NTP

National Toxicology Program

U.S. Department of Health and Human Services

NTP Technical Report on the Toxicology and Carcinogenesis Studies of

p-Chloro-α,α,α-trifluorotoluene (CASRN 98-56-6) in Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice (Inhalation Studies)

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NTP Technical Report on the Toxicology and Carcinogenesis Studies of *p*-Chloro-α,α,α-trifluorotoluene (CASRN 98-56-6) in Sprague Dawley (Hsd: Sprague Dawley® SD®) Rats and B6C3F1/N Mice (Inhalation Studies)

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Foreword

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection per se is not an indicator of a substance's carcinogenic potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

The NTP Technical Reports are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these studies are included in NTP's Chemical Effects in Biological Systems database.

For questions about the reports and studies, please email NTP or call 984-287-3211.

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This report has been reformatted to meet new NTP publishing requirements;
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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 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 on 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 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.

Peer Review

The members of the Peer Review Panel who evaluated the draft *NTP Technical Report on the Toxicology and Carcinogenesis Studies of p-Chloro-α,α,α-trifluorotoluene (CASRN 98-56-6) in Sprague Dawley (Hsd: Sprague Dawley® SD®) Rats and B6C3F1/N Mice (Inhalation Studies)* on July 13, 2017, are listed below. Panel members served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members had 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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Abstract

p-Chloro-α,α,α-trifluorotoluene is a solvent used in paints and coatings and as an industrial intermediate in the production of other chemicals (e.g., herbicides, dyes, pharmaceuticals).*p*-Chloro-α,α,α-trifluorotoluene was nominated for study by the National Cancer Institute and Kowa American Corporation for study because of its high import volume and lack of occupational exposure limits. Male and female Hsd:Sprague Dawley® SD® rats and B6C3F1/N mice were exposed to *p*-chloro-α,α,α-trifluorotoluene (purity greater than 99.5%) by inhalation for 3 months or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and rat and mouse peripheral blood erythrocytes.

Three-month Study in Rats

Groups of 10 male and 10 female rats were exposed by whole body inhalation to *p*-chloro-α,α,α-trifluorotoluene vapor at concentrations of 0, 125, 250, 500, 1,000, or 2,000 ppm for 6 hours plus T_{90} (15 minutes) per day, 5 days per week for 14 weeks. There was no exposure-related effect on survival. The final mean body weights and mean body weight gains of females exposed to 500 ppm or greater were significantly greater than those of the chamber controls. Alanine aminotransferase and sorbitol dehydrogenase activities were significantly increased in 2,000 ppm males and females. Alkaline phosphatase activities were significantly increased in all exposed male groups and in females exposed to 250 ppm or greater, and bile salt concentrations were significantly increased in females exposed to 500 ppm or greater. There were significant exposure concentration-dependent increases in cholesterol and triglyceride concentrations in males exposed to 500 ppm or greater. In females, cholesterol concentrations were significantly increased in groups exposed to 250 ppm or greater and triglyceride concentrations were significantly increased in the 1,000 and 2,000 ppm groups. There were significant exposure concentration-related increases in the absolute and relative liver weights of males exposed to 250 ppm or greater and of females exposed to 500 ppm or greater. The absolute kidney weights were significantly increased in all exposed groups of males, and relative kidney weights were significantly increased in males exposed to 250 ppm or greater.

In the liver, there were significantly increased incidences of centrilobular hepatocyte hypertrophy in males exposed to 250 ppm or greater and in females exposed to 1,000 or 2,000 ppm. In the Harderian gland, there were significantly increased incidences of degeneration in males and females exposed to 250 ppm or greater. There were significantly increased incidences of cytoplasmic vacuolization in the adrenal cortex of 2,000 ppm males and 1,000 and 2,000 ppm females. In the kidney of males, the severities of hyaline droplet accumulation and chronic nephropathy generally increased with increasing exposure concentration. There were significantly increased incidences of mammary gland hyperplasia in 1,000 and 2,000 ppm females.

Males exposed to 2,000 ppm had significantly decreased left cauda and left epididymis weights and numbers of sperm per cauda epididymis. Sperm motility was also significantly decreased in 1,000 and 2,000 ppm males. These findings were associated with histopathologic changes in the testes and epididymides. Female rats exposed to 2,000 ppm had decreased frequency of estrus and increased frequency of diestrus, a significantly higher probability of extended diestrus, a fewer number of cycles, and a fewer number of rats exhibiting cycles. Based on these findings, *p*-chloro-α,α,α-trifluorotoluene exhibited the potential to be a reproductive toxicant in both male and female rats.

Three-month Study in Mice

Groups of 10 male and 10 female mice were exposed by whole body inhalation to *p*-chloro-α,α,α-trifluorotoluene vapor at concentrations of 0, 125, 250, 500, 1,000, or 2,000 ppm for 6 hours plus T_{90} (15 minutes) per day, 5 days per week for 14 weeks. All mice survived to the end of the study. The final mean body weights of males exposed to 500 ppm or greater and females exposed to 250 ppm or greater were significantly greater than those of the chamber control groups. The absolute liver weights were significantly increased in an exposure concentration-dependent manner in males and females exposed to 250 ppm or greater. Relative liver weights were significantly increased in males exposed to 250 ppm or greater and females exposed to 500 ppm or greater. In males, absolute kidney weights were significantly increased in groups exposed to 500 ppm or greater, and relative kidney weights were significantly increased in the 1,000 and 2,000 ppm groups. The absolute thymus weight was significantly decreased in 2,000 ppm females, and the relative thymus weights were signifi-cantly decreased in 2,000 ppm males and females.

In the liver, there were significantly increased incidences of centrilobular hepatocyte hypertrophy in males exposed to 250 ppm or greater and females exposed to 500 ppm or greater. There were also significantly increased inci-dences of centrilobular hepatocyte necrosis and multinucleated hepatocytes in males exposed to 500 ppm or greater and females exposed to 1,000 or 2,000 ppm. There were significantly increased incidences of epithelium hyperplasia in the forestomach of males and females exposed to 500 ppm or greater. In 2,000 ppm males and females, there were significantly increased incidences of granulomatous inflammation in the forestomach. In the adrenal cortex, there were significantly increased incidences of zona fasciculata hypertrophy in 2,000 ppm males and females and X-zone degeneration in 2,000 ppm females. In the red pulp of the spleen, there were significantly increased incidences of megakaryocyte and erythrocytic hematopoietic cell proliferation in females exposed to 250 ppm or greater. In males, there were significantly increased incidences of megakaryocyte hematopoietic cell proliferation at 1,000 and 2,000 ppm, and a significantly increased incidence of erythrocytic hematopoietic cell proliferation at 2,000 ppm.

All exposed groups of males evaluated displayed significant exposure concentration-related decreases in sperm motility. In 1,000 and 2,000 ppm females, there were significant increases in estrous cycle length. In all exposed female groups evaluated, there were significantly higher probabilities of extended estrus. Based on these findings, *p*-chloro-α,α,α-trifluorotoluene exhibited the potential to be a reproductive toxicant in both male and female mice.

Two-year Study in Rats

Groups of 50 male and 50 female rats were exposed by whole body inhalation to *p*-chloro-α,α,α-trifluorotoluene at concentrations of 0, 100, 300, or 1,000 ppm for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 104 to 105 weeks. There was a significant negative trend in survival of males and survival of 1,000 ppm males was significantly less than that of the chamber controls; survival of other exposed groups of males and all exposed groups of females was similar to that of the chamber controls. Mean body weights of 100 and 300 ppm males and females were similar to those of the chamber controls throughout the study. The mean body weights of 1,000 ppm males were approximately 10% less than the chamber controls at the beginning of the study and subsequently were within 5% to 10% of the chamber controls for the remainder of the study. Female body weights in the 1,000 ppm group were within 10% of the

chamber controls until week 72 and were approximately 10% less than the chamber controls for the remainder of the study.

In the thyroid gland, there were significantly increased incidences of C-cell adenoma in 1,000 ppm males and 100 and 1,000 ppm females.

There were occurrences of alveolar/bronchiolar adenoma and alveolar/bronchiolar carcinoma in the 100 and 1,000 ppm groups of male rats; these neoplasms were not observed in the chamber controls. There were significantly increased incidences of chronic inflammation of the lung in all exposed groups of males and females. There were significantly increased incidences of fibrosis of the lung in all exposed male groups and in 300 and 1,000 ppm females and of hemorrhage in all groups of exposed males and 1,000 ppm females.

In the adrenal medulla, there was a significantly increased incidence of benign pheochromocytoma in 1,000 ppm females, and the incidences of medullary hyperplasia were significantly increased in 300 and 1,000 ppm females.

In the uterus, there were positive trends in the incidences of adenocarcinoma and atypical hyperplasia of the endometrium and a significantly increased incidence of stromal polyp in 300 ppm females.

In the liver, there were significantly increased incidences of centrilobular hepatocyte hypertrophy in all exposed groups of males and in 300 and 1,000 ppm females and of fatty change in 300 and 1,000 ppm males and females. There were significantly increased incidences of eosinophilic focus in 1,000 ppm males and mixed cell focus and clear cell focus in 1,000 ppm females.

The severity of kidney nephropathy increased with increasing exposure concentration in male rats.

There was a significantly increased incidence of exudate in the nose of 1,000 ppm males.

Two-year Study in Mice

Groups of 50 male and 50 female mice were exposed by whole body inhalation to *p*-chloro-α,α,α-trifluorotoluene at concentrations of 0, 100, 200, or 400 ppm for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 104 to 105 weeks. Survival of 400 ppm males was significantly less than that of the chamber controls; survival of 400 ppm females was similar to that of 400 ppm males, but was not significantly different from the chamber controls. Survival of other exposed groups of males and females was similar to that of the chamber controls. Mean body weights of exposed groups of males were similar to those of the chamber control group throughout the study. The mean body weights of 100 ppm females were at least 10% greater than those of the chamber control group generally after week 69. The mean body weights of 200 and 400 ppm females were at least 10% greater than those of the chamber controls after weeks 13 and 2, respectively. Clinical observations included increased occurrences of distended abdomen at removal. Thinness also occurred in 400 ppm males and females.

In the liver of 200 and 400 ppm groups, there were significantly increased incidences of multiple hepatocellular adenoma in males and hepatocellular adenoma (including multiples) in females. There were significantly increased incidences of hepatocellular carcinoma in all exposed groups of males and in 400 ppm females. There were significantly increased incidences of hepatoblastoma in 400 ppm males and females. There were significantly increased incidences of eosinophilic focus in 400 ppm males and in 200 and 400 ppm females. There were significantly

increased incidences of centrilobular hepatocyte hypertrophy in all exposed groups of males and in 200 and 400 ppm females. There were significantly increased incidences of multinucleated hepatocytes in 200 and 400 ppm males and in 400 ppm females. The incidences of hepatocyte necrosis were significantly increased in 400 ppm males and females. There were also significantly increased incidences of intrahepatocellular erythrocytes in 200 and 400 ppm males.

In the Harderian gland, there was a significantly increased incidence of adenoma in 400 ppm females, and significantly increased incidences of adenoma or adenocarcinoma (combined) in 200 and 400 ppm females.

There were significantly increased incidences of alveolar/bronchiolar epithelium hyperplasia and peribronchiolar fibrosis of the lungs in all exposed groups of males and females.

There was a significantly increased incidence of epithelium hyperplasia in the forestomach of 400 ppm females. In males, there were significantly increased incidences of inflammation in the 100 and 400 ppm groups.

In the larynx, there was a significantly increased incidence of squamous epithelium hyperplasia in 400 ppm males.

Genetic Toxicology

p-Chloro-α,α,α-trifluorotoluene was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 or in *E. coli* strain WP2 *uvr*A/pKM101 when tested without exogenous metabolic activation or with 10% induced hamster or rat liver S9 mix (only activation system used for the *E. coli* strain). In vivo, no significant increases in micronucleated immature or mature erythrocytes were observed in peripheral blood samples from male and female rats exposed to *p*-chloro-α,α,α-trifluorotoluene by inhalation for 3 months. In male and female mice, small but statistically significant increases in micronucleated mature erythrocytes were seen at the highest exposure concentration (2,000 ppm), but the observed values for the female mice were within historical control ranges and were not considered to be biologically significant. For male mice, the observed response was judged to be positive. An exposure concentration-related increase in the percentage of immature erythrocytes in peripheral blood was seen only in female mice, suggesting that *p*-chloro-α,α,α-trifluorotoluene may have stimulated erythropoiesis in female mice. No significant changes in the percentage of immature erythrocytes were seen in male or female rats or male mice.

Conclusions

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity* of *p*-chloro-α,α,α-trifluorotoluene in male Hsd:Sprague Dawley® SD® rats based on increased incidences of C-cell adenoma in the thyroid gland (see Explanation of Levels of Evidence of Carcinogenic Activity; see summary of the Peer Review Panel comments and the public discussion on this Technical Report in Appendix M). The combined occurrences of alveolar/bronchiolar adenoma or carcinoma in the lung of male rats may have been related to treatment. There was *some evidence of carcinogenic activity* of *p*-chloro-α,α,α-trifluorotoluene in female Hsd:Sprague Dawley® SD® rats based on increased incidences of C-cell adenoma in the thyroid gland, increased incidences of benign pheochromocytoma in the adrenal medulla, increased incidences of adenocarcinoma in the uterus, and increased incidences of stromal polyp in the uterus. There was *clear evidence of carcinogenic activity* of *p*-chloro-α,α,α-trifluorotoluene in male B6C3F1/N mice based on increased incidences of hepatocellular carcinoma and hepatoblastoma in the liver. There was *clear evidence of carcinogenic activity* of

p-chloro-α,α,α-trifluorotoluene in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma in the liver. The combined incidences of adenoma or adenocarcinoma in the Harderian gland of female mice were also considered to be related to treatment.

Exposure to *p*-chloro-α,α,α-trifluorotoluene caused increased incidences of nonneoplastic lesions in the lung and liver of male and female rats and mice, in the nose of male rats, in the adrenal medulla and uterus of female rats, in the forestomach of male and female mice, and in the larynx in male mice. Exposure to *p*-chloro-α,α,α-trifluorotoluene caused increased severity of nonneoplastic lesions in the kidney of male rats.

Synonyms: Benzene, 1-chloro-4-(trifluoromethyl)-; p-chlorobenzotrifluoride; 4-chlorobenzotrifluoride; 1-chloro-4-(trifluoromethyl)benzene; 4-trifluoromethylchlorobenzene

Summary of the Two-year Carcinogenesis and Genetic Toxicology Studies of *p*-Chloro-α,α,α-trifluorotoluene

	Male Hsd:Sprague Dawley® SD® Rats	Female Hsd:Sprague Dawley® SD® Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in air	0, 100, 300, or 1,000 ppm	0, 100, 300, or 1,000 ppm	0, 100, 200, or 400 ppm	0, 100, 200, or 400 ppm
Survival rates	25/50, 21/50, 15/50, 5/50	23/50, 21/50, 25/50, 30/50	40/50, 40/50, 35/50, 28/50	38/50, 33/50, 37/50, 27/50
Body weights	1,000 ppm group at least 10% less than the chamber control group during weeks 2 through 4	1,000 ppm group at least 10% less than the chamber control group generally after week 69	Exposed groups similar to the chamber control group	Exposed groups at least 10% greater than the chamber control group: 100 ppm generally after week 69; 200 ppm after week 13; 400 ppm after week 2
Nonneoplastic effects	Lung: inflammation, chronic (32/50, 42/50, 47/50, 45/50); fibrosis (8/50, 22/50, 28/50, 24/50); hemorrhage (11/50, 23/50, 28/50, 28/50) Liver: centrilobular, hepatocyte, hypertrophy (2/50, 17/50, 39/50, 47/50); fatty change (0/50, 3/50, 7/50, 26/50); eosinophilic focus (1/50, 5/50, 6/50, 8/50) Kidney: severity of nephropathy (2.5, 2.7, 3.3, 3.4) Nose: exudate (8/50, 12/50, 12/50, 18/50)	Lung: inflammation, chronic (35/50, 42/50, 48/50, 46/50); fibrosis (11/50, 17/50, 24/50, 28/50); hemorrhage (12/50, 11/50, 18/50, 26/50) Liver: centrilobular, hepatocyte, hypertrophy (0/50, 1/50, 10/50, 45/50); fatty change (2/50, 4/50, 11/50, 10/50); mixed cell focus (6/50, 6/50, 8/50, 18/50); clear cell focus (16/50, 15/50, 23/50, 38/50) Adrenal medulla: hyperplasia (17/49, 25/50, 34/50, 36/50) Uterus: endometrium, atypical hyperplasia (0/50, 0/50, 1/50, 3/50)	Lung: alveolar/bronchiolar epithelium, hyperplasia (0/50, 49/50, 50/50, 48/50); peribronchiolar, fibrosis (0/50, 45/50, 47/50, 44/50) Liver: eosinophilic focus (11/50, 14/50, 18/50, 21/50); centrilobular, hepatocyte, hypertrophy (0/50, 8/50, 19/50, 49/50); hepatocyte, multinucleated (2/50, 8/50, 19/50, 49/50); hepatocyte, necrosis (3/50, 4/50, 3/50, 15/50); intrahepatocellular erythrocytes (0/50, 1/50, 6/50, 15/50) Forestomach: inflammation (4/50, 12/50, 7/50, 12/48) Larynx: squamous epithelium, hyperplasia (3/50, 4/50, 6/50, 11/50)	Lung: alveolar/bronchiolar epithelium, hyperplasia (0/50, 49/50, 49/50, 50/50); peribronchiolar, fibrosis (0/50, 44/50, 44/50, 48/50) Liver: eosinophilic focus (4/50, 8/50, 24/50, 31/50); centrilobular, hepatocyte, hypertrophy (0/50, 4/50, 5/50, 40/50); hepatocyte, multinucleated (0/50, 2/50, 2/50, 25/50); hepatocyte, necrosis (2/50, 1/50, 3/50, 10/50) Forestomach: epithelium, hyperplasia (1/50, 3/50, 5/50, 14/50)

	Male Hsd:Sprague Dawley® SD® Rats	**Female Hsd:Sprague Dawley® SD® Rats**	**Male B6C3F1/N Mice**	**Female B6C3F1/N Mice**
Neoplastic effects	Thyroid gland: C-cell adenoma (2/50, 5/49, 3/49, 12/50)	Thyroid gland: C-cell adenoma (2/50, 8/50, 8/50, 14/50) Adrenal medulla: benign pheochromocytoma (0/49, 3/50, 4/50, 6/50) Uterus: adenocarcinoma (1/50, 1/50, 0/50, 5/50); stromal polyp (7/50, 9/50, 16/50, 12/50)	Liver: hepatocellular carcinoma (8/50, 19/50, 16/50, 35/50); hepatoblastoma (1/50, 1/50, 1/50, 15/50)	Liver: hepatocellular adenoma (12/50, 14/50, 24/50, 34/50); hepatocellular carcinoma (7/50, 8/50, 12/50, 34/50); hepatoblastoma (0/50, 0/50, 1/50, 8/50) Harderian gland: adenoma (2/50, 6/50, 6/50, 8/50); adenoma or adenocarcinoma (2/50, 6/50, 9/50, 8/50)
Equivocal findings	Lung: alveolar/bronchiolar adenoma or carcinoma (0/50, 2/50, 0/50, 3/50)	None	None	None
Level of evidence of carcinogenic activity	Some evidence	Some evidence	Clear evidence	Clear evidence
Genetic toxicology				
Bacterial gene mutations:	Negative in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 with or without S9 and negative in *E. coli* WP2 uvrA/pKM101			
Micronucleated erythrocytes				
Rat peripheral blood in vivo:	Negative in males and females			
Mouse peripheral blood in vivo:	Positive in males; negative in females			

Introduction

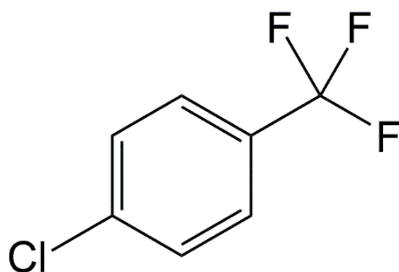


Figure 1. *p*-Chloro-α,α,α-trifluorotoluene (CASRN 98-56-6; Chemical Formula: $C_7H_4ClF_3$; Molecular Weight: 180.56)

Synonyms: Benzene, 1-chloro-4-(trifluoromethyl)-; p-chlorobenzotrifluoride; 4-chlorobenzotrifluoride; 1-chloro-4-(trifluoromethyl)benzene; 4-trifluoromethylchlorobenzene.

Chemical and Physical Properties

p-Chloro-α,α,α-trifluorotoluene is part of a group of structurally related compounds, benzotrifluorides (BTFs). *p*-Chloro-α,α,α-trifluorotoluene is a combustible, clear, colorless liquid with an aromatic odor[1; 2]. *p*-Chloro-α,α,α-trifluorotoluene has a melting point of –36°C, a flash point of 43°C, a boiling point of 139°C, and a vapor pressure of 7.63 mm Hg at 25°C[1-4]. *p*-Chloro-α,α,α-trifluorotoluene reacts with photochemically produced hydroxyl radicals following prolonged exposure to air[5].

Production, Use, and Human Exposure

p-Chloro-α,α,α-trifluorotoluene is a solvent used in paints and coatings (e.g., epoxy, acrylics) and as an industrial intermediate in the production of other chemicals (e.g., herbicides, dyes, pharmaceuticals)[4]. Domestic production and import of *p*-chloro-α,α,α-trifluorotoluene was reported to be 10 to 50 million pounds in 1986, 1990, 1994, and 1998; in 2011, the reported production/import was 29 million pounds, over 95% of which was imported[6]. *p*-Chloro-α,α,α-trifluorotoluene is considered an exempt volatile organic compound with non-ozone depleting status, resulting in higher end-user applications than other solvents[4]. End-user applications of *p*-chloro-α,α,α-trifluorotoluene, including automobile body coating and part cleaning, likely lead to higher inhalation and dermal exposure than use in industrial settings.

Occupational exposure has been assessed by the National Institute for Occupational Safety and Health (NIOSH) in vehicle and paint manufacturing plants using industrial hygiene sampling methods across a number of job tasks in each industry[7]. The geometric mean of personal exposures was 2.1 ppm and 0.7 ppm at the vehicle and painting manufacturing plants, respectively. Most personal exposures were lower than 10 ppm, with the exception of a single interior refurbishment worker in one of the vehicle manufacturing plants (12.2 ppm). This study did not assess exposures in other occupational settings (e.g., autobody repair), which may be higher than was observed by Lee et al.[7].

Environmental exposure to *p*-chloro-α,α,α-trifluorotoluene may result following spillage or improper disposal in industrial settings. Industrial releases of *p*-chloro-α,α,α-trifluorotoluene by

Occidental Chemical Corporation in the early 1990s in Niagara Falls, NY, resulted in detection of *p*-chloro-α,α,α-trifluorotoluene in groundwater up to 4.6 mg/L[8]. *p*-Chloro-α,α,α-trifluorotolu-ene was detected in various media near the Occidental Chemical Corporation in the 1980s, including fish (0.17 to 2.0 ppm) and Niagara River sediment (up to 2 ppm) from the Bloody Run Creek, Niagara River Watershed[9; 10]. Near the Diaz Chemical Corporation in Holley, NY, groundwater levels of *p*-chloro-α,α,α-trifluorotoluene were reported to be 49 mg/L[11]. *p*-Chloro-α,α,α-trifluorotolu-ene was detected in groundwater samples in Vicenza, Italy, at concentrations up to 1 mg/L due to industrial contamination[12]. Water samples from Love Canal, NY, have qualitatively tested positive for *p*-chloro-α,α,α-trifluorotoluene[13].

Regulatory Status

The United States Environmental Protection Agency set *p*-chloro-α,α,α-trifluorotoluene preliminary remediation goals for noncancer endpoints for residential soil (1.2×10^3 mg/kg), industrial soil (1.2×10^4 mg/kg), air (73 µg/m^3), and drinking water (7.3×10^2 µg/L) contamination related to superfund sites[14]. These values were based on a chronic reference dose of 20 µg/kg body weight per day[15] generated from an oral gavage study in rats that reported renal tube degeneration with a no-observed-adverse-effect-level of 15 mg/kg per day[16]. There is currently no federal occupational exposure guideline; however, a United States importer of *p*-chloro-α,α,α-trifluorotoluene has issued a permissible exposure limit of 20 ppm (8-hour time-weighted average)[17]. New York has implemented a water quality limit of 5 µg/L *p*-chloro-α,α,α-trifluorotoluene in groundwater[18]. No federal regulations exist for occupational exposure, drinking water, or air contamination.

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Partition coefficients for blood, fat, brain, and muscle have been reported in the literature for *p*-chloro-α,α,α-trifluorotoluene following inhalation exposure[19]; however, no other absorption, distribution, metabolism or excretion studies have been reported for inhalation exposure.

Following a single oral gavage dose of 1 mg [^{14}C] *p*-chloro-α,α,α-trifluorotoluene/kg body weight, expiration of the parent compound was the major route of excretion (62% to 68%, 96 hours post-gavage) in male and female Sprague Dawley rats and urinary and fecal excretion accounted for 14% to 15% and 3% to 5%, respectively[20]. Only 1% of the administered dose was detected in the carcass 96 hours post-gavage. In the same study, at a higher single oral gavage dose (104 mg/kg, female rats only), expiration accounted for 82% of the administered dose. In urine, the major compounds detected were glucuronide conjugates of hydroxyl-metabolites; mercapturic acid metabolites were also detected, accounting for less than 0.5% of the administered dose for both doses and sexes. The highest tissue concentration of radioactivity was found in abdominal adipose, with female adipose levels 17 times higher than those in males administered the same dose.

The absorption half-life of *p*-chloro-α,α,α-trifluorotoluene in male F344/N rats following a single oral gavage dose (0, 10, 50, or 400 mg/kg) has been determined using different vehicles[21]. The vehicle used for oral gavage influenced time to maximum blood concentration (T_{max}) and the absorption rate constant; α-cyclodextrin had a larger absorption rate constant and shorter T_{max}

than corn oil in all doses examined. However, maximum concentration and area under the curve were similar between vehicles and increased proportionally to the dose administered. Similarly, there was no difference in elimination rate constant, volume of distribution, or total clearance rate between dose or vehicle. Bioavailability estimated following administration was approximately 100% despite the vehicle used and suggests very little or no first pass metabolism. Following repeated dosing with *p*-chloro-α,α,α-trifluorotoluene in corn oil at 10, 50, 400, or 1,000 mg/kg, levels of *p*-chloro-α,α,α-trifluorotoluene in blood and liver were similar in male and female rats; however, male rats had much higher concentrations of *p*-chloro-α,α,α-trifluorotoluene in the kidney, compared to females at all doses examined.

p-Chloro-α,α,α-trifluorotoluene inhalation exposure for 13 weeks, at doses up to 250 ppm in Sprague Dawley rats, has been reported to cause increases in various cytochrome P450 (CYP) isoforms; a substantial increase in CYP2B activity was reported, along with moderate, statistically significant increases in activity for CYP2E1 (males only), CYP3A (females only), CYP1A1, CYP1A2, and total CYP[22].

Humans

Human partition coefficients for blood, liver, fat, brain, and kidney have been estimated based on inhalation data from rats, predicting the highest percentage of inhaled dose in fat, regardless of exposure concentration[23]. No other published data are available regarding the absorption, distribution, metabolism, or excretion of *p*-chloro-α,α,α-trifluorotoluene in humans.

Toxicity

Experimental Animals

Short-term studies of *p*-chloro-α,α,α-trifluorotoluene following oral dosing indicate that liver, kidney, and adrenal cortex are common target organs. Short-term oral gavage dosing of *p*-chloro-α,α,α-trifluorotoluene, up to 1,000 mg/kg per day, resulted in increased kidney and liver weights in rats and mice[21; 24]. Male and female F344/N rats and $B6C3F_1$ mice administered 1,000 mg/kg per day for 14 days had consistently higher liver weights compared to controls, ranging from 55% to 82% higher than controls[21]. Higher liver weights were also observed in Sprague Dawley rats (1,000 mg/kg per day) following 28 days of dosing[24]. Histologic findings in the liver were not consistent between the studies, possibly due to differences in the strains/stocks of animals used. Hepatocyte hypertrophy was observed in the NTP[21] study (all 1,000 mg/kg animals), while no histologic liver findings were reported by Macri et al.[24]. Subchronic (3-month) oral gavage dosing of Fischer 344 rats with *p*-chloro-α,α,α-trifluorotoluene (up to 500 mg/kg per day) also resulted in increased liver weights with accompanying centrilobular hypertrophy[25].

In a 14-day study[21], oral dosing of *p*-chloro-α,α,α-trifluorotoluene caused kidney nephropathy in all male rats (doses of 50 mg/kg per day or greater), accompanied by a dose-dependent increase in kidney α2u-globulin (five-fold higher at 1,000 mg/kg). This was consistent with the Macri et al.[24] study that reported hyaline droplet necrosis of the proximal convoluted tubules in all 1,000 mg/kg male rats and some male rats at lower doses. Adrenal cortex cytoplasmic vacuolization was observed in male rats receiving 400 mg/kg or greater and female rats receiving 1,000 mg/kg in the NTP[21] study and in male rats receiving 1,000 mg/kg in the Macri et al.[24]

study. Clinical chemistry endpoints including increased serum cholesterol, triglycerides (males), and lactate dehydrogenase (females) were also affected in Sprague Dawley rats[24].

Similar to oral dosing, short-term (4-week) whole body inhalation exposure of Sprague Dawley rats to *p*-chloro-α,α,α-trifluorotoluene (up to 1,044 ppm) resulted in changes in clinical chemistry parameters and liver and kidney weights[26]. Increased liver weights occurred in males and females exposed to 492 ppm or greater (25% to 48%) and increased kidney weights also occurred, with less consistency, in males exposed to 492 ppm and females exposed to 492 ppm or greater. Increased incidences and severities of hepatocyte hypertrophy occurred in a concentration-dependent manner in males and females. During exposure, increased activity at exposures of 262 ppm or greater and hyperactivity and tremors at exposures of 1,044 ppm occurred in both sexes, more often in females. These findings prompted a more comprehensive 13-week neurotoxicity study involving whole body inhalation exposure of Sprague Dawley rats to *p*-chloro-α,α,α-trifluorotoluene concentrations of 0, 10, 31, or 252 ppm[27]. No treatment-related effects were noted in clinical observations or function assessments during the study, and organ weight changes that occurred at 4 weeks were not repeated (except increased male kidney weights). Hepatocyte hypertrophy was present in males and females (3/10) in the highest exposure concentration group.

Acute oral gavage and inhalation studies have reported neurologic and behavioral effects. A 4-hour inhalation exposure in Sprague Dawley rats, up to approximately 9,000 ppm, was reported to cause muscle spasms, labored breathing, excessive salivation, limb ataxia, and hypersensitivity to touch; upon necropsy, these animals exhibited dark lungs with white foci and hemorrhagic staining of the thymus[28]. A single oral gavage dose (~6.8 g/kg) in Sprague Dawley rats caused hypoactivity, tremors, ataxia, decreased limb tone, and loss of the righting reflex; at necropsy, lung discoloration with red foci and kidney discoloration were noted[29]. Acute dermal exposure (2 mL/kg body weight) in New Zealand White rabbits caused skin erythema and edema that resolved following exposure, as well as macroscopic effects in the kidneys and lungs (red foci) and abscesses that were noted at necropsy[30].

Humans

Human exposure to *p*-chloro-α,α,α-trifluorotoluene may cause irritation at sites of contact (skin, eye, respiratory tract, mucous membranes), and inhalation exposure may cause labored breathing, dizziness, and drowsiness according to the safety data sheet[17]. Other effects have been reported following inhalation exposure including coughing, shortness of breath, chest pain, and edema[31].

Reproductive and Developmental Toxicity

Experimental Animals

One study has investigated the reproductive toxicity of *p*-chloro-α,α,α-trifluorotoluene[16]. Male and female Sprague Dawley rats (20/sex per group) received oral gavage doses of *p*-chloro-α,α,α-trifluorotoluene (up to 45 mg/kg per day) for 4 weeks prior to mating and throughout gestation and lactation until weaning of the F_1 pups, for a total of 76 to 83 days. F_1 pups were then dosed daily, at the same dose as their dams, beginning at weaning (postnatal day 21) and continuing for 90 days postweaning. Significant findings included increased weight gain in male

pups in all dosed groups and decreased weight gain in high-dose female pups, hematologic changes (decreased erythrocytes and hemoglobin, increased mean cell volume), and increased incidences of nonneoplastic lesions in the lung. Fecundity (pups per litter and pup survival) was higher in dosed animals compared to controls, and there was no observed effect on fertility.

Humans

No reports on the effect of *p*-chloro-α,α,α-trifluorotoluene on reproduction or development in humans were found in the literature.

Carcinogenicity

Experimental Animals

No reports on the carcinogenic potential of *p*-chloro-α,α,α-trifluorotoluene in experimental animals were found in the literature.

Humans

An epidemiologic assessment of a small cohort of workers (4,000) at the Occidental Chemical Corporation plant in Niagara, NY, reported higher than expected rates of respiratory system and stomach cancers[32]. However, it is likely that this cohort of individuals was exposed to a large mixture of chemicals, with more than 80 CAS numbers listed as keywords on the submission report.

Genetic Toxicity

There are few published reports on the genotoxicity of *p*-chloro-α,α,α-trifluorotoluene, but the available data indicate that the compound is not genotoxic. No evidence of mutagenicity was observed for *p*-chloro-α,α,α-trifluorotoluene at doses up to 1,000 µg/plate in any of several strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) in tests conducted with and without Aroclor 1254-induced rat or hamster liver S9 mix[4; 33]. Bignami and Crebelli[34] also reported negative results with *p*-chloro-α,α,α-trifluorotoluene in a test for induction of 8-azoguanine resistance in *S. typhimurium*. In addition to these two published reports, an absence of mutagenic activity was reported in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 using ß-glucoronidase-treated or untreated urine from mice exposed to up to 500 mg/kg *p*-chloro-α,α,α-trifluorotoluene for 2 days by oral gavage[5].

Generally negative results from unreviewed, unpublished studies have been reported for *p*-chloro-α,α,α-trifluoro-toluene in mammalian cell test systems[5]. No induction of trifluorothymidine resistance was seen in mouse lymphoma L5178Y cells exposed to concentrations up to 50 nL/mL *p*-chloro-α,α,α-trifluorotoluene, with or without rat liver S9 mix[5]. However, *p*-chloro-α,α,α-trifluorotoluene was reported to induce sister chromatid exchanges in L5178Y mouse lymphoma cells with and without S9 activation, but no increases in chromosomal aberrations were seen in cultured Chinese hamster ovary cells treated with *p*-chloro-α,α,α-trifluorotoluene, with or without rat liver S9 mix[5]. Furthermore, negative results were reported in an in vivo bone marrow chromosomal aberrations test in male and female Sprague Dawley rats administered a single dose of *p*-chloro-α,α,α-trifluorotoluene by oral gavage up to 5 mL/kg[5].

Study Rationale

p-Chloro-α,α,α-trifluorotoluene was nominated to NTP in 1981 by the National Cancer Institute (NCI) due to its high production volume and suspicion of carcinogenic potential. Subsequently, NTP conducted 14-day gavage studies in F344/N rats and B6C3F$_1$ mice[21]. In 2001, public comments submitted to the NTP Center for the Evaluation of Risks to Human Reproduction, suggested an evaluation of the *p*-chloro-α,α,α-trifluorotoluene literature to determine if chronic toxicity testing was warranted based on increased use of *p*-chloro-α,α,α-trifluorotoluene as a replacement for volatile organic compounds in certain solvent applications. In 2006, a representative from Kowa American Corporation, a *p*-chloro-α,α,α-trifluorotoluene importer, nominated *p*-chloro-α,α,α-trifluorotoluene for toxicologic characterization due to expanding use and lack of occupational exposure limits from the Occupational Safety and Health Administration, the NIOSH, or the American Conference of Governmental Industrial Hygienists. Additionally, the NCI and Kowa American Corporation nominated the benzotrifluoride (BTF) compound class to NTP. NTP evaluated various BTF compounds for potential testing, including benzotrifluoride and 3,4-dichlorobenzotrifluoride. Benzotrifluoride had no available production or import data for the United States, and the use of 3,4-dichlorobenzotrifluoride was restricted to industrial settings with less potential for exposure compared to *p*-chloro-α,α,α-trifluorotoluene. After evaluating the exposure and use information and the toxicology literature for several BTF compounds, NTP proposed and subsequently conducted inhalation prechronic and chronic toxicity and carcinogenicity studies of *p*-chloro-α,α,α-trifluorotoluene.

Materials and Methods

Procurement and Characterization of *p*-Chloro-α,α,α-trifluorotoluene

p-Chloro-α,α,α-trifluorotoluene was obtained from Special Materials Company (Cherry Hill, NJ, and New York, NY) in two lots. Lot 2009-01-05 was used in the 3-month studies and lot 2010-05-24 was used in the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Chemir Analytical Services (Maryland Heights, MO) and the study laboratory at Battelle Toxicology Northwest (Richland, WA) (Appendix I). Reports on analyses performed in support of the *p*-chloro-α,α,α-trifluorotoluene studies are on file at the National Institute of Environmental Health Sciences.

Both lots were clear liquid and were identified as *p*-chloro-α,α,α-trifluorotoluene by proton nuclear magnetic resonance spectroscopy and Fourier transform infrared spectroscopy. Purity of both lots was determined by elemental analyses and gas chromatography (GC) with flame ionization detection (FID). Karl Fischer titration indicated a water content of 82.4 (lot 2009-01-05) or 109.8 (lot 2010-05-24) ppm. Elemental analyses of both lots for carbon and hydrogen were consistent with theoretical values. Inductively coupled plasma spectroscopic analysis of lot 2010-05-24 indicated less than 0.002% sulfur. GC/FID indicated one major peak (peak area percents of 99.68% for lot 2009-01-05, 99.57% for lot 2010-05-24) and two impurities with areas greater than or equal to 0.1% relative to the major peak area; the two peaks were identified as 3-chlorobenzotrifluoride (0.2%, lot 2009-01-05; 0.3%, lot 2010-05-24) and 2-chlorobenzotrifluoride (0.1%, lot 2009-01-05; 0.2%, lot 2010-05-24). Analysis of both lots by high performance liquid chromatography (HPLC) with an ultraviolet light/visible light detector showed that 3,4-dihydroxybenzoic acid, a peroxide scavenger, was not present. The overall purity of each lot was determined to be greater than 99.5%.

The bulk chemical was stored in the original shipping containers at room temperature. To ensure stability, periodic reanalyses of the bulk chemical were performed during the 3-month and 2-year studies by the study laboratory using GC/FID. No degradation of the bulk chemical was detected.

Vapor Generation and Exposure System

p-Chloro-α,α,α-trifluorotoluene was pumped from a stainless steel reservoir into a heated glass column. Preheated nitrogen entered the column and assisted in vaporizing the chemical while conveying it from the generator into a vapor distribution manifold. Concentration in the manifold was determined by the chemical pump rate, dilution air flow, and nitrogen flow rates. Pressure in the distribution manifold was kept fixed to ensure constant flows through the manifold and into all chambers as the flow of vapor to each chamber was adjusted.

Heated (approximately 130°F) individual Teflon® delivery lines carried the vapor from the manifold to three-way exposure valves at the chamber inlets. The exposure valves diverted vapor delivery to the exposure chamber exhaust until the generation system stabilized and exposure could proceed. The flow rate to each chamber was controlled by a metering valve at the manifold. To initiate exposure, the chamber exposure valves were rotated to allow the

p-chloro-α,α,α-trifluorotoluene vapor to flow to each exposure chamber inlet duct where it was diluted with conditioned chamber air to achieve the desired exposure concentration.

The study laboratory designed the inhalation exposure chamber so that uniform vapor concentrations could be maintained throughout the chamber with the catch pans in place. The total active mixing volume of each chamber was 1.7 m^3. A small particle detector (Model 3022A; TSI Inc., St. Paul, MN) was used with and without animals in the exposure chambers to ensure that *p*-chloro-α,α,α-trifluorotoluene vapor, and not aerosol, was produced. No particle counts above the minimum resolvable level (approximately 200 particles/cm^3) were detected.

Vapor Concentration Monitoring

Summaries of the chamber vapor concentrations are given in Table I-2 and Table I-3. Chamber and room concentrations of *p*-chloro-α,α,α-trifluorotoluene were monitored by an on-line gas chromatograph with FID. Samples were drawn from each exposure chamber approximately every 20 minutes for the 3-month and 2-year studies during each 6-hour exposure period using Hastelloy®-C stream-select and gas-sampling valves in a separate, heated oven. The sample lines composing each sample loop were made from Teflon® tubing and were connected to the exposure chamber relative humidity sampling lines at a location close to the gas chromatograph. A vacuum regulator maintained a constant vacuum in the sample loop to compensate for variations in sample line pressure. An in-line flow meter between the vacuum regulator and the gas chromatograph allowed digital measurement of sample flow.

The on-line gas chromatograph was checked throughout each exposure day for instrument drift against an on-line standard vapor of 4-chlorotoluene in nitrogen supplied by a standard generator. The on-line gas chromatograph was recalibrated as required to meet acceptance criteria. Calibration was performed by a comparison of chamber concentration data to data from grab samples collected with sorbent tubes, extracted with toluene containing an internal standard of 4-chlorotoluene and analyzed using an off-line gas chromatograph equipped with a FID. Known volumes of chamber atmosphere were sampled at a constant flow rate ensured by a calibrated critical orifice. The off-line gas chromatograph was calibrated with gravimetrically prepared standard solutions of the test chemical containing 4-chlorotoluene as an internal standard in toluene.

Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined with and without animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 9.2 minutes. Based on experimental data, a T_{90} value of 15 minutes was selected for the 3-month studies, and a T_{90} value of 12 minutes was selected for the 2-year studies.

The uniformity of *p*-chloro-α,α,α-trifluorotoluene vapor concentration in the inhalation exposure chambers without animals present was evaluated before the 3-month and 2-year studies began; in addition, concentration uniformity with animals present in the chambers was measured once

during the 3-month studies and approximately every 3 months during the 2-year studies. The vapor concentration was measured using the on-line gas chromato-graph. Chamber concentration uniformity was maintained throughout the studies.

The persistence of *p*-chloro-α,α,α-trifluorotoluene in the chambers after vapor delivery ended was determined by monitoring the vapor concentration in the 2,000 ppm chambers in the 3-month studies and the 1,000 ppm rat and 400 ppm mouse chambers in the 2-year studies with and without animals present in the chambers. In the 3-month studies, the concentration decreased to 1% of the target concentration within 24 minutes without animals present and within 30 minutes with animals present. For the 2-year rat studies, the concentration decreased to 1% of the target concentration within 24 minutes without animals present and within 46 minutes with animals present. For the 2-year mouse studies, the concentration decreased to 1% of the target concentration within 23 minutes without animals present and within 31 minutes with animals present.

Samples of the test atmosphere from the distribution lines and the low and high exposure concentration chambers for each species were collected prior to the study without animals present and at the beginning and end of one generation day with animals present during the 3-month and 2-year studies. Additional samples were collected from the generator reservoir, and all of the samples were analyzed using GC/FID to measure the stability and purity of *p*-chloro-α,α,α-trifluorotoluene in the generation and delivery system. To assess whether impurities or degradation products co-eluted with *p*-chloro-α,α,α-trifluoro-toluene or the solvent, a second GC/FID analysis of samples from the distribution line, 125 ppm and 2,000 ppm chambers (3-month studies), 100 ppm and 1,000 ppm rat chambers (2-year studies), 100 ppm and 400 ppm mouse chambers (2-year studies), and the generator reservoir was performed. The chamber atmosphere was monitored for 3,4-dihydroxybenzoic acid, a potential degradation product of *p*-chloro-α,α,α-trifluorotoluene, using HPLC prior to and during the 3-month and 2-year studies.

No evidence of degradation of *p*-chloro-α,α,α-trifluoro-toluene was noted in any part of the exposure system in any of the samples collected prior to or during the 3-month and 2-year studies. During the 3-month studies, 3-chlorobenzotrifluoride (0.2%) was the only impurity detected in atmosphere or generator reservoir samples with an area greater than 0.1% of the total peak area. During the 2-year studies, 3-chlorobenzotrifluoride (0.3%) and 2-chlorobenzotrifluoride (0.2%) were the only impurities detected in the atmosphere or generator reservoir samples with areas greater than 0.1% of the total peak area. No 3,4-dihydroxybenzoic acid was detected in the exposure chamber atmosphere samples at greater than or equal to 0.1%.

Animal Source

Male and female Hsd:Sprague Dawley® SD® rats were obtained from Harlan Laboratories, Inc. (Livermore, CA), and B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc. (Germantown, NY), for the 3-month and 2-year studies.

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Studies were approved by the Battelle Toxicology Northwest Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Three-month Studies

The 3-month studies were conducted to evaluate the cumu-lative toxic effects of repeated exposure to *p*-chloro-α,α,α-trifluorotoluene and to determine the appropriate exposure concentrations to be used in the 2-year studies.

On receipt, the rats and mice were 4 to 5 weeks old. Animals were quarantined for 13 (rats) or 12 (mice) days and were 6 weeks (rats) or 5 to 6 weeks (mice) old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K). All test results were negative.

Groups of 10 male and 10 female rats and mice were exposed by whole body inhalation to *p*-chloro-α,α,α-trifluorotoluene vapor at concentrations of 0, 125, 250, 500, 1,000, or 2,000 ppm, 6 hours plus T_{90} (15 minutes) per day, 5 days per week for 14 weeks. Exposure concentrations used in the 3-month studies were selected based on the available inhalation literature. Newton et al.[27] reported on 4-week and 3-month inhalation toxicity studies in Sprague Dawley rats. In the 4-week study, relatively little toxicity was observed at exposure concentrations up to 1,000 ppm; however, the highest concentration selected for the 3-month study was 250 ppm. In the 3-month study, a low incidence of hepatocyte hypertrophy was the only histopathologic finding reported. No prechronic mouse studies were available for use in selecting exposure concentrations. Based on the available information, 2,000 ppm was selected as the highest exposure concentration in these NTP 3-month studies for both rats and mice, with twofold exposure concentration spacing. Feed was available ad libitum except during exposure periods, and water was available ad libitum. Rats and mice were housed individually. Clinical observations were recorded on day 8, weekly thereafter, and at study termination. The animals were weighed initially, on day 8, weekly thereafter, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 3-month studies, blood was collected from the retroorbital plexus of rats and the retroorbital sinus of mice for hematology and clinical chemistry (rats only) analyses. For the hematology samples, blood was collected in tubes containing potassium EDTA; for the clinical chemistry samples, the blood was collected in a tube devoid of anticoagulant but containing a separator gel for serum. An ADVIA 120 (Siemans Medical Solutions Diagnostics, Tarrytown, NY) was used to determine packed cell volume; hemoglobin concentration; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; and leukocyte counts and differential. Manual hematocrit values were determined using a microcentri-fuge (Heraeus Haemofuge, Heraeus Holding GmbH; Hanau, Germany) and a Damon/IEC capillary reader (International Equipment Company, Needham Heights, MA) for comparison to ADVIA 120 values for packed cell volume. Blood smears were stained with a Romanosky-type aqueous stain in a Wescor 7120 aerospray slide stainer (Wescor, Inc., Logan, UT). Cell morphology was assessed using the blood smears. Manual leukocyte differentials were performed on the blood smears when populations were flagged by the ADVIA 120. Blood

samples for clinical chemistry analyses were analyzed using a Cobas c 311 analyzer (Roche Diagnostic Corporation, Indianapolis, IN). The parameters measured are listed in Table 1.

Table 1. Experimental Design and Materials and Methods in the Inhalation Studies of *p*-Chloro-α,α,α-trifluorotoluene

Three-month Studies	Two-year Studies
Study Laboratory	
Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)
Strain and Species	
Hsd:Sprague Dawley® SD® rats B6C3F1/N mice	Hsd:Sprague Dawley® SD® rats B6C3F1/N mice
Animal Source	
Rats: Harlan Laboratories, Inc. (Livermore, CA) Mice: Taconic Farms, Inc. (Germantown, NY)	Rats: Harlan Laboratories, Inc. (Livermore, CA) Mice: Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
Rats: 13 days Mice: 12 days	12 days
Average Age When Studies Began	
Rats: 6 weeks Mice: 5 to 6 weeks	Rats: 6 weeks Mice: 5 to 6 weeks
Date of First Exposure	
October 26, 2009	Rats: January 17, 2011 Mice: January 31, 2011
Duration of Exposure	
6 hours plus T_{90} (15 minutes) per day, 5 days per week, for 14 weeks	6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 104 to 105 weeks
Date of Last Exposure	
Rats: January 25 (males) or 26 (females), 2010 Mice: January 27 (males) or 28 (females), 2010	Rats: January 13-14 (males) or 15-16 (females), 2013 Mice: January 27-29 (males) or 29-31 (females), 2013
Necropsy Dates	
Rats: January 26 (males) or 27 (females), 2010 Mice: January 28 (males) or 29 (females), 2010	Rats: January 14-15 (males) or 16-17 (females), 2013 Mice: January 28-30 (males) or January 30-February 1 (females), 2013
Average Age at Necropsy	
Rats: 19 weeks Mice: 19 to 20 weeks	Rats: 110 to 111 weeks Mice: 109 to 111 weeks
Size of Study Groups	
10 males and 10 females	50 males and 50 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies

Three-month Studies	Two-year Studies
Animals per Cage	
1	1
Method of Animal Identification	
Tail tattoo	Tail tattoo
Diet	
Irradiated NTP-2000 wafers (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, except during exposure periods, changed weekly	Same as 3-month studies
Water	
Tap water (Richland, WA, municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available ad libitum	Same as 3-month studies
Cages	
Stainless steel wire bottom (Lab Products, Inc., Seaford, DE), changed and rotated weekly	Same as 3-month studies
Cageboard	
Techboard® Ultra untreated paper excreta pan liner (Shepherd Specialty Papers, Watertown, TN), changed daily	Same as 3-month studies
Chamber Air Supply Filters	
Single HEPA (open stock), charcoal (RSE, Inc., New Baltimore, MI), and Purafil (Environmental Systems, Lynnwood, WA), all new at study start	Same as 3-month studies, except HEPA changed annually
Chambers	
Stainless steel, excreta pan at each of six levels (Lab Products, Inc., Seaford, DE), changed weekly, excreta pans changed daily	Same as 3-month studies
Chamber Environment	
Temperature: 75° ± 3°F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15 ± 2/hour	Same as 3-month studies
Exposure Concentrations	
0, 125, 250, 500, 1,000, or 2,000 ppm	Rats: 0, 100, 300, or 1,000 ppm Mice: 0, 100, 200, or 400 ppm
Type and Frequency of Observation	
Observed twice daily; animals were weighed initially, on day 8, weekly thereafter, and at the end of the studies; clinical observations were recorded on day 8, weekly thereafter, and at the end of the studies.	Observed twice daily. Mice and female rats were weighed initially, weekly for 13 weeks, every 4 weeks thereafter, and at the end of the studies; clinical observations were recorded at week 5, every 4 weeks thereafter, and at the end of the studies. Male rats were weighed initially, weekly for 13 weeks, every 4 weeks through week 93, every 2 weeks thereafter, and at the

Three-month Studies	Two-year Studies
	end of the study; clinical observations were recorded at week 5, every 4 weeks through week 93, every 2 weeks thereafter, and at the end of the study.
Method of Kill	
Carbon dioxide asphyxiation (70% in air)	Same as 3-month studies
Necropsy	
Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology	
Blood was collected from the retroorbital plexus of rats and the retroorbital sinus of mice at the end of the studies for hematology and clinical chemistry (rats only). *Hematology:* spun hematocrit; packed cell volume; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differential *Clinical chemistry:* urea nitrogen, creatinine, glucose, total protein, albumin, globulin, cholesterol, triglycerides, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile salts	None
Histopathology	
Complete histopathology was performed on chamber control and 2,000 ppm rats and mice. 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), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with bronchus), lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus. The adrenal gland, Harderian gland, kidney, liver, and thymus of rats and mice; testis with epididymis of male rats; mammary gland of female rats; and the forestomach and spleen of male and female mice were also examined in the remaining exposure groups.	Complete histopathology was performed on all rats and mice. 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), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung (with bronchus), lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus.

Three-month Studies	Two-year Studies
Sperm Motility and Vaginal Cytology	
At the end of the studies, spermatid and sperm samples were collected from male animals in the 0, 500, 1,000, and 2,000 ppm groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 16 consecutive days prior to the end of the studies from females exposed to 0, 500, 1,000, or 2,000 ppm for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None
Evaluation of *Hras* and *Ctnnb1* Mutations in Hepatocellular Carcinomas	
None	At necropsy, male and female mouse hepatocellular carcinomas (HCC) and normal liver tissues were obtained as formalin-fixed paraffin-embedded blocks. Hot-spot mutations in *Hras* and *Ctnnb1* genes were evaluated in HCCs arising from all *p*-chloro-α,α,α-trifluorotoluene-exposed groups (n = 62) and those arising spontaneously in chamber controls (n = 15). In addition, age-matched non-tumor livers (n = 10) from chamber controls were analyzed. There were no differences in the incidences of mutations between male and female mice, therefore the combined data from male and female mice are presented.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on rats and mice exposed to 0, 500, 1,000, or 2,000 ppm. The parameters evaluated are listed in Table 1. For 16 consecutive days prior to scheduled terminal kill, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution, and testes, vaginal tunics, and epididymides were first fixed in modified Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed by the study laboratory pathologist on chamber control and 2,000 ppm groups of rats and mice. Table 1 lists the tissues and organs routinely examined.

The sperm motility and vaginal cytology evaluations results suggested an effect on spermatogenesis, but there was no microscopic correlation found during the routine pathology examination of the tissues from the 3-month studies. Therefore, a detailed qualitative histopathologic examination was conducted on H&E-stained slides of testes and epididymides from the chamber control and 2,000 ppm groups of male rats and mice from the 3-month studies. In rats, findings in the 2,000 ppm group prompted examination of testes and epididymides from all exposure groups. In mice, no microscopic findings were recorded in the chamber control or the 2,000 ppm groups. Hence, no other exposed groups of mice were reviewed. Tubular stages of the spermatogenic cycle were taken into account in order to identify findings.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to a NTP Pathology Peer Review (PPR) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP PPR process. Final diagnoses for reviewed lesions represent a consensus of the PPR or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PPR coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman[35] and Boorman et al.[36].

Two-year Studies

Study Design

Groups of 50 male and 50 female rats were exposed by whole body inhalation to *p*-chloro-α,α,α-trifluorotoluene vapor at concentrations of 0, 100, 300, or 1,000 ppm, and 50 male and 50 female mice were exposed to concen-trations of 0, 100, 200, or 400 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 104 to 105 weeks.

Rats and mice were quarantined for 12 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 6 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 K). All test results were negative.

Rats and mice were housed individually. Feed was available ad libitum except during exposure periods, and water was available ad libitum. Cages were rotated weekly within the chambers; cages and chambers were changed weekly. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. For mice and female rats, clinical observations were recorded at week 5, every 4 weeks thereafter, and at study termination; body weights were recorded on day 1, weekly for 13 weeks, every 4 weeks thereafter, and at study termination. For male rats, clinical observations were recorded at week 5, every 4 weeks through week 93, every 2 weeks thereafter, and at study termination; body weights were recorded on day 1, weekly for 13 weeks, every 4 weeks through week 93, every 2 weeks thereafter, and at study termination.

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 (except eyes were first fixed in Davidson's solution, and testes, vaginal tunics, and epididymides were first fixed in modified Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin 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 1.

At necropsy, normal liver samples and hepatocellular carcinomas (HCC) from chamber control and B6C3F1/N mice exposed to *p*-chloro-α,α,α-trifluorotoluene were fixed in 10% neutral buffered formalin and processed into paraffin blocks. The formalin-fixed paraffin-embedded normal liver tissues from chamber controls (n = 10; five males and five females) and HCCs arising spontaneously (n = 15; eight males and seven females) or from *p*-chloro-α,α,α-trifluorotoluene-exposed groups (n = 62; 100 ppm, 10 males and eight females; 200 ppm, 10 males and 10 females; 400 ppm, 12 males and 12 females) were examined for hot-spot mutations in *Hras* and *Ctnnb1* genes (Appendix L).

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent QA 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 QA pathologist evaluated slides from all tumors and all potential target organs, which included the adrenal gland, forestomach, kidney, liver, lung, and thyroid gland in male and female rats; uterus in female rats; and forestomach, Harderian gland, small intestine, kidney, larynx, liver, and lung in male and female mice.

The QA 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 QA pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and QA pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the QA pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups. 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[35] and Boorman et al.[36]. 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.[37].

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier[38] and is presented in the form of graphs. Animals found dead of other than natural causes were censored; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's[39] method for testing two groups for equality and Tarone's[40] 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 Table A-1, Table A-3, Table B-1, Table B-3, Table C-1, Table C-4, Table D-1, and Table D-5 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 (Table A-2, Table B-2, Table C-2, and Table D-2) 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. Table A-2, Table B-2, Table C-2, and Table D-2 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 kill.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test[41-43] 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 kill; if the animal died prior to terminal kill 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[41]. 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[41] following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F$_1$ mice[44]. Bailer and Portier[41] 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[45].

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 = 0.01 N).

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[46] and Williams[47; 48]. Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[49] (as modified by Williams[50]) and Dunn[51]. Jonckheere's test[52] 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[53] were examined by NTP personnel, and implausible values were eliminated from the analysis. Tests for extended periods of estrus, diestrus, metestrus, and proestrus, as well as skipped estrus and skipped diestrus, were constructed based on a Markov chain model proposed by Girard and Sager[54]. For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within each stage as well as for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

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 control database must be generally similar. Significant factors affecting the background incidences of neoplasms at a variety of sites are diet, sex, strain/stock, and route of exposure. The NTP historical control database contains all 2-year studies for each species, sex, and strain/stock with histopathology findings in control animals completed within the most recent 5-year period[55-57]. In general, the historical control database for a given study includes studies using the same route of administration, and the overall incidences of neoplasms in controls for all routes of administration are included for comparison, including the current mouse study. Historical control

data for rats in this Technical Report are limited to all routes of administration because the current study is the only 2-year inhalation study in Hsd:Sprague Dawley® SD® rats conducted by NTP in the most recent 5-year period and include the concurrent controls and control animals from one other study.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations[58]. In addition, the 3-month and 2-year study reports were audited retrospectively by an independent QA contractor against study records submitted to the NTP Archives. 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.

Genetic Toxicology

The genetic toxicity of *p*-chloro-α,α,α-trifluorotoluene was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increases in the frequency of micronucleated erythrocytes in rat and mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division[59; 60]. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term in vitro and in vivo genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity[61] and the somatic mutation theory of cancer[62; 63]. However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity corresponds well with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites[64]. A positive response in the *Salmonella* test was shown to be the most predictive in vitro (89% of the *Salmonella* mutagens are rodent carcinogens)[65; 66]. Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute in vivo bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test[67; 68]. However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent

carcinogenicity studies[69]. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of in vivo genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

Results

Data Availability

The National Toxicology Program (NTP) evaluated all study data. Data relevant for evaluating toxicological findings are presented here. All study data are available in the NTP Chemical Effects in Biological Systems (CEBS) database: https://doi.org/10.22427/NTP-DATA-TR-594.

Rats

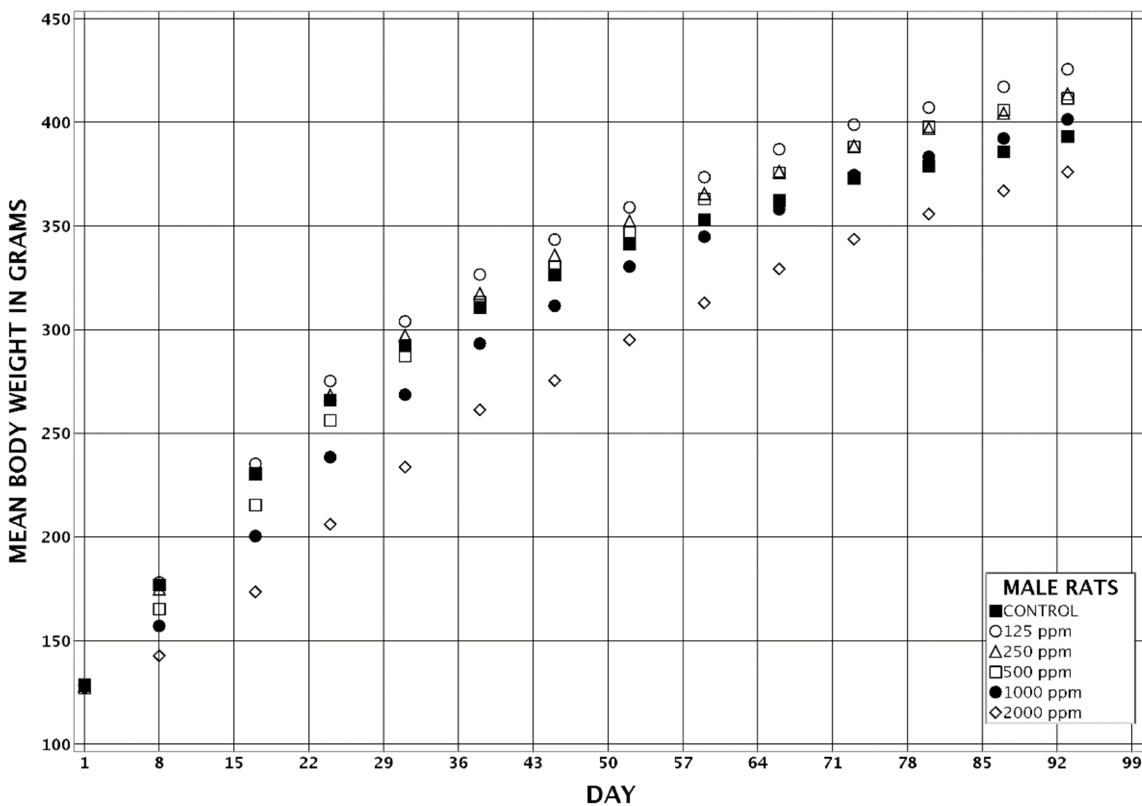
Three-month Study

All animals survived to the end of the study except one 2,000 ppm female that was removed early due to a sublingual abscess unrelated to chemical exposure (Table 2); the animal also had clinical observations of abnormal breathing, thinness, and nasal/eye discharge. The final mean body weights and mean body weight gains of females exposed to 500 ppm or greater were significantly greater than those of the chamber controls (Table 2; Figure 2). The increases in the final mean body weight and mean body weight gain of males exposed to 125 ppm were not considered biologically relevant because they were within 10% of chamber controls and only occurred at the lowest exposure concentration. Clinical observations occurred primarily in 2,000 ppm males and females and included nasal and eye discharge, tremors, and lethargy after exposure. Clinical observations resolved by the mornings following exposure and were no longer apparent by week 4.

Table 2. Survival and Body Weights of Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

Concentration (ppm)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	129 ± 2	393 ± 7	264 ± 8	–
125	10/10	128 ± 2	426 ± 5*	298 ± 6*	108
250	10/10	128 ± 2	414 ± 11	286 ± 10	105
500	10/10	127 ± 2	412 ± 11	285 ± 10	105
1,000	10/10	129 ± 2	401 ± 7	273 ± 7	102
2,000	10/10	129 ± 2	376 ± 6	247 ± 6	96
Female					
0	10/10	118 ± 1	240 ± 5	122 ± 4	–
125	10/10	119 ± 2	249 ± 5	130 ± 5	104
250	10/10	119 ± 2	255 ± 6	136 ± 4	106
500	10/10	118 ± 2	259 ± 5*	141 ± 5*	108
1,000	10/10	118 ± 1	267 ± 9**	149 ± 8**	111
2,000	9/10[c]	117 ± 2	294 ± 8**	177 ± 8**	123

*Significantly different ($P \leq 0.05$) from the chamber control group by Williams' or Dunnett's test.
**$P \leq 0.01$.
[a]Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[b]Number of animals surviving at 14 weeks/number initially in group.
[c]Week of death: 7.



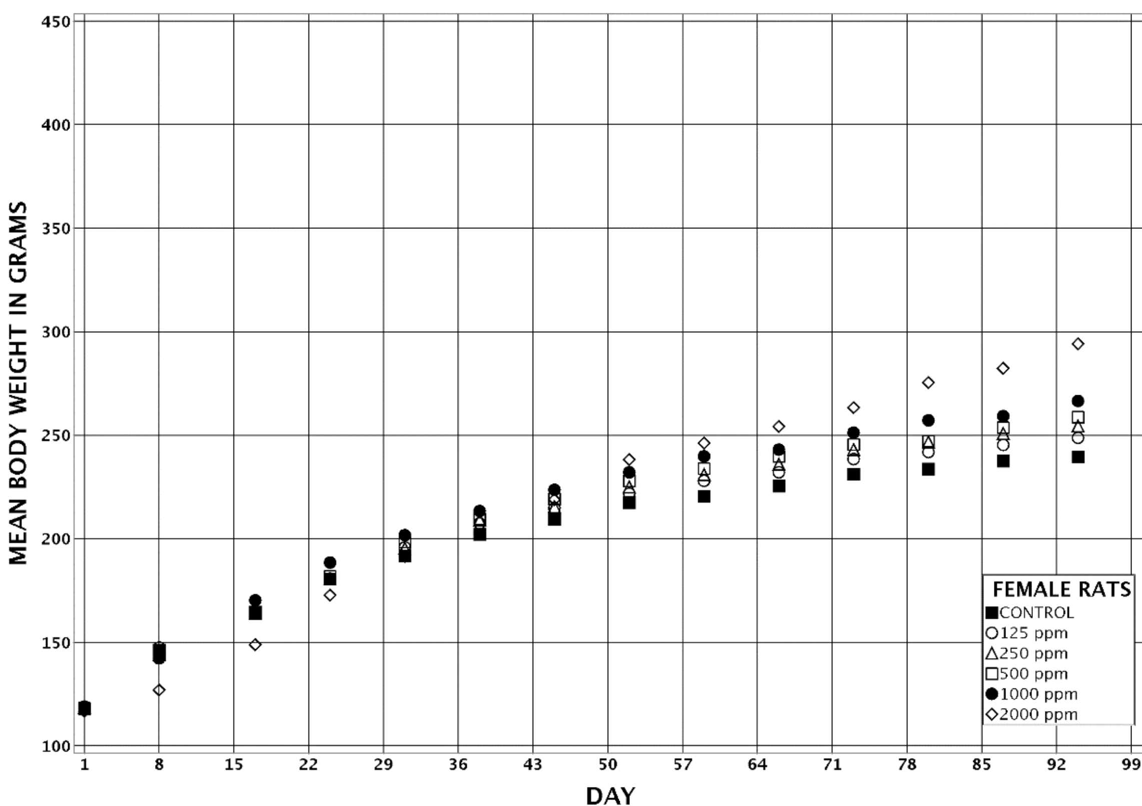


Figure 2. Growth Curves for Rats Exposed to *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Three Months

The leukocyte count was significantly decreased in 2,000 ppm females, and the lymphocyte counts were significantly decreased in 2,000 ppm males and females (Table F-1). In addition, the neutrophil counts were significantly increased in males exposed to 500 ppm or greater. This combination of changes is consistent with a stress leukogram, which is due to the chronic production of endogenous corticosteroids. It is also plausible that the increases in male neutrophil counts were due to mild inflammation secondary to the observed hepatic necrosis; this is supported by the significantly increased globulin concentrations in 1,000 and 2,000 ppm males. Total protein and globulin concentrations were also significantly increased in 2,000 ppm females.

Alanine aminotransferase and sorbitol dehydrogenase activities were significantly increased in 2,000 ppm males and females (Table 3 and Table F-1). Alkaline phosphatase activities were significantly increased in all exposed male groups and in females exposed to 250 ppm or greater, and bile salt concentrations were significantly increased in females exposed to 500 ppm or greater.

There were significant exposure concentration-dependent increases in cholesterol and triglyceride concentrations in males exposed to 500 ppm or greater (Table 3 and Table F-1). In females, cholesterol concentrations were significantly increased in groups exposed to 250 ppm or greater and triglyceride concentrations were significantly increased in the 1,000 and 2,000 ppm groups. Although the exact mechanism is not known, these increases were most likely due to alterations in lipid metabolism and the toxicologic significance is unknown.

Table 3. Selected Clinical Chemistry Data for Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
Male						
n	10	10	10	10	10	10
Alanine aminotransferase (IU/L)	55 ± 2	53 ± 2	52 ± 2	52 ± 2	59 ± 3	73 ± 4*
Sorbitol dehydrogenase (IU/L)	13 ± 1	12 ± 1	14 ± 1	15 ± 0	16 ± 1	18 ± 1**
Alkaline phosphatase (IU/L)	165 ± 6	192 ± 9*	229 ± 10**	261 ± 15**	238 ± 13**	247 ± 17**
Cholesterol (mg/dL)	132 ± 6	135 ± 4	137 ± 6	149 ± 5*	182 ± 6**	268 ± 15**
Triglycerides (mg/dL)	71 ± 6	82 ± 5	74 ± 4	96 ± 8*	118 ± 11**	137 ± 6**
Female						
n	10	10	10	10	10	9
Alanine aminotransferase (IU/L)	49 ± 1	48 ± 2	48 ± 3	50 ± 1	51 ± 2	62 ± 4**
Sorbitol dehydrogenase (IU/L)	11 ± 1	10 ± 0	12 ± 1	12 ± 0	13 ± 1	18 ± 2**
Alkaline phosphatase (IU/L)	12 1± 6	147 ± 12	185 ± 10**	201 ± 12**	227 ± 12**	216 ± 15**
Bile salts (μmol/L)	12.5 ± 5.5	16.0 ± 2.5	13.6 ± 4.0	33.8 ± 6.5**	33.5 ± 6.0**	14.7 ± 3.1**
Cholesterol (mg/dL)	110 ± 6	117 ±2	128 ± 3*	141 ± 5**	189 ± 6**	261 ± 10**
Triglycerides (mg/dL)	51 ± 4	53 ± 6	44 ± 4	58 ± 4	83 ± 5**	115 ± 9**

*Significantly different ($P \leq 0.05$) from the chamber control group by Shirley's test.
**$P \leq 0.01$.
[a]Data are given as mean ± standard error. Statistical tests were performed on unrounded data.

There were significant exposure concentration-related increases in the absolute and relative liver weights of males exposed to 250 ppm or greater; the absolute liver weight of the 2,000 ppm group was 180% of that of the chamber controls (Table 4 and Table G-1). The absolute liver weight of 125 ppm males was also significantly increased, but the relative liver weight was not significantly increased. The absolute and relative liver weights of females exposed to 500 ppm or greater were significantly increased compared to those of the chamber controls. Among females, the mean absolute liver weight was highest in the 2,000 ppm group (240% of that in the chamber controls).

Absolute kidney weights were significantly increased in all exposed groups of males (up to 126% of the chamber control group in the 1,000 ppm group), and relative kid-ney weights were significantly increased in males exposed to 250 ppm or greater (Table 4 and Table G-1). In females, the absolute kidney weights were significantly increased in groups exposed to 250 ppm or greater, but the relative kidney weights were not significantly increased.

There was a significant decrease in the relative thymus weight in 2,000 ppm females, but there was no change in absolute thymus weight and no histologic lesions associated with the change in relative weight (Table 4 and Table G-1).

Absolute heart weights were significantly increased in 125 ppm males and in females exposed to 500 ppm or greater, but there were no changes in relative heart weights and these increases were not considered to be related to chemical exposure (Table G-1).

Table 4. Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	393 ± 7	426 ± 5*	414 ± 11	412 ± 11	401 ± 7	376 ± 6
R. Kidney						
Absolute	1.28 ± 0.04	1.46 ± 0.02**	1.48 ± 0.03**	1.57 ± 0.04**	1.61 ± 0.04**	1.58 ± 0.06**
Relative	3.24 ± 0.05	3.44 ± 0.06	3.57 ± 0.06**	3.82 ± 0.07**	4.00 ± 0.05**	4.20 ± 0.14**
Liver						
Absolute	12.22 ± 0.38	13.74 ± 0.21*	14.41 ± 0.56**	16.56 ± 0.65**	19.34 ± 0.49**	22.02 ± 0.69**
Relative	31.03 ± 0.56	32.32 ± 0.50	34.79 ± 0.83**	40.18 ± 1.10**	48.25 ± 1.26**	58.48 ± 1.29**
Female						
n	10	10	10	10	10	9
Necropsy body wt	240 ± 5	249 ± 5	255 ± 6	259 ± 5*	267 ± 9**	294 ± 8**
R. Kidney						
Absolute	0.84 ± 0.01	0.89 ± 0.02	0.94 ± 0.03*	0.94 ± 0.02*	0.94 ± 0.04*	1.06 ± 0.03**
Relative	3.51 ± 0.05	3.58 ± 0.05	3.67 ± 0.06	3.64 ± 0.06	3.50 ± 0.07	3.62 ± 0.08
Liver						
Absolute	7.07 ± 0.19	7.47 ± 0.26	7.93 ± 0.21	8.82 ± 0.23**	11.10 ± 0.51**	16.95 ± 0.52**
Relative	29.50 ± 0.39	30.00 ± 0.72	31.15 ±0 .48	34.05 ± 0.51**	41.58 ± 1.27**	57.75 ± 1.47**
Thymus						
Absolute	0.254 ± 0.012	0.258 ± 0.013	0.265 ± 0.014	0.241 ± 0.017	0.257 ± 0.015	0.238 ± 0.020
Relative	1.062 ± 0.049	1.037 ± 0.049	1.038 ± 0.049	0.925 ± 0.056	0.964 ± 0.050	0.804 ± 0.059**

*Significantly different ($P \leq 0.05$) from the chamber 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).

There were no gross lesions associated with exposure to *p*-chloro-α,α,α-trifluorotoluene. Significant histologic lesions were recorded in the liver, Harderian gland, adrenal cortex, and kidney of males and females, in the mammary gland of females, and in the testes and epididymides of males.

In the liver, incidences of centrilobular hepatocyte hypertrophy were significantly increased in males exposed to 250 ppm or greater and in females exposed to 1,000 or 2,000 ppm compared to those of the respective chamber control groups (Table 5). Two 2,000 ppm males and one 1,000 ppm male had necrosis of the liver. Hepatocyte hypertrophy was centrilobular to midzonal in location and was characterized by an increase in the size of the hepatocytes. Hepatocellular hypertrophy corresponded to increased liver weights in males exposed to 250 ppm or greater. In females, hepatocellular hypertrophy occurred in the 1,000 and 2,000 ppm groups. Hypertrophy was not recorded in the 500 ppm group despite the 15% increase in relative liver weight in this group. Due to the natural variation in the size of hepatocytes, hypertrophy is sometimes difficult to discern histologically until liver weights are increased more than 20%[70]. Necrosis was characterized by small foci of pale-staining hepatocytes lacking cellular detail or brightly eosinophilic individual hepatocytes with pyknotic nuclei.

Degeneration of the Harderian gland occurred in all exposure groups, and the incidences were significantly increased in males and females exposed to 250 ppm or greater (Table 5). Degeneration was characterized by clusters of shrunken, hypereosinophilic cells lacking cellular detail and containing pyknotic nuclei (Figure 8 and Figure 9). Occasionally, sloughed cells could be found in the lumens of acini or ducts. Enlarged nuclei and cellular piling could be seen as evidence of regeneration, which was seen occasionally as part of the degenerative process.

In the adrenal cortex, there were significantly increased incidences of cytoplasmic vacuolization in 2,000 ppm males and 1,000 and 2,000 ppm females (Table 5). Focal areas of cells of the zona fasciculata contained numerous small, round, cytoplasmic vacuoles. At times, this vacuolization caused some enlargement of the cytoplasm, and subsequently the adrenal cortex, but the primary lesion was considered to be the microvesicular droplets within the cytoplasm.

In the kidney, the severities of hyaline droplet accumulation and chronic nephropathy generally increased with increasing exposure concentration in males (Table 5). In females, there were more occurrences of chronic nephropathy in the 1,000 and 2,000 ppm groups, although there was no pairwise significance between the incidences of nephropathy in groups of exposed females and the chamber controls. Hyaline droplet accumulation was characterized by fine eosinophilic droplets randomly dispersed throughout the cytoplasm of renal tubule cells. These droplets stained prominently with Mallory-Heidenhain stain, consistent with α2u-globulin (Figure 10 and Figure 11). In 2,000 ppm males, the droplets were increased in number, and occasionally in size, with the presence of some angular and crystalline forms. The appearance of more, larger, and angular or crystalline forms of hyaline droplets is consistent with hyaline droplet nephropathy, however, other changes typically observed in clear cases of hyaline droplet nephropathy (such as degeneration, necrosis, and granular casts) were lacking, suggesting that this might be a mild response. Chronic progressive nephropathy was typical of that seen in rats, and was characterized by regenerating tubules, thickened basement membranes, interstitial inflammation, and hyaline casts. Increases in the severities of hyaline droplet accumulation and chronic progressive nephropathy corresponded to increases in kidney weights.

The incidences of mammary gland hyperplasia were significantly increased in 1,000 and 2,000 ppm females (Table 5). Hyperplasia consisted of an increase in the number and size of the glandular acini. Glandular epithelial cells were often increased in size and contained multiple small, clear vacuoles.

Incidences of seminiferous tubule, spermatid retention in the testis were significantly increased in males exposed to 1,000 or 2,000 ppm, and germ cell degeneration in the testis and exfoliated germ cell in the epididymis duct were significantly increased in 2,000 ppm males (Table 5). Spermatid retention, or delayed spermiation, was evaluated by examining stage IX through XII tubules at high power. Spermatid retention was characterized by the presence of step 19 spermatids at the luminal surface, within the germinal epithelium, or at the base in stage IX through XII tubules. These mature spermatids should normally be released during stage VIII. A number of changes comprised the diagnosis of germ cell degeneration in the testes. These primarily involved tubular vacuolation and apoptosis of germ cells. Vacuolation was seen as both macrovacuolation (a single large, well-demarcated vacuole) and microvacuolation (multiple, small, sometimes poorly delineated vacuoles with pale, wispy cytoplasm). Microvacuolation was often associated with focal germinal epithelium disorganization or a focal cohort of germ cell loss. Apoptotic germ cells were characterized by cytoplasmic eosinophilia and nuclear pyknosis. Germ cell degeneration was a multifocal lesion, affecting scattered tubules and numerous stages. Less often, degenerating germ cells included multinucleated giant cell forms. Partial depletion of germ cells, segmental germ cell loss, disordered arrangement of the germ cell layers, and decreased elongated spermatids each occurred less frequently then apoptosis and vacuolation, but were also considered to be components of degeneration. Exfoliated germ cells in the epididymis were characterized by the presence of intact, round cells intermixed with cell debris and mature sperm within the epididymal duct lumina that exceeded the level of occasional sloughed cells in chamber control animals.

Table 5. Incidences of Selected Nonneoplastic Lesions in Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
Male						
Adrenal Cortex[a]	10	10	10	10	10	10
Vacuolization Cytoplasmic[b]	0	0	0	0	0	7** (1.0)[c]
Epididymis	10	10	10	10	10	10
Duct, Exfoliated Germ Cell	0	0	1 (4.0)	0	1 (1.0)	6** (1.5)
Harderian Gland	10	10	10	10	10	10
Degeneration	0	1 (1.0)	9** (1.1)	10** (1.4)	10** (1.1)	10** (2.0)
Kidney	10	10	10	10	10	10
Accumulation, Hyaline Droplet	10 (1.0)	10 (1.0)	10 (1.6)	10 (2.0)	10 (3.0)	10 (3.0)
Nephropathy, Chronic	10 (1.6)	10 (1.3)	10 (1.5)	10 (1.7)	10 (1.7)	10 (2.6)
Liver	10	10	10	10	10	10
Centrilobular, Hepatocyte, Hypertrophy	0	0	8** (1.0)	10** (1.0)	10** (1.9)	10** (2.0)
Necrosis	0	0	0	0	1 (1.0)	2 (2.0)
Testes	10	10	10	10	10	10
Germ Cell, Degeneration	0	0	1 (4.0)	0	2 (1.0)	6** (1.3)
Seminiferous Tubule, Spermatid Retention	0	0	0	3 (1.3)	4* (1.5)	4* (1.5)
Female						
Adrenal Cortex	10	10	10	10	10	10
Vacuolization Cytoplasmic	0	0	0	0	4* (1.0)	10** (1.0)
Harderian Gland	10	10	10	10	10	10
Degeneration	0	3 (1.0)	5* (1.0)	9** (1.1)	10** (1.5)	10** (2.0)
Kidney	10	10	10	10	10	10
Nephropathy, Chronic	7 (1.0)	4 (1.0)	7 (1.0)	9 (1.2)	10 (1.1)	10 (1.6)
Liver	10	10	10	10	10	10
Centrilobular, Hepatocyte, Hypertrophy	0	0	0	0	10** (1.3)	10** (2.0)
Mammary Gland	10	10	10	10	10	10
Hyperplasia	1 (1.0)	0	0	4 (1.3)	6* (1.3)	10** (1.9)

*Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact 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.

Male rats exposed to 2,000 ppm had significantly decreased left cauda and left epididymis weights and numbers of sperm per cauda epididymis (Table 6 and Table H-1). Sperm motility was also significantly decreased in 1,000 and 2,000 ppm males. Female rats exposed to 2,000 ppm had decreased frequency of estrus and increased frequency of diestrus, a significantly higher probability of extended diestrus, a fewer number of cycles, and a fewer number of rats exhibiting cycles compared to the chamber control group (Table 6, Table H-2, Table H-3, and Figure H-1). *p*-Chloro-α,α,α-trifluorotoluene exposure via inhalation exhibited the potential to be a reproductive toxicant in male and female Hsd:Sprague Dawley® SD® rats.

Table 6. Summary of Reproductive Tissue Evaluations for Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	500 ppm	1,000 ppm	2,000 ppm
Male				
n	10	10	10	10
Weights (g)				
L. Cauda epididymis	0.273 ± 0.0146	0.283 ± 0.0066	0.256 ± 0.0065	0.212 ± 0.0065**
L. Epididymis	0.648 ± 0.0280	0.669 ± 0.0147	0.625 ± 0.0164	0.555 ± 0.0128**
L. Testis	1.920 ± 0.0896	2.069 ± 0.0361	1.976 ± 0.0503	1.978 ± 0.0233
Spermatid measurements				
Spermatid heads (10^6/testis)	270.92 ± 22.197	303.18 ± 19.790	263.56 ± 10.265	265.95 ± 13.848
Spermatid heads (10^6/g testis)	138.97 ± 7.762	146.82 ± 10.025	133.51 ± 4.364	134.67 ± 7.351
Epididymal spermatozoal measurements				
Sperm motility (%)	76.9 ± 3.06	74.9 ± 3.33	68.7 ± 1.51*	68.4 ± 1.67*
Sperm (10^6/cauda epididymis)	158.6 ± 7.14	164.8 ± 9.11	144.0 ± 10.22	123.6 ± 5.62**
Female				
n	10	10	10	3[b]
Number of estrous cycles	2.2 ± 0.25	2.0 ± 0.26	1.9 ± 0.18	1.3 ± 0.33
Estrous stages (% of cycle)				
Diestrus	55.0	53.1	48.1	78.5
Proestrus	10.6	5.6	7.5	0.7
Estrus	33.8	37.5	42.5	14.6
Metestrus	0.0	3.1	1.9	6.3

*Significantly different (P ≤ 0.05) from the chamber control group by Shirley's test.
**Significantly different (P ≤ 0.01) from the chamber control group by Williams' test (left cauda epididymis and left epididymis weights) or Shirley's test (sperm/cauda epididymis).
[a]Data for organ weights, epididymal spermatozoal measurements, and number of estrous cycles are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunn's test (numbers of estrous cycles). Tests for equality of transition probability matrices among all groups and between the chamber control group and each exposed group indicated that 2,000 ppm females spent significantly more time in extended diestrus (P < 0.001) than the chamber control group.
[b]Estrous cyclicity samples were evaluated for 9 female rats; however, in 6 of 9 animals estrous cycles were longer than 16 days or unclear and were not included in these analyses.

Exposure Concentration Selection Rationale: Statistically significant exposure-related increases in liver weights (approximately 80% or greater) in 2,000 ppm males and females precluded the use of this concentration in the 2-year study. Due to logistical considerations regarding the number of whole body inhalation chambers that can be included in a study, the same *p*-chloro-α,α,α-trifluorotoluene exposure concentrations were selected for males and females: 100, 300, and 1,000 ppm. These concentrations were selected to accommodate logistical challenges while adequately challenging both males and females; half-log (base 10) spacing of exposure concentrations was selected to capture a wider exposure range.

Two-year Study

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 7 and in the Kaplan-Meier survival curves (Figure 3). There was a significant negative trend of survival in males and survival of the 1,000 ppm males was significantly less than that of the chamber controls, with only five animals surviving to the end of the study. Most of the early deaths occurred relatively late in the study and were due to nephropathy. Survival of exposed groups of females was similar to that of the chamber controls.

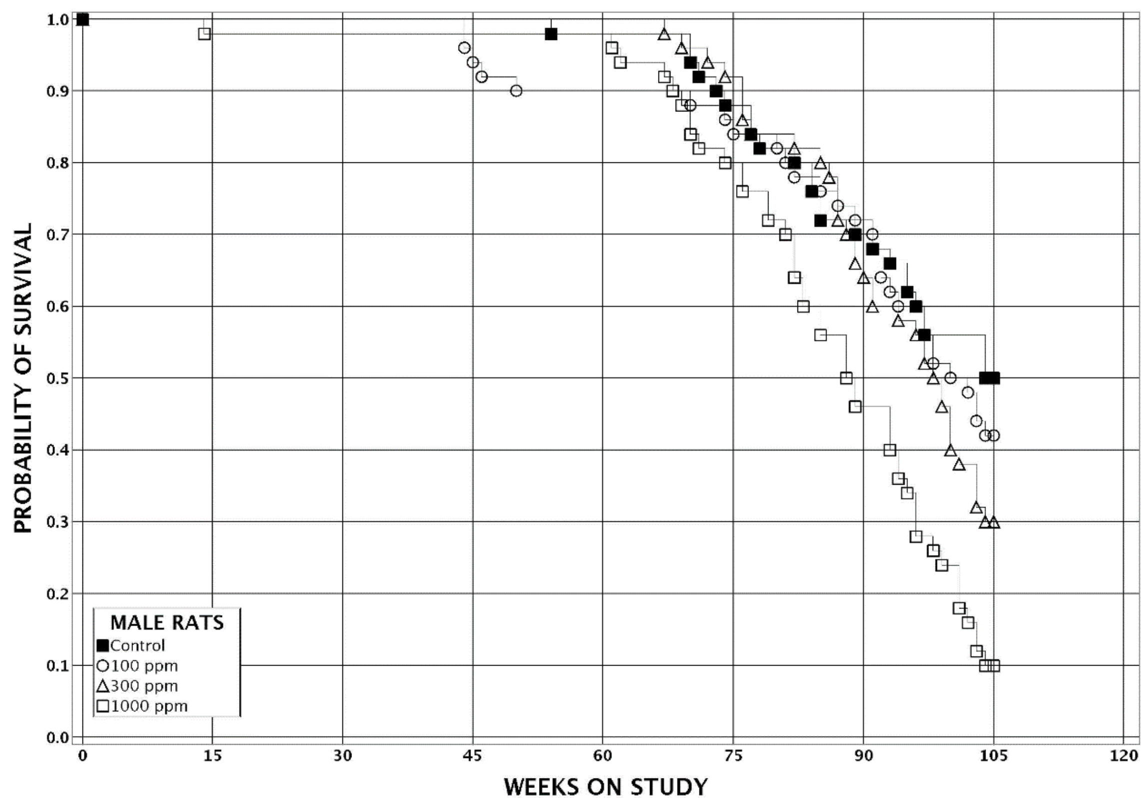
Body Weights and Clinical Observations

Mean body weights of exposed groups of males were within 10% of the chamber controls with the exception of the 1,000 ppm group that was at least 10% less than the chamber controls for 3 weeks very early in the study (Figure 4; Table 8). Subsequent mean body weights for the 1,000 ppm group were within 10% of the chamber controls. Mean body weights of females exposed to 100 or 300 ppm were similar to those of the chamber controls throughout the study (Figure 4; Table 9). The mean body weights of 1,000 ppm females were similar to those of the chamber controls for most of the study and were approximately 10% less than the chamber controls from around day 506. There were no clinical observations that were clearly attributable to exposure, although there were more occurrences of abnormal breathing in the 100 and 300 ppm males and the 1,000 ppm females than in the chamber control groups.

Table 7. Survival of Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	16	19	21	27
Natural deaths	9	10	14	18
Animals surviving to study termination	25	21	15	5
Percent probability of survival at end of study[a]	50	42	30	10
Mean survival (days)[b]	661	641	655	602
Survival analysis[c]	P < 0.001	P = 0.568	P = 0.137	P < 0.001
Female				
Animals initially in study	50	50	50	50
Accidental death[d]	0	1	0	0
Moribund	22	27	18	18
Natural deaths	5	1	7	2
Animals surviving to study termination	23[e]	21	25	30
Percent probability of survival at end of study	44	43	50	60
Mean survival (days)	626	643	664	694
Survival analysis	P = 0.029N	P = 0.884N	P = 0.419N	P = 0.045N

[a]Kaplan-Meier determinations.
[b]Mean of all deaths (uncensored, censored, and terminal kill).
[c]The result of the life table trend test[40] is in the chamber control column, and the results of the life table pairwise comparisons[39] with the chamber controls are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by N.
[d]Censored in the survival analysis.
[e]Includes one animal that died during the last week of the study.



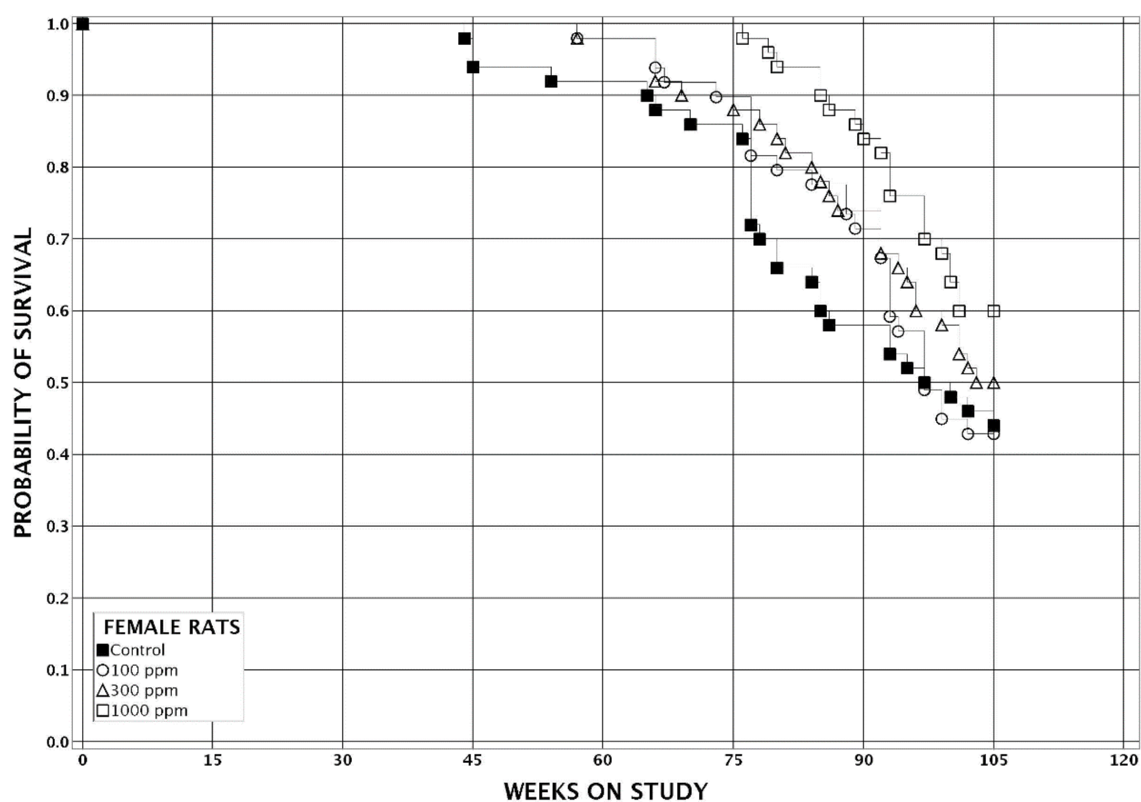
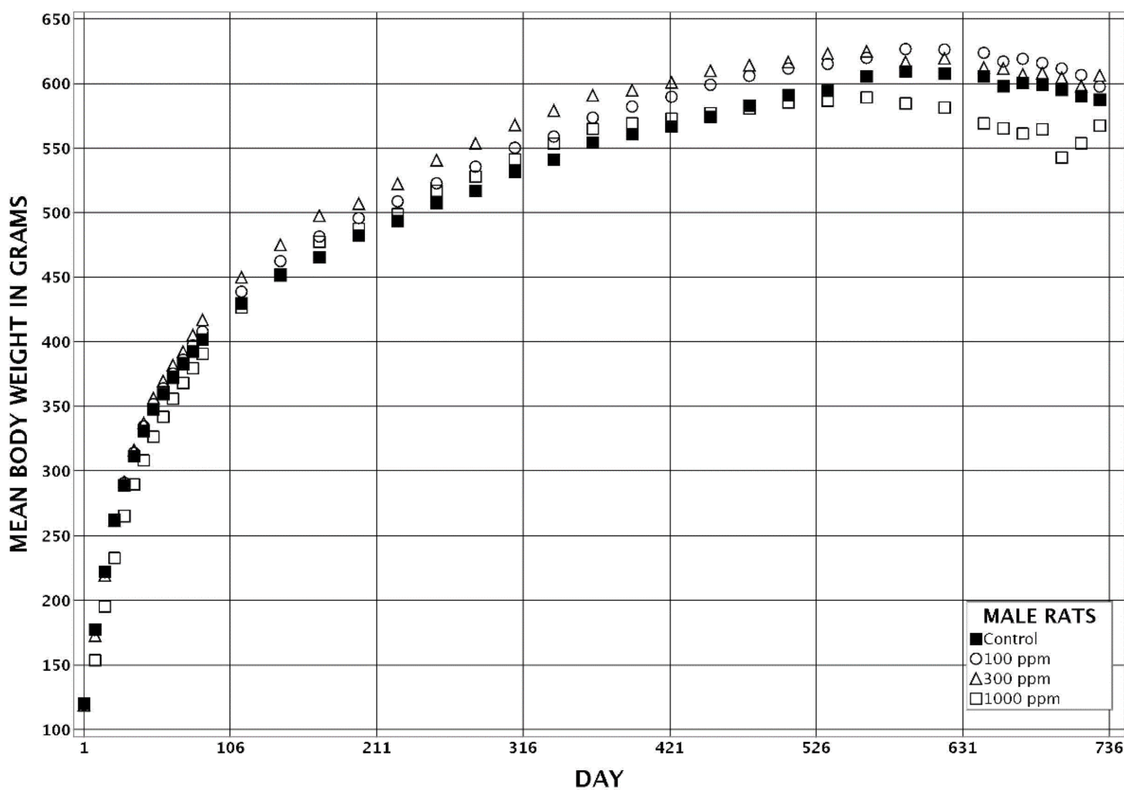


Figure 3. Kaplan-Meier Survival Curves for Rats Exposed to *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years



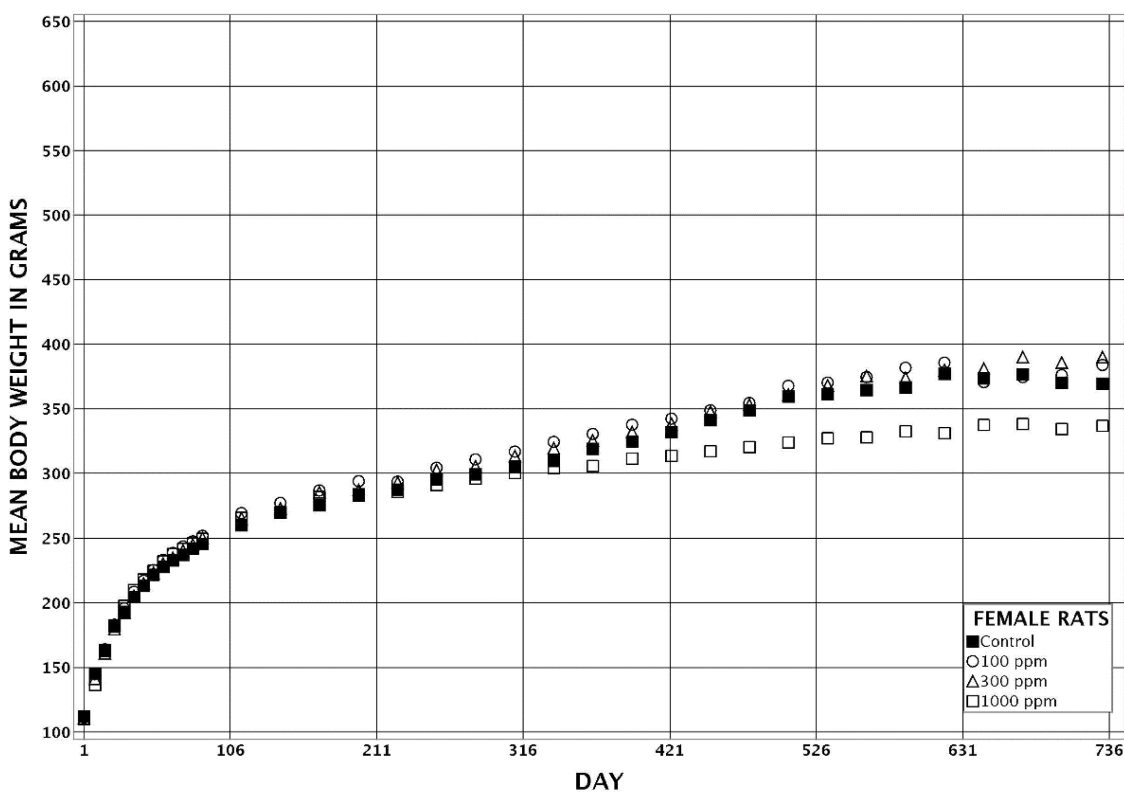


Figure 4. Growth Curves for Rats Exposed to *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years

Table 8. Mean Body Weights and Survival of Male Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control		100 ppm			300 ppm			1,000 ppm		
Day	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	120	50	120	100	50	119	99	50	119	99	50
9	177	50	177	100	50	173	97	50	154	87	50
16	222	50	222	100	50	220	99	50	195	88	50
23	263	50	263	100	50	262	100	50	233	89	50
30	289	50	291	101	50	292	101	50	265	92	50
37	312	50	314	101	50	316	102	50	290	93	50
44	331	50	335	101	50	337	102	50	308	93	50
51	348	50	352	101	50	356	103	50	327	94	50
58	360	50	364	101	50	370	103	50	342	95	50
65	372	50	376	101	50	382	103	50	356	96	50
72	382	50	386	101	50	392	103	50	368	96	50
79	393	50	397	101	50	405	103	50	380	97	50
86	402	50	408	102	50	417	104	50	391	97	50
114	430	50	439	102	50	450	105	50	427	99	49
142	452	50	462	102	50	475	105	50	451	100	49
170	465	50	482	104	50	498	107	50	478	103	49
198	482	50	496	103	50	507	105	50	488	101	49
226	494	50	509	103	50	522	106	50	499	101	49
254	508	50	523	103	50	541	107	50	517	102	49
282	517	50	536	104	50	554	107	50	528	102	49
310	532	50	550	104	47	568	107	50	542	102	49
338	541	50	559	103	46	579	107	50	554	102	49
366	554	50	574	104	45	591	107	50	565	102	49
394	561	49	582	104	45	595	106	50	569	102	49
422	567	49	590	104	45	601	106	50	573	101	48
450	574	49	599	104	45	610	106	50	577	101	47
478	583	49	606	104	45	614	105	48	581	100	45
506	591	45	612	104	44	617	104	47	586	99	41
534	594	43	615	104	42	623	105	42	587	99	38
562	605	41	620	102	41	625	103	42	590	97	35
590	609	36	627	103	38	617	101	40	585	96	29
618	608	35	626	103	36	620	102	33	582	96	24
646	605	34	624	103	32	612	101	30	570	94	22
660	598	33	617	103	30	612	102	29	565	95	18
674	601	29	619	103	29	607	101	27	561	93	14
688	599	28	616	103	26	608	102	25	565	94	12
702	595	28	612	103	25	605	102	20	543	91	12
716	591	28	607	103	23	598	101	19	554	94	7
Mean for Weeks											
1–13	305	–	308	101	–	311	102	–	287	94	–
14–52	491	–	506	103	–	522	106	–	498	101	–
53–103	590	–	609	103	–	610	103	–	572	97	–

Table 9. Mean Body Weights and Survival of Female Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control		100 ppm			300 ppm			1,000 ppm		
Day	**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	112	50	112	99	50	111	99	50	110	98	50
9	145	50	145	100	50	141	97	50	137	94	50
16	164	50	164	100	50	161	98	50	163	100	50
23	182	50	183	101	50	180	99	50	183	101	50
30	193	50	196	101	50	193	100	50	198	103	50
37	205	50	209	102	50	206	101	50	210	103	50
44	213	50	217	102	50	215	101	50	218	102	50
51	222	50	226	102	50	224	101	50	225	102	50
58	228	50	233	102	50	230	101	50	232	102	50
65	233	50	239	103	49	235	101	50	238	102	50
72	237	50	244	103	49	241	101	50	242	102	50
79	242	50	248	102	49	245	101	50	246	102	50
86	246	50	252	103	49	250	102	50	250	102	50
114	260	50	269	104	49	265	102	50	266	102	50
142	270	50	277	103	49	273	101	50	271	100	50
170	275	50	287	104	49	285	104	50	282	102	50
198	284	50	294	104	49	287	101	50	283	100	50
226	288	50	294	102	49	293	102	50	286	99	50
254	295	50	305	103	49	303	103	50	291	99	50
282	300	50	311	104	49	306	102	50	296	99	50
310	305	49	317	104	49	313	103	50	300	98	50
338	310	47	324	105	49	320	103	50	304	98	50
366	319	47	331	104	49	326	102	50	306	96	50
394	325	46	338	104	49	332	102	50	312	96	50
422	332	46	343	103	48	339	102	49	314	95	50
450	341	45	349	102	48	348	102	49	317	93	50
478	349	44	355	102	45	353	101	45	321	92	50
506	360	43	368	102	45	361	100	45	324	90	50
534	362	38	371	102	41	368	102	44	327	91	49
562	365	33	375	103	39	375	103	42	328	90	47
590	367	31	382	104	38	374	102	39	333	91	45
618	377	29	386	102	35	380	101	37	331	88	43
646	374	27	371	99	30	381	102	34	338	91	39
674	377	25	375	99	24	390	104	30	338	90	35
702	370	24	376	102	22	386	104	27	335	90	30
Mean for Weeks											
1–13	202	–	205	101	–	202	100	–	204	101	–
14–52	287	–	298	104	–	294	102	–	287	100	–
53–101	355	–	363	102	–	363	102	–	325	92	–

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the thyroid gland, lung, adrenal medulla, uterus, liver, kidney, nose, adrenal cortex, small intestine, mammary gland, pituitary gland, and brain. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for male rats and Appendix B for female rats.

Thyroid Gland: Compared to those of the chamber controls, there were significantly increased incidences of C-cell adenoma and C-cell adenoma or carcinoma (combined) in 1,000 ppm males and females; the combined incidence in 100 ppm females was also significantly increased (Table 10, Table A-1, Table A-2, Table B-1, and Table B-2). The incidences of C-cell hyperplasia in exposed groups of males and females were similar to those in the chamber control groups (Table 10, Table A-3 and Table B-3). C-cell adenomas consisted of discrete proliferations of C-cells that were larger than five follicles in diameter and caused compression of surrounding follicles (Figure 12). C-cell carcinomas tended to be large, and were diagnosed when there was evidence of invasion of the thyroid gland capsule or surrounding tissue (Figure 13).

Table 10. Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Male				
Number Examined Microscopically	50	49	49	50
C-cell, Hyperplasia[a]	16 (1.4)[b]	12 (1.7)	14 (1.6)	15 (1.5)
Bilateral, C-cell, Adenoma	0	1	1	1
C-cell Adenoma (includes bilateral)[c]				
Overall rate[d]	2/50 (4%)	5/49 (10%)	3/49 (6%)	12/50 (24%)
Adjusted rate[e]	5.1%	13.4%	7.9%	36.3%
Terminal rate[f]	2/25 (8%)	4/21 (19%)	1/15 (7%)	3/5 (60%)
First incidence (days)	729 (T)	680	669	568
Poly-3 test[g]	P < 0.001	P = 0.192	P = 0.482	P < 0.001
C-cell, Carcinoma[h]	1	0	1	1
C-cell Adenoma or Carcinoma[i]				
Overall rate	3/50 (6%)	5/49 (10%)	4/49 (8%)	13/50 (26%)
Adjusted rate	7.6%	13.4%	10.6%	39.2%
Terminal rate	3/25 (12%)	4/21 (19%)	1/15 (7%)	3/5 (60%)
First incidence (days)	729 (T)	680	669	568
Poly-3 test	P < 0.001	P = 0.326	P = 0.481	P < 0.001
Female				
Number Examined Microscopically	50	50	50	50

	Chamber Control	**100 ppm**	**300 ppm**	**1,000 ppm**
C-cell, Hyperplasia	16 (1.4)	15 (1.2)	21 (1.4)	13 (1.9)
Bilateral, C-cell, Adenoma	0	0	0	3
C-cell Adenoma (includes bilateral)[j]				
Overall rate	2/50 (4%)	8/50 (16%)	8/50 (16%)	14/50 (28%)
Adjusted rate	5.5%	20.4%	20.2%	31.6%
Terminal rate	0/22 (0%)	3/21 (14%)	8/25 (32%)	11/30 (37%)
First incidence (days)	459	534	731 (T)	646
Poly-3 test	P = 0.008	P = 0.056	P = 0.057	P = 0.003
C-cell, Carcinoma[k]	0	2	0	1
C-cell Adenoma or Carcinoma[j]				
Overall rate	2/50 (4%)	10/50 (20%)	8/50 (16%)	15/50 (30%)
Adjusted rate	5.5%	25.5%	20.2%	33.6%
Terminal rate	0/22 (0%)	5/21 (24%)	8/25 (32%)	11/30 (37%)
First incidence (days)	459	534	731 (T)	646
Poly-3 test	P = 0.009	P = 0.017	P = 0.057	P = 0.002

(T) Terminal kill.
[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 all routes of 2-year studies (incidence per study): 15/100 (2/50, 13/50).
[d]Number of animals with neoplasm per number of animals with thyroid gland examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[h]Historical control incidence: 1/100 (1/50, 0/50).
[i]Historical control incidence: 16/100 (3/50, 13/50).
[j]Historical control incidence: 13/99 (2/50, 11/49).
[k]Historical control incidence: 0/99 (0/50, 0/49).

Lung: In males, alveolar/bronchiolar adenoma and alveolar/bronchiolar carcinoma were only observed in the 100 and 1,000 ppm groups, although the incidences were not significantly different from those of the chamber controls (Table 11 and Table A-1). No alveolar/bronchiolar neoplasms were observed in females (Table B-1). Alveolar/bronchiolar adenomas were well demarcated, densely cellular proliferations of cuboidal to columnar cells forming short projections into alveolar spaces and causing compression of the surrounding parenchyma. Alveolar/bronchiolar carcinomas were larger, and often poorly demarcated and composed of pleomorphic cells forming different patterns of growth (Figure 14 and Figure 15).

Although the chamber control groups had fairly high incidences of chronic inflammation, there were significantly increased incidences of chronic inflammation in all exposed groups of males and females (Table 11, Table A-3, and Table B-3). There were significantly increased incidences of fibrosis in all exposed male groups and in 300 and 1,000 ppm females, and significantly increased incidences of hemorrhage in all groups of exposed males and in 1,000 ppm females. Chronic inflammation of collections of large, foamy macrophages, with fewer neutrophils and lymphocytes, within the alveolar spaces and the interstitium. Multinucleated giant cells were seen in many areas of inflammation, often containing phagocytosed cells or debris. Hemorrhage was characterized by red blood cells and fibrin within the alveolar spaces or the interstitium, often in perivascular areas (Figure 16). Evidence of the antemortem nature of the hemorrhage was provided by the presence of erythrophagocytosis. Thickening of the interstitium by fibrous connective tissue, which on occasion progressed to fibrous nodules that protruded into alveolar and bronchiolar spaces, was diagnosed as fibrosis (Figure 17). This diagnosis was recorded only when the amount of fibrosis exceeded that which would be expected in a typical focus of chronic inflammation. Fibrosis was often associated with areas of hemorrhage and fibrin, with the fibrosis almost appearing to be forming as part of the organization of fibrin. Inflammation, hemorrhage and fibrosis were usually seen together, with the degree of severity of inflammation generally corresponding to the amount of hemorrhage and fibrosis.

Table 11. Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Male				
Number Examined Microscopically	50	50	50	50
Fibrosis[a]	8 (1.4)[b]	22** (1.5)	28** (1.4)	24** (1.8)
Hemorrhage	11 (1.2)	23* (1.7)	28** (1.5)	28** (1.7)
Inflammation, Chronic	32 (1.2)	42* (1.5)	47** (1.4)	45** (1.8)
Alveolar/bronchiolar Adenoma[c]	0	2	0	1
Alveolar/bronchiolar Carcinoma[c]				
Overall rate[d]	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adjusted rate[e]	0.0%	0.0%	0.0%	6.4%
Terminal rate[f]	0/25 (0%)	0/21 (0%)	0/15 (0%)	0/5 (0%)
First incidence (days)	–[h]	–	–	549
Poly-3 test[g]	P = 0.032	–[i]	–	P = 0.191
Alveolar/bronchiolar Adenoma or Carcinoma[c]				
Overall rate	0/50 (0%)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	5.3%	0.0%	9.3%
Terminal rate	0/25 (0%)	1/21 (5%)	0/15 (0%)	0/5 (0%)
First incidence (days)	–	650	–	516
Poly-3 test	P = 0.073	P = 0.228	–	P = 0.086
Female				
Number Examined Microscopically	50	50	50	50
Fibrosis	11 (1.0)	17 (1.1)	24** (1.4)	28** (1.4)
Hemorrhage	12 (1.1)	11 (1.5)	18 (1.4)	26* (1.4)
Inflammation, Chronic	35 (1.2)	42* (1.2)	48** (1.4)	46* (1.4)

*Significantly different ($P \leq 0.05$) from the chamber 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]Historical control incidence for all routes of 2-year studies (incidence per study): 0/99 (0/50, 0/49).
[d]Number of animals with neoplasm per number of animals with lung examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[h]Not applicable; no neoplasms in animal group.
[i]Value of statistic cannot be computed.

Adrenal Medulla: There was a significantly increased incidence of benign pheochromocytoma in 1,000 ppm females (Table 12, Table B-1, and Table B-2). The incidences of medullary hyperplasia were also significantly increased in 300 and 1,000 ppm females (Table 12 and Table B-3). Benign pheochromocytoma was diagnosed when there was a well-demarcated, expansile proliferation of mildly pleomorphic medullary cells arranged in large trabeculae or solid clusters (Figure 18). Hyperplasia was characterized by a focal area of increased cellularity with no compression of the surrounding tissue; hyperplastic cells were typically smaller and more basophilic than normal medullary cells.

Table 12. Incidences of Neoplasms and Nonneoplastic Lesions of the Adrenal Medulla in Female Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Number Examined Microscopically	49	50	50	50
Hyperplasia[a]	17 (1.1)[b]	25 (1.6)	34** (1.1)	36** (1.5)
Benign Pheochromocytoma[c]				
Overall rate[d]	0/49 (0%)	3/50 (6%)	4/50 (8%)	6/50 (12%)
Adjusted rate[e]	0.0%	8.1%	9.9%	13.5%
Terminal rate[f]	0/21 (0%)	2/21 (10%)	2/25 (8%)	4/30 (13%)
First incidence (days)	–[h]	691	478	530
Poly-3 test[g]	P = 0.066	P = 0.135	P = 0.085	P = 0.035

**Significantly different (P ≤ 0.01) from the chamber 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]Historical control incidence for all routes of 2-year studies (incidence per study): 0/99 (0/49, 0/50).
[d]Number of animals with neoplasm per number of animals with adrenal medulla examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[h]Not applicable; no neoplasms in animal group.

Uterus: There was a positive trend in the incidences of adenocarcinoma (Table 13, Table B-1, and Table B-2). Adenocarcinomas were characterized by poorly circumscribed proliferations of cuboidal to columnar epithelial cells that displayed pleomorphism and atypia. The cells were arranged in papillary or glandular structures, and there was effacement of the normal parenchyma and invasion into the underlying musculature. Atypical hyperplasia occurred in the 300 and 1,000 ppm groups, but the incidences were not significantly increased compared to the chamber control group (Table 13 and Table B-3). It has been reported that atypical hyperplasia can progress to adenocarcinoma[71]. Atypical hyperplasia was characterized by clusters of enlarged glands lined by thickened, disorganized epithelium; epithelial cells displayed loss of nuclear polarization, karyomegaly, mitoses, and cellular pleomorphism.

There was a significantly increased incidence of stromal polyp in 300 ppm females (Table 13, Table B-1, and Table B-2). Histologically, stromal polyps were exophytic nodules that projected into the uterine lumen. They were covered by normal-appearing endometrial surface epithelium and supported by a broad stalk of endometrial stroma with blood vessels and occasionally endometrial glands. A single stromal sarcoma occurred in the 300 ppm group. This sarcoma was composed of spindle-shaped cells with elongated, hyperchromatic nuclei. The sarcoma was poorly demarcated with indistinct borders and infiltrated surrounding tissues.

Table 13. Incidences of Neoplasms and Nonneoplastic Lesions of the Uterus in Female Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Number Necropsied	50	50	50	50
Endometrium, Atypical Hyperplasia[a]	0	0	1 (2.0)[b]	3 (2.3)
Adenocarcinoma[c]				
Overall rate[d]	1/50 (2%)	1/50 (2%)	0/50 (0%)	5/50 (10%)
Adjusted rate[e]	2.9%	2.7%	0.0%	11.3%
Terminal rate[f]	1/22 (5%)	1/21 (5%)	0/25 (0%)	3/30 (10%)
First incidence (days)	731 (T)	731 (T)	–[h]	530
Poly-3 test[g]	P = 0.017	P = 0.748N	P = 0.475N	P = 0.166
Stromal Polyp, Multiple	0	1	4	2
Stromal Polyp (includes multiple)				
Overall rate	7/50 (14%)	9/50 (18%)	16/50 (32%)	12/50 (24%)
Adjusted rate	19.6%	23.8%	39.3%	27.2%
Terminal rate	5/22 (23%)	6/21 (29%)	12/25 (48%)	10/30 (33%)
First incidence (days)	590	643	558	646
Poly-3 test	P = 0.419	P = 0.440	P = 0.047	P = 0.298
Stromal Sarcoma	0	0	1	0
Stromal Polyp or Stromal Sarcoma				
Overall rate	7/50 (14%)	9/50 (18%)	17/50 (34%)	12/50 (24%)
Adjusted rate	19.6%	23.8%	41.8%	27.2%
Terminal rate	5/22 (23%)	6/21 (29%)	13/25 (52%)	10/30 (33%)
First incidence (days)	590	643	558	646
Poly-3 test	P = 0.439	P = 0.440	P = 0.028	P = 0.298

[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[c]No historical controls are available for uterine findings due to a protocol change for uterine pathology sectioning.
[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 chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in an exposure group is indicated by N.
[h]Not applicable; no neoplasms in animal groupendometrial glands. A single stromal sarcoma occurred in the 300 ppm group. This sarcoma was composed of spindle-shaped cells with elongated, hyperchromatic nuclei. The sarcoma was poorly demarcated with indistinct borders and infiltrated surrounding tissues.

Liver: There were significantly increased incidences of centrilobular hepatocyte hypertrophy in all exposed male groups and in 300 and 1,000 ppm females and significantly increased incidences of fatty change in 300 and 1,000 ppm males and females (Table 14, Table A-3, and Table B-3). There were significantly increased incidences of eosinophilic focus in 1,000 ppm males and mixed cell focus and clear cell focus in 1,000 ppm females; in all exposed male groups, there were significantly decreased incidences of clear cell focus. Hepatocellular adenoma or carcinoma (combined) occurred in three 100 ppm males, hepatocellular carcinoma occurred in one 1,000 ppm male, and cholangiocarcinoma occurred in two 100 ppm females (Table A-1, Table A-2, and Table B-1). These sporadic occurrences of liver neoplasms were not considered related to exposure.

Table 14. Incidences of Nonneoplastic Lesions of the Liver in Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Males				
Number Examined Microscopically	50	50	50	50
Centrilobular, Hepatocyte, Hypertrophy[a]	2 (1.0)[b]	17** (1.2)	39** (1.5)	47** (1.8)
Clear Cell Focus	40	31*	29**	10**
Eosinophilic Focus	1	5	6	8**
Fatty Change	0	3 (1.3)	7** (1.3)	26** (1.2)
Females				
Number Examined Microscopically	50	50	50	50
Centrilobular, Hepatocyte, Hypertrophy	0	1 (2.0)	10** (1.1)	45** (1.9)
Clear Cell Focus	16	15	23	38**
Eosinophilic Focus	7	6	7	15
Fatty Change	2 (1.0)	4 (1.0)	11* (1.2)	10* (1.3)
Mixed Cell Focus	6	6	8	18*

*Significantly different ($P \leq 0.05$) from the chamber 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.

Centrilobular hepatocellular hypertrophy was characterized by an increased amount of granular cytoplasm causing an increase in the size of hepatocytes; occasionally nuclei of affected hepatocytes were also enlarged. Severity was based upon the amount of the hepatic lobule involved, with minimal hypertrophy diagnosed when hepatocytes adjacent to central veins were enlarged. Mild and moderate hypertrophy were diagnosed when a central veins were enlarged. Mild and moderate hypertrophy were diagnosed when a greater percentage of each lobule was involved, typically around 20% to 50% for mild and greater than 50% for moderate. Severe hypertrophy was recorded when the entire lobule was involved throughout the liver, giving the appearance of diffuse hypertrophy. Fatty change was primarily microvesicular in nature, consisting of scattered areas of hepatocytes with multiple small lipid vacuoles. Eosinophilic foci were areas of hepatocytes that were more eosinophilic than surrounding hepatocytes. The involved hepatocytes were occasionally larger, causing some compression of adjacent parenchyma, although not typically as much as would be seen with a hepatocellular adenoma. Also unlike a hepatocellular adenoma, foci did not tend to disrupt the lobular architecture of the liver. Mixed cell foci were made up of cells that resembled those of two different kinds of foci, typically eosinophilic and clear cell in this study. Clear cell foci were typically composed of small hepatocytes with clear cytoplasm, due to either multiple small vacuoles or a single large vacuole.

Kidney: Grossly, there were increased occurrences of granular kidneys in exposed male groups compared to the chamber controls. These changes corresponded histologically with increased severities of nephropathy in the exposed groups (Table 15 and Table A-3). Histologically, nephropathy was characterized by protein casts; dilated tubules with basophilic and attenuated to hypertrophic renal tubule epithelium; hyper- or hypocellular glomeruli; interstitial inflammation and fibrosis; and hyperplasia of the transitional epithelium of the renal pelvis. The severity of nephropathy was graded on a scale of 1 (minimal) to 4 (marked). Minimal nephropathy consisted of one to 15 tubules with the tubule changes listed above; mild nephropathy was diagnosed when 16 to 50 tubules were affected. Moderate nephropathy was diagnosed when there were coalescing areas of affected tubules and marked nephropathy was diagnosed when no or nearly no unaffected renal tubules were observed. There were more males in the 1,000 ppm group with marked severity than in the chamber control group (11, 13, 27, and 30 in the chamber control, 100, 300, and 1,000 ppm groups, respectively).

Table 15. Incidences of Selected Nonneoplastic Lesions in Male Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Kidney[a]	50	50	50	50
Nephropathy[b]	49 (2.5)[c]	49 (2.7)	50 (3.3)	50 (3.4)
Nose	50	50	50	50
Exudate	8 (1.3)	12 (1.0)	12 (1.0)	18** (1.1)

**Significantly different ($P \leq 0.01$) from the chamber control group by the Poly-3 test.
[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.

Nose: There was a significantly increased incidence of exudate in 1,000 ppm males (Table 15 and Table A-3). Histologically, this lesion was characterized by clusters of neutrophils within the lumen of the nasal cavity.

Other Tissues: In the adrenal cortex of females, low incidences of adenoma were observed in exposed groups, but the incidences were not statistically different from that of the chamber control group (Table B-1). There were also increased incidences of hyperplasia in exposed female groups, but the increases were not statistically significant (Table B-3). In the small intestine of males, adenocarcinoma occurred in three 100 ppm animals and one 300 ppm animal (Table A-1 and Table A-2). While not statistically significant, these lesions are unusual. In females, there were decreased, but generally not significantly, incidences of mammary gland fibroadenoma (1,000 ppm), adenoma (300 and 1,000 ppm), and adenocarcinoma (all exposed groups) compared to the chamber control group incidences (Table B-1 and Table B-2). In 1,000 ppm females, there was a significantly decreased incidence of pars distalis adenoma in the pituitary gland and a concomitant significantly decreased incidence of compression in the brain (hypothalamus) (Table B-1, Table B-2, and Table B-3). The biological significance of the findings described in this paragraph is unknown.

Mice

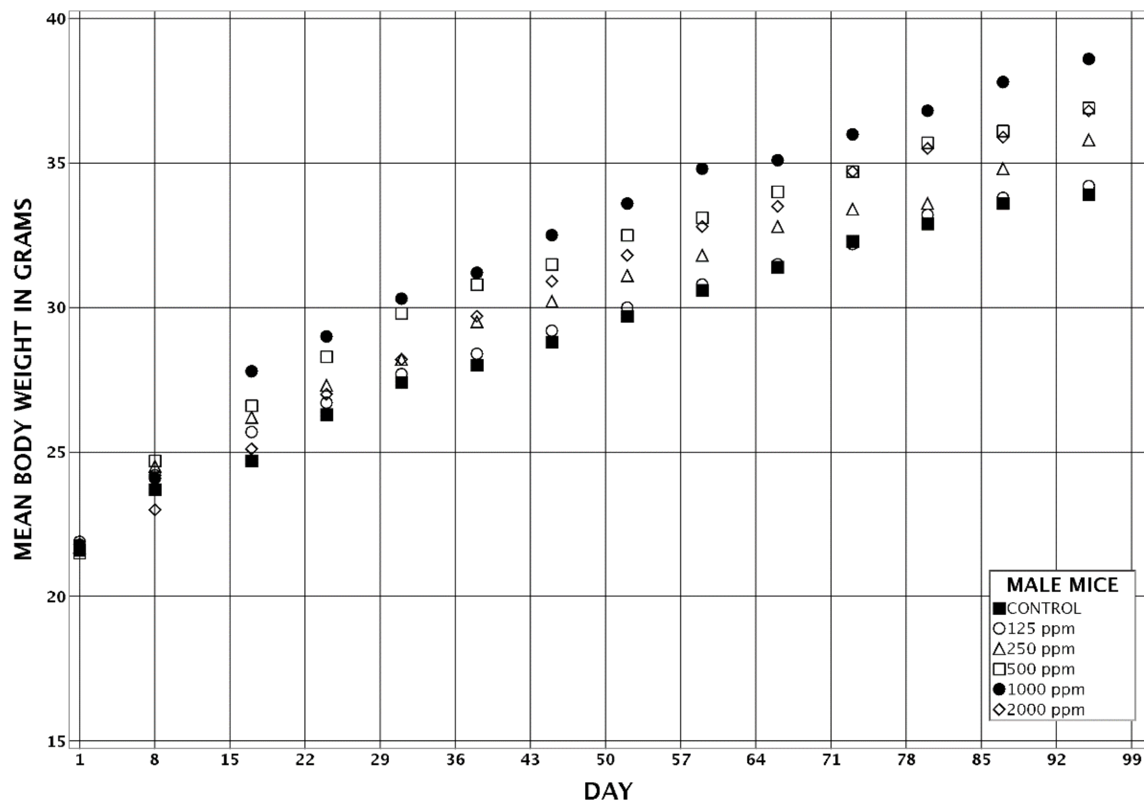
Three-month Study

All mice survived to the end of the study, and the final mean body weights and mean body weight gains of males exposed to 500 ppm or greater and females exposed to 250 ppm or greater were significantly greater than those of the chamber control groups; the mean body weight gain of 250 ppm males was also significantly increased (Table 16, Figure 5). The highest final mean body weights were found in 1,000 ppm males and females, which were 14% and 20% greater than those of the chamber control groups, respectively. A clinical observation of excitability was observed on days 8 and 17 of the study in the 2,000 ppm groups, but was not observed as the study continued.

Table 16. Survival and Body Weights of Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

Concentration (ppm)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	21.6 ± 0.3	33.9 ± 0.6	12.2 ± 0.4	–
125	10/10	21.9 ± 0.3	34.2 ± 1.0	12.3 ± 0.9	101
250	10/10	21.7 ± 0.3	35.8 ± 0.9	14.2 ± 0.7*	106
500	10/10	21.5 ± 0.3	36. 9 ± 0.8**	15.4 ± 0.6**	109
1,000	10/10	21.8 ± 0.3	38.6 ± 0.8**	16.7 ± 0.7**	114
2,000	10/10	21.8 ± 0.3	36.8 ± 0.4**	15.0 ± 0.4**	109
Female					
0	10/10	18.4 ± 0.3	28.2 ± 0.4	9.8 ± 0.3	–
125	10/10	19.0 ± 0.3	29.9 ± 0.8	11.0 ± 0.7	106
250	10/10	18.5 ± 0.2	31.0 ± 0.7**	12.5 ± 0.6**	110
500	10/10	19.0 ± 0.3	32.2 ± 0.7**	13.2 ± 0.7**	114
1,000	10/10	18.7 ± 0.2	33.7 ± 0.8**	15.0 ± 0.7**	120
2,000	10/10	18.8 ± 0.3	29.7 ± 0.7**	10.9 ± 0.6**	106

*Significantly different ($P \leq 0.05$) from the chamber control group by Williams' test.
**$P \leq 0.01$.
[a]Weights and weight changes are given as mean ± standard error.
[b]Number of animals surviving at 14 weeks/number initially in group.



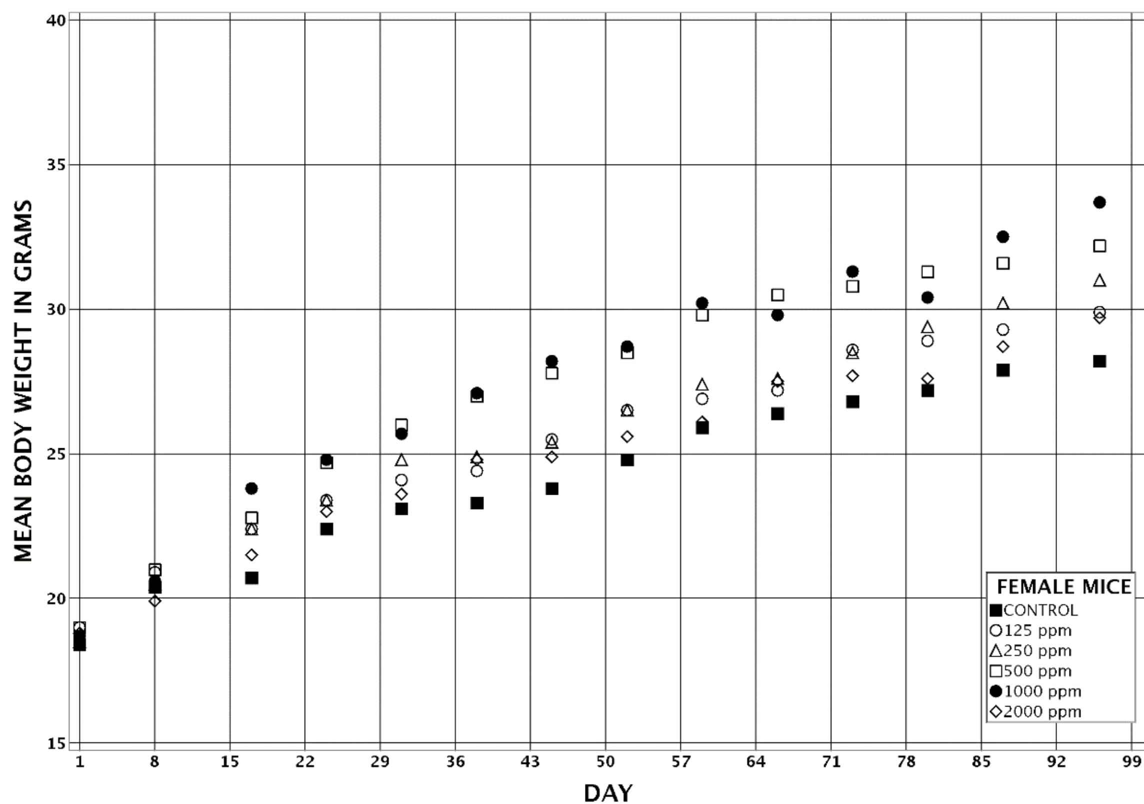


Figure 5. Growth Curves for Mice Exposed to *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Three Months

Erythrocyte counts, hematocrit values, and packed cell volumes were significantly decreased in 2,000 ppm males and 1,000 and 2,000 ppm females compared to those in the chamber control groups (Table F-2). Hemoglobin concentration was also significantly decreased in the 2,000 ppm males and females. In males, reticulocyte counts were significantly decreased in the 1,000 and 2,000 ppm groups. These changes are consistent with mild erythroid suppression secondary to inflammation (forestomach and liver). Platelet counts in males exposed to 500 ppm or greater and in 1,000 and 2,000 ppm females were significantly increased; the mechanism is not known but may reflect increased production or altered peripheral distribution. Absolute liver weights were significantly increased in an exposure concentration-dependent manner in males and females exposed to 250 ppm or greater (Table 17 and Table G-2). Absolute liver weights in 2,000 ppm males and females were 277% and 212% of the chamber control liver weights, respectively. Relative liver weights were also significantly increased in males exposed to 250 ppm or greater and females exposed to 500 ppm or greater. In males, absolute kidney weights were significantly increased in groups exposed to 500 ppm or greater, with the greatest weight (131% of that of the chamber controls) in the 2,000 ppm group (Table 17 and Table G-2). Relative kidney weights were significantly increased in the 1,000 and 2,000 ppm groups of males. In females, absolute kidney weights were significantly increased in the 1,000 and 2,000 ppm groups, and relative kidney weight was significantly increased in the 2,000 ppm group. The increases in females were not as great as those in the males, with the greatest absolute kidney weight (115% of that of the chamber controls) occurring in the 2,000 ppm group. The increases in kidney weights were not associated with corresponding histologic changes in males or females.

Table 17. Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	33.9 ± 0.6	34.2 ± 1.0	35.8 ± 0.9	36.9 ± 0.8**	38.6 ± 0.8**	36.8 ± 0.4**
R. Kidney						
Absolute	0.29 ± 0.01	0.30 ± 0.01	0.31 ± 0.01	0.32 ± 0.01*	0.36 ± 0.01**	0.38 ± 0.01**
Relative	8.65 ± 0.14	8.86 ± 0.19	8.74 ± 0.20	8.75 ± 0.23	9.36 ± 0.23*	10.28 ± 0.22**
Liver						
Absolute	1.30 ± 0.04	1.44 ± 0.06	1.65 ± 0.07**	2.09 ± 0.07**	3.07 ± 0.07**	3.60 ± 0.05**
Relative	38.43 ± 0.87	42.11 ± 1.30	46.01 ± 1.36**	56.73 ± 1.84**	79.75 ± 1.16**	97.85 ± 1.59**
Thymus						
Absolute	0.038 ± 0.002	0.041 ± 0.003	0.044 ± 0.002	0.043 ± 0.003	0.043 ± 0.002	0.032 ± 0.002
Relative	1.121 ± 0.056	1.196 ± 0.074	1.242 ± 0.044	1.173 ± 0.065	1.108 ± 0.053	0.859 ± 0.053*
Female						
Necropsy body wt	28.2 ± 0.4	29.9 ± 0.8	31.0 ± 0.7**	32.2 ± 0.7**	33.7 ± 0.8**	29.7 ± 0.7**
R. Kidney						
Absolute	0.20 ± 0.01	0.21 ± 0.00	0.22 ± 0.01	0.21 ± 0.00	0.22 ± 0.01*	0.23 ± 0.01**
Relative	6.97 ± 0.20	6.98 ± 0.13	7.01 ± 0.17	6.55 ± 0.17	6.48 ± 0.12	7.69 ± 0.29*
Liver						
Absolute	1.23 ± 0.05	1.33 ± 0.05	1.51 ± 0.05*	1.81 ± 0.08**	2.44 ± 0.08**	2.61 ± 0.14**
Relative	43.51 ± 1.66	44.28 ± 0.91	48.73 ± 0.79	56.18 ± 1.98**	72.49 ± 1.95**	87.58 ± 3.36**
Thymus						
Absolute	0.055 ± 0.002	0.058 ± 0.001	0.060 ± 0.004	0.065 ± 0.003	0.062 ± 0.001	0.041 ± 0.004**
Relative	1.956 ± 0.100	1.938 ± 0.054	1.925 ± 0.108	2.011 ± 0.068	1.858 ± 0.044	1.371 ± 0.102**

*Significantly different ($P \leq 0.05$) from the chamber 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).

Absolute thymus weight was significantly decreased in 2,000 ppm females (75% of the chamber controls) and relative thymus weights were significantly decreased in 2,000 ppm males and females (Table 17 and Table G-2). There were no corresponding histologic lesions in the thymus.

There were significantly increased absolute heart weights in males exposed to 500 ppm or greater and significantly decreased relative heart and relative lung weights in females exposed to 500 ppm or greater (Table G-2).

There were no gross lesions associated with exposure to *p*-chloro-α,α,α-trifluorotoluene. Significant histologic lesions were recorded in the liver, forestomach, adrenal cortex, and spleen of male and female mice.

In the liver, there were significantly increased incidences of centrilobular hepatocyte hypertrophy in males exposed to 250 ppm or greater and females exposed to 500 ppm or greater compared to their respective chamber control groups (Table 18); severities also generally increased with increasing exposure concentration, with most 1,000 and 2,000 ppm males and most 2,000 ppm females having marked hypertrophy. Increased incidences of hepatocyte hypertrophy correspond with the higher liver weights. There were also significantly increased incidences of centrilobular hepatocyte necrosis and multinucleated hepatocytes in males exposed to 500 ppm or greater and females exposed to 1,000 or 2,000 ppm.

Hepatocyte hypertrophy was characterized by enlargement of hepatocytes up to two or three times normal size (Figure 19). The hypertrophic hepatocytes had increased amounts of cytoplasm, which was often eosinophilic or granular in appearance, and enlarged nuclei. Severity grading of hepatocyte hypertrophy was based primarily on the amount of each hepatic lobule that was involved; lesions of minimal severity affected only the centrilobular hepatocytes, whereas with mild and moderate lesions, an increasing area of each hepatic lobule was affected. With marked severity, the hypertrophy was essentially a diffuse change, but it was still recorded as centrilobular as it was considered the same process as that seen with lesser severities. Also, as the severity increased, the size of the individual hepatocytes tended to increase as well. Centrilobular hepatocyte necrosis was characterized by a loss of cellular detail and differential staining, with replacement by eosinophilic cellular and karyorrhectic debris, in the centrilobular zone. Typically, there was a lack of associated inflammation. Multinucleated hepatocytes were large hepatocytes containing three or more nuclei, often up to eight or 10. Severity grading of multinucleated hepatocytes was based primarily on the number of multinucleated hepatocytes observed within a given section of liver.

In the forestomach, there were significantly increased incidences of epithelium hyperplasia in males and females exposed to 500 ppm or greater (Table 18). In 2,000 ppm males and females, there were significantly increased incidences of granulomatous inflammation. Epithelium hyperplasia was characterized by increased numbers of squamous epithelial cells that caused an increased thickness of the mucosal epithelium up to three times as thick as that seen in chamber control animals (Figure 20). Granulomatous inflammation consisted of focal to multifocal infiltrates of histiocytes, with fewer multinucleated giant cells, within the submucosa.

In the adrenal cortex, there were significantly increased incidences of zona fasciculata hypertrophy in 2,000 ppm males and females (Table 18). In 2,000 ppm females, there was also a significantly increased incidence of X-zone degeneration. Hypertrophy was characterized by eosinophilic cells with increased amounts of cytoplasm, within the zona fasciculata. X-zone

degeneration was characterized by a narrow X-zone, with fewer cells, condensed nuclei, and increased intercellular spaces. Degeneration of the X-zone is a normal physiological process that takes place several weeks after puberty in female mice. When degeneration of the X-zone is diagnosed, it typically reflects a shift in the normal time course of degeneration of the X-zone (a process influenced by hormones) rather than a unique pathological process.

In the red pulp of the spleen, there were significantly increased incidences of megakaryocyte and erythrocytic hematopoietic cell proliferation in females exposed to 250 ppm or greater (Table 18). In males, there were significantly increased incidences of megakaryocyte hematopoietic cell proliferation at 1,000 and 2,000 ppm, and a significantly increased incidence of erythrocytic hematopoietic cell proliferation at 2,000 ppm. Hematopoietic cell proliferation was characterized by increased numbers of megakaryocytes (megakaryocyte hematopoietic cell proliferation) or red blood cell precursors (erythrocytic hematopoietic cell proliferation) within the red pulp of the spleen.

Table 18. Incidences of Selected Nonneoplastic Lesions in Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
Male						
Adrenal Cortex[a]	10	10	10	10	10	9
Zona Fasciculata, Hypertrophy[b]	1 (1.0)[c]	0	0	0	1 (1.0)	6* (1.0)
Forestomach	10	10	10	10	10	10
Epithelium, Hyperplasia	0	0	1 (1.0)	4* (1.0)	7** (1.3)	9** (2.0)
Inflammation, Granulomatous	0	0	0	1 (1.0)	2 (1.0)	10** (1.5)
Liver	10	10	10	10	10	10
Centrilobular, Hepatocyte, Hypertrophy	0	0	10** (1.5)	10** (2.7)	10** (3.9)	10** (4.0)
Centrilobular, Hepatocyte, Necrosis	0	0	2 (1.0)	10** (1.2)	10** (1.5)	10** (2.0)
Hepatocyte, Multinucleated	0	0	1 (1.0)	8** (1.6)	10** (3.5)	10** (4.0)
Spleen	10	10	10	10	10	10
Red Pulp, Hematopoietic Cell Proliferation Erythrocytic	0	0	0	1 (1.0)	2 (1.0)	10** (1.0)
Red Pulp, Hematopoietic Cell Proliferation, Megakaryocyte	0	0	0	1 (1.0)	6** (1.3)	10** (2.0)
Female						
Adrenal Cortex	10	10	10	10	10	10
X-zone, Degeneration	0	0	0	0	0	10** (3.7)
Zona Fasciculata, Hypertrophy	0	0	0	0	0	7** (1.6)
Forestomach	10	9	9	10	10	10
Epithelium, Hyperplasia	0	2 (1.0)	3 (1.0)	5* (1.0)	10** (1.6)	10** (2.1)
Inflammation, Granulomatous	0	0	0	0	0	8** (1.8)
Liver	10	10	10	10	10	10
Centrilobular, Hepatocyte, Hypertrophy	0	0	2 (1.5)	4* (1.0)	10** (2.2)	10** (3.9)
Centrilobular, Hepatocyte, Necrosis	0	0	1 (1.0)	2 (1.0)	8** (1.1)	10** (1.6)
Hepatocyte, Multinucleated	0	0	0	0	6** (1.5)	10** (3.3)
Spleen	10	10	10	10	10	10
Red Pulp, Hematopoietic Cell Proliferation Erythrocytic	0	0	5* (1.0)	4* (1.0)	9** (1.0)	10** (1.0)
Red Pulp, Hematopoietic Cell Proliferation, Megakaryocyte	0	0	5* (1.0)	6** (1.2)	10** (1.5)	10** (2.0)

*Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact 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.

All exposed groups of males evaluated displayed significant exposure concentration-related decreases in sperm motility (Table 19 and Table H-4). The number of sperm/cauda epididymis was increased in these exposed groups; however, the value in the chamber control group was on the lower side of NTP historical experience. In 1,000 and 2,000 ppm females, there were significant increases in estrous cycle length compared to the chamber control group (Table 19 and Table H-5). All exposed female groups evaluated had significantly higher probabilities of extended estrus (Table 19, Table H-5, Table H-6, and Figure H-2). *p*-Chloro-α,α,α-trifluorotoluene exposure via inhalation exhibited the potential to be a reproductive toxicant in male and female B6C3F1/N mice.

Exposure Concentration Selection Rationale: Statistically significant exposure-related increases in liver weight (approximately 50% or greater) and increased incidences of liver necrosis and other liver lesions precluded the use of 500 ppm in male mice and 1,000 ppm in female mice. The *p*-chloro-α,α,α-trifluorotoluene exposure concentrations selected for the 2-year inhalation study in mice were 100, 200, and 400 ppm. Similar to rats, these concentrations were selected to accommodate logistical challenges while adequately challenging both males and females.

Table 19. Summary of Reproductive Tissue Evaluations for Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	500 ppm	1,000 ppm	2,000 ppm
n	10	10	10	10
Male				
Spermatid measurements				
Spermatid heads (10^6/testis)	19.72 ± 1.004	22.31 ± 0.656	19.99 ± 1.324	17.72 ± 1.321
Spermatid heads (10^6/g testis)	191.87 ± 8.230	206.29 ± 7.450	192.82 ± 11.952	172.12 ±12.644
Epididymal spermatozoal measurements				
Sperm motility (%)	79.9 ± 0.89	67.1 ± 3.33**	63.2 ± 1.42**	60.1 ± 1.31**
Sperm (10^6/cauda epididymis)	11.3 ± 0.55	14.8 ± 0.66*	14.2 ± 1.29	15.0 ± 1.15*
Female				
Estrous cycle length (days)	4.1 ± 0.10	4.5 ± 0.16	4.6 ± 0.14*	5.3 ± 0.65**
Estrous stages (% of cycle)				
Diestrus	36.9	32.5	28.8	30.0
Proestrus	0.0	2.5	4.4	6.9
Estrus	47.5	48.1	44.4	45.0
Metestrus	15.6	16.3	21.3	16.9

*Significantly different (P ≤ 0.05) from the chamber control group by Shirley's test (sperm/cauda epididymis) or Dunn's test (estrous cycle length).
**Significantly different (P ≤ 0.01) from the chamber control group by Shirley's test (sperm motility) or Dunn's test (estrous cycle length).
[a]Data for sperm motility, sperm/cauda epididymis, and estrous cycle length are presented as mean ± standard error. Differences from the control group for spermatid measurements are not significant by Dunn's test. Tests for equality of transition probability matrices among all groups and between the chamber control group and each exposed group indicated that all exposed groups evaluated spent significantly more time in extended estrus (P ≤ 0.001) than the chamber control group.

Two-year Study

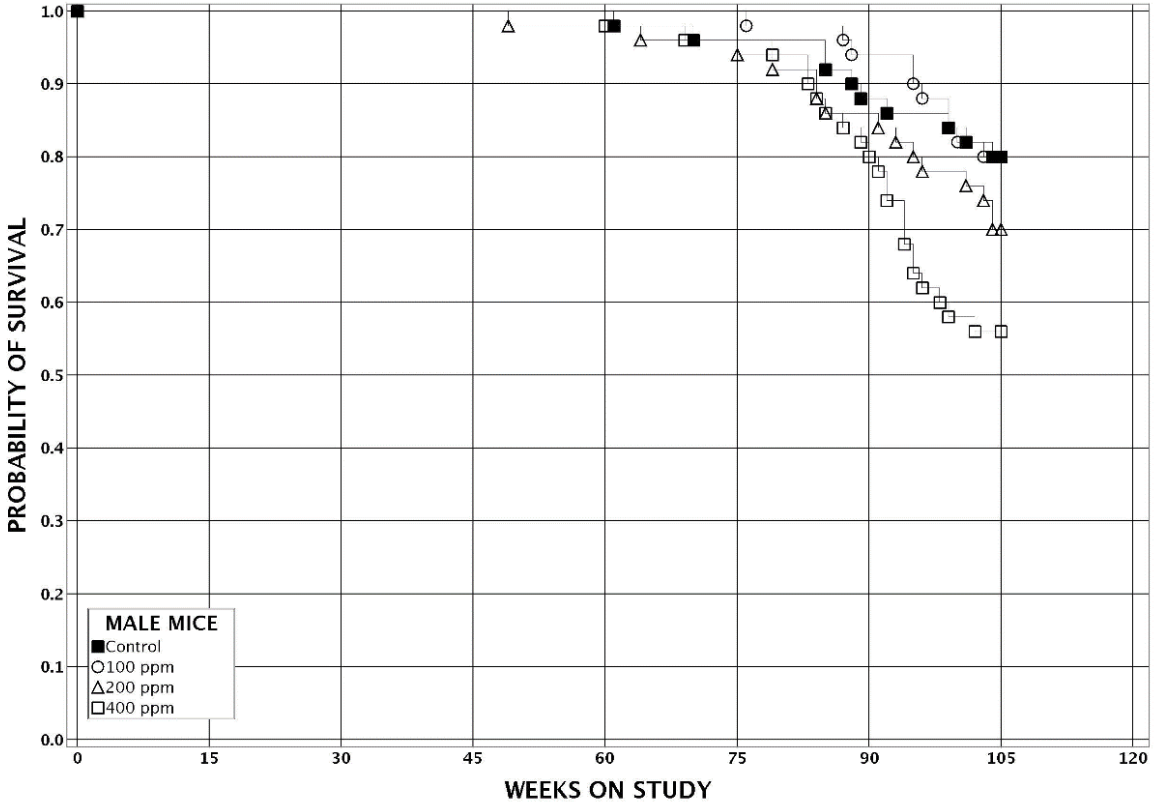
Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 20 and in the Kaplan-Meier survival curves (Figure 6). Survival of 400 ppm males was significantly decreased compared to the chamber control group. Survival of 400 ppm females was less than the chamber control group, but the difference was not statistically significant. Decreased survival in the 400 ppm groups occurred very late in the study. Sixteen of the 22 male and 11 of the 23 female early deaths and moribund removals were attributable to liver neoplasms.

Table 20. Survival of Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	200 ppm	400 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	8	5	11	12
Natural deaths	2	5	4	10
Animals surviving to study termination	40	40	35	28
Percent probability of survival at end of study[a]	80	80	70	56
Mean survival (days)[b]	705	714	692	680
Survival analysis[c]	P = 0.002	P = 1.000	P = 0.348	P = 0.018
Female				
Animals initially in study	50	50	50	50
Accidental death[d]	0	0	1	0
Moribund	7	12	9	16
Natural deaths	5	5	3[e]	7
Animals surviving to study termination	38	33	37	27[f]
Percent probability of survival at end of study	76	66	76	54
Mean survival (days)	700	688	687	688
Survival analysis	P = 0.059	P = 0.398	P = 1.000	P = 0.051

[a]Kaplan-Meier determinations.
[b]Mean of all deaths (uncensored, censored, and terminal kill).
[c]The result of the life table trend test[40] is in the chamber control column, and the results of the life table pairwise comparisons[39] with the chamber controls are in the exposed group columns.
[d]Censored in the survival analysis.
[e]Includes two animals that died during the last week of the study.
[f]Includes one animal that died during the last week of the study.



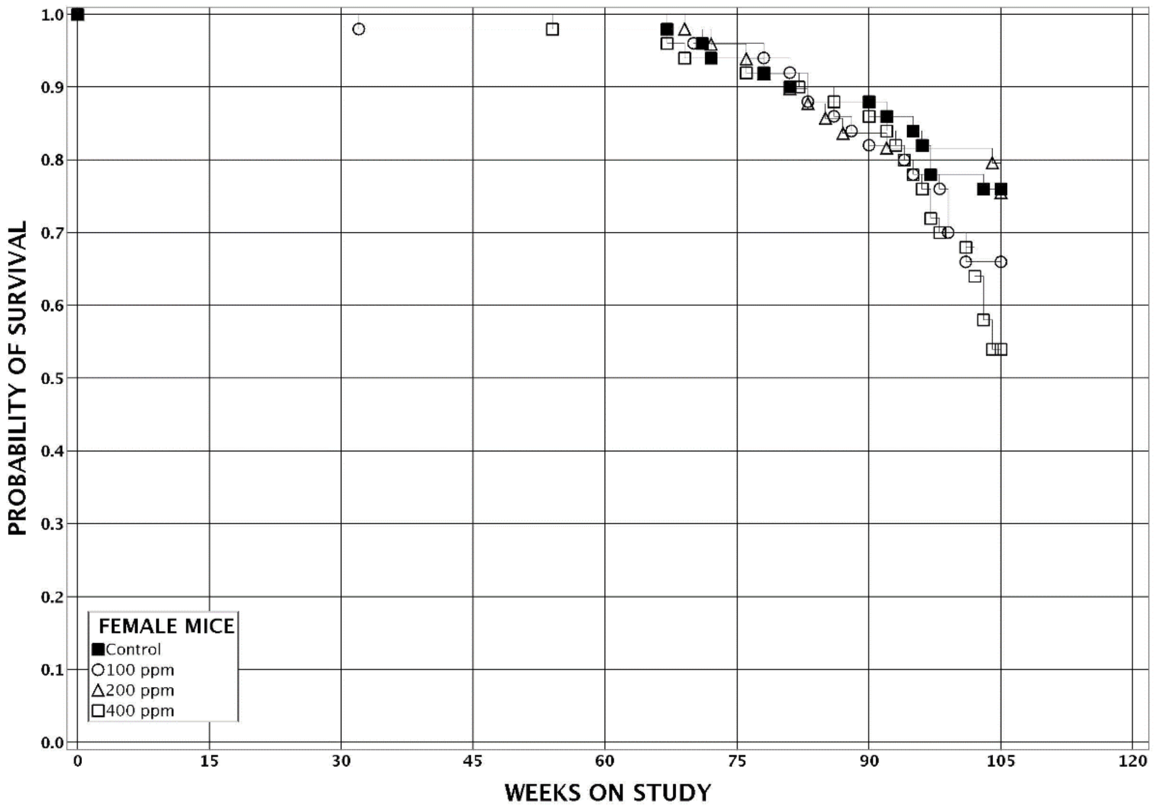


Figure 6. Kaplan-Meier Survival Curves for Mice Exposed to *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years

Body Weights and Clinical Observations

Mean body weights of exposed groups of males were within 10% of those of the chamber controls throughout the study (Figure 7, Table 21). The mean body weights of 100 ppm females were at least 10% greater than those of the chamber controls generally after week 69 (Figure 7, Table 22). The mean body weights of 200 and 400 ppm females were at least 10% greater than those of the chamber controls after weeks 13 and 2, respectively. Clinical observations included increased occurrences of distended abdomen at removal, which were associated with liver neoplasms. There were also increased occurrences of thinness in 400 ppm males and females.

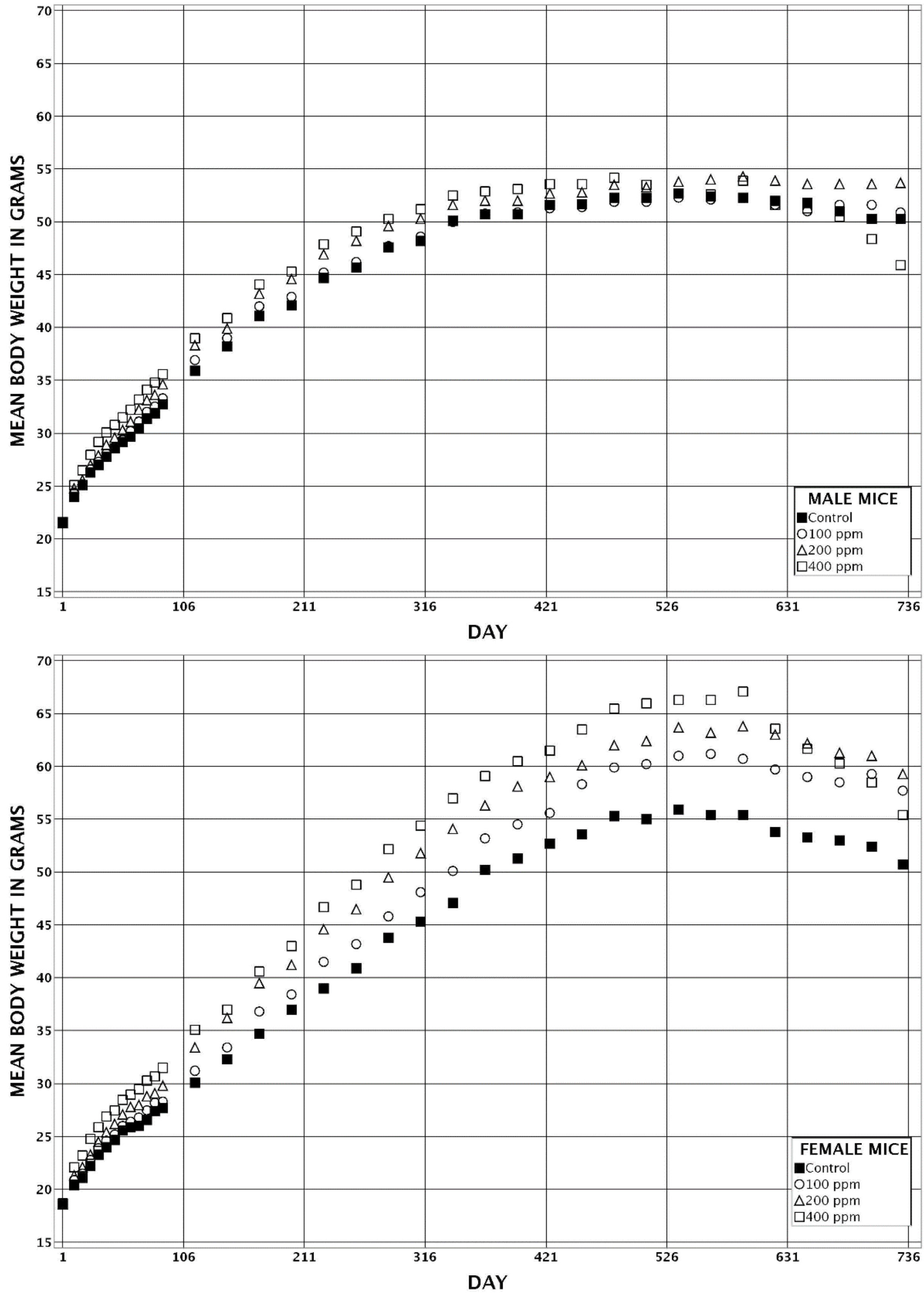


Figure 7. Growth Curves for Mice Exposed to *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years

Table 21. Mean Body Weights and Survival of Male Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control		100 ppm			200 ppm			1,000 ppm		
Day	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	21.6	50	21.5	100	50	21.5	100	50	21.5	100	50
11	24.0	50	24.4	102	50	24.8	103	50	25.1	105	50
18	25.1	50	25.2	101	50	25.6	102	50	26.5	106	50
25	26.3	50	26.6	101	50	27.0	103	50	28.0	107	50
32	27.0	50	27.3	101	50	27.9	104	50	29.2	108	50
39	27.8	50	28.1	101	50	28.9	104	50	30.1	108	50
46	28.6	50	28.7	100	50	29.6	104	50	30.8	108	50
53	29.2	50	29.4	101	50	30.3	104	50	31.5	108	50
60	29.7	50	30.2	102	50	31.1	105	50	32.2	108	50
67	30.5	50	31.1	102	50	32.2	106	50	33.2	109	50
74	31.4	50	32.0	102	50	33.1	105	50	34.1	109	50
81	31.9	50	32.5	102	50	33.6	105	50	34.8	109	50
88	32.7	50	33.3	102	50	34.6	106	50	35.6	109	50
116	35.9	50	36.9	103	50	38.3	107	50	39.0	109	50
144	38.2	50	39.0	102	50	39.9	105	50	40.9	107	50
172	41.1	50	42.0	102	50	43.2	105	50	44.1	107	50
200	42.1	50	42.9	102	50	44.6	106	50	45.3	108	50
228	44.7	50	45.2	101	50	46.9	105	50	47.9	107	50
256	45.7	50	46.2	101	50	48.2	106	50	49.1	107	50
284	47.6	50	47.7	100	50	49.6	104	50	50.3	106	50
312	48.2	50	48.6	101	50	50.3	105	50	51.2	106	50
340	50.1	50	50.0	100	50	51.6	103	49	52.5	105	50
368	50.7	50	50.8	100	50	52.0	103	49	52.9	104	50
396	50.7	50	50.9	100	50	52.0	103	49	53.1	105	50
424	51.6	49	51.3	100	50	52.7	102	49	53.6	104	49
452	51.7	49	51.4	99	50	52.8	102	48	53.6	104	49
480	52.3	49	51.9	99	50	53.5	102	48	54.2	104	48
508	52.3	48	51.9	99	50	53.3	102	48	53.5	102	48
536	52.7	48	52.3	99	49	53.8	102	47	52.7	100	48
564	52.4	48	52.1	100	49	54.0	103	46	52.6	100	47
592	52.3	47	52.3	100	49	54.3	104	43	53.9	103	43
620	52.0	44	51.6	99	47	53.9	104	43	51.6	99	42
648	51.8	43	51.0	99	47	53.6	104	41	51.3	99	37
676	51.0	43	51.6	101	44	53.6	105	39	50.5	99	31
704	50.3	41	51.6	103	41	53.6	106	38	48.4	96	29
Mean for Weeks											
1–13	28.1	–	28.5	101	–	29.2	104	–	30.2	107	–
14–52	43.7	–	44.3	101	–	45.8	105	–	46.7	107	–
53–101	51.7	–	51.6	100	–	53.3	103	–	52.5	102	–

Table 22. Mean Body Weights and Survival of Female Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control		100 ppm			200 ppm			1,000 ppm		
Day	**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	18.6	50	18.6	100	50	18.6	100	50	18.7	101	50
11	20.4	50	20.9	103	50	21.3	105	50	22.1	109	50
18	21.1	50	21.5	102	50	22.1	105	50	23.2	110	50
25	22.2	50	22.8	103	50	23.3	105	50	24.8	112	50
32	23.3	50	23.9	103	50	24.5	105	50	25.9	111	50
39	24.0	50	24.6	103	50	25.4	106	50	26.9	112	50
46	24.7	50	25.2	102	50	26.2	106	50	27.5	112	50
53	25.6	50	26.0	102	50	27.1	106	50	28.5	112	50
60	25.9	50	26.4	102	50	27.8	107	50	29.0	112	50
67	26.0	50	26.8	103	50	28.0	108	50	29.5	114	50
74	26.6	50	27.5	103	50	28.8	108	50	30.3	114	50
81	27.4	50	28.2	103	50	29.1	106	50	30.7	112	50
88	27.7	50	28.3	102	50	29.8	107	50	31.5	113	50
116	30.1	50	31.2	104	50	33.4	111	49	35.1	117	50
144	32.3	50	33.4	103	50	36.2	112	49	37.0	115	50
172	34.7	50	36.8	106	50	39.5	114	49	40.6	117	50
200	37.0	50	38.4	104	50	41.2	111	49	43.0	116	50
228	39.0	50	41.5	106	49	44.6	114	49	46.7	120	50
256	40.9	50	43.2	105	49	46.5	114	49	48.8	119	50
284	43.8	50	45.8	105	49	49.5	113	49	52.2	119	50
312	45.3	50	48.1	106	49	51.8	114	49	54.4	120	50
340	47.1	50	50.1	106	49	54.1	115	49	57.0	121	50
368	50.2	50	53.2	106	49	56.3	112	49	59.1	118	50
396	51.3	50	54.5	106	49	58.1	113	49	60.5	118	49
424	52.7	50	55.6	106	49	59.0	112	49	61.5	117	49
452	53.6	50	58.3	109	49	60.1	112	49	63.5	118	49
480	55.3	49	59.9	108	49	62.0	112	48	65.5	118	47
508	55.0	47	60.2	110	48	62.4	114	47	66.0	120	47
536	55.9	47	61.0	109	48	63.7	114	46	66.3	119	46
564	55.4	45	61.2	111	46	63.2	114	44	66.3	120	46
592	55.4	45	60.7	110	44	63.8	115	43	67.1	121	45
620	53.8	45	59.7	111	42	63.0	117	41	63.6	118	44
648	53.3	43	59.0	111	41	62.2	117	40	61.7	116	41
676	53.0	39	58.5	110	39	61.3	116	40	60.3	114	36
704	52.4	39	59.3	113	33	61.0	116	40	58.5	112	34
Mean for Weeks											
1–13	24.1	–	24.7	102	–	25.5	106	–	26.8	111	–
14–52	38.9	–	40.9	105	–	44.1	113	–	46.1	119	–
53–101	53.6	–	58.5	109	–	61.2	114	–	63.1	118	–

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the liver, Harderian gland, lung, larynx, and forestomach. 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.

Liver: The overall incidences of hepatocellular adenoma were significantly increased in 200 and 400 ppm females, and the overall incidences of hepatocellular carcinoma were significantly increased in all exposed male groups and 400 ppm females (Table 23, Table C-1, Table C-2, Table D-1, and Table D-2). In addition, there were significantly increased incidences of multiple hepatocellular adenoma in 200 and 400 ppm males and females and multiple hepatocellular carcinoma in 400 ppm males and females compared to the chamber control groups. There were significantly increased incidences of hepatoblastoma in 400 ppm males and females. The incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) were significantly increased in 200 and 400 ppm males and females.

Hepatocellular adenomas were discrete, well-circumscribed lesions that compressed surrounding parenchyma and had hepatic cords generally oriented perpendicularly to surrounding hepatic cords. Central veins and portal areas were generally absent. Hepatocytes were well differentiated with variable size and tinctoral characteristics. Hepatocellular carcinomas were large lesions, frequently with areas of necrosis, which caused compression of, and invasion into, surrounding parenchyma. Typically, hepatocellular carcinomas were characterized by hepatocytes forming trabeculae that were at least three cells thick, although some of the areas of carcinomas were of a solid pattern of growth (Figure 21). Cells within the hepatocellular carcinomas ranged from eosinophilic to basophilic in staining, and displayed marked pleomorphism and an increased mitotic rate. Nucleoli tended to be distinct and larger than normal. Hepatoblastomas were composed of small cells with scant cytoplasm and hyperchromatic, oval nuclei (Figure 22). Cells were often arranged in rows around variably sized vascular spaces. Hepatoblastomas often arose from within a hepatocellular carcinoma, and when this occurred, only the hepatoblastoma was recorded, but several hepatoblastomas were observed with no connection to hepatocellular tumors.

There were significantly increased incidences of eosinophilic focus in 400 ppm males and 200 and 400 ppm females (Table 23, Table C-4, and Table D-4). There were significantly increased incidences of centrilobular hepatocyte hypertrophy in all exposed groups of males and in 200 and 400 ppm females. There were significantly increased incidences of multinucleated hepatocytes in 200 and 400 ppm males and 400 ppm females. The incidences of hepatocyte necrosis were significantly increased in 400 ppm males and females. Intrahepatocellular erythrocytes only occurred in exposed males, and the incidences were significantly increased in the 200 and 400 ppm groups. There was a significantly increased incidence of clear cell focus in 200 ppm males, but this was considered biological variation due to the lack of exposure concentration response and the fact that clear cell foci are fairly common as a background change.

Eosinophilic foci were discrete groups of enlarged hepatocytes with brightly eosinophilic cytoplasm. Some foci caused compression of some of the surrounding parenchyma, but not to the extent of hepatocellular adenomas. Hepatocytes within foci were generally aligned with

hepatocytes in the normal liver, and portal triads and hepatic veins were present within foci, in contrast to hepatocellular adenomas. Foci typically lacked cellular atypia and mitotic figures. Centrilobular hepatocyte hypertrophy was characterized by an accentuated lobular pattern due to the presence of enlarged hepatocytes, primarily in the centrilobular zone, but expanding into the midzonal and periportal zones within increasing severity (Figure 23). Involved hepatocytes had abundant granular eosinophilic cytoplasm containing clumped basophilic material (Figure 24). Nuclei were frequently enlarged and had stippled chromatin, prominent nucleoli, and occasional bright eosinophilic inclusions. Severity grading was based upon the amount of hepatic lobule involved, with minimal, mild, or moderate being recorded when 1% to 20%, 20% to 50%, or more than 50% of each hepatic lobule was affected, respectively (no marked lesions were recorded.) Multinucleated hepatocytes were characterized by enlarged hepatocytes containing three or more, often up to 10 and occasionally as many as 20, nuclei (Figure 25). Severity grading was based on the number of affected hepatocytes, with only minimal and mild lesions being recorded. Minimal severity typically consisted of one to five affected hepatocytes within a section of liver. Hepatocyte necrosis was characterized by randomly scattered clusters of three to 15 necrotic hepatocytes; necrosis was not diagnosed if it occurred within a tumor. Severity grading was based upon the amount of hepatic parenchyma involved, with 1% to 3%, 4% to 10%, or 11% to 50% involvement being recorded as minimal, mild, or moderate, respectively. Intrahepatocellular erythrocytes, when present, were randomly scattered throughout the liver with no particular zonality.

Table 23. Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	200 ppm	400 ppm
Male				
Number Examined Microscopically	50	50	50	50
Centrilobular, Hepatocyte, Hypertrophy[a]	0	8** (1.0)[b]	19** (1.1)	49** (1.5)
Clear Cell Focus	15	16	23*	15
Eosinophilic Focus	11	14	18	21*
Hepatocyte, Multinucleated	2 (1.0)	8 (1.0)	19** (1.1)	49** (1.4)
Hepatocyte, Necrosis	3 (1.0)	4 (2.5)	3 (2.0)	15** (1.5)
Intrahepatocellular Erythrocytes	0	1 (1.0)	6* (1.0)	15** (1.3)
Hepatocellular Adenoma, Multiple	9	15	19*	21**
Hepatocellular Adenoma (includes multiple)[c]				
Overall rate[d]	25/50 (50%)	24/50 (48%)	31/50 (62%)	29/50 (58%)
Adjusted rate[e]	53.0%	50.2%	68.1%	64.3%
Terminal rate[f]	23/40 (58%)	21/40 (53%)	27/35 (77%)	18/28 (64%)
First incidence (days)	424	660	551	477
Poly-3 test[g]	P = 0.079	P = 0.473N	P = 0.096	P = 0.181
Hepatocellular Carcinoma, Multiple	2	5	7	30**
Hepatocellular Carcinoma (includes multiple)[h]				
Overall rate	8/50 (16%)	19/50 (38%)	16/50 (32%)	35/50 (70%)
Adjusted rate	17.0%	38.6%	34.1%	75.3%
Terminal rate	6/40 (15%)	12/40 (30%)	8/35 (23%)	22/28 (79%)
First incidence (days)	488	605	523	548
Poly-3 test	P < 0.001	P = 0.015	P = 0.046	P < 0.001
Hepatoblastoma, Multiple	0	0	0	5*
Hepatoblastoma (includes multiple)[i]				
Overall rate	1/50 (2%)	1/50 (2%)	1/50 (2%)	15/50 (30%)
Adjusted rate	2.1%	2.1%	2.3%	34.6%
Terminal rate	0/40 (0%)	1/40 (3%)	1/35 (3%)	8/28 (29%)
First incidence (days)	591	729 (T)	729 (T)	638
Poly-3 test	P < 0.001	P = 0.757N	P = 0.749	P < 0.001
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma[j]				
Overall rate	31/50 (62%)	37/50 (74%)	40/50 (80%)	48/50 (96%)
Adjusted rate	64.1%	74.9%	84.3%	98.6%
Terminal rate	26/40 (65%)	28/40 (70%)	30/35 (86%)	28/28 (100%)
First incidence (days)	424	605	523	477
Poly-3 test	P < 0.001	P = 0.169	P = 0.017	P < 0.001
Female				
Number Examined Microscopically	50	50	50	50
Centrilobular, Hepatocyte, Hypertrophy	0	4 (1.0)	5* (1.2)	40** (1.2)
Eosinophilic Focus	4	8	24**	31**
Hepatocyte, Multinucleated	0	2 (1.0)	2 (1.0)	25** (1.0)
Hepatocyte, Necrosis	2 (2.0)	1 (1.0)	3 (1.7)	10* (1.3)
Number Examined Microscopically	50	50	50	50
Hepatocellular Adenoma, Multiple	3	5	15**	25**
Hepatocellular Adenoma (includes multiple)[k]				

	Chamber Control	100 ppm	200 ppm	400 ppm
Overall rate	12/50 (24%)	14/50 (28%)	24/50 (48%)	34/50 (68%)
Adjusted rate	26.1%	31.9%	54.5%	72.6%
Terminal rate	9/38 (24%)	11/33 (33%)	24/37 (65%)	18/27 (67%)
First incidence (days)	544	685	731 (T)	530
Poly-3 test	P < 0.001	P = 0.355	P = 0.004	P < 0.001
Hepatocellular Carcinoma, Multiple	2	3	7	28**
Hepatocellular Carcinoma (includes multiple)[l]				
Overall rate	7/50 (14%)	8/50 (16%)	12/50 (24%)	34/50 (68%)
Adjusted rate	15.3%	18.1%	26.9%	75.9%
Terminal rate	4/38 (11%)	5/33 (15%)	9/37 (24%)	23/27 (85%)
First incidence (days)	664	578	576	642
Poly-3 test	P < 0.001	P = 0.472	P = 0.136	P < 0.001
Hepatoblastoma[m]				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	8/50 (16%)
Adjusted rate	0.0%	0.0%	2.3%	18.3%
Terminal rate	0/38 (0%)	0/33 (0%)	1/37 (3%)	4/27 (15%)
First incidence (days)	–[n]	–	731 (T)	673
Poly-3 test	P < 0.001	–[o]	P = 0.495	P = 0.003
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma[p]				
Overall rate	18/50 (36%)	18/50 (36%)	29/50 (58%)	46/50 (92%)
Adjusted rate	38.8%	40.4%	65.0%	97.6%
Terminal rate	13/38 (34%)	13/33 (39%)	26/37 (70%)	27/27 (100%)
First incidence (days)	554	578	576	530
Poly-3 test	P < 0.001	P = 0.523	P = 0.008	P < 0.001

*Significantly different (P ≤ 0.05) from the chamber control group by the Poly-3 test.
**P ≤ 0.01.
(T) Terminal kill.
[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 inhalation studies with chamber control groups (mean ± standard deviation): 147/300 (49.0% ± 9.0%), range 34%–60%; all routes: 302/550 (54.9% ± 12.4%), range 34%–78%.
[d]Number of animals with neoplasm per number of animals with liver examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in an exposure group is indicated by N.
[h]Historical incidence for inhalation studies: 97/300 (32.3% ± 12.6%), range 16%–50%; all routes: 165/550 (30.0% ± 10.0%), range 16%–50%.
[i]Historical incidence for inhalation studies: 6/300 (2.0% ± 1.8%), range 0%–4%; all routes: 18/550 (3.3% ± 2.4%), range 0%–8%.
[j]Historical incidence for inhalation studies: 207/300 (69.0% ± 7.2%), range 62%–78%; all routes: 395/550 (71.8% ± 8.1%), range 62%–84%.
[k]Historical incidence for inhalation studies: 71/300 (23.7% ± 8.9%), range 12%–38%; all routes: 141/549 (25.8% ± 15.9%), range 10%–67%.
[l]Historical incidence for inhalation studies: 45/300 (15.0% ± 4.2%), range 10%–20%; all routes: 71/549 (12.9% ± 5.1%), range 4%–20%.
[m]Historical incidence for inhalation studies: 3/300 (1.0% ± 1.1%), range 0%–2%; all routes: 3/549 (0.6% ± 0.9%), range 0%–2%.
[n]Not applicable; no neoplasms in animal group.
[o]Value of statistic cannot be computed.
[p]Historical incidence for inhalation studies: 104/300 (34.7% ± 9.4%), range 24%–50%; all routes: 189/549 (34.5% ± 15.8%), range 16%–73%.

The number of erythrocytes within hepatocytes varied from one to too numerous to count. Affected hepatocytes were often increased in size, with the increase in size usually correlating to the number of intracellular erythrocytes, and the cytoplasm and nuclei of these hepatocytes was usually peripheralized (Figure 26). Minimal severity grade was recorded when less than three or four hepatocytes with intracellular erythrocytes were noted throughout the liver sections examined, while mild was used when several areas containing multiple affected hepatocytes were noted.

Liver Molecular Pathology: Hras mutations in rodent hepatocellular carcinomas (HCCs) are commonly observed within codon 61[72; 73]. In the current study, the incidence of *Hras* mutations in mouse HCCs examined from all *p*-chloro-α,α,α-trifluorotoluene-exposed groups was 37% (23/62) and the incidence in chamber controls was 73% (11/15) (Appendix L); these mutations were located within codon 61. There were significant trend and pairwise differences for *Hras* mutation incidence in the negative direction between HCCs occurring spontaneously in chamber controls and HCCs resulting from chronic exposure to *p*-chloro-α,α,α-trifluorotoluene. There were no statistically significant differences in *Ctnnb1* mutations in mouse HCCs resulting from chronic exposure to *p*-chloro-α,α,α-trifluoro-toluene compared to the chamber control groups. Four of the HCCs arising spontaneously harbored *Ctnnb1* mutations all of which were point mutations in codon 15-46. There was a deletion of codons 27-35 in five HCCs from *p*-chloro-α,α,α-trifluorotoluene-exposed groups. None of the spontaneous HCCs had deletions in the *Ctnnb1* gene. There were no differences in the incidences of mutations between male and female mice (data not presented) and hence, the combined data from both male and female mice are presented in Appendix L.

Harderian Gland: There was a significantly increased incidence of adenoma in 400 ppm females, and significantly increased incidences of adenoma or adenocarcinoma (combined) in 200 and 400 ppm females (Table 24, Table D-1, and Table D-2). Adenomas were fairly well-circumscribed nodules that caused compression of surrounding parenchyma. The cells were cuboidal to tall columnar epithelium, similar to that found in a normal gland, but patterns of growth included papillary and cystic (Figure 27). Adenocarcinomas were expansile and invasive, and had a solid, cystic, or papillary growth pattern or a combination of different growth patterns (Figure 28). Cells were typically smaller than normal, and often had a single large clear cytoplasmic vacuole, in contrast to the numerous small vacuoles usually seen in the cytoplasm of normal Harderian gland cells.

Table 24. Incidences of Neoplasms of the Harderian Gland in Female Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	200 ppm	400 ppm
Adenoma[a]				
Overall rate[b]	2/50 (4%)	6/50 (12%)	6/50 (12%)	8/50 (16%)
Adjusted rate[c]	4.4%	13.6%	13.5%	18.1%
Terminal rate[d]	2/38 (5%)	5/33 (15%)	5/37 (14%)	4/27 (15%)
First incidence (days)	731 (T)	576	562	642
Poly-3 test[e]	P = 0.049	P = 0.124	P = 0.129	P = 0.041
Adenocarcinoma[f,g]	0	0	3	0
Adenoma or Adenocarcinoma[g,h]				
Overall rate	2/50 (4%)	6/50 (12%)	9/50 (18%)	8/50 (16%)
Adjusted rate	4.4%	13.6%	19.7%	18.1%
Terminal rate	2/38 (5%)	5/33 (15%)	6/37 (16%)	4/27 (15%)
First incidence (days)	731 (T)	576	480	642
Poly-3 test	P = 0.046	P = 0.124	P = 0.026	P = 0.041

(T) Terminal kill.

[a]Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 14/300 (4.7% ± 1.6%), range 2%–6%; all routes: 35/550 (6.4% ± 4.4%), range 2%–18%.

[b]Number of animals with neoplasm per number of animals necropsied.

[c]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.

[d]Observed incidence at terminal kill.

[e]Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.

[f]Historical incidence for inhalation studies: 8/300 (2.7% ± 3.5%), range 0%–8%; all routes: 12/550 (2.2% ± 2.8%), range 0%–8%.

[g]Adenocarcinoma of the Harderian gland was recorded as carcinoma in the historical control database.

[h]Historical incidence for inhalation studies: 22/300 (7.3% ± 3.7%), range 4%–14%; all routes: 47/550 (8.6% ± 4.7%), range 4%–20%.

Lung: There were significantly increased incidences of alveolar/bronchiolar epithelium hyperplasia and peribronchiolar fibrosis in all exposed groups of males and females (Table 25, Table C-4, and Table D-5). Alveolar/bronchiolar epithelium hyperplasia was characterized by a proliferation of crowded, plump, and piled-up epithelial cells of the terminal bronchioles, alveolar ducts, and adjacent alveoli (Figure 29 and Figure 30). The epithelial cells were well organized and did not display atypia. A slight increase in the prominence of fibroblasts was observed in the lamina propria of affected areas, but inflammatory cells were not noted. In lesions of marked severity, the hyperplastic cells extended into the adjacent alveolar septa and occasionally bridged adjacent bronchioles within the same lung lobule. When the hyperplasia involved less than 10%, 10% to 25%, 25% to 75%, or more than 75% of bronchioles, it was considered minimal, mild, moderate, or marked, respectively. Peribronchiolar fibrosis was characterized by a minimal increase in spindle-shaped mesenchymal cells in the lamina propria beneath the hyperplastic epithelial cells in the areas of alveolar/bron-chiolar hyperplasia. Associated with these spindle-shaped cells was an increase in pale eosinophilic fibrillar matrix that stained positive for collagen with Masson's trichrome special stain (Figure 31 and Figure 32). The severity of fibrosis was considered minimal in all exposed groups and no inflammatory cell infiltrate was associated with the fibrosis. There were no significant changes in the incidences of neoplasms in exposed groups.

Table 25. Incidences of Nonneoplastic Lesions of the Lung in Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	200 ppm	400 ppm
Male				
Number Examined Microscopically	50	50	50	50
Alveolar/bronchiolar Epithelium, Hyperplasia[a]	0	49** (1.8)[b]	50** (2.4)	48** (2.8)
Peribronchiolar, Fibrosis	0	45** (1.0)	47** (1.0)	44** (1.0)
Female				
Number Examined Microscopically	50	50	50	50
Alveolar/bronchiolar Epithelium, Hyperplasia	0	49** (2.1)	49** (2.3)	50** (2.9)
Peribronchiolar, Fibrosis	0	44** (1.0)	44** (1.0)	48** (1.0)

**Significantly different ($P \leq 0.01$) from the chamber 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.

Larynx: There was a significantly increased incidence of squamous epithelium hyperplasia in 400 ppm males (Table 26 and Table C-4). Hyperplasia of the squamous epithelium was characterized by increased cellularity of the squamous epithelial cells with slight disorganization of cell layers. This change was limited to the medial aspect of the arytenoid cartilage. Nearly all cases were of minimal severity, and consisted of just one or two additional layers of squamous cells in the squamous epithelium.

Forestomach: There was a significantly increased incidence of epithelium hyperplasia in 400 ppm females (Table 26 and Table D-4). The incidences of epithelium hyperplasia in exposed male groups were increased compared to that in the chamber controls, but the increases were not statistically significant (Table 26 and Table C-4). There were significantly increased incidences of inflammation in 100 and 400 ppm males; and while there was a positive trend (P = 0.034) in the incidences of this lesion in females, there were no statistically significant increases in the exposed groups. Epithelium hyperplasia was characterized by focal areas of thickened squamous epithelium due to increased numbers of squamous epithelial cells. Most cases were minimal and were characterized by slight crowding of nuclei with minimal disorganization and cellular pleomorphism of basal cells. Inflammation was composed primarily of lymphocytes, with fewer neutrophils, and was present primarily within the submucosa. It was often associated with areas of hyperplasia in the overlying epithelium. Focal submucosal inflammation consisting of a cluster of up to 20 inflammatory cells was coded as minimal. Multifocal submucosal inflammation of up to 20 cells in each focus, or focal accumulations of 20 to 100 cells, was coded as mild. One moderate case consisted of two areas of more than 100 inflammatory cells each.

Table 26. Incidences of Selected Nonneoplastic Lesions in Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	200 ppm	400 ppm
Male				
Larynx[a]	50	50	50	50
Squamous Epithelium, Hyperplasia[b]	3 (1.0)[c]	4 (1.0)	6 (1.2)	11* (1.1)
Stomach, Forestomach	50	50	50	48
Epithelium, Hyperplasia	5 (1.0)	9 (1.6)	9 (1.7)	10 (2.2)
Inflammation	4 (1.0)	12* (1.2)	7 (1.3)	12* (1.4)
Female				
Stomach, Forestomach	50	50	50	50
Epithelium, Hyperplasia	1 (1.0)	3 (1.7)	5 (1.0)	14** (1.1)
Inflammation	3 (1.0)	5 (1.0)	5 (1.2)	9 (1.2)

*Significantly different (P ≤ 0.05) from the chamber 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.

Genetic Toxicology

In an earlier bacterial mutagenicity assay, *p*-chloro-α,α,α-trifluorotoluene (10 to 1,000 µg/plate) was not mutagenic in any of four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) when tested without exogenous metabolic activation or with 10% Aroclor 1254-induced hamster or rat liver S9 mix (Table E-1; Haworth et al.[33]). In a second bacterial mutagenicity assay conducted with the same lot of *p*-chloro-α,α,α-tri-fluorotoluene that was tested in the 2-year rodent bioassay, no evidence of mutagenicity was observed in *S. typhimurium* strains TA98 or TA100 or in *Escherichia coli* strain WP2 *uvr*A/pKM101, with or without induced rat liver S9 mix (Table E-2). Doses in this second assay were significantly higher than those that were achievable in the initial assay (up to 5,000 µg/plate without S9 and 6,000 µg/plate in the presence of S9).

In vivo, no significant increases in micronucleated immature (polychromatic erythrocytes, PCEs) or mature (normochromatic erythrocytes, NCEs) erythrocytes were observed in peripheral blood samples from rats in the 3-month study (Table E-3). The small increase in micronucleated immature erythrocytes observed in the male rats was within historical control ranges (<1 standard deviation of the mean) and was therefore judged not to be biologically significant. In mice from the 3-month study, small but statistically significant increases in micronucleated mature erythrocytes were seen at the highest exposure concentration (2,000 ppm), but the observed values for the female mice were within historical control ranges (<1 standard deviation of the mean) and were not considered to be biologically significant (Table E-4). For male mice, the observed response was outside the historical control range for the laboratory and was therefore judged to be positive. An exposure concentration-related increase in the percentage of immature erythrocytes in peripheral blood was seen only in female mice, suggesting that *p*-chloro-α,α,α-trifluorotoluene may have stimulated erythropoiesis in female mice. No significant changes in the percentage of immature erythrocytes were seen in male or female rats, or male mice.

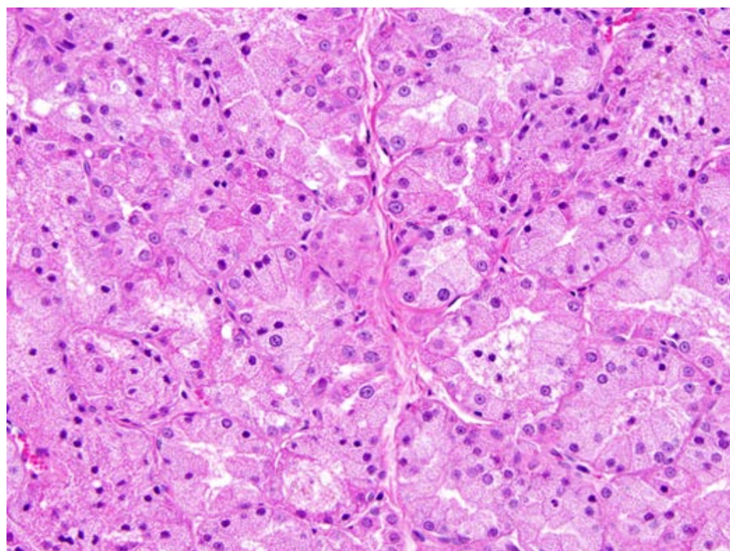


Figure 8. Normal Harderian Gland in a Chamber Control Male Rat in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene (H&E)

The cells of the gland are large and have abundant, foamy cytoplasm due to numerous vacuoles.

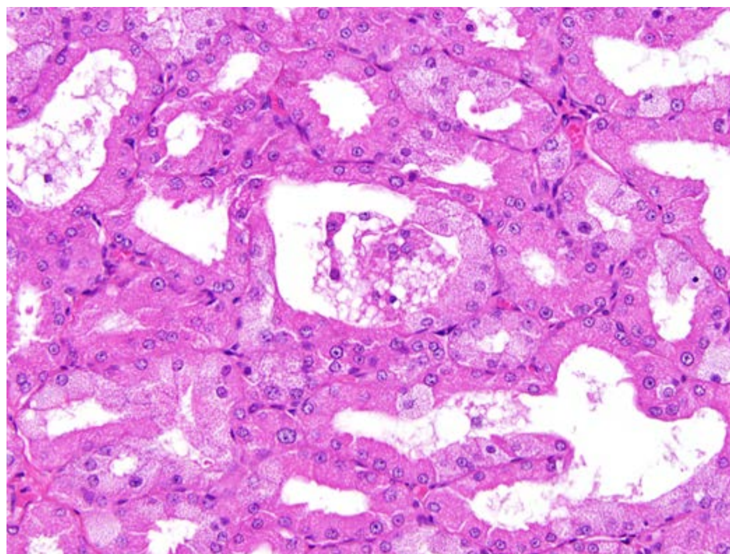


Figure 9. Degeneration in the Harderian Gland of a Male Rat Exposed to 2,000 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Three Months (H&E)

Compared to the normal Harderian gland in Figure 8, the epithelial cells are smaller and more eosinophilic.

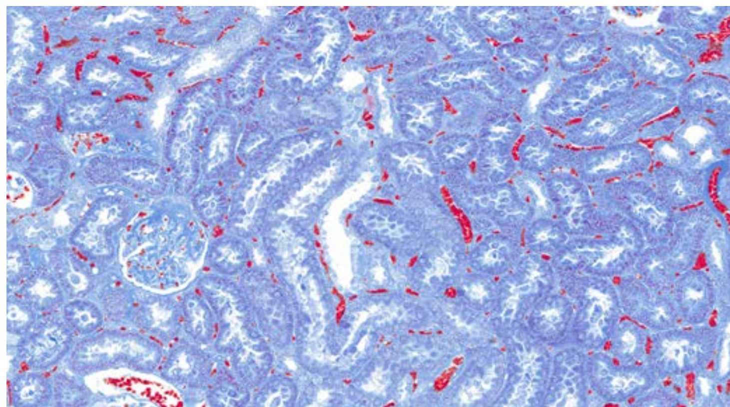


Figure 10. Normal Kidney (Stained with Mallory-Heidenhain Stain) in a Chamber Control Male Rat in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

Hyaline droplets stain bright red, and compared to the exposed animal (Figure 11), there are not many droplets found in the renal tubule epithelium. Erythrocytes also stain very bright red, and are found in vessels located between renal tubules.

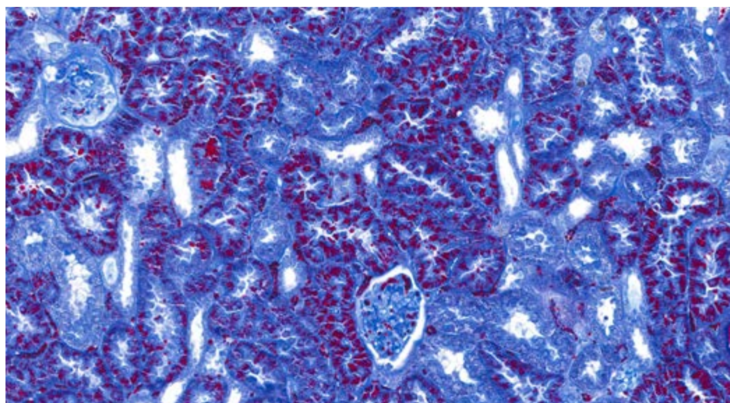


Figure 11. Hyaline Droplet Accumulation in the Kidney (Stained with Mallory-Heidenhain Stain) of a Male Rat Exposed to 2,000 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Three Months

Within the proximal tubule epithelial cells there are numerous small, bright red droplets consistent with alpha-2u-globulin protein.

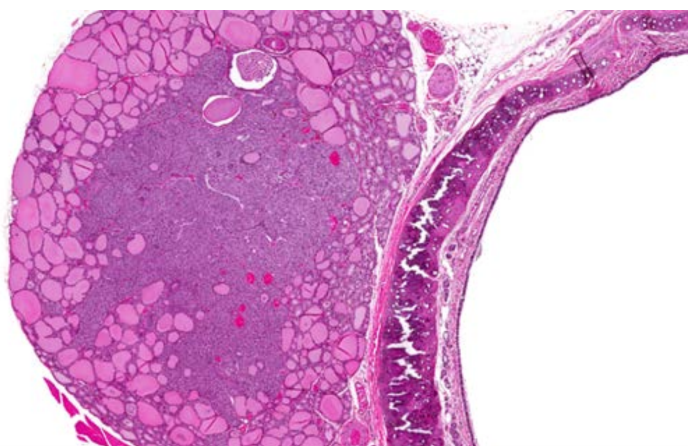


Figure 12. C-cell Adenoma in the Thyroid Gland of a Female Rat Exposed to 1,000 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

The adenoma is a discrete mass, typically larger than five average sized follicles, composed of proliferations of C-cells.

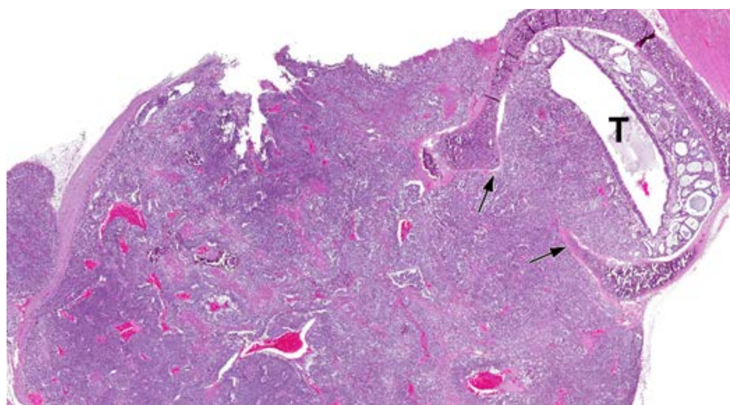


Figure 13. C-cell carcinoma in the Thyroid Gland of a Female Rat Exposed to 1,000 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

The large mass has completely effaced the normal architecture of the thyroid gland and has invaded through the capsule of the gland and into the trachea (arrows), causing compression of the tracheal lumen (T).

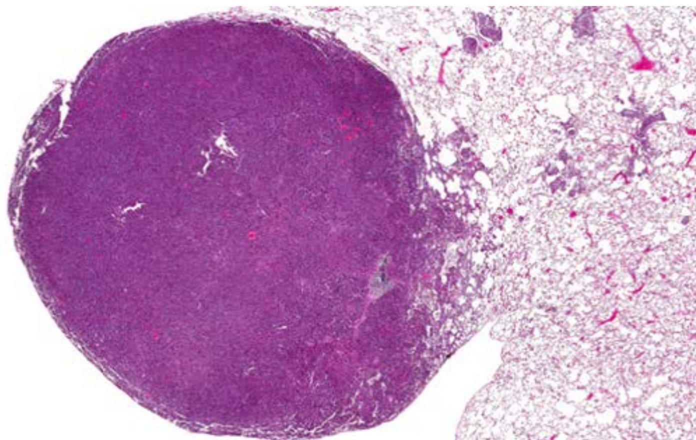


Figure 14. Alveolar/Bronchiolar Carcinoma in the Lung of a Male Rat Exposed to 1,000 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

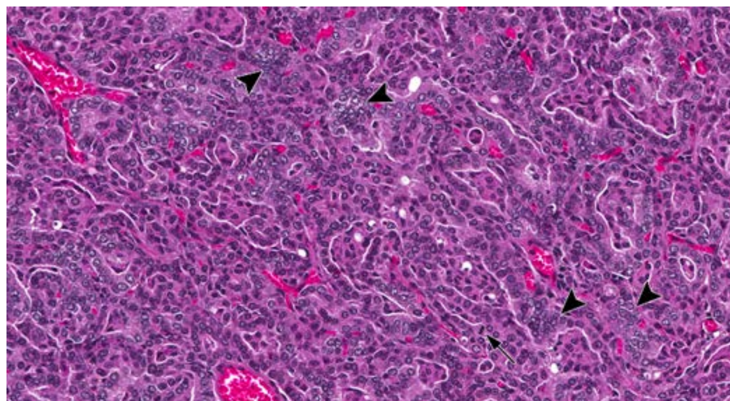


Figure 15. Higher Magnification of Figure 14 (H&E)

Cells have lost their orderly arrangement and are piling up (arrowheads). Note also the variation in nuclear size and the mitotic figure (arrow).

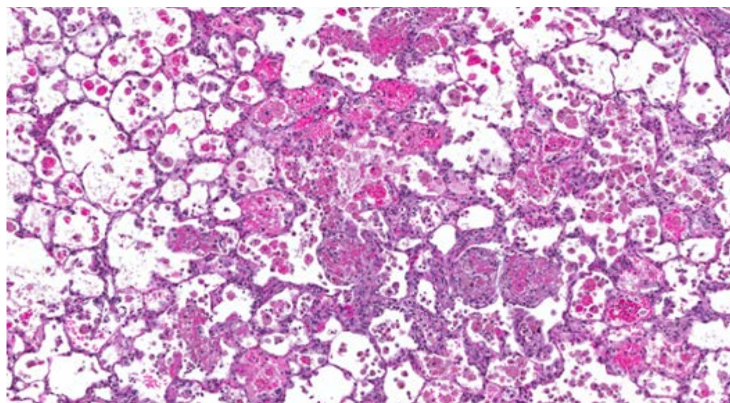


Figure 16. Hemorrhage in the Lung of a Male Rat Exposed to 1,000 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

Hemorrhage is within the alveolar spaces, along with erythrophagocytosis, thrombin, and inflammatory cells. Alveolar septae are thickened due to inflammation and fibrosis.

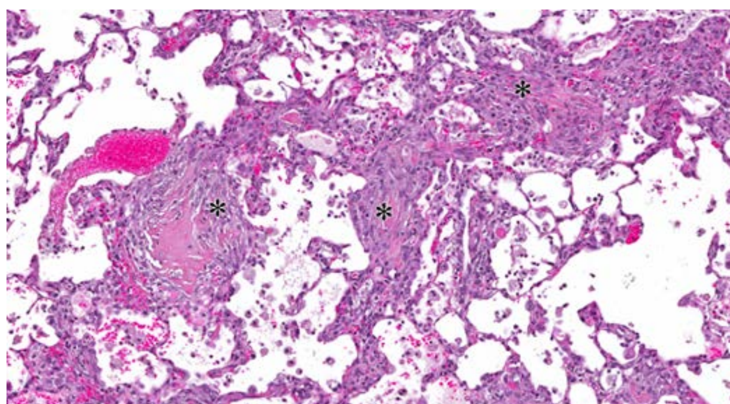


Figure 17. Fibrosis in the Lung of a Female Rat Exposed to 300 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

Note the fibrous connective tissue (asterisks) within the interstitium.

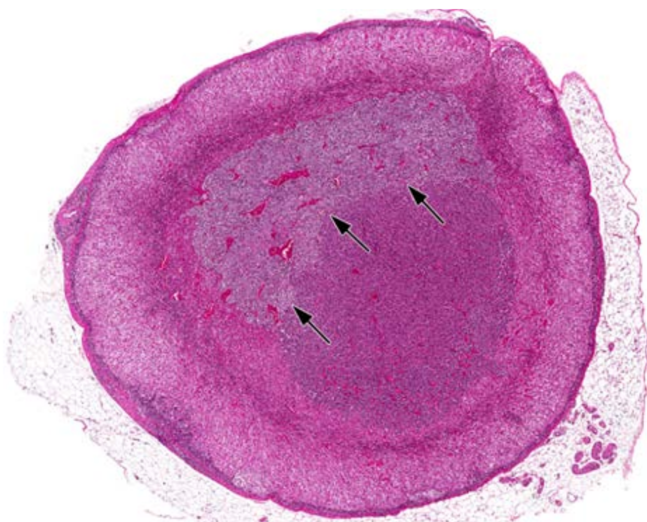


Figure 18. Benign Pheochromocytoma in the Adrenal Medulla of a Female Rat Exposed to 1,000 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

The well demarcated mass is compressing the adjacent, normal medulla (arrows).

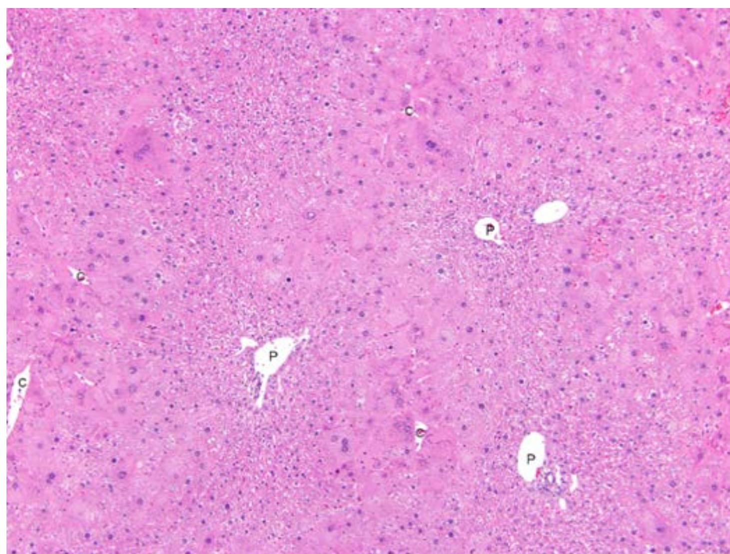


Figure 19. Centrilobular Hepatocyte Hypertrophy in the Liver of a Male Mouse Exposed to 2,000 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Three Months (H&E)

The hepatocytes around the central veins (C) are much larger than those around the portal veins (P). The hypertrophied hepatocytes contain large amounts of eosinophilic cytoplasm, often with a granular appearance, and lack the typical glycogen content found in periportal hepatocytes. Nuclei in affected cells are also enlarged.

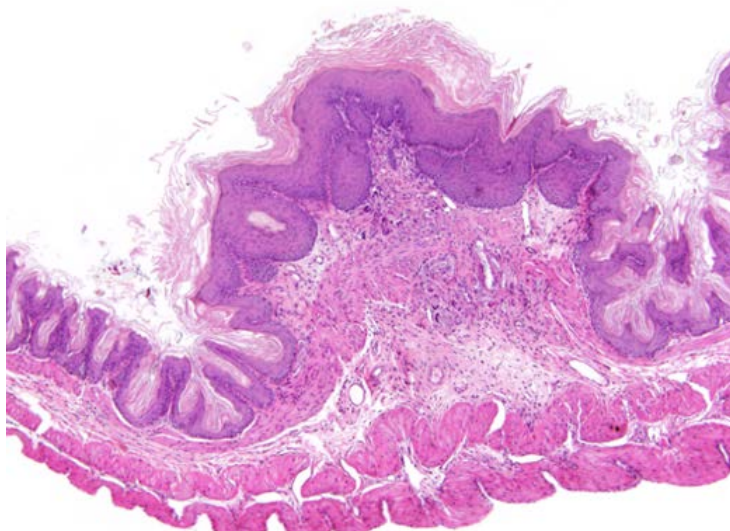


Figure 20. Epithelium Hyperplasia in the Forestomach of a Female Mouse Exposed to 2,000 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Three Months (H&E)

The squamous epithelium of the normal forestomach is typically three to five cell layers thick, while the epithelium in this example is irregular, with downward projections, and up to three times thicker than normal. Overlying the hyperplastic epithelium is a thick layer of keratin, and in the submucosa, there is a mixed inflammatory cell infiltrate.

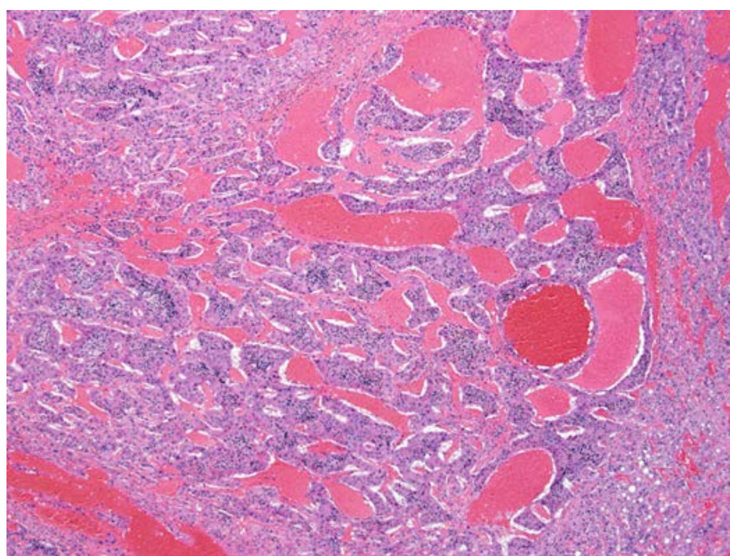


Figure 21. Hepatocellular Carcinoma in the Liver of a Male Mouse Exposed to 400 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

Trabeculae are three cell layers or more thick and separated by blood-filled spaces. The cells within the trabeculae are often small and crowded together and do not maintain the normal arrangement of hepatocellular cords.

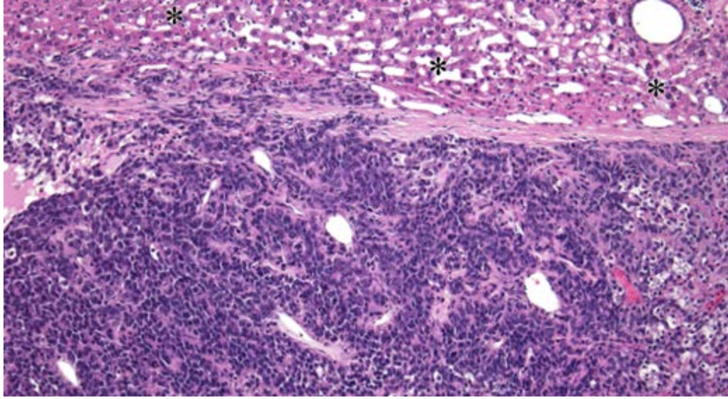


Figure 22. Hepatoblastoma in the Liver of a Male Mouse Exposed to 400 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

The tumor is well demarcated from the surrounding liver parenchyma (asterisks) and composed of tightly packed cells with scant cytoplasm and basophilic oval nuclei.

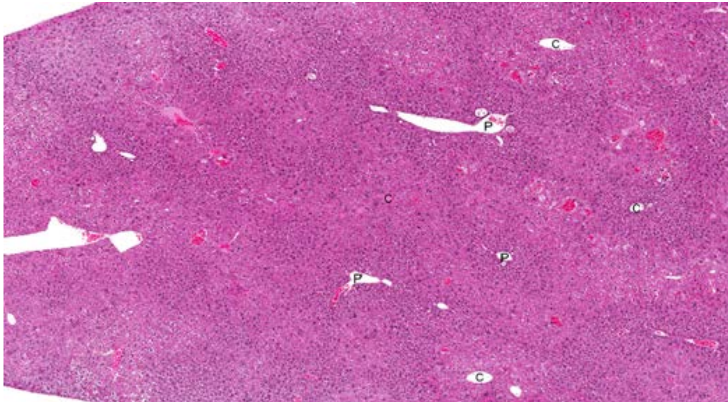


Figure 23. Centrilobular Hepatocyte Hypertrophy in the Liver of a Male Mouse Exposed to 400 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

A zonal pattern is apparent due to enlarged hepatocytes in the centrilobular region. (C = central vein; P = portal vein).

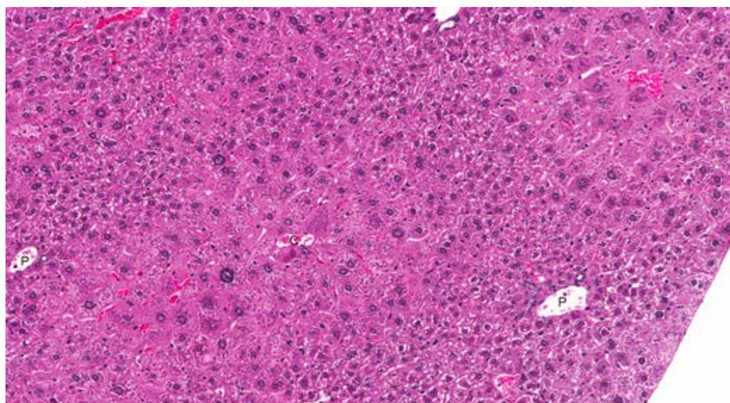


Figure 24. Higher Magnification of Figure 23 (H&E)

The hepatocytes in the centrilobular region are enlarged, with abundant granular eosinophilic cytoplasm containing clumped basophilic material. Nuclei are frequently enlarged, with stippled chromatin and prominent nucleoli. (C = central vein; P = portal vein).

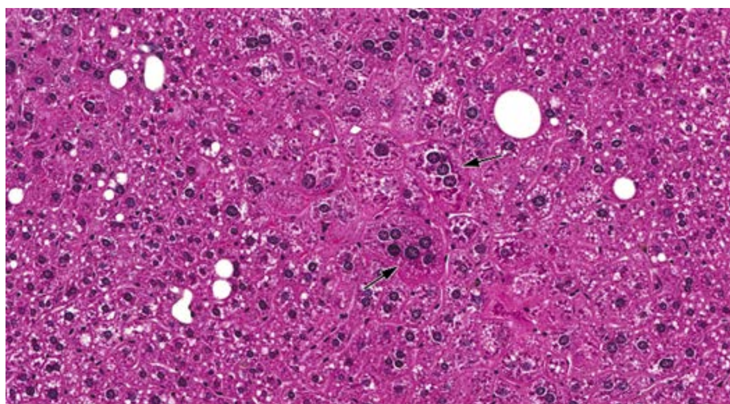


Figure 25. Multinucleated Hepatocytes in the Liver of a Male Mouse Exposed to 400 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

At high magnification, two examples of hepatocytes containing multiple nuclei are noted (arrows); the surrounding hepatocytes are also enlarged.

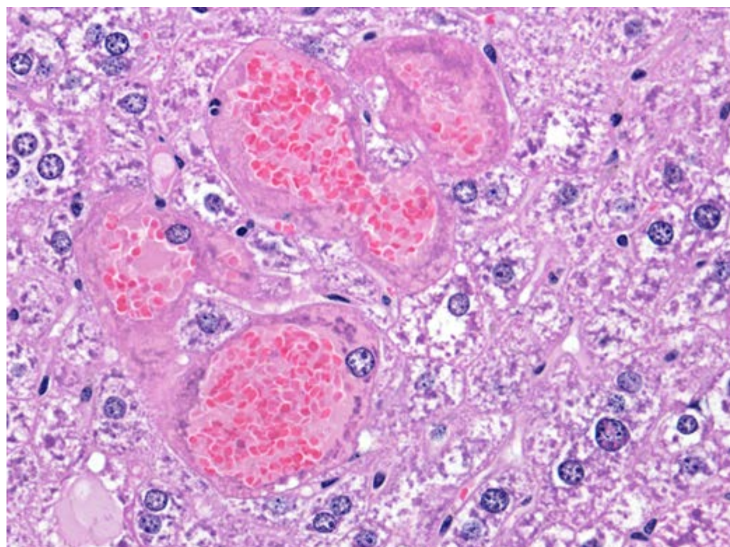


Figure 26. Intrahepatocellular Erythrocytes in the Liver of a Male Mouse Exposed to 400 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

At high magnification, several markedly enlarged hepatocytes containing abundant erythrocytes are noted; the remaining cytoplasm and nuclei are displaced to the periphery of the enlarged cells.

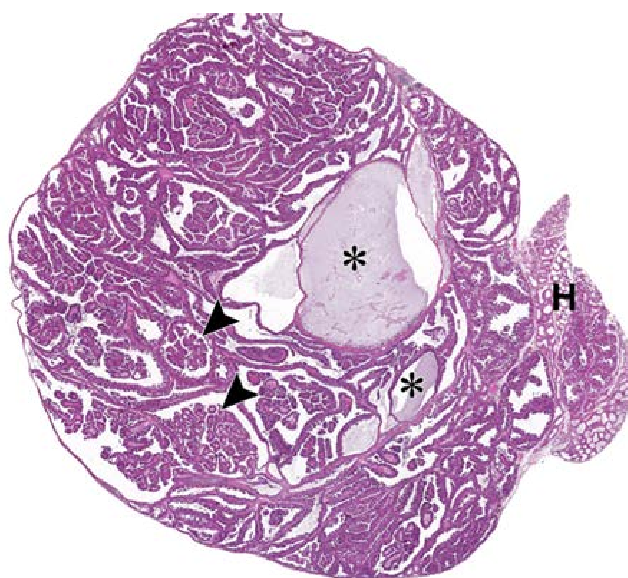


Figure 27. Adenoma in the Harderian Gland of a Female Mouse Exposed to 400 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

The adenoma is large and involves almost the entire gland; a small area of uninvolved Harderian gland is visible on the right (H). Within the adenoma, there is a combination of cystic (asterisks) and papillary (arrowheads) growth patterns.

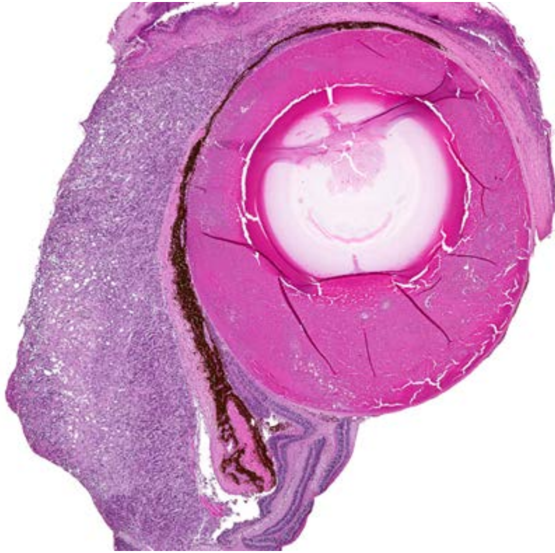


Figure 28. Adenocarcinoma in the Harderian Gland of a Female Mouse Exposed to 200 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

The adenocarcinoma has invaded into the back of the eye.

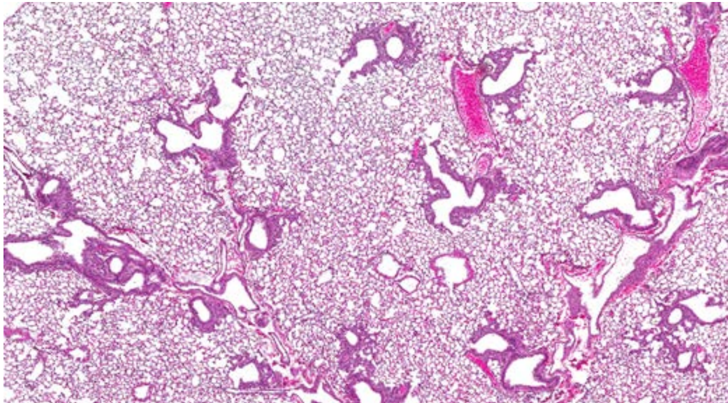


Figure 29. Alveolar/Bronchiolar Epithelium Hyperplasia in the Lung of a Male Mouse Exposed to 400 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

The lesion affected the terminal bronchioles, alveolar ducts, and adjacent alveoli.

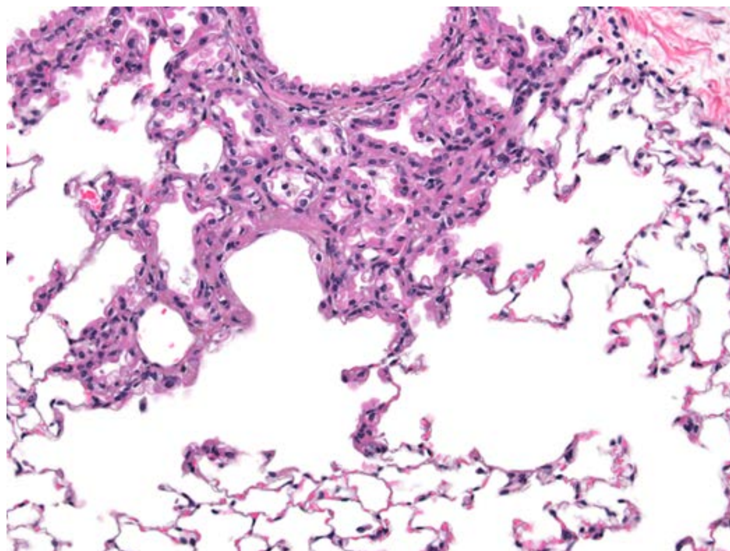


Figure 30. Alveolar/Bronchiolar Epithelium Hyperplasia in the Lung of a Male Mouse Exposed to 400 ppm *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Two Years (H&E)

The epithelial cells lining the terminal bronchioles and adjacent alveoli are plump, crowded, and piled up. There is a fine network of fibrous connective tissue in the lamina propria.

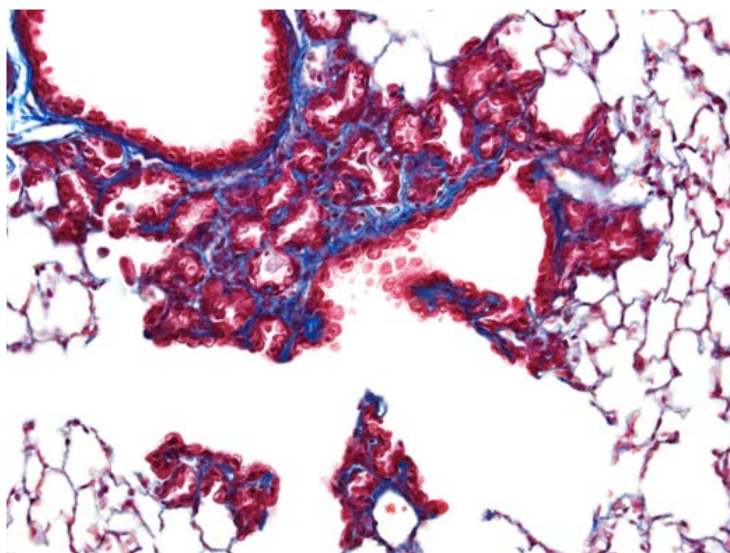


Figure 31. Peribronchiolar Fibrosis in the Terminal Bronchioles and Adjacent Alveoli of the Mouse Described in Figure 30

The Masson's trichrome stain highlights the presence of fibrous connective tissue, which stains bright blue. (Compare to Figure 30).

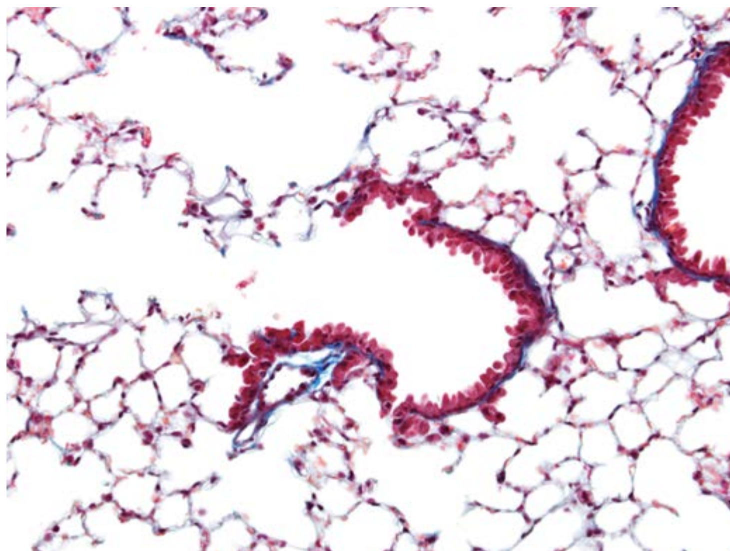


Figure 32. A Masson's Trichrome Stain of the Region Surrounding a Terminal Bronchiole in the Lung of a Chamber Control Male Mouse in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

There is a lack of the fine network of bright blue-stained fibrous connective tissue seen in Figure 31.

Discussion

p-Chloro-α,α,α-trifluorotoluene is a volatile organic compound-exempt solvent used in paints and coatings and for automobile parts and body cleaning. As a solvent with non-ozone depleting status, *p*-chloro-α,α,α-trifluo-rotoluene also has greater use in end-user applications, potentially resulting in higher exposure compared to industrial settings. *p*-Chloro-α,α,α-trifluorotoluene was nominated by Kowa American Corporation for study due to the lack of occupational exposure limits from the Occupational Safety and Health Administration, the National Institute for Occupational Safety and Health, or the American Conference of Governmental Industrial Hygienists. In addition, there are no chronic toxicity or carcinogenicity studies available in the literature. To address these data gaps, the National Toxicology Program (NTP) conducted 3-month toxicity and 2-year toxicity and carcinogenicity studies of *p*-chloro-α,α,α-trifluorotoluene, via whole body inhalation, in Hsd:Sprague Dawley® SD® rats and B6C3F1/N mice.

In the 3-month studies, higher body weights were observed fairly consistently in female rats (500 ppm or greater) and male (500 ppm or greater) and female (250 ppm or greater) mice. Clinical signs were transiently observed in the 2,000 ppm rats and mice, including nasal and ocular discharge, tremors and lethargy in rats, and excitability in mice; these signs were no longer observed after several weeks on study. Exposure-related increases in liver weights were observed in both sexes and species and increased kidney weights were observed in male rats and male and female mice. Other studies, via various routes of exposure, have also consistently reported effects on liver and kidney weights following exposure to *p*-chloro-α,α,α-trifluorotoluene[21; 24; 25; 27].

The liver was one of the primary target organs for toxicity observed in the 3-month studies. Histopathologic lesions of the liver were observed in male and female rats and mice; centrilobular hepatocyte hypertrophy was commonly seen in rats and female mice at higher exposure concentrations and in all male mice exposed to 250 ppm or greater. Low incidences of hepatocellular necrosis were observed in 2,000 ppm male rats and in male and female mice exposed to 250 ppm or greater. *p*-Chloro-α,α,α-trifluorotoluene has been shown to induce cytochrome P450 enzymes, specifically CYP2B[22], and this suggests that the liver weight changes and hepatocyte hypertrophy observed in the current study could be due to enzyme induction.

Clinical chemistry alterations, consistent with the observed hepatic histopathology, were also observed in male and female rats in the 3-month study. Mild increases in alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) activities observed in 2,000 ppm male rats were consistent with the hepatocellular necrosis. Mild increases in ALT and SDH activities in 2,000 ppm females may also indicate mild hepatic injury, although hepatocellular necrosis was not observed. Small increases (below twofold) in ALT activities have been reported with the administration of CYP2B inducers (among others) at dose levels where centrilobular hypertrophy was present without accompanying degenerative changes[70; 74]. Alkaline phosphatase (ALP) activities were mildly elevated in most exposed groups of males and females, while bile salt levels were elevated in females. Bile salt levels and ALP activities are used as markers of cholestasis. The increase in ALP activity was relatively mild and no histologic changes associated with cholestasis were observed. Thus, the increases in ALP activities and bile salt levels may be related to the hepatocyte hypertrophy (enzyme induction) or altered hepatic metabolism, respectively[74].

The kidney was a target organ for toxicity in the 3-month rat study, with an exposure-related increase in the severity of nephropathy in males. Kidney toxicity was also observed in the NTP 2-week oral gavage study of *p*-chloro-α,α,α-trifluorotoluene, where treatment was associated with an accumulation of α2u-protein in the F344/N male rat kidney: α2u accounted for 11%, 17%, 34%, and 55% of total protein in the kidney at doses of 0, 50, 400, and 1,000 mg *p*-chloro-α,α,α-trifluoro-toluene/kg body weight per day, respectively[21]. Hyaline droplet accumulation was confirmed in the current 3-month study using a Mallory-Heindenhain stain.

In the current 2-year study, the kidney was also a major target organ for toxicity in male rats: nephropathy was the cause of death for 18%, 30%, 56%, and 78% of animals in the 0, 100, 300, and 1,000 ppm groups, respectively. Based on α2u-protein measurements in the NTP 2-week gavage study in F344/N rats[21] and Mallory-Heindenhain staining in the current 3-month inhalation study it is possible that the observed nephropathy in the current 2-year study may have been, in part due to an α2u-mediated mechanism. However, in the 3-month study, there was a lack of degeneration, necrosis and granular casts that are typically observed in α2u-mediated nephropathy along with slight increases in the incidences and severities of nephropathy in female rats and increased kidney weights in mice, suggesting additional mechanisms of action for *p*-chloro-α,α,α-trifluorotoluene-induced kidney toxicity. Neoplastic and nonneoplastic outcomes in the male rat kidney that are considered α2u-mediated are frequently not duplicated in other sexes/species and are not considered relevant to humans in risk assessments[75]. In the current study, the lack of renal tubule mineralization in the presence of renal nephropathy-induced mortality and kidney effects in other sexes/species, along with the lack of other typical features of α2u-nephropathy, may indicate that the severe nephropathy observed in male rats was predominantly an exacerbation of the back-ground chronic progressive nephropathy commonly seen in rats.

In the 3-month studies, *p*-chloro-α,α,α-trifluorotoluene exposure resulted in effects on the male reproductive system in rats and mice. Lower sperm motility was observed in rats at 1,000 and 2,000 ppm and in all groups of exposed mice evaluated. Male rats also had lower epididymal weights and lower epididymal sperm counts per cauda. In rats, the effects on sperm parameters were supported by histopathologic findings in the epididymal duct (exfoliated germ cells) and the testes (germ cell degeneration and spermatid retention), suggesting disruption of spermatogenesis. Spermatid retention is considered a sensitive endpoint with which to measure testicular toxicity, often occurring at lower exposure levels than other findings[76; 77]. For example, exposure to boric acid at lower concentrations leads to spermatid retention, while at higher concentrations germ cell degeneration/exfoliation and testicular atrophy are also observed[78]. Effects on spermiation and germ cell exfoliation have been associated with Sertoli cell toxicants; however, the exact target of toxicity is difficult to discern without more targeted mechanistic studies[79; 80].

p-Chloro-α,α,α-trifluorotoluene exposure in the 3-month studies also produced alterations in estrus cycling in female rats and mice. In rats, extended diestrus was observed in animals exposed to 2,000 ppm and in mice, extended estrus was observed in all exposed groups. Also observed in the 3-month study of mice was degeneration of the X-zone in the adrenal gland. Although regression of the X-zone is a normal occurrence in mice that typically occurs several weeks after puberty in females, the diagnosis of degeneration of the X-zone represents an alteration in the temporal sequence of the normal regression. The regression of the X-zone is mediated by gonadal and thyroid gland hormones and alterations in hormone levels can alter the

normal regression of the X-zone[81]. The estrus cycle deregulation observed in rats may have played a role in the uterine histopathologic findings observed in the 2-year study. Based on these findings, *p*-chloro-α,α,α-trifluorotoluene exposure via inhalation exhibits the potential to be a reproductive toxicant in both male and female Hsd:Sprague Dawley® SD® rats and B6C3F1/N mice.

In the 2-year studies, lower survival was observed in male rats and mice. Mortality in male rats was largely attributed to nephropathy. Decreases in survival were observed relatively late in the studies, beginning at approximately week 89 in male rats and after week 90 in male mice. In mice, a majority of male and approximately half of female moribund removals and early deaths were attributed to liver neoplasms, corresponding with clinical observations of distended abdomens. Final body weights were within 10% of chamber controls for male and female rats and male mice. Similar to the 3-month study, higher, yet nonsignificant, body weights were observed in *p*-chloro-α,α,α-trifluorotoluene-exposed female mice. Overall in the 2-year studies, the primary target organs for carcinogenicity differed between rats and mice, with some concordance between males and females within a species. The primary target organs were the thyroid gland (C-cell), adrenal gland (females), and uterus (females) of rats and the liver and Harderian gland (females) of mice. While carcinogenicity targets differed between rats and mice, exposure in all sexes and species resulted in strong nonneoplastic responses in the lung and liver.

Along with other criteria, NTP takes historical control tumor incidences into account when interpreting the results of a study. NTP historical control database consists of 2-year studies that were started within a 5-year time period of the study being evaluated. As previously mentioned, there are no Hsd:Sprague Dawley® SD® historical control studies for the inhalation route that can provide context to interpret the findings of the current study. There is one Hsd:Sprague Dawley® SD® study via the gavage route that fits the criteria for historical controls of all routes of exposure[82] and has been included for reference. The limited nature of the available data and the notable differences in study design between inhalation and noninhalation studies limit the utility of this historical control reference for interpretation of the current study.

p-Chloro-α,α,α-trifluorotoluene displayed carcinogenic activity in the thyroid gland C-cells of male and female rats exposed via whole body inhalation for 2 years. In males, there were significant trend and pairwise increases ($P < 0.001$) in C-cell adenoma at 1,000 ppm (2/50 at 0 ppm, 12/50 at 1,000 ppm) indicating an exposure-related effect. The incidence of C-cell adenoma in controls (26%) in the other NTP study with Hsd:Sprague Dawley® SD® rats[82] is similar to the incidence in 1,000 ppm males from the current study. In females, there were significant trend ($P < 0.008$) and pairwise ($P < 0.003$) increases in the incidence of C-cell adenoma at 1,000 ppm, with contribution from animals with bilateral lesions. The incidence of C-cell adenoma in 1,000 ppm female rats was higher than the incidence in control females in the other recent NTP study with Hsd:Sprague Dawley® SD® rats[82]. Due to limitations cited above for these historical controls, the weight of interpretation for these findings was placed on the concurrent controls, as they were considered to be most relevant. Taken together, the increased incidences of C-cell adenoma were considered to be some evidence of carcinogenic activity in male and female rats.

Proliferative lesions of the C-cells are considered to represent a biological continuum with potential for progression from hyperplasia to adenoma to carcinoma. There were no increases in the incidences of C-cell hyperplasia in male or female rats. Low incidences of C-cell carcinoma

were sporadically observed in male and female rats; in female rats, C-cell carcinoma was only observed in animals exposed to *p*-chloro-α,α,α-trifluorotoluene. Treatment-related increases in the incidences of C-cell lesions have not been observed recently in NTP 2-year studies and have only been reported in four other NTP Technical Reports in rats: 2,4-diaminoanisole sulfate, tetrachlorvinphos, ziram, and 4,4′-methylenedianiline dihydrochloride[83-86]. C-cells of the thyroid gland are responsible for the production and release of calcitonin in order to regulate plasma calcium levels. Calcitonin functions to increase renal clearance of calcium, as well as decrease bone desorption of calcium. Calcitonin is continuously secreted; however, elevated plasma calcium will signal large releases of stored calcitonin. If hypercalcemia is persistent, C-cell proliferation and associated lesions (hyperplasia, adenoma, and carcinoma) will arise to sustain calcitonin demands[87]. Pharmacologic agents have been shown to cause C-cell proliferation and C-cell adenoma via the glucagon-like peptide-1 receptor[88]. Serum cal-cium levels were not assessed in the current study, and the cause of the increased incidences of observed C-cell adenoma is unknown.

In the lung of male rats following *p*-chloro-α,α,α-trifluorotoluene exposure for 2 years, there were higher incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in the 100 and 1,000 ppm groups compared to the chamber controls. In 1,000 ppm males, there were two occurrences of alveolar/bronchiolar carcinoma with a positive trend statistic, but the combined incidence of adenoma or carcinoma was not statistically significant by trend or pairwise comparison. No alveolar/bronchio-lar neoplasms were observed in the lung of the concurrent chamber controls or control male rats from the recent NTP gavage study in Hsd:Sprague Dawley® SD® rats[82]. NTP has limited recent experience with Hsd:Sprague Dawley® SD® rats, but based on more extensive historical control experience with F344/N and Wistar Han rats, neoplastic lesions of the lung are considered rare in rats. Due to the rarity of lung neoplasms, the combined occurrence of alveolar/bronchiolar adenoma or carcinoma in the lung of male rats may have been related to treatment. There were high incidences of nonneoplastic lesions in the lung of male and female rats in the current study, but they were not considered preneoplastic (e.g., fibrosis). Although not preneoplastic, fibrosis is an important lesion with relevance to chronic lung disease in humans and is discussed in more depth below.

In the adrenal medulla of female rats, the incidences of benign pheochromocytoma increased with increasing exposure concentration and there was a significant pairwise ($P < 0.05$) increased incidence in the 1,000 ppm group. In addition, there were significant trend and pairwise increased incidences of hyperplasia of the adrenal medulla, considered preneoplastic, at 300 and 1,000 ppm. Pheochromocytoma, benign or malignant, was not observed in control animals in the current study or the other NTP study in Hsd:Sprague Dawley® SD® rats[82]. The increased incidences of benign pheochromocytoma were considered to be related to treatment. These effects were not observed in male rats.

In the uterus of female rats, there was a significant increasing trend in the incidence of adenocarcinoma (chamber control, 1/50; 1,000 ppm, 5/50). Atypical hyperplasia of the endometrium, considered preneoplastic, was also observed in the 1,000 ppm group at a low incidence, and was considered supporting evidence for carcinogenic activity. The increased incidence of adenocarcinoma in the uterus of female rats was considered to be related to treatment. In the other NTP study with Hsd:Sprague Dawley® SD® rats[82], the uterus was not sectioned longitudinally and cannot be used as a reference to interpret these findings.

Also in the uterus of female rats, there was a significant pairwise increase in the incidence of stromal polyp (16/50) at 300 ppm. The highest incidence of multiple polyps (4/50) also occurred in the 300 ppm group. There was not a significant trend due to a lower response at 1,000 ppm (12/50) compared to 300 ppm. While the neoplasm response did not increase with exposure concentration at 1,000 ppm, all exposed groups had increased incidences of stromal polyp compared to the chamber controls, and the observed responses were considered to be related to *p*-chloro-α,α,α-trifluorotoluene exposure. One stromal sarcoma was observed in the 300 ppm group; stromal sarcoma can occasionally arise from within a stromal polyp[71] and are often considered together with stromal polyps. In female rats, the increased incidences of C-cell adenoma in the thyroid gland, benign pheochromocytoma in the adrenal medulla, adenocarcinoma in the uterus, and stromal polyp in the uterus were all considered to contribute to the conclusion of some evidence of carcinogenic activity.

Spontaneous uterine lesions in rats are largely associated with natural changes in hormone regulation. Stromal polyps are considered a common background lesion in many strains of rats and their occurrence is thought to coincide with natural estrous cycle deregulation in rats and prolonged progesterone stimulation[89]. Conversely, others have suggested that rodent stromal polyps are not hormone sensitive[90]. In the current study, there was evidence that exposure to *p*-chloro-α,α,α-trifluorotoluene for 3 months resulted in estrous cycle alterations in female rats and mice. In the 2-year study, the occurrence of stromal polyps in rats may be related to prolonged diestrus, observed at 3 months, and progesterone stimulation. The hormonal stimulation of uterine adenocarcinomas is not as well understood, but prolonged or constant estrous is considered a potential factor[91].

In mice exposed to *p*-chloro-α,α,α-trifluorotoluene for 2 years, the primary target organ for toxicity and carcinogenicity was the liver. In males, there were trend and pairwise increases in the incidences of hepatocellular carcinoma (including multiple) in all exposed groups, with a large contribution of animals with multiple neoplasms. The incidence of hepatocellular carcinoma at 400 ppm (35/50, 70%) exceeded the historical control ranges for inhalation studies (16% to 50%) and all routes of exposure (16% to 50%). There was also a significant trend and a pairwise increased incidence of hepatoblastoma (including multiple) in 400 ppm males (15/50, 30%), exceeding the historical control ranges for inhalation studies (0% to 4%) and all routes of exposure (0% to 8%). In male mice, the occurrence of hepatocellular carcinoma and hepatoblastoma were separately considered to contribute to the conclusion of clear evidence of carcinogenic activity. The incidences of hepatocellular adenoma did not increase with increasing *p*-chloro-α,α,α-trifluoro-toluene exposure; however, the contribution of animals with multiple adenoma did increase in an exposure concentration-dependent manner with significantly higher incidences of multiple adenoma at 200 and 400 ppm.

In female mice, there were significantly increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma, separately. There were trend and pairwise increases in the incidences of hepatocellular adenoma, with exposure-related contribution of multiples at 200 and 400 ppm. The incidence of hepatocellular adenoma at 400 ppm (34/50, 68%) exceeded the historical control ranges for inhalation studies (12% to 38%) and all routes of exposure (10% to 67%). There were trend and pairwise significant increases in the incidences of hepatocellular carcinoma (including multiple) at 400 ppm. The incidences of hepatocellular carcinoma at 200 ppm (12/50, 24%) and 400 ppm (34/50, 68%) exceeded the historical control ranges for inhalation studies (10% to 20%) and all routes of exposure (4% to 20%). The

incidence of hepatoblastoma at 400 ppm (8/50, 16%) was significantly greater than that of the chamber controls and exceeded the historical control ranges for inhalation studies (0% to 2%) and all routes of exposure (0% to 2%). In female mice, the increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma were all separately considered to contribute to the conclusion of clear evidence of carcinogenic activity.

There were statistically significant trend and pairwise differences in the negative direction for *Hras* mutations at codon 61 between spontaneous hepatocellular carcinomas (HCCs) in chamber control mice (males and females combined) and HCCs resulting from chronic *p*-chloro-α,α,α-trifluorotoluene exposure (Appendix L). The *Hras* mutations in spontaneous and chemically exposed rodent hepatocellular tumors are frequently localized within codon 61[72; 73]. *p*-Chloro-α,α,α-trifluorotoluene is nongenotoxic (Ames assay negative, chromosomal aberration assay negative) and may not directly cause mutations and initiate carcinogenesis. However, further mechanistic studies are needed to better understand the *p*-chloro-α,α,α-trifluorotoluene-induced hepatocellular carcinogenesis.

There is evidence that *p*-chloro-α,α,α-trifluorotoluene inhalation exposure can lead to CYP2B induction in the liver of Sprague-Dawley rats[22]; liver microsomes from male rats exposed to 250 ppm *p*-chloro-α,α,α-trifluorotoluene had approximately six times higher CYP2B activity compared to controls, with little activity seen at lower exposure concentrations or in females. Other CYP isoforms evaluated also showed higher activity in exposed animals; however the strongest induction was CYP2B. CYP2B activation via the constitutive androstane receptor (CAR) is a known mechanism of tumor promotion activity in the liver of rodents[92]. The potential for *p*-chloro-α,α,α-trifluorotoluene to activate CAR was evaluated in the Tox21 screening program but results were inconclusive[93]. Liver weights and nonneoplastic lesions observed in the current 3-month and 2-year studies are also consistent with a potential CAR-mechanism of action and similar responses have been observed in other studies with CAR/CYP2B inducers[94; 95].

In the Harderian gland of female mice following 2 years of *p*-chloro-α,α,α-trifluorotoluene exposure, there were trend and pairwise significant increases in the incidences of adenoma or adenocarcinoma (combined) in the 200 (9/50) and 400 (8/50) ppm groups compared to concurrent controls (2/50). The incidences at 200 and 400 ppm exceeded the historical control range for inhalation studies (4% to 14%; n = 6 studies) but not for all routes of exposure (4% to 20%; n = 11 studies). The average incidence of these neoplasms in control B6C3F1/N mice observed in NTP studies by all routes of exposure is 8.6% and all except one study (e.g., 10 of 11 studies) had control incidences lower than those observed in the 200 and 400 ppm groups in the current study. Although not observed in mice, Harderian gland degeneration was observed in rats in the 3-month study. The combined incidences of adenoma or adenocarcinoma in the Harderian gland of female mice were considered to be related to treatment.

Consistent patterns of nonneoplastic lesions were observed in the liver and lung of rats and mice in the 2-year studies. In the lung, there were increased incidences of fibrosis in both sexes of rats and mice, as well as hemorrhage and chronic inflammation in rats and alveolar/bronchiolar epithelium hyperplasia in mice. The exposure response for fibrosis was flat in rats with approximately one-half of the exposed rats in each group being diagnosed. In mice, nearly all animals in each exposure group were diagnosed with fibrosis, indicating a maximal response even at 100 ppm. There was no indication of this lesion in the 3-month studies and a no-

observed-effect level was not identified in the current study for this lesion in any sex or species. The lung fibrosis response observed in the current study may be of particular interest to humans exposed to *p*-chloro-α,α,α-trifluorotoluene. While fibrosis is not considered a preneoplastic lesion, it can critically alter pulmonary function and contribute to chronic lung diseases in humans.

In the liver, there were increased incidences of eosinophilic foci and centrilobular hepatocyte hypertrophy in all sexes/species. Low incidences of liver necrosis were observed in male rats and male and female mice. Increased incidences of fatty change, clear cell focus (females only) and mixed cell focus (females only) were also observed in rats following 2 years of exposure to *p*-chloro-α,α,α-trifluorotoluene. In mice, multinucleated hepatocytes and intrahepatocellular erythrocytes (males only) were also observed. Despite a similar response in nonneoplastic lesions of the liver, a robust neoplastic response was only observed in mice.

Other nonneoplastic lesions observed following 2 years of inhalation exposure to *p*-chloro-α,α,α-trifluorotoluene included adrenal cortex hyperplasia in female rats, and hyperplasia of the forestomach (males and females) and larynx (males) in mice. Mammary gland hyperplasia was observed in female rats in both the 3-month and 2-year studies; however, decreased trends for mammary gland fibroadenoma, adenoma, and adenocarcinoma were observed in the 2-year studies.

p-Chloro-α,α,α-trifluorotoluene was negative in several bacterial mutagenicity assays (*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A/pKM101). A small but statistically significant increase in micronucleated mature erythrocytes was observed in male mice after 3 months of inhalation exposure to the highest concentration of *p*-chloro-α,α,α-trifluorotoluene (2,000 ppm) and was judged to be a positive result. No induction of micronuclei was observed in female mice or male or female rats. Overall these results suggest that while *p*-chloro-α,α,α-trifluorotoluene may be capable of inducing chromosomal damage at high levels of inhalation exposure in male mice, the mode of action for the carcinogenicity of *p*-chloro-α,α,α-trifluorotoluene observed in rats and mice is unlikely to be driven by genotoxicity.

Conclusions

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity*[a] of *p*-chloro-α,α,α-trifluorotoluene in male Hsd:Sprague Dawley® SD® rats based on increased incidences of C-cell adenoma in the thyroid gland. The combined occurrences of alveolar/bronchiolar adenoma or carcinoma in the lung of male rats may have been related to treatment. There was *some evidence of carcinogenic activity* of *p*-chloro-α,α,α-trifluorotoluene in female Hsd:Sprague Dawley® SD® rats based on increased incidences of C-cell adenoma in the thyroid gland, increased incidences of benign pheochromocytoma in the adrenal medulla, increased incidences of adenocarcinoma in the uterus, and increased incidences of stromal polyp in the uterus. There was *clear evidence of carcinogenic activity* of *p*-chloro-α,α,α-trifluorotoluene in male B6C3F1/N mice based on increased incidences of hepatocellular carcinoma and hepatoblastoma in the liver. There was *clear evidence of carcinogenic activity* of *p*-chloro-α,α,α-trifluorotoluene in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma in the liver. The combined incidences of adenoma or adenocarcinoma in the Harderian gland of female mice were also considered to be related to treatment.

Exposure to *p*-chloro-α,α,α-trifluorotoluene caused increased incidences of nonneoplastic lesions in the lung and liver of male and female rats and mice, in the nose of male rats, in the adrenal medulla and uterus of female rats, in the forestomach of male and female mice, and in the larynx in male mice. Exposure to *p*-chloro-α,α,α-trifluorotoluene caused increased severity of nonneoplastic lesions in the kidney of male rats.

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[a]See Explanation of Levels of Evidence of Carcinogenic Activity. See summary of the peer review panel comments and the public discussion on this Technical Report in Appendix M.

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Appendix A. Summary of Lesions in Male Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

Tables

Table A-1. Summary of the Incidence of Neoplasms in Male Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene A-2
Table A-2. Statistical Analysis of Primary Neoplasms in Male Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene A-7
Table A-3. Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene A-11

Table A-1. Summary of the Incidence of Neoplasms in Male Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	16	19	21	27
Natural deaths	9	10	14	18
Survivors				
Terminal kill	25	21	15	5
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)
Leiomyoma	1 (2%)	–	–	–
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Adenocarcinoma	–	1 (2%)	–	–
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Adenocarcinoma	–	2 (4%)	1 (2%)	–
Leiomyosarcoma	1 (2%)	–	–	–
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	–	1 (2%)	–	–
Hepatocellular carcinoma	–	2 (4%)	–	1 (2%)
Schwannoma malignant, metastatic, heart	–	–	–	1 (2%)
Mesentery	(2)	(2)	(1)	(1)
Pancreas	(50)	(50)	(50)	(50)
Acinus, adenoma	–	1 (2%)	1 (2%)	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Carcinoma	–	1 (2%)	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	–	2 (4%)	–	–
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(1)	(0)	(0)
Tooth	(1)	(0)	(0)	(0)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Odontoma	1 (100%)	–	–	–
Cardiovascular System				
Blood vessel	(50)	(50)	(49)	(50)
Heart	(50)	(50)	(50)	(50)
Sarcoma	–	–	–	1 (2%)
Endocardium, schwannoma malignant	–	–	1 (2%)	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	1 (2%)	–	1 (2%)
Carcinoma	–	–	1 (2%)	–
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	8 (16%)	8 (16%)	12 (24%)	10 (20%)
Pheochromocytoma malignant	2 (4%)	–	2 (4%)	2 (4%)
Bilateral, pheochromocytoma benign	4 (8%)	5 (10%)	4 (8%)	2 (4%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	8 (16%)	5 (10%)	2 (4%)	–
Carcinoma	–	4 (8%)	–	1 (2%)
Parathyroid gland	(48)	(48)	(48)	(46)
Adenoma	–	–	–	2 (4%)
Carcinoma, metastatic, thyroid gland	–	–	–	1 (2%)
Pituitary gland	(50)	(50)	(49)	(50)
Carcinoma, metastatic, Zymbal's gland		1 (2%)	–	–
Pars distalis, adenoma	11 (22%)	6 (12%)	7 (14%)	6 (12%)
Pars intermedia, adenoma	–	–	–	1 (2%)
Thyroid gland	(50)	(49)	(49)	(50)
Bilateral, C-cell, adenoma		1 (2%)	1 (2%)	1 (2%)
C-cell, adenoma	2 (4%)	4 (8%)	2 (4%)	11 (22%)
C-cell, carcinoma	1 (2%)	–	1 (2%)	1 (2%)
Follicular cell, adenoma	–	–	1 (2%)	1 (2%)
General Body System				
None	–	–	–	–
Genital System				
Coagulating gland	(1)	(0)	(0)	(0)
Epididymis	(50)	(50)	(50)	(50)
Sarcoma	1 (2%)	–	–	–

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Preputial gland	(50)	(50)	(50)	(50)
Carcinoma	–	–	1 (2%)	–
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma	–	2 (4%)	2 (4%)	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(7)	(7)	(10)	(4)
Lymph node, bronchial	(30)	(26)	(29)	(26)
Lymph node, mandibular	(50)	(50)	(50)	(50)
Lymph node, mediastinal	(50)	(49)	(50)	(50)
Lymph node, mesenteric	(49)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(48)	(50)	(48)	(49)
Integumentary System				
Mammary gland	(43)	(38)	(38)	(37)
Adenoma	–	1 (3%)	–	–
Fibroadenoma	2 (5%)	1 (3%)	2 (5%)	–
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	–	–	2 (4%)	–
Keratoacanthoma	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Keratoacanthoma, multiple	1 (2%)	–	1 (2%)	–
Squamous cell papilloma	1 (2%)	2 (4%)	–	1 (2%)
Sebaceous gland, adenoma	1 (2%)	2 (4%)	2 (4%)	–
Subcutaneous tissue, fibroma	–	4 (8%)	2 (4%)	–
Subcutaneous tissue, fibrous histiocytoma	1 (2%)	1 (2%)	–	–
Subcutaneous tissue, hemangioma	1 (2%)	–	1 (2%)	–
Subcutaneous tissue, lipoma	1 (2%)	1 (2%)	1 (2%)	–
Subcutaneous tissue, schwannoma malignant	–	–	2 (4%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma	–	–	1 (2%)	–
Skeletal muscle	(7)	(6)	(4)	(2)
Hemangiosarcoma	–	1 (17%)	–	–

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Schwannoma malignant	–	1 (17%)	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Glioma malignant	2 (4%)	1 (2%)	1 (2%)	–
Peripheral nerve	(7)	(5)	(4)	(2)
Spinal cord	(7)	(5)	(4)	(2)
Schwannoma malignant, metastatic, skeletal muscle	–	1 (20%)	–	–
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	–	2 (4%)	–	1 (2%)
Alveolar/bronchiolar carcinoma	–	–	–	2 (4%)
Carcinoma, metastatic, lung	1 (2%)	–	–	–
Carcinoma, metastatic, salivary glands	–	1 (2%)	–	–
Carcinoma, metastatic, Zymbal's gland	–	1 (2%)	–	–
Pheochromocytoma malignant, metastatic, adrenal medulla	–	–	1 (2%)	–
Artery, schwannoma malignant, metastatic, heart	–	–	–	1 (2%)
Mediastinum, paraganglioma	–	1 (2%)	–	–
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Iris, leiomyoma	1 (2%)	–	–	–
Harderian gland	(50)	(50)	(50)	(50)
Pheochromocytoma malignant, metastatic, adrenal medulla	–	–	–	1 (2%)
Zymbal's gland	(1)	(2)	(0)	(0)
Adenoma	–	1 (50%)	–	–
Carcinoma	1 (100%)	1 (50%)	–	–
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Lipoma	–	–	1 (2%)	–
Renal tubule, adenoma	–	–	2 (4%)	–
Urinary bladder	(50)	(50)	(50)	(50)
Urothelium, papilloma	–	–	1 (2%)	–

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	–	–	1 (2%)	–
Leukemia granulocytic	1 (2%)	–	–	–
Leukemia mononuclear	3 (6%)	2 (4%)	–	1 (2%)
Lymphoma malignant	–	–	1 (2%)	–
Mesothelioma malignant	1 (2%)	–	1 (2%)	–
Neoplasm Summary				
Total animals with primary neoplasms[c]	37	41	39	35
Total primary neoplasms	62	70	62	51
Total animals with benign neoplasms	30	32	32	30
Total benign neoplasms	48	53	48	41
Total animals with malignant neoplasms	12	16	12	10
Total malignant neoplasms	14	17	14	10
Total animals with metastatic neoplasms	1	3	1	3
Total metastatic neoplasms	1	4	1	4

[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 A-2. Statistical Analysis of Primary Neoplasms in Male Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	3/50 (6%)	1/50 (2%)	0/50 (0%)	1/50 (2%)
Adjusted rate[b]	7.5%	2.7%	0.0%	3.2%
Terminal rate[c]	1/25 (4%)	1/21 (5%)	0/15 (0%)	0/5 (0%)
First incidence (days)	516	729 (T)	–[e]	645
Poly-3 test[d]	P = 0.390N	P = 0.329N	P = 0.129N	P = 0.400N
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate	12/50 (24%)	13/50 (26%)	16/50 (32%)	12/50 (24%)
Adjusted rate	29.7%	32.5%	37.9%	35.0%
Terminal rate	8/25 (32%)	6/21 (29%)	4/15 (27%)	2/5 (40%)
First incidence (days)	374	485	500	476
Poly-3 test	P = 0.392	P = 0.489	P = 0.288	P = 0.403
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	14/50 (28%)	13/50 (26%)	18/50 (36%)	14/50 (28%)
Adjusted rate	34.2%	32.5%	42.1%	39.7%
Terminal rate	9/25 (36%)	6/21 (29%)	4/15 (27%)	2/5 (40%)
First incidence (days)	374	485	500	476
Poly-3 test	P = 0.321	P = 0.531N	P = 0.296	P = 0.395
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	0.0%	7.9%	0.0%	3.2%
Terminal rate	0/25 (0%)	2/21 (10%)	0/15 (0%)	0/5 (0%)
First incidence (days)	–	631	–	612
Poly-3 test	P = 0.653	P = 0.111	–[f]	P = 0.455
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	0/50 (0%)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	5.3%	0.0%	9.3%
Terminal rate	0/25 (0%)	1/21 (5%)	0/15 (0%)	0/5 (0%)
First incidence (days)	–	650	–	516
Poly-3 test	P = 0.073	P = 0.228	–	P = 0.086
Pancreatic Islets: Adenoma				
Overall rate	8/50 (16%)	5/50 (10%)	2/50 (4%)	0/50 (0%)
Adjusted rate	19.8%	13.2%	5.2%	0.0%
Terminal rate	4/25 (16%)	4/21 (19%)	1/15 (7%)	0/5 (0%)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
First incidence (days)	533	655	607	–
Poly-3 test	P = 0.008N	P = 0.316N	P = 0.052N	P = 0.011N
Pancreatic Islets: Carcinoma				
Overall rate	0/50 (0%)	4/50 (8%)	0/50 (0%)	1/50 (2%)
Adjusted rate	0.0%	10.6%	0.0%	3.2%
Terminal rate	0/25 (0%)	3/21 (14%)	0/15 (0%)	0/5 (0%)
First incidence (days)	–	674	–	686
Poly-3 test	P = 0.569N	P = 0.054	–	P = 0.453
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	8/50 (16%)	9/50 (18%)	2/50 (4%)	1/50 (2%)
Adjusted rate	19.8%	23.7%	5.2%	3.2%
Terminal rate	4/25 (16%)	7/21 (33%)	1/15 (7%)	0/5 (0%)
First incidence (days)	533	655	607	686
Poly-3 test	P = 0.012N	P = 0.443	P = 0.052N	P = 0.041N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	11/50 (22%)	6/50 (12%)	7/49 (14%)	6/50 (12%)
Adjusted rate	28.0%	15.8%	18.8%	18.1%
Terminal rate	10/25 (40%)	4/21 (19%)	5/15 (33%)	1/5 (20%)
First incidence (days)	723	607	717	430
Poly-3 test	P = 0.333N	P = 0.150N	P = 0.245N	P = 0.233N
Skin: Keratoacanthoma				
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)	3/50 (6%)
Adjusted rate	7.6%	5.3%	5.3%	9.3%
Terminal rate	2/25 (8%)	2/21 (10%)	2/15 (13%)	1/5 (20%)
First incidence (days)	618	729 (T)	729 (T)	467
Poly-3 test	P = 0.426	P = 0.525N	P = 0.523N	P = 0.563
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	4/50 (8%)	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted rate	10.1%	8.0%	5.3%	9.3%
Terminal rate	2/25 (8%)	3/21 (14%)	2/15 (13%)	1/5 (20%)
First incidence (days)	618	729 (T)	729 (T)	467
Poly-3 test	P = 0.599N	P = 0.530N	P = 0.359N	P = 0.614N
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Basal Cell Adenoma				
Overall rate	4/50 (8%)	3/50 (6%)	4/50 (8%)	3/50 (6%)
Adjusted rate	10.1%	8.0%	10.6%	9.3%

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Terminal rate	2/25 (8%)	3/21 (14%)	4/15 (27%)	1/5 (20%)
First incidence (days)	618	729 (T)	729 (T)	467
Poly-3 test	P = 0.592	P = 0.530N	P = 0.617	P = 0.614N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	0/50 (0%)	4/50 (8%)	2/50 (4%)	0/50 (0%)
Adjusted rate	0.0%	10.6%	5.2%	0.0%
Terminal rate	0/25 (0%)	3/21 (14%)	0/15 (0%)	0/5 (0%)
First incidence (days)	–	680	590	–
Poly-3 test	P = 0.299N	P = 0.054	P = 0.233	–
Skin (Subcutaneous Tissue): Fibroma or Fibrous Histiocytoma				
Overall rate	1/50 (2%)	5/50 (10%)	2/50 (4%)	0/50 (0%)
Adjusted rate	2.5%	12.9%	5.2%	0.0%
Terminal rate	0/25 (0%)	3/21 (14%)	0/15 (0%)	0/5 (0%)
First incidence (days)	584	318	590	–
Poly-3 test	P = 0.167N	P = 0.093	P = 0.490	P = 0.550N
Small Intestine (Duodenum or Jejunum): Adenocarcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	7.9%	2.6%	0.0%
Terminal rate	0/25 (0%)	2/21 (10%)	0/15 (0%)	0/5 (0%)
First incidence (days)	–	525	611	–
Poly-3 test	P = 0.350N	P = 0.113	P = 0.494	–
Thyroid Gland (C-cell): Adenoma				
Overall rate	2/50 (4%)	5/49 (10%)	3/49 (6%)	12/50 (24%)
Adjusted rate	5.1%	13.4%	7.9%	36.3%
Terminal rate	2/25 (8%)	4/21 (19%)	1/15 (7%)	3/5 (60%)
First incidence (days)	729 (T)	680	669	568
Poly-3 test	P < 0.001	P = 0.192	P = 0.482	P < 0.001
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	5/49 (10%)	4/49 (8%)	13/50 (26%)
Adjusted rate	7.6%	13.4%	10.6%	39.2%
Terminal rate	3/25 (12%)	4/21 (19%)	1/15 (7%)	3/5 (60%)
First incidence (days)	729 (T)	680	669	568
Poly-3 test	P < 0.001	P = 0.326	P = 0.481	P < 0.001
All Organs: Mononuclear Cell Leukemia				
Overall rate	3/50 (6%)	2/50 (4%)	0/50 (0%)	1/50 (2%)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Adjusted rate	7.5%	5.3%	0.0%	3.2%
Terminal rate	0/25 (0%)	0/21 (0%)	0/15 (0%)	0/5 (0%)
First incidence (days)	590	710	–	589
Poly-3 test	P = 0.316N	P = 0.530N	P = 0.129N	P = 0.399N
All Organs: Benign Neoplasms				
Overall rate	30/50 (60%)	32/50 (64%)	32/50 (64%)	30/50 (60%)
Adjusted rate	68.2%	76.0%	71.6%	73.0%
Terminal rate	17/25 (68%)	18/21 (86%)	12/15 (80%)	5/5 (100%)
First incidence (days)	374	485	500	430
Poly-3 test	P = 0.477	P = 0.273	P = 0.452	P = 0.394
All Organs: Malignant Neoplasms				
Overall rate	12/50 (24%)	16/50 (32%)	12/50 (24%)	10/50 (20%)
Adjusted rate	27.2%	38.3%	29.6%	28.9%
Terminal rate	1/25 (4%)	8/21 (38%)	3/15 (20%)	0/5 (0%)
First incidence (days)	374	306	463	528
Poly-3 test	P = 0.458N	P = 0.189	P = 0.500	P = 0.534
All Organs: Benign or Malignant Neoplasms				
Overall rate	37/50 (74%)	41/50 (82%)	39/50 (78%)	35/50 (70%)
Adjusted rate	79.2%	88.5%	84.0%	81.4%
Terminal rate	18/25 (72%)	19/21 (91%)	13/15 (87%)	5/5 (100%)
First incidence (days)	374	306	463	430
Poly-3 test	P = 0.475N	P = 0.158	P = 0.360	P = 0.502

(T) Terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, pancreatic islets, 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 chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by N.
[e]Not applicable; no neoplasms in animal group.
[f]Value of statistic cannot be computed.

Table A-3. Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	16	19	21	27
Natural deaths	9	10	14	18
Survivors				
Terminal kill	25	21	15	5
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation, acute	–	1 (2%)	–	–
Artery, inflammation	1 (2%)	1 (2%)	–	–
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Serosa, inflammation	1 (2%)	–	–	–
Intestine small, duodenum	(50)	(50)	(50)	(50)
Hemorrhage	–	–	–	1 (2%)
Peyer’s patch, hyperplasia, lymphoid	–	1 (2%)	1 (2%)	–
Intestine small, ileum	(50)	(50)	(50)	(50)
Cyst	–	–	–	1 (2%)
Hemorrhage	–	–	–	1 (2%)
Artery, inflammation	1 (2%)	–	–	–
Peyer’s patch, hyperplasia, lymphoid	–	–	1 (2%)	–
Intestine small, jejunum	(50)	(50)	(50)	(50)
Inflammation	–	–	–	1 (2%)
Artery, inflammation	1 (2%)	–	–	1 (2%)
Peyer’s patch, hyperplasia, lymphoid	–	1 (2%)	1 (2%)	–
Liver	(50)	(50)	(50)	(50)
Angiectasis	–	–	–	3 (6%)
Basophilic focus	2 (4%)	3 (6%)	4 (8%)	2 (4%)
Cholangiofibrosis	–	–	–	1 (2%)
Clear cell focus	40 (80%)	31 (62%)	29 (58%)	10 (20%)
Degeneration, cystic	–	2 (4%)	1 (2%)	1 (2%)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Eosinophilic focus	1 (2%)	5 (10%)	6 (12%)	8 (16%)
Fatty change	–	3 (6%)	7 (14%)	26 (52%)
Hematopoietic cell proliferation	1 (2%)	1 (2%)	1 (2%)	–
Hemorrhage	1 (2%)	1 (2%)	–	–
Hepatodiaphragmatic nodule	2 (4%)	–	–	–
Hyperplasia, nodular	1 (2%)	–	–	–
Hypertrophy	–	–	–	1 (2%)
Inflammation, chronic active	2 (4%)	1 (2%)	–	
Mixed cell focus	2 (4%)	6 (12%)	3 (6%)	2 (4%)
Vacuolization cytoplasmic	–	1 (2%)	–	–
Bile duct, cyst	–	1 (2%)	2 (4%)	–
Bile duct, hyperplasia	32 (64%)	26 (52%)	24 (48%)	23 (46%)
Centrilobular, hepatocyte, hypertrophy	2 (4%)	17 (34%)	39 (78%)	47 (94%)
Centrilobular, hepatocyte, necrosis	–	–	1 (2%)	2 (4%)
Hepatocyte, degeneration	1 (2%)	–	–	–
Hepatocyte, necrosis	3 (6%)	3 (6%)	4 (8%)	4 (8%)
Mesentery	(2)	(2)	(1)	(1)
Artery, inflammation	–	–	–	1 (100%)
Fat, necrosis	1 (50%)	2 (100%)	1 (100%)	–
Pancreas	(50)	(50)	(50)	(50)
Inflammation	–	–	1 (2%)	–
Acinus, atrophy	8 (16%)	6 (12%)	6 (12%)	4 (8%)
Acinus, hyperplasia	7 (14%)	11 (22%)	12 (24%)	11 (22%)
Acinus, vacuolization cytoplasmic	–	–	–	1 (2%)
Artery, inflammation	17 (34%)	19 (38%)	19 (38%)	19 (38%)
Duct, cyst	–	–	1 (2%)	–
Salivary glands	(50)	(50)	(50)	(50)
Atrophy, focal	–	–	–	1 (2%)
Inflammation	–	–	2 (4%)	1 (2%)
Parotid gland, atrophy	1 (2%)	–	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, chronic active	–	2 (4%)	–	1 (2%)
Mineralization	–	1 (2%)	–	–
Ulcer	–	1 (2%)	–	4 (8%)
Epithelium, hyperplasia	1 (2%)	4 (8%)	–	4 (8%)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Stomach, glandular	(50)	(50)	(50)	(50)
Mineralization	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Ulcer	1 (2%)	1 (2%)	–	–
Tongue	(0)	(1)	(0)	(0)
Artery, inflammation	–	1 (100%)	–	–
Tooth	(1)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(49)	(50)
Aorta, inflammation	1 (2%)	–	–	–
Aorta, mineralization	3 (6%)	1 (2%)	2 (4%)	3 (6%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	41 (82%)	39 (78%)	45 (90%)	40 (80%)
Congestion	–	–	1 (2%)	–
Atrium, thrombosis	–	2 (4%)	1 (2%)	4 (8%)
Atrium, myocardium, inflammation	–	–	1 (2%)	–
Epicardium, inflammation	1 (2%)	–	–	–
Myocardium, mineralization	–	–	–	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	–	–	1 (2%)	–
Degeneration, cystic	9 (18%)	5 (10%)	10 (20%)	6 (12%)
Hyperplasia	11 (22%)	15 (30%)	19 (38%)	8 (16%)
Hypertrophy	18 (36%)	11 (22%)	13 (26%)	14 (28%)
Necrosis	–	2 (4%)	2 (4%)	2 (4%)
Thrombosis	–	2 (4%)	1 (2%)	1 (2%)
Vacuolization cytoplasmic	1 (2%)	1 (2%)	–	–
Zona fasciculata, atrophy	–	–	–	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	28 (56%)	37 (74%)	35 (70%)	30 (60%)
Necrosis	–	–	–	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Parathyroid gland	(48)	(48)	(48)	(46)
Hyperplasia	4 (8%)	1 (2%)	4 (8%)	1 (2%)
Hyperplasia, focal	–	–	–	1 (2%)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Pituitary gland	(50)	(50)	(49)	(50)
Angiectasis	–	1 (2%)	1 (2%)	–
Cyst	2 (4%)	3 (6%)	5 (10%)	1 (2%)
Pars distalis, hyperplasia	13 (26%)	15 (30%)	12 (24%)	8 (16%)
Pars distalis, necrosis	–	–	1 (2%)	–
Pars intermedia, hyperplasia	1 (2%)	–	–	–
Thyroid gland	(50)	(49)	(49)	(50)
Ultimobranchial cyst	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Ultimobranchial cyst, multiple	1 (2%)	–	–	–
C-cell, hyperplasia	16 (32%)	12 (24%)	14 (29%)	15 (30%)
Follicular cell, hyperplasia	1 (2%)	1 (2%)		1 (2%)
Follicular cell, hypertrophy	–	1 (2%)	–	–
General Body System				
None	–	–	–	–
Genital System				
Coagulating gland	(1)	(0)	(0)	(0)
Inflammation	1 (100%)	–	–	–
Epididymis	(50)	(50)	(50)	(50)
Atrophy	–	–	–	1 (2%)
Artery, inflammation	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Preputial gland	(50)	(50)	(50)	(50)
Cyst	4 (8%)	2 (4%)	3 (6%)	1 (2%)
Inflammation	26 (52%)	26 (52%)	14 (28%)	17 (34%)
Prostate	(50)	(50)	(50)	(50)
Atrophy	–	–	–	1 (2%)
Inflammation	1 (2%)	5 (10%)	1 (2%)	2 (4%)
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy	–	–	–	1 (2%)
Inflammation	3 (6%)	2 (4%)	–	2 (4%)
Testes	(50)	(50)	(50)	(50)
Artery, inflammation	22 (44%)	26 (52%)	25 (50%)	28 (56%)
Germinal epithelium, atrophy	5 (10%)	6 (12%)	12 (24%)	6 (12%)
Interstitial cell, hyperplasia	1 (2%)	1 (2%)	–	2 (4%)
Tunic, inflammation	–	–	1 (2%)	–

	Chamber Control	**100 ppm**	**300 ppm**	**1,000 ppm**
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	–	–	1 (2%)	–
Lymph node	(7)	(7)	(10)	(4)
Axillary, congestion	–	1 (14%)	–	–
Deep cervical, hyperplasia	1 (14%)	–	–	–
Iliac, ectasia	–	–	1 (10%)	–
Iliac, hyperplasia	1 (14%)	–	–	–
Inguinal, ectasia	–	1 (14%)	–	–
Lumbar, congestion	–	1 (14%)	–	–
Lumbar, ectasia	–	1 (14%)	–	–
Lumbar, hyperplasia	–	1 (14%)	–	–
Renal, congestion	1 (14%)	4 (57%)	9 (90%)	3 (75%)
Renal, ectasia	–	2 (29%)	–	–
Renal, hyperplasia	1 (14%)	–	–	–
Lymph node, bronchial	(30)	(26)	(29)	(26)
Lymph node, mandibular	(50)	(50)	(50)	(50)
Congestion	–	1 (2%)	2 (4%)	1 (2%)
Ectasia	–	–	1 (2%)	1 (2%)
Hyperplasia	3 (6%)	1 (2%)	1 (2%)	–
Lymph node, mediastinal	(50)	(49)	(50)	(50)
Congestion	3 (6%)	2 (4%)	5 (10%)	8 (16%)
Hyperplasia	–	1 (2%)	1 (2%)	–
Lymph node, mesenteric	(49)	(50)	(50)	(50)
Congestion	1 (2%)	2 (4%)	–	–
Hemorrhage	–	–	1 (2%)	–
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	29 (58%)	30 (60%)	30 (60%)	22 (44%)
Hemorrhage	1 (2%)	–	–	–
Necrosis	–	1 (2%)	–	–
Lymphoid follicle, hyperplasia	–	–	1 (2%)	–
Red pulp, congestion	–	1 (2%)	–	1 (2%)
Thymus	(48)	(50)	(48)	(49)
Atrophy	45 (94%)	44 (88%)	46 (96%)	45 (92%)
Cyst	–	–	1 (2%)	1 (2%)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Hematopoietic cell proliferation	–	–	1 (2%)	–
Integumentary System				
Mammary gland	(43)	(38)	(38)	(37)
Skin	(50)	(50)	(50)	(50)
Cyst, squamous	1 (2%)	–	–	–
Cyst epithelial inclusion	3 (6%)	4 (8%)	4 (8%)	3 (6%)
Edema	–	1 (2%)	1 (2%)	–
Inflammation	2 (4%)	1 (2%)	1 (2%)	–
Ulcer	–	–	1 (2%)	3 (6%)
Sebaceous gland, cyst	–	–	1 (2%)	
Sebaceous gland, inflammation, suppurative	1 (2%)	–	–	–
Subcutaneous tissue, fibrosis	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(7)	(6)	(4)	(2)
Degeneration	1 (14%)	–	–	–
Inflammation	2 (29%)	–	–	–
Regeneration	–	1 (17%)	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Angiectasis	–	–	–	1 (2%)
Gliosis	1 (2%)	–	–	–
Hemorrhage	–	–	–	1 (2%)
Artery, inflammation	–	1 (2%)	–	–
Cerebellum, necrosis	1 (2%)	–	–	–
Cerebrum, necrosis	1 (2%)	–	–	2 (4%)
Glial cell, hyperplasia	1 (2%)	–	–	–
Hypothalamus, compression	–	2 (4%)	–	1 (2%)
Unilateral, compression	–	–	1 (2%)	–
Peripheral nerve	(7)	(5)	(4)	(2)
Axon, degeneration	3 (43%)	–	–	–
Spinal cord	(7)	(5)	(4)	(2)
Axon, degeneration	–	1 (20%)	–	–
Nerve, gliosis	1 (14%)	–	–	–

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Inflammation	5 (10%)	6 (12%)	6 (12%)	4 (8%)
Metaplasia, squamous	–	–	1 (2%)	–
Lung	(50)	(50)	(50)	(50)
Edema	–	1 (2%)	1 (2%)	–
Fibrosis	8 (16%)	22 (44%)	28 (56%)	24 (48%)
Foreign body	–	–	–	2 (4%)
Hemorrhage	11 (22%)	23 (46%)	28 (56%)	28 (56%)
Infiltration cellular, histiocyte	1 (2%)	–	–	–
Inflammation, chronic	32 (64%)	42 (84%)	47 (94%)	45 (90%)
Metaplasia, osseous	12 (24%)	17 (34%)	17 (34%)	9 (18%)
Alveolar epithelium, hyperplasia	1 (2%)	2 (4%)	–	–
Alveolus, infiltration cellular, histiocyte	27 (54%)	26 (52%)	27 (54%)	22 (44%)
Bronchiole, inflammation, suppurative	–	–	–	1 (2%)
Mediastinum, hemorrhage	–	1 (2%)	–	–
Nose	(50)	(50)	(50)	(50)
Exudate	8 (16%)	12 (24%)	12 (24%)	18 (36%)
Foreign body	–	1 (2%)	–	–
Hemorrhage	–	–	1 (2%)	–
Inflammation, chronic active	2 (4%)	–	–	–
Nasopharyngeal duct, inflammation, suppurative	–	–	–	1 (2%)
Olfactory epithelium, accumulation, hyaline droplet	37 (74%)	31 (62%)	34 (68%)	30 (60%)
Olfactory epithelium, degeneration	1 (2%)	–	2 (4%)	2 (4%)
Olfactory epithelium, inflammation	–	–	–	1 (2%)
Olfactory epithelium, metaplasia, respiratory	1 (2%)	–	–	–
Trachea	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	–	–	–
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Degeneration	–	1 (2%)	–	–
Hemorrhage	–	1 (2%)	–	–
Anterior chamber, inflammation, suppurative	1 (2%)	1 (2%)	–	–
Cornea, hyperplasia	–	–	1 (2%)	–
Cornea, inflammation	4 (8%)	5 (10%)	9 (18%)	1 (2%)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Harderian gland	(50)	(50)	(50)	(50)
Atrophy	–	2 (4%)	1 (2%)	–
Hyperplasia	1 (2%)	–	–	–
Inflammation	–	3 (6%)	1 (2%)	2 (4%)
Zymbal's gland	(1)	(2)	(0)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	3 (6%)	2 (4%)	2 (4%)	3 (6%)
Hemorrhage	1 (2%)	–	–	–
Infarct	1 (2%)	–	–	–
Inflammation, chronic active	1 (2%)	–	–	–
Nephropathy	49 (98%)	49 (98%)	50 (100%)	50 (100%)
Thrombosis	–	–	–	1 (2%)
Pelvis, dilatation	–	2 (4%)	–	–
Renal tubule, mineralization	1 (2%)	1 (2%)	–	–
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation	–	2 (4%)	–	–
Necrosis	–	1 (2%)	–	–
Ulcer	1 (2%)	–	–	1 (2%)
Urothelium, hyperplasia	1 (2%)	–	–	–

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

Appendix B. Summary of Lesions in Female Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

Tables

Table B-1. Summary of the Incidence of Neoplasms in Female Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene....B-2
Table B-2. Statistical Analysis of Primary Neoplasms in Female Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene....B-7
Table B-3. Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene....B-11

Table B-1. Summary of the Incidence of Neoplasms in Female Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	1	–	–
Moribund	22	27	18	18
Natural deaths	5	1	7	2
Survivors				
Died last week of study	1	–	–	–
Terminal kill	22	21	25	30
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(49)	(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)
Leiomyoma	–	–	–	1 (2%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Cholangiocarcinoma	–	2 (4%)	–	–
Hemangioma	–	–	1 (2%)	–
Hemangiosarcoma	–	–	–	1 (2%)
Hepatocellular adenoma	2 (4%)	2 (4%)	1 (2%)	4 (8%)
Hepatocellular carcinoma	–	–	1 (2%)	
Mesentery	(1)	(1)	(1)	(2)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (50%)
Schwannoma malignant, metastatic, skeletal muscle	–	1 (100%)	–	–
Yolk sac carcinoma, metastatic, ovary	–	–	1 (100%)	–
Pancreas	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Acinus, adenoma	–	1 (2%)	–	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Tooth	(0)	(1)	(0)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	–	1 (2%)	2 (4%)	1 (2%)
Capsule, schwannoma malignant, metastatic, skeletal muscle	–	1 (2%)	–	–
Adrenal medulla	(49)	(50)	(50)	(50)
Pheochromocytoma benign	–	3 (6%)	4 (8%)	6 (12%)
Pheochromocytoma malignant	–	1 (2%)	–	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)	–	1 (2%)
Carcinoma	1 (2%)	–	–	–
Parathyroid gland	(45)	(44)	(48)	(42)
Adenoma	–	–	2 (4%)	–
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	18 (36%)	14 (28%)	16 (32%)	5 (10%)
Pars intermedia, adenoma	–	–	–	2 (4%)
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, c-cell, adenoma	–	–	–	3 (6%)
C-cell, adenoma	2 (4%)	8 (16%)	8 (16%)	11 (22%)
C-cell, carcinoma	–	2 (4%)	–	1 (2%)
Follicular cell, adenoma	–	1 (2%)	–	–
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(50)	(50)	(49)	(50)
Adenoma	–	–	1 (2%)	–
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor benign	3 (6%)	–	–	–
Granulosa-theca tumor benign	–	–	1 (2%)	–
Thecoma benign	1 (2%)	–	–	–

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Yolk sac carcinoma	–	–	1 (2%)	–
Uterus	(50)	(50)	(50)	(50)
Adenocarcinoma	1 (2%)	1 (2%)	–	5 (10%)
Adenoma	–	1 (2%)	–	–
Leiomyosarcoma	–	–	–	1 (2%)
Polyp stromal	7 (14%)	7 (14%)	12 (24%)	10 (20%)
Polyp stromal, multiple	–	1 (2%)	4 (8%)	2 (4%)
Sarcoma stromal	–	–	1 (2%)	
Schwannoma malignant	–	–	–	1 (2%)
Squamous cell carcinoma	1 (2%)	–	–	–
Yolk sac carcinoma, metastatic, ovary	–	–	1 (2%)	–
Cervix, polyp stromal	–	1 (2%)	–	–
Vagina	(46)	(48)	(48)	(38)
Granular cell tumor benign	1 (2%)	2 (4%)	–	–
Paraganglioma	–	–	1 (2%)	–
Schwannoma malignant	1 (2%)	–	1 (2%)	1 (3%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(2)	(6)	(5)	(3)
Deep cervical, carcinoma, metastatic, thyroid gland	–	–	–	1 (33%)
Lymph node, bronchial	(32)	(27)	(22)	(20)
Lymph node, mandibular	(49)	(50)	(50)	(50)
Lymph node, mediastinal	(50)	(50)	(50)	(49)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Carcinoma, metastatic, thyroid gland	–	–	–	1 (2%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Schwannoma malignant, metastatic, skeletal muscle	–	1 (2%)	–	–
Yolk sac carcinoma, metastatic, ovary	–	–	1 (2%)	–
Thymus	(50)	(48)	(49)	(49)
Thymoma benign	–	1 (2%)	–	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenocarcinoma	5 (10%)	3 (6%)	3 (6%)	1 (2%)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Adenocarcinoma, multiple	–	1 (2%)	–	–
Adenoma	4 (8%)	4 (8%)	–	1 (2%)
Fibroadenoma	17 (34%)	20 (40%)	20 (40%)	13 (26%)
Fibroadenoma, multiple	3 (6%)	8 (16%)	11 (22%)	5 (10%)
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma	1 (2%)	–	–	–
Squamous cell papilloma	–	–	–	1 (2%)
Sebaceous gland, adenoma	–	–	–	1 (2%)
Subcutaneous tissue, fibroma	–	1 (2%)	–	–
Subcutaneous tissue, fibrous histiocytoma	–	1 (2%)	–	–
Subcutaneous tissue, hibernoma	–	–	1 (2%)	–
Subcutaneous tissue, lipoma	–	1 (2%)	–	–
Subcutaneous tissue, schwannoma malignant	–	1 (2%)	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(3)	(6)	(1)	(3)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (33%)
Lipoma	–	–	–	1 (33%)
Liposarcoma	1 (33%)	–	–	–
Schwannoma malignant	–	1 (17%)	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Glioma malignant	1 (2%)	1 (2%)	–	–
Granular cell tumor benign	–	1 (2%)	–	–
Cranial nerve, schwannoma malignant	1 (2%)	1 (2%)	–	–
Peripheral nerve	(1)	(4)	(2)	(1)
Spinal cord	(1)	(4)	(1)	(1)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Basal cell carcinoma, metastatic, skin	1 (2%)	–	–	–
Carcinoma, metastatic, thyroid gland	–	1 (2%)		1 (2%)
Cystic keratinizing epithelioma	–	1 (2%)	1 (2%)	–
Fibrous histiocytoma, metastatic, skin	–	1 (2%)	–	–

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Yolk sac carcinoma, metastatic, ovary	–	–	1 (2%)	–
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Schwannoma malignant	–	–	1 (2%)	–
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(0)	(1)	(1)	(2)
Carcinoma	–	1 (100%)	–	1 (50%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, adenoma	1 (2%)	–	–	1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	–	–	2 (4%)	–
Lymphoma malignant	–	–	1 (2%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	41	42	43	42
Total primary neoplasms	73	96	98	83
Total animals with benign neoplasms	39	42	41	38
Total benign neoplasms	60	80	86	70
Total animals with malignant neoplasms	11	13	12	12
Total malignant neoplasms	13	16	12	13
Total animals with metastatic neoplasms	1	3	1	2
Total metastatic neoplasms	1	5	4	10

[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 B-2. Statistical Analysis of Primary Neoplasms in Female Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	0/49 (0%)	3/50 (6%)	4/50 (8%)	6/50 (12%)
Adjusted rate[b]	0.0%	8.1%	9.9%	13.5%
Terminal rate[c]	0/21 (0%)	2/21 (10%)	2/25 (8%)	4/30 (13%)
First incidence (days)	–[e]	691	478	530
Poly-3 test[d]	P = 0.066	P = 0.135	P = 0.085	P = 0.035
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	0/49 (0%)	4/50 (8%)	4/50 (8%)	6/50 (12%)
Adjusted rate	0.0%	10.7%	9.9%	13.5%
Terminal rate	0/21 (0%)	3/21 (14%)	2/25 (8%)	4/30 (13%)
First incidence (days)	–	691	478	530
Poly-3 test	P = 0.106	P = 0.071	P = 0.085	P = 0.035
Liver: Hepatocellular Adenoma				
Overall rate	2/50 (4%)	2/50 (4%)	1/50 (2%)	4/50 (8%)
Adjusted rate	5.7%	5.3%	2.5%	9.1%
Terminal rate	2/22 (9%)	0/21 (0%)	1/25 (4%)	3/30 (10%)
First incidence (days)	731 (T)	648	731 (T)	642
Poly-3 test	P = 0.256	P = 0.668N	P = 0.457N	P = 0.446
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	2/50 (4%)	4/50 (8%)
Adjusted rate	5.7%	5.3%	5.1%	9.1%
Terminal rate	2/22 (9%)	0/21 (0%)	2/25 (8%)	3/30 (10%)
First incidence (days)	731 (T)	648	731 (T)	642
Poly-3 test	P = 0.298	P = 0.668N	P = 0.650N	P = 0.446
Mammary Gland: Fibroadenoma				
Overall rate	20/50 (40%)	28/50 (56%)	31/50 (62%)	18/50 (36%)
Adjusted rate	49.6%	62.7%	69.3%	38.5%
Terminal rate	10/22 (46%)	12/21 (57%)	17/25 (68%)	9/30 (30%)
First incidence (days)	311	395	395	530
Poly-3 test	P = 0.015N	P = 0.149	P = 0.041	P = 0.202N
Mammary Gland: Adenoma				
Overall rate	4/50 (8%)	4/50 (8%)	0/50 (0%)	1/50 (2%)
Adjusted rate	11.2%	10.7%	0.0%	2.3%
Terminal rate	2/22 (9%)	2/21 (10%)	0/25 (0%)	1/30 (3%)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
First incidence (days)	459	646	–	731 (T)
Poly-3 test	P = 0.084N	P = 0.618N	P = 0.047N	P = 0.124N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	23/50 (46%)	31/50 (62%)	31/50 (62%)	19/50 (38%)
Adjusted rate	55.9%	69.3%	69.3%	40.6%
Terminal rate	11/22 (50%)	14/21 (67%)	17/25 (68%)	10/30 (33%)
First incidence (days)	311	395	395	530
Poly-3 test	P = 0.004N	P = 0.133	P = 0.131	P = 0.105N
Mammary Gland: Adenocarcinoma				
Overall rate	5/50 (10%)	4/50 (8%)	3/50 (6%)	1/50 (2%)
Adjusted rate	13.6%	10.7%	7.5%	2.3%
Terminal rate	1/22 (5%)	2/21 (10%)	1/25 (4%)	1/30 (3%)
First incidence (days)	303	674	584	731 (T)
Poly-3 test	P = 0.051N	P = 0.489N	P = 0.307N	P = 0.067N
Mammary Gland: Adenoma or Adenocarcinoma				
Overall rate	9/50 (18%)	8/50 (16%)	3/50 (6%)	2/50 (4%)
Adjusted rate	24.0%	21.1%	7.5%	4.6%
Terminal rate	3/22 (14%)	4/21 (19%)	1/25 (4%)	2/30 (7%)
First incidence (days)	303	646	584	731 (T)
Poly-3 test	P = 0.007N	P = 0.492N	P = 0.042N	P = 0.012N
Mammary Gland: Fibroadenoma, Adenoma, or Adenocarcinoma				
Overall rate	27/50 (54%)	34/50 (68%)	33/50 (66%)	20/50 (40%)
Adjusted rate	63.3%	75.3%	73.0%	42.8%
Terminal rate	12/22 (55%)	15/21 (71%)	18/25 (72%)	11/30 (37%)
First incidence (days)	303	395	395	530
Poly-3 test	P < 0.001N	P = 0.145	P = 0.213	P = 0.036N
Ovary: Benign Granulosa Cell Tumor				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	8.5%	0.0%	0.0%	0.0%
Terminal rate	2/22 (9%)	0/21 (0%)	0/25 (0%)	0/30 (0%)
First incidence (days)	646	–	–	–
Poly-3 test	P = 0.145N	P = 0.108N	P = 0.098N	P = 0.085N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	18/50 (36%)	14/50 (28%)	16/50 (32%)	5/50 (10%)
Adjusted rate	46.6%	35.8%	37.6%	11.2%

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Terminal rate	7/22 (32%)	5/21 (24%)	7/25 (28%)	1/30 (3%)
First incidence (days)	528	643	541	626
Poly-3 test	P < 0.001N	P = 0.226N	P = 0.273N	P < 0.001N
Thyroid Gland (C-cell): Adenoma				
Overall rate	2/50 (4%)	8/50 (16%)	8/50 (16%)	14/50 (28%)
Adjusted rate	5.5%	20.4%	20.2%	31.6%
Terminal rate	0/22 (0%)	3/21 (14%)	8/25 (32%)	11/30 (37%)
First incidence (days)	459	534	731 (T)	646
Poly-3 test	P = 0.008	P = 0.056	P = 0.057	P = 0.003
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	10/50 (20%)	8/50 (16%)	15/50 (30%)
Adjusted rate	5.5%	25.5%	20.2%	33.6%
Terminal rate	0/22 (0%)	5/21 (24%)	8/25 (32%)	11/30 (37%)
First incidence (days)	459	534	731 (T)	646
Poly-3 test	P = 0.009	P = 0.017	P = 0.057	P = 0.002
Uterus: Stromal Polyp				
Overall rate	7/50 (14%)	9/50 (18%)	16/50 (32%)	12/50 (24%)
Adjusted rate	19.6%	23.8%	39.3%	27.2%
Terminal rate	5/22 (23%)	6/21 (29%)	12/25 (48%)	10/30 (33%)
First incidence (days)	590	643	558	646
Poly-3 test	P = 0.419	P = 0.440	P = 0.047	P = 0.298
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	7/50 (14%)	9/50 (18%)	17/50 (34%)	12/50 (24%)
Adjusted rate	19.6%	23.8%	41.8%	27.2%
Terminal rate	5/22 (23%)	6/21 (29%)	13/25 (52%)	10/30 (33%)
First incidence (days)	590	643	558	646
Poly-3 test	P = 0.439	P = 0.440	P = 0.028	P = 0.298
Uterus: Adenocarcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	0/50 (0%)	5/50 (10%)
Adjusted rate	2.9%	2.7%	0.0%	11.3%
Terminal rate	1/22 (5%)	1/21 (5%)	0/25 (0%)	3/30 (10%)
First incidence (days)	731 (T)	731 (T)	–	530
Poly-3 test	P = 0.017	P = 0.748N	P = 0.475N	P = 0.166
All Organs: Benign Neoplasms				
Overall rate	39/50 (78%)	42/50 (84%)	41/50 (82%)	38/50 (76%)

	Chamber Control	**100 ppm**	**300 ppm**	**1,000 ppm**
Adjusted rate	87.4%	90.3%	88.0%	77.6%
Terminal rate	19/22 (86%)	18/21 (86%)	23/25 (92%)	22/30 (73%)
First incidence (days)	311	395	395	530
Poly-3 test	P = 0.036N	P = 0.451	P = 0.604	P = 0.152N
All Organs: Malignant Neoplasms				
Overall rate	11/50 (22%)	13/50 (26%)	12/50 (24%)	12/50 (24%)
Adjusted rate	28.8%	33.5%	29.0%	26.1%
Terminal rate	4/22 (18%)	7/21 (33%)	6/25 (24%)	5/30 (17%)
First incidence (days)	303	585	564	530
Poly-3 test	P = 0.337N	P = 0.421	P = 0.594	P = 0.484N
All Organs: Benign or Malignant Neoplasms				
Overall rate	41/50 (82%)	42/50 (84%)	43/50 (86%)	42/50 (84%)
Adjusted rate	88.6%	90.3%	90.8%	84.8%
Terminal rate	19/22 (86%)	18/21 (86%)	23/25 (92%)	23/30 (77%)
First incidence (days)	303	395	395	530
Poly-3 test	P = 0.238N	P = 0.532	P = 0.494	P = 0.396N

(T) Terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, ovary, 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 chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by N.
[e]Not applicable; no neoplasms in animal group.

Table B-3. Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	1	–	–
Moribund	22	27	18	18
Natural deaths	5	1	7	2
Survivors				
Died last week of study	1	–	–	–
Terminal kill	22	21	25	30
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(49)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation	–	1 (2%)	–	–
Artery, inflammation	–	1 (2%)	–	–
Intestine large, colon	(50)	(50)	(50)	(50)
Inflammation	–	1 (2%)	–	–
Artery, inflammation	–	1 (2%)	–	–
Artery, serosa, inflammation	–	–	1 (2%)	–
Intestine large, rectum	(50)	(50)	(50)	(50)
Inflammation	–	1 (2%)	–	–
Artery, inflammation	–	1 (2%)	–	–
Intestine small, duodenum	(50)	(50)	(50)	(50)
Artery, inflammation	–	1 (2%)	–	–
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Artery, inflammation	–	1 (2%)	–	1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis	–	5 (10%)	5 (10%)	1 (2%)
Basophilic focus	7 (14%)	3 (6%)	2 (4%)	7 (14%)
Cholangiofibrosis	1 (2%)	–	–	–
Clear cell focus	16 (32%)	15 (30%)	23 (46%)	38 (76%)
Eosinophilic focus	7 (14%)	6 (12%)	7 (14%)	15 (30%)
Fatty change	2 (4%)	4 (8%)	11 (22%)	10 (20%)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Fibrosis	1 (2%)	1 (2%)	–	1 (2%)
Hematopoietic cell proliferation	7 (14%)	1 (2%)	4 (8%)	3 (6%)
Hepatodiaphragmatic nodule	2 (4%)	1 (2%)	1 (2%)	–
Inflammation, chronic active	1 (2%)	–	–	1 (2%)
Mixed cell focus	6 (12%)	6 (12%)	8 (16%)	18 (36%)
Pigmentation	1 (2%)	–	–	–
Bile duct, cyst	4 (8%)	11 (22%)	5 (10%)	5 (10%)
Bile duct, cyst, multiple	2 (4%)	–	1 (2%)	2 (4%)
Bile duct, dilatation	–	–	1 (2%)	–
Bile duct, hyperplasia	14 (28%)	13 (26%)	17 (34%)	13 (26%)
Centrilobular, hepatocyte, hypertrophy	–	1 (2%)	10 (20%)	45 (90%)
Hepatocyte, necrosis	2 (4%)	–	2 (4%)	5 (10%)
Hepatocyte, periportal, hypertrophy	–	–	1 (2%)	–
Serosa, inflammation, chronic active	–	–	–	1 (2%)
Vein, thrombosis	–	–	–	1 (2%)
Mesentery	(1)	(1)	(1)	(2)
Artery, inflammation	–	–	–	1 (50%)
Fat, necrosis	1 (100%)	–	–	–
Pancreas	(50)	(50)	(50)	(50)
Inflammation, chronic	–	–	–	1 (2%)
Acinus, atrophy	2 (4%)	4 (8%)	–	2 (4%)
Acinus, basophilic focus	–	–	1 (2%)	–
Acinus, hyperplasia	1 (2%)	1 (2%)	–	4 (8%)
Artery, inflammation	3 (6%)	7 (14%)	5 (10%)	5 (10%)
Duct, cyst	–	1 (2%)	–	–
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	–	–	–	1 (2%)
Inflammation	1 (2%)	–	–	–
Parotid gland, hyperplasia	1 (2%)	–	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, chronic active	–	2 (4%)	–	1 (2%)
Epithelium, hyperplasia	–	–	1 (2%)	3 (6%)
Serosa, inflammation, chronic active	–	–	–	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Ectopic tissue	1 (2%)	–	–	–

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Artery, inflammation	–	–	1 (2%)	–
Tooth	(0)	(1)	(0)	(0)
Inflammation, suppurative	–	1 (100%)	–	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, inflammation	–	2 (4%)	–	–
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	16 (32%)	12 (24%)	15 (30%)	17 (34%)
Artery, inflammation	–	–	–	1 (2%)
Lymphatic, pericardium, inflammation	–	1 (2%)	–	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	9 (18%)	4 (8%)	8 (16%)	7 (14%)
Degeneration, cystic	9 (18%)	12 (24%)	12 (24%)	14 (28%)
Hematopoietic cell proliferation	1 (2%)	–	1 (2%)	–
Hyperplasia	4 (8%)	7 (14%)	8 (16%)	12 (24%)
Hypertrophy	15 (30%)	17 (34%)	11 (22%)	20 (40%)
Necrosis	3 (6%)	–	2 (4%)	2 (4%)
Thrombosis	4 (8%)	–	2 (4%)	2 (4%)
Adrenal medulla	(49)	(50)	(50)	(50)
Angiectasis	1 (2%)	–	–	–
Degeneration	1 (2%)	–	–	–
Hyperplasia	17 (35%)	25 (50%)	34 (68%)	36 (72%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	–	1 (2%)	–	–
Parathyroid gland	(45)	(44)	(48)	(42)
Fibrosis	–	–	1 (2%)	4 (10%)
Hyperplasia	–	–	1 (2%)	1 (2%)
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)	–	2 (4%)
Cyst	–	–	1 (2%)	1 (2%)
Pars distalis, hyperplasia	15 (30%)	14 (28%)	16 (32%)	17 (34%)
Pars distalis, vacuolization cytoplasmic	–	–	1 (2%)	–
Pars intermedia, hyperplasia	1 (2%)	1 (2%)	–	–
Thyroid gland	(50)	(50)	(50)	(50)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Inflammation, chronic active	–	1 (2%)	–	–
Ultimobranchial cyst	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Ultimobranchial cyst, multiple	–	–	1 (2%)	–
C-cell, hyperplasia	16 (32%)	15 (30%)	21 (42%)	13 (26%)
Follicular cell, hyperplasia	–	1 (2%)	1 (2%)	–
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(50)	(50)	(49)	(50)
Cyst	3 (6%)	4 (8%)	2 (4%)	7 (14%)
Inflammation	13 (26%)	21 (42%)	15 (31%)	16 (32%)
Ovary	(50)	(50)	(50)	(50)
Cyst	11 (22%)	7 (14%)	8 (16%)	10 (20%)
Fibrosis	1 (2%)	–	–	–
Inflammation	1 (2%)	–	–	–
Inflammation, suppurative	–	–	1 (2%)	–
Artery, inflammation	1 (2%)	1 (2%)	–	–
Granulosa cell, hyperplasia	–	–	–	1 (2%)
Mesothelium, hyperplasia	1 (2%)	–	–	–
Uterus	(50)	(50)	(50)	(50)
Adenomyosis	1 (2%)	–	2 (4%)	3 (6%)
Bacterium	1 (2%)	–	–	–
Dilatation	1 (2%)	3 (6%)	3 (6%)	–
Hemorrhage	2 (4%)	–	2 (4%)	–
Inflammation, suppurative	1 (2%)	–	2 (4%)	2 (4%)
Inflammation, chronic active	2 (4%)	3 (6%)	1 (2%)	–
Metaplasia, squamous	4 (8%)	6 (12%)	10 (20%)	1 (2%)
Necrosis	–	–	1 (2%)	–
Prolapse	1 (2%)	–	–	–
Thrombosis	–	–	1 (2%)	
Artery, inflammation, chronic	–	–	–	1 (2%)
Artery, necrosis, fibrinoid	–	–	–	1 (2%)
Cervix, cyst, squamous, multiple	1 (2%)	–	–	–
Cervix, cyst, squamous	–	1 (2%)	–	1 (2%)
Cervix, hyperplasia, squamous	–	2 (4%)	–	–

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Cervix, hypertrophy, stromal	1 (2%)	–	–	1 (2%)
Endometrium, atypical hyperplasia	–	–	1 (2%)	3 (6%)
Endometrium, erosion	–	1 (2%)	–	–
Endometrium, hyperplasia, cystic	41 (82%)	34 (68%)	31 (62%)	22 (44%)
Serosa, fibrosis	–	1 (2%)	–	–
Serosa, inflammation, chronic active	–	–	–	1 (2%)
Vagina	(46)	(48)	(48)	(38)
Cyst, squamous	–	–	1 (2%)	–
Hyperplasia, stromal	–	1 (2%)	–	–
Hyperplasia, granular cell	1 (2%)	–	–	–
Inflammation, suppurative	–	1 (2%)	1 (2%)	2 (5%)
Inflammation, chronic	–	–	1 (2%)	–
Ulcer	–	–	1 (2%)	–
Artery, inflammation, chronic active	–	1 (2%)	–	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy	–	1 (2%)	–	–
Lymph node	(2)	(6)	(5)	(3)
Congestion	–	1 (17%)	–	–
Hyperplasia	–	1 (17%)	–	–
Iliac, congestion	–	–	1 (20%)	–
Iliac, ectasia	–	–	–	1 (33%)
Iliac, hyperplasia	–	1 (17%)	2 (40%)	–
Lumbar, congestion	1 (50%)	1 (17%)	–	–
Lumbar, hyperplasia	–	1 (17%)	–	–
Pancreatic, hyperplasia	–	1 (17%)	–	–
Renal, congestion	1 (50%)	1 (17%)	2 (40%)	–
Renal, ectasia	–	1 (17%)	–	–
Lymph node, bronchial	(32)	(27)	(22)	(20)
Lymph node, mandibular	(49)	(50)	(50)	(50)
Hyperplasia	2 (4%)	–	–	–
Lymph node, mediastinal	(50)	(50)	(50)	(49)
Congestion	–	1 (2%)	–	–
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Congestion	1 (2%)	–	–	–

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Artery, inflammation	–	–	–	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	31 (62%)	31 (62%)	38 (76%)	36 (72%)
Inflammation	–	1 (2%)	–	–
Capsule, fibrosis	–	–	–	1 (2%)
Red pulp, infiltration cellular, histiocyte	–	–	–	1 (2%)
Thymus	(50)	(48)	(49)	(49)
Atrophy	44 (88%)	44 (92%)	43 (88%)	48 (98%)
Cyst	1 (2%)	–	1 (2%)	–
Hyperplasia, lymphoid	–	1 (2%)	–	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Cyst	–	–	1 (2%)	1 (2%)
Hyperplasia, cystic	2 (4%)	7 (14%)	1 (2%)	1 (2%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	–	3 (6%)	–
Inflammation	–	–	–	1 (2%)
Ulcer	3 (6%)	–	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(3)	(6)	(1)	(3)
Hemorrhage	1 (33%)	–	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hypothalamus, compression	7 (14%)	7 (14%)	6 (12%)	1 (2%)
Unilateral, inflammation, granulomatous	–	1 (2%)	–	–
Unilateral, necrosis	–	1 (2%)	–	–
Peripheral nerve	(1)	(4)	(2)	(1)
Inflammation	–	–	1 (50%)	–
Spinal cord	(1)	(4)	(1)	(1)
Cyst epithelial inclusion	–	1 (25%)	–	–
Axon, nerve, degeneration	–	1 (25%)	–	–
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Bacterium	–	–	–	1 (2%)

	Chamber Control	**100 ppm**	**300 ppm**	**1,000 ppm**
Fibrosis	–	–	–	1 (2%)
Foreign body	–	–	–	1 (2%)
Inflammation	1 (2%)	2 (4%)	2 (4%)	4 (8%)
Inflammation, chronic active	–	–	–	1 (2%)
Necrosis	–	–	–	1 (2%)
Squamous epithelium, hyperplasia	2 (4%)	–	–	1 (2%)
Lung	(50)	(50)	(50)	(50)
Fibrosis	11 (22%)	17 (34%)	24 (48%)	28 (56%)
Hemorrhage	12 (24%)	11 (22%)	18 (36%)	26 (52%)
Inflammation, chronic	35 (70%)	42 (84%)	48 (96%)	46 (92%)
Metaplasia, osseous	5 (10%)	4 (8%)	4 (8%)	2 (4%)
Metaplasia, squamous	1 (2%)	2 (4%)	2 (4%)	–
Alveolar epithelium, hyperplasia	2 (4%)	–	1 (2%)	1 (2%)
Alveolus, infiltration cellular, histiocyte	35 (70%)	31 (62%)	36 (72%)	33 (66%)
Artery, mediastinum, inflammation, chronic	–	–	–	1 (2%)
Artery, mediastinum, necrosis, fibrinoid	–	–	–	1 (2%)
Bronchiole, hyperplasia	–	–	1 (2%)	–
Mediastinum, inflammation, chronic	–	1 (2%)	–	–
Nose	(50)	(50)	(50)	(50)
Foreign body	–	1 (2%)	–	1 (2%)
Inflammation	2 (4%)	3 (6%)	6 (12%)	2 (4%)
Inflammation, acute	–	1 (2%)	–	–
Nasopharyngeal duct, inflammation, suppurative	–	–	1 (2%)	–
Olfactory epithelium, accumulation, hyaline droplet	40 (80%)	36 (72%)	40 (80%)	30 (60%)
Olfactory epithelium, degeneration	–	1 (2%)	2 (4%)	–
Olfactory epithelium, hemorrhage	–	–	–	1 (2%)
Respiratory epithelium, accumulation, hyaline droplet	–	–	–	1 (2%)
Respiratory epithelium, inflammation	–	–	–	1 (2%)
Sinus, polyp, inflammatory	1 (2%)	–	–	–
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cornea, inflammation	1 (2%)	1 (2%)	–	–
Harderian gland	(50)	(50)	(50)	(50)
Atrophy	2 (4%)	–	–	1 (2%)

	Chamber Control	100 ppm	300 ppm	1,000 ppm
Hyperplasia	–	–	–	3 (6%)
Inflammation	1 (2%)	1 (2%)	4 (8%)	2 (4%)
Zymbal's gland	(0)	(1)	(1)	(2)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Infarct	–	–	1 (2%)	–
Inflammation	1 (2%)	–	–	–
Nephropathy	44 (88%)	49 (98%)	49 (98%)	47 (94%)
Artery, inflammation	–	–	–	1 (2%)
Interstitium, inflammation, chronic active	1 (2%)	–	1 (2%)	–
Pelvis, inflammation, chronic active	1 (2%)	–	1 (2%)	–
Renal tubule, hyperplasia	–	–	2 (4%)	–
Urinary bladder	(50)	(50)	(50)	(50)
Polyp, inflammatory	–	–	–	1 (2%)

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

Appendix C. Summary of Lesions in Male Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

Tables

Table C-1. Summary of the Incidence of Neoplasms in Male Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene....C-2
Table C-2. Statistical Analysis of Primary Neoplasms in Male Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene....C-8
Table C-3. Historical Incidence of Liver Neoplasms in Control Male B6C3F1/N Mice....C-12
Table C-4. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene....C-13

Table C-1. Summary of the Incidence of Neoplasms in Male Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	100 ppm	200 ppm	400 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	8	5	11	12
Natural deaths	2	5	4	10
Survivors				
Terminal kill	40	40	35	28
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(50)	(49)	(44)	(44)
Intestine large, cecum	(50)	(50)	(50)	(50)
Adenoma	–	–	–	1 (2%)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Intestine large, colon	(50)	(50)	(50)	(50)
Adenoma	–	1 (2%)	–	–
Intestine large, rectum	(50)	(50)	(50)	(49)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Adenoma	–	–	1 (2%)	–
Hepatocellular carcinoma, metastatic, liver	–	–	–	1 (2%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Adenoma	–	–	–	1 (2%)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Adenocarcinoma	1 (2%)	–	–	1 (2%)
Adenoma	–	–	–	1 (2%)
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	–	2 (4%)	1 (2%)	1 (2%)
Hepatoblastoma	1 (2%)	1 (2%)	1 (2%)	10 (20%)
Hepatoblastoma, multiple	–	–	–	5 (10%)
Hepatocellular adenoma	16 (32%)	9 (18%)	12 (24%)	8 (16%)
Hepatocellular adenoma, multiple	9 (18%)	15 (30%)	19 (38%)	21 (42%)
Hepatocellular carcinoma	6 (12%)	14 (28%)	9 (18%)	5 (10%)
Hepatocellular carcinoma, multiple	2 (4%)	5 (10%)	7 (14%)	30 (60%)
Hepatocholangiocarcinoma	3 (6%)	2 (4%)	–	–

	Chamber Control	**100 ppm**	**200 ppm**	**400 ppm**
Rhabdomyosarcoma, metastatic, skeletal muscle	1 (2%)	–	–	–
Mesentery	(6)	(5)	(2)	(5)
Hepatoblastoma, metastatic, liver	–	–	–	1 (20%)
Hepatocellular carcinoma, metastatic, liver	–	1 (20%)	–	–
Hepatocholangiocarcinoma, metastatic, liver	2 (33%)	–	–	–
Mast cell tumor malignant	–	1 (20%)	–	–
Pancreas	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	–
Hepatocholangiocarcinoma, metastatic, liver	2 (4%)	–	–	–
Salivary glands	(50)	(50)	(50)	(49)
Stomach, forestomach	(50)	(50)	(50)	(48)
Adenocarcinoma, metastatic, mammary gland	1 (2%)	–	–	–
Hemangioma	–	–	1 (2%)	–
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Squamous cell papilloma	1 (2%)	–	–	1 (2%)
Squamous cell papilloma, multiple	–	–	1 (2%)	–
Stomach, glandular	(50)	(50)	(49)	(49)
Adenoma	–	1 (2%)	–	
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	–
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Tongue	(1)	(0)	(0)	(0)
Squamous cell papilloma	1 (100%)	–	–	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, hepatoblastoma, metastatic, liver	1 (2%)	–	–	–
Heart	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	–
Hepatocholangiocarcinoma, metastatic, liver	2 (4%)	–	–	–
Sarcoma, metastatic, skin	–	–	1 (2%)	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver	–	–	–	1 (2%)
Hepatocellular carcinoma, metastatic, liver	–	–	–	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	–	–	1 (2%)	–

	Chamber Control	100 ppm	200 ppm	400 ppm
Islets, pancreatic	(50)	(49)	(50)	(50)
Adenoma	1 (2%)	–	–	–
Carcinoma	–	1 (2%)	–	–
Parathyroid gland	(36)	(28)	(39)	(38)
Pituitary gland	(50)	(50)	(50)	(49)
Pars distalis, adenoma	–	–	1 (2%)	–
Pars intermedia, schwannoma malignant	1 (2%)	–	–	–
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	–	–	1 (2%)	–
General Body System				
Peritoneum	(0)	(0)	(1)	(0)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	–
Penis	(0)	(0)	(1)	(0)
Preputial gland	(50)	(50)	(49)	(50)
Hemangiosarcoma	–	–	1 (2%)	–
Prostate	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver	–	–	–	1 (2%)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Seminal vesicle	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	–
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Testes	(50)	(50)	(50)	(50)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	–	1 (2%)	–	–
Mast cell tumor malignant	–	1 (2%)	–	–
Lymph node	(4)	(0)	(3)	(4)
Pancreatic, hepatocellular carcinoma, metastatic, liver	–	–	–	1 (25%)
Pancreatic, hepatocholangiocarcinoma, metastatic, liver	1 (25%)	–	–	–
Lymph node, bronchial	(49)	(47)	(47)	(48)
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	1 (2%)
Hepatocellular carcinoma, metastatic, liver	–	1 (2%)	–	–
Hepatocholangiocarcinoma, metastatic, liver	2 (4%)	–	–	–

	Chamber Control	100 ppm	200 ppm	400 ppm
Lymph node, mandibular	(36)	(41)	(43)	(45)
Lymph node, mediastinal	(46)	(47)	(44)	(42)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	–
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	1 (2%)
Hepatocellular carcinoma, metastatic, liver	–	1 (2%)	–	–
Hepatocholangiocarcinoma, metastatic, liver	2 (4%)	–	–	–
Lymph node, mesenteric	(49)	(50)	(49)	(49)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	–	1 (2%)	–
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Spleen	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	–
Hemangiosarcoma	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	–
Mast cell tumor malignant	–	1 (2%)	1 (2%)	–
Thymus	(48)	(48)	(48)	(48)
Hepatoblastoma, metastatic, liver	–	–	–	1 (2%)
Hepatocholangiocarcinoma, metastatic, liver	2 (4%)	–	–	–
Integumentary System				
Mammary gland	(2)	(1)	(3)	(1)
Adenocarcinoma	1 (50%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Subcutaneous tissue, fibrous histiocytoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Subcutaneous tissue, hemangiosarcoma	2 (4%)	–	–	–
Subcutaneous tissue, sarcoma	–	–	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hemangiosarcoma	–	–	–	1 (2%)
Skeletal muscle	(2)	(1)	(1)	(2)
Hepatoblastoma, metastatic, liver	1 (50%)	–	–	1 (50%)
Hepatocholangiocarcinoma, metastatic, liver	1 (50%)	–	–	–
Mast cell tumor malignant	–	1 (100%)	–	–
Rhabdomyosarcoma	1 (50%)	–	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)

	Chamber Control	100 ppm	200 ppm	400 ppm
Schwannoma malignant, metastatic, pituitary gland	1 (2%)	–	–	–
Peripheral nerve	(0)	(0)	(1)	(0)
Spinal cord	(0)	(0)	(1)	(0)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, Harderian gland	–	1 (2%)	–	–
Adenocarcinoma, metastatic, mammary gland	1 (2%)	–	–	–
Alveolar/bronchiolar adenoma	8 (16%)	11 (22%)	9 (18%)	3 (6%)
Alveolar/bronchiolar adenoma, multiple	3 (6%)	–	–	–
Alveolar/bronchiolar carcinoma	4 (8%)	3 (6%)	3 (6%)	3 (6%)
Alveolar/bronchiolar carcinoma, multiple	2 (4%)	1 (2%)	–	–
Hemangiosarcoma	1 (2%)	–	–	–
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	5 (10%)
Hepatoblastoma, metastatic, uncertain primary site	–	–	–	1 (2%)
Hepatocellular carcinoma, metastatic, liver	5 (10%)	5 (10%)	5 (10%)	8 (16%)
Hepatocholangiocarcinoma, metastatic, liver	2 (4%)	1 (2%)	–	–
Mediastinum, sarcoma, metastatic, skin	–	–	1 (2%)	–
Nose	(50)	(50)	(50)	(50)
Mast cell tumor malignant	–	–	1 (2%)	–
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenocarcinoma	1 (2%)	3 (6%)	–	–
Adenoma	5 (10%)	4 (8%)	4 (8%)	8 (16%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	–
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Mast cell tumor malignant	–	–	1 (2%)	–
Renal tubule, adenoma	–	–	–	1 (2%)
Urethra	(0)	(0)	(0)	(1)
Urinary bladder	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	–

	Chamber Control	100 ppm	200 ppm	400 ppm
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	–	–	1 (2%)	–
Lymphoma malignant	6 (12%)	1 (2%)	5 (10%)	3 (6%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	44	47	46	49
Total primary neoplasms	78	82	86	106
Total animals with benign neoplasms	32	28	35	34
Total benign neoplasms	44	41	50	45
Total animals with malignant neoplasms	25	32	27	44
Total malignant neoplasms	34	41	36	61
Total animals with metastatic neoplasms	9	8	6	16
Total metastatic neoplasms	47	12	8	24
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 C-2. Statistical Analysis of Primary Neoplasms in Male Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	200 ppm	400 ppm
Harderian Gland: Adenoma				
Overall rate[a]	5/50 (10%)	4/50 (8%)	4/50 (8%)	8/50 (16%)
Adjusted rate[b]	10.7%	8.4%	8.9%	18.9%
Terminal rate[c]	3/40 (8%)	4/40 (10%)	3/35 (9%)	6/28 (21%)
First incidence (days)	640	729 (T)	631	643
Poly-3 test[d]	P = 0.125	P = 0.491N	P = 0.526N	P = 0.216
Harderian Gland: Adenocarcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	2.2%	6.3%	0.0%	0.0%
Terminal rate	1/40 (3%)	2/40 (5%)	0/35 (0%)	0/28 (0%)
First incidence (days)	729 (T)	721	–[e]	–
Poly-3 test	P = 0.166N	P = 0.315	P = 0.508N	P = 0.520N
Harderian Gland: Adenoma or Adenocarcinoma				
Overall rate	6/50 (12%)	7/50 (14%)	4/50 (8%)	8/50 (16%)
Adjusted rate	12.9%	14.8%	8.9%	18.9%
Terminal rate	4/40 (10%)	6/40 (15%)	3/35 (9%)	6/28 (21%)
First incidence (days)	640	721	631	643
Poly-3 test	P = 0.302	P = 0.512	P = 0.396N	P = 0.314
Small Intestine: Adenoma or Adenocarcinoma				
Overall rate	1/50 (2%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	2.2%	0.0%	2.3%	7.0%
Terminal rate	1/40 (3%)	0/40 (0%)	1/35 (3%)	2/28 (7%)
First incidence (days)	729 (T)	–	729 (T)	415
Poly-3 test	P = 0.084	P = 0.495N	P = 0.752	P = 0.277
Liver: Hepatocellular Adenoma				
Overall rate	25/50 (50%)	24/50 (48%)	31/50 (62%)	29/50 (58%)
Adjusted rate	53.0%	50.2%	68.1%	64.3%
Terminal rate	23/40 (58%)	21/40 (53%)	27/35 (77%)	18/28 (64%)
First incidence (days)	424	660	551	477
Poly-3 test	P = 0.079	P = 0.473N	P = 0.096	P = 0.181
Liver: Hepatocellular Carcinoma				
Overall rate	8/50 (16%)	19/50 (38%)	16/50 (32%)	35/50 (70%)
Adjusted rate	17.0%	38.6%	34.1%	75.3%
Terminal rate	6/40 (15%)	12/40 (30%)	8/35 (23%)	22/28 (79%)

	Chamber Control	100 ppm	200 ppm	400 ppm
First incidence (days)	488	605	523	548
Poly-3 test	P < 0.001	P = 0.015	P = 0.046	P < 0.001
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	30/50 (60%)	36/50 (72%)	40/50 (80%)	48/50 (96%)
Adjusted rate	62.6%	72.9%	84.3%	98.6%
Terminal rate	26/40 (65%)	27/40 (68%)	30/35 (86%)	28/28 (100%)
First incidence (days)	424	605	523	477
Poly-3 test	P < 0.001	P = 0.189	P = 0.011	P < 0.001
Liver: Hepatoblastoma				
Overall rate	1/50 (2%)	1/50 (2%)	1/50 (2%)	15/50 (30%)
Adjusted rate	2.1%	2.1%	2.3%	34.6%
Terminal rate	0/40 (0%)	1/40 (3%)	1/35 (3%)	8/28 (29%)
First incidence (days)	591	729 (T)	729 (T)	638
Poly-3 test	P < 0.001	P = 0.757N	P = 0.749	P < 0.001
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	9/50 (18%)	20/50 (40%)	16/50 (32%)	41/50 (82%)
Adjusted rate	19.0%	40.6%	34.1%	86.4%
Terminal rate	6/40 (15%)	13/40 (33%)	8/35 (23%)	24/28 (86%)
First incidence (days)	488	605	523	548
Poly-3 test	P < 0.001	P = 0.016	P = 0.074	P < 0.001
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	31/50 (62%)	37/50 (74%)	40/50 (80%)	48/50 (96%)
Adjusted rate	64.1%	74.9%	84.3%	98.6%
Terminal rate	26/40 (65%)	28/40 (70%)	30/35 (86%)	28/28 (100%)
First incidence (days)	424	605	523	477
Poly-3 test	P < 0.001	P = 0.169	P = 0.017	P < 0.001
Liver: Hepatocholangiocarcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	0/50 (0%)	0/50 (0%)
Adjusted rate	6.3%	4.2%	0.0%	0.0%
Terminal rate	0/40 (0%)	1/40 (3%)	0/35 (0%)	0/28 (0%)
First incidence (days)	591	531	–	–
Poly-3 test	P = 0.044N	P = 0.495N	P = 0.131N	P = 0.143N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	11/50 (22%)	11/50 (22%)	9/50 (18%)	3/50 (6%)
Adjusted rate	23.8%	23.2%	20.1%	7.2%

	Chamber Control	100 ppm	200 ppm	400 ppm
Terminal rate	10/40 (25%)	11/40 (28%)	8/35 (23%)	3/28 (11%)
First incidence (days)	723	729 (T)	647	729 (T)
Poly-3 test	P = 0.023N	P = 0.570N	P = 0.433N	P = 0.031N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	6/50 (12%)	4/50 (8%)	3/50 (6%)	3/50 (6%)
Adjusted rate	13.0%	8.4%	6.8%	7.1%
Terminal rate	6/40 (15%)	3/40 (8%)	3/35 (9%)	2/28 (7%)
First incidence (days)	729 (T)	698	729 (T)	632
Poly-3 test	P = 0.229N	P = 0.352N	P = 0.262N	P = 0.289N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	17/50 (34%)	14/50 (28%)	11/50 (22%)	6/50 (12%)
Adjusted rate	36.8%	29.5%	24.6%	14.2%
Terminal rate	16/40 (40%)	13/40 (33%)	10/35 (29%)	5/28 (18%)
First incidence (days)	723	698	647	632
Poly-3 test	P = 0.009N	P = 0.297N	P = 0.150N	P = 0.013N
Spleen: Hemangiosarcoma				
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.2%	4.2%	6.7%	2.4%
Terminal rate	1/40 (3%)	2/40 (5%)	2/35 (6%)	1/28 (4%)
First incidence (days)	729 (T)	729 (T)	647	729 (T)
Poly-3 test	P = 0.564	P = 0.509	P = 0.294	P = 0.738
All Organs: Hemangiosarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	5/50 (10%)	2/50 (4%)
Adjusted rate	4.3%	6.3%	11.2%	4.8%
Terminal rate	1/40 (3%)	3/40 (8%)	4/35 (11%)	2/28 (7%)
First incidence (days)	424	729 (T)	647	729 (T)
Poly-3 test	P = 0.490	P = 0.504	P = 0.196	P = 0.651
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	6/50 (12%)	2/50 (4%)
Adjusted rate	4.3%	6.3%	13.4%	4.8%
Terminal rate	1/40 (3%)	3/40 (8%)	5/35 (14%)	2/28 (7%)
First incidence (days)	424	729 (T)	647	729 (T)
Poly-3 test	P = 0.460	P = 0.504	P = 0.117	P = 0.651
All Organs: Malignant Lymphoma				
Overall rate	6/50 (12%)	1/50 (2%)	5/50 (10%)	3/50 (6%)

	Chamber Control	100 ppm	200 ppm	400 ppm
Adjusted rate	12.7%	2.1%	11.1%	7.0%
Terminal rate	3/40 (8%)	0/40 (0%)	3/35 (9%)	0/28 (0%)
First incidence (days)	612	691	551	606
Poly-3 test	P = 0.396N	P = 0.055N	P = 0.533N	P = 0.293N
All Organs: Benign Neoplasms				
Overall rate	32/50 (64%)	28/50 (56%)	35/50 (70%)	34/50 (68%)
Adjusted rate	67.4%	58.6%	76.3%	74.8%
Terminal rate	28/40 (70%)	25/40 (63%)	29/35 (83%)	22/28 (79%)
First incidence (days)	424	660	551	477
Poly-3 test	P = 0.113	P = 0.246N	P = 0.227	P = 0.282
All Organs: Malignant Neoplasms				
Overall rate	25/50 (50%)	32/50 (64%)	27/50 (54%)	44/50 (88%)
Adjusted rate	50.0%	64.0%	56.0%	90.2%
Terminal rate	15/40 (38%)	22/40 (55%)	16/35 (46%)	24/28 (86%)
First incidence (days)	424	531	444	415
Poly-3 test	P < 0.001	P = 0.113	P = 0.348	P < 0.001
All Organs: Benign or Malignant Neoplasms				
Overall rate	44/50 (88%)	47/50 (94%)	46/50 (92%)	49/50 (98%)
Adjusted rate	88.0%	94.0%	94.6%	99.0%
Terminal rate	34/40 (85%)	37/40 (93%)	33/35 (94%)	28/28 (100%)
First incidence (days)	424	531	444	415
Poly-3 test	P = 0.021	P = 0.243	P = 0.207	P = 0.030

(T) Terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and spleen; 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 chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by N.
[e]Not applicable; no neoplasms in animal group.

Table C-3. Historical Incidence of Liver Neoplasms in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Inhalation Studies				
Antimony trioxide (October 2008)	30/50	15/50	2/50	39/50
2,3-Butanedione (August 2009)	17/50	17/50	1/50	32/50
p-Chloro-α,α,α-trifluorotoluene (January 2011)	25/50	8/50	1/50	31/50
CIMSTAR 3800 (May 2008)	24/50	11/50	0/50	32/50
Cobalt metal (May 2006)	28/50	25/50	2/50	39/50
TRIM VX (August 2009)	23/50	21/50	0/50	34/50
Total (%)	147/300 (49.0%)	97/300 (32.3%)	6/300 (2.0%)	207/300 (69.0%)
Mean ± standard deviation	49.0% ± 9.0%	32.3% ± 12.6%	2.0% ± 1.8%	69.0% ± 7.2%
Range	34%–60%	16%–50%	0%–4%	62%–78%
Overall Historical Incidence: All Routes				
Total (%)	302/550 (54.9%)	165/550 (30.0%)	18/550 (3.3%)	395/550 (71.8%)
Mean ± standard deviation	54.9% ± 12.4%	30.0% ± 10.0%	3.3% ± 2.4%	71.8% ± 8.1%
Range	34%–78%	16%–50%	0%–8%	62%–84%

[a]Data as of August 2016.

Table C-4. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	100 ppm	200 ppm	400 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	8	5	11	12
Natural deaths	2	5	4	10
Survivors				
Terminal kill	40	40	35	28
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(50)	(49)	(44)	(44)
Inflammation	–	1 (2%)	–	–
Epithelium, vacuolization cytoplasmic	–	1 (2%)	1 (2%)	–
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation	–	2 (4%)	–	–
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(49)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Inflammation	–	–	–	1 (2%)
Necrosis	–	–	1 (2%)	–
Thrombosis	–	–	1 (2%)	1 (2%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Perforation	1 (2%)	–	–	–
Peyer's patch, hyperplasia	–	–	1 (2%)	1 (2%)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Perforation	1 (2%)	–	–	–
Peyer's patch, hyperplasia	1 (2%)	–	–	1 (2%)
Liver	(50)	(50)	(50)	(50)
Basophilic focus	4 (8%)	10 (20%)	10 (20%)	1 (2%)
Clear cell focus	15 (30%)	16 (32%)	23 (46%)	15 (30%)
Cyst	–	1 (2%)	–	–
Eosinophilic focus	11 (22%)	14 (28%)	18 (36%)	21 (42%)
Fatty change	–	–	1 (2%)	–
Fibrosis	–	–	1 (2%)	–

	Chamber Control	100 ppm	200 ppm	400 ppm
Hepatodiaphragmatic nodule	–	–	1 (2%)	–
Inflammation	3 (6%)	1 (2%)	–	–
Intrahepatocellular erythrocytes	–	1 (2%)	6 (12%)	15 (30%)
Mixed cell focus	15 (30%)	15 (30%)	13 (26%)	11 (22%)
Pigmentation	–	1 (2%)	–	–
Regeneration	2 (4%)	–	–	–
Centrilobular, hepatocyte, hypertrophy	–	8 (16%)	19 (38%)	49 (98%)
Hepatocyte, multinucleated	2 (4%)	8 (16%)	19 (38%)	49 (98%)
Hepatocyte, necrosis	3 (6%)	4 (8%)	3 (6%)	15 (30%)
Mesentery	(6)	(5)	(2)	(5)
Angiectasis	–	1 (20%)	–	–
Thrombosis	–	1 (20%)	–	–
Fat, necrosis	4 (67%)	2 (40%)	1 (50%)	4 (80%)
Pancreas	(50)	(50)	(50)	(50)
Inflammation	2 (4%)	–	–	–
Acinus, atrophy	1 (2%)	–	2 (4%)	3 (6%)
Acinus, hypertrophy	–	1 (2%)	–	–
Duct, cyst	–	–	1 (2%)	–
Salivary glands	(50)	(50)	(50)	(49)
Atrophy	–	1 (2%)	–	–
Inflammation	–	1 (2%)	–	–
Stomach, forestomach	(50)	(50)	(50)	(48)
Inflammation	4 (8%)	12 (24%)	7 (14%)	12 (25%)
Mineralization	2 (4%)	–	1 (2%)	1 (2%)
Epithelium, cyst, squamous	–	–	–	1 (2%)
Epithelium, hyperplasia	5 (10%)	9 (18%)	9 (18%)	10 (21%)
Epithelium, ulcer	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Stomach, glandular	(50)	(50)	(49)	(49)
Hyperplasia	–	–	–	1 (2%)
Inflammation	1 (2%)		1 (2%)	2 (4%)
Mineralization	–	1 (2%)	–	2 (4%)
Tongue	(1)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation	–	–	2 (4%)	–

	Chamber Control	100 ppm	200 ppm	400 ppm
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	10 (20%)	14 (28%)	13 (26%)	11 (22%)
Inflammation	1 (2%)	1 (2%)	3 (6%)	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	–	–	1 (2%)	–
Hyperplasia	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Hypertrophy	20 (40%)	34 (68%)	24 (48%)	17 (34%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)	–	1 (2%)
Islets, pancreatic	(50)	(49)	(50)	(50)
Hyperplasia	4 (8%)	1 (2%)	5 (10%)	–
Necrosis	1 (2%)	–	–	–
Parathyroid gland	(36)	(28)	(39)	(38)
Pituitary gland	(50)	(50)	(50)	(49)
Thyroid gland	(50)	(50)	(50)	(50)
General Body System				
Peritoneum	(0)	(0)	(1)	(0)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Exfoliated germ cell	1 (2%)	4 (8%)	–	2 (4%)
Granuloma sperm	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Penis	(0)	(0)	(1)	(0)
Congestion	–	–	1 (100%)	–
Preputial gland	(50)	(50)	(49)	(50)
Ectasia	2 (4%)	5 (10%)	6 (12%)	4 (8%)
Inflammation	2 (4%)	1 (2%)	2 (4%)	–
Prostate	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	–	1 (2%)	1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation	–	1 (2%)	2 (4%)	–
Inflammation	–	–	2 (4%)	1 (2%)
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, degeneration	7 (14%)	10 (20%)	10 (20%)	8 (16%)

	Chamber Control	100 ppm	200 ppm	400 ppm
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy	–	1 (2%)	1 (2%)	–
Fibrosis	–	–	1 (2%)	–
Hyperplasia	24 (48%)	26 (52%)	27 (54%)	27 (54%)
Necrosis	–	–	–	1 (2%)
Lymph node	(4)	(0)	(3)	(4)
Pancreatic, angiectasis	–	–	–	1 (25%)
Lymph node, bronchial	(49)	(47)	(47)	(48)
Ectasia	–	–	–	1 (2%)
Hyperplasia, lymphoid	1 (2%)	–	1 (2%)	–
Lymph node, mandibular	(36)	(41)	(43)	(45)
Hyperplasia, lymphoid	2 (6%)	1 (2%)	–	–
Lymph node, mediastinal	(46)	(47)	(44)	(42)
Necrosis, fatty	–	1 (2%)	–	–
Lymph node, mesenteric	(49)	(50)	(49)	(49)
Angiectasis	1 (2%)	–	–	1 (2%)
Ectasia	1 (2%)	–	–	–
Hematopoietic cell proliferation	–	2 (4%)	1 (2%)	–
Hyperplasia, lymphoid	3 (6%)	2 (4%)	–	1 (2%)
Infiltration cellular, histiocyte	–	–	2 (4%)	–
Inflammation	–	–	–	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Angiectasis	–	–	1 (2%)	–
Hematopoietic cell proliferation	17 (34%)	16 (32%)	10 (20%)	18 (36%)
Lymphoid follicle, hyperplasia	8 (16%)	4 (8%)	6 (12%)	4 (8%)
Red pulp, infiltration cellular, mast cell	–	–	1 (2%)	–
Thymus	(48)	(48)	(48)	(48)
Atrophy	21 (44%)	20 (42%)	15 (31%)	22 (46%)
Ectopic parathyroid gland	12 (25%)	9 (19%)	15 (31%)	9 (19%)
Integumentary System				
Mammary gland	(2)	(1)	(3)	(1)
Skin	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Ulcer	–	–	1 (2%)	3 (6%)

	Chamber Control	100 ppm	200 ppm	400 ppm
Epidermis, hyperplasia	1 (2%)	–	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	2 (4%)	–	2 (4%)	2 (4%)
Skeletal muscle	(2)	(1)	(1)	(2)
Degeneration	–	–	1 (100%)	–
Inflammation	–	–	1 (100%)	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Degeneration	–	–	2 (4%)	–
Gliosis	–	–	1 (2%)	–
Inflammation	1 (2%)	–	2 (4%)	–
Peripheral nerve	(0)	(0)	(1)	(0)
Degeneration	–	–	1 (100%)	–
Spinal cord	(0)	(0)	(1)	(0)
Inflammation	–	–	1 (100%)	–
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Epiglottis, inflammation	–	–	1 (2%)	–
Squamous epithelium, hyperplasia	3 (6%)	4 (8%)	6 (12%)	11 (22%)
Squamous epithelium, inflammation	–	1 (2%)	3 (6%)	2 (4%)
Squamous epithelium, necrosis	–	–	–	1 (2%)
Squamous epithelium, ulcer	–	–	1 (2%)	2 (4%)
Lung	(50)	(50)	(50)	(50)
Hemorrhage	2 (4%)	–	1 (2%)	–
Hyperplasia, lymphoid	–	–	1 (2%)	–
Inflammation	–	2 (4%)	–	2 (4%)
Metaplasia, osseous	–	–	1 (2%)	–
Pigmentation, hemosiderin	1 (2%)	–	–	–
Thrombosis	–	2 (4%)	1 (2%)	1 (2%)
Alveolar/bronchiolar epithelium, hyperplasia	–	49 (98%)	50 (100%)	48 (96%)
Alveolar epithelium, hyperplasia	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Alveolus, infiltration cellular, histiocyte	2 (4%)	2 (4%)	3 (6%)	3 (6%)
Bronchiole, epithelium, hyperplasia	2 (4%)	–	–	–
Peribronchiolar, fibrosis	–	45 (90%)	47 (94%)	44 (88%)

	Chamber Control	100 ppm	200 ppm	400 ppm
Pleura, inflammation	1 (2%)	–	–	–
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative	1 (2%)	3 (6%)	5 (10%)	4 (8%)
Necrosis	–	–	–	1 (2%)
Polyp, inflammatory	–	–	1 (2%)	–
Olfactory epithelium, accumulation, hyaline droplet	11 (22%)	9 (18%)	7 (14%)	13 (26%)
Olfactory epithelium, atrophy	–	–	1 (2%)	–
Olfactory epithelium, hyperplasia	–	–	1 (2%)	–
Olfactory epithelium, mineralization	–	1 (2%)	–	–
Respiratory epithelium, accumulation, hyaline droplet	9 (18%)	8 (16%)	3 (6%)	12 (24%)
Respiratory epithelium, metaplasia, squamous	–	1 (2%)	2 (4%)	1 (2%)
Turbinate, atrophy	–	–	–	1 (2%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Atrophy	–	1 (2%)	–	2 (4%)
Cataract	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Inflammation	–	3 (6%)	1 (2%)	1 (2%)
Cornea, hyperplasia	2 (4%)	–	–	–
Retina, pigmentation, melanin	–	–	–	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	5 (10%)	1 (2%)	1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	–	1 (2%)	1 (2%)	2 (4%)
Infarct	6 (12%)	4 (8%)	6 (12%)	4 (8%)
Metaplasia, osseous	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Nephropathy	43 (86%)	41 (82%)	38 (76%)	41 (82%)
Pigmentation	–	1 (2%)	–	4 (8%)
Pelvis, dilatation	–	–	–	1 (2%)
Urethra	(0)	(0)	(0)	(1)
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation	–	–	–	1 (2%)

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

Appendix D. Summary of Lesions in Female Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

Tables

Table D-1. Summary of the Incidence of Neoplasms in Female Mice in the Two-year Inhalation Study of p-Chloro-α,α,α-trifluorotoluene D-2
Table D-2. Statistical Analysis of Primary Neoplasms in Female Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene D-7
Table D-3. Historical Incidence of Liver Neoplasms in Control Female B6C3F1/N Mice D-11
Table D-4. Historical Incidence of Harderian Gland Neoplasms in Control Female B6C3F1/N Mice D-12
Table D-5. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene D-13

Table D-1. Summary of the Incidence of Neoplasms in Female Mice in the Two-year Inhalation Study of p-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	100 ppm	200 ppm	400 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	–	1	–
Moribund	7	12	9	16
Natural deaths	5	5	1	7
Survivors				
Died last week of study	–	–	2	1
Terminal kill	38	33	37	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(47)	(49)	(48)	(47)
Intestine large, cecum	(50)	(50)	(50)	(50)
Adenoma	–	–	1 (2%)	–
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(48)	(50)	(48)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	–	–	–
Intestine small, jejunum	(50)	(50)	(50)	(50)
Adenoma	–	–	–	1 (2%)
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	–	–	–	1 (2%)
Hepatoblastoma	–	–	1 (2%)	8 (16%)
Hepatocellular adenoma	9 (18%)	9 (18%)	9 (18%)	9 (18%)
Hepatocellular adenoma, multiple	3 (6%)	5 (10%)	15 (30%)	25 (50%)
Hepatocellular carcinoma	5 (10%)	5 (10%)	5 (10%)	6 (12%)
Hepatocellular carcinoma, multiple	2 (4%)	3 (6%)	7 (14%)	28 (56%)
Osteosarcoma, metastatic, uncertain primary site	–	–	–	1 (2%)
Mesentery	(9)	(13)	(15)	(12)
Hemangiosarcoma	1 (11%)	2 (15%)	–	–
Rhabdomyosarcoma, metastatic, skeletal muscle	1 (11%)	–	–	–
Pancreas	(50)	(50)	(50)	(50)

	Chamber Control	100 ppm	200 ppm	400 ppm
Salivary glands	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)	–	–	–
Rhabdomyosarcoma	–	–	1 (2%)	–
Sarcoma, metastatic, skin	1 (2%)	–	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Hemangioma	–	–	–	1 (2%)
Squamous cell papilloma	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Stomach, glandular	(50)	(50)	(50)	(50)
Adenoma	–	–	1 (2%)	–
Tongue	(0)	(0)	(0)	(1)
Squamous cell papilloma	–	–	–	1 (100%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver	–	–	–	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	1 (2%)	1 (2%)	–	–
Islets, pancreatic	(50)	(50)	(49)	(50)
Adenoma	–	1 (2%)	–	–
Parathyroid gland	(36)	(29)	(30)	(34)
Pituitary gland	(50)	(50)	(50)	(50)
Parts distalis, adenoma	4 (8%)	4 (8%)	–	3 (6%)
Pars intermedia, adenoma	–	1 (2%)	1 (2%)	1 (2%)
Thyroid gland	(50)	(50)	(49)	(49)
Follicular cell, adenoma	–	1 (2%)	–	1 (2%)
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(48)	(46)	(49)	(48)
Ovary	(50)	(50)	(50)	(50)
Cystadenoma	2 (4%)	1 (2%)	2 (4%)	–
Granulosa cell tumor benign	–	–	–	1 (2%)
Granulosa-theca tumor malignant	1 (2%)	–	–	–

	Chamber Control	100 ppm	200 ppm	400 ppm
Hemangioma	–	1 (2%)	–	–
Hemangiosarcoma	–	–	–	1 (2%)
Luteoma	2 (4%)	–	1 (2%)	
Teratoma benign	–	1 (2%)	–	–
Tubulostromal adenoma	–	–	1 (2%)	1 (2%)
Oviduct	(1)	(0)	(0)	(0)
Uterus	(50)	(50)	(50)	(50)
Adenocarcinoma	–	–	–	1 (2%)
Hemangiosarcoma	1 (2%)	–	–	1 (2%)
Polyp stromal	–	–	1 (2%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	–	1 (2%)	–	–
Lymph node	(2)	(3)	(4)	(7)
Lymph node, bronchial	(48)	(48)	(50)	(49)
Alveolar/bronchioles carcinoma, metastatic, lung	–	–	–	1 (2%)
Hepatocellular carcinoma, metastatic, liver	–	–	–	1 (2%)
Lymph node, mandibular	(47)	(42)	(49)	(46)
Sarcoma, metastatic, skin	1 (2%)	–	–	–
Lymph node, mediastinal	(48)	(49)	(49)	(47)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (2%)
Lymph node, mesenteric	(50)	(49)	(49)	(50)
Hemangiosarcoma	–	–	–	1 (2%)
Hepatocellular carcinoma, metastatic, liver	–	1 (2%)	–	–
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	–	–	1 (2%)
Thymus	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (2%)
Sarcoma, metastatic, bone	–	–	–	1 (2%)
Integumentary System				
Mammary gland	(49)	(50)	(50)	(50)
Adenocarcinoma	–	1 (2%)	1 (2%)	–
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibrosarcoma	–	–	–	2 (4%)
Subcutaneous tissue, hemangiosarcoma	–	–	1 (2%)	–

	Chamber Control	100 ppm	200 ppm	400 ppm
Subcutaneous tissue, rhabdomyosarcoma	–	1 (2%)	–	–
Subcutaneous tissue, sarcoma, multiple	1 (2%)	–	–	–
Subcutaneous tissue, schwannoma malignant	–	2 (4%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hemangiosarcoma	–	–	–	1 (2%)
Rib, sarcoma	–	–	–	1 (2%)
Vertebra, osteosarcoma	–	1 (2%)	–	1 (2%)
Skeletal muscle	(4)	(3)	(1)	(3)
Hemangiosarcoma	–	1 (33%)	–	–
Rhabdomyosarcoma	1 (25%)	–	–	–
Rhabdomyosarcoma, multiple	1 (25%)	–	–	–
Sarcoma, metastatic, bone	–	–	–	1 (33%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Plasma cell tumor malignant	–	1 (2%)	–	–
Peripheral nerve	(1)	(2)	(1)	(2)
Spinal cord	(1)	(2)	(1)	(2)
Respiratory System				
Larynx	(50)	(50)	(49)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	4 (8%)	5 (10%)	6 (12%)
Alveolar/bronchiolar carcinoma	–	–	2 (4%)	2 (4%)
Alveolar/bronchiolar carcinoma, multiple	–	1 (2%)	–	1 (2%)
Fibrosarcoma, metastatic, skin	–	–	–	1 (2%)
Hemangiosarcoma	–	1 (2%)	–	–
Hepatoblastoma, metastatic, liver	–	–	–	1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Osteosarcoma, metastatic, bone	–	1 (2%)	–	–
Osteosarcoma, metastatic, uncertain primary site	–	–	–	1 (2%)
Mediastinum, sarcoma, metastatic, bone	–	–	–	1 (2%)
Nose	(50)	(50)	(50)	(50)
Pleura	(0)	(0)	(0)	(2)
Trachea	(50)	(50)	(50)	(49)

	Chamber Control	100 ppm	200 ppm	400 ppm
Special Senses System				
Ear	(1)	(0)	(0)	(0)
Eye	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, Harderian gland	–	–	1 (2%)	–
Harderian gland	(50)	(50)	(50)	(50)
Adenocarcinoma	–	–	3 (6%)	–
Adenoma	2 (4%)	6 (12%)	6 (12%)	8 (16%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hemangiosarcoma	–	1 (2%)	–	–
Renal tubule, adenoma	1 (2%)	–	–	–
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Lymphoma malignant	9 (18%)	6 (12%)	11 (22%)	3 (6%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	38	37	41	49
Total primary neoplasms	57	65	77	124
Total animals with benign neoplasms	24	28	31	40
Total benign neoplasms	31	37	44	62
Total animals with malignant neoplasms	24	22	26	42
Total malignant neoplasms	26	28	33	62
Total animals with metastatic neoplasms	3	4	2	10
Total metastatic neoplasms	4	4	2	15
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 D-2. Statistical Analysis of Primary Neoplasms in Female Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	200 ppm	400 ppm
Harderian Gland: Adenoma				
Overall rate[a]	2/50 (4%)	6/50 (12%)	6/50 (12%)	8/50 (16%)
Adjusted rate[b]	4.4%	13.6%	13.5%	18.1%
Terminal rate[c]	2/38 (5%)	5/33 (15%)	5/37 (14%)	4/27 (15%)
First incidence (days)	731 (T)	576	562	642
Poly-3 test[d]	P = 0.049	P = 0.124	P = 0.129	P = 0.041
Harderian Gland: Adenocarcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	6.6%	0.0%
Terminal rate	0/38 (0%)	0/33 (0%)	1/37 (3%)	0/27 (0%)
First incidence (days)	–[e]	–	480	–
Poly-3 test	P = 0.515	–[f]	P = 0.119	–
Harderian Gland: Adenoma or Adenocarcinoma				
Overall rate	2/50 (4%)	6/50 (12%)	9/50 (18%)	8/50 (16%)
Adjusted rate	4.4%	13.6%	19.7%	18.1%
Terminal rate	2/38 (5%)	5/33 (15%)	6/37 (16%)	4/27 (15%)
First incidence (days)	731 (T)	576	480	642
Poly-3 test	P = 0.046	P = 0.124	P = 0.026	P = 0.041
Liver: Hepatocellular Adenoma				
Overall rate	12/50 (24%)	14/50 (28%)	24/50 (48%)	34/50 (68%)
Adjusted rate	26.1%	31.9%	54.5%	72.6%
Terminal rate	9/38 (24%)	11/33 (33%)	24/37 (65%)	18/27 (67%)
First incidence (days)	544	685	731 (T)	530
Poly-3 test	P < 0.001	P = 0.355	P = 0.004	P < 0.001
Liver: Hepatocellular Carcinoma				
Overall rate	7/50 (14%)	8/50 (16%)	12/50 (24%)	34/50 (68%)
Adjusted rate	15.3%	18.1%	26.9%	75.9%
Terminal rate	4/38 (11%)	5/33 (15%)	9/37 (24%)	23/27 (85%)
First incidence (days)	664	578	576	642
Poly-3 test	P < 0.001	P = 0.472	P = 0.136	P < 0.001
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	18/50 (36%)	18/50 (36%)	29/50 (58%)	46/50 (92%)
Adjusted rate	38.8%	40.4%	65.0%	97.6%
Terminal rate	13/38 (34%)	13/33 (39%)	26/37 (70%)	27/27 (100%)

	Chamber Control	100 ppm	200 ppm	400 ppm
First incidence (days)	544	578	576	530
Poly-3 test	P < 0.001	P = 0.523	P = 0.008	P < 0.001
Liver: Hepatoblastoma				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	8/50 (16%)
Adjusted rate	0.0%	0.0%	2.3%	18.3%
Terminal rate	0/38 (0%)	0/33 (0%)	1/37 (3%)	4/27 (15%)
First incidence (days)	–	–	731 (T)	673
Poly-3 test	P < 0.001	–	P = 0.495	P = 0.003
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	7/50 (14%)	8/50 (16%)	12/50 (24%)	36/50 (72%)
Adjusted rate	15.3%	18.1%	26.9%	79.8%
Terminal rate	4/38 (11%)	5/33 (15%)	9/37 (24%)	23/27 (85%)
First incidence (days)	664	578	576	642
Poly-3 test	P < 0.001	P = 0.472	P = 0.136	P < 0.001
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	18/50 (36%)	18/50 (36%)	29/50 (58%)	46/50 (92%)
Adjusted rate	38.8%	40.4%	65.0%	97.6%
Terminal rate	13/38 (34%)	13/33 (39%)	26/37 (70%)	27/27 (100%)
First incidence (days)	544	578	576	530
Poly-3 test	P < 0.001	P = 0.523	P = 0.008	P < 0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	5/50 (10%)	4/50 (8%)	5/50 (10%)	6/50 (12%)
Adjusted rate	11.1%	9.0%	11.2%	13.7%
Terminal rate	5/38 (13%)	3/33 (9%)	3/37 (8%)	3/27 (11%)
First incidence (days)	731 (T)	224	562	628
Poly-3 test	P = 0.360	P = 0.509N	P = 0.624	P = 0.482
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	0/50 (0%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	0.0%	2.3%	4.5%	6.8%
Terminal rate	0/38 (0%)	1/33 (3%)	2/37 (5%)	1/27 (4%)
First incidence (days)	–	731 (T)	731 (T)	570
Poly-3 test	P = 0.059	P = 0.493	P = 0.232	P = 0.114
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	5/50 (10%)	5/50 (10%)	7/50 (14%)	9/50 (18%)
Adjusted rate	11.1%	11.2%	15.7%	20.1%

	Chamber Control	100 ppm	200 ppm	400 ppm
Terminal rate	5/38 (13%)	4/33 (12%)	5/37 (14%)	4/27 (15%)
First incidence (days)	731 (T)	224	562	570
Poly-3 test	P = 0.110	P = 0.622	P = 0.371	P = 0.187
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	4/50 (8%)	4/50 (8%)	0/50 (0%)	3/50 (6%)
Adjusted rate	8.8%	9.1%	0.0%	6.8%
Terminal rate	3/38 (8%)	3/33 (9%)	0/37 (0%)	1/27 (4%)
First incidence (days)	664	564	–	530
Poly-3 test	P = 0.326N	P = 0.627	P = 0.064N	P = 0.518N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	1/50 (2%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	2.2%	4.6%	2.3%	7.0%
Terminal rate	1/38 (3%)	1/33 (3%)	1/37 (3%)	3/27 (11%)
First incidence (days)	731 (T)	703	731 (T)	731 (T)
Poly-3 test	P = 0.226	P = 0.488	P = 0.755	P = 0.290
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	4/50 (8%)
Adjusted rate	6.6%	4.6%	2.3%	9.1%
Terminal rate	2/38 (5%)	1/33 (3%)	1/37 (3%)	1/28 (4%)
First incidence (days)	676	597	731 (T)	480
Poly-3 test	P = 0.365	P = 0.514N	P = 0.315N	P = 0.485
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	3/50 (6%)	3/50 (6%)	1/50 (2%)	5/50 (10%)
Adjusted rate	6.6%	6.8%	2.3%	11.3%
Terminal rate	2/38 (5%)	2/33 (6%)	1/37 (3%)	2/27 (7%)
First incidence (days)	676	597	731 (T)	480
Poly-3 test	P = 0.265	P = 0.648	P = 0.315N	P = 0.341
All Organs: Histiocytic Sarcoma				
Overall rate	2/50 (4%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	4.3%	2.3%	2.2%	6.9%
Terminal rate	0/38 (0%)	0/33 (0%)	0/37 (0%)	2/27 (7%)
First incidence (days)	493	488	562	673
Poly-3 test	P = 0.311	P = 0.516N	P = 0.513N	P = 0.470
All Organs: Malignant Lymphoma				
Overall rate	9/50 (18%)	6/50 (12%)	11/50 (22%)	3/50 (6%)

	Chamber Control	**100 ppm**	**200 ppm**	**400 ppm**
Adjusted rate	19.6%	13.5%	24.8%	6.9%
Terminal rate	7/38 (18%)	3/33 (9%)	8/37 (22%)	2/27 (7%)
First incidence (days)	502	576	641	570
Poly-3 test	P = 0.107N	P = 0.312N	P = 0.366	P = 0.071N
All Organs: Benign Neoplasms				
Overall rate	24/50 (48%)	28/50 (56%)	31/50 (62%)	40/50 (80%)
Adjusted rate	51.5%	60.2%	69.5%	85.0%
Terminal rate	19/38 (50%)	21/33 (64%)	29/37 (78%)	23/27 (85%)
First incidence (days)	544	224	562	530
Poly-3 test	P < 0.001	P = 0.259	P = 0.056	P < 0.001
All Organs: Malignant Neoplasms				
Overall rate	24/50 (48%)	22/50 (44%)	26/50 (52%)	42/50 (84%)
Adjusted rate	49.1%	46.5%	55.7%	87.9%
Terminal rate	14/38 (37%)	10/33 (30%)	18/37 (49%)	24/27 (89%)
First incidence (days)	493	488	480	374
Poly-3 test	P < 0.001	P = 0.480N	P = 0.328	P < 0.001
All Organs: Benign or Malignant Neoplasms				
Overall rate	38/50 (76%)	37/50 (74%)	41/50 (82%)	49/50 (98%)
Adjusted rate	77.1%	74.9%	87.9%	99.5%
Terminal rate	27/38 (71%)	22/33 (67%)	33/37 (89%)	27/27 (100%)
First incidence (days)	493	224	480	374
Poly-3 test	P < 0.001	P = 0.489N	P = 0.126	P < 0.001

(T) Terminal kill.
[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 chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by N.
[e]Not applicable; no neoplasms in animal group.
[f]Value of statistic cannot be computed.

Table D-3. Historical Incidence of Liver Neoplasms in Control Female B6C3F1/N Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Inhalation Studies				
Antimony trioxide (October 2008)	11/50	6/50	0/50	14/50
2,3-Butanedione (August 2009)	6/50	5/50	1/50	12/50
p-Chloro-α,α,α-trifluorotoluene (January 2011)	12/50	7/50	0/50	18/50
CIMSTAR 3800 (May 2008)	14/50	10/50	1/50	20/50
Cobalt metal (May 2006)	19/50	10/50	0/50	25/50
TRIM VX (August 2009)	9/50	7/50	1/50	15/50
Total (%)	71/300 (23.7%)	45/300 (15.0%)	3/300 (1.0%)	104/300 (34.7%)
Mean ± standard deviation	23.7% ± 8.9%	15.0% ± 4.2%	1.0% ± 1.1%	34.7% ± 9.4%
Range	12%–38%	10%–20%	0%–2%	24%–50%
Overall Historical Incidence: All Routes				
Total (%)	141/549 (25.7%)	71/549 (12.9%)	3/549 (0.6%)	189/549 (34.4%)
Mean ± standard deviation	25.8% ± 15.9%	12.9% ± 5.1%	0.6% ± 0.9%	34.5% ± 15.8%
Range	10%–67%	4%–20%	0%–2%	16%–73%

[a]Data as of August 2016.

Table D-4. Historical Incidence of Harderian Gland Neoplasms in Control Female B6C3F1/N Mice[a]

Study (Study Start)	Adenoma	Adenocarcinoma	Adenoma or Adenocarcinoma
Historical Incidence: Inhalation Studies			
Antimony trioxide (October 2008)	3/50	4/50	7/50
2,3-Butanedione (August 2009)	3/50	1/50	4/50
p-Chloro-α,α,α-trifluorotoluene (January 2011)	2/50	0/50	2/50
CIMSTAR 3800 (May 2008)	2/50	0/50	2/50
Cobalt metal (May 2006)	3/50	0/50	3/50
TRIM VX (August 2009)	1/50	3/50	4/50
Total (%)	14/300 (4.7%)	8/300 (2.7%)	22/300 (7.3%)
Mean ± standard deviation	4.7% ± 1.6%	2.7% ± 3.5%	7.3% ± 3.7%
Range	2%–6%	0%–8%	4%–14%
Overall Historical Incidence: All Routes			
Total (%)	35/550 (6.4%)	12/550 (2.2%)	47/550 (8.6%)
Mean ± standard deviation	6.4% ± 4.4%	2.2% ± 2.8%	8.6% ± 4.7%
Range	2%–18%	0%–8%	4%–20%

[a]Data as of August 2016.

Table D-5. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

	Chamber Control	100 ppm	200 ppm	400 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	–	1	–
Moribund	7	12	9	16
Natural deaths	5	5	1	7
Survivors				
Died last week of study	–	–	2	1
Terminal kill	38	33	37	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(47)	(49)	(48)	(47)
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation	–	–	2 (4%)	1 (2%)
Epithelium, hyperplasia	1 (2%)	–	–	–
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(48)	(50)	(48)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Inflammation	–	–	–	1 (2%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Inflammation	–	–	1 (2%)	–
Liver	(50)	(50)	(50)	(50)
Angiectasis	–	1 (2%)	–	–
Basophilic focus	1 (2%)	4 (8%)	6 (12%)	1 (2%)
Clear cell focus	3 (6%)	3 (6%)	5 (10%)	5 (10%)
Cyst	–	–	1 (2%)	–
Eosinophilic focus	4 (8%)	8 (16%)	24 (48%)	31 (62%)
Fatty change	–	–	1 (2%)	–
Fibrosis	–	1 (2%)	1 (2%)	–
Inflammation	1 (2%)	–	2 (4%)	–
Mixed cell focus	10 (20%)	5 (10%)	6 (12%)	5 (10%)
Bile duct, hyperplasia	–	1 (2%)	–	–

	Chamber Control	100 ppm	200 ppm	400 ppm
Capsule, inflammation, suppurative	–	–	1 (2%)	–
Centrilobular, hepatocyte, hypertrophy	–	4 (8%)	5 (10%)	40 (80%)
Hepatocyte, multinucleated	–	2 (4%)	2 (4%)	25 (50%)
Hepatocyte, necrosis	2 (4%)	1 (2%)	3 (6%)	10 (20%)
Mesentery	(9)	(13)	(15)	(12)
Fat, necrosis	8 (89%)	9 (69%)	15 (100%)	12 (100%)
Pancreas	(50)	(50)	(50)	(50)
Acinus, atrophy	1 (2%)	1 (2%)	4 (8%)	2 (4%)
Acinus, hypertrophy	–	2 (4%)	–	1 (2%)
Duct, cyst	–	–	2 (4%)	–
Salivary glands	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	–	–	–	1 (2%)
Duct, hyperplasia	–	1 (2%)	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Angiectasis	–	–	1 (2%)	–
Inflammation	3 (6%)	5 (10%)	5 (10%)	9 (18%)
Mineralization	–	–	1 (2%)	–
Epithelium, hyperplasia	1 (2%)	3 (6%)	5 (10%)	14 (28%)
Epithelium, ulcer	–	1 (2%)	–	2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Hyperplasia, mast cell	–	–	–	1 (2%)
Inflammation	–	1 (2%)	–	–
Tongue	(0)	(0)	(0)	(1)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	14 (28%)	13 (26%)	8 (16%)	12 (24%)
Inflammation	2 (4%)	3 (6%)	5 (10%)	5 (10%)
Necrosis	–	1 (2%)	1 (2%)	2 (4%)
Thrombosis	–	2 (4%)	–	2 (4%)
Endocardium, fibrosis	–	–	–	1 (2%)
Valve, angiectasis	–	–	–	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	–	–	1 (2%)	–

	Chamber Control	100 ppm	200 ppm	400 ppm
Cyst	–	–	1 (2%)	–
Degeneration	2 (4%)	–	–	1 (2%)
Hyperplasia	1 (2%)	1 (2%)	–	1 (2%)
Hypertrophy	12 (24%)	12 (24%)	10 (20%)	16 (32%)
Vacuolization cytoplasmic	2 (4%)	2 (4%)	2 (4%)	3 (6%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	6 (12%)	6 (12%)	8 (16%)	1 (2%)
Vacuolization cytoplasmic	–	1 (2%)	–	–
Islets, pancreatic	(50)	(50)	(49)	(50)
Hyperplasia	–	–	–	1 (2%)
Parathyroid gland	(36)	(29)	(30)	(34)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, angiectasis	–	2 (4%)	–	1 (2%)
Pars distalis, hyperplasia	12 (24%)	4 (8%)	5 (10%)	6 (12%)
Thyroid gland	(50)	(50)	(49)	(49)
Follicular cell, hyperplasia	–	2 (4%)	1 (2%)	–
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(48)	(46)	(49)	(48)
Ovary	(50)	(50)	(50)	(50)
Angiectasis	–	–	1 (2%)	–
Cyst	8 (16%)	8 (16%)	7 (14%)	4 (8%)
Hemorrhage	–	–	1 (2%)	–
Inflammation, chronic active	–	–	1 (2%)	–
Thrombosis	–	1 (2%)	–	–
Oviduct	(1)	(0)	(0)	(0)
Uterus	(50)	(50)	(50)	(50)
Angiectasis	–	2 (4%)	–	–
Hyperplasia, reticulum cell	–	–	–	1 (2%)
Inflammation, chronic active	–	–	1 (2%)	–
Neovascularization	–	–	1 (2%)	–
Endometrium, hyperplasia, cystic	47 (94%)	48 (96%)	46 (92%)	45 (90%)
Serosa, cyst	–	1 (2%)	–	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)

	Chamber Control	100 ppm	200 ppm	400 ppm
Atrophy	1 (2%)	1 (2%)	–	–
Hyperplasia	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Necrosis	1 (2%)	–	1 (2%)	–
Lymph node	(2)	(3)	(4)	(7)
Iliac, angiectasis	–	–	–	2 (29%)
Iliac, ectasia	–	1 (33%)	–	1 (14%)
Lumbar, ectasia	–	–	1 (25%)	–
Renal, angiectasis	–	1 (33%)	–	–
Renal, ectasia	–	–	–	2 (29%)
Lymph node, bronchial	(48)	(48)	(50)	(49)
Hyperplasia	–	–	1 (2%)	–
Hyperplasia, lymphoid	–	–	–	1 (2%)
Inflammation	–	–	–	1 (2%)
Lymph node, mandibular	(47)	(42)	(49)	(46)
Hyperplasia, lymphoid	2 (4%)	–	2 (4%)	2 (4%)
Lymph node, mediastinal	(48)	(49)	(49)	(47)
Angiectasis	–	–	1 (2%)	–
Hyperplasia, lymphoid	–	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular	1 (2%)	–	–	–
Pigmentation, hemosiderin	–	–	1 (2%)	–
Lymph node, mesenteric	(50)	(49)	(49)	(50)
Angiectasis	–	2 (4%)	1 (2%)	–
Ectasia	1 (2%)	–	–	–
Erythrophagocytosis	–	–	–	1 (2%)
Hyperplasia, lymphoid	3 (6%)	1 (2%)	2 (4%)	2 (4%)
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	14 (28%)	12 (24%)	13 (26%)	16 (32%)
Lymphoid follicle, hyperplasia	17 (34%)	18 (36%)	15 (30%)	19 (38%)
Red pulp, hyperplasia, mast cell	–	–	–	1 (2%)
Thymus	(50)	(50)	(50)	(50)
Atrophy	10 (20%)	9 (18%)	11 (22%)	13 (26%)
Ectopic parathyroid gland	20 (40%)	13 (26%)	13 (26%)	18 (36%)
Hyperplasia, lymphoid	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Integumentary System				
Mammary gland	(49)	(50)	(50)	(50)
Hyperplasia	5 (10%)	7 (14%)	2 (4%)	1 (2%)

	Chamber Control	100 ppm	200 ppm	400 ppm
Inflammation	1 (2%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Dysplasia	–	1 (2%)	–	–
Inflammation	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Ulcer	1 (2%)	–	–	–
Epidermis, hyperplasia	1 (2%)	–	–	–
Eyelid, sebaceous gland, hyperplasia	–	–	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	13 (26%)	15 (30%)	6 (12%)	10 (20%)
Vertebra, fracture	–	–	1 (2%)	–
Skeletal muscle	(4)	(3)	(1)	(3)
Degeneration	–	1 (33%)	–	1 (33%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	–	3 (6%)	–	–
Degeneration	–	1 (2%)	1 (2%)	1 (2%)
Gliosis	–	1 (2%)	–	–
Inflammation	–	1 (2%)	2 (4%)	–
Peripheral nerve	(1)	(2)	(1)	(2)
Degeneration	–	1 (50%)	1 (100%)	2 (100%)
Spinal cord	(1)	(2)	(1)	(2)
Respiratory System				
Larynx	(50)	(50)	(49)	(50)
Inflammation, suppurative	–	1 (2%)	–	–
Squamous epithelium, hyperplasia	9 (18%)	9 (18%)	5 (10%)	5 (10%)
Squamous epithelium, inflammation	1 (2%)	4 (8%)	1 (2%)	2 (4%)
Squamous epithelium, ulcer	–	2 (4%)	–	–
Lung	(50)	(50)	(50)	(50)
Hemorrhage	–	3 (6%)	–	1 (2%)
Hyperplasia, lymphoid	–	1 (2%)	1 (2%)	–
Inflammation	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Pigmentation	–	–	–	1 (2%)
Thrombosis	–	3 (6%)	2 (4%)	1 (2%)
Alveolar/bronchiolar epithelium, hyperplasia	–	49 (98%)	49 (98%)	50 (100%)
Alveolar epithelium, hyperplasia	–	2 (4%)	2 (4%)	–

	Chamber Control	100 ppm	200 ppm	400 ppm
Alveolus, infiltration cellular, histiocyte	–	3 (6%)	3 (6%)	2 (4%)
Peribronchiolar, fibrosis	–	44 (88%)	44 (88%)	48 (96%)
Nose	(50)	(50)	(50)	(50)
Hemorrhage	–	1 (2%)	–	–
Inflammation, suppurative	5 (10%)	5 (10%)	5 (10%)	6 (12%)
Pigmentation, hemosiderin	–	–	1 (2%)	–
Ulcer	–	1 (2%)	–	–
Olfactory epithelium, accumulation, hyaline droplet	15 (30%)	14 (28%)	14 (28%)	8 (16%)
Respiratory epithelium, accumulation, hyaline droplet	21 (42%)	26 (52%)	24 (48%)	25 (50%)
Respiratory epithelium, atrophy	–	1 (2%)	–	–
Respiratory epithelium, metaplasia, squamous	1 (2%)	1 (2%)	1 (2%)	5 (10%)
Pleura	(0)	(0)	(0)	(2)
Inflammation	–	–	–	2 (100%)
Trachea	(50)	(50)	(50)	(49)
Special Senses System				
Ear	(1)	(0)	(0)	(0)
Eye	(50)	(50)	(50)	(50)
Atrophy	–	–	1 (2%)	1 (2%)
Cataract	1 (2%)	–	2 (4%)	1 (2%)
Inflammation	–	1 (2%)	2 (4%)	2 (4%)
Cornea, hyperplasia	–	–	–	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	1 (2%)	–	2 (4%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Amyloid deposition	–	1 (2%)	–	–
Infarct	7 (14%)	4 (8%)	5 (10%)	4 (8%)
Inflammation, suppurative	–	–	–	1 (2%)
Metaplasia, osseous	1 (2%)	–	–	2 (4%)
Necrosis	–	–	1 (2%)	–
Nephropathy	17 (34%)	17 (34%)	15 (30%)	19 (38%)
Pigmentation	–	–	1 (2%)	–
Urinary bladder	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid	–	–	–	1 (2%)

Appendix E. Genetic Toxicology

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E.1. Bacterial Mutagenicity Test Protocol

p-Chloro-α,α,α-trifluorotoluene was tested in two independent bacterial gene mutation assays. In the first assay, testing procedures followed protocols reported by Haworth et al.[33]. Briefly, a commercially obtained sample of *p*-chloro-α,α,α-trifluorotoluene was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver) for 20 minutes at 37ºC. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37ºC.

In the second assay, a sample of the same lot of *p*-chloro-α,α,α-trifluorotoluene that was used in the 2-year bioassay was sent to the testing laboratory for assessment of mutagenicity in *S. typhimurium* strains TA98 and TA100 and *Escherichia coli* strain WP2 *uvr*A/pKM101. Incubation in either buffer or S9 mix (from induced Sprague Dawley rat liver) and plating on minimal glucose agar plates was carried out as described above. Histidine-independent (for the *S. typhimurium* strains) or tryptophan-independent (for the *E. coli* strain) mutant colonies arising on these plates were counted following incubation for 2 days at 37ºC.

For all strains, each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of the test article. The high dose was limited by toxicity and all trials were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any single strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

E.2. Peripheral Blood Micronucleus Test Protocol

At the termination of the 3-month toxicity studies with *p*-chloro-α,α,α-trifluorotoluene, blood samples (~200 µL) were collected from male and female rats and mice, placed in EDTA-coated tubes, and shipped overnight to the testing laboratory. Upon arrival, blood samples were fixed in ultracold methanol using a MicroFlow^PLUS^ Kit (Litron Laboratories, Rochester, NY) according to the manufacturer's instructions. Fixed samples were stored in a -80°C freezer until analysis. Thawed blood samples were analyzed for frequency of micronucleated immature erythrocytes (polychromatic erythrocytes, PCEs, reticulocytes) and mature erythrocytes (normochromatic erythrocytes, NCEs) using a flow cytometer[96]; both the mature and the immature erythrocyte populations can be analyzed separately by employing special cell surface markers to differentiate the two cell types. Because the very young reticulocyte subpopulation (CD71-positive cells) can be targeted using this technique, rat blood samples can be analyzed for damage that occurred in the bone marrow within the past 24 to 48 hours, before the rat spleen appreciably alters the

percentage of micronucleated reticulocytes in circulation[97]. In mice, both the immature and mature erythrocyte populations can be evaluated for micronucleus frequency because the mouse spleen does not sequester and eliminate damaged erythrocytes. Damaged erythrocytes achieve steady state in the peripheral blood of mice following 4 weeks of continuous exposure. Approximately 20,000 reticulocytes and 1×10^6 erythrocytes were analyzed per animal for frequency of micronucleated cells, and the percentage of immature erythrocytes (%PCE) was calculated as a measure of bone marrow toxicity resulting from chemical exposure.

Based on prior experience with the large number of cells scored using flow cytometric scoring techniques[98], it is reasonable to assume that the proportion of micronucleated reticulocytes is approximately normally distributed. The statistical tests selected for trend and for pairwise comparisons with the control group depend on whether the variances among the groups are equal. Levene's test at $\alpha = 0.05$ is used to test for equal variances. In the case of equal variances, linear regression is used to test for a linear trend with dose and Williams' test is used to test for pairwise differences between each treatment group and the control group. In the case of unequal variances, Jonckheere's test is used to test for linear trend and Dunn's test is used for pairwise comparisons of each treatment group with the control group. To correct for multiple pairwise comparisons, the P value for each comparison with the control group is multiplied by the number of comparisons made. In the event that this product is greater than 1.00, it is replaced with 1.00. Trend tests and pairwise comparisons with the controls are considered statistically significant at $P \leq 0.025$.

In the micronucleus test, a positive result is preferably based on the presence of both a significant trend as well as at least one significantly elevated dose group compared with the corresponding control group. In addition, historical control data are used to evaluate the biological significance of any observed response. Both statistical significance and biological significance are considered when arriving at a call. The presence of either a significant trend or a single significant dose group generally results in an equivocal call. The absence of both a trend and a significant dose group results in a negative call. Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed (in acute studies), and the magnitudes of those effects.

E.3. Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple samples of a chemical were tested in the same assay, and different results were obtained among these samples and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the in vitro assays have another variable that must be considered in arriving at an overall test result. In vitro assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report

presents a result that represents a scientific judgment of the overall evidence for activity of the chemical in an assay.

E.4. Results

In an earlier bacterial mutagenicity assay, *p*-chloro-α,α,α-trifluorotoluene (10 to 1,000 µg/plate) was not mutagenic in any of four strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537) when tested without exogenous metabolic activation or with 10% Aroclor 1254-induced hamster or rat liver S9 mix (Table E-1; Haworth et al.[33]). In a second bacterial mutagenicity assay conducted with the same lot of *p*-chloro-α,α,α-trifluorotoluene that was tested in the 2-year rodent bioassay, no evidence of mutagenicity was observed in *S. typhimurium* strains TA98 or TA100 or in *E. coli* strain WP2 *uvr*A/pKM101, with or without induced rat liver S9 mix (Table E-2). Doses in this second assay were significantly higher than those that were achievable in the initial assay (up to 5,000 µg/plate without S9 and 6,000 µg/plate in the presence of S9).

In vivo, no significant increases in micronucleated immature or mature erythrocytes were observed in peripheral blood samples from rats in the 3-month study (Table E-3). The small increase in micronucleated immature erythrocytes observed in the male rats was within historical control ranges (<1 standard deviation of the mean) and was therefore judged not to be biologically significant. In mice from the 3-month study, small but statistically significant increases in micronucleated mature erythrocytes were seen at the highest exposure concentration (2,000 ppm), but the observed values for the female mice were within historical control ranges (<1 standard deviation of the mean) and were not considered to be biologically significant (Table E-4). For male mice, the observed response was outside the historical control range for the laboratory and was therefore judged to be positive. An exposure concentration-related increase in the percentage of immature erythrocytes in peripheral blood was seen only in female mice, suggesting that *p*-chloro-α,α,α-trifluorotoluene may have stimulated erythropoiesis in female mice. No significant changes in the percentage of immature erythrocytes were seen in male or female rats or male mice.

Table E-1. Mutagenicity of *p*-Chloro-α,α,α-trifluorotoluene in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% hamster S9	With 10% hamster S9	With 10% rat S9	With 10% rat S9
TA100							
	0	111 ± 6	92 ± 13	99 ± 3	98 ± 7	111 ± 13	123 ± 5
	10	113 ± 5	90 ± 11	104 ± 3	96 ± 10	115 ± 6	101 ± 8
	33	105 ± 5	–	–	–	–	–
	33.3	–	99 ± 8	106 ± 4	88 ± 10	101 ± 1	103 ± 11
	100	100 ± 5	103 ± 16	98 ± 4	106 ± 4	102 ± 4	88 ± 2
	333.3	110 ± 1	82 ± 4[b]	74 ± 5	101 ± 3	73 ± 2	72 ± 11[b]
	1,000	Toxic	Toxic	64 ± 6[b]	92 ± 9	64 ± 5[b]	67 ± 1[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		483 ± 7	416 ± 11	1,119 ± 120	2,115 ± 15	1,075 ± 30	549 ± 71
TA98							
	0	29 ± 1	34 ± 5	47 ± 8	33 ± 2	40 ± 3	43 ± 1
	10	31 ± 7	29 ± 2	52 ± 4	37 ± 2	53 ± 1	39 ± 3
	33.3	21 ± 2	29 ± 1	50 ± 9	35 ± 3	44 ± 6	35 ± 4
	100	22 ± 4	25 ± 7	48 ± 5	33 ± 4	32 ± 3	38 ± 4
	333.3	22 ± 2	8 ± 1[b]	28 ± 5	33 ± 3	22 ± 3	30 ± 5[b]
	1,000	Toxic	Toxic	35 ± 2[b]	25 ± 6	22 ± 4[b]	27 ± 2[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		691 ± 10	671 ± 58	570 ± 58	1,271 ± 8	574 ± 22	365 ± 23
TA1535							
	0	32 ± 2	20 ± 4	12 ± 2	10 ± 2	14 ± 1	8 ± 2
	10	38 ± 1	16 ± 1	16 ± 2	15 ± 1	15 ± 1	12 ± 0
	33.3	33 ± 4	14 ± 2	12 ± 2	12 ± 3	16 ± 3	9 ± 1
	100	36 ± 4	15 ± 3	15 ± 3	10 ± 2	12 ± 2	14 ± 1
	333.3	28 ± 2[b]	13 ± 3[b]	8 ± 1	9 ± 3	11 ± 3	8 ± 2[b]
	1,000	Toxic	8 ± 0[b]	7 ± 1[b]	11 ± 1	11 ± 3[b]	11 ± 2[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		412 ± 9	346 ± 14	257 ± 14	266 ± 10	314 ± 15	167 ± 5
TA1537							
	0	16 ± 4	5 ± 2	16 ± 2	8 ± 1	16 ± 2	6 ± 1
	10	10 ± 3	7 ± 2	23 ± 1	6 ± 0	19 ± 1	6 ± 1
	33.3	11 ± 1	3 ± 1	21 ± 2	6 ± 1	16 ± 0	7 ± 0
	100	9 ± 2	4 ± 1	19 ± 1	6 ± 1	15 ± 1	6 ± 1
	333.3	8 ± 0	4 ± 1	15 ± 1	6 ± 2	10 ± 4	5 ± 1
	1,000	3 ± 3[b]	Toxic	15 ± 3[b]	3 ± 1[b]	7 ± 1[b]	1 ± 1[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		329 ± 159	847 ± 54	459 ± 52	411 ± 10	495 ± 53	239 ± 25

[a]Study was performed at SRI International. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol and these data are presented by Haworth et al.[33]. 0 µg/plate was the solvent control.
[b]Slight toxicity.
[c]The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-o-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table E-2. Mutagenicity of *p*-Chloro-α,α,α-trifluorotoluene in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100					
	DMSO	104.7 ± 2.7	96.7 ± 9.3	114.0 ± 1.5	105.7 ± 9.6
	125	86.3 ± 13.7	86.7 ± 6.6	–	–
	250	66.0 ± 4.0	74.7 ± 2.0	96.7 ± 10.7	100.7 ± 8.3
	500	67.0 ± 6.1[b]	43.3 ± 6.4[b]	76.0 ± 7.2	78.3 ± 2.2
	1,000	53.3 ± 6.0[b]	45.0 ± 8.9[b]	75.3 ± 1.9[b]	69.7 ± 8.6[b]
	2,000	28.7 ± 1.9[b]	53.7 ± 13.7[b]	53.0 ± 17.5[b]	46.0 ± 4.0[b]
	3,000	–	–	36.7 ± 19.9[b]	32.7 ± 6.7[b]
	5,000	6.0 ± 2.3[b]	5.5 ± 2.5[b]	–	–
	6,000	–	–	Toxic	21.3 ± 5.8[b]
Trial summary	–	Negative	Negative	Negative	Negative
Positive controlc	–	589.7 ± 35.5	666.0 ± 30.0	664.7 ± 54.5	653.0 ± 30.4
TA98					
	DMSO	17.0 ± 2.3	11.0 ± 1.2	21.0 ± 2.9	16.7 ± 3.0
	125	11.0 ± 1.7	14.7 ± 4.6		
	250	12.3 ± 1.3	10.3 ± 2.0	18.3 ± 2.4	20.3 ± 4.4
	500	9.0 ± 2.6	10.0 ± 1.7[b]	19.7 ± 2.3	14.0 ± 8.4
	1,000	10.7 ± 2.3	9.3 ± 2.8[b]	11.3 ± 2.6	17.0 ± 1.0[b]
	2,000	11.0 ± 2.0[b]	12.3 ± 1.2[b]	13.0 ± 2.5	18.0 ± 3.1[b]
	3,000	–	–	18.0 ± 1.5	17.3 ± 2.7[b]
	5,000	Toxic	Toxic	–	–
	6,000	–	–	16.3 ± 1.5	3.3 ± 0.9[b]
Trial summary	–	Negative	Negative	Negative	Negative
Positive control	–	664.0 ± 15.7	698.0 ± 26.4	1,378.3 ± 89.5	1,611.7 ± 116.2
***Escherichia coli* WP2 *uvr*A/pKM101**					
	DMSO	97.3 ± 15.6	109.7 ± 4.4	147.0 ± 21.1	170.3 ± 5.8
	125	83.7 ± 9.3	122.7 ± 11.3	–	–
	250	71.3 ± 3.5	108.0 ± 2.1	179.3 ± 18.4	155.7 ± 11.3
	500	72.3 ± 1.3	106.0 ± 4.0[b]	122.7 ± 4.8	125.0 ± 15.0
	1,000	73.7 ± 2.6	78.7 ± 20.9[b]	93.3 ± 7.7[b]	119.0 ± 12.2[b]
	2,000	62.3 ± 8.1[b]	84.3 ± 10.7[b]	95.3 ± 7.5[b]	120.3 ± 5.0[b]
	3,000	–	–	78.7 ± 17.6[b]	97.7 ± 20.8[b]
	5,000	13.0 ± 3.6[b]	29.5 ± 24.5[b]	–	–
	6,000	–	–	45.0 ± 20.5[b]	106.7 ± 25.7[b]
Trial summary	–	Negative	Negative	Negative	Negative
Positive control	–	1,518.7 ± 61.0	2,208.0 ± 27.5	994.3 ± 2.7	1,321.3 ± 5.6

[a]Study was performed at ILS, Inc. Data are presented as revertants/plate (mean ± standard error) from three plates. Dimethyl sulfoxide (DMSO) was the solvent control.
[b]Slight toxicity
[c]The positive controls in the absence of metabolic activation were sodium azide (TA100), 2-nitrofluroene (TA98), and 4-nitroquinoline N-oxide (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene, except benzo(a)pyrene was used for TA100.

Table E-3. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Rats Following Treatment with *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Three Months[a]

	Exposure Concentration (ppm)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P value[d]	PCEs[b] (%)	P value[d]
Male								
Air[e]	0	5	0.52 ± 0.05	–	0.14 ± 0.02	–	0.916 ± 0.04	–
p-Chloro-α,α,α-trifluorotoluene								
	125	5	0.53 ± 0.06	0.4831	0.14 ± 0.02	0.7538	0.838 ± 0.05	1.0000
	250	5	0.52 ± 0.04	0.5629	0.10 ± 0.01	0.8340	0.945 ± 0.05	0.9999
	500	5	0.67 ± 0.10	0.1856	0.11 ± 0.02	0.8632	0.902 ± 0.08	1.0000
	1,000	5	0.60 ± 0.11	0.1904	0.10 ± 0.02	0.8758	0.925 ± 0.05	0.9985
	2,000	5	0.71 ± 0.05	0.0585	0.13 ± 0.04	0.7864	1.062 ± 0.07	0.1852
	–	–	P = 0.023[f]	–	P = 0.590[f]	–	P = 0.037[f]	–
Female								
Air	0	5	0.49 ± 0.13	–	0.09 ± 0.01	–	0.847 ± 0.07	–
p-Chloro-α,α,α-trifluorotoluene								
	125	5	0.60 ± 0.15	0.2167	0.06 ± 0.00	1.0000	0.895 ± 0.07	1.0000
	250	5	0.77 ± 0.09	0.1872	0.13 ± 0.04	1.0000	1.038 ± 0.18	0.8613
	500	5	0.62 ± 0.08	0.2014	0.11 ± 0.03	1.0000	1.113 ± 0.05	0.2213
	1,000	5	0.53 ± 0.06	0.2069	0.10 ± 0.02	1.0000	1.020 ± 0.05	0.9798
	2,000	5	0.61 ± 0.04	0.2115	0.16 ± 0.06	0.9229	1.072 ± 0.10	0.6569
	–	–	P = 0.525[f]	–	P = 0.112[g]	–	P = 0.099[g]	–

[a]Study was performed at ILS, Inc. The detailed protocol is presented by Witt et al.[96]. NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the chamber control group; exposed group values are significant at P ≤ 0.025 by William's test.
[d]Pairwise comparison with the chamber control group; exposed group values are significant at P ≤ 0.025 by Williams's test (males) or Dunn's test (females).
[e]Chamber control.
[f]Exposure concentration-related trend significant at P ≤ 0.025 by linear regression.
[g]Exposure concentration-related trend significant at P ≤ 0.025 by Jonckheere's test.

Table E-4. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Three Months[a]

	Exposure Concentration (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P value[d]	PCEs[b] (%)	P value[d]
Male								
Air[e]	0	5	2.18 ± 0.13	–	1.46 ± 0.03	–	1.543 ± 0.06	–
p-Chloro-α,α,α-trifluorotoluene								
	125	5	2.31 ± 0.13	1.0000	1.44 ± 0.03	0.5848	1.595 ± 0.04	1.0000
	250	5	2.46 ± 0.21	1.0000	1.45 ± 0.04	0.6700	1.464 ± 0.06	0.6653
	500	5	2.21 ± 0.09	1.0000	1.42 ± 0.02	0.7043	1.285 ± 0.07	0.7099
	1,000	5	2.43 ± 0.16	0.8739	1.48 ± 0.05	0.5151	1.516 ± 0.09	0.7318
	2,000	5	2.93 ± 0.55	0.4432	1.80 ± 0.09	0.0000	1.715 ± 0.09	0.1610
	–	–	P = 0.105[f]	–	P = 0.000[g]	–	P = 0.096[g]	–
Female								
Air	0	5	1.74 ± 0.19	–	1.07 ± 0.02	–	0.985 ± 0.10	–
p-Chloro-α,α,α-trifluorotoluene								
	125	5	2.15 ± 0.22	0.2655	1.07 ± 0.04	0.8172	1.169 ± 0.10	0.1569
	250	5	1.90 ± 0.20	0.3173	1.04 ± 0.03	0.8874	1.392 ± 0.11	0.0156
	500	5	1.79 ± 0.07	0.3400	0.94 ± 0.03	0.9102	1.336 ± 0.16	0.0161
	1,000	5	1.94 ± 0.14	0.3515	1.04 ± 0.03	0.8880	1.395 ± 0.08	0.0073
	2,000	5	1.69 ± 0.16	0.3609	1.19 ± 0.03	0.0085	1.614 ± 0.09	0.0004
	–	–	P = 0.832[g]	–	P = 0.007[g]	–	P = 0.001[g]	–

[a]Study was performed at ILS, Inc. The detailed protocol is presented by Witt et al.[96]. NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the chamber control group; exposed group values are significant at P ≤ 0.025 by Dunn's test (males) or William's test (females).
[d]Pairwise comparison with the chamber control group; exposed group values are significant at P ≤ 0.025 by Williams's test.
[e]Chamber control.
[f]Exposure concentration-related trend significant at P ≤ 0.025 by Jonckheere's test.
[g]Exposure concentration-related trend significant at P ≤ 0.025 by linear regression.

Appendix F. Clinical Pathology Results

Tables

Table F-1. Hematology and Clinical Chemistry Data for Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene F-2
Table F-2. Hematology Data for Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene F-5

Table F-1. Hematology and Clinical Chemistry Data for Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
Male						
Hematology						
n	10	9	10	10	10	10
Hematocrit (spun) (%)	51.1 ± 0.5	50.8 ± 0.4	49.8 ± 0.4	49.6 ± 0.4	49.9 ± 0.4	50.7 ± 0.5
Packed cell volume (%)	50.8 ± 0.6	50.3 ± 0.5	49.8 ± 0.4	49.2 ± 0.5	50.0 ± 0.5	50.8 ± 0.6
Hemoglobin (g/dL)	16.0 ± 0.1	15.9 ± 0.2	15.7 ± 0.1	15.6 ± 0.1	15.6 ± 0.1	16.0 ± 0.2
Erythrocytes (10^6/µL)	8.92 ± 0.09	9.07 ± 0.08	8.88 ± 0.12	8.73 ± 0.09	8.67 ± 0.11	8.78 ± 0.11
Reticulocytes (10^3/µL)	229 ± 8	222 ± 5	220 ± 7	228 ± 6	235 ± 7	247 ± 9
Mean cell volume (fL)	56.9 ± 0.4	55.5 ± 0.4	56.2 ± 0.5	56.4 ± 0.4	57.7 ± 0.4	57.9 ± 0.4
Mean cell hemoglobin (pg)	18.0 ± 0.1	17.5 ± 0.2	17.7 ± 0.2	17.8 ± 0.1	18.0 ± 0.1	18.2 ± 0.2
Mean cell hemoglobin concentration (g/dL)	31.6 ± 0.2	31.5 ± 0.1	31.6 ± 0.2	31.6 ± 0.1	31.3 ± 0.1	31.5 ± 0.2
Platelets (10^3/µL)	813 ± 46	872 ± 27	846 ± 39	874 ± 45	831 ± 55	722 ± 42
Leukocytes (10^3/µL)	10.30 ± 0.56	11.18 ± 0.76	10.76 ± 0.40	11.97 ± 0.78	11.10 ± 0.81	7.70 ± 0.47
Segmented neutrophils (10^3/µL)	1.06 ± 0.04	1.27 ± 0.12	1.17 ± 0.10	1.41 ± 0.11**	1.32 ± 0.05**	1.66 ± 0.08**
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
Lymphocytes (10^3/µL)	8.73 ± 0.53	9.28 ± 0.70	9.09 ± 0.40	9.96 ± 0.69	9.05 ± 0.75	5.49 ± 0.42**
Monocytes (10^3/µL)	0.29 ± 0.02	0.37 ± 0.03	0.29 ± 0.02	0.35 ± 0.03	0.45 ± 0.05*	0.38 ± 0.04
Basophils (10^3/µL)	0.030 ± 0.003	0.043 ± 0.005	0.040 ± 0.004	0.046 ± 0.006	0.052 ± 0.011	0.029 ± 0.004
Eosinophils (10^3/µL)	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.08 ± 0.01
Large unstained cells (10^3/µL)	0.094 ± 0.008	0.100 ± 0.008	0.099 ± 0.008	0.115 ± 0.011	0.131 ± 0.018	0.070 ± 0.006
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	16.9 ± 0.8	14.5 ± 0.5	15.6 ± 0.6	17.0 ± 0.4	17.2 ± 0.6	18.4 ± 0.9
Creatinine (mg/dL)	0.40 ± 0.02	0.39 ± 0.01	0.36 ± 0.02	0.35 ± 0.02	0.38 ± 0.01	0.45 ± 0.03

	Chamber Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
Glucose (mg/dL)	117 ± 5	120 ± 5	114 ± 2	120 ± 6	114 ± 2	117 ± 6
Total protein (g/dL)	7.3 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.2 ± 0.1	7.4 ± 0.1	7.7 ± 0.1
Albumin (g/dL)	4.5 ± 0.0	4.5 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	4.7 ± 0.1
Globulin (g/dL)	2.7 ± 0.0	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	3.0 ± 0.1**	3.0 ± 0.1**
Albumin/globulin ratio	1.7 ± 0.0	1.7 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.5 ± 0.1**	1.6 ± 0.0*
Cholesterol (mg/dL)	132 ± 6	135 ± 4	137 ± 6	149 ± 5*	182 ± 6**	268 ± 15**
Triglycerides (mg/dL)	71 ± 6	82 ± 5	74 ± 4	96 ± 8*	118 ± 11**	137 ± 6**
Alanine aminotransferase (IU/L)	55 ± 2	53 ± 2	52 ± 2	52 ± 2	59 ± 3	73 ± 4*
Alkaline phosphatase (IU/L)	165 ± 6	192 ± 9*	229 ± 10**	261 ± 15**	238 ± 13**	247 ± 17**
Creatine kinase (IU/L)	247 ± 55	230 ± 55	158 ± 22	151 ± 18	143 ± 16	151 ± 15
Sorbitol dehydrogenase (IU/L)	13 ± 1	12 ± 1	14 ± 1	15 ± 0	16 ± 1	18 ± 1**
Bile salts (µmol/L)	22.4 ± 5.7	17.9 ± 3.8	8.5 ± 2.4	17.7 ± 3.3	11.1 ± 1.5	7.2 ± 1.1
Female						
n	10	10	10	10	10	9
Hematology						
Hematocrit (spun) (%)	48.3 ± 0.3	47.0 ± 0.5	47.2 ± 0.4	47.5 ± 0.2	46.7 ± 0.7	46.9 ± 0.5
Packed cell volume (%)	47.7 ± 0.6	46.8 ± 0.4	47.0 ± 0.5	46.9 ± 0.4	46.5 ± 0.6	46.9 ± 0.6
Hemoglobin (g/dL)	15.3 ± 0.2	15.1 ± 0.1	15.1 ± 0.1	15.0 ± 0.1	14.9 ± 0.2	15.0 ± 0.1
Erythrocytes (10^6/µL)	8.19 ± 0.12	8.14 ± 0.07	8.14 ± 0.10	7.98 ± 0.09	7.87 ± 0.09	8.02 ± 0.08
Reticulocytes (10^3/µL)	234 ± 13	239 ± 13	255 ± 16	262 ± 15	241 ± 15	222 ± 14
Mean cell volume (fL)	58.3 ± 0.4	57.4 ± 0.3	57.7 ± 0.3	58.8 ± 0.5	59.1 ± 0.6	58.5 ± 0.4
Mean cell hemoglobin (pg)	18.7 ± 0.2	18.5 ± 0.1	18.5 ± 0.2	18.9 ± 0.2	19.0 ± 0.2	18.7 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.1 ± 0.2	32.3 ± 0.1	32.1 ± 0.2	32.1 ± 0.2	32.1 ± 0.1	32.0 ± 0.2
Platelets (10^3/µL)	840 ± 60[b]	823 ± 36	889 ± 35[b]	849 ± 71	866 ± 64	763 ± 42
Leukocytes (10^3/µL)	9.05 ± 0.81	7.75 ± 0.44	8.01 ± 0.39	10.03 ± 0.80	9.47 ± 0.69	5.96 ± 0.38*
Segmented neutrophils (10^3/µL)	1.05 ± 0.09	1.09 ± 0.10	1.31 ± 0.14	1.30 ± 0.06	1.17 ± 0.12	1.18 ± 0.07

	Chamber Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
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
Lymphocytes (10^3/µL)	7.51 ± 0.73	6.22 ± 0.33	6.24 ± 0.31	8.16 ± 0.72	7.83 ± 0.59	4.34 ± 0.29**
Monocytes (10^3/µL)	0.22 ± 0.02	0.22 ± 0.03	0.22 ± 0.02	0.28 ± 0.04	0.26 ± 0.03	0.26 ± 0.03
Basophils (10^3/µL)	0.036 ± 0.005	0.026 ± 0.003	0.032 ± 0.002	0.037 ± 0.006	0.031 ± 0.004	0.019 ± 0.003*
Eosinophils (10^3/µL)	0.14 ± 0.02	0.12 ± 0.02	0.14 ± 0.01	0.16 ± 0.02	0.09 ± 0.01	0.11 ± 0.01
Large unstained cells (10^3/µL)	0.082 ± 0.014	0.065 ± 0.007	0.068 ± 0.006	0.087 ± 0.011	0.078 ± 0.007	0.044 ± 0.005*
Clinical Chemistry						
Urea nitrogen (mg/dL)	15.8 ± 1.0	15.6 ± 0.6	15.8 ± 0.4	16.0 ± 0.5	14.0 ± 0.5	13.9 ± 0.8
Creatinine (mg/dL)	0.40 ± 0.01	0.41 ± 0.01	0.42 ± 0.01	0.40 ± 0.00	0.39 ± 0.01	0.36 ± 0.05
Glucose (mg/dL)	123 ± 3	131 ± 4	125 ± 3	116 ± 3	115 ± 2	124 ± 5
Total protein (g/dL)	7.3 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.1 ± 0.1	7.5 ± 0.1	7.7 ± 0.1**
Albumin (g/dL)	5.0 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	5.0 ± 0.1	4.9 ± 0.1
Globulin (g/dL)	2.3 ± 0.1	2.1 ± 0.0	2.3 ± 0.0	2.3 ± 0.0	2.5 ± 0.1	2.8 ± 0.1**
Albumin/globulin ratio	2.2 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.1 ± 0.0	2.1 ± 0.0	1.8 ± 0.0**
Cholesterol (mg/dL)	110 ± 6	117 ± 2	128 ± 3*	141 ± 5**	189 ± 6**	261 ± 10**
Triglycerides (mg/dL)	51 ± 4	53 ± 6	44 ± 4	58 ± 4	83 ± 5**	115 ± 9**
Alanine aminotransferase (IU/L)	49 ± 1	48 ± 2	48 ± 3	50 ± 1	51 ± 2	62 ± 4**
Alkaline phosphatase (IU/L)	121 ± 6	147 ± 12	185 ± 10**	201 ± 12**	227 ± 12**	216 ± 15**
Creatine kinase (IU/L)	146 ± 9	173 ± 21	180 ± 22	134 ± 11	134 ± 19	221 ± 38
Sorbitol dehydrogenase (IU/L)	11 ± 1	10 ± 0	12 ± 1	12 ± 0	13 ± 1	18 ± 2**
Bile salts (µmol/L)	12.5 ± 5.5	16.0 ± 2.5	13.6 ± 4.0	33.8 ± 6.5**	33.5 ± 6.0**	14.7 ± 3.1**

*Significantly different (P ≤ 0.05) from the chamber control group by Dunn's or Shirley's test.
**P ≤ 0.01.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b]n = 9.

Table F-2. Hematology Data for Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
Male						
n	10	10	10	9	10	10
Hematocrit (spun) (%)	48.8 ± 0.3	49.7 ± 0.2	49.0 ± 0.1	48.6 ± 0.5	48.6 ± 0.4	46.4 ± 0.5**
Packed cell volume (%)	46.1 ± 0.3	46.9 ± 0.3	46.0 ± 0.2	45.9 ± 0.4	46.1 ± 0.4	43.7 ± 0.4**
Hemoglobin (g/dL)	16.2 ± 0.1	16.4 ± 0.1	16.2 ± 0.1	16.1 ± 0.1	16.0 ± 0.1	15.0 ± 0.1**
Erythrocytes (10^6/µL)	10.59 ± 0.05	10.81 ± 0.07	10.67 ± 0.05	10.52 ± 0.14	10.41 ± 0.04	9.80 ± 0.07**
Reticulocytes (10^3/µL)	296 ± 6	315 ± 5	285 ± 6	288 ± 13	264 ± 6**	258 ± 8**
Howell-Jolly bodies (% erythrocytes)	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Mean cell volume (fL)	43.5 ± 0.2	43.4 ± 0.2	43.2 ± 0.2	43.6 ± 0.4	44.2 ± 0.4	44.6 ± 0.2*
Mean cell hemoglobin (pg)	15.3 ± 0.1	15.2 ± 0.1	15.2 ± 0.1	15.3 ± 0.1	15.4 ± 0.1	15.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	35.3 ± 0.1	35.1 ± 0.1	35.1 ± 0.2	35.1 ± 0.3	34.9 ± 0.3	34.3 ± 0.1**
Platelets (10^3/µL)	1,180 ± 28	1,243 ± 35	1,245 ± 39	1,351 ± 28**	1,433 ± 28**	1,577 ± 44**
Leukocytes (10^3/µL)	3.14 ± 0.53	2.17 ± 0.31	2.04 ± 0.22	2.71 ± 0.30	3.23 ± 0.32	2.58 ± 0.30
Segmented neutrophils (10^3/µL)	0.44 ± 0.08	0.26 ± 0.04	0.26 ± 0.03	0.31 ± 0.04	0.45 ± 0.06	0.76 ± 0.21
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
Lymphocytes (10^3/µL)	2.56 ± 0.44	1.79 ± 0.26	1.68 ± 0.18	2.26 ± 0.25	2.66 ± 0.25	1.71 ± 0.17
Monocytes (10^3/µL)	0.05 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
Basophils (10^3/µL)	0.010 ± 0.004	0.010 ± 0.003	0.007 ± 0.002	0.010 ± 0.003	0.011 ± 0.002	0.005 ± 0.002
Eosinophils (10^3/µL)	0.06 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	0.07 ± 0.02	0.05 ± 0.01	0.05 ± 0.01
Large unstained cells (10^3/µL)	0.022 ± 0.008	0.014 ± 0.003	0.013 ± 0.003	0.013 ± 0.004	0.023 ± 0.004	0.022 ± 0.004
Female						
n	10	10	10	10	10	10
Hematocrit (spun) (%)	50.6 ± 0.3	51.2 ± 0.3	50.4 ± 0.6	50.2 ± 0.4	49.2 ± 0.2**	47.4 ± 0.4**
Packed cell volume (%)	51.6 ± 0.2	52.2 ± 0.4	51.6 ± 0.4	51.6 ± 0.6	50.2 ± 0.2**	48.0 ± 0.5**

	Chamber Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
Hemoglobin (g/dL)	16.5 ± 0.1	16.6 ± 0.1	16.5 ± 0.1	16.5 ± 0.1	16.2 ± 0.1	15.2 ± 0.2**
Erythrocytes (10^6/µL)	10.69 ± 0.05	10.76 ± 0.06	10.62 ± 0.09	10.62 ± 0.11	10.27 ± 0.03**	9.74 ± 0.12**
Reticulocytes (10^3/µL)	276 ± 17	292 ± 8	275 ± 16	270 ± 14	258 ± 10	283 ± 13
Howell-Jolly bodies (% erythrocytes)	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0*	0.1 ± 0.0	0.1 ± 0.0
Mean cell volume (fL)	48.3 ± 0.1	48.5 ± 0.2	48.6 ± 0.1	48.6 ± 0.3	48.9 ± 0.2*	49.3 ± 0.2**
Mean cell hemoglobin (pg)	15.5 ± 0.1	15.5 ± 0.1	15.5 ± 0.1	15.6 ± 0.1	15.8 ± 0.1**	15.6 ± 0.0*
Mean cell hemoglobin concentration (g/dL)	32.0 ± 0.1	31.9 ± 0.2	31.9 ± 0.1	32.0 ± 0.2	32.3 ± 0.1	31.6 ± 0.1
Platelets (10^3/µL)	1,099 ± 29	1,101 ± 24	1,163 ± 53	1,209 ± 53	1,228 ± 33*	1,415 ± 25**
Leukocytes (10^3/µL)	3.46 ± 0.35	3.46 ± 0.45	2.96 ± 0.38	3.18 ± 0.33	2.97 ± 0.28	2.70 ± 0.17
Segmented neutrophils (10^3/µL)	0.53 ± 0.09	0.47 ± 0.05	0.44 ± 0.06	0.44 ± 0.10	0.38 ± 0.04	0.64 ± 0.09
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
Lymphocytes (10^3/µL)	2.76 ± 0.28	2.83 ± 0.38	2.40 ± 0.31	2.63 ± 0.27	2.46 ± 0.24	1.92 ± 0.11
Monocytes (10^3/µL)	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
Basophils (10^3/µL)	0.013 ± 0.002	0.018 ± 0.005	0.007 ± 0.003	0.010 ± 0.003	0.011 ± 0.003	0.013 ± 0.003
Eosinophils (10^3/µL)	0.08 ± 0.01	0.07 ± 0.02	0.07 ± 0.02	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
Large unstained cells (10^3/µL)	0.027 ± 0.005	0.038 ± 0.012	0.023 ± 0.008	0.017 ± 0.006	0.016 ± 0.005	0.028 ± 0.005

*Significantly different (P ≤ 0.05) from the chamber control group by Dunn's or Shirley's test.
**Significantly different (P ≤ 0.01) from the chamber control group by Shirley's test.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

Appendix G. Organ Weights and Organ-Weight-to-Body Ratios

Tables

Table G-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene G-2
Table G-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene G-3

Table G-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	393 ± 7	426 ± 5*	414 ± 11	412 ± 11	401 ± 7	376 ± 6
Heart						
Absolute	1.32 ± 0.03	1.48 ± 0.03*	1.41 ± 0.04	1.40 ± 0.04	1.37 ± 0.04	1.34 ± 0.03
Relative	3.36 ± 0.04	3.47 ± 0.05	3.41 ± 0.08	3.40 ± 0.06	3.41 ± 0.07	3.56 ± 0.08
R. Kidney						
Absolute	1.28 ± 0.04	1.46 ± 0.02**	1.48 ± 0.03**	1.57 ± 0.04**	1.61 ± 0.04**	1.58 ± 0.06**
Relative	3.24 ± 0.05	3.44 ± 0.06	3.57 ± 0.06**	3.82 ± 0.07**	4.00 ± 0.05**	4.20 ± 0.14**
Liver						
Absolute	12.22 ± 0.38	13.74 ± 0.21*	14.41 ± 0.56**	16.56 ± 0.65**	19.34 ± 0.49**	22.02 ± 0.69**
Relative	31.03 ± 0.56	32.32 ± 0.50	34.79 ± 0.83**	40.18 ± 1.10**	48.25 ± 1.26**	58.48 ± 1.29**
Lung						
Absolute	2.05 ± 0.07	2.30 ± 0.08	2.04 ± 0.06	2.17 ± 0.07	2.27 ± 0.10	2.22 ± 0.15
Relative	5.23 ± 0.18	5.41 ± 0.18	4.94 ± 0.12	5.26 ± 0.12	5.65 ± 0.23	5.93 ± 0.43
R. Testis						
Absolute	1.800 ± 0.173	2.020 ± 0.015	1.991 ± 0.039	1.922 ± 0.138	2.055 ± 0.046	1.999 ± 0.029
Relative	4.564 ± 0.427	4.751 ± 0.048	4.830 ± 0.114	4.717 ± 0.373	5.124 ± 0.106	5.328 ± 0.108*
Thymus						
Absolute	0.309 ± 0.024	0.369 ± 0.027	0.342 ± 0.030	0.351 ± 0.031	0.312 ± 0.022	0.305 ± 0.024
Relative	0.788 ± 0.061	0.865 ± 0.060	0.817 ± 0.051	0.845 ± 0.056	0.778 ± 0.051	0.809 ± 0.060
Female						
n	10	10	10	10	10	9
Necropsy body wt	240 ± 5	249 ± 5	255 ± 6	259 ± 5*	267 ± 9**	294 ± 8**
Heart						
Absolute	0.91 ± 0.02	0.96 ± 0.02	0.97 ± 0.02	0.99 ± 0.03*	1.00 ± 0.03*	1.10 ± 0.02**
Relative	3.79 ± 0.06	3.86 ± 0.10	3.79 ± 0.05	3.84 ± 0.08	3.75 ± 0.08	3.75 ± 0.11
R. Kidney						
Absolute	0.84 ± 0.01	0.89 ± 0.02	0.94 ± 0.03*	0.94 ± 0.02*	0.94 ± 0.04*	1.06 ± 0.03**
Relative	3.51 ± 0.05	3.58 ± 0.05	3.67 ± 0.06	3.64 ± 0.06	3.50 ± 0.07	3.62 ± 0.08
Liver						
Absolute	7.07 ± 0.19	7.47 ± 0.26	7.93 ± 0.21	8.82 ± 0.23**	11.10 ± 0.51**	16.95 ± 0.52**
Relative	29.50 ± 0.39	30.00 ± 0.72	31.15 ± 0.48	34.05 ± 0.51**	41.58 ± 1.27**	57.75 ± 1.47**
Lung						
Absolute	1.73 ± 0.05	1.83 ± 0.06	1.83 ± 0.08	1.86 ± 0.06	1.90 ± 0.10	2.01 ± 0.15
Relative	7.24 ± 0.31	7.38 ± 0.23	7.22 ± 0.30	7.18 ± 0.21	7.15 ± 0.35	6.85 ± 0.53
Thymus						
Absolute	0.254 ± 0.012	0.258 ± 0.013	0.265 ± 0.014	0.241 ± 0.017	0.257 ± 0.015	0.238 ± 0.020
Relative	1.062 ± 0.049	1.037 ± 0.049	1.038 ± 0.049	0.925 ± 0.056	0.964 ± 0.050	0.804 ± 0.059**

*Significantly different ($P \leq 0.05$) from the chamber control group by Williams' or Dunnett's test.
**Significantly different ($P \leq 0.01$) from the chamber control group by William's test.
[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).

Table G-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	33.9 ± 0.6	34.2 ± 1.0	35.8 ± 0.9	36.9 ± 0.8**	38.6 ± 0.8**	36.8 ± 0.4**
Heart						
Absolute	0.15 ± 0.00	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01*	0.17 ± 0.01*	0.16 ± 0.00*
Relative	4.41 ± 0.08	4.60 ± 0.14	4.46 ± 0.09	4.50 ± 0.19	4.33 ± 0.10	4.43 ± 0.05
R. Kidney						
Absolute	0.29 ± 0.01	0.30 ± 0.01	0.31 ± 0.01	0.32 ± 0.01*	0.36 ± 0.01**	0.38 ± 0.01**
Relative	8.65 ± 0.14	8.86 ± 0.19	8.74 ± 0.20	8.75 ± 0.23	9.36 ± 0.23*	10.28 ± 0.22**
Liver						
Absolute	1.30 ± 0.04	1.44 ± 0.06	1.65 ± 0.07**	2.09 ± 0.07**	3.07 ± 0.07**	3.60 ± 0.05**
Relative	38.43 ± 0.87	42.11 ± 1.30	46.01 ± 1.36**	56.73 ± 1.84**	79.75 ± 1.16**	97.85 ± 1.59**
Lung						
Absolute	0.21 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.21 ± 0.01
Relative	6.05 ± 0.10	6.10 ± 0.16	6.22 ± 0.22	6.21 ± 0.23	5.58 ± 0.10	5.58 ± 0.15
R. Testis						
Absolute	0.106 ± 0.003	0.111 ± 0.003	0.115 ± 0.003	0.114 ± 0.002	0.109 ± 0.004	0.109 ± 0.002
Relative	3.134 ± 0.078	3.271 ± 0.109	3.223 ± 0.111	3.087 ± 0.078	2.834 ± 0.125	2.976 ± 0.067
Thymus						
Absolute	0.038 ± 0.002	0.041 ± 0.003	0.044 ± 0.002	0.043 ± 0.003	0.043 ± 0.002	0.032 ± 0.002
Relative	1.121 ± 0.056	1.196 ± 0.074	1.242 ± 0.044	1.173 ± 0.065	1.108 ± 0.053	0.859 ± 0.053*
Female						
Necropsy body wt	28.2 ± 0.4	29.9 ± 0.8	31.0 ± 0.7**	32.2 ± 0.7**	33.7 ± 0.8**	29.7 ± 0.7**
Heart						
Absolute	0.14 ± 0.00	0.14 ± 0.00	0.15 ± 0.00	0.15 ± 0.01	0.14 ± 0.00	0.14 ± 0.00
Relative	4.87 ± 0.12	4.83 ± 0.09	4.70 ± 0.10	4.54 ± 0.13*	4.26 ± 0.08**	4.58 ± 0.07**
R. Kidney						
Absolute	0.20 ± 0.01	0.21 ± 0.00	0.22 ± 0.01	0.21 ± 0.00	0.22 ± 0.01*	0.23 ± 0.01**
Relative	6.97 ± 0.20	6.98 ± 0.13	7.01 ± 0.17	6.55 ± 0.17	6.48 ± 0.12	7.69 ± 0.29*
Liver						
Absolute	1.23 ± 0.05	1.33 ± 0.05	1.51 ± 0.05*	1.81 ± 0.08**	2.44 ± 0.08**	2.61 ± 0.14**
Relative	43.51 ± 1.66	44.28 ± 0.91	48.73 ± 0.79	56.18 ± 1.98**	72.49 ± 1.95**	87.58 ± 3.36**
Lung						
Absolute	0.22 ± 0.00	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.22 ± 0.00
Relative	7.68 ± 0.15	7.79 ± 0.21	7.34 ± 0.21	7.06 ± 0.15*	6.70 ± 0.16*	7.37 ± 0.21*
Thymus						
Absolute	0.055 ± 0.002	0.058 ± 0.001	0.060 ± 0.004	0.065 ± 0.003	0.062 ± 0.001	0.041 ± 0.004**
Relative	1.956 ± 0.100	1.938 ± 0.054	1.925 ± 0.108	2.011 ± 0.068	1.858 ± 0.044	1.371 ± 0.102**

*Significantly different ($P \leq 0.05$) from the chamber 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).

Appendix H. Reproductive Tissue Evaluations and Estrous Cycle Characterization

Tables

Table H-1. Summary of Reproductive Tissue Evaluations for Male Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene H-2
Table H-2. Estrous Cycle Characterization for Female Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene H-2
Table H-3. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Rats Exposed to *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Three Months H-3
Table H-4. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene H-4
Table H-5. Estrous Cycle Characterization for Female Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene H-4
Table H-6. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Mice Exposed to *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Three Months H-5

Figures

Figure H-1. Vaginal Cytology Plots for Female Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene H-6
Figure H-2. Vaginal Cytology Plots for Female Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene H-7

Table H-1. Summary of Reproductive Tissue Evaluations for Male Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	500 ppm	1,000 ppm	2,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	393 ± 7	412 ± 11	401 ± 7	376 ± 6
L. Cauda epididymis	0.273 ± 0.0146	0.283 ± 0.0066	0.256 ± 0.0065	0.212 ± 0.0065**
L. Epididymis	0.648 ± 0.0280	0.669 ± 0.0147	0.625 ± 0.0164	0.555 ± 0.0128**
L. Testis	1.920 ± 0.0896	2.069 ± 0.0361	1.976 ± 0.0503	1.978 ± 0.0233
Spermatid measurements				
Spermatid heads (10^6/testis)	270.92 ± 22.197	303.18 ± 19.790	263.56 ± 10.265	265.95 ± 13.848
Spermatid heads (10^6/g testis)	138.97 ± 7.762	146.82 ± 10.025	133.51 ± 4.364	134.67 ± 7.351
Epididymal spermatozoal measurements				
Sperm motility (%)	76.9 ± 3.06	74.9 ± 3.33	68.7 ± 1.51*	68.4 ± 1.67*
Sperm (10^6/cauda epididymis)	158.6 ± 7.14	164.8 ± 9.11	144.0 ± 10.22	123.6 ± 5.62**
Sperm (10^6/g cauda epididymis)	587.5 ± 21.28	582.3 ± 30.21	562.7 ± 37.98	583.2 ± 23.31

*Significantly different (P ≤ 0.05) from the chamber control group by Shirley's test.
**Significantly different (P ≤ 0.01) from the chamber control group by Williams' test (left cauda epididymis and left epididymis weights) or Shirley's test (sperm/cauda epididymis).
[a]Data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test (body and left testis weights) or Dunn's test (spermatid measurements and sperm/g cauda epididymis).

Table H-2. Estrous Cycle Characterization for Female Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	500 ppm	1,000 ppm	2,000 ppm
n	10	10	10	9
Necropsy body wt (g)	240 ± 5	259 ± 5*	267 ± 9**	294 ± 8**
Number of estrous cycles	2.2 ± 0.25	2.0 ± 0.26	1.9 ± 0.18	1.3 ± 0.33[b]
Estrous cycle length (days)	5.5 ± 0.87	4.6 ± 0.22	4.7 ± 0.11	4.3 ± 0.33[b]
Estrous stages (% of cycle)[b]				
Diestrus	55.0	53.1	48.1	78.5
Proestrus	10.6	5.6	7.5	0.7
Estrus	33.8	37.5	42.5	14.6
Metestrus	0.0	3.1	1.9	6.3

*Significantly different (P ≤ 0.05) from the chamber control group by Williams' test.
**P ≤ 0.01.
[a]Necropsy body weights, number of estrous cycles, and estrous cycle length data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunn's test (estrous cycle number and length). Tests for equality of transition probability matrices among all groups and between the chamber control group and each exposed group indicated that 2,000 ppm females spent significantly more time in extended diestrus (P < 0.001) than the chamber control group.
[b]Estrous cyclicity samples were evaluated for 9 females exposed to 2,000 ppm; however, in 6 of 9 animals estrous cycles were longer than 16 days or unclear and were not included in these analyses.

Table H-3. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Rats Exposed to *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Three Months

Stage	Comparison	P value	Trend[a]
Overall Tests	Overall	<0.001	–
Overall Tests	500 ppm vs. Chamber Controls	0.722	–
Overall Tests	1,000 ppm vs. Chamber Controls	0.91	–
Overall Tests	2,000 ppm vs. Chamber Controls	<0.001	–
Extended Estrus	Overall	0.008	–
Extended Estrus	500 ppm vs. Chamber Controls	0.315	–
Extended Estrus	1,000 ppm vs. Chamber Controls	0.064	–
Extended Estrus	2,000 ppm vs. Chamber Controls	0.008	N
Extended Diestrus	Overall	<0.001	–
Extended Diestrus	500 ppm vs. Chamber Controls	0.233	N
Extended Diestrus	1,000 ppm vs. Chamber Controls	0.498	N
Extended Diestrus	2,000 ppm vs. Chamber Controls	<0.001	–
Extended Metestrus	Overall	0.917	–
Extended Metestrus	500 ppm vs. Chamber Controls	1	–
Extended Metestrus	1,000 ppm vs. Chamber Controls	1	–
Extended Metestrus	2,000 ppm vs. Chamber Controls	0.362	–
Extended Proestrus	Overall	1	–
Extended Proestrus	500 ppm vs. Chamber Controls	1	–
Extended Proestrus	1,000 ppm vs. Chamber Controls	1	–
Extended Proestrus	2,000 ppm vs. Chamber Controls	1	–
Skipped Estrus	Overall	0.216	–
Skipped Estrus	500 ppm vs. Chamber Controls	0.154	N
Skipped Estrus	1,000 ppm vs. Chamber Controls	0.152	N
Skipped Estrus	2,000 ppm vs. Chamber Controls	0.539	N
Skipped Diestrus	Overall	1	–
Skipped Diestrus	500 ppm vs. Chamber Controls	1	–
Skipped Diestrus	1,000 ppm vs. Chamber Controls	1	–
Skipped Diestrus	2,000 ppm vs. Chamber Controls	1	–
Summary of Significant Groups			
Overall Tests	2,000 ppm vs. Chamber Controls	<0.001	–
Extended Estrus	2,000 ppm vs. Chamber Controls	0.008	N
Extended Diestrus	2,000 ppm vs. Chamber Controls	<0.001	–

[a]N indicates that the exposed group had fewer departures from normal than did the chamber control group. Having no N indicates that the exposed group had more departures from normal than did the chamber control group.

Table H-4. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	500 ppm	1,000 ppm	2,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	33.9 ± 0.59	36.9 ± 0.81**	38.6 ± 0.83**	36.8 ± 0.37**
L. Cauda epididymis	0.017 ± 0.0006	0.019 ± 0.0011	0.018 ± 0.0005	0.021 ± 0.0023
L. Epididymis	0.042 ± 0.0009	0.044 ± 0.0012	0.045 ± 0.0018	0.044 ± 0.0017
L. Testis	0.103 ± 0.0020	0.108 ± 0.0015	0.104 ± 0.0041	0.103 ± 0.0017
Spermatid measurements				
Spermatid heads (10^6/testis)	19.72 ± 1.004	22.31 ± 0.656	19.99 ± 1.324	17.72 ± 1.321
Spermatid heads (10^6/g testis)	191.87 ± 8.230	206.29 ± 7.450	192.82 ± 11.952	172.12 ± 12.644
Epididymal spermatozoal measurements				
Sperm motility (%)	79.9 ± 0.89	67.1 ± 3.33**	63.2 ± 1.42**	60.1 ± 1.31**
Sperm (10^6/cauda epididymis)	11.3 ± 0.55	14.8 ± 0.66*	14.2 ± 1.29	15.0 ± 1.15*
Sperm (10^6/g cauda epididymis)	679.0 ± 33.92	787.8 ± 37.63	794.9 ± 58.81	779.1 ± 65.92

*Significantly different ($P \leq 0.05$) from the chamber control group by Shirley's test.
**Significantly different ($P \leq 0.01$) from the chamber control group by Williams' test (body weights) or Shirley's test (sperm motility).
[a]Data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid measurements and sperm/g cauda epididymis).

Table H-5. Estrous Cycle Characterization for Female Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

	Chamber Control	500 ppm	1,000 ppm	2,000 ppm
n	10	10	10	10
Necropsy body wt (g)	28.2 ± 0.41	32.2 ± 0.72**	33.7 ± 0.79**	29.7 ± 0.74**
Number of estrous cycles	2.5 ± 0.22	2.2 ± 0.20	2.2 ± 0.13	2.4 ± 0.22
Estrous cycle length (days)	4.1 ± 0.10	4.5 ± 0.16	4.6 ± 0.14*	5.3 ± 0.65**
Estrous stages (% of cycle)				
Diestrus	36.9	32.5	28.8	30.0
Proestrus	0.0	2.5	4.4	6.9
Estrus	47.5	48.1	44.4	45.0
Metestrus	15.6	16.3	21.3	16.9

*Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's test.
**Significantly different ($P \leq 0.01$) from the chamber control group by Williams' test (body weights) or Dunn's test (estrous cycle length).
[a]Necropsy body weights, number of estrous cycles, and estrous cycle length data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunn's test (number of estrous cycles). Tests for equality of transition probability matrices among all groups and between the chamber control group and each exposed group indicated that all exposed groups spent significantly more time in extended estrus ($P \leq 0.001$) than the chamber control group.

Table H-6. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Mice Exposed to *p*-Chloro-α,α,α-trifluorotoluene by Inhalation for Three Months

Stage	Comparison	P value	Trend[a]
Overall Tests	Overall	<0.001	–
Overall Tests	500 ppm vs. Chamber Controls	<0.001	–
Overall Tests	1,000 ppm vs. Chamber Controls	<0.001	–
Overall Tests	2,000 ppm vs. Chamber Controls	<0.001	–
Extended Estrus	Overall	<0.001	–
Extended Estrus	500 ppm vs. Chamber Controls	<0.001	–
Extended Estrus	1,000 ppm vs. Chamber Controls	0.001	–
Extended Estrus	2,000 ppm vs. Chamber Controls	<0.001	–
Extended Diestrus	Overall	0.091	–
Extended Diestrus	500 ppm vs. Chamber Controls	0.311	–
Extended Diestrus	1,000 ppm vs. Chamber Controls	0.223	–
Extended Diestrus	2,000 ppm vs. Chamber Controls	0.061	–
Extended Metestrus	Overall	0.174	–
Extended Metestrus	500 ppm vs. Chamber Controls	0.223	–
Extended Metestrus	1,000 ppm vs. Chamber Controls	0.082	–
Extended Metestrus	2,000 ppm vs. Chamber Controls	0.607	–
Extended Proestrus	Overall	1	–
Extended Proestrus	500 ppm vs. Chamber Controls	1	–
Extended Proestrus	1,000 ppm vs. Chamber Controls	1	–
Extended Proestrus	2,000 ppm vs. Chamber Controls	1	–
Skipped Estrus	Overall	0.998	–
Skipped Estrus	500 ppm vs. Chamber Controls	1	–
Skipped Estrus	1,000 ppm vs. Chamber Controls	0.934	–
Skipped Estrus	2,000 ppm vs. Chamber Controls	0.869	–
Skipped Diestrus	Overall	0.988	–
Skipped Diestrus	500 ppm vs. Chamber Controls	0.805	–
Skipped Diestrus	1,000 ppm vs. Chamber Controls	0.934	–
Skipped Diestrus	2,000 ppm vs. Chamber Controls	0.804	–
Summary of Significant Groups			
Overall Tests	500 ppm vs. Chamber Controls	<0.001	–
Overall Tests	1,000 ppm vs. Chamber Controls	<0.001	–
Overall Tests	2,000 ppm vs. Chamber Controls	<0.001	–
Extended Estrus	500 ppm vs. Chamber Controls	<0.001	–
Extended Estrus	1,000 ppm vs. Chamber Controls	0.001	–
Extended Estrus	2,000 ppm vs. Chamber Controls	<0.001	–

[a]N indicates that the exposed group had fewer departures from normal than did the chamber control group. Having no N indicates that the exposed group had more departures from normal than did the chamber control group.

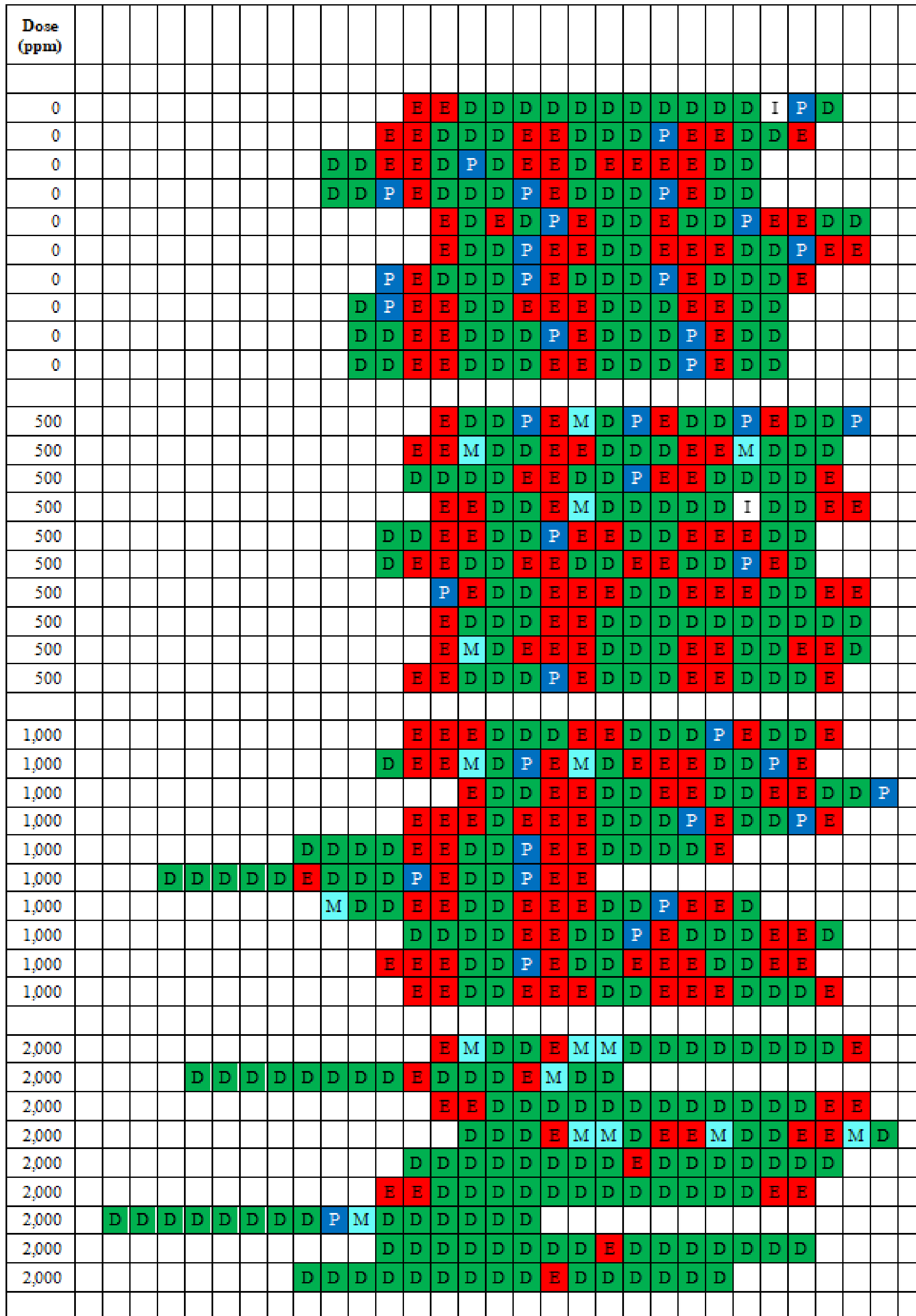

Figure H-1. Vaginal Cytology Plots for Female Rats in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

I = insufficient number of cells to determine stage, D = diestrus, P = proestrus, E = estrus, M = metestrus.

Dose (ppm)																													
0												E	E	M	D	E	E	M	D	E	E	M	D	E	E	M	D		
0										M	D	E	E	D	D	E	E	D	D	E	E	D	D	E	E				
0											D	E	E	M	D	E	E	M	D	E	E	M	D	E	E	M			
0						D	D	D	D	D	D	E	E	D	D	E	E	D	D	E	E								
0											D	E	E	M	D	E	E	M	D	E	E	D	D	E	E	D			
0													E	M	D	E	E	M	D	E	E	M	D	E	E	M	D	D	
0											D	E	E	M	D	E	E	M	D	E	E	D	D	E	E	M			
0											D	E	E	M	D	E	E	E	D	D	E	E	E	M	D	D			
0											D	E	E	M	D	E	M	D	D	E	E	D	D	E	E	D			
0										M	D	E	E	M	D	E	E	M	D	E	E	D	D	E	E				
500										E	E	E	M	D	E	E	E	D	D	E	E	E	M	D	P				
500													E	M	D	E	E	E	M	D	P	E	E	M	D	E	E	E	
500										D	E	E	E	D	D	E	E	E	M	D	E	E	E	D	D				
500											D	E	E	M	D	E	E	E	D	D	E	E	E	D	D	E			
500										M	D	E	E	M	D	E	E	M	D	E	E	M	D	E	E				
500										M	D	E	E	M	D	E	E	D	D	E	E	M	D	P	E				
500											D	E	E	M	D	E	M	D	D	E	E	D	D	E	E	D			
500													E	M	M	E	E	D	D	P	E	E	D	D	E	E	E	D	
500													E	M	M	E	E	M	D	E	E	M	D	E	E	M	I	E	
500													E	M	M	E	E	M	D	D	D	D	D	D	D	D	D	D	
1,000									M	D	D	E	E	D	D	E	E	D	D	E	E	M	D	E					
1,000													E	M	D	E	E	M	M	D	P	E	E	M	D	E	E	M	
1,000												E	E	M	D	D	E	D	D	I	E	E	E	D	D	P	E		
1,000												E	M	D	E	E	E	M	D	P	E	E	M	D	P	E	E		
1,000												D	D	M	M	E	E	E	D	D	E	E	M	D	E	E	D		
1,000												M	M	D	D	D	E	E	M	I	E	M	M	D	P	E	M		
1,000											E	E	M	D	D	E	E	M	D	D	E	E	M	D	P	E			
1,000												E	M	D	D	E	E	M	D	D	E	E	M	D	E	E	M		
1,000													E	M	D	E	E	M	M	D	E	E	E	M	D	E	E	E	
1,000										D	E	E	E	M	D	E	E	E	M	D	P	E	E	M	D				
2,000										E	M	P	E	M	P	E	E	M	D	E	E	E	M	D	E				
2,000													E	M	D	E	E	E	M	D	P	E	M	M	D	E	E	M	
2,000												E	M	D	E	E	E	D	D	P	E	E	M	D	I	E	D		
2,000													E	M	D	E	E	E	M	D	E	E	E	E	M	D	P	E	
2,000													E	M	D	E	E	M	D	E	E	M	D	E	E	D	D	P	
2,000													E	M	D	E	E	M	D	E	E	D	M	E	E	D	D	E	
2,000		D	E	E	E	D	D	M	D	I	D	D	D	D	D	E	M												
2,000										D	E	E	M	D	P	E	E	M	D	P	E	E	D	D	P				
2,000												E	M	D	E	E	E	M	D	P	E	E	M	D	P	E	E		
2,000												E	E	D	D	E	E	E	D	D	D	E	E	E	D	D	D		

Figure H-2. Vaginal Cytology Plots for Female Mice in the Three-month Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene

I = insufficient number of cells to determine stage, D = diestrus, P = proestrus, E = estrus, M = metestrus.

Appendix I. Chemical Characterization and Generation of Chamber Concentrations

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I.1. Procurement and Characterization of *p*-Chloro-α,α,α-trifluorotoluene

p-Chloro-α,α,α-trifluorotoluene was obtained from Special Materials Company (Cherry Hill, NJ, and New York, NY) in two lots. Lot 2009-01-05 was used in the 3-month studies and lot 2010-05-24 was used in the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Chemir Analytical Services (Maryland Heights, MO) and the study laboratory at Battelle Toxicology Northwest (Richland, WA). Reports on analyses performed in support of the *p*-chloro-α,α,α-trifluorotoluene studies are on file at the National Institute of Environmental Health Sciences.

Both lots were clear liquid, were identified as *p*-chloro-α,α,α-trifluorotoluene by the analytical chemistry laboratory using proton nuclear magnetic resonance (NMR) spectroscopy and by the study laboratory using Fourier transform infrared (FTIR) spectroscopy. NMR spectra were consistent with a *p*-chloro-α,α,α-trifluorotoluene standard (Sigma Aldrich, Lot 08507BO) and the structure of *p*-chloro-α,α,α-trifluorotoluene. FTIR spectra were consistent with the literature spectra[99] and the structure of *p*-chloro-α,α,α-trifluorotoluene. FTIR and proton NMR spectra are presented in Figure I-1 and Figure I-2, respectively.

For lots 2009-01-05 and 2010-05-24, the analytical chemistry laboratory determined the water content using Karl Fischer titration, conducted elemental analyses to determine the carbon and hydrogen content, and for lot 2010-05-24, determined the sulfur content using inductively coupled plasma (ICP) spectroscopy. The purity was determined by the study laboratory using gas chromatography (GC) with flame ionization detection (FID). The presence of 3,4-dihydroxybenzoic acid was determined using high performance liquid chromatography (HPLC) with an ultraviolet light/visible light detector. The HPLC system consisted of an Agilent Zorbax Eclipse XDB-Phenyl column (4.6 mm × 150 mm), with monitoring at 240 nm, and mobile phases of water:methanol (10:90, 10:90, 0:100, and 10:90) with a flow rate of 1 mL/minute.

Karl Fischer titration indicated a water content of 82.4 (lot 2009-01-05) or 109.8 (lot 2010-05-24) ppm. Elemental analyses of both lots for carbon and hydrogen were consistent with theoretical values. ICP analysis of lot 2010-05-24 indicated <0.002% sulfur. GC/FID by system A (Table I-1), relative to an independent standard purchased from Aldrich Chemical Co. (St. Louis, MO), indicated a relative purity of 100.3% (lot 2009-01-05) or 99.6% (lot 2010-05-24). GC/FID by system B indicated one major peak (peak area percents of 99.68% for lot 2009-01-05, 99.57% for lot 2010-05-24) and two impurities with areas ≥0.1% relative to the major peak area: 3-chlorobenzotrifluoride (0.2%, lot 2009-01-05; 0.3%, lot 2010-05-24) and 2-chlorobenzotrifluoride (0.1%, lot 2009-01-05; 0.2%, lot 2010-05-24). No 3,4-dihydroxybenzoic acid was detected by HPLC. The overall purity of each lot was determined to be greater than 99.5%.

The bulk chemical was stored in the original shipping containers at room temperature. To ensure stability, periodic reanalyses of the bulk chemical were performed during the 3-month and 2-year studies by the study laboratory using the same GC/FID (systems A and B) assays used in the initial bulk chemical purity assays, and no degradation of the bulk chemical was detected.

I.2. Vapor Generation and Exposure System

A diagram of the vapor generation and delivery system used in the studies is shown in Figure I-3. *p*-Chloro-α,α,α-trifluorotoluene was pumped from a stainless steel 8 gallon reservoir into a heated vaporizer glass column filled with glass beads and completely wrapped with heat tape. A waste collection flask was connected to the bottom of the column to collect residual chemical not completely vaporized in the generator column. Preheated nitrogen entered the column from below, vaporized the chemical, and carried the vapor from the generator cabinet to the distribution manifold through a heated chemical transport line. The nitrogen-chemical mixture was diluted with heated air before entering the distribution manifold. Concentration in the manifold was determined by the chemical pump rate, dilution air flow rates, and nitrogen flow rate. Pressure in the distribution manifold was kept fixed to ensure constant flows through the manifold and into all chambers as the flow of vapor to each chamber was adjusted.

Individual heated (approximately 130°F) Teflon® delivery lines carried the vapor from the manifold to three-way exposure valves at the chamber inlets. The exposure valves diverted vapor delivery to the exposure chamber exhaust until the generation system stabilized and exposure could proceed. The flow rate to each chamber was controlled by a metering valve at the manifold. To initiate exposure, the chamber exposure valves were rotated to allow the *p*-chloro-α,α,α-trifluorotoluene vapor to flow to each exposure chamber inlet duct where it was diluted with conditioned chamber air to achieve the desired exposure concentration. Conditioned air was defined as the mix of air derived from each exposure chamber's wet and dry air duct supplies. The temperature of the resultant mixture of air was adjusted by passage over a temperature-controlled radiator after sequential treatment with Purafil, charcoal, and HEPA filters. Target dewpoint temperatures of the wet and dry ducts were 60° and 40°F, respectively. Air for the ducts was obtained from the building air supply and was either passed over chillers to lower the dewpoint (dry duct) or injected with steam to raise it (wet duct).

The study laboratory designed the inhalation exposure chamber (Lab Products, Inc., Seaford, DE) so that uniform vapor concentrations could be maintained throughout the chamber with the catch pans in place. The total active mixing volume of each chamber was 1.7 m^3. A small particle detector (Model 3022A; TSI, Inc., St. Paul, MN) was used with and without animals in the exposure chambers to ensure that *p*-chloro-α,α,α-trifluorotoluene vapor, and not aerosol, was produced. No particle counts above the minimum resolvable level (approximately 200 particles/cm^3) were detected.

I.3. Vapor Concentration Monitoring

Summaries of the chamber vapor concentrations are given in Table I-2 and Table I-3. Chamber and room concentrations of *p*-chloro-α,α,α-trifluorotoluene were monitored by an on-line gas chromatograph (system C, Table I-1). Samples were drawn from each exposure chamber approximately every 20 minutes for the 3-month and 2-year studies during each 6-hour exposure period using Hastelloy®-C stream-select and gas-sampling valves (VALCO Instruments Company, Houston, TX) in a separate, heated oven. The sample lines composing each sample loop were made from Teflon® tubing and were connected to the exposure chamber relative humidity sampling lines at a location close to the gas chromatograph. A vacuum regulator maintained a constant vacuum in the sample loop to compensate for variations in sample line

pressure. An in-line flow meter between the vacuum regulator and the gas chromatograph allowed digital measurement of sample flow.

The on-line gas chromatograph was checked throughout each exposure day for instrument drift against an on-line standard vapor of 4-chlorotoluene in nitrogen supplied by a standard generator (Kin-Tek; Precision Calibration Systems, La Marque, TX). The on-line gas chromatograph was recalibrated as required to meet acceptance criteria. Calibration was performed by a comparison of chamber concentration data to data from grab samples collected with sorbent tubes (ORBO™-52; Supelco Inc., Bellefonte, PA), extracted with toluene containing an internal standard of 4-chlorotoluene and analyzed using an off-line gas chromatograph equipped with a FID (system D). Known volumes of chamber atmosphere were sampled at a constant flow rate ensured by a calibrated critical orifice. The off-line gas chromatograph was calibrated with gravimetrically prepared standard solutions of the test chemical containing 4-chlorotoluene as an internal standard in toluene.

I.4. Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined with and without animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 9.2 minutes. For rats and mice in the 3-month studies, T_{90} values ranged from 8 to 15 minutes without animals present and from 10 to 15 minutes with animals; T_{10} values were 8 to 11 minutes without animals present and 9 to 12 minutes with animals. For rats and mice in the 2-year studies, T_{90} values ranged from 9 to 10 minutes without animals present and from 10 to 12 minutes with animals; T_{10} values ranged from 9 to 10 minutes without animals present and from 11 to 13 minutes with animals. A T_{90} value of 15 minutes was selected for the 3-month studies, and a T_{90} value of 12 minutes was selected for the 2-year studies.

The uniformity of *p*-chloro-α,α,α-trifluorotoluene vapor concentration in the inhalation exposure chambers without animals present was evaluated before the 3-month and 2-year studies began; in addition, concentration uniformity with animals present in the chambers was measured once during the 3-month studies and approximately every 3 months during the 2-year studies. The vapor concentration was measured using the on-line gas chromatograph (system C, Table I-1) with the stream-selection valve fixed in one position to allow continuous monitoring from a single input line. Prior to the 3-month and 2-year studies, concentrations were measured at 12 chamber positions, one in front and one in back for each of the six possible animal cage unit positions per chamber. During the 3-month and 2-year studies, concentrations were measured at the regular monitoring port and from sample ports at levels where animals were present. Chamber concentration uniformity was maintained throughout the studies.

The persistence of *p*-chloro-α,α,α-trifluorotoluene in the chambers after vapor delivery ended was determined by monitoring the vapor concentration in the 2,000 ppm chambers in the 3-month studies and the 1,000 ppm rat and 400 ppm mouse chambers in the 2-year studies with and without animals present in the chambers. In the 3-month studies, the concentration decreased to 1% of the target concentration within 24 minutes without animals present and within

30 minutes with animals present. For the 2-year rat studies, the concentration decreased to 1% of the target concentration within 24 minutes without animals present and within 46 minutes with animals present. For the 2-year mouse studies, the concentration decreased to 1% of the target concentration within 23 minutes without animals present and within 31 minutes with animals present.

Samples of the test atmosphere from the distribution lines and the low and high exposure concentration chambers for each species were collected prior to the study without animals present and at the beginning and end of one generation day with animals present during the 3-month and 2-year studies. The atmosphere samples were collected with adsorbent gas sampling tubes containing silica gel (ORBO™-52; Supelco, Inc.) followed by a tube containing activated coconut charcoal (ORBO™-32), and extracted with methylene chloride. Additional samples were collected from the generator reservoir, and all of the samples were analyzed using GC/FID by system B to measure the stability and purity of *p*-chloro-α,α,α-trifluorotoluene in the generation and delivery system. To assess whether impurities or degradation products co-eluted with *p*-chloro-α,α,α-trifluorotoluene or the solvent, a second GC/FID analysis of samples from the distribution line, 125 ppm and 2,000 ppm chambers (3-month studies), 100 ppm and 1,000 ppm rat chambers (2-year studies), 100 ppm and 400 ppm mouse chambers (2-year studies), and the generator reservoir was performed using system B. 3,4-Dihydroxybenzoic acid was measured in atmosphere samples collected in ORBO™-52 tubes followed by ORBO™-32 tubes prior to and during the 3-month and 2-year studies.

No evidence of degradation of *p*-chloro-α,α,α-trifluorotoluene was noted in any part of the exposure system in any of the samples collected prior to or during the 3-month and 2-year studies. During the 3-month studies, 3-chlorobenzotrifluoride (0.2%) was the only impurity detected in atmosphere or generator reservoir samples with an area greater than 0.1% of the total peak area. During the 2-year studies, 3-chlorobenzotrifluoride (0.3%) and 2-chlorobenzotrifluoride (0.2%) were the only impurities detected in the atmosphere or generator reservoir samples with areas greater than 0.1% of the total peak area. No 3,4-dihydroxybenzoic acid was detected in the exposure chamber atmosphere samples at ≥ 0.1%.

Table I-1. Gas Chromatography Systems Used in the Inhalation Studies of *p*-Chloro-α,α,α-trifluorotoluene[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	DB-WAXETR, 30 m × 530 µm, 1 µm film (J&W Scientific, Folsom, CA)	Helium at 6 psi head pressure	40°C for 1 minute, then 10°C/minute to 120°C, then 25°C/minute to 230°C
System B			
Flame ionization	DB-5MS UI, 30 m × 250 µm, 0.25 µm film (J&W Scientific)	Helium at 12 psi head pressure	40°C for 4 minutes, then 8°C/minute to 260°C, held for 5 minutes
System C			
Flame ionization	Rtx®-5, 15 m × 0.53 mm, 1.5 µm film (Restek, Bellefonte, PA)	Nitrogen at 25 mL/minute	Isothermal at 80°C
System D			
Flame ionization	DB-WAXETR, 30 m × 530 µm, 1 µm film (J&W Scientific)	Helium at 6 psi head pressure	80°C for 5 minutes, then 10°C/minute to 120°C, then 25°C/minute to 230°C, held for 1 minute

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

Table I-2. Summary of Chamber Concentrations in the Three-month Inhalation Studies of *p*-Chloro-α,α,α-trifluorotoluene

	Total Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers			
	125	1,196	124.6 ± 2.9
	250	1,199	249.9 ± 5.0
	500	1,202	495.4 ± 11.8
	1,000	1,203	996.2 ± 21.0
	2,000	1,215	1,984.9 ± 41.2
Mouse Chambers			
	125	1,233	124.6 ± 2.9
	250	1,236	250.0 ± 5.0
	500	1,239	495.2 ± 11.8
	1,000	1,240	996.2 ± 21.0
	2,000	1,252	1,984.4 ± 40.9

[a]Mean ± standard deviation.

Table I-3. Summary of Chamber Concentrations in the Two-year Inhalation Studies of *p*-Chloro-α,α,α-trifluorotoluene

	Total Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers			
	100	7,905	100 ± 2
	300	7,967	300 ± 4
	1,000	8,031	999 ± 15
Mouse Chambers			
	100	8,081	100 ± 3
	200	7,718	201 ± 3
	400	7,841	401 ± 7

[a]Mean ± standard deviation.

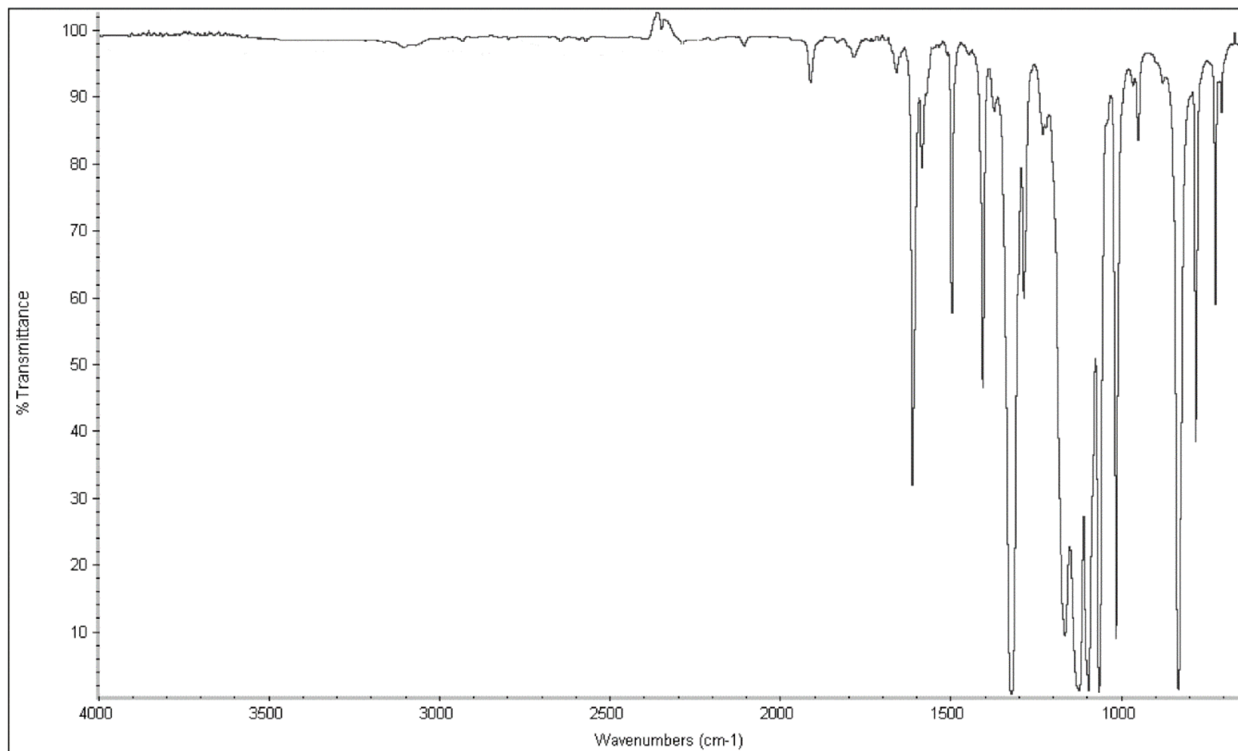

Figure I-1. Fourier Transform Infrared Absorption Spectrum of *p*-Chloro-α,α,α-trifluorotoluene

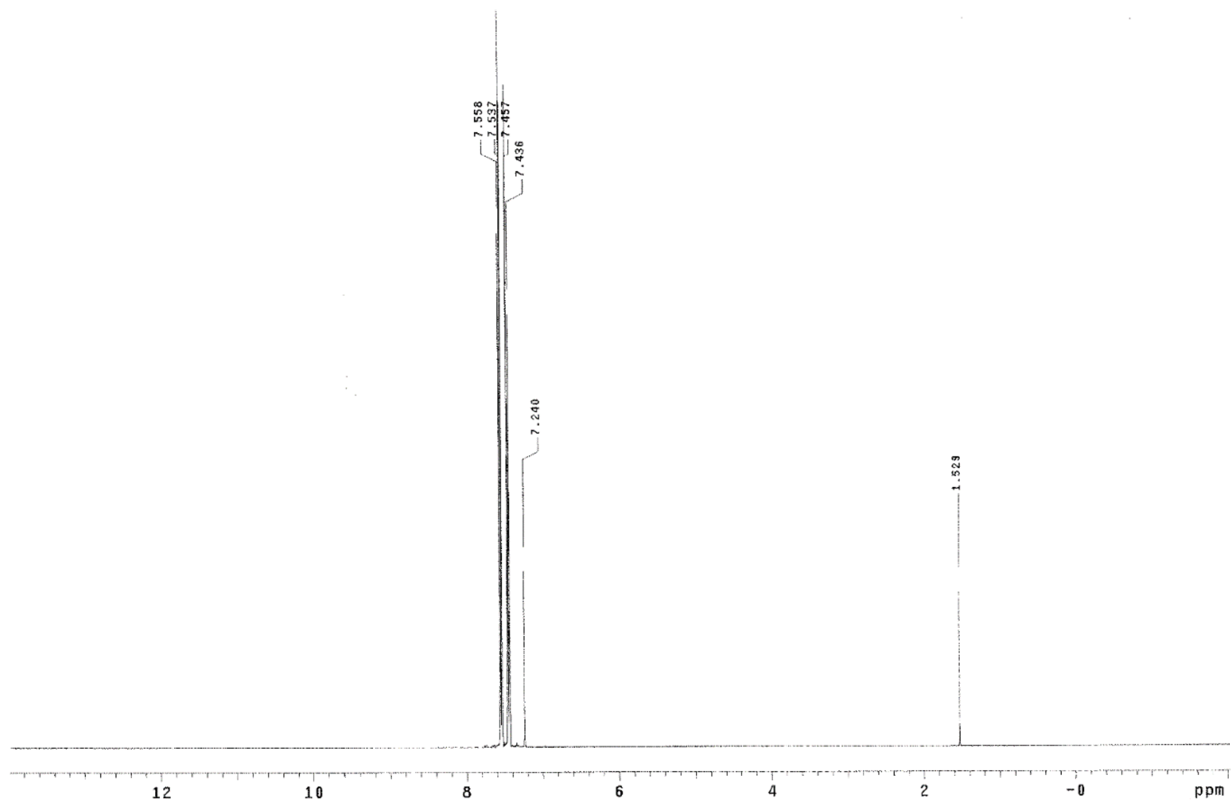

Figure I-2. Proton Nuclear Magnetic Resonance Spectrum of *p*-Chloro-α,α,α-trifluorotoluene

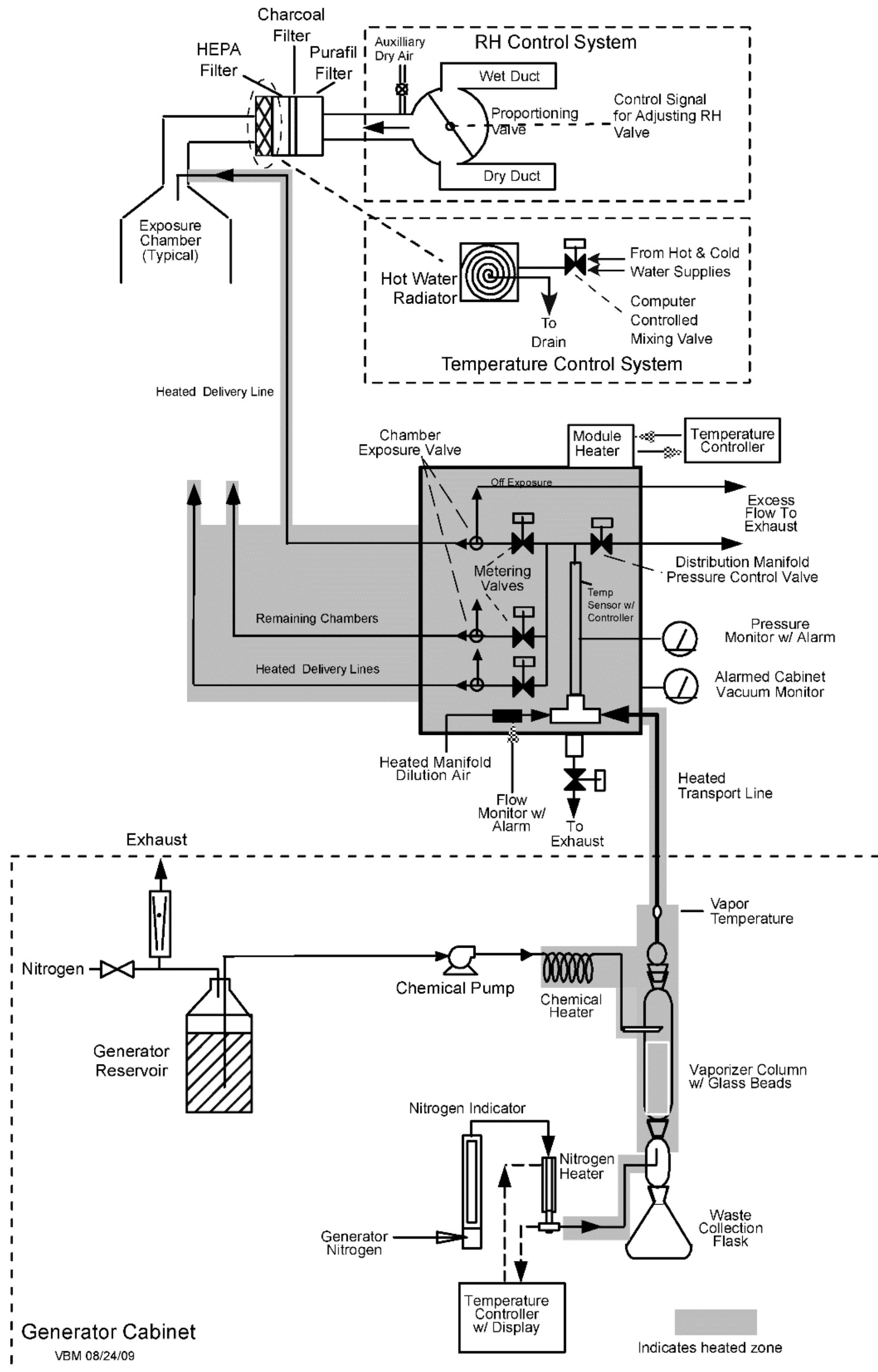

Figure I-3. Schematic of the Vapor Generation and Delivery System in the Inhalation Studies of *p*-Chloro-α,α,α-trifluorotoluene

Appendix J. Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration

Tables

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Table J-1. 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 J-2. 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	–
α-Pantothenic acid	10 mg	*α*-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 J-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.7 ± 0.53	13.9–16.5	29
Crude fat (% by weight)	8.6 ± 0.33	8.0–9.2	29
Crude fiber (% by weight)	9.6 ± 0.61	8.4–11.1	29
Ash (% by weight)	4.9 ± 0.13	4.7–5.2	29
Amino Acids (% of total diet)			
Arginine	0.794 ± 0.070	0.67–0.97	26
Cystine	0.220 ± 0.022	0.15–0.25	26
Glycine	0.700 ± 0.038	0.62–0.80	26
Histidine	0.344 ± 0.074	0.27–0.68	26
Isoleucine	0.546 ± 0.041	0.43–0.66	26
Leucine	1.092 ± 0.063	0.96–1.24	26
Lysine	0.700 ± 0.110	0.31–0.86	26
Methionine	0.408 ± 0.043	0.26–0.49	26
Phenylalanine	0.621 ± 0.048	0.47–0.72	26
Threonine	0.508 ± 0.040	0.43–0.61	26
Tryptophan	0.153 ± 0.028	0.11–0.20	26
Tyrosine	0.413 ± 0.063	0.28–0.54	26
Valine	0.663 ± 0.040	0.55–0.73	26
Essential Fatty Acids (% of total diet)			
Linoleic	3.92 ± 0.307	2.99–4.55	26
Linolenic	0.31 ± 0.030	0.21–0.35	26
Vitamins			
Vitamin A (IU/kg)	3,766 ± 66	2,110–5,450	29
Vitamin D (IU/kg)	1,000[a]	–	–
α-Tocopherol (ppm)	77 ± 24.82	7.81–124.0	26
Thiamine (ppm)[b]	8.3 ± 1.50	6.4–12.3	29
Riboflavin (ppm)	8.1 ± 2.91	4.20–17.50	26
Niacin (ppm)	78.9 ± 8.52	66.4–98.2	26
Pantothenic acid (ppm)	26.7 ± 11.63	17.4–81.0	26
Pyridoxine (ppm)[b]	9.7 ± 2.09	6.44–14.3	26
Folic acid (ppm)	1.59 ± 0.45	1.15–3.27	26
Biotin (ppm)	0.32 ± 0.10	0.20–0.704	26
Vitamin B_{12} (ppb)	54.9 ± 37.2	18.3–174.0	26
Choline (ppm)[b]	2,665 ± 631	1,160–3,790	26

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.892 ± 0.038	0.810–0.973	29
Phosphorus (%)	0.558 ± 0.020	0.520–0.594	29
Potassium (%)	0.669 ± 0.030	0.626–0.733	26
Chloride (%)	0.386 ± 0.037	0.300–0.474	26
Sodium (%)	0.193 ± 0.024	0.160–0.283	26
Magnesium (%)	0.216 ± 0.057	0.185–0.490	26
Sulfur (%)	0.170 ± 0.029	0.116–0.209	14
Iron (ppm)	190.5 ± 38.0	135–311	26
Manganese (ppm)	50.7 ± 9.72	21.0–73.1	26
Zinc (ppm)	58.2 ± 26.89	43.3–184.0	26
Copper (ppm)	7.44 ± 2.60	3.21–16.3	26
Iodine (ppm)	0.514 ± 0.195	0.158–0.972	26
Chromium (ppm)	0.674 ± 0.265	0.330–1.380	26
Cobalt (ppm)	0.235 ± 0.157	0.094–0.864	26

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

Table J-4. Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.21 ± 0.052	0.15–0.42	29
Cadmium (ppm)	0.07 ± 0.084	0.015–0.50	29
Lead (ppm)	0.2 ± 0.252	0.06–1.19	29
Mercury (ppm)	<0.02	–	29
Selenium (ppm)	0.17 ± 0.057	0.029–0.34	29
Aflatoxins (ppb)	<5.00	–	29
Nitrate nitrogen (ppm)[c]	15.48 ± 6.051	10.0–28.0	29
Nitrite nitrogen (ppm)[c]	0.61	–	29
BHA (ppm)[d]	<1.0	–	29
BHT (ppm)[d]	<1.0	–	29
Aerobic plate count (CFU/g)	15.86 ± 29.70	10–170	29
Coliform (MPN/g)	3.0 ± 0.0	3.0–3.0	29
Escherichia coli (MPN/g)	<10	–	29
Salmonella (MPN/g)	Negative	–	29
Total nitrosoamines (ppb)[e]	10.22 ± 4.99	1.6–19.9	29
N-Nitrosodimethylamine (ppb)[e]	2.4 ± 1.61	0.0–7.4	29
N-Nitrosopyrrolidine (ppb)[e]	7.99 ± 4.82	1.01–18.6	29
Pesticides (ppm)			
α-BHC	<0.01	–	29
β-BHC	<0.02	–	29
γ-BHC	<0.01	–	29
δ-BHC	<0.01	–	29
Heptachlor	<0.01	–	29
Aldrin	<0.01	–	29
Heptachlor epoxide	<0.01	–	29
DDE	<0.01	–	29
DDD	<0.01	–	29
DDT	<0.01	–	29
HCB	<0.01	–	29
Mirex	<0.01	–	29
Methoxychlor	<0.05	–	29
Dieldrin	<0.01	–	29
Endrin	<0.01	–	29
Telodrin	<0.01	–	29

	Mean ± Standard Deviation[b]	Range	Number of Samples
Chlordane	<0.05	–	29
Toxaphene	<0.10	–	29
Estimated PCBs	<0.20	–	29
Ronnel	<0.01	–	29
Ethion	<0.02	–	29
Trithion	<0.05	–	29
Diazinon	<0.10	–	29
Methyl chlorpyrifos	0.137 ± 0.131	0.020–0.553	29
Methyl parathion	<0.02	–	29
Ethyl parathion	<0.02	–	29
Malathion	0.101 ± 0.102	0.020–0.395	29
Endosulfan I	<0.01	–	29
Endosulfan II	<0.01	–	29
Endosulfan sulfate	<0.03	–	29

[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 K. Sentinel Animal Program

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K.1. Methods

Rodents used in 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 test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or dosed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

Blood samples were collected from each animal and allowed to clot and the serum was separated. Additionally, fecal samples were collected and tested for *Helicobacter* species. All samples were processed appropriately and evaluated for the presence of pathogens. Samples were tested in-house or sent to the IDEXX BioResearch (formerly, at the time of testing, Research Animal Diagnostic Laboratory (RADIL)), University of Missouri (Columbia, MO). The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Blood was collected from five animals per sex per time point except for the 18-month collection which consisted of 4 male rats and 5 female rats.

Table K-1. Laboratory Methods and Agents Tested for in the Sentinel Animal Program

Method and Test	Time of Collection
Rats	
Three-month Study	
Multiplex Fluorescent Immunoassay	
Kilham’s rat virus (KRV)	3 weeks, study termination
Mycoplasma pulmonis	3 weeks, study termination
Parvo NS-1	3 weeks, study termination
Pneumonia virus of mice (PVM)	3 weeks, study termination
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	3 weeks, study termination
Rat minute virus (RMV)	3 weeks, study termination
Rat parvovirus (RPV)	3 weeks, study termination
Rat theilovirus (RTV)	3 weeks, study termination
Sendai	3 weeks, study termination
Theiler’s murine encephalomyelitis virus (TMEV)	3 weeks, study termination
Toolan’s H-1 virus (H-1)	3 weeks, study termination
Two-year Study	
Multiplex Fluorescent Immunoassay	
Encephalitozoon cuniculi	6 months
H-1	3 weeks, 6, 12, and 18 months, study termination

Method and Test	Time of Collection
KRV	3 weeks, 6, 12, and 18 months, study termination
M. pulmonis	3 weeks, 6, 12, and 18 months, study termination
Parvo NS-1	3 weeks, 6, 12, and 18 months, study termination
PVM	3 weeks, 6, 12, and 18 months, study termination
RCV/SDA	3 weeks, 6, 12, and 18 months, study termination
RMV	3 weeks, 6, 12, and 18 months, study termination
RPV	3 weeks, 6, 12, and 18 months, study termination
RTV	3 weeks, 6, 12, and 18 months, study termination
Sendai	3 weeks, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Pneumocystis carnii	6 months
M. pulmonis	12 months
RTV	12 months
Mice	
Three-month Study	
Multiplex Fluorescent Immunoassay	
Ectromelia virus	3 weeks, study termination
Epizootic diarrhea of infant mice (EDIM)	3 weeks, study termination
Lymphocytic choriomeningitis virus (LCMV)	3 weeks, study termination
M. pulmonis	3 weeks, study termination
Mouse hepatitis virus (MHV)	3 weeks, study termination
Mouse norovirus (MNV)	3 weeks, study termination
Mouse parvovirus (MPV)	3 weeks, study termination
Minute virus of mice (MVM)	3 weeks, study termination
Parvo NS-1	3 weeks, study termination
PVM	3 weeks, study termination
Reovirus 3 (REO)	3 weeks, study termination
Theiler's murine encephalomyelitis virus — mouse poliovirus, strain GDVII (TMEV GDVII)	3 weeks, study termination
Sendai	3 weeks, study termination
Two-year Study	
Multiplex Fluorescent Immunoassay	
Ectromelia virus	3 weeks, 6, 12, and 18 months, study termination
EDIM	3 weeks, 6, 12, and 18 months, study termination
LCMV	3 weeks, 6, 12, and 18 months, study termination
M. pulmonis	3 weeks, 6, 12, and 18 months, study termination

Method and Test	Time of Collection
MHV	3 weeks, 6, 12, and 18 months, study termination
MNV	3 weeks, 6, 12, and 18 months, study termination
MPV	3 weeks, 6, 12, and 18 months, study termination
MVM	3 weeks, 6, 12, and 18 months, study termination
Parvo NS-1	3 weeks, 6, 12, and 18 months, study termination
PVM	3 weeks, 6, 12, and 18 months, study termination
REO	3 weeks, 6, 12, and 18 months, study termination
TMEV GDVII	3 weeks, 6, 12, and 18 months, study termination
Sendai	3 weeks, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Sendai	18 months
Polymerase Chain Reaction	
Helicobacter species	18 months

K.2. Results

All test results were negative.

Appendix L. Evaluation of *Hras* and *Ctnnb1* Mutations in Hepatocellular Carcinomas from B6C3F1/N Mice Chronically Exposed To p-Chloro-α,α,α-trifluorotoluene

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L.1. Introduction

Hepatocellular adenoma and hepatocellular carcinoma (HCC) are the most common primary liver tumors that arise either spontaneously or due to chemical exposure in the B6C3F1/N mice and occur more commonly in males than in females[100]. Evaluation of genetic mutations in cancer genes from HCCs from control and exposed groups can provide some insight into the mechanisms of chemical-induced carcinogenesis. Previous studies have shown that *Ctnnb1* (β-catenin) mutations and *Hras* mutations are common in mouse hepatocellular tumors[72; 101-103]. Examination of genetic mutations in the hepatocellular tumors of mice resulting from chronic whole body inhalation exposure to *p*-chloro-α,α,α-trifluorotoluene might provide some understanding of *p*-chloro-α,α,α-trifluorotoluene-induced hepatocellular tumorigenesis.

L.2. Materials and Methods

L.2.1. Animals and Tissue Sampling

HCCs arising either spontaneously or due to chemical exposure as well as chamber control normal liver samples from B6C3F1/N mice were obtained from the current *p*-chloro-α,α,α-trifluorotoluene 2-year bioassay. Male and female B6C3F1/N mice were exposed by whole body inhalation to *p*-chloro-α,α,α-trifluorotoluene at concentrations of 0, 100, 200, or 400 ppm for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 104 to 105 weeks. At necropsy, hepatocellular tumors were fixed in 10% neutral buffered formalin and processed into paraffin blocks, sectioned, and stained with hematoxylin and eosin for microscopic analysis. The formalin-fixed paraffin-embedded (FFPE) normal non-tumor liver tissue from chamber controls (n = 10; five males, five females) and HCCs from chamber control (spontaneous HCC) (n = 15; eight males, seven females) and *p*-chloro-α,α,α-trifluorotoluene-exposed mice (n = 62) (100 ppm, 10 males and eight females; 200 ppm, 10 males and 10 females; 400 ppm, 12 males and 12 females) were used for mutation analyses. The HCCs chosen for molecular biology analysis were based on their overall size and viability (minimal to no necrosis, autolysis, or hemorrhage observed microscopically) in order to maximize the amount and quality of DNA obtained from FFPE tissue sections. DNA quality was measured using a NanoDrop® spectrophotometer (Thermo Fischer Scientific, Inc., Wilmington, DE) to calculate the ratio of absorbances at 260 and 280 nm, with Qubit 3.0 for double stranded DNA mass, and DNA samples with a purity range of 1.7 to 2.0 were used for analysis.

L.2.2. DNA Extraction, Polymerase Chain Reaction (PCR), Sanger Sequencing, and Mutation Analysis

HCCs representing all *p*-chloro-α,α,α-trifluorotoluene-exposed groups (n = 62) and spontaneous HCCs from B6C3F1/N mice from chamber controls (n = 15) were evaluated for hot-spot mutations in *Hras* and *Ctnnb1* genes that are relevant in hepatocellular carcinogenesis. In addition, age-matched non-tumor livers from chamber control mice (n = 10, five males and five females) were also analyzed for mutations. FFPE sections at 10 µm thickness were collected into screwcap tubes for DNA extraction. DNA was isolated from these FFPE-dissected tissue sections with the DNeasy® Blood and Tissue Kit (QIAGEN, Valencia, CA). Amplification reactions were carried out by a semi-nested PCR using primer sets designed for *Hras* and *Ctnnb1* genes for mice (Table L-1). Controls lacking template DNA were run with all sets of reactions.

PCR products were purified using an ExoSAP PCR purification protocol. The purified PCR products were cycled with a Terminal Ready Reaction Mix-BigDye® (PerkinElmer Applied Biosystems, Foster City, CA), and the extension products were purified with the DyeEx 2.0 Spin Kit (QIAGEN). The lyophilized PCR products were sequenced with an automatic sequencer (PerkinElmer Applied Biosystems ABI Model 3100). The resulting electropherograms were compared to identify mutations in HCCs from the chamber control groups and *p*-chloro-α,α,α-trifluorotoluene-exposed groups. The mutations were confirmed by sequencing with both forward and reverse primers, and the positive mutations were verified by repeat analysis, starting from amplification of the original DNA extracts.

Table L-1. Primers Used to Amplify the Hot-Spot Regions of Mouse *Hras* and *Ctnnb1* Genes

Exon	Codon	Primer	Strand	Sequence
2	*Hras*-61	MH61OS	Sense	5′-CCACTAAGCCTGTTGTGTTTTGCAG-3′
		MH611s	Sense	5′-GGACTCCTAGCGGAAACAGG-3′
		MH61OA	Antisense	5′-CTGTACTGATGGATGTCCTCGAAGGA-3′
		MH611A	Antisense	5′-GGTGTTGTTGATGGCAAATACA-3′
3	*Ctnnb1*-8-55	MBCF17130	Sense	5′-GATGGAGTTGGACATGGC-3′
		MBCOR17294	Antisense	5′-ACTTGGGAGGTGTCAACA-3′
		MBCIR17257	Antisense	5′-TTCTTCCTCAGGGTTGCC-3′

L.2.3. Statistical Analysis of Mutation Incidences in Hepatocellular Tumors

To compare total mutation incidences in each exposed group to the incidences in the chamber control groups, one-sided Fisher exact test was used. Exact one-sided Cochran-Armitage trend test was used to test for exposure concentration-related trends in the incidences of mutations across all exposure groups.

L.3. Results

In this study, the incidence of *Hras* mutations in mouse HCCs examined from *p*-chloro-α,α,α-trifluorotoluene-exposed groups was 37% (23/62) and were located within codon 61 mainly C to A or A to T transversions (Table L-2). There were significant trend and pairwise differences for *Hras* mutation incidence in the negative direction between HCCs occurring either spontaneously in chamber controls or resulting from chronic exposure to *p*-chloro-α,α,α-trifluorotoluene. There were no statistically significant differences in *Ctnnb1* mutations in mouse HCCs resulting from chronic exposure to *p*-chloro-α,α,α-trifluorotoluene compared to the chamber control group. Four of the HCCs arising spontaneously harbored *Ctnnb1* mutations, all of which were point mutations in codons 15-46 and contained a mixture of transitions and transversions similar to those observed in the HCCs from *p*-chloro-α,α,α-trifluorotoluene-exposed groups. The deletion of codons 27-35 were noted only in the HCCs (n = 5) resulting from the *p*-chloro-α,α,α-trifluorotoluene-exposed groups and none of the spontaneous HCCs had deletions in *Ctnnb1* gene. There were no differences in the incidences of *Hras* or *Ctnnb1* mutations in HCCs from male and female mice (data not presented) and hence the combined data from both male and female mice are presented in Table L-2.

Table L-2. Summary of *Hras* and *Ctnnb1* Mutations in Non-tumor Liver Tissue and Hepatocellular Carcinomas (HCCs) from B6C3F1/N Mice in the Two-year Inhalation Study of *p*-Chloro-α,α,α-trifluorotoluene[a]

Tissue	Dose (ppm)	Mutation Frequency		*Hras* Codon 61 (CAA, Glu)			*Ctnnb1* (Codon 15-46)	
		Hras[b] (Codon 61)	*Ctnnb1*[b] (Codon 15-46)	AAA (Lys)	GCA (Arg)	CTA, CTT (Leu)	Point mutation	Deletion of Codon 27-35
Non-tumor Liver	0	0/10 (0)	0/10 (0)	0	0	0	0	0
HCCs	0	11/15 (73)#N	4/15 (27)	5	3	3	4	0#
	100	7/18 (39)	5/18 (28)	2	3	2	5	0
	200	9/20 (45)	10/20 (50)	4	3	2	8	2
	400	7/24 (29)**	6/24 (25)	4	2	1	3	3
Historical Spontaneous HCCs[c]		289/545 (53)	5/111 (4.5)	174	83	32	5	0
p-Chloro-α,α,α-trifluorotoluene-treated combined HCCs		23/62 (37)	21/62 (34)	10	8	5	16	5

**Significantly different ($P < 0.01$) from the spontaneous hepatocellular carcinomas (HCCs) in the chamber controls by the Fisher exact test.

#Significant ($P < 0.05$) exposure concentration-related trend. N indicates a negative trend.

[a]Male and female B6C3F1/N mice were exposed to 0, 100, 200, or 400 ppm p-chloro-α,α,α-trifluorotoluene by whole body inhalation for 2 years. Silent mutations were not included. Non-tumor Liver: 0 ppm (5 males + 5 females); HCCs: 0 ppm (8 males + 7 females), 100 ppm (10 males + 8 females), 200 ppm (10 males + 10 females), 400 ppm (12 males + 12 females).

[b]Number of tissues with mutations/number of tissues assayed (% with mutation).

[c]Historical database for *Hras* and *Ctnnb1* mutations in spontaneous HCCs (Sills et al.[104]; Hayashi et al.[105]; unpublished data).

L.4. Discussion

The *Hras* mutations in HCCs arising either spontaneously or due to chemical exposure are frequently localized within codon 61[72; 73]. There was a statistically significant trend and pairwise differences in the negative direction for *Hras* mutations between spontaneous HCCs in chamber control mice and HCCs resulting from chronic exposure to *p*-chloro-α,α,α-trifluorotoluene. *p*-Chloro-α,α,α-trifluorotoluene has been shown to increase CYP1A1 and CYP1A2 activity by twofold and CYP2B activity by fivefold[22]. CYP2B activation via constitutive androstane receptor is a well-known mechanism of tumor promotion activity in the liver of rodents[92]. *p*-Chloro-α,α,α-trifluorotoluene is nongenotoxic (Ames assay negative, chromosomal aberration assay negative) and may not directly cause mutations and initiate carcinogenesis. However, further mechanistic studies are needed to better understand *p*-chloro-α,α,α-trifluorotoluene-induced hepatocellular carcinogenesis.

Appendix M. Summary of Peer Review Panel Comments

On July 13, 2017, the draft Technical Report on the toxicology and carcinogenesis studies of *p*-chloro-α,α,α-trifluorotoluene received public review by the National Toxicology Program's Technical Reports Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. G.K. Roberts, NIEHS, introduced the toxicology and carcinogenicity studies of *p*-chloro-α,α,α-trifluorotoluene by discussing the uses of the chemical and the rationale for the studies, describing the experimental design of the 3-month and 2-year studies, reporting on survival and body weight effects, and commenting on chemical-related neoplasms and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year studies were *some evidence of carcinogenic activity* in male and female Hsd:Sprague Dawley® SD® rats and *clear evidence of carcinogenic activity* in male and female B6C3F1/N mice.

Dr. Gordon, the first reviewer, stated that the study design was excellent, and that based on the 3-month studies, the appropriate choices were made for the 2-year exposure concentrations for both rodent species. He also approved of the generation and monitoring of the exposure chamber concentrations and the pathologic survey of neoplastic and nonneoplastic changes. His only methodological concern was why the sperm endpoints were not included in the 2-year studies, because positive findings were observed for male reproductive endpoints in the 3-month rat studies. He found the data presentation very clear in both text and tables. He felt that appropriate conclusions were delineated, except that the correct conclusion for carcinogenesis in the female rats was somewhere between some and clear evidence. He suggested several additions to the report.

Dr. A.E. Brix, the NIEHS study pathologist, said that in the 2-year studies, the animals were too old and there were so many background changes, it would not be possible to detect subtle changes in male reproductive endpoints.

Dr. Conner, the second reviewer, stated that the design and conduct of the studies was appropriate for the questions raised. He recommended the inclusion of an estimate of dose, even if it is not precise. He felt that the conclusion for C-cell adenoma in male and female rats should have been equivocal evidence rather than some evidence. He also suggested that the conclusion for increased incidences of stromal polyp in the uterus of female rats should have been *equivocal evidence* rather than *some evidence*.

Dr. Roberts said it was not feasible to take accurate measurements during exposure, which would be needed for an accurate estimate of dose, due to several factors. Dr. Conner reiterated that that information is important in the regulatory world, and an estimate of dose, even with caveats, would be useful. Dr. Roberts discussed the reasons for the conclusions Dr. Conner mentioned.

Dr. Ludewig, the third reviewer, said that the studies were very well-planned and well-executed. She agreed with the NTP findings with respect to neoplasms. She said she would like to see if there were any difference in the chamber control animals from this inhalation study and controls from other types of studies, because the housing conditions for the inhalation studies were different. She noted a decrease in mammary gland adenomas and carcinomas in the exposed animals compared to chamber controls, indicating a hormonal aspect. She suggested further

discussion of mode of action parameters in the report. She noted that the word “obesogen” was not used in the report, despite the clear evidence of body weight gain. She suggested some reference as a trigger for future research.

Dr. Brix said that before labeling *p*-chloro-α,α,α-trifluorotoluene an obesogen, further studies would need to be conducted to determine whether the body weight gain was due to fat versus increased hypertrophy or liver neoplasms. Dr. Roberts noted that *p*-chloro-α,α,α-trifluoro-toluene was included in Tox21 evaluations. Regarding the housing used, she said that differences in controls from inhalation studies compared to controls from other routes was one reason there had been so much focus on the concurrent controls. Dr. N.J. Walker, NIEHS, added that it was always an issue in inhalation studies.

Dr. Peterson, the fourth reviewer, concurred that the studies were well-conducted, with appropriate study design and clear reasoning for the concentrations used in the 2-year studies. Overall, he agreed with the conclusions.

Dr. Gordon asked Dr. Conner which conclusions he felt should be changed from some evidence to equivocal evidence. Dr. Conner reiterated changing the conclusion from some evidence to equivocal evidence for the thyroid gland C-cell tumors and uterine stromal polyps, but said he would defer to the NTP calls and would not present a motion to change them.

Dr. Cattley asked for a motion to accept the NTP conclusions as written. Dr. Gordon moved and Dr. Peterson seconded the motion. The panel voted unanimously (6 yes votes) to accept the conclusions as written.

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