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

NTP Technical Report on the Toxicity Studies of Acetoin (CASRN 513-86-0) and 2,3-Pentanedione (CASRN 600-14-6) Administered by Inhalation to Wistar Han [Crl:WI(Han)] Rats and B6C3F1/N Mice

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Foreword

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

The Toxicity Report series began in 1991. The studies described in the NTP Toxicity Report series are designed and conducted to characterize and evaluate the toxicological potential of selected substances in laboratory animals (usually two species, rats and mice). Substances (e.g., chemicals, physical agents, and mixtures) selected for NTP toxicity studies are chosen primarily on the basis of human exposure, level of commercial production, and chemical structure. The interpretive conclusions presented in the Toxicity Reports are derived solely from the results of these NTP studies, and extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection for study per se is not an indicator of a substance's toxic potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and Food and Drug Administration Good Laboratory Practice Regulations and meets or exceeds 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 Laboratory Animals. Studies are subjected to retrospective quality assurance audits before they are presented for public review. Draft reports undergo external peer review before they are finalized and published.

NTP Toxicity 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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Peer Review

The National Toxicology Program (NTP) conducted a peer review of the draft *NTP Technical Report on the Toxicity Studies of Acetoin (CASRN 513-86-0) and 2,3-Pentanedione (CASRN 600-14-6) Administered by Inhalation to Wistar Han [Crl:WI(Han)] Rats and B6C3F1/N Mice* by letter in May 2022 by the experts listed below. Reviewer selection and document review followed established NTP practices. The reviewers were charged to:

(1) Peer review the draft *NTP Technical Report on the Toxicity Studies of Acetoin (CASRN 513-86-0) and 2,3-Pentanedione (CASRN 600-14-6) Administered by Inhalation to Wistar Han [Crl:WI(Han)] Rats and B6C3F1/N Mice*.

(2) Comment on NTP's interpretations of the data.

NTP carefully considered reviewer comments in finalizing this report.

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Abstract

Acetoin and 2,3-pentanedione are highly volatile components of artificial butter flavoring (ABF). Concerns over the inhalation toxicity of these compounds originate from the association between occupational exposures to ABF and adverse fibrotic lung effects, specifically obliterative bronchiolitis (OB) in the distal airways. 2,3-Pentanedione has been used as a replacement for 2,3-butanedione (diacetyl) in some ABF due to concerns about the respiratory toxicity of 2,3-butanedione. However, 2,3-pentanedione is structurally similar to 2,3-butanedione and has been shown to exhibit potency similar to 2,3-butanedione regarding airway toxicity following acute inhalation (whole-body) exposure. This report describes a series of studies to evaluate the 2-week inhalation toxicity of acetoin and the 3-month inhalation toxicity of acetoin and 2,3-pentanedione.

In the 2-week studies, groups of five male and five female Wistar Han [Crl:WI(Han)] rats and B6C3F1/N mice were exposed via whole-body inhalation to acetoin vapors at concentrations up to 800 ppm for 6 hours per day, 5 days per week, for 2 weeks (plus 2 to 3 days). In the 3-month study of acetoin, groups of 10 male and 10 female rats and mice were exposed via whole-body inhalation to vapor concentrations up to 800 ppm for 6 hours per day, 5 days per week, for 13 to 14 weeks. There were no significant exposure-related adverse effects in rats or mice exposed to acetoin for either 2 weeks or 3 months.

In the 3-month 2,3-pentanedione study, groups of 10 male and 10 female rats and mice were exposed via whole-body inhalation to vapor concentrations up to 100 ppm for 6 hours per day, 5 days per week, for 13 to 14 weeks. All rats and mice survived until scheduled euthanasia. Clinical observations in rats and mice exposed to 50 or 100 ppm included abnormal breathing, eye abnormality, and sneezing. There were no significant exposure-related changes in terminal mean body weights of rats, and mean body weights remained within 8% of the control group for the duration of the study. Absolute and relative lung weights and relative heart weights were significantly increased in female rats exposed to 100 ppm compared to those of the control group.

In male and female mice exposed to 50 or 100 ppm 2,3-pentanedione for 3 months, terminal mean body weights were significantly decreased compared to control mice. Relative lung weights of male mice exposed to ≥50 ppm and female mice exposed to 100 ppm were significantly increased, whereas absolute lung weights were significantly decreased in female mice exposed to ≥50 ppm 2,3-pentanedione. Absolute heart, kidney, and liver weights of male and female mice and absolute spleen weights of female mice exposed to ≥50 ppm, as well as absolute spleen weights of male mice and relative spleen weights of female mice exposed to 100 ppm, were significantly decreased. Relative heart and spleen weights of male mice and relative kidney weights of female mice exposed to 100 ppm were significantly increased.

On day 23 and at the end of the 3-month 2,3-pentanedione study, the white blood cell, lymphocyte, monocyte, and/or neutrophil counts were significantly increased in various exposed male and female rats but most consistently at 50 and 100 ppm. The one exception was a significant decrease in the lymphocyte count in the 100 ppm male group at study termination. The leukocyte increases were consistent with inflammation, and the decrease in lymphocytes was most likely due to chronic stress. In addition, at study termination, globulin concentration was significantly increased, and albumin concentration was significantly decreased in the

100 ppm female rats. Related to these changes, the albumin/globulin (A/G) ratio was significantly decreased in male rats exposed to 50 or 100 ppm and in female rats exposed to 100 ppm. These changes were also consistent with an exposure-related proinflammatory response.

Exposure to 2,3-pentanedione, but not acetoin, for 3 months caused adverse nonneoplastic histopathological effects in the nose, larynx, trachea, and lungs of rats and mice.

Significant effects in the nose with exposure to ≥50 ppm 2,3-pentanedione included, among others, an increase in the incidences of suppurative inflammation, respiratory metaplasia of the olfactory epithelium, and hyperplasia and squamous metaplasia of the respiratory epithelium in male and female rats and mice (≥25 ppm for hyperplasia in male rats and squamous metaplasia in male and female mice). Significant effects in the larynx with exposure to 50 and/or 100 ppm included an increase in the incidences of hyperplasia of the squamous epithelium in male and female rats, hyperplasia and squamous metaplasia of the respiratory epithelium in male rats, and chronic active inflammation and squamous metaplasia of the respiratory epithelium (≥25 ppm) in female rats. In male and female mice with exposure to 50 and/or 100 ppm, there were significant increases in the incidences of suppurative inflammation, atypical squamous metaplasia of the respiratory epithelium, and atypical hyperplasia of the squamous epithelium.

Significant effects in the trachea with exposure to 50 and/or 100 ppm 2,3-pentanedione included an increase in the incidences of hyperplasia and regeneration of the epithelium (and squamous metaplasia by positive trend only) in male and female rats and inflammation, regeneration, and atypical squamous metaplasia of the epithelium in male and female mice (and atypical hyperplasia in females by positive trend only). Significant effects in the lung with exposure to 100 ppm 2,3-pentanedione included an increase in the incidences of inflammation and hyperplasia of the bronchial and bronchiolar epithelium and bronchial epithelial regeneration in male and female rats (and squamous or goblet cell metaplasia of the bronchial epithelium by positive trend only). In mice with exposure to 50 and/or 100 ppm 2,3-pentanedione, there were significant increases in the incidences of inflammation, atypical squamous metaplasia, and regeneration of the bronchial epithelium in males and females, atypical hyperplasia of the bronchial epithelium in males, and hyperplasia of the bronchiolar epithelium in males.

Exposure of female rats to 50 and/or 100 ppm 2,3-pentanedione caused an increase in the incidence of acute inflammation in the cornea and ciliary body of the eye. Acute inflammation in the cornea was also observed in male rats and female mice (by positive trend only).

Acetoin was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA1535 when tested with and without 10% or 30% induced rat or hamster liver S9. Results of the peripheral blood micronucleus tests with acetoin and 2,3-pentanedione were negative in male and female rats and mice following 3 months of exposure via whole-body inhalation. In the acetoin study, an exposure concentration-related increase in the percentage of immature erythrocytes (% PCE) was observed in male, but not female, rats, suggesting a mild perturbation in erythropoiesis in male rats. In the 2,3-pentanedione study, a small exposure concentration-related increase in % PCE was seen in female rats but not in male rats. No alterations in % PCE values were observed in mice in either study.

Under the conditions of this inhalation study, there were no significant exposure-related adverse effects in rats or mice exposed to acetoin for 2 weeks or 3 months. Exposure to 2,3-pentanedione via whole-body inhalation for 3 months caused significant adverse effects primarily in the

respiratory tract, but also in the eyes, of rats and mice. These airway findings attributed to 2,3-pentanedione included exposure-related inflammation, injury (degeneration), regeneration, squamous metaplasia, and/or hyperplasia of the tracheal, bronchial, and bronchiolar epithelium. Interestingly, the hyperplasia and squamous metaplasia of the tracheal and bronchial epithelium observed in the mice were considered atypical and therefore potentially preneoplastic. The no-observed-effect level (NOEL) for the bronchial and bronchiolar adverse effects in the lung was 25 ppm in rats and 12.5 ppm in mice after 3 months of exposure to 2,3-pentanedione. These lesions are most relevant because the distal bronchi/bronchioles are the target sites for OB, and hyperplasia/squamous metaplasia could accompany or be precursors to fibrotic lesions (e.g., if the tested exposure concentration was higher) and the morphology of the bronchial/bronchiolar lesions is similar to OB. Regarding eye toxicity, inflammation and degenerative lesions of the cornea were observed, which extended into other ocular compartments in some animals, including the anterior chamber, ciliary body, sclera, conjunctiva, and iris. The NOEL of 2,3-pentanedione for this study overall was 12.5 ppm on the basis of adverse respiratory tract effects in rats and mice. These 3-month inhalation exposure data, including NOELs for adverse respiratory tract effects, can inform regulatory agencies to help mitigate exposure risks to 2,3-pentanedione vapors in the workplace.

Synonyms: 3-hydroxy-2-butanone, butan-2-ol-3-one, acetylmethylcarbinol (acetoin), acetyl propionyl (2,3-pentanedione)

Summary of Findings Considered Toxicologically Relevant in Male and Female Rats and Mice Exposed to Acetoin by Inhalation for Three Months

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in Air	0, 50, 100, 200, 400, or 800 ppm	0, 50, 100, 200, 400, or 800 ppm	0, 50, 100, 200, 400, or 800 ppm	0, 50, 100, 200, 400, or 800 ppm
Survival Rates	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10
Body Weights	Similar to the control group at necropsy	Similar to the control group at necropsy	Similar to the control group at necropsy	Similar to the control group at necropsy
Clinical Observations	None[a]	None	None	None
Organ Weights	None	None	None	None
Nonneoplastic Effects	None	None	None	None
Hematology	None	None	None	None
Clinical Chemistry	None	None	Not assessed[b]	Not assessed
Genetic Toxicology				
Bacterial Gene Mutations		Negative in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA1535 with and without rat and hamster liver S9		
Micronucleated Erythrocytes (In Vivo)				
Rat peripheral blood:		Negative in males and females		
Mouse peripheral blood:		Negative in males and females		

[a]None = no toxicologically relevant effects for this endpoint.
[b]Not assessed = this endpoint was not measured in this study.

Summary of Findings Considered Toxicologically Relevant in Male and Female Rats and Mice Exposed to 2,3-Pentanedione by Inhalation for Three Months

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in Air	0, 6.25, 12.5, 25, 50, or 100 ppm	0, 6.25, 12.5, 25, 50, or 100 ppm	0, 6.25, 12.5, 25, 50, or 100 ppm	0, 6.25, 12.5, 25, 50, or 100 ppm
Survival Rates	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10
Body Weights	Similar to the control group at necropsy	Similar to the control group at necropsy	50 and 100 ppm: 12% and 30% lower, respectively, than the control group at necropsy	50 and 100 ppm: 14% and 30% lower, respectively, than the control group at necropsy
Clinical Observations	Abnormal breathing, eye abnormality, sneezing	Abnormal breathing, eye abnormality, sneezing	Abnormal breathing, sneezing	Abnormal breathing, sneezing, eye abnormality

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Organ Weights	None[a]	↑ Absolute and relative lung weight ↑ Relative heart weight	↓ Absolute heart weight ↑ Relative heart weight ↓ Absolute kidney weight ↓ Absolute liver weight ↑ Relative lung weight ↓ Absolute spleen weight ↑ Relative spleen weight	↓ Absolute heart weight ↓ Absolute kidney weight ↑ Relative kidney weight ↓ Absolute liver weight ↓ Absolute lung weight ↑ Relative lung weight ↓ Absolute and relative spleen weight
Nonneoplastic Effects	Nose: hyperplasia, lymphoid (0/10, 1/10, 1/10, 1/10, 7/10, 5/10); inflammation, suppurative (0/10, 0/10, 0/10, 2/10, 10/10, 10/10); olfactory epithelium, atrophy (1/10, 1/10, 0/10, 4/10, 10/10, 10/10); olfactory epithelium, degeneration (2/10, 0/10, 0/10, 2/10, 10/10, 3/10); olfactory epithelium, metaplasia, respiratory (2/10, 0/10, 2/10, 1/10, 8/10, 10/10); olfactory epithelium, metaplasia, squamous (0/10, 0/10, 0/10, 0/10, 0/10, 2/10); respiratory epithelium, hyperplasia (0/10, 1/10, 2/10, 9/10, 10/10, 10/10); respiratory epithelium, metaplasia, squamous (0/10, 0/10, 1/10, 0/10, 10/10, 10/10); respiratory epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 2/10, 10/10); respiratory epithelium, regeneration (0/10, 0/10, 0/10, 0/10, 0/10, 10/10); turbinate, atrophy (0/10, 0/10, 0/10, 0/10, 2/10, 10/10) Larynx: inflammation, suppurative (0/10, 0/10, 0/10, 0/10, 2/10, 0/10); inflammation, chronic active (1/10, 0/10, 0/10, 0/10, 0/10, 4/10);	Nose: hyperplasia, lymphoid (0/10, 0/10, 0/10, 1/10, 0/10, 3/10); inflammation, suppurative (0/10, 0/10, 0/10, 0/10, 10/10, 10/10); olfactory epithelium, atrophy (1/10, 0/10, 1/10, 1/10, 10/10, 9/10); olfactory epithelium, degeneration (0/10, 1/10, 0/10, 0/10, 4/10, 5/10); olfactory epithelium, metaplasia, respiratory (0/10, 0/10, 1/10, 1/10, 8/10, 9/10); respiratory epithelium, hyperplasia (1/10, 0/10, 0/10, 3/10, 10/10, 10/10); respiratory epithelium, metaplasia, squamous (0/10, 0/10, 0/10, 0/10, 10/10, 10/10); respiratory epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 1/10, 10/10); respiratory epithelium, regeneration (0/10, 0/10, 0/10, 0/10, 3/10, 10/10); respiratory epithelium, ulcer (0/10, 0/10, 0/10, 0/10, 0/10, 1/10); turbinate, atrophy (0/10, 0/10, 0/10, 0/10, 2/10, 9/10) Larynx: inflammation, chronic active (0/10, 0/10, 1/10, 1/10, 0/10, 5/10); respiratory epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 2/10, 1/10); respiratory epithelium, metaplasia, squamous (0/10, 0/10,	Nose: inflammation, suppurative (0/10, 0/10, 0/10, 0/10, 10/10, 10/10); olfactory epithelium, accumulation, hyaline droplet (0/10, 0/10, 0/10, 0/10, 1/10, 6/10); olfactory epithelium, atrophy (0/10, 0/10, 0/10, 0/10, 10/10, 10/10); olfactory epithelium, metaplasia, respiratory (0/10, 0/10, 0/10, 0/10, 10/10, 9/10); respiratory epithelium, accumulation, hyaline droplet (0/10, 0/10, 0/10, 0/10, 4/10, 7/10); respiratory epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 3/10, 4/10); respiratory epithelium, metaplasia, squamous (0/10, 0/10, 0/10, 4/10, 8/10, 9/10); respiratory epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 1/10, 4/10); respiratory epithelium, regeneration (0/10, 0/10, 0/10, 6/10, 9/10, 10/10); septum, necrosis (0/10, 0/10, 0/10, 0/10, 0/10, 7/10); septum, perforation (0/10, 0/10, 0/10, 0/10, 2/10, 4/10); turbinate, atrophy (0/10, 0/10, 0/10, 0/10, 9/10,	Nose: inflammation, suppurative (0/10, 0/10, 0/10, 0/10, 10/10, 10/10); olfactory epithelium, accumulation, hyaline droplet (0/10, 0/10, 0/10, 0/10, 7/10, 10/10); olfactory epithelium, atrophy (0/10, 1/10, 0/10, 0/10, 10/10, 9/10); olfactory epithelium, metaplasia, respiratory (0/10, 0/10, 0/10, 0/10, 7/10, 8/10); respiratory epithelium, accumulation, hyaline droplet (1/10, 0/10, 0/10, 0/10, 5/10, 10/10); respiratory epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 8/10, 4/10); respiratory epithelium, metaplasia, squamous (0/10, 0/10, 0/10, 8/10, 10/10, 10/10); respiratory epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 1/10, 2/10); respiratory epithelium, regeneration (0/10, 0/10, 0/10, 1/10, 5/10, 9/10); septum, necrosis (0/10, 0/10, 0/10, 0/10, 0/10, 8/10); septum perforation (0/10, 0/10, 0/10, 0/10, 1/10, 3/10); turbinate, atrophy (0/10, 0/10, 0/10, 0/10, 10/10, 10/10); turbinate, necrosis (0/10, 0/10, 0/10, 0/10, 9/10, 10/10) Larynx: inflammation, acute (0/10, 0/10, 2/10,

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
	respiratory epithelium, hyperplasia (0/10, 1/10, 0/10, 1/10, 7/10, 3/10); respiratory epithelium, metaplasia, squamous (1/10, 1/10, 1/10, 5/10, 10/10, 10/10); respiratory epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 0/10, 2/10); respiratory epithelium, regeneration (0/10, 0/10, 0/10, 0/10, 1/10, 2/10); respiratory epithelium, ulcer (0/10, 0/10, 0/10, 0/10, 0/10, 1/10); squamous epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 0/10, 7/10) Trachea: inflammation, acute (0/10, 0/10, 0/10, 1/10, 0/10, 3/10); epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 0/10, 8/10); epithelium, metaplasia, squamous (0/10, 0/10, 0/10, 0/10, 0/10, 3/10); epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 0/10, 1/10); epithelium, regeneration (0/10, 1/10, 1/10, 0/10, 8/10, 10/10) Lung: fibrosis, focal (0/10, 0/10, 0/10, 0/10, 0/10, 1/10); inflammation, eosinophil (3/10, 3/10, 1/10, 5/10, 3/10, 10/10); alveolus, infiltration, cellular, polymorphonuclear (0/10, 0/10, 0/10, 0/10, 0/10, 1/10); bronchiole, epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 3/10, 10/10); bronchus, epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 0/10, 5/10); bronchus, epithelium, regeneration (0/10, 0/10, 0/10, 0/10, 1/10, 9/10); bronchus,	0/10, 4/10, 10/10, 10/10); respiratory epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 0/10, 1/10); respiratory epithelium, regeneration (0/10, 0/10, 0/10, 0/10, 0/10, 1/10); respiratory epithelium, ulcer (0/10, 0/10, 0/10, 1/10, 0/10, 0/10); squamous epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 0/10, 9/10); squamous epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 0/10, 5/10); squamous epithelium, ulcer (0/10, 0/10, 0/10, 0/10, 0/10, 4/10) Trachea: inflammation, acute (0/10, 0/10, 0/10, 0/10, 0/10, 2/10); epithelium, hyperplasia (0/10, 0/10, 0/10,1/10, 0/10, 4/10); epithelium, metaplasia, squamous (0/10, 0/10, 0/10, 0/10, 0/10, 2/10); epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 0/10, 2/10); epithelium, regeneration (0/10, 3/10, 0/10, 0/10, 7/10, 10/10) Lung: fibrosis, focal (0/10, 0/10, 0/10, 0/10, 0/10, 2/10); inflammation, eosinophil (4/10, 3/10, 4/10, 3/10, 5/10, 10/10); alveolus, infiltration, cellular, polymorphonuclear (0/10, 0/10, 0/10, 0/10, 0/10, 2/10); bronchiole, epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 0/10, 7/10); bronchus, epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 0/10, 4/10); bronchus, epithelium, regeneration (0/10, 0/10, 0/10, 0/10, 0/10, 8/10); bronchus, epithelium, metaplasia, goblet cell (0/10, 0/10, 0/10, 0/10, 0/10, 3/10)	10/10); turbinate, necrosis (0/10, 0/10, 0/10, 0/10, 9/10, 10/10) Larynx: inflammation, acute (0/10, 0/10, 1/10, 0/10, 2/10, 0/10); inflammation, chronic active (1/10, 0/10, 0/10, 1/10, 2/10, 10/10); respiratory epithelium, hyperplasia, atypical (0/10, 0/10, 0/10, 0/10, 1/10, 0/10); respiratory epithelium, metaplasia, squamous, atypical (0/10, 0/10, 0/10, 0/10, 8/10, 10/10); respiratory epithelium, metaplasia, squamous (0/10, 1/10, 0/10, 2/10, 1/10, 0/10); respiratory epithelium necrosis (0/10, 0/10, 1/10, 0/10, 0/10, 3/10); respiratory epithelium, regeneration (0/10, 1/10, 2/10, 2/10, 9/10, 0/10); respiratory, epithelium, ulcer (0/10, 0/10, 1/10, 0/10, 0/10, 0/10); squamous epithelium, hyperplasia, (0/10, 1/10, 1/10, 2/10, 0/10, 2/10); squamous epithelium, hyperplasia, atypical (0/10, 0/10, 0/10, 0/10, 9/10, 8/10); squamous epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 1/10, 2/10); squamous epithelium, regeneration (0/10, 0/10, 0/10, 3/10, 1/10, 0/10); squamous epithelium, ulcer (0/10, 0/10, 1/10, 0/10, 0/10, 2/10) Trachea: inflammation, suppurative (0/10,	0/10, 0/10, 0/10); inflammation, chronic active (0/10, 0/10, 1/10, 1/10, 2/10, 10/10); lumen, inflammation, suppurative (0/10, 0/10, 0/10, 0/10, 0/10, 1/10); respiratory epithelium, hyperplasia (0/10, 1/10, 0/10, 0/10, 1/10, 0/10); respiratory epithelium, hyperplasia, atypical (0/10, 0/10, 0/10, 0/10, 0/10, 1/10); respiratory epithelium, metaplasia, squamous, atypical (0/10, 0/10, 0/10, 0/10, 9/10, 10/10); respiratory epithelium, metaplasia, squamous (0/10, 1/10, 0/10, 0/10, 0/10, 0/10); respiratory epithelium, necrosis (0/10, 0/10, 2/10, 0/10, 0/10, 2/10); respiratory epithelium, regeneration (0/10, 0/10, 0/10, 1/10, 7/10, 0/10); respiratory epithelium, ulcer (0/10, 0/10, 0/10, 0/10, 1/10, 0/10); squamous epithelium, hyperplasia (0/10, 0/10, 0/10, 4/10, 1/10, 0/10); squamous epithelium, hyperplasia, atypical (0/10, 0/10, 0/10, 0/10, 9/10, 10/10); squamous epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 3/10, 3/10); squamous epithelium regeneration (0/10, 0/10, 2/10, 0/10, 0/10, 0/10); squamous epithelium, ulcer (0/10, 0/10, 0/10, 0/10, 0/10, 1/10) Trachea: inflammation, suppurative (0/10, 0/10, 0/10, 0/10, 0/10, 5/8); inflammation, chronic active (0/10, 0/10, 0/10, 0/10, 0/10, 3/8); epithelium, hyperplasia, atypical (0/10, 0/10, 0/10, 0/10, 0/10, 3/8); epithelium, metaplasia, squamous, atypical

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
	epithelium, metaplasia, squamous (0/10, 0/10, 0/10, 0/10, 0/10, 3/10); bronchus, epithelium, metaplasia, goblet cell (0/10, 0/10, 0/10, 0/10, 0/10, 1/10) Eye: anterior chamber, inflammation, acute (0/10, 0/10, 0/9, 0/10, 2/10, 0/10); ciliary body, inflammation, acute (0/10, 0/10, 0/9, 0/10, 3/10, 0/10); conjunctiva, inflammation, acute (0/10, 0/10, 2/9, 0/10, 1/10, 0/10); cornea, inflammation, acute (0/10, 0/10, 0/9, 0/10, 3/10, 3/10); cornea, neovascularization (0/10, 0/10, 0/9, 0/10, 0/10, 2/10); cornea, ulcer (0/10, 0/10, 0/9, 0/10, 1/10, 0/10); cornea, epithelium, hyperplasia (0/10, 0/10, 0/9, 0/10, 0/10, 1/10); cornea, epithelium vacuolation (0/10, 0/10, 0/9, 0/10, 0/10, 2/10); iris, inflammation, acute (0/10, 0/10, 0/9, 0/10, 2/10, 0/10)	Eye: anterior chamber, inflammation, acute (0/10, 0/10, 0/10, 0/9, 3/9, 0/10); ciliary body, inflammation, acute (0/10, 0/10, 0/10, 0/9, 5/9, 2/10); conjunctiva, inflammation, acute (0/10, 0/10, 1/10, 0/9, 1/9, 0/10); cornea, inflammation, acute (0/10, 0/10, 1/10, 0/9, 5/9, 6/10); cornea, ulcer (0/10, 0/10, 0/10, 0/9, 0/9, 1/10); cornea, epithelium, hyperplasia (0/10, 0/10, 0/10, 0/9, 0/9, 1/10); cornea, epithelium, vacuolation (0/10, 0/10, 0/10, 0/9, 1/9, 0/10); sclera, inflammation, acute (0/10, 0/10, 0/10, 0/9, 2/9, 0/10)	0/10, 1/9, 0/10, 0/10, 5/10); inflammation, chronic active (0/10, 0/10, 0/9, 0/10, 0/10, 5/10); epithelium, hyperplasia, atypical (0/10, 0/10, 0/9, 0/10, 1/10, 1/10); epithelium, metaplasia, squamous atypical (0/10, 0/10, 0/9, 0/10, 1/10, 10/10); epithelium, necrosis (0/10, 0/10, 0/9, 0/10, 0/10, 1/10); epithelium, regeneration (0/10, 0/10, 0/9, 0/10, 8/10, 0/10) Lung: bronchiole, infiltration, cellular, polymorphonuclear (0/10, 0/10, 0/10, 0/10, 0/10, 5/10); bronchiole, epithelium, degeneration (0/10, 0/10, 0/10, 0/10, 0/10, 4/10); bronchiole, epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 1/10, 4/10); bronchus, infiltration, cellular, polymorphonuclear (0/10, 0/10, 0/10, 0/10, 0/10, 9/10); bronchus, inflammation, chronic (0/10, 0/10, 0/10, 1/10, 6/10, 9/10); bronchus, ulcer (0/10, 0/10, 0/10, 0/10, 0/10, 1/10); bronchus, epithelium, degeneration (0/10, 0/10, 0/10, 2/10, 9/10, 8/10); bronchus, epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 2/10, 0/10); bronchus, epithelium, hyperplasia, atypical (0/10, 0/10, 0/10, 0/10, 7/10, 0/10); bronchus, epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 0/10, 3/10); bronchus,	(0/10, 0/10, 0/10, 0/10, 1/10, 8/8); epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 1/10, 1/8); epithelium, regeneration (1/10, 0/10, 0/10, 0/10, 10/10, 0/8) Lung: bronchiole, infiltration, cellular, polymorphonuclear (0/10, 0/10, 0/10, 0/10, 0/10, 3/10); bronchiole, epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 1/10, 1/10); bronchus, infiltration, cellular, polymorphonuclear (0/10, 0/10, 0/10, 0/10, 0/10, 9/10); bronchus, inflammation, chronic (0/10, 0/10, 0/10, 0/10, 4/10, 9/10); bronchus, epithelium, degeneration (0/10, 0/10, 0/10, 0/10, 9/10, 8/10); bronchus, epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 1/10, 0/10); bronchus, epithelium, hyperplasia, atypical (0/10, 0/10, 0/10, 0/10, 1/10, 0/10); bronchus, epithelium, metaplasia, squamous, atypical (0/10, 0/10, 0/10, 0/10, 0/10, 10/10); bronchus, epithelium, necrosis (0/10, 0/10, 0/10, 0/10, 0/10, 2/10); bronchus, epithelium, regeneration (0/10, 0/10, 0/10, 0/10, 9/10, 9/10); bronchus, epithelium, ulcer (0/10, 0/10, 0/10, 0/10, 0/10, 2/10) Eye: cornea, inflammation, acute (0/10, 0/10, 0/10, 0/10, 0/10, 2/10); cornea, mineralization (0/10, 0/10, 0/10, 0/10, 0/10, 6/10); cornea, ulcer (0/10, 0/10, 0/10, 0/10, 0/10, 2/10); cornea, epithelium, cellular alteration, focal (0/10, 0/10, 0/10, 0/10, 3/10,

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
			epithelium, metaplasia, squamous, atypical (0/10, 0/10, 0/10, 0/10, 0/10, 10/10); bronchus, epithelium, regeneration (0/10, 1/10, 0/10, 1/10, 8/10, 7/10) Eye: cornea, inflammation, acute (0/10, 0/10, 0/10, 0/10, 0/10, 1/10); cornea, epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 0/10, 1/10); sclera, inflammation, acute (0/10, 0/10, 0/10, 1/10, 0/10, 0/10)	0/10); cornea, epithelium, hyperplasia (0/10, 0/10, 0/10, 0/10, 0/10, 3/10)
Hematology[b] [Day 3, Day 23, Day 93 (males) or Day 94 (females)]	WBC (–,–,–); Lymphocytes (–,–,↓); Monocytes (–,–,–); Neutrophils (–,↑,↑)	WBC (–,↑,–); Lymphocytes (–,↑,–); Monocytes (–,↑,↑); Neutrophils (–,↑,↑)	Not assessed[c]	Not assessed
Clinical Chemistry[b] [Day 3, Day 23, Day 93 (males) or Day 94 (females)]	Albumin (–,–,–); Globulin (–,–,–); A/G Ratio (–,–,↓)	Albumin (–,–,↓); Globulin (–,–,↑); A/G Ratio (–,–,↓)	None	None
Genetic Toxicology				
Micronucleated Erythrocytes (In Vivo)				
Rat peripheral blood:		Negative in males and females		
Mouse peripheral blood:		Negative in males and females		

WBC = white blood cells; A/G = albumin/globulin.
[a]None = no toxicologically relevant effects for this endpoint.
[b]↑, ↓, and – represent an increase, a decrease, and no biologically significant change in a parameter, respectively, compared to the control group.
[c]Not assessed = this endpoint was not measured in this study.

Overview

Occupational exposure to volatile components of artificial butter flavoring (ABF) (including 2,3-butanedione [diacetyl]) via inhalation has been reported to be associated with airway fibrosis in the form of bronchiolitis obliterans, also known as obliterative bronchiolitis (OB), mostly in workers in the microwave popcorn packaging and flavoring industry[1-6] but also in other food and beverage industries.[7] OB is a potentially fatal lung disease that is frequently found in lung transplant patients and is characterized by bronchiolar wall inflammation and fibrosis resulting in constrictive bronchiolitis with restricted airflow.[8] ABF was nominated to the National Toxicology Program (NTP) by The United Food and Commercial Workers International Union for inhalation studies. The nomination included a request to study potentially toxic and highly volatile compounds found in ABF, including 2,3-butanedione and acetoin.

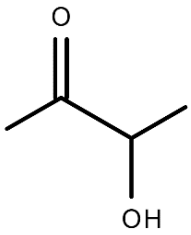
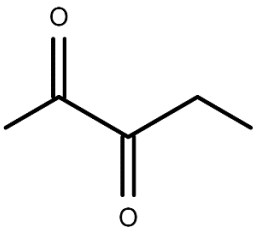
Intratracheal instillation or oropharyngeal aspiration of 2,3-butanedione, or inhalation (whole-body) exposure to 2,3-butanedione vapors, has been shown to cause respiratory toxicity in rats and mice,[9-13] including airway epithelial injury with OB-like fibrotic lesions in rats[12-14] that are similar to the OB lesions observed in occupational exposures. Inhalation (whole-body) exposure to 150 or 200 ppm 2,3-butanedione vapors for 2 weeks, or via a single intratracheal instillation of 2,3-butanedione (125 mg/kg body weight), was shown to induce bronchial fibrosis in rats.[12; 13] Three-month and 2-year inhalation toxicity and carcinogenicity testing of 2,3-butanedione (using whole-body exposure) was also conducted in rats and mice.[15]

Due to concerns about the respiratory toxicity of inhaled 2,3-butanedione, 2,3-pentanedione has been used as a replacement in some ABF.[16] However, 2,3-pentanedione is structurally similar to 2,3-butanedione (both are vicinal diketones) and has been shown to exhibit similar potency to 2,3-butanedione for airway toxicity following inhalation exposure. Inhalation of 2,3-pentanedione vapors by whole-body exposure has been shown to cause respiratory toxicity in rats similar to that of 2,3-butanedione,[13; 17-19] including OB-like airway (bronchial) fibrosis in rats exposed to 150 or 200 ppm for 2 weeks.[13; 17; 19]

To date, the inhalation toxicity of 2,3-pentanedione has been reported for 2-week and shorter whole-body exposure studies in rats with a main focus on adverse respiratory tract effects. The primary objective of the NTP study reported herein was to evaluate the 3-month inhalation toxicity of 2,3-pentanedione vapors within the respiratory tract, the eyes, and other target organs in Wistar Han [Crl:WI(Han)] rats and B6C3F1/N mice, including airway/lung lesion formation following 3 months of whole-body exposure and the potential mechanism(s) of chemical-induced pathogenesis within the airways/lung. In addition, the 2-week and 3-month inhalation toxicity of acetoin—which, like 2,3-butanedione, was originally found to be a major volatile component of ABF[9]—was evaluated in Wistar Han rats and B6C3F1/N mice.

Introduction

Table 1. Acetoin and 2,3-Pentanedione

Chemical Name	Chemical Formula	CASRN	Molecular Weight (g/mol)	Structure
Acetoin	$C_4H_8O_2$	513-86-0	88.11	
2,3-Pentanedione	$C_5H_8O_2$	600-14-6	100.12	

Images generated with ChemSpider.[20]
Synonyms: 3-hydroxy-2-butanone, butan-2-ol-3-one, acetylmethylcarbinol (acetoin), acetyl propionyl (2,3-pentanedione).

Chemical and Physical Properties

3-Hydroxy-2-butanone (acetoin) and 2,3-pentanedione (acetyl propionyl)—referred to as acetoin and 2,3-pentanedione, respectively, throughout this report—are both volatile, water-soluble ketones used as flavoring agents.[21] Acetoin is a yellow liquid monomer or solid crystal dimer with a pleasant butter aroma and a "sweet, buttery, creamy, sour, fatty, vanilla" organoleptic profile.[22; 23] 2,3-Pentanedione is a clear yellow liquid with a "creamy, penetrating, cheese, oily, sweet, buttery," almond-like organoleptic profile.[22; 24] 2,3-Pentanedione, a five-carbon diketone, is more water-soluble and volatile than acetoin, a four-carbon hydroxyl-keto compound.

Production, Use, and Human Exposure

Acetoin occurs widely in nature and is produced by microorganisms, plants, and animals. Naturally occurring acetoin contributes to the flavors of butter, honey, cocoa, fruits, vegetables, and flours.[21; 25; 26] Almost all alcoholic beverages contain acetoin that results from yeast or bacterial fermentation.[27] Acetoin is also an abundant volatile compound in fresh human sweat (9.5%).[28]

Chemical synthesis of acetoin can be performed by three main methods: partial reduction of 2,3-butanedione (diacetyl) with zinc and acid, halogenation of 2-butanone and hydrolysis to acetoin, and condensation of acetaldehyde with catalysis by triazolium ylide or thiazolium ylide.[21; 25] Annual production levels of acetoin in the United States increased from 116,000 pounds in 1995 to 216,000 pounds in 2015.[29]

2,3-Pentanedione is produced naturally as a fermentation product in foods and beverages, such as beer, wine, and yogurt.[30] Chemical synthesis of 2,3-pentanedione is mainly via oxidation of methyl propyl ketone when in the presence of hydroxylamine hydrochloride and catalyzed by sodium nitrite and diluted hydrochloric acid; it can also be synthesized by condensation of acetol with hexanal catalyzed by acid.[31; 32] The annual production of 2,3-pentanedione in the United States increased from 2,600 pounds in 1995 to 87,500 pounds in 2015.[29]

Acetoin and 2,3-pentanedione are used primarily as ingredients in synthetic flavoring formulations used as food additives.[15] Acetoin is also used as a fragrance carrier in the preparation of perfumes and essences. 2,3-Pentanedione is a common constituent of synthetic flavorings and is used as a 2,3-butanedione substitute in some flavor manufacturing and food production operations, including artificial butter flavorings (ABF).[33; 34] Another use of 2,3-pentanedione is as a raw material in the preparation of alkyl-substituted pyrazines.[22]

Human exposure to acetoin and 2,3-pentanedione occurs primarily by ingestion and inhalation of vapors, but dermal absorption is another potential route of exposure. Ingestion of low amounts of these flavorings typically present in foods and beverages has not been reported to cause adverse health effects.[34] Nonoccupational inhalation exposure to acetoin or 2,3-pentanedione can occur through exposure to fragrance vapors, use of e-cigarettes, and flavored tobacco in hookah water pipes.[35; 36] Cigarette smoke also has been reported to contain 2,3-pentanedione and 2,3-butanedione,[37; 38] and therefore can contribute to nonoccupational inhalation exposure. Occupational inhalation exposure to vapors can occur in workers involved in the production of microwave popcorn, coffee, baked goods, and other industries in which flavorings containing acetoin or 2,3-pentanedione, such as ABF, are heated.[34] Exposure levels for the related compound, 2,3-butanedione, were reported to be 57.2 ppm in the mixing room of a microwave popcorn production plant where some workers developed obliterative bronchiolitis (OB),[39] and the peak level of 2,3-butanedione measured in the headspace of mixing vats containing ABF was reported to be as high as 1,230 ppm.[1] Exposure levels for 2,3-pentanedione have been reported in food production facilities (range: 15.14–172.07 ppb),[16] in buttermilk flavoring for bakeries (up to 91 ppb),[33] and in coffee production including roasting, grinding, and flavoring (up to 3.82 ppm).[40-42] Duling et al.[41] reported the peak 15-minute exposure to 2,3-butanedione and 2,3-pentanedione in a coffee flavoring room to be 14.3 and 13.8 ppm, respectively.[41]

Regulatory Status

Currently there are no Occupational Safety and Health Administration (OSHA) occupational exposure limits (OELs) for acetoin or 2,3-pentanedione vapors. Acetoin is regulated by the U.S. Food and Drug Administration (FDA) as a synthetic flavoring substance or adjuvant for human consumption[43] and for animal drugs, feeds, and related products.[43; 44] The addition of acetoin as a synthetic flavoring to human food in the United States is generally recognized as safe (GRAS), as defined by the FDA (21 CFR 182.60).[43] 2,3-Pentanedione is also GRAS and approved by FDA for use in flavoring.[45] The National Institute for Occupational Safety and Health (NIOSH) recommends that inhalation exposure to 2,3-pentanedione be kept below a time-weighted average concentration of 9.3 ppb (recommended exposure limits [RELs]) during a 40-hour work week.[34] NIOSH also recommends short-term exposure limits (STELs) of 31 ppb 2,3-pentanedione during a 15-minute period.

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Acetoin fed to dogs was reported to be partially excreted in the urine,[46] along with a metabolite, butane-2,3-diol.[47] Additional reports have described the metabolism of acetoin by liver preparations in vitro and in laboratory animals fed acetoin. Doisy and Westerfeld[48] and Lipmann[49] reported an increase of acetylation of sulfanilamide upon feeding acetoin to rabbits or incubating it with liver homogenates. Gabriel et al.[50] demonstrated that cell-free extracts from mammalian liver could reduce acetoin to butane-2,3-diol. In subsequent studies, it was reported that ^{14}C-labeled acetoin was oxidized to carbon dioxide in Sprague Dawley rats and in rat liver preparations and that oxidation probably proceeded through the formation of acetate from acetoin prior to oxidation.[51] More recent studies on the metabolism of acetoin have not been identified. The literature contains no studies on the absorption, distribution, metabolism, and excretion (ADME) of inhaled 2,3-pentanedione in laboratory animals.

Humans

The literature contains no studies on the ADME of inhaled acetoin or 2,3-pentanedione vapors in humans. 2,3-Pentanedione and other α-dicarbonyls are metabolized by dicarbonyl/L-xylulose reductase (DCXR) in cell-free liver and kidney preparations.[52; 53] DCXR and its counterparts are enzymes found in the lung, kidney, liver, and epididymis of various species.[53] Zaccone et al.[54] demonstrated the ability of intact primary human tracheobronchial epithelial cells in vitro to metabolize 2,3-pentanedione to 2-hydroxy-3-pentanone after exposure to 2,3-pentanedione vapors.

Toxicity

Experimental Animals

The literature contains no studies on the toxicity of inhaled acetoin vapors in laboratory animals. The effects of acetoin administered in dosed drinking water were evaluated in male and female CFE rats.[55] Rats received drinking water containing 0 (control), 750, 3,000, or 12,000 ppm acetoin for 13 weeks. Exposure to 750 or 3,000 ppm had no adverse effects on body weight gain, hematological findings, serum chemistry, renal cell excretion, urinary concentration tests, organ weights, or histopathology. Exposure to 12,000 ppm acetoin resulted in a reduced rate of body weight gain, which was associated with a reduction in feed and water consumption. Relative liver weight was increased at 12,000 ppm, and animals exhibited slight anemia. The no-adverse-effect level was 3,000 ppm in drinking water, equivalent to an intake of approximately 330 mg acetoin/kg body weight/day or almost 700 times the calculated maximum daily intake in humans.[55]

Inhalation (whole-body) exposure of male Sprague Dawley [Hla®(SD)CVF®] rats or male and female Wistar Han rats to 2,3-pentanedione vapors caused significant respiratory tract toxicity, similar to that caused by 2,3-butanedione, including obliterative bronchiolitis (OB)-like airway fibrosis.[13; 17-19] Morgan et al.[13] compared the toxicity of 2,3-pentanedione in the respiratory tract with that of 2,3-butanedione and 2,3-hexanedione in a 2-week inhalation study in rats. Male Wistar Han rats were evaluated the day after 2 weeks of whole-body exposure (6 hours/day,

5 days/week [plus 2 days], for 12 exposures total) 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 200 ppm 2,3-butanedione and 2,3-pentanedione rats and in most 150 ppm 2,3-butanedione and all 2,3-pentanedione rats in the postexposure groups. For the recovery groups, bronchial fibrosis was observed in all surviving rats exposed to 200 ppm 2,3-butanedione or 2,3-pentanedione, some rats exposed to 150 ppm 2,3-butanedione, and most rats exposed to 150 ppm 2,3-pentanedione. Patchy interstitial fibrosis in the lungs of recovery group animals exposed to 150 or 200 ppm 2,3-pentanedione or 2,3-butanedione correlated with deficits in pulmonary function. Chemical reactivity of the α-diketones with an arginine substrate decreased with increasing chain length (2,3-butanedione ≥ 2,3-pentanedione > 2,3-hexanedione). Morgan et al.[13] concluded that 2,3-butanedione and 2,3-pentanedione vapors were of similar reactivity and potency in causing bronchial fibrosis in rats.

Increased airway reactivity to methacholine with bronchial constriction was shown in rats exposed to 2,3-pentanedione, and increased hypersensitivity (measured by increased lymphocyte proliferation in draining lymph nodes) was observed in a murine model of dermal sensitization to diacetyl alternatives, including 2,3-pentanedione.[56]

Humans

The literature contains no studies on the toxicity of acetoin and is limited for 2,3-pentanedione in humans. Acetoin has been detected in many of the workplaces where OB occurred in workers who make or use 2,3-butanedione.[3; 5] 2,3-Pentanedione has been used as a primary substitute for 2,3-butanedione in some workplace settings[34; 56; 57]; therefore, there is potential for occupational exposure to 2,3-pentanedione vapors.[42; 58]

Abnormal (decreased) lung function (suggestive of undiagnosed or subclinical OB), increased dyspnea, and asthma-like and other respiratory symptoms have been reported in workers exposed to components of flavorings, including ABF (e.g., 2,3-pentanedione).[56; 57; 59]

Eye irritation was frequently reported in NIOSH medical surveys at microwave popcorn and flavoring manufacturing plants.[34]

Reproductive and Developmental Toxicity

The literature contains no studies on the reproductive and developmental toxicity of acetoin or 2,3-pentanedione.

Carcinogenicity

The literature contains no studies on the carcinogenicity of acetoin or 2,3-pentanedione. Chronic inhalation exposure to the related α-diketone, 2,3-butanedione, resulted in a low incidence of rare nasal cavity tumors (squamous cell carcinomas) in male and female Wistar Han [Crl:WI(Han)] rats; a low incidence of adenocarcinoma of the nose was also observed in female B6C3F1/N mice exposed to 2,3-butanedione.[15]

Genetic Toxicity

Published reports on the genetic toxicity of acetoin or 2,3-pentanedione are limited. Negative results were observed when these chemicals were tested in bacterial mutagenicity assays. Acetoin (identified by the authors as butan-2-ol-3-one), tested at several concentrations ranging from 5 nmol to 0.5 mmol per plate, was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, and TA102 in the absence or presence of rat metabolic liver enzymes (S9 mix).[60] The literature contains no additional peer-reviewed publications on acetoin genotoxicity. A book chapter authored by Garst et al.,[61] however, reported bacterial mutation test results for a series of structurally related α-hydroxy ketones, which included acetoin, identified by the authors as acetylmethylcarbinol; although no data were shown, acetoin was listed as nonmutagenic in TA100 in the absence or presence of S9 mix.

2,3-Pentanedione, tested at several concentrations ranging from 9 nmol to 0.9 mmol per plate, was not mutagenic in *S. typhimurium* strains TA98, TA100, or TA102 in the absence or presence of S9 mix.[60] In addition, no mutagenicity was observed with 2,3-pentanedione tested at a single concentration of 3 μmol per plate in strains TA98, TA100, TA1535, or TA1537, with or without S9 mix.[62] A recent study using in silico QSAR (quantitative structure-activity relationship) modeling, bacterial mutagenicity testing, and in vitro micronucleus tests with human TK6 cells to characterize the toxicity potential for a group of data-poor chemicals confirmed the absence of mutagenicity for 2,3-pentanedione in *S. typhimurium* strains.[63] In the in vitro micronucleus test in TK6 cells, the authors called the responses observed with 2,3-pentanedione inconclusive, with and without S9, because the dose range tested was suboptimal (too high without S9 and too low with S9).[63]

A structurally related α-diketone, 2,3-butanedione, was shown to be mutagenic in *S. typhimurium* base-substitution strains TA100, TA102, and TA104, with and without rat liver S9 activation[60; 64; 65]; no mutagenicity was shown in the frameshift strain TA98, with or without S9.[60; 64] 2,3-Butanedione was also tested in two independent bacterial gene mutation assays as part of an NTP study.[15] In the first set of assays, conducted with and without hamster and rat liver S9, 2,3-butanedione was mutagenic in *S. typhimurium* strain TA97 with and without hamster and rat S9, equivocal in strain TA100 with hamster and rat S9, and negative in strains TA98, TA100, and TA1535. In the second set of assays, 2,3-butanedione, tested with and without rat liver S9, was mutagenic in strain TA97 without S9 and in *Escherichia coli* strain WP2 *uvrA* (pKM101) with and without S9; equivocal results were obtained in strain TA97 with S9 and in strain TA100 with and without S9, while no mutagenicity was observed in strain TA98. Results of a bone marrow micronucleus test conducted in male B6C3F1/N mice following intraperitoneal injection of 2,3-butanedione were negative. Results of a peripheral blood micronucleus test conducted in male and female Wistar Han [Crl:WI(Han)] rats and B6C3F1/N mice following 3 months of inhalation exposure were also negative.[15]

Study Rationale

The inhalation toxicities of acetoin and 2,3-pentanedione were investigated due to extensive occupational and nonoccupational exposures to the vapors of these chemicals and because both are structurally similar to 2,3-butanedione, a known profibrotic respiratory tract toxicant. To date, the inhalation toxicity of 2,3-pentanedione has only been reported for 2-week and shorter whole-body exposure studies in rats with a main focus on adverse respiratory tract effects. The

primary objective of the NTP study reported herein was to evaluate the 3-month inhalation toxicity of 2,3-pentanedione vapors within the respiratory tract, the eyes, and other target organs in Wistar Han [Crl:WI(Han)] rats and B6C3F1/N mice, including airway/lung lesion formation following 3 months of whole-body exposure and potential mechanism(s) of chemical-induced pathogenesis within the airways/lung. The inhalation toxicity of acetoin, which like 2,3-butanedione was originally found to be a major volatile component of ABF,[9] has not been investigated. Therefore, this NTP study also evaluated the 2-week and 3-month inhalation toxicity of acetoin in Wistar Han rats and B6C3F1/N mice. Additional toxicological data for 2,3-pentanedione and acetoin are needed by regulatory agencies to help inform inhalation exposure limits to mitigate occupational exposure risks.

NTP subchronic and chronic inhalation studies typically and historically have used whole-body exposure instead of nose-only exposure. Whole-body exposure has been shown to be less stressful than nose-only exposure (due to animal immobilization and increased heating within the nose-only exposure tubes), especially for repeated exposures, whereas nose-only exposure is more amenable to acute exposures.[18; 66] Whole-body exposure is also a more occupationally relevant exposure route to a volatile chemical for which, in addition to inhalation, there is potential for dermal and ocular exposure (which would be similar to exposed workers without proper PPE). Furthermore, initial testing of ABF (including the related compound 2,3-butanedione) reported in NTP studies[15] and others (e.g., Hubbs et al. 2002[9]) used whole-body exposure. As part of NTP's evaluation of ABF, 2,3-butanedione was also tested in 3-month and 2-year inhalation toxicity studies using whole-body exposure; therefore, the exposure method and route for the 2,3-pentanedione 3-month study were matched to that of 2,3-butanedione for comparative purposes. It is also known from several 2-week studies that whole-body exposure of rats to 2,3-pentanedione vapors consistently induces obliterative bronchiolitis (OB)-like fibrotic airway lesions in the lung, which reflect those observed occupationally in workers,[39] suggesting that 2,3-pentanedione permeates into the distal airways of the lung with whole-body exposure.

Materials and Methods

Procurement and Characterization of Acetoin and 2,3-Pentanedione

Acetoin was obtained from Sigma-Aldrich, Inc. (Milwaukee, WI) in one lot (09118JH). 2,3-Pentanedione also was obtained from Sigma-Aldrich, Inc. (Milwaukee, WI) in one lot (MKBB7504). Identity and purity analyses were conducted by the analytical chemistry and animal study laboratories at Battelle Toxicology Northwest (Richland, WA) (Appendix A). Reports on analyses performed in support of the acetoin and 2,3-pentanedione studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

Lot 09118JH, a solid white powder, was identified as acetoin by Chemir Analytical Services (Maryland Heights, MO) using Fourier transform infrared (IR) and ^{1}H nuclear magnetic resonance (NMR) spectroscopies. Lot MKBB7504, a clear yellow liquid, was identified as 2,3-pentanedione by the study laboratory using IR and by Chemir Analytical Services using ^{1}H NMR. Lot 09118JH was consistent with comparison spectra of IR and NMR from commercially procured reference standards. All spectra for both lot 09118JH and lot MKBB7504 matched the anticipated structures of the test articles (Appendix A). Elemental analysis was performed by Galbraith Laboratories, Inc. (Knoxville, TN) to aid in identification. The elemental compositions matched acetoin and 2,3-pentanedione.

Acetoin was received as a solid dimer and was thermally cracked to form the liquid monomer used as the exposure material. The purity of lot 09118JH in the solid and liquid form was determined by the study laboratory using gas chromatography (GC) with flame ionization detection (FID), and impurities were identified using GC with mass spectrometry (MS) detection (Table A-1). The purity of the solid dimer was determined to be 99.4%, and the purity of the liquid monomer was determined to be 99.2%. Three reportable impurities with peak areas ≥0.1% of the total integrated peak area were detected in the solid form, and two of the three reportable impurities were detected in the liquid form of acetoin. Karl Fischer titration, performed by Chemir Analytical Services, indicated <0.1% water in the solid test article. The overall purity of lot 09118JH was determined to be >99%.

The purity of lot MKBB7504 of 2,3-pentanedione was determined by the study laboratory using GC/FID (Table A-1). GC/FID determined a purity of 98.2% and detected four reportable impurities with peak areas ≥0.1%. Karl Fischer titration, performed by Chemir Analytical Services, yielded a water content of 0.75%. The overall purity of lot MKBB7504 was determined to be >98%.

To ensure stability, bulk acetoin was stored in sealed white plastic buckets at 5°C, and bulk 2,3-pentanedione was stored in sealed metal pails at 5°C. Periodic analyses of the bulk chemicals were performed by the study laboratory using GC/FID, and no degradation of either bulk chemical was detected.

Vapor Generation and Exposure System

A diagram of the vapor generation and delivery system used in the studies is shown in Figure A-5. The test chemical, cracked acetoin or 2,3-pentanedione, was pumped from a 4-liter

glass reservoir into a heated glass vaporizer column filled with glass beads and wrapped with heat tape. For the acetoin studies, formation of solid dimer was prevented by maintaining the generator reservoir at approximately 90°F and by daily rinsing of the liquid pump with water from an additional reservoir. For all studies, a waste collection flask was connected to the bottom of the column to collect residual chemical not completely vaporized.

Preheated nitrogen entered the vaporizer column from below, vaporized the test chemical, and carried the vapor from the generator cabinet to the distribution manifold through a heated chemical transport line. The nitrogen-chemical mixture was diluted with heated air before entering the distribution manifold. Concentration in the manifold was determined by the chemical pump rate, nitrogen flow rate, and dilution airflow rate. Pressure in the distribution manifold was kept fixed to ensure constant flow rates through the manifold and into all exposure chambers as the flow of vapor to each chamber was adjusted.

Individual heated Teflon® delivery lines carried the diluted acetoin or 2,3-pentanedione vapor from the distribution manifold to three-way exposure valves at the chamber inlets. The chamber exposure valves diverted vapor delivery to the 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 distribution manifold. To initiate exposure, the chamber exposure valves were rotated to allow the acetoin or 2,3-pentanedione vapor to flow to each exposure chamber inlet duct, where it was diluted with conditioned air to achieve the desired exposure concentration. Conditioned air was a temperature-controlled and filtered mix of air derived from each exposure chamber's wet and dry air duct supplies.

The study laboratory designed the inhalation exposure chamber (built by Lab Products, Inc., Seaford, DE) so that uniform vapor concentrations could be maintained throughout the chamber with catch pans in place. The total active mixing volume of each chamber was 1.7 m^3. A small particle detector (Model 3022A) was used with and without animals in the exposure chambers to ensure that acetoin or 2,3-pentanedione vapors, and not aerosols, were produced. No particle counts above the minimum resolvable level (approximately 200 particles/cm^3) were detected.

Vapor Concentration Monitoring

Exposure chamber and room concentrations of acetoin and 2,3-pentanedione were monitored using online GC/FID (Table A-1). Approximately every 20 minutes during each 6-hour exposure period, samples were drawn through stainless steel (acetoin) or Teflon (2,3-pentanedione) tubing connected to each exposure chamber's sampling line using a 16-port Hastelloy®-C stream-select valve that directs a continuous stream of sampled atmosphere to a 6-port Hastelloy-C gas-sampling valve with a 1-mL Silicosteel® sample loop. Both valves and the sampling loop were mounted in a dedicated valve oven. 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 GC allowed for digital measurement of sample flow. Summaries of the chamber vapor concentrations are given in Table A-2 through Table A-4. The mean relative errors were within the acceptance criteria of 10% for all exposure groups for all studies.

Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined with (all studies) and without (3-month studies) animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 9 minutes. A T_{90} value of 12 minutes was selected for both chemicals for all studies.

The persistence of the test chemical in the chambers was determined with (all studies) and without (3-month studies) animals present in the chambers. The time for the chamber concentration to decay to <1% of the target concentration after vapor generation was terminated (T_1) was measured in the 800 ppm chambers for acetoin and in the 100 ppm chambers for 2,3-pentanedione. In the 2-week acetoin studies, T_1 was approximately 47 minutes with animals present. In the 3-month acetoin studies, T_1 was approximately 22 minutes without animals present; when animals were present, the chamber concentration did not drop below 1% of the target concentration until after the chamber doors were opened to perform animal care activities (up to 134 minutes after shutdown). In the 3-month 2,3-pentanedione studies, T_1 was approximately 23 minutes without animals present and approximately 32 minutes with animals present. The presence of animals had a moderate effect on the decay of chamber concentration.

The uniformity of acetoin or 2,3-pentanedione vapor concentration was evaluated with (all studies) and without (3-month studies) animals present in the chambers. Concentrations were measured at 12 chamber positions, one in front and one in back, for each of the six possible animal cage positions per chamber, except for during the 3-month 2,3-pentanedione studies wherein concentrations were measured only at the regular monitoring port and from chamber positions where animals were present. Chamber concentration uniformity was maintained throughout the studies.

To measure stability and purity of the test chemicals in the generation and delivery system, samples of the test atmosphere from the distribution lines and low- and high-exposure concentration chambers for each species were collected during the first and last hours of generation with (all studies) and without (3-month studies) animals present in the chambers. The atmosphere samples were collected with sorbent gas-sampling tubes containing silica gel followed by a tube containing activated coconut charcoal. Grab samples were collected from the bulk chemical and generator reservoir for all studies of both test articles. To measure stability of the test chemicals in the exposure system, concentrations in all exposure chambers were monitored using online GC during 3 days of test generation prior to the 3-month studies.

Acetoin and 2,3-pentanedione were stable under the generation and exposure conditions used during the studies. During the 2-week and 3-month studies of acetoin, the impurities 2,3-butanedione, acetic acid, 3-methyl-2,4-pentanedione, and isomers of di-2-butan-3-one ether were detected in the atmosphere and generator reservoir samples. During the 3-month studies of 2,3-pentanedione, the impurities 2,3-butanedione, 3,4-hexanedione, acetaldehyde, paraldehyde, acetic acid, and five unknown peaks were detected in the atmosphere and generator reservoir samples.

Animal Source

Male and female Wistar Han [Crl:WI(Han)] rats were obtained from Charles River Laboratories, Inc. (Raleigh, NC). Male and female B6C3F1/N mice were obtained from the National Toxicology Program (NTP) colony maintained by Taconic Biosciences, Inc. (Germantown, NY).

Animal Welfare

Animal care and use were 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 AAALAC International. Studies were approved by the Battelle (Richland, WA) Animal Care and Use Committee and conducted in accordance with all relevant National Institutes of Health and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Exposure Concentration Selection Rationale

The exposure concentrations used for 2,3-pentanedione matched those used for 2,3-butanedione (diacetyl), which is structurally similar (also a vicinal diketone), in the NTP 3-month inhalation toxicity study in rats and mice.[15] The highest exposure concentration for 2,3-pentanedione (100 ppm) was also previously used for 3-month inhalation toxicity testing of 2,3-butanedione in C57BL/6 mice.[11] When the 3-month toxicity study of 2,3-pentanedione was initiated, it was expected that the potencies of 2,3-butanedione and 2,3-pentanedione would be similar due to their structural similarity (and, therefore, the exposure concentrations could be matched), which has since been shown to be true on the basis of comparative 2-week studies.[13] Acetoin is a less reactive hydroxyl-ketone compared to the diketones 2,3-butanedione and 2,3-pentanedione and is thought to be of relatively lower toxicological potency[34]; thus, higher exposure concentrations were selected.

Two-week Studies

Rats and mice were approximately 4 weeks old on receipt. Animals were quarantined for 13 (rats) or 12 (mice) days and were approximately 6 weeks old (rats and mice) on the first day of the studies. Rats and mice were randomly assigned to one of six exposure groups before the start of the study. Randomization was stratified by body weight that produced similar group mean weights using PATH/TOX SYSTEM software (Xybion Medical Systems Corporation, Cedar Knolls, NJ).

Before the studies began, five male and five female rats and mice were randomly selected for necropsy, parasite evaluation, and selected histopathology for evidence of disease. At study termination, sera collected from five male rats and mice, and five female rats and mice were analyzed according to the protocols of the NTP Sentinel Animal Program (Appendix C). All test results were negative.

Groups of five male and five female rats and mice were exposed to acetoin vapors at concentrations of 0 (air), 6.25, 25, 100, 400, or 800 ppm for 6 hours plus T_{90} per day, 5 days per week, for 2 weeks plus 2 (rats) or 3 (mice) additional exposure days for a total of 12 (rats) or 13 (mice) exposures over a period of 16 (rats) or 17 (mice) days.

Feed was available ad libitum except during exposure periods; water was available ad libitum. Rats and mice were housed individually. Rats and mice were observed twice daily for signs of mortality or moribundity. Clinical observations were made twice daily. Body weights were recorded before exposure on day 1, on days 6 and 13, and at study termination. Details of the study design and animal maintenance are summarized in Table 2. Information on feed composition and contaminants is provided in Appendix B.

Complete necropsies were performed on all animals. Organ weights were determined for the heart, right kidney, liver, lung, right testis, and thymus from all animals. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes, which were first fixed in Davidson's solution, and testes, vaginal tunics, and epididymides, which were first fixed in modified Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μm, and stained with hematoxylin and eosin (H&E). Lung, nasal cavity, trachea, larynx, mediastinal and tracheobronchial lymph nodes, and organs/tissues showing gross evidence of exposure-related lesions were examined microscopically to a no-effect level. Table 2 lists the tissues and organs examined.

Three-month Studies

Rats and mice were 3 to 4 weeks old on receipt. Animals in the acetoin study were quarantined for 13 (male rats), 14 (female rats), or 12 (male and female mice) days. Animals in the 2,3-pentanedione study were quarantined for 12 (male rats) or 13 (female rats; male and female mice) days. Rats were approximately 6 weeks old, and mice were approximately 5 to 6 weeks old on the first day of the studies. Rats and mice were randomly assigned to one of six exposure groups before the start of the study. Randomization was stratified by body weight that produced similar group mean weights using PATH/TOX SYSTEM software (Xybion Medical Systems Corporation, Cedar Knolls, NJ).

Before the studies began, five male and five female rats and mice were randomly selected for necropsy, parasite evaluation, and selected histopathology for evidence of disease. Serologic analyses and parasite evaluations were performed on five male rats and mice and five female rats and mice 3 weeks after arrival and at study termination (only sera were analyzed at the end of the acetoin study). Health monitoring was performed using the protocols of the NTP Sentinel Animal Program (Appendix C). All test results were negative.

Groups of 10 male and 10 female rats and mice in the 3-month study group and 10 male and 10 female rats in the clinical pathology group were exposed to acetoin vapors at concentrations of 0 (air), 50, 100, 200, 400, or 800 ppm for 6 hours plus T_{90} per day, 5 days per week, for approximately 13 to 14 weeks. Groups of 10 male and 10 female rats and mice in the 3-month study group and 10 male and 10 female rats in the clinical pathology group were exposed to 2,3-pentanedione vapors at concentrations of 0 (air), 6.25, 12.5, 25, 50, or 100 ppm for 6 hours plus T_{90} per day, 5 days per week, for approximately 13 to 14 weeks.

Feed was available ad libitum except during exposure periods; water was available ad libitum. Rats and mice were housed individually. Rats and mice were observed twice daily for signs of mortality or moribundity. Clinical observations were recorded on day 8, weekly thereafter, and at study termination. Body weights were recorded before exposure on day 1, on day 8, weekly thereafter, and at study termination. Details of the study design and animal maintenance are

summarized in Table 2. Information on feed composition and contaminants is provided in Appendix B.

Animals were anesthetized with carbon dioxide, and blood was collected for hematology (rats and mice) and clinical chemistry (rats). For the acetoin studies, blood was collected from the retroorbital plexus of clinical pathology rats on days 4 and 23 and from 3-month rats at study termination for hematology and clinical chemistry analyses; blood was collected from the supraorbital sinus of all 3-month mice at study termination for hematology. For the 2,3-pentanedione studies, blood was collected from the retroorbital plexus of clinical pathology rats on days 3 and 23 and from 3-month rats at study termination for hematology and clinical chemistry analyses; blood was collected from the supraorbital sinus of all mice at study termination for hematology. Blood was collected in tubes containing a potassium ethylenediaminetetraacetic acid (K^+-EDTA) anticoagulant for hematology (rats and mice) or into serum separator tubes for clinical chemistry (rats only). The following hematology parameters were measured using the ADVIA 120 analyzer (Siemens Medical Solutions Diagnostics, Tarrytown, NY): erythrocyte, platelet, and reticulocyte counts; hematocrit; hemoglobin concentration; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and white blood cell count and differential. Manual hematocrit values were determined using a microcentrifuge (Heraeus haemofuge, Heraeus Holding GmbH, Hanau, Germany) and a Damon/IEC capillary reader (International Equipment Co., Needham Heights, MA). Blood smears were stained with Romanowsky-type aqueous stain in a Wescor 7120 aerospray slide stainer (Wescor, Inc., Logan, UT), and the cell morphology and number of nucleated red blood cells were assessed. Blood samples for clinical chemistry were allowed to clot and centrifuged, and the serum was harvested. Clinical chemistry parameters were determined using a Cobas c311 analyzer (Roche Diagnostics Corp., Indianapolis, IN). Clinical chemistry parameters measured are listed in Table 2.

Complete necropsies were performed on all animals. Organ weights were determined for the heart, right kidney, liver, lung, spleen, right testis, and thymus from all animals. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes, which were first fixed in Davidson's solution, and testes, vaginal tunics, and epididymides, which were first fixed in modified Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μm, and stained with H&E. Complete histopathological examinations were performed on all 0 and 800 ppm 3-month rats and mice in the acetoin studies and in all 0 and 100 ppm 3-month rats and mice in the 2,3-pentanedione studies. Table 2 lists the tissues and organs 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 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 Division of Translational Toxicology (DTT) pathology peer-review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, DTT pathologist, QA pathologist(s), and PWG coordinator. Details of these review procedures have been described, in part, by Maronpot[67] and Boorman.[68]

Table 2. Experimental Design and Materials and Methods in the Two-week Inhalation Studies of Acetoin and Three-month Inhalation Studies of Acetoin and 2,3-Pentanedione

Two-week Studies	Three-month Studies
Study Laboratory	
Battelle Toxicology Northwest (Richland, WA)	Same as 2-week studies
Strain and Species	
Wistar Han [Crl:WI(Han)] rats	Same as 2-week studies
B6C3F1/N mice	
Animal Source	
Rats: Charles River Laboratories, Inc. (Raleigh, NC)	Same as 2-week studies
Mice: Taconic Biosciences, Inc. (Germantown, NY)	
Time Held before Studies	
Rats: 13 days	Rats (acetoin): 13 (males) or 14 (females) days Rats (2,3-pentanedione): 12 (males) or 13 (females) days
Mice: 12 days	Mice (acetoin): 12 days Mice (2,3-pentanedione): 13 days
Average Age When Studies Began	
Rats: 6 weeks	Rats: 6 weeks
Mice: 6 weeks	Mice: 5–6 weeks
Date of First Exposure	
October 27, 2008	Rats (acetoin): June 29 (males) or 30 (females), 2009 Rats (2,3-pentanedione): September 13 (males) or 14 (females), 2010 Mice (acetoin): June 29, 2009 Mice (2,3-pentanedione): September 13, 2010
Duration of Exposure	
Rats: 6 hours plus T_{90} (12 minutes)/day, 5 days/week, for 2 weeks plus 2 additional exposure days for a total of 12 exposures over a period of 16 days	6 hours plus T_{90} (12 minutes)/day, 5 days/week, for 3 months
Mice: 6 hours plus T_{90} (12 minutes)/day, 5 days/week, for 2 weeks plus 3 additional exposure days for a total of 13 exposures over a period of 17 days	
Date of Last Exposure	
Rats: November 11, 2008	Rats (acetoin): September 28 (males) or 29 (females), 2009 Rats (2,3-pentanedione): December 13 (males) or 14 (females), 2010
Mice: November 12, 2008	Mice (acetoin): September 30 (males) or October 1 (females), 2009 Mice (2,3-pentanedione): December 15 (males) or 16 (females), 2010
Necropsy Dates	
Rats: November 12, 2008	Rats (acetoin): September 29 (males) or 30 (females), 2009 Rats (2,3-pentanedione): December 14 (males) or 15 (females), 2010
Mice: November 13, 2008	Mice (acetoin): October 1 (males) or 2 (females), 2009 Mice (2,3-pentanedione): December 16 (males) or 17 (females), 2010

Two-week Studies	Three-month Studies
Average Age at Necropsy	
8 weeks	19–20 weeks
Size of Study Groups	
5 males and 5 females	3-month study: 10 males and 10 females Clinical pathology (rats only): 10 males and 10 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies
Animals per Cage	
1	Same as 2-week studies
Method of Animal Identification	
Tail tattoo	Same as 2-week studies
Diet	
Irradiated NTP-2000 wafer feed (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, except during exposure, changed weekly	Same as 2-week studies
Water	
Tap water (City of Richland, WA, municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available ad libitum	Same as 2-week studies
Cages	
Rats: R-24 stainless steel wire-bottom (Lab Products, Inc., Seaford, DE), changed weekly	Rats: Same as 2-week studies
Mice: M-40 stainless steel wire-bottom cage units (Lab Products, Inc.), changed weekly	Mice: Same as 2-week studies
Rack Filters/Excreta Liner	
Single HEPA (Environmental Filter, Santa Rosa, CA); charcoal (RSE, Inc., New Baltimore, MI); Purafil (Environmental Systems, Lynnwood, WA). Excreta pans and the cage board lining the pans (Techboard Ultra, Shepherd Specialty Papers, Watertown, TN) were changed daily.	Same as 2-week studies
Chambers	
Stainless steel with excreta pan suspended below each cage unit, changed weekly	Same as 2-week studies
Chamber Environment	
Temperature: 75°F ± 3°F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15 ± 2/hour	Same as 2-week studies
Exposure Concentrations	
0, 6.25, 25, 100, 400, or 800 ppm by whole-body inhalation	Acetoin: 0, 50, 100, 200, 400, or 800 ppm by whole-body inhalation 2,3-Pentanedione: 0, 6.25, 12.5, 25, 50, or 100 ppm by whole-body inhalation

Two-week Studies	Three-month Studies
Type and Frequency of Observation	
Observed twice daily; animals were weighed before exposure on day 1; animals were weighed, and clinical observations were recorded on days 6 and 13 and at study termination.	Observed twice daily; animals were weighed before exposure on day 1; animals were weighed, and clinical observations were recorded on day 8, weekly thereafter, and at study termination.
Method of Euthanasia	
Carbon dioxide	Same as 2-week studies
Necropsy	
Complete necropsies were performed on all animals. Organs weighed at study termination were heart, right kidney, liver, lung, right testis, and thymus.	Complete necropsies were performed on all animals. Organs weighed at study termination were heart, right kidney, liver, lung, spleen, right testis, and thymus.
Clinical Pathology	
None	Acetoin: Blood was collected from the retroorbital plexus of clinical pathology rats on days 4 and 23, from 3-month core study rats at study termination, and from the supraorbital sinus of all mice at study termination for clinical chemistry (rats only) and hematology. 2,3-Pentanedione: Blood was collected from the retroorbital plexus of clinical pathology rats on days 3 and 23, from 3-month core study rats at study termination, and from the supraorbital sinus of all mice at study termination for clinical chemistry (rats only) and hematology. *Hematology*: hematocrit; hemoglobin; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and white blood cell count and differentials. *Clinical chemistry (rats only)*: urea nitrogen, creatinine, glucose, total protein, albumin, globulin, A/G ratio, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, cholesterol, triglyceride, and bile salt/acids.
Histopathology	
Histopathology was performed on 0, 6.25, 25, 100, 400, and 800 ppm rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined to the no-effect level: larynx, lung, lymph nodes (mediastinal and tracheobronchial), nasal cavity, and trachea.	Acetoin: Complete histopathology was performed on 0 and 800 ppm 3-month study rats and mice. 2,3-Pentanedione: Complete histopathology was performed on 0 and 100 ppm 3-month 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 and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicles, thymus, thyroid gland, trachea, urinary bladder, and uterus.

A/G = albumin/globulin.

Statistical Methods

Statistical methods were chosen based on distributional assumptions. Unless specifically mentioned, all endpoints were tested for a trend across exposure groups, followed by pairwise tests for each exposed group against the control group. Significance of all trend and pairwise tests is determined by a p value of ≤0.05 and is reported at both 0.05 and 0.01 levels.

Calculation and Analysis of Nonneoplastic Lesion Incidences

The incidences of nonneoplastic lesions are presented as the numbers of animals bearing such lesions at a specific anatomical site and the numbers of animals with that site examined microscopically. Fisher's exact test,[69] a procedure that uses the overall proportion of affected animals, was used to determine statistical significance between exposed and vehicle control animals, and the Cochran-Armitage trend test was used to test for significant trends.[70]

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed 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[71] and Williams.[72; 73] Hematology and clinical chemistry data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[74] (as modified by Williams[75]) and Dunn.[76] The Jonckheere test[77] was used to assess the significance of the exposure concentration-related trends and to determine whether a trend-sensitive test (the Williams or Shirley test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic exposure concentration-related trend (the Dunnett or Dunn test). Before statistical analysis, extreme values identified by the outlier test of Dixon and Massey[78] were examined by DTT personnel, and implausible values were eliminated from the analysis.

Quality Assurance Methods

The 2-week and 3-month studies were conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice Regulations.[79] In addition, the 2-week and 3-month 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 Toxicity Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by DTT staff, and all comments were resolved or otherwise addressed during the preparation of this Toxicity Report.

Genetic Toxicology

The genetic toxicity of acetoin and 2,3-pentanedione was assessed by testing whether each chemical induces mutations in various strains of *Salmonella typhimurium* or increases the frequency of micronucleated erythrocytes in rat and mouse peripheral blood. The protocol for these studies, their analyses, and the results are given in Appendix D.

The genetic toxicity studies have evolved from an earlier effort to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals that is based on numerous considerations, including the relationship between 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 developed originally to clarify proposed mechanisms of chemical-induced DNA damage, given the relationship between electrophilicity and mutagenicity,[80] and the somatic mutation theory of cancer.[81; 82] Not all cancers, however, arise through genotoxic mechanisms.

Bacterial Mutagenicity (Acetoin)

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites.[83] 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).[84; 85] Additionally, no battery of tests that included the *Salmonella* test improved predictivity over the *Salmonella* test alone. Other tests, however, can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

Peripheral Blood Micronucleus Test (Acetoin and 2,3-Pentanedione)

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.[86; 87] Acute in vivo bone marrow chromosome aberration and micronucleus tests appear to be less predictive of carcinogenicity than the *Salmonella* test.[87; 88] 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.[89] Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, determination of in vivo genetic effects is important to overall understanding of risks associated with exposure to a particular chemical.

Results

Data Availability

All study data were evaluated. Data relevant for evaluating toxicological findings are presented here. All study data are available in the National Toxicology Program (NTP) Chemical Effects in Biological Systems (CEBS) database: https://doi.org/10.22427/NTP-DATA-TOX-98.[90]

Acetoin

Two-week Studies in Rats and Mice

All rats and mice survived to the end of the 2-week studies, and mean body weights at study termination of animals exposed to acetoin remained within 5% of those of control animals for both rats and mice (Table 3). No histopathological changes related to acetoin exposure were observed (Appendix E).

Table 3. Summary of Survival and Mean Body Weights of Male and Female Rats and Mice in the Two-week Inhalation Studies of Acetoin

Study Day[a]	0 ppm		6.25 ppm			25 ppm			100 ppm			400 ppm			800 ppm		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
Rats																	
Male																	
1	126.6	5	124.6	98.4	5	128.1	101.2	5	127.3	100.6	5	127.9	101.1	5	124.8	98.6	5
6	148.2	5	153.0	103.2	5	157.8	106.4	5	155.8	105.1	5	155.8	105.1	5	153.3	103.4	5
13	191.7	5	191.9	100.1	5	200.2	104.4	5	196.8	102.7	5	193.6	101.0	5	192.3	100.3	5
EOS	**215.9**	**5**	**216.0**	**100.1**	**5**	**224.9**	**104.2**	**5**	**224.1**	**103.8**	**5**	**217.3**	**100.7**	**5**	**217.7**	**100.8**	**5**
Female																	
1	106.5	5	106.8	100.3	5	108.6	101.9	5	105.7	99.2	5	107.4	100.8	5	107.2	100.6	5
6	119.8	5	123.6	103.1	5	125.4	104.7	5	121.9	101.7	5	120.3	100.4	5	124.6	104.0	5
13	141.6	5	137.6	97.2	5	140.1	99.0	5	138.9	98.1	5	135.5	95.7	5	140.1	98.9	5
EOS	**151.3**	**5**	**148.2**	**97.9**	**5**	**151.1**	**99.9**	**5**	**149.3**	**98.7**	**5**	**144.5**	**95.5**	**5**	**151.4**	**100.1**	**5**
Mice																	
Male																	
1	21.8	5	22.0	100.9	5	21.6	99.1	5	21.8	100.3	5	21.9	100.5	5	22.0	101.2	5
6	23.0	5	23.1	100.4	5	23.0	100.0	5	23.1	100.4	5	23.1	100.8	5	23.2	101.1	5
13	24.2	5	24.6	101.6	5	24.1	99.6	5	24.4	100.5	5	24.6	101.4	5	24.5	101.1	5
EOS	**25.2**	**5**	**25.6**	**101.6**	**5**	**25.4**	**100.6**	**5**	**25.5**	**101.2**	**5**	**25.4**	**100.7**	**5**	**25.6**	**101.4**	**5**
Female																	
1	18.6	5	19.0	102.5	5	18.8	101.4	5	19.1	102.7	5	19.3	103.9	5	19.4	104.3	5
6	20.0	5	19.9	99.9	5	20.5	102.8	5	19.8	99.1	5	20.1	100.8	5	20.3	101.6	5
13	21.3	5	20.5	96.6	5	21.3	100.2	5	20.8	97.9	5	20.6	97.0	5	21.3	100.2	5
EOS	**22.1**	**5**	**21.7**	**98.0**	**5**	**22.3**	**101.0**	**5**	**21.1**	**95.7**	**5**	**21.5**	**97.4**	**5**	**22.0**	**99.6**	**5**

No trend or pairwise statistical tests were performed on these data.
EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

Three-month Study in Rats

All rats survived to the end of the 3-month study (Table 4), and there were no exposure-related clinical observations (Appendix E). Mean body weights of male and female rats exposed to acetoin were slightly lower but were within 7% of those of the respective control groups throughout the study (Table 4; Figure 1).

At necropsy, there were no noteworthy gross lesions observed in male or female rats exposed to acetoin for 3 months (Appendix E). There were no significant differences in relative organ weights at study termination relative to control animals (Appendix E). The only exceptions were a significant increase in relative heart weight of males exposed to 50 ppm and in relative liver weight of males exposed to 800 ppm. Those differences were considered sporadic (heart) with no effect noted at higher concentrations or secondary to marginal differences in body weight (liver) and not toxicologically significant.

On day 23, there was a mild (≤8%) but significant decrease in the hematocrit and hemoglobin concentration in the 100 ppm and higher male rats (Appendix E). This change, which ameliorated by day 93, indicated a transient mild suppression in erythropoiesis. In addition, on day 23, neutrophil counts were significantly decreased in most male exposed groups but were unchanged compared to those of the control group by day 93 (Appendix E). On day 93, the white blood cell and lymphocyte counts were significantly decreased (19%–30%) in male rats exposed to 200 ppm and higher, and the monocyte count was decreased (≤29%) in the 400 and 800 ppm male groups (Appendix E). The day 93 leukocyte changes were most consistent with a stress leukogram (i.e., effects of a chronic increase in endogenous glucocorticoids).[91] All other statistically significant hematology changes were mild, inconsistent, and/or considered due to biological variability and, thus, not considered related to acetoin exposure.

On day 4, urea nitrogen (BUN) was significantly decreased (25%) in the 800 ppm male rats, and creatinine concentration was significantly increased in the 800 ppm male (194%) and female (55%) rats (Appendix E). The BUN remained mildly decreased in all male rats at day 23 with a significant increase in creatinine (47%) observed in the 800 ppm male rats. By day 93, the creatinine changes ameliorated, whereas the BUN was minimally increased (15%) in 800 ppm male rats. The combination of these changes most likely represented a physiological response related to decreased feed or water consumption as the animals adjusted to exposure. All other statistically significant changes were minimal, inconsistent, and/or not considered biologically relevant and, thus, not considered due to acetoin exposure.

Histopathology

There were no significant differences in exposure-related microscopic lesion incidences in male or female rats relative to control animals. A few microscopic lesions were observed in the heart, kidney, larynx, lymph nodes, lung, liver, pancreas, and preputial gland. Severity ranged from minimal to marked, and the lesions were present in both control and exposed animals. The microscopic lesions, therefore, were considered incidental findings and not related to exposure to acetoin (Appendix E).

Table 4. Summary of Survival and Mean Body Weights of Male and Female Rats in the Three-month Inhalation Study of Acetoin

Study Day[a]	0 ppm		50 ppm			100 ppm			200 ppm			400 ppm			800 ppm		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
Male																	
1	135.2	10	136.0	100.6	10	135.2	100.0	10	133.4	98.6	10	134.4	99.4	10	135.2	100.0	10
8	179.4	10	180.0	100.3	10	176.4	98.3	10	172.6	96.2	10	176.2	98.2	10	178.8	99.6	10
17	234.6	10	230.7	98.3	10	227.2	96.9	10	221.0	94.2	10	227.3	96.9	10	228.9	97.6	10
24	269.1	10	265.0	98.5	10	260.6	96.8	10	253.6	94.2	10	260.9	96.9	10	260.5	96.8	10
31	295.4	10	289.9	98.1	10	284.7	96.4	10	278.6	94.3	10	284.9	96.4	10	285.3	96.6	10
38	319.2	10	312.3	97.8	10	306.6	96.1	10	301.6	94.5	10	309.2	96.9	10	307.5	96.3	10
45	337.2	10	330.3	98.0	10	324.5	96.2	10	320.5	95.1	10	328.5	97.4	10	324.0	96.1	10
52	354.5	10	346.3	97.7	10	340.7	96.1	10	335.4	94.6	10	342.1	96.5	10	338.3	95.4	10
59	369.8	10	359.7	97.3	10	354.0	95.7	10	348.9	94.4	10	356.2	96.3	10	349.7	94.6	10
66	381.0	10	370.1	97.1	10	364.1	95.6	10	359.1	94.2	10	368.9	96.8	10	360.6	94.7	10
73	390.8	10	380.3	97.3	10	372.0	95.2	10	370.2	94.7	10	380.2	97.3	10	370.2	94.7	10
80	400.6	10	388.6	97.0	10	380.7	95.0	10	378.6	94.5	10	388.2	96.9	10	377.5	94.2	10
87	410.7	10	394.8	96.1	10	388.2	94.5	10	387.0	94.2	10	395.3	96.3	10	384.8	93.7	10
EOS	415.5	10	400.9	96.5	10	395.2	95.1	10	391.2	94.2	10	403.2	97.0	10	389.7	93.8	10
Female																	
1	121.6	10	119.9	98.6	10	121.3	99.8	10	120.4	99.0	10	121.7	100.1	10	122.2	100.5	10
8	142.9	10	141.1	98.8	10	142.4	99.7	10	141.4	99.0	10	143.9	100.7	10	142.0	99.4	10
16	163.2	10	161.8	99.2	10	163.3	100.1	10	162.2	99.4	10	165.2	101.2	10	162.5	99.6	10
23	180.3	10	177.9	98.7	10	177.2	98.3	10	177.5	98.4	10	182.7	101.3	10	176.8	98.1	10
30	192.2	10	187.1	97.3	10	187.4	97.5	10	188.7	98.2	10	193.0	100.4	10	185.0	96.2	10
37	202.1	10	196.1	97.0	10	196.6	97.3	10	198.2	98.1	10	203.4	100.6	10	197.3	97.6	10
44	207.9	10	206.2	99.2	10	205.9	99.0	10	206.0	99.1	10	211.2	101.6	10	204.1	98.2	10
51	215.5	10	215.2	99.8	10	212.5	98.6	10	213.4	99.0	10	219.0	101.6	10	211.5	98.2	10
58	222.7	10	218.9	98.3	10	217.3	97.6	10	219.7	98.6	10	224.2	100.6	10	215.1	96.6	10
65	225.4	10	223.3	99.0	10	224.0	99.4	10	223.6	99.2	10	228.8	101.5	10	221.2	98.1	10
72	228.3	10	229.8	100.7	10	229.6	100.6	10	228.8	100.2	10	235.3	103.1	10	226.5	99.2	10
79	231.3	10	233.7	101.0	10	231.2	99.9	10	231.3	100.0	10	240.2	103.8	10	232.6	100.6	10
86	237.3	10	236.5	99.7	10	235.7	99.3	10	234.0	98.6	10	244.0	102.8	10	234.0	98.6	10
EOS	238.5	10	239.6	100.5	10	238.3	99.9	10	234.0	98.1	10	244.5	102.5	10	237.1	99.4	10

No trend or pairwise statistical tests were performed on these data.
EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

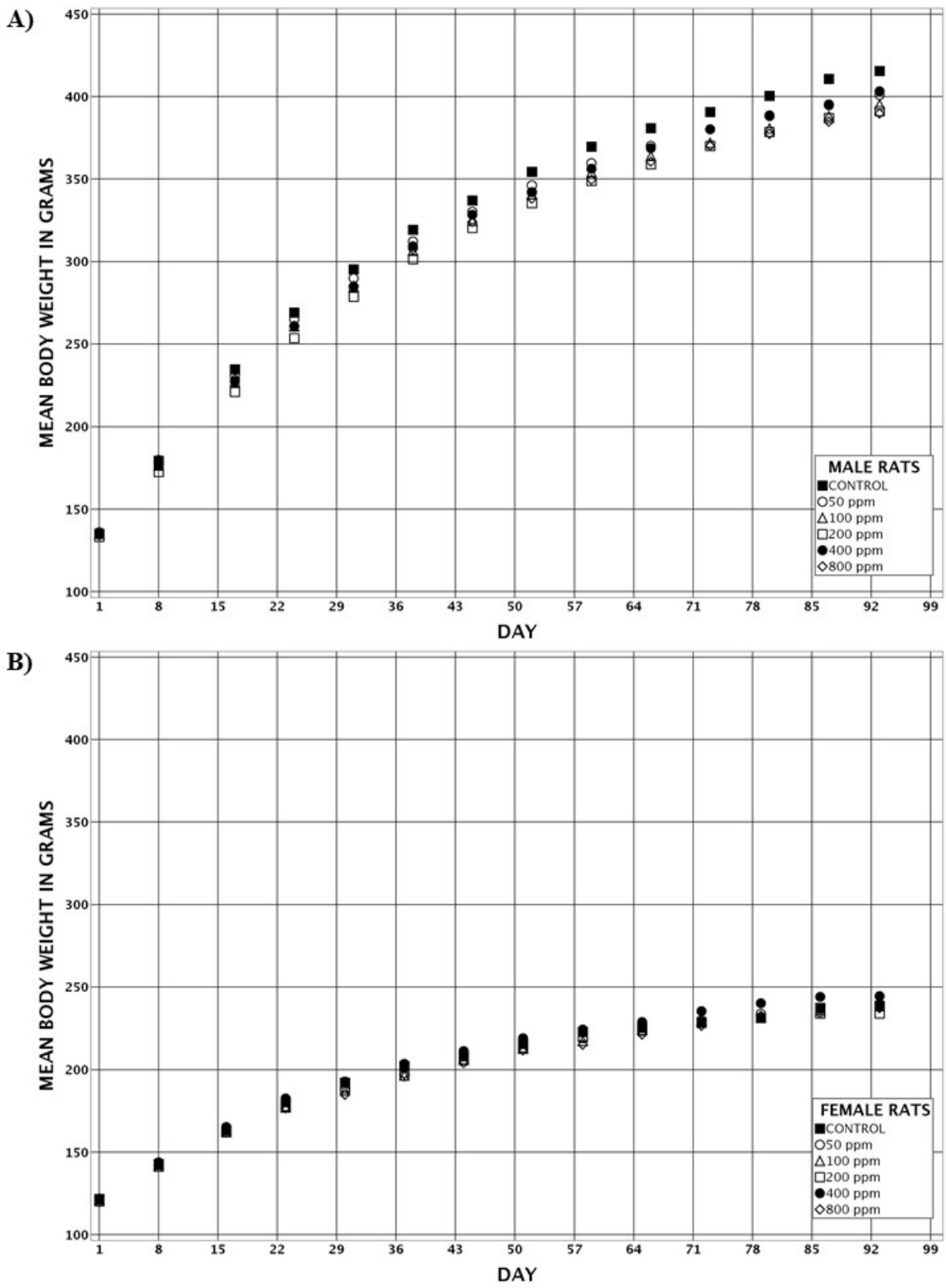


Figure 1. Growth Curves for Male and Female Rats in the Three-month Inhalation Study of Acetoin

Growth curves are shown for (A) males and (B) females.

Three-month Study in Mice

All mice survived to scheduled termination (Table 5). Mean body weights of male and female mice exposed to acetoin were within 8% of those of the respective control groups throughout the study (Table 5; Figure 2).

There were no clinical observations or gross lesions associated with exposure to acetoin up to 800 ppm (Appendix E). There were no significant exposure concentration-response alterations associated with absolute or relative organ weights at study termination (Appendix E). The only exceptions were significant increases in relative kidney weight and relative lung weight of males exposed to 100 ppm, but these likely have no toxicological relevance because differences were not noted at higher exposure concentrations or at any exposure concentration for female mice.

There were no exposure-related changes in the hematology of the mice exposed to acetoin (Appendix E).

Histopathology

There were no significant exposure-related microscopic alterations in any of the tissues evaluated from mice (Appendix E).

Table 5. Summary of Survival and Mean Body Weights of Male and Female Mice in the Three-month Inhalation Study of Acetoin

Study Day[a]	0 ppm		50 ppm			100 ppm			200 ppm			400 ppm			800 ppm		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
Male																	
1	23.6	10	23.7	100.5	10	23.7	100.5	10	23.7	100.5	10	23.3	98.7	10	23.5	99.7	10
8	25.4	10	25.5	100.3	10	25.0	98.2	10	25.3	99.6	10	24.6	96.9	10	24.9	98.2	10
17	27.1	10	26.9	99.5	10	26.6	98.3	10	26.8	99.1	10	26.6	98.0	10	27.0	99.6	10
24	28.2	10	27.9	98.8	10	27.5	97.5	10	27.9	98.9	10	27.2	96.5	10	28.0	99.1	10
31	28.7	10	28.7	100.0	10	28.3	98.6	10	28.7	99.9	10	28.2	98.3	10	28.8	100.2	10
38	30.1	10	29.8	98.8	10	29.1	96.7	10	29.6	98.4	10	29.1	96.5	10	29.8	99.0	10
45	31.1	10	30.3	97.5	10	30.2	97.1	10	30.4	97.7	10	29.9	96.3	10	30.5	98.3	10
52	31.9	10	31.4	98.5	10	30.9	96.9	10	31.3	98.3	10	30.5	95.7	10	31.6	99.1	10
59	32.8	10	32.0	97.5	10	31.8	97.0	10	32.3	98.4	10	31.6	96.4	10	32.6	99.6	10
66	33.9	10	33.1	97.6	10	32.5	96.0	10	32.8	96.9	10	32.4	95.6	10	33.6	99.2	10
73	34.8	10	34.1	97.9	10	33.3	95.5	10	33.3	95.5	10	33.7	96.9	10	34.4	98.7	10
80	35.6	10	35.1	98.6	10	33.9	95.3	10	34.6	97.1	10	34.4	96.5	10	35.5	99.7	10
87	36.7	10	36.0	98.3	10	34.8	94.8	10	35.7	97.3	10	35.2	96.0	10	36.4	99.2	10
EOS	37.4	10	36.9	98.6	10	35.6	95.3	10	36.6	97.8	10	35.9	95.9	10	37.5	100.3	10
Female																	
1	20.1	10	19.9	99.1	10	19.8	98.4	10	19.7	98.1	10	19.6	97.8	10	19.5	97.2	10
8	21.7	10	21.9	100.9	10	20.8	95.9	10	21.1	97.5	10	21.3	98.5	10	21.2	97.7	10
17	22.6	10	22.4	99.1	10	22.3	98.6	10	22.7	100.2	10	22.6	100.0	10	22.6	99.8	10
24	23.6	10	23.4	99.0	10	23.5	99.3	10	23.6	99.7	10	23.9	101.3	10	23.9	101.2	10
31	24.6	10	24.0	97.6	10	24.2	98.3	10	25.1	102.0	10	24.7	100.4	10	25.0	101.6	10
38	25.3	10	24.8	98.0	10	24.9	98.4	10	26.0	102.8	10	25.6	101.1	10	25.5	100.8	10
45	26.1	10	25.2	96.7	10	25.8	99.0	10	26.7	102.5	10	26.4	101.2	10	26.1	100.0	10
52	26.4	10	26.2	99.1	10	26.3	99.4	10	27.1	102.5	10	27.3	103.3	10	27.9	105.6	10
59	28.0	10	26.4	94.0	10	27.5	98.1	10	28.3	100.9	10	28.3	100.8	10	28.1	100.4	10
66	28.6	10	27.1	94.5	10	28.0	97.6	10	29.2	102.0	10	29.1	101.6	10	29.1	101.6	10
73	29.7	10	27.7	93.1	10	28.5	96.0	10	29.6	99.7	10	30.0	100.8	10	29.2	98.2	10
80	30.2	10	28.6	94.6	10	29.2	96.7	10	30.8	101.8	10	30.5	101.0	10	30.7	101.7	10
87	31.5	10	29.8	94.5	10	30.2	95.7	10	30.7	97.5	10	31.9	101.1	10	31.7	100.5	10
EOS	32.9	10	30.4	92.4	10	31.8	96.5	10	33.0	100.2	10	32.4	98.5	10	32.7	99.2	10

No trend or pairwise statistical tests were performed on these data.
EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

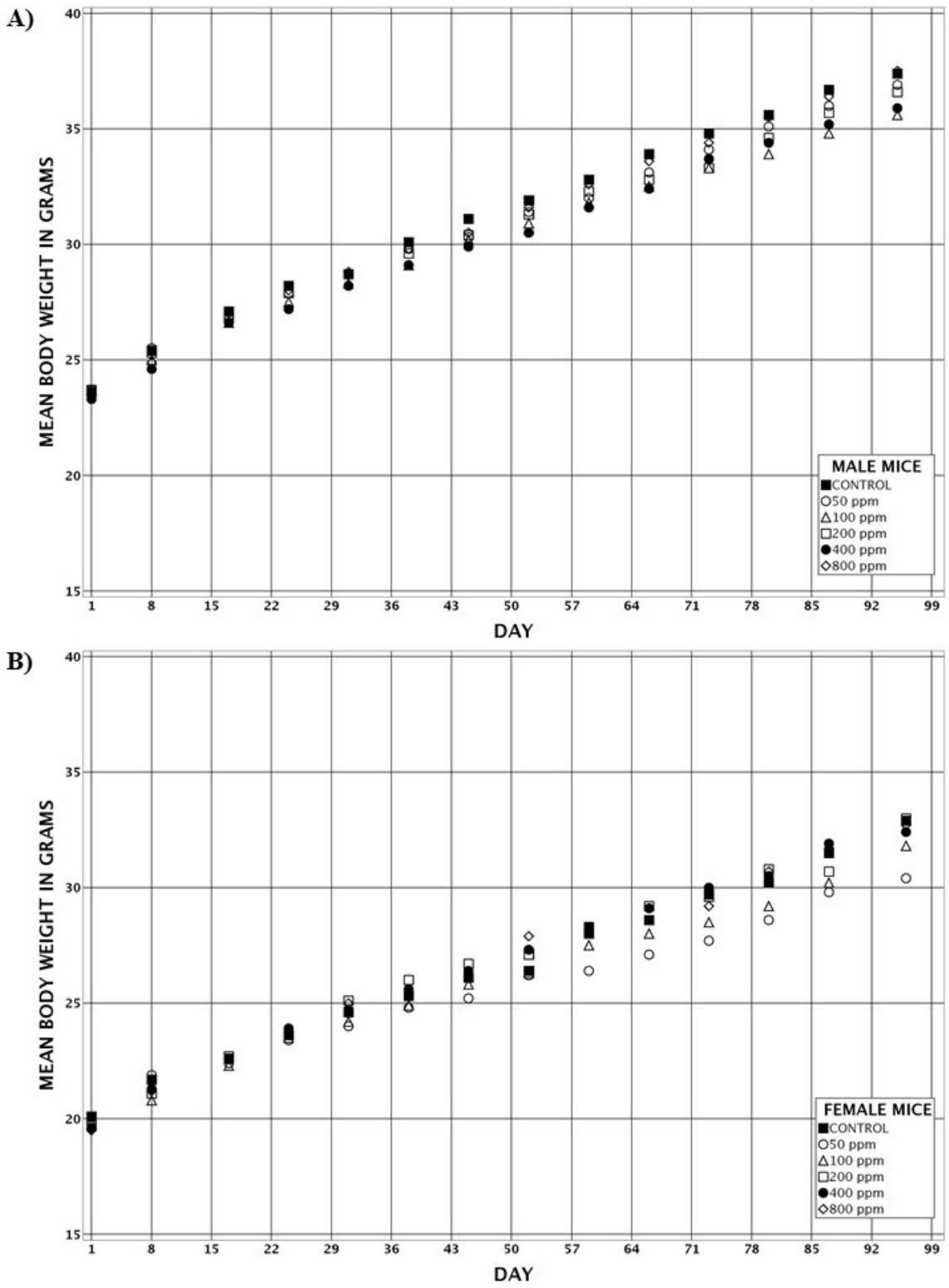


Figure 2. Growth Curves for Male and Female Mice in the Three-month Inhalation Study of Acetoin

Growth curves are shown for (A) males and (B) females.

2,3-Pentanedione

Three-month Study in Rats

All rats exposed to 2,3-pentanedione for 3 months survived to study termination (Table 6). Exposure-related clinical observations in the 50 and 100 ppm groups of both sexes included abnormal breathing, eye abnormality, and sneezing (Table 7). The mean body weight of males exposed to 100 ppm was lower (up to 8% lower) than that of the control group beginning on day 8 (Table 6; Figure 3). Although terminal mean body weights were not significantly different from the control group, the mean body weight of the 100 ppm group remained lower (within 8%) than that of the control group for the duration of the study (Appendix E).

Table 6. Summary of Survival and Mean Body Weights of Male and Female Rats in the Three-month Inhalation Study of 2,3-Pentanedione

Study Day[a]	0 ppm		6.25 ppm			12.5 ppm			25 ppm			50 ppm			100 ppm		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
Male																	
1	139.5	10	140.1	100.5	10	139.7	100.2	10	138.6	99.4	10	138.6	99.4	10	138.8	99.5	10
8	181.2	10	181.0	99.9	10	180.4	99.6	10	179.7	99.2	10	179.5	99.1	10	171.9	94.8	
19	241.1	10	240.7	99.8	10	239.5	99.3	10	243.6	101.1	10	235.9	97.8	10	222.7	92.4	
26	268.5	10	266.0	99.0	10	268.7	100.1	10	272.5	101.5	10	258.2	96.2	10	250.6	93.3	10
33	292.7	10	289.4	98.8	10	292.6	100.0	10	298.2	101.9	10	281.2	96.1	10	276.1	94.3	10
40	311.5	10	305.2	98.0	10	312.4	100.3	10	315.4	101.3	10	301.7	96.8	10	292.0	93.7	10
47	329.1	10	326.8	99.3	10	328.5	99.8	10	331.7	100.8	10	316.8	96.3	10	310.4	94.3	10
54	341.9	10	339.1	99.2	10	341.5	99.9	10	344.8	100.8	10	330.6	96.7	10	320.7	93.8	10
61	352.4	10	348.6	98.9	10	351.8	99.8	10	356.8	101.2	10	338.9	96.1	10	328.1	93.1	10
68	358.4	10	356.9	99.6	10	358.6	100.1	10	367.0	102.4	10	347.7	97.0	10	335.1	93.5	10
75	367.4	10	365.2	99.4	10	367.7	100.1	10	376.6	102.5	10	356.4	97.0	10	344.7	93.8	10
82	373.6	10	372.9	99.8	10	376.4	100.8	10	385.2	103.1	10	366.5	98.1	10	353.5	94.6	10
89	381.5	10	380.9	99.9	10	384.0	100.7	10	392.7	103.0	10	375.8	98.5	10	360.7	94.6	10
EOS	383.8	10	383.2	99.8	10	386.5	100.7	10	396.1	103.2	10	378.6	98.6	10	363.7	94.8	10
Female																	
8	141.1	10	143.0	101.4	10	145.7	103.3	10	146.6	103.9	10	144.3	102.2	10	144.6	102.5	10
18	165.5	10	166.0	100.3	10	171.1	103.4	10	168.0	101.6	10	163.6	98.9	10	165.9	100.3	10
25	178.2	10	175.1	98.2	10	181.5	101.8	10	181.0	101.6	10	175.2	98.3	10	176.5	99.0	10
32	185.8	10	186.2	100.2	10	194.1	104.5	10	193.1	103.9	10	185.6	99.9	10	185.8	100.0	10
39	196.9	10	194.4	98.7	10	203.4	103.3	10	200.9	102.1	10	193.9	98.5	10	190.9	97.0	10
46	202.1	10	201.0	99.4	10	211.2	104.5	10	206.4	102.1	10	200.5	99.2	10	200.1	99.0	10
53	209.7	10	206.0	98.2	10	214.8	102.4	10	214.0	102.0	10	206.0	98.2	10	205.6	98.0	10
60	214.1	10	211.6	98.8	10	220.6	103.0	10	220.0	102.8	10	209.1	97.7	10	209.3	97.8	10
67	219.3	10	216.9	98.9	10	224.6	102.4	10	225.3	102.8	10	214.1	97.6	10	211.4	96.4	10
74	223.4	10	219.4	98.2	10	228.5	102.3	10	225.9	101.1	10	215.2	96.3	10	214.0	95.8	10
81	226.6	10	222.0	98.0	10	229.1	101.1	10	229.3	101.2	10	219.4	96.8	10	218.2	96.3	10
88	228.0	10	225.6	98.9	10	234.7	102.9	10	234.0	102.6	10	224.1	98.3	10	222.6	97.6	10
EOS	227.7	10	225.5	99.0	10	234.9	103.2	10	235.1	103.2	10	224.6	98.6	10	223.7	98.2	10

No trend or pairwise statistical tests were performed on these data.
EOS = end of study.
[a]Study day 1 is the day animals were placed in study.

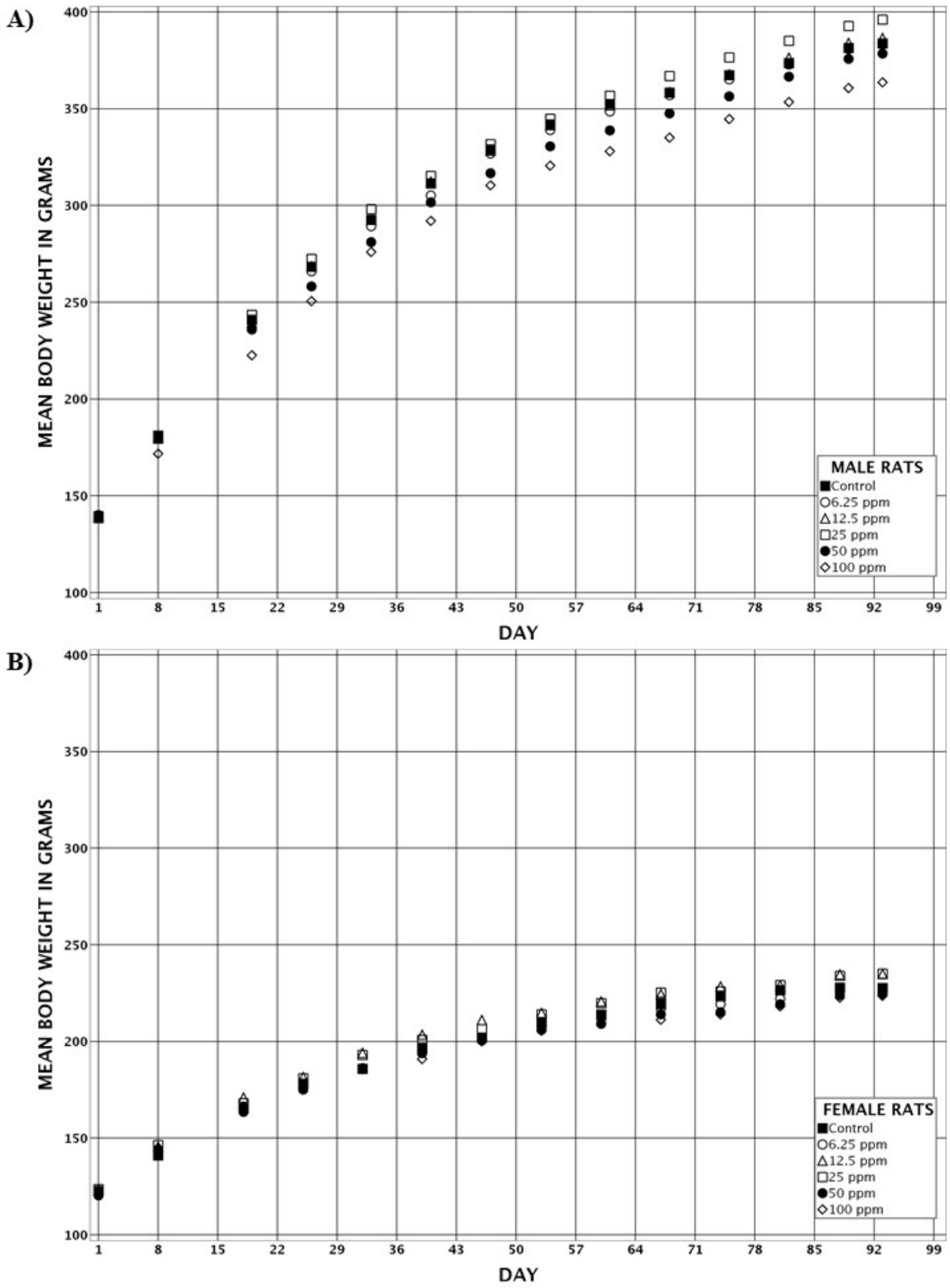


Figure 3. Growth Curves for Male and Female Rats in the Three-month Inhalation Study of 2,3-Pentanedione

Growth curves are shown for (A) males and (B) females.

Table 7. Summary of Clinical Observations for Male and Female Rats in the Three-month Inhalation Study of 2,3-Pentanedione

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Male[a]						
Abnormal Breathing	0/10	0/10	0/10	0/10	2/10 SD 19	4/10 SD 40
Eye Abnormality	0/10	0/10	0/10	0/10	4/10 SD 61	7/10 SD 61
Sneezing	0/10	0/10	0/10	0/10	9/10 SD 26	10/10 SD 26
Female						
Abnormal Breathing	0/10	0/10	0/10	0/10	2/10 SD 67	3/10 SD 46
Eye Abnormality	0/10	0/10	0/10	0/10	5/10 SD 60	9/10 SD 46
Sneezing	0/10	0/10	0/10	0/10	7/10 SD 32	10/10 SD 25

No trend or pairwise statistical tests were performed on these data.
SD = study day.
[a]Upper row displays cumulative number of animals with observation/total animals started on study. Lower row displays SD of observation onset.

Absolute and relative lung weights and relative heart weight were significantly increased in the 100 ppm females compared to those of the control group (Table 8). Grossly, some male and female rats in the 50 and 100 ppm groups had unilateral or bilateral corneal opacities, and two female rats exposed to 100 ppm had one or more 1- to 3-mm pale lung foci (Appendix E).

Table 8. Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats in the Three-month Inhalation Study of 2,3-Pentanedione[a,b]

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
n	10	10	10	10	10	10
Terminal Body Wt. (g)	227.7 ± 3.4	225.5 ± 6.1	234.9 ± 3.3	235.1 ± 4.5	224.6 ± 4.6	223.7 ± 4.1
Lung						
Absolute (g)	1.33 ± 0.04**	1.37 ± 0.06	1.45 ± 0.06	1.39 ± 0.04	1.41 ± 0.06	1.65 ± 0.08**
Relative (mg/g)[c]	5.85 ± 0.14**	6.09 ± 0.21	6.16 ± 0.26	5.93 ± 0.15	6.26 ± 0.22	7.39 ± 0.40**
Heart						
Absolute (g)	0.68 ± 0.01	0.71 ± 0.02	0.71 ± 0.01	0.71 ± 0.02	0.70 ± 0.02	0.72 ± 0.02
Relative (mg/g)	2.97 ± 0.06*	3.14 ± 0.05	3.00 ± 0.03	3.03 ± 0.07	3.12 ± 0.06	3.22 ± 0.05*

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

On day 23, white blood cell and monocyte counts were significantly increased in the 50 and 100 ppm female groups, and lymphocyte counts were significantly increased in groups of females exposed to ≥12.5 ppm; neutrophil counts were significantly increased in the 100 ppm group of both male and female rats (Table 9). At study termination, neutrophil counts were significantly increased in the 50 and 100 ppm male and female rats. Additionally, in females, the monocyte count was significantly increased in the 100 ppm group, whereas in males, the lymphocyte count was significantly decreased in the 100 ppm group. These changes were consistent with an exposure-related proinflammatory response. In addition, the decrease in lymphocyte count in male rats indicated a possible stress response to exposure (i.e., effects of a chronic increase in endogenous glucocorticoids).[91] All other statistically significant hematology changes were mild, inconsistent, and/or considered due to biological variability and, thus, not considered related to 2,3-pentanedione exposure.

On day 3, triglyceride concentrations were significantly decreased in the 25 and 50 ppm female rats; these changes were most likely due to changes in feed consumption while acclimating to exposure (Appendix E). At study termination, globulin concentration was significantly increased, and albumin concentration was significantly decreased in the 100 ppm female rats in the absence of significant changes in total protein (Table 10). In addition, the A/G ratio was significantly decreased in male rats exposed to 50 and 100 ppm and in female rats exposed to 100 ppm. These changes are consistent with the exposure-related inflammation in the respiratory tract because globulin is a positive acute phase protein and albumin a negative acute phase protein. All other statistically significant changes were minimal, inconsistent, and/or not considered biologically relevant and, thus, not considered due to 2,3-pentanedione exposure.

Table 9. Summary of Select Hematology Data for Male and Female Rats in the Three-month Inhalation Study of 2,3-Pentanedione[a,b]

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
n	10	10	10[c]	10	10[d]	10
Male (10^3/µL)						
White Blood Cells						
Day 3	10.86 ± 0.77	10.38 ± 0.92	9.24 ± 0.60	10.07 ± 0.61	8.43 ± 0.32*	9.40 ± 0.84
Day 23	10.00 ± 0.51	9.18 ± 0.65	8.90 ± 0.27	9.09 ± 0.39	9.76 ± 0.45	9.78 ± 0.94
Day 93	8.82 ± 0.69	8.50 ± 0.58	7.85 ± 0.56	7.66 ± 0.68	8.88 ± 0.83	7.36 ± 0.41
Lymphocytes						
Day 3	9.30 ± 0.72	8.92 ± 0.85	7.85 ± 0.49	8.58 ± 0.61	7.39 ± 0.31	7.94 ± 0.80
Day 23	8.81 ± 0.49	8.00 ± 0.70	7.83 ± 0.26	7.88 ± 0.40	8.07 ± 0.31	7.29 ± 0.76
Day 93	7.31 ± 0.62**	6.90 ± 0.54	6.50 ± 0.51	6.33 ± 0.61	6.61 ± 0.56	5.04 ± 0.28**
Monocytes						
Day 3	0.33 ± 0.03	0.30 ± 0.03	0.34 ± 0.05	0.31 ± 0.03	0.25 ± 0.02	0.27 ± 0.03
Day 23	0.24 ± 0.01	0.19 ± 0.02	0.22 ± 0.01	0.22 ± 0.02	0.28 ± 0.03	0.28 ± 0.03
Day 93	0.16 ± 0.01	0.17 ± 0.01	0.13 ± 0.01	0.15 ± 0.02	0.21 ± 0.03	0.13 ± 0.01

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Neutrophils						
Day 3	1.11 ± 0.12	1.06 ± 0.10	0.95 ± 0.12	1.08 ± 0.15	0.71 ± 0.05*	1.09 ± 0.12
Day 23	0.85 ± 0.06**	0.90 ± 0.12	0.75 ± 0.09	0.89 ± 0.09	1.31 ± 0.17	2.06 ± 0.64**
Day 93	1.25 ± 0.07**	1.31 ± 0.11	1.12 ± 0.06	1.09 ± 0.10	1.95 ± 0.28*	2.08 ± 0.31*
Female (10^3/μL)						
White Blood Cells						
Day 3	6.65 ± 0.17	6.91 ± 0.44	7.91 ± 0.31	6.88 ± 0.55	8.12 ± 0.68	7.03 ± 0.32
Day 23	5.69 ± 0.32**	6.91 ± 0.59	7.30 ± 0.41	6.09 ± 0.24	8.59 ± 0.50**	9.02 ± 0.64**
Day 93	6.14 ± 0.60	6.76 ± 0.63	7.26 ± 0.45	5.70 ± 0.37	6.74 ± 0.84	6.77 ± 0.49
Lymphocytes						
Day 3	5.64 ± 0.19	5.99 ± 0.37	6.85 ± 0.25*	5.90 ± 0.50	6.99 ± 0.64	6.11 ± 0.26
Day 23	4.74 ± 0.24**	5.95 ± 0.54	6.53 ± 0.39**	5.25 ± 0.24*	6.97 ± 0.41**	6.85 ± 0.51**
Day 93	4.94 ± 0.55*	5.40 ± 0.57	5.83 ± 0.42	4.42 ± 0.34	4.67 ± 0.70	4.02 ± 0.31
Monocytes						
Day 3	0.21 ± 0.02	0.19 ± 0.02	0.24 ± 0.03	0.21 ± 0.03	0.24 ± 0.03	0.20 ± 0.03
Day 23	0.14 ± 0.01**	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.22 ± 0.03**	0.25 ± 0.04**
Day 93	0.12 ± 0.01*	0.13 ± 0.02	0.13 ± 0.01	0.10 ± 0.01	0.15 ± 0.02	0.20 ± 0.03*
Neutrophils						
Day 3	0.68 ± 0.05	0.62 ± 0.06	0.70 ± 0.08	0.66 ± 0.08	0.74 ± 0.04	0.62 ± 0.07
Day 23	0.70 ± 0.07**	0.67 ± 0.05	0.51 ± 0.03	0.63 ± 0.05	1.29 ± 0.34	1.79 ± 0.27*
Day 93	1.00 ± 0.08**	1.12 ± 0.07	1.20 ± 0.05	1.09 ± 0.12	1.83 ± 0.21**	2.42 ± 0.20**

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[c]For the day 3 data, n = 9 for the male 12.5 ppm group.
[d]For the day 23 data, n = 9 for the female 50 ppm group.

Table 10. Summary of Select Clinical Chemistry Data for Male and Female Rats in the Three-month Inhalation Study of 2,3-Pentanedione[a,b]

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
n	10	10	10	10	10[c]	10
Male						
Total Protein (g/dL)						
Day 3	5.94 ± 0.09	5.83 ± 0.07	5.84 ± 0.04	5.70 ± 0.06	5.78 ± 0.07	5.83 ± 0.07
Day 23	6.39 ± 0.07	6.45 ± 0.06	6.33 ± 0.09	6.36 ± 0.05	6.38 ± 0.09	6.42 ± 0.08
Day 93	6.77 ± 0.07	7.05 ± 0.09	6.83 ± 0.09	6.96 ± 0.13	6.96 ± 0.10	6.84 ± 0.06

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Albumin (g/dL)						
Day 3	4.29 ± 0.05*	4.23 ± 0.05	4.18 ± 0.04	4.15 ± 0.07	4.13 ± 0.05	4.19 ± 0.05
Day 23	4.37 ± 0.05	4.41 ± 0.05	4.28 ± 0.04	4.38 ± 0.04	4.27 ± 0.05	4.32 ± 0.05
Day 93	4.50 ± 0.04	4.57 ± 0.07	4.53 ± 0.06	4.55 ± 0.07	4.47 ± 0.06	4.40 ± 0.05
Globulin (g/dL)						
Day 3	1.65 ± 0.07	1.60 ± 0.05	1.66 ± 0.05	1.55 ± 0.05	1.65 ± 0.03	1.64 ± 0.03
Day 23	2.02 ± 0.06	2.04 ± 0.05	2.05 ± 0.07	1.98 ± 0.06	2.11 ± 0.08	2.10 ± 0.06
Day 93	2.27 ± 0.07	2.48 ± 0.07	2.30 ± 0.04	2.41 ± 0.10	2.49 ± 0.08	2.44 ± 0.04
A/G Ratio						
Day 3	2.63 ± 0.09	2.66 ± 0.08	2.55 ± 0.11	2.71 ± 0.12	2.51 ± 0.04	2.56 ± 0.04
Day 23	2.18 ± 0.06	2.17 ± 0.06	2.11 ± 0.07	2.23 ± 0.08	2.05 ± 0.08	2.07 ± 0.06
Day 93	2.00 ± 0.06**	1.85 ± 0.06	1.97 ± 0.04	1.92 ± 0.09	1.81 ± 0.07*	1.81 ± 0.04*
Female						
Total Protein (g/dL)						
Day 3	6.24 ± 0.11	6.16 ± 0.08	6.24 ± 0.12	6.07 ± 0.09	6.05 ± 0.07	6.08 ± 0.07
Day 23	6.68 ± 0.10	6.62 ± 0.11	6.68 ± 0.14	6.53 ± 0.06	6.66 ± 0.12	6.64 ± 0.13
Day 93	7.26 ± 0.14	7.29 ± 0.13	7.24 ± 0.10	7.41 ± 0.11	7.40 ± 0.08	7.24 ± 0.11
Albumin (g/dL)						
Day 3	4.60 ± 0.06	4.57 ± 0.06	4.61 ± 0.07	4.50 ± 0.07	4.55 ± 0.05	4.53 ± 0.07
Day 23	4.78 ± 0.05	4.79 ± 0.07	4.82 ± 0.08	4.75 ± 0.06	4.77 ± 0.10	4.60 ± 0.10
Day 93	5.36 ± 0.09	5.40 ± 0.19	5.27 ± 0.06	5.51 ± 0.18	5.38 ± 0.08	5.03 ± 0.05*
Globulin (g/dL)						
Day 3	1.64 ± 0.05	1.59 ± 0.04	1.63 ± 0.07	1.57 ± 0.05	1.50 ± 0.04	1.55 ± 0.03
Day 23	1.90 ± 0.05	1.83 ± 0.05	1.86 ± 0.06	1.78 ± 0.03	1.89 ± 0.06	2.04 ± 0.06
Day 93	1.90 ± 0.06**	1.89 ± 0.10	1.97 ± 0.05	1.90 ± 0.09	2.02 ± 0.03	2.21 ± 0.09**
A/G Ratio						
Day 3	2.82 ± 0.06	2.89 ± 0.06	2.87 ± 0.13	2.89 ± 0.09	3.05 ± 0.07	2.93 ± 0.07
Day 23	2.53 ± 0.05*	2.63 ± 0.07	2.61 ± 0.06	2.68 ± 0.06	2.54 ± 0.09	2.27 ± 0.07
Day 93	2.84 ± 0.08**	3.02 ± 0.38	2.69 ± 0.06	3.03 ± 0.32	2.67 ± 0.07	2.30 ± 0.08**

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
A/G = albumin/globulin.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[c]For the day 23 data, n = 9 for the female 50 ppm group.

Histopathology

This section describes statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions in the respiratory tract (nose, larynx, trachea, lung) and the eyes of rats (Appendix E).

Nose: The exposure-related lesions observed in the nose were similar in males and females, and the incidences and average severities of these lesions tended to increase with increasing exposure concentration. The exposure-related lesions observed in both sexes were olfactory epithelial atrophy, olfactory epithelial degeneration, olfactory epithelial respiratory metaplasia, respiratory epithelial hyperplasia, respiratory epithelial squamous metaplasia, respiratory epithelial necrosis, respiratory epithelial regeneration, lymphoid hyperplasia (by positive trend only in females), suppurative inflammation, and turbinate atrophy (Table 11). Most of these lesions were seen in the 50 and/or 100 ppm groups, but a few were seen in groups exposed to lower concentrations. Significant increases in incidence were seen for these lesions in the 50 and/or 100 ppm groups compared to their respective control groups. In males, a significant increase in the incidence of respiratory epithelial hyperplasia was seen at exposure concentrations ≥25 ppm relative to control animals. Increased incidences of necrosis and regeneration of the respiratory epithelium, as well as turbinate atrophy, were seen in the 100 ppm male and female groups relative to control animals. In males, a significantly increased incidence of olfactory epithelial degeneration was seen in the 50 ppm group relative to the control group; the incidence in the 100 ppm group was lower than it was in the 50 ppm group and not statistically significant. This observation is presumably because the lesion progressed beyond degeneration in most of the animals in this group to necrosis, atrophy, or metaplasia—all of which are considered representative of a more severe manifestation of epithelial damage. Olfactory epithelial squamous metaplasia was seen in two males in the 100 ppm group, and respiratory epithelial ulceration was seen in one female exposed to 100 ppm. These increases did not reach statistical significance (except for the olfactory epithelial squamous metaplasia with a positive trend) but are considered related to exposure because they are consistent with the spectrum of 2,3-pentanedione-induced epithelial lesions observed in the nasal cavity.

Table 11. Incidences of Select Nonneoplastic Lesions of the Respiratory Tract in Male and Female Rats in the Three-month Inhalation Study of 2,3-Pentanedione

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
n[a]	10	10	10	10	10	10
Male						
Nose						
Hyperplasia, lymphoid[b]	0**	1 (1.0)[c]	1 (1.0)	1 (1.0)	7** (1.1)	5* (1.6)
Inflammation, suppurative	0**	0	0	2 (1.0)	10** (2.3)	10** (3.0)
Olfactory epithelium, atrophy	1** (1.0)	1 (1.0)	0	4 (1.0)	10** (1.0)	10** (1.0)
Olfactory epithelium, degeneration	2* (1.0)	0	0	2 (1.0)	10** (1.4)	3 (1.7)
Olfactory epithelium, metaplasia, respiratory	2** (1.0)	0	2 (1.0)	1 (1.0)	8* (1.0)	10** (1.0)
Olfactory epithelium, metaplasia, squamous	0**	0	0	0	0	2 (1.0)
Respiratory epithelium, hyperplasia	0**	1 (1.0)	2 (1.0)	9** (1.0)	10** (2.1)	10** (2.9)

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Respiratory epithelium, metaplasia, squamous	0**	0	1 (1.0)	0	10** (1.1)	10** (2.0)
Respiratory epithelium, necrosis	0**	0	0	0	2 (1.0)	10** (2.1)
Respiratory epithelium, regeneration	0**	0	0	0	0	10** (1.5)
Turbinate, atrophy	0**	0	0	0	2 (1.5)	10** (2.0)
Larynx						
Inflammation, suppurative	0	0	0	0	2 (1.0)	0
Inflammation, chronic active	1** (1.0)	0	0	0	0	4 (1.0)
Respiratory epithelium, hyperplasia	0**	1 (1.0)	0	1 (1.0)	7** (1.0)	3 (1.7)
Respiratory epithelium, metaplasia, squamous	1** (1.0)	1 (1.0)	1 (1.0)	5 (1.0)	10** (1.7)	10** (3.0)
Respiratory epithelium, necrosis	0**	0	0	0	0	2 (1.0)
Respiratory epithelium, regeneration	0*	0	0	0	1 (1.0)	2 (1.0)
Respiratory epithelium, ulcer	0	0	0	0	0	1 (1.0)
Squamous epithelium, hyperplasia	0**	0	0	0	0	7** (1.1)
Trachea						
Inflammation, acute	0**	0	0	1 (1.0)	0	3 (1.0)
Epithelium, hyperplasia	0**	0	0	0	0	8** (2.5)
Epithelium, metaplasia, squamous	0**	0	0	0	0	3 (1.7)
Epithelium, necrosis	0	0	0	0	0	1 (1.0)
Epithelium, regeneration	0**	1 (1.0)	1 (1.0)	0	8** (1.4)	10** (2.8)
Lung						
Fibrosis, focal	0	0	0	0	0	1 (1.0)
Inflammation, eosinophil	3** (1.0)	3 (1.0)	1 (1.0)	5 (1.0)	3 (1.0)	10** (2.0)
Alveolus, infiltration, cellular, polymorphonuclear	0	0	0	0	0	1 (1.0)
Bronchiole, epithelium, hyperplasia	0**	0	0	0	3 (1.0)	10** (2.2)
Bronchus, epithelium, hyperplasia	0**	0	0	0	0	5* (1.6)
Bronchus, epithelium, regeneration	0**	0	0	0	1 (1.0)	9** (2.8)
Bronchus, epithelium, metaplasia, squamous	0**	0	0	0	0	3 (1.3)
Bronchus, epithelium, metaplasia, goblet cell	0	0	0	0	0	1 (1.0)
Female						
Nose						
Hyperplasia, lymphoid	0**	0	0	1 (1.0)	0	3 (1.0)
Inflammation, suppurative	0**	0	0	0	10** (1.9)	10** (3.0)
Olfactory epithelium, atrophy	1** (1.0)	0	1 (1.0)	1 (1.0)	10** (1.0)	9** (1.0)
Olfactory epithelium, degeneration	0**	1 (1.0)	0	0	4* (1.3)	5* (1.6)
Olfactory epithelium, metaplasia, respiratory	0**	0	1 (1.0)	1 (1.0)	8** (1.0)	9** (1.2)

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Respiratory epithelium, hyperplasia	1** (1.0)	0	0	3 (1.0)	10** (2.0)	10** (3.0)
Respiratory epithelium, metaplasia, squamous	0**	0	0	0	10** (1.1)	10** (2.0)
Respiratory epithelium, necrosis	0**	0	0	0	1 (1.0)	10** (2.1)
Respiratory epithelium, regeneration	0**	0	0	0	3 (1.0)	10** (1.9)
Respiratory epithelium, ulcer	0	0	0	0	0	1 (1.0)
Turbinate, atrophy	0**	0	0	0	2 (1.0)	9** (2.0)
Larynx						
Inflammation, chronic active	0**	0	1 (1.0)	1 (1.0)	0	5* (1.4)
Respiratory epithelium, hyperplasia	0	0	0	0	2 (1.0)	1 (1.0)
Respiratory epithelium, metaplasia, squamous	0**	0	0	4* (1.0)	10** (1.5)	10** (3.0)
Respiratory epithelium, necrosis	0	0	0	0	0	1 (1.0)
Respiratory epithelium, regeneration	0	0	0	0	0	1 (1.0)
Respiratory epithelium, ulcer	0	0	0	1 (1.0)	0	0
Squamous epithelium, hyperplasia	0**	0	0	0	0	9** (1.0)
Squamous epithelium, necrosis	0**	0	0	0	0	5* (1.2)
Squamous epithelium, ulcer	0**	0	0	0	0	4* (2.3)
Trachea[d]						
Inflammation, acute	0*	0	0	0	0	2 (1.0)
Epithelium, hyperplasia	0**	0	0	1 (2.0)	0	4* (3.0)
Epithelium, metaplasia, squamous	0*	0	0	0	0	2 (1.0)
Epithelium, necrosis	0*	0	0	0	0	2 (1.0)
Epithelium, regeneration	0**	3 (1.0)	0	0	7** (1.6)	10** (3.0)
Lung						
Fibrosis, focal	0**	0	0	0	0	2 (1.0)
Inflammation, eosinophil	4** (1.3)	3 (1.0)	4 (1.0)	3 (1.0)	5 (1.0)	10** (1.7)
Alveolus, infiltration, cellular, polymorphonuclear	0**	0	0	0	0	2 (1.0)
Bronchiole, epithelium, hyperplasia	0**	0	0	0	0	7** (1.1)
Bronchus, epithelium, hyperplasia	0**	0	0	0	0	4* (1.5)
Bronchus, epithelium, regeneration	0**	0	0	0	0	8** (1.8)
Bronchus, epithelium, metaplasia, goblet cell	0**	0	0	0	0	3 (2.3)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Cochran-Armitage (trend) or Fisher's exact (pairwise) test; **$p \leq 0.01$.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[d]For the trachea data, n = 9 for the female 12.5 ppm group.

Olfactory epithelial atrophy consisted of thinning (attenuation) of the olfactory epithelium. Olfactory epithelium degeneration consisted of swollen and/or vacuolated olfactory epithelial cells (Figure 4A). The thickness of the epithelium was also decreased in some cases of degeneration. Respiratory metaplasia of the olfactory epithelium was characterized by replacement of the olfactory epithelium by ciliated, columnar epithelium resembling respiratory epithelium. Squamous metaplasia of the olfactory epithelium was characterized by replacement of the olfactory epithelium by squamous epithelium (Figure 4B). The metaplastic lesions were seen predominantly in the dorsal meatus of nasal sections II and III. These lesions are thought to be part of the same pathological process with degeneration of the olfactory epithelium leading to atrophy and further damage leading to respiratory and squamous metaplasia (respiratory and squamous epithelial types are thought to be more resistant to damage).

Respiratory epithelial hyperplasia was characterized by increased numbers of cells in the respiratory epithelium. The cells were crowded along the basement membrane and were narrower and taller than typical respiratory epithelial cells. In some cases, the cells were more cuboidal and were piled up into three or more layers. The respiratory epithelium lining the nasal septum occasionally formed rugal folds. Squamous metaplasia of the respiratory epithelium consisted of replacement of the respiratory epithelium by the more resistant squamous epithelium. Necrosis of the respiratory epithelium was characterized by an attenuated or denuded epithelium often bordered by hypereosinophilic, shrunken cells with pyknotic nuclei and cellular debris. Ulceration is generally considered a sequel to necrosis and was diagnosed when there was complete, focal loss of the epithelium. Regeneration of the respiratory epithelium was diagnosed when the necrotic epithelium was replaced by flattened epithelium that extended from the adjacent nonnecrotic epithelium. While squamous metaplasia of the respiratory epithelium is thought to be a protective response of the respiratory epithelium (as are metaplastic lesions in the olfactory epithelium), regeneration and hyperplasia are thought to be reparative responses after necrosis of the epithelium.

Turbinate atrophy was characterized by blunted, shortened, and/or thinned turbinates with increased airway space between the tips of the turbinates. Turbinate atrophy was primarily evident in the maxilloturbinates of level I, less frequently in maxilloturbinates of level II, and rarely in the nasoturbinates of level II.

Suppurative inflammation (Figure 4C) was characterized by variable numbers of neutrophils in the nasal cavity lumen admixed with cellular debris and eosinophilic proteinaceous material, neutrophils in the nasal mucosa lamina propria, and neutrophils transmigrating the nasal epithelium, generally involving all levels of the nose.

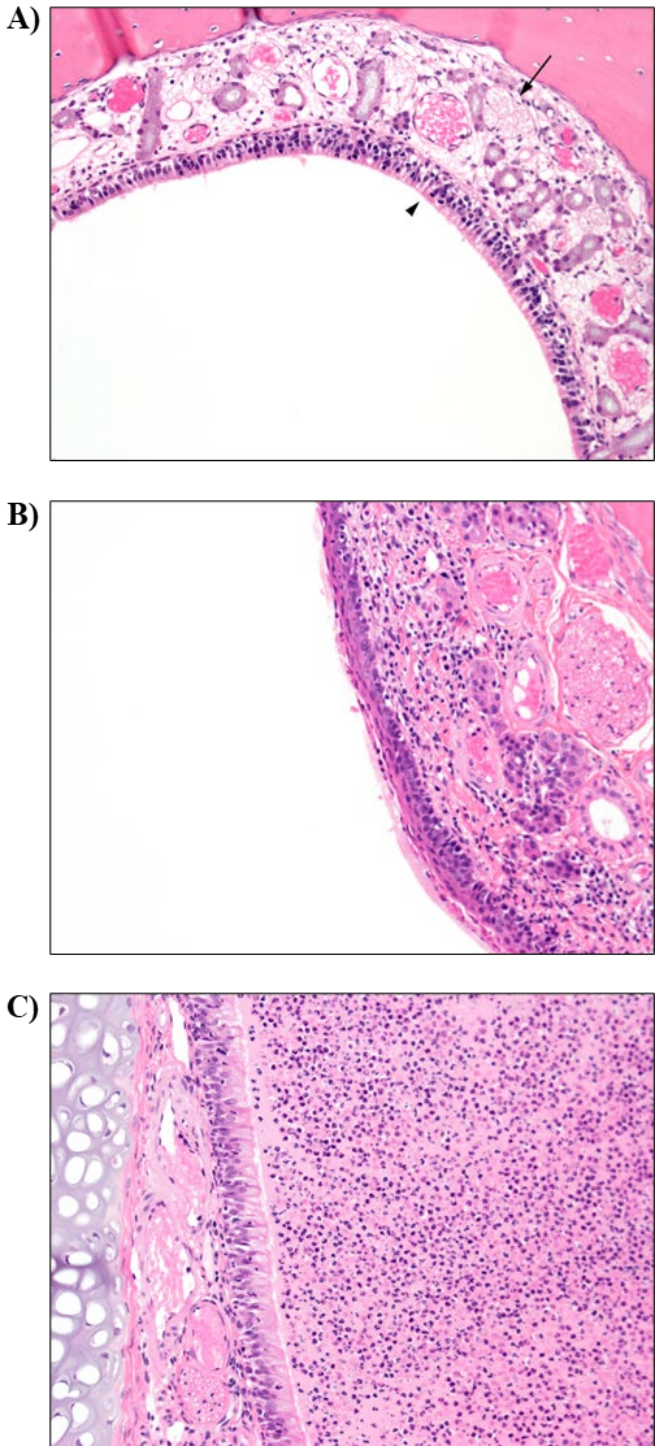


Figure 4. Representative Images of Atrophy, Squamous Metaplasia, and Suppurative Inflammation in the Nasal Cavity of Male Rats in the Three-month Inhalation Study of 2,3-Pentanedione (H&E)

(A) Atrophy of the olfactory epithelium (arrowhead) and olfactory nerves (arrow) at level I of the nasal cavity of a male rat exposed to 100 ppm 2,3-pentanedione (20×). (B) Squamous metaplasia of the epithelium at level I of the nasal cavity of a male rat exposed to 100 ppm 2,3-pentanedione (20×). (C) Suppurative inflammation at level II of the nasal cavity of a male rat exposed to 100 ppm 2,3-pentanedione. There are numerous degenerate neutrophils amid cellular debris and proteinaceous fluid on the right side of the photo (20×). H&E = hematoxylin and eosin stain.

Larynx: Most of the exposure-related lesions in the larynx were similar in male and female rats (but to a varying extent), and the incidences and average severities of these lesions either increased with increasing exposure concentration or were only seen in the highest exposure group (Table 11). The exposure-related lesions observed in both sexes were hyperplasia, squamous metaplasia, necrosis, ulceration, and regeneration of the respiratory epithelium; hyperplasia of the squamous epithelium; and chronic active inflammation. Two males in the 50 ppm group exhibited suppurative inflammation, and five and four females in the 100 ppm group had significantly increased incidences of necrosis and ulceration, respectively, of the squamous epithelium. Significant increases in the incidences of respiratory epithelial squamous metaplasia were seen in the 50 and 100 ppm groups of males and females compared to the control groups. In females, the incidence of this lesion was also significantly increased in the 25 ppm group relative to the control group. Hyperplasia of the squamous epithelium and of the respiratory epithelium was significantly increased in the 100 ppm groups of males and females and in the 50 ppm group of males, respectively, compared to the control groups. Chronic active inflammation of the larynx was significantly increased in 100 ppm group of females (and in males by positive trend only).

Chronic active inflammation of the larynx was characterized by an increased number of lymphocytes, macrophages, and neutrophils present in the laryngeal subepithelial tissue and occasionally extending into the mucosa and laryngeal lumen. Suppurative inflammation was characterized mainly by the presence of neutrophils in the lumen, many of which were degenerate, with fewer macrophages and lymphocytes. Some of the inflammatory cells were also present in the mucosa and submucosa. Regeneration of the respiratory epithelium consisted of flattened epithelial cells that stretched across the basement membrane and individualized epithelial cells with loss of cilia or an increase in thickness that did not exceed three cell layers. Hyperplasia of the respiratory and squamous epithelia were characterized by an increase in the thickness of the epithelium that was greater than three cell layers (Figure 5A). Respiratory epithelial squamous metaplasia was diagnosed when the normal ciliated columnar respiratory epithelium was replaced by stratified squamous epithelium, which was sometimes keratinized (Figure 5B). Necrosis of the respiratory or squamous epithelium was characterized by loss of the epithelial cell layer and replacement by necrotic cellular debris admixed with variable numbers of inflammatory cells and eosinophilic proteinaceous material or, rarely, individual cells with increased cytoplasmic eosinophilia and pyknotic nuclei. Ulceration of the respiratory and squamous epithelia was characterized by the absence of the epithelial cells, exposing the underlying connective tissue.

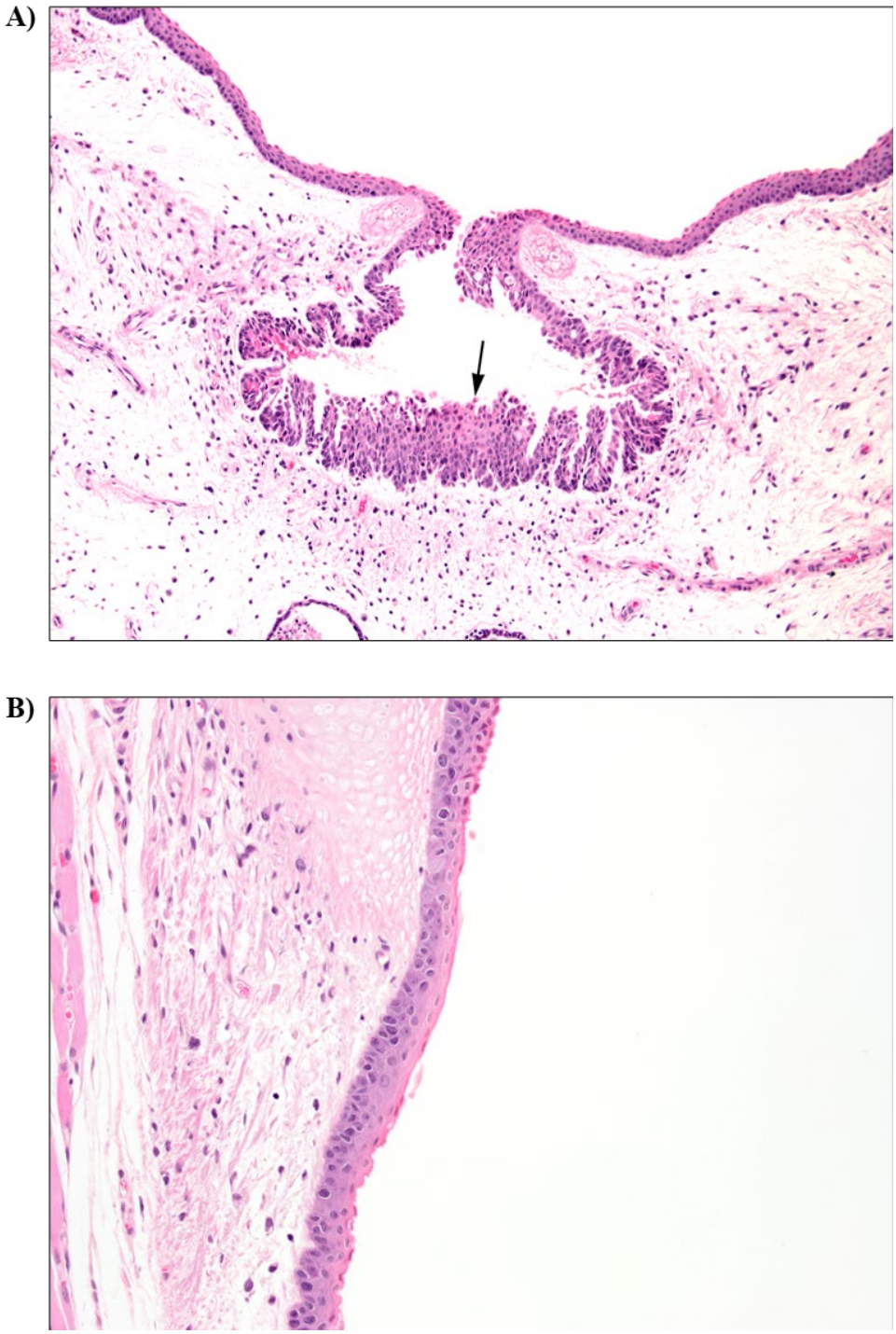


Figure 5. Representative Images of Hyperplasia and Squamous Metaplasia in the Larynx of Male Rats in the Three-month Inhalation Study of 2,3-Pentanedione (H&E)

(A) Hyperplasia and squamous metaplasia in the laryngeal pouch at level I of the larynx in a male rat exposed to 100 ppm 2,3-pentanedione. The squamous metaplasia is exemplified by the sheet-like cells in the center of the lesion (arrow), and the undulations and multiple layers of cells exemplify the hyperplasia (10×). (B) Squamous metaplasia of the epithelium on the lateral wall at level II of the larynx in a male rat exposed to 100 ppm 2,3-pentanedione. Note the keratinizing surface of the thickened epithelium (20×). H&E = hematoxylin and eosin stain.

Trachea: The exposure-related lesions in the trachea were consistent with the same general processes that were observed in the other respiratory tract organs. Lesion type, incidence, and significant increases in incidence were similar in males and females (Table 11). In both sexes, acute inflammation, epithelial squamous metaplasia, and epithelial hyperplasia were seen in the 100 ppm group, but only epithelial hyperplasia showed a significant increase in incidence over the respective control group by pairwise test. There were significant increases in the incidences of epithelial regeneration (Figure 6) in the male and female 50 and 100 ppm groups compared to the control groups. One male and two females in the 100 ppm groups were diagnosed with epithelial necrosis. Although not statistically significant (by pairwise test as there was a positive trend in females), the necrosis was considered related to exposure due to the presence of regeneration, a sequel to necrosis, in all animals in the 100 ppm groups. All of these lesions, except for acute inflammation, are similar to those described in the nose and larynx.

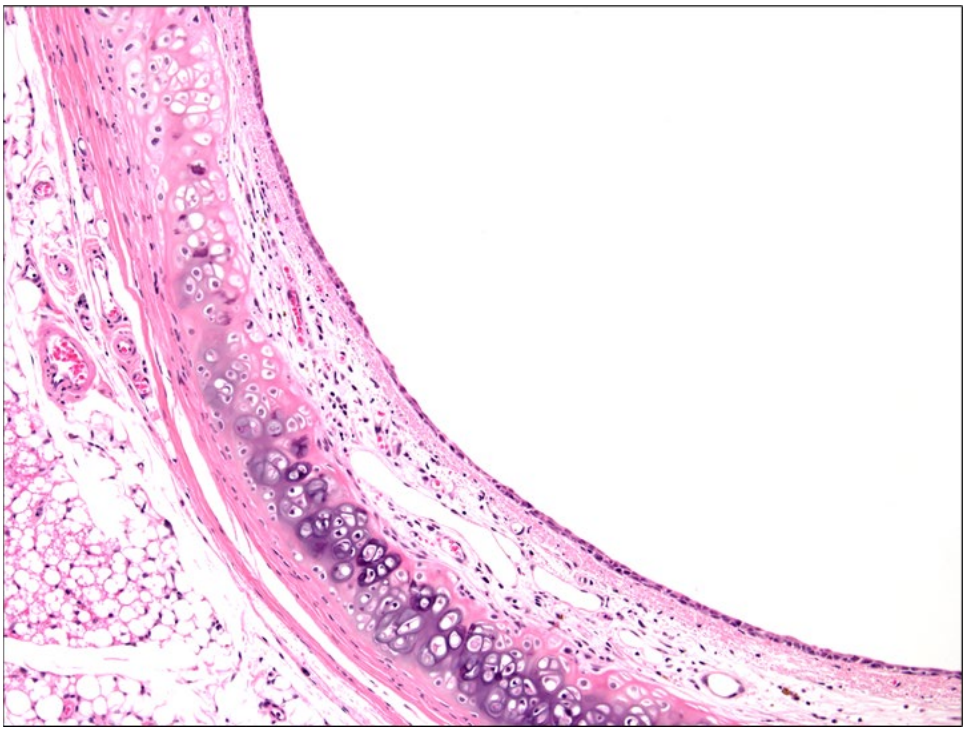


Figure 6. Representative Image of Regeneration in the Trachea of a Male Rat in the Three-month Inhalation Study of 2,3-Pentanedione (H&E)

Regeneration of the respiratory epithelium in the trachea of a male rat exposed to 100 ppm 2,3-pentanedione (10×).
H&E = hematoxylin and eosin stain.

Lung: Most exposure-related lesions in the lungs were similar in males and females. In both sexes, there were significant increases in the incidences of eosinophilic inflammation, bronchial epithelial hyperplasia, bronchiolar epithelial hyperplasia, and bronchial epithelial regeneration in the 100 ppm groups compared to the control groups (Table 11). Other exposure-related lesions for which the incidences in exposed groups were not significantly different from control animals, or there was only a positive trend, included polymorphonuclear cell infiltration of the alveoli, goblet cell metaplasia of the bronchi, and focal fibrosis (with positive trends in females only) and squamous metaplasia of the bronchi (with a positive trend in males only). These lesions were

considered exposure related because they are not typically seen in control animals; a few of them had slightly higher incidences in the females, and they are common responses to exposure.

Focal fibrosis consisted of small foci of fibrous connective tissue entrapping alveoli with low numbers of macrophages, with fewer eosinophils and lymphocytes and with occasional mast cells. The macrophages rarely contained golden-brown pigment, which was presumed to be hemosiderin.

Microscopically, eosinophilic inflammation was characterized mainly by eosinophils with fewer macrophages and occasional lymphocytes and neutrophils cuffing vessels and airways. The eosinophils were sometimes seen in the wall and epithelium of smaller airways and occasionally larger airways. Macrophages occasionally contained intracytoplasmic, golden-brown material (presumed to be hemosiderin).

In the bronchus, epithelial regeneration consisted of either flattening and elongation of the epithelium with loss of cilia or piling up of epithelium to three or more cell layers thick.

Epithelial hyperplasia in the bronchi was characterized by an increased thickness of the epithelium due to piling up of cells more than three layers thick (Figure 7A). Rarely, single cell necrosis was noted in these areas of high cell turnover. Squamous metaplasia in the bronchi consisted of replacement of the normal respiratory epithelium with flattened, squamous epithelium or with goblet cells, which had abundant, slightly basophilic, and vacuolated cytoplasm (Figure 7B).

Goblet cell metaplasia was noted in secondary bronchi, and occasionally there was accumulation of mucus within smaller, peripheral airways that was admixed with polymorphonuclear inflammatory cells and very rare sloughed epithelial cells.

Hyperplasia of the bronchiolar epithelium consisted of epithelial cells that were enlarged, piling up, crowding along the basement membrane, and often protruding into the airway lumen. Occasional cellular characteristics of slightly vacuolated cytoplasm, hyperchromatic nuclei, and reverse polarity (where the nucleus was closer to the lumen than the basement membrane) were present.

Polymorphonuclear cellular infiltration consisted of variable numbers of histiocytes infiltrating the alveoli.

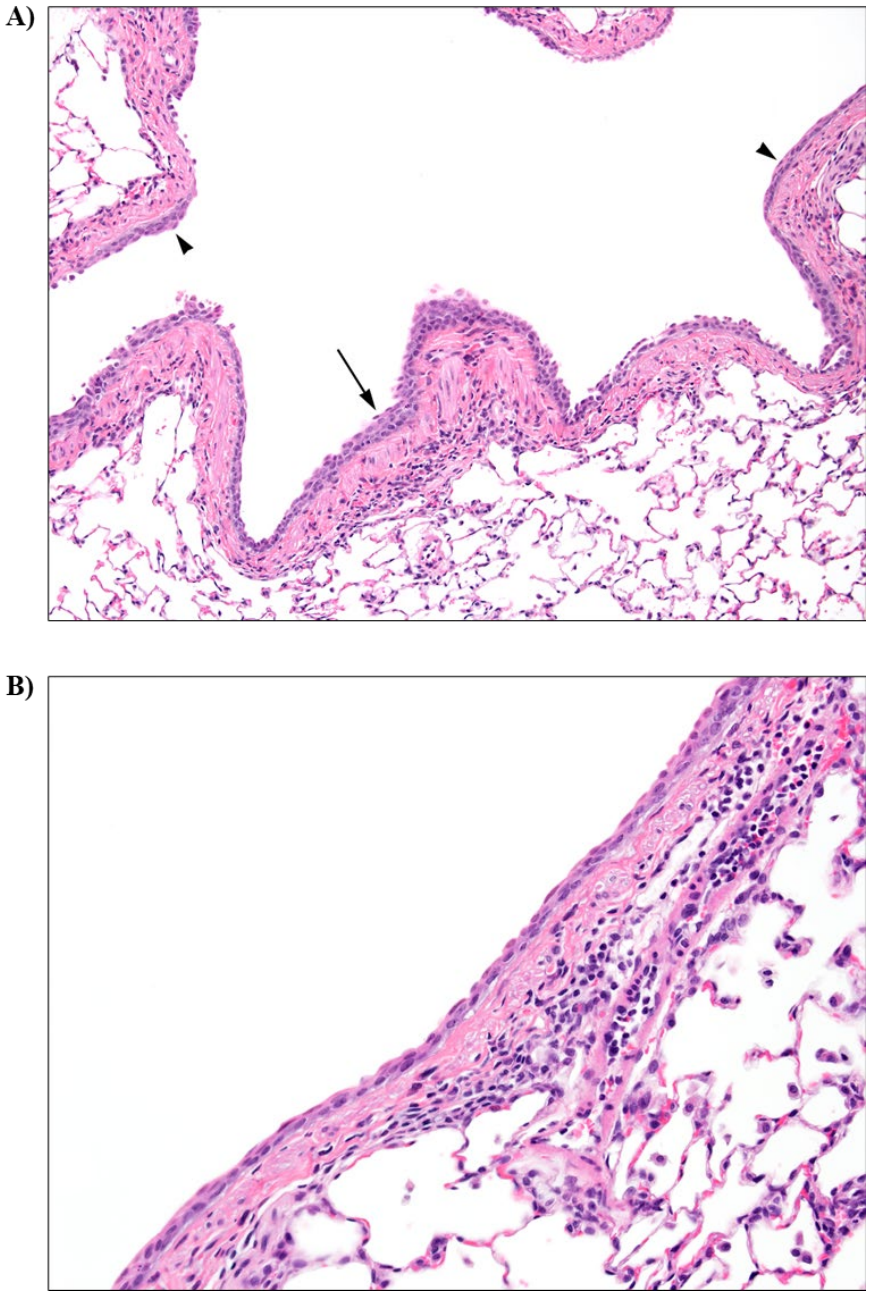


Figure 7. Representative Images of Hyperplasia and Squamous Metaplasia in the Lung of Male Rats in the Three-month Inhalation Study of 2,3-Pentanedione (H&E)

(A) Hyperplasia (arrow) and squamous metaplasia (arrowheads) of the epithelium of a bronchus in the lung of a male rat exposed to 100 ppm 2,3-pentanedione (10×). (B) Squamous metaplasia of the epithelium of a bronchus in the lung of a male rat exposed to 100 ppm 2,3-pentanedione (20×). H&E = hematoxylin and eosin stain.

Eyes: There was acute inflammation in the eyes (cornea, ciliary body, anterior chamber, sclera, and/or iris), which was significantly increased in the cornea of males (positive trend only), in the cornea of females exposed to 50 and 100 ppm, and in the ciliary body of females exposed to 50 ppm (Table 12). Three male rats and two female rats had minimal inflammation of the conjunctiva (two males and one female in the 12.5 ppm group and one male and one female in

the 50 ppm group). The inflammatory cells were predominantly neutrophils and were present within the cornea, ciliary body, anterior chamber, iris, and/or sclera. The overlying corneal epithelium was occasionally hyperplastic, vacuolated, ulcerated, and/or neovascularized (there was a positive trend for neovascularization and vacuolation in males).

Table 12. Incidences of Select Nonneoplastic Lesions of the Eye in Male and Female Rats in the Three-month Inhalation Study of 2,3-Pentanedione

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Male						
Eye[a]	10	10	9	10	10	10
Anterior chamber, inflammation, acute[b]	0	0	0	0	2 (1.0)[c]	0
Ciliary body, inflammation, acute	0	0	0	0	3 (1.3)	0
Conjunctiva, inflammation, acute	0	0	2 (1.0)	0	1 (1.0)	0
Cornea, inflammation, acute	0**	0	0	0	3 (1.0)	3 (1.3)
Cornea, neovascularization	0*	0	0	0	0	2 (1.0)
Cornea, ulcer	0	0	0	0	1 (2.0)	0
Cornea, epithelium, hyperplasia	0	0	0	0	0	1 (3.0)
Cornea, epithelium, vacuolation	0*	0	0	0	0	2 (2.5)
Iris, inflammation, acute	0	0	0	0	2 (1.0)	0
Female						
Eye	10	10	10	9	9	10
Anterior chamber, inflammation, acute	0	0	0	0	3 (1.0)	0
Ciliary body, inflammation, acute	0**	0	0	0	5* (1.2)	2 (1.0)
Conjunctiva, inflammation, acute	0	0	1 (1.0)	0	1 (1.0)	0
Cornea, inflammation, acute	0**	0	1 (1.0)	0	5* (1.2)	6** (1.3)
Cornea, ulcer	0	0	0	0	0	1 (2.0)
Cornea, epithelium, hyperplasia	0	0	0	0	0	1 (1.0)
Cornea, epithelium, vacuolation	0	0	0	0	1 (2.0)	0
Sclera, inflammation, acute	0	0	0	0	2 (1.5)	0

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Cochran-Armitage (trend) or Fisher's exact (pairwise) test; **$p \leq 0.01$.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Three-month Study in Mice

All 3-month study mice survived to study termination. In the 100 ppm exposed mice, group mean body weight at day 8 was lower than the day 1 pre-exposure mean body weight and did not exceed the day 1 mean body weight until day 68 in males and day 40 in females (Table 13; Figure 8). Body weight gain and mean body weights were lower in males and females exposed to 50 or 100 ppm relative to controls (Figure 8). Terminal mean body weights were significantly decreased for male and female mice exposed to 50 and 100 ppm (Appendix E). Exposure-related clinical observations in the 50 and 100 ppm groups included abnormal breathing and sneezing in both sexes and eye abnormality in females (Table 14).

Table 13. Summary of Survival and Mean Body Weights of Male and Female Mice in the Three-month Inhalation Study of 2,3-Pentanedione

Study Day[a]	0 ppm		6.25 ppm			12.5 ppm			25 ppm			50 ppm			100 ppm		
	Av. Wt. (g)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N	Av. Wt. (g)	Wt. (% of Controls)	N
Male																	
1	22.5	10	22.3	99.3	10	22.6	100.8	10	22.4	99.7	10	22.3	99.2	10	22.4	99.6	10
8	24.3	10	24.0	98.5	10	24.1	99.1	10	24.1	98.9	10	23.9	98.2	10	19.9	82.0	10
19	25.7	10	25.5	99.1	10	25.6	99.5	10	25.3	98.5	10	24.4	94.8	10	21.4	83.0	10
26	26.5	10	26.3	99.4	10	26.2	99.0	10	26.1	98.5	10	24.9	94.2	10	21.7	82.1	10
33	27.1	10	27.0	99.7	10	27.1	100.1	10	27.1	100.3	10	25.5	94.3	10	21.8	80.6	10
40	27.9	10	27.6	99.2	10	28.1	101.0	10	28.0	100.6	10	26.3	94.5	10	21.8	78.2	10
47	28.4	10	28.3	99.7	10	28.8	101.2	10	28.8	101.3	10	26.7	93.9	10	22.0	77.4	10
54	29.4	10	29.2	99.1	10	29.7	100.9	10	29.6	100.4	10	27.5	93.3	10	22.0	74.7	10
61	29.9	10	30.0	100.2	10	30.6	102.3	10	30.4	101.7	10	27.7	92.8	10	22.1	73.9	10
68	30.7	10	30.5	99.6	10	31.4	102.3	10	30.9	100.7	10	28.3	92.3	10	22.6	73.7	10
75	31.6	10	31.5	99.6	10	32.2	101.8	10	31.9	101.0	10	28.5	90.1	10	23.4	74.0	10
82	32.5	10	32.4	99.7	10	33.0	101.5	10	32.8	100.7	10	29.1	89.4	10	23.3	71.6	10
89	33.1	10	33.2	100.3	10	33.9	102.2	10	33.6	101.5	10	29.3	88.4	10	23.3	70.2	10
EOS	33.7	10	33.9	100.7	10	34.4	102.1	10	34.1	101.2	10	29.6	87.8	10	23.3	69.2	10
Female																	
1	19.7	10	19.3	98.0	10	19.5	98.8	10	19.5	98.7	10	19.5	98.9	10	19.0	96.2	10
8	21.3	10	21.0	98.6	10	21.0	98.6	10	20.9	98.4	10	20.8	97.9	10	18.0	84.5	10
19	22.2	10	21.8	98.2	10	22.0	99.4	10	21.3	96.3	10	21.0	94.7	10	18.6	84.0	10
27	23.4	10	23.0	98.1	10	23.3	99.7	10	22.7	97.0	10	21.6	92.2	10	18.3	78.3	10
33	24.2	10	23.8	98.3	10	23.4	96.6	10	23.5	97.2	10	21.8	90.2	10	18.5	76.5	10
40	24.8	10	24.8	100.2	10	24.9	100.5	10	24.3	98.2	10	22.6	91.5	10	19.1	77.1	10
47	25.0	10	25.3	101.0	10	25.2	100.5	10	25.3	101.2	10	23.1	92.1	10	19.7	78.5	10
54	26.3	10	26.4	100.5	10	26.0	98.7	10	26.3	99.9	10	23.2	88.4	10	19.8	75.3	10
61	26.5	10	26.8	101.2	10	26.3	99.2	10	26.2	98.8	10	23.7	89.5	10	19.7	74.3	10
68	26.5	10	27.4	103.2	10	26.9	101.3	10	27.1	102.3	10	24.0	90.3	10	20.4	77.1	10
75	27.4	10	27.6	100.9	10	26.9	98.4	10	27.4	100.2	10	24.5	89.5	10	20.7	75.7	10
82	28.0	10	28.4	101.3	10	27.8	99.1	10	28.1	100.4	10	25.3	90.3	10	21.1	75.3	10
89	28.2	10	28.9	102.3	10	28.2	99.9	10	28.9	102.5	10	25.2	89.2	10	20.1	71.1	10
EOS	29.1	10	29.7	102.2	10	28.9	99.5	10	29.4	101.1	10	25.1	86.2	10	20.3	69.7	10

No trend or pairwise statistical tests were performed on these data.
EOS = end of study.
[a]Study day 1 is the day animals were placed on study.

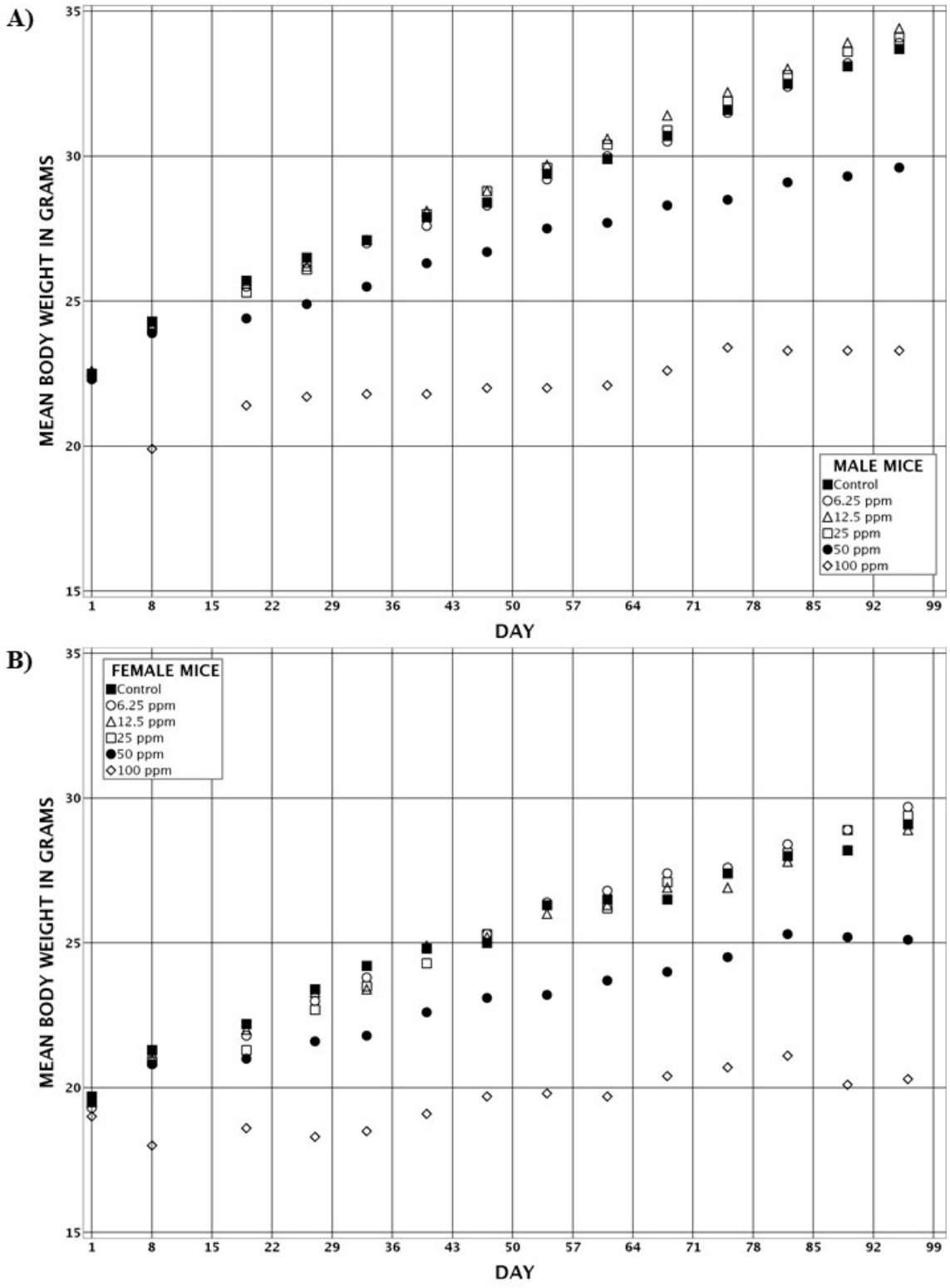


Figure 8. Growth Curves for Male and Female Mice in the Three-month Inhalation Study of 2,3-Pentanedione

Growth curves are shown for (A) males and (B) females.

Table 14. Summary of Clinical Observations for Male and Female Mice in the Three-month Inhalation Study of 2,3-Pentanedione

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Male[a]						
Abnormal Breathing	0/10	0/10	0/10	0/10	0/10	9/10 SD 33
Sneezing	0/10	0/10	0/10	0/10	10/10 SD 26	10/10 SD 33
Female						
Abnormal Breathing	0/10	0/10	0/10	0/10	0/10	5/10 SD 33
Sneezing	0/10	0/10	0/10	0/10	10/10 SD 27	10/10 SD 27
Eye Abnormality	0/10	0/10	0/10	0/10	0/10	2/10 SD 68

No trend or pairwise statistical tests were performed on these data.
SD = study day.
[a]Upper row displays cumulative number of animals with observation/total animals started on study. Lower row displays SD of observation onset.

There were significant increases in relative lung weights of males exposed to ≥50 ppm and females exposed to 100 ppm, whereas absolute lung weights were significantly decreased in females exposed to ≥50 ppm relative to control groups (Table 15). There were significant decreases in absolute heart, kidney, and liver weights of males and females and absolute spleen weights of females exposed to ≥50 ppm relative to control groups, as well as significant decreases in absolute spleen weights of males and relative spleen weights of females exposed to 100 ppm relative to control groups. There were significant increases in relative heart and spleen weights of males and relative kidney weights of females exposed to 100 ppm relative to control groups. Other statistically significant changes in absolute or relative organ weights were considered to be of uncertain biological significance and were likely due to decreased terminal body weights of groups exposed to ≥50 ppm. Grossly, two female mice exposed to 100 ppm had corneal lesions described as “pale” or “opaque” (Appendix E).

Table 15 Summary of Select Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male and Female Mice in the Three-month Inhalation Study of 2,3-Pentanedione[a,b]

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
n	10	10	10	10	10	10
Male						
Terminal Body Wt. (g)	33.7 ± 0.4**	33.9 ± 0.7	34.4 ± 0.5	34.1 ± 0.7	29.6 ± 0.4**	23.3 ± 0.5**
Heart						
Absolute (g)	0.14 ± 0.00**	0.14 ± 0.00	0.14 ± 0.00	0.15 ± 0.00	0.13 ± 0.00**	0.11 ± 0.00**
Relative (mg/g)[c]	4.22 ± 0.08**	4.26 ± 0.08	4.17 ± 0.09	4.29 ± 0.08	4.23 ± 0.06	4.82 ± 0.12**
Right Kidney						
Absolute (g)	0.30 ± 0.01**	0.29 ± 0.01	0.29 ± 0.01	0.29 ± 0.01	0.26 ± 0.01**	0.22 ± 0.00**

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Relative (mg/g)	8.77 ± 0.23	8.67 ± 0.24	8.52 ± 0.18	8.61 ± 0.16	8.66 ± 0.12	9.24 ± 0.20
Liver						
Absolute (g)	1.44 ± 0.02**	1.50 ± 0.04	1.42 ± 0.03	1.48 ± 0.05	1.24 ± 0.03**	1.01 ± 0.03**
Relative (mg/g)	42.70 ± 0.86	44.21 ± 0.99	41.46 ± 1.13	43.41 ± 1.05	42.04 ± 1.02	43.10 ± 0.85
Lung						
Absolute (g)	0.20 ± 0.00	0.21 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.21 ± 0.00	0.20 ± 0.01
Relative (mg/g)	6.04 ± 0.13**	6.16 ± 0.16	5.94 ± 0.13	5.85 ± 0.14	6.99 ± 0.20**	8.59 ± 0.18**
Spleen						
Absolute (g)	0.061 ± 0.002**	0.062 ± 0.002	0.062 ± 0.001	0.060 ± 0.003	0.057 ± 0.002	0.049 ± 0.002**
Relative (mg/g)	1.82 ± 0.07*	1.84 ± 0.10	1.80 ± 0.03	1.76 ± 0.07	1.93 ± 0.05	2.11 ± 0.08*
Female						
Terminal Body Wt. (g)	29.1 ± 0.6**	29.7 ± 0.7	28.9 ± 0.6	29.4 ± 0.7	25.1 ± 0.5**	20.3 ± 0.5**
Heart						
Absolute (g)	0.13 ± 0.00**	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.12 ± 0.00**	0.10 ± 0.00**
Relative (mg/g)	4.52 ± 0.11	4.53 ± 0.13	4.51 ± 0.09	4.53 ± 0.11	4.59 ± 0.10	4.70 ± 0.09
Right Kidney						
Absolute (g)	0.20 ± 0.00**	0.21 ± 0.01	0.20 ± 0.00	0.21 ± 0.00	0.18 ± 0.00**	0.15 ± 0.01**
Relative (mg/g)	6.95 ± 0.11**	7.03 ± 0.14	6.93 ± 0.17	7.05 ± 0.15	7.19 ± 0.12	7.43 ± 0.10*
Liver						
Absolute (g)	1.36 ± 0.05 **	1.36 ± 0.03	1.29 ± 0.02	1.39 ± 0.08	1.08 ± 0.03**	0.87 ± 0.03**
Relative (mg/g)	46.66 ± 1.22**	45.88 ± 0.73	44.71 ± 1.09	47.04 ± 2.41	43.02 ± 0.80	42.92 ± 0.84
Lung						
Absolute (g)	0.22 ± 0.01**	0.21 ± 0.01	0.21 ± 0.00	0.21 ± 0.00	0.19 ± 0.00**	0.19 ± 0.00**
Relative (mg/g)	7.49 ± 0.26**	7.12 ± 0.22	7.34 ± 0.11	7.27 ± 0.15	7.68 ± 0.25	9.28 ± 0.17**
Spleen						
Absolute (g)	0.096 ± 0.006**	0.095 ± 0.003	0.088 ± 0.002	0.089 ± 0.003	0.074 ± 0.003**	0.058 ± 0.002**
Relative (mg/g)	3.30 ± 0.18**	3.20 ± 0.11	3.05 ± 0.11	3.03 ± 0.09	2.96 ± 0.11	2.85 ± 0.08 **

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

There were no significant exposure-related changes in the hematology endpoints of mice exposed to 2,3-pentanedione (Appendix E).

Histopathology

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions in the respiratory tract (nose, larynx, trachea, lungs) and the eyes of mice (Appendix E).

Nose: The lesions in the nose were similar in males and females, and the incidences and average severities of these lesions tended to increase with increasing exposure concentration (Table 16). With the exception of respiratory epithelial squamous metaplasia and regeneration, which were observed in males and females in the 25 ppm groups, lesions were limited to animals exposed to ≥50 ppm 2,3-pentanedione. A complex array of lesions was observed that was similar to those in other organs of the respiratory tract. Lesions included inflammation, degenerative and regenerative lesions in the olfactory and/or respiratory epithelium, necrosis and perforation of the nasal septum, necrosis and atrophy of the nasal turbinates, and accumulation of hyaline droplets in the olfactory and respiratory epithelia. One or more of these lesions were present in most animals exposed to ≥50 ppm (Table 16).

Table 16. Incidences of Select Nonneoplastic Lesions of the Respiratory Tract in Male and Female Mice in the Three-month Inhalation Study of 2,3-Pentanedione

	0 ppm	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** (1.1)[c]	10** (2.4)
Olfactory epithelium, accumulation, hyaline droplet	0**	0	0	0	1 (1.0)	6** (1.7)
Olfactory epithelium, atrophy	0**	0	0	0	10** (2.2)	10** (1.9)
Olfactory epithelium, metaplasia, respiratory	0**	0	0	0	10** (1.3)	9** (1.3)
Respiratory epithelium, accumulation, hyaline droplet	0**	0	0	0	4* (1.0)	7** (1.1)
Respiratory epithelium, hyperplasia	0**	0	0	0	3 (1.7)	4* (2.0)
Respiratory epithelium, metaplasia, squamous	0**	0	0	4* (1.0)	8** (1.9)	9** (2.8)
Respiratory epithelium, necrosis	0**	0	0	0	1 (1.0)	4* (1.8)
Respiratory epithelium, regeneration	0**	0	0	6** (1.0)	9** (1.4)	10** (2.2)
Septum, necrosis	0**	0	0	0	0	7** (1.6)
Septum, perforation	0**	0	0	0	2 (3.0)	4* (2.8)
Turbinate, atrophy	0**	0	0	0	9** (1.3)	10** (1.8)
Turbinate, necrosis	0**	0	0	0	9** (1.4)	10** (2.3)
Larynx	10	10	10	10	10	10
Inflammation, acute	0	0	1 (1.0)	0	2 (1.5)	0
Inflammation, chronic active	1** (1.0)	0	0	1 (1.0)	2 (1.0)	10** (1.4)
Respiratory epithelium, hyperplasia, atypical	0	0	0	0	1 (3.0)	0

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Respiratory epithelium, metaplasia, squamous, atypical	0**	0	0	0	8** (2.8)	10** (2.6)
Respiratory epithelium, metaplasia, squamous	0	1 (1.0)	0	2 (1.0)	1 (3.0)	0
Respiratory epithelium, necrosis	0**	0	1 (1.0)	0	0	3 (1.0)
Respiratory epithelium, regeneration	0	1 (2.0)	2 (1.0)	2 (1.5)	9** (1.9)	0
Respiratory epithelium, ulcer	0	0	1 (1.0)	0	0	0
Squamous epithelium, hyperplasia	0	1 (1.0)	1 (1.0)	2 (1.0)	0	2 (1.0)
Squamous epithelium, hyperplasia, atypical	0**	0	0	0	9** (2.3)	8** (1.8)
Squamous epithelium, necrosis	0*	0	0	0	1 (1.0)	2 (1.0)
Squamous epithelium, regeneration	0	0	0	3 (1.0)	1 (3.0)	0
Squamous epithelium, ulcer	0	0	1 (1.0)	0	0	2 (1.0)
Trachea	10	10	9	10	10	10
Inflammation, suppurative	0**	0	1 (2.0)	0	0	5* (1.6)
Inflammation, chronic active	0**	0	0	0	0	5* (1.2)
Epithelium, hyperplasia, atypical	0	0	0	0	1 (1.0)	1 (3.0)
Epithelium, metaplasia, squamous, atypical	0**	0	0	0	1 (2.0)	10** (3.3)
Epithelium, necrosis	0	0	0	0	0	1 (2.0)
Epithelium, regeneration	0**	0	0	0	8** (2.9)	0
Lung	10	10	10	10	10	10
Bronchiole, infiltration, cellular, polymorphonuclear	0**	0	0	0	0	5* (1.0)
Bronchiole, epithelium, degeneration	0**	0	0	0	0	4* (1.5)
Bronchiole, epithelium, hyperplasia	0**	0	0	0	1 (1.0)	4* (1.0)
Bronchus, infiltration, cellular, polymorphonuclear	0**	0	0	0	0	9** (1.1)
Bronchus, inflammation, chronic	0**	0	0	1 (1.0)	6** (1.2)	9** (1.2)
Bronchus, ulcer	0	0	0	0	0	1 (1.0)
Bronchus, epithelium, degeneration	0**	0	0	2 (1.0)	9** (3.0)	8** (2.5)
Bronchus, epithelium, hyperplasia	0	0	0	0	2 (2.5)	0
Bronchus, epithelium, hyperplasia, atypical	0	0	0	0	7** (2.1)	0
Bronchus, epithelium, necrosis	0**	0	0	0	0	3 (2.0)
Bronchus, epithelium, metaplasia, squamous, atypical	0**	0	0	0	0	10** (2.6)
Bronchus, epithelium, regeneration	0**	1 (1.0)	0	1 (1.0)	8** (3.5)	7** (2.3)

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Female						
Nose	10	10	10	10	10	10
Inflammation, suppurative	0**	0	0	0	10** (1.8)	10** (2.5)
Olfactory epithelium, accumulation, hyaline droplet	0**	0	0	0	7** (1.1)	10** (1.5)
Olfactory epithelium, atrophy	0**	1 (1.0)	0	0	10** (2.0)	9** (1.8)
Olfactory epithelium, metaplasia, respiratory	0**	0	0	0	7** (1.1)	8** (1.3)
Respiratory epithelium, accumulation, hyaline droplet	1** (1.0)	0	0	0	5 (1.0)	10** (1.5)
Respiratory epithelium, hyperplasia	0**	0	0	0	8** (1.8)	4* (1.8)
Respiratory epithelium, metaplasia, squamous	0**	0	0	8** (1.0)	10** (2.0)	10** (2.1)
Respiratory epithelium, necrosis	0*	0	0	0	1 (1.0)	2 (1.0)
Respiratory epithelium, regeneration	0**	0	0	1 (1.0)	5* (1.2)	9** (2.4)
Septum, necrosis	0**	0	0	0	0	8** (2.1)
Septum, perforation	0**	0	0	0	1 (3.0)	3 (3.0)
Turbinate, atrophy	0**	0	0	0	10** (1.0)	10** (1.9)
Turbinate, necrosis	0**	0	0	0	9** (2.3)	10** (2.4)
Larynx	10	10	10	10	10	10
Inflammation, acute	0	0	2 (1.5)	0	0	0
Inflammation, chronic active	0**	0	1 (1.0)	1 (1.0)	2 (1.0)	10** (1.6)
Lumen, inflammation, suppurative	0	0	0	0	0	1 (3.0)
Respiratory epithelium, hyperplasia	0	1 (1.0)	0	0	1 (4.0)	0
Respiratory epithelium, hyperplasia, atypical	0	0	0	0	0	1 (2.0)
Respiratory epithelium, metaplasia, squamous, atypical	0**	0	0	0	9** (2.2)	10** (3.0)
Respiratory epithelium, metaplasia, squamous	0	1 (2.0)	0	0	0	0
Respiratory epithelium, necrosis	0	0	2 (1.0)	0	0	2 (1.0)
Respiratory epithelium, regeneration	0	0	0	1 (1.0)	7** (2.0)	0
Respiratory epithelium, ulcer	0	0	0	0	1 (1.0)	0
Squamous epithelium, hyperplasia	0	0	0	4* (1.5)	1 (1.0)	0
Squamous epithelium, hyperplasia, atypical	0**	0	0	0	9** (2.2)	10** (2.4)
Squamous epithelium, necrosis	0**	0	0	0	3 (1.0)	3 (1.0)
Squamous epithelium, regeneration	0	0	2 (1.0)	0	0	0
Squamous epithelium, ulcer	0	0	0	0	0	1 (1.0)

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Trachea	10	10	10	10	10	8
Inflammation, suppurative	0**	0	0	0	0	5** (1.6)
Inflammation, chronic active	0**	0	0	0	0	3 (2.0)
Epithelium, hyperplasia, atypical	0**	0	0	0	0	3 (2.0)
Epithelium, metaplasia, squamous, atypical	0**	0	0	0	1 (1.0)	8** (3.1)
Epithelium, necrosis	0	0	0	0	1 (1.0)	1 (1.0)
Epithelium, regeneration	1 (1.0)	0	0	0	10** (2.8)	0
Lung	10	10	10	10	10	10
Bronchiole, infiltration, cellular, polymorphonuclear	0**	0	0	0	0	3 (1.0)
Bronchiole, epithelium, hyperplasia	0	0	0	0	1 (1.0)	1 (1.0)
Bronchus, infiltration, cellular, polymorphonuclear	0**	0	0	0	0	9** (1.3)
Bronchus, inflammation, chronic	0**	0	0	0	4* (1.0)	9** (1.6)
Bronchus, epithelium, degeneration	0**	0	0	0	9** (1.8)	8** (2.8)
Bronchus, epithelium, hyperplasia	0	0	0	0	1 (2.0)	0
Bronchus, epithelium, hyperplasia, atypical	0	0	0	0	1 (3.0)	0
Bronchus, epithelium, metaplasia, squamous, atypical	0**	0	0	0	0	10** (2.1)
Bronchus, epithelium, necrosis	0**	0	0	0	0	2 (1.0)
Bronchus, epithelium, regeneration	0**	0	0	0	9** (2.4)	9** (2.1)
Bronchus, epithelium, ulcer	0**	0	0	0	0	2 (1.0)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Cochran-Armitage (trend) or Fisher's exact (pairwise) test; **$p \leq 0.01$.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Suppurative inflammation was present in all levels of the nose and was characterized by the presence of variable numbers of neutrophils in the nasal mucosa and the lumen of the nasal cavity. In the lumen, the neutrophils were admixed with cellular debris and eosinophilic proteinaceous material. Rarely, foreign bodies, bone fragments, and bacterial cocci were also present. In the olfactory epithelium, there was significantly increased atrophy in all mice exposed to ≥50 ppm relative to control animals (except one female at 100 ppm). This change was most prominent in the dorsal meatus of levels II and III and was characterized by decreased thickness of the olfactory epithelium due to loss of epithelial cells. Respiratory metaplasia of the olfactory epithelium, defined as the replacement of the normal olfactory epithelium by ciliated, columnar epithelium (respiratory epithelium), was seen in all male and seven female mice exposed to 50 ppm and nine male and eight female mice exposed to 100 ppm 2,3-pentanedione. Hyaline droplet accumulation was also noted in the olfactory epithelium and was characterized by the presence of eosinophilic, globular material within the cytoplasm of affected epithelial cells.

The finding of respiratory epithelial regeneration described either flattening and elongation of epithelial cells with loss of cilia or enlarged cuboidal epithelial cells and karyomegaly piled up to three cell layers thick. Hyperplasia was characterized by epithelial cells that were taller than normal or were piling up more than three cell layers thick with resultant increased thickness of the epithelium. Necrosis of the respiratory epithelium consisted of loss of epithelium and replacement by cellular and karyorrhectic debris. This finding primarily affected the lateral aspects of the nasal cavity in levels I and II. Squamous metaplasia of the respiratory epithelium was characterized by replacement of the normal respiratory epithelium by squamous epithelium. Hyaline droplet accumulation in the respiratory epithelium was characterized by the presence of small, red, hyaline droplets within the cytoplasm of respiratory epithelial cells.

Septal necrosis was present in seven male and eight female mice from the 100 ppm groups. Septal perforation was present in two males and one female exposed to 50 ppm and in four males and three females exposed to 100 ppm 2,3-pentanedione. Septal necrosis was characterized by the absence of osteocytes from lacunae; absence of the osteoblast lining cell layer; and infrequent thickening of the affected bone, scalloping of the edge of the affected bone, and clusters of neutrophils in proximity to the affected bone. Septal perforation was seen in level I of the nose and was characterized by loss of the central portion of the septum. Turbinate necrosis and atrophy affected the maxillo- and nasoturbinates in levels I and II. Turbinate necrosis was characterized by loss of osteocytes from lacunae and the osteoblast layer and fragmentation of the affected bone with occasional extrusion of the necrotic bone into the nasal cavity lumen. Turbinate atrophy was characterized by shortened, blunted naso- and maxilloturbinates (Figure 9).

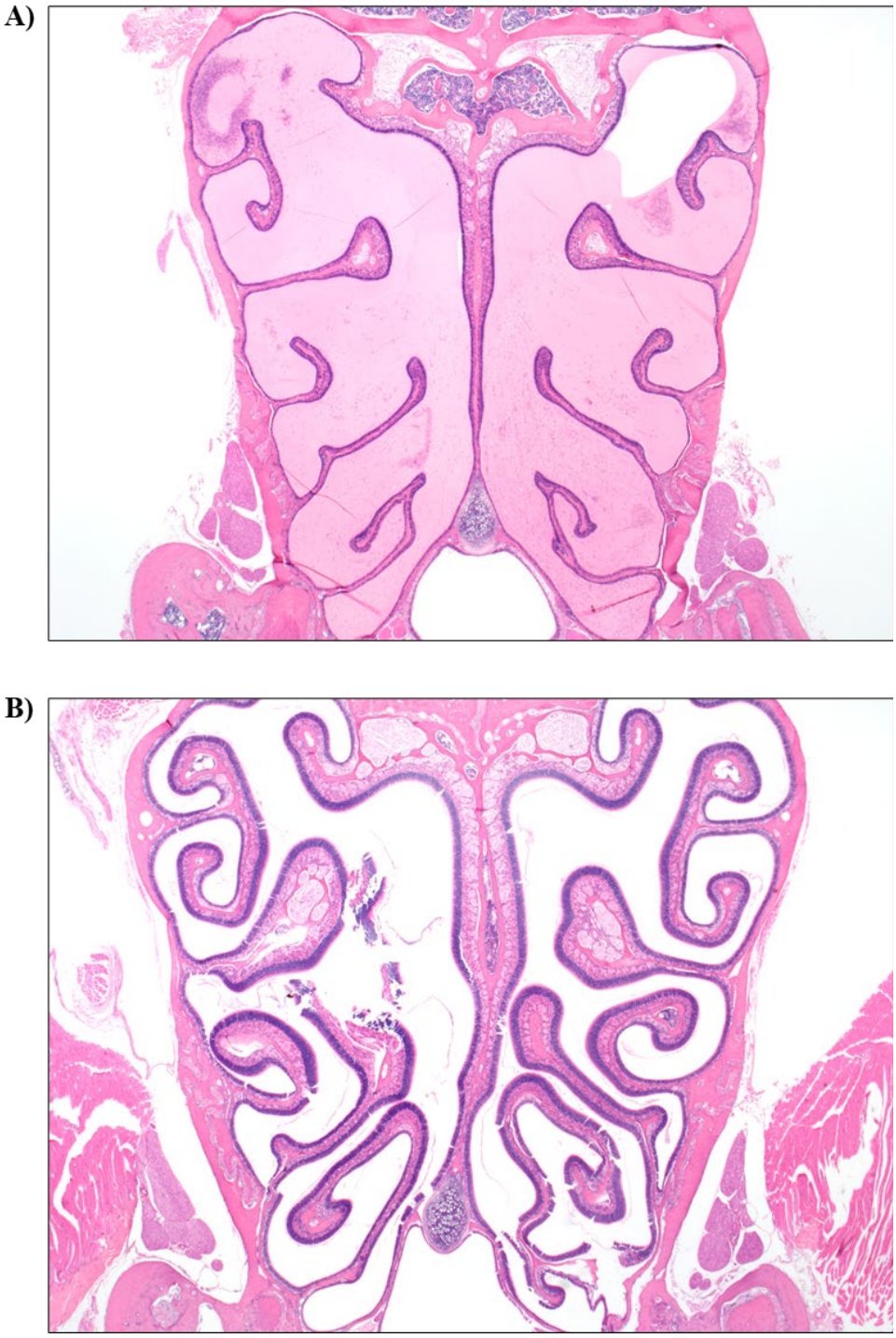


Figure 9. Representative Images of Turbinate Atrophy and Control Turbinate in the Nose of Male Mice in the Three-month Inhalation Study of 2,3-Pentanedione (H&E)

(A) Turbinate atrophy in the nose of a male mouse exposed to 100 ppm 2,3-pentanedione (compare to control in Panel B) (2×). (B) Turbinates in the nose of a male mouse exposed to 0 ppm 2,3-pentanedione (control mouse) (2×). H&E = hematoxylin and eosin stain.

Larynx: The lesions in the larynx were similar in male and female mice and included inflammation and epithelial necrosis, ulceration of the epithelium, regeneration and hyperplasia of the epithelium, atypical squamous metaplasia of the respiratory epithelium, and atypical hyperplasia of the squamous epithelium (Table 16). These lesions were seen at all levels of the larynx. They were most commonly seen in groups exposed to ≥50 ppm, in which the differences in incidence from the control group often reached statistical significance. A few of these lesions were seen at lower incidences in the groups exposed to lower concentrations (Table 16). In females, the incidence of squamous epithelial hyperplasia was significantly increased only in the 25 ppm group relative to control females. In general, these lesions were most common in the ventral and cranial portions of the larynx, and with increasing severity, the more caudal portions and lateral walls were increasingly affected.

Atypical hyperplasia of the squamous epithelium and atypical squamous metaplasia of the respiratory epithelium were observed in male and female mice exposed to ≥50 ppm 2,3-pentanedione. Squamous metaplasia of the respiratory epithelium is defined as replacement of the normally ciliated, columnar epithelium by squamous epithelium. These atypical metaplastic or hyperplastic lesions had histologic features that included cytomegaly, karyomegaly, hyperchromasia, multiple prominent nucleoli, and disorganized epithelial cell layers (Figure 10). Many of the mice with atypical hyperplasia or atypical squamous metaplasia of the larynx also had nonatypical squamous epithelial hyperplasia or squamous metaplasia in other portions of the larynx.

Chronic active inflammation was the most commonly observed type of inflammation in the larynx and consisted of a mixed population of macrophages, lymphocytes, and neutrophils in the subepithelial tissue. Occasional neutrophils were seen within the submucosal glands. Acute inflammation was composed of neutrophils in the laryngeal lamina propria. Suppurative inflammation, present at all laryngeal levels in one 100 ppm female mouse, consisted of intraluminal neutrophils that entrapped layers of sloughed keratin. Regeneration, hyperplasia, and squamous metaplasia of the respiratory epithelium in the larynx were similar to the changes in the nose. Infrequently, individual necrotic cells were noted in the respiratory epithelium in these areas of high cell turnover. Regeneration of the respiratory epithelium was characterized by cuboidal, hyperchromatic epithelial cells up to three cell layers thick. Hyperplasia of the squamous epithelium consisted of an increased thickness and piling up of the squamous epithelium overlying the arytenoid cartilage of level I with occasional keratinization. Necrosis of the respiratory or squamous epithelium was characterized by nuclear pyknosis and hypereosinophilic cytoplasm with varying degrees of cell sloughing and loss, hemorrhage, and neutrophilic inflammation. Respiratory or squamous epithelial ulceration was characterized by loss of the epithelial cells with exposure of the subepithelial tissue; this change was generally noted overlying the distal tips of the arytenoid cartilage.

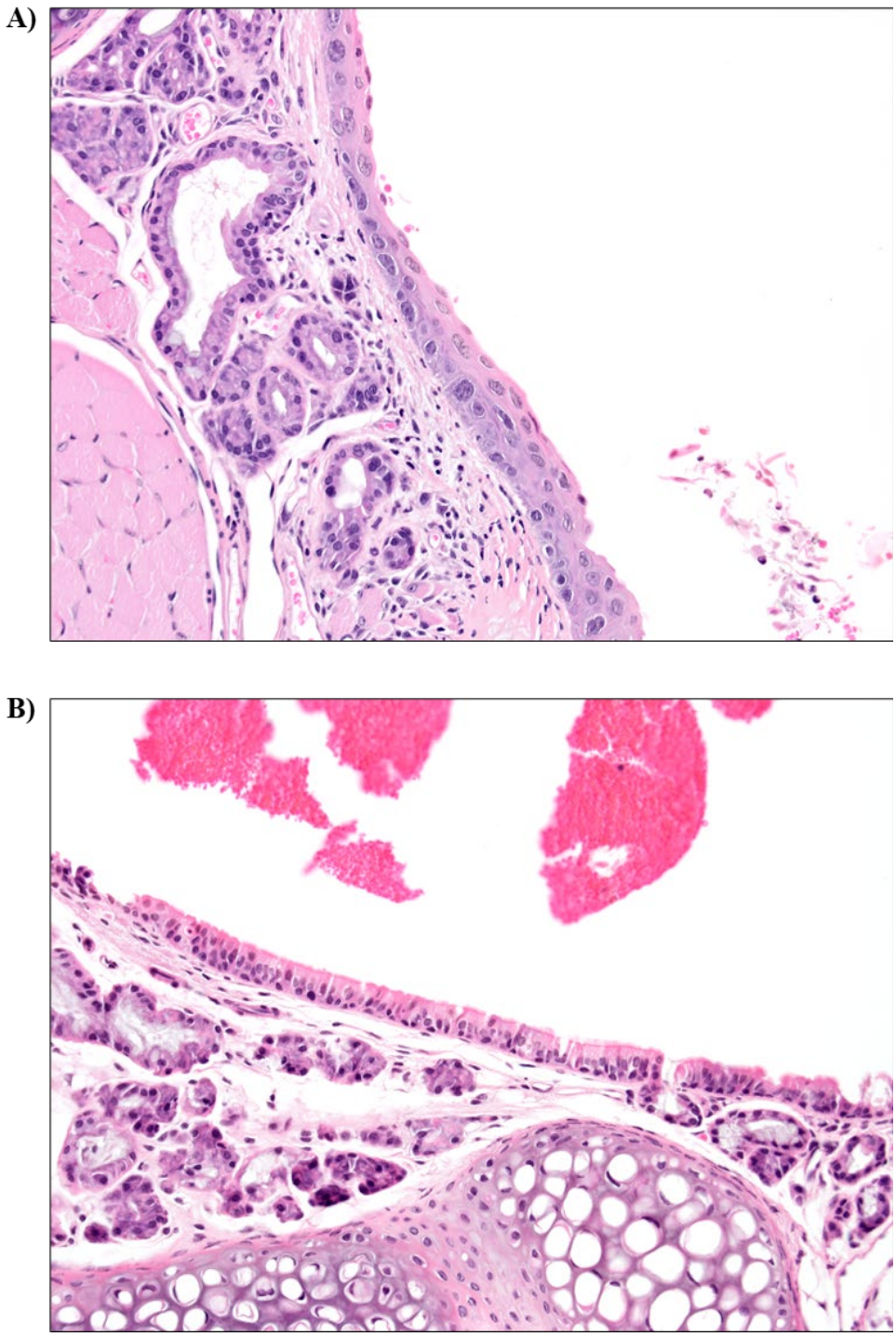


Figure 10. Representative Images of Atypical Squamous Metaplasia and Control Respiratory Epithelium in the Larynx of Male Mice in the Three-month Inhalation Study of 2,3-Pentanedione (H&E)

(A) Atypical squamous metaplasia of the respiratory epithelium in the larynx of a male mouse exposed to 100 ppm 2,3-pentanedione. The cells, particularly at the base of the epithelium, have enlarged nuclei (compare to control in Panel B) (20×). (B) Respiratory epithelium in the larynx of a male mouse exposed to 0 ppm 2,3-pentanedione (control mouse) (20×). H&E = hematoxylin and eosin stain.

Trachea: As with the other organs of the upper respiratory tract, lesions in the trachea included inflammation and degenerative and regenerative lesions in the epithelium. These lesions were seen in the 50 and 100 ppm groups and were similar in males and females (Table 16). Similar to the larynx, there was evidence of atypia in the regenerating epithelial cells. In both sexes, atypical squamous metaplasia and regeneration of the epithelium were significantly increased in the 100 ppm and 50 ppm groups, respectively.

Microscopically, chronic active inflammation consisted of a mixed population of inflammatory cells in the tracheal submucosa, similar to that in the larynx. Suppurative inflammation was characterized by the presence of neutrophil aggregates in the tracheal lumen admixed with sloughed epithelial cells. Atypical epithelial hyperplasia was seen in three female mice exposed to 100 ppm and in two male mice (one exposed to 50 ppm and the other exposed to 100 ppm) and was characterized by an increase in epithelial cell layers to more than three. Atypical squamous metaplasia consisted of replacement of the respiratory epithelium with stratified squamous, occasionally keratinized epithelium. Atypical epithelial hyperplastic and squamous metaplastic changes were characterized by nuclear atypia, karyomegaly, cytomegaly, prominent and often multiple nucleoli, and general disorganization of the epithelial cell layers. Epithelial necrosis was seen infrequently but was considered the result of exposure to 2,3-pentanedione. It was characterized by individual, or rafts of epithelial cells detached from the basement membrane and sloughed into the tracheal lumen; these epithelial cells sometimes exhibited hypereosinophilic cytoplasm and shrunken, pyknotic nuclei. Epithelial regeneration was seen only in the 50 ppm groups of mice and was characterized either by elongation and flattening of epithelial cells in areas of necrosis or by increased thickness of the epithelium up to three cell layers thick.

Lung: The lesions in the lungs were limited to the airways and were similar to what was seen in the other organs of the respiratory tract: inflammation, hyperplasia/squamous metaplasia, degeneration/necrosis, and regeneration of the airway epithelium. As with the other organs, the majority of the lesions were similar in male and female mice and were most commonly seen at $\geq$50 ppm. Bronchial inflammation and bronchial epithelial degeneration and regeneration were seen in a few males in the 25 ppm group (Table 16).

The airway epithelium exhibited multiple changes. Chronic bronchial inflammation was observed in males and females in the 50 and 100 ppm groups of mice and in one male in the 25 ppm group. Chronic inflammation was characterized by increased numbers and aggregates of lymphocytes and plasma cells in the bronchial adventitia. Increased numbers of polymorphonuclear cells were also seen in the bronchi and bronchioles of some males and females in the 100 ppm groups. Atypical squamous metaplasia of the bronchial epithelium, characterized by replacement of the respiratory bronchial epithelium by squamous epithelium, was seen in males and females exposed to 100 ppm 2,3-pentanedione. Atypical bronchial epithelial hyperplasia, seen as an increase in the number of epithelial cell layers with atypical cellular features, was present in seven males and one female in the 50 ppm group. The atypical features of both these lesions were the same as those seen in the trachea and larynx. Bronchial and bronchiolar epithelial cell degeneration was characterized by enlarged cells with colorless cytoplasm that occasionally projected into the airway lumen (ballooning degeneration). Bronchial epithelial degeneration was significantly increased in the 50 and 100 ppm groups in both sexes of mice. Bronchiolar epithelial degeneration was significantly increased in the

100 ppm group in males but was not observed in female mice. Epithelial regeneration was seen only in the bronchi in both sexes in the 50 and 100 ppm groups. In the males, one animal each from the 6.25 and 25 ppm groups was also diagnosed with regeneration. It was characterized both by enlarged cells that occasionally lacked cilia with enlarged nuclei forming a single layer of cells and by increased numbers of epithelial cells piling up and extending into the airway lumen. Bronchial epithelial hyperplasia (Figure 11), seen in two males and one female in the 50 ppm groups, and bronchiolar epithelial hyperplasia, seen in one male and one female in the 50 ppm groups and in four males and one female in the 100 ppm groups, were characterized by the increased thickness of the epithelium due to the presence of more than three layers of epithelial cells. There also were scattered necrotic epithelial cells in these areas of high cell turnover in the bronchi. Ulceration of the epithelium in the bronchus was present, as well, and was characterized by the absence of the epithelium, exposing the underlying tissue, and by infiltration by low numbers of neutrophils on the surface.

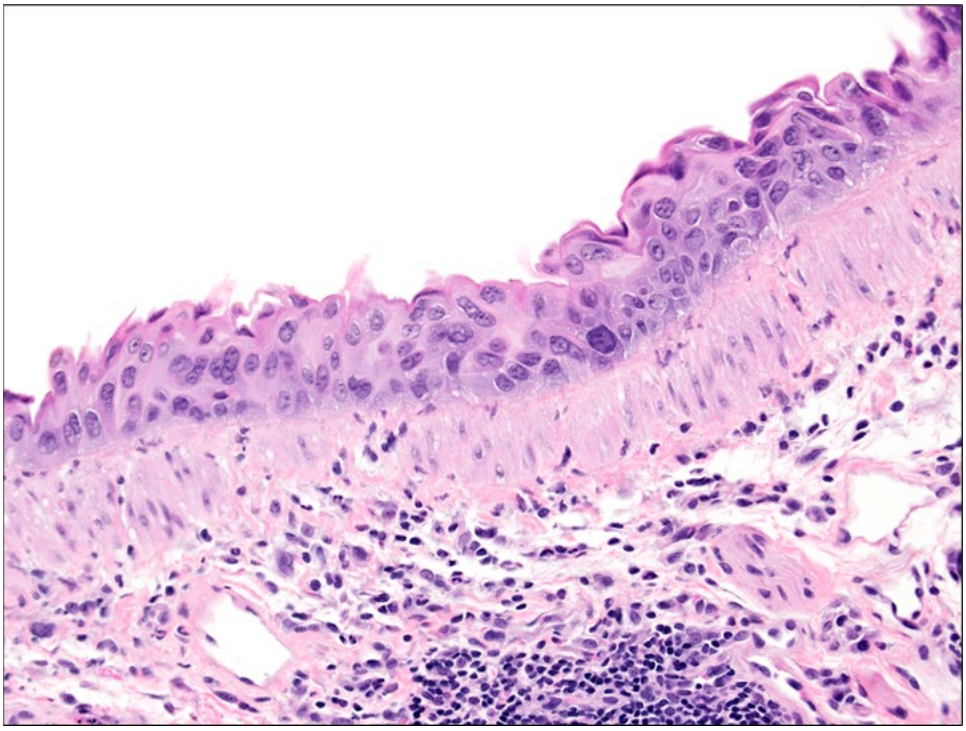


Figure 11. Representative Image of Hyperplasia in the Lung of a Male Mouse in the Three-month Inhalation Study of 2,3-Pentanedione (H&E)

Hyperplasia of the respiratory epithelium in the bronchus of the lung from a male mouse exposed to 50 ppm 2,3-pentanedione. (20×).

Eyes: There was mineralization in the cornea in six female mice exposed to 100 ppm 2,3-pentanedione (Table 17). Three of these six animals also had hyperplasia of the corneal epithelium, two had acute corneal inflammation, and two had corneal epithelial ulceration. In the 50 ppm females, three animals had minimal focal cellular alteration of the cornea. Only the increase in corneal mineralization incidence was statistically significant (with a positive trend for the other lesions except the focal cellular alteration). In male mice exposed to 100 ppm, one animal was diagnosed with corneal epithelial hyperplasia, and one had acute corneal inflammation. In the 25 ppm group, one male had acute scleral inflammation.

Microscopically, corneal hyperplasia consisted of multiple layers of epithelial cells with resultant increased corneal thickness. Corneal ulceration was characterized by loss of the corneal epithelium. Deposition of basophilic granular material, interpreted as mineralization, was observed in the corneal stroma beneath areas of corneal hyperplasia or ulceration. Acute inflammation consisted of varying numbers of neutrophils within the corneal stroma or at the base of the ciliary body. Cellular alteration was characterized by disorganized, slightly atypical, and slightly enlarged corneal epithelial cells that generally were associated with slight thinning of the corneal epithelium.

Table 17. Incidences of Select Nonneoplastic Lesions of the Eyes in Male and Female Mice in the Three-month Inhalation Study of 2,3-Pentanedione

	0 ppm	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
n[a]	10	10	10	10	10	10
Male						
Eye						
Cornea, inflammation, acute[b]	0	0	0	0	0	1 (1.0)[c]
Cornea, epithelium, hyperplasia	0	0	0	0	0	1 (1.0)
Sclera, inflammation, acute	0	0	0	1 (1.0)	0	0
Female						
Eye						
Cornea, inflammation, acute	0**	0	0	0	0	2 (1.5)
Cornea, mineralization	0**	0	0	0	0	6** (2.3)
Cornea, ulcer	0**	0	0	0	0	2 (1.5)
Cornea, epithelium, cellular alteration, focal	0	0	0	0	3 (1.0)	0
Cornea, epithelium, hyperplasia	0**	0	0	0	0	3 (1.0)

Statistical significance for an exposed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Cochran-Armitage (trend) or Fisher's exact (pairwise) test; **$p \leq 0.01$.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Genetic Toxicology

Data from all NTP genetic toxicity tests with acetoin and 2,3-pentanedione are available in the NTP Chemical Effects in Biological Systems database: https://doi.org/10.22427/NTP-DATA-TOX-98.[90]

Acetoin (tested up to 10,000 µg/plate) was not mutagenic when tested in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535, in the presence or absence of 10% or 30% induced rat or hamster liver S9 (Table D-1). Both acetoin (up to 800 ppm) and 2,3-pentanedione (up to 100 ppm) were evaluated for induction of micronucleated erythrocytes following 3 months of exposure via inhalation in male and female Wistar Han [Crl:WI(Han)] rats (Table D-2, Table D-3) and B6C3F1/N mice (Table D-4, Table D-5). Results of the micronucleus tests were

negative in both rats and mice for both chemicals, and no alterations in the percentage of immature erythrocytes (% PCE) were observed in mice exposed to either acetoin or 2,3-pentanedione. In the acetoin study, a small but significant exposure concentration-related increase in % PCE was observed in male, but not female, rats, suggesting a mild perturbation in erythropoiesis in male rats. In the study with 2,3-pentanedione, small increases in micronucleated normochromatic erythrocytes (NCEs) were observed at the highest concentration tested (100 ppm) in male and female rats. These increases were not considered indicative of a biological response as they were in the NCE population—which is not the appropriate cell population to evaluate for frequency of micronuclei in rats due to the efficient removal by the spleen of damaged erythrocytes soon after they enter the blood stream—and because the values were well within the laboratory historical control ranges. A small exposure concentration-related increase in % PCE was observed in female rats exposed to 2,3-pentanedione.

Discussion

Occupational inhalation exposure to volatile components of artificial butter flavoring (ABF) (including 2,3-butanedione [diacetyl]) has been reported to be associated with airway fibrosis in the form of obliterative bronchiolitis (OB).[1-6] ABF was subsequently nominated to the National Toxicology Program (NTP) by the United Food and Commercial Workers International Union for inhalation studies to assess the potentially toxic and highly volatile constituents of ABF, including 2,3-butanedione and acetoin. Due to concerns about the respiratory toxicity of inhaled 2,3-butanedione as indicated by workplace exposure assessments and in vivo studies showing the induction of OB-like fibrotic airway lesions (similar to those observed in occupational exposures)[14] in exposed rats, 2,3-pentanedione has been used as a replacement for 2,3-butanedione in some ABF.[16] However, 2,3-pentanedione is a vicinal diketone, like 2,3-butanedione, and has been shown to exhibit reactivity and toxicological potency that is similar to that of 2,3-butanedione.

The toxicity and fibrogenicity of inhaled 2,3-pentanedione has been reported for 2-week and shorter whole-body exposure studies in rats with a focus on adverse effects in the respiratory tract. The primary objective of this NTP study was to evaluate the 3-month inhalation toxicity of 2,3-pentanedione vapors within the respiratory tract, including airway lesion formation, and other target organs (including the eyes) in whole-body exposed rats and mice. The 2-week and 3-month inhalation toxicity of acetoin—which, like 2,3-butanedione, was originally found to be in ABF[9]—was also evaluated in rats and mice.

In the current studies, with the exception of a mild, but significant, effect on erythropoiesis observed in male rats exposed for 3 months, there were no significant exposure-related adverse effects in rats or mice exposed to acetoin vapor by whole-body inhalation for 2 weeks or 3 months at concentrations of up to 800 ppm in air for approximately 6 hours per day, 5 days per week.

In male and female rats exposed by whole body inhalation to 50 or 100 ppm 2,3-pentanedione vapor for up to 3 months (approximately 6 hours per day, 5 days per week), clinical observations included abnormal breathing, eye abnormalities (including unilateral or bilateral corneal opacities in some animals), and sneezing. Absolute and relative lung weights and relative heart weight were significantly increased in female rats exposed to 100 ppm for 3 months compared to those of the control group. A small exposure concentration-related increase in the percentage of immature erythrocytes (% PCE) was also observed in female rats exposed to 2,3-pentanedione.

Exposure to 2,3-pentanedione for 3 months caused significant adverse effects primarily in the respiratory tract (nose, larynx, trachea, and lung), but also in the eyes of rats. In the nose, suppurative inflammation, atrophy, and respiratory metaplasia of the olfactory epithelium and hyperplasia and squamous metaplasia of the respiratory epithelium were significantly increased in male and female rats exposed to 50 or 100 ppm. Lymphoid hyperplasia was significantly increased in male rats exposed to 50 or 100 ppm. Olfactory epithelial degeneration in the nose was significantly increased in male and female rats exposed to 50 ppm and female rats exposed to 100 ppm. Necrosis and regeneration of the respiratory epithelium and turbinate atrophy were also significantly increased in males and females exposed to 100 ppm. In the larynx, squamous metaplasia of the respiratory epithelium was significantly increased in males exposed to 50 or

100 ppm and females exposed to 25, 50, or 100 ppm. Hyperplasia of the squamous epithelium was significantly increased in male and female rats exposed to 100 ppm. Chronic active inflammation and necrosis and ulceration of the squamous epithelium were significantly increased in female rats exposed to 100 ppm; hyperplasia of the respiratory epithelium was significantly increased in male rats exposed to 50 ppm. In the trachea, regeneration of the epithelium was significantly increased in males and females exposed to 50 or 100 ppm relative to the control groups. Hyperplasia of the epithelium (and squamous metaplasia by positive trend only) was also increased in males and females exposed to 100 ppm. In the lung, (eosinophilic) inflammation, hyperplasia and regeneration of the bronchial epithelium, and hyperplasia of the bronchiolar epithelium were significantly increased in male and female rats exposed to 100 ppm (and squamous or goblet cell metaplasia of the bronchial epithelium by positive trend only). Focal fibrosis in the lung was also increased in female rats (by positive trend only).

In the eye, 2,3-pentanedione caused a significant increase in acute inflammation of the cornea (50 and 100 ppm) and ciliary body (50 ppm only) in female rats. The incidences of acute inflammation, neovascularization, and epithelial vacuolation of the cornea occurred with a positive trend in male rats. The increases in leukocyte counts (inflammatory leukogram) at day 23 and study termination in both male and female rats correlated with the observed histological changes in the respiratory tract.

In mice exposed to 50 or 100 ppm 2,3-pentanedione for 3 months, clinical observations included abnormal breathing and sneezing in males and females, as well as eye abnormalities in females. Terminal mean body weights were significantly decreased in male and female mice exposed for 3 months to 50 and 100 ppm. On day 8 after the start of exposure to 100 ppm, male and female mice exhibited dramatic body weight loss, but the exposure for this group was not stopped because the weight loss was <20% of the pre-exposure starting body weight and the body weights gradually increased thereafter for the duration of the study. Relative lung weights were significantly increased in male and female mice exposed to 100 ppm and in male mice exposed to 50 ppm. Absolute heart, kidney, and liver weights of male and female mice and absolute spleen weights of female mice exposed to ≥50 ppm 2,3-pentanedione, as well as absolute spleen weights of male mice and relative spleen weights of female mice exposed to 100 ppm, were significantly decreased. Relative heart and spleen weights of male mice and relative kidney weights of female mice exposed to 100 ppm 2,3-pentanedione were significantly increased.

Inhalation (whole-body) exposure to 2,3-pentanedione for 3 months caused significant adverse effects primarily in the respiratory tract (nose, larynx, trachea, and lung), but also in the eyes of mice. In the nose, suppurative inflammation and atrophy and respiratory metaplasia of the olfactory epithelium, regeneration and squamous metaplasia of the respiratory epithelium, and turbinate atrophy and necrosis were significantly increased in males and females exposed to 50 or 100 ppm. Olfactory and respiratory epithelial hyaline droplet formation, hyperplasia of the respiratory epithelium, and septum necrosis were significantly increased in male and female mice exposed to 100 ppm. Necrosis of the respiratory epithelium and perforation of the septum were also significantly increased in male mice exposed to 100 ppm. In the larynx, hyperplasia of the squamous epithelium and squamous metaplasia of the respiratory epithelium, which were considered atypical, were significantly increased in males and females exposed to 50 and 100 ppm. Chronic active inflammation was also significantly increased in males and females exposed to 100 ppm, as was regeneration of the respiratory epithelium in males and females exposed to 50 ppm. Squamous epithelial hyperplasia was also increased in females exposed to

25 ppm. In the trachea, inflammation and atypical squamous metaplasia of the epithelium were significantly increased in males and females exposed to 100 ppm (and atypical hyperplasia in females by positive trend only). Regeneration of the epithelium was also significantly increased in males and females exposed to 50 ppm. In the lung, hyperplasia and degeneration of the bronchiolar epithelium and polymorphonuclear cellular infiltration of the bronchiole were significantly increased in male mice exposed to 100 ppm. Chronic inflammation of the bronchi and degeneration and regeneration of the bronchial epithelium were significantly increased in male and female mice exposed to 50 and 100 ppm, and polymorphonuclear cellular infiltration of the bronchus was significantly increased in male and female mice exposed to 100 ppm. Atypical squamous metaplasia of the bronchial epithelium was also significantly increased in males and females exposed to 100 ppm as was atypical hyperplasia of the bronchial epithelium in males exposed to 50 ppm.

In the eye, acute inflammation, ulceration, and epithelial hyperplasia of the cornea were significantly increased in exposed female mice (by positive trend only), whereas cornea mineralization was significantly increased in female mice exposed to 100 ppm.

OB has been shown to be a significant clinical finding in occupational exposures to the volatile components of ABF including 2,3-butanedione.[39] In this NTP study, 3-month inhalation (whole-body) exposure to 2,3-pentanedione caused significant adverse airway effects in mice and rats, including hyperplasia of the bronchial and (more distal) bronchiolar epithelium and squamous or goblet cell metaplasia of the bronchial epithelium, but no bronchial or bronchiolar fibrosis was observed. Two-week inhalation (whole-body) exposure to 2,3-pentanedione has been shown previously to cause bronchial fibrosis (OB-like lesions), similar to 2,3-butanedione, in rats exposed to 150 or 200 ppm (with higher morbidity/mortality and decreased pulmonary function at 200 ppm) but not at exposure levels <150 ppm.[13; 17; 19] Therefore, despite 3 months of exposure, 100 ppm 2,3-pentanedione was probably not high enough to induce airway fibrosis in the rats. Regarding the mechanism(s) of OB-like fibrotic lesion formation, the data suggest that the initial injury to the airway epithelium is critical to damage the basement membrane and induce fibrosis[14] (which might not occur at exposure concentrations <150 ppm, even after a prolonged (3-month) exposure period). Of note, this 3-month study was initiated before there was confidence that 2-week inhalation (whole-body) exposure to 2,3-pentanedione at concentrations >150 ppm induced airway fibrosis (OB-like effects) in rats. Therefore, the exposure concentrations for the 3-month study were matched to those tested for 2,3-butanedione.[15]

The absence of airway fibrosis in the mice exposed to 100 ppm 2,3-pentanedione for 3 months is not surprising considering that mice have been shown to be much less susceptible than rats to 2,3-pentanedione-induced airway fibrosis in prior 2-week inhalation (whole-body) exposure studies (unpublished data). Thus, the induction of fibrosis by 2,3-pentanedione might be a species-specific effect. In the 2-year bioassay with 2,3-butanedione, which has also been previously shown to induce OB-like airway fibrosis in rats, some tracheal and bronchial fibroses were observed in rats and mice exposed to the highest tested concentration of 50 ppm,[15] suggesting that a much longer (chronic) exposure duration (of up to 2 years) to lower exposure concentrations may be sufficient to induce airway fibrosis. Interestingly, inhalation exposure of mice to 2,3-pentanedione vapors caused hyperplasia of the squamous epithelium and squamous metaplasia of the respiratory epithelium in the larynx and hyperplasia and squamous metaplasia

of the tracheal and bronchial epithelium, which were considered atypical and therefore potentially preneoplastic.

In summary, due to concerns regarding the association between occupational exposure to highly volatile components (acetoin, 2,3-butanedione, and 2,3-pentanedione) of ABF and adverse fibrotic lung effects (specifically, OB in the distal airways), 3-month inhalation toxicity testing of acetoin and 2,3-pentanedione vapors was conducted in Wistar Han [Crl:WI(Han)] rats and B6C3F1/N mice using whole-body exposure to evaluate the respiratory toxicity, as well as the toxicity to other target organs (including the eyes), of these compounds. A 2-week inhalation toxicity study was also performed for acetoin.

Under the conditions of this inhalation study, there were no significant exposure-related adverse effects in rats or mice exposed to acetoin for 2 weeks or 3 months. Exposure to 2,3-pentanedione via whole-body inhalation for 3 months caused significant adverse effects primarily in the respiratory tract, but also in the eyes, of rats and mice. These airway findings attributed to 2,3-pentanedione included exposure-related inflammation, injury (degeneration), regeneration, squamous metaplasia, and/or hyperplasia of the tracheal, bronchial, and bronchiolar epithelium. Interestingly, the hyperplasia and squamous metaplasia of the tracheal and bronchial epithelium observed in the mice were considered atypical and therefore potentially preneoplastic. The no-observed-effect level (NOEL) for the bronchial and bronchiolar adverse effects in the lung was 25 ppm in rats and 12.5 ppm in mice after 3 months of exposure to 2,3-pentanedione. These lesions are most relevant because the distal bronchi/bronchioles are the target sites for OB, and hyperplasia/squamous metaplasia could accompany or be precursors to fibrotic lesions (e.g., if the tested exposure concentration was higher) and the morphology of the bronchial/bronchiolar lesions is similar to OB. Regarding eye toxicity, inflammation and degenerative lesions of the cornea were observed, which extended into other ocular compartments in some animals, including the anterior chamber, ciliary body, sclera, conjunctiva, and iris. The NOEL of 2,3-pentanedione for this study overall was 12.5 ppm on the basis of adverse respiratory tract effects in rats and mice. These 3-month inhalation exposure data, including NOELs for adverse respiratory tract effects, can inform regulatory agencies to help mitigate exposure risks to 2,3-pentanedione vapors in the workplace.

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Appendix A. Chemical Characterization and Generation of Chamber Concentrations

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A.1. Procurement and Characterization

Acetoin was obtained from Sigma-Aldrich, Inc. (Milwaukee, WI) in one lot (09118JH) and was used in the 2-week and 3-month inhalation studies of acetoin. 2,3-Pentanedione, obtained from Sigma-Aldrich, Inc. (Milwaukee, WI) in one lot (MKBB7504), was used in the 3-month inhalation studies of 2,3-pentanedione. Identity and purity analyses of the two test chemicals were conducted by the analytical chemistry and animal study laboratories at Battelle Toxicology Northwest (Richland, WA). Reports on analyses performed in support of the acetoin and 2,3-pentanedione studies are on file at the National Institute of Environmental Health Sciences.

A.1.1. Acetoin

Lot 09118JH, a solid white powder, was identified as acetoin by Chemir Analytical Services (Maryland Heights, MO) using Fourier transform infrared (IR) and ^{1}H nuclear magnetic resonance (NMR) spectroscopies (Figure A-1, Figure A-2). All spectra were consistent with the reference spectra from a commercially procured standard (Sigma-Aldrich, Inc.; lot 01296MH) and matched the anticipated dimer structure of acetoin. Elemental analysis was performed by Galbraith Laboratories, Inc. (Knoxville, TN) to aid in identification. The relative amount of carbon (54.33%) and hydrogen (9.09%) were within 1% of the theoretical values (54.53% and 9.15%, respectively). Additionally, sulfur was detected (0.09%) and was within 1% of the theoretical value (0.00%).

Acetoin was received as a solid dimer and was thermally cracked to form the liquid monomer used as the exposure material. Monomer test materials were stored heated (approximately 90°F) to prevent reformation of solid dimer. Expiration dates of 14 days (2-week studies) or 26 days (3-month studies) after cracking were assigned to batches of prepared liquid monomer. The purity of lot 09118JH in the solid and liquid form was determined by the study laboratory using gas chromatography (GC) with flame ionization detection (FID), and impurities were identified using GC with mass spectrometry (MS) detection. The moisture content of the solid dimer was determined by Chemir Analytical Services using Karl Fischer titration.

For lot 09118JH, the purity of the solid dimer was determined to be 99.4% ± 0.2(sd)%, and the purity of the liquid monomer was determined to be 99.2% ± 0.2% using GC/FID (Table A-1, System A). Three reportable impurities with peak areas ≥0.1% of the total integrated peak area were detected in the solid form, and two of the three impurities were detected in the liquid form of acetoin. Using GC/MS, the impurities were identified as 2,3-butanedione (approximately 0.1%), acetic acid (approximately 0.1%, solid only), and di-2-butan-3-one ether (0.3% to 0.4%) (Table A-1, System B). Karl Fischer titration indicated <0.1% water in the solid test article. The overall purity of lot 09118JH was determined to be >99%.

To ensure stability, bulk acetoin was stored in sealed white plastic buckets at 5°C. Reanalyses of the bulk chemical were performed by the study laboratory prior to the 2-week studies; no degradation was detected by GC/FID (Table A-1, System A). Prior to the 3-month studies, the solid dimer appeared moist compared to its appearance prior to the 2-week studies; the chemical was dried with nitrogen before cracking and reanalyzing. Reanalyses of the bulk chemical were performed by the study laboratory prior to and after the 3-month studies; no degradation was detected by GC/FID (Table A-1, System A).

A.1.2. 2,3-Pentanedione

Lot MKBB7504, a clear yellow liquid, was identified as 2,3-pentanedione by the study laboratory using IR spectroscopy and by Chemir Analytical Services using ^{1}H NMR spectroscopy (Figure A-3, Figure A-4). All spectra were consistent with the anticipated structure of 2,3-pentanedione. Elemental analysis was performed by Galbraith Laboratories, Inc. (Knoxville, TN) to aid in identification. The relative amount of carbon (58.00%) and hydrogen (7.95%) were within 2% of the theoretical values (59.98% and 8.05%, respectively).

The purity of lot MKBB7504 was determined by the study laboratory using GC/FID. The moisture content was determined by Chemir Analytical Services using Karl Fischer titration.

For lot MKBB7504, the purity was determined to be 98.20% ± 0.02% using GC/FID (Table A-1, System C). Four reportable impurities with areas ≥0.1% were detected. Two of these were identified as 2,3-butanedione (0.2%) and 3,4-hexanedione (0.3%); no match was found for the other two impurities present at 0.1% and 0.7%. There was some indication that the impurity present at 0.7% might have been the enol form of 2,3-pentanedione. Karl Fischer titration yielded a water content of 0.75%. The overall purity of lot MKBB7504 was determined to be >98%.

To ensure stability, bulk 2,3-pentanedione was stored in sealed metal pails at 5°C. Reanalyses of the bulk chemical were performed by the study laboratory prior to and after the 3-month studies; no degradation was detected by GC/FID (Table A-1, System C).

A.2. Vapor Generation and Exposure System

A diagram of the vapor generation and delivery system used in the studies is shown in Figure A-5. The test chemical, cracked acetoin or 2,3-pentanedione, was pumped from a 4-liter glass reservoir into a heated glass vaporizer column (approximately 250°F for acetoin, approximately 120°F for 2,3-pentanedione) filled with glass beads and wrapped with heat tape. For the acetoin studies, formation of solid dimer was prevented by maintaining the generator reservoir at approximately 90°F and by daily rinsing of the liquid pump with water from an additional reservoir. For all studies, a waste collection flask was connected to the bottom of the vaporizer column to collect residual chemical not completely vaporized in the column.

Preheated nitrogen (approximately 255°F for acetoin, approximately 130°F for 2,3-pentanedione) entered the vaporizer column from below, vaporized the test chemical, and carried the vapor from the generator cabinet to the distribution manifold through a heated chemical transport line (approximately 110°F for acetoin, approximately 120°F for 2,3-pentanedione). The nitrogen-chemical mixture was diluted with heated air (approximately 110°F for acetoin, approximately 120°F for 2,3-pentanedione) before entering the distribution manifold. Concentration in the manifold was determined by the chemical pump rate, nitrogen flow rate, and dilution airflow rate. Pressure in the distribution manifold was kept fixed to ensure constant flow rates through the manifold and into all exposure chambers as the flow of vapor to each chamber was adjusted.

Individual heated Teflon delivery lines carried the diluted acetoin or 2,3-pentanedione vapor from the distribution manifold to three-way exposure valves at the chamber inlets. The chamber 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 distribution manifold. To initiate exposure, the chamber exposure valves were rotated to allow the acetoin or 2,3-pentanedione vapor to flow to each exposure chamber inlet duct, where it was diluted with conditioned air to achieve the desired exposure concentration. Conditioned air was a temperature-controlled and filtered mix of air derived from each exposure chamber's wet and dry air duct supplies. The temperature was adjusted by passage over a temperature-controlled radiator after sequential treatment with Purafil, charcoal, and HEPA (high efficiency particulate air) filters. Target dew point temperatures of the wet and dry ducts were 60°F and 40°F, respectively. Air for the ducts was obtained from the building air supply and was either passed over chillers to lower the dew point (dry duct) or injected with steam to raise it (wet duct).

The study laboratory designed the inhalation exposure chamber (built by Lab Products, Inc., Seaford, DE) so that uniform vapor concentrations could be maintained throughout the chamber with catch pans in place. The total active mixing volume of each chamber was 1.7 m^3. A small particle detector (Model 3022A; TSI, Inc., St. Paul, MN) was used with and without animals in the exposure chambers to ensure that acetoin or 2,3-pentanedione vapors, and not aerosols, were produced. Particle counts <200 particles/cm^3 are typical of an exposure atmosphere when no generation is occurring. Particle counts above this level, especially if the counts increase with exposure concentration and are above the level during the off-exposure period, suggest a contribution to the aerosol concentration from the generation system. No particle counts above the minimum resolvable level were detected.

A.3. Vapor Concentration Monitoring

Exposure chamber and room concentrations of acetoin and 2,3-pentanedione were monitored using online GC/FID for the 2-week acetoin studies, the 3-month acetoin studies, and the 3-month 2,3-pentanedione studies (Table A-1, System D, E, and F, respectively). Approximately every 20 minutes during each 6-hour exposure period, samples were drawn through stainless steel (acetoin) or Teflon (2,3-pentanedione) tubing connected to each exposure chamber's sampling line using a 16-port Hastelloy-C stream-select valve that directs a continuous stream of sampled atmosphere to a 6-port Hastelloy-C gas-sampling valve with a 1-mL Silicosteel sample loop. Both valves and the sampling loop were mounted in a dedicated valve oven (approximately 150°C for acetoin, approximately 175°C for 2,3-pentanedione). 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 GC allowed for digital measurement of sample flow.

The online GCs were checked throughout each exposure day for instrument drift against online standard vapors of acetoin or 2,3-pentanedione supplied by a permeation tube standard generator (KIN-TEK Models 491 or C0395, KIN-TEK Analytical, Inc., La Marque, TX). The online GCs were calibrated as required to meet acceptance criteria. Calibration was performed by correlating the peak area at the time of sampling with grab sample concentration data collected with silica gel sorbent gas-sampling tubes [ORBO™–53 (acetoin) or ORBO™–52 (2,3-pentanedione), Supelco, Inc., Bellefonte, PA]. The known volumes of chamber atmospheres were sampled from each chamber at a constant flow rate ensured by a calibrated critical orifice. Adsorbed test chemicals were extracted with acetone:water:methanol (95:4:1) (acetoin) or acetone

(2,3-pentanedione) and analyzed using off-line GC/FID for acetoin (Table A-1, System G) or 2,3-pentanedione (Table A-1, System H); 2-methyl-1-propanol was used as an internal standard (ISTD) in both extraction vehicles. The off-line GCs were calibrated with gravimetrically prepared standard solutions of acetoin or 2,3-pentanedione containing the ISTD in the appropriate extraction vehicle.

Summaries of the chamber vapor concentrations are given in Table A-2 through Table A-4. The mean relative errors (RE) were within the acceptance criteria of 10% for all exposure groups for all studies. For the 2-week acetoin studies, the mean exposure concentrations were within 3% of the respective target concentrations, and the number of acceptable samples ranged from 94% to 100% (Table A-2). For the 3-month acetoin studies, the mean exposure concentrations were within 1% of the respective target concentrations, and the number of acceptable samples ranged from 96% to 100% (Table A-3). For the 3-month 2,3-pentanedione studies, the mean exposure concentrations were within 1% of the respective target concentrations, and the number of acceptable samples ranged from 99% to 100% (Table A-4).

A.4. Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined with (all studies) and without (3-month studies) animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 9 minutes. For rats and mice in the 2-week acetoin studies with animals present, T_{90} values ranged from 13 to 19 minutes; T_{10} values ranged from 10 to 11 minutes. For rats and mice in the 3-month acetoin studies, T_{90} values ranged from 8 to 10 minutes without animals present and from 9 to 11 minutes with animals; T_{10} values ranged from 8 to 11 minutes without animals present and from 11 to 14 minutes with animals. For rats and mice in the 3-month 2,3-pentanedione studies, T_{90} values ranged from 9 to 10 minutes without animals present and from 9 to 12 minutes with animals; T_{10} values ranged from 9 to 10 minutes without animals present and from 11 to 12 minutes with animals. A T_{90} value of 12 minutes was selected for both chemicals for all studies.

The persistence of acetoin in the rat and mouse chambers after vapor delivery ended was determined by monitoring the concentration in the 800 ppm chambers in the 2-week and 3-month studies with (all studies) and without (3-month studies) animals present in the chambers. In the 2-week studies, the time for the chamber concentration to decay to <1% of the target concentration after vapor generation was terminated (T_1) was approximately 47 minutes with animals present. In the 3-month studies, T_1 was approximately 22 minutes without animals present; when animals were present, the chamber concentration did not drop to below 1% of the target concentration until after the chamber doors were opened to perform animal care activities (up to 134 minutes after shutdown).

The persistence of 2,3-pentanedione in the rat and mouse chambers after vapor delivery ended was determined by monitoring the concentration in the 100 ppm chambers in the 3-month studies, with and without animals present. In the 3-month studies, the concentration decreased to

1% of the target concentration within approximately 23 minutes without animals present and within approximately 32 minutes with animals present.

The uniformity of acetoin or 2,3-pentanedione vapor concentration without animals present in the chambers was evaluated before the 3-month studies began. In addition, concentration uniformity with animals present in the chambers was measured once during the 2-week and 3-month studies. The vapor concentration was measured using an online GC with the stream-selection valve fixed in one position to allow continuous monitoring from a single input line for the 2-week acetoin studies, 3-month acetoin studies, and 3-month 2,3-pentanedione studies (Table A-1, System D, E, and F, respectively). For all acetoin studies, and prior to the 3-month 2,3-pentanedione studies, concentrations were measured at 12 chamber positions, one in front and one in back for each of the six possible animal cage positions per chamber. During the 3-month 2,3-pentanedione studies, concentrations were measured only at the regular monitoring port and from chamber positions where animals were present. Chamber concentration uniformity was maintained throughout the studies; uniformity measurements were all within the acceptable criterion of <5% of the relative standard deviation (RSD).

To measure stability and purity of the test chemicals in the generation and delivery system, samples of the test atmosphere from the distribution lines and low- and high-exposure concentration chambers for each species were collected during the first and last hours of generation prior to the 3-month studies without animals present and during the 2-week and 3-month studies with animals present. The atmosphere samples were collected with sorbent gas-sampling tubes containing silica gel (ORBO™–53, Supelco, Inc., Bellefonte, PA) followed by a tube containing activated coconut charcoal (ORBO™–32, Supelco, Inc., Bellefonte, PA). Grab samples were collected from the bulk chemical and generator reservoir for all studies of both test articles. Acetoin and 2,3-pentanedione were stable under the generation and exposure conditions used during the studies. No evidence of degradation of acetoin or 2,3-pentanedione was noted in any part of the exposure system in any of the samples collected prior to or during the 2-week and 3-month studies. Stability in the generator reservoir was determined to be up to 15 days for acetoin (after cracking) and up to 21 days for 2,3-pentanedione.

For acetoin, the test chemical was extracted from the sorbent tubes with acetone:water:methanol (95:4:1) and analyzed using GC/FID (Table A-1, System I). To assess whether impurities or degradation products co-eluted with acetoin or the solvent mixture, a second GC/FID analysis was performed using System I with a less polar column (DB-5, 30 m × 0.53 mm internal diameter, 1.5 µm film, J&W Scientific, Folsom, CA) than the polar DB WAXetr column to provide alternate separation characteristics. The additional purity analyses of the bulk chemical and the test article in the generator reservoir were conducted by GC/FID (Table A-1, System K). During the 2-week and 3-month studies of acetoin, the impurities 2,3-butanedione, acetic acid, 3-methyl-2,4-pentanedione, and isomers of di-2-butan-3-one ether were detected in the atmosphere and generator reservoir samples with areas generally >0.1% and <1% of the total peak area. No additional impurities were detected with analyses using the less polar column.

For 2,3-pentanedione, the test chemical was extracted from the sorbent tubes with acetone and analyzed using GC/FID (Table A-1, System J). Duplicate samples were collected and extracted with dimethylformamide to assess for impurities or degradation products that co-elute with acetone. The additional purity analyses of the bulk chemical and the test article in the generator reservoir were conducted by GC/FID (Table A-1, System H). During the 3-month studies of

2,3-pentanedione, the impurities 2,3-butanedione, 3,4-hexanedione, acetaldehyde, paraldehyde, acetic acid, and five unknowns were detected in the atmosphere and generator reservoir samples with areas generally >0.1% and <1% of the total peak area, except for one unknown peak with an area of approximately 2.2% to 3.5%.

To measure stability of the test chemicals in the exposure system, concentrations in all exposure chambers were monitored during 3 days of test generation prior to the 3-month studies of acetoin and 2,3-pentanedione using online GC/FID (Table A-1, System E and F, respectively). The mean concentrations for all chambers for all studies met the acceptance criteria of being within ±10% of the target concentration with an RSD of ≤10%. For acetoin, the mean exposure concentrations were within 2% of the target concentrations with an RSD ranging from 3% to 5%. For 2,3-pentanedione, the mean exposure concentrations were within 2% of the target concentrations with an RSD ranging from 2% to 4%.

Table A-1. Gas Chromatography Systems Used in the Two-week and Three-month Inhalation Studies of Acetoin and 2,3-Pentanedione

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization (250°C)	Restek Stabilwax®-DA (30 m × 0.25 mm ID, 0.25 µm film thickness)	Helium at 1.5 mL/minute	40°C for 3 minutes, then increasing 6°C/minute to 240°C, then held for 5 minutes at 240°C
System B			
Mass spectrometry with electron impact ionization (selective ion monitoring)	Restek Stabilwax®-DA, (30 m × 0.25 mm ID, 0.50 µm film thickness)	Helium at 1.5 mL/minute	40°C for 3 minutes, then 6°C/minute to 240°C, held for 5 minutes
System C			
Flame ionization (250°C)	Restek Rtx®-Wax, (30 m × 0.25 mm ID, 0.50 µm film thickness)	Helium at 1.0 mL/minute	40°C for 2 minutes, then 6°C/minute to 230°C, held for 10 minutes
System D			
Flame ionization[a]	J&W DB-5, (15 m × 0.53 mm ID, 1.5 µm film thickness)	Nitrogen at 25 mL/minute	Isothermal at 45°C
System E			
Flame ionization (250°C)	J&W DB-WAXetr, (15 m × 0.53 mm ID, 1.0 µm film thickness)	Nitrogen at 25 mL/minute	Isothermal at 80°C
System F			
Flame ionization (250°C)	Restek Stabilwax®, (15 m × 0.53 mm ID, 2.0 µm film thickness)	Nitrogen at 25 mL/minute	Isothermal at 70°C or lowered to 35°C for better separation

Detection System	Column	Carrier Gas	Oven Temperature Program
System G			
Flame ionization (250°C)	J&W DB-WAXetr, (30 m × 0.53 mm ID, 1.5 µm film thickness)	Helium at 7 mL/minute	45°C for 1 minute, then 6°C/minute to 120°C, then 15°C/minute to 180°C, held for 2 minutes
System H			
Flame ionization (250°C)	Restek Rtx®-Wax, (30 m × 0.25 mm ID, 0.25 µm film thickness)	Helium at 1.5 mL/minute	40°C for 2 minutes, then 5°C/minute to 65°C, then 20°C/minute to 180°C, held for 2 minutes
System I			
Flame ionization (250°C)	J&W DB-WAXetr, (30 m × 0.53 mm ID, 1.5 µm film thickness)	Helium at 7 mL/minute	40°C for 4 (2-week studies) or 2 (3-month studies) minutes, then 6°C/minute to 235°C, held for 5 minutes
System J			
Flame ionization (250°C)	Restek Rtx®-Wax, (30 m × 0.25 mm ID, 0.25 µm film thickness)	Helium at 1.5 mL/minute	40°C for 3 minutes, then 4°C/minute to 65°C, then 6°C/minute to 230°C, held for 5 minutes
System K			
Flame ionization (250°C)	Restek Stabilwax®-DA, (30 m × 0.25 mm ID, 0.25 µm film thickness)	Helium at 1.0 mL/minute	45°C for 1 minute, then 6°C/minute to 120°C, then 15°C/minute to 180°C

ID = internal diameter.
[a]Temperature for this system was not recorded.

Table A-2. Summary of Chamber Concentrations in the Two-week Inhalation Studies of Acetoin

Exposure Date	Target Concentration (ppm)	Total Number of Readings	Determined Concentration (ppm)[a]	Difference from Target (%)	Acceptable Samples (%)[b]
Rat Chambers					
October 27, 2008–November 11, 2008	0 (Room)	247	ND	NA	99
	0	246	ND	NA	100
	6.25	229	6.23 ± 0.5	−0.3	97
	25	235	24.4 ± 1.4	−2.4	94
	100	226	100 ± 3.9	0	98
	400	249	397 ± 21	−0.8	96
	800	234	796 ± 40	−0.5	97
Mouse Chambers					
	0 (Room)	267	ND	NA	99

Exposure Date	Target Concentration (ppm)	Total Number of Readings	Determined Concentration (ppm)[a]	Difference from Target (%)	Acceptable Samples (%)[b]
October 27, 2008–November 12, 2008	0	266	ND	NA	100
	6.25	247	6.23 ± 0.5	−0.3	97
	25	253	24.4 ± 1.3	−2.4	94
	100	244	100 ± 3.9	0	98
	400	267	397 ± 21	−0.8	95
	800	252	795 ± 40	−0.6	97

ND = not detectable; NA = not applicable.
[a]Data shown as mean of readings ± standard deviation.
[b]Acceptable range: target concentration ± 10%, except for room and 0 ppm chamber: <limit of detection (~0.3 ppm).

Table A-3. Summary of Chamber Concentrations in the Three-month Inhalation Studies of Acetoin

Exposure Date	Target Concentration (ppm)	Total Number of Readings	Determined Concentration (ppm)[a]	Difference from Target (%)	Acceptable Samples (%)[b]
Rat Chambers					
June 29, 2009–September 28, 2009 (Males)	0 (Room)	1,252	ND	NA	100
	0 (Acclimation)	18	ND	NA	100
June 30, 2009–September 29, 2009 (Females)	0	1,236	ND	NA	100
	50	1,261	49.6 ± 2.0	−0.8	97
	100	1,268	99.0 ± 4.7	−1.0	96
	200	1,287	197.9 ± 9.3	−1.1	98
	400	1,301	397.3 ± 19.6	−0.7	97
	800	1,283	794.2 ± 29.0	−0.7	98
Mouse Chambers					
June 29, 2009–September 30, 2009 (Males)	0 (Room)	1,291	ND	NA	100
	0	1,274	ND	NA	100
June 29, 2009–October 1, 2009 (Females)	50	1,299	49.6 ± 2.0	−0.8	97
	100	1,306	99.0 ± 4.6	−1.0	96
	200	1,325	198.0 ± 9.2	−1.0	98
	400	1,339	397.5 ± 19.4	−0.6	97
	800	1,321	795.0 ± 29.0	−0.6	98

ND = not detectable; NA = not applicable.
[a]Data shown as mean of readings ± standard deviation.
[b]Acceptable range: target concentration ± 10%, except for room, acclimation, and 0 ppm chamber: <limit of detection (~0.2 ppm).

Table A-4. Summary of Chamber Concentrations in the Three-month Inhalation Studies of 2,3-Pentanedione

Exposure Date	Target Concentration (ppm)	Total Number of Readings	Determined Concentration (ppm)[a]	Difference from Target (%)	Acceptable Samples (%)[b]
Rat Chambers					
September 13, 2010–December 13, 2010 (Males)	0 (Room)	1,290	ND	NA	100
	0 (Acclimation)	18	ND	NA	100
September 14, 2010–December 14, 2010 (Females)	0	1,280	ND	NA	100
	6.25	1,254	6.28 ± 0.13	0.5	>99[c]
	12.5	1,263	12.5 ± 0.2	0.0	100
	25	1,285	25.1 ± 0.5	0.4	100
	50	1,303	50.1 ± 1.0	0.2	>99[c]
	100	1,306	100.8 ± 2.0	0.8	>99[c]
Mouse Chambers					
September 13, 2010–December 15, 2010 (Males)	0 (Room)	1,330	ND	NA	100
	0	1,320	ND	NA	100
September 13, 2010–December 16, 2010 (Females)	6.25	1,292	6.28 ± 0.13	0.5	>99[c]
	12.5	1,301	12.5 ± 0.2	0.0	100
	25	1,323	25.1 ± 0.5	0.4	100
	50	1,341	50.1 ± 1.1	0.2	>99[c]
	100	1,344	100.8 ± 2.0	0.8	>99[c]

ND = not detectable; NA = not applicable.
[a]Data shown as mean of readings ± standard deviation.
[b]Acceptable range: target concentration ± 10%, except for room, acclimation, and 0 ppm chamber: <limit of detection (~0.2 ppm).
[c]>99.5% but <100%.

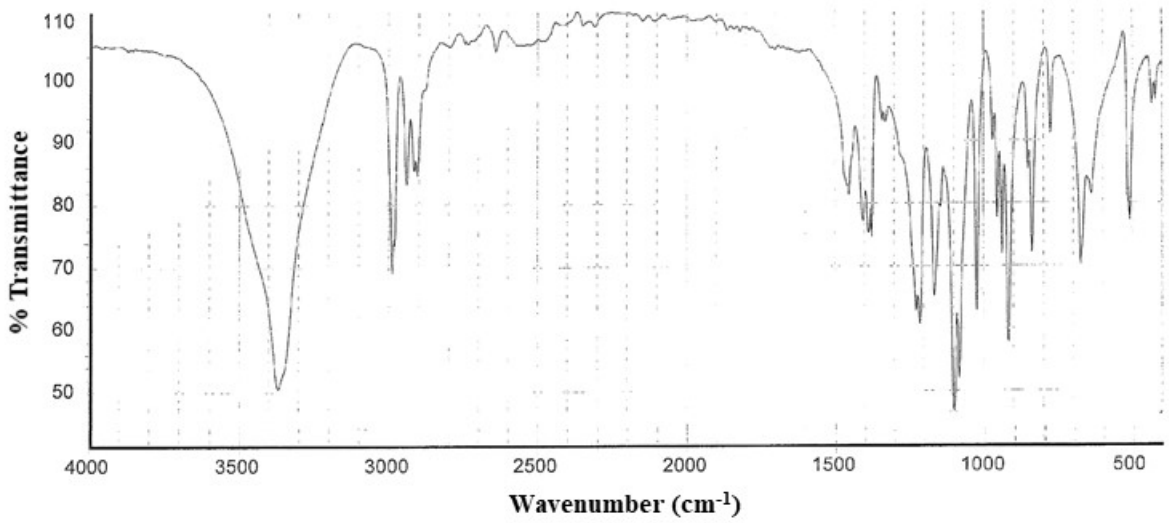

Figure A-1. Infrared Absorption Spectrum of Acetoin

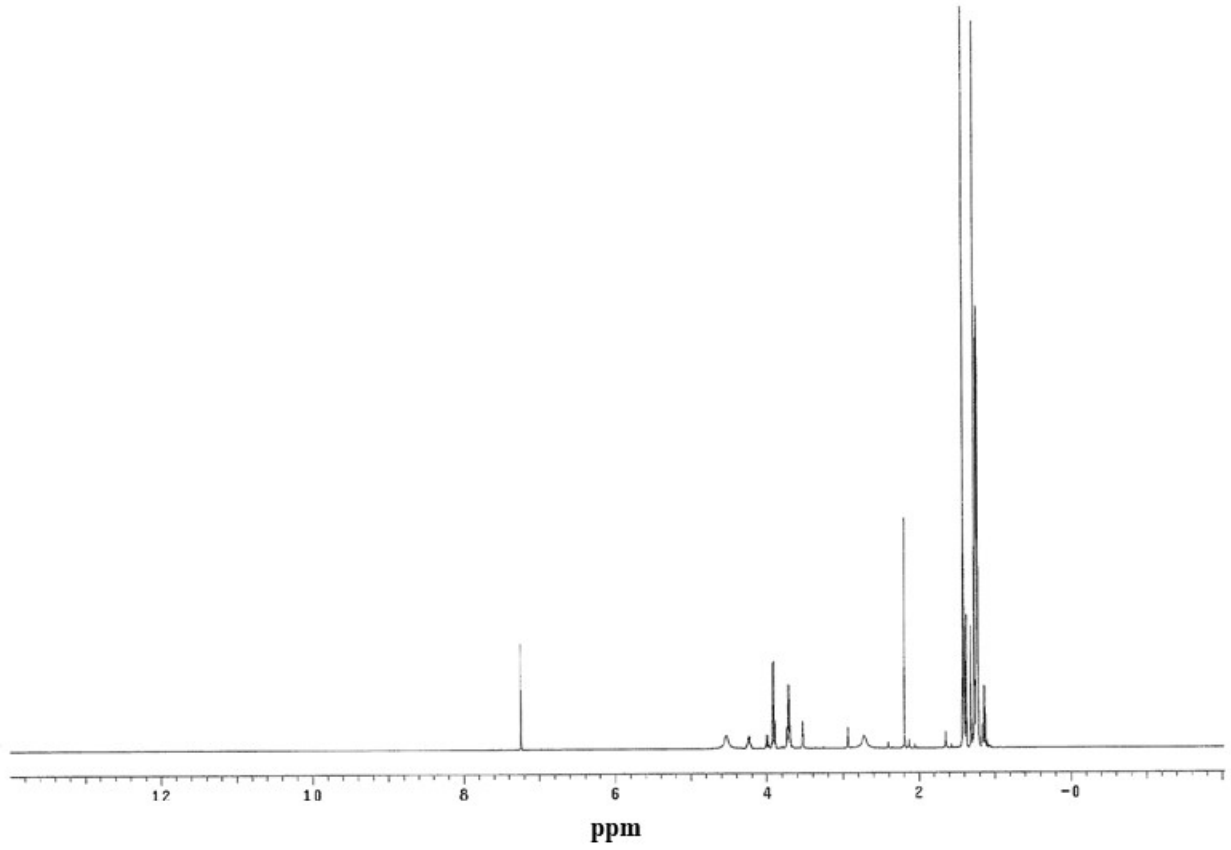

Figure A-2. ^{1}H Nuclear Magnetic Resonance Spectrum of Acetoin

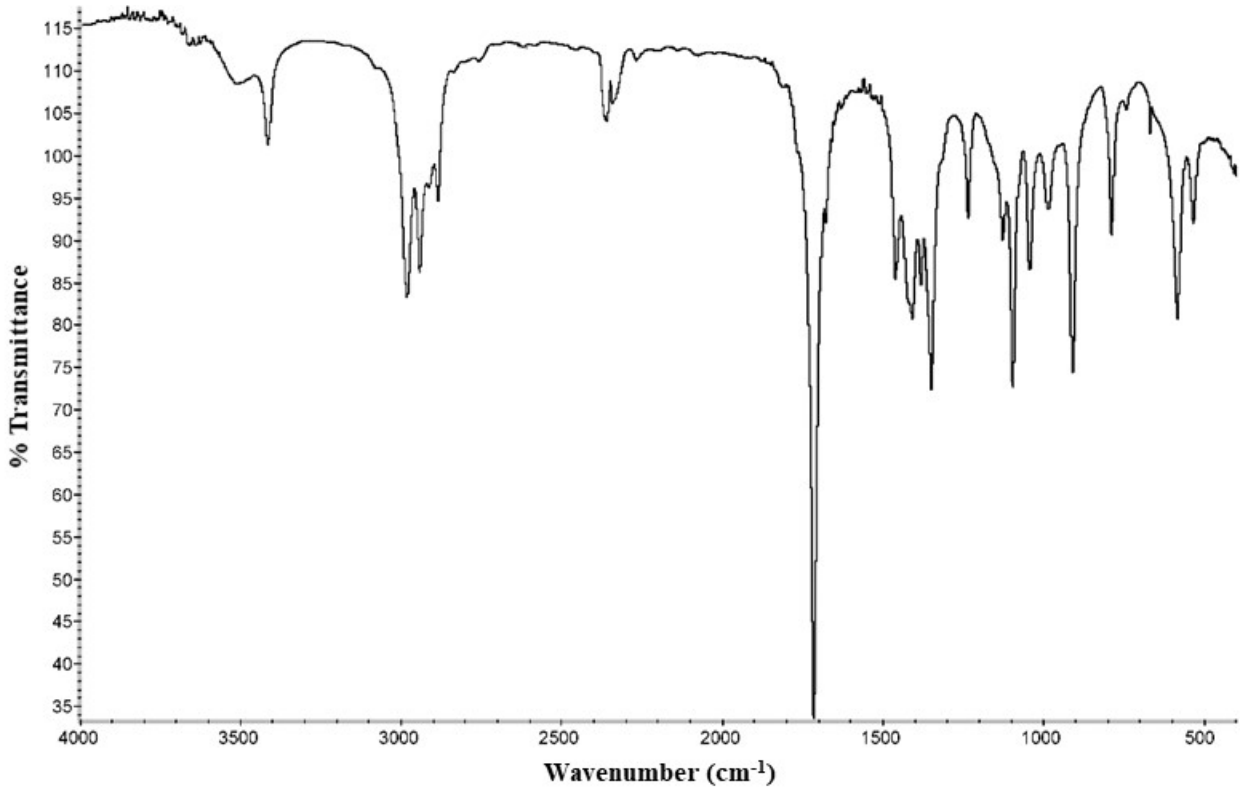

Figure A-3. Infrared Absorption Spectrum of 2,3-Pentanedione

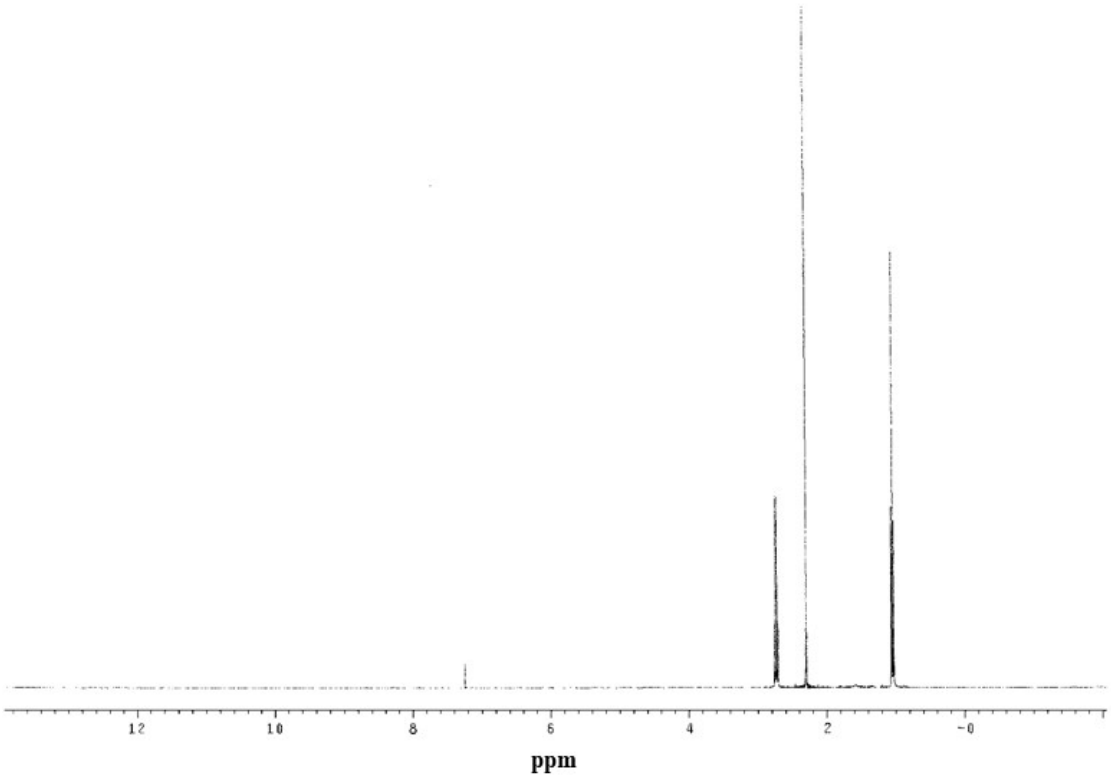

Figure A-4. ^{1}H Nuclear Magnetic Resonance Spectrum of 2,3-Pentanedione

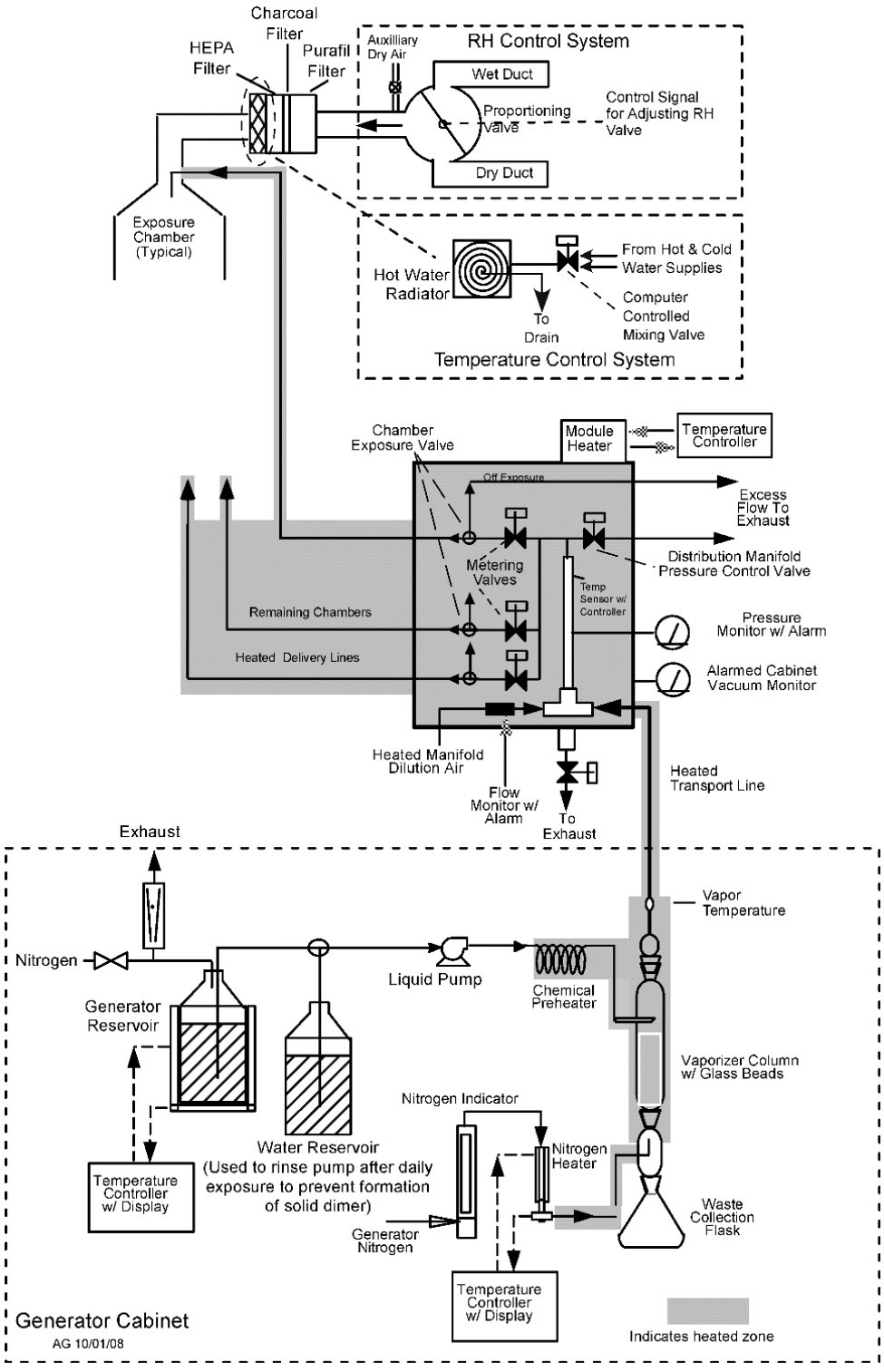

Figure A-5. Schematic of the Vapor Generation and Delivery System in the Inhalation Studies of Acetoin and 2,3-Pentanedione

The water reservoir used to rinse the liquid pump and prevent the formation of solid dimer acetoin was not used in the generation system for 2,3-pentanedione.

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

Tables

Table B-1. Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground Hard Winter Wheat	23.00
Ground #2 Yellow Shelled Corn	22.44
Wheat Middlings	15.0
Oat Hulls	8.5
Alfalfa Meal (Dehydrated, 17% Protein)	7.5
Purified Cellulose	5.5
Soybean Meal (49% Protein)	4.0
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

USP = United States Pharmacopeia.
[a]Wheat middlings as carrier.
[b]Calcium carbonate as carrier.

Table B-2. Vitamins and Minerals in NTP-2000 Rat and Mouse Ration

	Amount[a]	Source
Vitamins		
Vitamin A	4,000 IU	Stabilized vitamin A palmitate or acetate
Vitamin D	1,000 IU	D-activated animal sterol
Vitamin K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl Acetate	100 IU	–
Niacin	23 mg	–
Folic Acid	1.1 mg	–
α-Pantothenic Acid	10 mg	α-Calcium pantothenate
Riboflavin	3.3 mg	–
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	–
Pyridoxine	6.3 mg	Pyridoxine hydrochloride

	Amount[a]	Source
Biotin	0.2 mg	α-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a]Per kg of finished product.

Table B-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration in the Two-week and Three-month Studies of Acetoin

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	14.73 ± 0.498	14.1–15.7	8
Crude Fat (% by Weight)	8.2 ± 0.251	7.7–8.5	8
Crude Fiber (% by Weight)	9.01 ± 0.995	7.1–10.6	8
Ash (% by Weight)	5.189 ± 0.158	4.99–5.43	8
Amino Acids (% of Total Diet)			
Arginine	0.806 ± 0.074	0.67–0.97	30
Cystine	0.220 ± 0.021	0.15–0.25	30
Glycine	0.702 ± 0.037	0.62–0.8	30
Histidine	0.341 ± 0.069	0.27–0.68	30
Isoleucine	0.548 ± 0.039	0.43–0.66	30
Leucine	1.096 ± 0.062	0.96–1.24	30
Lysine	0.070 ± 0.103	0.31–0.86	30
Methionine	0.409 ± 0.041	0.26–0.49	30
Phenylalanine	0.623 ± 0.046	0.471–0.72	30
Threonine	0.513 ± 0.041	0.43–0.61	30
Tryptophan	0.156 ± 0.026	0.11–0.2	30
Tyrosine	0.423 ± 0.065	0.28–0.54	30
Valine	0.666 ± 0.039	0.55–0.73	30
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.939 ± 0.233	3.49–4.55	30
Linolenic	0.306 ± 0.030	0.21–0.368	30
Vitamins			

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Vitamin A (IU/kg)	3,700 ± 80.96	2,780–4,780	8
α-Tocopherol (ppm)	2,376 ± 12,602	13.6–69,100	30
Thiamine (ppm)[a]	7.6 ± 1.80	5.3–11.0	8
Riboflavin (ppm)	8.17 ± 2.792	4.2–17.5	30
Niacin (ppm)	79.19 ± 8.497	66.4–98.2	30
Pantothenic Acid (ppm)	26.33 ± 10.87	17.4–81.0	30
Pyridoxine (ppm)[a]	9.719 ± 2.018	6.44–14.3	30
Folic Acid (ppm)	1.60 ± 0.440	1.15–3.27	30
Biotin (ppm)	0.330 ± 0.097	0.2–0.704	30
B_{12} (ppb)	50.06 ± 34.34	18.3–174.0	30
Choline (as chloride) (ppm)	2,572 ± 634	1,160–3,790	30
Minerals			
Calcium (%)	0.904 ± 0.032	0.857–0.96	8
Phosphorus (%)	0.548 ± 0.026	0.504–0.588	8
Potassium (%)	0.668 ± 0.287	0.626–0.733	30
Chloride (%)	0.391 ± 0.044	0.3–0.517	30
Sodium (%)	0.194 ± 0.027	0.153–0.283	30
Magnesium (%)	0.217 ± 0.053	0.185–0.49	30
Iron (ppm)	190.43 ± 36.11	135–311	30
Manganese (ppm)	50.02 ± 9.27	21.0–73.1	30
Zinc (ppm)	56.81 ± 25.25	42.5–184	30
Copper (ppm)	7.61 ± 2.46	3.21–16.3	30
Iodine (ppm)	0.514 ± 0.217	0–0.972	30
Chromium (ppm)	1.119 ± 1.157	0.33–3.97	30
Cobalt (ppm)	0.219 ± 0.150	0.086–0.864	30

[a]As hydrochloride.

Table B-4. Nutrient Composition of NTP-2000 Rat and Mouse Ration in the Three-month Studies of 2,3-Pentanedione

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	14.7 ± 0.383	14.2–15.0	4
Crude Fat (% by Weight)	8.45 ± 0.173	8.3–8.7	4
Crude Fiber (% by Weight)	9.13 ± 0.606	8.57–9.96	4
Ash (% by Weight)	5.168 ± 0.118	5.0–5.26	4
Amino Acids (% of Total Diet)			
Arginine	0.806 ± 0.074	0.67–0.97	30
Cystine	0.220 ± 0.021	0.15–0.25	30
Glycine	0.702 ± 0.037	0.62–0.8	30
Histidine	0.341 ± 0.069	0.27–0.68	30
Isoleucine	0.548 ± 0.039	0.43–0.66	30
Leucine	1.096 ± 0.062	0.96–1.24	30
Lysine	0.070 ± 0.103	0.31–0.86	30
Methionine	0.409 ± 0.041	0.26–0.49	30
Phenylalanine	0.623 ± 0.046	0.471–0.72	30
Threonine	0.513 ± 0.041	0.43–0.61	30
Tryptophan	0.156 ± 0.026	0.11–0.2	30
Tyrosine	0.423 ± 0.065	0.28–0.54	30
Valine	0.666 ± 0.039	0.55–0.73	30
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.939 ± 0.233	3.49–4.55	30
Linolenic	0.306 ± 0.030	0.21–0.368	30
Vitamins			
Vitamin A (IU/kg)	3,800 ± 56.20	3,350–4,620	4
α-Tocopherol (ppm)	2,376 ± 12,602	13.6–69,100	30
Thiamine (ppm)[a]	7.65 ± 0.802	6.7–8.6	4
Riboflavin (ppm)	8.17 ± 2.792	4.2–17.5	30
Niacin (ppm)	79.19 ± 8.497	66.4–98.2	30
Pantothenic Acid (ppm)	26.33 ± 10.87	17.4–81.0	30
Pyridoxine (ppm)[a]	9.719 ± 2.018	6.44–14.3	30
Folic Acid (ppm)	1.56 ± 0.440	1.15–3.27	30
Biotin (ppm)	0.330 ± 0.097	0.2–0.704	30
B_{12} (ppb)	50.06 ± 34.34	18.3–174.0	30
Choline (as Chloride) (ppm)	2,572 ± 634	1,160–3,790	30

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.940 ± 0.063	0.856–0.994	4
Phosphorus (%)	0.587 ± 0.042	0.531–0.63	4
Potassium (%)	0.668 ± 0.287	0.626–0.733	30
Chloride (%)	0.391 ± 0.044	0.3–0.517	30
Sodium (%)	0.194 ± 0.027	0.153–0.283	30
Magnesium (%)	0.217 ± 0.053	0.185–0.49	30
Iron (ppm)	190.43 ± 36.11	135–311	30
Manganese (ppm)	50.02 ± 9.27	21.0–73.1	30
Zinc (ppm)	56.81 ± 25.25	42.5–184	30
Copper (ppm)	7.61 ± 2.46	3.21–16.3	30
Iodine (ppm)	0.514 ± 0.217	0–0.972	30
Chromium (ppm)	1.119 ± 1.157	0.33–3.97	30
Cobalt (ppm)	0.219 ± 0.150	0.086–0.864	30

[a]As hydrochloride.

Table B-5. Contaminant Levels in NTP-2000 Rat and Mouse Ration in the Two-week and Three-month Studies of Acetoin

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.262 ± 0.037	0.183–0.307	8
Cadmium (ppm)	0.066 ± 0.012	0.056–0.0957	8
Lead (ppm)	0.190 ± 0.282	0.0789–0.887	8
Mercury (ppm)	0.017 ± 0.003	0.012–0.021	8
Selenium (ppm)	0.211 ± 0.039	0.165–0.272	8
Aflatoxins (ppb)[a]	<5.0	–	8
Nitrate Nitrogen (ppm)[b]	22.68 ± 9.82	10.0–35.9	8
Nitrite Nitrogen (ppm)[a,b]	<0.61	–	8
BHA (ppm)[a,c]	<1.0	–	8
BHT (ppm)[a,c]	<1.0	–	8
Aerobic Plate Count (CFU/g)	<10	–	8
Coliform (MPN/g)	<3	–	8
Escherichia coli (MPN/g)	<10	–	8
Salmonella (MPN/g)	Negative	–	8
Total Nitrosamines (ppb)[d]	10.58 ± 4.97	2.0–16.2	8
N-Nitrosodimethylamine (ppb)[d]	4.75 ± 3.785	1.0–11.1	8
N-Nitrosopyrrolidine (ppb)[d]	6.96 ± 3.137	1.0–11.0	8

	Mean ± Standard Deviation	Range	Number of Samples
Pesticides (ppm)			
α-BHC[a]	<0.01	–	8
β-BHC[a]	<0.02	–	8
γ-BHC[a]	<0.01	–	8
δ-BHC[a]	<0.01	–	8
Heptachlor[a]	<0.01	–	8
Aldrin[a]	<0.01	–	8
Heptachlor Epoxide[a]	<0.01	–	8
DDE[a]	<0.01	–	8
DDD[a]	<0.01	–	8
DDT[a]	<0.01	–	8
HCB[a]	<0.01	–	8
Mirex[a]	<0.01	–	8
Methoxychlor[a]	<0.05	–	8
Dieldrin[a]	<0.01	–	8
Endrin[a]	<0.01	–	8
Telodrin[a]	<0.01	–	8
Chlordane[a]	<0.05	–	8
Toxaphene[a]	<0.01	–	8
Estimated PCBs[a]	<0.20	–	8
Ronnel[a]	<0.01	–	8
Ethion[a]	<0.02	–	8
Trithion[a]	<0.05	–	8
Diazinon[a]	<0.10	–	8
Methyl Chlorpyrifos	0.133 ± 0.102	0.02–0.3	8
Methyl Parathion[a]	<0.02	–	8
Ethyl Parathion[a]	<0.02	–	8
Malathion	0.073 ± 0.078	0.02–0.234	8
Endosulfan I[a]	<0.01	–	8
Endosulfan II[a]	<0.01	–	8
Endosulfane Sulfate[a]	<0.03	–	8

All samples were irradiated. BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride;
DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane;
HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.
[a]All values were below the detection limit. The detection limit is given as the mean.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

Table B-6. Contaminant Levels in NTP-2000 Rat and Mouse Ration in the Three-month Studies of 2,3-Pentanedione

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.267 ± 0.048	0.222–0.313	4
Cadmium (ppm)	0.051 ± 0.002	0.222–0.313	4
Lead (ppm)	0.090 ± 0.008	0.079–0.098	4
Mercury (ppm)	0.018 ± 0.004	0.012–0.022	4
Selenium (ppm)	0.182 ± 0.017	0.166–0.202	4
Aflatoxins (ppb)[a]	<5.0	–	4
Nitrate Nitrogen (ppm)[b]	10.38 ± 0.75	10.0–11.5	4
Nitrite Nitrogen (ppm)[a,b]	<0.61	–	4
BHA (ppm)[a,c]	<1.0	–	4
BHT (ppm)[c]	1.51 ± 1.02	1.0–3.04	4
Aerobic Plate Count (CFU/g)	12.5 ± 5.0	10.0–20.0	4
Coliform (MPN/g)	<3	–	4
Escherichia coli (MPN/g)	<10	–	4
Salmonella (MPN/g)	Negative	–	4
Total Nitrosamines (ppb)[d]	7.12 ± 2.71	3.68–9.7	4
N-Nitrosodimethylamine (ppb)[d]	2.01 ± 1.93	1.0–4.9	4
N-Nitrosopyrrolidine (ppb)[d]	5.12 ± 2.61	2.66–8.7	4
Pesticides (ppm)			
α-BHC[a]	<0.01	–	4
β-BHC[a]	<0.02	–	4
γ-BHC[a]	<0.01	–	4
δ-BHC[a]	<0.01	–	4
Heptachlor[a]	<0.01	–	4
Aldrin[a]	<0.01	–	4
Heptachlor Epoxide[a]	<0.01	–	4
DDE[a]	<0.01	–	4
DDD[a]	<0.01	–	4
DDT[a]	<0.01	–	4
HCB[a]	<0.01	–	4
Mirex[a]	<0.01	–	4
Methoxychlor[a]	<0.05	–	4
Dieldrin[a]	<0.01	–	4
Endrin[a]	<0.01	–	4

	Mean ± Standard Deviation	Range	Number of Samples
Telodrin[a]	<0.01	–	4
Chlordane[a]	<0.05	–	4
Toxaphene[a]	<0.01	–	4
Estimated PCBs[a]	<0.20	–	4
Ronnel[a]	<0.01	–	4
Ethion[a]	<0.02	–	4
Trithion[a]	<0.05	–	4
Diazinon[a]	<0.10	–	4
Methyl Chlorpyrifos	0.044 ± 0.020	0.02–0.63	4
Methyl Parathion[a]	<0.02	–	4
Ethyl Parathion[a]	<0.02	–	4
Malathion	0.112 ± 0.065	0.067–0.207	4
Endosulfan I[a]	<0.01	–	4
Endosulfan II[a]	<0.01	–	4
Endosulfane Sulfate[a]	<0.03	–	4

All samples were irradiated. BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride;
DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.
[a]All values were below the detection limit. The detection limit is given as the mean.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

Appendix C. Sentinel Animal Program

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Tables

C.1. Methods

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that might 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 exposed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

Blood samples were collected from each sentinel animal and allowed to clot, and the serum was separated. All samples were processed appropriately with serology testing performed in-house (acetoin) or by IDEXX BioResearch (formerly Rodent Animal Diagnostic Laboratory [RADIL], University of Missouri), Columbia, MO, (both chemicals) for determination of the presence of pathogens. Evaluation for endoparasites was performed in-house by the testing laboratory.

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 (Table C-1, Table C-2, Table C-3, Table C-4).

C.2. Results

Rats: All test results were negative for acetoin and 2,3-pentanedione.

Mice: All test results were negative for acetoin and 2,3-pentanedione.

Table C-1. Methods and Results for Sentinel Animal Testing in Male and Female Rats in the Two-week and Three-month Studies of Acetoin

Collection Time Points	Two-week Study	Three-month Study	
	Study Termination	Three Weeks	Study Termination
Number Examined (Males/Females)	5/5	5/5	5/5
Method/Test			
Multiplex Fluorescent Immunoassay (MFI)			
Kilham rat virus (KRV)	NT	–	–
Mycoplasma pulmonis	NT	–	–
Parvo NS-1	NT	–	–
Pneumonia virus of mice (PVM)	NT	–	–
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	NT	–	–
Rat minute virus (RMV)	NT	–	–
Rat parvo virus (RPV)	NT	–	–
Rat theilovirus (RTV)	NT	–	–
Sendai	NT	–	–

Collection Time Points	Two-week Study	Three-month Study	
	Study Termination	Three Weeks	Study Termination
Theiler's murine encephalomyelitis virus (TMEV)	NT	−	−
Toolan's H-1	NT	−	−
In-house Evaluation			
Mycoplasma pulmonis	−	NT	NT
Pneumonia virus of mice (PVM)	−	NT	NT
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	−	NT	NT
Rat parvo virus (RPV)	−	NT	NT
Sendai	−	NT	NT
Endoparasite evaluation (evaluation of perianal surface for *Syphacia* sp.)	−	−	−

NT = not tested; − = negative.

Table C-2. Methods and Results for Sentinel Animal Testing in Male and Female Mice in the Two-week and Three-month Studies of Acetoin

Collection Time Points	Two-week Study	Three-month Study	
	Study Termination	Three Weeks	Study Termination
Number Examined (Males/Females)	5/5	5/5	5/5
Method/Test			
Multiplex Fluorescent Immunoassay (MFI)			
Ectromelia virus	NT	−	−
Epizootic diarrhea of infant mice (EDIM)	NT	−	−
Lymphocytic choriomeningitis virus (LCMV)	NT	−	−
Mycoplasma pulmonis	NT	−	−
Mouse hepatitis virus (MHV)	NT	−	−
Mouse norovirus (MNV)	NT	−	−
Parvo NS-1	NT	−	−
Mouse parvovirus (MPV)	NT	−	−
Minute virus of mice (MVM)	NT	−	−
Pneumonia virus of mice (PVM)	NT	−	−
Reovirus (REO3)	NT	−	−
Sendai	NT	−	−
Theiler's murine encephalomyelitis virus (TMEV) GDVII	NT	−	−

Collection Time Points	Two-week Study	Three-month Study	
	Study Termination	Three Weeks	Study Termination
In-house Evaluation			
Mycoplasma pulmonis	−	NT	NT
Mouse hepatitis virus (MHV)	−	NT	NT
Mouse parvovirus (MPV)	−	NT	NT
Pneumonia virus of mice (PVM)	−	NT	NT
Sendai	−	NT	NT
Theiler's murine encephalomyelitis virus (TMEV) GDVII	−	NT	NT
Endoparasite evaluation (evaluation of perianal surface for *Syphacia* sp.)	−	−	−

NT = not tested; − = negative.

Table C-3. Methods and Results for Sentinel Animal Testing in Male and Female Rats in the Three-month Study of 2,3-Pentanedione

Collection Time Points	Three Weeks	Study Termination
Number Examined (Males/Females)	5/5	5/5
Method/Test		
Multiplex Fluorescent Immunoassay (MFI)		
Kilham rat virus (KRV)	–	–
Mycoplasma pulmonis	–	–
Pneumonia virus of mice (PVM)	–	–
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	–	–
Rat minute virus (RMV)	–	–
Rat parvo virus (RPV)	–	–
Rat theilovirus (RTV)	–	–
Sendai	–	–
Toolan's H-1	–	–
In-house Evaluation		
Endoparasite evaluation (evaluation of perianal surface for *Syphacia* sp.)	–	–

– = negative.

Table C-4. Methods and Results for Sentinel Animal Testing in Male and Female Mice in the Three-month Study of 2,3-Pentanedione

Collection Time Points	Three Weeks	Study Termination
Number Examined (Males/Females)	5/5	5/5
Method/Test		
Multiplex Fluorescent Immunoassay (MFI)		
Ectromelia virus	–	–
Epizootic diarrhea of infant mice (EDIM)	–	–
Lymphocytic choriomeningitis virus (LCMV)	–	–
Mycoplasma pulmonis	–	–
Mouse hepatitis virus (MHV)	–	–
Mouse norovirus (MNV)	–	–
Mouse parvovirus (MPV)	–	–
Minute virus of mice (MVM)	–	–
Pneumonia virus of mice (PVM)	–	–
Reovirus (REO3)	–	–
Sendai	–	–
Theiler's murine encephalomyelitis virus (TMEV) GDVII	–	–
In-house Evaluation		
Endoparasite evaluation (evaluation of perianal surface for *Syphacia* sp.)	–	–

– = negative.

Appendix D. Genetic Toxicology

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Tables

D.1. Evaluation Protocol

The National Toxicology Program (NTP) considers biological as well as statistical factors to determine an overall assay result. For an individual assay, the statistical procedures for data analysis are described in the following 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. In such cases, all the data are critically evaluated with attention given to possible protocol variations in determining the weight of evidence for an overall conclusion of chemical activity in an assay. For in vitro assays conducted with and without exogenous metabolic activation, results obtained in the absence of activation are analyzed separately from results obtained in the presence of activation. The summary table in the abstract of this toxicity report presents the Division of Translational Toxicology's scientific judgment regarding the overall evidence for activity of the chemical in an assay.

D.2. Bacterial Mutagenicity

D.2.1. Bacterial Mutagenicity Test Protocol

Testing procedures were modified from those originally reported by Zeiger et al.[85] Coded samples of acetoin (lot 02209CS) were incubated with the *Salmonella typhimurium* (TA97, TA98, TA100, TA1535) tester strains, either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat liver and male 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 after incubation for 2 days at 37°C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of acetoin. The highest concentration tested was 10,000 µg/plate.

In this assay, 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. No minimum percentage or fold-increase is 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.

D.2.2. Results

Acetoin (tested up to 10,000 µg/plate) was not mutagenic when tested in *S. typhimurium* strains TA97, TA98, TA100, and TA1535 in the presence or absence of 10% or 30% induced rat or hamster liver S9 (Table D-1).

Table D-1. Mutagenicity of Acetoin in Bacterial Tester Strains[a]

Strain	Dose (ppm)	Without S9	Without S9	With 10% Rat S9	With 30% Rat S9	With 10% Hamster S9	With 30% Hamster S9
TA97							
	0	127 ± 4.4	118 ± 5.2	164 ± 3.5	216 ± 11.0	145 ± 11.2	152 ± 10.9
	100	129 ± 10.6	104 ± 4.8	167 ± 5.2	197 ± 12.7	135 ± 8.2	178 ± 3.5
	333	145 ± 5.7	117 ± 3.8	160 ± 13.9	222 ± 12.8	136 ± 4.6	173 ± 0.9
	1,000	136 ± 1.9	104 ± 4.0	159 ± 14.2	210 ± 9.2	160 ± 10.1	175 ± 7.6
	3,333	143 ± 2.5	126 ± 7.5	162 ± 12.0	222 ± 9.8	126 ± 7.0	180 ± 13.7
	10,000	129 ± 5.9	122 ± 2.3	167 ± 1.7	216 ± 10.1	162 ± 4.5	158 ± 7.7
Trial Summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive Control[b]		306 ± 36.4	242 ± 15.9	1,432 ± 27.6	524 ± 25.1	551 ± 7.8	808 ± 10.2
TA98							
	0	13 ± 1.5	17 ± 3.2	20 ± 4.5	17 ± 2.3	19 ± 2.2	20 ± 3.0
	100	11 ± 0.3	16 ± 2.7	30 ± 1.8	18 ± 1.5	28 ± 3.8	15 ± 2.6
	333	11 ± 2.7	14 ± 1.0	20 ± 1.2	18 ± 2.8	20 ± 0.9	20 ± 0.9
	1,000	10 ± 1.2	12 ± 1.0	19 ± 2.0	16 ± 4.2	19 ± 3.8	18 ± 0.9
	3,333	9 ± 3.2	11 ± 1.5	17 ± 2.3	14 ± 1.9	20 ± 3.1	19 ± 2.3
	10,000	9 ± 1.2	17 ± 2.7	23 ± 3.4	17 ± 1.2	22 ± 1.8	18 ± 1.5
Trial Summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive Control		80 ± 9.6	74 ± 5.5	227 ± 37.9	124 ± 5.2	527 ± 20.9	151 ± 6.8
TA100							
	0	184 ± 8.8	156 ± 2.5	183 ± 14.0	191 ± 15.0	161 ± 5.6	137 ± 10.9
	100	187 ± 13.0	159 ± 10.7	171 ± 4.4	196 ± 25.6	160 ± 6.9	143 ± 1.9
	333	165 ± 9.5	155 ± 13.7	182 ± 15.2	193 ± 11.3	158 ± 14.4	139 ± 6.0
	1,000	165 ± 7.2	151 ± 6.3	198 ± 1.5	211 ± 12.7	165 ± 13.5	127 ± 8.4
	3,333	189 ± 9.5	147 ± 7.7	178 ± 1.2	215 ± 15.5	171 ± 5.8	151 ± 6.0
	10,000	168 ± 5.8	153 ± 1.7	165 ± 10.5	228 ± 17.3	192 ± 12.1	138 ± 9.2
Trial Summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive Control		398 ± 45.0	343 ± 10.7	469 ± 6.8	704 ± 58.2	520 ± 40.1	806 ± 20.5
TA1535							
	0	10 ± 1.2	10 ± 1.9	13 ± 1.2	10 ± 1.2	17 ± 3.2	9 ± 2.7
	100	7 ± 1.9	9 ± 1.2	13 ± 3.1	8 ± 2.1	14 ± 2.0	12 ± 0.9
	333	7 ± 1.7	8 ± 0.3	10 ± 1.5	10 ± 1.7	9 ± 1.2	6 ± 0.0
	1,000	9 ± 1.3	9 ± 1.5	10 ± 1.7	10 ± 0.0	9 ± 2.4	8 ± 2.5
	3,333	9 ± 1.2	9 ± 2.3	11 ± 4.5	11 ± 3.0	12 ± 0.3	9 ± 1.5

Strain	Dose (ppm)	Without S9	Without S9	With 10% Rat S9	With 30% Rat S9	With 10% Hamster S9	With 30% Hamster S9
	10,000	5 ± 1.2	10 ± 0.6	15 ± 0.7	12 ± 0.9	15 ± 1.5	11 ± 3.4
Trial Summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive Control		233 ± 5.2	219 ± 13.3	202 ± 5.5	205 ± 4.4	45 ± 4.5	78 ± 4.8

[a]Studies performed at BioReliance Corporation. Data are presented as revertants/plate (mean ± standard error) from three plates; 0 µg/plate served as the solvent control.
[b]The positive controls in the absence of metabolic activation were 9-aminiacridine (TA97), 4-nitro-*o*-phenylenediamine (TA98), and sodium azide (TA100, TA1535). The positive control for metabolic activation with all strains was 2-aminoanthracene.

D.3. Micronucleus Assay

D.3.1. Peripheral Blood Micronucleus Test Protocol

At termination of the 3-month inhalation toxicity studies of acetoin and 2,3-pentanedione, blood samples were collected from male and female Wistar Han [Crl:WI(Han)] rats and B6C3F1/N mice, placed in ethylenediaminetetraacetic acid (EDTA)-coated tubes, and shipped overnight to the testing laboratory. Upon arrival, blood samples were fixed in ultracold methanol using a MicroFlowPLUS Kit (Litron Laboratories, Rochester, NY), according to the manufacturer's instructions. Fixed samples were stored in a −80°C freezer until analysis. Thawed blood samples were analyzed for frequency of micronucleated immature reticulocytes (i.e., reticulocytes or polychromatic erythrocytes [PCEs]) and mature erythrocytes (i.e., normochromatic erythrocytes [NCEs]) using a flow cytometer[92]; both the mature and immature erythrocyte populations can be analyzed separately by employing special cell surface markers to differentiate the two cell types. Because the very young reticulocyte subpopulation (CD71+ cells) can be targeted using this technique, rat blood samples can be analyzed for damage that occurred in the bone marrow within the past 24–48 hours, before the rat spleen appreciably alters the percentage of PCEs in circulation.[93] In mice, both the mature and immature erythrocyte populations can be evaluated for micronucleus frequency because the mouse spleen does not sequester and eliminate damaged erythrocytes. Damaged erythrocytes achieve steady state in the peripheral blood of mice after 4 weeks of continuous exposure. Approximately 20,000 PCEs and 1×10^6 NCEs were analyzed per animal for frequency of micronucleated cells, and the percentage of immature erythrocytes (% PCE) was calculated as a measure of bone marrow toxicity resulting from chemical exposure.

Prior experience with the large number of cells scored using flow cytometric scoring techniques[94] suggests it is reasonable to assume that the proportion of micronucleated reticulocytes is approximately normally distributed. The statistical tests selected for trend and for pairwise comparisons with the control group depend on whether the variances among the groups are equal. The Levene 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 exposure concentration, and the Williams test is used to test for pairwise differences between each exposed group and the control group. In the case of unequal variances, the Jonckheere test is used to test for linear trend, and the Dunn test is used for pairwise comparisons of each exposed 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. If this product is >1.00, it is replaced with 1.00. Trend tests and pairwise comparisons with the control group are considered statistically significant at $p \leq 0.025$.

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

D.3.2. Results

Both acetoin (up to 800 ppm) and 2,3-pentanedione (up to 100 ppm) were evaluated for induction of micronucleated erythrocytes following 3 months of exposure via inhalation in male and female Wistar Han rats (Table D-2, Table D-3) and B6C3F1/N mice (Table D-4, Table D-5). Results of the micronucleus tests were negative in both rats and mice for both chemicals, and no alterations in % PCE were observed in mice exposed to either acetoin or 2,3-pentanedione. In the acetoin study, a small but significant exposure concentration-related increase in % PCE was observed in male, but not female, rats, suggesting a mild perturbation in erythropoiesis in male rats. In the study with 2,3-pentanedione, small increases in micronucleated NCEs were observed at the highest concentration tested (100 ppm) in male and female rats. These increases were not considered indicative of a biological response as they were in the NCE population—which is not the appropriate cell population to evaluate for frequency of micronuclei in rats due to the efficient removal by the spleen of damaged erythrocytes soon after they enter the blood stream—and because the values were well within the laboratory historical control ranges. A small exposure concentration-related increase in % PCE was observed in female rats exposed to 2,3-pentanedione.

Table D-2. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Rats in the Three-month Inhalation Study of Acetoin[a]

	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							
Exposure Concentration (ppm)							
0	5	0.960 ± 0.147		0.042 ± 0.007		0.648 ± 0.064	
50	5	0.580 ± 0.044	0.8081	0.042 ± 0.009	0.4823	0.717 ± 0.067	0.4083
100	5	0.770 ± 0.125	0.8799	0.052 ± 0.007	0.4692	0.831 ± 0.068	0.0563
200	5	0.920 ± 0.138	0.8332	0.045 ± 0.013	0.4998	0.871 ± 0.034	0.0209
400	5	0.750 ± 0.174	0.8481	0.040 ± 0.014	0.5179	0.877 ± 0.074	0.0201
800	5	0.980 ± 0.221	0.6072	0.050 ± 0.006	0.3649	1.014 ± 0.099	0.0016
Trend[d]		p = 0.1866		p = 0.3409		p = 0.0016	
Female							
Exposure Concentration (ppm)							
0	5	0.810 ± 0.168		0.073 ± 0.034		1.053 ± 0.182	
50	5	0.790 ± 0.111	0.5632	0.036 ± 0.007	1.0000	1.101 ± 0.138	1.0000
100	5	0.860 ± 0.109	0.6483	0.079 ± 0.014	0.3520	1.150 ± 0.147	1.0000
200	5	0.784 ± 0.143	0.6821	0.041 ± 0.010	1.0000	0.797 ± 0.060	0.5300
400	5	0.680 ± 0.096	0.7014	0.038 ± 0.007	1.0000	1.068 ± 0.023	1.0000
800	5	0.760 ± 0.157	0.7149	0.046 ± 0.008	1.0000	1.045 ± 0.086	1.0000
Trend		p = 0.7122		p = 0.5505		p = 0.9278	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the vehicle control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Table D-3. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Rats in the Three-month Inhalation Study of 2,3-Pentanedione[a]

	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							
Exposure Concentration (ppm)							
0	5	1.337 ± 0.140		0.102 ± 0.015		0.764 ± 0.043	
6.25	5	0.920 ± 0.117	0.9299	0.087 ± 0.014	0.7629	0.883 ± 0.042	0.4678
12.5	5	1.290 ± 0.040	0.9666	0.096 ± 0.014	0.8421	0.860 ± 0.071	0.5614
25.0	5	0.840 ± 0.117	0.9769	0.061 ± 0.008	0.8706	0.798 ± 0.047	0.6005
50.0	5	1.060 ± 0.117	0.9808	0.085 ± 0.016	0.8829	0.845 ± 0.098	0.6182
100.0	5	1.060 ± 0.144	0.9836	0.145 ± 0.022	0.0385	0.961 ± 0.133	0.1836
Trend[d]		p = 0.7620		p = 0.0114		p = 0.2653	
Female							
Exposure Concentration (ppm)							
0	5	0.749 ± 0.167		0.040 ± 0.012		0.925 ± 0.078	
6.25	5	0.960 ± 0.143	0.3763	0.058 ± 0.013	0.4978	0.851 ± 0.053	1.0000
12.5	5	0.840 ± 0.033	0.4456	0.046 ± 0.013	0.5792	0.958 ± 0.201	1.0000
25.0	5	0.730 ± 0.089	0.4754	0.027 ± 0.006	0.6123	0.952 ± 0.050	1.0000
50.0	5	0.660 ± 0.066	0.4929	0.031 ± 0.006	0.6322	1.207 ± 0.131	0.8062
100.0	5	0.800 ± 0.087	0.4954	0.080 ± 0.016	0.0155	1.366 ± 0.117	0.1703
Trend		p = 0.7173		p = 0.0357		p = 0.0044	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the vehicle control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Table D-4. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Mice in the Three-month Inhalation Study of Acetoin[a]

	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							
Exposure Concentration (ppm)							
0	5	2.390 ± 0.212		1.511 ± 0.018		1.359 ± 0.051	
50	5	2.380 ± 0.159	0.5939	1.435 ± 0.024	0.9217	1.443 ± 0.038	0.7267
100	5	2.420 ± 0.164	0.6801	1.436 ± 0.043	0.9619	1.394 ± 0.034	0.8502
200	5	2.380 ± 0.072	0.7145	1.459 ± 0.030	0.9735	1.429 ± 0.094	0.8921
400	5	2.120 ± 0.152	0.7333	1.434 ± 0.022	0.9776	1.381 ± 0.065	0.9120
800	5	2.570 ± 0.142	0.2868	1.481 ± 0.016	0.9053	1.304 ± 0.054	0.6758
Trend[d]		p = 0.2949		p = 0.4292		p = 0.1579	
Female							
Exposure Concentration (ppm)							
0	5	1.790 ± 0.233		1.044 ± 0.030		1.246 ± 0.123	
50	5	1.970 ± 0.160	0.3328	1.069 ± 0.028	1.0000	1.072 ± 0.226	1.0000
100	5	1.830 ± 0.117	0.3962	0.984 ± 0.026	1.0000	1.599 ± 0.204	0.6199
200	5	2.060 ± 0.185	0.2340	1.022 ± 0.013	1.0000	1.452 ± 0.039	0.6631
400	5	1.990 ± 0.230	0.2403	1.048 ± 0.030	1.0000	1.305 ± 0.071	0.6837
800	5	2.020 ± 0.097	0.2457	1.057 ± 0.055	1.0000	1.427 ± 0.203	0.6956
Trend		p = 0.2092		p = 0.5790		p = 0.4946	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the vehicle control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure concentration-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$).

Table D-5. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Mice in the Three-month Inhalation Study of 2,3-Pentanedione[a]

	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							
Exposure Concentration (ppm)							
0	5	2.180 ± 0.046		1.416 ± 0.030		1.363 ± 0.057	
6.25	5	2.600 ± 0.130	0.0379	1.457 ± 0.026	0.5393	1.555 ± 0.032	0.1182
12.5	5	2.750 ± 0.164	0.0457	1.455 ± 0.027	0.6220	1.540 ± 0.035	0.2410
25.0	5	2.650 ± 0.127	0.0479	1.382 ± 0.044	0.6572	1.495 ± 0.035	0.9191
50.0	5	2.310 ± 0.166	0.0477	1.416 ± 0.039	0.6765	1.372 ± 0.065	1.0000
100.0	5	2.460 ± 0.180	0.0488	1.344 ± 0.027	0.6895	1.636 ± 0.195	0.2213
Trend[d]		p = 0.6263		p = 0.9876		p = 0.6767	
Female							
Exposure Concentration (ppm)							
0	5	2.100 ± 0.376		1.055 ± 0.009		1.545 ± 0.142	
6.25	5	2.000 ± 0.092	0.7300	1.005 ± 0.016	0.9148	1.681 ± 0.086	0.8417
12.5	5	1.780 ± 0.086	0.8133	0.967 ± 0.026	0.9580	1.634 ± 0.142	0.9446
25.0	5	1.930 ± 0.291	0.8441	1.074 ± 0.043	0.9695	1.440 ± 0.149	0.9712
50.0	5	1.880 ± 0.174	0.8577	1.057 ± 0.017	0.9746	1.670 ± 0.097	0.7210
100.0	5	1.760 ± 0.110	0.8687	0.875 ± 0.023	0.9784	1.940 ± 0.403	0.3353
Trend		p = 0.8296		p = 0.9987		p = 0.2414	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[a]Study was performed at Integrated Laboratory Systems, LLC.
[b]Data are presented as mean ± standard error.
[c]Pairwise comparisons with the vehicle control group performed using the Williams or Dunn test ($p \leq 0.025$).
[d]Exposure-related trends evaluated by linear regression or the Jonckheere test ($p \leq 0.025$)

Appendix E. Supplemental Data

Tables with supplemental data can be found here: https://doi.org/10.22427/NTP-DATA-TOX-98.

E.1. Two-week Acetoin Study – Rats

E.1.1. Data Tables

E03 – Growth Curves
2072401_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
2072401_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
2072401_E05_Clinical_Observations_Summary.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
2072401_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
2072401_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)
2072401_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Sites_Systemic_Lesions_Abridged.pdf

P08 – Statistical Analysis of Primary Tumors
2072401_P08_Statistical_Analysis_of_Primary_Tumors.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
2072401_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
2072401_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
2072401_P14_Individual_Animal_Pathology_Data.pdf

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)
2072401_P17_Neoplasms_by_Individual_Animal_Systemic_Lesions_Abridged.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
2072401_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
2072401_P40_Survival_Curves.pdf

E.1.2. Individual Animal Data

Female Individual Animal Body Weight Data
2072401_Female_Individual_Animal_Body_Weight_Data.xls

Female Individual Animal Non-Neoplastic Pathology Data
2072401_Female_Individual_Animal_Non-Neoplastic_Pathology_Data.xls

Female Individual Animal Organ Weight Data
2072401_Female_Individual_Animal_Organ_Weight_Data.xls

Female Individual Animal Survival Data
2072401_Female_Individual_Animal_Survival_Data.xls

Male Individual Animal Body Weight Data
2072401_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data
2072401_Male_Individual_Animal_Non-Neoplastic_Pathology_Data.xls

Male Individual Animal Organ Weight Data
2072401_Male_Individual_Animal_Organ_Weight_Data.xls

Male Individual Animal Survival Data
2072401_Male_Individual_Animal_Survival_Data.xls

E.2. Two-week Acetoin Study – Mice

E.2.1. Data Tables

E03 – Growth Curves
2072402_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
2072402_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
2072402_E05_Clinical_Observations_Summary.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
2072402_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
2072402_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)
2072402_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Sites_Systemic_Lesions_Abridged.pdf

P08 – Statistical Analysis of Primary Tumors
2072402_P08_Statistical_Analysis_of_Primary_Tumors.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
2072402_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
2072402_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
2072402_P14_Individual_Animal_Pathology_Data.pdf

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)
2072402_P17_Neoplasms_by_Individual_Animal_Systemic_Lesions_Abridged.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
2072402_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
2072402_P40_Survival_Curves.pdf

E.2.2. Individual Animal Data

Female Individual Animal Body Weight Data
2072402_Female_Individual_Animal_Body_Weight_Data.xls

Female Individual Animal Organ Weight Data
2072402_Female_Individual_Animal_Organ_Weight_Data.xls

Female Individual Animal Survival Data
2072402_Female_Individual_Animal_Survival_Data.xls

Male Individual Animal Body Weight Data
2072402_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Organ Weight Data
2072402_Male_Individual_Animal_Organ_Weight_Data.xls

Male Individual Animal Survival Data
2072402_Male_Individual_Animal_Survival_Data.xls

E.3. Three-month Acetoin Study – Rats

E.3.1. Data Tables

E03 – Growth Curves
2072403_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
2072403_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
2072403_E05_Clinical_Observations_Summary.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
2072403_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
2072403_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)
2072403_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Sites_Systemic_Lesions_Abridged.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
2072403_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
2072403_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
2072403_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
2072403_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
2072403_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
C2072403_Organ_Weight_Summary.pdf

PA41 – Clinical Chemistry Summary
C2072403_Clinical_Chemistry_Summary.pdf

PA43 – Hematology Summary
C2072403_Hematology_Summary.pdf

E.3.2. Individual Animal Data

Female Individual Animal Body Weight Data
2072403_Female_Individual_Animal_Body_Weight_Data.xls

Female Individual Animal Non-Neoplastic Pathology Data
2072403_Female_Individual_Animal_Non-Neoplastic_Pathology_Data.xls

Female Individual Animal Organ Weight Data
2072403_Female_Individual_Animal_Organ_Weight_Data.xls

Female Individual Animal Survival Data
2072403_Female_Individual_Animal_Survival_Data.xls

Male Individual Animal Body Weight Data
2072403_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data
2072403_Male_Individual_Animal_Non-Neoplastic_Pathology_Data.xls

Male Individual Animal Organ Weight Data
2072403_Male_Individual_Animal_Organ_Weight_Data.xls

Male Individual Animal Survival Data
2072403_Male_Individual_Animal_Survival_Data.xls

Individual Animal Clinical Chemistry Data
C2072403_Individual_Animal_Clinical_Chemistry_Data.xlsx

Individual Animal Hematology Data
C2072403_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
C2072403_Individual_Animal_Organ_Weight_Data.xlsx

E.4. Three-month Acetoin Study – Mice

E.4.1. Data Tables

E03 – Growth Curves
2072404_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
2072404_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
2072404_E05_Clinical_Observations_Summary.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
2072404_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
2072404_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)
2072404_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Sites_Systemic_Lesions_Abridged.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
2072404_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
2072404_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
2072404_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
2072404_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
2072404_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
C2072404_Organ_Weights_Summary.pdf

PA43 – Hematology Summary
C2072404_Hematology_Summary.pdf

E.4.2. Individual Animal Data

Female Individual Animal Body Weight Data
2072404_Female_Individual_Animal_Body_Weight_Data.xls

Female Individual Animal Non-Neoplastic Pathology Data
2072404_Female_Individual_Animal_Non-Neoplastic_Pathology_Data.xls

Female Individual Animal Organ Weight Data
2072404_Female_Individual_Animal_Organ_Weight_Data.xls

Female Individual Animal Survival Data
2072404_Female_Individual_Animal_Survival_Data.xls

Male Individual Animal Body Weight Data
2072404_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data
2072404_Male_Individual_Animal_Non-Neoplastic_Pathology_Data.xls

Male Individual Animal Organ Weight Data
2072404_Male_Individual_Animal_Organ_Weight_Data.xls

Male Individual Animal Survival Data
2072404_Male_Individual_Animal_Survival_Data.xls

Individual Animal Hematology Data
C2072404_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
C2072404_Individual_Animal_Organ_Weight_Data.xlsx

E.5. Three-month 2,3-Pentanedione Study – Rats

E.5.1. Data Tables

E03 – Growth Curves
0801001_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0801001_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0801001_E05_Clinical_Observations_Summary.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0801001_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
0801001_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)
0801001_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Sites_Systemic_Lesions_Abridged.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0801001_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0801001_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
0801001_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0801001_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
0801001_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
C0801001_Organ_Weights_Summary.pdf

PA41 – Clinical Chemistry Summary
C0801001_Clinical_Chemistry_Summary.pdf

PA43 – Hematology Summary
C0801001_Hematology_Summary.pdf

E.5.2. Individual Animal Data

Female Individual Animal Body Weight Data
0801001_Female_Individual_Animal_Body_Weight_Data.xls

Female Individual Animal Non-Neoplastic Pathology Data
0801001_Female_Individual_Animal_Non-Neoplastic_Pathology_Data.xls

Female Individual Animal Organ Weight Data
0801001_Female_Individual_Animal_Organ_Weight_Data.xls

Female Individual Animal Survival Data
0801001_Female_Individual_Animal_Survival_Data.xls

Female Individual Clinical Observations
0801001_Female_Individual_Clinical_Observations.xls

Male Individual Animal Body Weight Data
0801001_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data
0801001_Male_Individual_Animal_Non-Neoplastic_Pathology_Data.xls

Male Individual Animal Organ Weight Data
0801001_Male_Individual_Animal_Organ_Weight_Data.xls

Male Individual Animal Survival Data
0801001_Male_Individual_Animal_Survival_Data.xls

Male Individual Clinical Observations
0801001_Male_Individual_Clinical_Observations.xls

Individual Animal Clinical Chemistry Data
C0801001_Individual_Animal_Clinical_Chemistry_Data.xlsx

Individual Animal Hematology Data
C0801001_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
C0801001_Individual_Animal_Organ_Weight_Data.xlsx

E.6. Three-month 2,3-Pentanedione Study – Mice

E.6.1. Data Tables

E03 – Growth Curves
0801002_E03_Growth_Curves.pdf

E04 – Mean Body Weights and Survival Table
0801002_E04_Mean_Body_Weights_and_Survival_Table.pdf

E05 – Clinical Observations Summary
0801002_E05_Clinical_Observations_Summary.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
0801002_P03_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal
0801002_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Sites (Systemic Lesions Abridged)
0801002_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Sites_Systemic_Lesions_Abridged.pdf

P09 – Non-Neoplastic Lesions by Individual Animal
0801002_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions
0801002_P10_Statistical_Analysis_of_Non-Neoplastic_Lesions.pdf

P14 – Individual Animal Pathology Data
0801002_P14_Individual_Animal_Pathology_Data.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades
0801002_P18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves
0801002_P40_Survival_Curves.pdf

PA06 – Organ Weights Summary
C0801002_Organ_Weights_Summary.pdf

PA43 – Hematology Summary
C0801002_Hematology_Summary.pdf

E.6.2. Individual Animal Data

Female Individual Animal Body Weight Data
0801002_Female_Individual_Animal_Body_Weight_Data.xls

Female Individual Animal Non-Neoplastic Pathology Data
0801002_Female_Individual_Animal_Non-Neoplastic_Pathology_Data.xls

Female Individual Animal Organ Weight Data
0801002_Female_Individual_Animal_Organ_Weight_Data.xls

Female Individual Animal Survival Data
0801002_Female_Individual_Animal_Survival_Data.xls

Female Individual Clinical Observations
0801002_Female_Individual_Clinical_Observations.xls

Male Individual Animal Body Weight Data
0801002_Male_Individual_Animal_Body_Weight_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data
0801002_Male_Individual_Animal_Non-Neoplastic_Pathology_Data.xls

Male Individual Animal Organ Weight Data
0801002_Male_Individual_Animal_Organ_Weight_Data.xls

Male Individual Animal Survival Data
0801002_Male_Individual_Animal_Survival_Data.xls

Male Individual Clinical Observations
0801002_Male_Individual_Clinical_Observations.xls

Individual Animal Hematology Data
C0801002_Individual_Animal_Hematology_Data.xlsx

Individual Animal Organ Weight Data
C0801002_Individual_Animal_Organ_Weight_Data.xlsx

E.7. Genetic Toxicology

E.7.1. Acetoin Micronucleus Study in Wistar Han Rats

G04 – In Vivo Micronucleus Summary Data
G99018_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G99018_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.7.2. Acetoin Micronucleus Study in B6C3F1 Mice

G04 – In Vivo Micronucleus Summary Data
G99018B_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G99018B_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.7.3. Acetoin *Salmonella/E.coli* Mutagenicity Test or Ames Test

G06 – Ames Summary Data
A17696_G06_Ames_Summary_Data.pdf

E.7.4. 2,3-Pentanedione Micronucleus Study in Wistar Han Rats

G04 – In Vivo Micronucleus Summary Data
G08010_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G08010_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.7.5. 2,3-Pentanedione Micronucleus Study in B6C3F1 Mice

G04 – In Vivo Micronucleus Summary Data
G08010B_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G08010B_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

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