (4%) | | | |
| Pleura | (1) | (1) | (1) | (0) |
| Trachea | (50) | (50) | (49) | (50) |
| Epithelium, mineralization | | | | 1 (2%) |
| **Special Senses System** | | | | |
| Eye | (50) | (50) | (49) | (50) |
| Atrophy | | | 1 (2%) | 2 (4%) |
| Anterior chamber, cornea, inflammation, suppurative | 1 (2%) | | | |
| Cornea, inflammation, chronic active | 1 (2%) | | | |
| Cornea, pigmentation | | | 1 (2%) | |
| Optic nerve, inflammation, chronic active | | 1 (2%) | | |
| Harderian gland | (50) | (50) | (49) | (50) |
| Degeneration | | | | 1 (2%) |
| Hyperplasia | 3 (6%) | 2 (4%) | 1 (2%) | |
| Infiltration cellular, lymphoid | 33 (66%) | 26 (52%) | 7 (14%) | 1 (2%) |
| Inflammation, chronic active | | 1 (2%) | | |
| Necrosis | | | 1 (2%) | |

**TABLE C4**
**Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Urinary System** | | | | |
| Kidney | (50) | (50) | (49) | (50) |
| Hydronephrosis | 1 (2%) | 5 (10%) | 17 (35%) | 29 (58%) |
| Infarct | 4 (8%) | | 1 (2%) | |
| Infiltration cellular, lymphoid | 39 (78%) | 38 (76%) | 12 (24%) | 3 (6%) |
| Inflammation, acute | | | | 3 (6%) |
| Inflammation, chronic active | | | 5 (10%) | 2 (4%) |
| Metaplasia, osseous | 2 (4%) | 3 (6%) | 1 (2%) | 1 (2%) |
| Mineralization | 40 (80%) | 43 (86%) | 40 (82%) | 42 (84%) |
| Nephropathy | 47 (94%) | 45 (90%) | 28 (57%) | 24 (48%) |
| Thrombosis | | | | 4 (8%) |
| Artery, inflammation, chronic active | 2 (4%) | 1 (2%) | | |
| Cortex, cyst | 11 (22%) | 6 (12%) | 2 (4%) | |
| Cortex, cyst, multiple | | | 1 (2%) | |
| Glomerulus, amyloid deposition | | 6 (12%) | 4 (8%) | 1 (2%) |
| Papilla, necrosis | | 3 (6%) | 3 (6%) | |
| Pelvis, hyperplasia | 1 (2%) | | | 1 (2%) |
| Renal tubule, dilatation | 1 (2%) | 8 (16%) | 31 (63%) | 43 (86%) |
| Renal tubule, hyperplasia | 1 (2%) | | | |
| Ureter | (43) | (45) | (47) | (50) |
| Dilatation | | 6 (13%) | 22 (47%) | 42 (84%) |
| Inflammation, chronic active | | 3 (7%) | 24 (51%) | 39 (78%) |
| Urethra | (48) | (50) | (49) | (50) |
| Dilatation | | 1 (2%) | 2 (4%) | 2 (4%) |
| Inflammation, chronic active | 1 (2%) | | 1 (2%) | |
| Artery, hyperplasia | 1 (2%) | | | |
| Artery, inflammation, chronic active | 1 (2%) | | | |
| Transitional epithelium, hyperplasia | | 17 (34%) | 2 (4%) | |
| Transitional epithelium, inflammation, chronic active | | 1 (2%) | | |
| Urinary bladder | (50) | (50) | (49) | (50) |
| Infiltration cellular, lymphoid | 25 (50%) | 25 (50%) | 4 (8%) | |
| Inflammation, chronic active | | 1 (2%) | 1 (2%) | 2 (4%) |
| Transitional epithelium, hyperplasia | | 1 (2%) | | 4 (8%) |

# APPENDIX D
# SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF TCAB

**TABLE D1**
**Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of TCAB[a]**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Accidental deaths | 1 | | 1 | 1 |
| Moribund | 6 | 9 | 9 | 21 |
| Natural deaths | 8 | 11 | 8 | 8 |
| Survivors | | | | |
| Died last week of study | | | | 1 |
| Terminal sacrifice | 35 | 30 | 32 | 19 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Esophagus | (49) | (50) | (50) | (50) |
| Fibrosarcoma, metastatic, skin | | | 1 (2%) | |
| Schwannoma malignant, metastatic, skin | | | 1 (2%) | |
| Gallbladder | (49) | (50) | (50) | (50) |
| Intestine large, cecum | (49) | (50) | (49) | (50) |
| Fibrosarcoma, metastatic, skin | | | | 1 (2%) |
| Squamous cell carcinoma, metastatic, stomach, forestomach | | | 1 (2%) | |
| Intestine large, colon | (49) | (50) | (49) | (50) |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (2%) |
| Intestine large, rectum | (49) | (50) | (50) | (50) |
| Intestine small, duodenum | (49) | (50) | (50) | (50) |
| Carcinoma | | | | 1 (2%) |
| Intestine small, jejunum | (49) | (50) | (50) | (50) |
| Carcinoma | | | | 1 (2%) |
| Leiomyosarcoma | | 1 (2%) | | |
| Schwannoma malignant, metastatic, skin | | | | 1 (2%) |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (2%) |
| Liver | (49) | (50) | (50) | (50) |
| Carcinoma, metastatic, islets, pancreatic | 1 (2%) | | | |
| Fibrosarcoma, metastatic, skin | | | | 2 (4%) |
| Fibrous histiocytoma, metastatic, skin | | 1 (2%) | | |
| Hepatocellular adenoma | 3 (6%) | 9 (18%) | 2 (4%) | 3 (6%) |
| Hepatocellular adenoma, multiple | | | 1 (2%) | |
| Hepatocellular carcinoma | 2 (4%) | 2 (4%) | 1 (2%) | 2 (4%) |
| Schwannoma malignant, metastatic, skin | | 1 (2%) | | 1 (2%) |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (2%) |
| Mesentery | (5) | (12) | (9) | (15) |
| Carcinoma, metastatic, islets, pancreatic | 1 (20%) | | | |
| Fibrosarcoma, metastatic, skin | | | | 2 (13%) |
| Leiomyosarcoma, metastatic, intestine small, jejunum | | 1 (8%) | | |
| Schwannoma malignant, metastatic, skin | | 1 (8%) | | 2 (13%) |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (7%) |
| Squamous cell carcinoma, metastatic, stomach, forestomach | | | 1 (11%) | |
| Pancreas | (49) | (50) | (50) | (50) |
| Carcinoma, metastatic, islets, pancreatic | 1 (2%) | | | |
| Fibrosarcoma, metastatic, skin | | | | 2 (4%) |
| Schwannoma malignant, metastatic, skin | | 1 (2%) | | |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (2%) |
| Squamous cell carcinoma, metastatic, stomach, forestomach | | | 1 (2%) | |
| Acinus, adenoma | | 1 (2%) | | |

**TABLE D1**
**Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Alimentary System** *(continued)* | | | | |
| Salivary glands | (49) | (50) | (50) | (50) |
| Squamous cell carcinoma | | | 1 (2%) | |
| Stomach, forestomach | (49) | (50) | (50) | (50) |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (2%) |
| Squamous cell carcinoma | | 1 (2%) | 1 (2%) | 4 (8%) |
| Squamous cell papilloma | 2 (4%) | 1 (2%) | 1 (2%) | |
| Stomach, glandular | (49) | (50) | (50) | (50) |
| Carcinoma | | | | 1 (2%) |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (2%) |
| Squamous cell carcinoma, metastatic, stomach, forestomach | | | | 3 (6%) |
| Tongue | (0) | (1) | (0) | (1) |
| Squamous cell carcinoma | | | | 1 (100%) |
| Tooth | (2) | (0) | (0) | (0) |
| **Cardiovascular System** | | | | |
| Blood vessel | (49) | (50) | (50) | (50) |
| Heart | (49) | (50) | (50) | (50) |
| Schwannoma malignant, metastatic, skin | | | 1 (2%) | |
| **Endocrine System** | | | | |
| Adrenal cortex | (49) | (50) | (50) | (50) |
| Carcinoma, metastatic, islets, pancreatic | 1 (2%) | | | |
| Fibrosarcoma, metastatic, skin | | | | 1 (2%) |
| Leiomyosarcoma, metastatic, intestine small, jejunum | | 1 (2%) | | |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (2%) |
| Squamous cell carcinoma, metastatic, stomach, forestomach | | | 1 (2%) | |
| Adrenal medulla | (49) | (50) | (50) | (50) |
| Pheochromocytoma malignant | 1 (2%) | | | |
| Islets, pancreatic | (49) | (50) | (50) | (50) |
| Adenoma | 1 (2%) | | | |
| Carcinoma | 1 (2%) | | | |
| Schwannoma malignant, metastatic, skin | | 1 (2%) | | |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (2%) |
| Parathyroid gland | (43) | (47) | (48) | (41) |
| Pituitary gland | (49) | (49) | (50) | (50) |
| Pars distalis, adenoma | 1 (2%) | 1 (2%) | 3 (6%) | |
| Thyroid gland | (49) | (50) | (50) | (50) |
| Bilateral, follicular cell, carcinoma | 1 (2%) | | | |
| C-cell, adenoma | | | 1 (2%) | |
| Follicular cell, adenoma | | 2 (4%) | | |
| Follicular cell, carcinoma | 1 (2%) | | | 1 (2%) |
| **General Body System** | | | | |
| None | | | | |
| **Genital System** | | | | |
| Clitoral gland | (49) | (50) | (49) | (50) |
| Carcinoma | 1 (2%) | 1 (2%) | | |
| Fibrosarcoma, metastatic, skin | | | 1 (2%) | |
| Schwannoma malignant, metastatic, skin | | 1 (2%) | | |

**TABLE D1**
**Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Genital System** *(continued)* | | | | |
| Ovary | (49) | (50) | (50) | (50) |
| Cystadenoma | 2 (4%) | | | |
| Fibrosarcoma, metastatic, skin | | | | 1 (2%) |
| Hemangioma | 1 (2%) | | | |
| Luteoma | | 1 (2%) | | 1 (2%) |
| Sarcoma stromal, metastatic, uterus | | | 1 (2%) | |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (2%) |
| Squamous cell carcinoma, metastatic, stomach, forestomach | | | 1 (2%) | |
| Teratoma benign | 1 (2%) | | | |
| Oviduct | (0) | (0) | (1) | (0) |
| Uterus | (49) | (50) | (50) | (50) |
| Carcinoma | | | 1 (2%) | 1 (2%) |
| Fibrosarcoma, metastatic, skin | | | | 1 (2%) |
| Hemangiosarcoma | | 1 (2%) | | |
| Sarcoma stromal | | 2 (4%) | 1 (2%) | |
| Cervix, squamous cell carcinoma, metastatic, vagina | | | 1 (2%) | |
| Vagina | (49) | (50) | (50) | (50) |
| Carcinoma, metastatic, uterus | | | 1 (2%) | |
| Granular cell tumor, benign | | | 1 (2%) | |
| Sarcoma stromal, metastatic, uterus | | | 1 (2%) | |
| Schwannoma malignant, metastatic, skin | | 1 (2%) | | |
| Squamous cell carcinoma | | | 1 (2%) | |
| **Hematopoietic System** | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Hemangiosarcoma | | 1 (2%) | | |
| Lymph node | (7) | (8) | (9) | (16) |
| Fibrosarcoma, metastatic, skin | 1 (14%) | | | 1 (6%) |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (6%) |
| Inguinal, fibrosarcoma, metastatic, skin | | | | 1 (6%) |
| Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung | | | | 1 (6%) |
| Mediastinal, fibrosarcoma, metastatic, skin | | 1 (13%) | 1 (11%) | 2 (13%) |
| Mediastinal, schwannoma malignant, metastatic, skin | | 1 (13%) | | |
| Mediastinal, schwannoma malignant, metastatic, uncertain primary site | | | | 1 (6%) |
| Pancreatic, fibrosarcoma, metastatic, skin | | | | 1 (6%) |
| Pancreatic, schwannoma malignant, metastatic, skin | | 1 (13%) | | |
| Lymph node, mandibular | (49) | (49) | (50) | (49) |
| Lymph node, mesenteric | (48) | (49) | (48) | (49) |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (2%) |
| Spleen | (49) | (50) | (50) | (50) |
| Fibrosarcoma, metastatic, skin | | | | 2 (4%) |
| Hemangiosarcoma | | 1 (2%) | | |
| Thymus | (48) | (48) | (45) | (48) |
| Fibrosarcoma, metastatic, skin | | | | 1 (2%) |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (2%) |
| Thymoma benign | 1 (2%) | | | |

**TABLE D1**
**Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Integumentary System** | | | | |
| Mammary gland | (49) | (50) | (50) | (50) |
| Skin | (49) | (50) | (50) | (50) |
| Squamous cell papilloma | 1 (2%) | | | |
| Subcutaneous tissue, fibroma | | | 1 (2%) | |
| Subcutaneous tissue, fibrosarcoma | 1 (2%) | 6 (12%) | 5 (10%) | 7 (14%) |
| Subcutaneous tissue, fibrosarcoma, multiple | | | | 1 (2%) |
| Subcutaneous tissue, fibrous histiocytoma | | 1 (2%) | | |
| Subcutaneous tissue, mast cell tumor, benign | | | | 1 (2%) |
| Subcutaneous tissue, mast cell tumor, malignant | | | | 1 (2%) |
| Subcutaneous tissue, rhabdomyosarcoma, metastatic, skeletal muscle | 1 (2%) | | | |
| Subcutaneous tissue, schwannoma, malignant | 1 (2%) | 2 (4%) | 2 (4%) | 4 (8%) |
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Osteosarcoma | | 1 (2%) | | |
| Skeletal muscle | (4) | (5) | (1) | (8) |
| Fibrosarcoma, metastatic, skin | | 2 (40%) | | 3 (38%) |
| Leiomyosarcoma, metastatic, intestine small, jejunum | | 1 (20%) | | |
| Rhabdomyosarcoma | 2 (50%) | | | |
| Schwannoma malignant, metastatic, skin | 1 (25%) | | 1 (100%) | 2 (25%) |
| Schwannoma malignant, metastatic, uncertain primary site | | | | 1 (13%) |
| **Nervous System** | | | | |
| Brain | (49) | (50) | (50) | (50) |
| Peripheral nerve | (0) | (2) | (1) | (0) |
| Spinal cord | (0) | (1) | (1) | (0) |
| **Respiratory System** | | | | |
| Lung | (49) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | 3 (6%) | 6 (12%) | 4 (8%) | 6 (12%) |
| Alveolar/bronchiolar adenoma, multiple | | | | 1 (2%) |
| Alveolar/bronchiolar carcinoma | | 2 (4%) | 1 (2%) | 4 (8%) |
| Carcinoma, metastatic, clitoral gland | 1 (2%) | | | |
| Cystic keratinizing epithelioma | | | | 1 (2%) |
| Cystic keratinizing epithelioma, multiple | | | | 1 (2%) |
| Fibrosarcoma, metastatic, skin | | 2 (4%) | 1 (2%) | 3 (6%) |
| Hepatocellular carcinoma, metastatic, liver | 2 (4%) | | | 1 (2%) |
| Rhabdomyosarcoma, metastatic, skeletal muscle | 1 (2%) | | | |
| Schwannoma malignant, metastatic, skin | | | 1 (2%) | |
| Squamous cell carcinoma, metastatic, stomach, forestomach | | | 1 (2%) | |
| Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung | | | | 1 (2%) |
| Mediastinum, fibrosarcoma, metastatic, skin | | | 1 (2%) | 1 (2%) |
| Mediastinum, schwannoma malignant, metastatic, skin | | 1 (2%) | | |
| Mediastinum, schwannoma malignant, metastatic, uncertain primary site | | | | 1 (2%) |
| Nose | (50) | (50) | (50) | (50) |

**TABLE D1**
**Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Special Senses System** | | | | |
| Ear | (1) | (0) | (1) | (2) |
| Eye | (49) | (50) | (50) | (49) |
| Retina, melanoma benign | | | | 1 (2%) |
| Harderian gland | (49) | (50) | (49) | (49) |
| Adenoma | 14 (29%) | 6 (12%) | 9 (18%) | 5 (10%) |
| **Urinary System** | | | | |
| Kidney | (49) | (50) | (50) | (50) |
| Carcinoma, metastatic, ureter | | | | 1 (2%) |
| Fibrosarcoma, metastatic, skin | | | | 2 (4%) |
| Ureter | (0) | (1) | (0) | (2) |
| Transitional epithelium, carcinoma | | | | 1 (50%) |
| Urethra | (49) | (50) | (49) | (49) |
| Transitional epithelium, carcinoma | | | | 2 (4%) |
| Urinary bladder | (49) | (50) | (50) | (50) |
| Carcinoma, metastatic, urethra | | | | 1 (2%) |
| Carcinoma, metastatic, uterus | | | 1 (2%) | |
| Fibrosarcoma, metastatic, skin | | | | 1 (2%) |
| **Systemic Lesions** | | | | |
| Multiple organs[b] | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | 1 (2%) | 2 (4%) | 1 (2%) | 2 (4%) |
| Lymphoma malignant | 2 (4%) | 5 (10%) | 8 (16%) | 7 (14%) |
| Mesothelioma malignant | | | 1 (2%) | |
| **Neoplasm Summary** | | | | |
| Total animals with primary neoplasms[c] | 29 | 37 | 34 | 38 |
| Total primary neoplasms | 44 | 56 | 47 | 61 |
| Total animals with benign neoplasms | 24 | 23 | 20 | 17 |
| Total benign neoplasms | 30 | 27 | 23 | 20 |
| Total animals with malignant neoplasms | 13 | 23 | 21 | 33 |
| Total malignant neoplasms | 14 | 29 | 24 | 41 |
| Total animals with metastatic neoplasms | 7 | 6 | 8 | 12 |
| Total metastatic neoplasms | 11 | 18 | 22 | 58 |
| Total animals with malignant neoplasms of uncertain primary site | | | | 1 |

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

**TABLE D2**
**Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Harderian Gland: Adenoma** | | | | |
| Overall rate[a] | 14/50 (28%) | 6/50 (12%) | 9/50 (18%) | 5/50 (10%) |
| Adjusted rate[b] | 32.0% | 13.3% | 20.6% | 13.8% |
| Terminal rate[c] | 12/35 (34%) | 4/30 (13%) | 7/32 (22%) | 3/19 (16%) |
| First incidence (days) | 569 | 596 | 477 | 541 |
| Poly-3 test[d] | P = 0.131N | P = 0.029N | P = 0.165N | P = 0.048N |
| **Liver: Hepatocellular Adenoma** | | | | |
| Overall rate | 3/49 (6%) | 9/50 (18%) | 3/50 (6%) | 3/50 (6%) |
| Adjusted rate | 7.1% | 20.1% | 7.0% | 8.4% |
| Terminal rate | 2/35 (6%) | 7/30 (23%) | 2/32 (6%) | 2/19 (11%) |
| First incidence (days) | 667 | 661 | 623 | 652 |
| Poly-3 test | P = 0.312N | P = 0.071 | P = 0.655N | P = 0.581 |
| **Liver: Hepatocellular Adenoma or Carcinoma** | | | | |
| Overall rate | 4/49 (8%) | 11/50 (22%) | 4/50 (8%) | 4/50 (8%) |
| Adjusted rate | 9.4% | 24.4% | 9.3% | 11.2% |
| Terminal rate | 2/35 (6%) | 8/30 (27%) | 3/32 (9%) | 3/19 (16%) |
| First incidence (days) | 667 | 661 | 623 | 652 |
| Poly-3 test | P = 0.305N | P = 0.055 | P = 0.636N | P = 0.546 |
| **Lung: Alveolar/bronchiolar Adenoma** | | | | |
| Overall rate | 3/49 (6%) | 6/50 (12%) | 4/50 (8%) | 7/50 (14%) |
| Adjusted rate | 7.1% | 13.5% | 9.2% | 19.3% |
| Terminal rate | 2/35 (6%) | 5/30 (17%) | 2/32 (6%) | 4/19 (21%) |
| First incidence (days) | 667 | 703 | 536 | 603 |
| Poly-3 test | P = 0.117 | P = 0.265 | P = 0.514 | P = 0.100 |
| **Lung: Alveolar/bronchiolar Carcinoma** | | | | |
| Overall rate | 0/49 (0%) | 2/50 (4%) | 1/50 (2%) | 4/50 (8%) |
| Adjusted rate | 0.0% | 4.5% | 2.3% | 11.1% |
| Terminal rate | 0/35 (0%) | 2/30 (7%) | 1/32 (3%) | 1/19 (5%) |
| First incidence (days) | —[e] | 731 (T) | 731 (T) | 648 |
| Poly-3 test | P = 0.031 | P = 0.248 | P = 0.503 | P = 0.042 |
| **Lung: Alveolar/bronchiolar Adenoma or Carcinoma** | | | | |
| Overall rate | 3/49 (6%) | 8/50 (16%) | 5/50 (10%) | 10/50 (20%) |
| Adjusted rate | 7.1% | 18.0% | 11.5% | 27.3% |
| Terminal rate | 2/35 (6%) | 7/30 (23%) | 3/32 (9%) | 5/19 (26%) |
| First incidence (days) | 667 | 703 | 536 | 603 |
| Poly-3 test | P = 0.028 | P = 0.112 | P = 0.370 | P = 0.015 |
| **Pituitary Gland (Pars Distalis): Adenoma** | | | | |
| Overall rate | 1/49 (2%) | 1/49 (2%) | 3/50 (6%) | 0/50 (0%) |
| Adjusted rate | 2.4% | 2.3% | 7.0% | 0.0% |
| Terminal rate | 0/35 (0%) | 1/29 (3%) | 3/32 (9%) | 0/19 (0%) |
| First incidence (days) | 669 | 731 (T) | 731 (T) | — |
| Poly-3 test | P = 0.426N | P = 0.756N | P = 0.308 | P = 0.536N |
| **Skin: Fibrosarcoma** | | | | |
| Overall rate | 1/50 (2%) | 6/50 (12%) | 5/50 (10%) | 8/50 (16%) |
| Adjusted rate | 2.3% | 13.3% | 11.4% | 21.2% |
| Terminal rate | 0/35 (0%) | 3/30 (10%) | 2/32 (6%) | 2/19 (11%) |
| First incidence (days) | 606 | 661 | 477 | 477 |
| Poly-3 test | P = 0.023 | P = 0.062 | P = 0.105 | P = 0.008 |

**TABLE D2**
**Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Skin (Subcutaneous Tissue): Fibrous Histiocytoma or Fibrosarcoma** | | | | |
| Overall rate | 1/50 (2%) | 7/50 (14%) | 5/50 (10%) | 8/50 (16%) |
| Adjusted rate | 2.3% | 15.5% | 11.4% | 21.2% |
| Terminal rate | 0/35 (0%) | 3/30 (10%) | 2/32 (6%) | 2/19 (11%) |
| First incidence (days) | 606 | 661 | 477 | 477 |
| Poly-3 test | P = 0.035 | P = 0.034 | P = 0.105 | P = 0.008 |
| **Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, or Fibrosarcoma** | | | | |
| Overall rate | 1/50 (2%) | 7/50 (14%) | 6/50 (12%) | 8/50 (16%) |
| Adjusted rate | 2.3% | 15.5% | 13.6% | 21.2% |
| Terminal rate | 0/35 (0%) | 3/30 (10%) | 3/32 (9%) | 2/19 (11%) |
| First incidence (days) | 606 | 661 | 477 | 477 |
| Poly-3 test | P = 0.037 | P = 0.034 | P = 0.058 | P = 0.008 |
| **Skin (Subcutaneous Tissue): Malignant Schwannoma** | | | | |
| Overall rate | 1/50 (2%) | 2/50 (4%) | 2/50 (4%) | 4/50 (8%) |
| Adjusted rate | 2.3% | 4.5% | 4.6% | 10.7% |
| Terminal rate | 0/35 (0%) | 0/30 (0%) | 0/32 (0%) | 0/19 (0%) |
| First incidence (days) | 546 | 673 | 517 | 522 |
| Poly-3 test | P = 0.089 | P = 0.511 | P = 0.501 | P = 0.137 |
| **Skin (Subcutaneous Tissue): Fibrosarcoma or Malignant Schwannoma** | | | | |
| Overall rate | 2/50 (4%) | 8/50 (16%) | 7/50 (14%) | 12/50 (24%) |
| Adjusted rate | 4.6% | 17.6% | 15.6% | 30.2% |
| Terminal rate | 0/35 (0%) | 3/30 (10%) | 2/32 (6%) | 2/19 (11%) |
| First incidence (days) | 546 | 661 | 477 | 477 |
| Poly-3 test | P = 0.004 | P = 0.051 | P = 0.084 | P = 0.001 |
| **Stomach (Forestomach): Squamous Cell Carcinoma** | | | | |
| Overall rate | 0/50 (0%) | 1/50 (2%) | 1/50 (2%) | 4/50 (8%) |
| Adjusted rate | 0.0% | 2.3% | 2.3% | 11.2% |
| Terminal rate | 0/35 (0%) | 0/30 (0%) | 0/32 (0%) | 2/19 (11%) |
| First incidence (days) | — | 722 | 677 | 680 |
| Poly-3 test | P = 0.011 | P = 0.507 | P = 0.501 | P = 0.040 |
| **Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma** | | | | |
| Overall rate | 2/50 (4%) | 2/50 (4%) | 2/50 (4%) | 4/50 (8%) |
| Adjusted rate | 4.7% | 4.5% | 4.6% | 11.2% |
| Terminal rate | 1/35 (3%) | 1/30 (3%) | 0/32 (0%) | 2/19 (11%) |
| First incidence (days) | 669 | 722 | 623 | 680 |
| Poly-3 test | P = 0.150 | P = 0.683N | P = 0.691N | P = 0.257 |
| **All Organs: Malignant Lymphoma** | | | | |
| Overall rate | 2/50 (4%) | 5/50 (10%) | 8/50 (16%) | 7/50 (14%) |
| Adjusted rate | 4.7% | 11.1% | 18.2% | 18.7% |
| Terminal rate | 2/35 (6%) | 1/30 (3%) | 5/32 (16%) | 3/19 (16%) |
| First incidence (days) | 731 (T) | 664 | 541 | 50 |
| Poly-3 test | P = 0.065 | P = 0.236 | P = 0.049 | P = 0.050 |
| **All Organs: Fibrosarcoma or Malignant Schwannoma** | | | | |
| Overall rate | 2/50 (4%) | 8/50 (16%) | 7/50 (14%) | 12/50 (24%) |
| Adjusted rate | 4.6% | 17.6% | 15.6% | 30.2% |
| Terminal rate | 0/35 (0%) | 3/30 (10%) | 2/32 (6%) | 2/19 (11%) |
| First incidence (days) | 546 | 661 | 477 | 477 |
| Poly-3 test | P = 0.004 | P = 0.051 | P = 0.084 | P = 0.001 |

**TABLE D2**
**Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **All Organs: Benign Neoplasms** | | | | |
| Overall rate | 24/50 (48%) | 23/50 (46%) | 20/50 (40%) | 17/50 (34%) |
| Adjusted rate | 52.2% | 49.9% | 44.6% | 44.7% |
| Terminal rate | 17/35 (49%) | 16/30 (53%) | 15/32 (47%) | 9/19 (47%) |
| First incidence (days) | 269 | 596 | 477 | 541 |
| Poly-3 test | P = 0.286N | P = 0.497N | P = 0.302N | P = 0.320N |
| **All Organs: Malignant Neoplasms** | | | | |
| Overall rate | 13/50 (26%) | 23/50 (46%) | 21/50 (42%) | 34/50 (68%) |
| Adjusted rate | 28.5% | 47.7% | 44.1% | 74.6% |
| Terminal rate | 6/35 (17%) | 8/30 (27%) | 10/32 (31%) | 10/19 (53%) |
| First incidence (days) | 546 | 459 | 477 | 50 |
| Poly-3 test | P < 0.001 | P = 0.043 | P = 0.086 | P < 0.001 |
| **All Organs: Benign or Malignant Neoplasms** | | | | |
| Overall rate | 29/50 (58%) | 37/50 (74%) | 34/50 (68%) | 39/50 (78%) |
| Adjusted rate | 60.9% | 76.0% | 70.6% | 85.1% |
| Terminal rate | 18/35 (51%) | 21/30 (70%) | 21/32 (66%) | 14/19 (74%) |
| First incidence (days) | 269 | 459 | 477 | 50 |
| Poly-3 test | P = 0.017 | P = 0.081 | P = 0.213 | P = 0.006 |

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and pituitary gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

**TABLE D3a**
**Historical Incidence of Lung Neoplasms in Control Female B6C3F1 Mice[a]**

| Study (Study Start) | Incidence in Controls | | | |
|---|---|---|---|---|
| | Cystic Keratinizing Epithelioma | Alveolar/bronchiolar Adenoma | Alveolar/bronchiolar Carcinoma | Alveolar/bronchiolar Adenoma or Carcinoma |
| **Historical Incidence: Corn Oil Gavage Studies** | | | | |
| Isoeugenol (May, 2002) | 0/48 | 4/48 | 0/48 | 4/48 |
| β-Myrcene (April, 2002) | 0/50 | 4/50 | 2/50 | 6/50 |
| Pulegone (April, 2003) | 0/49 | 1/49 | 2/49 | 3/49 |
| TCAB (February, 2003) | 0/49 | 3/49 | 0/49 | 3/49 |
| Total (%) | 0/196 | 12/196 (6.1%) | 4/196 (2.0%) | 16/196 (8.2%) |
| Mean ± standard deviation | | 6.1% ± 2.9% | 2.0% ± 2.3% | 8.1% ± 2.8% |
| Range | | 2% – 8% | 0% – 4% | 6% – 12% |
| **Overall Historical Incidence: All Routes** | | | | |
| Total (%) | 0/1,496 | 72/1,496 (4.8%) | 59/1,496 (3.9%) | 127/1,496 (8.5%) |
| Mean ± standard deviation | | 4.8% ± 3.5% | 3.9% ± 3.4% | 8.5% ± 4.0% |
| Range | | 0% – 12% | 0% – 12% | 2% – 18% |

[a] Data as of November 19, 2008

**TABLE D3b**
**Historical Incidence of Squamous Cell Carcinoma in the Forestomach of Control Female B6C3F1 Mice[a]**

| Study (Study Start) | Incidence in Controls |
|---|---|
| **Historical Incidence: Corn Oil Gavage Studies** | |
| Isoeugenol (May, 2002) | 0/49 |
| β-Myrcene (April, 2002) | 0/50 |
| Pulegone (April, 2003) | 0/49 |
| TCAB (February, 2003) | 0/50 |
| Total | 0/198 |
| **Overall Historical Incidence: All Routes** | |
| Total (%) | 3/1,498 (0.2%) |
| Mean ± standard deviation | 0.2% ± 0.6% |
| Range | 0% – 2% |

[a] Data as of November 19, 2008

**TABLE D3c**
**Historical Incidence of Skin Neoplasms in Control Female B6C3F1 Mice[a]**

| Study (Study Start) | Incidence in Controls | |
|---|---|---|
| | Fibrosarcoma | Malignant Schwannoma |
| **Historical Incidence: Corn Oil Gavage Studies** | | |
| Isoeugenol (May, 2002) | 0/49 | 0/49 |
| β-Myrcene (April, 2002) | 1/50 | 0/50 |
| Pulegone (April, 2003) | 1/49 | 2/49 |
| TCAB (February, 2003) | 1/50 | 1/50 |
| Total (%) | 3/198 (1.5%) | 3/198 (1.5%) |
| Mean ± standard deviation | 1.5% ± 1.0% | 1.5% ± 2.0% |
| Range | 0% – 2% | 0% – 4% |
| **Overall Historical Incidence: All Routes** | | |
| Total (%) | 30/1,498 (2.0%) | 10/1,498 (0.7%) |
| Mean ± standard deviation | 2.0% ± 2.6% | 0.7% ± 1.3% |
| Range | 0% – 8% | 0% – 4% |

[a] Data as of November 19, 2008

**TABLE D3d**
**Historical Incidence of Malignant Lymphoma in Control Female B6C3F1 Mice[a]**

| Study (Study Start) | Incidence in Controls |
|---|---|
| **Historical Incidence: Corn Oil Gavage Studies** | |
| Isoeugenol (May, 2002 | 11/49 |
| β-Myrcene (April, 2002) | 7/50 |
| Pulegone (April, 2003) | 6/49 |
| TCAB (February, 2003) | 2/50 |
| Total (%) | 26/198 (13.1%) |
| Mean ± standard deviation | 13.2% ± 7.6% |
| Range | 4% – 22% |
| **Overall Historical Incidence: All Routes** | |
| Total (%) | 307/1,498 (20.5%) |
| Mean ± standard deviation | 20.5% ± 9.7% |
| Range | 4% – 54% |

[a] Data as of November 19, 2008; includes data for histiocytic, lymphocytic, mixed, unspecified, or undifferentiated cell types

**TABLE D4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of TCAB[a]**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Disposition Summary** | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Accidental deaths | 1 | | 1 | 1 |
| Moribund | 6 | 9 | 9 | 21 |
| Natural deaths | 8 | 11 | 8 | 8 |
| Survivors | | | | |
| Died last week of study | | | | 1 |
| Terminal sacrifice | 35 | 30 | 32 | 19 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| **Alimentary System** | | | | |
| Esophagus | (49) | (50) | (50) | (50) |
| Perforation | 1 (2%) | | 1 (2%) | 1 (2%) |
| Gallbladder | (49) | (50) | (50) | (50) |
| Infiltration cellular, lymphoid | 3 (6%) | 3 (6%) | 2 (4%) | 1 (2%) |
| Inflammation, chronic active | | | 1 (2%) | |
| Intestine large, cecum | (49) | (50) | (49) | (50) |
| Inflammation, chronic active | | 1 (2%) | | |
| Ulcer | | 1 (2%) | | |
| Intestine large, colon | (49) | (50) | (49) | (50) |
| Intestine large, rectum | (49) | (50) | (50) | (50) |
| Inflammation, chronic active | 1 (2%) | | | |
| Intestine small, duodenum | (49) | (50) | (50) | (50) |
| Intestine small, jejunum | (49) | (50) | (50) | (50) |
| Peyer's patch, hyperplasia, lymphoid | | | 1 (2%) | 3 (6%) |
| Liver | (49) | (50) | (50) | (50) |
| Amyloid deposition | | | 2 (4%) | 4 (8%) |
| Basophilic focus | 1 (2%) | 6 (12%) | 3 (6%) | 1 (2%) |
| Clear cell focus | 2 (4%) | 1 (2%) | 1 (2%) | |
| Eosinophilic focus | 2 (4%) | | 1 (2%) | 1 (2%) |
| Hematopoietic cell proliferation | 3 (6%) | 9 (18%) | 9 (18%) | 21 (42%) |
| Infarct | | | | 3 (6%) |
| Infiltration cellular, histiocyte | | | | 1 (2%) |
| Infiltration cellular, lymphoid | 38 (78%) | 40 (80%) | 38 (76%) | 28 (56%) |
| Inflammation, chronic active | 39 (80%) | 37 (74%) | 37 (74%) | 32 (64%) |
| Mineralization | | 1 (2%) | | 1 (2%) |
| Mixed cell focus | 3 (6%) | 3 (6%) | 4 (8%) | 1 (2%) |
| Tension lipidosis | 4 (8%) | 6 (12%) | 4 (8%) | 4 (8%) |
| Bile duct, hyperplasia | | 1 (2%) | | 2 (4%) |
| Bile duct, inflammation, chronic active | | | | 1 (2%) |
| Hepatocyte, necrosis | 1 (2%) | 1 (2%) | 3 (6%) | 3 (6%) |
| Hepatocyte, vacuolization cytoplasmic | 33 (67%) | 33 (66%) | 35 (70%) | 20 (40%) |
| Mesentery | (5) | (12) | (9) | (15) |
| Congestion | | | 1 (11%) | |
| Fat, cyst | 1 (20%) | | | |
| Fat, fibrosis | 3 (60%) | 6 (50%) | 5 (56%) | 6 (40%) |
| Fat, inflammation, chronic active | 2 (40%) | 6 (50%) | 4 (44%) | 7 (47%) |
| Fat, mineralization | | 1 (8%) | 3 (33%) | |
| Fat, necrosis | 3 (60%) | 5 (42%) | 5 (56%) | 8 (53%) |

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

**TABLE D4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Alimentary System (continued)** | | | | |
| Pancreas | (49) | (50) | (50) | (50) |
| Basophilic focus | 1 (2%) | | | |
| Congestion | | | | 1 (2%) |
| Infiltration cellular, lymphoid | 12 (24%) | 5 (10%) | 6 (12%) | 3 (6%) |
| Inflammation, chronic active | | | 2 (4%) | 1 (2%) |
| Mineralization | | | | 1 (2%) |
| Acinus, atrophy | | 1 (2%) | | |
| Duct, cyst | | 1 (2%) | | |
| Duct, cyst, multiple | | | | 1 (2%) |
| Salivary glands | (49) | (50) | (50) | (50) |
| Infiltration cellular, lymphoid | 36 (73%) | 23 (46%) | 13 (26%) | 1 (2%) |
| Mineralization | | 1 (2%) | | |
| Stomach, forestomach | (49) | (50) | (50) | (50) |
| Infiltration cellular, lymphoid | 2 (4%) | | 2 (4%) | 10 (20%) |
| Inflammation, chronic active | 1 (2%) | 4 (8%) | 1 (2%) | 1 (2%) |
| Mineralization | | | | 1 (2%) |
| Epithelium, cyst | | 1 (2%) | 2 (4%) | |
| Epithelium, hyperplasia | 8 (16%) | 27 (54%) | 38 (76%) | 43 (86%) |
| Epithelium, ulcer | 1 (2%) | 3 (6%) | 1 (2%) | |
| Stomach, glandular | (49) | (50) | (50) | (50) |
| Infiltration cellular, lymphoid | 1 (2%) | 14 (28%) | 30 (60%) | 28 (56%) |
| Infiltration cellular, mast cell | 1 (2%) | | | |
| Inflammation, chronic active | 2 (4%) | | 1 (2%) | 1 (2%) |
| Mineralization | 1 (2%) | 4 (8%) | 3 (6%) | 3 (6%) |
| Epithelium, erosion | | 1 (2%) | | |
| Epithelium, hyperplasia, focal | 1 (2%) | 19 (38%) | 26 (52%) | 28 (56%) |
| Epithelium, necrosis | | | 1 (2%) | |
| Epithelium, ulcer | | 1 (2%) | | 1 (2%) |
| Epithelium, glands, cyst | 8 (16%) | 19 (38%) | 13 (26%) | 22 (44%) |
| Tongue | (0) | (1) | (0) | (1) |
| Tooth | (2) | (0) | (0) | (0) |
| Malformation | 2 (100%) | | | |
| **Cardiovascular System** | | | | |
| Blood vessel | (49) | (50) | (50) | (50) |
| Aorta, mineralization | 1 (2%) | 2 (4%) | | 1 (2%) |
| Heart | (49) | (50) | (50) | (50) |
| Cardiomyopathy | 3 (6%) | 5 (10%) | 4 (8%) | 9 (18%) |
| Hyperplasia, atypical | | | | 1 (2%) |
| Inflammation, chronic active | | 1 (2%) | | 1 (2%) |
| Mineralization | 3 (6%) | 3 (6%) | 3 (6%) | 2 (4%) |
| Valve, inflammation, chronic active | | 1 (2%) | | |
| **Endocrine System** | | | | |
| Adrenal cortex | (49) | (50) | (50) | (50) |
| Accessory adrenal cortical nodule | 2 (4%) | 2 (4%) | 1 (2%) | 1 (2%) |
| Cyst | 1 (2%) | | | |
| Degeneration, fatty | | | | 1 (2%) |
| Hematopoietic cell proliferation | | 2 (4%) | 1 (2%) | |
| Hyperplasia | 1 (2%) | | | |
| Hypertrophy | 2 (4%) | | | |
| Inflammation, chronic active | | | | 1 (2%) |
| Subcapsular, hyperplasia | 48 (98%) | 50 (100%) | 47 (94%) | 48 (96%) |

**TABLE D4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Endocrine System** *(continued)* | | | | |
| Adrenal medulla | (49) | (50) | (50) | (50) |
| Hyperplasia | 2 (4%) | | 2 (4%) | |
| Islets, pancreatic | (49) | (50) | (50) | (50) |
| Hyperplasia | 1 (2%) | | | |
| Parathyroid gland | (43) | (47) | (48) | (41) |
| Cyst | | 1 (2%) | | |
| Cyst, multiple | | 1 (2%) | | 1 (2%) |
| Pituitary gland | (49) | (49) | (50) | (50) |
| Pars distalis, angiectasis | 2 (4%) | 1 (2%) | 3 (6%) | 2 (4%) |
| Pars distalis, cyst | | | | 1 (2%) |
| Pars distalis, hyperplasia | 6 (12%) | 5 (10%) | 8 (16%) | 5 (10%) |
| Thyroid gland | (49) | (50) | (50) | (50) |
| Ectopic thymus | 1 (2%) | | 2 (4%) | |
| Infiltration cellular, lymphoid | 1 (2%) | | | |
| C-cell, hyperplasia | | | 1 (2%) | |
| Follicle, cyst | 1 (2%) | 1 (2%) | | 1 (2%) |
| Follicle, degeneration | 17 (35%) | 28 (56%) | 24 (48%) | 20 (40%) |
| **General Body System** | | | | |
| None | | | | |
| **Genital System** | | | | |
| Clitoral gland | (49) | (50) | (49) | (50) |
| Atrophy | 25 (51%) | 44 (88%) | 37 (76%) | 40 (80%) |
| Fibrosis | | | | 1 (2%) |
| Infiltration cellular, lymphoid | | 5 (10%) | 6 (12%) | 3 (6%) |
| Inflammation, chronic active | 3 (6%) | 4 (8%) | 17 (35%) | 25 (50)% |
| Duct, cyst | 5 (10%) | 46 (92%) | 43 (88%) | 43 (86%) |
| Ovary | (49) | (50) | (50) | (50) |
| Angiectasis | 3 (6%) | | | |
| Atrophy | 29 (59%) | 44 (88%) | 47 (94%) | 45 (90%) |
| Cyst | 11 (22%) | 8 (16%) | 5 (10%) | 4 (8%) |
| Cyst, multiple | 1 (2%) | | | |
| Infiltration cellular, lymphoid | 2 (4%) | | | |
| Inflammation, chronic active | | 1 (2%) | | |
| Mineralization | 1 (2%) | | | |
| Pigmentation | 2 (4%) | | | |
| Bilateral, cyst | | 1 (2%) | | |
| Oviduct | (0) | (0) | (1) | (0) |
| Inflammation, chronic active | | | 1 (100%) | |
| Uterus | (49) | (50) | (50) | (50) |
| Cyst | | 1 (2%) | 1 (2%) | |
| Infiltration cellular, lymphoid | 1 (2%) | | | |
| Inflammation, chronic active | | | 1 (2%) | |
| Endometrium, hyperplasia, cystic | 45 (92%) | 45 (90%) | 45 (90%) | 41 (82%) |
| Vagina | (49) | (50) | (50) | (50) |
| Infiltration cellular, lymphoid | 1 (2%) | | | 1 (2%) |
| Infiltration cellular, polymorphonuclear | 11 (22%) | 21 (42%) | 15 (30%) | 18 (36%) |
| Inflammation, chronic active | 2 (4%) | | 3 (6%) | |
| Epithelium, atrophy | 3 (6%) | 2 (4%) | 3 (6%) | 3 (6%) |
| Epithelium, hyperplasia | | 1 (2%) | | |

**TABLE D4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Hematopoietic System** | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Hyperplasia | 2 (4%) | 8 (16%) | 9 (18%) | 16 (32%) |
| Myelofibrosis | 16 (32%) | 30 (60%) | 33 (66%) | 15 (30%) |
| Lymph node | (7) | (8) | (9) | (16) |
| Bronchial, hyperplasia, lymphoid | 1 (14%) | | | |
| Inguinal, hyperplasia, lymphoid | 1 (14%) | | | |
| Inguinal, pigmentation | | | | 1 (6%) |
| Mediastinal, hematopoietic cell proliferation | | 1 (13%) | | |
| Mediastinal, hyperplasia, lymphoid | | 1 (13%) | 2 (22%) | 1 (6%) |
| Mediastinal, inflammation, chronic active | | | 1 (11%) | |
| Mediastinal, necrosis, lymphoid | 1 (14%) | | | |
| Pancreatic, hematopoietic cell proliferation | | | | 1 (6%) |
| Pancreatic, hyperplasia, lymphoid | | | | 1 (6%) |
| Pancreatic, pigmentation | | | | 1 (6%) |
| Renal, hyperplasia, lymphoid | | | | 1 (6%) |
| Lymph node, mandibular | (49) | (49) | (50) | (49) |
| Hyperplasia, lymphoid | 10 (20%) | 8 (16%) | 9 (18%) | 15 (31%) |
| Necrosis, lymphoid | 1 (2%) | | | 1 (2%) |
| Pigmentation | | 1 (2%) | 1 (2%) | 2 (4%) |
| Lymph node, mesenteric | (48) | (49) | (48) | (49) |
| Hyperplasia, lymphoid | 3 (6%) | | 4 (8%) | 2 (4%) |
| Mineralization | | 1 (2%) | | |
| Artery, mineralization | | 1 (2%) | | |
| Spleen | (49) | (50) | (50) | (50) |
| Amyloid deposition | | | 1 (2%) | 4 (8%) |
| Atrophy | 1 (2%) | 3 (6%) | 6 (12%) | 3 (6%) |
| Fibrosis | | 1 (2%) | | |
| Hematopoietic cell proliferation | 10 (20%) | 25 (50%) | 21 (42%) | 33 (66%) |
| Hyperplasia, lymphoid | | | 1 (2%) | |
| Infarct | | | | 1 (2%) |
| Lymphoid follicle, hyperplasia | 8 (16%) | | 1 (2%) | 1 (2%) |
| Thymus | (48) | (48) | (45) | (48) |
| Atrophy | 7 (15%) | 15 (31%) | 12 (27%) | 25 (52%) |
| Cyst | 9 (19%) | 5 (10%) | 4 (9%) | 3 (6%) |
| Cyst, multiple | 21 (44%) | 31 (65%) | 33 (73%) | 37 (77%) |
| Ectopic parathyroid gland | 10 (21%) | 11 (23%) | 20 (44%) | 5 (10%) |
| Ectopic thyroid | 1 (2%) | | | |
| Hyperplasia, lymphoid | 2 (4%) | | | |
| Infiltration cellular, histiocyte | | 1 (2%) | | |
| Inflammation, chronic active | | 5 (10%) | 12 (27%) | 5 (10%) |
| Thymocyte, necrosis | 1 (2%) | 1 (2%) | | |
| **Integumentary System** | | | | |
| Mammary gland | (49) | (50) | (50) | (50) |
| Skin | (49) | (50) | (50) | (50) |
| Inflammation, chronic active | | 2 (4%) | 6 (12%) | 11 (22%) |
| Dermis, fibrosis | | 1 (2%) | 4 (8%) | 11 (22%) |
| Epidermis, hyperplasia | 1 (2%) | 2 (4%) | 4 (8%) | 11 (22%) |
| Epidermis, ulcer | | 1 (2%) | 4 (8%) | 11 (22%) |
| Hair follicle, dilatation | 2 (4%) | | 11 (22%) | 23 (46%) |
| Sebaceous gland, atrophy | 6 (12%) | 10 (20%) | 11 (22%) | 15 (30%) |
| Subcutaneous tissue, inflammation, chronic active | | | 2 (4%) | |
| Subcutaneous tissue, metaplasia, osseous | | | 1 (2%) | |

**TABLE D4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Musculoskeletal System** | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Osteopetrosis | | | | 1 (2%) |
| Skeletal muscle | (4) | (5) | (1) | (8) |
| Infiltration cellular, lymphoid | 1 (25%) | | | |
| Inflammation, chronic active | | 1 (20%) | | |
| Mineralization | | | | 1 (13%) |
| **Nervous System** | | | | |
| Brain | (49) | (50) | (50) | (50) |
| Compression | 1 (2%) | 1 (2%) | | |
| Hydrocephalus | 1 (2%) | | | |
| Infiltration cellular, lymphoid | 1 (2%) | | | |
| Meninges, hemorrhage | | 1 (2%) | | |
| Neuron, necrosis | 2 (4%) | | | |
| Peripheral nerve | (0) | (2) | (1) | (0) |
| Sciatic, degeneration | | 1 (50%) | | |
| Spinal cord | (0) | (1) | (1) | (0) |
| Degeneration | | 1 (100%) | 1 (100%) | |
| **Respiratory System** | | | | |
| Lung | (49) | (50) | (50) | (50) |
| Hemorrhage | | | | 1 (2%) |
| Infiltration cellular, lymphoid | 4 (8%) | 2 (4%) | | 2 (4%) |
| Infiltration cellular, polymorphonuclear | | 1 (2%) | | |
| Inflammation, chronic active | | 3 (6%) | 5 (10%) | 7 (14%) |
| Alveolar epithelium, hyperplasia | 1 (2%) | 1 (2%) | 1 (2%) | 4 (8%) |
| Alveolar epithelium, metaplasia, squamous | | | | 1 (2%) |
| Alveolus, infiltration cellular, histiocyte | | 3 (6%) | 1 (2%) | 6 (12%) |
| Artery, mineralization | | 1 (2%) | | |
| Artery, mediastinum, mineralization | 1 (2%) | 1 (2%) | | |
| Bronchiole, metaplasia, squamous | | | | 1 (2%) |
| Mediastinum, inflammation | 1 (2%) | | | |
| Nose | (50) | (50) | (50) | (50) |
| Inflammation, chronic active | 4 (8%) | | | |
| Glands, olfactory epithelium, dilatation | | | | 2 (4%) |
| Glands, olfactory epithelium, hyperplasia | 1 (2%) | | | |
| Olfactory epithelium, metaplasia | 1 (2%) | | | |
| **Special Senses System** | | | | |
| Ear | (1) | (0) | (1) | (2) |
| Inflammation, chronic active | 1 (100%) | | | |
| Eye | (49) | (50) | (50) | (49) |
| Atrophy | 1 (2%) | | | |
| Cornea, inflammation, chronic active | | | | 1 (2%) |
| Optic nerve, infiltration cellular, lymphoid | | | 1 (2%) | 1 (2%) |
| Optic nerve, pigmentation | | | | 1 (2%) |
| Harderian gland | (49) | (50) | (49) | (49) |
| Atrophy | 1 (2%) | | | |
| Hyperplasia | 2 (4%) | 3 (6%) | 2 (4%) | 2 (4%) |
| Infiltration cellular, lymphoid | 32 (65%) | 13 (26%) | 7 (14%) | 4 (8%) |
| Inflammation, chronic active | 1 (2%) | | 2 (4%) | 3 (6%) |

**TABLE D4**
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of TCAB**

| | Vehicle Control | 3 mg/kg | 10 mg/kg | 30 mg/kg |
|---|---|---|---|---|
| **Urinary System** | | | | |
| Kidney | (49) | (50) | (50) | (50) |
| Hydronephrosis | | | 5 (10%) | 3 (6%) |
| Infarct | 3 (6%) | 6 (12%) | 11 (22%) | 2 (4%) |
| Infiltration cellular, lymphoid | 39 (80%) | 35 (70%) | 37 (74%) | 26 (52%) |
| Inflammation, chronic active | | | | 2 (4%) |
| Metaplasia, osseous | | 2 (4%) | | |
| Mineralization | 6 (12%) | 14 (28%) | 13 (26%) | 14 (28%) |
| Nephropathy | 29 (59%) | 32 (64%) | 38 (76%) | 41 (82%) |
| Cortex, cyst | 1 (2%) | | | |
| Cortex, cyst, multiple | 1 (2%) | | | |
| Glomerulus, amyloid deposition | | | 1 (2%) | 5 (10%) |
| Papilla, necrosis | | 1 (2%) | 8 (16%) | 2 (4%) |
| Pelvis, metaplasia, osseous | | | | 1 (2%) |
| Renal tubule, accumulation, hyaline droplet | 1 (2%) | 2 (4%) | 2 (4%) | 2 (4%) |
| Renal tubule, dilatation | 1 (2%) | 5 (10%) | 6 (12%) | 7 (14%) |
| Renal tubule, pigmentation | 1 (2%) | | | |
| Ureter | (0) | (1) | (0) | (2) |
| Transitional epithelium, hyperplasia | | | | 1 (50%) |
| Urethra | (49) | (50) | (49) | (49) |
| Dilatation | | | 1 (2%) | |
| Infiltration cellular, lymphoid | | | | 1 (2%) |
| Inflammation, suppurative | | | 1 (2%) | |
| Transitional epithelium, hyperplasia | | 4 (8%) | | 3 (6%) |
| Urinary bladder | (49) | (50) | (50) | (50) |
| Infiltration cellular, lymphoid | 41 (84%) | 30 (60%) | 27 (54%) | 25 (50%) |
| Inflammation, chronic active | | | | 2 (4%) |
| Transitional epithelium, hyperplasia | | | | 4 (8%) |

# APPENDIX E
# CLINICAL PATHOLOGY RESULTS

**TABLE E1**
**Hematology, Clinical Chemistry, and Thyroid Hormone Data for Rats in the 3-Month Gavage Study of TCAB[a]**

| | 0 mg/kg | 0.1 mg/kg | 0.3 mg/kg | 1 mg/kg | 3 mg/kg | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|---|---|---|---|
| **Male** | | | | | | | | |
| **Hematology (Week 13)** | | | | | | | | |
| n | 7 | 9 | 8 | 7 | 9 | 10 | 9 | 10 |
| Hematocrit (%) | 47.8 ± 0.6 | 48.3 ± 0.4 | 47.8 ± 0.3 | 47.3 ± 0.8 | 48.0 ± 0.7 | 45.6 ± 0.5* | 44.7 ± 0.7** | 43.8 ± 0.5** |
| Hemoglobin (g/dL) | 16.0 ± 0.2 | 16.2 ± 0.2 | 16.0 ± 0.1 | 15.9 ± 0.3 | 16.1 ± 0.2 | 15.2 ± 0.1** | 14.9 ± 0.2** | 14.6 ± 0.2** |
| Erythrocytes ($10^6$/μL) | 8.60 ± 0.11 | 8.84 ± 0.11 | 9.00 ± 0.08 | 8.87 ± 0.13 | 9.06 ± 0.11 | 8.61 ± 0.12 | 8.40 ± 0.14 | 8.26 ± 0.07 |
| Reticulocytes ($10^6$/μL) | 0.8 ± 0.1 | 1.4 ± 0.2 | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.0 ± 0.1 | 1.7 ± 0.1** | 1.7 ± 0.2** | 1.6 ± 0.2** |
| Nucleated erythrocytes (%) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Mean cell volume (fL) | 55.7 ± 0.6 | 54.7 ± 0.4 | 53.1 ± 0.3** | 53.3 ± 0.4** | 53.0 ± 0.4** | 53.1 ± 0.5** | 53.2 ± 0.5** | 53.0 ± 0.5** |
| Mean cell hemoglobin (pg) | 18.7 ± 0.2 | 18.3 ± 0.2 | 17.8 ± 0.1** | 18.0 ± 0.2* | 17.7 ± 0.1** | 17.7 ± 0.1** | 17.7 ± 0.2** | 17.7 ± 0.1** |
| Mean cell hemoglobin concentration (g/dL) | 33.5 ± 0.1 | 33.5 ± 0.2 | 33.4 ± 0.1 | 33.7 ± 0.2 | 33.5 ± 0.1 | 33.4 ± 0.1 | 33.3 ± 0.2 | 33.4 ± 0.1 |
| Platelets ($10^3$/μL) | 823.7 ± 26.0 | 783.3 ± 41.7 | 770.6 ± 38.5 | 836.4 ± 29.0 | 742.8 ± 25.7 | 780.5 ± 29.2 | 776.1 ± 42.8 | 717.2 ± 28.1 |
| Leukocytes ($10^3$/μL) | 8.67 ± 0.37 | 12.01 ± 0.50** | 9.84 ± 0.60 | 10.73 ± 0.65 | 12.44 ± 0.63** | 10.65 ± 0.33 | 11.57 ± 0.58* | 11.56 ± 0.72* |
| Segmented neutrophils ($10^3$/μL) | 0.85 ± 0.16 | 0.88 ± 0.10 | 0.88 ± 0.09 | 1.08 ± 0.09 | 1.20 ± 0.14 | 0.98 ± 0.09 | 1.08 ± 0.13 | 1.09 ± 0.16 |
| Bands ($10^3$/μL) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Lymphocytes ($10^3$/μL) | 7.63 ± 0.40 | 10.92 ± 0.45** | 8.81 ± 0.53 | 9.56 ± 0.60 | 11.13 ± 0.57** | 9.57 ± 0.32 | 10.38 ± 0.52* | 10.37 ± 0.74* |
| Monocytes ($10^3$/μL) | 0.12 ± 0.03 | 0.15 ± 0.03 | 0.08 ±0.04 | 0.06 ± 0.03 | 0.09 ± 0.03 | 1.00 ± 0.03 | 0.09 ± 0.03 | 0.10 ± 0.03 |
| Basophils ($10^3$/μL) | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| Eosinophils ($10^3$/μL) | 0.08 ± 0.04 | 0.07 ± 0.03 | 0.06 ± 0.03 | 0.04 ± 0.02 | 0.03 ± 0.02 | 0.01 ± 0.01 | 0.02 ± 0.02 | 0.00 ± 0.00* |
| Methemoglobin (g/dL) | 0.40 ± 0.00[b] | 0.41 ± 0.10 | 0.40 ± 0.00[b] | 0.40 ± 0.00[c] | 0.40 ± 0.01[b] | 0.41 ± 0.01 | 0.40 ± 0.00 | 0.44 ± 0.02*[c] |
| Osmotic fragility (%) | 0.265 ± 0.020[b] | 0.214 ± 0.023[d] | 0.268 ± 0.019[c] | 0.262 ± 0.034 | 0.283 ± 0.025[e] | 0.189 ± 0.017[c] | 0.159 ± 0.017* | 0.227 ± 0.023 |

**TABLE E1**
**Hematology, Clinical Chemistry, and Thyroid Hormone Data for Rats in the 3-Month Gavage Study of TCAB**

| | 0 mg/kg | 0.1 mg/kg | 0.3 mg/kg | 1 mg/kg | 3 mg/kg | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|---|---|---|---|
| **Male** *(continued)* | | | | | | | | |
| **Clinical Chemistry (Week 14)** | | | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Urea nitrogen (mg/dL) | 15.0 ± 0.4 | 15.4 ± 0.6 | 15.5 ± 0.9 | 15.3 ± 0.6 | 16.5 ± 0.6 | 15.0 ± 0.4 | 14.3 ± 0.8 | 15.4 ± 0.6 |
| Creatinine (mg/dL) | 0.56 ± 0.02 | 0.60 ± 0.00 | 0.59 ± 0.10 | 0.61 ± 0.03 | 0.59 ± 0.02 | 0.59 ± 0.02 | 0.61 ± 0.01 | 0.57 ± 0.02 |
| Total protein (g/dL) | 7.0 ± 0.1 | 7.1 ± 0.1 | 7.3 ± 0.1 | 7.2 ± 0.1 | 7.5 ± 0.1** | 7.2 ± 0.1 | 7.1 ± 0.1 | 7.1 ± 0.1 |
| Albumin (g/dL) | 4.3 ± 0.0 | 4.4 ± 0.0 | 4.4 ± 0.0 | 4.4 ± 0.1 | 4.5 ± 0.1* | 4.4 ± 0.1 | 4.4 ± 0.1 | 4.4 ± 0.0 |
| Cholesterol (mg/dL) | 110 ± 4 | 120 ± 5 | 122 ± 5 | 115 ± 3 | 132 ± 3** | 138 ± 6** | 147 ± 5** | 144 ± 4** |
| Triglycerides (mg/dL) | 80 ± 6 | 95 ± 7 | 92 ± 7 | 96 ± 9 | 103 ± 15 | 86 ± 9 | 85 ± 6 | 80 ± 7 |
| Alanine aminotransferase (IU/L) | 56 ± 3 | 60 ± 2 | 55 ± 1 | 53 ± 3 | 56 ± 3 | 63 ± 6 | 62 ± 3 | 56 ± 3 |
| Alkaline phosphatase (IU/L) | 201 ± 17 | 253 ± 16 | 240 ± 12 | 199 ± 18 | 253 ± 14* | 305 ± 13** | 347 ± 14** | 348 ± 22** |
| Creatine kinase (IU/L) | 147 ± 31 | 119 ± 28 | 128 ± 29 | 121 ± 25 | 59 ± 4** | 87 ± 10c | 91 ± 17 | 183 ± 61 |
| Sorbitol dehydrogenase (IU/L) | 15 ± 1 | 16 ± 1 | 16 ± 1 | 20 ± 1** | 21 ± 1** | 23 ± 4**c | 20 ± 1** | 22 ± 3** |
| Bile acids (µmol/L) | 37.1 ± 4.9 | 31.6 ± 3.2 | 42.0 ± 4.1 | 31.5 ± 2.9 | 39.7 ± 2.8 | 41.0 ± 3.4 | 43.1 ± 6.0 | 47.6 ± 3.5* |
| **Thyroid Hormone (Week 13)** | | | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Total thyroxine ($T_4$) (µg/dL) | 4.86 ± 0.100 | 1.57 ± 0.150** | 0.72 ± 0.110** | 0.31 ± 0.070** | 0.19 ± 0.040** | 0.15 ± 0.000** | 0.15 ± 0.000** | 0.15 ± 0.000** |
| Free thyroxine ($T_4$) (ng/dL) | 2.59 ± 0.120 | 1.12 ± 0.090** | 0.77 ± 0.060** | 0.37 ± 0.030** | 0.42 ± 0.040** | 0.30 ± 0.040** | 0.18 ± 0.020** | 0.15 ± 0.000** |
| Total triiodothyronine ($T_3$) (ng/dL) | 109.8 ± 3.8 | 96.6 ± 7.5 | 98.2 ± 2.9 | 92.5 ± 5.5* | 87.7 ± 2.7** | 104.3 ± 5.4 | 90.5 ± 4.2* | 100.2 ± 3.6 |
| Thyroid stimulating hormone (TSH) (ng/mL) | 10.3 ± 0.7 | 12.9 ± 1.6 | 11.3 ± 1.1 | 10.9 ± 1.8 | 10.0 ± 1.4 | 11.6 ± 1.6 | 14.5 ± 2.8 | 10.1 ± 1.0 |

**TABLE E1**
**Hematology, Clinical Chemistry, and Thyroid Hormone Data for Rats in the 3-Month Gavage Study of TCAB**

| | 0 mg/kg | 0.1 mg/kg | 0.3 mg/kg | 1 mg/kg | 3 mg/kg | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|---|---|---|---|
| **Female** | | | | | | | | |
| **Hematology (Week 13)** | | | | | | | | |
| n | 8 | 9 | 7 | 9 | 10 | 10 | 10 | 9 |
| Hematocrit (%) | 43.6 ± 0.7 | 44.2 ± 0.7 | 45.0 ± 0.7 | 43.9 ± 0.5 | 43.8 ± 0.3 | 42.3 ± 0.5 | 41.7 ± 0.6 | 42.3 ± 0.5 |
| Hemoglobin (g/dL) | 14.7 ± 0.2 | 14.8 ± 0.2 | 15.1 ± 0.2 | 14.7 ± 0.2 | 14.7 ± 0.1 | 14.2 ± 0.1 | 13.8 ± 0.2* | 14.0 ± 0.2* |
| Erythrocytes ($10^6$/μL) | 7.85 ± 0.13 | 7.97 ± 0.14 | 8.01 ± 0.13 | 7.95 ± 0.13 | 7.80 ± 0.07 | 7.66 ± 0.10 | 7.37 ± 0.12 | 7.64 ± 0.11 |
| Reticulocytes ($10^6$/μL) | 1.2 ± 0.1 | 1.4 ± 0.1 | 1.3 ± 0.1 | 1.4 ± 0.2 | 1.2 ± 0.1 | 1.2 ± 0.1 | 1.5 ± 0.1 | 1.5 ± 0.1 |
| Nucleated erythrocytes (%) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Mean cell volume (fL) | 55.6 ± 0.5 | 55.5 ± 0.5 | 56.2 ± 0.7 | 55.2 ± 0.4 | 56.2 ± 0.3 | 55.3 ± 0.3 | 56.6 ± 0.4 | 55.3 ± 0.4 |
| Mean cell hemoglobin (pg) | 18.8 ± 0.2 | 18.6 ± 0.2 | 18.9 ± 0.2 | 18.6 ± 0.2 | 18.8 ± 0.1 | 18.6 ± 0.2 | 18.7 ± 0.1 | 18.2 ± 0.2 |
| Mean cell hemoglobin concentration (g/dL) | 33.8 ± 0.1 | 33.5 ± 0.2 | 33.6 ± 0.1 | 33.7 ± 0.2 | 33.4 ± 0.1 | 33.6 ± 0.1 | 33.1 ± 0.1** | 33.0 ± 0.1** |
| Platelets ($10^3$/μL) | 784.4 ± 89.2 | 802.1 ± 52.9 | 730.3 ± 48.7 | 745.0 ± 44.3 | 675.9 ± 43.1 | 732.6 ± 48.7 | 758.2 ± 32.5 | 673.7 ± 47.7 |
| Leukocytes ($10^3$/μL) | 10.06 ± 0.83 | 9.27 ± 0.58 | 9.70 ± 0.69 | 8.98 ± 0.55 | 8.75 ± 0.64 | 8.29 ± 0.42 | 10.01 ± 0.37 | 12.03 ± 0.72 |
| Segmented neutrophils ($10^3$/μL) | 1.64 ± 0.24 | 0.93 ± 0.18 | 0.81 ± 0.09 | 1.14 ± 0.22 | 0.82 ± 0.12* | 0.89 ± 0.14 | 1.00 ± 0.15 | 1.56 ± 0.18 |
| Bands ($10^3$/μL) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Lymphocytes ($10^3$/μL) | 8.05 ± 0.53 | 8.14 ± 0.53 | 8.76 ± 0.70 | 7.72 ± 0.40 | 7.75 ± 0.54 | 7.25 ± 0.34 | 8.82 ± 0.37 | 10.25 ± 0.52 |
| Monocytes ($10^3$/μL) | 0.17 ± 0.07 | 0.14 ± 0.05 | 0.06 ± 0.03 | 0.09 ± 0.02 | 0.16 ± 0.04 | 0.13 ± 0.03 | 0.18 ± 0.04 | 0.21 ± 0.07 |
| Basophils ($10^3$/μL) | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 |
| Eosinophils ($10^3$/μL) | 0.20 ± 0.11 | 0.06 ± 0.02 | 0.06 ± 0.02 | 0.04 ± 0.02 | 0.02 ± 0.01* | 0.02 ± 0.01* | 0.01 ± 0.01** | 0.02 ± 0.02* |
| Methemoglobin (g/dL) | 0.48 ± 0.01[b] | 0.30 ± 0.00**[b] | 0.30 ± 0.00**[b] | 0.50 ± 0.10[b] | 0.49 ± 0.01 | 0.30 ± 0.00**[c] | 0.27 ± 0.02** | 0.50 ± 0.00[b] |
| Osmotic fragility (%) | 0.285 ± 0.016[b] | 0.126 ± 0.010** | 0.285 ± 0.013[c] | 0.296 ± 0.020[b] | 0.267 ± 0.015 | 0.252 ± 0.016[c] | 0.196 ± 0.015* | 0.314 ± 0.013[b] |

**TABLE E1**
**Hematology, Clinical Chemistry, and Thyroid Hormone Data for Rats in the 3-Month Gavage Study of TCAB**

| | 0 mg/kg | 0.1 mg/kg | 0.3 mg/kg | 1 mg/kg | 3 mg/kg | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|---|---|---|---|
| **Female** *(continued)* | | | | | | | | |
| **Clinical Chemistry (Week 14)** | | | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Urea nitrogen (mg/dL) | 18.5 ± 0.8 | 17.5 ± 0.6 | 18.2 ± 0.7 | 17.5 ± 0.6 | 15.9 ± 0.9 | 16.8 ± 0.8 | 22.5 ± 1.7 | 19.5 ± 1.2 |
| Creatinine (mg/dL) | 0.73 ± 0.02 | 0.76 ± 0.02 | 0.71 ± 0.01 | 0.71 ± 0.02 | 0.73 ± 0.02 | 0.75 ± 0.02 | 0.79 ± 0.02 | 0.73 ± 0.02 |
| Total protein (g/dL) | 7.3 ± 0.1 | 7.3 ± 0.2 | 7.3 ± 0.1 | 7.6 ± 0.1 | 7.4 ± 0.1 | 7.7 ± 0.1* | 7.6 ± 0.1* | 7.7 ± 0.1* |
| Albumin (g/dL) | 4.9 ± 0.1 | 4.8 ± 0.1 | 4.9 ± 0.1 | 5.1 ± 0.1 | 5.0 ± 0.1 | 5.1 ± 0.1 | 5.1 ± 0.1 | 5.1 ± 0.1 |
| Cholesterol (mg/dL) | 101 ± 5 | 102 ± 5 | 112 ± 4 | 118 ± 5 | 110 ± 5 | 130 ± 5** | 146 ± 7** | 166 ± 6** |
| Triglycerides (mg/dL) | 51 ± 6 | 49 ± 4 | 54 ± 4 | 60 ± 5 | 50 ± 6 | 58 ± 7 | 57 ± 4 | 69 ± 6 |
| Alanine aminotransferase (IU/L) | 56 ± 4 | 52 ± 3 | 61 ± 3 | 54 ± 3 | 53 ± 4 | 52 ± 2 | 55 ± 4 | 58 ± 4 |
| Alkaline phosphatase (IU/L) | 165 ± 19 | 176 ± 16 | 183 ± 12 | 182 ± 8 | 195 ± 15 | 245 ± 12** | 247 ± 22** | 240 ± 19** |
| Creatine kinase (IU/L) | 291 ± 82 | 336 ± 65 | 180 ± 38 | 128 ± 24 | 202 ± 45 | 222 ± 50 | 126 ± 25 | 166 ± 39 |
| Sorbitol dehydrogenase (IU/L) | 16 ± 1 | 18 ± 1 | 18 ± 0 | 15 ± 1 | 18 ± 1 | 18 ± 1 | 20 ± 1* | 18 ± 1 |
| Bile acids (µmol/L) | 38.1 ± 5.2 | 37.2 ± 6.5 | 42.9 ± 6.4 | 43.0 ± 6.0 | 38.3 ± 7.1 | 45.3 ± 6.4 | 44.4 ± 6.3 | 61.1 ± 5.4 |
| **Thyroid Hormone (Week 13)** | | | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Total thyroxine ($T_4$) (µg/dL) | 5.25 ± 0.280 | 3.28 ± 0.240** | 1.68 ± 0.180** | 1.40 ± 0.120** | 1.23 ± 0.080** | 0.71 ± 0.060** | 0.51 ± 0.070** | 0.48 ± 0.080** |
| Free thyroxine ($T_4$) (ng/dL) | 2.18 ± 0.140 | 1.22 ± 0.100** | 0.65 ± 0.060** | 0.58 ± 0.030** | 0.39 ± 0.030** | 0.21 ± 0.030** | 0.17 ± 0.020** | 0.33 ± 0.030** |
| Total triiodothyronine ($T_3$) (ng/dL) | 95.7 ± 4.3 | 108.5 ± 6.3 | 63.1 ± 10.3* | 79.1 ± 5.5 | 64.0 ± 4.9** | 74.3 ± 6.2 | 89.3 ± 5.3 | 76.5 ± 7.6 |
| Thyroid stimulating hormone (TSH) (ng/mL) | 9.4 ± 1.0 | 8.7 ± 0.6 | 7.7 ± 0.9 | 10.6 ± 1.4 | 10.6 ± 0.9 | 8.6 ± 0.9 | 8.3 ± 0.6 | 8.4 ± 0.7 |

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n = 10
[c] n = 9
[d] n = 7
[e] n = 8

# APPENDIX F
# ORGAN WEIGHTS AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

**TABLE F1**
**Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of TCAB[a]**

| | 0 mg/kg | 0.1 mg/kg | 0.3 mg/kg | 1 mg/kg | 3 mg/kg | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Male** | | | | | | | | |
| Necropsy body wt | 421 ± 8 | 443 ± 4 | 413 ± 6 | 422 ± 5 | 416 ± 5 | 411 ± 8 | 404 ± 8 | 414 ± 7 |
| Heart | | | | | | | | |
| Absolute | 1.49 ± 0.03 | 1.54 ± 0.02 | 1.45 ± 0.02 | 1.48 ± 0.04 | 1.45 ± 0.02 | 1.49 ± 0.04 | 1.54 ± 0.03 | 1.57 ± 0.04 |
| Relative | 3.528 ± 0.062 | 3.474 ± 0.047 | 3.513 ± 0.029 | 3.497 ± 0.067 | 3.497 ± 0.050 | 3.623 ± 0.043 | 3.813 ± 0.066** | 3.788 ± 0.085** |
| R. Kidney | | | | | | | | |
| Absolute | 1.18 ± 0.02 | 1.32 ± 0.02* | 1.26 ± 0.02* | 1.23 ± 0.02* | 1.24 ± 0.02* | 1.30 ± 0.04** | 1.34 ± 0.02** | 1.32 ± 0.04** |
| Relative | 2.814 ± 0.054 | 2.992 ± 0.057* | 3.056 ± 0.034* | 2.907 ± 0.059 | 2.985 ± 0.043 | 3.160 ± 0.053** | 3.334 ± 0.080** | 3.186 ± 0.074** |
| Liver | | | | | | | | |
| Absolute | 13.44 ± 0.38 | 15.59 ± 0.34* | 14.17 ± 0.28* | 14.57 ± 0.29* | 14.90 ± 0.31* | 16.79 ± 0.57** | 17.87 ± 0.48** | 18.97 ± 0.70** |
| Relative | 31.898 ± 0.689 | 35.232 ± 0.739** | 34.264 ± 0.458** | 34.502 ± 0.386* | 35.811 ± 0.537** | 40.742 ± 0.731** | 44.202 ± 0.684** | 45.687 ± 1.250** |
| Lung | | | | | | | | |
| Absolute | 2.27 ± 0.12 | 2.12 ± 0.09 | 2.04 ± 0.07 | 1.90 ± 0.03 | 2.08 ± 0.04 | 2.30 ± 0.09 | 2.50 ± 0.14 | 2.80 ± 0.09** |
| Relative | 5.369 ± 0.216 | 4.791 ± 0.200 | 4.933 ± 0.169 | 4.499 ± 0.054 | 5.015 ± 0.103 | 5.592 ± 0.197 | 6.184 ± 0.285** | 6.768 ± 0.273** |
| Spleen | | | | | | | | |
| Absolute | 0.695 ± 0.013 | 0.831 ± 0.021* | 0.740 ± 0.022* | 0.816 ± 0.037** | 0.790 ± 0.026** | 0.843 ± 0.018** | 0.851 ± 0.019** | 0.830 ± 0.035** |
| Relative | 1.650 ± 0.026 | 1.878 ± 0.046* | 1.794 ± 0.059* | 1.931 ± 0.074** | 1.899 ± 0.049** | 2.050 ± 0.025** | 2.106 ± 0.034** | 2.005 ± 0.084** |
| L. Testis | | | | | | | | |
| Absolute | 1.984 ± 0.034 | 1.979 ± 0.031 | 2.097 ± 0.034 | 2.003 ± 0.032 | 2.029 ± 0.066 | 2.018 ± 0.030 | 2.010 ± 0.050 | 1.968 ± 0.063 |
| Relative | 4.725 ± 0.127 | 4.473 ± 0.080 | 5.081 ± 0.102 | 4.747 ± 0.060 | 4.885 ± 0.165 | 4.918 ± 0.100 | 4.986 ± 0.143 | 4.745 ± 0.114 |
| Thymus | | | | | | | | |
| Absolute | 0.417 ± 0.025 | 0.383 ± 0.025 | 0.333 ± 0.020 | 0.441 ± 0.015 | 0.397 ± 0.009 | 0.350 ± 0.021* | 0.314 ± 0.013** | 0.359 ± 0.018** |
| Relative | 0.992 ± 0.059 | 0.862 ± 0.052 | 0.807 ± 0.049* | 1.047 ± 0.037 | 0.956 ± 0.026 | 0.848 ± 0.049 | 0.779 ± 0.034** | 0.865 ± 0.035 |
| Thyroid gland | | | | | | | | |
| Absolute | 0.030 ± 0.003 | 0.024 ± 0.001 | 0.027 ± 0.001 | 0.028 ± 0.001 | 0.025 ± 0.002 | 0.029 ± 0.001 | 0.028 ± 0.001 | 0.030 ± 0.001 |
| Relative | 0.070 ± 0.006 | 0.055 ± 0.003 | 0.065 ± 0.003 | 0.066 ± 0.002 | 0.059 ± 0.004 | 0.070 ± 0.003 | 0.069 ± 0.003 | 0.073 ± 0.002 |

**TABLE F1**
**Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of TCAB[a]**

| | 0 mg/kg | 0.1 mg/kg | 0.3 mg/kg | 1 mg/kg | 3 mg/kg | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|---|---|---|---|
| n | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Female** | | | | | | | | |
| Necropsy body wt | 285 ± 2 | 275 ± 4 | 276 ± 4 | 274 ± 4 | 274 ± 5 | 271 ± 5* | 263 ± 4** | 270 ± 3** |
| Heart | | | | | | | | |
| Absolute | 1.01 ± 0.02 | 1.03 ± 0.03 | 1.05 ± 0.01 | 1.05 ± 0.02 | 1.06 ± 0.02 | 1.07 ± 0.04 | 1.07 ± 0.02 | 1.09 ± 0.03 |
| Relative | 3.552 ± 0.085 | 3.765 ± 0.075 | 3.814 ± 0.057* | 3.827 ± 0.049* | 3.863 ± 0.048** | 3.942 ± 0.109** | 4.054 ± 0.071** | 4.053 ± 0.102** |
| R. Kidney | | | | | | | | |
| Absolute | 0.83 ± 0.02 | 0.79 ± 0.01 | 0.82 ± 0.02 | 0.82 ± 0.02 | 0.86 ± 0.02 | 0.84 ± 0.02 | 0.88 ± 0.01 | 0.87 ± 0.02 |
| Relative | 2.926 ± 0.057 | 2.862 ± 0.034 | 2.959 ± 0.071 | 3.006 ± 0.049 | 3.135 ± 0.054* | 3.108 ± 0.086* | 3.332 ± 0.057** | 3.220 ± 0.073** |
| Liver | | | | | | | | |
| Absolute | 9.01 ± 0.25 | 8.72 ± 0.28 | 9.95 ± 0.36 | 9.40 ± 0.21 | 9.73 ± 0.32 | 10.60 ± 0.24** | 11.17 ± 0.27** | 11.93 ± 0.22** |
| Relative | 31.596 ± 0.805 | 31.756 ± 0.879 | 35.974 ± 1.038** | 34.331 ± 0.528** | 35.500 ± 0.650** | 39.158 ± 0.617** | 42.395 ± 0.713** | 44.262 ± 0.879** |
| Lung | | | | | | | | |
| Absolute | 1.59 ± 0.04 | 1.71 ± 0.06 | 1.60 ± 0.04 | 1.70 ± 0.04 | 1.86 ± 0.12* | 1.75 ± 0.06* | 1.99 ± 0.11** | 2.07 ± 0.06** |
| Relative | 5.567 ± 0.154 | 6.215 ± 0.199 | 5.775 ± 0.099 | 6.209 ± 0.137 | 6.756 ± 0.343** | 6.484 ± 0.256** | 7.556 ± 0.399** | 7.660 ± 0.183** |
| L. Ovary | | | | | | | | |
| Absolute | 0.062 ± 0.003 | 0.062 ± 0.002 | 0.059 ± 0.003 | 0.069 ± 0.004 | 0.061 ± 0.004 | 0.056 ± 0.002 | 0.057 ± 0.003 | 0.060 ± 0.001 |
| Relative | 0.217 ± 0.012 | 0.227 ± 0.007 | 0.213 ± 0.010 | 0.253 ± 0.011 | 0.223 ± 0.011 | 0.206 ± 0.009 | 0.218 ± 0.009 | 0.223 ± 0.007 |
| Spleen | | | | | | | | |
| Absolute | 0.645 ± 0.025 | 0.660 ± 0.035 | 0.623 ± 0.019 | 0.660 ± 0.021 | 0.653 ± 0.043 | 0.662 ± 0.026 | 0.659 ± 0.032 | 0.657 ± 0.13 |
| Relative | 2.261 ± 0.086 | 2.410 ± 0.142 | 2.254 ± 0.054 | 2.406 ± 0.052 | 2.384 ± 0.142 | 2.439 ± 0.055 | 2.499 ± 0.100 | 2.438 ± 0.049 |
| Thymus | | | | | | | | |
| Absolute | 0.334 ± 0.010 | 0.312 ± 0.015 | 0.296 ± 0.018 | 0.273 ± 0.016* | 0.246 ± 0.021** | 0.276 ± 0.019** | 0.258 ± 0.019** | 0.267 ± 0.012** |
| Relative | 1.170 ± 0.036 | 1.135 ± 0.048 | 1.071 ± 0.061 | 0.992 ± 0.051* | 0.900 ± 0.076* | 1.015 ± 0.061* | 0.977 ± 0.067* | 0.994 ± 0.049* |
| Thyroid gland | | | | | | | | |
| Absolute | 0.020 ± 0.001 | 0.020 ± 0.001 | 0.023 ± 0.002 | 0.023 ± 0.002 | 0.020 ± 0.001 | 0.020 ± 0.001 | 0.020 ± 0.001 | 0.021 ± 0.001 |
| Relative | 0.070 ± 0.004 | 0.072 ± 0.003 | 0.083 ± 0.007 | 0.084 ± 0.008 | 0.072 ± 0.005 | 0.075 ± 0.003 | 0.077 ± 0.004 | 0.076 ± 0.005 |

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

# APPENDIX G
# CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

# CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

## Procurement and Characterization

### *TCAB*

TCAB was obtained from AccuStandard (New Haven, CT) in two lots (110199MT-AC-1 and 10009-52-01 RTI). Lot 110199MT-AC-1 was used in the 3-month study and lot 10009-52-01 RTI was used in the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Research Triangle Institute (Research Triangle Park, NC) and the study laboratory at Battelle Columbus Operations (Columbus, OH); the analytical chemistry laboratory also conducted stability analyses. Melting point determination, elemental analyses, and Karl Fischer titration were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the TCAB studies are on file at the National Institute of Environmental Health Sciences.

Both lots of the chemical, a fine, bright orange powder, were identified as TCAB by the analytical chemistry laboratory using infrared (IR) and proton nuclear magnetic resonance (NMR) spectroscopy; in addition, lot 10009-52-01 RTI was identified as TCAB using gas chromatography (GC) coupled with mass spectrometry (MS). Both lots of the chemical were identified as TCAB by the study laboratory using IR spectroscopy. All spectra were consistent with the structure of TCAB and reference spectra of the same lots; GC/MS spectra matched a literature spectrum (NIST, 2000). Representative IR and NMR spectra are presented in Figures G1 and G2. The melting point of lot 10009-52-01 RTI was 160.3° C to 160.6° C, which is consistent with the literature value of 158° C (Hsia and Burant, 1979).

For lot 10009-52-01 RTI, moisture content and elemental analysis were determined prior to the start of the 2-year studies. The analytical chemistry laboratory determined the purity of lot 110199MT-AC-1 using GC by system A and the purity of lot 10009-52-01 RTI by systems B and C (Table G1).

For lot 110199MT-AC-1, GC by system A indicated one major peak and one minor impurity with a peak area accounting for approximately 0.1% of the total peak area. GC/MS was used by the analytical chemistry laboratory in an attempt to confirm the presence and identity of the impurities using total ion and selected ion methods. Total ion chromatograms indicated no single impurity greater than or equal to 0.1% of the total peak area. Selected ion monitoring was used to look for TCDD, tetrachlorodibenzofuran, and several polychlorinated biphenyls (PCBs); none were detected. The overall purity of lot 110199MT-AC-1 was determined to be 99.7% or greater.

For lot 10009-52-01 RTI, Karl Fischer titration indicated a water content of 255 ppm and elemental analyses for carbon, hydrogen, nitrogen, and chlorine were in agreement with the theoretical values for TCAB. GC by both systems B and C indicated one major peak and 3 minor impurities, each with a peak area less than 0.1% of the total peak area. The overall purity of lot 10009-52-01 RTI was determined to be 99.8% or greater.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC by system D. These studies indicated that TCAB was stable as a bulk chemical for 2 weeks when stored in sealed amber glass vials at temperatures up to 25° C. To ensure stability of the bulk chemical, lot 110199MT-AC-1 was stored at approximately 5° C in amber glass bottles. Lot 10009-52-01 RTI was stored in a –20° C freezer in clear glass bottles to decrease the possibility of reagglomeration of TCAB particles. Periodic reanalyses of the bulk chemical were performed by the study laboratory during the 3-month and 2-year studies using system E, and no degradation of the bulk chemical was detected.

### *Formulation Materials*

Acetone was used with corn oil as the vehicle in all studies. For the 3-month study, one lot of USP-grade acetone was obtained from Spectrum Quality Products (Gardena, CA) and for the 2-year studies, one lot was obtained from Spectrum Quality Products and two lots from EM Science (Gibbstown, NJ). The identity of each lot was confirmed by the study laboratory using IR spectroscopy prior to its use. Acetone purity was determined prior to the start of each study and at intervals of no more than 6 months thereafter using GC by system F. All acetone lots had a purity greater than 99.9% except one lot that had a single impurity of 0.19% relative to the major peak. USP-grade corn oil for all studies was obtained from Spectrum Quality Products; periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations less than 3 mEq/kg.

## Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing TCAB with corn oil containing 1% acetone to give the required concentrations (Table G2). The dose formulations were stored at room temperature in clear glass bottles with screw-cap Teflon®-lined lids for up to 43 days.

Homogeneity studies of a 40 mg/mL dose formulation and stability studies of a 0.01 mg/mL dose formulation were conducted by the analytical chemistry laboratory on a lot of TCAB not used for dosing; samples were analyzed using GC by a system similar to system E (Table G1). Homogeneity studies of 3, 4, and 40 mg/mL dose formulations were conducted by the study laboratory prior to the start of the 3-month and 2-year studies and during the 2-year studies when it became necessary to increase the volume prepared for the formulations; these formulations were all analyzed using GC by system E. Homogeneity was confirmed and stability was confirmed for at least 43 days for dose formulations stored in sealed amber glass containers at 5° C and room temperature and for up to 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of TCAB were conducted by the study laboratory using GC by system E. During the 3-month study, the dose formulations were analyzed at the beginning, midpoint, and end of the study; animal room samples of these dose formulations were also analyzed (Table G3). Of the dose formulations analyzed, all 21 were within 10% of the target concentrations; all 21 of the animal room samples were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed at least every 3 months; all 60 of the dose formulations analyzed and used for rats (Table G4) and all 36 for mice (Table G5) were within 10% of the target concentrations. Animal room samples were also analyzed; 11 of 12 for rats and all 12 for mice were within 10% of the target concentrations.

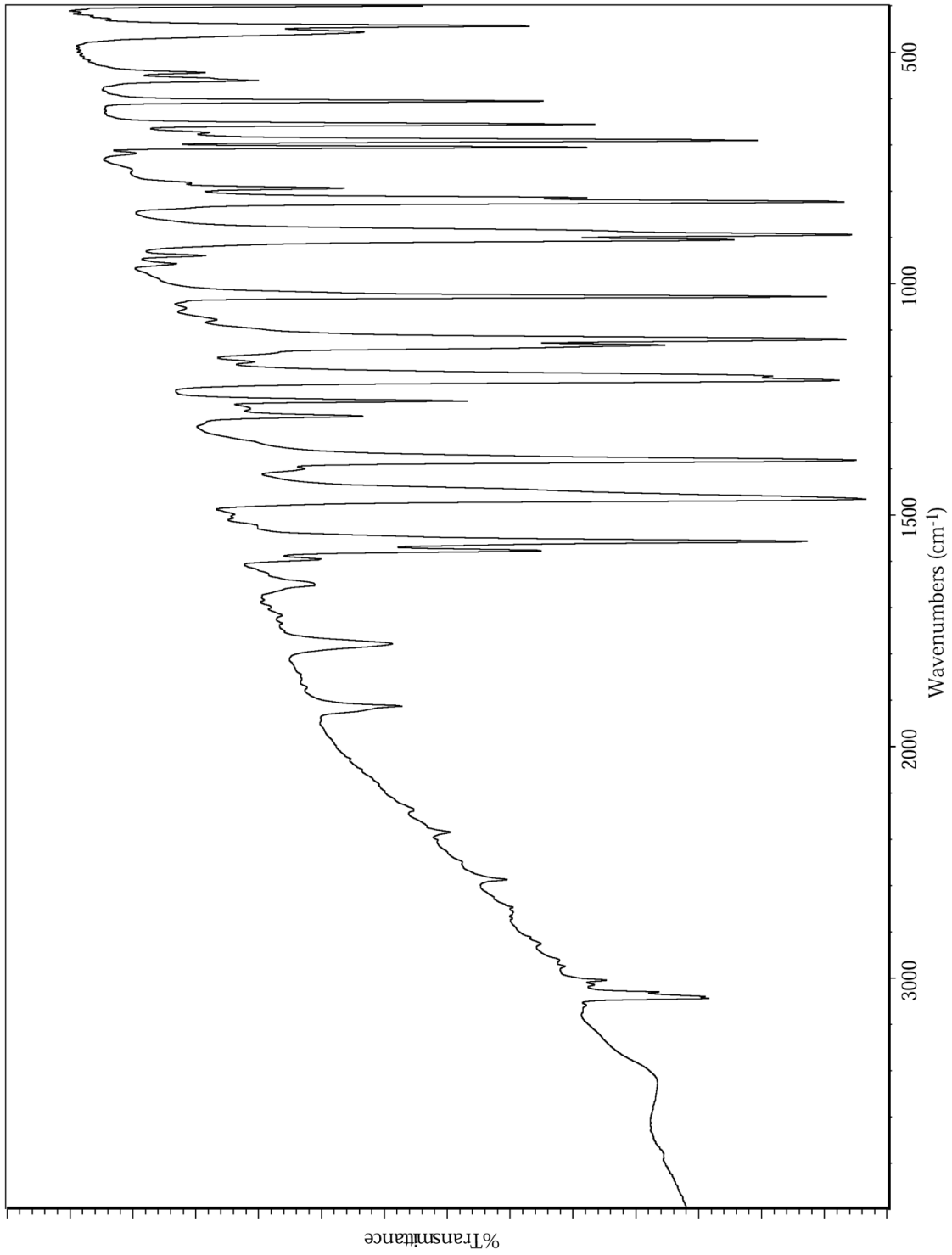


**FIGURE G1**
**Infrared Absorption Spectrum of TCAB**

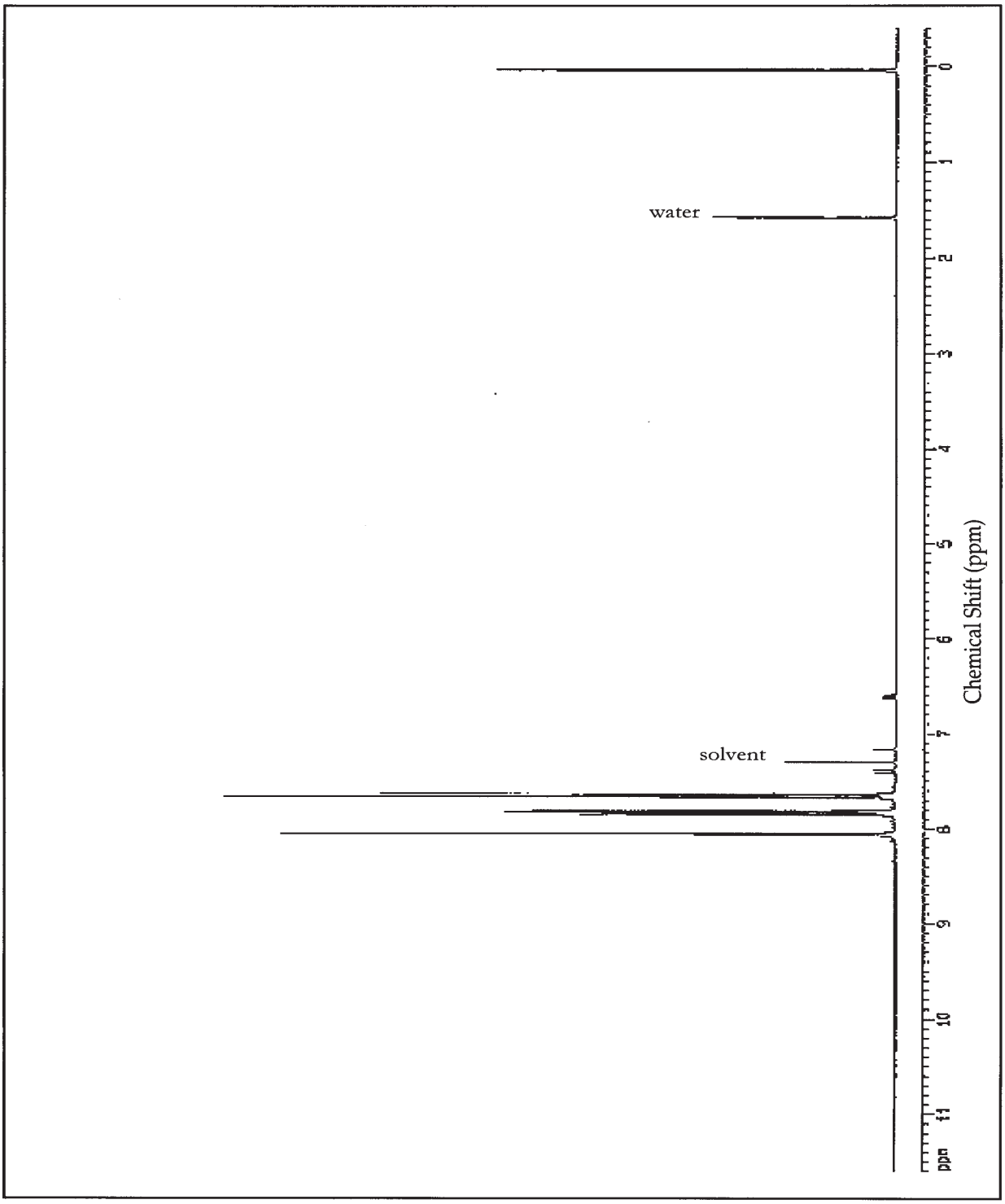


**FIGURE G2**
**Proton Nuclear Magnetic Resonance Spectrum of TCAB**

**TABLE G1**
**Gas Chromatography Systems Used in the Gavage Studies of TCAB[a]**

| Detection System | Column | Carrier Gas | Oven Temperature Program |
|---|---|---|---|
| **System A** | | | |
| Flame ionization | J&W DB-5 MS,<br>30 m × 0.32 mm, 0.5-µm film thickness<br>(J&W Scientific, Folsom, CA) | Helium at 1 mL/minute | 100° C to 300° C at 10° C/minute,<br>then held for 15 minutes |
| **System B** | | | |
| Flame ionization | Rtx-5,<br>30 m × 0.32 mm, 1.0-µm film thickness<br>(Restek, Bellefonte, PA) | Nitrogen at 1 mL/minute | 250° C for 5 minutes,<br>then 10° C/minute to 300° C,<br>then held for 30 minutes |
| **System C** | | | |
| Flame ionization | J&W DB-17,<br>30 m × 0.25 mm, 0.25-µm film thickness<br>(J&W Scientific) | Nitrogen at 1 mL/minute | 200° C to 260° C at 10° C/minute,<br>then held for 34 minutes |
| **System D** | | | |
| Electron capture | Rtx-5,<br>30 m × 0.32 mm, 1.0-µm film thickness<br>(Restek) | Nitrogen at 1 mL/minute | 220° C to 300° C at 10° C/minute,<br>then held for 12 minutes |
| **System E** | | | |
| Electron capture | Rtx-5,<br>30 m × 0.32 mm, 0.5- or 1.0-µm film thickness<br>(Restek) | Helium at 1.5 mL/minute | 220° C to 300° C at 5° C/minute,<br>then held for 4 minutes |
| **System F** | | | |
| Flame ionization | Supelcowax-10,<br>30 m × 0.53 mm, 0.5-µm film thickness<br>(Supelco, Inc., Bellefonte, PA) | Helium at 10 mL/minute | 40° C for 5 minutes,<br>then 10° C/minute to 220° C |

[a] The gas chromatographs were manufactured by Hewlett-Packard (Palo Alto, CA) (systems A, B, C, D, and F) or Agilent (Palo Alto, CA) (system E).

## TABLE G2
## Preparation and Storage of Dose Formulations in the Gavage Studies of TCAB

| | 3-Month Study | 2-Year Studies |
|---|---|---|
| **Preparation** | For the 0.04 mg/mL dose formulation, a 4.0 mg/mL stock solution was prepared by dissolving the specified amount of TCAB in acetone in a volumetric flask that was capped, shaken thoroughly, vortexed for approximately 2 minutes, sonicated, and inverted at least 10 times. A specified amount of this stock solution was added to a calibrated glass mixing bottle that was half-filled with corn oil, and the bottle was capped and vigorously hand shaken. The bottle was filled to volume with corn oil (diluting the stock solution 1:100), capped, shaken vigorously by hand, and shaken on a paint shaker for approximately 15 minutes. The 0.04 mg/mL dose formulation was transferred into clear glass screw-cap storage bottles that contained stir bars for dose administration and were capped with Teflon®-lined lids.<br><br>For the 0.12, 0.4, and 1.2 mg/mL dose formulations, the appropriate amount of TCAB was ground into a small amount of corn oil in a mortar to create a paste. The paste and several corn oil rinses of the mortar and pestle were transferred into a calibrated glass mixing bottle half-filled with corn oil, and the specified amount of acetone was added. The bottle was capped, hand shaken, filled to volume with corn oil, recapped, hand shaken, and shaken on a paint shaker for approximately 15 minutes. The 0.12, 0.4, and 1.2 mg/mL dose formulations were transferred into clear glass screw-cap storage bottles that contained stir bars and were capped with Teflon®-lined lids.<br><br>For the 4.0, 12, and 40 mg/mL dose formulations, a ground paste of TCAB and corn oil was prepared as above, but the paste and corn oil rinses were transferred into a glass beaker half-filled with corn oil. The beaker was filled to volume with corn oil, and the contents were stirred with a vigorous vortex for approximately 15 minutes using an overhead stirrer. Specified volumes of the TCAB suspensions and acetone were transferred into clear glass screw-cap storage bottles that contained stir bars and were capped with Teflon®-lined lids, and the resultant dose formulations were thoroughly shaken.<br><br>The dose formulations were prepared approximately monthly. | For the 0.3 and 1 mg/mL dose formulations prepared through September 23, 2003, the appropriate amounts of TCAB (including several corn oil rinses of the weighing container) and acetone were transferred to a calibrated glass mixing bottle half-filled with corn oil, and the bottle was capped and vigorously hand shaken, filled to volume with corn oil, recapped, hand shaken, and shaken on a paint shaker for at least 15 minutes. When these dose formulations were prepared on October 29, 2003, the glass mixing bottles were shaken on a paint shaker for 15 minutes, followed by sonication of the contents for approximately 15 minutes and subsequent shaking on a paint shaker for approximately 5 minutes. When these dose formulations were prepared after October 29, 2003, vigorous hand shaking (approximately 2 minutes) of the mixing bottle was substituted for mixing on the paint shaker. Aliquots of these dose formulations were transferred into clear glass screw-cap storage bottles that contained stir bars for dose administration and were capped with Teflon®-lined lids.<br><br>The 3, 4, 12, and 40 mg/mL dose formulations were prepared by transferring the appropriate amount of TCAB and several corn oil rinses of the weighing container to a glass beaker, filling the beaker to volume with corn oil, and mixing the contents with a vigorous vortex for approximately 15 minutes using an overhead stirrer. Appropriate amounts of acetone were added to these TCAB suspensions after they were transferred to clear glass screw-cap storage bottles; the storage bottles contained stir bars, were capped with Teflon®-lined lids, and were thoroughly shaken to mix the contents. Beginning September 23, 2003 (rats) and October 29, 2003 (mice), the beaker contents were stirred for 15 minutes, sonicated for approximately 15 minutes, and stirred for an additional 5 minutes using an overhead stirrer prior to transfer to the glass storage bottles, the addition of acetone, and thorough shaking.<br><br>The dose formulations were prepared approximately every 4 weeks. |
| **Chemical Lot Number** | 110199MT-AC-1 | 10009-52-01 RTI |
| **Maximum Storage Time** | 43 days | 43 days |
| **Storage Conditions** | Stored in clear glass screw-cap bottles with Teflon®-lined lids at room temperature. | Stored in clear glass screw-cap bottles with Teflon®-lined lids at room temperature. |
| **Study Laboratory** | Battelle Columbus Operations (Columbus, OH) | Battelle Columbus Operations (Columbus, OH) |

**TABLE G3**
**Results of Analyses of Dose Formulations Administered to Rats in the 3-Month Gavage Study of TCAB**

| Date Prepared | Date Analyzed | Target Concentration (mg/mL) | Determined Concentration[a] (mg/mL) | Difference from Target (%) |
|---|---|---|---|---|
| December 4, 2000 | December 5-6, 2000 | 0.04 | 0.04163 | +4 |
| | | 0.12 | 0.1164 | –3 |
| | | 0.4 | 0.3972 | –1 |
| | | 1.2 | 1.190 | –1 |
| | | 12 | 12.54 | +5 |
| | | 40 | 41.34 | +3 |
| | January 15-16, 2001[b] | 0.04 | 0.03832 | –4 |
| | | 0.12 | 0.1147 | –4 |
| | | 0.4 | 0.3804 | –5 |
| | | 1.2 | 1.156 | –4 |
| | | 12 | 11.25 | –6 |
| | | 40 | 40.89 | +2 |
| December 5, 2000 | December 5-6, 2000 | 4 | 4.293 | +7 |
| | January 15-16, 2001[b] | 4 | 3.969 | –1 |
| January 29, 2001 | February 1, 2001 | 0.04 | 0.03900 | –3 |
| | | 0.12 | 0.1119 | –7 |
| | | 0.4 | 0.3899 | –3 |
| | | 1.2 | 1.176 | –2 |
| | | 4 | 3.952 | –1 |
| | | 12 | 12.18 | +2 |
| | | 40 | 38.39 | –4 |
| | March 8, 2001[b] | 0.04 | 0.03984 | 0 |
| | | 0.12 | 0.1202 | 0 |
| | | 0.4 | 0.4089 | +2 |
| | | 1.2 | 1.206 | +1 |
| | | 4 | 4.158 | +4 |
| | | 12 | 12.54 | +5 |
| | | 40 | 40.36 | +1 |
| February 15, 2001 | February 19-20, 2001 | 0.04 | 0.03870 | –3 |
| | | 0.12 | 0.1171 | –2 |
| | | 0.4 | 0.3832 | –4 |
| | | 1.2 | 1.178 | –2 |
| | | 4 | 3.831 | –4 |
| | | 12 | 12.34 | +3 |
| | | 40 | 39.83 | 0 |
| | March 28-29, 2001[b] | 0.04 | 0.03865 | –3 |
| | | 0.12 | 0.1179 | –2 |
| | | 0.4 | 0.3923 | –2 |
| | | 1.2 | 1.194 | –1 |
| | | 4 | 4.040 | +1 |
| | | 12 | 11.05 | –8 |
| | | 40 | 41.29 | +3 |

[a] Results of duplicate analyses. Dosing volume = 2.5 mL/kg; 0.04 mg/mL = 0.1 mg/kg, 0.12 mg/mL = 0.3 mg/kg, 0.4 mg/mL = 1 mg/kg, 1.2 mg/mL = 3 mg/kg, 4.0 mg/mL = 10 mg/kg, 12 mg/mL = 30 mg/kg, 40 mg/mL = 100 mg/kg
[b] Animal room samples

**TABLE G4**
**Results of Analyses of Dose Formulations Administered to Rats in the 2-Year Gavage Study of TCAB**

| Date Prepared | Date Analyzed | Target Concentration (mg/mL) | Determined Concentration[a] (mg/mL) | Difference from Target (%) |
|---|---|---|---|---|
| January 17, 2003 | January 21-23, 2003 | 4 | 4.164[b] | +4 |
| | | 12 | 12.57 | +5 |
| | | 40 | 38.68 | –3 |
| | March 3-4, 2003[c] | 4 | 3.951 | –1 |
| | | 12 | 12.31 | +3 |
| | | 40 | 41.23 | +3 |
| April 1, 2003 | April 1-3, 2003 | 4 | 4.131 | +3 |
| | | 12 | 12.17 | +1 |
| | | 40 | 39.36 | –2 |
| June 10, 2003 | June 12, 2003 | 4 | 4.172 | +4 |
| | | 12 | 13.18 | +10 |
| | | 40 | 42.74 | +7 |
| August 19, 2003 | August 21-22, 2003 | 4 | 4.134 | +3 |
| | | 12 | 13.14 | +10 |
| | | 40 | 42.03 | +5 |
| | October 10-11, 2003[c] | 4 | 3.888 | –3 |
| | | 12 | 11.99 | 0 |
| | | 40 | 38.66 | –3 |
| September 23, 2003 | September 30-October 1, 2003 | 4 | 3.890 | –3 |
| | | 4 | 3.951 | –1 |
| | | 12 | 12.03 | 0 |
| | | 12 | 11.91 | –1 |
| | | 40 | 39.78 | –1 |
| | | 40 | 31.60[d] | –21 |
| October 8, 2003 | October 11, 2003 | 40 | 39.99[e] | 0 |
| October 29, 2003 | October 29-30, 2003 | 4 | 4.283 | +7 |
| | | 4 | 4.003 | 0 |
| | | 12 | 12.29 | +2 |
| | | 12 | 12.29 | +2 |
| | | 40 | 40.68 | +2 |
| | | 40 | 40.83 | +2 |
| January 7, 2004 | January 9-11, 2004 | 4 | 4.168 | +4 |
| | | 4 | 4.203 | +5 |
| | | 12 | 12.90 | +8 |
| | | 12 | 12.75 | +6 |
| | | 40 | 42.85 | +7 |
| | | 40 | 41.89 | +5 |

**TABLE G4**
**Results of Analyses of Dose Formulations Administered to Rats in the 2-Year Gavage Study of TCAB**

| Date Prepared | Date Analyzed | Target Concentration (mg/mL) | Determined Concentration[a] (mg/mL) | Difference from Target (%) |
|---|---|---|---|---|
| March 18, 2004 | March 18-19, 2004 | 4 | 3.937 | –2 |
| | | 4 | 3.961 | –1 |
| | | 12 | 12.02 | 0 |
| | | 12 | 11.88 | –1 |
| | | 40 | 39.67 | –1 |
| | | 40 | 39.53 | –1 |
| | May 3-4, 2004[c] | 4 | 4.064 | +2 |
| | | 12 | 15.25[b] | +27 |
| | | 40 | 39.36 | –2 |
| May 25, 2004 | May 26-27, 2004 | 4 | 4.033 | 0 |
| | | 4 | 4.005 | 0 |
| | | 12 | 12.32 | +3 |
| | | 12 | 12.05 | 0 |
| | | 40 | 39.21 | –2 |
| | | 40 | 38.70 | –3 |
| August 3, 2004 | August 5, 2004 | 4 | 3.794 | –5 |
| | | 4 | 3.861 | –3 |
| | | 12 | 12.02 | 0 |
| | | 12 | 11.59 | –3 |
| | | 40 | 36.98 | –8 |
| | | 40 | 37.78 | –6 |
| October 13, 2004 | October 15-16, 2004 | 4 | 4.201 | +5 |
| | | 4 | 4.004 | 0 |
| | | 12 | 12.18 | +2 |
| | | 12 | 12.11 | +1 |
| | | 40 | 39.28 | –2 |
| | | 40 | 39.21 | –2 |
| | December 2-3, 2004[c] | 4 | 4.121 | +3 |
| | | 12 | 12.33 | +3 |
| | | 40 | 41.08 | +3 |
| December 28, 2004 | December 30, 2004-January 3, 2005 | 4 | 4.003 | 0 |
| | | 4 | 4.232 | +6 |
| | | 12 | 11.79 | –2 |
| | | 12 | 12.51 | +4 |
| | | 40 | 41.07 | +3 |
| | | 40 | 42.21 | +6 |

[a] Results of duplicate analyses. Dosing volume = 2.5 mL/kg; 4 mg/mL = 10 mg/kg, 12 mg/mL = 30 mg/kg, 40 mg/mL = 100 mg/kg
[b] Results of quadruplicate analyses
[c] Animal room samples
[d] Remixed; not used in study
[e] Results of remix

**TABLE G5**
**Results of Analyses of Dose Formulations Administered to Mice in the 2-Year Gavage Study of TCAB**

| Date Prepared | Date Analyzed | Target Concentration (mg/mL) | Determined Concentration[a] (mg/mL) | Difference from Target (%) |
|---|---|---|---|---|
| January 17, 2003 | January 21-22, 2003 | 0.3 | 0.3062 | +2 |
| | | 1 | 0.9980 | 0 |
| | | 3 | 2.802 | –7 |
| | March 3-4, 2003[b] | 0.3 | 0.2959 | –1 |
| | | 1 | 0.9655 | –3 |
| | | 3 | 2.737 | –9 |
| April 1, 2003 | April 1-3, 2003 | 0.3 | 0.2988 | 0 |
| | | 1 | 0.9254 | –7 |
| | | 3 | 2.993 | 0 |
| June 10, 2003 | June 12, 2003 | 0.3 | 0.3138 | +5 |
| | | 1 | 1.032 | +3 |
| | | 3 | 3.103 | +3 |
| August 19, 2003 | August 21-22, 2003 | 0.3 | 0.3188 | +6 |
| | | 1 | 1.066 | +7 |
| | | 3 | 3.150 | +5 |
| | October 10-11, 2003[b] | 0.3 | 0.2977 | –1 |
| | | 1 | 0.9822 | –2 |
| | | 3 | 2.987 | 0 |
| September 23, 2003 | September 30-October 1, 2003 | 0.3 | 0.2982 | –1 |
| | | 1 | 1.032 | +3 |
| | | 3 | 2.971 | –1 |
| October 29, 2003 | October 29-30, 2003 | 0.3 | 0.3104 | +3 |
| | | 1 | 1.026 | +3 |
| | | 3 | 2.982 | –1 |
| January 7, 2004 | January 9-11, 2004 | 0.3 | 0.2945 | –2 |
| | | 1 | 1.046 | +5 |
| | | 3 | 3.200 | +7 |
| March 18, 2004 | March 18-19, 2004 | 0.3 | 0.2897 | –3 |
| | | 1 | 0.9717 | –3 |
| | | 3 | 3.017 | +1 |
| | May 3-4, 2004[b] | 0.3 | 0.2878 | –4 |
| | | 1 | 1.009 | +1 |
| | | 3 | 2.905 | –3 |
| May 25, 2004 | May 26-27, 2004 | 0.3 | 0.2916 | –3 |
| | | 1 | 0.9974 | 0 |
| | | 3 | 2.973 | –1 |
| August 3, 2004 | August 5, 2004 | 0.3 | 0.2843 | –5 |
| | | 1 | 0.9853 | –1 |
| | | 3 | 2.855 | –5 |

**TABLE G5**
**Results of Analyses of Dose Formulations Administered to Mice in the 2-Year Gavage Study of TCAB**

| Date Prepared | Date Analyzed | Target Concentration (mg/mL) | Determined Concentration[a] (mg/mL) | Difference from Target (%) |
|---|---|---|---|---|
| October 13, 2004 | October 15-16, 2004 | 0.3 | 0.2919 | –3 |
| | | 1 | 1.015 | +2 |
| | | 3 | 3.081 | +3 |
| | December 2-3, 2004[b] | 0.3 | 0.3005 | 0 |
| | | 1 | 1.027 | +3 |
| | | 3 | 3.189 | +6 |
| December 28, 2004 | December 30, 2004-January 3, 2005 | 0.3 | 0.3116 | +4 |
| | | 1 | 1.075 | +8 |
| | | 3 | 3.296 | +10 |

[a] Results of duplicate analyses. Dosing volume = 10 mL/kg; 0.3 mg/mL = 3 mg/kg, 1 mg/mL = 10 mg/kg, 3 mg/mL = 30 mg/kg
[b] Animal room samples

# APPENDIX H
# INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

**TABLE H1**
**Ingredients of NTP-2000 Rat and Mouse Ration**

| Ingredients | Percent by Weight |
|---|---|
| Ground hard winter wheat | 22.26 |
| Ground #2 yellow shelled corn | 22.18 |
| Wheat middlings | 15.0 |
| Oat hulls | 8.5 |
| Alfalfa meal (dehydrated, 17% protein) | 7.5 |
| Purified cellulose | 5.5 |
| Soybean meal (49% protein) | 5.0 |
| Fish meal (60% protein) | 4.0 |
| Corn oil (without preservatives) | 3.0 |
| Soy oil (without preservatives) | 3.0 |
| Dried brewer's yeast | 1.0 |
| Calcium carbonate (USP) | 0.9 |
| Vitamin premix[a] | 0.5 |
| Mineral premix[b] | 0.5 |
| Calcium phosphate, dibasic (USP) | 0.4 |
| Sodium chloride | 0.3 |
| Choline chloride (70% choline) | 0.26 |
| Methionine | 0.2 |

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

**TABLE H2**
**Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]**

| | Amount | Source |
|---|---|---|
| **Vitamins** | | |
| A | 4,000 IU | Stabilized vitamin A palmitate or acetate |
| D | 1,000 IU | D-activated animal sterol |
| K | 1.0 mg | Menadione sodium bisulfite complex |
| α-Tocopheryl acetate | 100 IU | |
| Niacin | 23 mg | |
| Folic acid | 1.1 mg | |
| *d*-Pantothenic acid | 10 mg | *d*-Calcium pantothenate |
| Riboflavin | 3.3 mg | |
| Thiamine | 4 mg | Thiamine mononitrate |
| $B_{12}$ | 52 µg | |
| Pyridoxine | 6.3 mg | Pyridoxine hydrochloride |
| Biotin | 0.2 mg | *d*-Biotin |
| **Minerals** | | |
| Magnesium | 514 mg | Magnesium oxide |
| Iron | 35 mg | Iron sulfate |
| Zinc | 12 mg | Zinc oxide |
| Manganese | 10 mg | Manganese oxide |
| Copper | 2.0 mg | Copper sulfate |
| Iodine | 0.2 mg | Calcium iodate |
| Chromium | 0.2 mg | Chromium acetate |

[a] Per kg of finished product

**TABLE H3**
**Nutrient Composition of NTP-2000 Rat and Mouse Ration**

| Nutrient | Mean ± Standard Deviation | Range | Number of Samples |
|---|---|---|---|
| Protein (% by weight) | 14.9 ± 0.53 | 13.8 – 16.1 | 25 |
| Crude fat (% by weight) | 8.0 ± 0.37 | 7.4 – 9.0 | 25 |
| Crude fiber (% by weight) | 9.2 ± 0.45 | 8.2 – 9.9 | 25 |
| Ash (% by weight) | 5.0 ± 0.21 | 4.4 – 5.4 | 25 |
| **Amino Acids (% of total diet)** | | | |
| Arginine | 0.770 ± 0.070 | 0.670 – 0.970 | 18 |
| Cystine | 0.225 ± 0.023 | 0.150 – 0.250 | 18 |
| Glycine | 0.706 ± 0.043 | 0.620 – 0.800 | 18 |
| Histidine | 0.362 ± 0.082 | 0.310 – 0.680 | 18 |
| Isoleucine | 0.542 ± 0.046 | 0.430 – 0.660 | 18 |
| Leucine | 1.087 ± 0.066 | 0.960 – 1.240 | 18 |
| Lysine | 0.712 ± 0.118 | 0.310 – 0.840 | 18 |
| Methionine | 0.407 ± 0.051 | 0.260 – 0.490 | 18 |
| Phenylalanine | 0.626 ± 0.043 | 0.540 – 0.720 | 18 |
| Threonine | 0.500 ± 0.046 | 0.430 – 0.610 | 18 |
| Tryptophan | 0.142 ± 0.024 | 0.110 – 0.200 | 18 |
| Tyrosine | 0.388 ± 0.058 | 0.280 – 0.540 | 18 |
| Valine | 0.667 ± 0.045 | 0.550 – 0.730 | 18 |
| **Essential Fatty Acids (% of total diet)** | | | |
| Linoleic | 3.92 ± 0.243 | 3.49 – 4.54 | 18 |
| Linolenic | 0.30 ± 0.035 | 0.21 – 0.35 | 18 |
| **Vitamins** | | | |
| Vitamin A (IU/kg) | 4,920 ± 1,210 | 3,360 – 8,900 | 25 |
| Vitamin D (IU/kg) | 1,000[a] | | |
| α-Tocopherol (ppm) | 84.2 ± 16.60 | 52.0 – 110.0 | 15 |
| Thiamine (ppm)[b] | 8.5 ± 3.66 | 5.9 – 25.2 | 25 |
| Riboflavin (ppm) | 6.8 ± 2.11 | 4.20 – 11.20 | 15 |
| Niacin (ppm) | 79.0 ± 10.50 | 66.4 – 98.2 | 15 |
| Pantothenic acid (ppm) | 23.9 ± 3.73 | 17.4 – 29.8 | 15 |
| Pyridoxine (ppm)[b] | 9.21 ± 2.20 | 6.4 – 13.7 | 15 |
| Folic acid (ppm) | 1.75 ± 0.54 | 1.20 – 3.27 | 15 |
| Biotin (ppm) | 0.332 ± 0.12 | 0.225 – 0.704 | 15 |
| Vitamin $B_{12}$ (ppb) | 60.5 ± 46.5 | 18.3 – 174.0 | 15 |
| Choline (ppm)[b] | 3,064 ± 270 | 2,700 – 3,790 | 15 |
| **Minerals** | | | |
| Calcium (%) | 0.959 ± 0.046 | 0.873 – 1.030 | 25 |
| Phosphorus (%) | 0.589 ± 0.028 | 0.538 – 0.641 | 25 |
| Potassium (%) | 0.665 ± 0.023 | 0.626 – 0.694 | 15 |
| Chloride (%) | 0.376 ± 0.041 | 0.300 – 0.474 | 15 |
| Sodium (%) | 0.191 ± 0.017 | 0.160 – 0.222 | 15 |
| Magnesium (%) | 0.201 ± 0.009 | 0.185 – 0.217 | 15 |
| Sulfur (%) | 0.170 ± 0.029 | 0.116 – 0.209 | 15 |
| Iron (ppm) | 182 ± 46.7 | 135 – 311 | 15 |
| Manganese (ppm) | 54.1 ± 7.89 | 42.1 – 73.1 | 15 |
| Zinc (ppm) | 55.0 ± 9.55 | 43.3 – 78.5 | 15 |
| Copper (ppm) | 6.65 ± 1.790 | 3.21 – 10.50 | 15 |
| Iodine (ppm) | 0.512 ± 0.221 | 0.233 – 0.972 | 15 |
| Chromium (ppm) | 0.604 ± 0.253 | 0.330 – 1.380 | 14 |
| Cobalt (ppm) | 0.25 ± 0.074 | 0.20 – 0.47 | 14 |

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

**TABLE H4**
**Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]**

| | Mean ± Standard Deviation[b] | Range | Number of Samples |
|---|---|---|---|
| **Contaminants** | | | |
| Arsenic (ppm) | 0.33 ± 0.158 | 0.14 – 0.50 | 25 |
| Cadmium (ppm) | 0.07 ± 0.021 | 0.04 – 0.10 | 25 |
| Lead (ppm) | 0.08 ± 0.026 | 0.05 – 0.13 | 25 |
| Mercury (ppm) | <0.02 | | 25 |
| Selenium (ppm) | 0.20 ± 0.057 | 0.14 – 0.45 | 25 |
| Aflatoxins (ppb) | <5.00 | | 25 |
| Nitrate nitrogen (ppm)[c] | 14.5 ± 4.33 | 10.00 – 24.4 | 25 |
| Nitrite nitrogen (ppm)[c] | <0.61 | | 25 |
| BHA (ppm)[d] | <1.0 | | 25 |
| BHT (ppm)[d] | <1.0 | | 25 |
| Aerobic plate count (CFU/g) | 10.0 ± 0 | 10.0 – 10.0 | 25 |
| Coliform (MPN/g) | 3.0 ± 0.0 | 3.0 – 3.0 | 25 |
| *Escherichia coli* (MPN/g) | <10 | | 25 |
| *Salmonella* (MPN/g) | Negative | | 25 |
| Total nitrosoamines (ppb)[e] | 4.4 ± 2.04 | 2.3 – 8.5 | 25 |
| *N*-Nitrosodimethylamine (ppb)[e] | 2.6 ± 1.74 | 1.1 – 6.9 | 25 |
| *N*-Nitrosopyrrolidine (ppb)[e] | 1.8 ± 0.79 | 0.9 – 4.1 | 25 |
| **Pesticides (ppm)** | | | |
| α-BHC | <0.01 | | 25 |
| β-BHC | <0.02 | | 25 |
| γ-BHC | <0.01 | | 25 |
| δ-BHC | <0.01 | | 25 |
| Heptachlor | <0.01 | | 25 |
| Aldrin | <0.01 | | 25 |
| Heptachlor epoxide | <0.01 | | 25 |
| DDE | <0.01 | | 25 |
| DDD | <0.01 | | 25 |
| DDT | <0.01 | | 25 |
| HCB | <0.01 | | 25 |
| Mirex | <0.01 | | 25 |
| Methoxychlor | <0.05 | | 25 |
| Dieldrin | <0.01 | | 25 |
| Endrin | <0.01 | | 25 |
| Telodrin | <0.01 | | 25 |
| Chlordane | <0.05 | | 25 |
| Toxaphene | <0.10 | | 25 |
| Estimated PCBs | <0.20 | | 25 |
| Ronnel | <0.01 | | 25 |
| Ethion | <0.02 | | 25 |
| Trithion | <0.05 | | 25 |
| Diazinon | <0.10 | | 25 |
| Methyl chlorpyrifos | 0.098 ± 0.111 | 0.020 – 0.416 | 25 |
| Methyl parathion | <0.02 | | 25 |
| Ethyl parathion | <0.02 | | 25 |
| Malathion | 0.189 ± 0.377 | 0.020 – 1.850 | 25 |
| Endosulfan I | <0.01 | | 25 |
| Endosulfan II | <0.01 | | 25 |
| Endosulfan sulfate | <0.03 | | 25 |

a All samples were irradiated. CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride
b For values less than the limit of detection, the detection limit is given as the mean.
c Sources of contamination: alfalfa, grains, and fish meal
d Sources of contamination: soy oil and fish meal
e All values were corrected for percent recovery.

# APPENDIX I
# SENTINEL ANIMAL PROGRAM

# SENTINEL ANIMAL PROGRAM

## METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Serum samples were collected from five male and five female sentinel rats at one month and at the end of the 3-month study. For the 2-year studies, serum samples were collected from five male and five female sentinel rats and mice at 1, 6, 12, and 18 months with the exception of only four male sentinel rats at 18 months. In addition, serum samples were also collected from three 30 mg/kg and two 100 mg/kg male rats, five 100 mg/kg female rats, five 10 mg/kg male mice, and five 30 mg/kg female mice at study termination. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to MA Bioservices/BioReliance (Rockville, MD) for determination of antibody titers. At 18 months, fecal samples were obtained for PCR analysis. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

| Method and Test | Time of Analysis |
|---|---|
| **Rats** | |
| ***3-Month Study*** | |
| ELISA | |
| *Mycoplasma arthritidis* | Study termination |
| *Mycoplasma pulmonis* | Study termination |
| PVM (pneumonia virus of mice) | 1 month and study termination |
| RCV/SDA (rat coronavirus/sialodacryoadenitis virus) | 1 month and study termination |
| Sendai | 1 month and study termination |
| Immunofluorescence Assay | |
| Parvovirus | 1 month and study termination |
| *Mycoplasma arthritidis* | Study termination |
| ***2-Year Study*** | |
| ELISA | |
| *M. arthritidis* | Study termination |
| *M. pulmonis* | Study termination |
| PVM | 1, 6, 12, and 18 months, study termination |
| RCV/SDA | 1, 6, 12, and 18 months, study termination |
| Sendai | 1, 6, 12, and 18 months, study termination |
| Immunofluorescence Assay | |
| Parvovirus | 1, 6, 12, and 18 months, study termination |
| RCV/SDA | 6 months |

| Method and Test | Time of Analysis |
|---|---|
| **Mice** | |
| ***2-Year Study*** | |
| ELISA | |
| Ectromelia virus | 1, 6, 12, and 18 months, study termination |
| EDIM (epizootic diarrhea of infant mice) | 1, 6, 12, and 18 months, study termination |
| GDVII (mouse encephalomyelitis virus) | 1, 6, 12, and 18 months, study termination |
| LCM (lymphocytic choriomeningitis virus) | 1, 6, 12, and 18 months, study termination |
| Mouse adenovirus-FL | 1, 6, 12, and 18 months, study termination |
| MHV (mouse hepatitis virus) | 1, 6, 12, and 18 months, study termination |
| MMV (mouse minute virus) | 18 months and study termination |
| MPV (mouse parvovirus) | 18 months and study termination |
| *M. arthritidis* | Study termination |
| *M. pulmonis* | Study termination |
| PVM | 1, 6, 12, and 18 months, study termination |
| Reovirus 3 | 1, 6, 12, and 18 months, study termination |
| Sendai | 1, 6, 12, and 18 months, study termination |
| Immunofluorescence Assay | |
| Parvovirus | 1, 6, and 12 months |
| MCMV (mouse cytomegalovirus) | Study termination |
| Sendai | 1 month |
| Mouse adinovirus – FL | 12 months |
| Reovirus 3 | 12 months |
| EDIM | 12 months |
| GDVII | 18 months |
| Ectromelia Virus | 18 months |
| Polymerase Chain Reaction (PCR) | |
| *Helicobacter* species | 18 months |

## RESULTS

All test results were negative.

# APPENDIX J
# TOXICOKINETIC STUDIES
# IN SPRAGUE-DAWLEY RATS

# TOXICOKINETIC STUDIES IN SPRAGUE-DAWLEY RATS

## INTRODUCTION

Time-course data were collected from special study female Sprague-Dawley rats for the determination of toxicokinetic parameters in whole blood and tissues at time points up to 120 hours after the last dose in the 3-month study. In core study animals, tissue samples were collected 24 hours after the last dose in the 3-month study and analyzed for TCAB concentrations.

## MATERIALS AND METHODS

TCAB (lot 110199MT-AC-1) was obtained from AccuStandard (New Haven, CT) and stored refrigerated in amber glass bottles. The bulk chemical was analyzed for identity and purity; the results and analytical systems are described in Appendix G.

Two groups of eight pilot study female rats were used to determine time points for blood collection from special study rats at the end of the 13-week gavage study. The two pilot study groups were given an intravenous dose of approximately 3 mg/kg TCAB; group A had received a daily gavage dose of 3 mg/kg for the 7 days prior to the intravenous dose, and group B had not received any prior dosing of TCAB. Samples were collected at 0, 1, 4, 8, 12, 16, 24, and 36 hours post-dosing via cardiac puncture following sacrifice of the animals with $CO_2$. The zero-hour sample was collected immediately prior to administration of the intravenous dose. Calculated toxicokinetic parameters for the pilot study included clearance (CL), area under the concentration-time curve (AUC), half-lives ($t_{½\alpha}$ and $t_{½\beta}$), elimination constants ($\alpha$ and $\beta$), volume of distribution at steady state ($V_{SS}$), and mean residence time (MRT).

Based on the results of the pilot study, groups of 30 (dosed groups) or 6 (vehicle controls) special study female rats were administered 0, 0.1, 3, or 100 mg/kg TCAB by gavage for 3 months and fat, liver, lung, and blood time-course concentrations of TCAB were measured during the interval of 6 to 120 hours (fat, liver, and lung) or 0 to 30 hours (blood) after the last dose on day 92; dosing included 2 consecutive days (Sunday and Monday) prior to sample collection. A non-compartmental model was fit to the blood concentration data obtained from the retroorbital blood samples from the 3 and 100 mg/kg special study groups. Non-compartmental toxicokinetic parameters were calculated using WinNonlin® (Pharsight Corp., Mountain View, CA).

Groups of 10 male and 10 female core study rats were administered 0, 0.1, 0.3, 1, 3, 10, 30, or 100 mg TCAB/kg body weight by gavage for 3 months and fat, liver, and lung concentrations of TCAB were measured 24 hours after the last oral dose. Additional details describing the core study and special study groups of the 3-month gavage study are presented in the Materials and Methods section of this Technical Report.

Methylene chloride or hexane extracts of the tissues were prepared and analyzed for TCAB content using the following procedures:

### *Fat*

Matrix standards were prepared by homogenizing a sufficient amount of control fat tissue to allow for the removal of 0.5 g of homogenate. Each standard aliquot was spiked with an appropriate volume of TCAB stock solution to prepare standards over the concentration ranges of 10 to 4,000 and 815 to 84,000 ng/g. Internal standard solution was added to 0.5 g samples and spiked matrix standards followed by 2 mL of 40% (w/w) potassium hydroxide (KOH) solution. Samples were vortexed and allowed to stand for 16 hours, after which they were vortexed again

for 2 minutes. The samples were extracted with 5 mL of methylene chloride, vortexed for 2 minutes, and cooled in an ice water bath prior to centrifugation to clarify the extracts. The solvent layer was removed and collected into a scintillation vial, which was then cooled to –17° C prior to sample clean-up using 60 to 200 mesh silica gel packed in 8-mL glass columns to remove dissolved fat. Clean-up columns were rinsed with 10 mL of methylene chloride on a vacuum manifold. Each sample extract was then pipetted onto each column followed by 5 mL of methylene chloride and collected in individual scintillation vials. Extracts were evaporated to 250 µL (low-range) or 1 mL (high-range) final volume and analyzed by gas chromatography/electron capture detection (GC/ECD) using system A (Table J1).

### *Liver and Lung*

Matrix standards were prepared by spiking 0.5 g of pooled control liver and lung tissue with an appropriate volume of TCAB stock solution to prepare standards over the range of 20.0 to 500 ng/g (liver) or 15.0 to 500 ng/g (lung). Samples over 500 ng/g were diluted into the calibration range by analyzing a smaller amount of tissue. Internal standard solution was added to 0.5 g samples and spiked matrix standards followed by 2.5 mL of 40% (w/v) chilled KOH solution and 1 mL of chilled 95% ethanol. Samples were vortexed and then mixed overnight (15 to 18 hours) on an orbital shaker, after which 2.5 mL of purified deionized, distilled water and 2.5 mL of 95% ethanol were added to each sample and vortexed. The saponified samples were extracted by adding 3 mL of hexane and mixing on an orbital shaker for 5 minutes and then on a vortex mixer for 30 seconds. The samples were clarified by standing and the hexane layer was transferred to a clean scintillation vial. This extraction procedure was repeated three more times. The four hexane extracts were combined and evaporated to dryness, reconstituted into 1.0 mL of hexane (liver) or 0.5 mL of acetone (lung), vortexed, and analyzed using system B (Table J1).

### *Blood*

Matrix standards were prepared by spiking 0.5 mL of control whole blood with an appropriate amount TCAB stock solution previously prepared in 2-propanol and 25 µL of internal standard solution. Samples (0.5 mL) and matrix standards were analyzed by adding 1 mL of 40% (w/w) KOH solution to each sample. Samples were vortexed and allowed to stand for 16 hours, after which they were extracted by adding 3 mL of methylene chloride and 0.3 mL of methanol, vortexing for 1 minute, and centrifuging to clarify the extract. The solvent layer was removed and passed through a microporous polyvinylidene fluoride membrane filter and collected in a scintillation vial. The extraction was repeated three more times with 2-mL aliquots of methylene chloride. The combined solvent layer was evaporated to dryness in a Savant SpeedVac® and subsequently reconstituted with 200 µL acetone. The samples and standards were analyzed by GC/ECD using system C (Table J1).

## RESULTS

### *Pilot Study*

A pilot study was conducted to select timepoints for collection of whole blood from special study rats at the end of the 3-month study. For the pilot study, two groups of eight female rats were given nominal intravenous doses of 3 mg/kg; group A received a daily oral dose of 3 mg/kg for the 7 days prior to the intravenous dose; group B did not receive any prior dosing of TCAB. Samples were collected at eight timepoints (0, 1, 4, 8, 12, 16, 24, and 36 hours) post-dosing via cardiac puncture after over-exposure to $CO_2$. The zero-hour sample was collected immediately prior to administration of the intravenous dose. The blood elimination profiles for groups A and B are shown in Figure J1. Calculated toxicokinetic parameters (Table J2) included clearance (CL), area under the concentration-time curve (AUC), half-lives ($t_{½\alpha}$, $t_{½\beta}$), elimination constants ($\alpha$, $\beta$), volume of distribution ($V_{ss}$), and mean residence time (MRT). Values for groups A and B were similar, indicating that 7 days of oral dosing with TCAB had no effect on the absorption or elimination kinetics of TCAB in these animals. The terminal elimination half-life ($t_{½\beta}$) was found to be 6 hours for both groups. Clearance was approximately 400 mL/hour per kg, while MRT was approximately 2 hours for both groups. Based on the terminal elimination half-life value of

approximately 6 hours from the pilot study, time points from 0 to 30 hours (5 half-lives) were selected for blood collection from the special study rats. Collection of solid tissues from the special study rats began 6 hours after dosing, based on the $t_{½\alpha}$ value of approximately 1 hour, which allowed time for TCAB to be distributed to the tissues.

## *3-Month Study*

### Special Study

Special study female rats in each of three dose groups (0.1, 3, and 100 mg/kg) were dosed through day 92 of the 3-month study and were dosed for two consecutive days (Sunday and Monday) prior to sample collection. Fat, liver, and lung samples were collected 6, 30, 60, 90, and 120 hours post-dosing on day 92, and blood samples were collected from the retroorbital sinus 0, 0.25, 0.5, 1, 3, 6, 9, 12, 20, and 30 hours post-dosing on day 92.

Time-course data for the elimination of TCAB from tissues of the special study rats are shown in Figure J2. Fat tissue showed a gradual decline over time in tissue concentrations of TCAB for each dose group and estimated half-lives of 115, 81, and 86 hours for the 0.1, 3, and 100 mg/kg groups, respectively. Liver tissue also showed gradual declines in tissue concentrations of TCAB, and half-lives were estimated to be 121, 86, and 88 hours for the 0.1, 3, and 100 mg/kg groups, respectively. Lung tissue showed a gradual decline in tissue concentrations and estimated half-lives of 51, 83, and 48 hours for the 0.1, 3, and 100 mg/kg groups, respectively. Blood concentrations for the 0.1 mg/kg group were largely below the limit of detection. Non-compartmental toxicokinetic parameters were calculated for the 3 and 100 mg/kg groups using WinNonlin® and are given in Table J3. Half-life and $T_{max}$ increased from 1 hour to approximately 3 hours when the dose was increased from 3 mg/kg to 100 mg/kg. $AUC_{\infty}$ normalized to dose decreased approximately 10-fold between the 3 and 100 mg/kg groups. $C_{max}$ normalized to dose also decreased 10-fold between the same dose groups, decreasing from 64 ng • kg/mL per mg for the 3 mg/kg group to 6.2 ng • kg/mL per mg for the 100 mg/kg group. These data indicated that the amount of TCAB absorbed decreased with increasing dose.

### Core Study

Tissue concentrations of TCAB in male and female rats were measured 24 hours after the last dose at the end of the 3-month gavage study of TCAB (Table J4). Mean fat concentrations of TCAB increased from 160 to 19,380 ng/g in males and from 136 to 10,940 ng/g in females as the dose increased from 0.1 to 100 mg/kg. Measured fat concentrations in the dosed groups were significantly different from those in the vehicle controls ($P<0.001$, except $P<0.05$ for the 0.1 mg/kg groups) at each dose for males and females. The increases in mean TCAB fat concentration with dose were less than dose proportional at all doses for males and females. Mean fat TCAB concentrations in females were less that those in males in the 1 ($P<0.02$), 10, 30, and 100 mg/kg groups ($P<0.001$). Mean liver concentrations of TCAB increased from 0.58 to 136 ng/g in males and from 0.71 to 154 ng/g in females as the dose increased from 0.1 to 100 mg/kg. Measured liver concentrations in the dosed groups were significantly different from those in the vehicle controls ($P<0.001$) at each dose for males and females. The increases in mean TCAB liver concentration with dose were less than dose proportional at all doses for males and females. Mean liver TCAB concentrations in 0.3, 1, 3, and 10 mg/kg females were greater than those in equivalently dosed males ($P<0.003$). Ratios of TCAB concentrations in liver to those in fat tissue were calculated for each dose group. Liver:fat ratios ranged from 0.00237 to 0.00699 for males and from 0.00454 to 0.0141 for females. Mean lung concentrations of TCAB increased from 0.85 to 40.9 ng/g over the dose range of 0.1 to 100 mg/kg in males, and from 0.69 to 115 ng/g over the dose range of 0.1 to 30 mg/kg in females. The measured 1 mg concentrations in the dosed groups were significantly different from those in the vehicle controls ($P<0.001$) at each dose for males and females. The increases in mean TCAB lung concentration with dose were less than dose proportional at all doses, except the 30 mg/kg groups of males and females and the 10 and 100 mg/kg groups of males. Mean lung TCAB concentrations in females were greater than those in males in the 1, 3, and 10 mg/kg groups ($P<0.05$). Ratios of TCAB concentrations in lung to those in fat tissue were calculated for each dose group. Lung:fat ratios ranged from 0.00038 to 0.00528 for males and from 0.00055 to 0.00814 for females.

**TABLE J1**
**Gas Chromatography Systems Used in the Toxicokinetic Studies of TCAB[a]**

| Detection System | Column | Carrier Gas | Oven Temperature Program |
|---|---|---|---|
| **System A** | | | |
| Electron capture | J&W DB-5ms, 30 m × 0.32 mm, 0.5-µm film thickness (J&W Scientific, Folsom, CA) | Helium at 1.8 mL/minute | 200° C to 280° C at 5° C/minute |
| **System B** | | | |
| Electron capture | J&W DB-5ms, 30 m × 0.32 mm, 0.5-µm film thickness (J&W Scientific) | Helium at 1.66 mL/minute | 180° C for 1 minute, then 10° C/minute to 220° C (liver) or 230° C (lung), held for 20 minutes, then 20° C/minute to 260° C, held for 13 minutes |
| **System C** | | | |
| Electron capture | J&W DB-17, 30 m × 0.32 mm, 0.5-µm film thickness (J&W Scientific) | Nitrogen at 1 mL/minute | 180° C for 0.5 minute, then 10° C/minute to 280° C, held for 10 to 25 minutes |

[a] The gas chromatographs were manufactured by Hewlett-Packard (Palo Alto, CA)

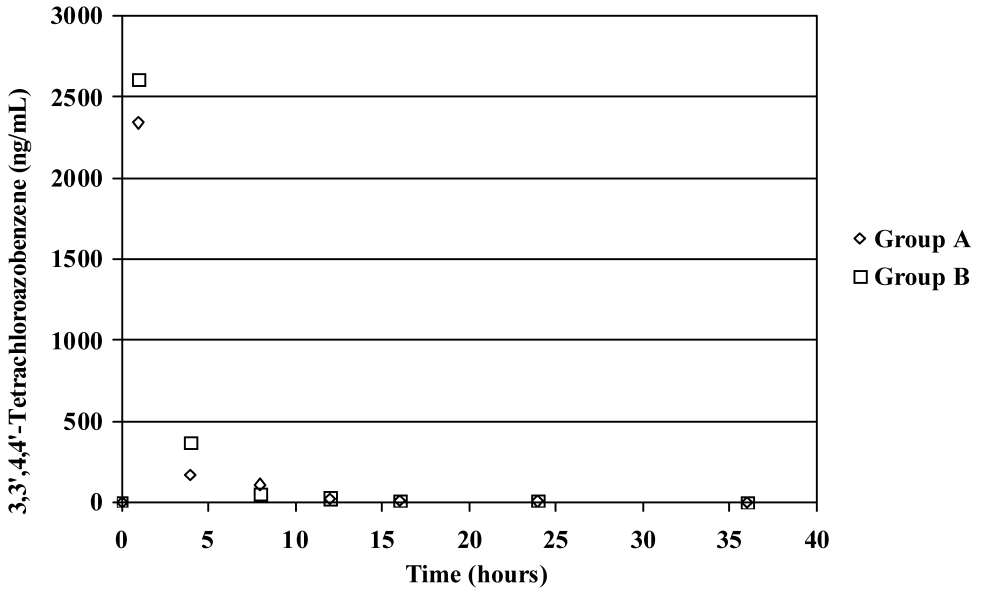


**FIGURE J1**
**Blood Elimination Profiles for Pilot Study Female Sprague-Dawley Rats Administered a Nominal 3 mg/kg Intravenous Dose of TCAB**
Group A received seven prior daily gavage doses of 3 mg/kg TCAB; Group B had no prior exposure to TCAB

**TABLE J2**
**Toxicokinetic Parameter Estimates for the Elimination of TCAB from the Blood of Pilot Study Female Sprague-Dawley Rats After an Intravenous Dose of TCAB[a]**

| Model Parameter | Group A[b] | Group B[c] |
|---|---|---|
| Intravenous Dose (mg/kg) | 2.86 | 2.87 |
| $C_{max}$ (ng/mL) | 5,674 | 5,419 |
| AUC (ng • hour/mL) | 6,624 | 8,162 |
| $\alpha$ ($hour^{-1}$) | 0.9313 | 0.7287 |
| $t_{½\alpha}$ (hour) | 0.7 | 1.0 |
| $\beta$ ($hour^{-1}$) | 0.1159 | 0.1198 |
| $t_{½\beta}$ (hour) | 6.0 | 5.8 |
| $V_{ss}$ (mL/kg) | 763 | 743 |
| CL (mL/hour per kg) | 432 | 352 |
| MRT (hour) | 1.8 | 2.1 |

[a] Blood samples were collected 0, 1, 4, 8, 12, 16, 24, and 36 hours after the intravenous dose of TCAB.
[b] Daily gavage administration (3 mg/kg) for 7 days prior to intravenous dose
[c] No prior TCAB exposure
$C_{max}$= maximum whole blood TCAB concentration; AUC = area under the blood concentration-time curve; $\alpha$ and $\beta$ = elimination constants; $t_{½\alpha}$ and $t_{½\beta}$ = half-lives; $V_{ss}$= volume of distribution at study state; CL = clearance; MRT = mean residence time

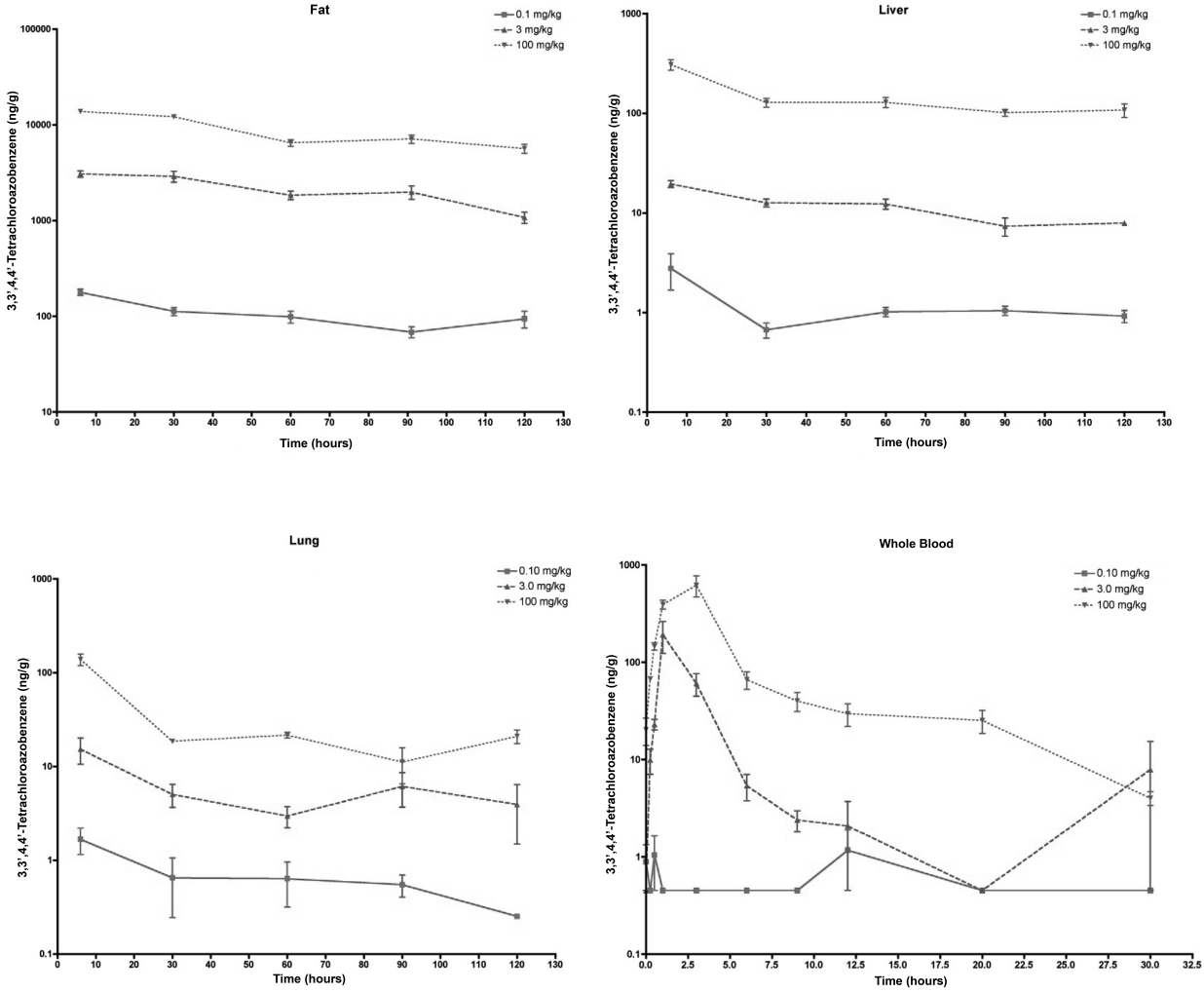


**FIGURE J2**
**Tissue Elimination Profiles for Special Study Female Sprague-Dawley Rats in the 3-Month Gavage Study of TCAB**

**TABLE J3**
**Non-compartmental Analysis of Whole Blood TCAB Concentration Versus Time Profiles for Special Study Female Sprague-Dawley Rats in the 3-Month Gavage Study of TCAB[a]**

| Model Parameter | 3 mg/kg | 100 mg/kg |
|---|---|---|
| $T_{max}$ (hour) | 1.0 | 3.0 |
| $C_{max}$ (ng/mL) | 192.3 | 619.8 |
| $C_{max}$/Dose (ng • kg/mL per mg) | 64.1 | 6.20 |
| $AUC_{\infty}$ (ng • hour/mL) | 998.4 | 2,868 |
| $AUC_{\infty}$/Dose (ng • hour • kg/mL per mg) | 332.8 | 28.7 |
| $\lambda_z$ ($hour^{-1}$) | 0.5741 | 0.1451 |
| $V_{ss}$ (mL/kg) | 5,680.5 | 240,507 |
| CL (mL/hour per kg) | 3,004.9 | 34,890 |
| MRT (hour) | 17.75 | 5.423 |
| $t_{½\beta}$ (hour) | 1.2 | 4.8 |

[a] Blood samples were collected 0.25 to 30 hours after the last gavage dose on day 92.
$T_{max}$= time at which $C_{max}$ was observed; $C_{max}$= maximum whole blood TCAB concentration;
$AUC_{\infty}$ = area under the blood concentration-time curve extrapolated to infinity; AUC/Dose = dose-normalized AUC;
$\lambda_z$= elimination rate constant; $V_{ss}$= volume of distribution at study state; CL = clearance; MRT = mean residence time; $t_{½\beta}$ = half-life

**TABLE J4**
**Tissue Concentrations of TCAB 24 Hours After the Last Dose in Core Study Sprague-Dawley Rats in the 3-Month Gavage Study of TCAB[a]**

| Vehicle Control | 0.1 mg/kg | 0.3 mg/kg | 1 mg/kg | 3 mg/kg | 10 mg/kg | 30 mg/kg | 100 mg/kg |
|---|---|---|---|---|---|---|---|
| n 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Male** | | | | | | | |
| Fat (ng/g) | | | | | | | |
| 3.06 ± 1.18[b] | 160.3 ± 16.7**[b] | 385.1 ± 44.6** | 768.0 ± 53.0**[b] | 3,524 ± 259** | 10,908 ± 541** | 19,078 ± 1,101**[b] | 19,380 ± 1,027** |
| Liver (ng/g) | | | | | | | |
| 0.025 ± 0.006 | 0.579 ± 0.071** | 1.230 ± 0.190** | 4.373 ± 1.539** | 8.348 ± 0.591** | 34.62 ± 12.55** | 69.71 ± 5.91** | 135.6 ± 23.6** |
| Lung (ng/g) | | | | | | | |
| 0.050 ± 0.000 | 0.847 ± 0.408** | 1.012 ± 0.371** | 0.802 ± 0.208** | 2.169 ± 0.161** | 4.107 ± 0.946** | 23.63 ± 1.75**[b] | 40.88 ± 6.98**[b] |
| **Female** | | | | | | | |
| Fat (ng/g) | | | | | | | |
| 3.09 ± 1.25 | 136 ± 12** | 390 ± 38** | 633 ± 28** | 3,699 ± 292** | 8,698 ± 650** | 14,140 ± 550** | 10,936 ± 822** |
| Liver (ng/g) | | | | | | | |
| 0.025 ± 0.006 | 0.706 ± 0.063** | 3.190 ± 0.541** | 6.204 ± 0.786** | 16.80 ± 2.50** | 47.55 ± 4.24** | 80.71 ± 6.30** | 154.1 ± 7.9** |
| Lung (ng/g) | | | | | | | |
| 0.056 ± 0.006[b] | 0.692 ± 0.389* | 0.792 ± 0.311** | 0.350 ± 0.115** | 3.551 ± 0.532** | 20.45 ± 6.91**[b] | 115.17 ± 90.11**[b] | 48.46 ± 13.52**[c] |

* Significantly different (P≤0.05) from the vehicle control group by Shirley's test
** P≤0.01
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n = 9
[c] n = 8


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