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NTP Technical Report on the Toxicology and Carcinogenesis Studies of

2,3-Butanedione (CASRN 431-03-8) in Wistar Han [Crl:WI (Han)] Rats and B6C3F1/N Mice (Inhalation Studies)

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NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2,3-Butanedione (CASRN 431-03-8) in Wistar Han [Crl:WI(Han)] 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;
its content has not changed.

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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 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 2,3-Butanedione (CASRN 431-03-8) in Wistar Han [Crl:WI (Han)] Rats and B6C3F1/N Mice (Inhalation Studies)* on July 13, 2017, are listed below. Panel members serve 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

2,3-Butanedione is commonly used by the flavor manufacturing industry for production of artificial flavor formulations. Examples of flavored food products include popcorn, cake mixes, flour, beer, wine, margarines and soft spreads, cheese, candy, bakery products, crackers, cookies, ice cream, frozen foods, and many other food and beverage products. 2,3-Butanedione was nominated by the United Food and Commercial Workers Union for long-term inhalation studies due to outbreaks of bronchiolitis obliterans in workers exposed to its vapors. Male and female Wistar Han [CRL:WI (Han)] rats and B6C3F1/N mice were exposed to 2,3-butanedione (greater than or equal to 98.5%) by inhalation for 3 months or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and *Escherichia coli*, mouse bone marrow cells, and rat and mouse peripheral blood erythrocytes.

Three-month Study in Rats

Groups of 10 male and 10 female rats were exposed to 2,3-butanedione vapor by whole body inhalation at concentrations of 0, 6.25, 12.5, 25, 50, or 100 ppm, 6 hours plus T_{90} (10 minutes) per day, 5 days per week for 14 weeks. Additional groups of 10 male and 10 female rats were exposed to the same concentrations for 23 days for clinical pathology analyses. Two male rats in the 100 ppm group died before the end of the study. All other rats survived to the end of the study. The mean body weights of 100 ppm males and females were significantly less than those of the chamber control groups. Clinical observations, noted only in the 50 and 100 ppm groups, included abnormal breathing, sneezing, and lethargy.

On day 23 and at study termination, neutrophil counts were significantly increased in 100 ppm females and were consistent with the inflammation observed in the respiratory tract. Significant increases in the erythron occurred most consistently in the 100 ppm male and female groups on day 23 and at study termination. These erythron increases were consistent with dehydration or a secondary erythrocytosis.

2,3-Butanedione exposure resulted in a significant increase of nonneoplastic lesions in the respiratory tract of male and female rats, primarily in the 50 and 100 ppm groups. The highest number of lesions occurred in the nose and included suppurative inflammation; necrosis, regeneration, squamous metaplasia and hyperplasia of the respiratory epithelium; necrosis, degeneration, and respiratory metaplasia of the olfactory epithelium; atrophy of the turbinate; and hyperplasia of the lymphoid tissue. In the larynx, lesions included respiratory epithelium squamous metaplasia and squamous epithelium hyperplasia in males and females and epithelium necrosis and chronic active inflammation in females. In the trachea, necrosis and regeneration occurred in the epithelium of males and females and hyperplasia occurred in the epithelium of females. In the lung, significantly increased incidences of nonneoplastic lesions only occurred in the 100 ppm groups of males and females and included hyperplasia and regeneration of the bronchus epithelium and bronchiole epithelium hyperplasia; in addition, the incidences of histiocyte cellular infiltration and bronchus epithelium necrosis were significantly increased in 100 ppm males, and the incidence of atypical hyperplasia of the bronchus epithelium was significantly increased in 100 ppm females.

Three-month Study in Mice

Groups of 10 male and 10 female mice were exposed to 2,3-butanedione vapor by whole body inhalation at concentrations of 0, 6.25, 12.5, 25, 50, or 100 ppm, 6 hours plus T_{90} (10 minutes)

per day, 5 days per week for 14 weeks. All mice survived to the end of the study. The mean body weights of males exposed to 50 or 100 ppm and females exposed to 12.5 ppm or greater were significantly less than those of the chamber control groups. Clinical observations in mice exposed to 50 or 100 ppm included sneezing and abnormal breathing.

Significant increases in neutrophil counts occurred in 50 and 100 ppm males and 100 ppm females and were consistent with inflammation. In all of these groups, mean cell volume and mean cell hemoglobin were significantly decreased, possibly indicating minimal alterations in iron metabolism or hemoglobin production.

Exposure-related significantly increased incidences of nonneoplastic lesions occurred in the respiratory tract of male and female mice, primarily in the 50 and 100 ppm groups. As in rats, the highest number of lesions occurred in the nose and included suppurative inflammation; necrosis, regeneration, and squamous metaplasia of the respiratory epithelium; necrosis and atrophy of the turbinate; and atrophy and respiratory metaplasia of the olfactory epithelium. In the larynx, lesions included necrosis of the epithelium; regeneration, hyperplasia, squamous metaplasia, and atypical squamous metaplasia of the respiratory epithelium; hyperplasia and atypical hyperplasia of the squamous epithelium; and chronic active inflammation. In the trachea, the incidences of atypical squamous metaplasia, hyperplasia, and degeneration in the epithelium were significantly increased in the 100 ppm groups, as were the incidences of chronic active inflammation. The incidences of regeneration of the tracheal epithelium were significantly increased in the 50 ppm groups. In the lung, the incidences of chronic inflammation and polymorphonuclear cellular infiltration of the bronchus were significantly increased in the 100 ppm groups. The incidences of atypical hyperplasia, atypical squamous metaplasia, and regeneration of the bronchus epithelium were significantly increased in the 100 ppm groups.

Two-year Study in Rats

Groups of 50 male and 50 female rats were exposed to 2,3-butanedione vapor by whole body inhalation at concentrations of 0, 12.5, 25, or 50 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. Survival of 50 ppm males was significantly less than that of the chamber control group. Survival was moderately reduced in 25 ppm females. At the end of the study, mean body weights of both sexes exposed to 50 ppm were decreased relative to the respective chamber control groups, with more of an effect in males (81% of chamber controls) than in females (91% of chamber controls). Exposure-related clinical observations included thinness, abnormal breathing, eye abnormality, and nasal/eye discharge in males and eye abnormality and abnormal breathing in females.

Three squamous cell carcinomas and one squamous cell papilloma of the nasal mucosa occurred in male rats exposed to 50 ppm, and three squamous cell carcinomas of the nasal mucosa occurred in females exposed to 50 ppm. No squamous cell carcinomas or papillomas of the nose occurred in the concurrent male or female chamber controls, and none are recorded in the NTP historical control database.

A spectrum of nonneoplastic lesions of the nose occurred in both the respiratory and olfactory epithelium, primarily in the 25 and 50 ppm groups. Nasal lesions with incidences significantly greater than the chamber control incidences included suppurative inflammation; respiratory epithelium hyperplasia and squamous meta-plasia; olfactory epithelium atrophy, respiratory meta-plasia, and necrosis (males); turbinate hyperostosis; and fibrosis of the lamina propria.

In the larynx, incidences of chronic active inflammation in the 50 ppm groups, hyperplasia of the squamous epithelium in the 25 and 50 ppm groups, focal areas of ulceration of the squamous epithelium in the 50 ppm groups, and squamous metaplasia of the respiratory epithelium in the 50 ppm groups were significantly greater than the chamber control incidences.

In the trachea, the incidences of chronic active inflammation, epithelium hyperplasia, and submucosa fibrosis were significantly increased in 50 ppm males and females. In 50 ppm males, the incidences of epithelium squamous metaplasia, regeneration, atrophy, and necro-sis were significantly increased. The incidence of epithelium regeneration was also significantly increased in 25 ppm males.

In the lung, the incidences of suppurative inflammation in 50 ppm males, granulomatous inflammation in 50 ppm females, and peribronchial chronic active inflammation in 50 ppm males and females were significantly increased. Significantly increased lesion incidences in the airways included bronchial and bronchiolar epithelium hyperplasia and bronchial epithelium atrophy in 50 ppm males and females and bronchial epithelium regeneration and submucosa fibrosis in 50 ppm males. The incidence of bronchiolar epithelium hyperplasia was also significantly increased in 25 ppm females. Lesions occurring in the lung parenchyma included histiocytic cellular infiltration in the alveolar spaces, alveolar epithelium hyperplasia, and interstitium fibrosis. The incidences of these lesions were significantly increased in 50 ppm rats, except the incidence of alveolar epithelium hyperplasia in females.

In the eye, chronic active inflammation of the cornea in 25 and 50 ppm rats, suppurative inflammation of the anterior chamber in 25 ppm rats, acute inflammation of the iris in 25 ppm females, cornea epithelium hyperplasia in 25 ppm females and 50 ppm males and females, cornea epithelium ulcer in 50 ppm rats, lens cataract in 25 ppm males and females and 50 ppm females, and unilateral phthisis bulbi in 50 ppm females occurred with significantly increased incidences.

Two-year Study in Mice

Groups of 50 male and 50 female mice were exposed to 2,3-butanedione vapor by whole body inhalation at concentrations of 0, 12.5, 25, or 50 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 105 weeks. Survival of 50 ppm males and females was significantly less than that of the chamber control groups. At the end of the study, the mean body weights of the 50 ppm groups were reduced to 65% (males) and 62% (females) of those of the respective chamber control groups. Clinical observations were most prominent in the 50 ppm groups and included abnormal breathing, thinness, sneezing, and eye abnormality in both sexes.

In the nose, adenocarcinomas occurred in two 50 ppm females. No nasal adenocarcinomas have been recorded in the NTP historical control database.

Compared to the chamber control group incidences, nonneoplastic lesions of the nose that were significantly increased in 50 ppm mice included suppurative inflammation; respiratory epithelium squamous metaplasia, hyperplasia (males), and necrosis; respiratory metaplasia of the Steno’s glands; regeneration of the mucosa epithelium; olfactory epithelium atrophy, respiratory metaplasia, and necrosis; turbinate atrophy and necrosis; perforation of the nasal septum; and fibrosis of the lamina propria. Most of these lesion incidences were also significantly increased in 25 ppm mice and sometimes in 12.5 ppm mice.

In the larynx, incidences of chronic active inflammation; lumen exudate (females); respiratory epithelium squamous metaplasia, hyperplasia (males), necrosis, and regeneration; and squamous epithelium hyperplasia were significantly increased in 50 ppm mice. Incidences of chronic active inflammation, squamous epithelium hyperplasia, and respiratory epithelium necrosis were also significantly increased in the 25 ppm groups. The incidence of squamous epithelium hyperplasia was significantly increased in 12.5 ppm females.

Incidences of tracheal lesions that were significantly increased in the 50 ppm groups included chronic active inflammation; lumen exudate; necrosis; epithelium regeneration, hyperplasia (males), and squamous metaplasia (males); submucosa fibrosis; and carina submucosa fibrosis, mineralization, and chronic active inflammation (males). The incidence of epithelium regeneration was also increased in 25 ppm females.

In the lung of 50 ppm mice, the most common bronchial lesion in both males and females was bronchus epithelium regeneration. Bronchus submucosa fibrosis occurred in five 50 ppm females. Incidences of suppurative inflammation of the lung, pleura, and mediastinum were significantly increased in 50 ppm females. Incidences of chronic active inflammation of the mediastinum were significantly increased in 50 ppm males and females.

In the cornea of the eye, incidences of acute inflammation, mineralization, and epithelium hyperplasia in 25 ppm females and 50 ppm males and females; epithelium ulcer in 25 and 50 ppm females; and necrosis in 50 ppm females were significantly greater than the chamber control incidences. The incidence of suppurative inflammation of the anterior chamber was increased in 50 ppm males.

Genetic Toxicology

2,3-Butanedione was mutagenic in two independent bacterial mutagenicity assays. In the initial assay, conducted with a different lot of the chemical than was tested in the NTP rodent studies, a weak positive response was observed in *S. typhimurium* strain TA97 with and without exogenous metabolic activation (S9 mix). No clear mutagenic activity was observed in any of the other strains tested (TA98, TA100, and TA1535). In the second bacterial mutation assay, conducted with the same lot of 2,3-butanedione that was used in the 2-year rodent bioassay, mutagenic activity was seen in *S. typhimurium* strain TA97a in the absence of S9 and in the *E. coli* strain WP2 *uvrA*/pKM101 with and without S9 mix. As with the initial test, no clear mutagenic activity was seen in any of the other strains tested (TA100, TA98) with or without S9.

To assess chromosomal damage, the frequency of micronucleated polychromatic erythrocytes (PCEs) was bone marrow samples obtained from male B6C3F1/N mice following intraperitoneal injection of 2,3-butanedione once daily for 3 days; no increases in micronucleated PCEs were observed at doses up to 500 mg 2,3-butanedione/kg body weight per day.

At the end of the 3-month inhalation studies, peripheral blood samples were obtained from male and female rats and mice and analyzed by flow cytometry for the frequency of micronucleated PCEs and mature (normochromatic) erythrocytes (NCEs). No increases in micronucleated PCEs or NCEs were seen in either sex or species. The percentage of PCEs among circulating red blood cells was unaffected by exposure to 2,3-butanedione, suggesting the chemical had no effect on erythropoiesis.

Conclusions

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity* of 2,3-butanedione in male Wistar Han rats based on the combined incidences of squamous cell papilloma and squamous cell carcinoma of the nose. There was *some evidence of carcinogenic activity* of 2,3-butanedione in female Wistar Han rats based on the incidences of squamous cell carcinoma of the nose (see Explanation of Levels of Evidence of Carcinogenic Activity; see a summary of the Peer Review Panel comments and the public discussion on this Technical Report in Appendix K). There was *no evidence of carcinogenic activity* of 2,3-butanedione in male B6C3F1/N mice exposed to 12.5, 25, or 50 ppm. There was *equivocal evidence of carcinogenic activity* of 2,3-butanedione in female B6C3F1/N mice based on the occurrences of adenocarcinoma of the nose.

Exposure to 2,3-butanedione resulted in increased incidences of nonneoplastic lesions of the nose, larynx, trachea, lung, and eye in male and female rats and mice.

Synonyms: Biacetyl; butane-2,3-dione; butanedione; diacetyl; dimethylglyoxal

Trade name: NSC 8750

Summary of the Two-year Carcinogenesis and Genetic Toxicology Studies of 2,3-Butanedione

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in air	0, 12.5, 25, or 50 ppm	0, 12.5, 25, or 50 ppm	0, 12.5, 25, or 50 ppm	0, 12.5, 25, or 50 ppm
Survival rates	36/50, 37/50, 33/50, 22/50	34/50, 31/50, 24/50, 31/50	35/50, 39/50, 37/50, 25/50	36/50, 40/50, 42/50, 18/50
Body weights	50 ppm males at least 10% less than chamber controls after week 57	50 ppm females at least 10% less than chamber controls after week 97	50 ppm males at least 10% less than chamber controls after week 11	50 ppm females at least 10% less than chamber controls after week 17
Nonneoplastic effects	Nose: inflammation, suppurative (3/50, 4/50, 35/50, 50/50); respiratory epithelium, hyperplasia (0/50, 2/50, 5/50, 50/50); respiratory epithelium, metaplasia, squamous (0/50, 0/50, 5/50, 34/50); olfactory epithelium, atrophy (0/50, 5/50, 27/50, 22/50); olfactory epithelium, metaplasia, respiratory (1/50, 3/50, 6/50, 50/50); olfactory epithelium, necrosis (0/50, 0/50, 0/50, 6/50); turbinate, hyperostosis (0/50, 0/50, 0/50, 10/50); lamina propria, fibrosis (0/50, 0/50, 28/50, 38/50) Larynx: inflammation, chronic active (14/50, 7/50, 7/50, 33/50); squamous epithelium, hyperplasia (2/50, 2/50, 8/50, 46/50); squamous epithelium, ulcer, focal (0/50, 0/50, 0/50, 15/50); respiratory epithelium, metaplasia, squamous (0/50, 1/50, 0/50, 45/50) Trachea: inflammation, chronic active (0/50, 0/50, 1/50, 8/50); epithelium,	Nose: inflammation, suppurative (4/50, 3/50, 11/50, 49/50); respiratory epithelium, hyperplasia (1/50, 0/50, 2/50, 44/50); respiratory epithelium, metaplasia, squamous (1/50, 0/50, 1/50, 44/50); olfactory epithelium, atrophy (1/50, 1/50, 14/50, 24/50); olfactory epithelium, metaplasia, respiratory (1/50, 0/50, 18/50, 46/50); turbinate, hyperostosis, (0/50, 0/50, 0/50, 8/50); lamina propria, fibrosis, (1/50, 1/50, 17/50, 46/50) Larynx: inflammation, chronic active (4/50, 2/50, 4/50, 25/50); squamous epithelium, hyperplasia (1/50, 1/50, 6/50, 48/50); squamous epithelium, ulcer, focal (0/50, 0/50, 0/50, 5/50); respiratory epithelium, metaplasia, squamous (0/50, 0/50, 0/50, 35/50) Trachea: inflammation, chronic active (0/50, 0/50, 0/50, 20/50); epithelium, hyperplasia (0/50, 0/50, 0/50, 30/50); submucosa, fibrosis	Nose: inflammation, suppurative (2/49, 4/48, 47/50, 50/50); respiratory epithelium, metaplasia, squamous (0/49, 6/48, 47/50, 50/50); respiratory epithelium, hyperplasia (1/49, 0/48, 0/50, 8/50); glands, sinus, metaplasia, respiratory (0/49, 0/48, 1/50, 13/50); respiratory epithelium, necrosis (0/49, 0/48, 34/50, 50/50); mucosa, regeneration (0/49, 0/48, 47/50, 47/50); olfactory epithelium, atrophy (0/49, 14/48, 48/50, 38/50); olfactory epithelium, metaplasia, respiratory (1/49, 0/48, 39/50, 45/50); olfactory epithelium, necrosis (0/49, 0/48, 0/50, 19/50); turbinate, atrophy (0/49, 8/48, 49/50, 50/50); turbinate, necrosis (0/49, 0/48, 4/50, 27/50); septum, perforation (0/49, 0/48, 3/50, 11/50); lamina propria, fibrosis (0/49, 0/48, 44/50, 50/50) Larynx: inflammation, chronic active (4/49, 2/49, 11/49, 42/50); respiratory epithelium,	Nose: inflammation, suppurative (3/50, 20/50, 50/50, 50/50); respiratory epithelium, metaplasia, squamous (1/50, 9/50, 48/50, 50/50); glands, sinus, metaplasia, respiratory (0/50, 0/50, 0/50, 12/50); respiratory epithelium, necrosis (1/50, 5/50, 33/50, 50/50); mucosa, regeneration (0/50, 0/50, 39/50, 48/50); olfactory epithelium, atrophy (0/50, 41/50, 49/50, 45/50); olfactory epithelium, metaplasia, respiratory (0/50, 22/50, 46/50, 49/50); olfactory epithelium, necrosis (0/50, 0/50, 1/50, 20/50); turbinate, atrophy (0/50, 32/50, 50/50, 50/50); turbinate, necrosis (0/50, 0/50, 1/50, 11/50); septum, perforation 0/50, 0/50, 6/50, 5/50); lamina propria, fibrosis (0/50, 0/50, 47/50, 49/50) Larynx: inflammation, chronic active (4/49, 5/50, 22/50, 36/49); lumen exudate (0/49, 0/50, 0/50, 4/49); respiratory epithelium, metaplasia, squamous (2/49, 0/50, 6/50,

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
	hyperplasia (0/50, 0/50, 1/50, 32/50); epithelium, metaplasia, squamous (0/50, 0/50, 0/50, 12/50); epithelium, regeneration, (0/50, 0/50, 5/50, 12/50); epithelium, atrophy, (0/50, 0/50, 0/50, 7/50); epithelium, necrosis (0/50, 0/50, 0/50, 6/50); submucosa, fibrosis (0/50, 0/50, 0/50, 27/50)	(0/50, 0/50, 0/50, 19/50)	metaplasia, squamous (3/49, 0/49, 6/49, 50/50); squamous epithelium, hyperplasia (3/49, 7/49, 15/49, 42/50); respiratory epithelium, hyperplasia (1/49, 0/49, 0/49, 11/50); respiratory epithelium, necrosis (2/49, 1/49, 9/49, 34/50); respiratory epithelium, regeneration (0/49, 0/49, 0/49, 32/50)	48/49); squamous epithelium, hyperplasia (4/49, 13/50, 34/50, 40/49); respiratory epithelium, necrosis (1/49, 1/50, 14/50, 32/49); respiratory epithelium, regeneration (0/49, 0/50, 3/50, 30/49)
	<u>Lung</u>: inflammation, suppurative (0/50, 0/49, 1/50, 15/50); peribronchial, inflammation, chronic active (0/50, 0/49, 0/50, 13/50); bronchus, epithelium, hyperplasia (0/50, 0/49, 2/50, 47/50); bronchiole, epithelium, hyperplasia (0/50, 0/49, 0/50, 33/50); bronchus, epithelium, atrophy (0/50, 0/49, 1/50, 23/50); bronchus, epithelium, regeneration (0/50, 0/49, 4/50, 9/50); bronchus, submucosa, fibrosis, (0/50, 0/49, 0/50, 5/50); alveolus, infiltration cellular, histiocyte (10/50, 14/49, 16/50, 34/50); alveolar epithelium, hyperplasia (1/50, 4/49, 2/50, 8/50); interstitium, fibrosis, (0/50, 1/49, 1/50, 11/50) <u>Eye</u>: cornea, inflammation, chronic active (1/50, 6/50, 16/49, 28/49); anterior chamber,	<u>Lung</u>: inflammation, granulomatous (2/50, 1/50, 3/50, 13/50); peribronchial, inflammation, chronic active (1/50, 2/50, 0/50, 27/50); bronchus, epithelium, hyperplasia (0/50, 0/50, 0/50, 46/50); bronchiole, epithelium, hyperplasia (0/50, 0/50, 8/50, 39/50); bronchus, epithelium, atrophy (0/50, 0/50, 0/50, 7/50); alveolus, infiltration cellular, histiocyte (13/50, 11/50, 10/50, 32/50); interstitium, fibrosis (1/50, 1/50, 1/50, 9/50) <u>Eye</u>: cornea, inflammation, chronic active (2/50, 6/50, 23/50, 31/50); anterior chamber, inflammation, suppurative (1/50, 0/50, 6/50, 5/50); iris, inflammation, acute (0/50, 0/50, 5/50, 4/50); cornea, epithelium, hyperplasia (0/50, 3/50, 8/50, 5/50); cornea, epithelium,	<u>Trachea</u>: inflammation, chronic active (0/48, 0/49, 2/49, 45/49); lumen, exudate (0/48, 0/49, 0/49, 4/49); necrosis (0/48, 0/49, 0/49, 47/49); epithelium, regeneration (0/48, 0/49, 0/49, 45/49); epithelium, hyperplasia (0/48, 0/49, 0/49, 6/49); epithelium, metaplasia, squamous 0/48, 0/49, 0/49, 5/49); submucosa, fibrosis (0/48, 0/49, 0/49, 46/49); carina, submucosa, fibrosis (0/48, 0/49, 0/49, 16/49); carina, submucosa, mineralization (0/48, 0/49, 0/49, 15/49); carina, submucosa, inflammation, chronic active (0/48, 0/49, 0/49, 4/49) <u>Lung</u>: bronchus, epithelium, regeneration (0/50, 0/49, 0/50, 34/50); mediastinum, inflammation, chronic active (1/50, 0/49,	<u>Trachea</u>: inflammation, chronic active (1/50, 0/49, 4/50, 42/50); lumen, exudate (0/50, 0/49, 0/50, 12/50); necrosis (0/50, 0/49, 3/50, 48/50); epithelium, regeneration (0/50, 0/49, 9/50, 45/50); submucosa, fibrosis (0/50, 0/49, 0/50, 44/50); carina, submucosa, fibrosis (0/50, 0/49, 0/50, 6/50); carina, submucosa, mineralization (0/50, 0/49, 0/50, 5/50) <u>Lung</u>: bronchus, epithelium, regeneration (2/50, 0/50, 0/50, 38/50); bronchus, submucosal fibrosis (0/50, 0/50, 0/50, 5/50); inflammation, suppurative (0/50, 1/50, 0/50, 5/50); pleura, inflammation, suppurative (0/50, 0/50, 0/50, 5/50); mediastinum, inflammation, suppurative (0/50, 1/50, 1/50, 8/50);

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
	inflammation, suppurative (0/50, 1/50, 6/49, 3/49); cornea, epithelium, hyperplasia (0/50, 2/50, 3/49, 6/49); cornea, epithelium, ulcer (0/50, 1/50, 4/49, 6/49); lens, cataract (1/50, 5/50, 6/49, 3/49)	ulcer (0/50, 1/50, 2/50, 13/50); lens, cataract (1/50, 1/50, 6/50, 9/50); unilateral, phthisis bulbi (0/50, 1/50, 0/50, 8/50)	0/50, 8/50) Eye: cornea, inflammation, acute (2/49, 0/49, 1/50, 17/50); cornea, mineralization (0/49, 0/49, 0/50, 5/50); cornea, epithelium, hyperplasia (1/49, 0/49, 0/50, 9/50); anterior chamber, inflammation, suppurative (0/49, 0/49, 0/50, 5/50)	mediastinum, inflammation, chronic active (0/50 0/50, 1/50, 7/50) Eye: cornea, inflammation, acute (1/50, 2/49, 20/50, 23/49); cornea, epithelium, ulcer (0/50, 0/49, 10/50, 10/49); cornea, necrosis (0/50, 0/49, 0/50, 6/49); cornea, mineralization (0/50, 0/49, 13/50, 16/49); cornea, epithelium, hyperplasia (2/50, 2/49, 10/50, 9/49)
	Nose: squamous cell carcinoma (0/50, 0/50, 0/50, 3/50); squamous cell papilloma or carcinoma (0/50, 0/50, 0/50, 4/50)	Nose: squamous cell carcinoma (0/50, 0/50, 0/50, 3/50)	None	None
Equivocal findings	None	None	None	Nose: adenocarcinoma (0/50, 0/50, 0/50, 2/50)
Level of evidence of carcinogenic activity	Some evidence	Some evidence	No evidence	Equivocal evidence

Genetic toxicology	
Bacterial gene mutations:	
Study 1	Positive in *S. typhimurium* strain TA97 with and without induced hamster and rat liver S9; equivocal in strain TA100 in the presence of S9; negative in strain TA100 without S9 and in strains TA1535 and TA98 with and without S9.
Study 2 (same lot used in the bioassay)	Positive in strain TA97a without rat liver S9 and equivocal in strain TA97a with rat liver S9; positive in *E. coli* with and without S9; equivocal in strain TA100 with and without S9; negative in strain TA98 with and without S9.
Micronucleated erythrocytes	
Mouse bone marrow in vivo:	Negative in males
Rat peripheral blood in vivo:	Negative in males and females
Mouse peripheral blood in vivo:	Negative in males and females

Introduction

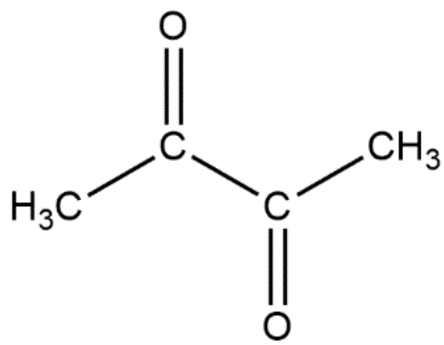


Figure 1. 2,3-Butanedione (CASRN 431-03-8; Chemical Formula: $C_4H_6O_2$; Molecular Weight: 86.09)

Synonyms: Biacetyl; butane-2,3-dione; butanedione; diacetyl; dimethylglyoxal.
Trade name: NSC 8750.

Chemical and Physical Properties

2,3-Butanedione is a yellow liquid at room temperature with an intense quinone-like odor. The vapor has a butter odor that at high concentrations has been described as a chlorine, or rancid butter odor[1]. The odor threshold for 2,3-butanedione has been reported to be approximately 50 ppb[2], although this value varies depending upon the method of assessment[3; 4]. 2,3-Butanedione is relatively water soluble (200 g/L at 15°C) and volatile (vapor pressure = 56.8 mm Hg at 25°C)[5].

2,3-Butanedione is classified as a 1,2-diketone and is the simplest member of this chemical class. The two ketone groups of the 1,2-diketones are located on adjacent or vicinal carbons. A distinctive feature of 2,3-butanedione and other 1,2-diketones is the long C-C bond linking the adjacent ketone groups. This bond distance is about 1.54 Å, compared to 1.45 Å for a molecule containing a conjugated system of double bonds. The unusual bond length of the 1,2-diketones is attributed to the high electronegativity of the oxygen atoms and the resulting repulsion between the partial positive charges of the carbonyl carbon atoms[6]. The positively charged carbonyl carbons are susceptible to attack by nucleophiles such as the amines. Resonance between the electron-deficient oxygen atoms on adjacent carbons contributes to the reactivity of 2,3-butanedione.

Production, Use, and Human Exposure

2,3-Butanedione is produced and supplied domestically in both bulk and smaller specialty quantities by many manufacturers and distributors; however, specific information on recent annual production volumes was not found in the available literature. The Flavor & Extract Manufacturers Association (FEMA) reported estimated production of 211,000 pounds in 1995, 228,000 pounds in 2005, 85,000 pounds in 2010[7], and about 30,200 pounds in 2015 (John Hallagan, personal communication). According to the United States Environmental Protection Agency (USEPA) Non-Confidential Inventory Updating Report, 2,3-butanedione had an aggregate production volume between 10,000 and 500,000 pounds in 2002[8]. 2,3-Butanedione is also imported, but little specific information on annual import volumes could be found in the available literature.

2,3-Butanedione occurs naturally in butter, various fruits, coffee, honey, and other foods and as a fermentation by-product in wine, beer, and dairy products. During fermentation, 2,3-butanedione is produced by decarboxylation of α-acetolactate by some species of the lactic acid bacteria family. The butter flavor in some foods is increased by adding 2,3-butanedione in the form of a concentrated starter distillate[9; 10]. Starter distillate is prepared by fermenting milk with bacterial starter cultures. The steam distillate contains 1% to 5% 2,3-butanedione[11].

2,3-Butanedione is commonly used by the flavor manufacturing industry for production of artificial flavor formulations. 2,3-Butanedione is typically used as a liquid component in flavoring solutions but can also be encapsulated in powders for addition to dry mixtures[12]. Examples of flavored food products include cake mixes, flour, beer, wine, margarines and soft spreads, cheese, candy, bakery products, crackers, pop-corn, cookies, ice cream, frozen foods, and many other food and beverage products[13].

2,3-Butanedione is also used as a chemical modifier of arginine residues in proteins in studying glycation (the nonenzymatic browning of foods or the nonenzymatic binding of sugar and protein molecules in the body)[14]. Other uses for 2,3-butanedione include reactant/starting material in chemical or biochemical reactions, analytical reagent, anti-microbial/preservative, electron stabilizing compound, modifier of radiation response for chemical and biological systems, and photoinitiator/photosensitizer in polymerizations[5].

2,3-Butanedione is an ingredient in many different food products, and nonoccupational exposure occurs primarily by ingestion. Consumption of 2,3-butanedione at low levels commonly added to food has not been reported to cause adverse health effects. Three consumers of butter-flavored microwave popcorn with biopsy-confirmed bronchiolitis obliterans have been reported[15]. Estimated exposures of 2 to 24 ppm 2,3-butanedione were based upon peak exposures and individual consumption habits. These cases likely represent a subgroup of susceptible individuals. Nonoccupational inhalation exposure to 2,3-butanedione also can occur from cigarette smoking[16], the use of electronic cigarettes[17-19], and from the use of flavored tobacco in hookah water pipes[20; 21].

Occupational exposure to 2,3-butanedione occurs primarily by inhalation of vapors, especially where artificial flavorings containing 2,3-butanedione are mixed or heated[22; 23]. Occupational exposure to 2,3-butanedione was first recognized as a health hazard at microwave popcorn production facilities that used artificial butter flavoring[22; 24].

Another group of exposed workers was identified in flavoring manufacturing facilities[23]. According to FEMA, whose members produce approximately 95% of all flavors in the United States, a total of 6,520 employees work directly in flavor manufacturing or laboratory activities in membership companies (Personal communication, J. Hallagan, FEMA General Counsel, to L. McKernan, CDC, October 19, 2010). In microwave popcorn plants and in flavoring manufacturing facilities, workers were exposed to open vessels of flavoring containing 2,3-butanedione, and in some cases, workers were exposed to heated vessels of flavoring mixtures. The headspace of a heated vessel containing heated flavoring mixture at a microwave popcorn plant was reported to contain a peak concentration of 1,230 ppm 2,3-butanedione[22]. The mean 2,3-butanedione air concentrations measured at this microwave popcorn facility were

57.2 ppm (range: 5.43 to 147 ppm) in the mixing room, followed by 2.8 ppm (range: 0.48 to 8.2 ppm) in the packaging area for machine operators. Workers in both areas of the plant had symptoms of obstructive lung disease[25].

Occupational exposure to 2,3-butanedione vapors has also been reported in coffee processing facilities. 2,3-Butanedione is released when coffee beans are roasted[26] and when roasted coffee beans are ground[27-29]. Worker exposure can also occur when adding artificial flavorings to some flavored coffee. Other food industries with potential worker exposure to 2,3-butanedione include snack food production plants, commercial and retail bakeries, baking mix production, margarine and other vegetable oil-based cooking products, butter and other dairy products, and candy manufacturers[30].

Dermal exposure of workers to toxic levels of 2,3-butanedione has been reported in the microwave popcorn packaging and flavor manufacturing industries. Reported dermatologic problems ranged from 12% at one of the six microwave popcorn plants evaluated to 36% among production workers at a flavoring plant[31]. Skin problems were reported by 60% of workers who primarily made liquid flavorings at this plant[32].

Regulatory Status

2,3-Butanedione is regulated by the FDA and was granted "generally recognized as safe" (GRAS) status when used as a direct food ingredient[11]. Following reports of respiratory disease in the popcorn and flavoring industries, California promulgated a regulation for occupational exposure to food flavorings containing 2,3-butanedione that requires installation of exposure controls to reduce exposures to the lowest feasible levels. In 2015, the American Conference of Governmental Industrial Hygienists (ACGIH) published a threshold limit value of 0.010 ppm 8-hour time weighted-average (TWA) with a short-term exposure limit (STEL) of 0.020 ppm for 2,3-butanedione[33]. NIOSH published its recommended exposure limits for 2,3-butanedione (5 ppb 8-hour TWA, 25 ppb STEL) in October 2016[34]. There currently is no established OSHA permissible exposure limit for 2,3-butanedione.

Absorption, Distribution, Metabolism, and Excretion

There were no data available on the absorption, distribution, metabolism, or excretion (ADME) of inhaled 2,3-butanedione. NTP conducted limited ADME studies following 2,3-butanedione administration by intratracheal instillation, oropharyngeal aspiration, and gavage. Uptake of ^{14}C-2,3-butanedione from the lung and binding to hemoglobin and albumin were determined following intratracheal instillation in Harlan Sprague Dawley® rats (100 mg/kg) and oropharyngeal aspiration in B6C3F1/N mice (157 mg/kg)[35]. Blood and plasma were collected 24 hours later and analyzed for ^{14}C content. In rats, 0.88% of the administered dose was in blood and 0.66% in plasma. Binding to albumin and hemoglobin accounted for 0.26% and 0.30% of the administered dose, respectively. In mice, 0.38% of the administered dose occurred in blood with 0.17% in plasma. Binding to albumin and hemoglobin accounted for 0.09% and 0.14%, respectively. ^{14}C-2,3-butanedione binding sites on albumin and hemoglobin were determined by mass spectroscopy to be arginine residues.

2,3-Butanedione distribution and excretion were evaluated after administration of a single gavage dose of 1.58, 15.8, or 158 mg ^{14}C-2,3-butanedione/kg body weight to rats (NTP, unpublished studies). The majority of the radioactivity (54% to 82%) was excreted as carbon dioxide at all doses. With increasing dose, the percentage excreted as carbon dioxide decreased and urinary excretion of radioactivity increased (7% to 34%), suggesting that metabolic pathways leading to carbon dioxide were saturated at higher doses. Elimination via fecal excretion accounted for up to 2.24%, and exhalation of volatile organics in breath accounted for 0.8% of the administered dose at 158 mg/kg. Analysis of urine samples obtained from rats administered 158 mg/kg yielded three major ^{14}C-labeled components, one of which coeluted with uric acid. Treatment of the urine samples with β-glucuronidase/sulfatase suggested the presence of conjugated metabolites. Identification of urinary metabolites was not pursued.

2,3-Butanedione uptake efficiency of the upper airways was measured in a surgically isolated rat upper respiratory tract model[36]. Uptake efficiency of the upper airways in this model was reported as moderate (25% to 75%) relative to uptake efficiencies in excess of 95% for water-soluble weak acids (e.g., acetic acid and butyric acid). 2,3-Butanedione metabolism was measured in homogenates of rat nasal olfactory mucosa, nasal respiratory mucosa, and tracheal mucosa. Metabolism was about fourfold greater in nasal olfactory tissue than in nasal respiratory or tracheal tissue. 2,3-Butanedione was metabolized in nasal and tracheal tissues by a NADPH-dependent pathway, presumably by dicarbonyl/L-xylulose reductase (DCXR). Computational modeling of these uptake and metabolism data was used to extrapolate to humans. The model predicted that 2,3-butanedione scrubbing was less efficient in the nose-breathing human than in the rat. The concentration of 2,3-butanedione reaching the bronchi in mouth-breathing humans was estimated to be 1.5-fold greater than in nose-breathing rats.

The inhalation dosimetry of 2,3-butanedione in the airways of rats and humans was investigated using a computational fluid dynamic and physiologically based pharmacokinetic model[37]. Metabolism kinetics and direct reaction rates were determined in vitro using rat respiratory tract tissue homogenates. In the absence of metabolism, the model estimated 40% to 50% of inspired 2,3-butanedione was scrubbed by the rat nose with 20% to 30% reaching the bronchioles. The effects of metabolism were dependent upon the 2,3-butanedione concentration. At 1 ppm, 20% was estimated to penetrate the nose with less than 2% reaching the bronchioles. At higher 2,3-butanedione concentrations, increased amounts reach distal sites, possibly due to saturation of metabolic pathways. Less than 2% of inspired 2,3-butanedione was estimated to reach the bronchioles of the rat, whereas 24% was estimated to penetrate to the bronchioles in lightly exercising humans. The amount of 2,3-butanedione in human bronchiolar tissue was estimated to be 40-fold greater than in rat bronchiolar tissue following exposure to the same concentration. Estimated tissue concentrations in the resting nose-breathing human were fivefold greater than in the nose-breathing rat. These results indicate that direct extrapolation of 2,3-butanedione data from the nose-breathing rat may underestimate the risks of small airway injury in humans.

Studies of 2,3-butanedione and acetoin metabolism in mammalian liver slices and extracts showed inter-conversion of acetoin with 2,3-butanedione and 2,3-butanediol[38]. In the perfused liver, 2,3-butanediol is metabolized to carbon dioxide and acetate[39]. 2,3-Butanedione is metabolized in rat and hamster hepatocytes to acetoin in a reaction catalyzed by DCXR with either NADH or NADPH as coenzymes[40-42]. Acetoin can be further reduced to 2,3-butanediol in a NADH-dependent manner[41]. DCXR also catalyzes the metabolism of several other dicarbonyl compounds, including 2,3-pentanedione, 2,3-hexanedione, 2,3-hep-tanedione and 3,4-hexanedione[42]. Low affinity, high capacity and high affinity, low capacity pathways for 2,3-butanedione metabolism were identified in the respiratory tract of the rat[37]. The high affinity pathway was inhibited by sodium benzoate indicating that it is DCXR. The low affinity pathway is not believed to play a major role at 2,3-butanedione concentrations associated with most exposures. 2,3-Butanedione metabolism in the rat lung can also be catalyzed by AKR1C15, an aldo-keto reductase that metabolizes α-diketones[43].

Toxicity

Experimental Animals

The oral toxicity of 2,3-butanedione was investigated in rats following gavage administration of a 20% 2,3-butanedione solution in water[44]. The LD_{50} for a single gavage dose of 2,3-butanedione was estimated to be 3 g/kg in female rats and 3.4 g/kg in male rats. Subchronic (90 day) gavage administration of 540 mg 2,3-butanedione/kg body weight per day to rats caused decreased body weight, increased water consumption, increased adrenal weight, increased relative kidney and liver weights (in females absolute kidney and liver weights were also increased), decreased blood hemoglobin concentration, and gastric ulceration. No adverse effects were noted at the next highest dose level, which was 90 mg/kg per day. On a mg/kg basis, 90 mg/kg was estimated to be roughly 500-fold greater than the estimated human maximum daily intake of 2,3-butanedione from foods consumed at that time, with 50 ppm 2,3-butanedione being the highest estimated concentration in any food.

Concerns over the toxicity of inhaled 2,3-butanedione vapors were prompted by the finding of obstructive lung disease (bronchiolitis obliterans) in microwave popcorn workers exposed to artificial butter flavoring vapors[22]. The acute inhalation toxicity of artificial butter flavoring vapors was investigated by exposing male Sprague Dawley [Hla: (SD) CVF] rats to artificial butter flavoring vapors containing 2,3-butanedione[45]. Rats were exposed for 6 hours to vapors containing average concentrations of 203, 285, or 352 ppm 2,3-butanedione. A fourth group received pulsed exposures to vapors containing an average of 371 ppm 2,3-butanedione. Animals were necropsied the day following exposure. The most severe lesions were present in the nasal cavity of exposed rats. Butter flavoring vapors containing 203 to 371 ppm 2,3-butanedione caused necrosuppurative rhinitis in all four levels of the nose. In the lung, vapors containing 285 to 371 ppm caused multifocal necrotizing bronchitis, and there was no effect on the alveoli. In a subsequent study, acute 6-hour exposure of rats to 2,3-butanedione alone also caused epithelial necrosis and inflammation in bronchi at concentrations greater than 295 ppm and caused epithelial necrosis and inflammation in the trachea and larynx at concentrations greater than or equal to 224 ppm[46]. Although bronchiolitis obliterans was not observed following these acute exposures, these results demonstrated that 2,3-butanedione in artificial butter flavoring vapors was associated with severe injury to the epithelium lining the respiratory tract.

The respiratory toxicity of 2,3-butanedione vapor was investigated in mice using several exposure profiles relevant to workplace conditions at microwave popcorn packaging plants[47]. Male C57Bl/6 mice were exposed to inhaled 2,3-butanedione across several concentrations and duration profiles or by direct oropharyngeal aspiration. Subacute exposure to 200 or 400 ppm 2,3-butanedione for 5 days caused deaths, necrotizing rhinitis, necrotizing laryngitis, and bronchitis. Reducing the exposure to 1 hour/day (100, 200, or 400 ppm) for 4 weeks resulted in less nasal and laryngeal toxicity but led to peribronchial and peribronchiolar lymphocytic inflammation. A similar pattern was observed with intermittent high-dose exposures at 1,200 ppm (15 minutes, twice a day, for 4 weeks). Subchronic exposures (6 hours/day for

12 weeks) of C57Bl/6 mice to 100 ppm 2,3-butanedione caused moderate nasal injury and lymphocytic bronchiolitis accompanied by epithelial atrophy, denudation, and regeneration. Subchronic (12 weeks) exposure to 2,3-butanedione did not cause bronchiolitis obliterans in mice; however, lymphocytic bronchiolitis is considered to be a potential precursor to bronchiolitis obliterans in lung transplant patients[48].

Because rodents are obligate nose breathers and because the rodent nasal cavity is highly efficient in scrubbing reactive components like 2,3-butanedione from the inspired air, some studies were conducted where 2,3-butanedione was administered by intratracheal instillation (rats) or oropharyngeal aspiration (mice) to bypass the nose. A single treatment of C57Bl/6 mice with 400 mg/kg 2,3-butanedione by oropharyngeal aspiration caused foci of fibrohistiocytic proliferation with little or no inflammation at the junction of the terminal bronchiole and alveolar duct[47]. A single intratracheal instillation of 2,3-butanedione (125 mg/kg) in male Sprague Dawley rats resulted in development of bronchiolitis obliterans[49]. In the rat, 2,3-butanedione-induced bronchiolitis obliterans was associated with necrosis of the bronchiolar epithelium followed by dysregulated repair of the injured epithelium and excessive deposition of the extracellular matrix component tenascin C. This study demonstrated that rats were more susceptible than mice to 2,3-butanedione-induced bronchiolitis obliterans and that 2,3-butanedione can cause bronchiolitis obliterans. In a subsequent inhalation study, Wistar Han rats exposed for 2 weeks to 200 ppm 2,3-butanedione or 2,3-pentane-dione, a related flavoring agent, developed bronchiolitis obliterans-like lesions similar to those occurring in humans[50].

Cutaneous sensitization by 2,3-butanedione may be initiated through haptenation of 2,3-butanedione with proteins containing the amino acids lysine and arginine[51]. Topical application of 2,3-butanedione caused sensitization based on a murine local lymph node assay[51-53]. Based on results of the local lymph node assay and immune cell phenotyping, it was suggested that 2,3-butanedione is a dermal sensitizer[52].

There is considerable interest in finding a safe chemical alternative to 2,3-butanedione because of the strong association with lung disease and the significant potential for human exposure. Only a limited number of chemicals produce butter flavor, and many of these chemicals are structurally related to 2,3-butanedione. 2,3-Pentanedione and 2,3-hexanedione are α-diketone flavoring ingredients that may be used as potential substitutes for 2,3-butanedione. Inhalation studies demonstrated that 2,3-pentanedione causes significant respiratory toxicity in rats and mice similar to that caused by 2,3-butanedione[50; 54].

The respiratory toxicity and chemical reactivity of 2,3-butanedione, 2,3-pentanedione, and 2,3-hexanedione were compared in a recent inhalation study[55]. Chemical reactivity of the diketones with an arginine substrate decreased with increasing chain length (2,3-butanedione ≥ 2,3-pentanedione > 2,3-hexanedione). Animals were evaluated the day after a 2-week exposure to 0, 100, 150, or 200 ppm 2,3-butanedione, 2,3-pentanedione, or 2,3-hexanedione (postexposure groups) or 2 weeks later (recovery groups). Bronchial fibrosis was observed in all 2,3-butanedione and 2,3-pentanedione rats at 200 ppm and in most 2,3-butanedione and all 2,3-pentanedione rats at 150 ppm in the postexposure groups. Bronchial fibrosis was not observed in 2,3-hexanedione-exposed rats, except for a minimal focus of fibrosis in two rats in the 200 ppm postexposure group. Patchy interstitial fibrosis in the lungs of recovery groups exposed to 150 or 200 ppm 2,3-pentanedione or 2,3-butanedione correlated with pulmonary function deficits. Minimal interstitial fibrosis occurred in two of the recovery group rats exposed to 200 ppm 2,3-hexanedione. These results indicated that 2,3-butanedione and 2,3-pentanedione were of similar reactivity and potency in causing bronchial fibrosis. 2,3-Hexanedione (and possibly longer chain α-diketones) exhibits toxicity, but it is less toxic than 2,3-butanedione or 2,3-pentanedione for the respiratory tract of rats.

Acetoin, the partial reduction product of 2,3-butanedione, is also used to produce butter flavor in foods. Acetoin was detected along with 2,3-butanedione in many of the workplaces where bronchiolitis obliterans occurred in workers who make or use 2,3-butanedione[23; 56]. Sub-chronic (13-week) inhalation studies of acetoin were conducted in male and female Wistar Han rats and B6C3F1/N mice (http://ntp.niehs.nih.gov/testing/status/agents/ts-m990018.html). No exposure-related effects on survival, body weights, organ weights, clinical pathology, or histopathology were observed in rats or mice following exposure to acetoin concentrations up to 800 ppm.

Humans

There are no epidemiological data on workers exposed to 2,3-butanedione alone. A retrospective study evaluated workers who were employed between 1960 and 2003 in a chemical plant producing 2,3-butanedione[23]. At least four cases of bronchiolitis obliterans were found in 206 workers employed in this 2,3-butanedione production facility. However, in addition to 2,3-butanedione, workers may also have been exposed to acetoin and acetaldehyde.

Bronchiolitis obliterans was initially diagnosed in a number of young, otherwise healthy, microwave popcorn workers[24]. Subsequent NIOSH medical and environmental surveys conducted at flavoring manufacturing plants and microwave popcorn packaging facilities have provided much of the information on the human health effects of artificial butter flavoring containing 2,3-butanedione[32; 57-60]. The main symptoms of affected workers at these sites were progressive shortness of breath on exertion, chronic nonproductive cough, and wheezing[22; 24]. Although skin, eye, nose, and throat irritation were reported by some microwave popcorn workers, the primary site of injury was the distal airways; the onset of symptoms was usually gradual over months or years[22]. Airway obstruction in affected workers was diagnosed as bronchiolitis obliterans, an uncommon lung disease characterized by pulmonary function changes that are associated with scarring and constriction of the small airways[61].

Reproductive and Developmental Toxicity

Experimental Animals

When given by gavage to pregnant mice for 10 days, 2,3-butanedione (1.6 g starter distillate/kg) had no effect on maternal or fetal survival or nidation. There were no statistically significant changes in the number of fetal abnormalities compared to controls. Tests in hamsters and rats gave similar results[1].

Humans

No case reports were found in the literature that reported reproductive or developmental toxicity of 2,3-butanedione in humans.

Carcinogenicity

Experimental Animals

Inhalation studies evaluating the carcinogenic potential of 2,3-butanedione vapor were not found in the literature. Administration of 2,3-butanedione by intraperitoneal injection (1.7 or 8.4 mg/kg) to male and female A/He mice once weekly for 24 weeks did not induce any lung tumors[62].

Furihata et al.[63] studied potential initiating and promoting activities of 2,3-butanedione in the rat glandular stomach. 2,3-Butanedione administered at 150 to 400 mg/kg by gavage to male F344 rats induced increases up to 100-fold in ornithine decarboxylase activity after 16 hours. Treatment with 2,3-butanedione also induced a greater than 10-fold increase in DNA synthesis. The authors concluded that 2,3-butanedione has potential tumor-promoting activity.

Humans

No epidemiological studies were found in the literature that evaluated the carcinogenic potential of 2,3-butanedione in humans.

Genetic Toxicity

2,3-Butanedione has been shown to be mutagenic in *Salmonella typhimurium* base-substitution strains TA100, TA102, and TA104, with and without rat liver S9 activation[64-66]; no mutagenicity was shown in the frameshift strain TA98, with or without S9[64; 66]. In tests carried out in the yeast *Saccharomyces cerevisiae*, 2,3-butanedione (173 to 393 µg/mL) did not induce chromosome loss, mitotic recombination, or respiratory deficient mutants when cultures were tested at 28°C or under conditions of cold shock, which has been shown to enhance the effects of some chemicals in this assay[67].

In mammalian cell test systems, significant increases in the number of sister chromatid exchanges (SCE) (suggestive of DNA damage) were observed in Chinese hamster ovary AUXB1 cells treated with 2,3-butanedione (125 or 250 µM)[68]. In a follow-up investigation, up to 82% of the SCE-inducing activity of 2,3-butanedione (at the 125 µM dose) was blocked in the presence of 1 mM bisulfite, which reacts with 1,2-dicarbonyl compounds to eliminate the carbonyl moiety, believed to be the active subgroup responsible for genotoxicity[68]. 2,3-Butanedione was also strongly mutagenic in mouse lymphoma L5178Y $TK^{+/-}$ cells at doses of 200 and 250 µg/mL when tested in the presence of pooled human S9 mix[69]; no testing was conducted in the absence of S9.

Study Rationale

Artificial butter flavoring, and two major volatile components, 2,3-butanedione and acetoin, were nominated by the United Food and Commercial Workers Union for long-term inhalation studies. This nomination was based on outbreaks of bronchiolitis obliterans, a severe fibroproliferative disease of the small airways, in workers exposed to artificial butter flavoring vapors. Occupational exposure limits and inhalation toxicity data for these chemicals did not exist. Because inhalation studies on the artificial butter flavor mixture were being conducted by NIOSH, NTP conducted separate studies on two major volatile components, 2,3-butanedione and acetoin, as well as 2,3-pentanedione, a potential replacement for 2,3-butanedione. The results of NTP Laboratory and NIOSH short-term studies investigating the mechanism(s) by which 2,3-butanedione and 2,3-pentanedione cause respiratory toxicity were published in the peer-reviewed literature. These studies demonstrated that short-term (2-week) exposure to 2,3-butanedione concentrations greater than or equal to 150 ppm caused bronchiolitis obliterans-like lesions in rats. However, there was no information available on the potential toxicity of subchronic or chronic exposure to lower concentrations of 2,3-butanedione. NTP 3-month inhalation studies were conducted on 2,3-butanedione, acetoin, and 2,3-pentanedione for comparison of toxicity. NTP chronic toxicology and carcinogenicity studies were only conducted on 2,3-butanedione because of the greater potential for human exposure relative to other components of artificial butter flavoring.

Table 1. Summary of NIOSH and NTP Studies of Artificial Butter Flavoring Constituents

Substance	Exposure Duration	Study
NIOSH Studies[a]		
Artificial butter flavoring	6 hours	Hubbs et al.[45]
2,3-Butanedione	6 hours constant, intermittent	Hubbs et al.[46]
2,3-Butanedione	NA	Morris and Hubbs[36]
2,3-Butanedione	6 hours	Hubbs et al.[70]
2,3-Butanedione	6 hours	Hubbs et al.[54]
2,3-Pentanedione	6 hours	Hubbs et al.[54]
NIEHS Studies		
NTP Guideline Studies		
Acetoin	2 weeks and 3 months	NTP[b] (in preparation)
2,3-Butanedione	3 months and 2 years	NTP (current Technical Report)
2,3-Pentanedione	3 months	NTP[c] (in preparation)
NTP Research Program[a]		
2,3-Butanedione	5 days to 12 weeks	Morgan et al.[47]
2,3-Butanedione	NA	Mathews et al.[71]
2,3-Butanedione	1 week (ITI)	Palmer et al.[49]
2,3-Butanedione	1 week (ITI)	Kelly et al.[72]
^{14}C-2,3-Butanedione	24 hours (ITI)	Fennell et al.[35]
2,3-Pentanedione	2 weeks	Morgan et al.[50]
2,3-Pentanedione	2 weeks	Morgan et al.[73]
2,3-Butanedione	2 weeks	Morgan et al.[55]
2,3-Hexanedione	2 weeks	Morgan et al.[55]
2,3-Pentanedione	2 weeks	Morgan et al.[55]

[a]Full citations appear in the reference list for this report.
[b]https://ntp.niehs.nih.gov/testing/status/agents/ts-m990018.html.
[c]https://ntp.niehs.nih.gov/testing/status/agents/ts-08010.html.
NA – not applicable; ITI – Single intratracheal instillation.

Materials and Methods

Procurement and Characterization of 2,3-Butanedione

2,3-Butanedione was obtained from Sigma-Aldrich (Aldrich Chemical Co., Inc., Sheboygan Falls, WI) in two lots (10815TD and 03798LJ). Lot 10815TD was used in the 3-month studies, and lot 03798LJ was used in the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Chemir Analytical Services (Maryland Heights, MO) and by the study laboratory at Battelle Toxicology Northwest (Richland, WA) (Appendix H). Reports on analyses performed in support of the 2,3-butanedione studies are on file at the National Institute of Environmental Health Sciences.

The test chemical, a yellow liquid, was identified as 2,3-butanedione by the analytical chemistry laboratory and the study laboratory using Fourier transform infrared spectroscopy and by the analytical chemistry laboratory using proton nuclear magnetic resonance spectroscopy. All spectra were consistent with the structure and composition of 2,3-butanedione.

Elemental analysis was performed by Galbraith Laboratories (Knoxville, TN), and water content was determined by the analytical chemistry laboratory using Karl Fischer titration. The relative purity and area percent purity were determined by the study laboratory using gas chromatography (GC) with flame ionization detection (FID).

For lot 10815TD, elemental analyses for carbon, hydrogen, nitrogen, and sulfur were consistent with theoretical values for 2,3-butanedione. Karl Fischer titration indicated 0.1% water content. In samples collected from the top, middle, and bottom of the drum, GC/FID indicated an average purity of 98.7% and four minor peaks with areas greater than 0.1% of the total peak area. These impurities were identified as ethyl acetate (0.39%), 2-butanone (0.51%), acetonitrile (0.24%), and acetic acid (0.12%), based on the retention time relative to authentic standards.

For lot 03798LJ, elemental analyses for carbon, hydrogen, nitrogen, and sulfur were consistent with theoretical values for 2,3-butanedione. Karl Fischer titration indicated 0.42% water content. Average purity in samples taken from the top, middle, and bottom of the drum was 99.1% using GC/FID, and four minor peaks with areas greater than or equal to 0.1% of the total peak area were indicated. Three of the four impurities were identified as acetaldehyde (0.1%), acetic acid (0.3%), and acetoin (0.3%).

To ensure stability, the test chemical was stored at refrigerated temperatures in metal drums under a nitrogen headspace. Periodic reanalyses of the test chemical were performed by the study laboratory using GC/FID prior to and after the 3-month and 2-year studies and approximately every 6 months during the 2-year studies; no degradation of the test chemical was detected.

Vapor Generation and Exposure Systems

2,3-Butanedione was pumped onto glass beads in a heated glass column where it was vaporized. Heated nitrogen flowed through the column and carried the vapor to a short vapor-distribution manifold, where concentration was controlled by the chemical pump and nitrogen flow rates. For the 2-year studies, the nitrogen-chemical mixture was diluted with heated air (~140°F) before entering the distribution manifold. Pressure in the distribution manifold was fixed to ensure constant flows through the manifold and into the chambers.

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 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 (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) 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 present in the exposure chambers to ensure that 2,3-butanedione vapor, and not aerosol, was produced. No particle counts above the minimum resolvable level (approximately 200 particles/cm^3) were detected.

Vapor Concentration Monitoring

Chamber concentrations of 2,3-butanedione were monitored using an online GC/FID. Samples were drawn from each exposure chamber approximately every 20 minutes during each 6-hour exposure period using Hasteloy-C stream-select and gas-sampling valves (VALCO Instruments Co., Houston, TX) in a separate, heated valve 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 chromato-graph allowed digital measurement of sample flow.

The on-line gas chromatograph was checked throughout the day for instrument drift against an on-line standard of 2,3-butanedione in nitrogen supplied by a standard generator (Kin-Tek; Precision Calibration Systems, La Marque, TX). The on-line gas chromatograph was calibrated prior to the start of each study and monthly during the 3-month and 2-year studies by a comparison of chamber concentration data to data from grab samples that were collected with sorbent gas sampling tubes containing silica gel (ORBO-53; Supelco, Bellefonte, PA) followed by a sampling tube containing activated coconut charcoal (ORBO-32; Supelco), extracted with acetone containing 2-methyl-1-propanol as an internal standard, and analyzed using an off-line gas chromatograph. The volumes of gas were sampled at a constant flow rate ensured by a calibrated critical orifice. The off-line gas chromatograph was calibrated with gravimetrically prepared standards of 2,3-butanedione and the internal standard 2-methyl-1-propanol in acetone.

Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined with and without animals present in the chambers. For rats and mice in the 3-month studies, T_{90} values ranged from 9 to 11 minutes without animals present and from 9 to 13 minutes with animals present; T_{10}

values ranged from 9 to 11 minutes without animals present and from 10 to 11 minutes with animals present. For rats and mice in the 2-year studies, T_{90} values ranged from 8 to 9 minutes without animals present and from 10 to 12 minutes with animals present; T_{10} values ranged from 8 to 10 minutes without animals present and from 10 to 11 minutes with animals present. A T_{90} value of 10 minutes was selected for the 3-month studies, and a T_{90} value of 12 minutes was selected for the 2-year studies.

The persistence of 2,3-butanedione in the chambers after vapor delivery ended was determined by monitoring the postexposure vapor concentration in the 100 ppm rat/mouse chamber in the 3-month studies and the 50 ppm chambers in the 2-year studies without animals present in the chambers. In the 3-month studies, the concentration decreased to 1% of the target concentration within 20 minutes without animals present. During the 2-year studies in the rat only chambers, the concentration decreased to 1% of the target concentration within 21 minutes without animals present and 35 minutes with animals present. In the rat/mouse chambers, the concentration decreased to 1% of the target concentration within 19 minutes without animals present and 95 minutes with animals present.

The uniformity of 2,3-butanedione vapor concentration in the inhalation exposure chambers without animals present was evaluated before the 3-month and 2-year studies began; 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 with the stream-selection valve fixed in one position to allow continuous monitoring from a single input line. During the 3-month 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 2-year studies, concentrations were measured at the regular monitoring port and from all sample ports where animals were present. Chamber concentration uniformity was maintained throughout the studies.

Samples of the test atmosphere from the distribution lines and low and high exposure concentration chambers were collected prior to the 3-month and 2-year studies without animals, and also at the beginning of the 3-month and 2-year studies with animals. The atmosphere samples were collected at the beginning and the end of the exposure day with sorbent gas sampling tubes containing silica gel (ORBO-53; Supelco) and extracted with acetone. All of the samples were analyzed using GC/FID to measure the stability and purity of 2,3-butanedione in the generation and delivery system. To assess whether impurities or degradation products co-eluted with 2,3-butanedione or the solvent, a second GC/FID analysis was performed on samples extracted with dimethyl formamide. In conjunction with the stability and purity measurements described above, the purity of 2,3-butanedione in the generator reservoir was measured using GC/FID. To demonstrate the resolution and sensitivity of the system to detect low levels of possible impurity or degradation products, a 0.1% solution of acetoin, 2-butanone, 2,3-butanediol, 3-methyl-2-4-pen-tanedione, ethyl acetate, acetonitrile, and acetic acid was analyzed. During the 2-year studies, GC/FID used to detect impurities was unable to determine the presence of ethyl acetate or 2-butanone due to a contaminant peak that eluted at the same retention times. GC with mass spectrometry detection was used, and results indicated that ethyl acetate and 2-butanone were less than 0.1% in the distribution line, 50 ppm chambers, generator reservoir, and test chemical samples and less than 0.5% in the 12.5 ppm chambers.

Animal Source

Male and female Wistar Han [Crl:WI (Han)] rats were obtained from Charles River Laboratories (Raleigh, NC) and male and female 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 cumulative toxic effects of repeated exposure to 2,3-butanedione and to determine the appropriate exposure concentrations to be used in the 2-year studies.

On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 12 or 13 days and were approximately 6 weeks 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 J). All test results were negative.

Groups of 10 male and 10 female rats and mice were exposed by whole body inhalation to 2,3-butanedione vapor at concentrations of 0, 6.25, 12.5, 25, 50, or 100 ppm, 6 hours plus T_{90} (10 minutes) per day, 5 days per week for 14 weeks. These exposure concentrations were selected based upon results of previous 2- and 12 week studies in rats and mice, respectively[47; 50]. Additional groups of 10 male and 10 female rats were exposed to the same concentrations for 23 days for clinical pathology analyses. Feed was available ad libitum except during exposure periods; water was available ad libitum. Rats and mice were housed individually. Clinical observations were recorded weekly beginning on day 8 (female rats) or day 9 (male rats; mice) and at the end of the studies. The animals were weighed initially, on day 8 (female rats) or 9 (male rats; mice), weekly thereafter, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Blood was collected from clinical pathology rats on days 3 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats) analyses. Blood was collected from the retroorbital plexus of rats and the retroorbital sinus of mice anesthetized with carbon dioxide. Blood for hematology was placed in tubes containing potassium EDTA (Microtainer; Becton Dickinson; Franklin Lakes, NJ), and blood for clinical chemistry was placed in tubes containing a separator gel (Vacutainer; Becton Dickinson; Franklin Lakes, NJ). Hematology analyses were performed on an Abbott Cell-Dyn 3700 analyzer (Abbott Diagnostics Systems, Abbott Park, IL), except manual hematocrit determinations were performed using a microcentrifuge (Heraeus Holding GmbH., Hanau, Germany) and a Damon/IEC capillary reader (International Equipment Co., Needham Heights, MA). Platelet, leukocyte, and erythrocyte morphology and nucleated erythrocytes were assessed using smears stained with a Romanowsky-type aqueous stain in a Wescor 7120 aerospray slide stainer (Wescor, Inc., Logan, UT). Reticulocytes were stained with new methylene blue and counted using the Miller disc method[74]. Samples for clinical chemistry analyses were centrifuged, and parameters were measured using a Roche Hitachi 912 system (Roche Diagnostic Corp., Indianapolis, IN). Table 2 lists the clinical pathology parameters measured.

Necropsies were performed on all animals. The heart, right kidney, liver, lung, spleen, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes, testes, vaginal tunics, and epididymides were first fixed in Davidson's solution or a 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 100 ppm groups of rats and mice; respiratory system tissues were examined to a no-effect level in the remaining exposed groups. Table 2 lists the tissues and organs routinely examined.

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 Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman[75] and Boorman et al.[76].

Two-year Studies

Study Design

Groups of 50 male and 50 female rats and mice were exposed by whole body inhalation to 2,3-butanedione vapor at concentrations of 0, 12.5, 25, or 50 ppm, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 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 and mice were 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 J). All test results were negative.

Animals were housed individually. Feed was available ad libitum except during exposure periods; water was available ad libitum. Cages were rotated within chambers weekly. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix I.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical observations were recorded at week 5, at 4-week intervals through week 93, every 2 weeks thereafter, and at terminal euthanasia. Body weights were recorded on day 1, weekly thereafter for the first 13 weeks, at 4-week intervals through week 93, every 2 weeks thereafter, and at terminal euthanasia.

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 epidymides were first fixed in modified Davidson's solution). All tissue samples were 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 2.

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 eye, lung, nose, larynx, and trachea of rats and mice; the adrenal gland of mice; and the uterus of female rats.

The QA report and the reviewed slides were submitted to the NTP 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[75] and Boorman et al.[76]. 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.[77].

Because a recent report in the literature More et al.[78] noted an exacerbation of β-amyloid cytotoxicity in cell cultures by 2,3-butanedione, the histologic sections of the brain of all rats and mice exposed to 50 ppm 2,3-butanedione were reviewed and compared to the brains of the chamber control animals. In addition, subsets of 10 randomly selected male and female rats exposed to 50 ppm and 10 male and female chamber control rats, as well as equal numbers of randomly chosen high concentration and chamber control mice were selected for Bielschowsky silver staining and immuno-histochemical staining for β-amyloid-40 and -42 on histologic sections of the brain. No neurofibrillary tangles, neuritic plaques, or vascular amyloid were identified.

Table 2. Experimental Design and Materials and Methods in the Inhalation Studies of 2,3-Butanedione

Three-month Studies	Two-year Studies
Study Laboratory	
Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)
Strain and Species	
Wistar Han [Crl:WI (Han)] rats B6C3F1/N mice	Wistar Han [Crl:WI (Han)] rats B6C3F1/N mice
Animal Source	
Rats: Charles River Laboratories (Raleigh, NC) Mice: Taconic Farms, Inc. (Germantown, NY)	Rats: Charles River Laboratories (Raleigh, NC) Mice: Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
Rats: 12 or 13 days Mice: 12 days	12 days
Average Age When Studies Began	
6 weeks	5 to 6 weeks
Date of First Exposure	
Rats: July 28 (males) or 29 (females), 2008 Mice: July 28, 2008	Rats: August 17, 2009 Mice: August 31, 2009
Exposure Duration	
6 hours plus T90 (10 minutes) per day, 5 days per week, for 14 weeks	6 hours plus T90 (12 minutes) per day, 5 days per week, for 105 weeks
Date of Last Exposure	
Rats: October 27 (males) or 28 (females), 2008 Mice: October 29 (males) or 30 (females), 2008	Rats: August 18, 2011 Mice: September 1, 2011
Necropsy Dates	
Rats: October 28 (males) or 29 (females), 2008 Mice: October 30 (males) or 31 (females), 2008	Rats: August 15 to 19, 2011 Mice: August 29 to September 2, 2011
Average Age at Necropsy	
19 weeks	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
Animals per Cage	
1	1

Three-month Studies	Two-year Studies
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	Same as 3-month studies
Water	
Tap water (City of Richland, WA) 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 pan liner (Shepherd Specialty Papers, Watertown, TN)	Same as 3-month studies
Chamber Air Supply Filters	
Single HEPA (open stock); charcoal (RSE, Inc., New Baltimore, MI); Purafil (Environmental Systems, Lynwood, WA), new at study start	Same as 3-month studies, except single HEPA changed annually
Chambers	
Stainless steel, excreta pan at each of six levels (Lab Products, Inc.), excreta pans changed daily, chambers changed weekly	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, 6.25, 12.5, 25, 50, and 100 ppm	0, 12.5, 25, and 50 ppm
Type and Frequency of Observation	
Observed twice daily; core study animals were weighed initially, and core study animals were weighed and clinical observations were recorded on day 8 (female rats), day 9 (male rats; mice), weekly thereafter, and at the end of the studies.	Observed twice daily; clinical findings were recorded at week 5, at 4-week intervals through week 93, every 2 weeks thereafter, and at terminal euthanasia; animals were weighed on day 1, weekly thereafter for the first 13 weeks, at 4-week intervals through week 93, every 2 weeks thereafter, and at terminal euthanasia.
Method of Euthanasia	
Carbon dioxide asphyxiation	Same as 3-month studies

Three-month Studies	Two-year Studies
Necropsy	
Necropsies were performed on all animals. Organs weighed from core study animals were heart, right kidney, liver, lung, spleen, right testis, and thymus	Necropsies were performed on all animals.
Clinical Pathology	
Blood was collected from the retroorbital plexus of clinical pathology rats on days 3 and 23 and of core study rats at the end of the study for hematology and clinical chemistry. Blood was collected from the retroorbital sinus of mice at the end of the study for hematology. Hematology: hematocrit; packed cell volume; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; Howell-Jolly bodies (mice only); mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials Clinical chemistry: urea nitrogen; creatinine, glucose, total protein, albumin, globulin, cholesterol, triglyceride, alanine aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, creatine kinase, bile acids	None
Histopathology	
Complete histopathology was performed on 0 and 100 ppm core study 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, mesenteric, and mediastinal), 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. Respiratory system tissues were examined to a no-effect level in the remaining exposed 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, mesenteric, and mediastinal), mammary gland (females only), 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.

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of[79] 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[80] method for testing two groups for equality and Tarone's life table test[81] 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-3, Table D-1, and Table D-3 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 euthanasia.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test[82-84] 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 euthanasia; if the animal died prior to terminal euthanasia 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[83]. 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[83] following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice[85]. Bailer and Portier[83] 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[86].

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P = 0.99 is presented as P = 0.01N).

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[87] and Williams[88; 89]. Hematology and clinical chemistry data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[90] (as modified by Williams[91]) and Dunn[92]. Jonckheere's test[93] 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[94] were examined by NTP personnel, and implausible values were eliminated from the analysis.

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[95-97]. 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 studies.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations[98]. 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 2,3-butanedione was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli*, micronucleated erythrocytes in mouse bone marrow, 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[99; 100]. 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[101] and the somatic mutation theory of cancer[102; 103]. However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites[104]. A positive response in the *Salmonella* test was shown to be the most predictive in vitro indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens)[105; 106]. 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[107; 108]. 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[109]. 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-593.

Rats

Three-month Study

Two male rats in the 100 ppm group were found dead on days 91 and 92, respectively (Table 3). All other rats survived to the end of the study. The final mean body weights and body weight gains of 100 ppm males and females were significantly less than those of the chamber control groups (Table 3 and Figure 2). Abnormal breathing was observed in 100 ppm males (5/10) and females (5/10) and 50 ppm females (6/10). Sneezing was observed in 50 ppm males (9/10) and females (6/10) and in 100 ppm males (9/10) and females (8/10). Lethargy was observed in one 100 ppm male.

Table 3. Survival and Body Weights of Rats in the Three-month Inhalation Study of 2,3-Butanedione[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	143 ± 3	410 ± 8	267 ± 8	
6.25	10/10	144 ± 3	401 ± 13	258 ± 11	98
12.5	10/10	142 ± 3	386 ± 8	244 ± 8	94
25	10/10	143 ± 3	407 ± 16	264 ± 15	99
50	10/10	144 ± 3	387 ± 8	243 ± 7	94
100	8/10[c]	141 ± 4	333 ± 12**	192 ± 10**	81
Female					
0	10/10	124 ± 2	236 ± 6	112 ± 5	
6.25	10/10	122 ± 2	235 ± 5	113 ± 4	99
12.5	10/10	123 ± 2	228 ± 4	106 ± 4	97
25	10/10	122 ± 2	226 ± 4	104 ± 4	96
50	10/10	120 ± 2	232 ± 6	112 ± 5	98
100	10/10	122 ± 2	208 ± 9**	86 ± 8**	88

**Significantly different ($P \leq 0.01$) from the chamber control group by Williams' or Dunnett's test.
[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]Weeks of death: 13, 14.

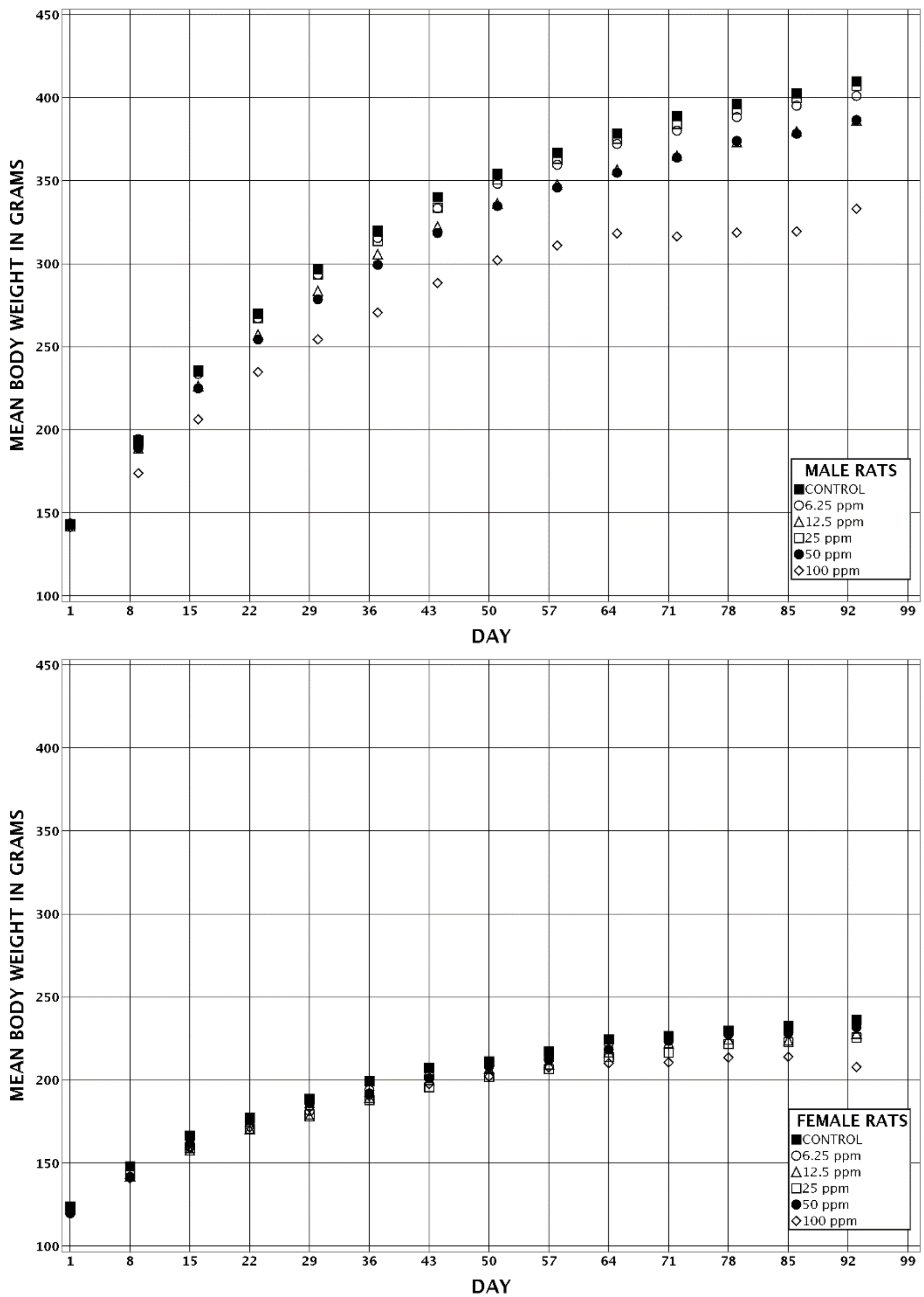


Figure 2. Growth Curves for Rats Exposed to 2,3-Butanedione by Inhalation for Three Months

On day 23 and at study termination, neutrophil counts in the blood were significantly increased in 100 ppm females and were consistent with the inflammation observed in the respiratory tract (Table 4 and Table F-1). Erythrocyte count, hemoglobin concentration, packed cell volume, and hematocrit were significantly increased on day 23 in 100 ppm females. At study termination, the erythrocyte count, hemoglobin concentration, and packed cell volume were significantly increased in 100 ppm males; hemoglobin concentration and packed cell volume were also significantly increased in the 50 ppm group of males. In female rats at study termination, the erythrocyte count was significantly increased in the 50 and 100 ppm groups, and the packed cell volume was significantly increased in the 100 ppm group. While it may be that the mild increase in the erythron was due to decreased water intake, other indicators of dehydration were unchanged (urea nitrogen and total protein concentrations). Another plausible explanation for the erythron alterations is that the extent of the respiratory lesions in these particular groups led to hypoxia and a mild secondary erythrocytosis. No other statistically significant changes in hematology parameters were considered toxicologically relevant.

Triglyceride concentrations were decreased in 100 ppm males and 100 ppm females on day 3 (Table F-1). These alterations were transient and may have been due to decreases in feed intake as the animals acclimated to exposure. All other statistically significant biochemical changes were mild, inconsistent, and not considered toxicologically relevant.

Table 4. Selected Hematology Data for Rats in the Three-month Inhalation Study of 2,3-Butanedione[a]

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Male						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	10	10	10	10	10	7
Packed cell volume (%)						
Day 3	44.1 ± 0.6	43.0 ± 0.5	41.4 ± 0.6*	42.1 ± 0.5	42.4 ± 0.4	42.7 ± 0.7
Day 23	46.8 ± 0.7	46.0 ± 0.6	46.5 ± 0.7	46.6 ± 0.9	45.9 ± 0.7	47.0 ± 0.8
Week 14	47.2 ± 0.7	47.3 ± 0.6	47.5 ± 0.7	48.6 ± 1.2	49.4 ± 0.8*	50.3 ± 1.1*
Hemoglobin (g/dL)						
Day 3	13.7 ± 0.2	13.4 ± 0.2	13.1 ± 0.2	13.3 ± 0.2	13.3 ± 0.1	13.4 ± 0.2
Day 23	14.7 ± 0.2	14.6 ± 0.2	14.7 ± 0.2	14.8 ± 0.3	14.4 ± 0.2	14.9 ± 0.2
Week 14	15.2 ± 0.2	15.3 ± 0.2	15.4 ± 0.2	15.7 ± 0.3	15.7 ± 0.2*	16.3 ± 0.4**
Erythrocytes ($10^6/\mu L$)						
Day 3	7.07 ± 0.10	6.86 ± 0.12	6.68 ± 0.11	6.89 ± 0.09	6.96 ± 0.09	6.96 ± 0.14
Day 23	7.80 ± 0.11	7.64 ± 0.15	7.67 ± 0.13	7.80 ± 0.12	7.75 ± 0.11	7.98 ± 0.16
Week 14	8.82 ± 0.14	8.86 ± 0.13	8.97 ± 0.10	8.93 ± 0.25	9.14 ± 0.16	9.57 ± 0.19**

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Female						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 3	45.2 ± 0.5	45.7 ± 0.3	45.4 ± 0.4	44.0 ± 0.7	45.2 ± 0.5	44.6 ± 0.9
Day 23	47.2 ± 0.2	47.3 ± 0.4	48.1 ± 0.7	47.6 ± 0.6	48.3 ± 0.5	48.9 ± 0.5**
Week 14	46.3 ± 0.5	45.8 ± 0.6	46.8 ± 0.7	46.1 ± 0.3	47.8 ± 0.4	48.1 ± 0.8
Packed cell volume (%)						
Day 3	43.7 ± 0.5	43.6 ± 0.4	43.0 ± 0.4	41.8 ± 0.5	43.3 ± 0.5	42.8 ± 0.8
Day 23	45.4 ± 0.2	45.8 ± 0.3	46.4 ± 0.6	45.9 ± 0.5	46.4 ± 0.5	47.5 ± 0.5**
Week 14	45.7 ± 0.3	45.7 ± 0.4	46.9 ± 0.6	45.7 ± 0.4	47.1 ± 0.5	47.6 ± 0.6*
Hemoglobin (g/dL)						
Day 3	13.7 ± 0.2	13.7 ± 0.1	13.6 ± 0.1	13.1 ± 0.2	13.8 ± 0.2	13.6 ± 0.2
Day 23	14.8 ± 0.1	14.9 ± 0.1	15.1 ± 0.1	14.9 ± 0.2	15.2 ± 0.2	15.4 ± 0.2**
Week 14	14.8 ± 0.1	14.9 ± 0.1	15.0 ± 0.2	14.7 ± 0.1	15.3 ± 0.1	15.3 ± 0.3
Erythrocytes (10^6/µL)						
Day 3	7.15 ± 0.13	7.10 ± 0.07	7.08 ± 0.12	6.93 ± 0.11	7.19 ± 0.05	7.12 ± 0.17
Day 23	7.68 ± 0.06	7.68 ± 0.05	7.88 ± 0.11	7.82 ± 0.11	7.90 ± 0.11	8.07 ± 0.14*
Week 14	8.17 ± 0.09	8.18 ± 0.09	8.36 ± 0.12	8.16 ± 0.07	8.54 ± 0.11*	8.67 ± 0.17*
Segmented neutrophils (10^3/µL)						
Day 3	0.90 ± 0.11	0.76 ± 0.10	0.65 ± 0.06	0.69 ± 0.08	0.77 ± 0.06	1.03 ± 0.14
Day 23	0.90 ± 0.09	0.82 ± 0.09	0.63 ± 0.08	0.75 ± 0.10	1.82 ± 0.35	1.59 ± 0.24*
Week 14	1.54 ± 0.21	1.27 ± 0.15	1.24 ± 0.08	1.32 ± 0.27	2.19 ± 0.27	3.48 ± 0.42**

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

Relative lung weight was significantly increased in 100 ppm females (Table G-1). Significant decreases in absolute organ weights were restricted to the 100 ppm groups. Absolute heart weights were significantly decreased in 100 ppm males, while relative heart weights were increased in both sexes at 100 ppm. Significant decreases occurred in absolute liver, thymus, and right kidney weights of 100 ppm males and absolute liver and thymus weights of 100 ppm females. These findings are of uncertain toxicologic significance given the body weight decreases in these groups. No treatment-related lesions were noted in these tissues.

Exposure-related histopathology findings occurred in the nose, larynx, trachea, lung, and eye of rats. In the nose, lesions occurred primarily in the 50 and 100 ppm groups, as well as in a few 25 ppm males and females (Table 5). Suppurative inflammation of mild to marked severity occurred in all males and females exposed to 50 or 100 ppm. Mild respiratory epithelium necrosis occurred in all males and females exposed to 100 ppm, and minimal necrosis occurred in a few of the 50 ppm animals. Respiratory epithelium regeneration occurred in most of the rats exposed to 100 ppm and in a few exposed to 50 ppm. Mild to marked squamous metaplasia of the respiratory epithelium occurred in all 50 and 100 ppm rats, and minimal squamous metaplasia occurred in most of those exposed to 25 ppm. Respiratory epithelium hyperplasia occurred in all females and most males exposed to 50 or 100 ppm, and minimal hyperplasia occurred in two 25 ppm males. In animals exposed to 100 ppm, mild olfactory epithelium necrosis occurred in nine males and minimal necrosis occurred in four females. Olfactory epithelium degeneration, characterized by cytoplasmic vacuolation, occurred in Level II of most of the 50 ppm rats and also occurred in some 25 ppm males and females and 100 ppm females. Minimal to moderate respiratory metaplasia of the olfactory epithelium occurred in all females and most of the males exposed to 50 or 100 ppm and also in two 25 ppm males. Olfactory epithelium atrophy occurred in four females and one male exposed to 100 ppm. Atrophy of olfactory epithelium may occur as a sequel to degeneration or necrosis of the olfactory epithelium. Mild to moderate turbinate atrophy, primarily affecting the nasoturbinate bones in Level I, occurred in all males and females exposed to 100 ppm; minimal to mild turbinate atrophy also occurred in a few of the 50 ppm rats. Necrosis of septal cartilage occurred in one male and two females in the 100 ppm groups. Hyperplasia of nasal-associated lymphoid tissue adjacent to the nasopharyngeal duct in Level III occurred in most of the rats exposed to 50 or 100 ppm, and minimal hyperplasia occurred in a few animals exposed to 25 ppm.

Table 5. Incidences of Nonneoplastic Lesions of the Respiratory System in Rats in the Three-month Inhalation Study of 2,3-Butanedione

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Male						
Nose[a]	10	10	10	10	10	10
Inflammation, Suppurative[b]	0	0	0	1 (1.0)[c]	10** (2.5)	10** (3.6)
Respiratory Epithelium, Necrosis	0	0	0	0	2 (1.0)	10** (2.2)
Respiratory Epithelium, Regeneration	0	0	0	0	4* (1.0)	10** (1.2)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	8** (1.0)	10** (2.3)	10** (3.5)
Respiratory Epithelium, Hyperplasia	0	0	0	2 (1.0)	10** (2.6)	9** (2.7)
Olfactory Epithelium, Necrosis	0	0	0	0	0	9** (2.1)
Olfactory Epithelium, Degeneration	0	0	0	3 (1.0)	9** (1.6)	0
Olfactory Epithelium, Metaplasia, Respiratory	0	0	0	2 (1.0)	7** (1.9)	9** (2.0)
Olfactory Epithelium, Atrophy	0	0	0	0	0	1 (2.0)
Turbinate, Atrophy	0	0	0	0	3 (1.0)	10** (2.2)

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Septum, Necrosis	0	0	0	0	0	1 (1.0)
Lymphoid Tissue, Hyperplasia	0	0	0	3 (1.0)	9** (1.7)	6** (2.3)
Larynx	10	10	10	10	10	10
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	8** (1.4)	10** (3.5)
Respiratory Epithelium, Hyperplasia	0	0	0	0	3 (1.3)	1 (2.0)
Squamous Epithelium, Hyperplasia	0	0	0	0	0	5* (2.0)
Squamous Epithelium, Hyperplasia, Atypical	0	0	0	0	0	2 (2.0)
Epithelium, Necrosis	0	0	0	0	0	2 (2.0)
Inflammation, Chronic Active	0	0	0	0	0	2 (1.0)
Trachea	10	10	10	10	10	10
Epithelium, Necrosis	0	0	0	0	0	10** (3.5)
Epithelium, Regeneration	0	0	0	0	0	9** (1.4)
Epithelium, Metaplasia, Squamous	0	0	0	0	0	4* (1.3)
Epithelium, Hyperplasia	0	0	0	0	2 (1.0)	2 (1.5)
Inflammation, Chronic Active	0	0	0	0	0	2 (1.5)
Lung	10	10	10	10	10	10
Bronchus, Epithelium, Hyperplasia	0	0	0	0	0	4* (1.8)
Bronchus, Epithelium, Hyperplasia, Atypical	0	0	0	0	0	3 (2.0)
Bronchus, Epithelium, Necrosis	0	0	0	0	0	4* (2.3)
Bronchus, Epithelium, Regeneration	0	0	0	0	0	5* (2.0)
Bronchiole, Epithelium, Hyperplasia	0	0	0	0	0	5* (1.2)
Inflammation, Eosinophil	2 (1.0)	0	3 (1.0)	1 (1.0)	0	6 (2.0)
Infiltration Cellular, Histiocyte	1 (1.0)	0	1 (1.0)	0	0	7** (1.4)
Female						
Nose	10	10	10	10	10	10

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Inflammation, Suppurative	0	0	0	0	10** (2.0)	10** (3.6)
Respiratory Epithelium, Necrosis	0	0	0	0	3 (1.0)	10** (2.2)
Respiratory Epithelium, Regeneration	0	0	0	0	3 (1.0)	9** (1.3)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	5** (1.0)	10** (2.8)	10** (3.7)
Respiratory Epithelium, Hyperplasia	0	0	0	0	10** (2.6)	10** (2.8)
Olfactory Epithelium, Necrosis	0	0	0	0	0	4* (1.0)
Olfactory Epithelium, Degeneration	0	0	0	4* (1.0)	9** (1.0)	4* (1.8)
Olfactory Epithelium, Metaplasia, Respiratory	0	0	0	0	10** (1.4)	10** (2.5)
Olfactory Epithelium, Atrophy	0	0	0	0	0	4* (1.5)
Turbinate, Atrophy	0	0	0	0	4* (1.3)	10** (2.0)
Septum, Necrosis	0	0	0	0	0	2 (1.5)
Lymphoid Tissue, Hyperplasia	0	1 (1.0)	0	2 (1.0)	8** (1.8)	6** (2.0)
Larynx	10	10	10	10	10	10
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	8** (1.0)	10** (3.0)
Squamous Epithelium, Hyperplasia	0	0	0	0	0	9** (2.0)
Respiratory Epithelium, Hyperplasia	0	0	0	0	0	2 (1.5)
Epithelium, Necrosis	0	0	0	0	0	6** (1.7)
Inflammation, Chronic Active	0	0	0	0	0	5** (1.0)
Trachea	10	10	10	10	10	10
Epithelium, Necrosis	0	0	0	0	1 (2.0)	10** (2.7)
Epithelium, Regeneration	0	0	0	0	2 (1.0)	10** (1.2)
Epithelium, Hyperplasia	0	0	0	0	0	9** (2.4)
Inflammation, Chronic Active	0	0	0	0	0	1 (1.0)
Lung	10	10	10	10	10	10
Bronchus, Epithelium, Hyperplasia	0	0	0	0	0	5* (1.8)

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Bronchus, Epithelium, Hyperplasia, Atypical	0	0	0	0	0	4* (2.0)
Bronchus, Epithelium, Necrosis	0	0	0	0	0	2 (2.0)
Bronchus, Epithelium, Regeneration	0	0	0	0	0	4* (1.8)
Bronchus, Epithelium, Metaplasia, Squamous	0	0	0	0	0	2 (2.0)
Bronchiole, Epithelium, Hyperplasia	0	0	0	0	0	8** (1.0)
Inflammation, Eosinophil	4 (1.0)	3 (1.0)	0*	1 (1.0)	1 (1.0)	7 (2.0)
Infiltration Cellular, Histiocyte	0	0	0	0	0	3 (1.0)

*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.

In the larynx, respiratory epithelium squamous metaplasia of moderate to marked severity occurred in all rats exposed to 100 ppm, and minimal to mild squamous metaplasia occurred in most of the 50 ppm animals (Table 5). The squamous epithelium lining the arytenoid cartilages was mildly hyperplastic in most of the 100 ppm animals, and two 100 ppm males exhibited mild atypical hyperplasia of the squamous epithelium. Minimal to mild respiratory epithelium hyperplasia occurred in a few 50 and 100 ppm males and two 100 ppm females. Foci of epithelium necrosis occurred in two males and six females exposed to 100 ppm. The foci of necrosis were small and sometimes located in more than one location in the same animal. The sites of necrosis were variable, but were most often seen either in the squamous epithelium lining the arytenoid cartilages or in the respiratory epithelium lining the lateral walls of Level III. Minimal chronic active inflammation occurred in a few of the 100 ppm rats.

In the trachea, mild to marked mucosal epithelium necrosis occurred in all 100 ppm males and females and mild epithelium necrosis occurred in one 50 ppm female rat (Table 5). The necrosis was a focal to multifocal change consisting of loss of the epithelium with the denuded tracheal surface sometimes covered by a layer of inflammatory exudate and cell debris. Minimal to mild epithelium regeneration occurred in most of the 100 ppm animals; this lesion also occurred with a minimal severity in two 50 ppm females. Regeneration was characterized by a single layer of cuboidal epithelium covering the tracheal surface and was considered to be a reparative change secondary to necrosis. Minimal to mild epithelium squamous metaplasia occurred in four 100 ppm males. In the distal portion of the trachea, hyperplasia of the mucosal epithelium occurred in nine 100 ppm females, two 100 ppm males, and two 50 ppm males. Minimal to mild chronic active inflammation occurred in a few 100 ppm animals, usually in areas of epithelium necrosis.

In the lung, bronchus epithelium changes occurred only in the large bronchi of the 100 ppm groups. Either minimal to mild bronchus epithelium hyperplasia or mild atypical bronchus epithelium hyperplasia occurred in the majority of the rats exposed to 100 ppm (Table 5). Hyperplasia of the bronchial epithelium consisted of irregular thickening of the epithelial layer due to an increase in the number of epithelial cells, while atypical hyperplasia was characterized by thickened epithelium with the cells having enlarged, hyperchromatic nuclei. The severity grading of the atypical hyperplasia was based upon both the severity of the atypical cellular changes and the extent of the lesion. Mild to moderate bronchus epithelium necrosis occurred in four 100 ppm males, and mild epithelium necrosis occurred in two 100 ppm females. The necrosis was characterized by areas of partial to total loss of the full thickness of the respiratory epithelium, sometimes with residual necrotic cells. Mild bronchus epithelium regeneration occurred in half of the 100 ppm males, and minimal to mild regeneration occurred in four of the 100 ppm females. In areas of regeneration, the normal respiratory epithelium was replaced by a single layer of flattened to cuboidal epithelial cells, presumably covering areas of previous epithelial loss due to necrosis. Mild squamous metaplasia of the bronchus epithelium occurred in two 100 ppm females. Minimal bronchiole epithelium hyperplasia occurred in most of the 100 ppm females, and minimal to mild bronchiole epithelium hyperplasia occurred in half of the 100 ppm males. Hyperplasia of the bronchiole epithelium consisted of an increase in epithelial thickness due to an increase in the number of epithelial cells as well as an increase in the size of some epithelial cells, often with increased size of the nuclei. Epithelial cells in affected bronchioles appeared crowded and sometimes disorganized, and in some cases, appeared to form more than one layer. A mild eosinophilic inflammatory infiltrate occurred in the lungs of most 100 ppm males and females, centered around the blood vessels and airways, with occasional extension into a few alveolar lumens. The infiltrate consisted primarily of eosinophils, often mixed with a few neutrophils, lymphocytes, and macrophages, and also occurred in a few of the animals exposed to lower concentrations, as well as in a few of the chamber controls. Minimal to mild cellular infiltrations of histiocytes, sometimes in aggregates, also occurred in the alveolar spaces of most of the 100 ppm males, and minimal infiltrations of alveolar histiocytes occurred in one 12.5 ppm male, one chamber control male, and three 100 ppm females.

A few exposed rats exhibited lesions of the cornea of the eye (Table 6). Two 100 ppm males and three 100 ppm females exhibited minimal to mild acute inflammation of the cornea; one 50 ppm female had mild acute corneal inflammation. Minimal neovascularization of the corneal stroma accompanied the inflammation in one male and one female each in the 100 ppm groups, and minimal vacuolation of the corneal epithelium occurred in one 100 ppm female. Minimal mineralization of the corneal stroma occurred in one 50 ppm male.

Exposure Concentration Selection Rationale: Based on significant reductions in body weights and increased incidences of nonneoplastic lesions of the respiratory tract at 100 ppm, 2,3-butanedione exposure concentrations selected for the 2-year inhalation study in rats were 12.5, 25, and 50 ppm. Nasal lesions were a concern at 50 ppm; however, this concentration was included because lower exposure concentrations were not expected to result in sufficient airway exposure and injury to potentially cause bronchiolitis obliterans. The 50 ppm exposure concentration was also included because it was considered occupationally relevant. Flavoring mixers were exposed to a mean concentration of 57.2 ppm 2,3-butanedione in a microwave popcorn manufacturing plant where some workers developed bronchiolitis obliterans[25].

Table 6. Incidences of Nonneoplastic Lesions of the Eye in Rats in the Three-month Inhalation Study of 2,3-Butanedione

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Male						
Number Examined Microscopically	10	0	0	0	10	10
Cornea, Inflammation, Acute[a]	0	–	–	–	0	2 (1.5)[b]
Cornea, Neovascularization	0	–	–	–	0	1 (1.0)
Cornea, Mineralization	0	–	–	–	1 (1.0)	0
Female						
Number Examined Microscopically	10	0	0	0	10	10
Cornea, Inflammation, Acute	0	–	–	–	1 (2.0)	3 (1.3)
Cornea, Neovascularization	0	–	–	–	0	1 (1.0)
Cornea, Epithelium, Vacuolation	0	–	–	–	0	1 (1.0)

[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

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). Survival of 50 ppm males was significantly less than that of the chamber control group. In males, 72% of the chamber control group survived to study termination but 66% and 44% of the 25 and 50 ppm animals, respectively, survived; survival of 12.5 ppm males was similar to that of the chamber controls. The primary cause of early deaths in male rats and one female rat exposed to 50 ppm was inflammation of the lungs.

Table 7. Survival of Rats in the Two-year Inhalation Study of 2,3-Butanedione

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	12	11	15	18
Natural deaths	2	2	2	10
Animals surviving to study termination	36	37[c]	33	22
Percent probability of survival at end of study[a]	72	74	66	44
Mean survival (days)[b]	692	696	676	666
Survival analysis[d]	P = 0.001	P = 0.986N	P = 0.626	P = 0.007
Female				
Animals initially in study	50	50	50	50
Moribund	15	18	26	15

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Natural deaths	1	1	0	4
Animals surviving to study termination	34	31	24	31c
Percent probability of survival at end of study	68	62	48	60
Mean survival (days)	688	681	665	691
Survival analysis	P = 0.507	P = 0.723	P = 0.077	P = 0.591

[a]Kaplan-Meier determinations.
[b]Mean of all deaths (uncensored, censored, and terminal euthanasia).
[c]Includes one animal that was euthanized moribund during the last week of the study.
[d]The result of the life table trend test[81] is in the chamber control column, and the results of the life table pairwise comparisons[80] with the chamber controls are in the exposed group columns. A lower mortality in an exposure group is indicated by **N**.

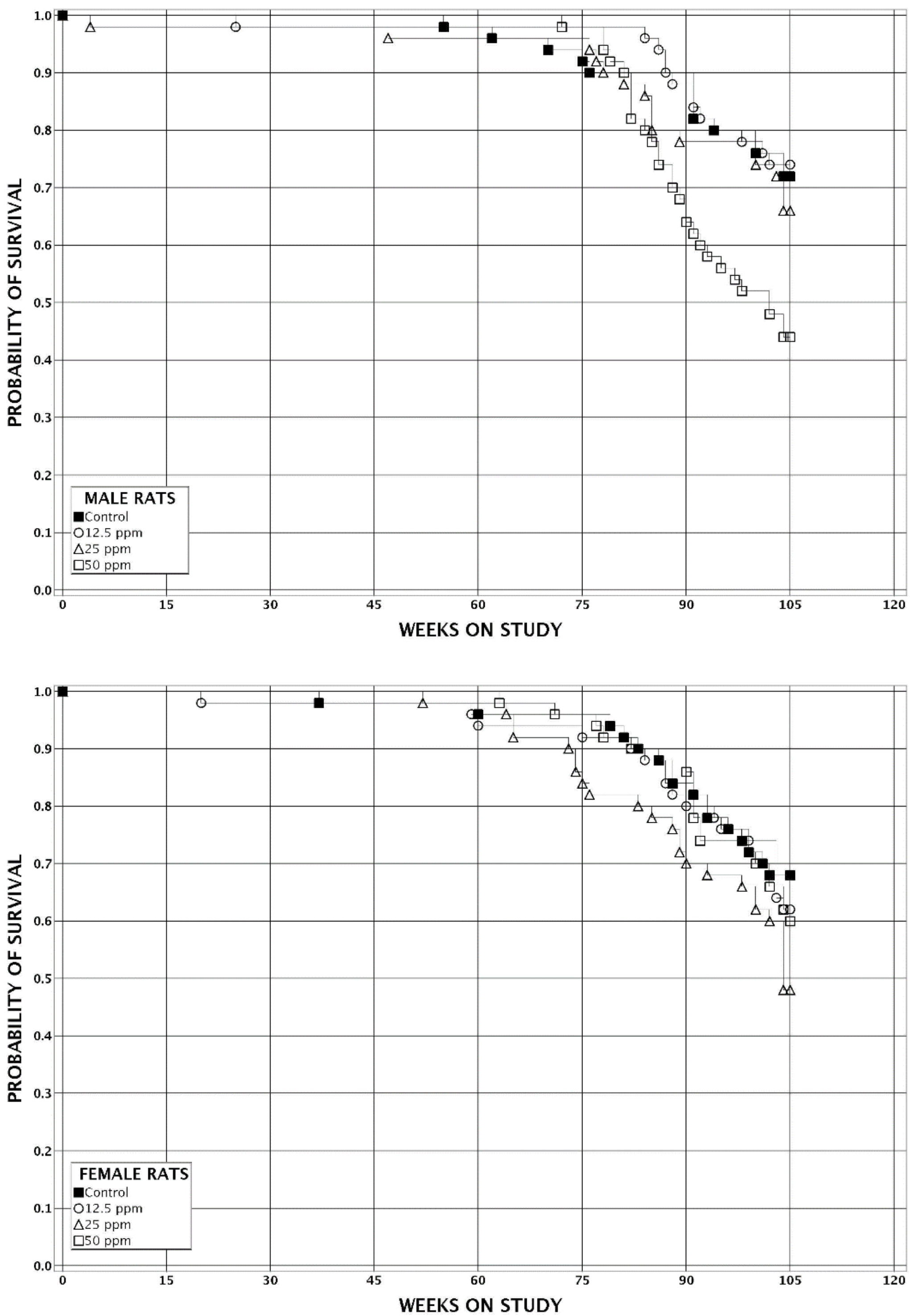


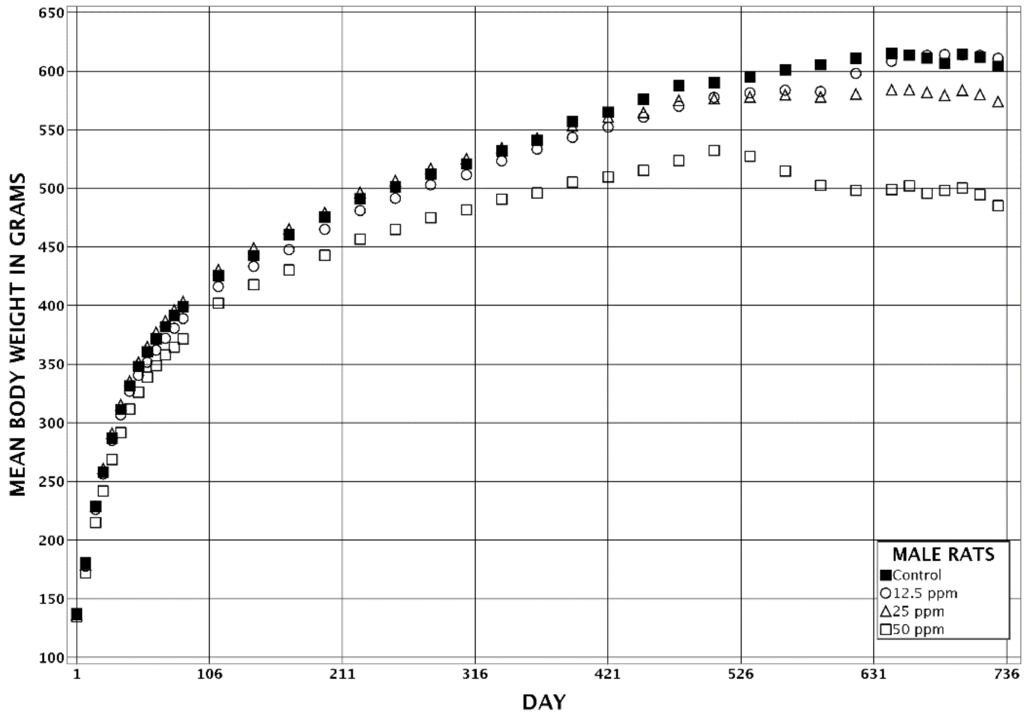
Figure 3. Kaplan-Meier Survival Curves for Rats Exposed to 2,3-Butanedione by Inhalation for Two Years

Body Weights and Clinical Observations

At the end of the study, mean body weights of both sexes exposed to 50 ppm were decreased relative to the respective chamber control groups, with more of an effect in males (81% of chamber controls) than in females (91% of chamber controls) (Figure 4; Table 8, and Table 9). Exposure-related clinical observations in males included thinness, abnormal breathing, eye abnormality, and nasal/eye discharge. Exposure-related clinical observations in females included eye abnormality and abnormal breathing. Eye abnormality was the most frequently noted clinical observation in both males and females exposed to 25 or 50 ppm. A number of appendage ulcer/abscesses were recorded in chamber control and exposed male rats that were attributed to housing heavy rats in wire caging.

Gross Observations

At terminal euthanasia, several rats exposed to 50 ppm had mottled lesions of the lungs, which often correlated histopathologically to acute inflammation of the bronchi and alveoli. Many rats exposed to 50 ppm and some rats exposed to 25 ppm had eye lesions or foci that were described as small, opaque, pale, cloudy, or as nodules, which generally corresponded histopathologically to inflammation of the cornea and associated structures. It was also noted that the nasal passages could not be well flushed in some animals.



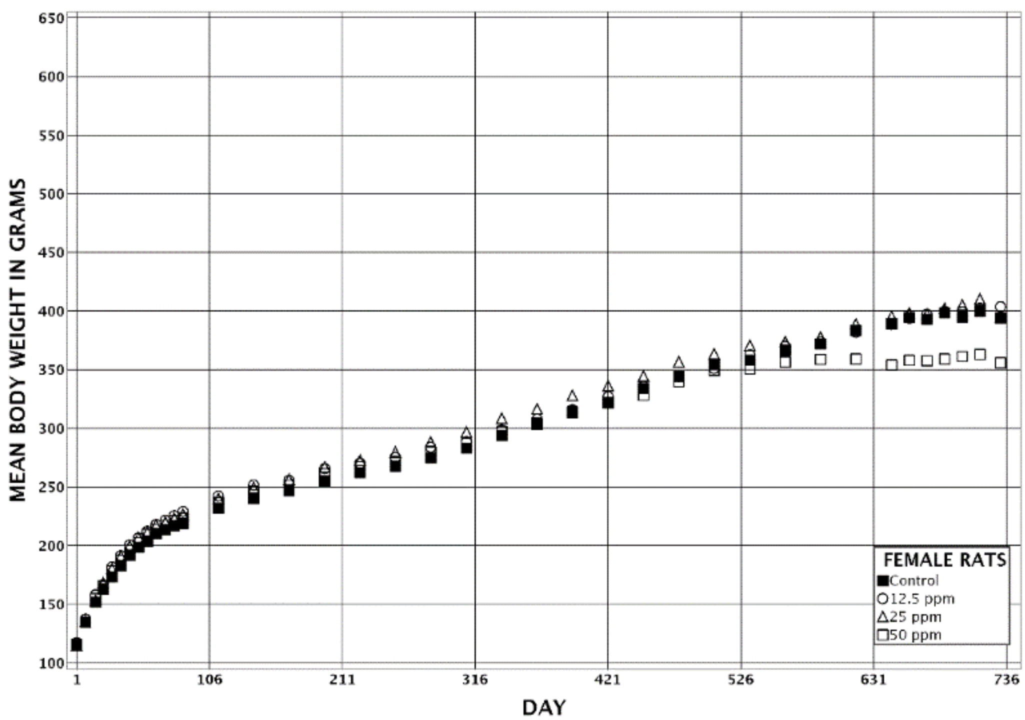


Figure 4. Growth Curves for Rats Exposed to 2,3-Butanedione by Inhalation for Two Years

Table 8. Mean Body Weights and Survival of Male Rats in the Two-year Inhalation Study of 2,3-Butanedione

Day	Chamber Control		12.5 ppm			25 ppm			50 ppm		
	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	137	50	136	99	50	137	99	50	135	98	50
8	181	50	178	98	50	179	99	50	172	95	50
16	229	50	226	99	50	229	100	50	215	94	50
22	258	50	256	99	50	261	101	50	242	94	50
29	287	50	285	99	50	291	101	49	269	94	50
36	312	50	307	99	50	316	101	49	292	94	50
43	332	50	327	99	50	336	101	49	312	94	50
50	348	50	341	98	50	352	101	49	326	94	50
57	361	50	352	98	50	365	101	49	339	94	50
64	372	50	362	97	50	377	101	49	349	94	50
71	382	50	372	97	50	387	101	49	358	94	50
78	392	50	381	97	50	396	101	49	365	93	50
85	399	50	389	98	50	403	101	49	372	93	50
113	426	50	416	98	50	431	101	49	402	95	50
141	443	50	434	98	50	449	101	49	418	94	50
169	461	50	448	97	50	465	101	49	431	94	50
197	476	50	465	98	49	480	101	49	443	93	50
225	491	50	481	98	49	497	101	49	457	93	50
253	501	50	492	98	49	507	101	49	465	93	50
281	512	50	503	98	49	517	101	49	475	93	50
309	521	50	512	98	49	525	101	49	482	93	50
337	532	50	523	98	49	535	101	48	491	92	50
365	541	50	533	99	49	543	100	48	496	92	50
393	557	49	544	98	49	553	99	48	505	91	50
421	565	49	552	98	49	561	99	48	510	90	50
449	576	48	561	97	49	565	98	48	516	90	50
477	588	48	570	97	49	575	98	48	524	89	50
505	590	47	578	98	49	577	98	48	533	90	49
533	595	45	582	98	49	578	97	47	527	89	49
561	601	45	584	97	49	580	96	44	515	86	46
589	606	45	583	96	48	578	95	42	503	83	39
617	611	45	598	98	44	581	95	39	499	82	35
645	615	41	609	99	41	584	95	39	499	81	30
659	614	40	614	100	40	584	95	39	503	82	28
673	611	40	613	100	40	582	95	39	496	81	28
687	607	40	614	101	39	579	96	39	498	82	26
701	615	38	614	100	38	584	95	37	501	82	26
715	612	38	614	100	37	580	95	36	495	81	24
Mean for Weeks											
1–13	307		301	98		310	101		288	94	
14–52	485		475	98		490	101		452	93	
53–103	594		585	99		574	97		508	86	

Table 9. Mean Body Weights and Survival of Female Rats in the Two-year Inhalation Study of 2,3-Butanedione

Day	Chamber Control		12.5 ppm			25 ppm			50 ppm		
	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	116	50	117	101	50	115	99	50	115	100	50
8	135	50	138	102	50	136	101	50	135	100	50
16	152	50	158	104	50	153	101	50	155	102	50
22	163	50	167	102	50	168	103	50	166	102	50
29	174	50	182	105	50	181	104	50	178	103	50
36	183	50	191	105	50	191	105	50	188	103	50
43	192	50	201	104	50	200	104	50	196	102	50
50	199	50	207	104	50	207	104	50	203	102	50
57	204	50	212	104	50	211	104	50	210	103	50
64	211	50	218	104	50	218	103	50	214	102	50
71	214	50	222	104	50	220	103	50	218	102	50
78	217	50	226	104	50	223	103	50	221	102	50
85	219	50	229	105	50	227	104	50	224	102	50
113	232	50	242	105	50	240	104	50	237	102	50
141	240	50	252	105	49	250	104	50	246	103	50
169	247	50	256	103	49	256	104	50	252	102	50
197	255	50	266	104	49	267	105	50	262	103	50
225	263	50	270	103	49	273	104	50	268	102	50
253	268	49	276	103	49	280	104	50	273	102	50
281	275	49	284	103	49	288	105	50	280	102	50
309	283	49	289	102	49	297	105	50	287	101	50
337	294	49	299	102	49	309	105	50	298	101	50
365	303	49	308	102	49	317	104	49	304	100	50
393	314	49	316	101	49	328	105	49	315	100	50
421	322	48	327	102	47	336	104	49	322	100	50
449	334	48	336	101	47	345	103	48	328	98	49
477	345	48	344	100	47	357	104	46	340	99	49
505	355	48	351	99	47	364	103	46	350	99	48
533	358	48	363	101	46	371	104	41	351	98	48
561	367	46	370	101	46	374	102	41	357	97	46
589	372	45	374	100	44	378	102	39	359	96	45
617	384	42	382	100	41	389	102	36	359	94	45
645	389	41	389	100	40	396	102	35	354	91	37
659	395	39	394	100	39	398	101	34	358	91	37
673	393	38	397	101	38	397	101	34	358	91	37
687	399	36	400	100	37	402	101	33	359	90	36
701	395	36	399	101	37	405	103	31	361	92	35
715	400	34	403	101	34	410	103	30	363	91	33
Mean for Weeks											
1–13	183		190	104		188	103		186	102	
14–52	262		270	103		273	104		267	102	
53–103	364		366	101		373	103		346	95	

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 nose, larynx, trachea, lung, eye, bone marrow, and skin. 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.

Nose: Three squamous cell carcinomas and one squamous cell papilloma of the nasal mucosa occurred in male rats exposed to 50 ppm, and three squamous cell carcinomas of the nasal mucosa occurred in females exposed to 50 ppm (Table 10, Table A-1, Table A-2, Table B-1, and Table B-2). All of these neoplasms arose from the lateral walls, the nasoturbinates, or the maxilloturbinates in Level I and/or Level II of the nasal cavity. In these locations, the normal respiratory and transitional mucosal epithelia were often replaced in part by squamous metaplastic epithelium, which was sometimes contiguous with the squamous cell carcinomas. The squamous metaplastic epithelium was frequently thickened and hyperkeratotic, and sometimes atypical, suggesting that it may have provided the preneoplastic background for development of the squamous cell carcinomas. Among the males, one of the squamous cell carcinomas arose in the distal portion of a nasoturbinate and formed a bulbous mass due to expansion of the lamina propria by neoplastic infiltration (Figure 8). The second squamous cell carcinoma formed a plaque-like mass along the lateral wall and adjacent maxilloturbinate, with invasion of the lamina propria adjacent to the alveolar process of the premaxilla bone (Figure 9). The third squamous cell carcinoma formed a large mass that filled and occluded one side of the nasal cavity, impinging upon and deviating the nasal septum towards the opposite lateral wall (Figure 10). Histologically, this latter mass displayed squamous cell carcinoma on the surface, while much of the infiltrating neoplasm exhibited a solid, spindle cell pattern that invaded the dorsal wall of the nose and extended through the frontal process of the premaxilla bone. A second smaller squamous carcinoma was also noted in the same animal on the nasoturbinate of the opposite side of the nasal cavity. The squamous cell papilloma noted in a fourth male rat of the 50 ppm group formed a largely necrotic polypoid structure extending from the dorsal end to the ventral end of the nasal cavity, partially filling the lumen on one side (Figure 11). Among the 50 ppm females with squamous cell carcinomas, one animal exhibited a lesion involving the entire lateral wall mucosa dorsal to the nasolacrimal duct on one side, with invasion of the lateral wall lamina propria and the nasopremaxillary suture, perineural invasion, and invasion of a Haversian canal within the premaxillary bone (Figure 12). In the other two females, each squamous cell carcinoma involved the lateral wall, with one of these irregularly nodular and infiltrating next to the alveolar process of the premaxillary bone (Figure 13), while the other was plaque-like and invaded more superficially into the lamina propria (Figure 14).

No squamous cell carcinomas or papillomas of the nose occurred in the concurrent male or female chamber controls, and none are recorded in the NTP historical control database (Table 10). The combined incidence of three squamous cell carcinomas and one squamous cell papilloma in the 50 ppm male rats was significantly increased. One adenoma occurred in Level III of a con-current chamber control male (Table 10 and Table A-1).

A spectrum of nonneoplastic lesions occurred in both the respiratory and olfactory epithelium, primarily in the 25 and 50 ppm groups (Table 10, Table A-3, and Table B-3). Suppurative inflammation, usually of marked severity, occurred in almost all 50 ppm males and females and also occurred, usually to a mild degree, in most of the 25 ppm males and some of the 25 ppm females. The inflammation consisted of both exudate in the nasal cavities and inflammatory infiltrate within the nasal mucosa. The respiratory epithelium in Levels I and II exhibited both moderate to marked epithelium hyperplasia and mild squamous metaplasia in most of the 50 ppm males and females; minimal respiratory epithelium hyperplasia and squamous metaplasia occurred in a few of the 25 ppm males. The respiratory epithelium hyperplasia occurred most often along the nasal septum of Levels I and II, and was characterized by increased cell density, numerous goblet cells, and increased thickness, often with folds. Minimal to mild olfactory epithelial atrophy occurred in many of the animals exposed to 25 or 50 ppm, and respiratory metaplasia of the olfactory epithelium occurred in almost all of the 50 ppm animals and in some of the 25 ppm animals. Both the atrophy and the respiratory metaplasia of the olfactory epithelium occurred most often in the dorsal meatus of Level II. In a few 50 ppm males and females, foci of necrosis occurred in either the olfactory or respiratory epithelia. Turbinate hyperostosis, characterized by increased thickness of the turbinate bone, occurred in a few of the 50 ppm rats, usually in the nasoturbinates.

In addition to the inflammation and spectrum of epithelial lesions, most of the animals exposed to 50 ppm, and many of those exposed to 25 ppm, exhibited fibrosis of the lamina propria of the nasal mucosa, primarily in Levels I and II (Figure 15) (Table 10, Table A-3, and Table B-3). The fibrosis was characterized by increased collagenous tissue immediately beneath the mucosal epithelium, with few or absent glands and vessels, most often present along the lateral walls of the nasal cavities and the tips of the nasoturbinates.

Table 10. Incidences of Neoplasms and Nonneoplastic Lesions of the Nose in Rats in the Two-year Inhalation Study of 2,3-Butanedione

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Male				
Number Examined Microscopically	50	50	50	50
Inflammation, Suppurative[a]	3 (1.0)[b]	4 (1.5)	35** (1.9)	50** (3.9)
Respiratory Epithelium, Hyperplasia	0	2 (1.0)	5* (1.2)	50** (3.7)
Respiratory Epithelium, Metaplasia, Squamous	0	0	5* (1.0)	34** (2.0)
Respiratory Epithelium, Necrosis	0	0	0	2 (1.0)
Olfactory Epithelium, Atrophy	0	5* (1.4)	27** (1.4)	22** (1.3)
Olfactory Epithelium, Metaplasia, Respiratory	1 (1.0)	3 (1.3)	6 (1.7)	50** (3.2)
Olfactory Epithelium, Necrosis	0	0	0	6* (1.3)
Turbinate, Hyperostosis	0	0	0	10** (1.2)
Lamina Propria, Fibrosis	0	0	28** (1.0)	38** (1.2)
Squamous Cell Papilloma[c]	0	0	0	1
Squamous Cell Carcinoma[c]				
Overall rate[d]	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate[e]	0.0%	0.0%	0.0%	7.5%
Terminal rate[f]	0/36 (0%)	0/37 (0%)	0/33 (0%)	1/22 (5%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
First incidence (days)	–[h]	–	–	616
Poly-3 test[g]	P = 0.010	–[i]	–	P = 0.101
Squamous Cell Papilloma or Carcinoma[c]	–	–	–	–
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	0.0%	9.9%
Terminal rate	0/36 (0%)	0/37 (0%)	0/33 (0%)	1/22 (5%)
First incidence (days)	–	–	–	589
Poly-3 test	P = 0.002	–	–	P = 0.049
Adenoma[j]	1	0	0	0
Female				
Number Examined Microscopically	50	50	50	50
Inflammation, Suppurative	4 (1.8)	3 (1.0)	11* (1.5)	49** (3.9)
Respiratory Epithelium, Hyperplasia	1 (2.0)	0	2 (1.0)	44** (3.4)
Respiratory Epithelium, Metaplasia, Squamous	1 (2.0)	0	1 (2.0)	44** (1.8)
Respiratory Epithelium, Necrosis	0	0	0	3 (3.3)
Olfactory Epithelium, Atrophy	1 (1.0)	1 (1.0)	14** (1.4)	24** (1.9)
Olfactory Epithelium, Metaplasia, Respiratory	1 (4.0)	0	18** (1.3)	46** (2.8)
Olfactory Epithelium, Necrosis	0	0	0	4 (2.8)
Turbinate, Hyperostosis	0	0	0	8** (1.0)
Lamina Propria, Fibrosis	1 (1.0)	1 (1.0)	17** (1.0)	46** (1.4)
Squamous Cell Carcinoma[c]				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	6.9%
Terminal rate	0/34 (0%)	0/31 (0%)	0/24 (0%)	2/30 (7%)
First incidence (days)	–	–	–	724
Poly-3 test	P = 0.011	–	–	P = 0.118

*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 squamous cell papilloma, squamous cell carcinoma, or squamous cell papilloma or carcinoma of the nose in Wistar Han rats in 2-year inhalation studies with chamber control groups: 0/200 males and 0/200 females; all routes: 0/349 males and 0/350 females.
[d]Number of animals with neoplasm per number of animals with nose examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal euthanasia.
[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 euthanasia.
[h]Not applicable; no neoplasms in animal group.
[i]Value of statistic cannot be computed.
[j]Historical incidence for 2-year inhalation studies (mean ± standard deviation): 1/200 (0.5% ± 1.0%), range 0%–2%; all routes 1/349 (0.3% ± 0.8%), range 0%–2%.

Larynx: Most of the lesions in the larynx were significantly increased in only the 50 ppm groups (Table 11, Table A-3, and Table B-3). Minimal to mild chronic active inflammation occurred in most of the 50 ppm males and in half of the 50 ppm females. The inflammation consisted primarily of neutrophils with lesser numbers of lymphocytes and plasma cells and was most often present in the ventral pouch and/or the submucosa of the arytenoid processes where the squamous epithelium was often hyperplastic or ulcerated. Hyperplasia of the squamous epithelium overlying the arytenoid cartilages, graded as mild to moderate, occurred in almost all 50 ppm males and females, and hyperplasia of lesser degree occurred in a few of the rats exposed to 25 ppm. Focal areas of ulceration of the squamous epithelium occurred in some of the rats exposed to 50 ppm. Squamous metaplasia of the respiratory epithelium occurred in most of the 50 ppm animals. Squamous metaplasia was most commonly noted to start at the base of the epiglottis in Level I in minimal cases, with extension along the sides of the larynx in cases of mild severity. Moderate severity was used when the entire base of the epiglottis and two levels of the larynx were involved, and marked severity was reserved for cases in which all three levels of larynx were involved. The average severity of respiratory epithelium squamous metaplasia was in the mild to moderate range for both males and females.

Trachea: Mild chronic active inflammation occurred in the mucosa and submucosa, and sometimes deeper structures, of many 50 ppm females, and minimal to mild inflammation occurred in a few 50 ppm males (Table 11, Table A-3, and Table B-3). A variety of epithelial lesions occurred in the mucosa, the most common being epithelium hyperplasia, which occurred in most 50 ppm males and females. Epithelium squamous metaplasia and/or epithelium regeneration occurred in some 50 ppm males and a few 50 ppm females; epithelium regeneration also occurred in a few 25 ppm males. Minimal epithelium atrophy occurred in a few 50 ppm rats, and focal epithelium necrosis occurred in a few 50 ppm males and one 50 ppm female. In addition to the epithelial lesions, submucosa fibrosis occurred in many 50 ppm males and females. The fibrosis was characterized by an increase in fibrous connective tissue that expanded a portion of the submucosa in minimal to mild cases and extended into the deeper tissues in moderate to marked cases (Figure 16). In severely affected animals, the fibrosis extended around the cartilage rings and was associated with loss of cartilage.

Table 11. Incidences of Nonneoplastic Lesions of the Respiratory System in Rats in the Two-year Inhalation Study of 2,3-Butanedione

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Male				
Larynx[a]	50	50	50	50
Inflammation, Chronic Active[b]	14 (1.1)[c]	7 (1.1)	7 (1.0)	33** (1.2)
Squamous Epithelium, Hyperplasia	2 (1.0)	2 (2.0)	8* (1.3)	46** (2.4)
Squamous Epithelium, Ulcer, Focal	0	0	0	15** (1.3)
Respiratory Epithelium, Metaplasia, Squamous	0	1 (1.0)	0	45** (2.3)
Trachea	50	50	50	50
Inflammation, Chronic Active	0	0	1 (1.0)	8** (1.3)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Epithelium, Hyperplasia	0	0	1 (1.0)	32** (1.2)
Epithelium, Metaplasia, Squamous	0	0	0	12** (1.2)
Epithelium, Regeneration	0	0	5* (1.0)	12** (1.1)
Epithelium, Atrophy	0	0	0	7** (1.1)
Epithelium, Necrosis	0	0	0	6** (1.0)
Submucosa, Fibrosis	0	0	0	27** (1.1)
Lung	50	49	50	50
Inflammation, Suppurative	0	0	1 (2.0)	15** (2.4)
Inflammation, Granulomatous	4 (1.0)	3 (1.0)	1 (1.0)	4 (1.0)
Peribronchial, Inflammation, Chronic Active	0	0	0	13** (1.5)
Bronchus, Epithelium, Hyperplasia	0	0	2 (1.0)	47** (1.2)
Bronchiole, Epithelium, Hyperplasia	0	0	0	33** (1.3)
Bronchus, Epithelium, Atrophy	0	0	1 (1.0)	23** (1.0)
Bronchus, Epithelium, Regeneration	0	0	4 (1.0)	9** (1.0)
Bronchus, Submucosa, Fibrosis	0	0	0	5* (1.0)
Alveolus, Infiltration Cellular, Histiocyte	10 (1.1)	14 (1.2)	16 (1.1)	34** (1.4)
Alveolar Epithelium, Hyperplasia	1 (2.0)	4 (1.0)	2 (1.0)	8** (1.1)
Interstitium, Fibrosis	0	1 (1.0)	1 (1.0)	11** (1.3)
Female				
Larynx	50	50	50	50
Inflammation, Chronic Active	4 (1.0)	2 (1.0)	4 (1.0)	25** (1.3)
Squamous Epithelium, Hyperplasia	1 (1.0)	1 (2.0)	6* (1.0)	48** (2.5)
Squamous Epithelium, Ulcer, Focal	0	0	0	5* (1.2)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	35** (2.3)
Trachea	50	50	50	50
Inflammation, Chronic Active	0	0	0	20** (2.0)
Epithelium, Hyperplasia	0	0	0	30** (1.5)
Epithelium, Metaplasia, Squamous	0	0	0	3 (2.0)
Epithelium, Regeneration	0	0	0	3 (1.0)
Epithelium, Atrophy	0	0	0	4 (1.0)
Epithelium, Necrosis	0	0	0	1 (2.0)
Submucosa, Fibrosis	0	0	0	19** (1.8)
Lung	50	50	50	50
Inflammation, Suppurative	0	0	0	3 (2.3)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Inflammation, Granulomatous	2 (1.0)	1 (1.0)	3 (1.0)	13** (1.0)
Peribronchial, Inflammation, Chronic Active	1 (1.0)	2 (1.0)	0	27** (1.5)
Bronchus, Epithelium, Hyperplasia	0	0	0	46** (1.4)
Bronchiole, Epithelium, Hyperplasia	0	0	8** (1.0)	39** (1.4)
Bronchus, Epithelium, Atrophy	0	0	0	7** (1.0)
Bronchus, Epithelium, Regeneration	0	0	1 (1.0)	2 (1.0)
Alveolus, Infiltration Cellular, Histiocyte	13 (1.1)	11 (1.0)	10 (1.0)	32** (1.3)
Alveolar Epithelium, Hyperplasia	1 (1.0)	1 (1.0)	1 (1.0)	3 (1.3)
Interstitium, Fibrosis	1 (1.0)	1 (1.0)	1 (2.0)	9** (1.2)

*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 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.

Lung: Inflammation of various types occurred in the airways and/or the alveolar parenchyma of the lung in many animals (Table 11, Table A-3, and Table B-3). Based on the cell type of the infiltrate and the location, most of these inflammatory lesions fell into one of three categories: suppurative, granulomatous, or peribronchial chronic active. Suppurative inflammation occurred mainly in the 50 ppm groups, primarily in the males, and was characterized by neutrophilic exudate within airway lumens, alveolar spaces, or both (Figure 17). Bacterial clusters were sometimes noted in areas of suppuration. Suppurative inflammation in the lungs may have been secondary to aspiration of inflammatory exudate from the upper respiratory tract, perhaps combined with impaired warming, humidifying, and filtering of the air by the inflamed nasal passages. Granulomatous inflammation occurred in a few animals in each exposed group, as well as in the chamber controls, and the incidence was significantly increased in 50 ppm females. This type of inflammation consisted of macrophages and giant cells often surrounding small fragments of foreign material, which was thought to have been aspirated from the upper respiratory tract. Peribronchial chronic active inflammation occurred primarily in the 50 ppm groups, occurring in several males and many females, and it consisted of mixed cell infiltrates surrounding bronchi, bronchioles, and blood vessels.

A variety of lesions occurred in the airways (Table 11, Table A-3, and Table B-3). Most common of these was epithelium hyperplasia, occurring in both the bronchi and the bronchioles of most of the 50 ppm males and females. Minimal bronchiolar epithelium hyperplasia also occurred in a few 25 ppm females and minimal bronchial epithelium hyperplasia in two 25 ppm males. Both bronchial and bronchiolar epithelium hyperplasia were characterized by increased epithelial height and cell density (Figure 18). The bronchiolar lesion sometimes included goblet cell metaplasia and occurred most often in areas of moderate to marked inflammation. Many 50 ppm males and a few 50 ppm females also exhibited areas of minimal epithelium atrophy within the extrapulmonary bronchi; the epithelium in these areas was reduced in height but retained some cilia. Retention of cilia was used to differentiate epithelium atrophy from epithelium regeneration, which also occurred in the extrapulmonary bronchi in a few 50 ppm males and females and was characterized by a single layer of flattened, nonciliated epithelial cells that appeared to have stretched to cover a denuded surface. Small areas of submucosa fibrosis occurred in the extrapulmonary bronchi just below the tracheal carinae in five 50 ppm males (Figure 18).

Lesions occurring in the lung parenchyma included histiocytic cellular infiltration in the alveolar spaces, alveolar epithelium hyperplasia, and interstitium fibrosis (Table 11, Table A-3, and Table B-3). Clusters of alveolar histiocytes occurred in most of the rats exposed to 50 ppm, as well as in some of the chamber controls and rats exposed to 12.5 or 25 ppm. Minimal alveolar epithelium hyperplasia occurred in a few chamber control and exposed animals and was increased in incidence in 50 ppm males. Minimal to mild interstitium fibrosis of alveolar septae occurred in 11 males and nine females in the 50 ppm groups. The interstitium fibrosis was characterized by thickening of alveolar septae by eosinophilic, collagenous material that caused the septae to take on a straightened or stiff appearance. In some animals the fibrosis formed nodular foci that expanded the alveolar walls and extended in tendrils into the surrounding alveolar septae (Figure 19).

Eye: Chronic active inflammation of the cornea occurred in most of the 50 ppm animals, many of those exposed to 25 ppm, and a few of the 12.5 ppm and chamber control rats (Table 12, Table A-3, and Table B-3). Grading of the inflammation was based upon intensity, extent of the cornea involved, and bilaterality. Most animals with inflammation fell into the minimal to mild categories. Moderate severity was diagnosed when involvement was at least 50% in both eyes or greater than 67% in one eye, and marked severity when perforation appeared imminent or had occurred. More females than males had moderate inflammation, and a few 12.5 and 25 ppm females were graded as marked. In a few 25 and 50 ppm rats, acute and/or suppurative inflammation occurred in the anterior chamber and/or the iris. Minimal to moderate cornea epithelium hyperplasia occurred in a few animals in each exposed group. Cornea epithelium ulcers, usually minimal to mild, occurred in a few animals in the 25 and 50 ppm groups and in one male and one female exposed to 12.5 ppm. Minimal corneal epithelial necrosis occurred in two 50 ppm males, and minimal corneal stromal necrosis occurred in one 25 ppm female. Focal areas of mineralization of the corneal stroma, probably related to previous injury, occurred in a few animals. Increased incidences of cataract of the lens occurred in all exposed groups of males and in 25 and 50 ppm females, and the increases were significant in the 25 ppm males and in 25 and 50 ppm females. Phthisis bulbi, characterized by shrinkage of the globe with structural disorganization, occurred in a few exposed males and females, and the incidence was significantly increased in 50 ppm females.

Bone Marrow: Significantly increased incidences of myeloid cell hyperplasia occurred in 50 ppm males and females (Table 12, Table A-3, and Table B-3). The increase in myeloid cells in the marrow was probably a secondary reactive response to the inflammatory changes in the respiratory tract.

Skin: Incidences of chronic active inflammation and ulcer of the skin of the foot occurred in both chamber control and exposed groups of males; these lesions occurred with increased incidences in 25 and 50 ppm males and also occurred in a few 50 ppm females (Table 12, Table A-3, and Table B-3). These lesions were thought to be secondary to the large size of the Wistar Han rats, particularly the males, and walking on the wire caging. The increased incidences in the higher exposure concentration groups may have been related to debilitation and chemical exposure.

Table 12. Incidences of Selected Nonneoplastic Lesions in Rats in the Two-year Inhalation Study of 2,3-Butanedione

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Male				
Eye[a]	50	50	49	49
Cornea, Inflammation, Chronic Active[b]	1 (1.0)[c]	6 (2.3)	16** (1.6)	28** (1.3)
Anterior Chamber, Inflammation, Suppurative	0	1 (1.0)	6* (1.3)	3 (2.0)
Iris, Inflammation, Acute	0	1 (1.0)	3 (1.0)	1 (2.0)
Cornea, Epithelium, Hyperplasia	0	2 (1.0)	3 (1.7)	6** (1.2)
Cornea, Epithelium, Ulcer	0	1 (1.0)	4 (1.3)	6** (1.5)
Cornea, Necrosis	0	0	0	2 (1.0)
Cornea, Mineralization	1 (1.0)	4 (1.0)	1 (1.0)	0
Lens, Cataract	1 (2.0)	5 (1.6)	6* (1.3)	3 (1.7)
Unilateral, Phthisis Bulbi	0	1 (4.0)	3 (3.7)	1 (3.0)
Bilateral, Phthisis Bulbi	0	0	1 (4.0)	0
Bone Marrow	50	50	50	50
Myeloid Cell, Hyperplasia	15 (2.9)	12 (2.8)	16 (2.8)	32** (2.9)
Skin	50	50	50	50
Foot, Inflammation, Chronic Active	11 (3.3)	7 (3.1)	17 (3.2)	23** (3.0)
Foot, Ulcer	14 (3.5)	7 (3.4)	17 (3.4)	22 (2.9)
Female				
Eye	50	50	50	50
Cornea, Inflammation, Chronic Active	2 (1.5)	6 (2.0)	23** (1.9)	31** (2.0)
Anterior Chamber, Inflammation, Suppurative	1 (1.0)	0	6* (2.5)	5 (1.8)
Iris, Inflammation, Acute	0	0	5* (1.8)	4 (1.0)
Cornea, Epithelium, Hyperplasia	0	3 (2.0)	8** (1.4)	5* (1.4)
Cornea, Epithelium, Ulcer	0	1 (2.0)	2 (2.5)	13** (1.8)
Cornea, Necrosis	0	0	1 (1.0)	0
Cornea, Mineralization	0	1 (1.0)	0	2 (1.0)
Lens, Cataract	1 (1.0)	1 (3.0)	6* (1.5)	9** (1.7)
Unilateral, Phthisis Bulbi	0	1 (2.0)	0	8** (3.1)
Bone Marrow	50	50	50	50
Myeloid Cell, Hyperplasia	12 (2.9)	19 (3.2)	20 (3.0)	36** (2.9)
Skin	50	50	50	50
Foot, Inflammation, Chronic Active	0	0	0	4 (2.8)
Foot, Ulcer, Focal	0	0	0	4 (3.0)

*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 tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Mice

Three-month Study

All mice survived to the end of the study (Table 13). The final mean body weights and body weight gains of males exposed to 50 or 100 ppm and females exposed to 12.5 ppm or greater were significantly less than those of the chamber control groups (Table 13 and Figure 5). Sneezing was observed in 50 ppm males (9/10) and females (8/10) and 100 ppm males (9/10) and females (9/10). Abnormal breathing was observed in 100 ppm males (2/10) and females (1/10).

Table 13. Survival and Body Weights of Mice in the Three-month Inhalation Study of 2,3-Butanedione[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	24.9 ± 0.4	37.8 ± 1.2	12.9 ± 1.1	
6.25	10/10	24.7 ± 0.3	38.0 ± 1.3	13.3 ± 1.0	101
12.5	10/10	24.9 ± 0.2	37.8 ± 0.7	13.0 ± 0.6	100
25	10/10	25.3 ± 0.3	39.1 ± 0.6	13.8 ± 0.6	103
50	10/10	24.4 ± 0.3	34.2 ± 1.1*	9.7 ± 0.8**	90
100	10/10	24.7 ± 0.2	27.9 ± 0.6**	3.2 ± 0.5**	74
Female					
0	10/10	20.1 ± 0.3	33.0 ± 0.9	12.9 ± 0.9	
6.25	10/10	20.6 ± 0.2	31.8 ± 0.7	11.2 ± 0.7	96
12.5	10/10	20.1 ± 0.2	30.9 ± 0.6*	10.8 ± 0.6*	94
25	10/10	20.0 ± 0.2	29.4 ± 0.7**	9.4 ± 0.5**	89
50	10/10	20.3 ± 0.3	27.6 ± 0.5**	7.4 ± 0.4**	84
100	10/10	20.4 ± 0.3	23.8 ± 0.5**	3.4 ± 0.6**	72

*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.

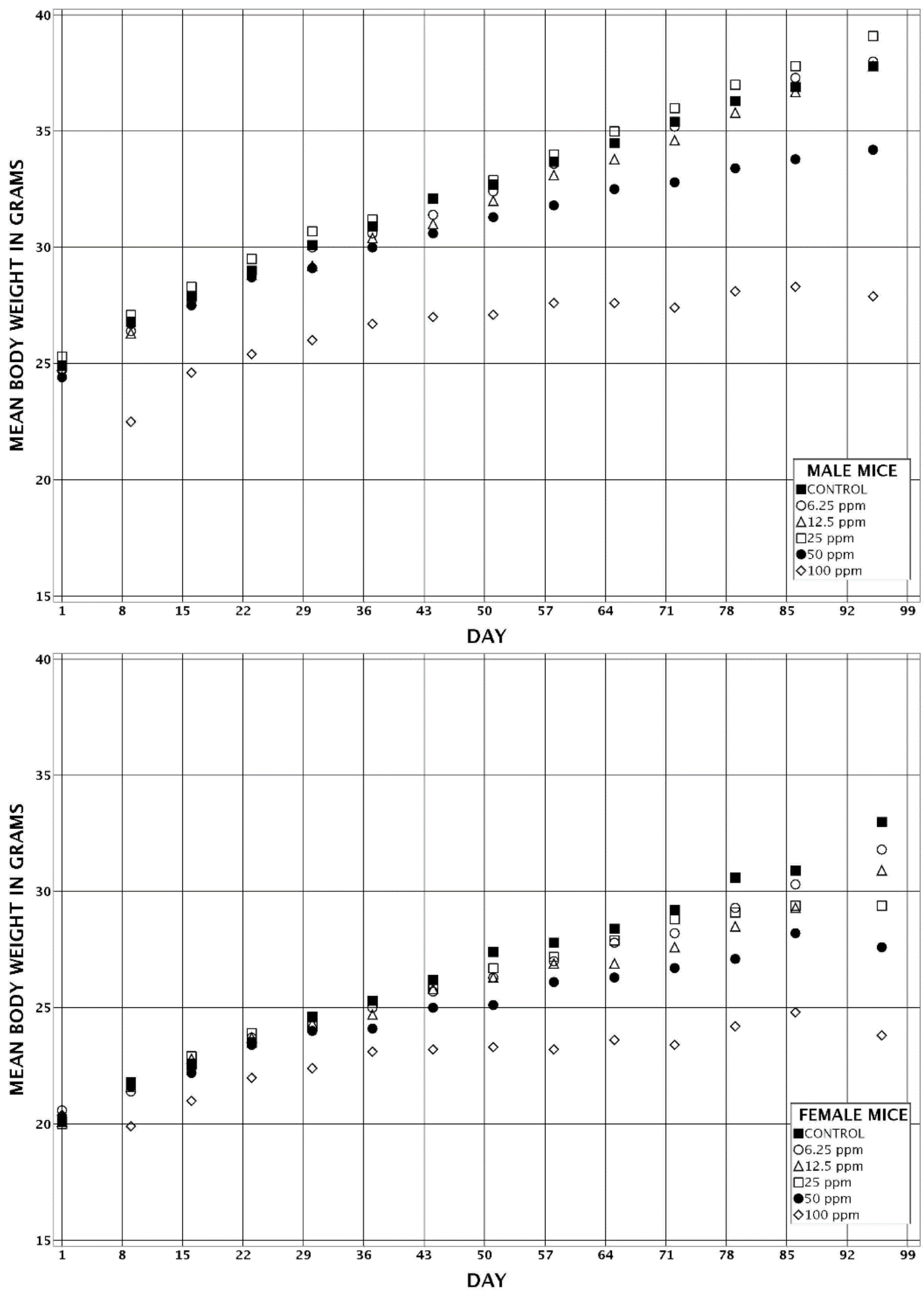


Figure 5. Growth Curves for Mice Exposed to 2,3-Butanedione by Inhalation for Three Months

At study termination, neutrophil counts in the blood were increased in 50 and 100 ppm male mice and 100 ppm female mice (Table 14 and Table F-2) and were consistent with inflammation. In 50 and 100 ppm males and 100 ppm females, mean cell volume and mean cell hemoglobin were significantly decreased, indicating a reduction in erythrocyte size. This may indicate some minimal alteration in iron metabolism or hemoglobin production during erythropoiesis. All other statistically significant changes in hematology parameters were minimal and not considered toxicologically relevant.

Significant changes in organ weights were restricted to the 50 and 100 ppm groups (Table G-2). Relative lung weights were significantly increased in 50 ppm males and 100 ppm males and females. Absolute heart, kidney, and liver weights of males and females were significantly decreased in the 50 and 100 ppm groups. Relative kidney weights in females were significantly increased at 25 ppm and higher. Relative spleen weights in males were increased at 100 ppm and absolute spleen weights of females were decreased at 50 and 100 ppm. Absolute testis weights were significantly decreased and relative testis weights were significantly increased in 100 ppm males. Relative thymus weights were significantly decreased in 100 ppm males. Absolute thymus weights in females were significantly deceased at all exposure concentrations. These findings are of uncertain toxicologic significance given the body weight decreases in the exposed groups and the lack of treatment-related lesions in these tissues.

No gross lesions associated with exposure to 2,3-butanedione were noted in mice at the time of necropsy. Exposure-related histopathologic findings occurred primarily in the respiratory tract. Lesions in the nose occurred predominantly in the 50 and 100 ppm groups (Table 15). Minimal to moderate suppurative inflammation occurred in all males and females in the 50 and 100 ppm groups. The inflammation usually involved all levels of the nose and consisted of neutrophilic exudate combined with varying amounts of eosinophilic proteinaceous material within the lumen of the nasal cavity. Occasionally, neutrophils were also present within the nasal mucosal epithelium and underlying lamina propria, particularly in areas of necrosis. Large quantities of the eosinophilic proteinaceous material were present in some noses and filled or nearly filled the nasal cavity, especially in Level III. Minimal to mild respiratory epithelium necrosis occurred in all mice in the 50 and 100 ppm groups, and minimal necrosis occurred in eight males and four females exposed to 25 ppm. Respiratory epithelium regeneration, usually characterized by a single layer of flattened, elongated cells covering the surface, occurred in almost all animals in the 50 and 100 ppm groups, presumably occurring in areas previously denuded by necrosis. Moderate turbinate atrophy, primarily affecting the nasoturbinate bones in Level I, occurred in all mice exposed to 50 or 100 ppm and consisted of variable loss of bone associated with shortening and blunting of the turbinate bone. In some of the animals exposed to 50 or 100 ppm, turbinate necrosis also occurred in which fragments of the nasoturbinate hooks were detached from the body of the turbinate and were lacking in both osteocytes and osteoblasts. Necrosis of the nasal septum cartilage occurred in one 100 ppm female, and perforation of the nasal septum occurred in two 100 ppm males. Mild to moderate squamous metaplasia of the respiratory epithelium occurred in all mice exposed to 50 or 100 ppm. In the dorsal portions of Levels II and III in all mice exposed to 50 or 100 ppm, the olfactory epithelium exhibited minimal to mild atrophy with variable degrees of thinning of the olfactory epithelium. Olfactory epithelium respiratory metaplasia also occurred in most of these 50 and 100 ppm mice, usually in Level II and sometimes also in Level III.

Table 14. Selected Hematology Data for Mice in the Three-month Inhalation Study of 2,3-Butanedione[a]

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
n	10	10	10	10	10	10
Male						
Mean cell volume (fL)	48.7 ± 0.2	48.3 ± 0.3	48.9 ± 0.3	48.8 ± 0.1	47.8 ± 0.3*	46.6 ± 0.3**
Mean cell hemoglobin (pg)	15.1 ± 0.1	14.9 ± 0.1	15.1 ± 0.1	15.1 ± 0.1	14.9 ± 0.1*	14.6 ± 0.1**
Segmented neutrophils (10^3/µL)	0.42 ± 0.05	0.38 ± 0.03	0.42 ± 0.06	0.42 ± 0.03	1.22 ± 0.57*	0.96 ± 0.20**
Female						
Mean cell volume (fL)	49.0 ± 0.2	49.1 ± 0.1	49.2 ± 0.2	49.1 ± 0.2	48.6 ± 0.1	47.2 ± 0.2**
Mean cell hemoglobin (pg)	15.4 ± 0.1	15.4 ± 0.0	15.4 ± 0.1	15.4 ± 0.1	15.3 ± 0.0	14.8 ± 0.1**
Segmented neutrophils (10^3/µL)	0.53 ± 0.08	0.47 ± 0.06	0.42 ± 0.06	0.59 ± 0.08	0.79 ± 0.16	1.04 ± 0.19*

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

Table 15. Incidences of Nonneoplastic Lesions of the Respiratory System in Mice in the Three-month Inhalation Study of 2,3-Butanedione

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Male						
Nose[a]	10	10	10	10	10	10
Inflammation, Suppurative[b]	0	0	0	0	10** (1.9)[c]	10** (3.0)
Respiratory Epithelium, Necrosis	0	0	0	8** (1.0)	10** (2.0)	10** (1.7)
Respiratory Epithelium, Regeneration	0	0	0	0	10** (1.0)	10** (1.0)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	1 (1.0)	10** (2.0)	10** (3.0)
Turbinate, Atrophy	0	0	0	0	10** (3.0)	10** (3.0)
Turbinate, Necrosis	0	0	0	0	3 (1.0)	5* (1.0)
Septum, Perforation	0	0	0	0	0	2 (2.5)
Olfactory Epithelium, Atrophy	0	0	0	0	10** (1.8)	10** (1.6)
Olfactory Epithelium, Metaplasia, Respiratory	0	0	0	0	6** (1.5)	7** (1.6)
Larynx	10	10	10	10	10	10
Epithelium, Necrosis	0	0	2 (1.0)	1 (1.0)	9** (1.0)	8** (1.0)
Respiratory Epithelium, Regeneration	0	0	0	0	5* (1.2)	0
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	1 (1.0)	3 (1.3)	0

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Respiratory Epithelium, Metaplasia, Atypical Squamous	0	0	0	0	7** (1.3)	10** (3.4)
Respiratory Epithelium, Hyperplasia	0	0	0	2 (1.0)	8** (1.5)	8** (2.4)
Squamous Epithelium, Hyperplasia	0	0	0	3 (1.0)	3 (1.3)	5* (2.6)
Squamous Epithelium, Hyperplasia, Atypical	0	0	0	0	7** (1.7)	5* (2.8)
Inflammation, Chronic Active	1 (1.0)	1 (1.0)	3 (1.0)	3 (1.7)	8** (1.1)	10** (1.2)
Trachea	10	10	10	10	10	10
Epithelium, Metaplasia, Atypical Squamous	0	0	0	0	0	10** (2.8)
Epithelium, Hyperplasia	0	0	0	0	2 (1.0)	9** (2.4)
Epithelium, Degeneration	0	0	0	1 (1.0)	3 (1.3)	8** (2.0)
Epithelium, Regeneration	0	0	0	0	7** (1.6)	0
Inflammation, Chronic Active	0	0	0	0	0	7** (1.1)
Lung	10	10	10	10	10	10
Bronchus, Inflammation, Chronic	1 (1.0)	0	0	0	7** (1.3)	10** (2.7)
Bronchus, Infiltration Cellular, Polymorphonuclear	0	0	0	0	0	10** (1.7)
Bronchus, Epithelium, Hyperplasia, Atypical	0	0	0	0	2 (1.5)	9** (2.1)
Bronchus, Epithelium, Metaplasia, Atypical Squamous	0	0	0	0	0	10** (2.2)
Bronchus, Epithelium, Regeneration	0	0	0	0	1 (1.0)	9** (2.6)
Female						
Nose	10	10	10	10	10	10
Inflammation, Suppurative	0	0	0	1 (1.0)	10** (2.1)	10** (3.0)
Respiratory Epithelium, Necrosis	0	0	0	4* (1.0)	10** (1.6)	10** (2.0)
Respiratory Epithelium, Regeneration	0	0	0	0	9** (1.1)	10** (1.6)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	10** (2.0)	10** (3.0)
Turbinate, Atrophy	0	0	0	0	10** (3.0)	10** (3.0)
Turbinate, Necrosis	0	0	0	0	4* (1.0)	8** (1.3)
Septum, Necrosis	0	0	0	0	0	1 (1.0)
Olfactory Epithelium, Atrophy	0	0	0	0	10** (2.0)	10** (2.0)
Olfactory Epithelium, Metaplasia, Respiratory	0	0	0	0	8** (1.6)	9** (1.7)
Larynx	10	10	10	10	9	10
Epithelium, Necrosis	0	0	0	1 (1.0)	5* (1.0)	9** (1.2)

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Respiratory Epithelium, Regeneration	0	0	0	2 (1.0)	8** (1.6)	0
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	1 (1.0)	9** (1.6)	0
Respiratory Epithelium, Metaplasia, Atypical Squamous	0	0	0	0	0	10** (3.4)
Respiratory Epithelium, Hyperplasia	0	0	0	4* (1.0)	8** (1.1)	9** (2.3)
Squamous Epithelium, Hyperplasia	0	0	0	2 (1.0)	4* (1.8)	1 (2.0)
Squamous Epithelium, Hyperplasia, Atypical	0	0	0	2 (1.0)	3 (1.3)	9** (2.9)
Inflammation, Chronic Active	0	3 (1.0)	1 (1.0)	4* (1.0)	4* (1.0)	10** (1.6)
Trachea	10	10	10	10	10	10
Epithelium, Metaplasia, Atypical Squamous	0	0	0	0	0	10** (3.2)
Epithelium, Hyperplasia	0	0	0	0	2 (1.0)	9** (2.2)
Epithelium, Degeneration	0	0	0	0	3 (1.7)	8** (1.9)
Epithelium, Regeneration	0	0	0	0	7** (2.1)	0
Inflammation, Chronic Active	0	0	0	0	0	7** (1.1)
Lung	10	10	10	10	10	10
Bronchus, Inflammation, Chronic	1 (1.0)	0	0	0	5 (1.6)	8** (2.4)
Bronchus, Infiltration Cellular, Polymorphonuclear	0	0	0	0	0	8** (1.6)
Bronchus, Epithelium, Hyperplasia, Atypical	0	0	0	0	0	7** (2.3)
Bronchus, Epithelium, Metaplasia, Atypical Squamous	0	0	0	0	0	7** (2.0)
Bronchus, Epithelium, Regeneration	0	0	0	0	6** (1.0)	7** (2.3)
Lung	10	10	10	10	10	10
Bronchus, Inflammation, Chronic	1 (1.0)	0	0	0	5 (1.6)	8** (2.4)
Bronchus, Infiltration Cellular, Polymorphonuclear	0	0	0	0	0	8** (1.6)
Bronchus, Epithelium, Hyperplasia, Atypical	0	0	0	0	0	7** (2.3)
Bronchus, Epithelium, Metaplasia, Atypical Squamous	0	0	0	0	0	7** (2.0)
Bronchus, Epithelium, Regeneration	0	0	0	0	6** (1.0)	7** (2.3)

*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.

In the larynx, epithelium necrosis, usually minimal, occurred in most of the males and females exposed to 50 or 100 ppm, in one male and one female in the 25 ppm groups, and in two males in the 12.5 ppm group (Table 15). The necrosis occurred primarily either in the squamous epithelium lining the arytenoid cartilages or in the respiratory epithelium lining the lateral walls of Levels II or III. Regenerative changes in the respiratory epithelium occurred in half of the 50 ppm males, most of the 50 ppm females, and two 25 ppm females and were considered to be reparative changes secondary to necrosis. The squamous epithelium lining the arytenoid cartilages exhibited either hyperplasia or atypical hyperplasia, minimal to moderate, in all 50 and 100 ppm males, all 100 ppm and most 50 ppm females, and a few 25 ppm males and females. Atypical squamous epithelium hyperplasia consisted of thickening of the epithelium by pleomorphic, atypical-appearing squamous epithelial cells, many of which were variably enlarged with enlarged nuclei and arranged in a disorganized pattern. Minimal to mild squamous metaplasia or minimal to marked atypical squamous metaplasia of the respiratory epithelium occurred in almost all 50 and 100 ppm males and females. Minimal to moderate respiratory epithelium hyperplasia occurred in most of the mice exposed to 50 or 100 ppm. These epithelial changes were accompanied by minimal to mild chronic active inflammation in all mice exposed to 100 ppm, many of those exposed to 50 ppm, and some animals in the lower exposure concentration groups.

In the trachea, atypical squamous metaplasia of the epithelium, ranging from mild to marked, occurred in all 100 ppm mice (Table 15). The atypical squamous metaplastic epithelium was characterized by varying degrees of cellular pleomorphism, with some of the cells quite large with enlarged nuclei and arranged in a disorganized pattern. Minimal to moderate hyperplasia of the respiratory epithelium occurred in most of the mice exposed to 100 ppm and a few of the mice exposed to 50 ppm. The mucosal epithelium also exhibited vacuolar degenerative changes in most of the 100 ppm mice and a few of the mice exposed to 50 ppm. Epithelium regenerative changes, composed generally of a single layer of enlarged cells with large nuclei, occurred in most of the 50 ppm mice. Chronic active inflammation, usually minimal in degree, occurred in most of the 100 ppm mice.

In the lung, minimal to moderate bronchus chronic inflammation occurred in most of the mice exposed to 50 or 100 ppm, and this was accompanied by minimal to mild polymorphonuclear cellular infiltration in most of the 100 ppm mice (Table 15). Atypical hyperplasia and atypical squamous metaplasia of the bronchus epithelium occurred in most of the male and female mice exposed to 100 ppm. These atypical epithelial changes were of mild to moderate severity and occurred primarily in the large bronchi. As in other tissues, such as the trachea, the epithelial atypia was characterized by the presence of large, atypical-appearing, irregularly arranged cells with enlarged, hyperchromatic nuclei. Regenerative changes of the bronchus epithelium, consisting of a single layer of flattened to cuboidal epithelium covering the bronchial surface, were also noted in most of the 100 ppm males and females and many of the 50 ppm females; such changes were interpreted as reparative changes following epithelial injury.

Exposure Concentration Selection Rationale: Based on significant reductions in body weights and increased incidences of nonneoplastic lesions of the respiratory tract at 100 ppm, 2,3-butanedione exposure concentrations selected for the 2-year inhalation study in mice were 12.5, 25, and 50 ppm. Although nasal lesions in mice were a concern at 50 ppm, this concentration was included because lower concentrations were not expected to result in sufficient airway exposure and injury to potentially cause bronchiolitis obliterans. In addition, the 50 ppm exposure concentration was selected because it was considered occupationally relevant. Flavoring mixers were exposed to a mean concentration of 57.2 ppm 2,3-butanedione in a microwave popcorn manufacturing plant where some workers developed bronchiolitis obliterans[25].

Two-year Study

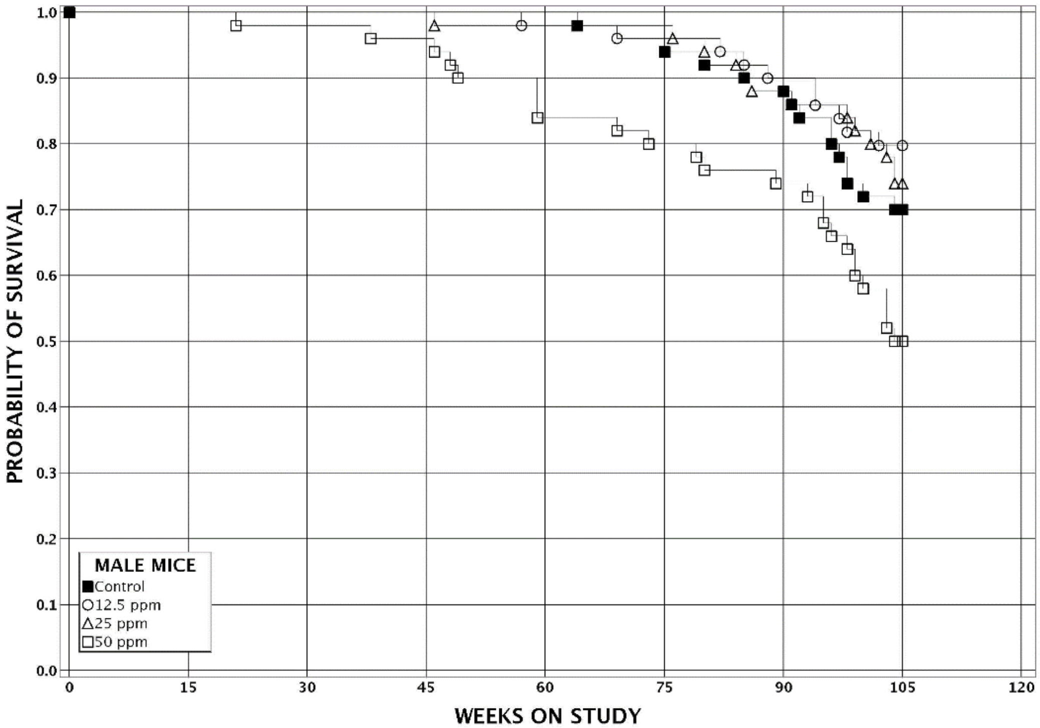
Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 16 and in the Kaplan-Meier survival curves (Figure 6). Survival of 50 ppm males and females was significantly less than that of the chamber control groups; 50% of males and 34% of females survived to the end of the study compared to 70% and 72% in the male and female chamber control groups, respectively. The mean survival days for 50 ppm males and females (637 and 589 days, respectively) were also reduced relative to that of the chamber control groups (697 days for both sexes). The primary cause of early death in mice exposed to 50 ppm was inflammation in the upper respiratory tract (nose, larynx, and/or trachea).

Table 16. Survival of Mice in the Two-year Inhalation Study of 2,3-Butanedione

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Male				
Animals initially in study	50	50	50	50
Accidental death[a]	0	1	0	0
Moribund	6	5	4	14
Natural deaths	9	5	9	11
Animals surviving to study termination	35	39	37	25
Percent probability of survival at end of study[b]	70	80	74	50
Mean survival (days)[c]	697	701	701	637
Survival analysis[d]	P = 0.007	P = 0.386N	P = 0.760N	P = 0.049
Female				
Animals initially in study	50	50	50	50
Moribund	10	6	4	15
Natural deaths	4	4	4	17
Animals surviving to study termination	36	40	42	18[e]
Percent probability of survival at end of study	72	80	84	34
Mean survival (days)	697	718	716	589
Survival analysis	P < 0.001	P = 0.415N	P = 0.224N	P < 0.001

[a]Censored in the survival analysis.
[b]Kaplan-Meier determinations.
[c]Mean of all deaths (uncensored, censored, and terminal euthanasia).
[d]The result of the life table trend test[81] is in the chamber control column, and the results of the life table pairwise comparisons[80] with the chamber controls are in the exposed group columns. A lower mortality in an exposure group is indicated by **N**.
[e]Includes one animal that was euthanized moribund during the last week of the study.



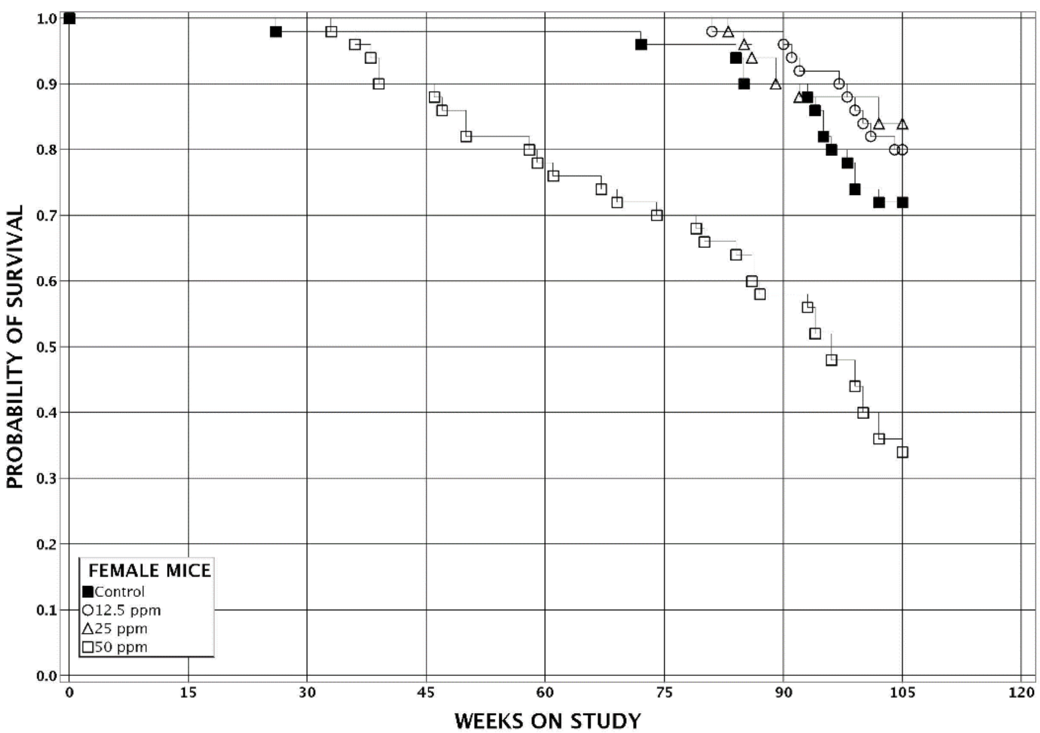


Figure 6. Kaplan-Meier Survival Curves for Mice Exposed to 2,3-Butanedione by Inhalation for Two Years

Body Weights and Clinical Observations

At the end of the study, mean body weights of the 50 ppm groups were reduced to 65% (males) and 62% (females) of those of the respective chamber control groups (Figure 7; Table 17, and Table 18). Males exposed to 25 ppm exhibited slightly reduced body weights at the end of the study (94% of chamber controls), while body weights of 12.5 ppm males were similar to those of the chamber controls throughout the study, and body weights of 12.5 and 25 ppm females were slightly increased relative to the chamber controls.

Clinical observations were most prominent in the 50 ppm groups and included abnormal breathing, thinness, sneezing, and eye abnormality in both sexes. Incidences were highest for thinness and eye abnormality. Thinness was observed in 22/50 males and 22/50 females in the 50 ppm groups, as compared to 6/50 and 8/50 in the respective chamber control groups. Eye abnormality was observed in 10/50 males and 35/50 females in the 50 ppm groups, compared to 3/50 and 2/50 in the respective chamber control groups.

Gross Observations

At terminal euthanasia, many mice exposed to 50 ppm and some females exposed to 25 ppm were noted to have opaque or pale eye lesions or foci.

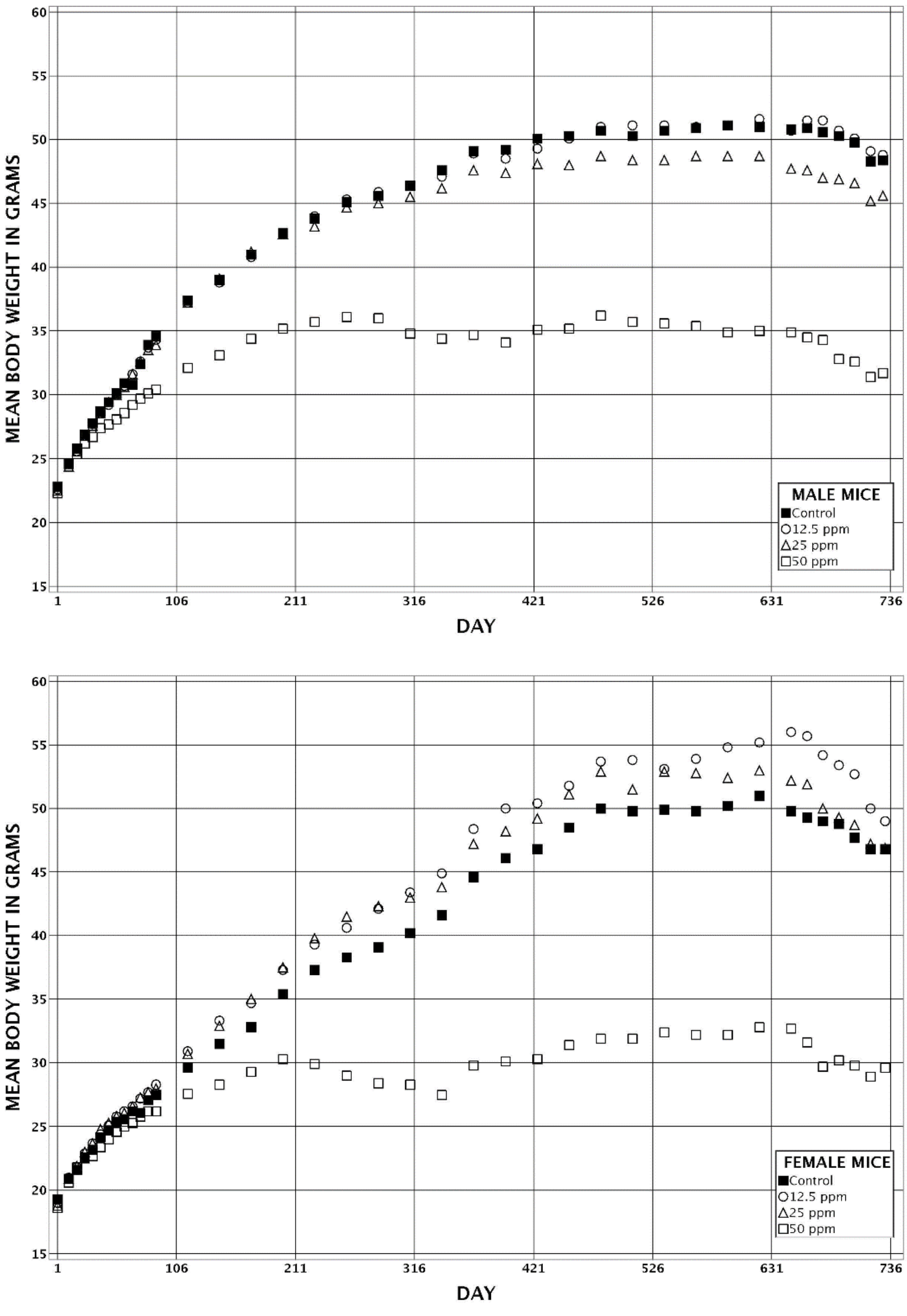


Figure 7. Growth Curves for Mice Exposed to 2,3-Butanedione by Inhalation for Two Years

Table 17. Mean Body Weights and Survival of Male Mice in the Two-year Inhalation Study of 2,3-Butanedione

Day	Chamber Control		12.5 ppm			25 ppm			50 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	22.8	50	22.6	99	50	22.5	99	50	22.3	98	50
11	24.6	50	24.6	100	50	24.4	99	50	24.6	100	50
18	25.8	50	25.6	100	50	25.6	99	50	25.4	99	50
25	26.9	50	26.8	100	50	26.8	100	50	26.2	97	50
32	27.8	50	27.7	100	50	27.6	99	50	26.7	96	50
39	28.7	50	28.6	100	50	28.6	100	50	27.4	95	50
46	29.4	50	29.2	99	50	29.4	100	50	27.7	94	50
53	30.1	50	30.0	100	50	30.0	100	50	28.1	94	50
60	30.9	50	30.7	99	50	30.6	99	50	28.6	93	50
67	30.8	50	31.6	103	50	31.6	103	50	29.2	95	50
74	32.4	50	32.6	101	50	32.6	101	50	29.7	92	50
81	33.9	50	33.7	100	50	33.5	99	50	30.1	89	50
88	34.6	50	34.3	99	50	33.9	98	50	30.4	88	50
116	37.4	50	37.2	100	50	37.2	100	50	32.1	86	50
144	39.0	50	38.8	99	50	39.1	100	50	33.1	85	49
172	41.0	50	40.8	99	50	41.2	101	50	34.4	84	49
200	42.7	50	42.7	100	50	42.6	100	50	35.2	82	49
228	43.8	50	44.0	100	50	43.2	99	50	35.7	82	49
256	45.1	50	45.3	101	50	44.7	99	50	36.1	80	49
284	45.6	50	45.9	101	50	45.0	99	50	36.0	79	48
312	46.4	50	46.4	100	50	45.5	98	50	34.8	75	48
340	47.6	50	47.1	99	50	46.2	97	49	34.4	72	45
368	49.1	50	48.9	100	50	47.6	97	49	34.7	71	45
396	49.2	50	48.5	99	49	47.4	96	49	34.1	69	45
424	50.1	50	49.3	99	49	48.1	96	49	35.1	70	42
452	50.3	49	50.1	100	49	48.0	95	49	35.2	70	42
480	50.7	49	51.0	101	48	48.7	96	49	36.2	71	41
508	50.3	49	51.1	102	48	48.4	96	49	35.7	71	40
536	50.7	47	51.1	101	48	48.4	95	48	35.6	70	40
564	50.9	46	51.0	100	48	48.7	96	47	35.4	70	38
592	51.1	45	51.1	100	46	48.7	95	46	34.9	68	38
620	51.0	45	51.6	101	44	48.7	96	44	35.0	69	37
648	50.8	42	50.7	100	44	47.7	94	43	34.9	69	37
662	50.9	42	51.5	101	42	47.6	94	43	34.5	68	35
676	50.6	39	51.5	102	41	47.0	93	43	34.3	68	33
690	50.3	37	50.7	101	40	46.9	93	41	32.8	65	30
704	49.8	36	50.1	101	40	46.6	94	41	32.6	66	29
718	48.3	36	49.1	102	39	45.2	94	40	31.4	65	26
Mean for Weeks											
1–13	29.1	–	29.1	100	–	29.0	100	–	27.4	95	–
14–52	43.2	–	43.1	100	–	42.7	99	–	34.6	81	–
53–103	50.3	–	50.5	101	–	47.7	95	–	34.5	69	–

Table 18. Mean Body Weights and Survival of Female Mice in the Two-year Inhalation Study of 2,3-Butanedione

Day	Chamber Control		12.5 ppm			25 ppm			50 ppm		
	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	19.3	50	19.0	98	50	18.8	98	50	18.6	97	50
11	20.9	50	21.0	101	50	20.9	100	50	20.6	99	50
18	21.6	50	21.8	101	50	21.9	101	50	21.8	101	50
25	22.5	50	22.9	102	50	23.0	102	50	22.6	100	50
32	23.2	50	23.7	102	50	23.7	102	50	22.7	98	50
39	24.2	50	24.4	101	50	24.8	102	50	23.4	97	50
46	24.7	50	25.1	102	50	25.3	102	50	24.0	97	50
53	25.4	50	25.8	102	50	25.8	102	50	24.6	97	50
60	25.6	50	26.2	103	50	26.1	102	50	25.0	98	50
67	26.2	50	26.6	102	50	26.6	102	50	25.3	97	50
74	26.1	50	27.2	104	50	27.3	105	50	25.8	99	50
81	27.1	50	27.7	102	50	27.7	102	50	26.2	97	50
88	27.5	50	28.3	103	50	28.0	102	50	26.2	95	50
116	29.6	50	30.9	104	50	30.7	104	50	27.6	93	50
144	31.5	50	33.3	106	50	32.9	105	50	28.3	90	50
172	32.8	50	34.7	106	50	35.0	106	50	29.3	89	50
200	35.4	49	37.3	105	50	37.5	106	50	30.3	86	50
228	37.3	49	39.3	105	50	39.8	107	50	29.9	80	49
256	38.3	49	40.6	106	50	41.5	108	50	29.0	76	48
284	39.1	49	42.1	108	50	42.3	108	50	28.4	73	45
312	40.2	49	43.4	108	50	43.0	107	50	28.3	70	45
340	41.6	49	44.9	108	50	43.8	105	50	27.5	66	43
368	44.6	49	48.4	109	50	47.2	106	50	29.8	67	41
396	46.1	49	50.0	109	50	48.2	105	50	30.1	65	41
424	46.8	49	50.4	108	50	49.2	105	50	30.3	65	38
452	48.5	49	51.8	107	50	51.1	105	50	31.4	65	38
480	50.0	49	53.7	108	50	52.9	106	50	31.9	64	36
508	49.8	48	53.8	108	50	51.5	104	50	31.9	64	36
536	49.9	48	53.1	106	50	52.9	106	50	32.4	65	35
564	49.8	48	53.9	108	49	52.8	106	50	32.2	65	33
592	50.2	46	54.8	109	49	52.4	104	48	32.2	64	32
620	51.0	45	55.2	108	49	53.0	104	45	32.8	64	29
648	49.8	44	56.0	113	46	52.2	105	44	32.7	66	28
662	49.3	43	55.7	113	46	51.9	105	44	31.6	64	26
676	49.0	40	54.2	111	45	50.0	102	44	29.7	61	24
690	48.8	37	53.4	109	43	49.3	101	44	30.2	62	22
704	47.7	37	52.7	110	41	48.7	102	44	29.8	63	20
718	46.8	36	50.0	107	41	47.2	101	42	28.9	62	18
Mean for Weeks											
1–13	24.2	–	24.6	102	–	24.6	102	–	23.6	98	–
14–52	36.2	–	38.5	106	–	38.5	106	–	28.7	80	–
53–103	48.6	–	52.9	109	–	50.7	104	–	31.1	64	–

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 nose, larynx, trachea, lung, eye, bone marrow, spleen, thyroid gland, heart, thymus, lymph nodes, and adrenal gland. 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 C for male mice and Appendix D for female mice.

Nose: Adenocarcinomas occurred in two 50 ppm females (Table 19 and Table D-1). Both neoplasms involved all three Levels of the nasal cavity but appeared to arise in Level III in a background of extensive respiratory metaplasia of the olfactory epithelium. One of these neoplasms infiltrated subtly within the lamina propria between the benign respiratory glands and the respiratory metaplastic glands from the dorsal end to the ventral end of the nasal cavity. This neoplasm exhibited little distortion of the architectural features of the nasal cavity, except for focal formation of a bridge between one or more ethmoid turbinates and the nasal septum in Level III (Figure 20). In Level I, the adenocarcinoma occurred within the inflamed soft tissue of the palate, having infiltrated around the free end of the palatal process of the premaxilla in Level II. The second animal with adenocarcinoma presented with a more obvious mass that largely filled the nasal cavity in Level III, encompassing the nasal septum and the ethmoid scrolls (Figure 21). Laterally, this neoplasm invaded through the maxillary or premaxillary bones on both the left and right sides, extending into adjacent soft tissue. Dorsally, the neoplasm appeared to have invaded through both the cribriform plate of the ethmoid bone and the frontal bone, resulting in a 0.5 cm diameter skull deformity that was noted at necropsy. Ventrally, the neoplasm infiltrated between the nasopharyngeal duct and the palatine process of the maxilla and exhibited foci of perineural and vascular wall invasion.

No nasal adenocarcinomas or other nasal neoplasms occurred in 12.5 or 25 ppm females, exposed males, or chamber controls of either sex (Table C-1 and Table D-1). No nasal adenocarcinomas have been recorded in the NTP historical control database (Table 19).

Nonneoplastic lesions of the nose occurred predominantly in the 25 and 50 ppm groups, but some lesions also occurred with lower incidences and severities in the 12.5 ppm groups (Table 19, Table C-3, and Table D-3). Suppurative inflammation occurred in all mice exposed to 50 ppm, in most 25 ppm mice, and in some 12.5 ppm mice. The inflammation was characterized by the presence of neutrophilic exudate and proteinaceous fluid within the nasal cavity and variably dense infiltrates of neutrophils, lymphocytes, and plasma cells within the underlying lamina propria (Figure 22). In association with the inflammation, a variety of epithelial lesions occurred. Squamous metaplasia of the respiratory epithelium in Levels I and II occurred in all 50 ppm mice, most 25 ppm mice, and a few 12.5 ppm mice. Minimal hyperplasia of the respiratory epithelium occurred in a few 50 ppm mice. Respiratory metaplasia of the Steno's glands surrounding the maxillary sinus occurred in some 50 ppm mice, usually occurring in several small areas of the glands. Necrosis of the respiratory epithelium, usually minimal to mild, occurred in all 50 ppm mice, most 25 ppm mice, a few 12.5 ppm females, and one female chamber control mouse. Regeneration of the mucosa epithelium, probably secondary to previous necrosis or ulceration, occurred in most 25 and 50 ppm mice. Within the olfactory epithelium of Levels II and III, atrophy occurred in most of the animals exposed to 25 or 50 ppm and also occurred in some 12.5 ppm males and most 12.5 ppm females. Respiratory metaplasia of the olfactory epithelium also occurred in most of the 25 and 50 ppm mice and many of the 12.5 ppm females. Minimal to moderate necrosis of the olfactory epithelium occurred in many of the 50 ppm males and females and in one 25 ppm female.

Turbinate atrophy, primarily involving the naso- and maxilloturbinates, occurred in all exposed groups, with exposure concentration-related increases in incidences and severities (Table 19, Table C-3, and Table D-3). The atrophy was characterized by loss of turbinate tissue, including the bone of the turbinates, resulting in shortening and blunting, with only the base remaining in some cases (Figure 23). Turbinate necrosis was diagnosed when fragments of residual turbinate bone lacking in both osteocytes and osteoblasts occurred; these fragments of necrotic bone were usually small and were sometimes surrounded by neutrophils and/or were being extruded into the nasal cavity (Figure 24A). Turbinate necrosis occurred in many 50 ppm males, some 50 ppm females, and a few 25 ppm mice. Perforation of the nasal septum occurred in 11 males and five females in the 50 ppm groups and in three males and six females in the 25 ppm groups. Septum perforation was characterized by total loss of tissue, including cartilage, in the central part of the nasal septum in Level I, with the exposed tissue edges covered by a layer of squamous epithelium (Figure 22A).

Table 19. Incidences of Neoplasms and Nonneoplastic Lesions of the Nose in Mice in the Two-year Inhalation Study of 2,3-Butanedione

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Male				
Number Examined Microscopically	49	48	50	50
Inflammation, Suppurative[a]	2 (1.0)[b]	4 (1.0)	47** (1.4)	50** (3.2)
Respiratory Epithelium, Metaplasia, Squamous	0	6* (1.0)	47** (1.5)	50** (2.4)
Respiratory Epithelium, Hyperplasia	1 (1.0)	0	0	8** (1.0)
Respiratory Epithelium, Necrosis	0	0	34** (1.1)	50** (1.6)
Glands, Sinus, Metaplasia, Respiratory	0	0	1 (2.0)	13** (1.0)
Mucosa, Regeneration	0	0	47** (1.2)	47** (1.6)
Olfactory Epithelium, Atrophy	0	14** (1.1)	48** (2.1)	38** (1.8)
Olfactory Epithelium, Metaplasia, Respiratory	1 (1.0)	0	39** (1.6)	45** (2.7)
Olfactory Epithelium, Necrosis	0	0	0	19** (2.2)
Turbinate, Atrophy	0	8** (1.1)	49** (1.2)	50** (2.3)
Turbinate, Necrosis	0	0	4 (1.0)	27** (1.0)
Septum, Perforation	0	0	3	11**
Lamina Propria, Fibrosis	0	0	44** (1.0)	50** (2.0)
Female				
Number Examined Microscopically	50	50	50	50
Inflammation, Suppurative	3 (1.0)	20** (1.2)	50** (1.6)	50** (3.3)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Respiratory Epithelium, Metaplasia, Squamous	1 (1.0)	9* (1.0)	48** (1.3)	50** (2.7)
Respiratory Epithelium, Hyperplasia	0	1 (1.0)	0	2 (1.5)
Respiratory Epithelium, Necrosis	1 (1.0)	5 (1.0)	33** (1.2)	50** (1.8)
Glands, Sinus, Metaplasia, Respiratory	0	0	0	12** (1.0)
Mucosa, Regeneration	0	0	39** (1.2)	48** (2.1)
Olfactory Epithelium, Atrophy	0	41** (1.6)	49** (2.2)	45** (1.4)
Olfactory Epithelium, Metaplasia, Respiratory	0	22** (1.2)	46** (1.7)	49** (3.3)
Olfactory Epithelium, Necrosis	0	0	1 (2.0)	20** (1.6)
Turbinate, Atrophy	0	32** (1.0)	50** (1.1)	50** (2.1)
Turbinate, Necrosis	0	0	1 (1.0)	11** (1.0)
Septum, Perforation	0	0	6*	5*
Lamina Propria, Fibrosis	0	0	47** (1.0)	49** (1.9)
Adenocarcinoma[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%	6.2%
Terminal rate[f]	0/36 (0%)	0/40 (0%)	0/42 (0%)	1/17 (6%)
First incidence (days)	–[h]	–	–	708
Poly-3 test[g]	P = 0.038	–[i]	–	P = 0.171

*Significantly different (P ≤ 0.05) from the chamber control group by the Poly-3 test.
**P ≤ 0.01.
[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[c]Historical control incidence for adenocarcinoma of the nose in B6C3F1/N female mice in 2-year inhalation studies with chamber control groups: 0/300; all routes: 0/548.
[d]Number of animals with neoplasm per number of animals with nose examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal euthanasia.
[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 euthanasia.
[h]Not applicable; no neoplasms in animal group.
[i]Value of statistic cannot be computed.

Fibrosis of the mucosal lamina propria occurred in all of the 50 ppm males and most of the 50 ppm females and 25 ppm males and females (Table 19, Table C-3, and Table D-3). The fibrosis was characterized by increased collagenous tissue immediately beneath the mucosal epithelium of the nasal cavity, primarily in Levels I and II (Figure 24B). The fibrosis was often noted in the tips of atrophic turbinates or beneath the epithelial lining of the lateral walls or septum, associated with obliteration of the glands normally occurring in these sites.

Larynx: Minimal to mild chronic active inflammation occurred in the submucosa of most 50 ppm and many 25 ppm mice (Table 20, Table C-3, and Table D-3). Variable amounts of neutrophilic exudate were sometimes noted in the lumen of the larynx (Figure 25), and in two males and four females in the 50 ppm groups the exudate was estimated to occupy 40% or more of the lumen space and was diagnosed separately as lumen exudate. Moderate squamous metaplasia of the respiratory epithelium occurred in almost all of the 50 ppm mice, and minimal to mild metaplasia occurred in a few 25 ppm mice. The squamous metaplasia occurred more commonly in the cranial portions of the larynx, and occurred consistently in the base of the epiglottis. In two of the female mice, one in the 50 ppm group and the other in the 12.5 ppm group, the squamous metaplastic changes were cytologically atypical and were diagnosed as atypical squamous metaplasia of the respiratory epithelium. Exposure concentration-related increases in incidences and severities of hyperplasia of the squamous epithelium overlying the arytenoid cartilages occurred, particularly in Level I. Hyperplasia of the respiratory epithelium also occurred in a few animals, primarily in the 50 ppm group of males. Foci of epithelial necrosis, often occurring in the respiratory epithelium of Levels II and III and sometimes in the squamous epithelium overlying the arytenoid cartilages, occurred in most of the mice exposed to 50 ppm and in some exposed to 25 ppm (Figure 25B). Regeneration of the respiratory epithelium, probably secondary to previous necrosis, occurred in most 50 ppm mice and in three 25 ppm female mice.

Table 20. Incidences of Nonneoplastic Lesions of the Respiratory System in Mice in the Two-year Inhalation Studyof 2,3-Butanedione

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Male				
Larynx[a]	49	49	49	50
Inflammation, Chronic Active[b]	4 (1.0)[c]	2 (1.0)	11* (1.2)	42** (1.6)
Lumen, Exudate	0	0	0	2 (3.5)
Respiratory Epithelium, Metaplasia, Squamous	3 (1.0)	0	6 (1.2)	50** (3.1)
Respiratory Epithelium, Hyperplasia	1 (1.0)	0	0	11** (1.3)
Respiratory Epithelium, Necrosis	2 (1.0)	1 (1.0)	9* (1.0)	34** (1.2)
Respiratory Epithelium, Regeneration	0	0	0	32** (1.8)
Squamous Epithelium, Hyperplasia	3 (1.3)	7 (1.3)	15** (1.4)	42** (1.8)
Trachea	48	49	49	49
Inflammation, Chronic Active	0	0	2 (1.0)	45** (1.6)
Lumen, Exudate	0	0	0	4* (3.0)
Necrosis	0	0	0	47** (1.5)
Epithelium, Regeneration	0	0	0	45** (2.9)
Epithelium, Hyperplasia	0	0	0	6* (2.0)
Epithelium, Metaplasia, Squamous	0	0	0	5* (1.0)
Submucosa, Fibrosis	0	0	0	46** (2.3)
Carina, Submucosa, Fibrosis	0	0	0	16** (1.7)
Carina, Submucosa, Mineralization	0	0	0	15** (1.9)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Carina, Submucosa, Inflammation, Chronic Active	0	0	0	4* (1.8)
Lung	50	49	50	50
Bronchus, Epithelium, Regeneration	0	0	0	34** (1.9)
Bronchus, Epithelium, Necrosis	0	0	0	2 (1.5)
Inflammation, Suppurative	0	0	0	3 (2.3)
Pleura, Inflammation, Suppurative	0	0	0	2 (1.5)
Mediastinum, Inflammation, Suppurative	0	0	0	3 (1.3)
Mediastinum, Inflammation, Chronic Active	1 (1.0)	0	0	8** (1.1)
Female				
Larynx	49	50	50	49
Inflammation, Chronic Active	4 (1.3)	5 (1.2)	22** (1.2)	36** (2.1)
Lumen, Exudate	0	0	0	4* (3.8)
Respiratory Epithelium, Metaplasia, Squamous	2 (1.0)	0	6 (1.7)	48** (3.0)
Respiratory Epithelium, Metaplasia, Atypical, Squamous	0	1 (3.0)	0	1 (3.0)
Respiratory Epithelium, Hyperplasia	0	0	4 (1.3)	1 (2.0)
Respiratory Epithelium, Necrosis	1 (1.0)	1 (1.0)	14** (1.1)	32** (1.5)
Respiratory Epithelium, Regeneration	0	0	3 (1.0)	30** (1.4)
Squamous Epithelium, Hyperplasia	4 (1.3)	13* (1.2)	34** (1.5)	40** (2.1)
Trachea	50	49	50	50
Inflammation, Chronic Active	1 (1.0)	0	4 (1.5)	42** (2.1)
Lumen, Exudate	0	0	0	12** (3.4)
Necrosis	0	0	3 (1.0)	48** (1.9)
Epithelium, Regeneration	0	0	9** (1.1)	45** (3.0)
Epithelium, Hyperplasia	0	0	1 (2.0)	1 (2.0)
Epithelium, Metaplasia, Squamous	0	0	0	2 (1.5)
Submucosa, Fibrosis	0	0	0	44** (2.3)
Carina, Submucosa, Fibrosis	0	0	0	6** (1.3)
Carina, Submucosa, Mineralization	0	0	0	5* (1.8)
Lung	50	50	50	50
Bronchus, Epithelium, Regeneration	2 (1.0)	0	0	38** (2.8)
Bronchus, Epithelium, Necrosis	2 (1.0)	0	0	5 (1.4)
Bronchus, Submucosa, Fibrosis	0	0	0	5* (1.2)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Inflammation, Suppurative	0	1 (1.0)	0	5* (1.8)
Pleura, Inflammation, Suppurative	0	0	0	5* (2.0)
Mediastinum, Inflammation, Suppurative	0	1 (1.0)	1 (1.0)	8** (2.0)
Mediastinum, Inflammation, Chronic Active	0	0	1 (1.0)	7** (1.7)

*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 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.

Trachea: Lesions occurred primarily in the 50 ppm groups, in a few 25 ppm mice, and did not occur in the 12.5 ppm groups (Table 20, Table C-3, and Table D-3). Chronic active inflammation occurred in most of the mice exposed to 50 ppm and in a few exposed to 25 ppm. Grading of the inflammation was based upon both the intensity and the intramural depth of the inflammatory cell infiltrates, ranging from minimal when localized to the submucosa, mild when the muscularis was involved, moderate for extension to the cartilage, and marked in those cases in which the inflammation extended through the tracheal wall and into the surrounding thyroid gland, connective tissue, and/or the periesophageal tissues. Some animals with marked inflammation also had neutrophilic exudate in the tracheal lumen. When it appeared that 40% or more of the lumen was occupied by exudate, a separate diagnosis of lumen exudate was made because the airflow would have been partially obstructed; four males and 12 females in the 50 ppm groups were found to have this level of exudate. Most of the mice exposed to 50 ppm and three females exposed to 25 ppm exhibited varying degrees of necrosis, usually graded as minimal to mild. However, in some of the 50 ppm animals the necrosis was marked, with ulceration and replacement of most or all of the epithelium by neutrophilic exudate and variable extension of the necrosis and suppurative inflammation into the sub-mucosa, cartilage, and peritracheal tissue. Epithelium regeneration, probably secondary to the necrosis, occurred in most 50 ppm mice and to a minimal extent in nine 25 ppm females. Minimal to mild epithelium hyperplasia and/or squamous metaplasia occurred in a few mice exposed to 50 ppm.

Mild to moderate submucosa fibrosis occurred in most of the mice in the 50 ppm groups and did not occur in the lower exposure concentration groups or in the chamber controls (Table 20, Table C-3, and Table D-3). In mildly affected mice, the fibrosis expanded the submucosa and replaced the glands. In some of the more severely affected animals, the fibrosis extended around the cartilage rings or completely replaced the cartilage and extended into the surrounding tissue (Figure 26). The fibrosis also resulted in narrowing of the tracheal lumen in some cases. In addition to the fibrosis noted in the proximal tracheal section, sections of the distal trachea revealed the occurrence of submucosa fibrosis of the carina in some 50 ppm mice (Figure 27). Fibrosis of the carina was diagnosed separately because the carina is the most distal portion of the trachea, situated at the point of bifurcation into the left and right extrapulmonary bronchi. The fibrosis in the carina partially replaced the smooth muscle in some cases and was often accompanied by submucosa mineralization (calcium deposits) and sometimes by submucosa chronic active inflammation.

Lung: Lesions of the bronchi and lung parenchyma occurred predominantly in the 50 ppm groups (Table 20, Table C-3, and Table D-3). The most common bronchial lesion in both male and female mice was minimal to moderate extrapulmonary bronchus epithelium regeneration that occurred in most of the animals exposed to 50 ppm. Regeneration was characterized by a single cell layer of elongated, flattened epithelial cells with the long axis parallel to the basement membrane, which was interpreted as cells attempting to cover a basement membrane that had probably been previously exposed by epithelial cell necrosis with denudation (Figure 28). Minimal to mild extrapulmonary bronchus epithelium necrosis occurred in two males and five females exposed to 50 ppm. Bronchus submucosa fibrosis, characterized by small, focal raised areas of dense collagenous tissue in the submucosa of the extrapulmonary bronchi, occurred in five 50 ppm females (Figure 29).

Lesions of the alveolar parenchyma of the lung were limited to a few animals, primarily in the 50 ppm groups (Table 20, Table C-3, and Table D-3). Suppurative inflammation was used as a combining term for the presence of neutrophilic exudate in the lung, whether located predominantly in the bronchi, bronchioles, alveolar spaces, perivascular tissue, or pleura with spillover into the lung. Bacteria were frequently present in areas of suppuration. The suppurative inflammation in the lung could have resulted from one or more factors, including aspiration of exudate from the upper respiratory tract, mediastinal and secondary pleural inflammation from peritracheal extension of suppurative tracheitis, and/or loss or reduction in the normal nasal function of warming, humidifying, and filtering of inspired air because of the nasal inflammation and other lesions. Minimal to moderate suppurative inflammation of the lung occurred in three males and five females in the 50 ppm groups. Suppurative inflammation of the pleura occurred in two males and five females exposed to 50 ppm. In the 50 ppm groups, suppurative inflammation of the mediastinum occurred in three males and eight females, and chronic active inflammation of the mediastinum occurred in eight males and seven females.

Eye: Minimal to mild acute inflammation of the cornea occurred in 17 males and 23 females exposed to 50 ppm, one male and 20 females exposed to 25 ppm, two females exposed to 12.5 ppm, and two male and one female chamber controls (Table 21, Table C-3, and Table D-3). Grading of the inflammation was based upon the estimated percentage of corneal stroma infiltrated by neutrophils. Minimal to mild epithelium ulcer of the cornea occurred in three males and 10 females exposed to 50 ppm and in 10 females exposed to 25 ppm. Necrosis of the cornea occurred in six females in the 50 ppm group in which necrotic corneal epithelial cells were still attached to or were lifting off from the underlying corneal basement membrane. Mineralization (presumptive calcium deposits) occurred in the superficial portion of the corneal stroma in a few males and several females in the 50 ppm groups and in several 25 ppm females. The mineral deposits were fairly well circumscribed and were often located immediately below areas of corneal ulceration. Epithelium hyperplasia of the cornea was diagnosed when there were six or more layers of epithelial nuclei; this lesion occurred in several mice in the 50 ppm groups, several 25 ppm females, two 12.5 ppm females, two chamber control females, and one chamber control male. The hyperplastic epithelium was sometimes seen at the edges of ulcers of the corneal epithelium. Suppurative inflammation of the anterior chamber occurred in a few of the 50 ppm mice and a few of the 25 ppm females.

Bone Marrow: Significantly increased incidences of myeloid cell hyperplasia occurred in the 50 ppm groups and in 25 ppm males (Table 21, Table C-3, and Table D-3). All stages of maturation were well represented. The erythroid cells often appeared decreased in number as the myeloid hyperplasia increased in severity, likely due to the space-occupying effect of the myeloid cell hyperplasia. The increase in myeloid cells probably represented a secondary reactive response of the marrow to the inflammatory changes in the respiratory tract.

Spleen: A significant increase in the incidence of hematopoietic cell proliferation occurred in 50 ppm males, and the lesion was thought to be a secondary reactive response to the inflammatory changes in the respiratory tract (Table 21 and Table C-3). The proliferation was characterized by an increase in myeloid cells, erythroid cells, and megakaryocytes in the red pulp of the spleen.

Thyroid Gland, Heart, Thymus, and Lymph Nodes: Significantly increased incidences of a few nonneoplastic lesions were considered to be secondary reactions to the respiratory tract inflammation in adjacent or regional tissues. The incidence of chronic active inflammation in the thyroid gland was significantly increased in 50 ppm females (0/50, 0/49, 0/48, 9/50; Table D-3) due to extension of the inflammatory process in the trachea through the tracheal wall and into the adjacent thyroid gland. Similarly, the incidence of chronic active inflammation was significantly increased in the heart of male (0/50, 0/50, 1/50, 5/49; Table C-3) and female (0/50, 2/50, 1/50, 7/50; Table D-3) 50 ppm mice as a result of extension of mediastinal inflammation into either the base of the heart or the wall of an atrium. In the thymus, the incidences of chronic active inflammation (0/47, 0/50, 0/49, 8/45) and atrophy (4/47, 5/50, 2/49, 12/45) were significantly increased in 50 ppm females (Table D-3). Two incidences of chronic inflammation of the thymus were noted in female chamber controls. Increased incidences of thymic inflammation appeared to be secondary to the mediastinal inflammation. Increased incidences of thymic atrophy may have been related to stress. Lymph nodes in the region of the respiratory tract sometimes exhibited lymphoid hyperplasia, as did the mandibular nodes in males (2/36, 5/33, 11/30, 10/32; Table C-3) and females (4/39, 5/47, 12/45, 12/39; Table D-3) and the deep cervical nodes in a few females (0/11, 0/10, 1/11, 4/7; Table D-3). Inflammatory infiltrates sometimes occurred in regional lymph nodes, such as chronic active inflammation in bronchial lymph nodes of a few 50 ppm females (0/40, 0/42, 0/43, 5/43; Table D-3) and plasma cell infiltrates in mandibular lymph nodes of 50 ppm males (1/36, 0/33, 2/30, 14/32; Table C-3).

Table 21. Incidences of Selected Nonneoplastic Lesions in Mice in the Two-year Inhalation Study of 2,3-Butanedione

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Male				
Eye[a]	49	49	50	50
Cornea, Inflammation, Acute[b]	2 (1.5)[c]	0	1 (1.0)	17** (1.4)
Cornea, Epithelium, Ulcer	0	0	0	3 (2.0)
Cornea, Mineralization	0	0	0	5* (1.4)
Cornea, Epithelium, Hyperplasia	1 (1.0)	0	0	9** (1.2)
Anterior Chamber, Inflammation, Suppurative	0	0	0	5* (2.0)
Bone Marrow	48	47	50	49
Myeloid Cell, Hyperplasia	9 (2.1)	8 (1.4)	20* (1.3)	39** (2.5)
Spleen	48	49	50	50

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Hematopoietic Cell Proliferation	11 (2.2)	12 (1.7)	15 (1.8)	25** (2.0)
Female				
Eye	50	49	50	49
Cornea, Inflammation, Acute	1 (1.0)	2 (1.0)	20** (1.2)	23** (1.1)
Cornea, Epithelium, Ulcer	0	0	10** (1.4)	10** (1.6)
Cornea, Necrosis	0	0	0	6** (1.3)
Cornea, Mineralization	0	0	13** (1.0)	16** (1.3)
Cornea, Epithelium, Hyperplasia	2 (1.5)	2 (1.0)	10* (1.1)	9** (1.1)
Anterior Chamber, Inflammation, Suppurative	0	0	4 (1.8)	3 (1.0)
Bone Marrow	50	50	50	49
Myeloid Cell, Hyperplasia	3 (2.3)	2 (1.0)	7 (2.1)	33** (2.2)

*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 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.

Adrenal Gland: The incidence of adrenal cortical adenoma was significantly increased in 12.5 ppm males (2/50, 8/49, 3/50, 2/50; Table C-1 and Table C-2), but there was no exposure concentration-response, and the increase in the 12.5 ppm males was within the historical control range. Adrenal cortical adenomas did not occur in exposed female mice.

Genetic Toxicology

2,3-Butanedione was tested in two bacterial reverse mutation assays, an acute-exposure mouse bone marrow micronucleus test, and subchronic exposure peripheral blood micronucleus tests in rats and mice. Results in both the bacterial assays were positive, and the rodent micronucleus tests were negative.

In the first bacterial mutation assay (Table E-1), a weak positive response (less than twofold increase in revertants, but a clear, reproducible, dose-related increase that approached the twofold level) was observed in *Salmonella typhimurium* strain TA97 in the absence of liver metabolic activation enzymes (S9 mix) and in the presence of 10% and 30% induced hamster and rat liver S9 mixes. The dose range over which the response was observed in TA97 ranged from 10 to 333 µg/plate. No mutagenic responses were observed in any other strain, although some trials were concluded to demonstrate an equivocal response. These equivocal responses were not easily replicated, unlike the weak positive responses seen in strain TA97.

In the second bacterial mutation assay, conducted with the same lot of 2,3-butanedione that was used in the 2-year rodent bioassay, a positive response was seen in *S. typhimurium* strain TA97a in the absence of exogenous metabolic activation, and a response concluded to be equivocal was seen with exogenous metabolic activation (10% induced rat liver S9 mix) (Table E-2). In the *Escherichia coli* strain (WP2 *uvrA*/pKM101) that was included in this second assay, clearly positive responses were seen in all trials conducted with and without S9 mix, suggesting that 2,3-butanedione is a direct-acting mutagen that is not detoxified by induced rat liver S9. The *E. coli* strain reverts via base substitution at the tryptophan locus at an AT base pair. Equivocal results were obtained in *S. typhimurium* strain TA100, with and without S9, and no mutagenicity was observed with strain TA98, with or without S9.

In an acute micronucleus test conducted in male mice, the frequency of micronucleated polychromatic erythrocytes (PCEs) was measured in bone marrow following intra-peritoneal injection of 2,3-butanedione once daily for 3 days (Table E-3). In this test, no increases in micro-nucleated PCEs were observed over the dose range of 7.812 to 500 mg 2,3-butanedione/kg body weight per day.

At the end of the 3-month inhalation studies, peripheral blood samples were obtained from male and female rats and mice and analyzed for the frequency of micronucleated PCEs and normochromatic erythrocytes (NCEs) (Table E-4 and Table E-5). In these studies, no increases in the frequencies of micronucleated PCEs or NCEs occurred in either sex or species exposed to 2,3-butanedione. The percentage of PCEs among circulating red blood cells was unaffected by exposure to 2,3-butanedione, suggesting the chemical had no effect on erythropoiesis.

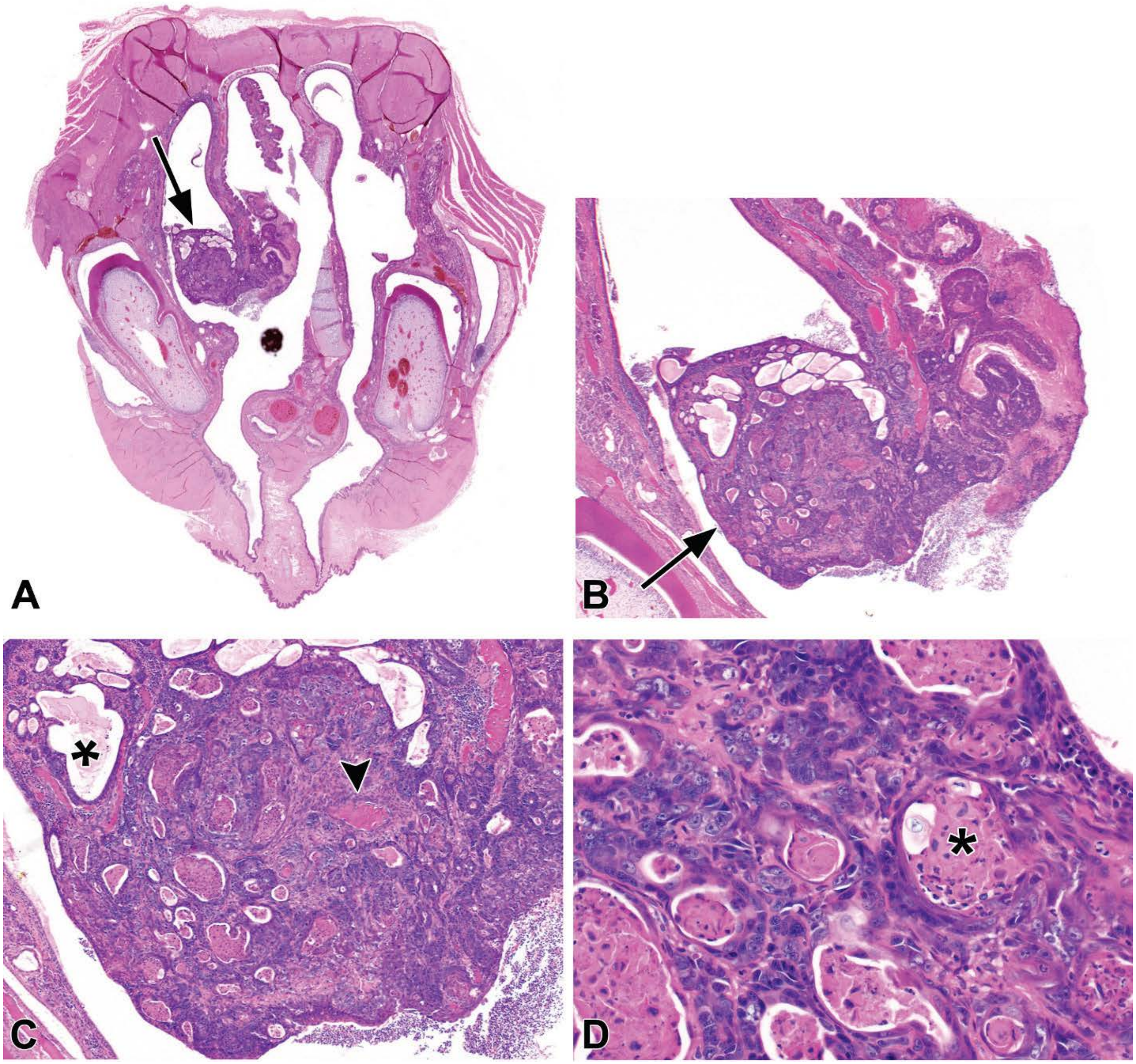


Figure 8. Squamous Cell Carcinoma in the Nose of a Male Wistar Han Rat Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A) The neoplasm forms a bulbous mass (arrow), arising from the tip of a nasoturbinate in Level II, and projects into the nasal cavity. B) The neoplasm invades into the fibrotic stroma of the nasoturbinate (arrow). C) Between the nests of neoplastic cells, a few remnants of bone (arrowhead) from the nasoturbinate hook are seen. Dilated respiratory glands (asterisk), probably secondary to outflow obstruction, are also noted. D) Many of the nests of neoplastic cells have central cystic spaces filled with keratin and cell debris (asterisk). Original objective magnification: A = 1×, B = 4×, C = 10×, D = 20×.

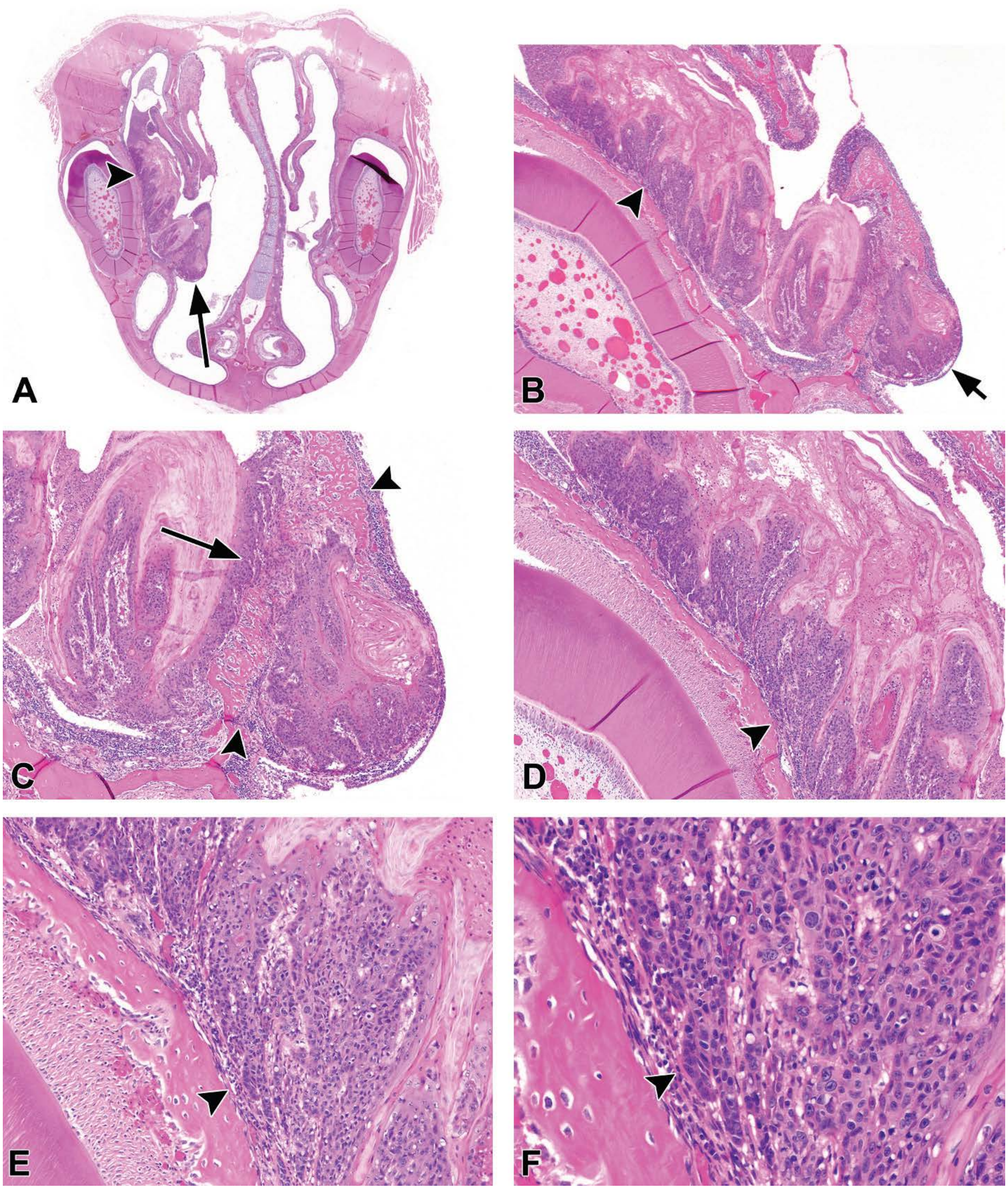


Figure 9. Squamous Cell Carcinoma in the Nose of a Male Wistar Han Rat Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A, B) The neoplasm forms a plaque-like mass, arising from the lateral wall (arrowhead) of the nasal cavity and the adjacent maxilloturbinate (arrow). C) The keratinizing squamous carcinoma (arrow) invades the stroma adjacent to the bone (arrowheads) of the maxilloturbinate. D, E, F) The lateral wall portion of the neoplasm exhibits foci of microinvasion (arrowheads) into the lamina propria close to the alveolar process of the premaxillary bone. Original objective magnification: A = 1×, B = 2×, C = 4×, D = 4×, E = 10×, F = 20×.

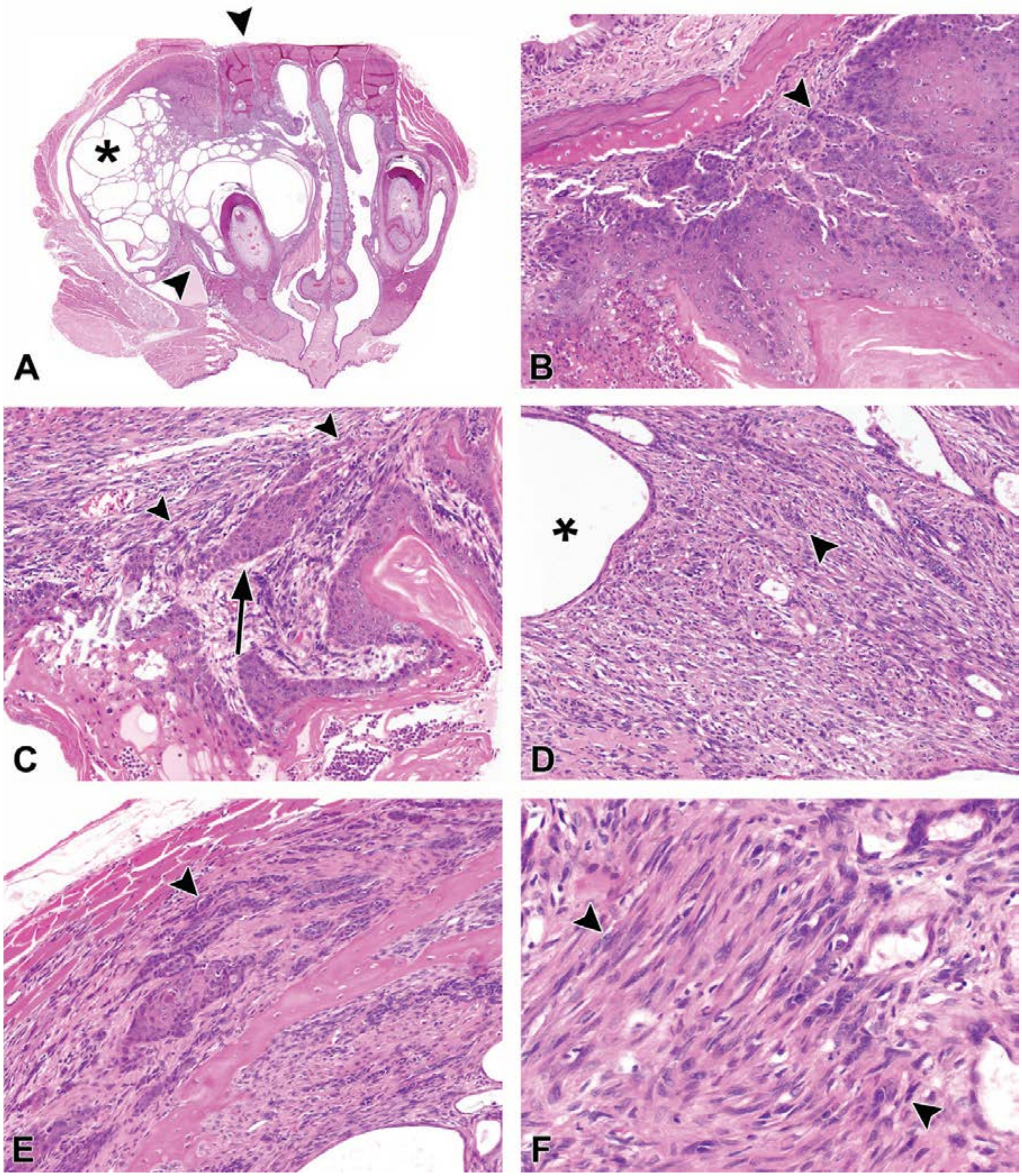


Figure 10. Squamous Cell Carcinoma in the Nose of a Male Wistar Han Rat Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A) The neoplasm forms a large solid and cystic mass, filling and expanding one side of the nasal cavity in Levels I and II, with deviation of the nasal septum to the opposite side. The main portion of the solid neoplasm is located in the dorsolateral wall (top arrowhead), but the infiltration also extends laterally and ventrally to invade the premaxilla bone (lower arrowhead). The cystic spaces (asterisk) are probably dilated respiratory glands with their ducts obstructed by the invasive neoplasm. B, C) The inner surface of the neoplasm shows a keratinizing squamous carcinoma (lower right), with buds (arrowheads) and nests (arrow in C) of invasion into the lamina propria. D) Much of the invasive neoplasm consists of a solid, spindle cell component with only scattered squamous cell nests (arrowhead). A cystically dilated respiratory gland is present in the upper left (asterisk). E) The neoplasm (arrowhead) invades through the frontal process of the premaxillary bone into the surrounding muscle. F) The spindle cell character of the invasive neoplasm is evident (between the arrowheads). Original objective magnification: A = 1×, B = 10×, C = 10×, D = 10×, E = 10×, F = 20×.

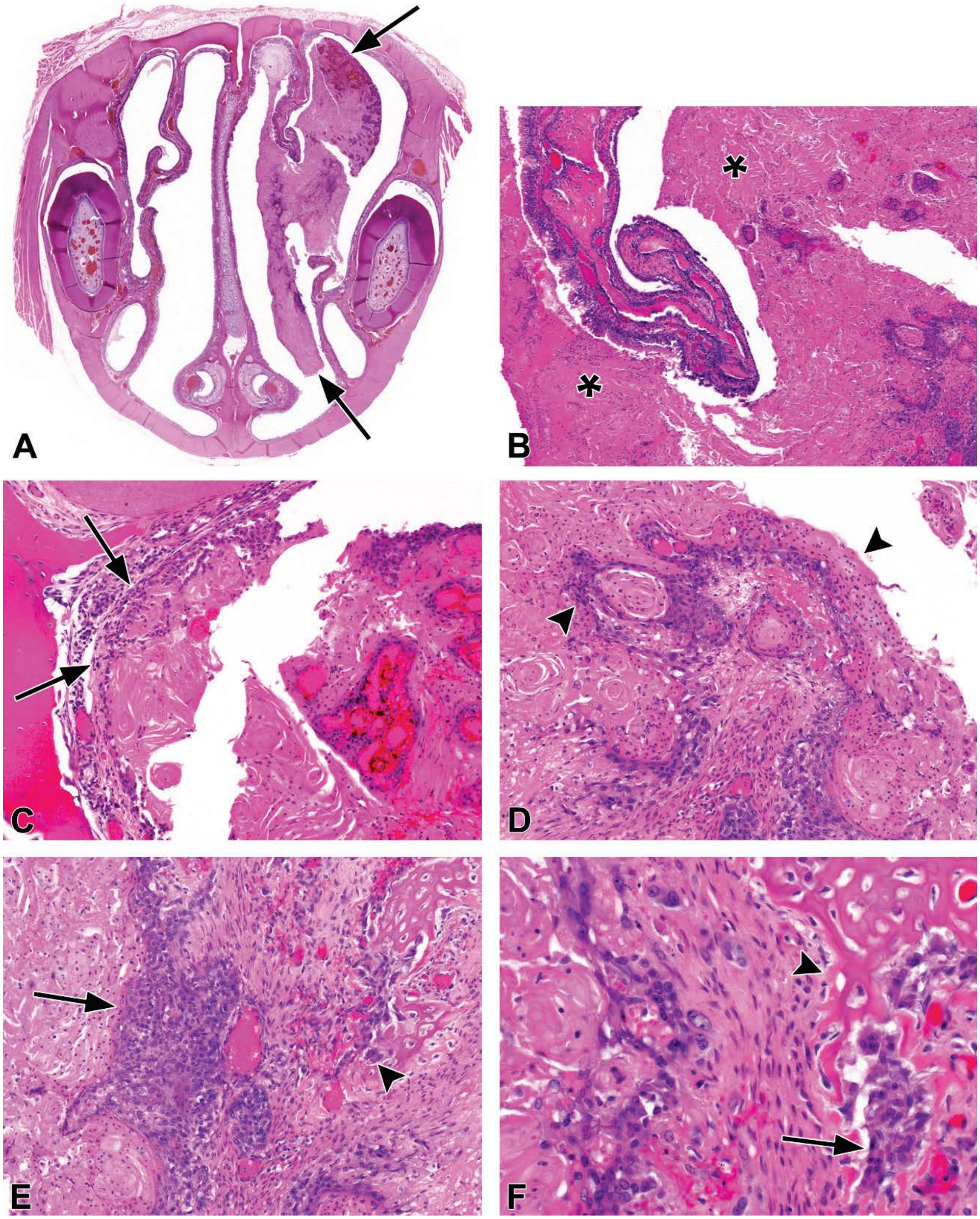


Figure 11. Squamous Cell Papilloma in the Nose of a Male Wistar Han Rat Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A) The necrotic, polypoid mass extends from the dorsal end (top arrow) of the nasal cavity almost to the ventral end (bottom arrow). B) Necrotic neoplasm largely fills the lateral meatus (upper asterisk), wraps around the nasoturbinate, and extends into the dorsal meatus (lower asterisk). C) Arrows indicate the pedicle of the neoplasm at the base of the nasoturbinate. D) Focal islands of residual squamous epithelium with keratin are noted (arrowheads). E) Residual nests of squamous epithelium are indicated by the arrow and the arrowhead, with the latter lying adjacent to bone fragments. F) A nest of neoplastic cells (arrow) lies adjacent to bone (arrowhead). Fibrotic stroma is present in the center. Original objective magnification: A = 1×, B = 4×, C = 10×, D = 10×, E = 10×, F = 20×.

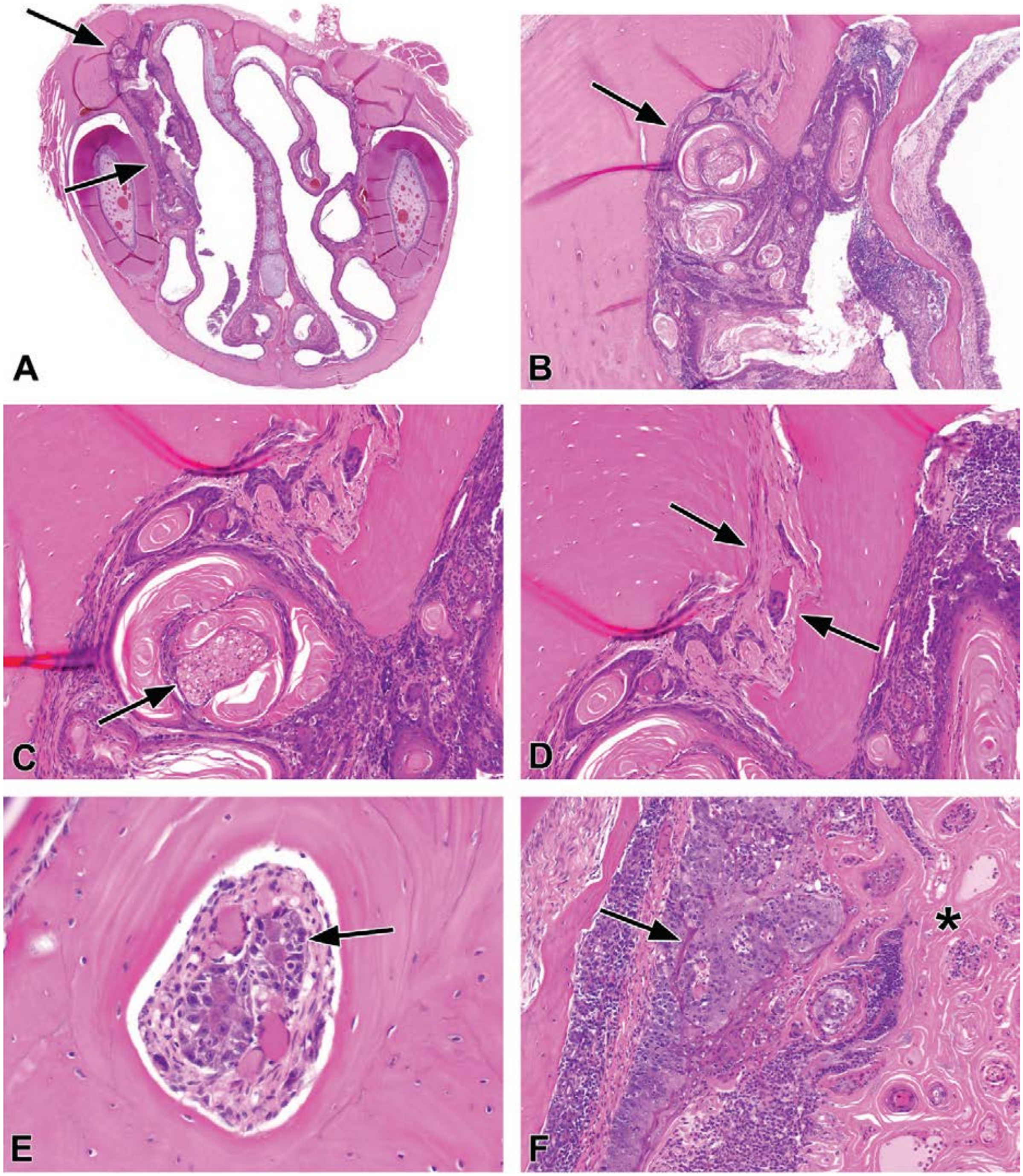


Figure 12. Squamous Cell Carcinoma in the Nose of a Female Wistar Han Rat Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A) The neoplasm involves much of the lateral wall (between the arrows) on one side of Level I. B) The neoplasm invades the lamina propria (arrow) close to the premaxillary bone. C) Perineural invasion is present (arrow). D) Nests of neoplastic cells invade the naso-premaxillary suture (arrows). E) A nest of neoplastic cells (arrow) within a Haversian canal in the premaxillary bone. The neoplasm probably reached the interior of the bone by invading through a Volkmann canal on the dorsomedial side of the bone. F) Atypical squamous metaplasia (arrow) with marked hyperkeratosis (asterisk), is replacing the respiratory epithelium of the lateral wall. This type of metaplasia is atypical both architecturally and cytologically and is a probable background precursor for the development of squamous carcinoma. Original objective magnification: A = 1×, B = 4×, C = 10×, D = 10×, E = 20×, F = 10×.

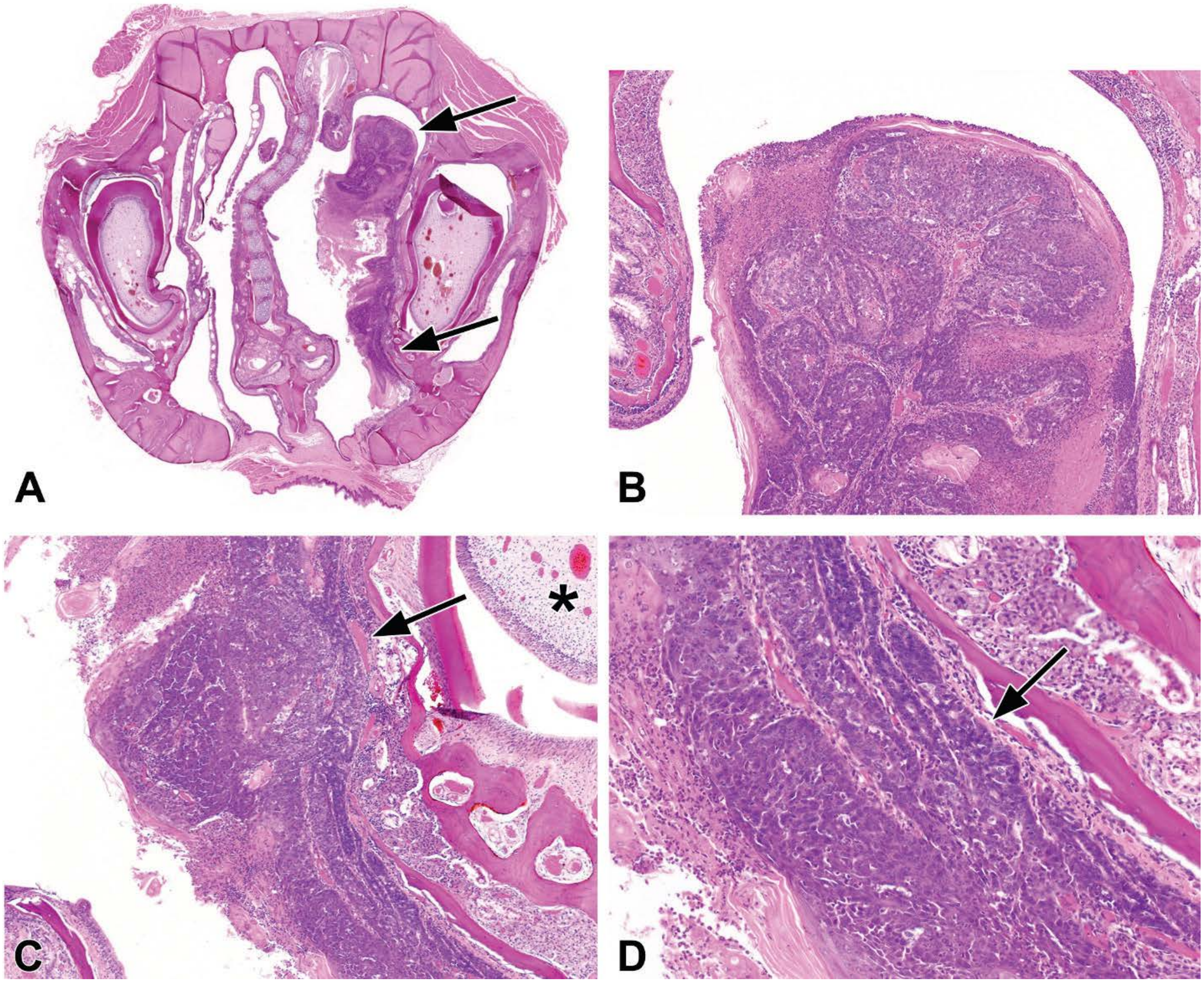


Figure 13. Squamous Cell Carcinoma in the Nose of a Female Wistar Han Rat Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A) The neoplasm extends from near the ventral end (lower arrow) of the lateral wall to the dorsal end (upper arrow). Note that the bulbous expansion of the neoplasm dorsally has caused the adjacent lateral hook of the nasoturbinate to deviate medially. B) Nests of neoplastic cells invade the lamina propria. C) The neoplasm invades the lamina propria (arrow) of the ventral meatus, adjacent to the incisor tooth (asterisk). D) Neoplasm invades the deeper portion of the lamina propria (arrow), adjacent to the alveolar process of the premaxillary bone. Original objective magnification: A = 1×, B = 4×, C = 4×, D = 10×.

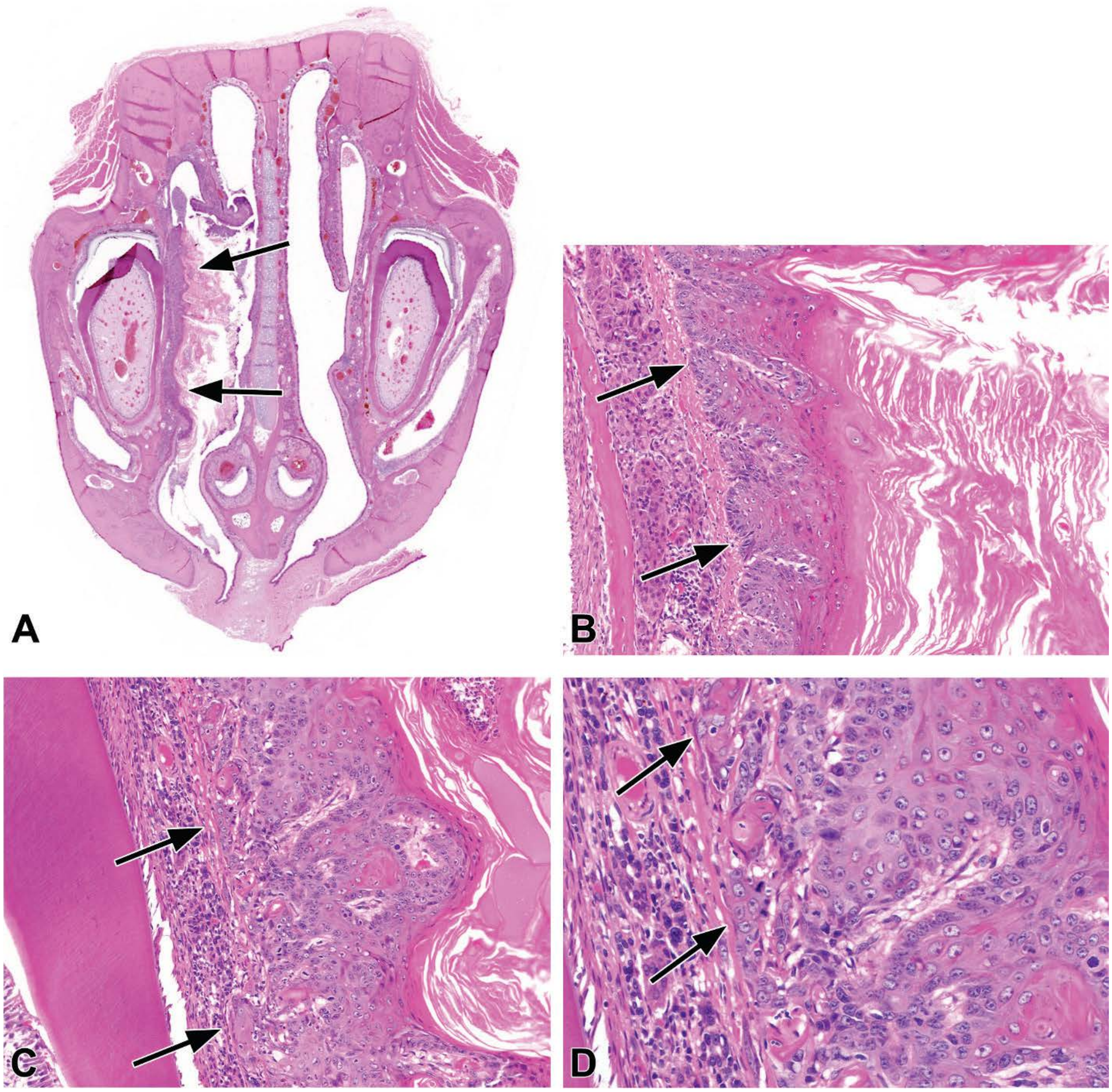


Figure 14. Squamous Cell Carcinoma in the Nose of a Female Wistar Han Rat Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A) The neoplasm forms a plaque-like lesion along the lateral wall of Level II (arrows). B) The neoplasm (arrows) is superficial, in situ, and hyperkeratotic in this region. C, D) Small buds of microinvasive neoplasm are noted in the lamina propria (arrows). Original objective magnification: A = 1×, B = 10×, C = 10×, D = 20×.

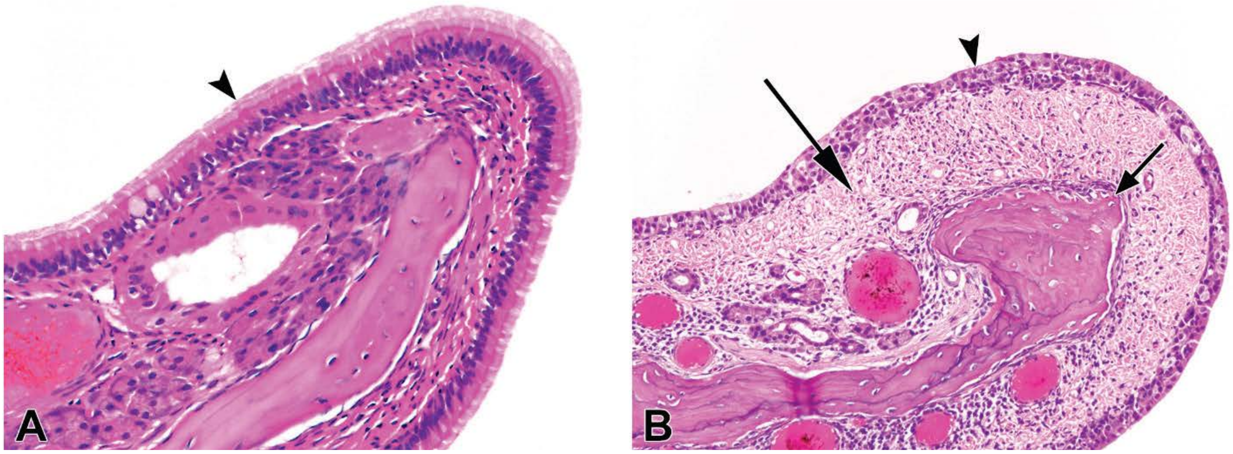


Figure 15. Normal Nasoturbinate, Level II, in the Nose of a Chamber Control Male Wistar Han Rat at Two Years (A), and Fibrosis in the Nose, Nasoturbinate Level II, of a Male Wistar Han Rat Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (B) (H&E)

B) The lamina propria exhibits fibrosis (long arrow) with replacement of the glands. The mucosal respiratory epithelium in A (arrowhead) covering the turbinate has been replaced by regenerative hyperplastic epithelium in B (arrowhead). The tip of the turbinate bone is hyperostotic (short arrow). Original objective magnification: A = 20×, B = 20×.

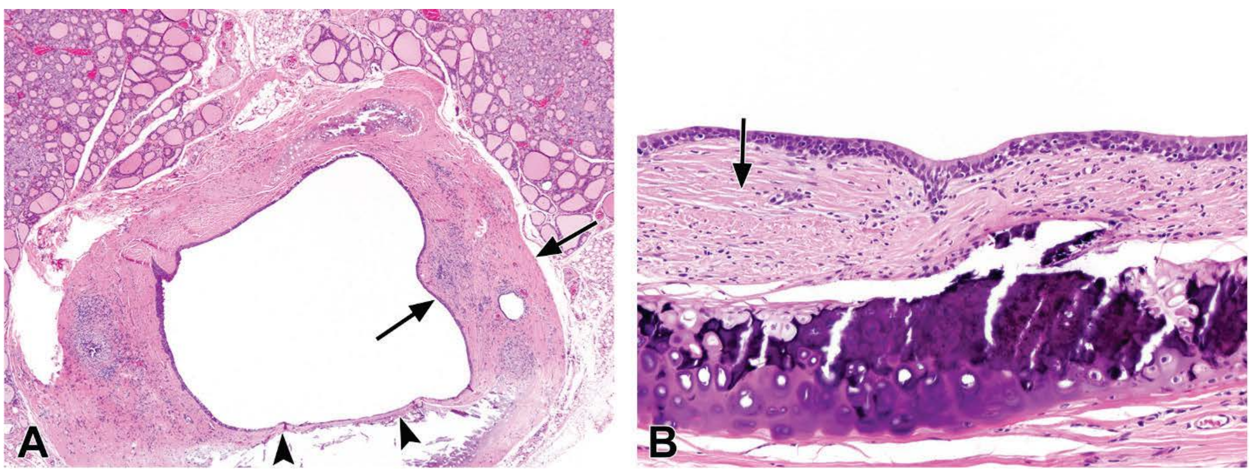


Figure 16. Fibrosis in the Trachea of a Female Wistar Han Rat (A) and A Male Wistar Han Rat (B), Both Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A) There is marked fibrotic thickening of the wall (arrows), except for one segment at the bottom (between the arrowheads). Much of the cartilage in the wall has been replaced by the fibrosis, and there is reduction in the size of the tracheal lumen. B) Marked fibrotic thickening of the tracheal submucosa (arrow). Original objective magnification: A = 4×, B = 20×.

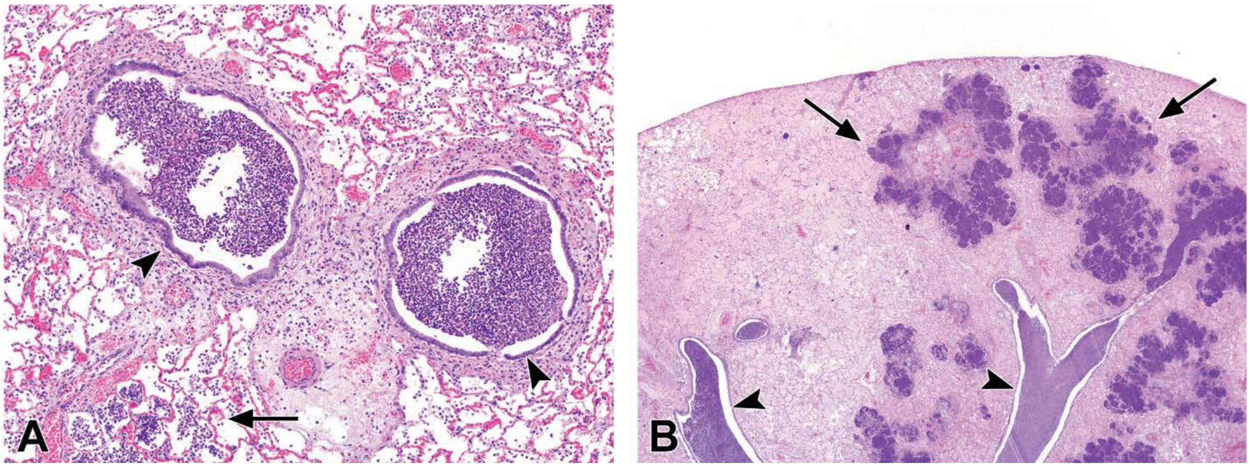


Figure 17. Suppurative Inflammation in the Lung of Two Male Wistar Han Rats Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

There is suppurative exudate in the bronchial lumens (arrowheads) (A and B). In A, acute inflammation is present around the bronchial branches and in the alveolar spaces of the lung (arrow). In B, the lung also exhibits dense aggregates (arrows) of inflammatory cells and bacterial colonies. Original objective magnification: A = 10×, B = 2×.

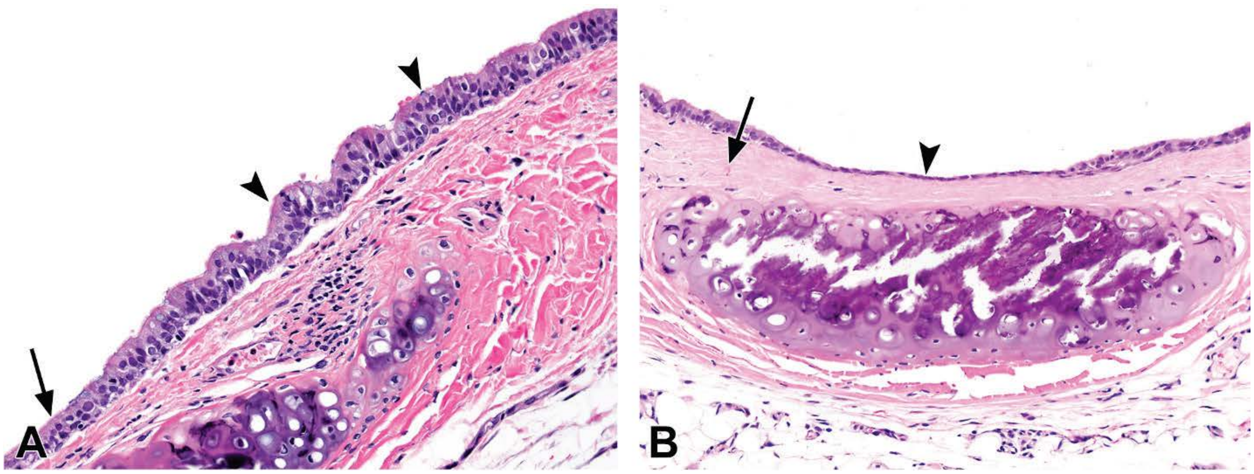


Figure 18. Bronchus Epithelium Hyperplasia (A) and Submucosa Fibrosis (B) in the Bronchi of Male Wistar Han Rats Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A) Much of the mucosal epithelium shows mild hyperplasia (arrowheads), characterized by thickening and increased cellularity (compare to normal columnar epithelium at arrow). B) There is hyaline fibrosis of the submucosa (arrow) in this extra-pulmonary bronchus, and replacement of the respiratory epithelium in the center by a flattened, regenerating epithelium (arrowhead). Original objective magnification: A = 20×, B = 20×.

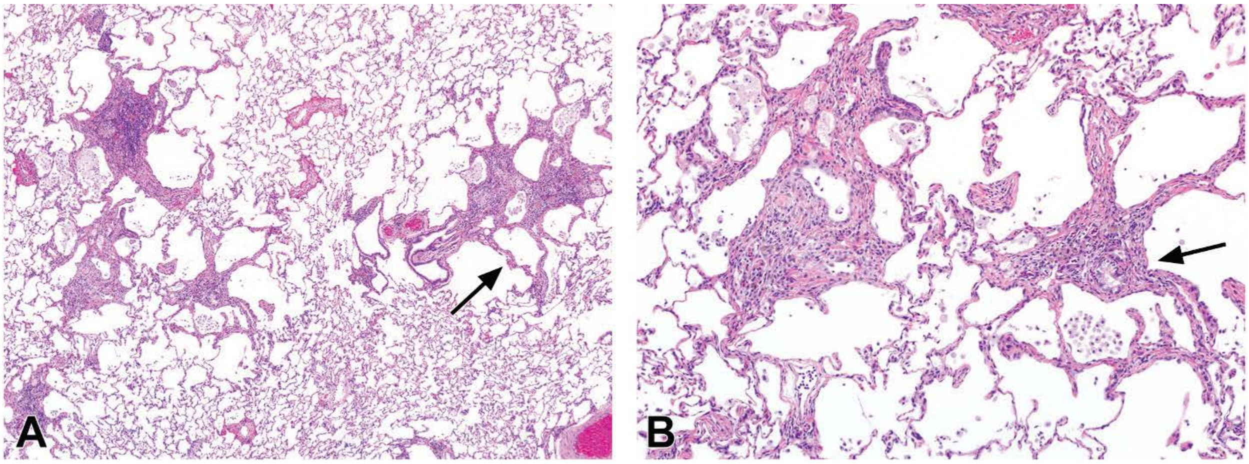


Figure 19. Interstitium Fibrosis in the Lung of a Male Wistar Han Rat Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (A and B) (H&E)

Nodular foci of interstitial fibrosis of the alveolar walls, accompanied by inflammatory infiltrate, extend irregularly into the surrounding alveolar septa (arrows). Original objective magnification: A = 4×, B = 10×.

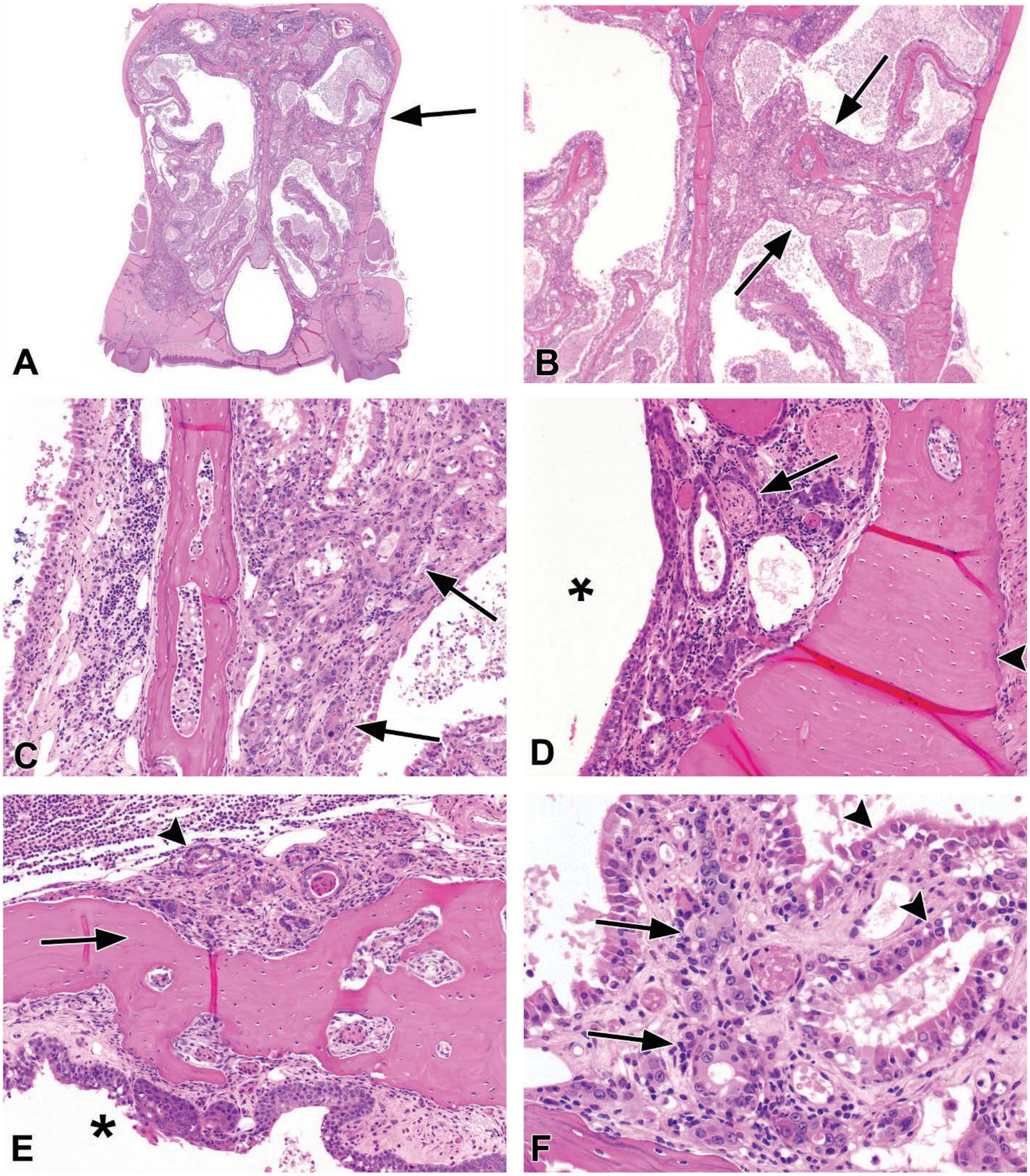


Figure 20. Adenocarcinoma in the Nose of a Female B6C3F1/N Mouse Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A, B) This neoplasm infiltrates the lamina propria of the nasal cavity, without mass formation, and without architectural distortion, except focally where there is bridging (arrows) of an ethmoid turbinate with the nasal septum. C) Infiltrating adenocarcinoma (arrows) on one side of the nasal septum in Level III. D) The neoplasm infiltrates between the nasopharyngeal duct (asterisk) and the palatine process of the maxillary bone (arrowhead), exhibiting focal perineural invasion (arrow). E) The neoplasm (arrowhead) invades the inflamed soft tissue of the palate on the ventral side of the premaxillary bone (arrow) in Level I. The nasal cavity is indicated by the asterisk. F) The adenocarcinoma (arrows) is arising in the background of extensive respiratory metaplasia (arrowheads) of the olfactory epithelium in Level III. Original objective magnification: A = 1×, B = 2×, C = 10×, D = 10×, E = 10×, F = 20×.

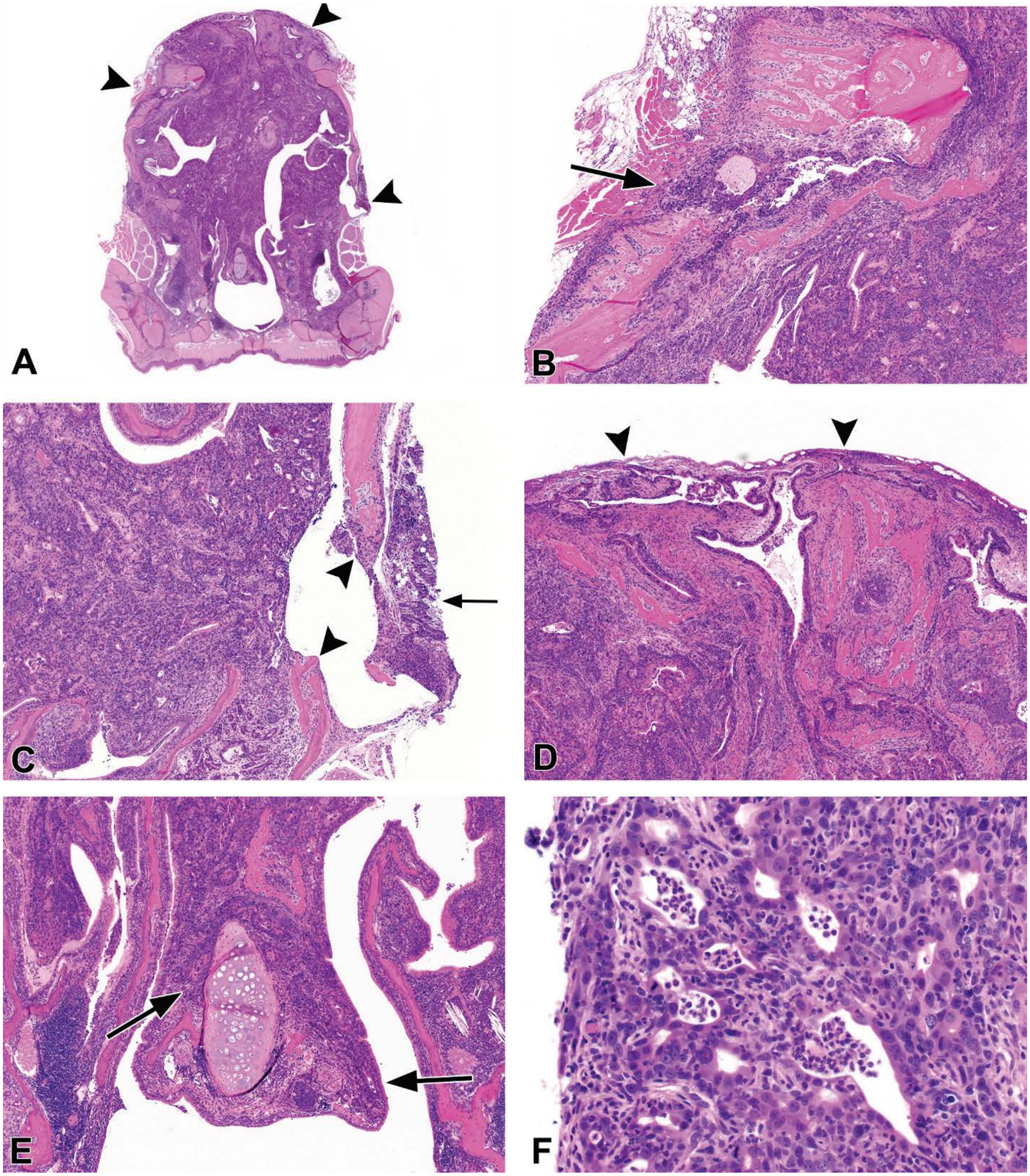


Figure 21. Adenocarcinoma in the Nose of a Female B6C3F1/N Mouse Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A) The neoplastic mass largely fills the nasal cavity in Level III and invades through the bony wall on both lateral sides, as well as dorsally (arrowheads). B) The neoplasm invades through the maxillary bone into periosteal soft tissue adjacent to striated muscle (arrow). C) The neoplasm invades through maxillary bone (arrowheads) on the opposite side from B), and into periosteal soft tissue (arrow). D) The neoplasm appears to have invaded dorsally through the cribriform plate of the ethmoid bone as well as the frontal bone, extending near the dorsal surface (arrowheads) in Level III. E) The neoplasm (arrows) invades into the ventral portion of the nasal septum adjacent to the nasopharyngeal duct (bottom of photo) in Level III. F) Higher magnification of the infiltrating neoplasm within an inflamed fibrous stroma. Original objective magnification: A = 1×, B = 4×, C = 4×, D = 4×, E = 4×, F = 20×.

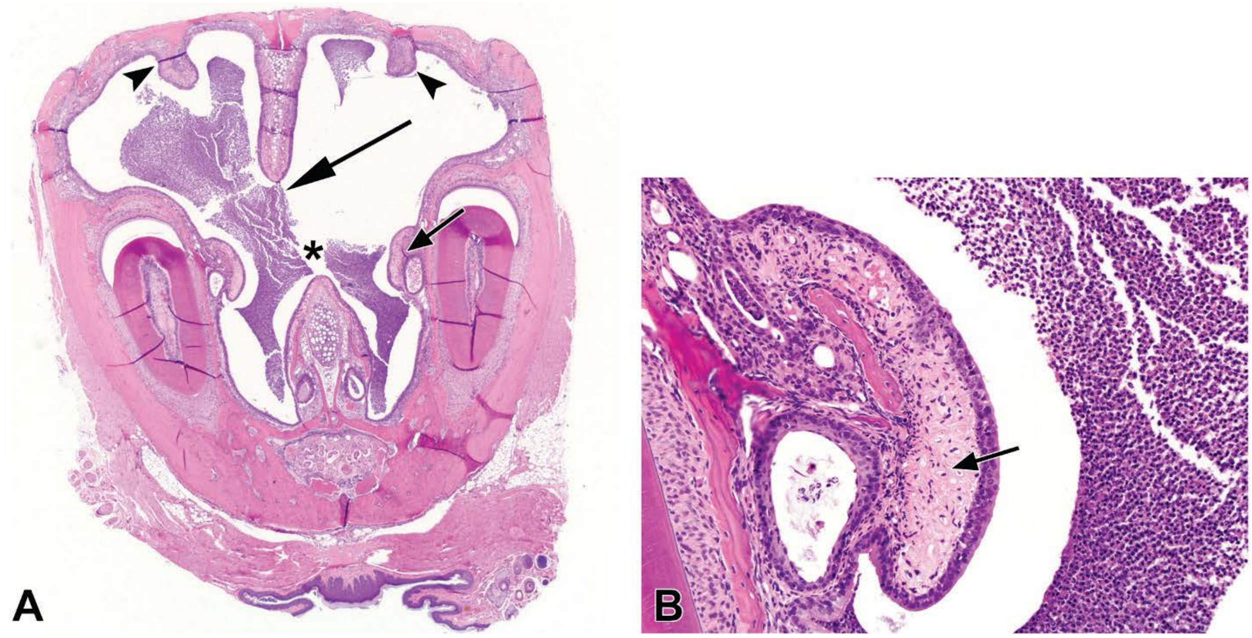


Figure 22. Suppurative Inflammation, Septum Perforation, Turbinate Atrophy, And Lamina Propria Fibrosis in the Nose of a Male B6C3F1/N Mouse Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A) This low power view of the nasal cavity, Level I, shows suppurative inflammation (long arrow) within the nasal cavity, a healed septal perforation (asterisk in gap), marked nasoturbinate atrophy (arrowheads), and fibrosis of the lamina propria of the naso- and maxilloturbinates (short arrow). B) Higher magnification of fibrosis (arrow) within the lamina propria of a maxilloturbinate. Original objective magnification: A = 2×, B = 10×.

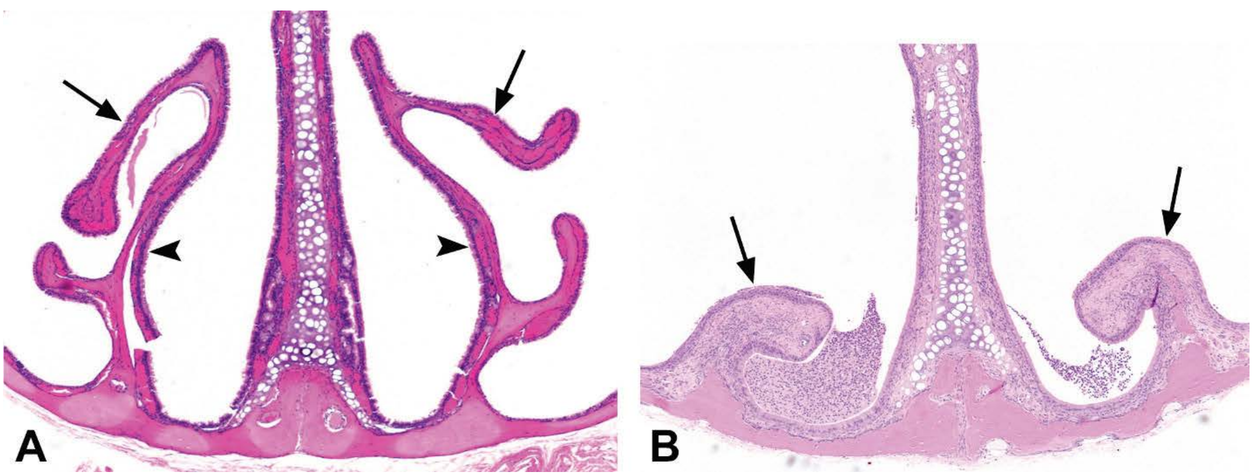


Figure 23. Normal Nasoturbinates, Level I, in the Nose of a Chamber Control Male B6C3F1/N Mouse at Two Years (A), and Turbinate Atrophy in the Nose of a Male B6C3F1/N Mouse Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (B) (H&E)

A) Long, slender nasoturbinates (arrowheads) with lateral hooks (arrows), in Level I of the nasal cavity. B) Markedly shortened, blunted, and fibrotic nasoturbinates (arrows) in Level I, taken at the same magnification as A. Original objective magnification: A = 4×, B = 4×.

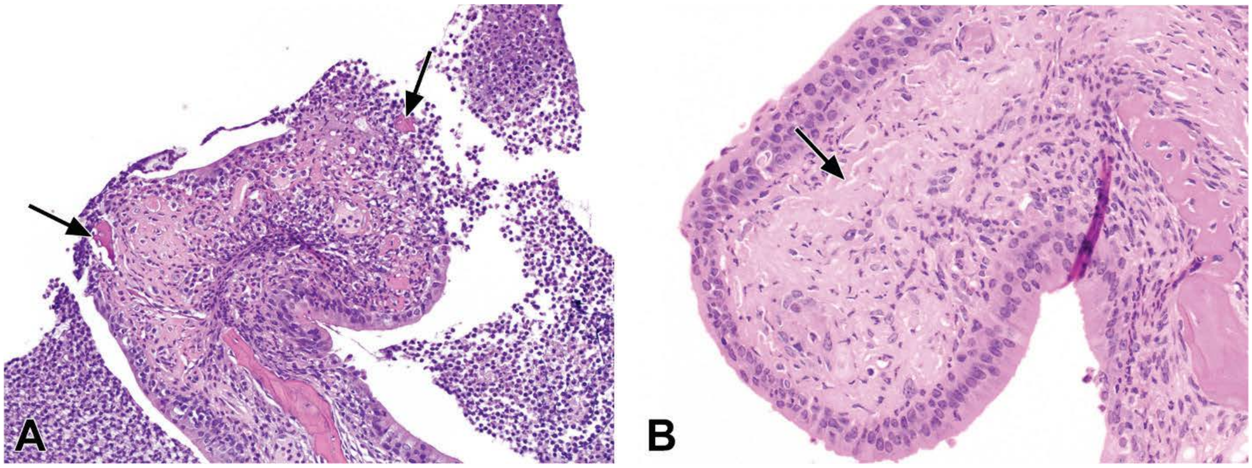


Figure 24. Turbinate Necrosis in the Nose of a Female B6C3F1/N Mouse (A) and Lamina Propria Fibrosis in the Nose of a Male B6C3F1/N Mouse (B) Both Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A) The nasoturbinate shows marked inflammation, foci of mucosal ulceration, and loss of the bony hook except for two remnants of necrotic and partially extruded bone (arrows). B) The bony hook of the nasoturbinate has been replaced by dense fibrocollagenous tissue (arrow) which has expanded the thickness of the lamina propria. Original objective magnification: A = 20×, B = 20×.

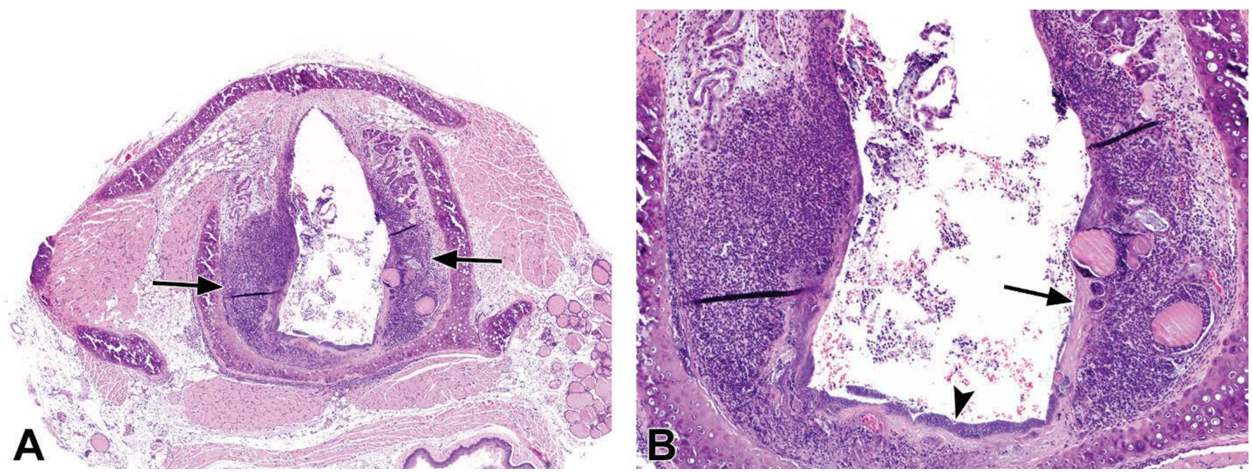


Figure 25. Lumen Exudate and Respiratory Epithelium Necrosis in the Larynx of a Female B6C3F1/N Mouse Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (H&E)

A) Much of the laryngeal wall is infiltrated by a dense infiltrate of inflammatory cells (arrows), and there is inflammatory exudate in the lumen. B) Higher magnification reveals necrosis and ulceration of the mucosal epithelium (arrow), with bacterial colonies (arrowhead) on the ulcerated surface. Original objective magnification: A = 4×, B = 10×.

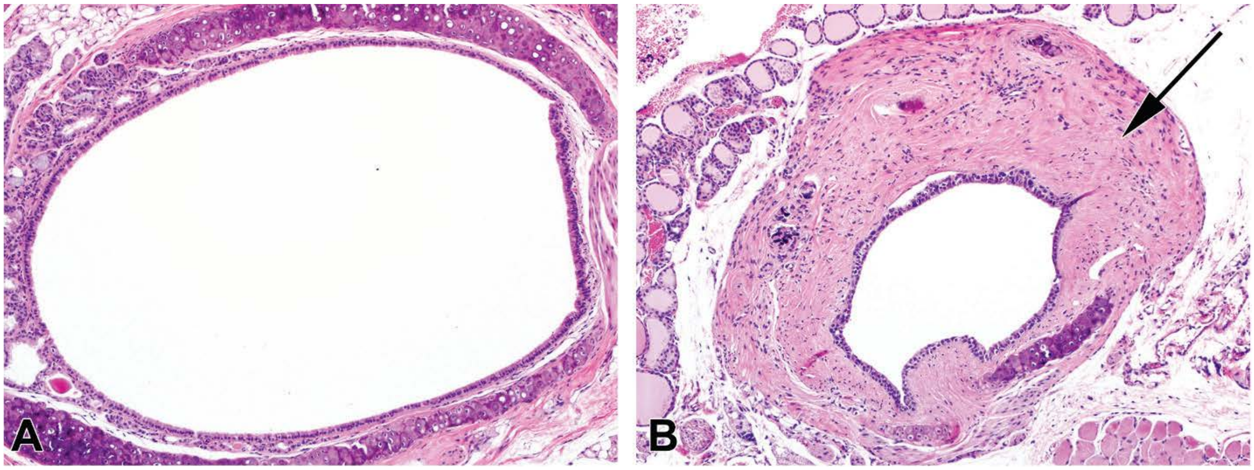


Figure 26. Normal Trachea of a Chamber Control Female B6C3F1/N Mouse at Two Years (A) and Submucosa Fibrosis in the Trachea of a Male B6C3F1/N Mouse Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (B) (H&E)

B) Marked fibrosis of the entire tracheal wall (arrow), with loss of most of the cartilage and marked narrowing of the lumen. The lumenal area of the trachea in this exposed B6C3F1/N mouse is approximately one-fifth of that of the chamber control B6C3F1/N mouse. Original objective magnification: A = 10×, B = 10×.

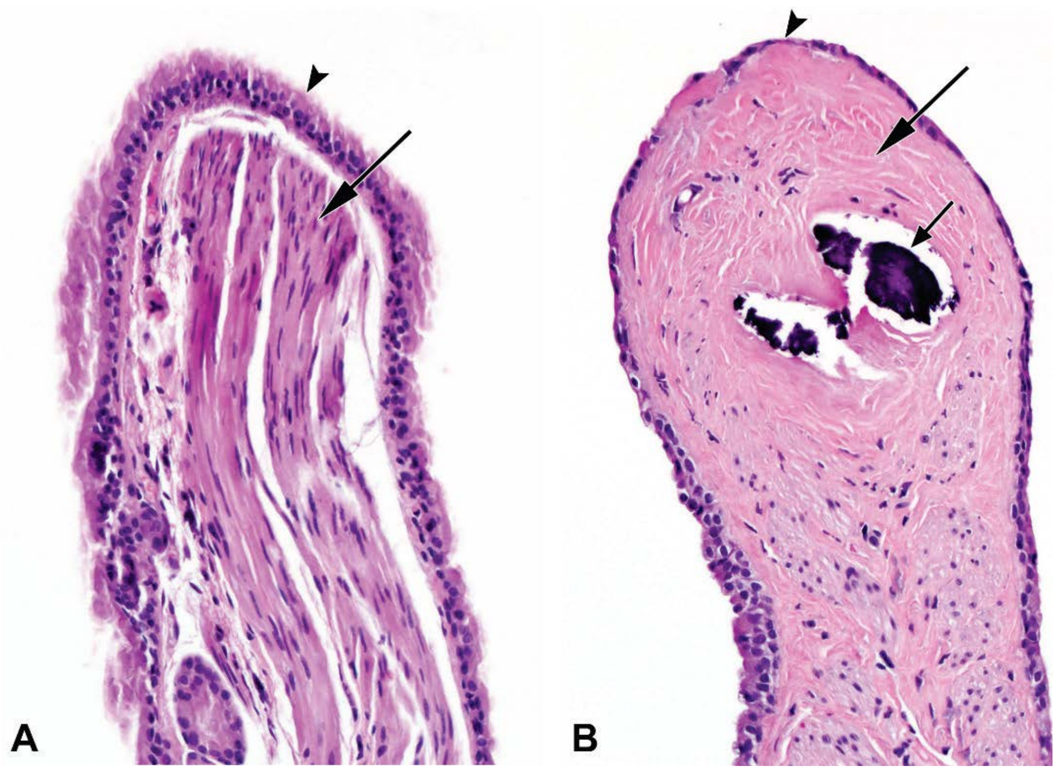


Figure 27. Normal Tracheal Carina of a Chamber Control Male B6C3F1/N Mouse at Two Years (A) and Submucosa Fibrosis and Mineralization in the Tracheal Carina of a Male B6C3F1/N Mouse Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (B) (H&E)

A) The carina shows subepithelial smooth muscle (arrow) and respiratory epithelium (arrowhead) on the surface. B) The subepithelial smooth muscle has been replaced by fibrosis (long arrow), containing foci of mineralization (short arrow). A flat regenerative epithelium (arrowhead) lines the surface of the carina. Original objective magnification: A = 20×, B = 20×.

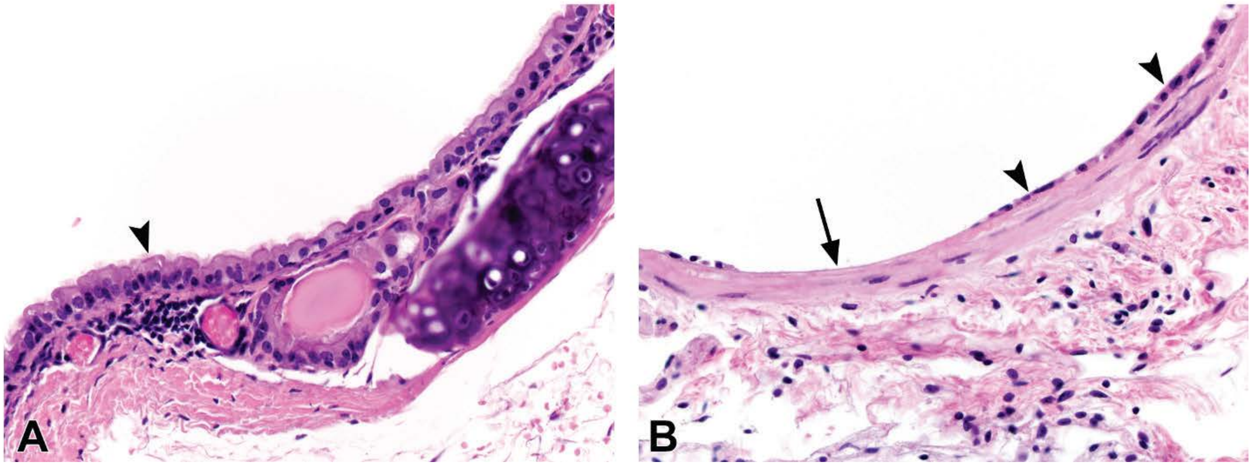


Figure 28. Normal Bronchus of a Chamber Control Male B6C3F1/N Mouse at Two Years (A) and Epithelium Regeneration in the Bronchus of a Male B6C3F1/N Mouse Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (B) (H&E)

A) An extrapulmonary bronchus lined by columnar, ciliated respiratory epithelium (arrowhead). B) The respiratory epithelium has been eroded (arrow) and is partially replaced by a flattened to cuboidal regenerating epithelium (arrowheads). Original objective magnification: A = 40×, B = 40×.

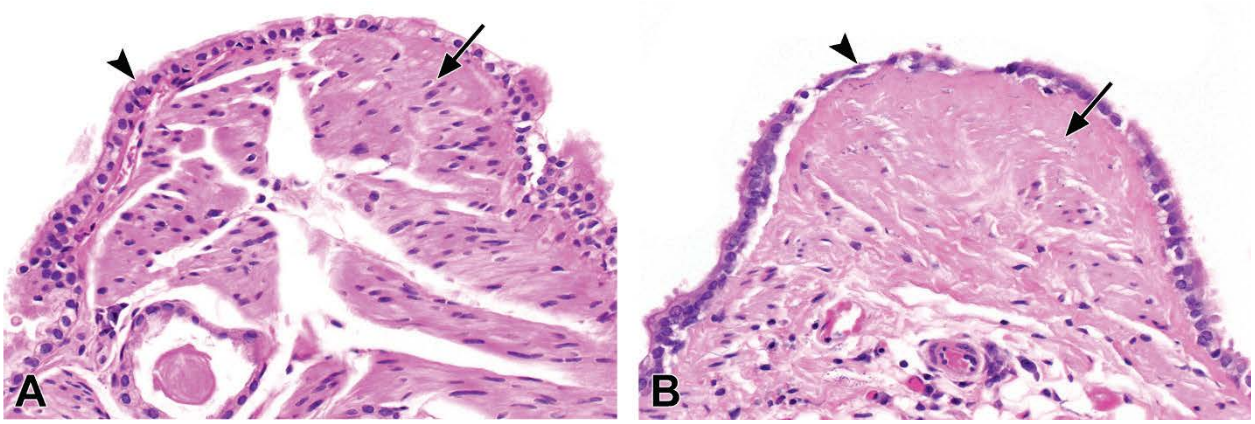


Figure 29. Normal Mainstem Bronchus and Bifurcation Knob of a Chamber Control Female B6C3F1/N Mouse at Two Years (A) and Submucosa Fibrosis in the Mainstem Bronchus and Bifurcation Knob of a Female B6C3F1/N Mouse Exposed to 50 ppm 2,3-Butanedione by Inhalation for Two Years (B) (H&E)

A) Respiratory epithelium is on the surface (arrowhead), with underlying smooth muscle (arrow). B) The surface epithelium is slightly attenuated (arrowhead), and the subepithelial muscle has been replaced by dense fibrous tissue (arrow). Original objective magnification: A = 20×, B = 20×.

Discussion

2,3-Butanedione is a volatile, direct acting, diketone that caused significant toxicity throughout the respiratory tract of rodents. The severity and distribution of lesions in the respiratory tract caused by direct-acting volatile toxicants is dependent upon the water solubility and reactivity of the chemical, the exposure concentration and duration, and regional differences in cellular susceptibility. 2,3-Butanedione is water soluble and can readily penetrate the aqueous mucous layer protecting the respiratory tract mucosa[36]. 2,3-Butanedione also is highly reactive, especially toward guanidinium groups on positively charged amino acids such as arginine, lysine, and histidine[35; 71; 110]. Arginyl residues are often located at the active sites of enzymes, and reaction with 2,3-butanedione and related diketones results in a loss of enzyme activity[55; 111-115]. Reaction of 2,3-butanedione with arginyl residues on proteins can result in modification of structure and function[116-118]. In a recent study, 2,3-butanedione-induced airway damage in mice was correlated with increased immunofluorescence for markers of protein turnover and autophagy[70]. Inhalation exposure of mice to 200 ppm 2,3-butanedione for 6 hours caused concentration-dependent increases in bronchial epithelial cells with increased accumulations of total ubiquitin and K63-ubiquitin, central mediators of protein turnover. Immunofluorescent colocalization of ubiquitin with lysosomal-associated membrane proteins 1 and 2, and with sequestosome-1, a multifunctional scaffolding protein, confirmed ubiquitin autophagy. Free ubiquitin was depleted, which is a concern because of its role in cellular homeostasis, including DNA repair and chromatin remodeling[119; 120].

Inhalation of 2,3-butanedione and other direct-acting irritants causes a characteristic sequence of events, beginning with injury to the epithelium lining the respiratory tract. Necrosis of epithelial cells typically is followed by inflammation and regenerative proliferation to repair the injured epithelium. Repeated exposure and injury to the epithelium can result in an adaptive response such as squamous metaplasia, and excessive cell proliferation can result in epithelial hyperplasia[121; 122]. Repetitive epithelial injury in the respiratory tract can also lead to fibrosis. Fibrosis in the small airways can progress to bronchiolitis obliterans, a debilitating obstructive airway disease characterized by epithelial degeneration and progressive fibroproliferation with eventual obliteration of the airway lumens. Occupational exposure to 2,3-butanedione was first associated with an increased prevalence of bronchiolitis obliterans in workers at a microwave popcorn packaging plant[22; 24]. The prevalence of bronchiolitis obliterans was highest for workers in the artificial butter flavor mixing room where a mean 2,3-butanedione concentration of 57.2 ppm was reported[25; 59].

Initial studies were conducted to better understand the association between 2,3-butanedione exposure and the development of bronchiolitis obliterans. Male Wistar Han rats were exposed 6 hours/day, 5 days/week for 2 weeks to 100, 150, 175, or 200 ppm 2,3-butanedione, and bronchiolitis obliterans occurred only in rats exposed to 150 ppm or greater[55]. These studies demonstrated that 2,3-butanedione has a steep concentration-response curve and suggest that peak exposure concentration may be more important than exposure duration in the pathogenesis of bronchiolitis obliterans. In these 2-week studies, airway epithelial injury caused by repeated exposure to 100 ppm 2,3-butanedione was insufficient to trigger the dysregulated fibroproliferation associated with bronchiolitis obliterans. It is possible that repeated exposure to 150 ppm 2,3-butanedione or greater not only causes epithelial necrosis, but may also eliminate progenitor cells thereby preventing reepithelialization[123]. Alternatively, denudation of both the epithelium and basement membrane exposes the underlying connective tissue to chemical irritation, potentially triggering the fibrotic response[124].

Two-week exposures to 50 and 100 ppm 2,3-butanedione caused significant lesions in the nasal cavity, but concentrations reaching the small airways may have been insufficient to cause loss of basal cells and basement membrane disruption. Absorption and reaction of inhaled 2,3-butanedione in the nose and upper respiratory tract reduce the concentration that reaches the distal airways. This effect is greater in rodents than humans because rodents are obligate nose breathers, and because their complex nasal anatomy allows more efficient scrubbing of 2,3-butanedione from the inhaled air. These species differences may result in greater nasal toxicity in 2,3-butanedione-exposed rodents, with less injury to the lower airways. Morris and Hubbs[36] estimated that in nose-breathing rats exposed to 100 ppm 2,3-butanedione, only 62 ppm reached the bronchi. In contrast, in mouth-breathing humans this concentration was predicted to be 97 ppm, about 1.5-fold higher, suggesting that the dose delivered to intrapulmonary airways in humans may be much greater than that in the rat.

The current 2-year studies were designed to evaluate whether chronic exposures to low concentrations of 2,3-butanedione would cause respiratory tract fibrosis or have carcinogenic effects. Small areas of submucosal fibrosis were present in the proximal bronchi of a few rats exposed to 50 ppm for 2 years; however, these minimal lesions were not progressive and did not cause bronchiolitis obliterans. Although a few incidences of fibrosis were present in the small airways, fibrosis was much more prevalent in the nose and trachea of rats and mice exposed for 2 years to 2,3-butanedione. Fibrosis of the lamina propria of the nasal mucosa occurred in most of the rats and mice exposed for 2 years to 50 ppm 2,3-butanedione and many of those exposed to 25 ppm. In most cases, fibrosis occurred along the lateral walls of the nasal cavities and the tips of the nasoturbinates, sites receiving high exposure to inhaled 2,3-butanedione vapors. Fibrosis of the nasal cavity has been reported in NTP chronic inhalation studies of other water-soluble, reactive chemicals such as chloroprene[125], furfuryl alcohol[126], ozone[127], vinylidene chloride[128], and tetrahydrofuran[129].

Chronic exposure to 50 ppm 2,3-butanedione also caused considerable submucosal fibrosis in the trachea of rats and mice. Mild to moderate tracheal fibrosis occurred in most of the male (94%) and female (88%) mice exposed to 50 ppm 2,3-butanedione for 2 years. The prevalence and severity of these tracheal lesions were considerably lower in rats [male rats (54%), female rats (38%)] than in mice. Tracheal fibrosis is uncommon in NTP inhalation studies and has only been reported for one other chemical. *o*-Phthalaldehyde inhalation caused minimal to mild tracheal fibrosis in male and female rats exposed for 3 months. Like, 2,3-butanedione, *o*-phthalaldehyde is water soluble, direct-acting, and highly reactive. Submucosal fibrosis in relatively large air passages such as the nasal cavity and trachea typically have minimal effects on pulmonary function. 2,3-Butanedione also caused minimal to mild interstitium fibrosis of alveolar septae in 11 male and nine female rats in the 50 ppm groups. In some animals the fibrosis formed nodular foci that expanded the alveolar walls and extended in tendrils into the surrounding alveolar septae.

2,3-Butanedione did not cause bronchiolitis obliterans in this 2-year study because the highest exposure concentration was limited to 50 ppm in an attempt to minimize adverse nasal effects over a 2-year period. However, the significant nasal and tracheal fibrosis and the foci of bronchial fibrosis in both species and foci of pulmonary fibrosis in the rats demonstrate the potent fibrogenicity of this chemical following inhalation exposure. Because rodents are nose-only breathers and absorb more of the 2,3-butanedione in the nose than do humans, the fibrosis that occurred in the nose and trachea of the rats and mice may be considered as a rodent surrogate for bronchiolitis obliterans in humans. Fibrosis of the trachea in the mice was particularly prominent in terms of the circumferential and intramural extent, the associated loss of cartilage in some tracheas, and the resulting reduction in size of the tracheal lumen. In addition, many of the male mice and a few of the female mice exhibited fibrosis of the tracheal carina, which is the distal end of the trachea at the point of bifurcation into the right and left mainstem bronchi. This was a unique lesion that was diagnosed separately to indicate that the fibrogenic effect of the chemical reached the distal end of the trachea. Because the carina is typically not represented in all mice and in very few rats, the true incidence of this carinal lesion in the study is probably underrepresented.

The fibrotic foci in the bronchi were identified in the large bronchi at bifurcation points, which are probably high impact areas receiving maximal airflow. The foci of pulmonary interstitial fibrosis in the rats suggest sensitivity of the alveolar epithelium to 2,3-butanedione. Pulmonary fibrosis has been seen previously in short-term studies at higher concentrations (e.g., 150 ppm) of this chemical[55].

Although the pathogenesis of bronchiolitis obliterans is unclear, airway epithelial injury appears to be a critical first step[130]. Considerable evidence suggests that immunological injury of the airway epithelium is a key factor in transplant-related bronchiolitis obliterans. 2,3-Butanedione-induced bronchiolitis obliterans may result from immunological reaction to 2,3-butanedione-arginine adducts on the airway epithelium membrane proteins[71]. Serum from rats and mice exposed to 0, 12.5, 25, or 50 ppm 2,3-butanedione in the current 2-year studies was evaluated for autoantibodies against nuclear antigens; however, none of the exposed groups had an incidence of ANA-positive responses that was significantly different from that of the chamber control group (data not presented). Inhalation exposure to 2,3-butanedione caused the most severe toxicity in the nasal cavity of rats and mice, with decreasing toxicity at more distal sites in the respiratory tract. Nasal toxicity often occurs in rodents during inhalation exposure to reactive chemicals because they are obligate nose breathers and the nasal cavity is exposed to the highest concentrations of inhaled toxicants. Nasal irritation was frequently reported by workers in NIOSH medical surveys at microwave popcorn plants and flavoring manufacturing plants[34]. 2,3-Butanedione and other water-soluble, reactive chemicals are rapidly scrubbed from the inhaled air passing over the moist nasal turbinates[131]. Once absorbed, 2,3-butanedione can readily penetrate the protective aqueous mucous layer and react with the nasal mucosa[36]. The nasal cavity is a common target site for rodents exposed by inhalation to many direct-acting, reactive chemicals such as chlorine[132], formaldehyde[133], ammonia[134], acetaldehyde[135], acrolein[136], diethylamine[137], and propargyl alcohol[138].

Although toxicity for the nasal cavity was expected, chronic exposure of rats to 2,3-butanedione also resulted in some evidence of carcinogenicity in the nasal cavity. Low incidences of squamous cell carcinoma of the nasal mucosa occurred in male (6%) and female (6%) rats exposed to 50 ppm 2,3-butanedione for 2 years. A squamous cell papilloma also occurred in one 50 ppm male rat and was considered a potential precursor lesion to squamous cell carcinoma. Although incidences of squamous cell carcinoma were not statistically significant, the combination of three squamous cell carcinomas and one squamous cell papilloma in 50 ppm male rats was statistically significant relative to concurrent chamber controls (0/50). Nasal neoplasms are rare in rodents and have not been observed in NTP historical controls (0/349 by all routes) for male Wistar Han rats. The spontaneous incidence of nasal neoplasms of all types is typically much less than 0.5% in Fischer 344[139; 140] and Wistar[141] rats.

Squamous metaplasia of the respiratory epithelium occurred in the nasal cavities of most rats exposed to 50 ppm 2,3-butanedione. The metaplastic squamous epithelium was frequently thickened, hyperkeratotic, and sometimes atypical. Squamous metaplasia is considered an adaptive change, because squamous epithelium is more resistant to injury than other epithelial types. Squamous metaplasia may be reversible with discontinuation of exposure, but with chronic exposure, squamous metaplasia may give rise to squamous cell papilloma or squamous cell carcinoma[121; 122]. Rats appear to be more susceptible than mice to squamous cell carcinoma caused by inhaled chemicals[140]. Squamous cell carcinomas have been reported in NTP inhalation studies for 10 chemicals. Of these 10 chemicals, eight caused squamous cell carcinomas in male rats, two in female rats, and only one in female mice.

The presence of adenocarcinoma in the nasal cavity of two (4%) female mice exposed to 50 ppm for 2 years may have been related to chronic 2,3-butanedione exposure. This incidence of adenocarcinoma was not statistically significant relative to concurrent chamber control mice and occurred only at the high concentration that did cause significant decreases in body weight gain and survival of mice. However, spontaneous adenocarcinoma of the nasal cavity is extremely rare and has not been observed in NTP historical controls for B6C3F1/N mice (0/548 by all routes). Treatment-related adenocarcinoma of the nose has only been reported in NTP inhalation studies of propylene oxide[142], 1,2-dibromoethane[143], and 1,2-dibromo-3-chloropropane[144]. Adenocarcinomas may arise from the respiratory epithelium of the anterior nasal cavity, the epithelium of the submucosal glands, or from a malignant change within an existing adenoma[122; 145-147]. Adenocarcinomas in the two 2,3-butanedione-exposed female mice in the current study appeared to arise in Level III, the olfactory portion of the nasal cavity. Exposure to 50 ppm 2,3-butanedione caused extensive respiratory metaplasia in the olfactory region of the nose in almost all mice, and this respiratory metaplasia of both the olfactory epithelium and the underlying Bowman’s glands may have been the background precursor lesion leading to the two adenocarcinomas.

Squamous cell carcinomas of the nasal cavity occurred in rats exposed to 50 ppm 2,3-butanedione, a concentration that caused significant respiratory epithelial hyperplasia, and squamous metaplasia. Repeated injury to the nasal mucosa caused by reactive inhaled toxicants may contribute to the induction of nasal tumors[148]. Formaldehyde, a known nasal carcinogen, causes squamous cell carcinoma in the nasal cavity of rats, but only at exposure concentrations that cause severe degeneration, hyperplasia, and squamous metaplasia in the nasal epithelium[149; 150]. Sustained cytotoxicity and cell proliferation in the nasal cavity caused by chronic 2,3-butanedione exposure may predispose to nasal carcinogenesis because cell division is required to convert DNA adducts to permanent mutations[133]. 2,3-Butanedione has been shown to form adducts with 2-deoxyguanosine and to disrupt DNA ternary structure[151]. In the current study 2,3-butanedione was shown to be mutagenic in bacterial assays. The related 1,2-dicarbonyl compounds, methylglyoxal and glyoxal, also form adducts with guanosine and are highly mutagenic[152-154].

Eye irritation was frequently reported in NIOSH medical surveys at microwave popcorn plants and flavoring manufacturing plants[34]. At one plant, several workers developed severe eye irritation and blurred vision after use of a new butter flavoring[155]. 2,3-Butanedione is a major volatile component of artificial butter flavorings and likely contributed to these ocular effects. In the current studies, chronic exposure to 2,3-butanedione vapors caused ocular lesions in rats and mice. Microscopic lesions included corneal inflammation, minimal to mild ulcer and hyperplasia of the corneal epithelium, and an increased incidence of cataracts. Inhalation studies of other contact irritant gases and vapors such as diethylamine[137], cumene[156], and propargyl alcohol[138] also reported eye irritation in rats and mice.

Conclusions

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity*[a] of 2,3-butanedione in male Wistar Han rats based on the combined incidences of squamous cell papilloma and squamous cell carcinoma of the nose. There was *some evidence of carcinogenic activity* of 2,3-butanedione in female Wistar Han rats based on the incidences of squamous cell carcinoma of the nose. There was *no evidence of carcinogenic activity* of 2,3-butanedione in male B6C3F1/N mice exposed to 12.5, 25, or 50 ppm. There was *equivocal evidence of carcinogenic activity* of 2,3-butanedione in female B6C3F1/N mice based on the occurrences of adenocarcinoma of the nose.

Exposure to 2,3-butanedione resulted in increased incidences of nonneoplastic lesions of the nose, larynx, trachea, lung, and eye in male and female rats and mice.

[a]See Explanation of Levels of Evidence of Carcinogenic Activity. See a summary of the Peer Review Panel comments and the public discussion on this Technical Report in Appendix K.

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Appendix A. Summary of Lesions in Male Rats in the Two-year Inhalation Study of 2,3-Butanedione

Tables

Table A-1. Summary of the Incidence of Neoplasms in Male Rats in the Two-year Inhalation Study of 2,3-Butanedione[a]

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths	–	–	–	–
Moribund	12	11	15	18
Natural deaths	2	2	2	10
Survivors	–	–	–	–
Died last week of study	–	1	–	–
Terminal euthanasia	36	36	33	22
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)
Carcinoma	–	–	–	1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Schwannoma malignant, metastatic, heart	–	1 (2%)	–	–
Intestine small, duodenum	(50)	(50)	(50)	(50)
Schwannoma malignant, metastatic, heart	–	1 (2%)	–	–
Intestine small, ileum	(50)	(50)	(50)	(50)
Carcinoid tumor benign	–	1 (2%)	–	–
Schwannoma malignant, metastatic, heart	–	1 (2%)	–	–
Intestine small, jejunum	(50)	(50)	(50)	(50)
Leiomyoma	1 (2%)	–	–	–
Liver	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)	–	–	–
Hepatocellular adenoma	–	1 (2%)	–	–
Schwannoma malignant, metastatic, heart	–	1 (2%)	–	–
Mesentery	(5)	(4)	(3)	(2)
Carcinosarcoma, metastatic, prostate	1 (20%)		–	–
Hemangiosarcoma	–	1 (25%)	–	–
Fat, liposarcoma, metastatic, kidney	–	1 (25%)	–	–
Oral mucosa	(1)	(1)	(0)	(0)
Squamous cell carcinoma	1 (100%)	1 (100%)		
Pancreas	(50)	(50)	(50)	(50)
Acinus, carcinosarcoma, metastatic, prostate	1 (2%)	–	–	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Salivary glands	(50)	(50)	(50)	(50)
Carcinoma	–	1 (2%)	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Sarcoma	–	–	1 (2%)	–
Squamous cell carcinoma	–	–	1 (2%)	–
Squamous cell papilloma	–	1 (2%)	1 (2%)	–
Stomach, glandular	(50)	(50)	(50)	(50)
Schwannoma malignant, metastatic, heart	–	1 (2%)	–	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Neural crest tumor, metastatic, ear	–	–	1 (2%)	–
Schwannoma malignant	–	2 (4%)	1 (2%)	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	1 (2%)	3 (6%)	1 (2%)	3 (6%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	–	1 (2%)	–	–
Parathyroid gland	(49)	(46)	(49)	(47)
Adenoma	1 (2%)	–	–	–
Bilateral, adenoma	–	1 (2%)	–	–
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	9 (18%)	14 (28%)	9 (18%)	5 (10%)
Pars intermedia, adenoma	2 (4%)	1 (2%)	–	1 (2%)
Pars nervosa, schwannoma malignant	–	–	–	1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, C-cell, adenoma	1 (2%)	–	–	–
C-cell, adenoma	5 (10%)	5 (10%)	3 (6%)	1 (2%)
C-cell, adenoma, multiple	–	–	1 (2%)	–
C-cell, carcinoma	–	1 (2%)	1 (2%)	–
Follicular cell, adenoma	2 (4%)	1 (2%)	2 (4%)	–
General Body System				
None	–	–	–	–
Genital System				
Epididymis	(50)	(50)	(50)	(50)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Adenoma	–	1 (2%)	–	–
Carcinosarcoma	1 (2%)	–	–	–
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma	3 (6%)	3 (6%)	–	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(6)	(4)	(9)	(7)
Lymph node, bronchial	(41)	(46)	(43)	(42)
Lymph node, mandibular	(48)	(44)	(49)	(48)
Squamous cell carcinoma, metastatic, skin	–	–	–	1 (2%)
Lymph node, mediastinal	(42)	(47)	(47)	(47)
Hemangiosarcoma	–	1 (2%)	–	–
Schwannoma malignant, metastatic, thymus	–	–	1 (2%)	–
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hemangioma	–	–	1 (2%)	1 (2%)
Hemangiosarcoma	2 (4%)	–	1 (2%)	–
Schwannoma malignant, metastatic, heart	–	1 (2%)	–	–
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	–	–	–
Thymus	(47)	(48)	(48)	(48)
Schwannoma malignant	–	–	1 (2%)	–
Thymoma benign	1 (2%)	–	1 (2%)	–
Integumentary System				–
Mammary gland	(12)	(9)	(14)	(22)
Carcinoma	1 (8%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Trichoepithelioma	–	1 (2%)	–	–
Epidermis, basal cell carcinoma	–	1 (2%)	–	–
Epidermis, keratoacanthoma	3 (6%)	2 (4%)	1 (2%)	–
Epidermis, pilomatrixoma	–	–	1 (2%)	–
Epidermis, squamous cell carcinoma	–	–	1 (2%)	1 (2%)
Epidermis, squamous cell papilloma	–	1 (2%)	–	–
Sebaceous gland, adenoma	1 (2%)	–	–	–
Subcutaneous tissue, fibrosarcoma	–	–	–	1 (2%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Subcutaneous tissue, fibrous histiocytoma	1 (2%)	1 (2%)	–	–
Subcutaneous tissue, hemangioma	–	–	1 (2%)	–
Subcutaneous tissue, myxoma	1 (2%)	–	–	–
Subcutaneous tissue, schwannoma malignant	–	–	–	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(6)	(3)	(4)	(6)
Hemangiosarcoma	–	–	1 (25%)	–
Squamous cell carcinoma, metastatic, oral mucosa	–	1 (33%)	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Glioma malignant	1 (2%)	1 (2%)	1 (2%)	–
Granular cell tumor benign	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Granular cell tumor malignant	1 (2%)	–	–	–
Squamous cell carcinoma, metastatic, oral mucosa	–	1 (2%)	–	–
Peripheral nerve	(6)	(1)	(3)	(6)
Spinal cord	(6)	(1)	(3)	(6)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(49)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	–	–	1 (2%)
Carcinosarcoma, metastatic, prostate	1 (2%)	–	–	–
Neural crest tumor, metastatic, ear	–	–	1 (2%)	–
Mediastinum, schwannoma malignant, metastatic, heart	–	1 (2%)	–	–
Nose	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	–	–	–
Squamous cell carcinoma	–	–	–	3 (6%)
Squamous cell papilloma	–	–	–	1 (2%)
Pleura	(0)	(1)	(0)	(0)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(0)	(0)	(1)	(0)
Neural crest tumor	–	–	1 (100%)	–
Eye	(50)	(50)	(49)	(49)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Schwannoma malignant, metastatic, Harderian gland	–	1 (2%)	–	–
Retina, melanoma malignant	–	1 (2%)	–	–
Harderian gland	(50)	(50)	(50)	(50)
Schwannoma malignant	–	1 (2%)	–	–
Lacrimal gland	(0)	(0)	(0)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Liposarcoma	–	1 (2%)	–	–
Ureter	(0)	(1)	(0)	(0)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)	–	–
Lymphoma malignant	3 (6%)	–	3 (6%)	1 (2%)
Mesothelioma malignant	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	35	36	30	22
Total primary neoplasms	53	57	39	27
Total animals with benign neoplasms	27	30	22	16
Total benign neoplasms	39	41	24	16
Total animals with malignant neoplasms	13	12	13	11
Total malignant neoplasms	14	16	13	11
Total animals with metastatic neoplasms	1	4	2	1
Total metastatic neoplasms	3	11	3	1
Total animals with uncertain neoplasms-benign or malignant	–	–	2	–
Total uncertain neoplasms	–	–	2	–

[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 2,3-Butanedione

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	1/50 (2%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
Adjusted rate[b]	2.3%	6.6%	2.3%	7.5%
Terminal rate[c]	0/36 (0%)	3/37 (8%)	1/33 (3%)	1/22 (5%)
First incidence (days)	724	729 (T)	729 (T)	628
Poly-3 test[d]	P = 0.266	P = 0.311	P = 0.754	P = 0.267
Brain: Benign Granular Cell Tumor				
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)	1/50 (2%)
Adjusted rate	6.8%	4.4%	4.6%	2.5%
Terminal rate	3/36 (8%)	0/37 (0%)	1/33 (3%)	0/22 (0%)
First incidence (days)	729 (T)	603	722	499
Poly-3 test	P = 0.267N	P = 0.486N	P = 0.514N	P = 0.343N
Nose: Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	7.5%
Terminal rate	0/36 (0%)	0/37 (0%)	0/33 (0%)	1/22 (5%)
First incidence (days)	–e	–	–	616
Poly-3 test	P = 0.010	–f	–	P = 0.101
Nose: Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	0.0%	9.9%
Terminal rate	0/36 (0%)	0/37 (0%)	0/33 (0%)	1/22 (5%)
First incidence (days)	–	–	–	589
Poly-3 test	P = 0.002	–	–	P = 0.049
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	9/50 (18%)	14/50 (28%)	9/50 (18%)	5/50 (10%)
Adjusted rate	19.4%	29.7%	19.3%	12.4%
Terminal rate	4/36 (11%)	7/37 (19%)	1/33 (3%)	2/22 (9%)
First incidence (days)	379	600	527	616
Poly-3 test	P = 0.141N	P = 0.179	P = 0.598N	P = 0.277N
Skin: Keratoacanthoma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	0/50 (0%)
Adjusted rate	6.8%	4.4%	2.3%	0.0%
Terminal rate	3/36 (8%)	2/37 (5%)	1/33 (3%)	0/22 (0%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
First incidence (days)	729 (T)	729 (T)	729 (T)	–
Poly-3 test	P = 0.073N	P = 0.492N	P = 0.316N	P = 0.141N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	3/50 (6%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	6.8%	6.6%	2.3%	0.0%
Terminal rate	3/36 (8%)	3/37 (8%)	1/33 (3%)	0/22 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	–
Poly-3 test	P = 0.065N	P = 0.655N	P = 0.316N	P = 0.141N
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	6.8%	6.6%	4.7%	2.5%
Terminal rate	3/36 (8%)	3/37 (8%)	2/33 (6%)	0/22 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	628
Poly-3 test	P = 0.231N	P = 0.655N	P = 0.514N	P = 0.346N
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Carcinoma, or Squamous Cell Carcinoma				
Overall rate	3/50 (6%)	5/50 (10%)	2/50 (4%)	1/50 (2%)
Adjusted rate	6.8%	11.1%	4.7%	2.5%
Terminal rate	3/36 (8%)	5/37 (14%)	2/33 (6%)	0/22 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	628
Poly-3 test	P = 0.173N	P = 0.365	P = 0.514N	P = 0.346N
Testes: Adenoma				
Overall rate	3/50 (6%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	6.7%	6.6%	0.0%	2.5%
Terminal rate	2/36 (6%)	3/37 (8%)	0/33 (0%)	1/22 (5%)
First incidence(days)	694	729 (T)	–	729 (T)
Poly-3 test	P = 0.152N	P = 0.656N	P = 0.125N	P = 0.350N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	6/50 (12%)	5/50 (10%)	4/50 (8%)	1/50 (2%)
Adjusted rate	13.4%	11.1%	9.3%	2.5%
Terminal rate	4/36 (11%)	5/37 (14%)	3/33 (9%)	1/22 (5%)
First incidence (days)	654	729 (T)	715	729 (T)
Poly-3 test	P = 0.059N	P = 0.495N	P = 0.394N	P = 0.079N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	6/50 (12%)	6/50 (12%)	5/50 (10%)	1/50 (2%)
Adjusted rate	13.4%	13.1%	11.6%	2.5%

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Terminal rate	4/36 (11%)	5/37 (14%)	4/33 (12%)	1/22 (5%)
First incidence (days)	654	586	715	729 (T)
Poly-3 test	P = 0.064N	P = 0.608N	P = 0.528N	P = 0.079N
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)	0/50 (0%)
Adjusted rate	6.8%	4.4%	4.7%	0.0%
Terminal rate	3/36 (8%)	2/37 (5%)	2/33 (6%)	0/22 (0%)
First incidence (days)	729 (T)	729 (T)	729 (T)	–
Poly-3 test	P = 0.106N	P = 0.492N	P = 0.514N	P = 0.141N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	4/50 (8%)	2/50 (4%)	4/50 (8%)	1/50 (2%)
Adjusted rate	9.0%	4.4%	9.3%	2.5%
Terminal rate	3/36 (8%)	2/37 (5%)	4/33 (12%)	0/22 (0%)
First incidence (days)	695	729 (T)	729 (T)	710
Poly-3 test	P = 0.232N	P = 0.331N	P = 0.625	P = 0.217N
All Organs: Malignant Lymphoma				
Overall rate	3/50 (6%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	6.6%	0.0%	6.8%	2.5%
Terminal rate	1/36 (3%)	0/37 (0%)	1/33 (3%)	0/22 (0%)
First incidence (days)	523	–	325	626
Poly-3 test	P = 0.412N	P = 0.121N	P = 0.650	P = 0.356N
All Organs: Benign Neoplasms				
Overall rate	27/50 (54%)	30/50 (60%)	22/50 (44%)	16/50 (32%)
Adjusted rate	56.7%	61.7%	47.0%	37.5%
Terminal rate	17/36 (47%)	19/37 (51%)	12/33 (36%)	7/22 (32%)
First incidence (days)	379	586	527	499
Poly-3 test	P = 0.017N	P = 0.384	P = 0.228N	P = 0.049N
All Organs: Malignant Neoplasms				
Overall rate	13/50 (26%)	12/50 (24%)	13/50 (26%)	11/50 (22%)
Adjusted rate	27.7%	25.9%	28.8%	25.8%
Terminal rate	8/36 (22%)	8/37 (22%)	8/33 (24%)	2/22 (9%)
First incidence (days)	432	586	325	549
Poly-3 test	P = 0.494N	P = 0.514N	P = 0.546	P = 0.515N
All Organs: Benign or Malignant Neoplasms				
Overall rate	35/50 (70%)	36/50 (72%)	30/50 (60%)	22/50 (44%)
Adjusted rate	70.0%	73.5%	61.3%	49.3%

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Terminal rate	21/36 (58%)	24/37 (65%)	16/33 (49%)	8/22 (36%)
First incidence (days)	379	586	325	499
Poly-3 test	P = 0.010N	P = 0.438	P = 0.242N	P = 0.028N

T = Terminal euthanasia
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, brain, nose, pituitary gland, testes, 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 euthanasia.
[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 euthanasia. 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 2,3-Butanedione[a]

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	12	11	15	18
Natural deaths	2	2	2	10
Survivors				
Died last week of study	–	1	–	–
Terminal euthanasia	36	36	33	22
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Metaplasia, squamous	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
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%)	2 (4%)	6 (12%)	1 (2%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Basophilic focus	12 (24%)	16 (32%)	18 (36%)	4 (8%)
Clear cell focus	27 (54%)	21 (42%)	27 (54%)	12 (24%)
Degeneration, cystic	1 (2%)	–	2 (4%)	–
Eosinophilic focus	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Fatty change, focal	2 (4%)	–	1 (2%)	–
Fatty change, diffuse	1 (2%)	–	–	–
Hematopoietic cell proliferation	2 (4%)	–	1 (2%)	1 (2%)
Hepatodiaphragmatic nodule	1 (2%)	–	3 (6%)	3 (6%)
Infiltration cellular, lymphocyte	1 (2%)	–	–	–
Infiltration cellular, mixed cell	5 (10%)	–	5 (10%)	4 (8%)
Mixed cell focus	2 (4%)	4 (8%)	1 (2%)	–
Necrosis, focal	1 (2%)	1 (2%)	1 (2%)	–
Pigmentation, hemosiderin	–	1 (2%)	1 (2%)	–
Tension lipidosis	–	–	1 (2%)	–
Bile duct, cyst	–	2 (4%)	–	1 (2%)
Bile duct, dilatation	–	–	–	1 (2%)
Bile duct, hyperplasia	5 (10%)	3 (6%)	4 (8%)	1 (2%)
Centrilobular, necrosis	1 (2%)	–	–	–
Serosa, fibrosis	–	1 (2%)	–	–
Mesentery	(5)	(4)	(3)	(2)
Fat, necrosis	3 (60%)	2 (50%)	2 (67%)	2 (100%)
Oral mucosa	(1)	(1)	(0)	(0)
Pancreas	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	7 (14%)	2 (4%)	4 (8%)
Basophilic focus	–	1 (2%)	–	–
Inflammation	–	–	–	1 (2%)
Acinus, hyperplasia	1 (2%)	1 (2%)	–	–
Salivary glands	(50)	(50)	(50)	(50)
Basophilic focus	–	–	–	1 (2%)
Inflammation	–	1 (2%)	1 (2%)	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	–	4 (8%)	–
Ulcer	–	–	1 (2%)	–
Epithelium, hyperplasia	1 (2%)	3 (6%)	4 (8%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	–	–	2 (4%)
Mineralization	–	1 (2%)	1 (2%)	–
Aorta, mineralization	–	1 (2%)	–	–
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	31 (62%)	38 (76%)	28 (56%)	25 (50%)
Inflammation, suppurative	–	–	1 (2%)	–
Epicardium, fibrosis, focal	–	–	–	1 (2%)
Septum interventricular, necrosis, acute	1 (2%)	–	–	–
Valve, degeneration	–	1 (2%)	–	–
Valve, inflammation, chronic	–	1 (2%)	–	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Degeneration, cystic	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Hematopoietic cell proliferation	–	–	2 (4%)	–
Infiltration cellular, lipocyte	–	–	–	1 (2%)
Bilateral, necrosis, multifocal	–	–	–	1 (2%)
Zona fasciculata, hyperplasia	2 (4%)	–	–	–
Zona fasciculata, hyperplasia, focal	4 (8%)	4 (8%)	3 (6%)	6 (12%)
Zona fasciculata, hyperplasia, multifocal	–	2 (4%)	1 (2%)	–
Zona fasciculata, hypertrophy	3 (6%)	2 (4%)	–	–
Zona fasciculata, hypertrophy, focal	12 (24%)	12 (24%)	11 (22%)	6 (12%)
Zona fasciculata, hypertrophy, multifocal	14 (28%)	9 (18%)	18 (36%)	11 (22%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	2 (4%)	–	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	5 (10%)	1 (2%)	5 (10%)	2 (4%)
Parathyroid gland	(49)	(46)	(49)	(47)
Hyperplasia, focal	1 (2%)	1 (2%)	1 (2%)	–
Pituitary gland	(50)	(50)	(50)	(50)
Fibrosis	–	–	–	1 (2%)
Pars distalis, hyperplasia	16 (32%)	18 (36%)	23 (46%)	10 (20%)
Pars intermedia, hyperplasia	1 (2%)	3 (6%)	1 (2%)	–
Thyroid gland	(50)	(50)	(50)	(50)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
C-cell, hyperplasia	8 (16%)	13 (26%)	8 (16%)	2 (4%)
Follicular cell, hyperplasia	4 (8%)	2 (4%)	1 (2%)	1 (2%)
Follicular cell, hypertrophy	2 (4%)	2 (4%)	5 (10%)	5 (10%)
General Body System				
None	–	–	–	–
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)	1 (2%)	–	–
Inflammation	–	–	–	1 (2%)
Preputial gland	(50)	(50)	(50)	(50)
Inflammation	–	1 (2%)	–	–
Prostate	(50)	(50)	(50)	(50)
Inflammation	21 (42%)	18 (36%)	18 (36%)	12 (24%)
Epithelium, ventral, hyperplasia	9 (18%)	9 (18%)	7 (14%)	10 (20%)
Seminal vesicle	(50)	(50)	(50)	(50)
Hyperplasia	–	1 (2%)	–	–
Inflammation	–	1 (2%)	–	–
Testes	(50)	(50)	(50)	(50)
Edema	4 (8%)	2 (4%)	–	–
Germinal epithelium, degeneration	5 (10%)	10 (20%)	4 (8%)	4 (8%)
Interstitial cell, hyperplasia	4 (8%)	1 (2%)	3 (6%)	1 (2%)
Rete testes, inflammation, granulomatous	–	–	1 (2%)	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Myeloid cell, hyperplasia	15 (30%)	12 (24%)	16 (32%)	32 (64%)
Lymph node	(6)	(4)	(9)	(7)
Axillary, infiltration cellular, plasma cell	1 (17%)	–	1 (11%)	–
Deep cervical, infiltration cellular, plasma cell	–	–	–	1 (14%)
Iliac, ectasia	–	1 (25%)	–	–
Iliac, infiltration cellular, plasma cell	–	–	–	2 (29%)
Inguinal, infiltration cellular, plasma cell	1 (17%)	–	–	–
Lumbar, ectasia	–	–	1 (11%)	–
Lumbar, hyperplasia, lymphoid	1 (17%)	–	–	–
Lumbar, infiltration cellular, plasma cell	5 (83%)	3 (75%)	6 (67%)	3 (43%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Pancreatic, inflammation	–	–	1 (11%)	–
Popliteal, hyperplasia, lymphoid	1 (17%)	–	2 (22%)	–
Popliteal, infiltration cellular, plasma cell	–	–	4 (44%)	2 (29%)
Popliteal, inflammation, chronic active	1 (17%)	–	–	–
Renal, ectasia	–	–	–	1 (14%)
Renal, infiltration cellular, plasma cell	2 (33%)	1 (25%)	1 (11%)	4 (57%)
Lymph node, bronchial	(41)	(46)	(43)	(42)
Atrophy	–	1 (2%)	2 (5%)	1 (2%)
Inflammation, suppurative	–	–	–	1 (2%)
Lymph node, mandibular	(48)	(44)	(49)	(48)
Ectasia	–	–	–	1 (2%)
Hemorrhage	–	–	1 (2%)	–
Hyperplasia, lymphoid	–	–	–	1 (2%)
Infiltration cellular, plasma cell	–	–	–	5 (10%)
Lymph node, mediastinal	(42)	(47)	(47)	(47)
Atrophy	1 (2%)	–	–	1 (2%)
Ectasia	1 (2%)	–	–	–
Infiltration cellular, plasma cell	–	–	1 (2%)	–
Inflammation	–	–	–	1 (2%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia	–	1 (2%)	–	–
Hyperplasia, lymphoid	–	1 (2%)	–	–
Inflammation	–	1 (2%)	–	–
Necrosis	1 (2%)	–	–	–
Spleen	(50)	(50)	(50)	(50)
Congestion	1 (2%)	–	–	–
Cyst	–	–	1 (2%)	–
Hematopoietic cell proliferation	41 (82%)	41 (82%)	36 (72%)	37 (74%)
Inflammation	–	–	1 (2%)	–
Pigmentation, hemosiderin	18 (36%)	22 (44%)	20 (40%)	20 (40%)
Lymphoid follicle, atrophy	6 (12%)	5 (10%)	4 (8%)	12 (24%)
Thymus	(47)	(48)	(48)	(48)
Atrophy	46 (98%)	47 (98%)	45 (94%)	47 (98%)
Hemorrhage	1 (2%)	–	–	–
Hyperplasia, lymphoid	1 (2%)	2 (4%)	1 (2%)	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Integumentary System				
Mammary gland	(12)	(9)	(14)	(22)
Galactocele	–	–	1 (7%)	1 (5%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	–	–	–	2 (4%)
Dorsal, inflammation, chronic active	–	–	1 (2%)	–
Dorsal, ulcer, focal	–	–	1 (2%)	–
Epidermis, cyst	–	–	1 (2%)	–
Epidermis, hyperplasia	2 (4%)	–	–	–
Foot, inflammation, acute	3 (6%)	–	–	1 (2%)
Foot, inflammation, chronic active	11 (22%)	7 (14%)	17 (34%)	23 (46%)
Foot, ulcer	14 (28%)	7 (14%)	17 (34%)	22 (44%)
Inguinal, inflammation, granulomatous	–	–	1 (2%)	–
Inguinal, ulcer, focal	–	–	1 (2%)	–
Prepuce, inflammation, acute	1 (2%)	–	–	–
Prepuce, ulcer, focal	1 (2%)	–	–	–
Tail, cyst epithelial inclusion	–	–	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis	–	–	1 (2%)	–
Osteosclerosis	–	–	–	1 (2%)
Skeletal muscle	(6)	(3)	(4)	(6)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Cerebrum, gray matter, hemorrhage, focal	1 (2%)	–	–	1 (2%)
Cranial nerve, glial cell, hyperplasia	1 (2%)	1 (2%)	–	–
Peripheral nerve	(6)	(1)	(3)	(6)
Axon, degeneration	–	–	–	1 (17%)
Spinal cord	(6)	(1)	(3)	(6)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Foreign body	2 (4%)	–	–	–
Inflammation, chronic active	14 (28%)	7 (14%)	7 (14%)	33 (66%)
Respiratory epithelium, metaplasia, squamous	–	1 (2%)	–	45 (90%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Squamous epithelium, hyperplasia	2 (4%)	2 (4%)	8 (16%)	46 (92%)
Squamous epithelium, ulcer, focal	–	–	–	15 (30%)
Squamous epithelium, ulcer, multifocal	–	–	–	3 (6%)
Lung	(50)	(49)	(50)	(50)
Inflammation, suppurative	–	–	1 (2%)	15 (30%)
Inflammation, granulomatous	4 (8%)	3 (6%)	1 (2%)	4 (8%)
Inflammation, chronic active	1 (2%)	2 (4%)	–	–
Metaplasia, osseous	–	1 (2%)	–	–
Thrombus	1 (2%)	–	–	–
Alveolar epithelium, hyperplasia	1 (2%)	4 (8%)	2 (4%)	8 (16%)
Alveolar epithelium, hyperplasia, focal	3 (6%)	3 (6%)	5 (10%)	1 (2%)
Alveolus, edema	–	–	–	1 (2%)
Alveolus, infiltration cellular, histiocyte	10 (20%)	14 (29%)	16 (32%)	34 (68%)
Bronchiole, epithelium, hyperplasia	–	–	–	33 (66%)
Bronchus, epithelium, atrophy	–	–	1 (2%)	23 (46%)
Bronchus, epithelium, hyperplasia	–	–	2 (4%)	47 (94%)
Bronchus, epithelium, metaplasia, squamous	–	–	–	2 (4%)
Bronchus, epithelium, regeneration	–	–	4 (8%)	9 (18%)
Bronchus, submucosa, fibrosis	–	–	–	5 (10%)
Interstitium, fibrosis		1 (2%)	1 (2%)	11 (22%)
Peribronchial, inflammation, chronic active	–	–	–	13 (26%)
Pleura, fibrosis	–	–	–	2 (4%)
Pleura, fibrosis, focal	1 (2%)	–	–	–
Nose	(50)	(50)	(50)	(50)
Foreign body	1 (2%)	1 (2%)	2 (4%)	–
Inflammation, suppurative	3 (6%)	4 (8%)	35 (70%)	50 (100%)
Inflammation, chronic active	1 (2%)	–	2 (4%)	–
Polyp, inflammatory	1 (2%)	–	–	–
Lamina propria, fibrosis	–	–	28 (56%)	38 (76%)
Nerve, hyperplasia	–	1 (2%)		
Olfactory epithelium, accumulation, hyaline droplet	10 (20%)	6 (12%)	4 (8%)	4 (8%)
Olfactory epithelium, atrophy	–	5 (10%)	27 (54%)	22 (44%)
Olfactory epithelium, metaplasia, respiratory	1 (2%)	3 (6%)	6 (12%)	50 (100%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Olfactory epithelium, necrosis	–	–	–	6 (12%)
Respiratory epithelium, hyperplasia	–	2 (4%)	5 (10%)	50 (100%)
Respiratory epithelium, metaplasia, squamous	–	–	5 (10%)	34 (68%)
Respiratory epithelium, necrosis	–	–	–	2 (4%)
Turbinate, atrophy	–	–	1 (2%)	1 (2%)
Turbinate, hyperostosis	–	–	–	10 (20%)
Pleura	(0)	(1)	(0)	(0)
Trachea	(50)	(50)	(50)	(50)
Inflammation, suppurative	–	–	–	1 (2%)
Inflammation, chronic active	–	–	1 (2%)	8 (16%)
Epithelium, atrophy	–	–	–	7 (14%)
Epithelium, hyperplasia	–	–	1 (2%)	32 (64%)
Epithelium, metaplasia, squamous	–	–	–	12 (24%)
Epithelium, necrosis	–	–	–	6 (12%)
Epithelium, regeneration	–	–	5 (10%)	12 (24%)
Submucosa, fibrosis	–	–	–	27 (54%)
Special Senses System				
Ear	(0)	(0)	(1)	(0)
Eye	(50)	(50)	(49)	(49)
Anterior chamber, inflammation, suppurative	–	1 (2%)	6 (12%)	3 (6%)
Bilateral, phthisis bulbi	–	–	1 (2%)	–
Cornea, inflammation, chronic active	1 (2%)	6 (12%)	16 (33%)	28 (57%)
Cornea, mineralization	1 (2%)	4 (8%)	1 (2%)	–
Cornea, necrosis	–	–	–	2 (4%)
Cornea, epithelium, hyperplasia	–	2 (4%)	3 (6%)	6 (12%)
Cornea, epithelium, ulcer	–	1 (2%)	4 (8%)	6 (12%)
Iris, inflammation, acute	–	1 (2%)	3 (6%)	1 (2%)
Lens, cataract	1 (2%)	5 (10%)	6 (12%)	3 (6%)
Retina, degeneration	8 (16%)	11 (22%)	11 (22%)	6 (12%)
Unilateral, inflammation, pyogranulomatous	–	–	–	1 (2%)
Unilateral, phthisis bulbi	–	1 (2%)	3 (6%)	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	–	1 (2%)	3 (6%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Metaplasia	1 (2%)	–	–	–
Lacrimal gland	(0)	(0)	(0)	(1)
Metaplasia	–	–	–	1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Calculus microscopic observation only	4 (8%)	10 (20%)	2 (4%)	7 (14%)
Fibrosis, focal	–	–	1 (2%)	–
Infarct	1 (2%)	1 (2%)	1 (2%)	–
Infiltration cellular, lymphoid	–	–	1 (2%)	–
Nephropathy	33 (66%)	40 (80%)	41 (82%)	30 (60%)
Thrombosis	–	–	1 (2%)	–
Cortex, cyst	–	–	2 (4%)	1 (2%)
Papilla, necrosis	1 (2%)	–	–	–
Pelvis, dilatation	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Pelvis, inflammation	10 (20%)	11 (22%)	8 (16%)	14 (28%)
Pelvis, transitional epithelium, hyperplasia	1 (2%)	1 (2%)	–	–
Renal tubule, dilatation	1 (2%)	–	–	–
Ureter	(0)	(1)	(0)	(0)
Inflammation	–	1 (100%)	–	–
Urinary bladder	(50)	(50)	(50)	(50)
Calculus gross observation	–	1 (2%)	–	–
Hemorrhage	–	–	1 (2%)	–
Inflammation	–	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 2,3-Butanedione

Tables

Table B-1. Summary of the Incidence of Neoplasms in Female Rats in the Two-year Inhalation Study of 2,3-Butanedione[a]

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	15	18	26	15
Natural deaths	1	1	–	4
Survivors				
Died last week of study	–	–	–	1
Terminal euthanasia	34	31	24	30
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	1 (2%)	–	–
Intestine small, jejunum	(50)	(50)	(50)	(50)
Leiomyosarcoma	–	1 (2%)	–	–
Liver	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	2 (4%)	–	1 (2%)
Cholangioma	3 (6%)	1 (2%)	–	–
Hepatocellular adenoma	–	1 (2%)	1 (2%)	–
Schwannoma malignant, metastatic, uterus	–	–	1 (2%)	–
Mesentery	(8)	(10)	(4)	(4)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (25%)
Cystadenocarcinoma, metastatic, ovary	–	1 (10%)	–	–
Fat, schwannoma malignant, metastatic, uterus	–	1 (10%)	–	–
Pancreas	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	1 (2%)	–	–
Schwannoma malignant, metastatic, uterus	–	–	–	1 (2%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Acinus, adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma, multiple	1 (2%)	–	–	–
Stomach, glandular	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	1 (2%)	–	1 (2%)
Adenoma	1 (2%)	–	1 (2%)	1 (2%)
Schwannoma malignant, metastatic, uterus	–	–	1 (2%)	–
Adrenal medulla	(50)	(50)	(49)	(50)
Pheochromocytoma benign	3 (6%)	–	–	–
Pheochromocytoma malignant	–	–	–	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Parathyroid gland	(46)	(44)	(46)	(42)
Adenoma	–	–	1 (2%)	–
Pituitary gland	(50)	(50)	(50)	(50)
Schwannoma malignant, metastatic, Harderian gland	–	1 (2%)	–	–
Pars distalis, adenoma	26 (52%)	24 (48%)	24 (48%)	23 (46%)
Pars intermedia, adenoma	1 (2%)	–	–	–
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	2 (4%)	4 (8%)	3 (6%)	3 (6%)
C-cell, carcinoma	1 (2%)	–	–	–
Follicular cell, adenoma	2 (4%)	4 (8%)	1 (2%)	–
Follicular cell, carcinoma	–	1 (2%)	–	–
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(49)	(50)	(50)	(49)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Ovary	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Cystadenocarcinoma	–	1 (2%)	–	–
Cystadenoma	–	1 (2%)	–	–
Granulosa cell tumor malignant	–	1 (2%)	2 (4%)	–
Granulosa-theca tumor malignant	1 (2%)	–	–	1 (2%)
Sertoli cell tumor benign	–	–	–	2 (4%)
Thecoma benign	–	–	–	1 (2%)
Germinal epithelium, tubulostromal adenoma	1 (2%)	–	–	–
Uterus	(50)	(50)	(50)	(50)
Adenocarcinoma	2 (4%)	4 (8%)	2 (4%)	3 (6%)
Adenoma	–	–	–	2 (4%)
Hemangioma	–	–	–	1 (2%)
Leiomyoma	–	1 (2%)	–	–
Polyp stromal	9 (18%)	10 (20%)	7 (14%)	13 (26%)
Polyp stromal, multiple	–	3 (6%)	–	–
Sarcoma stromal	–	–	3 (6%)	–
Schwannoma malignant	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Cervix, granular cell tumor benign	–	3 (6%)	–	–
Cervix, hemangioma	–	–	1 (2%)	–
Cervix, sarcoma stromal	1 (2%)	–	–	–
Cervix, schwannoma malignant	–	–	1 (2%)	–
Cervix, squamous cell carcinoma	–	–	1 (2%)	–
Serosa, cystadenocarcinoma, metastatic, ovary	–	1 (2%)	–	–
Vagina	(0)	(1)	(2)	(0)
Granular cell tumor benign	–	1 (100%)	–	–
Polyp	–	–	1 (50%)	–
Schwannoma malignant, metastatic, uterus	–	–	1 (50%)	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(1)	(3)	(2)	(5)
Lumbar, adenocarcinoma, metastatic, uterus	–	1 (33%)	–	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Renal, adenocarcinoma, metastatic, uterus	–	2 (67%)	–	–
Lymph node, bronchial	(43)	(44)	(43)	(42)
Lymph node, mandibular	(46)	(48)	(45)	(48)
Hemangioma	1 (2%)	–	–	–
Lymph node, mediastinal	(44)	(47)	(48)	(50)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)	–	–	–
Schwannoma malignant, metastatic, uterus	–	1 (2%)	1 (2%)	–
Spleen	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Thymus	(49)	(50)	(49)	(50)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	4 (8%)	3 (6%)	2 (4%)	1 (2%)
Adenoma, multiple	1 (2%)	–	–	–
Carcinoma	2 (4%)	4 (8%)	1 (2%)	8 (16%)
Carcinoma, multiple	2 (4%)	–	–	1 (2%)
Fibroadenoma	12 (24%)	10 (20%)	9 (18%)	8 (16%)
Fibroadenoma, multiple	1 (2%)	3 (6%)	3 (6%)	3 (6%)
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, adenocarcinoma, metastatic, uterus	–	1 (2%)	–	–
Subcutaneous tissue, hibernoma	–	–	–	2 (4%)
Subcutaneous tissue, lipoma	–	1 (2%)	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(3)	(7)	(5)	(5)
Adenocarcinoma, metastatic, uterus	–	2 (29%)	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Granular cell tumor benign	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Oligodendroglioma benign	1 (2%)	–	–	1 (2%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Schwannoma malignant, metastatic, Harderian gland	–	1 (2%)	–	–
Peripheral nerve	(3)	(5)	(5)	(5)
Trigeminal, schwannoma malignant, metastatic, Harderian gland	–	1 (20%)	–	–
Spinal cord	(3)	(5)	(5)	(5)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	–	1 (2%)	–	–
Alveolar/bronchiolar adenoma	–	–	–	1 (2%)
Schwannoma malignant, metastatic, uterus	–	–	–	1 (2%)
Mediastinum, adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Nose	(50)	(50)	(50)	(50)
Squamous cell carcinoma	–	–	–	3 (6%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Iris, melanoma malignant	–	–	1 (2%)	–
Optic nerve, schwannoma malignant, metastatic, Harderian gland	–	1 (2%)	–	–
Harderian gland	(50)	(50)	(50)	(50)
Schwannoma malignant	–	1 (2%)	–	–
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Urinary bladder	(50)	(50)	(50)	(50)
Schwannoma malignant, metastatic, uterus	–	–	1 (2%)	1 (2%)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	–	1 (2%)	–	–
Lymphoma malignant	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	40	45	44	40
Total primary neoplasms	83	89	73	83

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Total animals with benign neoplasms	39	42	38	37
Total benign neoplasms	70	72	56	63
Total animals with malignant neoplasms	12	14	17	19
Total malignant neoplasms	13	17	17	20
Total animals with metastatic neoplasms	–	6	3	2
Total metastatic neoplasms	–	20	5	12

[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 2,3-Butanedione

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	3/50 (6%)	0/50 (0%)	0/49 (0%)	0/50 (0%)
Adjusted rate[b]	6.8%	0.0%	0.0%	0.0%
Terminal rate[c]	2/34 (6%)	0/31 (0%)	0/23 (0%)	0/30 (0%)
First incidence (days)	599	–[e]	–	–
Poly-3 test[d]	P = 0.044N	P = 0.123N	P = 0.141N	P = 0.121N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	3/50 (6%)	0/50 (0%)	0/49 (0%)	1/50 (2%)
Adjusted rate	6.8%	0.0%	0.0%	2.3%
Terminal rate	2/34 (6%)	0/31 (0%)	0/23 (0%)	0/30 (0%)
First incidence (days)	599	–	–	711
Poly-3 test	P = 0.244N	P = 0.123N	P = 0.141N	P = 0.309N
Liver: Cholangioma				
Overall rate	3/50 (6%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Adjusted rate	6.8%	2.3%	0.0%	0.0%
Terminal rate	2/34 (6%)	1/31 (3%)	0/24 (0%)	0/30 (0%)
First incidence (days)	614	731 (T)	–	–
Poly-3 test	P = 0.043N	P = 0.313N	P = 0.136N	P = 0.120N
Mammary Gland: Fibroadenoma				
Overall rate	13/50 (26%)	13/50 (26%)	12/50 (24%)	11/50 (22%)
Adjusted rate	28.7%	30.0%	29.3%	24.5%
Terminal rate	8/34 (24%)	12/31 (39%)	7/24 (29%)	5/30 (17%)
First incidence (days)	561	630	442	544
Poly-3 test	P = 0.341N	P = 0.542	P = 0.571	P = 0.414N

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Mammary Gland: Adenoma				
Overall rate	5/50 (10%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	11.2%	6.9%	5.0%	2.3%
Terminal rate	2/34 (6%)	2/31 (7%)	2/24 (8%)	0/30 (0%)
First incidence (days)	614	687	731 (T)	544
Poly-3 test	P = 0.065N	P = 0.373N	P = 0.261N	P = 0.103N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	15/50 (30%)	15/50 (30%)	14/50 (28%)	11/50 (22%)
Adjusted rate	32.6%	34.4%	34.2%	24.5%
Terminal rate	8/34 (24%)	13/31 (42%)	9/24 (38%)	5/30 (17%)
First incidence (days)	561	630	442	544
Poly-3 test	P = 0.202N	P = 0.515	P = 0.526	P = 0.267N
Mammary Gland: Carcinoma				
Overall rate	4/50 (8%)	4/50 (8%)	1/50 (2%)	9/50 (18%)
Adjusted rate	9.0%	9.1%	2.5%	19.8%
Terminal rate	2/34 (6%)	3/31 (10%)	1/24 (4%)	4/30 (13%)
First incidence (days)	599	418	731 (T)	491
Poly-3 test	P = 0.053	P = 0.636	P = 0.212N	P = 0.123
Mammary Gland: Adenoma or Carcinoma				
Overall rate	8/50 (16%)	7/50 (14%)	3/50 (6%)	10/50 (20%)
Adjusted rate	17.8%	15.9%	7.5%	21.7%
Terminal rate	4/34 (12%)	5/31 (16%)	3/24 (13%)	4/30 (13%)
First incidence (days)	599	418	731 (T)	491
Poly-3 test	P = 0.344	P = 0.519N	P = 0.138N	P = 0.417
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	15/50 (30%)	17/50 (34%)	15/50 (30%)	18/50 (36%)
Adjusted rate	32.6%	38.3%	36.7%	38.7%
Terminal rate	8/34 (24%)	14/31 (45%)	10/24 (42%)	9/30 (30%)
First incidence (days)	561	418	442	491
Poly-3 test	P = 0.337	P = 0.362	P = 0.431	P = 0.343
Nose: Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	6.9%
Terminal rate	0/34 (0%)	0/31 (0%)	0/24 (0%)	2/30 (7%)
First incidence (days)	–	–	–	724
Poly-3 test	P = 0.011	–[f]	–	P = 0.118

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	26/50 (52%)	24/50 (48%)	24/50 (48%)	23/50 (46%)
Adjusted rate	53.2%	50.7%	51.6%	49.0%
Terminal rate	14/34 (41%)	9/31 (29%)	9/24 (38%)	10/30 (33%)
First incidence (days)	253	418	361	537
Poly-3 test	P = 0.389N	P = 0.485N	P = 0.517N	P = 0.418N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	2/50 (4%)	4/50 (8%)	3/50 (6%)	3/50 (6%)
Adjusted rate	4.6%	9.3%	7.5%	6.9%
Terminal rate	2/34 (6%)	3/31 (10%)	1/24 (4%)	3/30 (10%)
First incidence (days)	731 (T)	687	723	731 (T)
Poly-3 test	P = 0.493	P = 0.333	P = 0.462	P = 0.499
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	4/50 (8%)	3/50 (6%)	3/50 (6%)
Adjusted rate	6.9%	9.3%	7.5%	6.9%
Terminal rate	3/34 (9%)	3/31 (10%)	1/24 (4%)	3/30 (10%)
First incidence (days)	731 (T)	687	723	731 (T)
Poly-3 test	P = 0.527N	P = 0.495	P = 0.624	P = 0.662
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	2/50 (4%)	4/50 (8%)	1/50 (2%)	0/50 (0%)
Adjusted rate	4.6%	9.3%	2.5%	0.0%
Terminal rate	1/34 (3%)	4/31 (13%)	1/24 (4%)	0/30 (0%)
First incidence (days)	614	731 (T)	731 (T)	–
Poly-3 test	P = 0.092N	P = 0.327	P = 0.533N	P = 0.239N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	5/50 (10%)	1/50 (2%)	0/50 (0%)
Adjusted rate	4.6%	11.6%	2.5%	0.0%
Terminal rate	1/34 (3%)	5/31 (16%)	1/24 (4%)	0/30 (0%)
First incidence (days)	614	731 (T)	731 (T)	–
Poly-3 test	P = 0.077N	P = 0.206	P = 0.533N	P = 0.239N
Uterus: Granular Cell Tumor Benign				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	0.0%	6.9%	0.0%	0.0%
Terminal rate	0/34 (0%)	2/31 (7%)	0/24 (0%)	0/30 (0%)
First incidence (days)	–	630	–	–
Poly-3 test	P = 0.328N	P = 0.118	–	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Uterus: Stromal Polyp				
Overall rate	9/50 (18%)	13/50 (26%)	7/50 (14%)	13/50 (26%)
Adjusted rate	20.3%	28.3%	17.3%	29.1%
Terminal rate	6/34 (18%)	6/31 (19%)	6/24 (25%)	9/30 (30%)
First incidence (days)	614	409	589	491
Poly-3 test	P = 0.277	P = 0.259	P = 0.471N	P = 0.236
Uterus: Stromal Sarcoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	2.3%	0.0%	7.3%	0.0%
Terminal rate	0/34 (0%)	0/31 (0%)	1/24 (4%)	0/30 (0%)
First incidence (days)	579	–	516	–
Poly-3 test	P = 0.495N	P = 0.505N	P = 0.280	P = 0.503N
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	10/50 (20%)	13/50 (26%)	9/50 (18%)	13/50 (26%)
Adjusted rate	22.3%	28.3%	21.7%	29.1%
Terminal rate	6/34 (18%)	6/31 (19%)	6/24 (25%)	9/30 (30%)
First incidence (days)	579	409	516	491
Poly-3 test	P = 0.328	P = 0.336	P = 0.577N	P = 0.310
Uterus: Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	2/50 (4%)	3/50 (6%)
Adjusted rate	4.6%	9.3%	5.0%	6.9%
Terminal rate	2/34 (6%)	2/31 (7%)	2/24 (8%)	1/30 (3%)
First incidence (days)	731 (T)	687	731 (T)	687
Poly-3 test	P = 0.515	P = 0.333	P = 0.663	P = 0.502
Uterus: Malignant Schwannoma				
Overall rate	1/50 (2%)	2/50 (4%)	4/50 (8%)	2/50 (4%)
Adjusted rate	2.3%	4.6%	9.7%	4.6%
Terminal rate	0/34 (0%)	0/31 (0%)	0/24 (0%)	1/30 (3%)
First incidence (days)	635	418	528	633
Poly-3 test	P = 0.386	P = 0.500	P = 0.160	P = 0.499
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
Adjusted rate	6.9%	0.0%	2.5%	2.3%
Terminal rate	3/34 (9%)	0/31 (0%)	1/24 (4%)	1/30 (3%)
First incidence (days)	731 (T)	–	731 (T)	731 (T)
Poly-3 test	P = 0.291N	P = 0.121N	P = 0.336N	P = 0.306N

	Chamber Control	12.5 ppm	25 ppm	50 ppm
All Organs: Malignant Lymphoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.3%	2.3%	7.4%	2.3%
Terminal rate	0/34 (0%)	0/31 (0%)	0/24 (0%)	0/30 (0%)
First incidence (days)	710	715	614	435
Poly-3 test	P = 0.570	P = 0.758	P = 0.281	P = 0.758N
All Organs: Benign Neoplasms				
Overall rate	39/50 (78%)	42/50 (84%)	38/50 (76%)	37/50 (74%)
Adjusted rate	78.0%	86.6%	79.3%	77.4%
Terminal rate	23/34 (68%)	25/31 (81%)	17/24 (71%)	22/30 (73%)
First incidence (days)	253	409	361	491
Poly-3 test	P = 0.389N	P = 0.195	P = 0.537	P = 0.571N
All Organs: Malignant Neoplasms				
Overall rate	12/50 (24%)	14/50 (28%)	17/50 (34%)	19/50 (38%)
Adjusted rate	26.5%	30.7%	38.7%	40.0%
Terminal rate	7/34 (21%)	6/31 (19%)	4/24 (17%)	8/30 (27%)
First incidence (days)	579	137	516	435
Poly-3 test	P = 0.085	P = 0.414	P = 0.153	P = 0.120
All Organs: Benign or Malignant Neoplasms				
Overall rate	40/50 (80%)	45/50 (90%)	44/50 (88%)	40/50 (80%)
Adjusted rate	80.0%	91.0%	88.0%	81.8%
Terminal rate	24/34 (71%)	27/31 (87%)	18/24 (75%)	23/30 (77%)
First incidence (days)	253	137	361	435
Poly-3 test	P = 0.510N	P = 0.101	P = 0.208	P = 0.513

T = Terminal euthanasia
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, nose, 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 euthanasia.
[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 euthanasia. 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 B-3. Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Two-year Inhalation Study of 2,3-Butanedione[a]

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	15	18	26	15
Natural deaths	1	1	–	4
Survivors				
Died last week of study	–	–	–	1
Terminal euthanasia	34	31	24	30
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	–	–	–
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Peyer's patch, hyperplasia, lymphoid	–	–	1 (2%)	–
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Basophilic focus	34 (68%)	30 (60%)	29 (58%)	26 (52%)
Clear cell focus	6 (12%)	8 (16%)	5 (10%)	2 (4%)
Eosinophilic focus	3 (6%)	5 (10%)	7 (14%)	4 (8%)
Fatty change, diffuse	1 (2%)	1 (2%)	2 (4%)	–
Hematopoietic cell proliferation	2 (4%)	2 (4%)	3 (6%)	1 (2%)
Hepatodiaphragmatic nodule	3 (6%)	–	–	2 (4%)
Infiltration cellular, lymphoid	–	1 (2%)	–	–
Infiltration cellular, mixed cell	6 (12%)	6 (12%)	3 (6%)	3 (6%)
Mixed cell focus	–	2 (4%)	–	1 (2%)
Necrosis, multifocal	–	1 (2%)	2 (4%)	–
Pigmentation, hemosiderin	1 (2%)	1 (2%)	–	–
Bile duct, cyst	2 (4%)	1 (2%)	–	1 (2%)
Bile duct, dilatation	–	1 (2%)	–	–
Bile duct, hyperplasia	8 (16%)	12 (24%)	15 (30%)	10 (20%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Centrilobular, degeneration	–	1 (2%)	–	–
Kupffer cell, hyperplasia	–	–	1 (2%)	–
Oval cell, hyperplasia	–	1 (2%)	–	–
Mesentery	(8)	(10)	(4)	(4)
Fat, necrosis	8 (100%)	8 (80%)	4 (100%)	3 (75%)
Pancreas	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Basophilic focus	–	2 (4%)	1 (2%)	2 (4%)
Inflammation	–	–	2 (4%)	–
Salivary glands	(50)	(50)	(50)	(50)
Inflammation	–	1 (2%)	1 (2%)	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation	3 (6%)	5 (10%)	3 (6%)	1 (2%)
Mineralization	–	–	1 (2%)	–
Ulcer	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Epithelium, erosion	–	1 (2%)	–	–
Epithelium, hyperplasia	3 (6%)	6 (12%)	3 (6%)	2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Mineralization	–	–	1 (2%)	–
Epithelium, necrosis	–	–	–	1 (2%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation	–	–	1 (2%)	1 (2%)
Media, hypertrophy	–	–	1 (2%)	–
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	21 (42%)	28 (56%)	24 (48%)	27 (54%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Amyloid deposition	–	–	1 (2%)	–
Angiectasis	–	2 (4%)	1 (2%)	–
Degeneration, cystic	12 (24%)	7 (14%)	12 (24%)	12 (24%)
Hematopoietic cell proliferation	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Zona fasciculata, hyperplasia, focal	3 (6%)	2 (4%)	7 (14%)	7 (14%)
Zona fasciculata, hyperplasia, multifocal	4 (8%)	1 (2%)	–	3 (6%)
Zona fasciculata, hypertrophy	1 (2%)	–	–	–
Zona fasciculata, hypertrophy, focal	17 (34%)	9 (18%)	13 (26%)	15 (30%)
Zona fasciculata, hypertrophy, multifocal	5 (10%)	3 (6%)	2 (4%)	3 (6%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Adrenal medulla	(50)	(50)	(49)	(50)
Hyperplasia	1 (2%)	–	–	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	2 (4%)	1 (2%)	–
Parathyroid gland	(46)	(44)	(46)	(42)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, angiectasis, focal	–	–	–	1 (2%)
Pars distalis, hyperplasia	18 (36%)	16 (32%)	24 (48%)	18 (36%)
Pars intermedia, hyperplasia	–	–	1 (2%)	–
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, hyperplasia	20 (40%)	27 (54%)	22 (44%)	16 (32%)
Follicular cell, hyperplasia	2 (4%)	4 (8%)	1 (2%)	2 (4%)
Follicular cell, hypertrophy	–	–	2 (4%)	–
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(49)	(50)	(50)	(49)
Ectasia	1 (2%)	2 (4%)	2 (4%)	–
Inflammation	–	2 (4%)	–	–
Ovary	(50)	(50)	(50)	(50)
Atrophy	27 (54%)	22 (44%)	25 (50%)	27 (54%)
Cyst	18 (36%)	16 (32%)	20 (40%)	23 (46%)
Germinal epithelium, hyperplasia	–	–	–	1 (2%)
Thecal cell, hyperplasia	1 (2%)	–	–	–
Uterus	(50)	(50)	(50)	(50)
Adenomyosis	–	1 (2%)	–	–
Adenomyosis, focal	2 (4%)	–	–	–
Angiectasis, focal	–	–	–	1 (2%)
Fibrosis, focal	–	–	–	1 (2%)
Inflammation, chronic active	–	–	1 (2%)	–
Cervix, cyst, squamous	–	1 (2%)	–	–
Cervix, hyperplasia, squamous	7 (14%)	5 (10%)	8 (16%)	10 (20%)
Cervix, hypertrophy, stromal	–	–	2 (4%)	1 (2%)
Endometrial glands, hyperplasia, atypical	–	1 (2%)	–	–
Endometrial glands, hyperplasia, focal	–	–	–	1 (2%)
Endometrium, decidual reaction, focal	–	–	1 (2%)	–
Endometrium, hyperplasia, cystic	24 (48%)	16 (32%)	17 (34%)	25 (50%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Endometrium, hyperplasia, focal	–	–	3 (6%)	1 (2%)
Endometrium, metaplasia, squamous	5 (10%)	1 (2%)	7 (14%)	6 (12%)
Serosa, cyst multilocular	1 (2%)	–	–	–
Vagina	(0)	(1)	(2)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Myeloid cell, hyperplasia	12 (24%)	19 (38%)	20 (40%)	36 (72%)
Lymph node	(1)	(3)	(2)	(5)
Axillary, hyperplasia, lymphoid	–	–	–	1 (20%)
Deep cervical, infiltration cellular, plasma cell	–	–	–	2 (40%)
Iliac, infiltration cellular, plasma cell	–	–	1 (50%)	–
Lumbar, ectasia	1 (100%)	–	–	–
Lumbar, infiltration cellular, plasma cell	–	–	1 (50%)	1 (20%)
Popliteal, inflammation	–	1 (33%)	–	–
Renal, infiltration cellular, plasma cell	–	–	–	1 (20%)
Lymph node, bronchial	(43)	(44)	(43)	(42)
Lymph node, mandibular	(46)	(48)	(45)	(48)
Ectasia	–	–	1 (2%)	1 (2%)
Hyperplasia, atypical	–	1 (2%)	–	–
Infiltration cellular, plasma cell	–	1 (2%)	–	2 (4%)
Lymph node, mediastinal	(44)	(47)	(48)	(50)
Atrophy	–	–	–	1 (2%)
Hyperplasia, lymphoid	–	–	–	1 (2%)
Infiltration cellular, plasma cell	–	1 (2%)	–	1 (2%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy	–	2 (4%)	2 (4%)	5 (10%)
Fibrosis, focal	1 (2%)	–	–	–
Hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)	–
Infiltration cellular, plasma cell	–	–	1 (2%)	–
Inflammation	1 (2%)	–	–	–
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	37 (74%)	40 (80%)	38 (76%)	39 (78%)
Pigmentation, hemosiderin	42 (84%)	39 (78%)	38 (76%)	39 (78%)
Capsule, thrombosis	–	1 (2%)	–	–
Lymphoid follicle, atrophy	12 (24%)	6 (12%)	5 (10%)	7 (14%)
Lymphoid follicle, hyperplasia	–	1 (2%)	–	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Thymus	(49)	(50)	(49)	(50)
Atrophy	45 (92%)	42 (84%)	45 (92%)	49 (98%)
Cyst	–	–	1 (2%)	–
Hyperplasia, lymphoid	–	2 (4%)	–	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	4 (8%)	4 (8%)	2 (4%)	1 (2%)
Hyperplasia	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Inflammation	–	–	–	1 (2%)
Duct, dilatation	–	–	1 (2%)	–
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	1 (2%)	–	–
Dermis, thrombosis	1 (2%)	–	–	–
Dorsal, inflammation, acute	–	–	–	1 (2%)
Ear, inflammation, acute	1 (2%)	2 (4%)	–	–
Ear, inflammation, chronic active	–	1 (2%)	–	–
Ear, ulcer, multifocal	–	1 (2%)	–	–
Ear, epidermis, hyperplasia	–	1 (2%)	–	–
Epidermis, hyperplasia	–	1 (2%)	–	1 (2%)
Epidermis, ulcer, multifocal	1 (2%)	–	–	1 (2%)
Foot, inflammation, chronic active	–	–	–	4 (8%)
Foot, ulcer, focal	–	–	–	4 (8%)
Inguinal, inflammation, pyogranulomatous	–	1 (2%)	–	2 (4%)
Inguinal, inflammation, acute	–	–	–	1 (2%)
Lateral, inflammation, acute	–	1 (2%)	–	–
Lateral, ulcer, focal	–	1 (2%)	–	–
Neck, inflammation, chronic active	1 (2%)	–	–	–
Neck, ulcer, diffuse	1 (2%)	–	–	–
Other, inflammation, multifocal	1 (2%)	–	–	–
Subcutaneous tissue, abscess	–	1 (2%)	–	–
Tail, inflammation, chronic active	–	1 (2%)	–	–
Tail, ulcer, multifocal	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosclerosis	–	–	1 (2%)	1 (2%)
Tarsal, tendon, inflammation	–	–	1 (2%)	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Skeletal muscle	(3)	(7)	(5)	(5)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	–	1 (2%)	–	–
Hemorrhage	–	1 (2%)	–	–
Choroid plexus, hyperplasia	–	–	1 (2%)	–
Peripheral nerve	(3)	(5)	(5)	(5)
Spinal cord	(3)	(5)	(5)	(5)
Gliosis	–	1 (20%)	–	–
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Foreign body	–	–	1 (2%)	–
Inflammation, chronic active	4 (8%)	2 (4%)	4 (8%)	25 (50%)
Respiratory epithelium, hyperplasia	–	–	–	2 (4%)
Respiratory epithelium, metaplasia, squamous	–	–	–	35 (70%)
Respiratory epithelium, regeneration	–	–	–	2 (4%)
Squamous epithelium, hyperplasia	1 (2%)	1 (2%)	6 (12%)	48 (96%)
Squamous epithelium, ulcer, focal	–	–	–	5 (10%)
Squamous epithelium, ulcer, multifocal	–	–	–	1 (2%)
Lung	(50)	(50)	(50)	(50)
Inflammation, suppurative	–	–	–	3 (6%)
Inflammation, granulomatous	2 (4%)	1 (2%)	3 (6%)	13 (26%)
Inflammation, chronic active	2 (4%)	–	–	–
Thrombus	–	–	–	1 (2%)
Alveolar epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Alveolar epithelium, hyperplasia, focal	1 (2%)	1 (2%)		1 (2%)
Alveolus, infiltration cellular, histiocyte	13 (26%)	11 (22%)	10 (20%)	32 (64%)
Bronchiole, epithelium, hyperplasia	–	–	8 (16%)	39 (78%)
Bronchus, epithelium, atrophy	–	–	–	7 (14%)
Bronchus, epithelium, hyperplasia	–	–	–	46 (92%)
Bronchus, epithelium, metaplasia, squamous	–	–	–	1 (2%)
Bronchus, epithelium, necrosis	–	–	–	1 (2%)
Bronchus, epithelium, regeneration	–	–	1 (2%)	2 (4%)
Bronchus, submucosa, inflammation, suppurative	–	–	–	1 (2%)
Bronchus, submucosa, necrosis	–	–	–	1 (2%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Interstitium, fibrosis	1 (2%)	1 (2%)	1 (2%)	9 (18%)
Peribronchial, inflammation, chronic active	1 (2%)	2 (4%)	–	27 (54%)
Pleura, fibrosis	–	–	–	1 (2%)
Nose	(50)	(50)	(50)	(50)
Foreign body	–	–	–	1 (2%)
Inflammation, suppurative	4 (8%)	3 (6%)	11 (22%)	49 (98%)
Lamina propria, fibrosis	1 (2%)	1 (2%)	17 (34%)	46 (92%)
Olfactory epithelium, accumulation, hyaline droplet	5 (10%)	10 (20%)	3 (6%)	1 (2%)
Olfactory epithelium, atrophy	1 (2%)	1 (2%)	14 (28%)	24 (48%)
Olfactory epithelium, metaplasia, respiratory	1 (2%)	–	18 (36%)	46 (92%)
Olfactory epithelium, necrosis	–	–	–	4 (8%)
Respiratory epithelium, hyperplasia	1 (2%)	–	2 (4%)	44 (88%)
Respiratory epithelium, metaplasia, squamous	1 (2%)	–	1 (2%)	44 (88%)
Respiratory epithelium, necrosis	–	–	–	3 (6%)
Turbinate, hyperostosis	–	–	–	8 (16%)
Trachea	(50)	(50)	(50)	(50)
Inflammation, suppurative	–	–	–	1 (2%)
Inflammation, chronic active	–	–	–	20 (40%)
Epithelium, atrophy	–	–	–	4 (8%)
Epithelium, hyperplasia	–	–	–	30 (60%)
Epithelium, metaplasia, squamous	–	–	–	3 (6%)
Epithelium, necrosis	–	–	–	1 (2%)
Epithelium, regeneration	–	–	–	3 (6%)
Epithelium, ulcer	–	–	–	1 (2%)
Submucosa, fibrosis	–	–	–	19 (38%)
Submucosa, necrosis	–	–	–	1 (2%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Inflammation, chronic active	–	1 (2%)	1 (2%)	–
Retinal detachment	–	–	1 (2%)	–
Anterior chamber, inflammation, suppurative	1 (2%)	–	6 (12%)	5 (10%)
Cornea, inflammation, chronic active	2 (4%)	6 (12%)	23 (46%)	31 (62%)
Cornea, mineralization	–	1 (2%)	–	2 (4%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Cornea, necrosis	–	–	1 (2%)	–
Cornea, endothelium, rupture	–	1 (2%)	–	1 (2%)
Cornea, epithelium, hyperplasia	–	3 (6%)	8 (16%)	5 (10%)
Cornea, epithelium, ulcer	–	1 (2%)	2 (4%)	13 (26%)
Iris, inflammation, acute	–	–	5 (10%)	4 (8%)
Lens, cataract	1 (2%)	1 (2%)	6 (12%)	9 (18%)
Retina, degeneration	10 (20%)	7 (14%)	11 (22%)	15 (30%)
Unilateral, phthisis bulbi	–	1 (2%)	–	8 (16%)
Harderian gland	(50)	(50)	(50)	(50)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	–	1 (2%)	2 (4%)	–
Calculus gross observation	–	1 (2%)	1 (2%)	–
Calculus microscopic observation only	27 (54%)	29 (58%)	35 (70%)	27 (54%)
Infarct	1 (2%)	–	2 (4%)	–
Nephropathy	12 (24%)	11 (22%)	10 (20%)	11 (22%)
Thrombosis	–	1 (2%)	–	–
Cortex, cyst	1 (2%)	–	2 (4%)	–
Papilla, necrosis	–	1 (2%)	–	
Pelvis, dilatation	–	2 (4%)	–	2 (4%)
Pelvis, inflammation	5 (10%)	8 (16%)	6 (12%)	6 (12%)
Renal tubule, dilatation	–	1 (2%)	–	–
Renal tubule, necrosis	–	–	–	1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Calculus gross observation	–	1 (2%)	–	–
Hemorrhage	–	1 (2%)	–	–
Hyperplasia	–	1 (2%)	–	–
Inflammation	–	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 2,3-Butanedione

Tables

Table C-1. Summary of the Incidence of Neoplasms in Male Mice in the Two-year Inhalation Study of 2,3-Butanedione[a]

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	1	–	–
Moribund	6	5	4	14
Natural deaths	9	5	9	11
Survivors				
Terminal euthanasia	35	39	37	25
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(45)	(42)	(43)	(43)
Intestine large, cecum	(46)	(48)	(47)	(48)
Intestine large, colon	(46)	(47)	(48)	(47)
Intestine large, rectum	(46)	(46)	(45)	(49)
Intestine small, duodenum	(45)	(47)	(44)	(44)
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	–
Polyp adenomatous	1 (2%)	–	–	–
Intestine small, ileum	(45)	(47)	(44)	(46)
Sarcoma, metastatic, uncertain primary site	1 (2%)	–	–	–
Intestine small, jejunum	(44)	(46)	(43)	(43)
Sarcoma, metastatic, uncertain primary site	1 (2%)	–	–	–
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	–	–	–	1 (2%)
Hepatoblastoma	1 (2%)	–	1 (2%)	–
Hepatocellular adenoma	6 (12%)	9 (18%)	11 (22%)	3 (6%)
Hepatocellular adenoma, multiple	11 (22%)	7 (14%)	4 (8%)	1 (2%)
Hepatocellular carcinoma	10 (20%)	6 (12%)	5 (10%)	6 (12%)
Hepatocellular carcinoma, multiple	7 (14%)	5 (10%)	8 (16%)	–
Hepatocholangiocarcinoma	–	–	1 (2%)	–
Hepatocholangiocarcinoma, multiple	1 (2%)	–	–	–
Sarcoma, metastatic, uncertain primary site	1 (2%)	–	–	–
Mesentery	(4)	(1)	(5)	(0)
Hepatoblastoma, metastatic, liver	–	–	1 (20%)	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Hepatocellular carcinoma, metastatic, liver	–	–	1 (20%)	–
Hepatocholangiocarcinoma, metastatic, liver	–	–	1 (20%)	–
Sarcoma, metastatic, uncertain primary site	1 (25%)	–	–	–
Pancreas	(48)	(48)	(50)	(50)
Hepatoblastoma, metastatic, liver	–	–	1 (2%)	–
Hepatocholangiocarcinoma, metastatic, liver	–	–	1 (2%)	–
Sarcoma, metastatic, uncertain primary site	1 (2%)	–	–	–
Salivary glands	(50)	(49)	(50)	(50)
Stomach, forestomach	(48)	(48)	(49)	(50)
Sarcoma, metastatic, uncertain primary site	1 (2%)	–	–	–
Squamous cell papilloma	–	1 (2%)	–	–
Stomach, glandular	(47)	(48)	(49)	(49)
Hepatocholangiocarcinoma, metastatic, liver	–	–	1 (2%)	–
Tooth	(2)	(2)	(1)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (2%)	–
Hemangioma	–	–	–	1 (2%)
Hemangiosarcoma	1 (2%)	–	–	–
Hepatocellular carcinoma, metastatic, liver	1 (2%)	–	–	–
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Adenoma	–	1 (2%)	1 (2%)	–
Subcapsular, adenoma	2 (4%)	7 (14%)	3 (6%)	2 (4%)
Adrenal medulla	(50)	(48)	(50)	(50)
Islets, pancreatic	(48)	(48)	(49)	(50)
Adenoma	–	–	2 (4%)	–
Parathyroid gland	(40)	(39)	(44)	(37)
Pituitary gland	(50)	(49)	(50)	(50)
Thyroid gland	(50)	(49)	(50)	(50)
Follicular cell, adenoma	–	1 (2%)	1 (2%)	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
General Body System				
Tissue NOS	(0)	(0)	(0)	(1)
Genital System				
Epididymis	(49)	(49)	(50)	(49)
Hepatoblastoma, metastatic, liver	–	–	1 (2%)	–
Hepatocholangiocarcinoma, metastatic, liver	–	–	1 (2%)	–
Preputial gland	(50)	(49)	(50)	(49)
Prostate gland	(49)	(49)	(49)	(50)
Carcinoma, metastatic, urinary bladder	–	–	1 (2%)	–
Hepatocholangiocarcinoma, metastatic, liver	–	–	1 (2%)	–
Seminal vesicle	(49)	(49)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver	–	–	1 (2%)	–
Testes	(49)	(49)	(50)	(49)
Hematopoietic System				
Bone marrow	(48)	(47)	(50)	(49)
Hemangiosarcoma, metastatic, spleen	–	2 (4%)	–	–
Mast cell tumor malignant	1 (2%)	–	–	–
Lymph node	(5)	(1)	(3)	(8)
Pancreatic, hepatoblastoma, metastatic, liver	–	–	1 (33%)	–
Renal, hepatocholangiocarcinoma, metastatic, liver	1 (20%)	–	–	–
Lymph node, bronchial	(34)	(43)	(38)	(31)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (3%)	–
Hepatocholangiocarcinoma, metastatic, liver	1 (3%)	–	–	–
Lymph node, mandibular	(36)	(33)	(30)	(32)
Lymph node, mediastinal	(37)	(33)	(38)	(32)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (3%)	–	1 (3%)	–
Hepatoblastoma, metastatic, liver	–	–	1 (3%)	–
Hepatocholangiocarcinoma, metastatic, liver	1 (3%)	–	1 (3%)	–
Sarcoma, metastatic, uncertain primary site	1 (3%)	–	–	–
Lymph node, mesenteric	(47)	(47)	(49)	(47)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)	–	–	–
Hepatocellular carcinoma, metastatic, liver	1 (2%)	–	–	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Hepatocholangiocarcinoma, metastatic, liver	–	–	1 (2%)	–
Sarcoma, metastatic, uncertain primary site	1 (2%)	–	–	–
Spleen	(48)	(49)	(50)	(50)
Hemangiosarcoma	–	2 (4%)	–	–
Thymus	(43)	(42)	(44)	(30)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (2%)	–
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Sarcoma, metastatic, uncertain primary site	1 (2%)	–	–	–
Integumentary System				
Mammary gland	(1)	(0)	(0)	(1)
Skin	(50)	(49)	(50)	(49)
Carcinoma	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(2)	(4)	(5)	(2)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (50%)	–	1 (20%)	–
Hepatoblastoma, metastatic, liver	–	–	1 (20%)	–
Hepatocholangiocarcinoma, metastatic, liver	1 (50%)	–	1 (20%)	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(0)	(2)	(1)	(1)
Spinal cord	(0)	(2)	(1)	(1)
Respiratory System				
Larynx	(49)	(49)	(49)	(50)
Lung	(50)	(49)	(50)	(50)
Alveolar/bronchiolar adenoma	4 (8%)	5 (10%)	5 (10%)	4 (8%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)	–	–	–
Alveolar/bronchiolar carcinoma	4 (8%)	3 (6%)	4 (8%)	2 (4%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)	–	2 (4%)	1 (2%)
Hepatoblastoma, metastatic, liver	1 (2%)	–	1 (2%)	–
Hepatocellular carcinoma, metastatic, liver	4 (8%)	2 (4%)	7 (14%)	–
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	1 (2%)	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Nose	(49)	(48)	(50)	(50)
Turbinate, polyp	1 (2%)	–	–	–
Trachea	(48)	(49)	(49)	(49)
Special Senses System				
Eye	(49)	(49)	(50)	(50)
Harderian gland	(50)	(49)	(50)	(50)
Adenoma	3 (6%)	6 (12%)	2 (4%)	3 (6%)
Carcinoma	–	1 (2%)	–	1 (2%)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver	–	–	1 (2%)	–
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Urinary bladder	(49)	(48)	(50)	(49)
Carcinoma	–	–	1 (2%)	–
Hepatocholangiocarcinoma, metastatic, liver	–	–	1 (2%)	–
Systemic Lesions				
Multiple organsb	(50)	(50)	(50)	(50)
Histiocytic sarcoma	–	1 (2%)	2 (4%)	–
Lymphoma malignant	6 (12%)	4 (8%)	3 (6%)	2 (4%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	37	37	36	18
Total primary neoplasms	61	60	56	27
Total animals with benign neoplasms	22	27	21	11
Total benign neoplasms	29	37	29	14
Total animals with malignant neoplasms	27	20	24	12
Total malignant neoplasms	32	23	27	13
Total animals with metastatic neoplasms	8	5	11	–
Total metastatic neoplasms	28	6	33	–
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 2,3-Butanedione

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	2/50 (4%)	8/49 (16%)	3/50 (6%)	2/50 (4%)
Adjusted rate[b]	4.4%	17.6%	6.6%	5.2%
Terminal ratec	1/35 (3%)	7/39 (18%)	3/37 (8%)	2/25 (8%)
First incidence (days)	590	611	729 (T)	729 (T)
Poly-3 test[d]	P = 0.381N	P = 0.046	P = 0.505	P = 0.632
Harderian Gland: Adenoma				
Overall rate	3/50 (6%)	6/50 (12%)	2/50 (4%)	3/50 (6%)
Adjusted rate	6.7%	13.0%	4.4%	7.9%
Terminal rate	2/35 (6%)	5/39 (13%)	2/37 (5%)	2/25 (8%)
First incidence (days)	667	574	729 (T)	718
Poly-3 test	P = 0.471N	P = 0.255	P = 0.492N	P = 0.585
Harderian Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	7/50 (14%)	2/50 (4%)	4/50 (8%)
Adjusted rate	6.7%	15.2%	4.4%	10.4%
Terminal rate	2/35 (6%)	5/39 (13%)	2/37 (5%)	2/25 (8%)
First incidence (days)	667	574	729 (T)	650
Poly-3 test	P = 0.542	P = 0.168	P = 0.492N	P = 0.416
Liver: Hepatocellular Adenoma				
Overall rate	17/50 (34%)	16/50 (32%)	15/50 (30%)	4/50 (8%)
Adjusted rate	37.4%	34.7%	32.4%	10.5%
Terminal rate	15/35 (43%)	14/39 (36%)	13/37 (35%)	4/25 (16%)
First incidence (days)	590	611	554	729 (T)
Poly-3 test	P = 0.005N	P = 0.482N	P = 0.391N	P = 0.004N
Liver: Hepatocellular Carcinoma				
Overall rate	17/50 (34%)	11/50 (22%)	13/50 (26%)	6/50 (12%)
Adjusted rate	36.0%	22.6%	27.6%	15.7%
Terminal rate	8/35 (23%)	5/39 (13%)	8/37 (22%)	6/25 (24%)
First incidence (days)	519	396	527	729 (T)
Poly-3 test	P = 0.043N	P = 0.112N	P = 0.258N	P = 0.031N
Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma				
Overall rate	31/50 (62%)	27/50 (54%)	25/50 (50%)	8/50 (16%)
Adjusted rate	64.9%	55.0%	52.4%	21.0%
Terminal rate	21/35 (60%)	19/39 (49%)	18/37 (49%)	8/25 (32%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
First incidence (days)	519	396	527	729 (T)
Poly-3 test	P < 0.001N	P = 0.214N	P = 0.148N	P < 0.001N
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	18/50 (36%)	11/50 (22%)	14/50 (28%)	6/50 (12%)
Adjusted rate	37.6%	22.6%	29.7%	15.7%
Terminal rate	8/35 (23%)	5/39 (13%)	9/37 (24%)	6/25 (24%)
First incidence (days)	519	396	527	729 (T)
Poly-3 test	P = 0.035N	P = 0.082N	P = 0.275N	P = 0.021N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	32/50 (64%)	27/50 (54%)	26/50 (52%)	8/50 (16%)
Adjusted rate	66.2%	55.0%	54.5%	21.0%
Terminal rate	21/35 (60%)	19/39 (49%)	19/37 (51%)	8/25 (32%)
First incidence (days)	519	396	527	729 (T)
Poly-3 test	P < 0.001N	P = 0.175N	P = 0.165N	P < 0.001N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	5/50 (10%)	5/49 (10%)	5/50 (10%)	4/50 (8%)
Adjusted rate	11.2%	11.1%	10.9%	10.4%
Terminal rate	5/35 (14%)	5/39 (13%)	4/37 (11%)	2/25 (8%)
First incidence (days)	729 (T)	729 (T)	722	650
Poly-3 test	P = 0.511N	P = 0.625N	P = 0.616N	P = 0.591N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	5/50 (10%)	3/49 (6%)	6/50 (12%)	3/50 (6%)
Adjusted rate	11.2%	6.7%	13.0%	7.9%
Terminal rate	4/35 (11%)	3/39 (8%)	3/37 (8%)	3/25 (12%)
First incidence (days)	722	729 (T)	588	729 (T)
Poly-3 test	P = 0.481N	P = 0.351N	P = 0.523	P = 0.446N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	9/50 (18%)	8/49 (16%)	10/50 (20%)	7/50 (14%)
Adjusted rate	20.1%	17.7%	21.6%	18.1%
Terminal rate	8/35 (23%)	8/39 (21%)	7/37 (19%)	5/25 (20%)
First incidence (days)	722	729 (T)	588	650
Poly-3 test	P = 0.508N	P = 0.492N	P = 0.532	P = 0.519N
All Organs: Malignant Lymphoma				
Overall rate	6/50 (12%)	4/50 (8%)	3/50 (6%)	2/50 (4%)
Adjusted rate	13.2%	8.6%	6.5%	5.2%
Terminal rate	4/35 (11%)	2/39 (5%)	0/37 (0%)	1/25 (4%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
First incidence (days)	519	480	637	718
Poly-3 test	P = 0.133N	P = 0.356N	P = 0.236N	P = 0.197N
All Organs: Benign Neoplasms				
Overall rate	22/50 (44%)	27/50 (54%)	21/50 (42%)	11/50 (22%)
Adjusted rate	48.1%	58.0%	45.0%	28.4%
Terminal rate	19/35 (54%)	24/39 (62%)	18/37 (49%)	8/25 (32%)
First incidence (days)	590	574	554	650
Poly-3 test	P = 0.019N	P = 0.226	P = 0.462N	P = 0.049N
All Organs: Malignant Neoplasms				
Overall rate	27/50 (54%)	20/50 (40%)	24/50 (48%)	12/50 (24%)
Adjusted rate	55.1%	40.5%	49.9%	31.0%
Terminal rate	14/35 (40%)	11/39 (28%)	14/37 (38%)	9/25 (36%)
First incidence (days)	519	396	527	650
Poly-3 test	P = 0.038N	P = 0.105N	P = 0.379N	P = 0.019N
All Organs: Malignant or Benign Neoplasms				
Overall rate	37/50 (74%)	37/50 (74%)	36/50 (72%)	18/50 (36%)
Adjusted rate	75.5%	74.9%	73.3%	46.5%
Terminal rate	24/35 (69%)	28/39 (72%)	24/37 (65%)	14/25 (56%)
First incidence (days)	519	396	527	650
Poly-3 test	P = 0.003N	P = 0.565N	P = 0.493N	P = 0.003N

T = Terminal euthanasia

[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, and lung; for other tissues, denominator is number of animals necropsied.

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

[c]Observed incidence at terminal euthanasia.

[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 euthanasia. A negative trend or a lower incidence in an exposure group is indicated by **N**.

Table C-3. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Two-year Inhalation Study of 2,3-Butanedione[a]

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	1	–	–
Moribund	6	5	4	14
Natural deaths	9	5	9	11
Survivors				
Terminal euthanasia	35	39	37	25
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Epithelium, hyperplasia	–	1 (2%)	–	–
Periesophageal tissue, inflammation, chronic active	–	–	–	3 (6%)
Submucosa, inflammation, chronic active	–	–	–	1 (2%)
Gallbladder	(45)	(42)	(43)	(43)
Inflammation				1 (2%)
Intestine large, cecum	(46)	(48)	(47)	(48)
Inflammation	7 (15%)	–	2 (4%)	–
Intestine large, colon	(46)	(47)	(48)	(47)
Intestine large, rectum	(46)	(46)	(45)	(49)
Inflammation	–	–	–	1 (2%)
Intestine small, duodenum	(45)	(47)	(44)	(44)
Inflammation	1 (2%)	–	–	–
Necrosis	1 (2%)	–	–	–
Intestine small, ileum	(45)	(47)	(44)	(46)
Hemorrhage	–	–	–	1 (2%)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	2 (5%)	–
Inflammation	3 (7%)	–	1 (2%)	–
Intestine small, jejunum	(44)	(46)	(43)	(43)
Hyperplasia, lymphoid	1 (2%)	–	1 (2%)	1 (2%)
Inflammation	1 (2%)	1 (2%)	–	–
Liver	(50)	(50)	(50)	(50)
Basophilic focus	7 (14%)	5 (10%)	7 (14%)	6 (12%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Clear cell focus	2 (4%)	5 (10%)	5 (10%)	–
Cyst	1 (2%)	1 (2%)	–	–
Eosinophilic focus	4 (8%)	6 (12%)	4 (8%)	4 (8%)
Hematopoietic cell proliferation	–	–	–	1 (2%)
Hepatodiaphragmatic nodule	1 (2%)	–	–	–
Mixed cell focus	4 (8%)	7 (14%)	2 (4%)	1 (2%)
Pigmentation, ceroid	1 (2%)	–	–	–
Syncytial alteration	–	1 (2%)	–	–
Tension lipidosis	–	2 (4%)	–	1 (2%)
Thrombosis	–	1 (2%)	–	–
Hepatocyte, degeneration	2 (4%)	–	–	–
Hepatocyte, necrosis	4 (8%)	5 (10%)	1 (2%)	3 (6%)
Mesentery	(4)	(1)	(5)	(0)
Hemorrhage	1 (25%)	–	–	–
Artery, thrombosis	1 (25%)	–	–	–
Fat, necrosis	1 (25%)	1 (100%)	2 (40%)	–
Pancreas	(48)	(48)	(50)	(50)
Necrosis	–	–	1 (2%)	–
Acinus, atrophy	2 (4%)	–	1 (2%)	–
Salivary glands	(50)	(49)	(50)	(50)
Stomach, forestomach	(48)	(48)	(49)	(50)
Inflammation	2 (4%)	1 (2%)	1 (2%)	–
Necrosis	–	–	1 (2%)	–
Epithelium, hyperplasia	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Stomach, glandular	(47)	(48)	(49)	(49)
Inflammation	3 (6%)	4 (8%)	2 (4%)	–
Mineralization	–	–	1 (2%)	–
Epithelium, hyperplasia	1 (2%)	–	–	–
Tooth	(2)	(2)	(1)	(0)
Dysplasia	2 (100%)	2 (100%)	1 (100%)	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation	2 (4%)	–	–	1 (2%)
Heart	(50)	(50)	(50)	(49)
Cardiomyopathy	4 (8%)	1 (2%)	8 (16%)	3 (6%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Inflammation, acute	1 (2%)	–	1 (2%)	3 (6%)
Inflammation, chronic active	–	–	1 (2%)	5 (10%)
Mineralization	–	1 (2%)	–	–
Necrosis	1 (2%)	–	–	3 (6%)
Polyarteritis	3 (6%)	–	–	1 (2%)
Thrombosis	1 (2%)	–	–	–
Valve, inflammation, chronic active	1 (2%)	–	–	–
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Hyperplasia	1 (2%)	–	–	–
Inflammation, suppurative	–	–	–	1 (2%)
Vacuolization cytoplasmic	1 (2%)	–	–	–
Zona fasciculata, hypertrophy, focal	23 (46%)	26 (53%)	35 (70%)	9 (18%)
Adrenal medulla	(50)	(48)	(50)	(50)
Hyperplasia	–	–	3 (6%)	–
Islets, pancreatic	(48)	(48)	(49)	(50)
Hyperplasia	1 (2%)	1 (2%)	4 (8%)	–
Parathyroid gland	(40)	(39)	(44)	(37)
Pituitary gland	(50)	(49)	(50)	(50)
Pars distalis, hyperplasia	1 (2%)	2 (4%)	–	–
Thyroid gland	(50)	(49)	(50)	(50)
Cyst	1 (2%)	–	–	–
Inflammation, chronic active	1 (2%)	–	–	2 (4%)
Follicular cell, hyperplasia	–	1 (2%)	–	1 (2%)
General Body System				
Tissue NOS	(0)	(0)	(0)	(1)
Inflammation, chronic active	–	–	–	1 (100%)
Genital System				
Epididymis	(49)	(49)	(50)	(49)
Exfoliated germ cell	32 (65%)	23 (47%)	25 (50%)	26 (53%)
Granuloma sperm	1 (2%)	2 (4%)	–	1 (2%)
Infiltration cellular, lymphoid	1 (2%)	–	–	–
Inflammation	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Preputial gland	(50)	(49)	(50)	(49)
Ectasia	–	2 (4%)	2 (4%)	1 (2%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Inflammation	1 (2%)	3 (6%)	5 (10%)	3 (6%)
Prostate gland	(49)	(49)	(49)	(50)
Inflammation	–	2 (4%)	–	1 (2%)
Seminal vesicle	(49)	(49)	(50)	(50)
Dilatation	2 (4%)	–	–	–
Testes	(49)	(49)	(50)	(49)
Interstitial cell, hyperplasia	–	–	1 (2%)	1 (2%)
Seminiferous tubule, degeneration	14 (29%)	6 (12%)	13 (26%)	6 (12%)
Hematopoietic System				
Bone marrow	(48)	(47)	(50)	(49)
Infiltration cellular, mast cell	–	–	1 (2%)	–
Necrosis	1 (2%)	–	–	–
Myeloid cell, hyperplasia	9 (19%)	8 (17%)	20 (40%)	39 (80%)
Lymph node	(5)	(1)	(3)	(8)
Deep cervical, ectasia	–	–	–	1 (13%)
Deep cervical, hematopoietic cell proliferation	–	–	–	1 (13%)
Deep cervical, hyperplasia, lymphoid	–	–	–	4 (50%)
Deep cervical, infiltration cellular, plasma cell	–	–	–	1 (13%)
Pancreatic, ectasia	–	–	1 (33%)	–
Pancreatic, hyperplasia, lymphoid	–	–	–	1 (13%)
Renal, ectasia	1 (20%)	–	1 (33%)	–
Renal, hematopoietic cell proliferation	–	–	–	1 (13%)
Lymph node, bronchial	(34)	(43)	(38)	(31)
Ectasia	–	–	–	1 (3%)
Hematopoietic cell proliferation	–	–	1 (3%)	–
Hyperplasia, lymphoid	–	1 (2%)	3 (8%)	2 (6%)
Infiltration cellular, plasma cell	–	–	–	1 (3%)
Inflammation, chronic active	–	–	–	2 (6%)
Pigmentation	–	–	–	3 (10%)
Lymph node, mandibular	(36)	(33)	(30)	(32)
Ectasia	–	–	–	4 (13%)
Hematopoietic cell proliferation	–	–	–	3 (9%)
Hyperplasia, lymphoid	2 (6%)	5 (15%)	11 (37%)	10 (31%)
Infiltration cellular, plasma cell	1 (3%)	–	2 (7%)	14 (44%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Lymph node, mediastinal	(37)	(33)	(38)	(32)
Hematopoietic cell proliferation	–	–	1 (3%)	1 (3%)
Hyperplasia, lymphoid	–	–	–	2 (6%)
Infiltration cellular, plasma cell	–	–	1 (3%)	2 (6%)
Inflammation, chronic active	–	–	–	2 (6%)
Lymph node, mesenteric	(47)	(47)	(49)	(47)
Ectasia	1 (2%)	–	1 (2%)	–
Hematopoietic cell proliferation	4 (9%)	1 (2%)	–	2 (4%)
Hyperplasia, lymphoid	7 (15%)	1 (2%)	2 (4%)	–
Inflammation	–	–	1 (2%)	–
Spleen	(48)	(49)	(50)	(50)
Angiectasis	–	1 (2%)	–	–
Congestion	–	–	–	1 (2%)
Hematopoietic cell proliferation	11 (23%)	12 (24%)	15 (30%)	25 (50%)
Hyperplasia, lymphoid	–	4 (8%)	1 (2%)	3 (6%)
Inflammation	1 (2%)	–	–	2 (4%)
Necrosis	–	–	–	2 (4%)
Lymphoid follicle, atrophy	–	–	–	2 (4%)
Thymus	(43)	(42)	(44)	(30)
Atrophy	10 (23%)	5 (12%)	2 (5%)	5 (17%)
Cyst	1 (2%)	–	–	1 (3%)
Ectopic parathyroid gland	6 (14%)	4 (10%)	11 (25%)	4 (13%)
Inflammation, chronic active	1 (2%)	–	–	3 (10%)
Integumentary System				
Mammary gland	(1)	(0)	(0)	(1)
Skin	(50)	(49)	(50)	(49)
Hyperkeratosis	–	–	1 (2%)	–
Inflammation, chronic active	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Pigmentation, melanin	–	1 (2%)	–	–
Epidermis, hyperplasia	–	2 (4%)	1 (2%)	–
Epidermis, necrosis	–	–	1 (2%)	–
Epidermis, ulcer	1 (2%)	1 (2%)	1 (2%)	–
Subcutaneous tissue, cyst epithelial inclusion	–	–	1 (2%)	–
Subcutaneous tissue, inflammation, suppurative	–	–	1 (2%)	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Subcutaneous tissue, necrosis	–	–	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	1 (2%)	1 (2%)	3 (6%)	2 (4%)
Maxilla, osteomalacia	–	–	–	1 (2%)
Skeletal muscle	(2)	(4)	(5)	(2)
Degeneration	–	1 (25%)	–	1 (50%)
Hemorrhage, acute	–	2 (50%)	–	–
Inflammation	–	1 (25%)	–	1 (50%)
Necrosis, acute	–	1 (25%)	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte	–	–	2 (4%)	–
Inflammation, acute	1 (2%)	–	1 (2%)	–
Inflammation, chronic active	–	–	–	1 (2%)
Necrosis, chronic	1 (2%)	–	–	–
Peripheral nerve	(0)	(2)	(1)	(1)
Degeneration	–	1 (50%)	1 (100%)	1 (100%)
Spinal cord	(0)	(2)	(1)	(1)
Respiratory System				
Larynx	(49)	(49)	(49)	(50)
Inflammation, suppurative	2 (4%)	–	–	–
Inflammation, chronic active	4 (8%)	2 (4%)	11 (22%)	42 (84%)
Artery, inflammation, chronic active	–	–	–	1 (2%)
Lumen, exudate	–	–	–	2 (4%)
Respiratory epithelium, hyperplasia	1 (2%)	–	–	11 (22%)
Respiratory epithelium, metaplasia, squamous	3 (6%)	–	6 (12%)	50 (100%)
Respiratory epithelium, necrosis	2 (4%)	1 (2%)	9 (18%)	34 (68%)
Respiratory epithelium, regeneration	–	–	–	32 (64%)
Squamous epithelium, hyperplasia	3 (6%)	7 (14%)	15 (31%)	42 (84%)
Lung	(50)	(49)	(50)	(50)
Foreign body	–	–	–	1 (2%)
Inflammation, suppurative	–	–	–	3 (6%)
Inflammation, chronic active	1 (2%)	–	2 (4%)	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Mineralization	1 (2%)	–	–	–
Thrombosis	2 (4%)	1 (2%)	–	2 (4%)
Alveolar epithelium, hyperplasia	1 (2%)	1 (2%)	–	–
Alveolar epithelium, hyperplasia, focal	3 (6%)	3 (6%)	4 (8%)	–
Alveolus, infiltration cellular, histiocyte	7 (14%)	2 (4%)	3 (6%)	9 (18%)
Bronchiole, epithelium, hyperplasia	1 (2%)	–	1 (2%)	2 (4%)
Bronchus, inflammation, suppurative	–	–	–	1 (2%)
Bronchus, epithelium, necrosis	–	–	–	2 (4%)
Bronchus, epithelium, regeneration	–	–	–	34 (68%)
Interstitium, fibrosis	1 (2%)	–	1 (2%)	–
Mediastinum, inflammation, suppurative	–	–	–	3 (6%)
Mediastinum, inflammation, chronic active	1 (2%)	–	–	8 (16%)
Mediastinum, necrosis	–	–	–	1 (2%)
Pleura, inflammation, suppurative	–	–	–	2 (4%)
Pleura, inflammation, chronic active	–	–	1 (2%)	–
Nose	(49)	(48)	(50)	(50)
Foreign body	–	–	3 (6%)	–
Inflammation, suppurative	2 (4%)	4 (8%)	47 (94%)	50 (100%)
Glands, olfactory epithelium, inflammation, acute	–	1 (2%)	–	–
Glands, respiratory epithelium, cyst	–	1 (2%)	–	5 (10%)
Glands, sinus, metaplasia, respiratory	–	–	1 (2%)	13 (26%)
Lamina propria, fibrosis	–	–	44 (88%)	50 (100%)
Lateral wall, inflammation, chronic active	5 (10%)	8 (17%)	5 (10%)	2 (4%)
Mucosa, regeneration	–	–	47 (94%)	47 (94%)
Nasopharyngeal duct, polyp, inflammatory	–	–	–	1 (2%)
Olfactory epithelium, accumulation, hyaline droplet	1 (2%)	–	1 (2%)	2 (4%)
Olfactory epithelium, atrophy	–	14 (29%)	48 (96%)	38 (76%)
Olfactory epithelium, metaplasia, respiratory	1 (2%)	–	39 (78%)	45 (90%)
Olfactory epithelium, metaplasia, squamous	1 (2%)	–	–	–
Olfactory epithelium, necrosis	–	–	–	19 (38%)
Respiratory epithelium, accumulation, hyaline droplet	3 (6%)	1 (2%)	6 (12%)	7 (14%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Respiratory epithelium, cyst	–	1 (2%)	–	–
Respiratory epithelium, hyperplasia	1 (2%)	–	–	8 (16%)
Respiratory epithelium, metaplasia, squamous	–	6 (13%)	47 (94%)	50 (100%)
Respiratory epithelium, necrosis	–	–	34 (68%)	50 (100%)
Respiratory epithelium, regeneration	–	–	2 (4%)	–
Septum, perforation	–	–	3 (6%)	11 (22%)
Turbinate, atrophy	–	8 (17%)	49 (98%)	50 (100%)
Turbinate, necrosis	–	–	4 (8%)	27 (54%)
Trachea	(48)	(49)	(49)	(49)
Inflammation, chronic active	–	–	2 (4%)	45 (92%)
Necrosis	–	–	–	47 (96%)
Carina, epithelium, hyperplasia	–	–	–	1 (2%)
Carina, epithelium, necrosis	–	–	–	1 (2%)
Carina, submucosa, fibrosis	–	–	–	16 (33%)
Carina, submucosa, inflammation, chronic active	–	–	–	4 (8%)
Carina, submucosa, mineralization	–	–	–	15 (31%)
Epithelium, hyperplasia	–	–	–	6 (12%)
Epithelium, metaplasia, squamous	–	–	–	5 (10%)
Epithelium, regeneration	–	–	–	45 (92%)
Lumen, exudate	–	–	–	4 (8%)
Submucosa, fibrosis	–	–	–	46 (94%)
Special Senses System				
Eye	(49)	(49)	(50)	(50)
Phthisis bulbi	–	–	–	1 (2%)
Anterior chamber, inflammation, suppurative	–	–	–	5 (10%)
Cornea, edema	–	–	–	1 (2%)
Cornea, inflammation, acute	2 (4%)	–	1 (2%)	17 (34%)
Cornea, mineralization	–	–	–	5 (10%)
Cornea, neovascularization	–	–	–	1 (2%)
Cornea, epithelium, hyperplasia	1 (2%)	–	–	9 (18%)
Cornea, epithelium, ulcer	–	–	–	3 (6%)
Lens, cataract	1 (2%)	1 (2%)	–	2 (4%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Harderian gland	(50)	(49)	(50)	(50)
Hyperplasia	2 (4%)	3 (6%)	1 (2%)	2 (4%)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Cyst	1 (2%)	2 (4%)	1 (2%)	–
Infarct	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Infiltration cellular, lymphocyte	–	1 (2%)	–	–
Inflammation	1 (2%)	–	2 (4%)	1 (2%)
Metaplasia, osseous	2 (4%)	2 (4%)	4 (8%)	2 (4%)
Mineralization	–	1 (2%)	–	–
Nephropathy	34 (69%)	26 (52%)	25 (50%)	10 (20%)
Pigmentation, hemosiderin	–	–	1 (2%)	–
Papilla, necrosis	–	–	–	1 (2%)
Pelvis, dilatation	–	1 (2%)	1 (2%)	–
Urinary bladder	(49)	(48)	(50)	(49)
Angiectasis	1 (2%)	–	–	–
Hyperplasia, lymphoid	–	–	1 (2%)	–
Inflammation	–	1 (2%)	–	–
Mineralization	–	–	–	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 2,3-Butanedione

Tables

Table D-1. Summary of the Incidence of Neoplasms in Female Mice in the Two-year Inhalation Study of 2,3-Butanedione[a]

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	10	6	4	15
Natural deaths	4	4	4	17
Survivors				
Died last week of study	–	–	–	1
Terminal euthanasia	36	40	42	17
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(42)	(45)	(45)	(37)
Hemangiosarcoma, metastatic, liver	–	1 (2%)	–	–
Intestine large, cecum	(47)	(49)	(50)	(41)
Intestine large, colon	(47)	(49)	(50)	(46)
Intestine large, rectum	(49)	(49)	(46)	(43)
Intestine small, duodenum	(47)	(48)	(49)	(38)
Polyp adenomatous	–	–	1 (2%)	–
Intestine small, ileum	(47)	(48)	(49)	(39)
Intestine small, jejunum	(46)	(49)	(49)	(39)
Liver	(50)	(50)	(50)	(50)
Granulosa-theca tumor malignant, metastatic, ovary	1 (2%)	–	–	–
Hemangiosarcoma	–	1 (2%)	–	1 (2%)
Hemangiosarcoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Hepatoblastoma	1 (2%)	1 (2%)	–	–
Hepatocellular adenoma	4 (8%)	10 (20%)	7 (14%)	2 (4%)
Hepatocellular adenoma, multiple	2 (4%)	1 (2%)	–	1 (2%)
Hepatocellular carcinoma	4 (8%)	4 (8%)	3 (6%)	–
Hepatocellular carcinoma, multiple	1 (2%)	1 (2%)	–	–
Mesentery	(6)	(10)	(4)	(1)
Hemangiosarcoma, metastatic, skeletal muscle	–	–	–	1 (100%)
Hepatoblastoma, metastatic, liver	1 (17%)	–	–	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Hepatocellular carcinoma, metastatic, liver	–	1 (10%)	–	–
Pancreas	(50)	(50)	(50)	(47)
Granulosa-theca tumor malignant, metastatic, ovary	1 (2%)	–	–	–
Salivary glands	(49)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(49)
Granulosa-theca tumor malignant, metastatic, ovary	1 (2%)	–	–	–
Squamous cell papilloma	–	1 (2%)	–	–
Stomach, glandular	(49)	(50)	(50)	(46)
Granulosa-theca tumor malignant, metastatic, ovary	1 (2%)	–	–	–
Tooth	(1)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Hemangiosarcoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (2%)	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Capsule, hemangiosarcoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Subcapsular, adenoma	1 (2%)	–	–	–
Adrenal medulla	(50)	(50)	(49)	(50)
Pheochromocytoma benign	1 (2%)	–	–	–
Islets, pancreatic	(49)	(50)	(49)	(48)
Parathyroid gland	(38)	(39)	(46)	(46)
Pituitary gland	(49)	(49)	(50)	(48)
Pars distalis, adenoma	2 (4%)	4 (8%)	2 (4%)	–
Pars distalis, carcinoma	–	1 (2%)	1 (2%)	–
Pars intermedia, adenoma	–	1 (2%)	–	1 (2%)
Pars intermedia, carcinoma	1 (2%)	–	–	–
Thyroid gland	(50)	(49)	(48)	(50)
Carcinoma	–	–	1 (2%)	–
Follicular cell, adenoma	1 (2%)	–	–	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(47)	(49)	(48)	(44)
Hemangiosarcoma, metastatic, spleen	–	–	1 (2%)	–
Ovary	(49)	(50)	(49)	(50)
Cystadenoma		2 (4%)	3 (6%)	–
Granulosa-theca tumor malignant	1 (2%)	–	–	–
Hemangioma	–	–	1 (2%)	–
Hemangiosarcoma, metastatic, spleen	–	–	1 (2%)	–
Luteoma	–	1 (2%)	3 (6%)	–
Teratoma benign	–	1 (2%)	–	–
Periovarian tissue, hemangiosarcoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Periovarian tissue, hepatoblastoma, metastatic, liver	1 (2%)	–	–	–
Uterus	(50)	(50)	(50)	(50)
Granular cell tumor benign	–	–	1 (2%)	–
Hemangioma	–	–	–	1 (2%)
Polyp stromal	–	2 (4%)	2 (4%)	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Hemangiosarcoma, metastatic, spleen	–	1 (2%)	1 (2%)	–
Lymph node	(11)	(10)	(11)	(7)
Iliac, hepatoblastoma, metastatic, liver	1 (9%)	–	–	–
Pancreatic, hepatocellular carcinoma, metastatic, liver	–	1 (10%)	–	–
Lymph node, bronchial	(40)	(42)	(43)	(43)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (2%)	–
Lymph node, mandibular	(39)	(47)	(45)	(39)
Carcinoma, metastatic, Harderian gland	1 (3%)	–	–	–
Lymph node, mediastinal	(40)	(38)	(41)	(33)
Hemangiosarcoma, metastatic, spleen	–	–	1 (2%)	–
Hepatoblastoma, metastatic, liver	1 (3%)	–	–	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Lymph node, mesenteric	(49)	(50)	(50)	(45)
Granulosa-theca tumor malignant, metastatic, ovary	1 (2%)	–	–	–
Spleen	(50)	(50)	(50)	(48)
Hemangiosarcoma	–	1 (2%)	2 (4%)	–
Thymus	(47)	(50)	(49)	(45)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (2%)	–
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	–
Integumentary System				
Mammary gland	(49)	(50)	(50)	(49)
Adenoma	1 (2%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma	1 (2%)	–	–	–
Melanoma malignant	–	1 (2%)	–	–
Neural crest tumor	–	1 (2%)	–	–
Squamous cell carcinoma	–	–	–	1 (2%)
Subcutaneous tissue, fibrosarcoma	–	1 (2%)	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(6)	(1)	(3)	(4)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (33%)	–
Granulosa-theca tumor malignant, metastatic, ovary	1 (17%)	–	–	–
Hemangiosarcoma	–	–	–	1 (25%)
Sarcoma	1 (17%)	–	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland	–	1 (2%)	–	–
Peripheral nerve	(2)	(1)	(2)	(2)
Spinal cord	(2)	(1)	(2)	(2)
Respiratory System				
Larynx	(49)	(50)	(50)	(49)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	4 (8%)	2 (4%)	1 (2%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Alveolar/bronchiolar adenoma, multiple	–	1 (2%)	–	–
Alveolar/bronchiolar carcinoma	2 (4%)	1 (2%)	2 (4%)	–
Alveolar/bronchiolar carcinoma, multiple	1 (2%)	–	–	–
Carcinoma, metastatic, Harderian gland	1 (2%)	–	–	–
Hepatoblastoma, metastatic, liver	1 (2%)	–	–	–
Hepatocellular carcinoma, metastatic, liver	–	2 (4%)	–	–
Nose	(50)	(50)	(50)	(50)
Adenocarcinoma				2 (4%)
Carcinoma, metastatic, Harderian gland	1 (2%)	–	–	–
Pleura	(0)	(0)	(1)	(0)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (100%)	–
Trachea	(50)	(49)	(50)	(50)
Special Senses System				
Ear	(0)	(0)	(0)	(1)
Eye	(50)	(49)	(50)	(49)
Carcinoma, metastatic, Harderian gland	1 (2%)	–	–	–
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	3 (6%)	3 (6%)	2 (4%)
Carcinoma	1 (2%)	–	–	–
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Carcinoma	1 (2%)	–	–	–
Urinary bladder	(50)	(50)	(50)	(49)
Systemic Lesions				
Multiple organsb	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	3 (6%)	–	1 (2%)
Lymphoma malignant	9 (18%)	6 (12%)	16 (32%)	4 (8%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	34	33	34	13
Total primary neoplasms	43	53	51	18
Total animals with benign neoplasms	15	24	22	6
Total benign neoplasms	18	31	25	8
Total animals with malignant neoplasms	24	19	23	9
Total malignant neoplasms	25	21	26	10

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Total animals with metastatic neoplasms	3	6	3	1
Total metastatic neoplasms	16	8	9	5
Total animals with uncertain neoplasms benign or malignant	–	1	–	–
Total uncertain neoplasms	–	1	–	–

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

Table D-2. Statistical Analysis of Primary Neoplasms in Female Mice in the Two-year Inhalation Study of 2,3-Butanedione

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Harderian Gland: Adenoma				
Overall rate[a]	3/50 (6%)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted rate[b]	6.7%	6.3%	6.3%	6.1%
Terminal rate[c]	3/36 (8%)	3/40 (8%)	3/42 (7%)	0/17 (0%)
First incidence (days)	731 (T)	731 (T)	731 (T)	547
Poly-3 test[d]	P = 0.526N	P = 0.636N	P = 0.638N	P = 0.636N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted rate	8.9%	6.3%	6.3%	6.1%
Terminal rate	3/36 (8%)	3/40 (8%)	3/42 (7%)	0/17 (0%)
First incidence (days)	690	731 (T)	731 (T)	547
Poly-3 test	P = 0.387N	P = 0.471N	P = 0.474N	P = 0.488N
Liver: Hepatocellular Adenoma				
Overall rate	6/50 (12%)	11/50 (22%)	7/50 (14%)	3/50 (6%)
Adjusted rate	13.3%	23.1%	14.8%	9.2%
Terminal rate	5/36 (14%)	11/40 (28%)	7/42 (17%)	2/17 (12%)
First incidence (days)	653	731 (T)	731 (T)	585
Poly-3 test	P = 0.281N	P = 0.168	P = 0.534	P = 0.428N
Liver: Hepatocellular Carcinoma				
Overall rate	5/50 (10%)	5/50 (10%)	3/50 (6%)	0/50 (0%)
Adjusted rate	11.1%	10.5%	6.3%	0.0%
Terminal rate	5/36 (14%)	5/40 (13%)	3/42 (7%)	0/17 (0%)
First incidence (days)	731 (T)	731 (T)	731 (T)	–[e]
Poly-3 test	P = 0.047N	P = 0.594N	P = 0.329N	P = 0.073N

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma				
Overall rate	11/50 (22%)	15/50 (30%)	9/50 (18%)	3/50 (6%)
Adjusted rate	24.3%	31.5%	19.0%	9.2%
Terminal rate	10/36 (28%)	15/40 (38%)	9/42 (21%)	2/17 (12%)
First incidence (days)	653	731 (T)	731 (T)	585
Poly-3 test	P = 0.044N	P = 0.293	P = 0.358N	P = 0.083N
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	6/50 (12%)	5/50 (10%)	3/50 (6%)	0/50 (0%)
Adjusted rate	13.2%	10.5%	6.3%	0.0%
Terminal rate	5/36 (14%)	5/40 (13%)	3/42 (7%)	0/17 (0%)
First incidence (days)	590	731 (T)	731 (T)	–
Poly-3 test	P = 0.026N	P = 0.468N	P = 0.222N	P = 0.046N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	12/50 (24%)	15/50 (30%)	9/50 (18%)	3/50 (6%)
Adjusted rate	26.2%	31.5%	19.0%	9.2%
Terminal rate	10/36 (28%)	15/40 (38%)	9/42 (21%)	2/17 (12%)
First incidence (days)	590	731 (T)	731 (T)	585
Poly-3 test	P = 0.030N	P = 0.369	P = 0.279N	P = 0.058N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	3/50 (6%)	5/50 (10%)	2/50 (4%)	1/50 (2%)
Adjusted rate	6.7%	10.5%	4.2%	3.1%
Terminal rate	3/36 (8%)	5/40 (13%)	1/42 (2%)	1/17 (6%)
First incidence (days)	731 (T)	731 (T)	714	731 (T)
Poly-3 test	P = 0.229N	P = 0.388	P = 0.476N	P = 0.435N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)	0/50 (0%)
Adjusted rate	6.6%	2.1%	4.2%	0.0%
Terminal rate	2/36 (6%)	0/40 (0%)	1/42 (2%)	0/17 (0%)
First incidence (days)	663	697	639	–
Poly-3 test	P = 0.154N	P = 0.286N	P = 0.476N	P = 0.194N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	6/50 (12%)	6/50 (12%)	4/50 (8%)	1/50 (2%)
Adjusted rate	13.3%	12.6%	8.4%	3.1%
Terminal rate	5/36 (14%)	5/40 (13%)	2/42 (5%)	1/17 (6%)
First incidence (days)	663	697	639	731 (T)
Poly-3 test	P = 0.087N	P = 0.582N	P = 0.336N	P = 0.136N

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Ovary: Cystadenoma				
Overall rate	0/49 (0%)	2/50 (4%)	3/49 (6%)	0/50 (0%)
Adjusted rate	0.0%	4.2%	6.5%	0.0%
Terminal rate	0/35 (0%)	2/40 (5%)	3/41 (7%)	0/17 (0%)
First incidence (days)	–	731 (T)	731 (T)	–
Poly-3 test	P = 0.465	P = 0.255	P = 0.128	–[f]
Ovary: Luteoma				
Overall rate	0/49 (0%)	1/50 (2%)	3/49 (6%)	0/50 (0%)
Adjusted rate	0.0%	2.1%	6.5%	0.0%
Terminal rate	0/35 (0%)	1/40 (3%)	3/41 (7%)	0/17 (0%)
First incidence (days)	–	731 (T)	731 (T)	–
Poly-3 test	P = 0.390	P = 0.516	P = 0.128	–
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	2/49 (4%)	4/49 (8%)	2/50 (4%)	0/48 (0%)
Adjusted rate	4.5%	8.4%	4.2%	0.0%
Terminal rate	1/35 (3%)	2/39 (5%)	2/42 (5%)	0/17 (0%)
First incidence (days)	663	561	731 (T)	–
Poly-3 test	P = 0.199N	P = 0.371	P = 0.669N	P = 0.323N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	2/49 (4%)	5/49 (10%)	3/50 (6%)	0/48 (0%)
Adjusted rate	4.5%	10.5%	6.3%	0.0%
Terminal rate	1/35 (3%)	3/39 (8%)	3/42 (7%)	0/17 (0%)
First incidence (days)	663	561	731 (T)	–
Poly-3 test	P = 0.235N	P = 0.246	P = 0.531	P = 0.323N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	3/50 (6%)
Adjusted rate	0.0%	4.2%	6.3%	9.0%
Terminal rate	0/36 (0%)	1/40 (3%)	2/42 (5%)	0/17 (0%)
First incidence (days)	–	703	713	547
Poly-3 test	P = 0.049	P = 0.251	P = 0.128	P = 0.074
All Organs: Histiocytic Sarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	2.2%	6.3%	0.0%	3.0%
Terminal rate	0/36 (0%)	2/40 (5%)	0/42 (0%)	0/17 (0%)
First incidence (days)	499	644	–	252
Poly-3 test	P = 0.458N	P = 0.323	P = 0.493N	P = 0.682

	Chamber Control	12.5 ppm	25 ppm	50 ppm
All Organs: Malignant Lymphoma				
Overall rate	9/50 (18%)	6/50 (12%)	16/50 (32%)	4/50 (8%)
Adjusted rate	19.9%	12.6%	33.2%	12.0%
Terminal rate	7/36 (19%)	5/40 (13%)	14/42 (33%)	1/17 (6%)
First incidence (days)	687	680	592	325
Poly-3 test	P = 0.527	P = 0.249N	P = 0.110	P = 0.268N
All Organs: Benign Neoplasms				
Overall rate	15/50 (30%)	24/50 (48%)	22/50 (44%)	6/50 (12%)
Adjusted rate	32.7%	49.1%	46.1%	18.2%
Terminal rate	11/36 (31%)	20/40 (50%)	20/42 (48%)	4/17 (24%)
First incidence (days)	653	561	639	547
Poly-3 test	P = 0.148N	P = 0.076	P = 0.130	P = 0.121N
All Organs: Malignant Neoplasms				
Overall rate	24/50 (48%)	19/50 (38%)	23/50 (46%)	9/50 (18%)
Adjusted rate	50.7%	39.2%	47.3%	25.6%
Terminal rate	16/36 (44%)	14/40 (35%)	19/42 (45%)	3/17 (18%)
First incidence (days)	499	644	592	252
Poly-3 test	P = 0.039N	P = 0.176N	P = 0.451N	P = 0.018N
All Organs: Benign or Malignant Neoplasms				
Overall rate	34/50 (68%)	33/50 (66%)	34/50 (68%)	13/50 (26%)
Adjusted rate	71.0%	66.5%	69.9%	36.4%
Terminal rate	24/36 (67%)	25/40 (63%)	29/42 (69%)	6/17 (35%)
First incidence (days)	499	561	592	252
Poly-3 test	P = 0.002N	P = 0.396N	P = 0.540N	P < 0.001N

T = Terminal euthanasia
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, 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 euthanasia.
[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 euthanasia. 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. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Two-year Inhalation Study of 2,3-Butanedione[a]

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	10	6	4	15
Natural deaths	4	4	4	17
Survivors				
Died last week of study	–	–	–	1
Terminal euthanasia	36	40	42	17
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Epithelium, hyperplasia	–	1 (2%)	–	–
Periesophageal tissue, inflammation, chronic active	–	–	–	9 (18%)
Gallbladder	(42)	(45)	(45)	(37)
Hyperplasia	–	1 (2%)	–	–
Inflammation	–	–	–	3 (8%)
Ulcer	–	1 (2%)	–	–
Intestine large, cecum	(47)	(49)	(50)	(41)
Inflammation	2 (4%)	–	2 (4%)	–
Inflammation, granulomatous	1 (2%)	–	–	–
Necrosis	–	1 (2%)	–	–
Intestine large, colon	(47)	(49)	(50)	(46)
Intestine large, rectum	(49)	(49)	(46)	(43)
Inflammation	–	1 (2%)	–	–
Intestine small, duodenum	(47)	(48)	(49)	(38)
Inflammation	1 (2%)	2 (4%)	–	–
Necrosis	1 (2%)	–	–	–
Intestine small, ileum	(47)	(48)	(49)	(39)
Hyperplasia, lymphoid	–	1 (2%)	1 (2%)	–
Inflammation	1 (2%)	–	–	–
Necrosis	1 (2%)	1 (2%)	–	–
Ulcer	1 (2%)	–	–	–
Intestine small, jejunum	(46)	(49)	(49)	(39)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Liver	(50)	(50)	(50)	(50)
Amyloid deposition	–	–	1 (2%)	–
Angiectasis	1 (2%)	–	–	1 (2%)
Basophilic focus	1 (2%)	3 (6%)	3 (6%)	1 (2%)
Clear cell focus	1 (2%)	7 (14%)	3 (6%)	–
Cyst	1 (2%)	–	1 (2%)	2 (4%)
Eosinophilic focus	4 (8%)	4 (8%)	–	1 (2%)
Fatty change	–	1 (2%)	2 (4%)	–
Fibrosis, focal	–	1 (2%)	–	–
Hematopoietic cell proliferation	–	1 (2%)	1 (2%)	1 (2%)
Hepatodiaphragmatic nodule	–	–	–	1 (2%)
Hyperplasia, lymphoid	–	2 (4%)	4 (8%)	–
Infiltration cellular, lymphocyte	–	–	–	1 (2%)
Inflammation	–	–	1 (2%)	–
Mixed cell focus	2 (4%)	–	–	–
Tension lipidosis	–	–	–	1 (2%)
Centrilobular, hepatocyte, hypertrophy	–	–	–	1 (2%)
Hepatocyte, degeneration	–	1 (2%)	–	–
Hepatocyte, necrosis	3 (6%)	4 (8%)	1 (2%)	1 (2%)
Kupffer cell, hypertrophy	–	–	–	1 (2%)
Mesentery	(6)	(10)	(4)	(1)
Fat, necrosis	4 (67%)	9 (90%)	4 (100%)	–
Pancreas	(50)	(50)	(50)	(47)
Cyst	1 (2%)	–	–	1 (2%)
Inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)	–
Necrosis	–	1 (2%)	–	–
Acinus, atrophy	1 (2%)	2 (4%)	1 (2%)	–
Salivary glands	(49)	(50)	(50)	(50)
Inflammation, chronic active	–	–	–	3 (6%)
Stomach, forestomach	(50)	(50)	(50)	(49)
Infiltration cellular, lymphoid	–	–	1 (2%)	–
Inflammation	3 (6%)	1 (2%)	3 (6%)	–
Mineralization	1 (2%)	–	–	–
Epithelium, hyperplasia	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Stomach, glandular	(49)	(50)	(50)	(46)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Cyst	1 (2%)	–	–	–
Degeneration	–	1 (2%)	–	–
Infiltration cellular, lymphoid	1 (2%)	–	1 (2%)	–
Inflammation	5 (10%)	–	1 (2%)	2 (4%)
Mineralization	3 (6%)	–	–	1 (2%)
Necrosis	1 (2%)	–	–	–
Tooth	(1)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	1 (2%)	–	3 (6%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	4 (8%)	1 (2%)	2 (4%)	4 (8%)
Inflammation, acute	2 (4%)	–	–	2 (4%)
Inflammation, chronic active	–	2 (4%)	1 (2%)	7 (14%
Mineralization	–	1 (2%)	–	1 (2%)
Necrosis	1 (2%)	–	–	2 (4%)
Polyarteritis	1 (2%)	–	–	3 (6%)
Valve, inflammation, chronic active	–	–	–	1 (2%)
Valve, thrombosis	1 (2%)	–	–	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	1 (2%)	–	–	
Angiectasis	–	–	–	2 (4%)
Hematopoietic cell proliferation	–	–	–	1 (2%)
Hemorrhage	–	–	–	1 (2%)
Hyperplasia	–	–	1 (2%)	1 (2%)
Hypertrophy	–	–	–	2 (4%)
Inflammation, chronic active	–	–	1 (2%)	–
Necrosis	1 (2%)	–	1 (2%)	–
Vacuolization cytoplasmic	1 (2%)	1 (2%)	–	–
Zona fasciculata, hyperplasia, focal	–	1 (2%)	–	–
Zona fasciculata, hypertrophy, focal	2 (4%)	–	2 (4%)	3 (6%)
Adrenal medulla	(50)	(50)	(49)	(50)
Hyperplasia	2 (4%)	4 (8%)	1 (2%)	2 (4%)
Islets, pancreatic	(49)	(50)	(49)	(48)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Hyperplasia	–	1 (2%)	–	–
Parathyroid gland	(38)	(39)	(46)	(46)
Hyperplasia	–	–	1 (2%)	–
Pituitary gland	(49)	(49)	(50)	(48)
Pars distalis, angiectasis	3 (6%)	1 (2%)	1 (2%)	–
Pars distalis, hyperplasia	15 (31%)	7 (14%)	6 (12%)	1 (2%)
Pars distalis, inflammation, suppurative	–	–	1 (2%)	–
Pars intermedia, angiectasis	–	–	1 (2%)	–
Thyroid gland	(50)	(49)	(48)	(50)
Inflammation, chronic active	–	–	–	9 (18%)
Mineralization	–	–	–	1 (2%)
Follicular cell, hyperplasia	–	2 (4%)	1 (2%)	–
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(47)	(49)	(48)	(44)
Ovary	–	–	(49)	(50)
Angiectasis	–	–	1 (2%)	
Cyst	15 (31%)	17 (34%)	12 (24%)	12 (24%)
Hemorrhage	–	1 (2%)	1 (2%)	–
Hyperplasia, tubular	–	1 (2%)	–	–
Inflammation	2 (4%)	1 (2%)	3 (6%)	6 (12%)
Mineralization	–	1 (2%)	1 (2%)	–
Necrosis	–	1 (2%)	1 (2%)	–
Uterus	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	4 (8%)	2 (4%)	–
Hemorrhage	1 (2%)	–	–	–
Inflammation	4 (8%)	2 (4%)	–	–
Thrombosis	1 (2%)	3 (6%)	1 (2%)	–
Artery, necrosis	1 (2%)	–	–	–
Endometrium, hyperplasia, cystic	42 (84%)	43 (86%)	47 (94%)	30 (60%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Myeloid cell, hyperplasia	3 (6%)	2 (4%)	7 (14%)	33 (67%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Lymph node	(11)	(10)	(11)	(7)
Deep cervical, hyperplasia, lymphoid	–	–	1 (9%)	4 (57%)
Deep cervical, infiltration cellular, plasma cell	–	–	–	1 (14%)
Iliac, ectasia	–	1 (10%)	–	–
Lumbar, ectasia	1 (9%)	1 (10%)	1 (9%)	–
Lumbar, hyperplasia	1 (9%)	–	–	–
Lumbar, hyperplasia, lymphoid	1 (9%)	–	1 (9%)	–
Renal, amyloid deposition	1 (9%)	–	–	–
Renal, ectasia	–	–	1 (9%)	–
Renal, hyperplasia, lymphoid	2 (18%)	1 (10%)	–	–
Renal, necrosis	1 (9%)	–	–	–
Lymph node, bronchial	(40)	(42)	(43)	(43)
Ectasia	1 (3%)	–	–	–
Hematopoietic cell proliferation	–	1 (2%)	–	1 (2%)
Hyperplasia, lymphoid	5 (13%)	7 (17%)	4 (9%)	1 (2%)
Inflammation, chronic active	–	–	–	5 (12%)
Necrosis, lymphoid	–	1 (2%)	–	1 (2%)
Lymph node, mandibular	(39)	(47)	(45)	(39)
Amyloid deposition	1 (3%)	–	–	–
Ectasia	–	1 (2%)		3 (8%)
Hematopoietic cell proliferation	–	–	2 (4%)	1 (3%)
Hyperplasia, lymphoid	4 (10%)	5 (11%)	12 (27%)	12 (31%)
Hyperplasia, plasma cell	–	–	–	2 (5%)
Infiltration cellular, plasma cell	1 (3%)	–	–	5 (13%)
Infiltration cellular, polymorphonuclear	–	–	–	1 (3%)
Inflammation, suppurative	–	–	–	1 (3%)
Lymph node, mediastinal	(40)	(38)	(41)	(33)
Hematopoietic cell proliferation	–	1 (3%)	–	–
Hyperplasia, lymphoid	1 (3%)	2 (5%)	2 (5%)	1 (3%)
Hyperplasia, plasma cell	–	–	1 (2%)	1 (3%)
Inflammation, acute	1 (3%)	–	–	–
Inflammation, chronic active	–	–	–	2 (6%)
Necrosis, lymphoid	–	1 (3%)		1 (3%)
Lymph node, mesenteric	(49)	(50)	(50)	(45)
Ectasia	–	1 (2%)	1 (2%)	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Hematopoietic cell proliferation	–	–	–	2 (4%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Infiltration cellular, plasma cell	1 (2%)	–	–	–
Spleen	(50)	(50)	(50)	(48)
Amyloid deposition	–	–	1 (2%)	–
Angiectasis	–	–	–	1 (2%)
Hematopoietic cell proliferation	8 (16%)	14 (28%)	5 (10%)	11 (23%)
Hyperplasia, lymphoid	10 (20%)	15 (30%)	10 (20%)	4 (8%)
Inflammation	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Necrosis	–	1 (2%)	1 (2%)	3 (6%)
Lymphoid follicle, atrophy	–	–	–	1 (2%)
Thymus	(47)	(50)	(49)	(45)
Atrophy	4 (9%)	5 (10%)	2 (4%)	12 (27%)
Cyst	–	1 (2%)	–	–
Ectopic parathyroid gland	4 (9%)	8 (16%)	4 (8%)	3 (7%)
Hyperplasia, lymphoid	3 (6%)	4 (8%)	2 (4%)	1 (2%)
Inflammation, chronic	2 (4%)	–	–	–
Inflammation, chronic active	–	–	–	8 (18%)
Integumentary System				
Mammary gland	(49)	(50)	(50)	(49)
Hyperplasia	1 (2%)	–	–	–
Inflammation, chronic	1 (2%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Erosion	1 (2%)	–	–	–
Fibrosis	–	–	–	1 (2%)
Inflammation, chronic active	1 (2%)	2 (4%)	–	3 (6%)
Epidermis, hyperplasia	1 (2%)	–	–	1 (2%)
Subcutaneous tissue, necrosis	–	–	–	2 (4%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	13 (26%)	12 (24%)	17 (34%)	3 (6%)
Skeletal muscle	(6)	(1)	(3)	(4)
Degeneration	2 (33%)	–	1 (33%)	1 (25%)
Inflammation	–	1 (100%)	–	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Nervous System				
Brain	(50)	(50)	(50)	(50)
Infiltration cellular	–	1 (2%)	–	1 (2%)
Infiltration cellular, lymphocyte	2 (4%)	–	2 (4%)	5 (10%)
Inflammation, acute	1 (2%)	–	–	1 (2%)
Inflammation, chronic	–	–	1 (2%)	–
Peripheral nerve	(2)	(1)	(2)	(2)
Degeneration	1 (50%)	–	2 (100%)	1 (50%)
Spinal cord	(2)	(1)	(2)	(2)
Respiratory System				
Larynx	(49)	(50)	(50)	(49)
Inflammation, chronic active	4 (8%)	5 (10%)	22 (44%)	36 (73%)
Lumen, exudate	–	–	–	4 (8%)
Respiratory epithelium, hyperplasia	–	–	4 (8%)	1 (2%)
Respiratory epithelium, metaplasia, atypical, squamous	–	1 (2%)	–	1 (2%)
Respiratory epithelium, metaplasia, squamous	2 (4%)	–	6 (12%)	48 (98%)
Respiratory epithelium, necrosis	1 (2%)	1 (2%)	14 (28%)	32 (65%)
Respiratory epithelium, regeneration	–	–	3 (6%)	30 (61%)
Squamous epithelium, hyperplasia	4 (8%)	13 (26%)	34 (68%)	40 (82%)
Submucosa, mineralization	–	1 (2%)	–	–
Lung	(50)	(50)	(50)	(50)
Foreign body	–	–	–	1 (2%)
Hematopoietic cell proliferation	–	1 (2%)	–	–
Hemorrhage	1 (2%)	–	–	–
Hyperplasia, lymphoid	1 (2%)	1 (2%)	–	–
Inflammation, suppurative	–	1 (2%)	–	5 (10%)
Inflammation, chronic active	1 (2%)	–	1 (2%)	2 (4%)
Necrosis	–	–	–	1 (2%)
Alveolar epithelium, hyperplasia, focal	3 (6%)	–	–	–
Alveolus, infiltration cellular, histiocyte	2 (4%)	3 (6%)	3 (6%)	4 (8%)
Alveolus, inflammation, suppurative	–	–	–	1 (2%)
Alveolus, epithelium, hyperplasia	–	–	1 (2%)	–
Artery, mediastinum, inflammation, chronic active	–	–	–	1 (2%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Bronchiole, inflammation, suppurative	–	–	–	1 (2%)
Bronchiole, inflammation, chronic	–	–	–	1 (2%)
Bronchiole, epithelium, hyperplasia	1 (2%)	–	–	2 (4%)
Bronchus, inflammation, suppurative	–	–	–	2 (4%)
Bronchus, regeneration	–	–	–	1 (2%)
Bronchus, epithelium, degeneration	–	–	–	4 (8%)
Bronchus, epithelium, necrosis	2 (4%)	–	–	5 (10%)
Bronchus, epithelium, regeneration	2 (4%)	–	–	38 (76%)
Bronchus, smooth muscle, mineralization	–	–	–	3 (6%)
Bronchus, submucosa, fibrosis	–	–	–	5 (10%)
Interstitium, fibrosis	–	–	–	1 (2%)
Mediastinum, inflammation, suppurative	–	1 (2%)	1 (2%)	8 (16%)
Mediastinum, inflammation, chronic active	–	–	1 (2%)	7 (14%)
Pleura, inflammation, suppurative	–	–	–	5 (10%)
Pleura, inflammation, chronic active	–	–	1 (2%)	–
Nose	(50)	(50)	(50)	(50)
Foreign body	–	–	3 (6%)	3 (6%)
Inflammation, suppurative	3 (6%)	20 (40%)	50 (100%)	50 (100%)
Glands, respiratory epithelium, cyst	–	–	–	2 (4%)
Glands, sinus, metaplasia, respiratory	–	–	–	12 (24%)
Lamina propria, fibrosis	–	–	47 (94%)	49 (98%)
Lateral wall, inflammation, chronic active	1 (2%)	–	1 (2%)	–
Mucosa, regeneration	–	–	39 (78%)	48 (96%)
Olfactory epithelium, accumulation, hyaline droplet	11 (22%)	11 (22%)	17 (34%)	2 (4%)
Olfactory epithelium, atrophy	–	41 (82%)	49 (98%)	45 (90%)
Olfactory epithelium, metaplasia, respiratory	–	22 (44%)	46 (92%)	49 (98%)
Olfactory epithelium, necrosis	–	–	1 (2%)	20 (40%)
Respiratory epithelium, accumulation, hyaline droplet	18 (36%)	28 (56%)	28 (56%)	3 (6%)
Respiratory epithelium, hyperplasia	–	1 (2%)	–	2 (4%)
Respiratory epithelium, metaplasia, squamous	1 (2%)	9 (18%)	48 (96%)	50 (100%)
Respiratory epithelium, necrosis	1 (2%)	5 (10%)	33 (66%)	50 (100%)
Septum, perforation	–	–	6 (12%)	5 (10%)

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Turbinate, atrophy	–	32 (64%)	50 (100%)	50 (100%)
Turbinate, necrosis	–	–	1 (2%)	11 (22%)
Pleura	(0)	(0)	(1)	(0)
Trachea	(50)	(49)	(50)	(50)
Inflammation, chronic active	1 (2%)	–	4 (8%)	42 (84%)
Necrosis	–	–	3 (6%)	48 (96%)
Carina, inflammation, chronic active	–	–	–	1 (2%)
Carina, epithelium, regeneration	–	–	–	1 (2%)
Carina, submucosa, fibrosis	–	–	–	6 (12%)
Carina, submucosa, mineralization	–	–	–	5 (10%)
Epithelium, hyperplasia	–	–	1 (2%)	1 (2%)
Epithelium, metaplasia, squamous	–	–	–	2 (4%)
Epithelium, regeneration	–	–	9 (18%)	45 (90%)
Glands, hyperplasia	–	–	–	1 (2%)
Lumen, exudate	–	–	–	12 (24%)
Submucosa, fibrosis	–	–	–	44 (88%)
Special Senses System				
Ear	(0)	(0)	(0)	(1)
Inflammation, suppurative	–	–	–	1 (100%)
Eye	(50)	(49)	(50)	(49)
Anterior chamber, inflammation, suppurative	–	–	4 (8%)	3 (6%)
Cornea, fibrosis	–	–	1 (2%)	–
Cornea, inflammation, acute	1 (2%)	2 (4%)	20 (40%)	23 (47%)
Cornea, mineralization	–	–	13 (26%)	16 (33%)
Cornea, necrosis	–	–	–	6 (12%)
Cornea, epithelium, hyperplasia	2 (4%)	2 (4%)	10 (20%)	9 (18%)
Cornea, epithelium, ulcer	–	–	10 (20%)	10 (20%)
Lens, cataract	–	–	–	2 (4%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	4 (8%)	1 (2%)	4 (8%)
Inflammation	–	–	–	1 (2%)
Pigmentation, porphyrin	–	–	–	1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Amyloid deposition	–	–	1 (2%)	–

	Chamber Control	12.5 ppm	25 ppm	50 ppm
Cyst	1 (2%)	–	–	–
Hyperplasia, lymphoid	1 (2%)	3 (6%)	3 (6%)	–
Infarct	–	3 (6%)	3 (6%)	2 (4%)
Inflammation	–	–	–	1 (2%)
Metaplasia, osseous	1 (2%)	1 (2%)	1 (2%)	–
Necrosis	–	1 (2%)	–	–
Nephropathy	14 (28%)	15 (30%)	6 (12%)	6 (12%)
Capsule, inflammation	–	1 (2%)	–	–
Urinary bladder	(50)	(50)	(50)	(49)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)	–

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

Appendix E. Genetic Toxicology

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Tables

E.1. Bacterial Mutagenicity Test Protocol

2,3-Butanedione was tested in two independent bacterial gene mutation assays. In the first assay, testing procedures followed protocols reported by Zeiger et al.[157]. Briefly, a commercially obtained sample of 2,3-butanedione was sent to the laboratory under code. It was incubated with each of the *Salmonella typhimurium* tester strains (TA97, TA98, TA100, TA1535) 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 37°C. 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 37°C.

In the second assay, a sample of the same lot of 2,3-butanedione that was used in the 2-year bioassays was sent to the testing laboratory for assessment of mutagenicity in *S. typhimurium* strains TA100, TA97a, and TA98 and in *Escherichia coli* strain WP2 *uvrA*/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 (*S. typhimurium* strains) or tryptophan-independent (*E. coli* strain) mutant colonies arising on these plates were counted following incubation for 2 days at 37°C.

For all strains, each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of 2,3-butanedione. The high dose was limited by toxicity to 1,666 µg/plate in the first assay and 2,000 µg/plate in the second assay.

In these assays, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one 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, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

E.2. Mouse Bone Marrow Micronucleus Test Protocol

Published LD_{50} information was used to select the range of doses employed. The high dose was set at two times the stated LD_{50}, and six additional lower doses were tested, for a total of seven treated groups. The standard three-exposure protocol is described in detail by Shelby et al.[107]. Male B6C3F1/N mice were injected intraperitoneally (three times at 24-hour intervals) with 2,3-butanedione dissolved in phosphate buffered saline. Vehicle control animals were injected with phosphate buffered saline only. The positive control animals received injections of 15 mg/kg cyclophosphamide. The animals were euthanized 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs; reticulocytes) were scored for the frequency of micronucleated cells in each of five mice per dose group. In addition, the percentage of PCEs among the total erythrocyte population in the bone marrow was scored for each dose group as a measure of toxicity. There was no lethality in any of the seven treatment groups, but animals in the 500 mg/kg group showed clinical signs of toxicity, indicating that the maximum tolerated dose had been tested.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the vehicle control group. In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

E.3. Rat and Mouse Peripheral Blood Micronucleus Test Protocol

The procedures used for the rat and mouse peripheral blood micronucleus assay have been described in detail[158-160]. Briefly, at the termination of the 3-month studies, one to two drops of blood from five male and five female rats and mice exposed to 0 to 100 ppm 2,3-butanedione were collected in microtubes with EDTA and shipped on cool packs to the genetic toxicity testing laboratory for processing and fixation in ultracold methanol, as per procedures described in the MicroFlow^{PLUS} Kit for mouse or rat blood samples (Litron Laboratories, Rochester, NY). A FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) was used to analyze of the samples. PCEs were identified by the presence of an active transferrin receptor (CD71+) on the cell surface and normochromatic erythrocytes (NCEs; mature erythrocytes) were CD71–. For the rat samples, only PCEs with the highest CD71 activity were evaluated due to the speed and efficiency with which the rat spleen removes damaged PCEs from circulation. Thus, although micronucleus frequency was evaluated in both PCEs and NCEs, the appropriate cell population for this assessment in rats is the young PCE population. Micronuclei were detected using propidium iodide (a DNA stain) in conjunction with RNase treatment. Approximately 1×10^6 NCEs (CD71–), and 20,000 PCEs (CD71+) were evaluated per animal for the presence of micronuclei (propidium iodide-associated fluorescence). In addition, for each blood sample, the percentage of PCEs in 1×10^6 erythrocytes was determined as a measure of 2,3-butanedione-associated bone marrow toxicity.

Based on prior experience with the large number of cells scored using flow cytometric scoring techniques[161], it is reasonable to assume that the proportion of micronucleated cells 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 assay, a positive response is preferably based on the observation of both a significant trend as well an observation of at least one dose group significantly elevated over the concurrent control group. If only one statistical test (trend or pairwise) is significant, the micronucleus assay is judged to be equivocal. The absence of both a significant trend and a significant dose results in a negative call for the assay. Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed, and the magnitudes of those effects.

E.4. 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.5. Results

2,3-Butanedione was tested in bacterial reverse mutation assays, an acute-exposure mouse bone marrow micronucleus test, and subchronic exposure peripheral blood micronucleus tests. 2,3-Butanedione was mutagenic in both the bacterial mutagenicity assays, with and without S9 mix.

In the first bacterial mutation assay (Table E-1), a weak positive response (less than twofold increase in revertants, but a clear, reproducible, dose-related increase that approached the twofold level) was observed in *S. typhimurium* strain TA97 in the absence of S9 mix and in the presence of 10% and 30% induced hamster and rat liver S9 mixes. The dose range over which the response was observed in TA97 ranged from 10 to 333 µg/plate. No mutagenic responses were observed in any other strain, although some trials were concluded to demonstrate an equivocal response. These equivocal responses were not easily replicated, unlike the weak positive responses seen in strain TA97.

In the second bacterial mutation assay (Table E-2), conducted with the same lot of 2,3-butanedione that was used in the 2-year rodent bioassay, a positive response was seen in

S. typhimurium strain TA97a in the absence of exogenous metabolic activation (10% induced rat liver S9 mix), and a response that was equivocal was seen with metabolic activation. In the *E. coli* strain (WP2 *uvrA* pKM101) that was included in this second assay, clearly positive responses were seen in all trials conducted with and without S9 mix, suggesting that 2,3-butanedione is a direct-acting mutagen that is not detoxified by induced rat liver S9. The *E. coli* strain reverts via base substitution at the tryptophan locus at an AT base pair. Equivocal results were obtained in *S. typhimurium* strain TA100, with and without S9, and no mutagenicity was observed with strain TA98, with and without S9.

In an acute micronucleus test conducted in male mice, the frequency of micronucleated PCEs was measured in bone marrow following intraperitoneal injection of 2,3-butanedione once daily for 3 days (Table E-3). In this test, no increases in micronucleated PCEs were observed over the dose range of 7.812 to 500 mg 2,3-butanedione/kg body weight per day.

At the end of the 3-month studies, peripheral blood samples were obtained from male and female rats and mice and analyzed for the frequency of micronucleated PCEs and NCEs (Table E-4 and Table E-5). In these studies, no increases in the frequencies of PCEs or NCEs occurred in either sex or species exposed to 2,3-butanedione. The percentage of PCEs among circulating red blood cells was unaffected by exposure to 2,3-butanedione, suggesting the chemical had no effect on erythropoiesis.

Table E-1. Mutagenicity of 2,3-Butanedione in *Salmonella typhimurium*[a]

Strain	Dose (µg/ plate)	Without S9	Without S9	With 5% Hamster S9	With 5% Hamster S9	With 10% Hamster S9	With 10% Hamster S9	With 30% Hamster S9	With 30% Hamster S9	With 5% Rat S9	With 5% Rat S9	With 10% Rat S9	With 10% Rat S9	With 30% Rat S9	With 30% Rat S9
TA100	0	139 ± 5		109 ± 9	167 ± 16	95 ± 2	159 ± 4	135 ± 6	104 ± 6	100 ± 7	162 ± 6	103 ± 13	169 ± 8	125 ± 12	110 ± 16
	10	138 ± 2			158 ± 5		159 ± 0				156 ± 1		157 ± 5		
	33	131 ± 7		113 ± 9	161 ± 3	104 ± 6	164 ± 3		103 ± 7	105 ± 0	168 ± 6	98 ± 4	170 ± 7		113 ± 2
	66			113 ± 6	163 ± 8	123 ± 4	168 ± 5		100 ± 8	119 ± 6	148 ± 0	101 ± 1	168 ± 6		117 ± 4
	100	138 ± 7		129 ± 8	157 ± 1	111 ± 7	167 ± 8	164 ± 7	95 ± 5	102 ± 2	155 ± 7	107 ± 11	165 ± 4	166 ± 8	117 ± 2
	166			139 ± 4	167 ± 10	111 ± 12	155 ± 6		107 ± 10	126 ± 9	167 ± 5	95 ± 8	156 ± 8		133 ± 6
	333	120 ± 9[b]		156 ± 8	118 ± 6	145 ± 11	131 ± 9	179 ± 13	111 ± 8	142 ± 4	131 ± 2	139 ± 1	152 ± 10	195 ± 6	139 ± 2
	666	79 ± 18[b]						137 ± 4						95 ± 3	
	1,000							77 ± 5[b]						95 ± 3	
	1,666							0[c]						0[c]	
Trial summary		Negative		Equivocal	Negative	Equivocal	Negative	Equivocal	Negative	Equivocal	Negative	Equivocal	Negative	Equivocal	Equivocal
Positive control[d]		905 ± 20		819 ± 17	760 ± 68	560 ± 3	550 ± 23	508 ± 8	548 ± 11	919 ± 56	728 ± 49	811 ± 39	647 ± 30	394 ± 38	673 ± 41
TA97	0	138 ± 4	147 ± 9	161 ± 4		165 ± 7		167 ± 4	174 ± 8	158 ± 5		181 ± 3		200 ± 11	167 ± 4
	10	162 ± 6	175 ± 14	183 ± 5		156 ± 7		169 ± 11	182 ± 4	201 ± 6		171 ± 12		226 ± 6	176 ± 3
	33	187 ± 6	181 ± 4	209 ± 10		188 ± 1		178 ± 10	206 ± 7	221 ± 14		208 ± 4		254 ± 7	206 ± 10
	100	205 ± 9	227 ± 7	213 ± 1		212 ± 8		226 ± 14	250 ± 5	269 ± 16		235 ± 9		284 ± 19	227 ± 40
	333	237 ± 13	235 ± 8	211 ± 8		268 ± 16		270 ± 7	274 ± 4	284 ± 4		267 ± 5		282 ± 9	288 ± 1
	666	34 ± 16[c]	155 ± 18[c]	60 ± 17[b]		61 ± 12[b]			145 ± 22[b]	87 ± 12[b]		226 ± 18[b]			166 ± 24[b]
	1,000							50 ± 5[c]						40 ± 17[b]	
Trial summary		Weakly positive	Weakly positive	Equivocal		Weakly positive		Weakly positive	Weakly positive	Weakly positive		Weakly positive		Weakly positive	Weakly positive
Positive control		487 ± 28	512 ± 8	701 ± 8		655 ± 11		690 ± 25	589 ± 23	620 ± 21		630 ± 15		628 ± 11	548 ± 18
TA98	0	25 ± 7						20 ± 3						23 ± 3	
	10	25 ± 4													

Strain	Dose (µg/plate)	Without S9	Without S9	With 5% Hamster S9	With 5% Hamster S9	With 10% Hamster S9	With 10% Hamster S9	With 30% Hamster S9	With 30% Hamster S9	With 5% Rat S9	With 5% Rat S9	With 10% Rat S9	With 10% Rat S9	With 30% Rat S9	With 30% Rat S9
	33	22 ± 2													
	100	23 ± 4						24 ± 6						22 ± 1	
	333	20 ± 1						21 ± 4						23 ± 3	
	666	9 ± 5[b]						19 ± 2						18 ± 3	
	1,000							14 ± 3[b]						14 ± 3[b]	
	1,666							0[c]						0[c]	
Trial summary		Negative						Negative						Negative	
Positive control		394 ± 13						661 ± 32						495 ± 11	
TA1535	0	19 ± 0		10 ± 1		12 ± 1		15 ± 1		10 ± 1		13 ± 3		15 ± 1	
	10	16 ± 1		9 ± 1		12 ± 2				9 ± 0		11 ± 1			
	33	11 ± 1		9 ± 0		9 ± 1		12 ± 4		10 ± 1		11 ± 2		16 ± 1	
	66			8 ± 1		12 ± 1		13 ± 3		11 ± 1		11 ± 0		13 ± 3	
	100	13 ± 3		9 ± 1		12 ± 2		10 ± 1		9 ± 1		8 ± 0		14 ± 1	
	166			8 ± 1		10 ± 2		11 ± 2		10 ± 1		10 ± 1		13 ± 3	
	333	8 ± 1		11 ± 2				13 ± 0		7 ± 2		6 ± 1		20 ± 3	
	666	10 ± 2													
Trial summary		Negative		Negative		Negative		Negative		Negative		Negative		Negative	
Positive control		887 ± 36		347 ± 25		247 ± 20		264 ± 8		314 ± 12		130 ± 8		210 ± 18	

[a]Study was performed at SRI International. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol is presented by[157] 0 µg/plate was the solvent control.
[b]Slight toxicity.
[c]Slight toxicity and precipate on plate.
[d]The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table E-2. Mutagenicity of 2,3-Butanedione in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
TA100	0	109 ± 7	93 ± 7	130 ± 13	92 ± 13
	100	134 ± 15	106 ± 14	137 ± 4	101 ± 6
	200	165 ± 18	152 ± 26		
	250			164 ± 12	137 ± 15
	300	156 ± 5	144 ± 2		
	500	91 ± 9[b]	69 ± 11[b]	160 ± 10	176 ± 26
	750	2 ± 1[b]	Toxic	72 ± 21[b]	122 ± 19[b]
	1,000	Toxic	Toxic	18 ± 4[b]	49 ± 11[b]
	2,000			Toxic	Toxic
Trial summary		Equivocal	Equivocal	Negative	Weakly positive
Positive control[c]		590 ± 61	677 ± 63	665 ± 94	543 ± 35
TA97[a]	0	126 ± 26	102 ± 14	150 ± 8	127 ± 13
	100	145 ± 16	172 ± 26	155 ± 13	165 ± 11
	200	183 ± 22	148 ± 10		
	250			190 ± 31	195 ± 12
	300	212 ± 11	206 ± 20		
	500	170 ± 26[b]	164 ± 32	250 ± 18	197 ± 11
	750	166 ± 9[b]	123 ± 19[b]	164 ± 5[b]	100 ± 9[b]
	1,000	148 ± 9[b]	61 ± 22[b]	9 ± 2[b]	13 ± 10[b]
	2,000			Toxic	Toxic
Trial summary		Positive	Positive	Weakly Positive	Equivocal
Positive control		1,689 ± 54	2,207 ± 297	3,181 ± 499	2,082 ± 256
TA98	0	14 ± 4	17 ± 6	20 ± 5	21 ± 8
	100	17 ± 4	27 ± 4	19 ± 4	26 ± 10
	200	18 ± 0	20 ± 4		
	250			22 ± 8	26 ± 5
	300	16 ± 3	24 ± 5		
	500	18 ± 3	19 ± 3	15 ± 5	23 ± 4
	750	9 ± 2[b]	9 ± 6[b]	14 ± 1[b]	13 ± 6[b]
	1,000	3 ± 2[b]	6 ± 1[b]	2 ± 1[b]	7 ± 2[b]
	2,000			Toxic	Toxic
Trial summary		Negative	Negative	Negative	Negative
Positive control		609 ± 64	596 ± 77	811 ± 73	1,385 ± 139

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
***Escherichia coli* WP2 *uvrA*/pKM101 (analogous to TA102)**					
	0	141 ± 5	128 ± 14	175 ± 20	140 ± 4
	100	218 ± 22	206 ± 31	263 ± 32	280 ± 11
	200	279 ± 21	220 ± 40		
	250			319 ± 23	270 ± 26
	300	275 ± 24	260 ± 11		
	500	271 ± 57	278 ± 40	346 ± 38	298 ± 43
	750	165 ± 39[b]	98 ± 27[b]	342 ± 20	275 ± 23
	1,000	113 ± 32[b]	13 ± 3[b]	196 ± 57[b]	179 ± 14[b]
	2,000			Toxic	11 ± 4[b]
Trial summary		Positive	Positive	Positive	Positive
Positive control		2,111 ± 228	1,566 ± 137	1,063 ± 120	1,037 ± 58

[a]Study was performed at ILS, Inc. Data are presented as revertants/plate (mean ± standard deviation) from three plates. 0 µg/plate was the solvent control.
[b]Slight toxicity.
[c]The positive controls in the absence of metabolic activation were sodium azide (TA100), ICR191 (TA97a), 2-nitrofluorene (TA98), and 4-nitroquinoline-N-oxide (*E. coli*). The positive control for metabolic activation was 2-aminoanthracene (TA97a, TA98, *E. coli*) or benzo[a]pyrene (TA100).

Table E-3. Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Treated with 2,3-Butanedione by Intraperitoneal Injection for Three Days[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	PCEs[b] (%)
Phosphate buffered saline[d]	0	5	2.1 ± 0.29		45.50 ± 0.00
2,3-Butanedione	7.812	5	0.9 ± 0.33	0.9858	59.58 ± 0.82
	15.625	5	0.7 ± 0.30	0.9959	56.30 ± 2.43
	31.25	5	1.5 ± 0.45	0.8416	63.60 ± 1.94
	62.5	5	0.9 ± 0.19	0.9858	62.88 ± 1.97
	125	5	0.7 ± 0.25	0.9959	58.32 ± 2.50
	250	5	0.7 ± 0.20	0.9959	58.88 ± 1.87
	500	5	1.6 ± 0.29	0.7947	63.32 ± 1.54
			P = 0.361[e]		
Cyclophosphamide[f]	15	5	9.9 ± 1.18	0.0000	56.12 ± 1.26

[a]Study was performed at Environmental Health Research and Testing, Inc. (Lexington, KY). The detailed protocol is presented by Shelby et al.[107]. PCE = polychromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the vehicle control group; dosed group values are significant at P ≤ 0.0036; positive control values are significant at P ≤ 0.05.
[d]Vehicle control.
[e]Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test; significant at P ≤ 0.025.
[f]Positive control.

Table E-4. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Rats Following Treatment with 2,3-Butanedione 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[c]	PCEs[b] (%)	P Value[c]
Male								
Air[d]	0	5	1.08 ± 0.14		0.13 ± 0.05		0.765 ± 0.09	
2,3-Butanedione								
	6.25	5	0.79 ± 0.07	1.0000	0.08 ± 0.01	0.7622	0.737 ± 0.05	1.0000
	12.5	5	0.84 ± 0.19	1.0000	0.08 ± 0.01	0.8413	0.875 ± 0.04	0.4453
	25	5	0.98 ± 0.12	1.0000	0.09 ± 0.02	0.8700	0.895 ± 0.10	0.4764
	50	5	0.87 ± 0.07	1.0000	0.19 ± 0.07	0.4934	0.823 ± 0.06	0.4900
	100	5	0.79 ± 0.04	1.0000	0.11 ± 0.03	0.5034	0.828 ± 0.09	0.4994
			P = 0.809[e]		P = 0.236		P = 0.641	
Female								
Air	0	5	1.13 ± 0.23		0.17 ± 0.04		1.072 ± 0.14	
2,3-Butanedione								
	6.25	5	0.91 ± 0.09	1.0000	0.09 ± 0.02	0.7364	0.977 ± 0.06	1.0000
	12.5	5	0.95 ± 0.17	1.0000	0.21 ± 0.06	0.6615	0.961 ± 0.10	1.0000
	25	5	0.75 ± 0.14	1.0000	0.16 ± 0.04	0.6958	1.091 ± 0.15	1.0000
	50	5	1.01 ± 0.05	1.0000	0.11 ± 0.01	0.7147	0.936 ± 0.07	1.0000
	100	5	1.31 ± 0.26	0.8488	0.26 ± 0.06	0.0990	0.774 ± 0.18	1.0000
			P = 0.158		P = 0.054		P = 0.319	

[a]Study was performed at ILS, Inc. The detailed protocol is presented by Dertinger et al.[160], MacGregor et al.[159], and Witt et al.[158]. 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 or Williams' test.
[d]Chamber control.
[e]Exposure concentration-related trend; significant at P ≤ 0.025 by linear regression or Jonckheere's test.

Table E-5. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with 2,3-Butanedione 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[c]	PCEs[b] (%)	P Value[c]
Male								
Air[d]	0	5	2.42 ± 0.08		1.45 ± 0.03		1.442 ± 0.06	
2,3-Butanedione								
	6.25	5	2.58 ± 0.15	0.3212	1.50 ± 0.04	0.5605	1.540 ± 0.10	0.8501
	12.5	5	2.48 ± 0.13	0.3836	1.42 ± 0.02	0.6452	1.486 ± 0.06	0.9499
	25	5	2.53 ± 0.15	0.4091	1.47 ± 0.04	0.6794	1.495 ± 0.05	0.9751
	50	5	2.71 ± 0.25	0.3145	1.45 ± 0.05	0.6985	1.441 ± 0.20	0.9852

	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[c]	PCEs[b] (%)	P Value[c]
	100	5	2.47 ± 0.18	0.3231	1.37 ± 0.05	0.7120	1.547 ± 0.10	0.7518
			P = 0.431[e]		P = 0.979		P = 0.848	
Female								
Air	0	5	2.20 ± 0.33		1.08 ± 0.02		1.414 ± 0.15	
2,3-Butanedione								
	6.25	5	1.82 ± 0.13	1.0000	1.04 ± 0.03	0.8395	1.342 ± 0.12	1.0000
	12.5	5	2.06 ± 0.14	1.0000	1.11 ± 0.02	0.9049	1.401 ± 0.12	1.0000
	25	5	2.05 ± 0.19	1.0000	1.05 ± 0.03	0.9248	1.332 ± 0.09	1.0000
	50	5	2.00 ± 0.08	1.0000	1.04 ± 0.02	0.9357	1.472 ± 0.16	0.9074
	100	5	1.65 ± 0.11	1.0000	0.92 ± 0.03	0.9420	1.627 ± 0.07	0.2558
			P = 0.813		P = 1.000		P = 0.062	

[a]Study was performed at ILS, Inc. The detailed protocol is presented by Dertinger et al.[160], MacGregor et al.[159], and Witt et al.[158]. 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 or Williams' test.
[d]Chamber control.
[e]Exposure concentration-related trend; significant at P ≤ 0.025 by linear regression or Jonckheere's test.

Appendix F. Clinical Pathology Results

Tables

Table F-1. Hematology and Clinical Chemistry Data for Rats in the Three-month Inhalation Study of 2,3-Butanedione[a]

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Male						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	10	10	10	10	10	7
Hematocrit (%)						
Day 3	46.3 ± 0.5	45.2 ± 0.4	43.7 ± 0.6*	44.6 ± 0.6	44.2 ± 0.5	44.8 ± 0.8
Day 23	48.5 ± 0.8	47.6 ± 0.7	47.8 ± 0.6	48.1 ± 0.8	47.6 ± 0.6	48.5 ± 0.6
Week 14	49.0 ± 0.6	48.2 ± 0.5	48.2 ± 0.7	49.5 ± 1.2	50.4 ± 0.8	51.4 ± 1.1
Packed cell volume (%)						
Day 3	44.1 ± 0.6	43.0 ± 0.5	41.4 ± 0.6*	42.1 ± 0.5	42.4 ± 0.4	42.7 ± 0.7
Day 23	46.8 ± 0.7	46.0 ± 0.6	46.5 ± 0.7	46.6 ± 0.9	45.9 ± 0.7	47.0 ± 0.8
Week 14	47.2 ± 0.7	47.3 ± 0.6	47.5 ± 0.7	48.6 ± 1.2	49.4 ± 0.8*	50.3 ± 1.1*
Hemoglobin (g/dL)						
Day 3	13.7 ± 0.2	13.4 ± 0.2	13.1 ± 0.2	13.3 ± 0.2	13.3 ± 0.1	13.4 ± 0.2
Day 23	14.7 ± 0.2	14.6 ± 0.2	14.7 ± 0.2	14.8 ± 0.3	14.4 ± 0.2	14.9 ± 0.2
Week 14	15.2 ± 0.2	15.3 ± 0.2	15.4 ± 0.2	15.7 ± 0.3	15.7 ± 0.2*	16.3 ± 0.4**
Erythrocytes (10^6/µL)						
Day 3	7.07 ± 0.10	6.86 ± 0.12	6.68 ± 0.11	6.89 ± 0.09	6.96 ± 0.09	6.96 ± 0.14
Day 23	7.80 ± 0.11	7.64 ± 0.15	7.67 ± 0.13	7.80 ± 0.12	7.75 ± 0.11	7.98 ± 0.16
Week 14	8.82 ± 0.14	8.86 ± 0.13	8.97 ± 0.10	8.93 ± 0.25	9.14 ± 0.16	9.57 ± 0.19**
Reticulocytes (10^3/µL)						
Day 3	457.8 ± 15.8	435.9 ± 18.5	476.5 ± 8.9	477.3 ± 14.9	470.1 ± 20.8	468.8 ± 19.6
Day 23	176.1 ± 7.7	179.6 ± 10.2	183.2 ± 8.7	207.2 ± 12.6	201.1 ± 10.7	213.7 ± 19.3
Week 14	214.4 ± 9.1	175.4 ± 10.3	180.6 ± 12.5	182.1 ± 8.5	188.0 ± 7.8	177.4 ± 15.2
Reticulocytes/1,000 erythrocytes						
Day 3	64.70 ± 1.99	63.50 ± 2.35	71.50 ± 1.60	69.40 ± 2.27	67.40 ± 2.41	67.50 ± 2.94
Day 23	22.60 ± 0.93	23.50 ± 1.22	23.90 ± 1.15	26.60 ± 1.69	25.90 ± 1.24	26.80 ± 2.39
Week 14	24.30 ± 0.91	19.80 ± 1.16*	20.10 ± 1.29	20.40 ± 0.85	20.60 ± 0.81	18.57 ± 1.56*
Nucleated erythrocytes (10^3/µL)						
Day 3	9.28 ± 0.58	8.42 ± 0.60	8.63 ± 0.62	8.90 ± 0.40	8.43 ± 0.55	9.14 ± 0.45
Day 23	9.22 ± 0.64	7.53 ± 0.27	8.54 ± 0.42	9.27 ± 0.45	8.35 ± 0.78	9.53 ± 0.71
Week 14	7.33 ± 0.60	7.20 ± 0.37	7.68 ± 0.36	7.05 ± 0.60	9.02 ± 0.76	7.25 ± 0.65
Nucleated erythrocytes/100 leukocytes						
Day 4	0.4 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.2	0.0 ± 0.0	0.2 ± 0.1

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Day 23	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Week 14	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)						
Day 3	62.4 ± 0.6	62.8 ± 0.9	62.0 ± 0.6	61.2 ± 0.7	61.0 ± 0.6	61.4 ± 0.7
Day 23	60.1 ± 0.3	60.3 ± 0.9	60.6 ± 0.6	59.7 ± 0.6	59.2 ± 0.4	58.9 ± 0.7
Week 14	53.5 ± 0.5	53.5 ± 0.5	53.0 ± 0.6	54.5 ± 0.5	54.0 ± 0.4	52.6 ± 0.4
Mean cell hemoglobin (pg)						
Day 3	19.3 ± 0.2	19.5 ± 0.3	19.6 ± 0.2	19.3 ± 0.2	19.1 ± 0.2	19.2 ± 0.2
Day 23	18.9 ± 0.1	19.1 ± 0.3	19.2 ± 0.2	19.0 ± 0.2	18.6 ± 0.1	18.7 ± 0.2
Week 14	17.2 ± 0.2	17.2 ± 0.1	17.1 ± 0.1	17.6 ± 0.2	17.2 ± 0.1	17.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	30.9 ± 0.2	31.1 ± 0.1	31.6 ± 0.1**	31.5 ± 0.1*	31.3 ± 0.1	31.3 ± 0.1
Day 23	31.4 ± 0.1	31.7 ± 0.1	31.6 ± 0.1	31.8 ± 0.1	31.5 ± 0.2	31.7 ± 0.2
Week 14	32.2 ± 0.1	32.2 ± 0.1	32.3 ± 0.2	32.4 ± 0.2	31.8 ± 0.1	32.3 ± 0.3
Platelets ($10^3/\mu L$)						
Day 3	798 ± 30	898 ± 38	837 ± 30	821 ± 16	820 ± 36	870 ± 22
Day 23	697 ± 31	698 ± 20	692 ± 21	697 ± 24	712 ± 29	725 ± 34
Week 14	722 ± 29	726 ± 19	721 ± 36	710 ± 30	712 ± 20	692 ± 21
Leukocytes ($10^3/\mu L$)						
Day 3	9.24 ± 0.57	8.42 ± 0.59	8.62 ± 0.62	8.86 ± 0.39	8.43 ± 0.55	9.13 ± 0.45
Day 23	9.22 ± 0.64	7.53 ± 0.27	8.54 ± 0.42	9.27 ± 0.45	8.35 ± 0.78	9.53 ± 0.71
Week 14	7.33 ± 0.60	7.20 ± 0.37	7.68 ± 0.36	7.05 ± 0.60	9.02 ± 0.76	7.25 ± 0.65
Segmented neutrophils ($10^3/\mu L$)						
Day 3	1.03 ± 0.08	0.75 ± 0.07	0.84 ± 0.07	0.87 ± 0.05	0.93 ± 0.08	0.95 ± 0.09
Day 23	1.22 ± 0.11	0.86 ± 0.07	0.95 ± 0.10	1.02 ± 0.10	1.76 ± 0.38	1.65 ± 0.20
Week 14	1.47 ± 0.15	1.16 ± 0.10	1.29 ± 0.12	1.31 ± 0.11	2.48 ± 0.47	2.10 ± 0.21
Bands ($10^3/\mu L$)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	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 ($10^3/\mu L$)						
Day 3	7.92 ± 0.54	7.43 ± 0.51	7.54 ± 0.58	7.69 ± 0.38	7.27 ± 0.50	7.90 ± 0.45
Day 23	7.79 ± 0.55	6.49 ± 0.24	7.41 ± 0.40	8.07 ± 0.41	6.39 ± 0.58	7.71 ± 0.61
Week 14	5.66 ± 0.48	5.77 ± 0.33	6.06 ± 0.34	5.47 ± 0.62	6.37 ± 0.52	4.96 ± 0.50
Monocytes ($10^3/\mu L$)						
Day 3	0.17 ± 0.03	0.13 ± 0.03	0.13 ± 0.02	0.18 ± 0.02	0.13 ± 0.02	0.14 ± 0.02
Day 23	0.09 ± 0.03	0.08 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.11 ± 0.06	0.06 ± 0.01
Week 14	0.05 ± 0.02	0.10 ± 0.04	0.15 ± 0.05	0.10 ± 0.04	0.05 ± 0.01	0.05 ± 0.01

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Basophils ($10^3/\mu L$)						
Day 3	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
Day 23	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Week 14	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Eosinophils ($10^3/\mu L$)						
Day 3	0.11 ± 0.04	0.08 ± 0.01	0.10 ± 0.02	0.10 ± 0.01	0.07 ± 0.01	0.09 ± 0.01
Day 23	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.02
Week 14	0.14 ± 0.02	0.15 ± 0.03	0.17 ± 0.02	0.15 ± 0.02	0.12 ± 0.03	0.12 ± 0.02
Clinical Chemistry						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 14	10	10	10	10	10	8
Urea nitrogen (mg/dL)						
Day 3	8.9 ± 0.8	8.1 ± 0.6	9.3 ± 0.8	8.1 ± 0.6	9.1 ± 0.8	7.6 ± 0.3
Day 23	12.6 ± 0.7	11.7 ± 0.4	11.9 ± 0.5	10.8 ± 0.4	10.5 ± 0.4*	9.1 ± 0.5**
Week 14	16.3 ± 0.6	16.7 ± 0.6	16.3 ± 0.6	17.7 ± 0.6	16.4 ± 0.5	16.9 ± 1.0
Creatinine (mg/dL)						
Day 3	0.25 ± 0.02	0.25 ± 0.02	0.24 ± 0.02	0.22 ± 0.01	0.22 ± 0.02	0.24 ± 0.02
Day 23	0.32 ± 0.01	0.34 ± 0.03	0.31 ± 0.02	0.30 ± 0.01	0.32 ± 0.01	0.30 ± 0.01
Glucose (mg/dL)						
Day 3	149 ± 7	147 ± 6	139 ± 1	150 ± 7	141 ± 3	135 ± 2*
Day 23	140 ± 6	148 ± 7	130 ± 3	139 ± 6	147 ± 7	131 ± 5
Week 14	146 ± 7	135 ± 3	132 ± 4	128 ± 5	132 ± 6	144 ± 7
Total protein (g/dL)						
Day 3	6.0 ± 0.0	5.9 ± 0.1	5.9 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	6.0 ± 0.1
Day 23	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1
Week 14	7.0 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	7.2 ± 0.2	7.3 ± 0.1	7.1 ± 0.1
Albumin (g/dL)						
Day 3	4.4 ± 0.0	4.3 ± 0.1	4.4 ± 0.0	4.3 ± 0.0	4.3 ± 0.1	4.3 ± 0.0
Day 23	4.4 ± 0.0	4.4 ± 0.1	4.5 ± 0.0	4.4 ± 0.1	4.3 ± 0.1	4.5 ± 0.1
Week 14	4.7 ± 0.0	4.6 ± 0.0	4.6 ± 0.1	4.8 ± 0.1	4.7 ± 0.1	4.5 ± 0.1
Globulin (g/dL)						
Day 3	1.6 ± 0.0	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.0	1.6 ± 0.1
Day 23	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	1.9 ± 0.1
Week 14	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	2.6 ± 0.1
Albumin/globulin ratio						
Day 3	2.8 ± 0.1	2.8 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.7 ± 0.1

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Day 23	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.4 ± 0.1
Week 14	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
Cholesterol (mg/dL)						
Day 3	100 ± 5	91 ± 4	92 ± 5	92 ± 4	95 ± 4	91 ± 5
Day 23	81 ± 4	72 ± 3	75 ± 4	82 ± 4	80 ± 5	81 ± 6
Week 14	82 ± 7	93 ± 5	88 ± 6	93 ± 6	91 ± 4	77 ± 4
Triglycerides (mg/dL)						
Day 3	113 ± 18	80 ± 9	74 ± 9	75 ± 15	68 ± 8	57 ± 9**
Day 23	102 ± 15	91 ± 9	94 ± 13	114 ± 17	82 ± 11	83 ± 13
Week 14	115 ± 20	99 ± 9	105 ± 13	134 ± 25	101 ± 6	105 ± 21
Alanine aminotransferase (IU/L)						
Day 3	41 ± 2	45 ± 2	45 ± 3	42 ± 2	39 ± 1	39 ± 2
Day 23	32 ± 1	31 ± 1	33 ± 2	33 ± 2	31 ± 2	33 ± 2
Week 14	34 ± 1	31 ± 2	33 ± 2	33 ± 2	35 ± 2	37 ± 5
Alkaline phosphatase (IU/L)						
Day 3	269 ± 18	267 ± 19	239 ± 7	275 ± 16	269 ± 25	271 ± 18
Day 23	187 ± 13	190 ± 13	168 ± 6	200 ± 14	196 ± 14	205 ± 13
Week 14	123 ± 8	137 ± 7	119 ± 8	115 ± 7	131 ± 6	147 ± 12
Creatine kinase (IU/L)						
Day 3	368 ± 64	384 ± 46	256 ± 15	351 ± 45	298 ± 16	269 ± 21
Day 23	319 ± 26	503 ± 177	241 ± 18	341 ± 41	376 ± 39	344 ± 32
Week 14	155 ± 15	134 ± 8	161 ± 22	233 ± 33	151 ± 22	205 ± 24
Sorbitol dehydrogenase (IU/L)						
Day 3	14 ± 1	15 ± 0	13 ± 0	14 ± 0	14 ± 1	14 ± 1
Day 23	15 ± 1	14 ± 1	13 ± 1	15 ± 1	13 ± 1	13 ± 0
Week 14	13 ± 0	12 ± 1	f	11 ± 1	12 ± 1	13 ± 1
Bile acids (µmol/L)						
Day 3	27.6 ± 5.5	21.5 ± 2.1	30.5 ± 5.3	25.7 ± 3.5	20.1 ± 3.6	16.2 ± 3.2
Day 23	22.7 ± 3.9	20.9 ± 2.3	20.3 ± 4.0	20.3 ± 4.7	18.2 ± 4.3	10.2 ± 1.8*
Week 14	5.5 ± 1.8	4.7 ± 1.4	5.4 ± 1.7	3.3 ± 0.6	4.6 ± 1.0	6.4 ± 1.7
Female						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)						
Day 3	45.2 ± 0.5	45.7 ± 0.3	45.4 ± 0.4	44.0 ± 0.7	45.2 ± 0.5	44.6 ± 0.9
Day 23	47.2 ± 0.2	47.3 ± 0.4	48.1 ± 0.7	47.6 ± 0.6	48.3 ± 0.5	48.9 ± 0.5**
Week 14	46.3 ± 0.5	45.8 ± 0.6	46.8 ± 0.7	46.1 ± 0.3	47.8 ± 0.4	48.1 ± 0.8

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Packed cell volume (%)						
Day 3	43.7 ± 0.5	43.6 ± 0.4	43.0 ± 0.4	41.8 ± 0.5	43.3 ± 0.5	42.8 ± 0.8
Day 23	45.4 ± 0.2	45.8 ± 0.3	46.4 ± 0.6	45.9 ± 0.5	46.4 ± 0.5	47.5 ± 0.5**
Week 14	45.7 ± 0.3	45.7 ± 0.4	46.9 ± 0.6	45.7 ± 0.4	47.1 ± 0.5	47.6 ± 0.6*
Hemoglobin (g/dL)						
Day 3	13.7 ± 0.2	13.7 ± 0.1	13.6 ± 0.1	13.1 ± 0.2	13.8 ± 0.2	13.6 ± 0.2
Day 23	14.8 ± 0.1	14.9 ± 0.1	15.1 ± 0.1	14.9 ± 0.2	15.2 ± 0.2	15.4 ± 0.2**
Week 14	14.8 ± 0.1	14.9 ± 0.1	15.0 ± 0.2	14.7 ± 0.1	15.3 ± 0.1	15.3 ± 0.3
Erythrocytes (10^6/µL)						
Day 3	7.15 ± 0.13	7.10 ± 0.07	7.08 ± 0.12	6.93 ± 0.11	7.19 ± 0.05	7.12 ± 0.17
Day 23	7.68 ± 0.06	7.68 ± 0.05	7.88 ± 0.11	7.82 ± 0.11	7.90 ± 0.11	8.07 ± 0.14*
Week 14	8.17 ± 0.09	8.18 ± 0.09	8.36 ± 0.12	8.16 ± 0.07	8.54 ± 0.11*	8.67 ± 0.17*
Reticulocytes (10^3/µL)						
Day 3	386.3 ± 12.63	412.0 ± 21.9	398.0 ± 15.8	383.4 ± 22.7	397.7 ± 20.7	399.4 ± 16.2
Day 23	201.0 ± 9.5	191.1 ± 7.3	195.1 ± 9.0	195.8 ± 11.2	210.1 ± 12.1	226.7 ± 9.5
Week 14	225.7 ± 8.5	193.3 ± 8.9	199.6 ± 7.9	204.1 ± 12.5	204.5 ± 10.9	203.5 ± 10.6
Reticulocytes/1,000 erythrocytes						
Day 3	54.20 ± 2.13	58.00 ± 2.96	56.40 ± 2.61	55.20 ± 2.90	55.30 ± 2.80	56.10 ± 1.76
Day 23	26.20 ± 1.23	24.90 ± 0.96	24.70 ± 0.88	25.00 ± 1.30	26.60 ± 1.52	28.20 ± 1.40
Week 14	27.60 ± 0.92	23.60 ± 1.01	23.90 ± 0.95	25.10 ± 1.66	24.00 ± 1.34	23.50 ± 1.20
Nucleated erythrocytes (10^3/µL)						
Day 3	8.97 ± 0.50	7.45 ± 0.69	7.90 ± 0.62	7.52 ± 0.42	7.72 ± 0.74	8.31 ± 0.42
Day 23	6.91 ± 0.38	6.40 ± 0.62	6.59 ± 0.38	6.58 ± 0.47	8.10 ± 0.75	8.31 ± 0.36
Week 14	7.17 ± 0.57	6.25 ± 0.36	6.79 ± 0.30	6.19 ± 0.62	7.96 ± 0.73	7.88 ± 0.56
Nucleated erythrocytes/100 leukocytes						
Day 3	0.0 ± 0.0	0.0 ± 0.0	0.10 ± 0.1	0.10 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Day 23	0.0 ± 0.0	0.0 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Week 14	0.1 ± 0.1	0.0 ± 0.0	0.20 ± 0.1	0.00 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
Mean cell volume (fL)						
Day 3	61.2 ± 0.6	61.4 ± 0.3	60.8 ± 0.7	60.4 ± 0.7	60.2 ± 0.6	60.3 ± 0.8
Day 23	59.2 ± 0.4	59.7 ± 0.3	58.9 ± 0.7	58.8 ± 0.6	58.8 ± 0.7	58.9 ± 0.6
Week 14	56.0 ± 0.5	55.9 ± 0.5	56.1 ± 0.4	56.0 ± 0.2	55.3 ± 0.5	55.0 ± 0.5
Mean cell hemoglobin (pg)						
Day 3	19.2 ± 0.2	19.2 ± 0.2	19.2 ± 0.2	18.9 ± 0.2	19.2 ± 0.2	19.1 ± 0.3
Day 23	19.3 ± 0.1	19.4 ± 0.1	19.1 ± 0.2	19.1 ± 0.2	19.2 ± 0.2	19.1 ± 0.2
Week 14	18.1 ± 0.2	18.2 ± 0.2	18.0 ± 0.2	18.1 ± 0.1	17.9 ± 0.1	17.7 ± 0.3
Mean cell hemoglobin concentration (g/dL)						
Day 3	31.3 ± 0.1	31.3 ± 0.1	31.5 ± 0.2	31.3 ± 0.1	31.9 ± 0.1**	31.8 ± 0.2*

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Day 23	32.6 ± 0.1	32.5 ± 0.1	32.5 ± 0.1	32.4 ± 0.1	32.7 ± 0.1	32.5 ± 0.1
Week 14	32.3 ± 0.1	32.6 ± 0.1	32.1 ± 0.1	32.3 ± 0.1	32.4 ± 0.1	32.1 ± 0.5
Platelets (10^3/µL)						
Day 3	917 ± 38	884 ± 36	1,006 ± 32	909 ± 23	902 ± 20	906 ± 35
Day 23	732 ± 25	692 ± 27	760 ± 19	708 ± 17	768 ± 26	795 ± 23
Week 14	788 ± 32	732 ± 34	726 ± 25	734 ± 37	791 ± 24	772 ± 24
Leukocytes (10^3/µL)						
Day 3	8.97 ± 0.49	7.45 ± 0.69	7.89 ± 0.62	7.51 ± 0.42	7.72 ± 0.74	8.31 ± 0.42
Day 23	6.91 ± 0.38	6.40 ± 0.62	6.59 ± 0.38	6.58 ± 0.47	8.10 ± 0.75	8.31 ± 0.36
Week 14	7.16 ± 0.57	6.25 ± 0.36	6.78 ± 0.30	6.19 ± 0.62	7.95 ± 0.73	7.88 ± 0.56
Segmented neutrophils (10^3/µL)						
Day 3	0.90 ± 0.11	0.76 ± 0.10	0.65 ± 0.06	0.69 ± 0.08	0.77 ± 0.06	1.03 ± 0.14
Day 23	0.90 ± 0.09	0.82 ± 0.09	0.63 ± 0.08	0.75 ± 0.10	1.82 ± 0.35	1.59 ± 0.24*
Week 14	1.54 ± 0.21	1.27 ± 0.15	1.24 ± 0.08	1.32 ± 0.27	2.19 ± 0.27	3.48 ± 0.42**
Bands (10^3/µL)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	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)						
Day 3	7.72 ± 0.48	6.46 ± 0.65	7.05 ± 0.58	6.56 ± 0.37	6.69 ± 0.70	7.04 ± 0.35
Day 23	5.78 ± 0.33	5.36 ± 0.58	5.82 ± 0.38	5.66 ± 0.43	6.10 ± 0.70	6.51 ± 0.36
Week 14	5.38 ± 0.39	4.82 ± 0.29	5.31 ± 0.31	4.68 ± 0.47	5.46 ± 0.60	4.15 ± 0.41
Monocytes (10^3/µL)						
Day 3	0.19 ± 0.02	0.12 ± 0.02	0.09 ± 0.02*	0.16 ± 0.04	0.12 ± 0.02	0.13 ± 0.04
Day 23	0.07 ± 0.01	0.09 ± 0.03	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.03	0.08 ± 0.02
Week 14	0.13 ± 0.05	0.05 ± 0.01	0.12 ± 0.04	0.07 ± 0.02	0.16 ± 0.05	0.12 ± 0.04
Basophils (10^3/µL)						
Day 3	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
Day 23	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
Week 14	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
Eosinophils (10^3/µL)						
Day 3	0.14 ± 0.01	0.10 ± 0.01	0.09 ± 0.01*	0.08 ± 0.01**	0.13 ± 0.02	0.09 ± 0.01**
Day 23	0.15 ± 0.02	0.12 ± 0.02	0.07 ± 0.01**	0.12 ± 0.02	0.09 ± 0.02	0.11 ± 0.01
Week 14	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.02	0.11 ± 0.02	0.13 ± 0.02	0.12 ± 0.02

	Chamber Control	**6.25 ppm**	**12.5 ppm**	**25 ppm**	**50 ppm**	**100 ppm**
n	10	10	10	10	10	10
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 3	10.6 ± 1.0	9.9 ± 1.0	9.6 ± 0.7	10.4 ± 0.8	11.2 ± 0.4	8.1 ± 0.6
Day 23	14.5 ± 0.9	13.5 ± 1.2	12.3 ± 0.6	14.1 ± 1.0	14.7 ± 0.9	10.4 ± 0.9**
Week 14	18.1 ± 1.1	18.4 ± 0.7	17.1 ± 0.6	19.0 ± 0.7	17.5 ± 0.5	18.4 ± 1.8
Creatinine (mg/dL)						
Day 3	0.28 ± 0.02	0.27 ± 0.02	0.30 ± 0.01	0.28 ± 0.02	0.31 ± 0.02	0.27 ± 0.02
Day 23	0.35 ± 0.03	0.36 ± 0.02	0.38 ± 0.02	0.37 ± 0.03	0.38 ± 0.02	0.36 ± 0.02
Week 14	0.45 ± 0.02	0.46 ± 0.02	0.45 ± 0.02	0.45 ± 0.02	0.46 ± 0.03	0.40 ± 0.02
Glucose (mg/dL)						
Day 3	134 ± 4	132 ± 3	143 ± 5	131 ± 3	140 ± 6	135 ± 4
Day 23	127 ± 7	131 ± 5	123 ± 3	125 ± 2	128 ± 3	136 ± 6
Week 14	138 ± 8	142 ± 5	132 ± 3	139 ± 9	136 ± 3	139 ± 6
Total protein (g/dL)						
Day 3	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.1 ± 0.1
Day 23	6.7 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.9 ± 0.1	6.7 ± 0.1	6.6 ± 0.1
Week 14	7.5 ± 0.1	7.5 ± 0.1	7.3 ± 0.1	7.4 ± 0.1	7.6 ± 0.1	7.4 ± 0.2
Albumin (g/dL)						
Day 3	4.7 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.8 ± 0.0	4.6 ± 0.1
Day 23	4.9 ± 0.1	4.9 ± 0.1	5.0 ± 0.1	5.0 ± 0.0	4.9 ± 0.1	4.8 ± 0.1
Week 14	5.4 ± 0.1	5.4 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.1 ± 0.2
Globulin (g/dL)						
Day 3	1.6 ± 0.1	1.6 ± 0.0	1.4 ± 0.1	1.5 ± 0.0	1.6 ± 0.1	1.5 ± 0.0
Day 23	1.8 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
Week 14	2.1 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.0	2.3 ± 0.1	2.3 ± 0.1
Albumin/globulin ratio						
Day 3	3.0 ± 0.1	3.0 ± 0.1	3.3 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	3.2 ± 0.1
Day 23	2.8 ± 0.1	2.8 ± 0.1	3.0 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.8 ± 0.1
Week 14	2.6 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.3 ± 0.1
Cholesterol (mg/dL)						
Day 3	78 ± 3	81 ± 5	79 ± 5	73 ± 4	74 ± 3	74 ± 5
Day 23	65 ± 3	68 ± 5	66 ± 4	63 ± 4	63 ± 3	70 ± 5
Week 14	66 ± 4	62 ± 2	60 ± 2	62 ± 3	62 ± 4	55 ± 7
Triglycerides (mg/dL)						
Day 3	61 ± 4	63 ± 4	53 ± 4	55 ± 4	61 ± 6	43 ± 3*
Day 23	55 ± 7	52 ± 3	59 ± 6	76 ± 16	57 ± 7	55 ± 4
Week 14	50 ± 5	57 ± 6	45 ± 4	56 ± 4	55 ± 5	60 ± 10

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Alanine aminotransferase (IU/L)						
Day 3	29 ± 2	31 ± 1	34 ± 2	31 ± 2	34 ± 2	30 ± 2
Day 23	29 ± 4	28 ± 2	28 ± 2	25 ± 2	26 ± 2	25 ± 2
Week 14	36 ± 3	28 ± 2	32 ± 4	35 ± 4	34 ± 2	34 ± 3
Alkaline phosphatase (IU/L)						
Day 3	181 ± 9	193 ± 13	197 ± 16	159 ± 14	167 ± 10	168 ± 9
Day 23	100 ± 7	104 ± 8	114 ± 9	87 ± 7	97 ± 7	107 ± 7
Week 14	73 ± 8	74 ± 8	62 ± 7	68 ± 8	62 ± 8	89 ± 8
Creatine kinase (IU/L)						
Day 3	337 ± 25	340 ± 49	323 ± 42	337 ± 41	361 ± 46	380 ± 41
Day 23	331 ± 44	399 ± 75	331 ± 38	319 ± 31	328 ± 24	351 ± 29
Week 14	198 ± 18	271 ± 44	192 ± 25	341 ± 148	281 ± 48	251 ± 34
Sorbitol dehydrogenase (IU/L)						
Day 3	13 ± 1	14 ± 1	14 ± 1	13 ± 0	14 ± 1	13 ± 1
Day 23	13 ± 1	12 ± 1	15 ± 1	15 ± 1	16 ± 1	15 ± 1
Week 14	14 ± 1	12 ± 1	13 ± 1	13 ± 1	12 ± 1	11 ± 1
Bile acids (µmol/L)						
Day 3	16.3 ± 1.9	12.9 ± 1.8	18.5 ± 4.1	13.0 ± 1.7	23.6 ± 6.7	14.5 ± 2.8
Day 23	29.6 ± 8.0	14.9 ± 2.3	15.2 ± 2.6	19.0 ± 3.9	15.2 ± 2.6	12.5 ± 3.3*
Week 14	7.9 ± 2.0	6.9 ± 2.1	4.7 ± 0.7	14.3 ± 7.0	4.7 ± 0.7	11.5 ± 3.3

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

Table F-2. Hematology Data for Mice in the Three-month Inhalation Study of 2,3-Butanedione[a]

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
n	10	10	10	10	10	10
Male						
Hematocrit (%)	49.4 ± 0.4	50.0 ± 0.4	50.0 ± 0.4	49.5 ± 0.4	49.1 ± 0.5	48.8 ± 0.4
Packed cell volume (%)	50.3 ± 0.4	51.1 ± 0.3	50.8 ± 0.4	50.6 ± 0.3	49.8 ± 0.5	49.2 ± 0.4
Hemoglobin (g/dL)	15.6 ± 0.1	15.8 ± 0.1	15.7 ± 0.1	15.6 ± 0.1	15.5 ± 0.2	15.4 ± 0.1
Erythrocytes (10^6/µL)	10.33 ± 0.07	10.59 ± 0.06	10.38 ± 0.11	10.38 ± 0.07	10.42 ± 0.10	10.57 ± 0.08
Reticulocytes (10^6/µL)	286.1 ± 15.5	277.8 ± 13.3	277.4 ± 11.8	275.2 ± 11.7	275.8 ± 12.6	284.3 ± 10.5
Reticulocytes/1,000 erythrocytes	27.70 ± 1.51	26.20 ± 1.18	26.70 ± 1.10	26.50 ± 1.10	26.50 ± 1.22	26.90 ± 0.96
Nucleated erythrocytes (10^3/µL)	3.13 ± 0.30	3.05 ± 0.31	2.95 ± 0.18	3.03 ± 0.15	4.16 ± 0.76	3.67 ± 0.22
Nucleated erythrocytes /100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Howell-Jolly bodies (% erythrocytes)	0.2 ± 0.0	0.1 ± 0.0*	0.1 ± 0.0**	0.1 ± 0.0**	0.1 ± 0.0	0.1 ± 0.0*
Mean cell volume (fL)	48.7 ± 0.2	48.3 ± 0.3	48.9 ± 0.3	48.8 ± 0.1	47.8 ± 0.3*	46.6 ± 0.3**
Mean cell hemoglobin (pg)	15.1 ± 0.1	14.9 ± 0.1	15.1 ± 0.1	15.1 ± 0.1	14.9 ± 0.1*	14.6 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	31.1 ± 0.1	30.9 ± 0.1	30.9 ± 0.1	30.9 ± 0.1	31.1 ± 0.1	31.4 ± 0.1
Platelets (10^3/µL)	859 ± 11	856 ± 16	877 ± 16	874 ± 12	913 ± 33	925 ± 22*
Leukocytes (10^3/µL)	3.13 ± 0.30	3.05 ± 0.31	2.95 ± 0.18	3.03 ± 0.15	4.16 ± 0.76	3.67 ± 0.22
Segmented neutrophils (10^3/µL)	0.42 ± 0.05	0.38 ± 0.03	0.42 ± 0.06	0.42 ± 0.03	1.22 ± 0.57*	0.96 ± 0.20**
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)	2.61 ± 0.24	2.57 ± 0.29	2.40 ± 0.15	2.47 ± 0.15	2.79 ± 0.26	2.57 ± 0.11
Monocytes (10^3/µL)	0.05 ± 0.02	0.05 ± 0.02	0.07 ± 0.02	0.08 ± 0.02	0.06 ± 0.02	0.07 ± 0.02
Basophils (10^3/µL)	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.00
Eosinophils (10^3/µL)	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.08 ± 0.01	0.06 ± 0.01
Female						
Hematocrit (%)	49.4 ± 0.3	49.7 ± 0.3	50.6 ± 0.4	49.7 ± 0.4	49.2 ± 0.4	48.8 ± 0.4
Packed cell volume (%)	50.3 ± 0.5	50.5 ± 0.3	51.0 ± 0.4	50.6 ± 0.5	49.5 ± 0.3	49.3 ± 0.4
Hemoglobin (g/dL)	15.8 ± 0.2	15.9 ± 0.1	16.0 ± 0.1	15.9 ± 0.1	15.6 ± 0.1	15.5 ± 0.1
Erythrocytes (10^6/µL)	10.25 ± 0.10	10.28 ± 0.06	10.39 ± 0.09	10.30 ± 0.10	10.20 ± 0.07	10.43 ± 0.07
Reticulocytes (10^6/µL)	260.7 ± 11.6	269.6 ± 9.9	256.7 ± 8.1	262.5 ± 10.1	263.2 ± 5.0	270.3 ± 9.1
Reticulocytes/1,000 erythrocytes	25.40 ± 1.01	26.20 ± 0.92	24.70 ± 0.75	25.50 ± 1.02	25.80 ± 0.47	25.90 ± 0.85
Nucleated erythrocytes (10^3/µL)	3.92 ± 0.53	3.79 ± 0.30	3.60 ± 0.28	4.80 ± 0.47	3.86 ± 0.32	3.86 ± 0.40
Nucleated erythrocytes/100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Howell-Jolly bodies (% erythrocytes)	0.2 ± 0.0	0.1 ± 0.0*	0.1 ± 0.0*	0.1 ± 0.0	0.1 ± 0.0*	0.1 ± 0.0
Mean cell volume (fL)	49.0 ± 0.2	49.1 ± 0.1	49.2 ± 0.2	49.1 ± 0.2	48.6 ± 0.1	47.2 ± 0.2**
Mean cell hemoglobin (pg)	15.4 ± 0.1	15.4 ± 0.0	15.4 ± 0.1	15.4 ± 0.1	15.3 ± 0.0	14.8 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	31.4 ± 0.1	31.4 ± 0.1	31.3 ± 0.1	31.4 ± 0.2	31.5 ± 0.1	31.4 ± 0.2
Platelets (10^3/µL)	799 ± 19	808 ± 9	850 ± 9	786 ± 25	861 ± 16*	889 ± 26**
Leukocytes (10^3/µL)	3.92 ± 0.53	3.79 ± 0.30	3.60 ± 0.28	4.80 ± 0.47	3.86 ± 0.32	3.86 ± 0.40
Segmented neutrophils (10^3/µL)	0.53 ± 0.08	0.47 ± 0.06	0.42 ± 0.06	0.59 ± 0.08	0.79 ± 0.16	1.04 ± 0.19*
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)	3.29 ± 0.48	3.22 ± 0.24	3.08 ± 0.23	4.08 ± 0.41	2.91 ± 0.21	2.65 ± 0.27
Monocytes (10^3/µL)	0.06 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.09 ± 0.02	0.09 ± 0.02
Basophils (10^3/µL)	0.02 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.02
Eosinophils (10^3/µL)	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01

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

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

Tables

Table G-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Three-month Inhalation Study of 2,3-Butanedione[a]

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100ppm
Male						
n	10	10	10	10	10	8
Necropsy body wt	410 ± 8	401 ± 13	386 ± 8	407 ± 16	387 ± 8	333 ± 12**
Heart						
Absolute	1.11 ± 0.02	1.05 ± 0.02	1.03 ± 0.03	1.08 ± 0.03	1.12 ± 0.03	1.00 ± 0.03*
Relative	2.723 ± 0.047	2.621 ± 0.063	2.666 ± 0.046	2.651 ± 0.064	2.890 ± 0.054	3.001 ± 0.075**
R. Kidney						
Absolute	1.27 ± 0.02	1.24 ± 0.04	1.21 ± 0.03	1.31 ± 0.03	1.23 ± 0.03	1.10 ± 0.02**
Relative	3.105 ± 0.060	3.092 ± 0.075	3.131 ± 0.055	3.248 ± 0.087	3.182 ± 0.077	3.308 ± 0.066
Liver						
Absolute	12.50 ± 0.35	12.14 ± 0.53	11.83 ± 0.41	12.72 ± 0.81	11.68 ± 0.33	9.58 ± 0.36**
Relative	30.510 ± 0.684	30.186 ± 0.426	30.585 ± 0.719	31.023 ± 1.015	30.218 ± 0.685	28.822 ± 0.807
Lung						
Absolute	2.45 ± 0.08	2.40 ± 0.11	2.29 ± 0.08	2.46 ± 0.15	2.47 ± 0.10	2.25 ± 0.08
Relative	5.988 ± 0.162	6.005 ± 0.268	5.935 ± 0.164	6.036 ± 0.307	6.404 ± 0.270	6.810 ± 0.358
Spleen						
Absolute	0.570 ± 0.026	0.600 ± 0.035	0.570 ± 0.022	0.605 ± 0.023	0.606 ± 0.020	0.488 ± 0.026
Relative	1.389 ± 0.048	1.510 ± 0.097	1.478 ± 0.059	1.491 ± 0.048	1.568 ± 0.048	1.462 ± 0.057
R. Testis						
Absolute	1.819 ± 0.053	1.831 ± 0.058	1.778 ± 0.053	1.784 ± 0.057	1.826 ± 0.031	1.646 ± 0.055
Relative	4.448 ± 0.135	4.600 ± 0.185	4.604 ± 0.113	4.405 ± 0.133	4.735 ± 0.116	4.966 ± 0.185
Thymus						
Absolute	0.448 ± 0.027	0.449 ± 0.022	0.509 ± 0.030	0.467 ± 0.039	0.417 ± 0.020	0.340 ± 0.022*
Relative	1.088 ± 0.055	1.129 ± 0.073	1.324 ± 0.088	1.144 ± 0.083	1.076 ± 0.042	1.035 ± 0.085
Female						
n	10	10	10	10	10	10
Necropsy body wt	236 ± 6	235 ± 5	228 ± 4	226 ± 4	232 ± 6	208 ± 9**
Heart						
Absolute	0.77 ± 0.01	0.74 ± 0.02	0.74 ± 0.01	0.77 ± 0.05	0.74 ± 0.02	0.73 ± 0.02
Relative	3.257 ± 0.070	3.139 ± 0.049	3.242 ± 0.039	3.419 ± 0.203	3.206 ± 0.055	3.550 ± 0.087
R. Kidney						
Absolute	0.85 ± 0.02	0.82 ± 0.03	0.81 ± 0.02	0.80 ± 0.02	0.79 ± 0.03	0.77 ± 0.03

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100ppm
Relative	3.597 ± 0.085	3.468 ± 0.084	3.568 ± 0.069	3.531 ± 0.052	3.429 ± 0.110	3.747 ± 0.116
Liver						
Absolute	7.42 ± 0.25	7.21 ± 0.26	7.03 ± 0.17	6.93 ± 0.20	7.04 ± 0.16	6.41 ± 0.41*
Relative	31.390 ± 0.711	30.626 ± 0.700	30.802 ± 0.470	30.730 ± 0.872	30.446 ± 0.703	30.671 ± 1.153
Lung						
Absolute	1.63 ± 0.06	1.49 ± 0.06	1.53 ± 0.07	1.50 ± 0.07	1.72 ± 0.12	1.64 ± 0.06
Relative	6.943 ± 0.309	6.343 ± 0.228	6.740 ± 0.332	6.652 ± 0.252	7.370 ± 0.394	8.003 ± 0.395*
Spleen						
Absolute	0.413 ± 0.014	0.426 ± 0.012	0.408 ± 0.019	0.376 ± 0.017	0.422 ± 0.020	0.368 ± 0.025
Relative	1.756 ± 0.070	1.818 ± 0.053	1.786 ± 0.071	1.661 ± 0.058	1.822 ± 0.081	1.766 ± 0.084
Thymus						
Absolute	0.401 ± 0.020	0.406 ± 0.025	0.402 ± 0.011	0.409 ± 0.016	0.393 ± 0.019	0.304 ± 0.039*
Relative	1.701 ± 0.083	1.724 ± 0.090	1.763 ± 0.042	1.808 ± 0.053	1.702 ± 0.091	1.404 ± 0.165

*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).

Table G-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Inhalation Study of 2,3-Butanedione[a]

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	37.8 ± 1.2	38.0 ± 1.3	37.8 ± 0.7	39.1 ± 0.6	34.2 ± 1.1*	27.9 ± 0.6**
Heart						
Absolute	0.16 ± 0.00	0.16 ± 0.00	0.17 ± 0.00	0.17 ± 0.00	0.14 ± 0.00**	0.12 ± 0.00**
Relative	4.277 ± 0.095	4.200 ± 0.092	4.396 ± 0.122	4.303 ± 0.112	4.111 ± 0.075	4.395 ± 0.109
R. Kidney						
Absolute	0.32 ± 0.01	0.32 ± 0.01	0.33 ± 0.01	0.34 ± 0.01	0.29 ± 0.01**	0.23 ± 0.01**
Relative	8.591 ± 0.231	8.543 ± 0.200	8.796 ± 0.217	8.655 ± 0.213	8.444 ± 0.163	8.263 ± 0.178
Liver						
Absolute	1.57 ± 0.05	1.59 ± 0.05	1.68 ± 0.06	1.68 ± 0.05	1.36 ± 0.04**	1.12 ± 0.04**
Relative	41.525 ± 0.628	41.855 ± 0.688	44.455 ± 1.272	42.818 ± 1.033	39.908 ± 0.642	40.206 ± 0.985
Lung						
Absolute	0.21 ± 0.01	0.23 ± 0.01	0.22 ± 0.00	0.23 ± 0.01	0.21 ± 0.01	0.20 ± 0.00
Relative	5.632 ± 0.157	5.924 ± 0.162	5.925 ± 0.081	5.962 ± 0.140	6.302 ± 0.248*	7.243 ± 0.223**

	Chamber Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Spleen						
Absolute	0.067 ± 0.002	0.068 ± 0.002	0.073 ± 0.003	0.069 ± 0.002	0.065 ± 0.004	0.063 ± 0.003
Relative	1.786 ± 0.070	1.802 ± 0.064	1.939 ± 0.099	1.765 ± 0.055	1.916 ± 0.128	2.263 ± 0.101**
R. Testis						
Absolute	0.114 ± 0.002	0.116 ± 0.002	0.118 ± 0.002	0.116 ± 0.003	0.111 ± 0.001	0.108 ± 0.001*
Relative	3.044 ± 0.107	3.061 ± 0.076	3.116 ± 0.041	2.981 ± 0.082	3.289 ± 0.111	3.880 ± 0.070**
Thymus						
Absolute	0.053 ± 0.004	0.055 ± 0.003	0.050 ± 0.002	0.052 ± 0.005	0.046 ± 0.003	0.047 ± 0.002
Relative	1.383 ± 0.076	1.454 ± 0.100	1.324 ± 0.042	1.311 ± 0.116	1.352 ± 0.064	1.674 ± 0.047*
Female						
Necropsy body wt	33.0 ± 0.9	31.8 ± 0.7	30.9 ± 0.6*	29.4 ± 0.7**	27.6 ± 0.5**	23.8 ± 0.5**
Heart						
Absolute	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.14 ± 0.00	0.13 ± 0.00**	0.12 ± 0.00**
Relative	4.534 ± 0.160	4.662 ± 0.080	4.834 ± 0.123	4.772 ± 0.084	4.710 ± 0.096	4.847 ± 0.100
R. Kidney						
Absolute	0.22 ± 0.00	0.21 ± 0.01	0.22 ± 0.00	0.21 ± 0.00	0.19 ± 0.00**	0.18 ± 0.01**
Relative	6.569 ± 0.194	6.582 ± 0.140	7.012 ± 0.183	7.119 ± 0.120*	7.020 ± 0.097*	7.446 ± 0.170**
Liver						
Absolute	1.49 ± 0.05	1.49 ± 0.05	1.51 ± 0.04	1.38 ± 0.04	1.21 ± 0.03**	1.01 ± 0.04**
Relative	45.214 ± 1.240	46.788 ± 1.220	48.971 ± 0.700	46.724 ± 0.813	43.696 ± 0.758	42.284 ± 1.420
Lung						
Absolute	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.00	0.21 ± 0.00	0.21 ± 0.01	0.21 ± 0.01
Relative	6.959 ± 0.349	7.045 ± 0.206	7.271 ± 0.176	7.242 ± 0.103	7.565 ± 0.146	8.759 ± 0.186**
Spleen						
Absolute	0.099 ± 0.003	0.096 ± 0.003	0.094 ± 0.003	0.094 ± 0.002	0.081 ± 0.002**	0.074 ± 0.003**
Relative	3.017 ± 0.134	3.028 ± 0.095	3.056 ± 0.132	3.204 ± 0.087	2.938 ± 0.096	3.113 ± 0.123
Thymus						
Absolute	0.065 ± 0.004	0.056 ± 0.001*	0.056 ± 0.003*	0.054 ± 0.002**	0.048 ± 0.002**	0.048 ± 0.002**
Relative	1.954 ± 0.106	1.772 ± 0.056	1.819 ± 0.085	1.826 ± 0.055	1.747 ± 0.061	2.017 ± 0.076

*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. Chemical Characterization and Generation of Chamber Concentrations

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Figures

H.1. Procurement and Characterization of 2,3-Butanedione

2,3-Butanedione was obtained from Sigma-Aldrich (Aldrich Chemical Co., Inc., Sheboygan Falls, WI) in two lots (10815TD and 03798LJ). Lot 10815TD was used in the 3-month studies, and lot 03798LJ was used in the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Chemir Analytical Services (Maryland Heights, MO) and by the study laboratory at Battelle Toxicology Northwest (Richland, WA). Reports on analyses performed in support of the 2,3-butanedione studies are on file at the National Institute of Environmental Health Sciences.

Lots 10815TD and 03798LJ of the test chemical, a yellow liquid, were identified as 2,3-butanedione by the analytical chemistry laboratory and the study laboratory, respectively, using Fourier transform infrared (FTIR) spectroscopy and by the analytical chemistry laboratory using proton nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with the structure and composition of 2,3-butanedione. Representative FTIR and NMR spectra are presented in Figure H-1 and Figure H-2.

Elemental analysis was performed by Galbraith Laboratories (Knoxville, TN), and water content was determined by the analytical chemistry laboratory using Karl Fischer titration. The relative purity and area percent purity were determined by the study laboratory using gas chromatography (GC) with flame ionization detection (FID) by system A or B, respectively (Table H-1).

For lot 10815TD, elemental analyses for carbon, hydrogen, nitrogen, and sulfur were consistent with theoretical values for 2,3-butanedione. Karl Fischer titration indicated 0.1% water content. In samples taken from the top, middle, and bottom of the drum, GC/FID (System A) indicated an average purity of 98.7% and four minor peaks with areas greater than 0.1% of the total peak area. These impurities were identified as ethyl acetate (0.39%), 2-butanone (0.51%), acetonitrile (0.24%), and acetic acid (0.12%) by comparing to the retention times of authentic standards.

For lot 03798LJ, elemental analyses for carbon, hydrogen, nitrogen, and sulfur were consistent with theoretical values for 2,3-butanedione. Karl Fischer titration indicated 0.42% water content. Average purity in samples taken from the top, middle, and bottom of the drum was 99.1% using GC/FID system A and four minor peaks with areas greater than or equal to 0.1% of the total peak area were indicated. Three of the peaks were identified as acetaldehyde (0.1%), acetic acid (0.3%), and acetoin (0.3%) by comparing to the retention times of authentic standards.

To ensure stability, the test chemical was stored at refrigerated temperatures in metal drums under a nitrogen headspace. Periodic reanalyses of the test chemical were performed by the study laboratory using GC/FID by systems A or B prior to and after the 3-month and 2-year studies and approximately every 6 months during the 2-year studies; no degradation of the test chemical was detected.

H.2. Vapor Generation and Exposure Systems

Diagrams of the vapor generation and delivery systems used in the 3-month and 2-year studies are shown in Figure H-3 and Figure H-4, respectively. 2,3-Butanedione was pumped onto glass beads in a heated glass column where it was vaporized. Heated nitrogen flowed through the column and carried the vapor to a short vapor-distribution manifold, where concentration was controlled by the chemical pump and nitrogen flow rates. For the 2-year studies, the nitrogen-chemical mixture was diluted with heated air (~140°F) before entering the distribution manifold. Pressure in the distribution manifold was fixed to ensure constant flows through the manifold and into the chambers.

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 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 (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) 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 present in the exposure chambers to ensure that 2,3-butadione vapor, and not aerosol, was produced. No particle counts above the minimum resolvable level (approximately 200 particles/cm^3) were detected.

H.3. Vapor Concentration Monitoring

Summaries of the chamber vapor concentrations are given in Table H-2 and Table H-3. Chamber concentrations of 2,3-butanedione were monitored using an online gas chromatograph with FID (system C; Table H-1). Samples were drawn from each exposure chamber approximately every 20 minutes during each 6-hour exposure period using Hasteloy-C stream-select and gas-sampling valves (VALCO Instruments Co., Houston, TX) in a separate, heated valve 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 inline flow meter between the vacuum regulator and the gas chromatograph allowed digital measurement of sample flow.

The online gas chromatograph was checked throughout the day for instrument drift against an online standard of 2,3-butanedione in nitrogen supplied by a standard generator (Kin-Tek; Precision Calibration Systems, La Marque, TX). The online gas chromatograph was calibrated prior to the start of each study and monthly during the 3-month and 2-year studies by a comparison of chamber concentration data to data from grab samples that were collected with sorbent gas sampling tubes containing silica gel (ORBO-53; Supelco, Bellefonte, PA) followed by a sampling tube containing activated coconut charcoal (ORBO-32; Supelco), extracted with acetone containing 2-methyl-1-propanol as an internal standard, and analyzed using an offline gas chromatograph (system B, Table H-1). The volumes of gas were sampled at a constant flow rate ensured by a calibrated critical orifice. The offline gas chromatograph was calibrated with gravimetrically prepared standards of 2,3-butanedione and the internal standard 2-methyl-1-propanol in acetone.

H.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 values 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}) were approximately 9.2 minutes. For rats and mice in the 3-month studies, T_{90} values ranged from 9 to 11 minutes without animals present and from 9 to 13 minutes with animals present; T_{10} values ranged from 9 to 11 minutes without animals present and from 10 to 11 minutes with animals present. For rats and mice in the 2-year studies, T_{90} values ranged from 8 to 9 minutes without animals present and from 10 to 12 minutes with animals present; T_{10} values ranged from 8 to 10 minutes without animals present and from 10 to 11 minutes with animals present. A T_{90} value of 10 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 2,3-butanedione vapor concentration in the inhalation exposure chambers without animals present was evaluated before the 3-month and 2-year studies began; 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 (Table H-2 and Table H-3). The vapor concentration was measured using the on-line gas chromatograph (system B, Table H-1) with the stream-selection valve fixed in one position to allow continuous monitoring from a single input line. During the 3-month 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 2-year studies, concentrations were measured at the regular monitoring port and from all sample ports where animals were present. Chamber concentration uniformity was maintained throughout the studies.

The persistence of 2,3-butanedione in the chambers after vapor delivery ended was determined by monitoring the postexposure vapor concentration in the 100 ppm rat/mouse chamber in the 3-month studies and the 50 ppm chambers in the 2-year studies without animals present in the chambers. In the 3-month studies, the concentration decreased to 1% of the target concentration within 20 minutes without animals present. During the 2-year studies in the rat only chambers, the concentration decreased to 1% of the target concentration within 21 minutes without animals present and 35 minutes with animals present. In the rat/mouse chambers, the concentration decreased to 1% of the target concentration within 19 minutes without animals present and 95 minutes with animals present.

Samples of the test atmosphere from the distribution lines and low and high exposure concentration chambers were collected prior to the 3-month and 2-year studies and also at the beginning and end of each generation day during the 3-month and 2-year studies; the atmosphere samples were collected with sorbent gas sampling tubes containing silica gel (ORBO-53; Supelco) and extracted with acetone. All of the samples were analyzed using GC/FID by system D (Table H-1) to measure the stability and purity of 2,3-butanedione in the generation and delivery system. To assess whether impurities or degradation products co-eluted with

2,3-butanedione or the solvent, a second GC/FID analysis was performed on samples extracted with dimethyl formamide. In conjunction with the stability and purity measurements described above, the relative purity of 2,3-butanedione in the generator reservoir was measured using GC/FID by system D. To demonstrate the resolution and sensitivity of the system to detect low levels of possible impurity or degradation products, a 0.1% solution of acetoin, 2-butanone, 2,3-butanediol, 3-methyl-2-4-pentanedione, ethyl acetate, acetonitrile, and acetic acid was analyzed. During the 2-year studies, GC/FID used to detect impurities was unable to determine the presence of ethyl acetate or 2-butanone due to a contaminant peak that eluted at the same retention times. GC with mass spectrometry detection (system E; Table H-1) was used, and results indicated that ethyl acetate and 2-butanone were less than 0.1% in the distribution line, 50 ppm chambers, generator reservoir, and test chemical samples and less than 0.5% in the 12.5 ppm chambers.

Table H-1. Gas Chromatography Systems Used in the Inhalation Studies of 2,3-Butanedione[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	Stabilwax-DA®, 30 m × 250 µm, 0.50 µm film (Restek, Bellefonte, PA)	Helium at 1 mL/minute	40°C for 4 minutes, then 6°C/minute to 240°C, held for 5 minutes
System B			
Flame ionization	Stabilwax-DA®, 30 m × 250 µm, 0.50 µm film (Restek)	Helium at 1 mL/minute	45°C for 2 minutes, then 6°C/minute to 110°C, then 15°C/minute to 180°C
System C			
Flame ionization	Stabilwax ®, 15 m × 0.53 mm, 2.0 µm film (Restek)	Nitrogen at 25 mL/minute	65°C isothermal
System D			
Flame ionization	DB-Wax ETR, 30 m × 530 µm, 1.5 µm film (J&W Scientific, Folsom, CA)	Helium at 2.6 mL/minute	40°C for 2 minutes, then 6°C/minute to 235°C, held for 5 minutes
System E			
Mass spectrometry	DB-Wax ETR, 30 m × 250 µm, 0.25 µm film (J&W Scientific)	Helium at 0.7 mL/minute	40°C for 2 minutes, then 6°C/minute to 70°C, then 15°C/minute to 240°C, held for 3 minutes

[a]The gas chromatographs were manufactured by Agilent Technologies, Inc. (Santa Clara, CA).

Table H-2. Summary of Chamber Concentrations in the Three-month Inhalation Studies of 2,3-Butanedione

	Total Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Male Rat Chambers			
	6.25	1,280	6.2 ± 0.2
	12.5	1,295	12.3 ± 0.4
	25	1,293	24.9 ± 0.6
	50	1,308	49.8 ± 1.2
	100	1,317	99.5 ± 2.2
Female Rat and Mouse Chambers			
	6.25	1,319	6.2 ± 0.2
	12.5	1,334	12.3 ± 0.4
	25	1,332	24.9 ± 0.6
	50	1,347	49.8 ± 1.1
	100	1,356	99.5 ± 2.2

[a]Mean ± standard deviation.

Table H-3. Summary of Chamber Concentrations in the Two-year Inhalation Studies of 2,3-Butanedione

	Total Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Male Rat Chambers			
	12.5	8,003	12.5 ± 0.3
	25	8,040	25.0 ± 0.7
	50	8,084	50.1 ± 1.5
Female Rat and Mouse Chambers			
	12.5	8,241	12.5 ± 0.4
	25	8,073	24.9 ± 0.7
	50	8,098	49.9 ± 1.5

[a]Mean ± standard deviation.

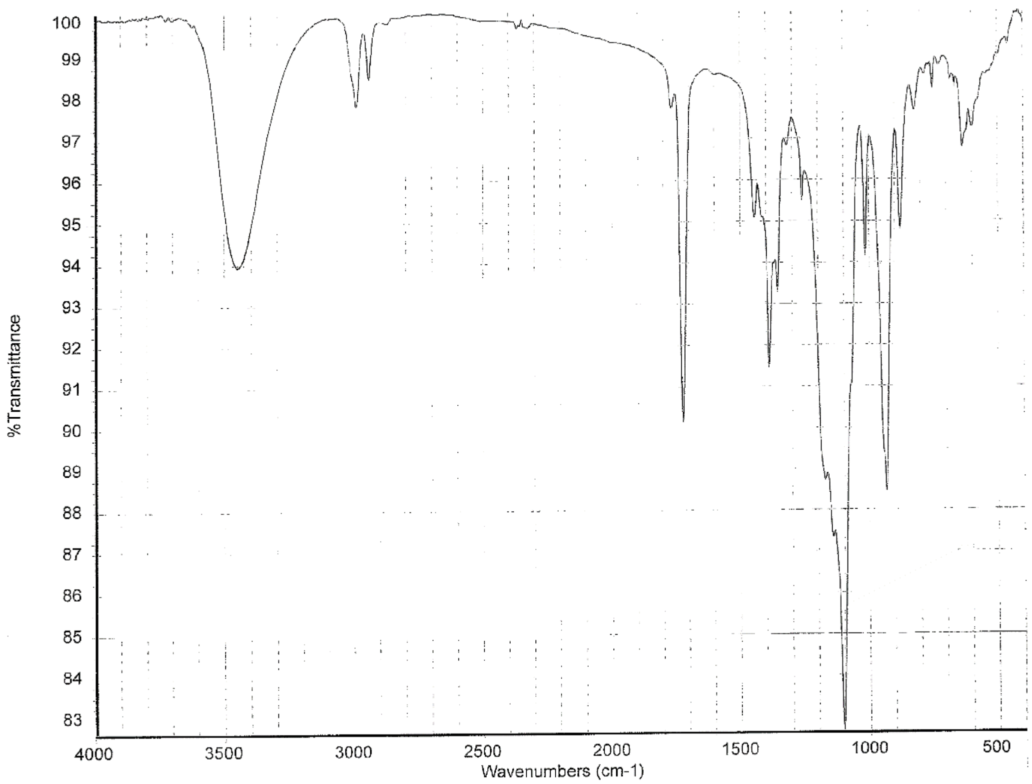

Figure H-1. Infrared Absorption Spectrum of 2,3-Butanedione

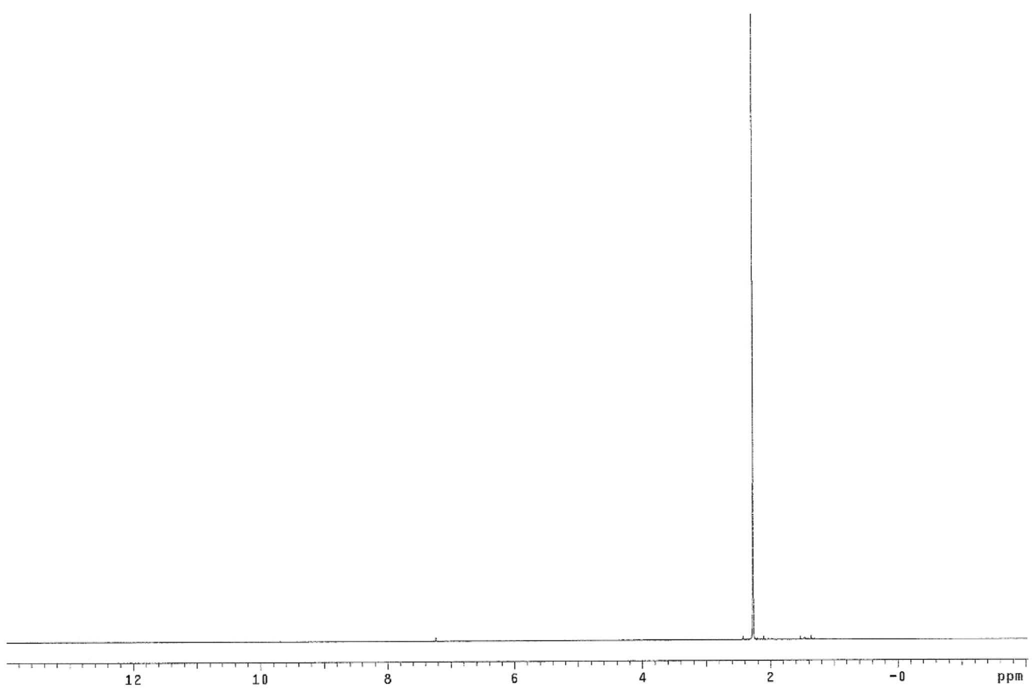

Figure H-2. Nuclear Magnetic Resonance Spectrum of 2,3-Butanedione

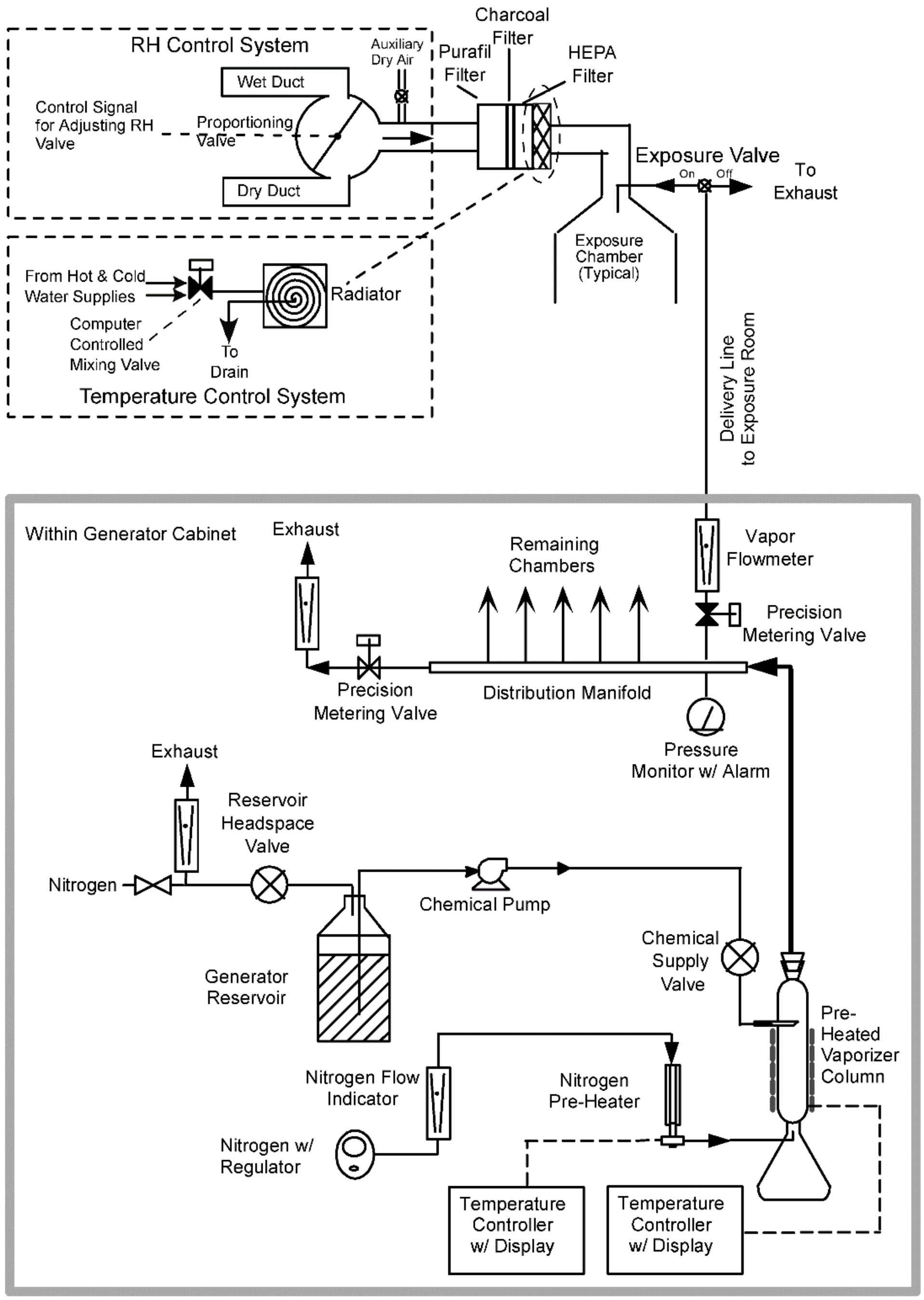

Figure H-3. Schematic of the Vapor Generation and Delivery System in the Three-month Inhalation Studies of 2,3-Butanedione

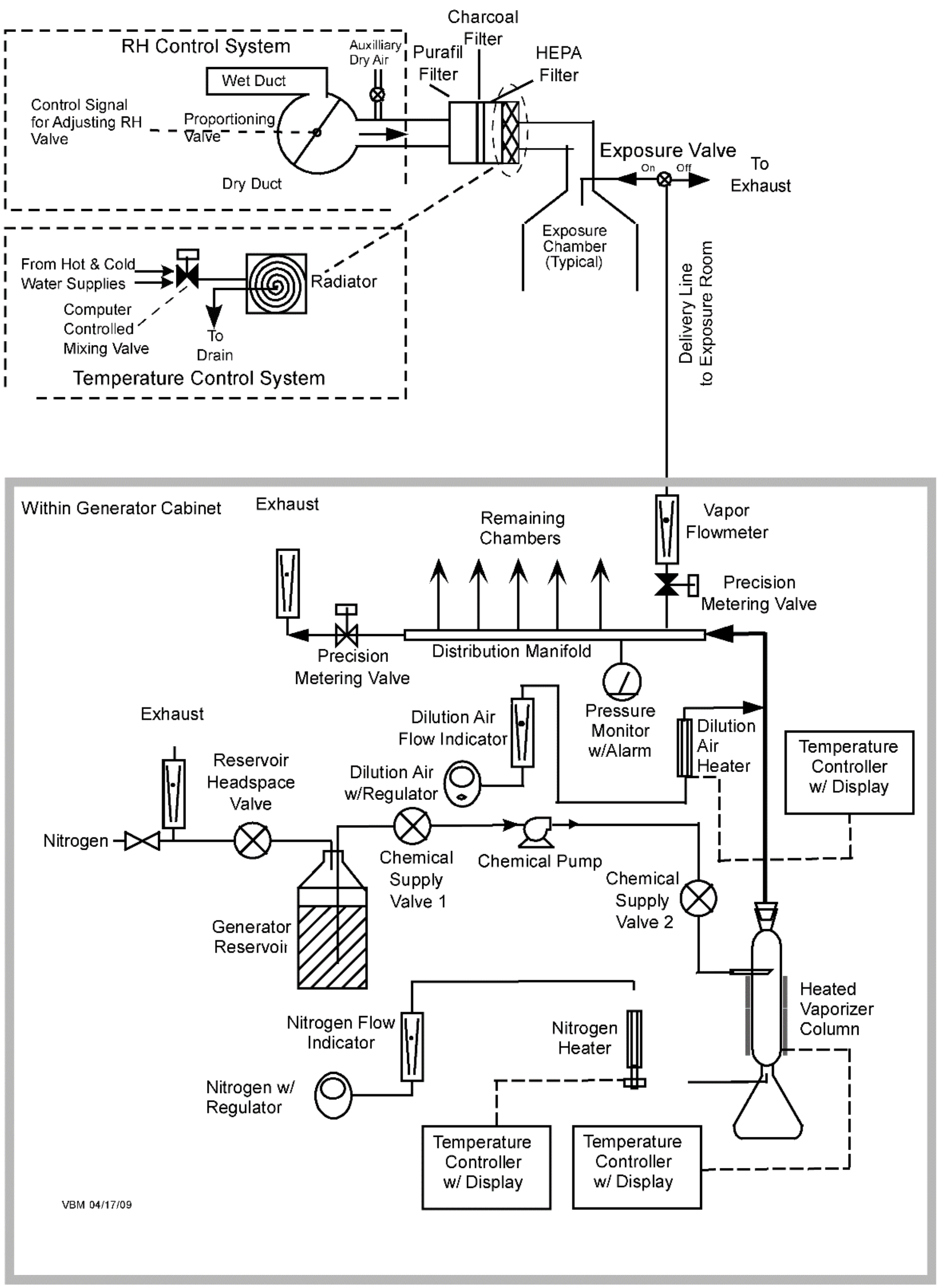

Figure H-4. Schematic of the Vapor Generation and Delivery System in the Two-year Inhalation Studies of 2,3-Butanedione

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

Tables

Table I-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 I-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
B12	52 µg	–
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	d-Biotin

	Amount	Source
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 I-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.7 ± 0.39	14.1–15.7	31
Crude fat (% by weight)	8.4 ± 0.28	7.7–9.0	31
Crude fiber (% by weight)	9.4 ± 0.95	7.1–11.8	31
Ash (% by weight)	5.1 ± 0.19	4.7–5.4	31
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)	3,768 ± 67	2,110–5,330	31
Vitamin D (IU/kg)	1,000[a]	–	–

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
α-Tocopherol (ppm)	77 ± 24.82	7.81–124.0	26
Thiamine (ppm)[b]	7.9 ± 1.58	5.3–12.3	31
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
Minerals			
Calcium (%)	0.899 ± 0.045	0.810–0.994	31
Phosphorus (%)	0.570 ± 0.054	0.504–0.822	31
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 I-4. Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.25 ± 0.047	0.17–0.42	31
Cadmium (ppm)	0.06 ± 0.012	0.04–0.10	31
Lead (ppm)	0.11 ± 0.146	0.06–0.89	31
Mercury (ppm)	<0.02	–	31
Selenium (ppm)	0.19 ± 0.048	0.09–0.34	31
Aflatoxins (ppb)	<5.00	–	31
Nitrate nitrogen (ppm)[c]	18.35 ± 8.46	10.0–42.3	31
Nitrite nitrogen (ppm)[c]	<0.61	–	31
BHA (ppm)[d]	<1.0	–	31
BHT (ppm)[d]	1.07 ± 0.370	1.0–3.04	31
Aerobic plate count (CFU/g)	15.48 ± 28.73	10–170	31
Coliform (MPN/g)	3.0 ± 0.0	3.0	31
Escherichia coli (MPN/g)	<10	–	31
Salmonella (MPN/g)	Negative	–	31
Total nitrosoamines (ppb)[e]	8.93 ± 4.47	2.0–19.46	31
N-Nitrosodimethylamine (ppb)[e]	2.7 ± 1.78	1.0–7.4	31
N-Nitrosopyrrolidine (ppb)[e]	6.4 ± 3.74	1.0–14.95	31
Pesticides (ppm)			
α-BHC	<0.01	–	31
β-BHC	<0.02	–	31
γ-BHC	<0.01	–	31
δ-BHC	<0.01	–	31
Heptachlor	<0.01	–	31
Aldrin	<0.01	–	31
Heptachlor epoxide	<0.01	–	31
DDE	<0.01	–	31
DDD	<0.01	–	31
DDT	0.01	–	31
HCB	<0.01	–	31
Mirex	<0.01	–	31
Methoxychlor	<0.05	–	31
Dieldrin	<0.01	–	31
Endrin	<0.01	–	31

	Mean ± Standard Deviation[b]	Range	Number of Samples
Telodrin	<0.01	–	31
Chlordane	<0.05	–	31
Toxaphene	<0.10	–	31
Estimated PCBs	<0.20	–	31
Ronnel	<0.01	–	31
Ethion	<0.02	–	31
Trithion	<0.05	–	31
Diazinon	<0.10	–	31
Methyl chlorpyrifos	0.11 ± 0.117	0.020–0.553	31
Methyl parathion	<0.02	–	31
Ethyl parathion	<0.02	–	31
Malathion	0.12 ± 0.097	0.020–0.395	31
Endosulfan I	<0.01	–	31
Endosulfan II	<0.01	–	31
Endosulfan sulfate	<0.03	–	31

[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 J. Sentinel Animal Program

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Tables

J.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 toxicological 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 and allowed to clot, and the serum was separated. Additionally, fecal samples were collected and tested for Helicobacter species. All samples were processed appropriately with serology testing performed in-house or sent to the Research Animal Diagnostic Laboratory (RADIL), University of Missouri, Columbia, MO, for determination of the presence of pathogens. 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 at the following times:18-month collection: five male rats and four female rats.

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

Method and Test	Time of Collection
Rats	
Three-month Study	
ELISA: In-House	
Mycoplasma pulmonis	3 weeks postarrival
Pneumonia virus of mice (PVM)	3 weeks postarrival
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	3 weeks postarrival
Rat parvovirus (RPV)	3 weeks postarrival
Sendai	3 weeks postarrival
Multiplex Fluorescent Immunoassay (MFI): RADIL	
Kilham’s rat virus (KRV)	Study termination
M. pulmonis	Study termination
Parvo NS-1	Study termination
PVM	Study termination
RCV/SDA	Study termination
Rat minute virus (RMV)	Study termination
RPV	Study termination
Rat theilovirus (RTV)	Study termination
Sendai	Study termination

Method and Test	Time of Collection
Theiler's murine encephalomyelitis virus (TMEV)	Study termination
Toolans H-1	Study termination
Two-year Study	
MFI: RADIL	
KRV	3 weeks postarrival, 6, 12, and 18 months, study termination
M. pulmonis	3 weeks postarrival, 6, 12, and 18 months, study termination
Parvo NS-1	3 weeks postarrival, 6 months
PVM	3 weeks postarrival, 6, 12, and 18 months, study termination
RCV/SDA	3 weeks postarrival, 6, 12, and 18 months, study termination
RMV	3 weeks postarrival, 6, 12, and 18 months, study termination
RPV	3 weeks postarrival, 6, 12, and 18 months, study termination
RTV	3 weeks postarrival, 6, 12, and 18 months, study termination
Sendai	3 weeks postarrival, 6, 12, and 18 months, study termination
TMEV	3 weeks postarrival, 6 months
Toolan's H-1	3 weeks postarrival, 6, 12, and 18 months, study termination
IFA: RADIL	
KRV	12 months
RMV	12 months
RPV	12 months, study termination
Toolan's H-1	12 months
M. pulmonis	18 months, study termination
Mice	
Three-month Study	
ELISA: In-House	
Mouse hepatitis virus (MHV)	3 weeks postarrival
Mouse parvovirus	3 weeks postarrival
M. pulmonis	3 weeks postarrival
Pneumonia virus of mice (PVM)	3 weeks postarrival
Sendai	3 weeks postarrival
Theiler's murine encephalomyelitis virus – Mouse poliovirus, strain GDVII (TMEV GDVII)	3 weeks postarrival
MFI: RADIL	
Ectromelia virus	Study termination
Epizootic diarrhea of infant mice (EDIM)	Study termination
Lymphocytic choriomeningitis virus (LCMV)	Study termination
M. pulmonis	Study termination

Method and Test	Time of Collection
MHV	Study termination
Mouse norovirus (MNV)	Study termination
Mouse parvovirus (MPV)	Study termination
Minute virus of mice (MVM)	Study termination
Parvo NS-1	Study termination
PVM	Study termination
Reovirus 3	Study termination
Sendai	Study termination
TMEV GDVII	Study termination
Two-year Study	
MFI: RADIL	
Ectromelia virus	3 weeks postarrival, 6, 12, and 18 months, study termination
EDIM	3 weeks postarrival, 6, 12, and 18 months, study termination
LCMV	3 weeks postarrival, 6, 12, and 18 months, study termination
M. pulmonis	3 weeks postarrival, 6, 12, and 18 months, study termination
MHV	3 weeks postarrival, 6, 12, and 18 months, study termination
MNV	3 weeks postarrival, 6, 12, and 18 months, study termination
Parvo NS-1	3 weeks postarrival, 6 months
MPV	3 weeks postarrival, 6, 12, and 18 months, study termination
MVM	3 weeks postarrival, 6, 12, and 18 months, study termination
PVM	3 weeks postarrival, 6, 12, and 18 months, study termination
Reovirus 3	3 weeks postarrival, 6, 12, and 18 months, study termination
Sendai	3 weeks postarrival, 6, 12, and 18 months, study termination
TMEV GDVII	3 weeks postarrival, 6, 12, and 18 months, study termination
IFA: RADIL	
EDIM	18 months, study termination
LCMV	Study termination
M. pulmonis	Study termination
MHV	Study termination
MNV	18 months, study termination
MPV	Study termination
MVM	Study termination
Reovirus 3	Study termination
TMEV GDVII	Study termination
Polymerase Chain Reaction	
Helicobacter species	18 months

J.2. Results

All test results were negative.

Appendix K. Summary of Peer Review Panel Comments

On July 13, 2017, the draft Technical Report on the toxicology and carcinogenesis studies of 2,3-butanedione 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. D.L. Morgan, NIEHS, introduced the toxicology and carcinogenesis studies of 2,3-butanedione by describing the uses of the chemical, the study designs, and the results of the studies in rats and mice, including nonneoplastic and neoplastic lesions in test animals. The proposed conclusions were *some evidence of carcinogenic activity* of 2,3-butanedione in male and female Wistar Han rats, *no evidence of carcinogenic activity* of 2,3-butanedione in male B6C3F1/N mice, and *equivocal evidence of carcinogenic activity* of 2,3-butanedione in female B6C3F1/N mice.

Dr. Gordon asked about the 10-day study where bronchiolitis obliterans had been found at 150 ppm. He found it surprising that the disease was not observed at the slightly lower doses used in the 3-month studies. Dr. Morgan said that the compound has a very steep dose-response curve, and it took some time to find the breaking point in terms of dosage. Dr. Gordon asked about the choice to use the vapor phase, as in the popcorn workers it seemed there was considerable dust. Dr. Morgan agreed but noted that dust would be an almost totally separate test agent, and would have to be studied separately.

Dr. Conner, the first primary reviewer, noted that the studies were complicated and reminiscent of formaldehyde studies, in which dosimetry was affected by breathing patterns. He suggested adding an estimate of dose to the report. He said the studies were well-conducted. He recommended upgrading the conclusion for combined incidences of squamous cell papilloma and squamous cell carcinoma of the nose in male rats from some evidence to clear evidence, based on the number of tumors seen. Similarly, he recommended upgrading the conclusion for adenocarcinoma of the nose in female mice from equivocal evidence to some evidence.

Dr. Morgan said that adding an estimate of dose would potentially be misleading in this instance. He discussed the reasoning behind the conclusions mentioned by Dr. Conner. Dr. Conner felt that the number of instances of such a rare tumor would justify the upgrade he suggested. Dr. Morgan agreed that interpretation of the incidences of rare tumors is challenging.

Dr. Gordon, the second primary reviewer, said the study design was excellent, with appropriate choices for the 2-year exposure concentrations. He felt that the data presentation was clear in both the text and the tables, with statistical comparisons appropriate as described. He agreed with the some evidence conclusion for the rare tumors.

Dr. Dybdal, the third primary reviewer, also felt that the study design was well done, and the experimental results were presented fully and clearly. She said she was concerned whether the rodent model was the correct model to look at the disease, given the compound's background and its effect on the lung. She noted that although bronchiolitis obliterans was not seen, a very clear impact on respiratory tract damage was seen, along with fibrosis. Thus, it is a shame within the confines of the reporting system that that point could not be emphasized more in the report, she observed, "Because I think there is a smoking gun in this data that supports that this compound could well be problematic to the workers." She agreed with the level of evidence conclusions.

Dr. Morgan noted that issues associated with use of rodents are inherent in all inhalation studies, but modelers have done well in extrapolation efforts. Dr. Flake, NIEHS, said that there had been an effort to highlight the fibrosis in the report, in that it was anticipated that the animals would not develop bronchiolitis obliterans at the dosages used. The finding of fibrosis in the nose was unusual in itself, he added. Dr. Walker, NIEHS, asked Dr. Dybdal if she was recommending adding detail to the discussion of the fibrotic responses. She said that would be advantageous.

Dr. Miles, the fourth primary reviewer, also thought that the study was well-designed and comprehensive and evaluated doses relevant to human exposures. She found interesting the sex difference seen in the studies and wondered if there were any reports of sex differences in the development of bronchiolitis obliterans in the literature. She suggested investigating whether there are any reports in the literature of nasal or respiratory cancer in the workers. She asked why the specific strains of rats and mice were chosen, and why data for female spleen was not included in one of the tables. She noted that she agreed with the conclusions stated in the report.

Dr. Morgan said there are no data available on nasal cancer in workers, nor is there reference to sex difference in the bronchiolitis obliterans literature. He explained that the Wistar Han rat was the strain being used at the time, and the mouse strain was the standard. Dr. Flake said that no alterations in the spleen were seen in the females.

Dr. Miles mentioned that it would be helpful to include concentrations reported from other types of processing facilities, such as coffee roasters. Dr. Morgan said that type of data should be available through NIOSH, and he would work to include it.

With respect to sex differences, Dr. Ludewig said the animals were very heavy at the end of the studies, especially the males, which could result in very shallow breathing. Day/night activity patterns with exposure during the resting phase could also play a role, she observed. Both could result in lower/less deep exposure by inhalation than expected. She said she was surprised to see that the ulcers and inflammation in the skin were rated as not related to exposure, because those issues had only occurred in the exposed group. She noted that there were also skin lesions in exposed workers, indicating that the compound damages the skin.

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