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

NTP Technical Report on the Toxicity Studies of o-Phthalaldehyde (CASRN 643-79-8) Administered by Inhalation to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice

NTP TOX 84

JANUARY 2018

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Toxicity Report 84

January 2018

National Toxicology Program
Public Health Service
U.S. Department of Health and Human Services
ISSN: 2378-8992

Research Triangle Park, North Carolina, USA

Foreword

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

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

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

NTP Technical Reports are indexed in the National Center for Biotechnology Information (NCBI) Bookshelf and are available free of charge electronically on the NTP website (http://ntp.niehs.nih.gov). Additional information regarding this study may be requested through Central Data Management (CDM) at cdm@niehs.nih.gov. Toxicity data are available through NTP's Chemical Effects in Biological Systems (CEBS) database: https://www.niehs.nih.gov/research/resources/databases/cebs/index.cfm.

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This report has been reformatted to meet new NTP publishing requirements;
its content has not changed.

About This Report

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The draft *NTP Technical Report on the Toxicity Studies of o-Phthalaldehyde (CASRN 643-79-8) Administered by Inhalation to Sprague Dawley (Hsd:Sprague® Dawley SD®) Rats and B6C3F1/N Mice* was evaluated by the reviewers listed below. These reviewers served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determined if the design and conditions of these NTP studies were appropriate and ensured that this Toxicity Study Report presented the experimental results and conclusions fully and clearly.

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Publication Details

Publisher: National Toxicology Program

Publishing Location: Research Triangle Park, NC

ISSN: 2378-8992

DOI: https://doi.org/10.22427/NTP-TOX-84

Report Series: NTP Toxicity Report Series

Report Series Number: 84

Official citation: National Toxicology Program (NTP). 2018. NTP technical report on the toxicity studies of *o*-phthaladehyde (CASRN 643-79-8) administered by inhalation to Sprague Dawley (Hsd:Sprague Dawley® SD®) rats and B6C3F1/N mice. Research Triangle Park, NC: National Toxicology Program. Toxicity Report 84.

Abstract

o-Phthalaldehyde is a high-level chemical disinfectant that is commonly used for disinfection of dental and medical instruments as an alternative to glutaraldehyde, which is a known skin and respiratory sensitizer. *o*-Phthalaldehyde was nominated by the National Institute for Occupational Safety and Health for toxicologic characterization based on its proposed use as a safer alternative to glutaraldehyde for chemical disinfection, its increasing use, the lack of adequate and publicly available toxicologic data, and because many human case reports document incidences of skin and respiratory sensitization following occupational exposure. Inhalation was chosen as the route of exposure for these studies because inhalation is a major route of human occupational exposure. Male and female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats and B6C3F1/N mice were exposed to *o*-phthalaldehyde (99.7% pure) by whole-body inhalation for 3 months.

Groups of 10 male and 10 female rats and mice were exposed to *o*-phthalaldehyde at concentrations of 0, 0.44, 0.88, 1.75, 3.5, or 7.0 ppm, 6 hours plus T_{90} (17 minutes) per day, 5 days per week for 14 weeks; additional groups of 10 male and 10 female clinical pathology study rats were exposed to the same concentrations for 23 days.

All rats exposed to 7.0 ppm died by the end of week 2 of the study, and seven males and two females exposed to 3.5 ppm died by week 7 of the study. All mice exposed to 7.0 ppm died during week 1 of the study, and five males and four females exposed to 3.5 ppm died by week 6 of the study. Clinical observations in rats and mice included abnormal breathing, sneezing, and thinness, with increasing frequency in higher exposure groups. In rats, clinical observations also included black discoloration of the appendages (pinnae and/or feet), which was noted throughout the study in male and female rats exposed to 3.5 ppm or greater. Clinical observations in mice also included alopecia. Mean body weights of all surviving exposed groups of male rats and 1.75 and 3.5 ppm female rats were significantly less than those of the chamber controls. Mean body weights of all surviving exposed groups of male and female mice were significantly less than those of the chamber controls, and 3.5 ppm males lost weight during the study.

In the hematopoietic system of rats, decreases in lymphocyte counts in males and females coincided with increases in neutrophil counts. These alterations in lymphocyte and neutrophil counts were consistent with stress and inflammation. Decreased lymphocyte counts corresponded to lymphoid atrophy in the thymus and spleen. Within the erythron, the erythrocyte counts, hemoglobin concentrations, hematocrit values, and packed cell volumes were significantly elevated in both male and female rats at all time points. Erythron increases at the earlier time points were consistent with a physiological hemoconcentration, while increases at study termination may have been due to hypoxia with a resultant secondary erythrocytosis.

In the hematopoietic system of mice, the total leukocyte and lymphocyte counts, as well as neutrophil and eosinophil counts, were increased in males at study termination. Similarly, female mice had increased total leukocyte, neutrophil, and eosinophil counts. The increases in the leukon were generally consistent with inflammation. Hemoglobin concentrations, erythrocyte counts, hematocrit values, and packed cell volumes were decreased in male and female mice. The decreases in the erythron were most likely due to bone marrow suppression as a result of the chronic inflammation in the respiratory tract.

Inhalation exposure to *o*-phthalaldehyde resulted in a spectrum of lesions at sites of contact within the respiratory tract (nose, larynx, trachea, and lung), skin, and eye that were generally consistent with an irritant effect. In general, exposure of rats and mice to *o*-phthalaldehyde resulted in lesions throughout the respiratory tract that included necrosis, inflammation, regeneration, hyperplasia, and metaplasia, ranging from minimal to moderate in severity. In general, histologic findings occurred at deeper sites within the respiratory tract with increasing exposure concentration. The first site of contact, the nose, was most affected, with many lesions occurring at the lowest exposure concentration (0.44 ppm) in male and female rats and mice. Laryngeal lesions occurred at all exposure concentrations in rats and at 0.88 ppm or greater in mice. Tracheal findings were first noted at a variety of exposure concentrations. Lung findings were most prevalent at the two highest exposure concentrations (3.5 and 7.0 ppm) in rats and mice. In the skin, there were significant increases in adnexa degeneration and epithelial parakeratosis in both male and female rats and mice. In the eye, there were significant increases in suppurative inflammation of the anterior chamber and cornea, as well as corneal necrosis in male and female rats.

Rats exposed to *o*-phthalaldehyde exhibited lower cauda epididymis, epididymis, and testis weights. In rats, total sperm/cauda exhibited a negative trend and sperm motility was lower. There were no histopathologic correlates identified that could explain the observed responses in sperm parameters, or the weight changes in the testis or epididymis. However, in the higher dose groups where morbidity and mortality were observed, testicular and epididymal histopathologic lesions were noted. In the testes, these lesions included significant increases in the incidences of elongated spermatid degeneration, apoptosis of the germinal epithelium, and interstitial cell atrophy. In the epididymis of male rats, there were significant increases in the incidences of exfoliated germ cells and apoptosis of the epithelium. The mice also displayed decreased sperm motility, and some testicular and epididymal histopathologic lesions, including significantly increased incidences of exfoliated germ cells of the epididymal duct, as well as multifocal cellular depletion of the germinal epithelium, and interstitial cell atrophy of the testis.

o-Phthalaldehyde was mutagenic in *Salmonella typhimurium* strain TA100 in the absence of exogenous metabolic activation (S9 mix); no mutagenicity was seen in TA100 with S9 or in TA98 or *Escherichia coli* WP2 *uvrA*/pKM101, with or without S9. Following 3 months of inhalation exposure to *o*-phthalaldehyde, no increases in the frequencies of micronucleated reticulocytes were observed in male or female Sprague Dawley rats. In B6C3F1/N mice following 3 months of inhalation exposure to *o*-phthalaldehyde, a small increase in micronucleated reticulocytes was seen in male mice exposed to 3.5 ppm, but no significant increases in micronuclei were seen in erythrocytes of male mice or in reticulocytes or erythrocytes of female mice. A small increase in the percentage of reticulocytes was seen in female mice at the highest dose tested (3.5 ppm).

Under the conditions of these 3-month inhalation studies, there were treatment-related lesions in male and female rats and mice. The major targets from *o*-phthalaldehyde exposure in rats and mice included the respiratory system (nasal cavity, larynx, trachea, and lung), skin, eye, testis, and epididymis. The most sensitive measure of *o*-phthalaldehyde inhalation toxicity in male and female rats and mice was significantly increased incidences of nasal cavity lesions (lowest-observable-effect concentration = 0.44 ppm). A no-observed-effect concentration was not reached in rats or mice of either sex.

Synonyms: 1,2-Benzenedicarboxaldehyde; *o*-phthaldialdehyde; phthalic aldehyde

Summary of Findings Considered Toxicologically Relevant in Rats and Mice Exposed to *o*-Phthalaldehyde for Three Months[a]

	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Exposure concentrations	0, 0.44, 0.88, 1.75, 3.5, 7.0 ppm	0, 0.44, 0.88, 1.75, 3.5, 7.0 ppm	0, 0.44, 0.88, 1.75, 3.5, 7.0 ppm	0, 0.44, 0.88, 1.75, 3.5, 7.0 ppm
Survival rates	10/10, 10/10, 10/10, 10/10, 3/10, 0/10	10/10, 10/10, 10/10, 10/10, 8/10, 0/10	10/10, 10/10, 10/10, 10/10, 5/10, 0/10	10/10, 10/10, 10/10, 10/10, 6/10, 0/10
Clinical findings	Abnormal breathing, sneezing, and thinness	Abnormal breathing, sneezing, and thinness	Abnormal breathing, sneezing, thinness, and alopecia	Abnormal breathing, sneezing, thinness, and alopecia
Body weights	0.44, 0.88, 1.75, and 3.5 ppm groups less than chamber control group	1.75 and 3.5 ppm groups less than chamber control group	0.44, 0.88, 1.75, and 3.5 ppm groups less than chamber control group	0.44, 0.88, 1.75, and 3.5 ppm groups less than chamber control group
Organ weights	↓ Cauda epididymis ↓ Epididymis ↓ Testis	None	None	None
Hematology				
Rats (Day 3, Day 23, Week 14) Mice (Week 14)	Leukocytes (↓,↓,↓); Lymphocytes (↓,↓,↓); Segmented neutrophils (↑,↑,↑); Erythrocytes (↑,↑,–); Hemoglobin (↑,↑,↑); Mean cell volume (↓,↓,–); Packed cell volume (↑,↑,↑); Hematocrit (↑,↑,↑); Platelets (↑,↓,↓); Reticulocytes (↑,–,–)	Leukocytes (↓,–,–); Lymphocytes (↓,↓,↓); Segmented neutrophils (↑,↑,↑); Erythrocytes (↑,↑,↑); Hemoglobin (↑,↑,↑); Mean cell volume (↓,–,–); Packed cell volume (↑,↑,↑); Hematocrit (↑,↑,↑); Platelets (↑,↓,↓); Reticulocytes (↑,↑,–)	Leukocytes (↑); Lymphocytes (↑); Segmented neutrophils (↑); Eosinophils (↑); Erythrocytes (↓); Hemoglobin (↓); Packed cell volume (↓); Hematocrit (↓); Mean cell hemoglobin (↓)	Leukocytes (↑); Segmented neutrophils (↑); Eosinophils (↑); Erythrocytes (↓); Hemoglobin (↓); Packed cell volume (↓); Hematocrit (↓); Mean cell hemoglobin (↓)
Clinical chemistry				
(Day 3, Day 23, Week 14)	Total protein (↑,↑,–); Albumin (↑,–,–); Globulin (↑,–,–); Alkaline phosphatase (↓,–,↑);Alanine aminotransferase (↓,↑,↑); Bile acids (↓,–,↑); Sorbitol dehydrogenase (↑,–,–);Urea nitrogen (↑,↑,–); Cholesterol (↓,–,↓); Triglycerides (↓,–,–); Glucose (↑,↑,↑); Creatinine kinase (–,↑,↑)	Total protein (↑,–,–); Albumin (–,–,↓); Albumin/globulin ratio (↓,↓,↓); Globulin (↑,–,↑); Alkaline phosphatase (↓,↑,↑); Alanine aminotransferase (↓,–,↑); Bile acids (–,–,↑); Sorbitol dehydrogenase (↑,–,–); Urea nitrogen (↑,↑,–); Cholesterol (↓,–,–); Triglycerides (–,–,↑); Glucose (↑,↑,–); Creatinine kinase (–,↑,↑)	Not assessed	Not assessed
Reproductive effects	↓ Total sperm/cauda ↓ Sperm motility	Not assessed	↓ Sperm motility	Not assessed

	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Nonneoplastic effects	Nose: inflammation, suppurative (0/10, 10/10, 10/10, 10/10, 10/10, 10/10); olfactory epithelium, atrophy (2/10, 10/10, 10/10, 10/10, 7/10, 6/10); olfactory epithelium, metaplasia, respiratory (1/10, 1/10, 6/10, 6/10, 2/10, 0/10); olfactory epithelium, regeneration (0/10, 0/10, 1/10, 0/10, 4/10, 0/10); respiratory epithelium, hyperplasia (3/10, 9/10, 9/10, 7/10, 3/10, 0/10); respiratory epithelium, squamous metaplasia (0/10, 10/10, 10/10, 10/10, 10/10, 8/10); respiratory epithelium, necrosis (0/10, 0/10, 3/10, 5/10, 10/10, 10/10); respiratory epithelium, regeneration (0/10, 0/10, 1/10, 0/10, 3/10, 6/10); turbinate, atrophy (0/10, 0/10, 7/10, 10/10, 0/10, 0/10) Larynx: inflammation, chronic active (1/10, 2/10, 8/10, 10/10, 10/10, 10/10); metaplasia, squamous (0/10, 1/10, 8/10, 10/10, 10/10, 10/10); necrosis (0/10, 0/10, 1/10, 5/10, 9/10, 10/10); regeneration (0/10, 0/10, 3/10, 2/10, 2/10, 6/10) Trachea: fibrosis (0/10, 0/10 0/10, 5/10, 3/10, 0/10); inflammation, chronic active (0/10, 0/10, 4/10, 8/10, 9/10, 10/10); metaplasia, squamous (0/10, 0/10, 4/10, 10/10, 6/10, 6/10); necrosis (0/10, 0/10, 0/10, 3/10, 8/10, 8/10); regeneration (0/10, 0/10, 0/10, 7/10, 7/10, 6/10)	Nose: inflammation, suppurative (0/10, 9/10, 10/10, 10/10, 10/10, 10/10); olfactory epithelium, atrophy (0/10, 9/10, 10/10, 10/10, 10/10, 7/10); olfactory epithelium, metaplasia, respiratory (0/10, 1/10, 2/10, 5/10, 7/10, 1/10); respiratory epithelium, hyperplasia (0/10, 9/10, 7/10, 4/10, 3/10, 3/10); respiratory epithelium, squamous metaplasia (0/10, 10/10, 10/10, 10/10, 10/10, 10/10); respiratory epithelium, necrosis (0/10, 2/10, 6/10, 9/10, 10/10, 10/10); respiratory epithelium, regeneration (0/10, 0/10, 2/10, 0/10, 1/10, 3/10); turbinate, atrophy (0/10, 2/10, 4/10, 10/10, 10/10, 0/10) Larynx: inflammation, chronic active (0/10, 1/10, 1/10, 9/10, 10/10, 10/10); metaplasia, squamous (0/10, 1/10, 4/10, 10/10, 10/10, 10/10); necrosis (0/10, 0/10, 0/10, 1/10, 7/10, 8/10) Trachea: fibrosis (0/10, 0/10, 0/10, 2/10, 6/10, 0/10); inflammation, chronic active (0/10, 0/10, 3/10, 5/10, 10/10, 10/10); metaplasia, squamous (0/10, 0/10, 3/10, 10/10, 7/10, 7/10); necrosis (0/10, 0/10, 0/10, 3/10, 3/10, 8/10); regeneration (0/10, 0/10, 1/10, 7/10, 10/10, 9/10)	Nose: inflammation, suppurative (0/10, 10/10, 10/10, 10/10, 10/10, 10/10); glands, olfactory epithelium, hyperplasia (0/10, 10/10, 10/10, 7/10, 6/10, 0/10); olfactory epithelium, atrophy (0/10, 10/10, 10/10, 10/10, 10/10, 10/10); olfactory epithelium, metaplasia, respiratory (0/10, 1/10, 8/10, 10/10, 4/10, 0/10); respiratory epithelium, metaplasia, squamous (0/10, 10/10, 10/10, 10/10, 10/10, 6/10); respiratory epithelium, necrosis (0/10, 2/10, 6/10, 5/10, 9/10, 10/10); respiratory epithelium, regeneration (0/10, 0/10, 1/10, 0/10, 1/10, 4/10); turbinate atrophy (0/10, 4/10, 6/10, 10/10, 8/10, 0/10) Larynx: inflammation, chronic active (0/10, 0/10, 0/10, 4/10, 10/10, 10/10); metaplasia, squamous (0/10, 0/10, 0/10, 3/10, 10/10, 3/10; necrosis (0/10, 0/10, 0/10, 0/10, 1/10, 10/10) Trachea: inflammation, chronic active (0/10, 0/10, 0/10, 1/10, 9/10, 10/10); metaplasia, squamous (0/10, 0/10, 0/10, 3/10, 10/10, 3/10); necrosis (0/10, 0/10, 0/10 0/10, 0/10, 9/10)	Nose: inflammation, suppurative (0/10, 10/10, 10/10, 10/10, 10/10, 6/10); glands, olfactory epithelium, hyperplasia (0/10, 10/10, 9/10, 10/10, 8/10, 0/10); olfactory epithelium, atrophy (0/10, 10/10, 10/10, 10/10, 9/10, 9/10); olfactory epithelium, metaplasia, respiratory (0/10, 3/10, 6/10, 3/10, 4/10, 0/10); respiratory epithelium, metaplasia, squamous (0/10, 10/10, 10/10, 10/10, 8/10, 0/10); respiratory epithelium, necrosis (0/10, 2/10, 7/10, 6/10, 8/10, 10/10); respiratory epithelium, regeneration (0/10, 0/10, 0/10, 1/10, 3/10, 6/10); turbinate atrophy (0/10, 7/10, 9/10, 10/10, 7/10, 0/10) Larynx: inflammation, chronic active (0/10, 0/10, 0/10, 0/10, 9/10, 10/10); metaplasia, squamous (0/10, 0/10, 3/10, 10/10, 10/10, 8/10); necrosis (0/10, 0/10, 0/10, 0/10, 3/10, 9/10) Trachea: inflammation, chronic active (0/10, 0/10, 0/10, 0/10, 10/10, 10/10); metaplasia, squamous (0/10, 0/10, 0/10, 0/10, 9/10, 2/10); necrosis (0/10, 0/10, 0/10, 0/10, 2/10, 10/10)

	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Nonneoplastic effects (continued)	Lung: alveolus, inflammation, suppurative (0/10, 0/10, 0/10, 0/10, 3/10, 6/10); bronchus, fibrosis (0/10, 0/10, 0/10, 1/10, 2/10, 1/10); bronchus, hyperplasia (0/10, 0/10, 0/10, 2/10, 5/10, 1/10); bronchus, inflammation, chronic active (0/10, 0/10, 0/10, 3/10, 9/10, 8/10); bronchus, metaplasia, squamous (0/10, 0/10, 0/10, 7/10, 6/10, 1/10); bronchus, necrosis (0/10, 0/10, 0/10, 0/10, 5/10, 7/10) Skin: adnexa, degeneration (0/10, 0/10, 0/10, 0/10, 4/10, 10/10); epithelium, hair follicle, parakeratosis (0/10, 7/10, 4/10, 7/10, 3/10, 10/10) Eye: anterior chamber, inflammation, suppurative (0/10, 0/10, 0/10, 0/10, 4/10, 5/10); cornea, inflammation, suppurative (1/10, 1/10, 1/10, 1/10, 5/10, 6/10); cornea, necrosis (0/10, 0/10, 1/10, 0/10, 2/10, 4/10) Epididymis: duct, exfoliated germ cell (0/10, 0/10, 1/10, 1/10, 5/10, 4/10); epithelium, apoptosis (0/10, 0/10, 0/10, 0/10, 3/10, 5/10) Testis: elongated spermatid, degeneration (0/10, 0/10, 0/10, 0/10, 4/10, 0/10); germinal epithelium, apoptosis (0/10, 0/10, 0/10, 0/10, 4/10, 6/10); interstitial cell, atrophy (0/10, 0/10 0/10, 0/10, 7/10, 10/10); seminiferous tubule, vacuolation (0/10, 0/10, 0/10, 0/10, 3/10, 0/10)	Lung: alveolus, inflammation, suppurative (0/10, 0/10, 0/10, 0/10, 2/10, 4/10); bronchus, fibrosis (0/10, 0/10, 0/10, 1/10, 1/10, 3/10); bronchus, hyperplasia (0/10, 0/10, 0/10, 1/10, 6/10, 1/10); bronchus, inflammation, chronic active (0/10, 0/10, 0/10, 2/10, 10/10, 10/10); bronchus, metaplasia, squamous (0/10, 0/10, 0/10, 2/10, 9/10, 0/10); bronchus, necrosis (0/10, 0/10, 0/10, 0/10, 2/10, 10/10); bronchus, regeneration (0/10, 0/10, 0/10, 0/10, 1/10, 5/10) Skin: adnexa, degeneration (0/10, 1/10, 0/10, 0/10, 1/10, 10/10); epithelium, hair follicle, parakeratosis (0/10, 1/10, 1/10, 3/10, 3/10, 6/10) Eye: anterior chamber, inflammation, suppurative (0/10, 0/10, 0/10, 0/10, 1/10, 5/10); cornea, inflammation, suppurative (0/10, 0/10, 1/10, 0/10, 1/10, 8/10); cornea, necrosis (0/10, 0/10, 0/10, 0/10, 0/10, 7/10)	Lung: bronchus, inflammation, chronic active (0/10, 0/10, 0/10, 0/10, 1/10, 6/10); bronchus, necrosis (0/10, 0/10, 0/10, 0/10, 2/10, 9/10) Skin: hyperplasia, squamous (0/10, 1/10, 0/10, 0/9, 9/10, 4/10); inflammation, chronic active (0/10, 0/10, 0/10, 1/9, 10/10, 9/10); adnexa, degeneration (0/10, 2/10, 0/10, 4/9, 1/10, 6/10); epithelium, hair follicle, parakeratosis (0/10, 3/10, 4/10, 2/9, 2/10, 10/10) Eye: cornea, inflammation, suppurative (0/10, 1/10, 0/10, 0/10, 1/10, 4/10) Epididymis: duct, exfoliated germ cell (0/10, 0/10, 0/10, 0/10, 4/10, 1/10); Testis: germinal epithelium, depletion, cellular, multifocal (0/10, 1/10, 1/10, 0/10, 7/10, 0/10); interstitial cell, atrophy (0/10, 0/10, 0/10, 0/10, 3/10, 0/10)	Lung: bronchus, inflammation, chronic active (0/10, 0/10, 0/10, 0/10, 6/10, 8/10); bronchus, necrosis (0/10, 0/10, 0/10, 0/10, 2/10, 8/10) Skin: hyperplasia, squamous (3/10, 0/10, 0/10, 6/10, 9/10, 6/10); inflammation, chronic active (1/10, 1/10, 3/10, 9/10, 9/10, 8/10); adnexa, degeneration (2/10, 0/10, 0/10, 1/10, 0/10, 9/10); epithelium, hair follicle, parakeratosis (0/10, 3/10, 7/10, 9/10, 10/10, 10/10)

	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Genetic toxicology				
Bacterial gene mutations (in vitro):	Positive in Salmonella typhimurium strain TA100 in the absence of exogenous metabolic activation. Negative in Salmonella typhimurium strain TA100 with exogenous activation and in TA98 and Escherichia coli WP2 uvrA/pKM101 with and without exogenous activation.			
Micronucleated reticulocytes (in vivo):				
Rat	Negative in males and females.			
Mouse	Equivocal in males and negative in females.			

[a]↑, ↓, and – represent an increase, a decrease, and no biologically significant change in a parameter, respectively, compared to the chamber control group.

Introduction

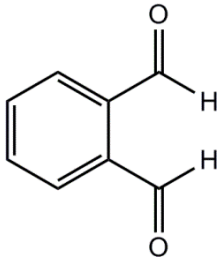

Figure 1. *o*-Phthalaldehyde (CASRN 643-79-8; Chemical Formula: $C_8H_6O_2$; Molecular Weight: 134.13)

Synonyms: 1,2-Benzenedicarboxaldehyde; *o*-phthaldialdehyde; phthalic aldehyde.

Chemical and Physical Properties

o-Phthalaldehyde is a pale yellow crystalline solid with a specific gravity of 0.63 ± 0.10 g/mL at 20°C, a melting point of 55° to 58°C, a boiling point of 266° ± 23°C at 760 mm Hg, and a vapor pressure of 0.0052 mm Hg at 21°C[1; 2]. The solubility of *o*-phthalaldehyde is 3 g/100 mL diisopropyl ether, 5 g/100 mL deionized water, 20 g/100 mL chloroform, or 20 g/100 mL acetone at 20°C.

Production, Use, and Human Exposure

A variety of processes for manufacturing *o*-phthalaldehyde have been reported in the literature. *o*-Phthalaldehyde is produced by heating pure benzaldehyde and chloroform with potassium hydroxide solution[3]. The resulting solution is further acidified with hydrochloric acid and cooled to yield a colorless powder of *o*-phthalaldehyde. *o*-Phthalaldehyde is also produced by ozonization of naphthalene in alcohol followed by catalytic hydrogenation[4]. Catalytic oxidation of various chemicals is also used in manufacturing *o*-phthalaldehyde. *o*-Phthalaldehyde can be manufactured by oxidation of phthalan by nitrogen monoxide in acetonitrile with *N*-hydroxyphthalimide as the catalyst to yield 80% to 90% *o*-phthalaldehyde[5]. Also, 76% to 99.9% *o*-phthalaldehyde can be produced by oxidation of *α*-dichloro-*o*-xylene with aqueous nitric acid solution in the presence of vanadium pentoxide as the catalyst[6]. Oxidation of *o*-xylylene oxide and/or *o*-xylylene glycol with nitric acid solution followed by a purification step yields 35% to 72% *o*-phthalaldehyde[7]. In addition, oxidation of *o*-phthalaldehyde tetraalkyl acetals by an electrochemical technique is also used to produce *o*-phthalaldehyde[8; 9].

o-Phthalaldehyde is mainly used as a high-level disinfectant (a low-temperature chemical method) for heat-sensitive medical and dental equipment such as endoscopes and thermometers[10]; in recent years, it has gained popularity as a safe and better alternative to glutaraldehyde. Glutaraldehyde is a widely used chemical disinfectant for heat-sensitive equipment, but it has been associated with health effects such as dermatitis and occupational asthma[11]. Along with its increasing popularity as a chemical sterilizer, *o*-phthalaldehyde has many applications in analytical methods and in diagnostic kits. *o*-Phthalaldehyde is also used as an intermediate in the synthesis of pharmaceuticals and as a reagent in the tanning industry, hair colorings, wood treatment, and antifouling paints. *o*-Phthalaldehyde was approved for use as an

indoor antimicrobial pesticide in 1997; however, it is no longer registered with the United States Environmental Protection Agency (USEPA) for this use[12].

The primary routes of human exposure to *o*-phthalaldehyde are by inhalation and through the skin, which may occur through accidental or occupational exposures. Many case reports document accidental exposure of patients to *o*-phthalaldehyde through use of medical equipment (e.g., transesophageal electroradiography probes and colonoscopes) that have been sterilized with the compound and inadequately rinsed. Occupational exposure to *o*-phthalaldehyde vapor or liquid occurs primarily in the health care industry, during chemical sterilization of medical equipment in hospital settings. *o*-Phthalaldehyde has been detected in air samples from several endoscopy units (1.0 to 13.5 ppb) in hospitals in Italy and the United States[13; 14]. Data from the 1981 to 1983 National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health[15] indicates that 3,253 workers such as geologists, geodesists, clinical laboratory technologists and technicians, engineering technicians, and chemical technicians were potentially exposed to *o*-phthalaldehyde in the workplace. During the same period, more than 318,362 workers were potentially exposed to glutaraldehyde in the healthcare field alone. Based on the rising popularity of *o*-phthalaldehyde as a replacement for glutaraldehyde, it is reasonable to assume that more than 300,000 healthcare workers are currently being exposed to *o*-phthalaldehyde. In 2009 and 2010, the Health Hazard Evaluation Program through the Centers for Disease Control and Prevention (CDC) and NIOSH, which evaluated eight health care facilities across the country for *o*-phthalaldehyde-specific health practices, assessed the exposures and potential health risks of employees to *o*-phthalaldehyde[16]. Across the eight facilities, the full shift, time-weighted average *o*-phthalaldehyde concentrations ranged from not detected (ND) to 38 $\mu g/m^3$ (0.007 ppm), which is higher than the range that was measured in the control group [ND to 0.67 $\mu g/m^3$ (0.0001 ppm)].

Regulatory Status

o-Phthalaldehyde is commercially available as Cidex® OPA concentrate (5.75% *o*-phthalaldehyde) or Cidex® OPA ready to use 0.55% aqueous solution (0.55% *o*-phthalaldehyde and 99.45% water) (Advanced Sterilization Products, Irvine, CA). Other solutions containing *o*-phthalaldehyde as a sterilization ingredient include Opaciden Solution (0.6%) (Ciden Technologies, LLC) and Rapicide OPA-28 (0.575%) (Minntech Corporation)[17]. *o*-Phthalaldehyde is also listed as 1,2-benzenedicarboxaldehyde in the active ingredients of UCARCIDE™ P200 Antimicrobial (99.8%) (Dow Chemical Company, Midland, MI) and Cidex® OPA Antimicrobial (99.7%) (Johnson & Johnson Medical, Inc.). The product registrations as pesticides with the USEPA for both the UCARCIDE™ P200 and Cidex® OPA Antimicrobial were cancelled in 2005 and 1998, respectively.

Currently, the above commercial products containing *o*-phthalaldehyde (0.55% to 5.75%) have received United States Food and Drug Administration market approval as high-level chemical disinfectants for chemical sterilization of medical devices. With regard to occupational exposures to *o*-phthalaldehyde, there are no existing Occupational Safety and Health Administration (OSHA) or NIOSH recommendations or promulgated standards for exposure limits to the chemical in occupational settings. Similarly, no recommended guidelines have been published by the American Conference of Governmental Industrial Hygienists (ACGIH) for *o*-phthalaldehyde. Though there is no personal exposure limit established by OSHA for *o*-phthalaldehyde,

glutaraldehyde has a NIOSH Recommended Exposure Limit of 0.2 ppm (0.8 mg/m^3) and the ACGIH recommends a Threshold Limit Value of 0.05 ppm in air[18].

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Limited information on the disposition and metabolism of *o*-phthalaldehyde in experimental animals was found in the literature. In male rats (strain not specified) following intratracheal or oral administration of [^{14}C]-*o*-phthalaldehyde, the absorption was rapid with peak plasma concentrations occurring at 0.8 and 1.7 hours, respectively[19]. The radioactivity was cleared at a similar rate following the two routes of exposure with a terminal phase half-life of approximately 94 hours. The excretion in urine and feces was 44% and 11%, respectively, 72 hours after intratracheal administration. Urinary excretion following oral administration was similar to intratracheal administration with 43% of the radiolabel excreted 72 hours after dosing; feces contained 50% of the radiolabel, suggesting incomplete absorption following oral administration. In addition, residual radioactivity in carcasses following intratracheal and oral administration was 41% and 2%, respectively. The major urinary products identified were 2-carboxybenzaldehyde (phthalaldehydic acid) (33%) and phthalic anhydride (4%); parent *o*-phthalaldehyde was not detected.

Humans

No data describing the in vivo fate of *o*-phthalaldehyde in humans were found in a review of the literature.

Toxicity

Experimental Animals

The oral LD_{50} of a commercially used solution containing 0.55% *o*-phthalaldehyde (Cidex® OPA) was reported to be greater than 5,000 mg/kg in rats[20]. The LD_{50} in mice after intraperitoneal injection of a 99.8% *o*-phthalaldehyde solution was 27 mg/kg[21] and the dermal LD_{50} of Cidex® OPA was reported to be greater than 2,000 mg/kg in rabbits[20]. Animal toxicity data are limited and no acute, subchronic, or chronic toxicity studies of *o*-phthalaldehyde are reported in the scientific literature. In rats, the no-observed-effect level of *o*-phthalaldehyde has been reported to be 5 mg/kg per day following oral administration; however, experimental details of the reporting study have not been published in the literature[20].

The immunotoxicity of *o*-phthalaldehyde has been investigated in numerous studies in mice. ICR mice were injected subcutaneously with 0.0025%, 0.0125%, 0.025%, 0.125% or 0.25% *o*-phthalaldehyde with or without ovalbumin (OVA) on days 0 and 5 and challenged with 10 µg of OVA on days 18 or 19[22]. Increased neutrophil infiltration in the bronchoalveolar fluid and production of OVA-specific IgE in *o*-phthalaldehyde-treated mice indicated that it is an effective immunological adjuvant. Furthermore, based on increases in IL-4 and IL-5 gene expression, the authors concluded that *o*-phthalaldehyde induces Th2-type immune responses. In another study, ICR mice were injected subcutaneously twice on days 0 and 5 with 0.125%, 0.25%, 0.3125%, 0.375%, or 0.5% *o*-phthalaldehyde and observed for 18 days[23]. The animals developed systemic

inflammation and liver injury due to acute toxicity. *o*-Phthalaldehyde acted as a hapten and induced immunological responses. A significant dose–dependent increase in *o*-phthalaldehyde-specific IgG and IgE was observed in the serum of mice treated with repeat doses of *o*-phthalaldehyde.

Dermal sensitization studies conducted in female Balb/c mice identified *o*-phthalaldehyde as an irritant and contact sensitizer[24]. The sensitizing potency of *o*-phthalaldehyde measured by the ear swelling test and local lymph node assay (LLNA) was demonstrated to be similar to the sensitizing potency of glutaraldehyde. In the LLNA, statistically significant increases in lymph node cell proliferation were observed with the top two test concentrations (0.1% and 0.75%). An EC3 value (the estimated concentration required to induce a threefold or greater increase in proliferative activity compared with concurrent vehicle treated controls) of 0.051% was calculated, suggesting that *o*-phthalaldehyde is a strong sensitizer. Mechanistically, *o*-phthalaldehyde exposure resulted in increased numbers of IgE-positive B cells in the draining lymph nodes of *o*-phthalaldehyde-treated mice. Increased serum levels of total IgE, *o*-phthalaldehyde-specific IgE and *o*-phthalaldehyde-specific IgG1, and OVA-specific serum IgE were found in *o*-phthalaldehyde-treated mice. Based on emerging incidences of bronchial asthma in hospital workers exposed to *o*-phthalaldehyde[25], another study evaluated the respiratory sensitization potential of *o*-phthalaldehyde following nose-only inhalation exposure in female C57Bl/6 mice[26]. The study demonstrated that inhalation exposure to 500 ppb or greater *o*-phthalaldehyde resulted in *o*-phthalaldehyde-specific IgG1. *o*-Phthalaldehyde-specific IgE was not detected in the exposed mice. Cytokine gene expression and phenotyping of the lymphocyte cell population in the respiratory mucosa and draining lymph nodes indicated that *o*-phthalaldehyde has the potential to cause respiratory sensitization in mice. A study by Katagiri et al.[27] investigated the potential neurotoxic effects of *o*-phthalaldehyde in female Wistar rats. The rats were exposed to 100 or 200 ppb *o*-phthalaldehyde by whole-body inhalation 5 days per week for 4 weeks. Dopamine levels were significantly decreased (approximately 50%) in the cerebrum but not in the other regions of the brain.

Humans

Since its introduction to the market in the United States in 1999 for use as a high-level disinfectant, numerous case reports have associated *o*-phthalaldehyde with a variety of adverse health effects. Some of the unintentional human health effects reported by patients directly exposed to *o*-phthalaldehyde are discoloration of the oral and esophageal mucosa[28], and chemical burning of the esophagus[29]. Four bladder cancer patients undergoing urologic procedures experienced nine episodes of anaphylactic shock that included urticaria, angioedema, and laryngeal edema[30]. Skin testing of these patients strongly confirmed the role of *o*-phthalaldehyde in the observed anaphylactic reactions. In another report, anaphylaxis was reported in three patients following a laryngoscopic procedure; detection of *o*-phthalaldehyde-specific IgE in serum and in vitro histamine release tests identified *o*-phthalaldehyde as the allergen[31].

A number of health problems have been reported by healthcare workers routinely using *o*-phthalaldehyde in occupational settings for cleaning endoscopes and devices for surgical procedures. Very low levels of *o*-phthalaldehyde have been reported to cause skin, eye, and respiratory symptoms. Cases of bronchial asthma and contact dermatitis associated with occupational exposures to *o*-phthalaldehyde have been frequently reported[25; 32; 33]. In a study investigating adverse health effects in 70 healthcare workers in hospital endoscopy units using

o-phthalaldehyde, 17 workers were diagnosed with contact dermatitis, occupational asthma, or eye irritation[34]. A urology resident developed facial discoloration after a cystoscopy performed using *o*-phthalaldehyde-disinfected instruments[35]. In contrast to these reports, the Health Hazard Evaluation Program run by the CDC and NIOSH did not find any significant evidence of skin irritation, skin discoloration, or symptoms of unusual shortness of breath in 74 *o*-phthalaldehyde-exposed workers when evaluated postshift for symptoms and compared to the non-*o*-phthalaldehyde exposed group of workers[16].

Reproductive and Developmental Toxicity

No information regarding the reproductive or developmental toxicity of *o*-phthalaldehyde in experimental animals or humans was found in the literature.

Carcinogenicity

No information on the carcinogenicity of *o*-phthalaldehyde in experimental animals or humans was found in a review of the literature.

Genetic Toxicity

No published reports on the genotoxicity of *o*-phthalaldehyde were identified in a search of the literature; however, in a summary of an industry-sponsored study, negative results were reported for *o*-phthalaldehyde (99.7% pure), tested over a concentration range of 1 to 30 µg/mL, in the HGPRT gene mutation assay in Chinese hamster ovary cells, with and without exogenous metabolic activation from rat liver S9 mix[36].

Study Rationale and Design

o-Phthalaldehyde was nominated by NIOSH to NTP for toxicological characterization based on its increasing use in sterilization of dental and medical equipment and due to lack of adequate and publicly available toxicologic data. Furthermore, human case reports in the literature document incidences of skin and respiratory system sensitization following occupational exposure to *o*-phthalaldehyde. *o*-Phthalaldehyde was also of interest because of its promotion as a safe alternative to the widely used disinfectant glutaraldehyde, a known skin and respiratory system sensitizer.

This toxicity report describes the results of toxicity studies in which Sprague Dawley rats and B6C3F1/N mice were exposed to *o*-phthalaldehyde by whole-body inhalation for 3 months. Inhalation was chosen as the route of exposure for these studies because inhalation is a major route of human exposure. The exposure concentrations for the 3-month studies were 0, 0.44, 0.88, 1.75, 3.5, and 7.0 ppm *o*-phthalaldehyde. The highest exposure concentration was selected based on NTP evaluations of the maximum achievable concentration without aerosolization (MACWA) under normal chamber environmental specifications. The lowest concentration was similar to the experimental limit of quantitation for the online monitor used in these studies. Although a lower limit of quantitation may have been achievable using this online monitor or available offline methods, exposure of animals to lower concentrations was not feasible under the conditions of these studies due to reactivity of the aldehyde moieties of *o*-phthalaldehyde with amines resulting from the presence of animals.

Materials and Methods

Procurement and Characterization of *o*-Phthalaldehyde

o-Phthalaldehyde was obtained from MP Biomedicals, LLC (Solon, OH), in one lot (8674J) that was used during the 3-month studies. Identity and purity analyses were conducted by the study laboratory at Battelle Toxicology Northwest (Richland, WA) and by the analytical chemistry laboratory at Chemir Analytical Services (Maryland Heights, MO) (Appendix F). Reports on analyses performed in support of the *o*-phthalaldehyde studies are on file at the National Institute of Environmental Health Sciences.

Lot 8674J of the chemical, a pale-yellow, coarse, crystalline material, was identified as *o*-phthalaldehyde by the study laboratory using infrared spectroscopy, and by the analytical chemistry laboratory using Fourier transform infrared and proton nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with literature spectra and the structure of *o*-phthalaldehyde.

The analytical chemistry laboratory determined the moisture content of lot 8674J using Karl Fischer titration and elemental analyses for carbon, hydrogen, nitrogen, and sulfur using inductively coupled plasma/optical emission spectroscopy. The study laboratory determined the purity of lot 8674J directly as well as relative to a commercial *o*-phthalaldehyde standard using gas chromatography (GC) with flame ionization detection (FID), and GC with mass spectrometry (MS) for identification of impurities.

For lot 8674J, Karl Fischer titration indicated approximately 0.12% water; elemental analyses for carbon and hydrogen were in agreement with the theoretical values for *o*-phthalaldehyde. GC/FID indicated one major peak that was 99% of the total peak area and three impurities that were each over 0.1% of the total peak area, with a combined total of approximately 1% of the total peak area. Two of the impurities were tentatively identified as toluene and phthalide by comparison of GC retention times to a chromatogram obtained from a standard solution containing possible impurities or degradation products that included toluene, phthalide, benzaldehyde, phthalan, *N*-hydroxyphthalimide, naphthalene, isophthalaldehyde, and terepthaldicarboxaldehyde. The third peak was not identified. GC/MS indicated that the phthalide peak also contained phthalic acid, which eluted at the same retention time. Different GC columns of varying polarity with FID were used but failed to resolve these two compounds. To ensure the absence of certain degradation products, GC/FID was used to determine the presence of acetonitrile, GC/MS was used for chloroform, and high-performance liquid chromatography with ultraviolet UV detection (HPLC/UV) was used for 2-carboxybenzaldehyde. Acetonitrile and chloroform were less than 0.1% and 2-carboxybenzaldehyde was approximately 0.4% by weight. The overall purity of lot 8674J was determined to be greater than 99%. The purity relative to the commercial standard was greater than 99.7%. To ensure stability, the test chemical was stored refrigerated in the original sealed plastic containers. Periodic reanalyses of the bulk chemical were performed during the 3-month studies using GC/FID, GC/MS, and HPLC/UV, and no degradation of the test chemical occurred.

Vapor Generation and Exposure System

o-Phthalaldehyde vapor was generated from a flask heated with a heating mantle, purged by a heated nitrogen flow entering above the flask area, blended with heated dilution air to obtain the vapor concentration desired, and transported into a distribution manifold located above the generator. Vapor concentration was determined by the reservoir temperature, nitrogen flow rate, and dilution air flow rate. Pressure in the distribution manifold was fixed to ensure constant flows through the manifold and into the chambers.

Due to the high boiling point of *o*-phthalaldehyde, all vapor transport lines and the on-line GC transport sample line of the 7.0 ppm chambers were heated above the minimum temperature needed to transport vapor without condensation. Individual Teflon®-delivery lines carried the vapor from the distribution manifold to three-way exposure valves at the chamber inlets. The exposure valves diverted vapor from the metering valves to exposure chamber exhaust until the generation system stabilized and exposure could proceed; an additional 60 minutes was added to the prestart stabilization time to purge residual toluene present in the test chemical to less than 1% before exposures began. To initiate exposure, the chamber exposure valves were rotated to allow the vapor to flow to each chamber exposure duct where it was diluted with conditioned chamber air to achieve the desired exposure concentration.

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

Vapor Concentration Monitoring

Summaries of the chamber vapor concentrations are given in Table F-2. Concentrations of *o*-phthalaldehyde in exposure chambers were monitored by an on-line GC/FID system. Samples were drawn from all exposure and control chambers approximately every 20 minutes during each exposure period using Hasteloy-C stream-select and gas-sampling valves (VALCO Instruments Company, Houston, TX) in a separate, heated oven. The sampling lines composing the sample loop were made from Teflon® tubing and were connected to the exposure chamber relative humidity sampling lines near the gas chromatograph. A vacuum regulator maintained a constant vacuum in the sample loop to compensate for variations in sample line pressure. An in-line flow meter between the vacuum regulator and the gas chromatograph allowed digital measurement of sample flow. The on-line gas chromatograph was checked throughout the day for instrument drift against an on-line standard vapor of *o*-phthalaldehyde in nitrogen supplied by a standard generator (Kin-Tek; Precision Calibration Systems, La Marque, TX). The on-line gas chromatograph was checked before the start of each exposure day and after every tenth sample throughout the exposure period. The on-line gas chromatograph was calibrated prior to the start of the study and at least monthly by a comparison of chamber concentration data to data from grab samples that were collected with adsorbent gas sampling tubes containing silica gel (ORBO-52™; Supelco, Bellefonte, PA), extracted with acetonitrile containing α-terpineol as an internal standard, and analyzed using an off-line GC/FID system. Originally, the grab samples

were located within the whole body exposure chambers; however, after animals were transferred into the chambers, ammonia produced by the action of fecal bacteria on urine[37] caused inaccurate measurements of exposure concentration; sampling tubes were moved within the inlet lines. Additional samples were collected from the on-line standard generator to bracket concentrations found in the exposure chambers. The off-line gas chromatograph was calibrated with gravimetrically prepared standards of *o*-phthalaldehyde and the internal standard (α-terpineol) in acrylonitrile.

Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined with and without animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 9.2 minutes. For rats and mice in the 3-month studies, T_{90} values ranged from 15 to 21 minutes without animals present and from 24 to 35 minutes with animals; T_{10} values ranged from 10 to 18 minutes without animals present and from 14 to 39 minutes with animals. A T_{90} value of 17 minutes was used for these studies.

The uniformity of vapor concentration in the inhalation exposure chambers without animals was evaluated before each of the studies began; in addition, concentration uniformity with animals present in the chambers was measured once during the 3-month studies. The vapor concentration was measured using the on-line GC/FID system. The automatic 12-port sample valve was disabled to allow continuous monitoring from a single input line. Samples were collected from 12 positions in each chamber. Chamber concentration uniformity was maintained throughout the studies.

The persistence of *o*-phthalaldehyde in the chambers after vapor delivery ended was determined by monitoring the vapor concentration overnight in the 7.0 ppm chamber without and with animals present in the chambers. The concentration decreased to 1% of the target concentration within 128 minutes without animals present and 279 minutes with animals present. Stability studies of the test chemical were performed by the study laboratory on the distribution line and 7.0 and 0.44 ppm exposure chambers without animals present and on the 3.5 and 0.44 ppm exposure chambers with animals present. Samples were collected with two adsorbent tubes in series during the first and last hours of generation, extracted with acetonitrile (internal standard, α-terpineol) and analyzed using GC/FID. Samples were also collected from the generator reservoir. The presence of chloroform, acetonitrile, and 2-carboxybenzaldehyde in the exposure atmosphere was investigated using samples collected on ORBO-52™ tubes by GC/FID (acetonitrile and chloroform) or HPLC/UV (2-carboxybenzaldehyde). These studies indicated that the *o*-phthalaldehyde test material was stable for 1 day.

Animal Source

Male and female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were obtained from Harlan Laboratories, Inc., (Livermore, CA), and B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Biosciences, Inc. (formerly Taconic Farms, Germantown, NY).

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Studies were approved by the Battelle Toxicology Northwest Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Three-month Studies

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

Groups of 10 male and 10 female rats and mice were exposed to *o*-phthalaldehyde via whole-body inhalation at concentrations of 0, 0.44, 0.88, 1.75, 3.5, or 7.0 ppm, 6 hours plus T_{90} (17 minutes) per day, 5 days per week for 14 weeks. In addition, groups of 10 male and 10 female clinical pathology rats were exposed to the same concentrations for 23 days. Feed was available ad libitum except during exposure periods; water was available ad libitum. Rats and mice were housed individually. Clinical findings were recorded on day 8, weekly thereafter, and at the end of the studies for core animals. Feed consumption was recorded weekly by cage. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1. Information on feed composition and contaminants are provided in Appendix G.

Animals were anesthetized with carbon dioxide, and blood was collected from the retroorbital plexus of 10 male and 10 female clinical pathology rats on days 3 and 23 and from core study rats at the end of the study for hematology and clinical chemistry analyses. Blood was collected from the retroorbital sinus of mice at the end of the study for hematology analyses. Blood samples for hematology analyses were collected into tubes containing potassium EDTA. Packed cell volume; hemoglobin concentration; erythrocyte, platelet, leukocyte, and leukocyte differential counts; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration were determined using an Abbott Cell-Dyn 3700 Analyzer (Abbott Diagnostics Systems, Abbott Park, IL). 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) for comparison to Cell-Dyn values for packed cell volume. Blood smears were stained with Romanowsky-type aqueous stain in a Wescor 7120 aerospray slide stainer (Wescor, Inc., Logan, UT), and blood cell morphologies were examined; the number of nucleated erythrocytes was also noted and the leukocyte count adjusted accordingly. Manual leukocyte differential counts were performed in the event of flagged automated counts. The manual leukocyte differential was based on classifying a minimum of 100 white cells. Reticulocytes were stained with New Methylene Blue and enumerated as a reticulocyte:erythrocyte ratio using the Miller disc method[38]. Blood samples for clinical chemistry analyses were placed in tubes without anticoagulant and containing a separator gel, allowed to clot, and centrifuged. Parameters were determined using a Roche Hitachi 912

System (Roche Diagnostic Corporation, Indianapolis, IN). Parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on rats and mice exposed to 0, 0.44, 0.88, or 1.75 ppm. The parameters evaluated are listed in Table 1. For 16 consecutive days prior to scheduled terminal euthanasia, the vaginal vaults of the females were moistened with saline, and samples of vaginal fluid and cells were collected and subsequently stained; however, due to inconsistent sample collection and slide staining, an assessment of estrous cyclicity could not be made. Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a Hamilton Thorne Bioscience Integrated Visual Optical System following homogenization in buffer using a Brinkman Polytron with generator.

Necropsies were performed on core study rats and mice. The heart, right kidney, liver, lungs, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were fixed in Davidson's solution and testes, vaginal tunics, and epididymides were first fixed in modified Davidson's solution) processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed by the study laboratory pathologist on core study chamber control rats and mice, male rats and mice exposed to 1.75, 3.5, or 7.0 ppm and female rats and mice exposed to 3.5 or 7.0 ppm. Table 1 lists the tissues and organs routinely examined.

In addition to the routinely examined tissues and organs, there were special histopathologic reviews of the skin, testes, and epididymides in these studies. Although clinical findings of black skin pigmentation were noted on distal appendages (pinnae and/or feet) during in-life exposure in rats, pinnae were not available for histopathology in rats but were available for mice. Therefore, histopathologic evaluation included the left pinna in chamber control and 7.0 ppm mice (both sexes) based on clinical findings of black discoloration on distal appendages in rats, lesions noted in routine inguinal skin sections in mice, and scientific literature reporting contact dermatitis/chemical burns associated with *o*-phthalaldehyde exposure in humans[25; 29]. As part of the special review of testes and epididymides, tissues from two untreated 45-day-old rats were examined to ascertain histopathologic features typical of peripuberty to compare to testes and epididymides from rats that died in the first 2 weeks of the study, prior to sexual maturity.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to a NTP

Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PWG coordinators. Special pathology peer reviews were convened following the initial PWG to evaluate the testes and epididymides. Details of these review procedures have been described, in part, by Maronpot and Boorman[39] and Boorman et al.[40].

Table 1. Experimental Design and Materials and Methods in the Three-month Inhalation Studies of *o*-Phthalaldehyde

Three-month Studies
Study Laboratory
Battelle Toxicology Northwest (Richland, WA)
Strain and Species
Sprague Dawley (Hsd:Sprague Dawley® SD®) rats
B6C3F1/N mice
Animal Source
Rats: Harlan Laboratories, Inc. (Livermore, CA)
Mice: Taconic Biosciences, Inc. (Germantown, NY)
Time Held Before Studies
Rats: 12 (males) or 13 (females) days Mice: 12 days
Average Age When Studies Began
Rats: 6 weeks Mice: 5 to 6 weeks
Date of First Exposure
Rats: January 19 (males) or 20 (females), 2009 Mice: January 19, 2009
Duration of Exposure
6 hours plus T_{90} (17 minutes), 5 days/week for 14 weeks
Date of Last Exposure
Rats: April 20 (males) or 21 (females), 2009 Mice: April 22 (males) or 23 (females), 2009
Necropsy Dates
Rats: April 21 (males) or 22 (females), 2009 Mice: April 23 (males) or 24 (females), 2009
Average Age at Necropsy
19 to 20 weeks
Size of Study Groups
10 males and 10 females
Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights.
Animals per Cage
1
Method of Animal Identification
Tail tattoo

Three-month Studies

Diet

NTP-2000 irradiated wafer diet (Zeigler Brothers, Inc., Gardners, PA), available ad libitum except during exposure periods, changed weekly

Water

Tap water (Richland municipal water supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available ad libitum

Cages

Stainless steel wire-bottom (Lab Products, Inc., Seaford, DE), changed and rotated weekly

Cageboard

Untreated paper (Shepherd Specialty Papers, Watertown, TN), changed daily

Chamber Air Supply Filters

Single HEPA, charcoal (RSE, Inc., New Baltimore, MI), Purafil (Environmental Systems, Lynnwood, WA); all new at study start

Chambers

Stainless steel, excreta pan at each of six levels (Lab Products, Inc., Seaford, DE), chambers changed weekly; excreta pans changed daily

Chamber Environment

Temperature: 75° ± 3°F
Relative humidity: 55% ± 15%
Room fluorescent light: 12 hours/day
Room air changes: 15 ± 2/hour

Exposure Concentrations

0, 0.44, 0.88, 1.75, 3.5, and 7.0 ppm

Type and Frequency of Observation

Observed twice daily; core study animals were weighed initially, on day 8, weekly, and at the end of the studies; clinical findings were recorded on day 8, weekly, and at the end of the studies.

Method of Euthanasia

Carbon dioxide asphyxiation

Necropsy

Necropsies were performed on core study animals. Organs weighed in the chamber control, 0.44, 0.88, 1.75, and 3.5 ppm groups were heart, right kidney, liver, lung, right testis, and thymus.

Clinical Pathology

Blood was collected via the retroorbital plexus from surviving clinical pathology rats on days 3 and 23 and from all core study rats surviving to the end of the study for hematology and clinical chemistry. Blood was collected from the retroorbital sinus of mice at the end of the study for hematology.
Hematology: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte, leukocyte, and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; leukocyte count and differentials, and packed cell volume.
Clinical chemistry: urea nitrogen, creatinine, glucose, total protein, albumin, cholesterol, globulin, albumin/globulin ratio, triglycerides, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids

Three-month Studies

Histopathology

Histopathology was performed on core study chamber control rats and mice, male rats and mice exposed to 1.75, 3.5, or 7.0 ppm, and female rats and mice exposed to 3.5 or 7.0 ppm. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, Harderian gland, gallbladder (mice), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pharynx, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), thymus, thyroid gland, trachea, urinary bladder, and uterus. Tissues were examined to a no-effect level in the remaining core study groups. In addition, the pinna was examined in chamber control and 7.0 ppm mice and the testis with epididymis was examined in all male rats and mice. The testis with epididymis from two untreated 45-day-old rats were also examined.

Sperm Motility

At the end of the studies, spermatid and sperm samples were collected from male rats and mice in the 0, 0.44, 0.88, and 1.75 ppm groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm motility, and sperm per cauda epididymis and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed.

Statistical Methods

Calculation and Analysis of Lesion Incidences

The incidences of lesions are presented in Appendix A as numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test[41], a procedure based on the overall proportion of affected animals, was used to determine significance between exposed and chamber control animals, and the Cochran-Armitage trend test was used to test for significant trends[42].

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett[43] and Williams[44; 45]. Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[46] (as modified by Williams[47]) and Dunn[48]. Jonckheere's test[49] was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey[50] were examined by NTP personnel, and implausible values were eliminated from the analysis.

Quality Assurance Methods

The 3-month studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations[51]. In addition, as records from the 3-month studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent QA contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Toxicity Study Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Toxicity Study Report.

Genetic Toxicology

Bacterial Mutagenicity Test Protocol

o-Phthalaldehyde was tested for bacterial mutagenicity using test protocols that were modified from Zeiger et al.[52]. Briefly, *o*-phthalaldehyde was incubated with *Salmonella typhimurium* tester strains TA98 and TA100 and an *Escherichia coli* strain WP2 *uvrA*/pKM101, either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat liver) for 20 minutes at 37º C. Top agar supplemented with L-histidine (for the *S. typhimurium* strains) or tryptophan (for the *E. coli* strain) and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates.

Histidine- or tryptophan-independent mutant colonies arising on these plates were counted following 2 days incubation at 37ºC.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of five doses of test article; the highest concentration tested was limited by toxicity.

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. There is no minimum percentage or fold-increase required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

Rat and Mouse Peripheral Blood Micronucleus Test Protocol

At the end of the 3-month studies, blood samples were collected from surviving rats and mice, placed into EDTA tubes, chilled, and shipped overnight to the testing laboratory At the testing laboratory, samples were fixed in ultracold methanol, and frozen at −80°C until analysis. Thawed blood samples were analyzed for frequency of micronucleated reticulocytes (PCEs) and erythrocytes (NCEs) using a flow cytometer[53]; both the mature erythrocyte population and the immature reticulocyte population can be accurately distinguished by employing special cell surface markers to differentiate the two cell types. Approximately 20,000 reticulocytes and 1 million erythrocytes were analyzed per animal; the percentage of reticulocytes among circulating erythrocytes was also determined as a measurement of bone marrow toxicity.

Based on prior experience with the large number of cells scored using flow cytometric scoring techniques[54], 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 chamber control group depend on whether the variances among the groups are equal. Levene's test at $\alpha = 0.05$ is used to test for equal variances. In the case of equal variances, linear regression is used to test for a linear trend with dose and Williams' test is used to test for pairwise differences between each treatment group and the control group. In the case of unequal variances, Jonckheere's test is used to test for linear trend and Dunn's test is used for pairwise comparisons of each treatment group with the control group. To correct for multiple pairwise comparisons, the P value for each comparison with the control group is multiplied by the number of comparisons made. In the event that this product is greater than 1.00, it is replaced with 1.00. Trend tests and pairwise comparisons with the controls are considered statistically significant at $P \leq 0.025$. Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed, and the magnitudes of those effects.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple samples of a chemical were

tested in the same assay, and different results were obtained among these samples and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the in vitro assays have another variable that must be considered in arriving at an overall test result. In vitro assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The results presented in the Abstract of this Toxicity Study Report represent a scientific judgment of the overall evidence for activity of the chemical in an assay.

Results

Rats

All rats exposed to 7.0 ppm died by the end of week 2 of the study, and seven males and two females exposed to 3.5 ppm died by week 7 of the study (Table 2). In males exposed to 7.0 ppm, eight rats were found dead in week 1 and two were euthanized in weeks 1 and 2. n females exposed to 7.0 ppm, nine rats were found dead, eight in week 1 and one in week 2, and one was euthanized in week 2. In males exposed to 3.5 ppm, four were found dead in weeks 1 and 2, three were euthanized in weeks 6 and 7, and three survived to study completion. In females exposed to 3.5 ppm, two were euthanized in weeks 3 and 7, and eight survived to study completion.

Table 2. Survival and Body Weights of Rats in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

Concentration (ppm)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	136 ± 2	409 ± 4	273 ± 4	
0.44	10/10	135 ± 3	377 ± 6**	242 ± 6**	92
0.88	10/10	134 ± 2	350 ± 5**	217 ± 6**	86
1.75	10/10	134 ± 2	309 ± 9**	175 ± 9**	76
3.5[c]	3/10	134 ± 3	222 ± 15**	92 ± 13**	54
7.0[d]	0/10	134 ± 2	–	–	–
Female					
0	10/10	118 ± 3	239 ± 5	121 ± 4	
0.44	10/10	120 ± 2	238 ± 5	118 ± 5	99
0.88	10/10	118 ± 2	230 ± 3	112 ± 4	96
1.75	10/10	116 ± 2	214 ± 5**	97 ± 4**	89
3.5[e]	8/10	119 ± 2	205 ± 6**	88 ± 6**	85
7.0[f]	0/10	118 ± 2	–	–	–

**Significantly different (P≤0.01) from the chamber control group by Williams' test.
[a]Body weights and weight changes are given as mean ± standard error.
[b]Number of animals surviving at 14 weeks/number initially in group. Subsequent calculations are based on animals surviving to the end of the study.
[c]Weeks of death: 1, 1, 1, 2, 6, 6, 7.
[d]Weeks of death: 1, 1, 1, 1, 1, 1, 1, 1, 1, 2.
[e]Weeks of death: 3, 7.
[f]Weeks of death: 1, 1, 1, 1, 1, 1 ,1 ,1, 2, 2.

Clinical findings in groups exposed to 0.88 ppm or greater generally increased with exposure concentration and included abnormal breathing, sneezing, and thinness. One or more of these clinical findings were present in animals that were euthanized prior to study completion. Of the

rats found dead in the two highest exposure groups (3.5 and 7.0 ppm), there were no clinical findings that preceded death and the probable cause of death was listed as undetermined. Based on microscopic findings, necrosis and inflammation in the respiratory tract may have led to respiratory compromise and death in animals prior to study completion. *o*-Phthalaldehyde exposure also induced black discoloration on the distal regions of appendages (pinnae and/or feet) of rats exposed to 0.88 ppm or greater and caused urine and feces within the exposure chambers to turn black.

The final mean body weights and body weight gains of all surviving exposed groups of males and 1.75 and 3.5 ppm females were significantly less than those of the chamber controls (Table 2 and Figure 2). Final body weights relative to controls of the 11 surviving rats in the 3.5 ppm groups were 46% and 15% lower in exposed males and females, respectively.

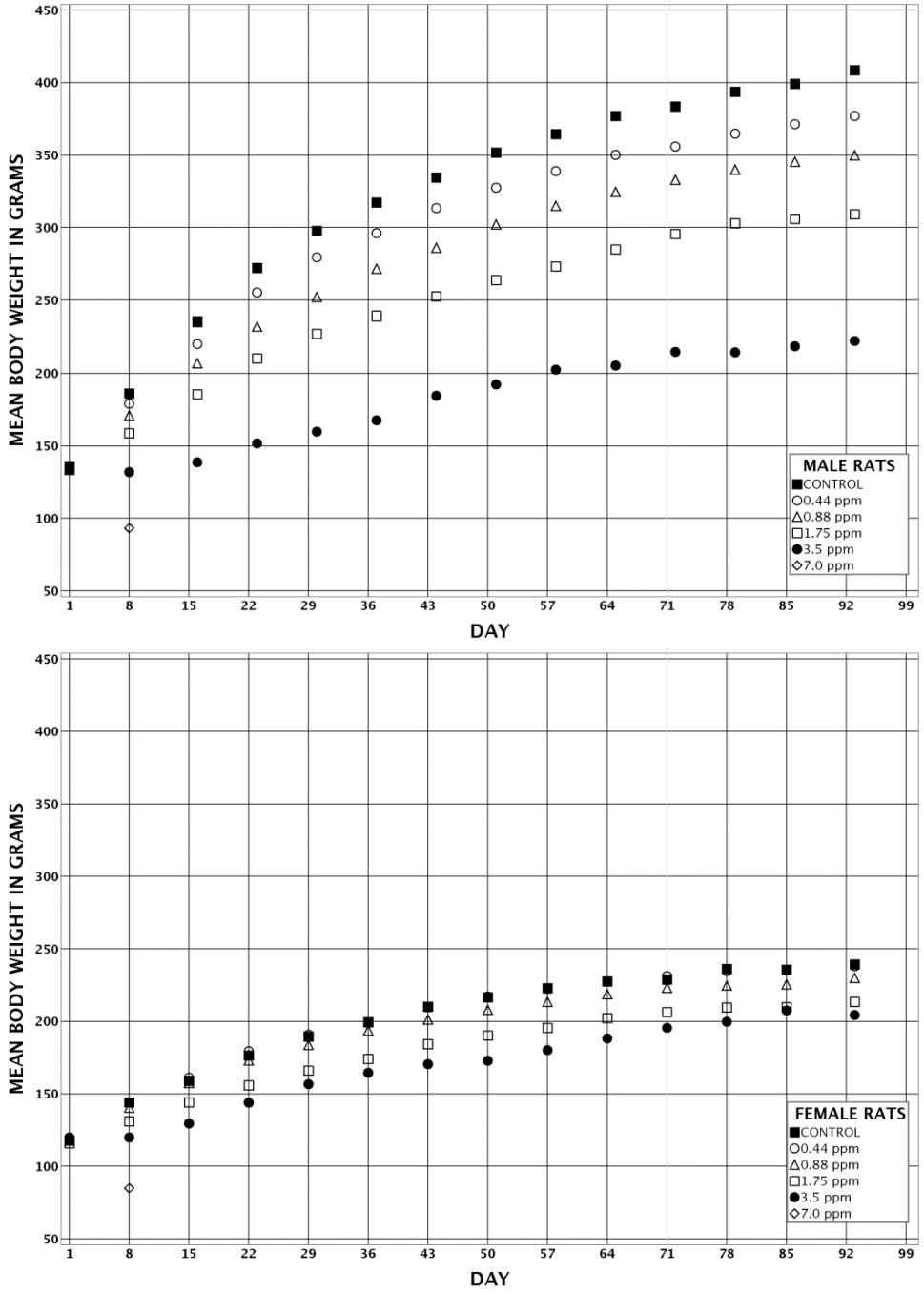

Figure 2. Growth Curves for Rats Exposed to *o*-Phthalaldehyde by Inhalation for Three Months

No hematology or clinical chemistry evaluations were performed on 7.0 ppm rats on day 23 or at week 14 due to mortality and early removal (Table 3, Table 4, and Table B-1). Total leukocyte and lymphocyte counts were significantly decreased in both male and female rats in various dose groups throughout the study, but most consistently in males exposed to 0.88 ppm or greater and females exposed to 3.5 or 7.0 ppm; neutrophil counts were elevated most consistently in 0.88 ppm males and females. These combinations of leukocyte changes were consistent with a stress response; the increase in neutrophils may have also been due to inflammation. Within the erythron, the erythrocyte counts, hemoglobin concentrations, hematocrit values, and packed cell

volumes were significantly elevated in both males and females at all time points and most consistently in those exposed to 0.88 ppm or greater. Reticulocytes were also observed to be significantly elevated at different time points in both sexes. In addition, blood urea nitrogen and total protein concentrations were significantly increased in the earlier time points. This combination of changes was consistent with a physiological hemoconcentration (i.e., dehydration). The increase in blood urea nitrogen and total protein ameliorated by the end of the study, but the erythron increases persisted. While the end of study erythron increases may have been due to decreased water intake, it is also plausible that the extent of the respiratory lesions led to hypoxia and a secondary erythrocytosis. Platelet numbers were significantly increased in the higher exposed groups of male and female rats early in the study, but were decreased by study termination. The increases in platelet numbers were consistent with hemoconcentration, while the decreases may have been due to altered peripheral distribution.

At the end of the study, albumin levels were significantly decreased and globulin levels significantly increased in various exposed female groups, which resulted in a significant decrease in the albumin:globulin ratio (Table 4 and Table B-1). These particular biochemical changes were consistent with inflammation as albumin is a negative acute phase protein and globulin a positive acute phase protein. Decreased feed consumption, as evidenced by the decreases in body weight, may have also contributed to the decreases in albumin.

Significant decreases in alkaline phosphatase (ALP) activity, alanine aminotransferase (ALT) activity, and bile acid levels on day 3 in most male groups and female groups exposed to 0.88 ppm or greater were most likely due to decreased feed consumption or altered hepatic metabolism (Table 4 and Table B-1). At study termination, bile acid concentrations were significantly elevated and exposure concentration-dependent increases in ALT and ALP activities were observed in all exposed male groups and in females exposed to 0.88 ppm or greater. Increases in ALT activity are used as a marker of hepatocellular injury, while increases in ALP activity and bile acids are used as markers of cholestasis. Sorbitol dehydrogenase, another marker of hepatocellular injury, was unchanged. The elevations of these liver parameters were relatively mild, and no hepatic lesions were identified on histopathology. Thus, the toxicologic significance of these increases is not known. The increases may suggest mild hepatocellular injury and cholestasis, but could also be related to enzyme induction or altered hepatic function. Creatine kinase activity was significantly elevated in both the male and female 3.5 ppm rats at the end of the study, indicating skeletal or cardiac muscle injury; the reason for muscle injury is not known.

Table 3. Selected Hematology Data for Rats in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Male						
n						
Day 3	10	10	10	10	10	9
Day 23	10	10	10	9	5	0
Week 14	10	10	10	10	3	0
Hematocrit (spun) (%)						
Day 3	41.4 ± 0.4	42.5 ± 0.4	42.1 ± 0.3	45.9 ± 0.7**	48.9 ± 0.8**	50.5 ± 0.5**
Day 23	45.1 ± 0.3	45.9 ± 0.5	47.1 ± 0.7*	48.4 ± 0.5**	55.9 ± 1.0**	–
Week 14	48.3 ± 0.4	48.8 ± 0.5	50.2 ± 0.4**	49.6 ± 0.6*	51.3 ± 1.1*	–
Packed cell volume (%)						
Day 3	39.6 ± 0.4	40.6 ± 0.4	40.0 ± 0.4	44.1 ± 0.6**	47.2 ± 0.9**	49.5 ± 0.5**
Day 23	43.8 ± 0.3	44.2 ± 0.5	45.5 ± 0.6*	47.1 ± 0.5**	53.9 ± 1.1**	–
Week 14	46.4 ± 0.4	47.4 ± 0.4	49.1 ± 0.4**	48.2 ± 0.5**	50.3 ± 1.7*	–
Hemoglobin (g/dL)						
Day 3	12.7 ± 0.1	13.1 ± 0.1	13.1 ± 0.1	14.4 ± 0.2**	15.3 ± 0.3**	16.2 ± 0.2**
Day 23	14.4 ± 0.1	14.5 ± 0.2	15.0 ± 0.2*	15.5 ± 0.2**	17.7 ± 0.3**	–
Week 14	15.6 ± 0.1	15.9 ± 0.1	16.3 ± 0.1**	16.1 ± 0.2**	17.0 ± 0.5**	–
Erythrocytes (10^6/μL)						
Day 3	6.03 ± 0.07	6.16 ± 0.06	6.23 ± 0.07	6.84 ± 0.11**	7.42 ± 0.15**	7.84 ± 0.11**
Day 23	7.19 ± 0.05	7.18 ± 0.07	7.52 ± 0.11*	7.85 ± 0.08**	9.16 ± 0.23**	–
Week 14	8.53 ± 0.08	8.62 ± 0.08	8.87 ± 0.09	8.71 ± 0.12	8.99 ± 0.27	–
Reticulocytes (10^3/μL)						
Day 3	446 ± 10	413 ± 18	393 ± 17	451 ± 13	515 ± 17*	507 ± 19*
Day 23	281 ± 15	255 ± 10	235 ± 13	265 ± 11	277 ± 28	–
Week 14	193 ± 13	205 ± 9	185 ± 13	196 ± 11	270 ± 13	–
Platelets (10^3/μL)						
Day 3	1,112 ± 29	1,164 ± 30	1,170 ± 30	1,259 ± 45**	1,328 ± 50**	1,355 ± 85**
Day 23	975 ± 33	936 ± 10	941 ± 24	935 ± 19	786 ± 42**	–
Week 14	823 ± 23	781 ± 13	770 ± 22	703 ± 23**	697 ± 51*	–
Leukocytes (10^3/μL)						
Day 3	14.58 ± 0.66	13.44 ± 1.04	13.56 ± 0.93	9.38 ± 0.83**	8.89 ± 1.08**	8.87 ± 0.69**
Day 23	12.02 ± 0.81	10.94 ± 0.44	11.14 ± 0.77	10.04 ± 0.68	7.98 ± 0.73**	–
Week 14	11.34 ± 0.49	11.81 ± 0.65	10.73 ± 0.74	9.27 ± 0.60*	6.69 ± 0.30**	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Segmented neutrophils (10^3/µL)						
Day 3	1.41 ± 0.12	1.33 ± 0.20	1.46 ± 0.16	1.12 ± 0.11	2.06 ± 0.50	3.28 ± 0.20**
Day 23	1.36 ± 0.20	1.38 ± 0.12	2.76 ± 0.57**	2.30 ± 0.17**	2.95 ± 0.34**	–
Week 14	1.35 ± 0.14	1.66 ± 0.11	2.05 ± 0.16**	2.39 ± 0.13**	2.42 ± 0.24**	–
Lymphocytes (10^3/µL)						
Day 3	12.92 ± 0.58	11.78 ± 0.82	11.81 ± 0.84	8.05 ± 0.83**	6.58 ± 0.61**	5.40 ± 0.69**
Day 23	10.45 ± 0.72	9.28 ± 0.42	8.14 ± 0.36*	7.52 ± 0.64**	4.80 ± 0.49**	–
Week 14	9.72 ± 0.39	9.87 ± 0.62	8.42 ± 0.66*	6.67 ± 0.54**	3.84 ± 0.46**	–
Female						
n						
Day 3	10	10	10	10	10	9
Day 23	10	10	9	10	7	0
Week 14	10	10	10	10	8	0
Hematocrit (spun) (%)						
Day 3	43.9 ± 0.4	44.2 ± 0.3	43.3 ± 0.8	45.3 ± 0.5	48.5 ± 0.7**	51.6 ± 0.7**
Day 23	46.8 ± 0.5	47.2 ± 0.4	48.1 ± 0.6	49.1 ± 0.5**	52.3 ± 1.3**	–
Week 14	46.2 ± 0.5	47.4 ± 0.6	47.6 ± 0.5	49.1 ± 0.6**	49.8 ± 1.0**	–
Packed cell volume (%)						
Day 3	41.7 ± 0.5	41.9 ± 0.4	40.9 ± 0.7	43.4 ± 0.4*	46.6 ± 0.7**	50.3 ± 0.6**
Day 23	45.2 ± 0.4	45.8 ± 0.4	46.9 ± 0.6*	47.6 ± 0.5**	51.3 ± 1.3**	–
Week 14	45.0 ± 0.4	45.6 ± 0.6	46.1 ± 0.5	47.7 ± 0.7**	48.6 ± 1.0**	–
Hemoglobin (g/dL)						
Day 3	13.5 ± 0.2	13.7 ± 0.1	13.4 ± 0.2	14.1 ± 0.1**	15.3 ± 0.2**	16.6 ± 0.2**
Day 23	14.9 ± 0.1	15.1 ± 0.1	15.4 ± 0.2*	15.7 ± 0.2**	16.9 ± 0.4**	–
Week 14	15.2 ± 0.1	15.4 ± 0.2	15.6 ± 0.1	16.0 ± 0.2**	16.4 ± 0.3**	–
Erythrocytes (10^6/µL)						
Day 3	6.34 ± 0.11	6.42 ± 0.05	6.42 ± 0.07	6.71 ± 0.10**	7.38 ± 0.13**	8.11 ± 0.10**
Day 23	7.31 ± 0.11	7.47 ± 0.09	7.79 ± 0.09**	7.80 ± 0.09**	8.53 ± 0.23**	–
Week 14	7.79 ± 0.10	8.17 ± 0.12*	8.11 ± 0.10*	8.37 ± 0.15**	8.39 ± 0.16**	–
Reticulocytes (10^3/µL)						
Day 3	423 ± 13	439 ± 25	431 ± 14	465 ± 17	460 ± 18	511 ± 28*
Day 23	182 ± 8	225 ± 10	196 ± 5	223 ± 12*	263 ± 30**	–
Week 14	192 ± 8	200 ± 4	206 ± 7	219 ± 9	216 ± 13	–
Platelets (10^3/µL)						
Day 3	1,309 ± 38	1,182 ± 57	1,309 ± 49	1,305 ± 50	1,435 ± 39	1,550 ± 85*

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Day 23	1,006 ± 34	934 ± 28	1,043 ± 45	996 ± 38	859 ± 30*	–
Week 14	919 ± 25	943 ± 15	807 ± 43*	829 ± 27*	801 ± 38*	–
Leukocytes (10^3/μL)						
Day 3	12.51 ± 0.49	12.62 ± 0.51	11.32 ± 0.47	12.62 ± 0.92	11.06 ± 0.97	7.95 ± 0.84**
Day 23	9.21 ± 0.65	7.93 ± 0.60	10.23 ± 0.56	10.42 ± 0.72	8.16 ± 0.79	–
Week 14	10.48 ± 0.51	11.59 ± 0.78	10.88 ± 0.56	11.15 ± 1.00	8.29 ± 0.84	–
Segmented neutrophils (10^3/μL)						
Day 3	1.15 ± 0.10	1.02 ± 0.10	0.98 ± 0.10	1.21 ± 0.12	1.44 ± 0.14	2.71 ± 0.23**
Day 23	1.19 ± 0.17	0.81 ± 0.06	1.61 ± 0.13	2.28 ± 0.29**	3.05 ± 0.75**	–
Week 14	1.38 ± 0.15	1.85 ± 0.11**	2.03 ± 0.19**	3.52 ± 0.43**	3.07 ± 0.57**	–
Lymphocytes (10^3/μL)						
Day 3	11.01 ± 0.51	11.27 ± 0.46	10.01 ± 0.46	11.10 ± 0.85	9.38 ± 0.89	5.11 ± 0.72**
Day 23	7.76 ± 0.64	6.92 ± 0.56	8.34 ± 0.48	7.82 ± 0.68	4.92 ± 0.28**	–
Week 14	8.82 ± 0.42	9.41 ± 0.69	8.57 ± 0.62	7.35 ± 0.69	4.88 ± 0.69**	–

*Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data. Due to 100% mortality in clinical pathology and core study rats exposed to 7.0 ppm, no data are available for these groups at day 23 or week 14.

Table 4. Selected Clinical Chemistry Data for Rats in the Three-month Inhalation Study of *o*-Phthalaldehyde

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Male						
n						
Day 3	10	10	10	10	10	9
Day 23	10	10	10	9	5	0
Week 14	10	10	10	10	3	0
Urea nitrogen (mg/dL)						
Day 3	8.5 ± 0.3	10.2 ± 0.7	9.9 ± 0.8	14.4 ± 0.9**	22.3 ± 1.5**	25.3 ± 0.7**
Day 23	9.3 ± 0.4	8.4 ± 0.4	8.5 ± 0.5	10.8 ± 0.5*	14.2 ± 1.6**	–
Week 14	15.4 ± 0.5	15.2 ± 0.4	14.5 ± 0.6	15.1 ± 0.4	18.0 ± 0.6	–
Glucose (mg/dL)						
Day 3	142 ± 4	133 ± 3	137 ± 4	157 ± 4*	170 ± 19*	176 ± 7**
Day 23	125 ± 4	136 ± 5	123 ± 7	146 ± 6**	144 ± 12*	–
Week 14	121 ± 2	129 ± 4	130 ± 6	128 ± 2*	153 ± 11**	–
Total protein (g/dL)						
Day 3	5.5 ± 0.0	5.6 ± 0.0	5.6 ± 0.1	6.1 ± 0.1**	6.3 ± 0.1**	6.0 ± 0.1**
Day 23	6.3 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.7 ± 0.1*	–
Week 14	7.1 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.0 ± 0.1	7.0 ± 0.2	–
Albumin (g/dL)						
Day 3	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.6 ± 0.1**	4.7 ± 0.1**	4.5 ± 0.1**
Day 23	4.5 ± 0.0	4.4 ± 0.0	4.5 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	–
Week 14	4.7 ± 0.0	4.8 ± 0.1	4.8 ± 0.0	4.7 ± 0.1	4.6 ± 0.1	–
Globulin (g/dL)						
Day 3	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.5 ± 0.0**	1.6 ± 0.1**	1.5 ± 0.1**
Day 23	1.8 ± 0.0	1.7 ± 0.0	1.9 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	–
Week 14	2.4 ± 0.0	2.4 ± 0.0	2.4 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	–
Albumin/globulin ratio						
Day 3	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.0	3.0 ± 0.1	3.0 ± 0.1	3.1 ± 0.1
Day 23	2.5 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	2.3 ± 0.1	–
Week 14	1.9 ± 0.0	2.0 ± 0.0	2.0 ± 0.1	2.1 ± 0.1*	1.9 ± 0.1	–
Cholesterol (mg/dL)						
Day 3	123 ± 3	117 ± 3	116 ± 3	119 ± 3	118 ± 4	107 ± 4*
Day 23	100 ± 4	93 ± 2	99 ± 3	104 ± 5	116 ± 5	–
Week 14	114 ± 4	105 ± 3	107 ± 4	98 ± 4**	86 ± 3**	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Triglycerides (mg/dL)						
Day 3	32 ± 2	28 ± 2	20 ± 1**	18 ± 1**	21 ± 3**	24 ± 2**
Day 23	29 ± 2	26 ± 3	25 ± 2	28 ± 3	39 ± 9	–
Week 14	52 ± 4	50 ± 5	48 ± 4	66 ± 5	78 ± 16	–
Alanine aminotransferase (IU/L)						
Day 3	72 ± 3	60 ± 3*	57 ± 2**	50 ± 2**	41 ± 2**	48 ± 4**
Day 23	44 ± 1	48 ± 2	51 ± 2**	49 ± 2*	64 ± 10**	–
Week 14	55 ± 2	69 ± 6**	69 ± 3**	73 ± 2**	92 ± 12**	–
Alkaline phosphatase (IU/L)						
Day 3	312 ± 9	274 ± 11*	265 ± 6**	246 ± 10**	186 ± 4**	172 ± 9**
Day 23	197 ± 7	205 ± 9	214 ± 9	233 ± 9*	206 ± 14	–
Week 14	156 ± 5	175 ± 6*	204 ± 7**	216 ± 6**	238 ± 24**	–
Creatine kinase (IU/L)						
Day 3	627 ± 111	526 ± 43	518 ± 45	573 ± 91	315 ± 26**	416 ± 39*
Day 23	271 ± 20	352 ± 39	351 ± 34	429 ± 62**	485 ± 63**	–
Week 14	196 ± 29	193 ± 21	223 ± 30	242 ± 26	1,014 ± 694**	–
Sorbitol dehydrogenase (IU/L)						
Day 3	11 ± 1	11 ± 1	11 ± 1	11 ± 2	15 ± 1*	15 ± 1*
Day 23	12 ± 0	12 ± 1	12 ± 1	11 ± 1	14 ± 1	–
Week 14	14 ± 1	12 ± 1	12 ± 1	12 ± 1	12 ± 0	–
Bile acids (µmol/L)						
Day 3	32.1 ± 4.4	27.4 ± 4.4	20.6 ± 4.2*	17.3 ± 3.4*	10.1 ± 1.6**	10.9 ± 1.1**
Day 23	12.2 ± 2.1	14.9 ± 2.3	23.4 ± 5.1	14.2 ± 2.8	35.3 ± 16.8	–
Week 14	10.6 ± 2.3	29.3 ± 7.1*	26.7 ± 3.7*	26.0 ± 2.3**	24.8 ± 13.8	–
Female						
n						
Day 3	10	10	10	10	10	9
Day 23	10	10	9	10	7	0
Week 14	10	10	10	10	8	0
Urea nitrogen (mg/dL)						
Day 3	10.4 ± 0.7	8.9 ± 0.7	11.2 ± 1.1	12.6 ± 1.1	18.1 ± 0.9**	24.4 ± 0.8**
Day 23	10.0 ± 0.3	10.5 ± 0.6	10.3 ± 0.5	14.1 ± 0.8**	17.3 ± 1.1**	–
Week 14	15.3 ± 0.7	15.5 ± 0.3	13.1 ± 0.5	16.9 ± 1.0	18.8 ± 1.5	–
Glucose (mg/dL)						
Day 3	137 ± 2	138 ± 4	145 ± 3	149 ± 3*	156 ± 4**	163 ± 9**

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Day 23	130 ± 5	131 ± 5	141 ± 3	143 ± 5	152 ± 9*	–
Week 14	129 ± 4	117 ± 1	127 ± 5	134 ± 4	145 ± 13	–
Total protein (g/dL)						
Day 3	5.8 ± 0.1	5.7 ± 0.1	6.0 ± 0.1	6.0 ± 0.0*	6.2 ± 0.1**	6.1 ± 0.1**
Day 23	6.4 ± 0.1	6.3 ± 0.0	6.5 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	–
Week 14	7.1 ± 0.1	7.2 ± 0.1	6.8 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	–
Albumin (g/dL)						
Day 3	4.6 ± 0.1	4.5 ± 0.1	4.7 ± 0.0	4.6 ± 0.0	4.7 ± 0.1	4.6 ± 0.1
Day 23	4.7 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.8 ± 0.1	–
Week 14	5.1 ± 0.1	5.1 ± 0.1	4.8 ± 0.1**	4.8 ± 0.1**	4.7 ± 0.1**	–
Globulin (g/dL)						
Day 3	1.2 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	1.4 ± 0.0**	1.5 ± 0.1**	1.5 ± 0.1**
Day 23	1.7 ± 0.1	1.6 ± 0.0	1.7 ± 0.1	1.8 ± 0.0	1.8 ± 0.1	–
Week 14	2.0 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.3 ± 0.1**	2.3 ± 0.1**	–
Albumin/globulin ratio						
Day 3	3.7 ± 0.1	3.7 ± 0.1	3.6 ± 0.1	3.4 ± 0.1*	3.2 ± 0.1**	3.1 ± 0.1**
Day 23	2.9 ± 0.2	3.1 ± 0.1	2.7 ± 0.1	2.6 ± 0.1**	2.6 ± 0.1*	–
Week 14	2.6 ± 0.1	2.4 ± 0.1*	2.4 ± 0.1*	2.1 ± 0.1**	2.0 ± 0.1**	–
Cholesterol (mg/dL)						
Day 3	114 ± 5	114 ± 4	111 ± 4	111 ± 5	109 ± 4	81 ± 5**
Day 23	102 ± 5	104 ± 5	109 ± 4	105 ± 6	87 ± 6	–
Week 14	98 ± 5	110 ± 5	102 ± 3	99 ± 5	80 ± 4	–
Triglycerides (mg/dL)						
Day 3	36 ± 2	33 ± 1	32 ± 3	29 ± 3	29 ± 2	31 ± 1
Day 23	32 ± 1	35 ± 3	40 ± 3	34 ± 3	34 ± 4	–
Week 14	37 ± 3	43 ± 2	43 ± 2	73 ± 9**	59 ± 8**	–
Alanine aminotransferase (IU/L)						
Day 3	57 ± 2	49 ± 3	44 ± 2**	44 ± 2**	35 ± 2**	39 ± 3**
Day 23	42 ± 4	36 ± 1	40 ± 3	45 ± 2	49 ± 4	–
Week 14	53 ± 3	54 ± 2	64 ± 6	66 ± 4	88 ± 6**	–
Alkaline phosphatase (IU/L)						
Day 3	245 ± 9	222 ± 7	218 ± 9*	197 ± 6**	158 ± 7**	134 ± 6**
Day 23	124 ± 5	121 ± 5	148 ± 8*	156 ± 8**	152 ± 4**	–
Week 14	127 ± 6	146 ± 6*	176 ± 23*	184 ± 9**	220 ± 17**	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Creatine kinase (IU/L)						
Day 3	540 ± 74	586 ± 113	651 ± 143	428 ± 81	476 ± 69	417 ± 50
Day 23	370 ± 123	269 ± 35	373 ± 51	316 ± 44	465 ± 52**	–
Week 14	188 ± 20	200 ± 26	212 ± 20	258 ± 43	449 ± 123*	–
Sorbitol dehydrogenase (IU/L)						
Day 3	10 ± 1	10 ± 1	11 ± 1	10 ± 1	10 ± 1	14 ± 1*
Day 23	14 ± 3	11 ± 0	11 ± 1	12 ± 1	12 ± 1	–
Week 14	12 ± 0	11 ± 1	12 ± 1	11 ± 1	11 ± 1	–
Bile acids (µmol/L)						
Day 3	19.4 ± 4.5	20.3 ± 5.6	13.9 ± 4.1	9.1 ± 1.5	7.8 ± 0.8*	10.2 ± 1.4
Day 23	9.6 ± 1.6	11.9 ± 1.3	9.4 ± 2.5	13.8 ± 4.2	12.7 ± 2.6	–
Week 14	12.5 ± 4.8	18.4 ± 4.0	43.4 ± 13.9**	25.5 ± 3.4**	24.2 ± 3.8**	–

*Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data. Due to 100% mortality in clinical pathology and core study rats exposed to 7.0 ppm, no data are available for these groups at day 23 or week 14.

Significant increases or decreases in cholesterol, glucose, and triglyceride concentrations were observed throughout the study in both male and female rats. In particular, glucose was elevated in males exposed to 1.75 ppm or greater and was most likely due to stress. Alterations in cholesterol and triglycerides were most likely due to changes in lipid metabolism or decreased food intake.

Compared to those of the chamber control groups, the absolute thymus weights were significantly decreased in 0.44 ppm females (18% lower) and 0.88, 1.75, and 3.5 ppm males (14%, 31%, and 56% lower, respectively) and females (10%, 37%, and 44% lower, respectively), as were the relative thymus weights of 1.75 and 3.5 ppm females (Table C-1). Lymphoid atrophy of the thymus, diagnosed in the 3.5 and 7.0 ppm groups, likely contributed to the decreased thymus weights. In male rats, there were significant decreases in absolute heart, kidney, and liver weights of groups exposed to 0.44 ppm or greater. These organ weight decreases tended to parallel the mean body weight decreases, and the relative organ weights were not significantly decreased at 0.44 or 0.88 ppm for the heart and kidney, or at any exposure concentration for the liver. The decreases in absolute heart, kidney, and liver weights were not considered to be related to chemical exposure, as there were no histopathologic findings in the heart, kidney, or liver corresponding to organ weight decreases. Organ weight data were not available for the 7.0 ppm groups due to 100% mortality.

Overall, exposure to *o*-phthalaldehyde resulted in statistically significant or biologically noteworthy histopathologic changes in the nose, larynx, trachea, lung, skin, eye, spleen, thymus, testis, and epididymis of rats. Inhalation exposure to *o*-phthalaldehyde resulted in a spectrum of lesions at sites of contact within the respiratory tract, skin, and eye that were generally consistent with an irritant effect. With increasing exposure concentration, lesions were observed at increasing depth within the respiratory tract. Another group of histopathologic changes, that were

generally consistent with stress, were present in the hematopoietic system. An additional group of findings were present in the male reproductive system. In general, some lesions occurred only in the two highest exposure groups. In contrast, some lesions had lower incidences in the 3.5 and/or 7.0 ppm groups, in which case they were likely associated with the limited exposure duration due to early deaths.

Nose: A main target of *o*-phthalaldehyde exposure was the respiratory tract. The first site of contact, the nose, was most affected. All exposed males and females (except one 0.44 ppm female) exhibited suppurative inflammation of the nose that, in general, increased in severity with increasing exposure concentration (Table 5, Table A-1 and Table A-2). This lesion did not occur in chamber controls. The incidences of turbinate atrophy were significantly increased in males exposed to 0.88 or 1.75 ppm and females exposed to 0.88, 1.75, or 3.5 ppm. Turbinate atrophy is a chronic change that likely did not occur in 3.5 and 7.0 ppm males or 7.0 ppm females due to early deaths.

Microscopically, suppurative inflammation was primarily present in the level I and II histologic sections of the nose and was characterized by variable numbers of viable and degenerate neutrophils within the lamina propria and the epithelium, and neutrophils often accumulated in necrotic debris within the nasal lumen. Turbinate atrophy was present as blunted, thinned, or misshapen turbinates and was most prominent in the level I and II histologic sections and occasionally appeared as shortening of the ethmoid turbinates in the level III section.

Several lesions in the olfactory epithelium of the nose were noted in exposed male and female rats (Table 5, Table A-1, and Table A-2). Mild necrosis of the olfactory epithelium was present in a few males exposed to 1.75 or 3.5 ppm, although these incidences were not statistically significant. Hyaline droplet accumulation of the olfactory epithelium, a common background finding, was present in chamber control rats and in rats exposed to 1.75 ppm or less, but absent at the two highest exposure concentrations in males and females, likely due to the early deaths. Alternatively, detection or formation of hyaline droplets could have been obscured or decreased by inflammation or reparative or adaptive changes in the higher exposure groups. Reparative or adaptive changes observed in the olfactory epithelium of the nose included hyperplasia, atrophy, metaplasia, and regeneration. There were low numbers of exposed males and females with minimal hyperplasia of the glands of the olfactory epithelium of the nose. All exposed groups of rats, except 7.0 ppm males, had significantly increased incidences of olfactory epithelium atrophy. The incidences of respiratory metaplasia of the olfactory epithelium in males exposed to 0.88 or 1.75 ppm and females exposed to 1.75 or 3.5 ppm were significantly increased. A few male rats exposed to 0.88 ppm or greater had squamous metaplasia or regeneration of the olfactory epithelium of the nose; the incidence of regeneration was significantly increased in 3.5 ppm males.

Table 5. Incidences of Nonneoplastic Lesions of the Respiratory System in Rats in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Male						
Nose[b]	10	10	10	10	10	10
Inflammation, Suppurative[c]	0	10** (1.6)[d]	10** (2.0)	10** (2.3)	10** (2.7)	10** (2.3)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Glands, Olfactory Epithelium, Hyperplasia	0	0	0	1 (1.0)	2 (1.0)	3 (1.7)
Olfactory Epithelium, Accumulation, Hyaline Droplet	9 (1.3)	10 (2.8)	9 (1.7)	1** (2.0)	0**	0**
Olfactory Epithelium, Atrophy	2 (1.0)	10** (1.9)	10** (2.0)	10** (2.0)	7* (2.0)	6 (1.3)
Olfactory Epithelium, Metaplasia, Respiratory	1 (1.0)	1 (1.0)	6* (1.0)	6* (1.0)	2 (2.0)	0
Olfactory Epithelium, Metaplasia, Squamous	0	0	0	1 (1.0)	1 (2.0)	1 (1.0)
Olfactory Epithelium, Necrosis	0	0	0	1 (2.0)	3 (2.0)	0
Olfactory Epithelium, Regeneration	0	0	1 (1.0)	0	4* (1.8)	0
Respiratory Epithelium, Hyperplasia	3 (1.0)	9** (1.0)	9** (1.2)	7 (1.0)	3 (1.0)	0
Goblet Cell, Respiratory Epithelium, Hyperplasia	0	4* (1.0)	6** (1.7)	2 (2.0)	0	1 (1.0)
Respiratory Epithelium, Metaplasia, Squamous	0	10** (1.9)	10** (2.0)	10** (2.0)	10** (1.7)	8** (1.8)
Respiratory Epithelium, Necrosis	0	0	3 (1.3)	5* (1.4)	10** (2.3)	10** (2.9)
Respiratory Epithelium, Regeneration	0	0	1 (1.0)	0	3 (1.3)	6** (1.5)
Turbinate, Atrophy	0	0	7** (1.0)	10** (1.0)	0	0
Larynx	10	10	10	10	10	10
Inflammation, Chronic Active	1 (1.0)	2 (1.0)	8** (1.4)	10** (1.6)	10** (2.5)	10** (3.0)
Metaplasia, Squamous	0	1 (1.0)	8** (1.0)	10** (1.9)	10** (2.5)	10** (2.7)
Necrosis	0	0	1 (1.0)	5* (1.0)	9** (1.9)	10** (2.6)
Regeneration	0	0	3 (1.7)	2 (1.5)	2 (2.0)	6** (2.0)
Trachea	10	10	10	10	10	10
Fibrosis	0	0	0	5* (1.0)	3 (1.7)	0
Inflammation, Chronic Active	0	0	4* (1.0)	8** (1.8)	9** (1.9)	10** (2.7)
Metaplasia, Squamous	0	0	4* (1.0)	10** (1.0)	6** (2.0)	6** (1.8)
Necrosis	0	0	0	3 (1.3)	8** (1.8)	8** (2.6)
Regeneration	0	0	0	7** (2.9)	7** (2.3)	6** (2.2)
Ulcer	0	0	0	0	1 (1.0)	2 (2.5)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Lung	10	10	10	10	10	10
Alveolus, Infiltration Cellular, Histiocyte	0	1 (1.0)	0	3 (1.3)	9** (1.4)	1 (1.0)
Alveolus, Inflammation, Suppurative	0	0	0	0	3 (1.0)	6** (1.0)
Bronchus, Fibrosis	0	0	0	1 (1.0)	2 (1.5)	1 (2.0)
Bronchus, Hyperplasia	0	0	0	2 (1.0)	5* (1.8)	1 (1.0)
Bronchus, Inflammation, Chronic Active	0	0	0	3 (1.3)	9** (1.7)	8** (2.4)
Bronchus, Metaplasia, Squamous	0	0	0	7** (1.0)	6** (1.5)	1 (1.0)
Bronchus, Necrosis	0	0	0	0	5* (1.6)	7** (2.7)
Bronchus, Regeneration	0	0	0	0	3 (1.0)	1 (2.0)
Female						
Nose	10	10	10	10	10	10
Inflammation, Suppurative	0	9** (1.1)	10** (1.5)	10** (2.4)	10** (2.9)	10** (2.4)
Glands, Olfactory Epithelium, Hyperplasia	0	2 (1.0)	0	2 (1.0)	2 (1.0)	2 (1.0)
Olfactory Epithelium, Accumulation, Hyaline Droplet	9 (1.2)	10 (2.6)	8 (2.5)	4* (1.5)	0**	0**
Olfactory Epithelium, Atrophy	0	9** (1.8)	10** (2.0)	10** (2.0)	10** (1.9)	7** (1.6)
Olfactory Epithelium, Metaplasia, Respiratory	0	1 (1.0)	2 (1.0)	5* (1.2)	7** (1.4)	1 (1.0)
Respiratory Epithelium, Hyperplasia	0	9** (1.0)	7** (1.1)	4* (1.3)	3 (1.0)	3 (1.0)
Respiratory Epithelium, Metaplasia, Squamous	0	10** (1.9)	10** (2.0)	10** (1.9)	10** (2.1)	10** (1.7)
Respiratory Epithelium, Necrosis	0	2 (1.0)	6** (1.2)	9** (1.1)	10** (1.8)	10** (2.3)
Respiratory Epithelium, Regeneration	0	0	2 (1.5)	0	1 (2.0)	3 (1.7)
Turbinate, Atrophy	0	2 (1.0)	4* (1.0)	10** (1.0)	10** (1.0)	0
Larynx	10	10	10	10	10	10
Inflammation, Chronic Active	0	1 (1.0)	1 (2.0)	9** (1.4)	10** (1.8)	10** (2.6)
Metaplasia, Squamous	0	1 (1.0)	4* (1.0)	10** (1.9)	10** (3.0)	10** (3.0)
Necrosis	0	0	0	1 (2.0)	7** (1.3)	8** (2.5)
Trachea	10	10	10	10	10	10

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Fibrosis	0	0	0	2 (1.0)	6** (1.2)	0
Inflammation, Chronic Active	0	0	3 (1.0)	5* (1.2)	10** (1.8)	10** (2.9)
Metaplasia, Squamous	0	0	3 (1.0)	10** (1.0)	7** (1.7)	7** (1.4)
Necrosis	0	0	0	3 (1.0)	3 (1.0)	8** (2.0)
Regeneration	0	0	1 (2.0)	7** (2.6)	10** (2.9)	9** (1.8)
Ulcer	0	0	0	0	1 (2.0)	2 (2.0)
Lung	10	10	10	10	10	10
Alveolus, Infiltration Cellular, Histiocyte	0	2 (1.0)	1 (1.0)	3 (1.3)	9** (2.0)	3 (1.0)
Alveolus, Inflammation, Suppurative	0	0	0	0	2 (1.5)	4* (1.5)
Bronchus, Fibrosis	0	0	0	1 (1.0)	1 (1.0)	3 (2.3)
Bronchus, Hyperplasia	0	0	0	1 (2.0)	6** (2.0)	1 (2.0)
Bronchus, Inflammation, Chronic Active	0	0	0	2 (1.5)	10** (1.6)	10** (2.0)
Bronchus, Metaplasia, Squamous	0	0	0	2 (1.0)	9** (1.6)	0
Bronchus, Necrosis	0	0	0	0	2 (1.0)	10** (2.3)
Bronchus, Regeneration	0	0	0	0	1 (1.0)	5* (1.6)
Interstitium, Inflammation, Granulomatous	0	0	0	0	0	2 (1.5)
Perivascular, Inflammation, Chronic Active	0	0	1 (1.0)	1 (2.0)	6** (1.7)	0

*Significantly different from the chamber control group ($P \leq 0.05$) by the Fisher exact test.
**$P \leq 0.01$.
[a]All males and females exposed to 7.0 ppm died by the end of week 2, and seven male and two female 3.5 ppm rats died by week 7. This limited exposure duration may have affected lesion incidence rates.
[b]Number of animals with tissue examined microscopically.
[c]Number of animals with lesion.
[d]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Microscopically, olfactory epithelial necrosis was noted as focal to focally extensive areas of shrunken, fragmented, or partially sloughed epithelium along either the dorsal aspect in level II histologic sections of the nose or along the dorsal aspect and ethmoid turbinates of level III. Hyaline droplet accumulation was microscopically noted as bright, eosinophilic material in the cytoplasm of olfactory epithelial cells. Hyperplasia of the glands of the olfactory epithelium, present along the nasal septum and the dorsal aspect of level II, was characterized by Bowman's glands within the lamina propria that were increased in size and number, with increased cytoplasm and cytoplasmic basophilia. Olfactory epithelium atrophy was noted as thinning or reduced numbers of layers of the olfactory epithelium, most often along the dorsal meatus of level II, but occasionally present along the ethmoid turbinates of level III. Respiratory metaplasia of the olfactory epithelium was noted as replacement of the normal olfactory epithelial cells by

cuboidal to tall columnar ciliated epithelium, generally along the lateral edges of the dorsal aspect of level II. Squamous metaplasia of the olfactory epithelium was characterized by replacement of the normal olfactory epithelial cells with multiple layers of stratified squamous epithelium that often progressed to keratinization along the superficial surface, primarily in the dorsal aspect of level II. Regeneration was characterized by a single layer of flattened cells that replaced the denuded olfactory epithelium.

Several lesions were also diagnosed in the respiratory epithelium of the nose of rats (Table 5, Table A-1, and Table A-2). Respiratory epithelium necrosis increased in severity with increasing exposure concentration in males and females, and incidences were significantly increased in females exposed to 0.88 ppm or greater and in males exposed to 1.75 ppm or greater. Significant increases in the incidences of respiratory epithelium hyperplasia were observed in males and females exposed to 0.44 or 0.88 ppm and in females exposed to 1.75 ppm. There were significantly increased incidences of respiratory epithelium goblet cell hyperplasia in male rats exposed to 0.44 or 0.88 ppm. All exposed females and males, except two males in the 7.0 ppm group, had respiratory epithelium squamous metaplasia; this finding was not present in chamber controls. The incidence of regeneration was significantly increased in males exposed to 7.0 ppm.

Microscopically, respiratory epithelium necrosis was comprised of shrunken, fragmented, or partially sloughed epithelial cells covering nasal turbinates and the dorsolateral walls in level I and II histologic sections of the nose. Often, the necrotic foci were associated with fibrin, debris, and neutrophils. Respiratory epithelium hyperplasia was characterized by focal to extensive areas of thickening of the epithelium with nuclear crowding, primarily lining the nasal septum. Goblet cell hyperplasia was primarily present in the respiratory epithelium along the nasal septum at levels I and II. This change was characterized by the proliferation of enlarged, tall epithelial cells containing abundant clear to pale basophilic cytoplasm. Respiratory epithelium squamous metaplasia was characterized by replacement of the normal ciliated respiratory epithelial cells with multiple layers of stratified squamous epithelium that often progressed to keratinization along the superficial surface. Respiratory epithelium regeneration was present as a thin single layer of elongate squamous epithelium that replaced the normal respiratory epithelium.

Larynx: Exposure-related lesions occurred in the laryngeal epithelium and included chronic active inflammation, squamous metaplasia, and necrosis (Table 5, Table A-1, and Table A-2). The incidences of chronic active inflammation were significantly increased in males exposed to 0.88 ppm or greater and females exposed to 1.75 ppm or greater. The incidences of squamous metaplasia were significantly increased in males and females exposed to 0.88 ppm or greater. The incidences of necrosis of the larynx were significantly increased in males exposed to 1.75 ppm or greater and in females exposed to 3.5 or 7.0 ppm. The incidence of regeneration in the larynx was significantly increased in 7.0 ppm males. In general, the severity of the laryngeal lesions increased with increasing exposure concentration.

Microscopically, chronic active inflammation was characterized by numerous neutrophils, lymphocytes, plasma cells, and macrophages that frequently infiltrated the submucosa subjacent to the areas of necrosis. Squamous metaplasia was characterized by replacement of the normal respiratory epithelium of the larynx with multiple thickened layers of cuboidal to flattened epithelium, which often progressed to keratinization along the luminal surface. Necrosis was characterized as partial to complete loss of the epithelium with partial involvement of the underlying lamina propria in some cases. Regeneration was characterized by focal to focally

extensive loss of the normal respiratory epithelial cells in the larynx and replacement by a single layer of elongate cells that stretched to cover the denuded area.

Trachea: Exposure-related lesions occurred in the trachea and included chronic active inflammation, squamous metaplasia, necrosis, regeneration, and fibrosis (Table 5, Table A-1, and Table A-2). The incidences of chronic active inflammation and squamous metaplasia were significantly increased in males exposed to 0.88 ppm or greater and females exposed to 1.75 ppm or greater. The incidences of necrosis were significantly increased in male rats exposed to 3.5 ppm or greater and in females exposed to 7.0 ppm. The incidences of regeneration were significantly increased in males and females exposed to 1.75 ppm or greater. The incidences of fibrosis were significantly increased in males exposed to 1.75 ppm and females exposed to 3.5 ppm. Tracheal ulcers were noted in the two highest exposure groups; however, the incidences were not statistically significant. In rats exposed to 3.5 ppm, there was one male and one female with tracheal ulceration; both were moribund sacrifices as a result of abnormal breathing. In rats exposed to 7.0 ppm, there were two males and two females with tracheal ulceration; one male was a moribund sacrifice due to abnormal breathing and the remaining male and both females were found dead. Microscopically, in the trachea, chronic active inflammation was characterized by mixed submucosal infiltrates of lymphocytes, plasma cells, neutrophils, and macrophages. Squamous metaplasia consisted of replacement of the normal respiratory epithelium of the trachea with multiple layers of flattened to cuboidal epithelial cells with increasing cytoplasmic eosinophilia (keratinization) towards the lumen. Necrosis was seen as hypereosinophilia and loss of differential staining in focal to diffuse areas of respiratory epithelium that often sloughed into the tracheal lumen. Regeneration was noted as focal to focally extensive areas of epithelial loss with replacement by a single layer of elongated thin epithelial cells that stretched to cover the denuded area. Fibrosis was noted as an increase in spindloid cells within the lamina propria of the trachea that distorted and partially occluded the tracheal lumen in some cases (Figure 4). Ulcer was characterized by a focal to focally extensive area of epithelial loss that lacked necrotic epithelial layers, fibrin, or necrotic debris overlying the denuded area.

Lung: A spectrum of lesions occurred in the lungs of exposed rats (Table 5, Table A-1, and Table A-2). The incidences of alveolar histiocytic cellular infiltration and alveolar suppurative inflammation were significantly increased in males and females exposed to 3.5 ppm and males and females exposed to 7.0 ppm, respectively. There were two occurrences of interstitial granulomatous inflammation in the lung of female rats exposed to 7.0 ppm. A significant increase in the incidence of perivascular chronic active inflammation occurred in females exposed to 3.5 ppm.

Microscopically, alveolar histiocytic cellular infiltration was comprised of focal accumulations of plump histiocytes in alveolar spaces, whereas alveolar suppurative inflammation was comprised of accumulations of low numbers of neutrophils. Interstitial granulomatous inflammation was characterized by multifocal accumulations of neutrophils, macrophages, and multinucleated giant cells within the interstitium of the lung. Perivascular chronic active inflammation was seen as variable expansion of perivascular spaces by minimal to mild numbers of neutrophils, lymphocytes, plasma cells, and macrophages.

In the bronchi, exposure-related lesions occurred in male and female rats only at exposures of 1.75 ppm or greater (Table 5, Table A-1, and Table A-2). Bronchus necrosis and regeneration were only seen in males and females at the two highest concentrations, 3.5 and 7.0 ppm.

Significantly increased incidences of bronchus lesions included chronic active inflammation in males and females exposed to 3.5 or 7.0 ppm, necrosis in males exposed to 3.5 ppm and in males and females exposed to 7.0 ppm, and regeneration in females exposed to 7.0 ppm. A few males exposed to 3.5 or 7.0 ppm also had regeneration, but the incidences were not significantly increased. Significantly increased lesion incidences in the bronchus also included hyperplasia in males and females exposed to 3.5 ppm and squamous metaplasia in males exposed to 1.75 or 3.5 ppm and females exposed to 3.5 ppm. Bronchus fibrosis was present in one male and one female exposed to 1.75 ppm, two males and one female exposed to 3.5 ppm, and one male and three females exposed to 7.0 ppm.

Microscopically, in the bronchi, chronic active inflammation was seen as variable numbers of neutrophils, lymphocytes, plasma cells, and macrophages within mucus in the bronchi and within the peribronchial connective tissue (Figure 5). Necrosis in the bronchus was noted as epithelium with loss of differential staining with accumulation of fibrin or necrotic cellular debris. Regeneration of the bronchus consisted of a single layer of elongate, thin squamous cells that stretched to cover an area of epithelial loss. In the bronchus, hyperplasia was noted as segmental areas of crowded epithelium with plump epithelial cells and prominent goblet cells. Squamous metaplasia consisted of replacement of the normal respiratory epithelial cells by nonciliated cuboidal to flattened squamous cells that rarely keratinized and were several layers thick. Bronchus fibrosis included intraluminal and intramural changes. Intraluminal fibrosis included large inflammatory fibrotic polyps or polyploid structures extending into and partially occluding the bronchial lumen (Figure 5), whereas intramural fibrosis was thickening of the bronchial wall by similar connective tissue without projection into the lumen.

Skin: Clinical observations of the skin included black discoloration of pinnae and/or feet in rats exposed to 0.88 ppm or greater. Although skin from pinnae and feet were not available for evaluation in rats, routine inguinal skin sections from all exposure groups were reviewed. Exposure-related lesions in the skin of males and females included adnexa degeneration and hair follicle epithelium parakeratosis (Table 6, Table A-1, and Table A-2). Significant increases in the incidences of adnexa degeneration were noted in males exposed to 3.5 ppm and males and females exposed to 7.0 ppm. Significantly increased incidences of hair follicle epithelium parakeratosis occurred in 0.44, 0.88, and 1.75 ppm males and 7.0 ppm males and females.

Microscopically, skin adnexa degeneration, which was less pronounced in rats than in mice, was seen primarily within the hair follicle epithelium and adnexal structures and was characterized by the intracytoplasmic accumulation of pale eosinophilic to amphophilic material that compressed the nucleus of hair follicle epithelial cells, or by an increase in the amount of individual cell death seen as multiple small, round, and dark pyknotic bodies (apoptotic debris) within areas of single cell epithelial loss. Hair follicle epithelium parakeratosis was minimal to mild and characterized by thickened plaques of keratinizing squamous epithelium with retention of keratinocyte nuclei that often extended down into hair follicles. In interfollicular areas, smaller plaques of material were seen that often did not contain nuclei.

Eye: The eye was another site of contact affected by *o*-phthalaldehyde exposure. Exposure-related lesions included anterior chamber suppurative inflammation, corneal suppurative inflammation, and corneal necrosis (Table 6, Table A-1, and Table A-2). The incidences of anterior chamber suppurative inflammation in 3.5 ppm males and 7.0 ppm males and females and suppurative inflammation and necrosis of the cornea in 7.0 ppm males and females were

significantly increased in comparison to the chamber controls. In addition, there were two occurrences of corneal hyperplasia in male rats exposed to 7.0 ppm.

Microscopically, anterior chamber suppurative inflammation was characterized by the presence of neutrophils within the anterior chamber of the eye that often collected subjacent to the corneal endothelium (Figure 6). Suppurative inflammation of the cornea was seen as few to numerous neutrophils present within the corneal epithelium and in rats was often accompanied by anterior chamber suppurative inflammation (Figure 6). Necrosis of the cornea was seen as focal to multifocal thinning of the corneal epithelium due to erosion or ulceration (Figure 6). Corneal hyperplasia was present as focal corneal thickening due to increased layers.

Hematopoietic System: The incidences of lymphoid atrophy of the spleen and thymus were significantly increased in males and females exposed to 3.5 and 7.0 ppm; these lesions were not noted in the three lowest exposure groups (except one 1.75 ppm female) (Table 6, Table A-1, Table A-2). The lesions were more severe in the 7.0 ppm groups than in the 3.5 ppm groups. Five of seven male rats exposed to 3.5 ppm that died within the first 7 weeks of the study had both splenic and thymic atrophy. Lymphoid atrophy of the spleen and thymus are likely attributable to glucocorticoid release-induced lymphocyte death because they occurred in the two highest exposure groups in which there were early deaths.

Table 6. Incidences of Selected Nonneoplastic Lesions in Rats in the Three-month Inhalation Study of *o*-Phthalaldehyde

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Male						
Skin[a]	10	10	10	10	10	10
Adnexa, Degeneration[b]	0	0	0	0	4* (1.3)[c]	10** (1.2)
Epithelium, Hair Follicle, Parakeratosis	0	7** (1.0)	4* (1.0)	7** (1.0)	3 (1.3)	10** (1.7)
Eye	10	10	10	10	10	10
Anterior Chamber, Inflammation, Suppurative	0	0	0	0	4* (1.0)	5* (1.0)
Cornea, Hyperplasia	0	0	0	0	0	2 (1.5)
Cornea, Inflammation, Suppurative	1 (1.0)	1 (1.0)	1 (2.0)	1 (2.0)	5 (2.0)	6* (2.3)
Cornea, Necrosis	0	0	1 (1.0)	0	2 (1.5)	4* (2.5)
Spleen	10	9	10	10	10	10
Atrophy, Lymphoid	0	0	0	0	6** (1.5)	10** (2.7)
Thymus	10	10	10	10	10	10
Atrophy, Lymphoid	0	0	0	0	8** (1.5)	7** (2.7)
Female						
Skin	10	10	10	10	10	10
Adnexa, Degeneration	0	1 (1.0)	0	0	1 (1.0)	10** (1.7)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Epithelium, Hair Follicle, Parakeratosis	0	1 (1.0)	1 (1.0)	31.0)	3 (1.0)	6** (1.5)
Eye	10	10	10	10	10	10
Anterior Chamber, Inflammation, Suppurative	0	0	0	0	1 (1.0)	5* (1.6)
Cornea, Inflammation, Suppurative	0	0	1 (1.0)	0	1 (1.0)	8**2.5)
Cornea, Necrosis	0	0	0	0	0	7** (2.4)
Spleen	10	10	10	10	10	10
Atrophy, Lymphoid	0	0	0	1 (1.0)	4* (1.3)	10** (3.5)
Thymus	10	10	10	10	9	10
Atrophy, Lymphoid	0	0	0	0	6** (1.0)	10** (3.0)

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

Microscopically, lymphoid atrophy in the spleen was seen as decreased numbers of lymphocytes within follicles, periarteriolar lymphoid sheaths, and mantle zones. Lymphoid atrophy in the thymus was characterized by a diffuse reduction in cortical lymphocytes resulting in an overall decrease in organ size, shrinkage of thymic lobules, and loss of the corticomedullary junction. Decreased thymus weights corresponded to histologic findings of lymphoid atrophy in males and females exposed to 3.5 ppm.

Male Reproductive System: Male rats in the 0.88 and 1.75 ppm groups exhibited lower cauda epididymis (16% and 23% lower), epididymis (14% and 19% lower) and testis (12% and 13% lower) weights compared to chamber controls (Table 7 and Table D-1). Total sperm/cauda exhibited a negative trend (14% and 18% lower in the 0.88 and 1.75 ppm groups, respectively). Sperm motility was lower (10% to 21% lower) in all exposed groups evaluated; the 3.5 and 7.0 ppm groups were not evaluated due to excessive mortality. No histopathologic correlates were identified at exposure concentrations below 3.5 ppm that could explain the observed responses in sperm parameters and decreased testicular and epididymal weights. At higher exposure concentrations (where morbidity and mortality were observed), testicular and epididymal histopathologic findings were noted as described below.

Because rats are not sexually mature at 6 weeks, which was the approximate age of rats at the start of the study, male rats exposed to 3.5 or 7.0 ppm that died or were euthanized in the first 10 days of the study (four 3.5 ppm males and all 7.0 ppm males) displayed some microscopic features consistent with sexual immaturity, prompting enhanced evaluations of the testes and epididymides to distinguish any treatment-related findings from findings consistent with sexual immaturity. Some of the features observed in rats that died early were consistent with peripuberty, as observed in the sample of age-matched (45-day-old) untreated rats. These peripubertal features included low numbers of elongated spermatids, especially in stage VII and VIII tubules; relatively small testicular size and tubular lumen diameters as compared to adults;

no or negligible sperm in the epididymis; relatively small lumen diameters of the ducts in the distal corpus and cauda of the epididymis; and sloughed germ cells and debris throughout the epididymis. Spermatogenic development in the rats that died early in the two highest exposure groups was also consistent with that described by Picut et al.[55] for 46-day-old rats.

Biologically relevant or statistically significant treatment-related microscopic findings were present in the testis or epididymis of rats in the two highest exposure groups (3.5 and 7.0 ppm) (Table 7 and Table A-1). Because animals in the two highest exposure groups died within the first 2 weeks, in addition to the aforementioned peripubertal findings, they exhibited some stage- or cell-specific findings in the testis. Therefore specific diagnoses for findings in the germinal epithelium were favored over the more general diagnosis of germinal epithelium degeneration. The diagnosis of germinal epithelium degeneration encompasses a spectrum of degenerative changes typically seen in animals that survive to the end of a study; such changes are not stage or cell specific.

Table 7. Reproductive System Parameters of Male Rats in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
n	10	10	10	10	0	0
L. Cauda Epididymis Weight	0.2555 ± 0.0092	0.2697 ± 0.0109	0.2140 ± 0.0103**	0.1956 ± 0.0072**	–	–
L. Epididymis Weight	0.6692 ± 0.0100	0.6559 ± 0.0197	0.5721 ± 0.0254**	0.5405 ± 0.0133**	–	–
L. Testis Weight	2.0070 ± 0.0294	1.9794 ± 0.0590	1.7603 ± 0.1211*	1.7549 ± 0.0316*	–	–
Sperm Motility (%)	83.1 ± 1.5	75.2 ± 1.5**	67.1 ± 8.1**	65.6 ± 2.5**	–	–
Sperm (10^6/cauda epididymis)	81.19 ± 2.44	87.24 ± 6.47	69.88 ± 10.56	66.68 ± 5.57	–	–
Epididymis[b]	10	10	10	10	10	10
Duct, Exfoliated Germ Cell[c]	0	0	1 (2.0)[d]	1 (2.0)	5▲ (1.6)	4▲ (2.5)
Epithelium, Apoptosis	0	0	0	0	3 (1.3)	5▲ (1.2)
Testes	10	10	10	10	10	10
Exfoliated Germ Cell	0	0	0	0	1 (2.0)	2 (2.0)
Elongated Spermatid, Degeneration	0	0	0	0	4▲ (1.3)	0
Germinal Epithelium, Apoptosis	0	0	0	0	4▲ (1.3)	6▲▲ (1.0)
Interstitial Cell, Atrophy	0	0	0	0	7▲▲ (2.9)	10▲▲ (2.9)
Seminiferous Tubule, Vacuolation	0	0	0	0	3 (1.3)	0

*Significantly different ($P \leq 0.05$) from the chamber control group by Williams' test.
**Significantly different ($P \leq 0.01$) from the chamber control group by Williams' (tissue weights) or Shirley's (motility) test.
▲Significantly different from the chamber control group ($P \leq 0.05$) by the Fisher exact test.
▲▲$P \leq 0.01$.
[a]Tissue weights and sperm data are presented as mean ± standard error. Tissue weights and sperm data were not available for the 3.5 and 7.0 ppm groups due to excessive mortality.
[b]Number of animals with tissue examined microscopically.
[c]Number of animals with lesion.
[d]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

In the testes of rats, incidences of germinal epithelium apoptosis and interstitial cell atrophy were significantly increased in the 3.5 and 7.0 ppm groups (Table 7 and Table A-1). Interstitial cell atrophy occurred in seven 3.5 ppm and all 7.0 ppm males. The incidence of elongated spermatid degeneration was significantly increased in the 3.5 ppm group, occurring in four males. Three 3.5 ppm males that survived to at least 39 days of exposure had unilateral or bilateral degenerative changes in the testes that were characterized by seminiferous tubule cytoplasmic vacuolation, although this increase was not statistically significant. One 3.5 ppm and two 7.0 ppm males had focal germ cell exfoliation in seminiferous tubules near the rete testis.

Microscopically, germinal epithelium apoptosis was minimal to mild and characterized by low numbers of round spermatids and pachytene spermatocytes with shrunken, deeply basophilic pyknotic nuclei and condensed hypereosinophilic cytoplasm, almost exclusively in stage VII and VIII seminiferous tubules. Interstitial cell atrophy was generally moderate in severity and noted as decreased numbers and size of interstitial cells; this finding exceeded low numbers/sizes expected due to sexual immaturity, based on comparison to a sample of age-matched untreated animals. Elongated spermatid degeneration was noted as minimal to mild decreases in numbers of elongated spermatids and with clubbing and/or misshapen heads. Seminiferous tubule cytoplasmic vacuolation was minimal to mild and seen as one or more large vacuoles near the periphery of the seminiferous tubule. The vacuolation was often accompanied by focal loss of germ cells from the Sertoli cell cytoplasm and/or evidence of degeneration/depletion of elongated spermatids. These two degenerative changes, seminiferous tubule vacuolation and elongated spermatid degeneration, were sometimes accompanied by exfoliated germ cells and debris in the ductular lumen of the epididymis. Focal germ cell exfoliation in the testis, which was characterized by the presence of rounded, isolated germ cells in the lumen in a few seminiferous tubules adjacent to the rete testis, was also accompanied in one 3.5 ppm rat and two 7.0 ppm rats by exfoliated germ cells and debris in the epididymis. However, the low incidence, focal distribution, and the frequently unilateral nature of this lesion near the rete testis make its relationship to *o*-phthalaldehyde administration uncertain.

In the epididymis, there were significant increases in the incidences of exfoliated germ cell within the duct lumen in males exposed to 3.5 or 7.0 ppm (Table 7 and Table A-1). The ductular lumen of the caput epididymis from five 3.5 ppm males and four 7.0 ppm males contained increased numbers of exfoliated germ cells and cell debris, a change that generally reflects the exfoliation of germ cells from the testis. Also within the epididymis, there were significantly increased incidences of epithelial apoptosis in 7.0 ppm males. Apoptosis of the epithelium of the epididymis was sometimes accompanied by two changes in the testis, germinal epithelium apoptosis and interstitial cell atrophy.

Microscopically, exfoliated germ cell within the epididymal duct lumen was seen as mild to moderate amounts of rounded germ cells and debris in the caput epididymis. Apoptosis of the epithelium of the epididymis was minimal to mild and characterized by single shrunken cells lining the duct of the epididymis with deeply basophilic, pyknotic nuclei and condensed, hypereosinophilic cytoplasm.

Some findings in rats exposed to *o*-phthalaldehyde are of unknown toxicologic significance or considered incidental findings. Two 7.0 ppm rats had degeneration of the round spermatids, noted as deeply basophilic ring-shaped nuclei, which indicates acute spermatid degeneration. However, because the change was only present in two rats and both rats were found dead, the

change may be associated with moribundity rather than *o*-phthalaldehyde administration. One 1.75 ppm rat had mild testis germinal epithelium degeneration accompanied by mild sloughed germ cells/debris in the epididymis. The diagnosis of germinal epithelium degeneration, rather than specific degenerative changes as diagnosed for males in the two highest exposure groups with early deaths, is more appropriate in this case because degeneration in males surviving to study completion typically encompasses a spectrum of degenerative changes. One 0.88 ppm rat had marked germinal epithelium atrophy associated with absence of sperm in the epididymis. Based on the single occurrences of germinal epithelium degeneration or atrophy in these two exposure groups and the absence of germinal epithelium atrophy in higher exposure groups, these are both considered to be incidental findings.

Mice

All mice exposed to 7.0 ppm died during week 1 of the study, and five males and four females exposed to 3.5 ppm died by week 6 of the study (Table 8). In males exposed to 7.0 ppm, eight mice were found dead and two were euthanized. In females exposed to 7.0 ppm, nine mice were found dead and one was euthanized. In males exposed to 3.5 ppm, five were euthanized (during weeks 5 and 6), and five survived to study completion. In females exposed to 3.5 ppm, four were euthanized (in weeks 1, 3, and 6), and six survived to study completion.

Clinical findings in males and females exposed to 3.5 or 7.0 ppm included abnormal breathing, sneezing, and thinness. One or more of these clinical findings were present in animals that were euthanized prior to study completion. Of the mice that were found dead, there were no clinical findings that preceded death. The probable cause of death for all mice that died in the two highest exposure groups was undetermined; however, it was noted for nine mice that the nasal cavity could not be flushed with formalin. Necrosis and inflammation in the respiratory tract may have led to respiratory compromise and death in mice prior to study completion, as for rats. Alopecia was also observed in 3.5 ppm mice on day 44, but the condition had resolved by the end of the study. Exposure to *o*-phthalaldehyde caused animal urine and feces within the exposure chambers to turn black, as seen in rats. However, unlike in rats, black discoloration of the appendages was not noted in *o*-phthalaldehyde-exposed mice, possibly due to the normal dark skin pigmentation and coat color of B6C3F1/N mice.

Table 8. Survival and Body Weights of Mice in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

Concentration (ppm)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	22.3 ± 0.3	35.6 ± 0.9	13.3 ± 0.7	
0.44	10/10	22.4 ± 0.2	29.5 ± 0.4**	7.1 ± 0.3**	83
0.88	10/10	22.4 ± 0.2	27.2 ± 0.4**	4.9 ± 0.3**	76
1.75	10/10	22.3 ± 0.2	25.8 ± 0.3**	3.5 ± 0.3**	73
3.5[c]	5/10	22.6 ± 0.3	21.6 ± 0.8**	−0.8 ± 0.5**	61
7.0[d]	0/10	22.4 ± 0.2	–	–	–

Concentration (ppm)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Female					
0	10/10	19.1 ± 0.4	29.8 ± 0.9	10.7 ± 0.7	
0.44	10/10	18.8 ± 0.1	25.5 ± 0.3**	6.7 ± 0.3**	86
0.88	10/10	19.1 ± 0.2	23.3 ± 0.3**	4.2 ± 0.3**	78
1.75	10/10	19.0 ± 0.3	22.8 ± 0.4**	3.7 ± 0.4**	76
3.5[e]	6/10	19.0 ± 0.2	20.7 ± 0.8**	1.6 ± 1.0**	69
7.0[d]	0/10	19.2 ± 0.2	–	–	–

**Significantly different ($P \leq 0.01$) from the chamber control group by Williams' test.
[a]Body weights and weight changes are given as mean ± standard error.
[b]Number of animals surviving at 14 weeks/number initially in group. Subsequent calculations are based on animals surviving to the end of the study.
[c]Weeks of death: 5, 5, 5, 6, 6.
[d]Week of deaths: 1 (all).
[e]Weeks of death: 1, 3, 3, 6.

The final mean body weights and body weight gains of all surviving exposed groups of mice were significantly less than those of the chamber controls, and 3.5 ppm males lost weight during the study (4%; Table 8 and Figure 3). Final body weights relative to controls of all surviving mice were up to 39% and 31% lower in exposed males and females, respectively.

Hematology data were not available for the 7.0 ppm groups due to 100% mortality. The total leukocyte counts were significantly increased in all exposed male groups and in the 3.5 ppm female group (Table 9 and Table B-2). Lymphocyte numbers were significantly increased in all exposed male groups, while segmented neutrophil and eosinophil counts were significantly increased in groups exposed to 1.75 ppm or greater. In female mice, the segmented neutrophil count was significantly increased in the 3.5 ppm group and the eosinophil counts increased in groups exposed to 1.75 ppm or greater. These alterations were consistent with an inflammatory leukogram.

Hemoglobin concentrations, erythrocyte counts, hematocrit values, and packed cell volumes were significantly decreased in 1.75 and 3.5 ppm male mice (Table 9 and Table B-2). Similarly, the hemoglobin concentrations, hematocrit values, and packed cell volumes were significantly decreased in females exposed to 0.88 ppm or greater, and the erythrocyte count was decreased in the 3.5 ppm group. This combination of hematologic alterations is consistent with erythron suppression secondary to inflammation (i.e., anemia of inflammatory disease). All other statistically significant changes were sporadic or minimal, and not considered toxicologically relevant.

Absolute thymus weights of surviving groups of exposed mice were significantly lower than those of the chamber controls (up to 51% lower in males and 46% lower in females); the relative thymus weight of 3.5 ppm females was also significantly decreased (Table C-2). Histopathologic findings of lymphoid atrophy of the thymus, observed in the 3.5 and 7.0 ppm groups, likely contributed to the decreased thymus weights. In males and females, there were significant decreases in absolute heart, kidney, and liver weights at all exposure concentrations that were, in general, unaccompanied by significant decreases in relative organ weights. There were no histopathologic findings in the heart, kidney, or liver corresponding to organ weight decreases. Because body weights were significantly decreased at all exposure concentrations in male and female mice, these organ weight decreases were considered to be related to decreased body weights rather than exposure to *o*-phthalaldehyde. Organ weight data were not available in the 7.0 ppm groups due 100% mortality.

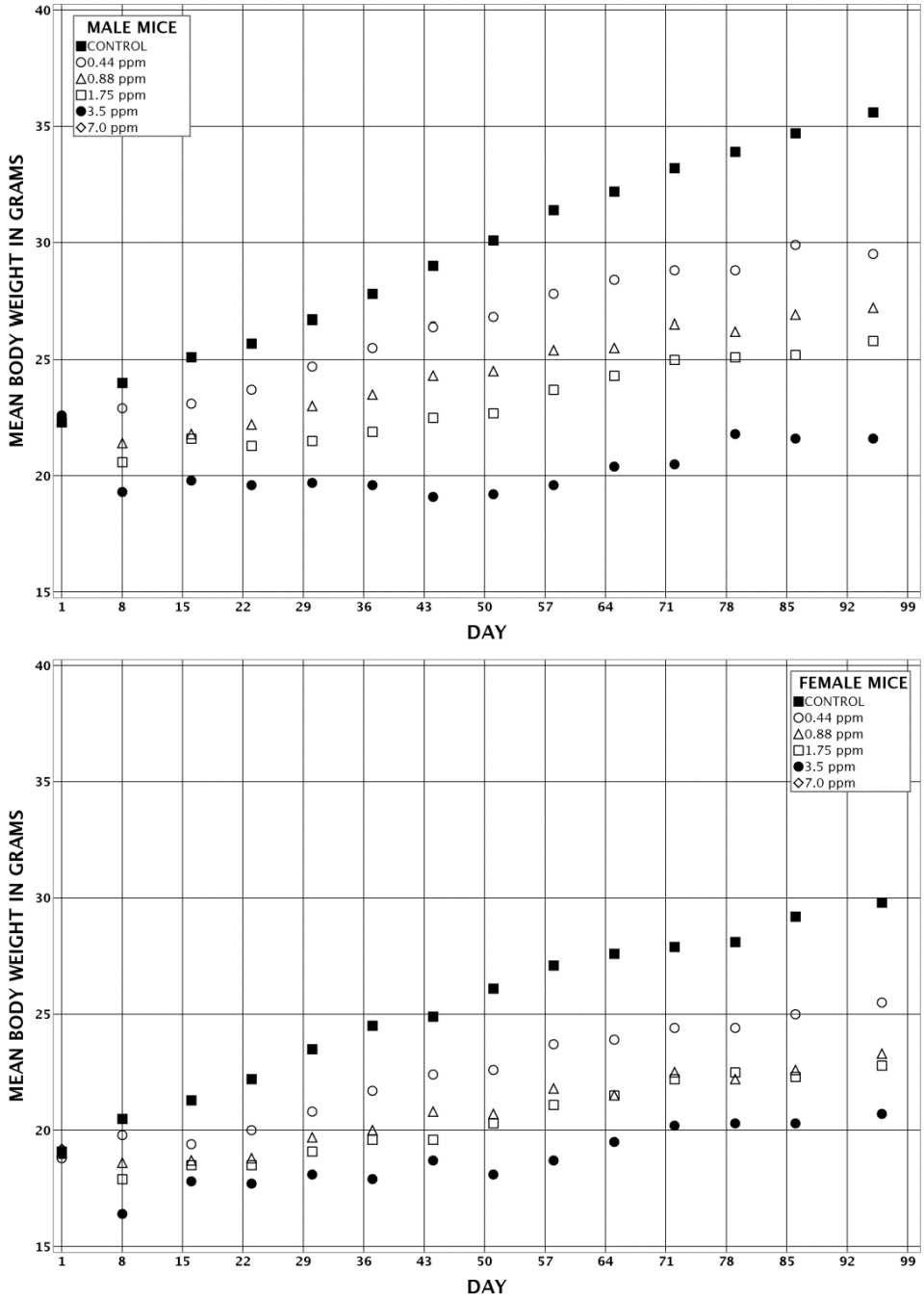

Figure 3. Growth Curves for Mice Exposed to *o*-Phthalaldehyde by Inhalation for Three Months

Table 9. Selected Hematology Data for Mice in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm
Male					
n	10	10	10	10	5
Hematocrit (spun) (%)	50.2 ± 0.4	50.3 ± 0.3	50.3 ± 0.4	49.0 ± 0.3*	47.5 ± 0.9*
Packed cell volume (%)	49.8 ± 0.4	50.3 ± 0.5	49.7 ± 0.3	47.9 ± 0.3**	46.4 ± 1.0**
Hemoglobin (g/dL)	16.0 ± 0.1	16.4 ± 0.1	16.1 ± 0.1	15.6 ± 0.1*	15.0 ± 0.3*
Erythrocytes (10^6/μL)	10.38 ± 0.07	10.66 ± 0.12	10.52 ± 0.08	10.04 ± 0.09*	9.72 ± 0.14**
Reticulocytes (10^3/μL)	256 ± 9	257 ± 14	258 ± 9	224 ± 10	228 ± 28
Mean cell volume (fL)	47.9 ± 0.2	47.2 ± 0.1	47.2 ± 0.2*	47.8 ± 0.2	47.7 ± 0.5
Mean cell hemoglobin (pg)	15.5 ± 0.1	15.4 ± 0.1	15.3 ± 0.1	15.5 ± 0.1	15.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.2 ± 0.1	32.6 ± 0.1	32.5 ± 0.2	32.5 ± 0.1	32.3 ± 0.1
Leukocytes (10^3/μL)	2.32 ± 0.19	3.32 ± 0.21**	3.08 ± 0.12**	4.61 ± 0.42**	6.15 ± 1.22**
Segmented neutrophils (10^3/μL)	0.30 ± 0.04	0.42 ± 0.02	0.41 ± 0.03	0.87 ± 0.08**	2.90 ± 1.14**
Lymphocytes (10^3/μL)	1.91 ± 0.15	2.82 ± 0.19**	2.57 ± 0.13**	3.57 ± 0.35**	2.83 ± 0.21**
Eosinophils (10^3/μL)	0.02 ± 0.00	0.02 ± 0.01	0.05 ± 0.01	0.12 ± 0.02**	0.19 ± 0.06**
Female					
n	10	10	10	10	6
Hematocrit (spun) (%)	50.7 ± 0.6	50.2 ± 0.3	48.8 ± 0.4**	49.3 ± 0.7*	46.0 ± 1.0**[b]
Packed cell volume (%)	50.1 ± 0.6	50.2 ± 0.3	48.6 ± 0.4**	48.7 ± 0.6*	47.1 ± 0.4**
Hemoglobin (g/dL)	16.5 ± 0.2	16.3 ± 0.1	15.8 ± 0.1**	15.9 ± 0.2**	15.2 ± 0.2**
Erythrocytes (10^6/μL)	10.33 ± 0.08	10.44 ± 0.05	10.10 ± 0.07	10.16 ± 0.11	9.70 ± 0.11**
Reticulocytes (10^3/μL)	285 ± 23	279 ± 16	299 ± 16	276 ± 13	243 ± 13
Mean cell volume (fL)	48.4 ± 0.3	48.1 ± 0.2	48.1 ± 0.1	48.0 ± 0.2	48.5 ± 0.3
Mean cell hemoglobin (pg)	15.9 ± 0.1	15.7 ± 0.1*	15.7 ± 0.0*	15.7 ± 0.1**	15.6 ± 0.1*
Mean cell hemoglobin concentration (g/dL)	32.9 ± 0.1	32.5 ± 0.1	32.6 ± 0.1	32.7 ± 0.1	32.2 ± 0.2*
Leukocytes (10^3/μL)	3.80 ± 0.51	2.78 ± 0.18	3.37 ± 0.34	4.01 ± 0.36	5.31 ± 0.36*
Segmented neutrophils (10^3/μL)	0.69 ± 0.23	0.47 ± 0.08	0.61 ± 0.06	0.84 ± 0.12	2.14 ± 0.31**
Lymphocytes (10^3/μL)	3.03 ± 0.32	2.27 ± 0.14	2.65 ± 0.30	3.04 ± 0.24	2.94 ± 0.37
Eosinophils (10^3/μL)	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.07 ± 0.01*	0.19 ± 0.05**

*Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data. Due to 100% mortality in mice exposed to 7.0 ppm, no data are available for this group.
[b]n = 5.

Statistically significant or biologically relevant histopathologic changes were noted in the nose, larynx, trachea, lung, skin, eye, spleen, thymus, bone marrow, testis, and epididymis of mice. As in rats, inhalation exposure to *o*-phthalaldehyde resulted in lesions at sites of contact within the respiratory system, skin, and eye that were generally consistent with an irritant effect; changes in the hematopoietic system that may be attributed to stress and inflammation; and changes in the male reproductive system. As in rats, in general, decreased incidences of some lesions in mice exposed to 3.5 or 7.0 ppm were likely associated with the limited exposure duration due to early deaths in these two highest exposure groups.

Nose: As in rats, a main target of *o*-phthalaldehyde exposure in mice was the respiratory system. In the nose, mice had many lesions occurring within the lowest exposure group, as seen in rats. All exposed male and female mice (except four 7.0 ppm females) exhibited minimal to marked suppurative inflammation (Table 10, Table A-3, and Table A-4). Significantly increased incidences of turbinate atrophy occurred in all exposed groups of mice, except 7.0 ppm males and females. Turbinate atrophy, a chronic change, may have been absent at 7.0 ppm due to decreased exposure duration because of the early deaths. Microscopically, suppurative inflammation in the nose and turbinate atrophy were similar to the same lesions observed in rats (Figure 7).

As in rats, several lesions were noted in the olfactory epithelium of the nose in exposed male and female mice (Table 10, Table A-3, and Table A-4). The incidences of olfactory epithelium hyaline droplet accumulation and glands hyperplasia were significantly increased in all exposed groups, except 7.0 ppm males and females. All exposed groups of male and female mice had increased incidences of olfactory epithelium atrophy as compared to the chamber controls. Incidences of respiratory metaplasia of the olfactory epithelium in 0.88, 1.75, and 3.5 ppm males and 0.88 and 3.5 ppm females were significantly increased. A few males in the 0.88, 1.75, and 3.5 ppm groups also had squamous metaplasia of the olfactory epithelium. The incidence of necrosis of the olfactory epithelium of the nose in males exposed to 7.0 ppm was significantly increased compared to that in the chamber controls.

Microscopically, olfactory epithelium hyaline droplet accumulation, atrophy, respiratory metaplasia, squamous metaplasia, and necrosis were similar to the same lesions observed in rats. Hyperplasia of the glands in the olfactory epithelium occurred predominantly in level III sections of the nose, subjacent to atrophic olfactory epithelium, and was seen as clusters of Bowman's glands with increased cell numbers with more densely staining cytoplasm and nuclei, often arranged around a distinct lumen, which sometimes contained neutrophils.

As in rats, lesions were also present in the respiratory epithelium of the nose (Table 10, Table A-3, and Table A-4). Exposed male and female mice exhibited significantly increased incidences of respiratory epithelium hyaline droplet accumulation, except in the highest exposure group (7.0 ppm), likely due to their limited exposure duration due to early deaths. Many of the mice with hyaline droplet accumulation in the respiratory epithelium also had significantly increased incidences of hyaline droplet accumulation in the underlying glands. All exposed groups, except for the 7.0 ppm females, exhibited significantly increased incidences of respiratory epithelium squamous metaplasia. There were significantly increased incidences in necrosis of the respiratory epithelium in males and females exposed to 0.88 ppm or greater compared to the chamber controls. The incidences of respiratory epithelium regeneration were

significantly increased in 7.0 ppm males and females. There were two incidences of respiratory epithelium ulcer in 7.0 ppm females, but the increases were not statistically significant.

Table 10. Incidences of Nonneoplastic Lesions of the Respiratory System in Mice in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Male						
Nose[b]	10	10	10	10	10	10
Inflammation, Suppurative[c]	0	10** (2.1)[d]	10** (2.7)	10** (4.0)	10** (3.2)	10** (1.9)
Glands, Olfactory Epithelium, Hyperplasia	0	10** (1.5)	10** (1.6)	7** (1.7)	6** (1.5)	0
Olfactory Epithelium, Accumulation, Hyaline Droplet	0	10** (2.9)	10** (3.0)	10** (2.2)	9** (2.2)	0
Olfactory Epithelium, Atrophy	0	10** (2.3)	10** (1.9)	10** (2.6)	10** (2.3)	10** (1.5)
Olfactory Epithelium, Metaplasia, Respiratory	0	1 (2.0)	8** (1.4)	10** (1.9)	4* (1.5)	0
Olfactory Epithelium, Metaplasia, Squamous	0	0	2 (2.0)	1 (1.0)	2 (1.5)	0
Olfactory Epithelium, Necrosis	0	0	0	0	0	5* (2.2)
Respiratory Epithelium, Accumulation, Hyaline Droplet	0	10** (3.0)	10** (3.5)	10** (3.8)	10** (2.0)	0
Glands, Respiratory Epithelium, Accumulation, Hyaline Droplet	0	7** (2.0)	10** (2.4)	6** (1.0)	0	0
Respiratory Epithelium, Metaplasia, Squamous	0	10** (1.7)	10** (2.1)	10** (2.7)	10** (2.4)	6** (1.2)
Respiratory Epithelium, Necrosis	0	2 (1.0)	6** (1.3)	5* (1.2)	9** (1.9)	10** (2.8)
Respiratory Epithelium, Regeneration	0	0	1 (1.0)	0	1 (2.0)	4* (1.3)
Turbinate Atrophy	0	4* (1.0)	6** (1.7)	10** (2.8)	8** (2.4)	0
Larynx	10	10	9	10	10	10
Inflammation, Chronic Active	0	0	0	4* (1.0)	10** (1.3)	10** (3.0)
Metaplasia, Squamous	0	0	1 (1.0)	10** (2.4)	10** (4.0)	8** (3.5)
Necrosis	0	0	0	0	1 (2.0)	10** (3.1)
Trachea	10	10	10	10	10	10
Inflammation, Chronic Active	0	0	0	1 (1.0)	9** (1.3)	10** (1.6)
Metaplasia, Squamous	0	0	0	3 (1.0)	10** (2.8)	3 (2.0)
Necrosis	0	0	0	0	0	9** (2.7)
Regeneration	0	0	0	0	0	5* (1.6)
Lung	10	10	10	10	10	10
Bronchiole, Goblet Cell, Hyperplasia	0	0	0	0	4* (1.0)	0

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Bronchus, Inflammation, Chronic Active	0	0	0	0	1 (2.0)	6** (1.5)
Bronchus, Necrosis	0	0	0	0	2 (1.0)	9** (2.4)
Female						
Nose	10	10	10	10	10	10
Inflammation, Suppurative	0	10** (2.0)	10** (3.2)	10** (4.0)	10** (3.0)	6** (1.5)
Glands, Olfactory Epithelium, Hyperplasia	0	10** (2.1)	9** (2.1)	10** (1.5)	8** (2.0)	0
Olfactory Epithelium, Accumulation, Hyaline Droplet	0	10** (3.0)	10** (2.8)	10** (2.0)	6** (1.3)	0
Olfactory Epithelium, Atrophy	0	10** (2.3)	10** (2.1)	10** (2.6)	9** (2.4)	9** (1.8)
Olfactory Epithelium, Metaplasia, Respiratory	0	3 (1.3)	6** (1.2)	3 (1.0)	4* (2.3)	0
Respiratory Epithelium, Accumulation, Hyaline Droplet	0	10** (3.0)	10** (3.3)	10** (3.6)	8** (2.0)	0
Glands, Respiratory Epithelium, Accumulation, Hyaline Droplet	0	9** (2.1)	10** (2.1)	6** (2.0)	2 (1.0)	0
Respiratory Epithelium, Metaplasia, Squamous	0	10** (2.0)	10** (2.7)	10** (2.7)	8** (2.6)	0
Respiratory Epithelium, Necrosis	0	2 (1.0)	7** (1.1)	6** (1.3)	8** (1.5)	10** (2.5)
Respiratory Epithelium, Regeneration	0	0	0	1 (1.0)	3 (2.0)	6** (1.7)
Respiratory Epithelium, Ulcer	0	0	0	0	0	2 (1.0)
Turbinate Atrophy	0	7** (1.0)	9** (2.1)	10** (3.3)	7** (3.1)	0
Larynx	10	10	10	10	10	10
Inflammation, Chronic Active	0	0	0	0	9** (1.9)	10** (3.1)
Metaplasia, Squamous	0	0	3 (1.3)	10** (2.1)	10** (3.9)	8** (3.8)
Necrosis	0	0	0	0	3 (2.0)	9** (2.6)
Regeneration	0	0	0	0	0	3 (1.7)
Trachea	10	10	10	10	10	10
Inflammation, Chronic Active	0	0	0	0	10** (1.9)	10** (1.3)
Metaplasia, Squamous	0	0	0	0	9** (3.1)	2 (1.5)
Necrosis	0	0	0	0	2 (1.5)	10** (3.5)
Regeneration	0	0	0	0	1 (1.0)	2 (2.0)
Lung	10	10	10	10	10	10
Bronchiole, Goblet Cell, Hyperplasia	0	0	0	0	6** (1.5)	0
Bronchus, Inflammation, Chronic Active	0	0	0	0	6** (1.2)	8** (1.4)
Bronchus, Necrosis	0	0	0	0	2 (2.0)	8** (2.8)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Bronchus, Ulcer	0	0	0	0	0	2 (2.5)

*Significantly different from the chamber control group ($P \leq 0.05$) by the Fisher exact test.
**$P \leq 0.01$.
[a]All mice exposed to 7.0 ppm died during week 1 of the study, and five males and four females exposed to 3.5 ppm died by week 6 of the study. This limited exposure duration may have affected lesion incidence rates.
[b]Number of animals with tissue examined microscopically.
[c]Number of animals with lesion.
[d]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Microscopically, respiratory epithelium hyaline droplet accumulation was similar to that observed in the olfactory epithelium. Hyaline droplet accumulation in the glands of the respiratory epithelium was characterized in levels I and II sections of the nose by robust enlargement of the glands in the lamina propria of the dorsal meatus and nasal septum by accumulations of bright eosinophilic, hyaline material that often compressed the nucleus. These glands were arranged around distinct lumens that were often filled with degenerate neutrophils. Necrosis and regeneration of the respiratory epithelium were similar to those observed in rats. Ulcers were noted as focal to focally extensive areas of loss of the normal respiratory epithelium.

Larynx: In the larynx, male and female mice exhibited significantly increased incidences of chronic active inflammation, squamous metaplasia, and necrosis (Table 10, Table A-3, and Table A-4). Significantly increased incidences of chronic active inflammation occurred in 1.75 ppm males as well as in almost all 3.5 and 7.0 ppm males and females, and severity increased with exposure concentration. Significantly increased incidences of squamous metaplasia occurred in males and females exposed to 1.75 ppm or greater. Significant increases in the incidences of necrosis occurred in male and female mice exposed to 7.0 ppm. Three 7.0 ppm females exhibited regeneration in the larynx, but this incidence was not statistically significant compared to the chamber control incidence.

Microscopically, chronic active inflammation of the larynx varied in that the animals with more necrosis demonstrated an infiltrate that was predominantly neutrophilic, whereas animals that developed squamous metaplasia had increased numbers of lymphocytes, plasma cells, and macrophages, in addition to neutrophils. Squamous metaplasia was characterized as replacement of the normal respiratory epithelium with multiple layers of flattened squamous epithelium that sometimes progressed to keratinization along the superficial surface. Necrosis and regeneration were similar to the lesions observed in rats.

Trachea: While lesions were noted in the nose beginning in the lowest exposure group, 0.44 ppm, and in the larynx beginning at 0.88 ppm, findings in the trachea were only noted beginning at exposures of 1.75 ppm for males and 3.5 ppm for females (Table 10, Table A-3, and Table A-4). In the trachea, male and female mice exhibited significantly increased incidences of chronic active inflammation, squamous metaplasia, necrosis, and regeneration (males only). The incidences of chronic active inflammation were significantly increased in 3.5 and 7.0 ppm males and females. Significant increases in the incidences of squamous metaplasia were observed in males and females exposed to 3.5 ppm. Significantly increased incidences of necrosis (Figure 8) occurred in 7.0 ppm males and females, and a significantly increased incidence of regeneration occurred in 7.0 ppm males. For comparison, there were six occurrences of tracheal ulceration in rats, whereas there was only one noted in mice (Table A-1 and Table A-3). Tracheal ulceration

was present in a 3.5 ppm female, which was a moribund sacrifice noted to have abnormal breathing (Table A-4). Microscopically, fibrosis and regeneration in the trachea in mice were similar to those described previously in rats (Figure 4).

Lung: Lesions in the lung in mice were notable only in the two highest exposure groups, 3.5 and 7.0 ppm (Table 10, Table A-3, and Table A-4). Thus, as observed in rats, inflammatory and degenerative or reparative findings in mice increased in depth within the respiratory tract from nose to lung with increased exposure concentration of *o*-phthalaldehyde. The occurrences of bronchiole goblet cell hyperplasia in 3.5 ppm males and females, chronic active inflammation of the bronchus in 3.5 ppm females and 7.0 ppm males and females, and necrosis in the bronchus of 7.0 ppm males and females were significantly increased compared to those in the chamber controls. Although not statistically significant, ulcer of the bronchus occurred in two female mice in the 7.0 ppm group.

Microscopically, goblet cell hyperplasia in the lung was characterized by epithelial cells with increased cytoplasm expanded by pale basophilic material, with mucus and occasional inflammatory cells in the lumen of bronchioles. Chronic active inflammation of the bronchus was characterized by neutrophils with rare plasma cells and lymphocytes within bronchi and peribronchial connective tissue. Rarely, this infiltrate extended into bronchioles. Bronchus necrosis was seen as a focal to focally extensive area of epithelium that displayed loss of differential staining and was associated with fibrin or necrotic cellular debris. Ulcer of the bronchus was noted when the epithelium was absent with no associated necrotic cellular debris or fibrin overlying the denuded epithelium.

Skin: In addition to the respiratory tract, the skin was also a target of *o*-phthalaldehyde inhalation exposure. In standard inguinal skin sections, male and female mice exhibited significantly increased incidences of squamous hyperplasia, chronic active inflammation, adnexa degeneration, epidermis necrosis (females only), and hair follicle epithelium parakeratosis (Table 11, Table A-3, and Table A-4). The incidences of squamous hyperplasia were significantly increased in 3.5 ppm males and females and 7.0 ppm males. The incidences of chronic active inflammation were significantly increased in females exposed to 1.75 ppm and males and females exposed to 3.5 or 7.0 ppm. Significant increases in the incidences of adnexa degeneration occurred in 1.75 ppm males and in 7.0 ppm males and females. Significantly increased incidences of epidermal necrosis occurred in 7.0 ppm females and significantly increased incidences of hair follicle epithelium parakeratosis occurred in males exposed to 0.88 or 7.0 ppm and in females exposed to 0.88 ppm or greater.

Microscopically, squamous hyperplasia in routine inguinal skin sections was observed as thickening of the epithelium that exceeded two to three cell layers. Chronic active inflammation consisted of neutrophils, plasma cells, and lymphocytes that were scattered throughout the superficial dermis and rarely extended into the overlying epidermis. In a few cases, small clusters of degenerate neutrophils (pustules) accumulated within the epidermis.

Table 11. Incidences of Selected Nonneoplastic Lesions in Mice in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Male						
Skin[b]	10	10	10	9	10	10
Hyperplasia, Squamous[c]	0	1 (2.0)[d]	0	0	9** (1.7)	4* (2.0)
Inflammation, Chronic Active	0	0	0	1 (1.0)	10** (1.8)	9** (1.1)
Adnexa, Degeneration	0	2 (1.5)	0	4* (2.0)	1 (1.0)	6** (2.0)
Epidermis, Necrosis	0	0	0	0	0	3 (2.0)
Epithelium, Hair Follicle, Parakeratosis	0	3 (1.0)	4* (1.0)	2 (1.0)	2 (1.5)	10** (1.8)
Skin, Pinna	10	0	0	0	0	10
Adnexa, Degeneration	0	–	–	–	–	10** (2.0)
Epithelium, Hair Follicle, Parakeratosis	0	–	–	–	–	10** (2.1)
Hyperplasia, Squamous	0	–	–	–	–	10** (2.0)
Inflammation, Chronic Active	0	–	–	–	–	10** (1.8)
Eye	10	10	10	10	10	10
Cornea, Inflammation, Suppurative	0	1 (2.0)	0	0	1 (1.0)	4* (1.0)
Bone Marrow	10	0	0	10	10	10
Hyperplasia	0	–	–	2 (1.0)	5* (1.4)	7** (1.9)
Spleen	10	0	0	10	10	10
Atrophy, Lymphoid	0	–	–	0	0	10** (1.9)
Thymus	10	10	10	10	9	10
Atrophy, Lymphoid	0	0	0	0	5* (1.4)	10** (2.7)
Female						
Skin	10	10	10	10	10	10
Hyperplasia, Squamous	3 (1.3)	0	0	6 (1.3)	9** (1.7)	6 (1.0)
Inflammation, Chronic Active	1 (1.0)	1 (1.0)	3 (1.0)	9** (1.1)	9** (1.9)	8** (1.3)
Adnexa, Degeneration	2 (1.0)	0	0	1 (1.0)	0	9** (1.3)
Epidermis, Necrosis	0	0	0	0	0	5* (2.2)
Epithelium, Hair Follicle, Parakeratosis	0	3 (1.0)	7** (1.0)	9** (1.1)	10** (1.1)	10** (2.1)
Skin, Pinna	10	0	0	0	0	10
Adnexa, Degeneration	0	–	–	–	–	10** (1.7)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Epithelium, Hair Follicle, Parakeratosis	0	–	–	–	–	10** (2.0)
Hyperplasia, Squamous	0	–	–	–	–	10** (1.5)
Inflammation, Chronic Active	0	–	–	–	–	10** (1.4)
Eye	10	10	10	10	10	8
Cornea, Inflammation, Suppurative	0	0	0	0	2 (1.0)	3 (1.0)
Cornea, Necrosis	0	0	0	0	0	2 (1.5)
Bone Marrow	10	0	0	0	10	7
Hyperplasia	0	–	–	–	8** (2.0)	0
Spleen	10	0	0	0	10	8
Atrophy, Lymphoid	0	–	–	–	1 (1.0)	8** (1.6)
Thymus	10	10	9	10	10	10
Atrophy, Lymphoid	0	0	0	0	6** (2.0)	10** (2.6)

*Significantly different from the chamber control group ($P \leq 0.05$) by the Fisher exact test.
**$P \leq 0.01$.
[a]All mice exposed to 7.0 ppm died during week 1 of the study, and five males and four females exposed to 3.5 ppm died by week 6 of the study. This limited exposure duration may have affected lesion incidence rates.
[b]Number of animals with tissue examined microscopically.
[c]Number of animals with lesion.
[d]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Adnexa degeneration was characterized by the accumulation of pale basophilic homogenous material in the cytoplasm of multiple follicular and adnexal epithelial cells that often surrounded and compressed the nucleus (Figure 9). Electron microscopy, performed on an affected skin sample, showed that the cytoplasmic accumulations were inconsistently membrane bound and varied from diffusely electron lucent particles to small dense bodies that resemble glycogen. In addition to the cytoplasmic accumulations, adnexa degeneration also encompassed an increase in apoptosis, seen as small cells with dark eosinophilic cytoplasm and multiple small round, dark pyknotic bodies (apoptotic debris) within scattered individual hair follicle epithelial cells. Necrosis in the epidermis was noted as decreased staining and loss of cellular detail involving the entire thickness of the epidermis, with occasional cleft formation between the epidermis and dermis. Hair follicle epithelium parakeratosis was characterized by increased layers of brightly eosinophilic material (keratin) with retention of nuclei (parakeratosis) that collected into plaques along the epithelial surface, with preferential involvement of follicular regions over interfollicular areas.

Evaluation of skin included additional evaluation of the left pinna in control and 7.0 ppm male and female mice (pinnae were not available in rats). The additional evaluation was based on lesions noted in routine inguinal skin sections in mice during peer review, clinical findings of black pigmentation noted on appendages (pinnae and/or feet) during in-life exposure in rats, and technical information and scientific literature on contact dermatitis/chemical burns associated with *o*-phthalaldehyde exposure in humans. Squamous hyperplasia, chronic active inflammation,

adnexa degeneration, and hair follicle epithelium parakeratosis were present in the pinnae of all male and female mice exposed to 7.0 ppm (Table 11, Table A-3, and Table A-4).

Microscopically, within skin of the pinna (Figure 10 and Figure 11), squamous hyperplasia was characterized by diffuse thickening of the epithelium, primarily involving the stratum spinosum, in some cases up to 10 cell layers thick. Chronic active inflammation was noted as minimal to mild collections of neutrophils, lymphocytes, plasma cells, and mast cells primarily within the superficial dermis. Adnexa degeneration was noted as irregular, poorly defined sebaceous glands with increased pyknotic and karyorrhectic debris evident both within the follicular epithelium and adnexal structures. Hair follicle epithelium parakeratosis was described as diffuse thickening of the stratum corneum with increased keratin within follicular regions predominantly characterized by retention of nuclei (parakeratosis), whereas interfollicular regions lacked nuclei (orthokeratosis) (Figure 11).

Eye: As was observed in the rats, in addition to the skin, the eye was another target of *o*-phthalaldehyde inhalation exposure in mice. In the eye, the incidence of cornea suppurative inflammation was significantly increased in 7.0 ppm males (Table 11, Table A-3, and Table A-4). Necrosis of the cornea was present in two 7.0 ppm females. Microscopically, suppurative inflammation and necrosis resembled the lesions observed in rats.

Hematopoietic System: A change observed in mice, but not in rats, was significantly increased incidences of bone marrow hyperplasia in 3.5 ppm males and females and 7.0 ppm males (Table 11, Table A-3, and Table A-4). Microscopically, bone marrow hyperplasia was seen as hypercellularity with a subjective increase in the myeloid:erythroid ratio, most noticeably within the diaphysis of the femur, but also within sections of the skull. The increase in the myeloid lineage was likely in response to inflammation in the respiratory tract, skin, and/or eye.

Lymphoid atrophy occurred in the spleen and thymus of male and female mice (Table 11, Table A-3, and Table A-4). Lymphoid atrophy of the spleen was significantly increased in 7.0 ppm males and females. The incidence of lymphoid atrophy of the thymus was significantly increased in 3.5 and 7.0 ppm males and females.

Microscopically, lymphoid atrophy in the spleen was similar to what was seen in rats. In the thymus, lymphoid atrophy consisted of a diffuse reduction in cortical lymphocytes with a resulting decrease in organ size, shrinkage of thymic lobules, loss of the corticomedullary junction, and increased prominence of centrilobular septae. Less affected animals had subtle lesions that often consisted only of increased numbers of apoptotic bodies and tingible body macrophages. In rats and mice exposed to *o*-phthalaldehyde, lymphoid atrophy in the spleen and thymus are likely attributable to glucocorticoid release because they were present in the two highest exposure groups, which had early deaths.

Male Reproductive System: Mice exposed to 0.44, 0.88, or 1.75 ppm *o*-phthalaldehyde exhibited lower sperm motility (10% to 21% lower) (Table 12 and Table D-2). No histopathologic lesions were attributed to exposure concentrations less than 3.5 ppm. However, as was observed in rats, at the two highest exposure concentrations (at which morbidity and mortality were observed), testicular and epididymal histopathologic lesions were noted. Sperm parameter data were not available in the 3.5 and 7.0 ppm groups due to excessive mortality.

A significantly increased incidence of cellular depletion of the germinal epithelium of the testis occurred in 3.5 ppm males (Table 12 and Table A-3). In addition, three of the early death mice from the 3.5 ppm group had mild interstitial cell atrophy. There was a significantly increased incidence of exfoliated germ cell in the epididymal duct in males exposed to 3.5 ppm. These changes in the testis and epididymis in the 3.5 ppm group (partial depletion of germ cells, interstitial cell atrophy, and exfoliated germ cells in the epididymis) could be due to decreased body weight gain secondary to treatment, but an effect of *o*-phthalaldehyde cannot be ruled out.

Microscopically, germinal epithelium cellular depletion of the testis was minimal to mild and characterized by small numbers of seminiferous tubules with partial depletion of one or more generations of germ cells. Four of the seven mice with germinal epithelium cellular depletion also had minimal to mild accumulations of exfoliated germ cells and debris present in the duct of the epididymis. Interstitial cell atrophy resembled the lesion in rats but was less severe in mice.

Table 12. Reproductive System Parameters of Male Mice in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
n	10	10	10	10	0	0
Sperm Motility (%)	74.7 ± 8.4	69.5 ± 7.8*	68.8 ± 7.7**	41.8 ± 9.6**	–	–
Epididymis[b]	10	10	10	10	10	10
Duct, Exfoliated Germ Cell[c]	0	0	0	0	4▲ (1.3)[d]	1 (1.0)
Testes	10	10	10	10	10	10
Germinal Epithelium, Depletion Cellular, Multifocal	0	1 (1.0)	1 (1.0)	0	7▲▲ (1.3)	0
Interstitial Cell, Atrophy	0	0	0	0	3 (2.0)	0

*Significantly different ($P \leq 0.05$) from the chamber control group by Shirley's test.
**$P \leq 0.01$.
▲Significantly different from the chamber control group ($P \leq 0.05$) by the Fisher exact test.
▲▲$P \leq 0.01$.
[a]Sperm motility is presented as mean ± standard error. Sperm motility was not available for the 3.5 and 7.0 ppm groups due to excessive mortality.
[b]Number of animals with tissue examined microscopically.
[c]Number of animals with lesion.
[d]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Genetic Toxicology

o-Phthalaldehyde was tested by NTP for mutagenicity in bacterial tester strains (Table E-1) and for induction of chromosomal damage, measured as micronuclei in normochromatic erythrocytes (NCEs) and polychromatic erythrocytes (PCEs; reticulocytes) of male and female rats (Table E-2) and mice (Table E-3).

o-Phthalaldehyde (0.5 to 50 µg/plate) was mutagenic in *Salmonella typhimurium* strain TA100 (this strain reverts via base substitution) in the absence of exogenous metabolic activation (induced rat liver S9 enzymes and cofactors). *o*-Phthalaldehyde (10 to 400 µg/plate) was not mutagenic in TA100 with S9, nor was it mutagenic in TA98 or the *Escherichia coli* tester strain

WP2 *uvrA*/pKM101, with or without S9, over a concentration range of 0.5 to 200 µg/plate (Table E-1).

o-Phthalaldehyde (0.44 to 3.5 ppm) did not increase the frequencies of micronucleated PCEs in male or female Sprague Dawley rats exposed to *o*-phthalaldehyde for 3 months by inhalation (Table E-2). No increases in micronucleated erythrocytes (NCEs) were seen in male or female rats following exposure to *o*-phthalaldehyde, and that is expected, because the rat spleen efficiently removes damaged reticulocytes soon after they emerge from the bone marrow. In addition, no significant effects on the percentage of PCEs (%PCE) were seen in either male or female rats. It should be noted that in the 3.5 ppm group of male rats, only two animals survived; the data from this group were not included in the pairwise comparisons or trend tests because a minimum of three animals is required for a valid data point.

o-Phthalaldehyde (0.44 to 3.5 ppm) did not increase the frequencies of micronucleated PCEs or micronucleated NCEs in female B6C3F1/N mice exposed to the chemical for 3 months by inhalation. In male mice, results of the micronucleus test were judged to be equivocal, based on an increased frequency of micronucleated PCEs in the 3.5 ppm group, which resulted in a significant trend ($P = 0.005$). A pairwise comparison (William's test) of this treatment group to the chamber control group was not significant ($P = 0.0289$ with 0.025 required for significance), and the increase in micronucleated PCEs seen in the male mice was not confirmed in the NCE population, where no increase in micronucleated NCEs was seen at any dose level. This difference in response in the male mice between the immature (PCE) and mature (NCE) erythrocyte populations is difficult to reconcile because, unlike in rats, the frequency of micronucleated erythrocytes reaches steady state in peripheral blood of mice after about 30 days of exposure. The %PCE was elevated (trend $P = 0.001$) in female mice exposed to *o*-phthalaldehyde but not in male mice.

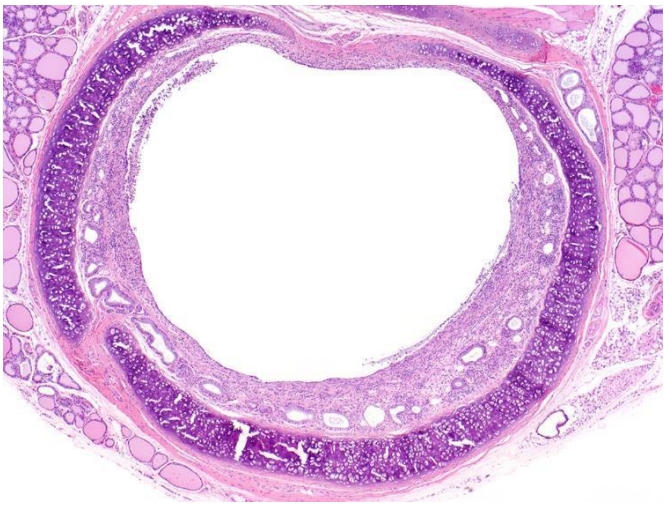

Figure 4. Fibrosis in the Trachea of a Male Sprague Dawley Rat Exposed to 3.5 ppm *o*-Phthalaldehyde by Inhalation for Three Months (H&E)

There is an increase in fibrous connective tissue that expands the lamina propria.

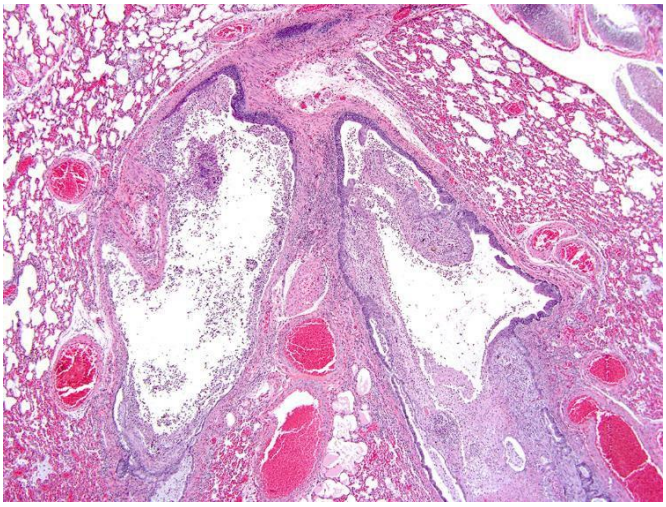

Figure 5. Chronic Active Inflammation, Necrosis, and Fibrosis (Intra-luminal) in the Bronchi of a Female Sprague Dawley Rat Exposed to 7.0 ppm *o*-Phthalaldehyde by Inhalation for Three Months (H&E)

Neutrophils, lymphocytes, plasma cells, and macrophages are present within mucus in the bronchi and the peribronchial connective tissue. The epithelium is focally lost and inflammatory fibrotic structures extend into the bronchial lumen.

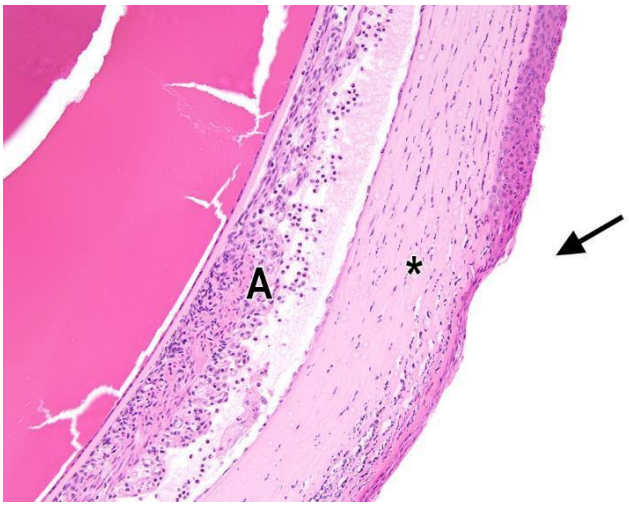

Figure 6. Necrosis of the Cornea (Arrow), Suppurative Inflammation of the Cornea (Asterisk), and Suppurative Inflammation of the Anterior Chamber (A) in the Eye of a Female Sprague Dawley Rat Exposed to 7.0 ppm *o*-Phthalaldehyde by Inhalation for Three Months (H&E)

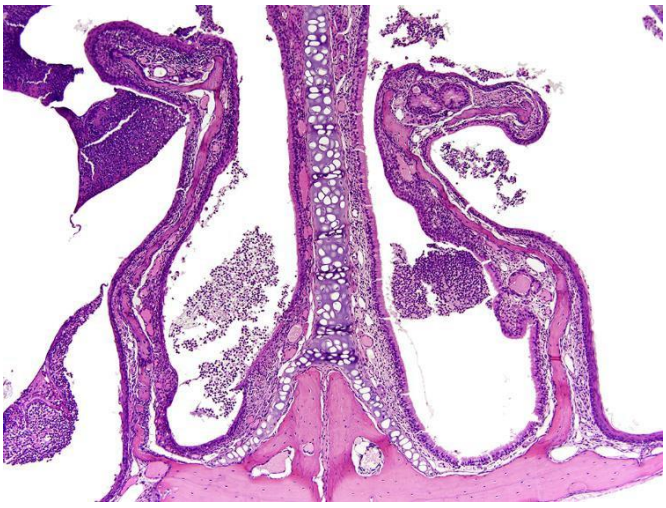

Figure 7. Suppurative Inflammation and Turbinate Atrophy in the Nose of a Female B6C3F1/N Mouse Exposed to 3.5 ppm *o*-Phthalaldehyde by Inhalation for Three Months (H&E)

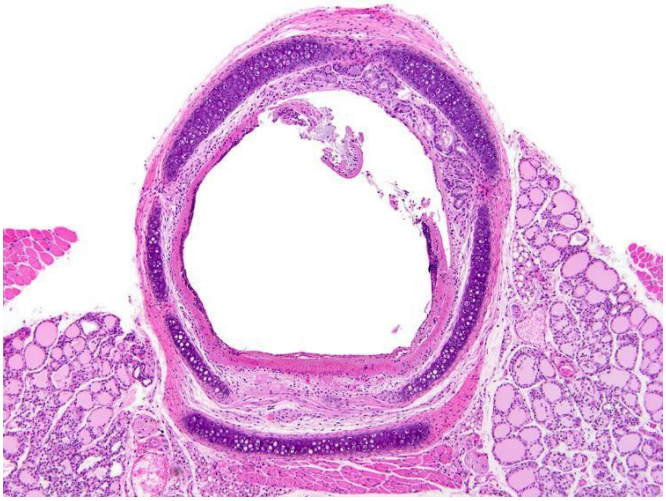

Figure 8. Necrosis in the Trachea of a Male B6C3F1/N Mouse Exposed to 7.0 ppm *o*-Phthalaldehyde by Inhalation for Three Months (H&E)

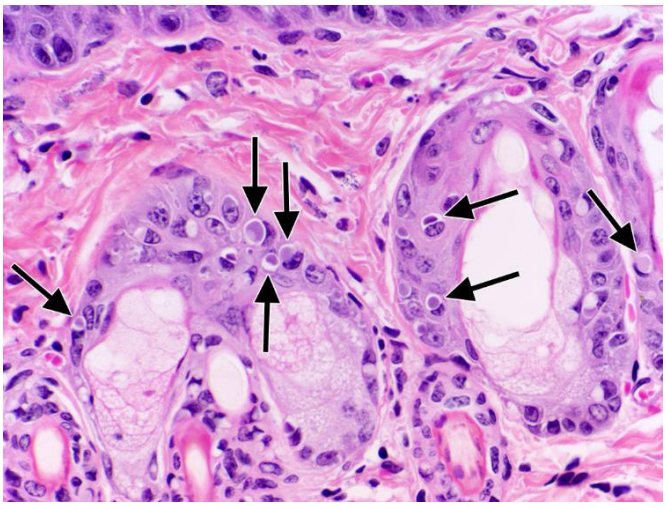

Figure 9. Adnexa Degeneration in the Skin of a Male B6C3F1/N Mouse Exposed to 7.0 ppm *o*-Phthalaldehyde by Inhalation for Three Months (H&E)

The sebaceous glands are irregular and poorly defined. Pale basophilic homogeneous material in the cytoplasm of multiple adnexal epithelial cells surrounds and compresses the nuclei (arrows).

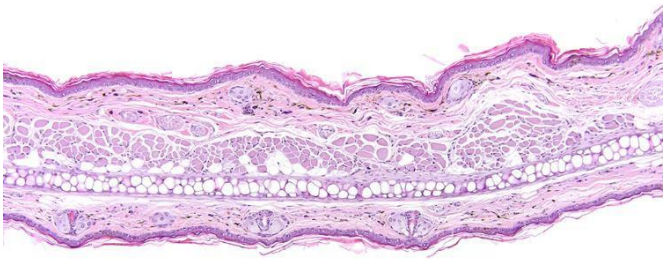

Figure 10. Normal Skin from the Pinna (Ear) of a Chamber Control Male B6C3F1/N Mouse in the Three-Month Inhalation Study of *o*-Phthalaldehyde (H&E)

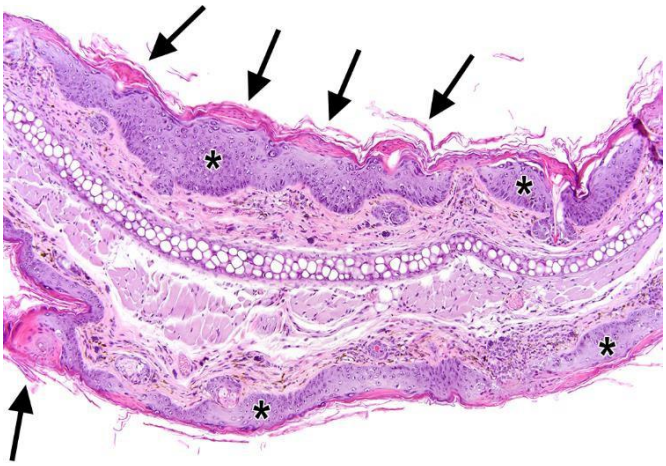

Figure 11. Epithelium Parakeratosis (Arrows) and Squamous Hyperplasia (Asterisks) in the Skin of the Pinna of a Male B6C3F1/N Mouse Exposed to 7.0 ppm *o*-Phthalaldehyde by Inhalation for Three Months (Compare to Figure 10 at the Same Magnification) (H&E)

There are collections of brightly eosinophilic material (keratin) with retention of nuclei (parakeratosis) along the epithelial surface (arrows). The epithelium is diffusely thickened (asterisks).

Discussion

o-Phthalaldehyde was introduced to the United States market in 1999 as a safer alternative high-level chemical disinfectant for medical equipment compared to glutaraldehyde. Glutaraldehyde has been used extensively to disinfect heat-sensitive medical equipment such as endoscopes and thermometers, but it has been associated with adverse health outcomes (e.g., dermatitis and occupational asthma)[11]. *o*-Phthalaldehyde is considered to be safer than glutaraldehyde due to its antimicrobial efficacy combined with its lower vapor pressure[11; 20]. However, the low vapor pressure and low odor of *o*-phthalaldehyde can present an occupational hazard, because its presence in room air would not be as noticeable to workers. There are limited studies assessing the safety of *o*-phthalaldehyde; however, dermal sensitization and inhalation studies have found *o*-phthalaldehyde to be a dermal irritant and contact-sensitizer that can also cause irritation to the eyes and the mucus membranes lining the respiratory tract[24; 26].

There are no existing personal exposure limits or recommended guidelines established by the Occupational Safety and Health Administration (OSHA) or the National Institute for Occupational Safety and Health (NIOSH) for *o*-phthalaldehyde in occupational settings. Though there is no personal exposure limit established by OSHA or NIOSH for *o*-phthalaldehyde, glutaraldehyde has a NIOSH Recommended Exposure Limit of 0.2 ppm (0.8 mg/m^3) and the American Conference of Governmental Industrial Hygienists recommends a Threshold Limit Value of 0.05 ppm in air[18]. In addition to its widespread occupational use as a disinfectant, *o*-phthalaldehyde has many other uses that include diagnostic tests[56-58], as a reagent in drinking water analysis[59], and as an intermediate in pharmaceutical or chemical synthesis. There is potential for occupational exposure in the health care industry during chemical disinfection of medical equipment; *o*-phthalaldehyde has been detected in air samples from hospital endoscopy units in Italy and the United States at levels ranging from 1.0 to 13.5 ppb[13; 14]. As a result of its increasing use and the lack of adequate and publicly available toxicity data, *o*-phthalaldehyde was nominated for study by NIOSH for toxicologic characterization. Given the occupational use pattern, whole-body inhalation was chosen as the route of exposure for the current studies.

The exposure concentrations for these 3-month inhalation studies in Sprague Dawley rats and B6C3F1/N mice were 0, 0.44, 0.88, 1.75, 3.5, and 7.0 ppm *o*-phthalaldehyde. The highest exposure concentration was selected based on NTP evaluations of the maximum achievable concentration without aerosolization under normal chamber environmental specifications. The lowest concentration was similar to the experimental limit of quantitation for the online monitor used in these studies. Although a lower limit of quantitation may have been achievable using this online monitor or available offline methods, exposure of animals to lower concentrations was not feasible under the conditions of these studies, due to reactivity of the aldehyde moieties of *o*-phthalaldehyde with amines resulting from the presence of animals. In the current studies, the main toxicity targets of *o*-phthalaldehyde exposure in rats and mice included the respiratory system (nasal cavity, larynx, trachea, lung), other sites of contact (skin, eye), and the male reproductive system (testis, epididymis).

Exposure to *o*-phthalaldehyde for 3 months caused overt toxicity at the two highest exposure concentrations in rats and mice, resulting in clinical findings of toxicity (e.g., abnormal breathing, sneezing, and thinness) and death. All of the rats and mice exposed to 7.0 ppm *o*-phthalaldehyde died within the first 2 weeks of study. Among animals that were exposed to

3.5 ppm *o*-phthalaldehyde, 70% of the male rats and 20% of the female rats died in the first 7 weeks of exposure and 50% of the male mice and 40% of the female mice died by week 6 of exposure. The decreased survival may be attributable to the significant extent of the respiratory lesions. Upon microscopic evaluation, suppurative inflammation in the nose was noted in almost all exposed animals. This finding in rats and mice, which are obligate nose-breathing animals, may have interfered with drinking and eating and may have contributed to respiratory insufficiency, and possibly death.

The most significant toxic response to *o*-phthalaldehyde inhalation occurred within the respiratory tract, including the nose, larynx, trachea, and lung, of rats and mice. In general, exposure of rats and mice to *o*-phthalaldehyde resulted in a spectrum of lesions throughout the respiratory tract that included necrosis, inflammation, regeneration, hyperplasia, and metaplasia, ranging from minimal to moderate in severity. In general, histologic findings occurred at deeper sites within the respiratory tract with increasing exposure concentration. The first site of contact, the nose, was most affected, with many lesions occurring at the lowest exposure concentration (0.44 ppm) in male and female rats and mice. Laryngeal lesions occurred at all exposure concentrations in rats and at 0.88 ppm or greater in mice. Tracheal findings were first noted at a variety of exposure concentrations. Lung findings were most prevalent at the two highest exposure concentrations (3.5 and 7.0 ppm) in rats and mice. Knowing the extent of the respiratory tract lesions, the increase in the erythron observed in the rats at study termination may be due, at least in part, to hypoxia with a resultant secondary appropriate erythrocytosis; decreased water intake may also have contributed.

In comparison to a previous study of glutaraldehyde by inhalation for 3 months, respiratory lesions were limited to the anterior region of the nose and did not extend further into the respiratory tract[60]. The mice in the 3-month glutaraldehyde study had lesions that extended past the nasal cavity and into the larynx, but only at the highest exposure concentration (1 ppm). From the studies with glutaraldehyde, the no-observed-adverse-effect concentration (NOAEC) was determined to be 0.125 ppm in rats and no no-observed-effect concentration (NOEC) was reached for the mice due to the occurrence of inflammation in the anterior nasal passage at the lowest concentration administered (0.0625 ppm).

The nasal cavity was a target of *o*-phthalaldehyde toxicity in the 3-month studies in both rats and mice. As rodents are obligate nose breathers, the nasal cavity is often a primary target site for direct-acting, reactive, and gaseous chemicals, including triethylamine[61] and diethylamine[62] and other aldehydes such as acetaldehyde[63] and glutaraldehyde[64]. The nasal epithelium response to toxicant injury often includes the co-occurrence of inflammation, atrophy, degeneration, or necrosis, as well as the reparative or adaptive responses of metaplasia, hyperplasia, or regeneration. Following *o*-phthalaldehyde inhalation, suppurative inflammation in the nasal cavity was observed in nearly all animals in all exposed groups. Necrosis of the respiratory epithelium occurred in male and female rats and mice, in an exposure concentration-dependent manner with increasing severity; however, this excludes the top two exposure groups (3.5 and 7.0 ppm) in which the animals died early in the study. Sequelae of the necrosis seen in male and female rats and mice in this study include regeneration of the original respiratory epithelium or metaplasia to a different histologic type of epithelium. Squamous metaplasia, observed in this study at increased incidences in either olfactory or respiratory epithelium, is a common adaptive response to prolonged or repeated injury to the epithelium and results in the replacement of injured epithelium with more resistant squamous epithelium[65]. A spectrum of similar changes

was also seen within the olfactory epithelium of both sexes of rats and mice in these studies. Additionally, all exposed male and female rats and mice (except male rats at 7.0 ppm) had significantly increased incidences of olfactory epithelium atrophy, which is a lesion that often occurs subsequent to either degeneration or necrosis[66]. As is often the case with atrophy of the olfactory epithelium, the underlying turbinate bones may also be atrophic with incidences and severity generally increasing with increasing exposure concentration. The absence of nasal turbinate atrophy in the 7.0 ppm groups of rats and mice and in the 3.5 ppm group of male rats is likely due to decreased exposure time due to early deaths. Similar nasal lesions were seen following a 3-month inhalation exposure to glutaraldehyde and included hyperplasia and squamous metaplasia of the respiratory epithelium lining the nasoturbinates/septum, as well as degeneration of the olfactory epithelium[60]. However, these lesions were mostly present only at the highest exposure concentration tested (1 ppm) and the extent of the lesions following glutaraldehyde exposure was also much less than that observed in these studies with *o*-phthalaldehyde. Inhalation exposure to diethylamine induced the same distribution of lesions within the nasal cavity at incidences that were significantly increased following inhalation exposure to 62 or 125 ppm for 3 months[62]. It would appear from the comparison of these studies that *o*-phthalaldehyde may not be a safer alternative to glutaraldehyde or diethylamine, with many of the *o*-phthalaldehyde-induced lesions extending throughout the entire respiratory tract.

o-Phthalaldehyde-induced lesions extended deeper into the respiratory tract, also targeting the larynx, trachea, bronchi, and lung. As in the nasal cavity, many of the lesions increased in incidence and severity with exposure concentration and covered a spectrum of injury as a result of continued *o*-phthalaldehyde exposure. In the larynx and trachea there were also exposure concentration-dependent increases in the incidences of squamous metaplasia, which was present in both sexes of rats and mice as well. Some of the male and female rats that died early in the high concentration exposure groups (3.5 and 7.0 ppm) had ulceration of the mucosal epithelium in the trachea. Singular incidences of trachea ulcerations have previously been reported in 2-year NTP oral gavage studies in rats with either 3-sulfolene, Elmiron®, or ginseng; however, these incidences were not considered test article related[67-69]. Another NTP study where this lesion was observed was a 2-year oral gavage study with 1,1,1-trichloroethane, in which the male Osborne-Mendel rats exhibited increased incidences of acute ulcers in the trachea (5%, 6%, and 14% at 0, 750, and 1,500 mg 1,1,1-trichloroethane/kg body weight, respectively)[70]. In response to the chronic active inflammation and/or necrosis within the trachea and larynx, there were also concentration-dependent increases in the incidences of regeneration and/or squamous metaplasia in rats and mice.

The lung was also a target of *o*-phthalaldehyde exposure and exhibited significantly increased incidences of histiocytic or suppurative inflammation of the alveolus in male and female rats. Given the ability of *o*-phthalaldehyde to induce neutrophil infiltration in the bronchoalveolar fluid in ICR mice following subcutaneous injection[22] and that *o*-phthalaldehyde has been identified as an irritant and contact sensitizer[24], it is not surprising to see inflammation, either suppurative or chronic active, throughout the respiratory tract. The apparent increased sensitivity of the rats in comparison to the mice with the lesions that extended deeper into the respiratory tract, with the lung in particular, may be due to differences in minute volume (respiratory rate × volume of inhaled air). In response to sensory irritant inhalation, mice have been shown to reduce their minute volume by 75% (in the case of formaldehyde exposure), whereas rats only reduced their minute volume by 45%[71]. In rats, chronic active inflammation and necrosis in the

bronchus at the higher exposure concentrations (1.75 ppm or greater) also occurred in conjunction with regeneration, hyperplasia, and/or squamous metaplasia. In the bronchus in mice, incidences of chronic active inflammation and necrosis were only present at the two highest exposure concentrations (3.5 and 7.0 ppm).

Ocular findings in male and female rats in the current study included significantly increased incidences of necrosis and suppurative inflammation of the cornea, as well as suppurative inflammation within the anterior chamber. Although these lesions are more severe than those found following occupational exposures, the rats were exposed for a longer period of time than humans occupationally exposed, and the significantly increased incidences of these lesions were limited to some rats exposed within the two highest exposure concentrations (3.5 and 7.0 ppm). There were also incidences of these cornea lesions in the mice, and although the increases were not statistically significant, their presence is likely treatment related given the significantly increased incidences in rats. Suppurative inflammation within the eye following whole-body inhalation exposures generally occurs in cases where the epithelium is compromised (e.g., abrasions or toxicant-induced necrosis). Mild to moderate suppurative inflammation of the corneas in male and female rats has previously been reported in NTP studies following a longer 2-year exposure to 125 ppm diethylamine[62].

In the current studies, treatment-related skin lesions were noted in both rats and mice, and clinical findings of black discoloration of the pinnae and feet were noted in rats during the in-life portion of the study. All the male and female mice in the highest exposure group (7.0 ppm) also had several lesions of the skin of the pinna, which included adnexa degeneration, chronic active inflammation, hair follicle epithelium parakeratosis, and squamous hyperplasia. The pinnae are not typically a protocol-required tissue for evaluation in NTP toxicity studies; however, because lesions in routine inguinal skin sections in rats and mice and discoloration of the appendages in the rats were noted in this study, pinnae were evaluated in mice in addition to the routine inguinal skin sections. The cause of *o*-phthalaldehyde-induced black discoloration is unknown; however, it has been shown that a condensation reaction occurs in humans between *o*-phthalaldehyde and ammonia, in the presence of water, resulting in the production of similar discoloration[72]. Additional dermal findings were identified in rats and mice, which are consistent with a dermal irritation response. Ocular and dermal effects, including eye irritation and contact dermatitis, have been documented in workers exposed to *o*-phthalaldehyde[25; 32-34]. There have also been specific isolated human case reports of skin damage and discoloration in response to *o*-phthalaldehyde residues on medical equipment following disinfection[35; 73].

Significantly increased incidences of lymphoid atrophy occurred in the thymus and spleen in both male and female rats and mice, and were limited to the top two exposure groups (3.5 and 7.0 ppm). The lymphoid atrophy in the thymus and spleen observed in the current studies is consistent with stress-related organ effects that are a common secondary finding in toxicity studies. The thymus is one of the most sensitive lymphoid organs to stress in toxicity studies[74]. Further support of stress within this study included the decreased body weights and the clinical findings of thinness. In addition, alterations observed in the leukon on days 3 and 23 and at week 14 (namely decreased lymphocyte counts) were also consistent with a stress response.

In mice, bone marrow hyperplasia was observed at the three highest exposure concentrations (1.75, 3.5, and 7.0 ppm) in the males and only at 3.5 ppm in females. The bone marrow hyperplasia, with subjectively observed increases in the myeloid series (i.e., increased

myeloid:erythroid ratio), is most likely due to an increased peripheral demand for leukocytes paired with an erythroid suppression (as indicated by the decreases in the erythron) due to the chronic inflammation. The increased peripheral demand for leukocytes likely stems from the inflammation in the respiratory tract and parallels the increases in the leukocyte counts observed in all exposed male groups and in the 3.5 ppm females.

Microscopic findings consistent with peripuberty were noted in the testis and epididymis of male rats exposed to the two highest exposure concentrations (3.5 and 7.0 ppm) that died early within the first 2 weeks, but not in male mice, as mice reach sexual maturity earlier than rats. In the *o*-phthalaldehyde-exposed rats, some of the testicular microscopic observations (e.g., low numbers of elongated spermatids, relatively small testicular size and tubule lumen diameters as compared to adults, no or negligible sperm in the epididymis) were consistent with those that were found in the age-matched controls, and therefore these effects in the rat were attributed to their peripubertal status and not to the inhalation of *o*-phthalaldehyde.

Inhalation of *o*-phthalaldehyde resulted in significantly decreased testis, epididymis, and cauda epididymis weights in rats exposed to 0.88 ppm and 1.75 ppm. Additionally, there were degenerative changes in the testes of rats exposed to 3.5 ppm that survived beyond 39 days. These changes included seminiferous tubule vacuolation with focal loss of germ cells from individual Sertoli cells and degeneration and depletion of elongated spermatids. Vacuolation is a common morphological response of the Sertoli cell to toxicant-induced injury[75]. As vacuolation is an early event, it is typically followed by degeneration, disorganization, or exfoliation of the germ cells, depending on the nature of the Sertoli cell functional disturbance[76]. This typically results in the appearance of these exfoliated germ cells within the epididymis[77]. In the current studies, some incidences of exfoliated germ cells were observed in the epididymis of rats and mice. Damage to epididymal sperm can have an immediate impact on fertility parameters. Percent sperm motility was significantly reduced in an exposure concentration-related manner in both rats and mice in all exposed groups evaluated, compared to the chamber control groups. Additionally, rats had significantly lower absolute weights of the left cauda epididymis, left epididymis, and left testis in the 0.88 and 1.75 ppm groups; exposed mice did not have decreased absolute testis or epididymis weights. There were no chemical-related microscopic changes in the testis or epididymis of rats or mice exposed to 0.44, 0.88, or 1.75 ppm that would explain the significant exposure concentration-dependent decreases in sperm motility in rats and mice. Either the changes were below the level of histologic detection, or there were physiological changes that resulted in decreased motility. Taken together, the changes in sperm parameters, testis and epididymis weights, and microscopic findings suggest that *o*-phthalaldehyde has the potential to be a male reproductive toxicant in rats and mice.

Under the conditions of these 3-month inhalation studies, there were treatment-related lesions in male and female rats and mice. The major targets from *o*-phthalaldehyde exposure in rats and mice included the respiratory system (nasal cavity, larynx, trachea, and lung), skin, eye, testis, and epididymis. The most sensitive measure of *o*-phthalaldehyde inhalation toxicity in male and female rats and mice was significantly increased incidences of nasal cavity lesions (lowest-observable-effect concentration = 0.44 ppm). A no-observed-effect concentration was not determined in rats or mice of either sex.

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Appendix A. Summary of Nonneoplastic Lesions in Rats and Mice

Tables

Table A-1. Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund	–	–	–	–	3	2
Natural deaths	–	–	–	–	4	8
Survivors						
Terminal euthanasia	10	10	10	10	3	–
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(0)	(0)	(9)	(10)	(10)
Intestine large, cecum	(10)	(0)	(0)	(10)	(10)	(8)
Intestine large, colon	(10)	(0)	(0)	(10)	(10)	(8)
Intestine large, rectum	(10)	(0)	(0)	(10)	(10)	(10)
Intestine small, duodenum	(10)	(0)	(0)	(10)	(10)	(10)
Intestine small, ileum	(10)	(0)	(0)	(10)	(10)	(8)
Diverticulum	–	–	–	–	–	1 (13%)
Intestine small, jejunum	(10)	(0)	(0)	(10)	(10)	(7)
Liver	(10)	(1)	(0)	(10)	(10)	(10)
Fibrosis	–	–	–	–	1 (10%)	–
Hepatodiaphragmatic nodule	–	1 (100%)	–	–	–	–
Hepatocyte, degeneration, focal	1 (10%)	–	–	–	–	–
Pancreas	(10)	(0)	(0)	(10)	(10)	(10)
Salivary glands	(10)	(0)	(0)	(10)	(10)	(10)
Stomach, forestomach	(10)	(0)	(0)	(10)	(10)	(10)
Cyst, squamous	–	–	–	–	–	1 (10%)
Hyperplasia, squamous	–	–	–	1 (10%)	–	–
Stomach, glandular	(10)	(0)	(0)	(10)	(10)	(10)
Cardiovascular System						
Blood vessel	(10)	(0)	(0)	(10)	(0)	(0)
Heart	(10)	(0)	(1)	(10)	(10)	(10)
Cardiomyopathy	1 (10%)	–	–	1 (10%)	–	–
Congestion	–	–	1 (100%)	–	–	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Endocrine System						
Adrenal cortex	(10)	(0)	(0)	(10)	(10)	(10)
Adrenal medulla	(10)	(0)	(0)	(10)	(10)	(10)
Islets, pancreatic	(10)	(0)	(0)	(10)	(10)	(10)
Parathyroid gland	(10)	(0)	(0)	(8)	(10)	(9)
Pituitary gland	(10)	(0)	(0)	(10)	(10)	(10)
Thyroid gland	(10)	(0)	(0)	(10)	(10)	(10)
General Body System						
None	–	–	–	–	–	–
Genital System						
Epididymis	(10)	(10)	(10)	(10)	(10)	(10)
Hypospermia	–	–	1 (10%)	–	1 (10%)	–
Infiltration cellular, mononuclear cell	–	–	–	–	–	1 (10%)
Duct, exfoliated germ cell	–	–	1 (10%)	1 (10%)	5 (50%)	4 (40%)
Epithelium, apoptosis	–	–	–	–	3 (30%)	5 (50%)
Penis	(0)	(0)	(0)	(0)	(1)	(0)
Inflammation, suppurative	–	–	–	–	1 (100%)	–
Preputial gland	(10)	(0)	(0)	(9)	(10)	(10)
Inflammation, chronic active	1 (10%)	–	–	–	1 (10%)	–
Prostate	(10)	(0)	(0)	(10)	(10)	(10)
Inflammation, chronic active	–	–	–	1 (10%)	–	–
Seminal vesicle	(10)	(0)	(0)	(10)	(10)	(10)
Atrophy	–	–	–	–	1 (10%)	–
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Exfoliated germ cell, focal	–	–	–	–	1 (10%)	2 (20%)
Immature	–	–	–	–	4 (40%)	10 (100%)
Elongated spermatid, degeneration	–	–	–	–	4 (40%)	–
Germinal epithelium, apoptosis	–	–	–	–	4 (40%)	6 (60%)
Germinal epithelium, atrophy, multifocal	–	–	1 (10%)	–	–	–
Germinal epithelium, degeneration, focal	1 (10%)	–	–	–	–	–
Germinal epithelium, degeneration, multifocal	–	–	–	1 (10%)	–	–
Interstitial cell, atrophy	–	–	–	–	7 (70%)	10 (100%)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Round spermatid, degeneration	–	–	–	–	–	2 (20%)
Seminiferous tubule, vacuolation	–	–	–	–	3 (30%)	–
Hematopoietic System						
Bone marrow	(10)	(0)	(0)	(10)	(10)	(10)
Lymph node	(0)	(1)	(0)	(0)	(0)	(0)
Pigmentation	–	1 (100%)	–	–	–	–
Lymph node, bronchial	(7)	(0)	(0)	(6)	(7)	(6)
Lymph node, mandibular	(9)	(0)	(0)	(10)	(10)	(10)
Lymph node, mediastinal	(8)	(0)	(0)	(4)	(9)	(7)
Lymph node, mesenteric	(10)	(0)	(0)	(10)	(10)	(10)
Spleen	(10)	(9)	(10)	(10)	(10)	(10)
Atrophy, lymphoid	–	–	–	–	6 (60%)	10 (100%)
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy, lymphoid	–	–	–	–	8 (80%)	7 (70%)
Integumentary System						
Mammary gland	(6)	(0)	(0)	(7)	(6)	(2)
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia, squamous	–	–	–	–	1 (10%)	1 (10%)
Inflammation, suppurative	–	–	–	–	1 (10%)	–
Adnexa, degeneration	–	–	–	–	4 (40%)	10 (100%)
Epithelium, hair follicle, parakeratosis	–	7 (70%)	4 (40%)	7 (70%)	3 (30%)	10 (100%)
Musculoskeletal System						
Bone	(10)	(0)	(0)	(10)	(10)	(10)
Maxilla, inflammation, chronic active	–	–	–	–	–	1 (10%)
Nervous System						
Brain	(10)	(0)	(0)	(10)	(10)	(10)
Respiratory System						
Larynx	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active	1 (10%)	2 (20%)	8 (80%)	10 (100%)	10 (100%)	10 (100%)
Metaplasia, squamous	–	1 (10%)	8 (80%)	10 (100%)	10 (100%)	10 (100%)
Necrosis	–	–	1 (10%)	5 (50%)	9 (90%)	10 (100%)
Regeneration	–	–	3 (30%)	2 (20%)	2 (20%)	6 (60%)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Alveolus, infiltration cellular, histiocyte	–	1 (10%)	–	3 (30%)	9 (90%)	1 (10%)
Alveolus, inflammation, suppurative	–	–	–	–	3 (30%)	6 (60%)
Bronchus, fibrosis	–	–	–	1 (10%)	2 (20%)	1 (10%)
Bronchus, hyperplasia	–	–	–	2 (20%)	5 (50%)	1 (10%)
Bronchus, inflammation, chronic active	–	–	–	3 (30%)	9 (90%)	8 (80%)
Bronchus, metaplasia, squamous	–	–	–	7 (70%)	6 (60%)	1 (10%)
Bronchus, necrosis	–	–	–	–	5 (50%)	7 (70%)
Bronchus, regeneration	–	–	–	–	3 (30%)	1 (10%)
Bronchus, goblet cell, hyperplasia	–	–	–	–	1 (10%)	–
Perivascular, inflammation, chronic active	–	–	–	–	2 (20%)	–
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, suppurative	–	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Glands, olfactory epithelium, hyperplasia	–	–	–	1 (10%)	2 (20%)	3 (30%)
Goblet cell, respiratory epithelium, hyperplasia	–	4 (40%)	6 (60%)	2 (20%)	–	1 (10%)
Olfactory epithelium, accumulation, hyaline droplet	9 (90%)	10 (100%)	9 (90%)	1 (10%)	–	–
Olfactory epithelium, atrophy	2 (20%)	10 (100%)	10 (100%)	10 (100%)	7 (70%)	6 (60%)
Olfactory epithelium, metaplasia, respiratory	1 (10%)	1 (10%)	6 (60%)	6 (60%)	2 (20%)	–
Olfactory epithelium, metaplasia, squamous	–	–	–	1 (10%)	1 (10%)	1 (10%)
Olfactory epithelium, necrosis	–	–	–	1 (10%)	3 (30%)	–
Olfactory epithelium, regeneration	–	–	1 (10%)	–	4 (40%)	–
Respiratory epithelium, accumulation, hyaline droplet	1 (10%)	–	1 (10%)	–	–	–
Respiratory epithelium, hyperplasia	3 (30%)	9 (90%)	9 (90%)	7 (70%)	3 (30%)	–
Respiratory epithelium, metaplasia, squamous	–	10 (100%)	10 (100%)	10 (100%)	10 (100%)	8 (80%)
Respiratory epithelium, necrosis	–	–	3 (30%)	5 (50%)	10 (100%)	10 (100%)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Respiratory epithelium, regeneration	–	–	1 (10%)	–	3 (30%)	6 (60%)
Respiratory epithelium, ulcer	–	1 (10%)	–	–	–	–
Turbinate, atrophy	–	–	7 (70%)	10 (100%)	–	–
Trachea	(10)	(10)	(10)	(10)	(10)	(10)
Fibrosis	–	–	–	5 (50%)	3 (30%)	–
Inflammation, chronic active	–	–	4 (40%)	8 (80%)	9 (90%)	10 (100%)
Metaplasia, squamous	–	–	4 (40%)	10 (100%)	6 (60%)	6 (60%)
Necrosis	–	–	–	3 (30%)	8 (80%)	8 (80%)
Regeneration	–	–	–	7 (70%)	7 (70%)	6 (60%)
Ulcer	–	–	–	–	1 (10%)	2 (20%)
Special Senses System						
Eye	(10)	(10)	(10)	(10)	(10)	(10)
Anterior chamber, inflammation, suppurative	–	–	–	–	4 (40%)	5 (50%)
Cornea, hyperplasia	–	–	–	–	–	2 (20%)
Cornea, inflammation, suppurative	1 (10%)	1 (10%)	1 (10%)	1 (10%)	5 (50%)	6 (60%)
Cornea, necrosis	–	–	1 (10%)	–	2 (20%)	4 (40%)
Harderian gland	(10)	(0)	(0)	(10)	(10)	(10)
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Nephropathy	10 (100%)	10 (100%)	9 (90%)	7 (70%)	1 (10%)	2 (20%)
Urinary bladder	(10)	(0)	(0)	(10)	(10)	(10)

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

Table A-2. Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund	–	–	–	–	2	1
Natural deaths	–	–	–	–	–	9
Survivors						
Terminal euthanasia	10	10	10	10	8	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(0)	(0)	(0)	(10)	(10)
Intestine large, cecum	(10)	(0)	(0)	(0)	(10)	(10)
Intestine large, colon	(10)	(0)	(0)	(0)	(10)	(10)
Intestine large, rectum	(10)	(0)	(0)	(0)	(10)	(10)
Intestine small, duodenum	(10)	(0)	(0)	(0)	(10)	(10)
Intestine small, ileum	(10)	(0)	(0)	(0)	(10)	(10)
Intestine small, jejunum	(10)	(0)	(0)	(0)	(10)	(6)
Liver	(10)	(0)	(0)	(0)	(10)	(10)
Mesentery	(0)	(0)	(1)	(0)	(0)	(0)
Fat, degeneration	–	–	1 (100%)	–	–	–
Pancreas	(10)	(0)	(0)	(0)	(10)	(10)
Salivary glands	(10)	(0)	(0)	(0)	(10)	(10)
Stomach, forestomach	(10)	(0)	(0)	(0)	(10)	(10)
Cyst, squamous	1 (10%)	–	–	–	–	–
Stomach, glandular	(10)	(9)	(9)	(10)	(10)	(10)
Ulcer	–	–	–	–	–	2 (20%)
Cardiovascular System						
Blood vessel	(10)	(0)	(0)	(0)	(10)	(0)
Heart	(10)	(0)	(0)	(0)	(10)	(10)
Cardiomyopathy	–	–	–	–	–	2 (20%)
Congestion	–	–	–	–	–	1 (10%)
Endocrine System						
Adrenal cortex	(10)	(0)	(0)	(0)	(10)	(10)
Adrenal medulla	(10)	(0)	(0)	(0)	(10)	(10)
Islets, pancreatic	(10)	(0)	(0)	(0)	(10)	(10)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Parathyroid gland	(8)	(0)	(0)	(0)	(9)	(9)
Pituitary gland	(10)	(0)	(0)	(0)	(10)	(10)
Thyroid gland	(10)	(0)	(0)	(0)	(10)	(10)
General Body System						
None	–	–	–	–	–	–
Genital System						
Clitoral gland	(10)	(0)	(0)	(0)	(10)	(10)
Ovary	(10)	(0)	(0)	(0)	(10)	(10)
Uterus	(10)	(0)	(0)	(1)	(10)	(10)
Decidual reaction	–	–	–	1 (100%)	–	–
Hematopoietic System						
Bone marrow	(10)	(0)	(0)	(0)	(10)	(10)
Lymph node, bronchial	(8)	(0)	(0)	(0)	(7)	(5)
Lymph node, mandibular	(9)	(0)	(0)	(0)	(10)	(10)
Lymph node, mediastinal	(8)	(0)	(0)	(0)	(6)	(10)
Lymph node, mesenteric	(10)	(0)	(0)	(0)	(10)	(9)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy, lymphoid	–	–	–	1 (10%)	4 (40%)	10 (100%)
Thymus	(10)	(10)	(10)	(10)	(9)	(10)
Atrophy, lymphoid	–	–	–	–	6 (67%)	10 (100%)
Integumentary System						
Mammary gland	(10)	(0)	(0)	(0)	(10)	(10)
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia, squamous	–	–	–	–	–	1 (10%)
Adnexa, degeneration	–	1 (10%)	–	–	1 (10%)	10 (100%)
Epithelium, hair follicle, parakeratosis	–	1 (10%)	1 (10%)	3 (30%)	3 (30%)	6 (60%)
Hair follicle, atrophy	–	–	–	–	1 (10%)	–
Musculoskeletal System						
Bone	(10)	(0)	(0)	(0)	(10)	(10)
Nervous System						
Brain	(10)	(0)	(0)	(0)	(10)	(10)
Respiratory System						
Larynx	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active	–	1 (10%)	1 (10%)	9 (90%)	10 (100%)	10 (100%)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Metaplasia, squamous	–	1 (10%)	4 (40%)	10 (100%)	10 (100%)	10 (100%)
Necrosis	–	–	–	1 (10%)	7 (70%)	8 (80%)
Regeneration	–	–	1 (10%)	3 (30%)	1 (10%)	2 (20%)
Ulcer	–	–	1 (10%)	1 (10%)	–	–
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Alveolus, infiltration cellular, histiocyte	–	2 (20%)	1 (10%)	3 (30%)	9 (90%)	3 (30%)
Alveolus, inflammation, suppurative	–	–	–	–	2 (20%)	4 (40%)
Bronchus, fibrosis	–	–	–	1 (10%)	1 (10%)	3 (30%)
Bronchus, hyperplasia	–	–	–	1 (10%)	6 (60%)	1 (10%)
Bronchus, inflammation, chronic active	–	–	–	2 (20%)	10 (100%)	10 (100%)
Bronchus, metaplasia, squamous	–	–	–	2 (20%)	9 (90%)	–
Bronchus, necrosis	–	–	–	–	2 (20%)	10 (100%)
Bronchus, regeneration	–	–	–	–	1 (10%)	5 (50%)
Interstitium, fibrosis	–	–	1 (10%)	–	2 (20%)	–
Interstitium, inflammation, granulomatous	–	–	–	–	–	2 (20%)
Perivascular, inflammation, chronic active	–	–	1 (10%)	1 (10%)	6 (60%)	–
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Foreign body	–	–	–	–	–	1 (10%)
Inflammation, suppurative	–	9 (90%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Synechia	–	–	–	1 (10%)	–	–
Glands, olfactory epithelium, hyperplasia	–	2 (20%)	–	2 (20%)	2 (20%)	2 (20%)
Goblet cell, respiratory epithelium, hyperplasia	–	–	–	1 (10%)	–	1 (10%)
Olfactory epithelium, accumulation, hyaline droplet	9 (90%)	10 (100%)	8 (80%)	4 (40%)	–	–
Olfactory epithelium, atrophy	–	9 (90%)	10 (100%)	10 (100%)	10 (100%)	7 (70%)
Olfactory epithelium, metaplasia, respiratory	–	1 (10%)	2 (20%)	5 (50%)	7 (70%)	1 (10%)
Olfactory epithelium, metaplasia, squamous	–	–	–	1 (10%)	–	–
Olfactory epithelium, necrosis	–	–	1 (10%)	–	1 (10%)	–
Olfactory epithelium, regeneration	–	–	–	–	1 (10%)	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Respiratory epithelium, accumulation, hyaline droplet	–	4 (40%)	–	–	–	–
Respiratory epithelium, hyperplasia	–	9 (90%)	7 (70%)	4 (40%)	3 (30%)	3 (30%)
Respiratory epithelium, metaplasia, squamous	–	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Respiratory epithelium, necrosis	–	2 (20%)	6 (60%)	9 (90%)	10 (100%)	10 (100%)
Respiratory epithelium, regeneration	–	–	2 (20%)	–	1 (10%)	3 (30%)
Respiratory epithelium, ulcer	–	–	–	1 (10%)	–	1 (10%)
Turbinate, atrophy	–	2 (20%)	4 (40%)	10 (100%)	10 (100%)	–
Trachea	(10)	(10)	(10)	(10)	(10)	(10)
Fibrosis	–	–	–	2 (20%)	6 (60%)	–
Inflammation, chronic active	–	–	3 (30%)	5 (50%)	10 (100%)	10 (100%)
Metaplasia, squamous	–	–	3 (30%)	10 (100%)	7 (70%)	7 (70%)
Necrosis	–	–	–	3 (30%)	3 (30%)	8 (80%)
Regeneration	–	–	1 (10%)	7 (70%)	10 (100%)	9 (90%)
Ulcer	–	–	–	–	1 (10%)	2 (20%)
Special Senses System						
Eye	(10)	(10)	(10)	(10)	(10)	(10)
Anterior chamber, inflammation, suppurative	–	–	–	–	1 (10%)	5 (50%)
Cornea, hyperplasia	–	–	–	–	–	1 (10%)
Cornea, inflammation, suppurative	–	–	1 (10%)	–	1 (10%)	8 (80%)
Cornea, necrosis	–	–	–	–	–	7 (70%)
Harderian gland	(10)	(0)	(0)	(0)	(10)	(10)
Urinary System						
Kidney	(10)	(10)	(9)	(10)	(10)	(10)
Nephropathy	6 (60%)	5 (50%)	4 (44%)	5 (50%)	1 (10%)	2 (20%)
Urinary bladder	(10)	(0)	(0)	(0)	(10)	(10)

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

Table A-3. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund	–	–	–	–	5	2
Natural deaths	–	–	–	–	–	8
Survivors						
Terminal euthanasia	10	10	10	10	5	–
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(0)	(0)	(10)	(10)	(10)
Inflammation, chronic active	–	–	–	–	–	1 (10%)
Necrosis	–	–	–	–	–	1 (10%)
Gallbladder	(10)	(0)	(0)	(9)	(9)	(3)
Inflammation, neutrophil	4 (40%)	–	–	–	–	–
Intestine large, cecum	(10)	(0)	(0)	(10)	(10)	(7)
Intestine large, colon	(10)	(0)	(0)	(10)	(10)	(8)
Intestine large, rectum	(10)	(0)	(0)	(10)	(10)	(7)
Intestine small, duodenum	(10)	(0)	(0)	(10)	(10)	(3)
Intestine small, ileum	(10)	(0)	(0)	(10)	(10)	(3)
Intestine small, jejunum	(10)	(0)	(0)	(10)	(10)	(3)
Liver	(10)	(0)	(0)	(10)	(10)	(10)
Necrosis	–	–	–	–	2 (20%)	–
Pancreas	(10)	(0)	(0)	(10)	(10)	(10)
Salivary glands	(10)	(0)	(0)	(10)	(10)	(9)
Stomach, forestomach	(10)	(0)	(0)	(10)	(10)	(9)
Hyperplasia, squamous	–	–	–	–	1 (10%)	–
Stomach, glandular	(10)	(0)	(0)	(10)	(10)	(8)
Cardiovascular System						
Blood vessel	(10)	(0)	(0)	(10)	(0)	(2)
Heart	(10)	(0)	(0)	(10)	(10)	(10)
Endocrine System						
Adrenal cortex	(10)	(0)	(0)	(10)	(10)	(10)
Adrenal medulla	(10)	(0)	(0)	(10)	(10)	(10)
Islets, pancreatic	(10)	(0)	(0)	(10)	(10)	(10)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Parathyroid gland	(7)	(0)	(0)	(7)	(6)	(4)
Pituitary gland	(10)	(0)	(0)	(9)	(10)	(9)
Thyroid gland	(10)	(0)	(0)	(10)	(9)	(10)
General Body System						
None	–	–	–	–	–	–
Genital System						
Epididymis	(10)	(10)	(10)	(10)	(10)	(10)
Duct, exfoliated germ cell	–	–	–	–	4 (40%)	1 (10%)
Preputial gland	(10)	(0)	(0)	(10)	(10)	(10)
Inflammation, chronic active	–	–	–	–	1 (10%)	–
Prostate	(10)	(0)	(0)	(10)	(10)	(9)
Seminal vesicle	(10)	(0)	(0)	(10)	(10)	(10)
Inflammation, chronic	–	–	–	–	1 (10%)	–
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Germinal epithelium, depletion cellular, focal	–	1 (10%)	–	–	–	–
Germinal epithelium, depletion cellular, multifocal	–	1 (10%)	1 (10%)	–	7 (70%)	–
Interstitial cell, atrophy	–	–	–	–	3 (30%)	–
Seminiferous tubule, dilation, focal	–	2 (20%)	–	–	–	–
Hematopoietic System						
Bone marrow	(10)	(0)	(0)	(10)	(10)	(10)
Hyperplasia	–	–	–	2 (20%)	5 (50%)	7 (70%)
Lymph node, bronchial	(7)	(0)	(0)	(4)	(5)	(6)
Lymph node, mandibular	(4)	(0)	(0)	(6)	(9)	(5)
Lymph node, mediastinal	(4)	(0)	(0)	(1)	(4)	(3)
Lymph node, mesenteric	(10)	(0)	(0)	(10)	(10)	(7)
Spleen	(10)	(0)	(0)	(10)	(10)	(10)
Atrophy, lymphoid	–	–	–	–	–	10 (100%)
Thymus	(10)	(10)	(10)	(10)	(9)	(10)
Atrophy, lymphoid	–	–	–	–	5 (56%)	10 (100%)
Integumentary System						
Skin	(10)	(10)	(10)	(9)	(10)	(10)
Hyperplasia, squamous	–	1 (10%)	–	–	9 (90%)	4 (40%)
Inflammation, chronic active	–	–	–	1 (11%)	10 (100%)	9 (90%)
Ulcer	–	–	–	–	1 (10%)	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Adnexa, degeneration	–	2 (20%)	–	4 (44%)	1 (10%)	6 (60%)
Adnexa, pinna, degeneration	–	–	–	–	–	10 (100%)
Epidermis, necrosis	–	–	–	–	–	3 (30%)
Epithelium, hair follicle, parakeratosis	–	3 (30%)	4 (40%)	2 (22%)	2 (20%)	10 (100%)
Epithelium, hair follicle, pinna, parakeratosis	–	–	–	–	–	10 (100%)
Pinna, hyperplasia, squamous	–	–	–	–	–	10 (100%)
Pinna, inflammation, chronic active	–	–	–	–	–	10 (100%)
Musculoskeletal System						
Bone	(10)	(0)	(0)	(10)	(10)	(10)
Nervous System						
Brain	(10)	(0)	(0)	(10)	(10)	(10)
Respiratory System						
Larynx	(10)	(10)	(9)	(10)	(10)	(10)
Inflammation, chronic active	–	–	–	4 (40%)	10 (100%)	10 (100%)
Metaplasia, squamous	–	–	1 (11%)	10 (100%)	10 (100%)	8 (80%)
Necrosis	–	–	–	–	1 (10%)	10 (100%)
Ulcer	–	–	–	1 (10%)	–	–
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Bronchiole, goblet cell, hyperplasia	–	–	–	–	4 (40%)	–
Bronchus, inflammation, chronic active	–	–	–	–	1 (10%)	6 (60%)
Bronchus, metaplasia, squamous	–	–	–	–	1 (10%)	–
Bronchus, necrosis	–	–	–	–	2 (20%)	9 (90%)
Bronchus, regeneration	–	–	–	–	1 (10%)	1 (10%)
Perivascular, inflammation, chronic active	–	–	–	–	1 (10%)	–
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, suppurative	–	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Glands, olfactory epithelium, hyperplasia	–	10 (100%)	10 (100%)	7 (70%)	6 (60%)	–
Glands, respiratory epithelium, accumulation, hyaline droplet	–	7 (70%)	10 (100%)	6 (60%)	–	–
Olfactory epithelium, accumulation, hyaline droplet	–	10 (100%)	10 (100%)	10 (100%)	9 (90%)	–
Olfactory epithelium, atrophy	–	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Olfactory epithelium, metaplasia, respiratory	–	1 (10%)	8 (80%)	10 (100%)	4 (40%)	–
Olfactory epithelium, metaplasia, squamous	–	–	2 (20%)	1 (10%)	2 (20%)	–
Olfactory epithelium, necrosis	–	–	–	–	–	5 (50%)
Respiratory epithelium, accumulation, hyaline droplet	–	10 (100%)	10 (100%)	10 (100%)	10 (100%)	–
Respiratory epithelium, metaplasia, squamous	–	10 (100%)	10 (100%)	10 (100%)	10 (100%)	6 (60%)
Respiratory epithelium, necrosis	–	2 (20%)	6 (60%)	5 (50%)	9 (90%)	10 (100%)
Respiratory epithelium, regeneration	–	–	1 (10%)	–	1 (10%)	4 (40%)
Respiratory epithelium, ulcer	–	–	3 (30%)	–	–	–
Turbinate, atrophy	–	4 (40%)	6 (60%)	10 (100%)	8 (80%)	–
Trachea	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active	–	–	–	1 (10%)	9 (90%)	10 (100%)
Metaplasia, squamous	–	–	–	3 (30%)	10 (100%)	3 (30%)
Necrosis	–	–	–	–	–	9 (90%)
Regeneration	–	–	–	–	–	5 (50%)
Special Senses System						
Eye	(10)	(10)	(10)	(10)	(10)	(10)
Cornea, inflammation, suppurative	–	1 (10%)	–	–	1 (10%)	4 (40%)
Cornea, necrosis	–	–	–	–	1 (10%)	–
Harderian gland	(10)	(0)	(0)	(10)	(10)	(10)
Urinary System						
Kidney	(10)	(0)	(0)	(10)	(10)	(10)
Inflammation, chronic active	–	–	–	–	1 (10%)	–
Nephropathy	2 (20%)	–	–	1 (10%)	1 (10%)	–
Urinary bladder	(10)	(0)	(0)	(10)	(10)	(10)

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

Table A-4. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund	–	–	–	–	4	1
Natural deaths	–	–	–	–	–	9
Survivors						
Terminal euthanasia	10	10	10	10	6	–
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(0)	(0)	(0)	(10)	(10)
Gallbladder	(10)	(0)	(0)	(0)	(9)	(1)
Intestine large, cecum	(10)	(0)	(0)	(0)	(10)	(4)
Intestine large, colon	(10)	(0)	(0)	(0)	(10)	(3)
Intestine large, rectum	(10)	(0)	(0)	(0)	(10)	(4)
Intestine small, duodenum	(10)	(0)	(0)	(0)	(10)	(2)
Intestine small, ileum	(10)	(0)	(0)	(0)	(10)	(2)
Intestine small, jejunum	(10)	(0)	(0)	(0)	(10)	(2)
Liver	(10)	(0)	(0)	(0)	(10)	(10)
Necrosis	–	–	–	–	1 (10%)	–
Pancreas	(10)	(0)	(0)	(0)	(10)	(8)
Cyst	–	–	–	–	1 (10%)	–
Salivary glands	(10)	(0)	(0)	(0)	(10)	(10)
Stomach, forestomach	(10)	(0)	(0)	(0)	(10)	(7)
Stomach, glandular	(10)	(0)	(0)	(0)	(10)	(7)
Cardiovascular System						
Blood vessel	(10)	(0)	(0)	(0)	(9)	(0)
Heart	(10)	(0)	(0)	(0)	(10)	(10)
Endocrine System						
Adrenal cortex	(10)	(0)	(0)	(1)	(10)	(10)
Adrenal medulla	(10)	(0)	(0)	(1)	(10)	(10)
Islets, pancreatic	(10)	(0)	(0)	(0)	(10)	(8)
Parathyroid gland	(6)	(0)	(0)	(0)	(5)	(4)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Pituitary gland	(10)	(0)	(0)	(0)	(10)	(7)
Thyroid gland	(10)	(0)	(0)	(0)	(10)	(10)
General Body System						
None	–	–	–	–	–	–
Genital System						
Clitoral gland	(9)	(0)	(0)	(0)	(8)	(8)
Ovary	(10)	(0)	(0)	(0)	(10)	(10)
Cyst	1 (10%)	–	–	–	–	–
Uterus	(10)	(0)	(0)	(0)	(10)	(8)
Hematopoietic System						
Bone marrow	(10)	(0)	(0)	(0)	(10)	(7)
Hyperplasia	–	–	–	–	8 (80%)	–
Lymph node	(0)	(0)	(0)	(1)	(0)	(0)
Deep cervical, hyperplasia, lymphoid	–	–	–	1 (100%)	–	–
Lymph node, bronchial	(4)	(0)	(0)	(0)	(6)	(7)
Lymph node, mandibular	(7)	(0)	(0)	(0)	(8)	(4)
Lymph node, mediastinal	(4)	(0)	(0)	(0)	(3)	(2)
Lymph node, mesenteric	(10)	(0)	(0)	(0)	(10)	(6)
Spleen	(10)	(0)	(0)	(0)	(10)	(8)
Atrophy, lymphoid	–	–	–	–	1 (10%)	8 (100%)
Thymus	(10)	(10)	(9)	(10)	(10)	(10)
Atrophy, lymphoid	–	–	–	–	6 (60%)	10 (100%)
Integumentary System						
Mammary gland	(10)	(0)	(0)	(0)	(9)	(9)
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia, squamous	3 (30%)	–	–	6 (60%)	9 (90%)	6 (60%)
Inflammation, chronic active	1 (10%)	1 (10%)	3 (30%)	9 (90%)	9 (90%)	8 (80%)
Adnexa, degeneration	2 (20%)	–	–	1 (10%)	–	9 (90%)
Adnexa, pinna, degeneration	–	–	–	–	–	10 (100%)
Epidermis, necrosis	–	–	–	–	–	5 (50%)
Epithelium, hair follicle, parakeratosis	–	3 (30%)	7 (70%)	9 (90%)	10 (100%)	10 (100%)
Epithelium, hair follicle, pinna, parakeratosis	–	–	–	–	–	10 (100%)
Pinna, hyperplasia, squamous	–	–	–	–	–	10 (100%)

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Pinna, inflammation, chronic active	–	–	–	–	–	10 (100%)
Musculoskeletal System						
Bone	(10)	(0)	(0)	(0)	(10)	(10)
Nervous System						
Brain	(10)	(0)	(0)	(0)	(10)	(10)
Respiratory System						
Larynx	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active	–	–	–	–	9 (90%)	10 (100%)
Metaplasia, squamous	–	–	3 (30%)	10 (100%)	10 (100%)	8 (80%)
Necrosis	–	–	–	–	3 (30%)	9 (90%)
Regeneration	–	–	–	–	–	3 (30%)
Ulcer	–	–	–	–	–	1 (10%)
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Bronchiole, goblet cell, hyperplasia	–	–	–	–	6 (60%)	–
Bronchus, inflammation, chronic active	–	–	–	–	6 (60%)	8 (80%)
Bronchus, necrosis	–	–	–	–	2 (20%)	8 (80%)
Bronchus, regeneration	–	–	–	–	–	1 (10%)
Bronchus, ulcer	–	–	–	–	–	2 (20%)
Nose	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, suppurative	–	10 (100%)	10 (100%)	10 (100%)	10 (100%)	6 (60%)
Glands, olfactory epithelium, hyperplasia	–	10 (100%)	9 (90%)	10 (100%)	8 (80%)	–
Glands, respiratory epithelium, accumulation, hyaline droplet	–	9 (90%)	10 (100%)	6 (60%)	2 (20%)	–
Olfactory epithelium, accumulation, hyaline droplet	–	10 (100%)	10 (100%)	10 (100%)	6 (60%)	–
Olfactory epithelium, atrophy	–	10 (100%)	10 (100%)	10 (100%)	9 (90%)	9 (90%)
Olfactory epithelium, metaplasia, respiratory	–	3 (30%)	6 (60%)	3 (30%)	4 (40%)	–
Olfactory epithelium, necrosis	–	1 (10%)	–	–	1 (10%)	–
Olfactory epithelium, regeneration	–	–	–	–	1 (10%)	–
Respiratory epithelium, accumulation, hyaline droplet	–	10 (100%)	10 (100%)	10 (100%)	8 (80%)	–
Respiratory epithelium, metaplasia,squamous	–	10 (100%)	10 (100%)	10 (100%)	8 (80%)	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Respiratory epithelium, necrosis	–	2 (20%)	7 (70%)	6 (60%)	8 (80%)	10 (100%)
Respiratory epithelium, regeneration	–	–	–	1 (10%)	3 (30%)	6 (60%)
Respiratory epithelium, ulcer	–	–	–	–	–	2 (20%)
Turbinate, atrophy	–	7 (70%)	9 (90%)	10 (100%)	7 (70%)	–
Trachea	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic active	–	–	–	–	10 (100%)	10 (100%)
Metaplasia, squamous	–	–	–	–	9 (90%)	2 (20%)
Necrosis	–	–	–	–	2 (20%)	10 (100%)
Regeneration	–	–	–	–	1 (10%)	2 (20%)
Ulcer	–	–	–	–	1 (10%)	–
Special Senses System						
Eye	(10)	(10)	(10)	(10)	(10)	(8)
Cornea, inflammation, suppurative	–	–	–	–	2 (20%)	3 (38%)
Cornea, necrosis	–	–	–	–	–	2 (25%)
Harderian gland	(10)	(0)	(0)	(0)	(10)	(9)
Urinary System						
Kidney	(10)	(0)	(0)	(0)	(10)	(10)
Nephropathy	1 (10%)	–	–	–	–	–
Urinary bladder	(10)	(0)	(0)	(0)	(10)	(9)

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

Appendix B. Clinical Pathology Results

Tables

Table B-1. Hematology and Clinical Chemistry Data for Rats in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Male						
n						
Day 3	10	10	10	10	10	9
Day 23	10	10	10	9	5	0
Week 14	10	10	10	10	3	0
Hematology						
Hematocrit (spun) (%)						
Day 3	41.4 ± 0.4	42.5 ± 0.4	42.1 ± 0.3	45.9 ± 0.7**	48.9 ± 0.8**	50.5 ± 0.5**
Day 23	45.1 ± 0.3	45.9 ± 0.5	47.1 ± 0.7*	48.4 ± 0.5**	55.9 ± 1.0**	–
Week 14	48.3 ± 0.4	48.8 ± 0.5	50.2 ± 0.4**	49.6 ± 0.6*	51.3 ± 1.1*	–
Packed cell volume (%)						
Day 3	39.6 ± 0.4	40.6 ± 0.4	40.0 ± 0.4	44.1 ± 0.6**	47.2 ± 0.9**	49.5 ± 0.5**
Day 23	43.8 ± 0.3	44.2 ± 0.5	45.5 ± 0.6*	47.1 ± 0.5**	53.9 ± 1.1**	–
Week 14	46.4 ± 0.4	47.4 ± 0.4	49.1 ± 0.4**	48.2 ± 0.5**	50.3 ± 1.7*	–
Hemoglobin (g/dL)						
Day 3	12.7 ± 0.1	13.1 ± 0.1	13.1 ± 0.1	14.4 ± 0.2**	15.3 ± 0.3**	16.2 ± 0.2**
Day 23	14.4 ± 0.1	14.5 ± 0.2	15.0 ± 0.2*	15.5 ± 0.2**	17.7 ± 0.3**	–
Week 14	15.6 ± 0.1	15.9 ± 0.1	16.3 ± 0.1**	16.1 ± 0.2**	17.0 ± 0.5**	–
Erythrocytes (10^6/μL)						
Day 3	6.03 ± 0.07	6.16 ± 0.06	6.23 ± 0.07	6.84 ± 0.11**	7.42 ± 0.15**	7.84 ± 0.11**
Day 23	7.19 ± 0.05	7.18 ± 0.07	7.52 ± 0.11*	7.85 ± 0.08**	9.16 ± 0.23**	–
Week 14	8.53 ± 0.08	8.62 ± 0.08	8.87 ± 0.09	8.71 ± 0.12	8.99 ± 0.27	–
Reticulocytes (10^3/μL)						
Day 3	446 ± 10	413 ± 18	393 ± 17	451 ± 13	515 ± 17*	507 ± 19*
Day 23	281 ± 15	255 ± 10	235 ± 13	265 ± 11	277 ± 28	–
Week 14	193 ± 13	205 ± 9	185 ± 13	196 ± 11	270 ± 13	–
Nucleated erythrocytes/100 leukocytes						
Day 3	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.5 ± 0.3	0.6 ± 0.2
Day 23	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	–
Week 14	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	–
Mean cell volume (fL)						
Day 3	65.7 ± 0.4	66.0 ± 0.6	64.3 ± 0.3	64.5 ± 0.5	63.6 ± 0.3**	63.1 ± 0.6**
Day 23	61.0 ± 0.2	61.6 ± 0.5	60.5 ± 0.3	60.0 ± 0.2*	58.9 ± 0.6**	–
Week 14	54.5 ± 0.5	55.0 ± 0.4	55.4 ± 0.7	55.3 ± 0.5	55.8 ± 0.3	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Mean cell hemoglobin (pg)						
Day 3	21.1 ± 0.1	21.3 ± 0.2	21.0 ± 0.1	21.0 ± 0.1	20.7 ± 0.2	20.7 ± 0.2
Day 23	20.0 ± 0.1	20.2 ± 0.2	19.9 ± 0.1	19.8 ± 0.1	19.4 ± 0.2*	–
Week 14	18.3 ± 0.1	18.5 ± 0.1	18.4 ± 0.2	18.5 ± 0.2	18.9 ± 0.1	–
Mean cell hemoglobin concentration (g/dL)						
Day 3	32.1 ± 0.2	32.3 ± 0.2	32.7 ± 0.1	32.5 ± 0.1	32.5 ± 0.1	32.8 ± 0.1**
Day 23	32.8 ± 0.1	32.9 ± 0.1	32.9 ± 0.1	32.9 ± 0.1	32.9 ± 0.1	–
Week 14	33.5 ± 0.1	33.6 ± 0.1	33.2 ± 0.2	33.4 ± 0.1	33.8 ± 0.1	–
Platelets (10^3/μL)						
Day 3	1,112 ± 29	1,164 ± 30	1,170 ± 30	1,259 ± 45**	1,328 ± 50**	1,355 ± 85**
Day 23	975 ± 33	936 ± 10	941 ± 24	935 ± 19	786 ± 42**	–
Week 14	823 ± 23	781 ± 13	770 ± 22	703 ± 23**	697 ± 51*	–
Leukocytes (10^3/μL)						
Day 3	14.58 ± 0.66	13.44 ± 1.04	13.56 ± 0.93	9.38 ± 0.83**	8.89 ± 1.08**	8.87 ± 0.69**
Day 23	12.02 ± 0.81	10.94 ± 0.44	11.14 ± 0.77	10.04 ± 0.68	7.98 ± 0.73**	–
Week 14	11.34 ± 0.49	11.81 ± 0.65	10.73 ± 0.74	9.27 ± 0.60*	6.69 ± 0.30**	–
Segmented neutrophils (10^3/μL)						
Day 3	1.41 ± 0.12	1.33 ± 0.20	1.46 ± 0.16	1.12 ± 0.11	2.06 ± 0.50	3.28 ± 0.20**
Day 23	1.36 ± 0.20	1.38 ± 0.12	2.76 ± 0.57**	2.30 ± 0.17**	2.95 ± 0.34**	–
Week 14	1.35 ± 0.14	1.66 ± 0.11	2.05 ± 0.16**	2.39 ± 0.13**	2.42 ± 0.24**	–
Bands (10^3/μL)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
Lymphocytes (10^3/μL)						
Day 3	12.92 ± 0.58	11.78 ± 0.82	11.81 ± 0.84	8.05 ± 0.83**	6.58 ± 0.61**	5.40 ± 0.69**
Day 23	10.45 ± 0.72	9.28 ± 0.42	8.14 ± 0.36*	7.52 ± 0.64**	4.80 ± 0.49**	–
Week 14	9.72 ± 0.39	9.87 ± 0.62	8.42 ± 0.66*	6.67 ± 0.54**	3.84 ± 0.46**	–
Monocytes (10^3/μL)						
Day 3	0.13 ± 0.03	0.16 ± 0.04	0.14 ± 0.03	0.07 ± 0.02	0.16 ± 0.10	0.07 ± 0.02
Day 23	0.09 ± 0.02	0.14 ± 0.02	0.10 ± 0.02	0.07 ± 0.02	0.06 ± 0.02	–
Week 14	0.10 ± 0.02	0.06 ± 0.01	0.04 ± 0.01*	0.07 ± 0.02	0.07 ± 0.03	–
Basophils (10^3/μL)						
Day 3	0.028 ± 0.005	0.024 ± 0.003	0.028 ± 0.004	0.021 ± 0.004	0.010 ± 0.003**	0.003 ± 0.002**
Day 23	0.021 ± 0.002	0.021 ± 0.002	0.024 ± 0.004	0.027 ± 0.004	0.002 ± 0.002*	–
Week 14	0.015 ± 0.005	0.024 ± 0.006	0.013 ± 0.004	0.011 ± 0.002	0.003 ± 0.003	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Eosinophils ($10^3/\mu L$)						
Day 3	0.11 ± 0.01	0.14 ± 0.02	0.12 ± 0.01	0.12 ± 0.02	0.08 ± 0.02	0.12 ± 0.04
Day 23	0.10 ± 0.01	0.12 ± 0.02	0.12 ± 0.01	0.12 ± 0.03	0.16 ± 0.09	–
Week 14	0.17 ± 0.03	0.20 ± 0.03	0.21 ± 0.05	0.13 ± 0.01	0.36 ± 0.23	–
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 3	8.5 ± 0.3	10.2 ± 0.7	9.9 ± 0.8	14.4 ± 0.9**	22.3 ± 1.5**	25.3 ± 0.7**
Day 23	9.3 ± 0.4	8.4 ± 0.4	8.5 ± 0.5	10.8 ± 0.5*	14.2 ± 1.6**	–
Week 14	15.4 ± 0.5	15.2 ± 0.4	14.5 ± 0.6	15.1 ± 0.4	18.0 ± 0.6	–
Creatinine (mg/dL)						
Day 3	0.21 ± 0.02	0.21 ± 0.02	0.19 ± 0.01	0.21 ± 0.01	0.27 ± 0.02	0.22 ± 0.02
Day 23	0.36 ± 0.02	0.31 ± 0.01	0.34 ± 0.02	0.33 ± 0.02	0.36 ± 0.04	–
Week 14	0.36 ± 0.02	0.35 ± 0.02	0.34 ± 0.02	0.34 ± 0.02	0.33 ± 0.03	–
Glucose (mg/dL)						
Day 3	142 ± 4	133 ± 3	137 ± 4	157 ± 4*	170 ± 19*	176 ± 7**
Day 23	125 ± 4	136 ± 5	123 ± 7	146 ± 6**	144 ± 12*	–
Week 14	121 ± 2	129 ± 4	130 ± 6	128 ± 2*	153 ± 11**	–
Total protein (g/dL)						
Day 3	5.5 ± 0.0	5.6 ± 0.0	5.6 ± 0.1	6.1 ± 0.1**	6.3 ± 0.1**	6.0 ± 0.1**
Day 23	6.3 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.7 ± 0.1*	–
Week 14	7.1 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.0 ± 0.1	7.0 ± 0.2	–
Albumin (g/dL)						
Day 3	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.6 ± 0.1**	4.7 ± 0.1**	4.5 ± 0.1**
Day 23	4.5 ± 0.0	4.4 ± 0.0	4.5 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	–
Week 14	4.7 ± 0.0	4.8 ± 0.1	4.8 ± 0.0	4.7 ± 0.1	4.6 ± 0.1	–
Globulin (g/dL)						
Day 3	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.5 ± 0.0**	1.6 ± 0.1**	1.5 ± 0.1**
Day 23	1.8 ± 0.0	1.7 ± 0.0	1.9 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	–
Week 14	2.4 ± 0.0	2.4 ± 0.0	2.4 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	–
Albumin/globulin ratio						
Day 3	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.0	3.0 ± 0.1	3.0 ± 0.1	3.1 ± 0.1
Day 23	2.5 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	2.3 ± 0.1	–
Week 14	1.9 ± 0.0	2.0 ± 0.0	2.0 ± 0.1	2.1 ± 0.1*	1.9 ± 0.1	–
Cholesterol (mg/dL)						
Day 3	123 ± 3	117 ± 3	116 ± 3	119 ± 3	118 ± 4	107 ± 4*
Day 23	100 ± 4	93 ± 2	99 ± 3	104 ± 5	116 ± 5	–
Week 14	114 ± 4	105 ± 3	107 ± 4	98 ± 4**	86 ± 3**	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Triglycerides (mg/dL)						
Day 3	32 ± 2	28 ± 2	20 ± 1**	18 ± 1**	21 ± 3**	24 ± 2**
Day 23	29 ± 2	26 ± 3	25 ± 2	28 ± 3	39 ± 9	–
Week 14	52 ± 4	50 ± 5	48 ± 4	66 ± 5	78 ± 16	–
Alanine aminotransferase (IU/L)						
Day 3	72 ± 3	60 ± 3*	57 ± 2**	50 ± 2**	41 ± 2**	48 ± 4**
Day 23	44 ± 1	48 ± 2	51 ± 2**	49 ± 2*	64 ± 10**	–
Week 14	55 ± 2	69 ± 6**	69 ± 3**	73 ± 2**	92 ± 12**	–
Alkaline phosphatase (IU/L)						
Day 3	312 ± 9	274 ± 11*	265 ± 6**	246 ± 10**	186 ± 4**	172 ± 9**
Day 23	197 ± 7	205 ± 9	214 ± 9	233 ± 9*	206 ± 14	–
Week 14	156 ± 5	175 ± 6*	204 ± 7**	216 ± 6**	238 ± 24**	–
Creatine kinase (IU/L)						
Day 3	627 ± 111	526 ± 43	518 ± 45	573 ± 91	315 ± 26**	416 ± 39*
Day 23	271 ± 20	352 ± 39	351 ± 34	429 ± 62**	485 ± 63**	–
Week 14	196 ± 29	193 ± 21	223 ± 30	242 ± 26	1,014 ± 694**	–
Sorbitol dehydrogenase (IU/L)						
Day 3	11 ± 1	11 ± 1	11 ± 1	11 ± 2	15 ± 1*	15 ± 1*
Day 23	12 ± 0	12 ± 1	12 ± 1	11 ± 1	14 ± 1	–
Week 14	14 ± 1	12 ± 1	12 ± 1	12 ± 1	12 ± 0	–
Bile acids (µmol/L)						
Day 3	32.1 ± 4.4	27.4 ± 4.4	20.6 ± 4.2*	17.3 ± 3.4*	10.1 ± 1.6**	10.9 ± 1.1**
Day 23	12.2 ± 2.1	14.9 ± 2.3	23.4 ± 5.1	14.2 ± 2.8	35.3 ± 16.8	–
Week 14	10.6 ± 2.3	29.3 ± 7.1*	26.7 ± 3.7*	26.0 ± 2.3**	24.8 ± 13.8	–
Female						
n						
Day 3	10	10	10	10	10	9
Day 23	10	10	9	10	7	0
Week 14	10	10	10	10	8	0
Hematology						
Hematocrit (spun) (%)						
Day 3	43.9 ± 0.4	44.2 ± 0.3	43.3 ± 0.8	45.3 ± 0.5	48.5 ± 0.7**	51.6 ± 0.7**
Day 23	46.8 ± 0.5	47.2 ± 0.4	48.1 ± 0.6	49.1 ± 0.5**	52.3 ± 1.3**	–
Week 14	46.2 ± 0.5	47.4 ± 0.6	47.6 ± 0.5	49.1 ± 0.6**	49.8 ± 1.0**	–
Packed cell volume (%)						
Day 3	41.7 ± 0.5	41.9 ± 0.4	40.9 ± 0.7	43.4 ± 0.4*	46.6 ± 0.7**	50.3 ± 0.6**
Day 23	45.2 ± 0.4	45.8 ± 0.4	46.9 ± 0.6*	47.6 ± 0.5**	51.3 ± 1.3**	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Week 14	45.0 ± 0.4	45.6 ± 0.6	46.1 ± 0.5	47.7 ± 0.7**	48.6 ± 1.0**	–
Hemoglobin (g/dL)						
Day 3	13.5 ± 0.2	13.7 ± 0.1	13.4 ± 0.2	14.1 ± 0.1**	15.3 ± 0.2**	16.6 ± 0.2**
Day 23	14.9 ± 0.1	15.1 ± 0.1	15.4 ± 0.2*	15.7 ± 0.2**	16.9 ± 0.4**	–
Week 14	15.2 ± 0.1	15.4 ± 0.2	15.6 ± 0.1	16.0 ± 0.2**	16.4 ± 0.3**	–
Erythrocytes (10^6/μL)						
Day 3	6.34 ± 0.11	6.42 ± 0.05	6.42 ± 0.07	6.71 ± 0.10**	7.38 ± 0.13**	8.11 ± 0.10**
Day 23	7.31 ± 0.11	7.47 ± 0.09	7.79 ± 0.09**	7.80 ± 0.09**	8.53 ± 0.23**	–
Week 14	7.79 ± 0.10	8.17 ± 0.12*	8.11 ± 0.10*	8.37 ± 0.15**	8.39 ± 0.16**	–
Reticulocytes (10^3/μL)						
Day 3	423 ± 13	439 ± 25	431 ± 14	465 ± 17	460 ± 18	511 ± 28*
Day 23	182 ± 8	225 ± 10	196 ± 5	223 ± 12*	263 ± 30**	–
Week 14	192 ± 8	200 ± 4	206 ± 7	219 ± 9	216 ± 13	–
Nucleated erythrocytes/100 leukocytes						
Day 3	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1
Day 23	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	–
Week 14	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.00 ± 0.0	–
Mean cell volume (fL)						
Day 3	65.7 ± 0.7	65.3 ± 0.5	63.6 ± 0.6	64.7 ± 0.5	63.2 ± 0.5**	62.0 ± 0.5**
Day 23	61.8 ± 0.5	61.3 ± 0.4	60.2 ± 0.5	61.1 ± 0.3	60.2 ± 0.6	–
Week 14	57.8 ± 0.4	55.8 ± 0.5*	56.9 ± 0.4	57.0 ± 0.5	57.9 ± 0.6	–
Mean cell hemoglobin (pg)						
Day 3	21.3 ± 0.2	21.3 ± 0.2	20.9 ± 0.2	21.1 ± 0.2	20.8 ± 0.2*	20.5 ± 0.2**
Day 23	20.3 ± 0.2	20.2 ± 0.1	19.8 ± 0.2	20.1 ± 0.1	19.8 ± 0.2	–
Week 14	19.5 ± 0.2	18.9 ± 0.2*	19.3 ± 0.1	19.1 ± 0.1	19.5 ± 0.2	–
Mean cell hemoglobin concentration (pg)						
Day 3	32.4 ± 0.2	32.7 ± 0.1	32.8 ± 0.1	32.6 ± 0.2	32.9 ± 0.1	33.1 ± 0.1**
Day 23	32.9 ± 0.1	33.0 ± 0.1	32.9 ± 0.1	32.9 ± 0.1	32.9 ± 0.1	–
Week 14	33.8 ± 0.1	33.8 ± 0.1	33.9 ± 0.1	33.6 ± 0.2	33.7 ± 0.1	–
Platelets (10^3/μL)						
Day 3	1,309 ± 38	1,182 ± 57	1,309 ± 49	1,305 ± 50	1,435 ± 39	1,550 ± 85*
Day 23	1,006 ± 34	934 ± 28	1,043 ± 45	996 ± 38	859 ± 30*	–
Week 14	919 ± 25	943 ± 15	807 ± 43*	829 ± 27*	801 ± 38*	–
Leukocytes (10^3/μL)						
Day 3	12.51 ± 0.49	12.62 ± 0.51	11.32 ± 0.47	12.62 ± 0.92	11.06 ± 0.97	7.95 ± 0.84**
Day 23	9.21 ± 0.65	7.93 ± 0.60	10.23 ± 0.56	10.42 ± 0.72	8.16 ± 0.79	–
Week 14	10.48 ± 0.51	11.59 ± 0.78	10.88 ± 0.56	11.15 ± 1.00	8.29 ± 0.84	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Segmented neutrophils ($10^3/\mu L$)						
Day 3	1.15 ± 0.10	1.02 ± 0.10	0.98 ± 0.10	1.21 ± 0.12	1.44 ± 0.14	2.71 ± 0.23**
Day 23	1.19 ± 0.17	0.81 ± 0.06	1.61 ± 0.13	2.28 ± 0.29**	3.05 ± 0.75**	–
Week 14	1.38 ± 0.15	1.85 ± 0.11**	2.03 ± 0.19**	3.52 ± 0.43**	3.07 ± 0.57**	–
Bands ($10^3/\mu L$)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	–
Lymphocytes ($10^3/\mu L$)						
Day 3	11.01 ± 0.51	11.27 ± 0.46	10.01 ± 0.46	11.10 ± 0.85	9.38 ± 0.89	5.11 ± 0.72**
Day 23	7.76 ± 0.64	6.92 ± 0.56	8.34 ± 0.48	7.82 ± 0.68	4.92 ± 0.28**	–
Week 14	8.82 ± 0.42	9.41 ± 0.69	8.57 ± 0.62	7.35 ± 0.69	4.88 ± 0.69**	–
Monocytes ($10^3/\mu L$)						
Day 3	0.18 ± 0.03	0.15 ± 0.03	0.17 ± 0.03	0.14 ± 0.03	0.12 ± 0.06*	0.09 ± 0.04*
Day 23	0.13 ± 0.02	0.09 ± 0.01	0.11 ± 0.02	0.16 ± 0.07	0.04 ± 0.01**	–
Week 14	0.07 ± 0.02	0.09 ± 0.02	0.07 ± 0.01	0.09 ± 0.05	0.06 ± 0.01	–
Basophils ($10^3/\mu L$)						
Day 3	0.022 ± 0.004	0.021 ± 0.002	0.032 ± 0.007	0.036 ± 0.006	0.031 ± 0.010	0.002 ± 0.001**
Day 23	0.012 ± 0.001	0.015 ± 0.006	0.031 ± 0.010	0.027 ± 0.014	0.010 ± 0.004	–
Week 14	0.015 ± 0.003	0.025 ± 0.007	0.015 ± 0.003	0.010 ± 0.003	0.009 ± 0.004	–
Eosinophils ($10^3/\mu L$)						
Day 3	0.14 ± 0.01	0.16 ± 0.02	0.13 ± 0.01	0.14 ± 0.02	0.10 ± 0.01	0.04 ± 0.02**
Day 23	0.12 ± 0.01	0.10 ± 0.01	0.14 ± 0.02	0.14 ± 0.01	0.14 ± 0.02	–
Week 14	0.19 ± 0.01	0.21 ± 0.02	0.20 ± 0.04	0.17 ± 0.03	0.26 ± 0.04	–
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 3	10.4 ± 0.7	8.9 ± 0.7	11.2 ± 1.1	12.6 ± 1.1	18.1 ± 0.9**	24.4 ± 0.8**
Day 23	10.0 ± 0.3	10.5 ± 0.6	10.3 ± 0.5	14.1 ± 0.8**	17.3 ± 1.1**	–
Week 14	15.3 ± 0.7	15.5 ± 0.3	13.1 ± 0.5	16.9 ± 1.0	18.8 ± 1.5	–
Creatinine (mg/dL)						
Day 3	0.34 ± 0.02	0.34 ± 0.02	0.34 ± 0.02	0.33 ± 0.02	0.34 ± 0.02	0.36 ± 0.02
Day 23	0.38 ± 0.01	0.37 ± 0.02	0.37 ± 0.02	0.41 ± 0.02	0.41 ± 0.03	–
Week 14	0.39 ± 0.01	0.41 ± 0.02	0.35 ± 0.02	0.39 ± 0.02	0.36 ± 0.02	–
Glucose (mg/dL)						
Day 3	137 ± 2	138 ± 4	145 ± 3	149 ± 3*	156 ± 4**	163 ± 9**
Day 23	130 ± 5	131 ± 5	141 ± 3	143 ± 5	152 ± 9*	–
Week 14	129 ± 4	117 ± 1	127 ± 5	134 ± 4	145 ± 13	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Total protein (g/dL)						
Day 3	5.8 ± 0.1	5.7 ± 0.1	6.0 ± 0.1	6.0 ± 0.0*	6.2 ± 0.1**	6.1 ± 0.1**
Day 23	6.4 ± 0.1	6.3 ± 0.0	6.5 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	–
Week 14	7.1 ± 0.1	7.2 ± 0.1	6.8 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	–
Albumin (g/dL)						
Day 3	4.6 ± 0.1	4.5 ± 0.1	4.7 ± 0.0	4.6 ± 0.0	4.7 ± 0.1	4.6 ± 0.1
Day 23	4.7 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.8 ± 0.1	–
Week 14	5.1 ± 0.1	5.1 ± 0.1	4.8 ± 0.1**	4.8 ± 0.1**	4.7 ± 0.1**	–
Globulin (g/dL)						
Day 3	1.2 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	1.4 ± 0.0**	1.5 ± 0.1**	1.5 ± 0.1**
Day 23	1.7 ± 0.1	1.6 ± 0.0	1.7 ± 0.1	1.8 ± 0.0	1.8 ± 0.1	–
Week 14	2.0 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.3 ± 0.1**	2.3 ± 0.1**	–
Albumin/globulin ratio						
Day 3	3.7 ± 0.1	3.7 ± 0.1	3.6 ± 0.1	3.4 ± 0.1*	3.2 ± 0.1**	3.1 ± 0.1**
Day 23	2.9 ± 0.2	3.1 ± 0.1	2.7 ± 0.1	2.6 ± 0.1**	2.6 ± 0.1*	–
Week 14	2.6 ± 0.1	2.4 ± 0.1*	2.4 ± 0.1*	2.1 ± 0.1**	2.0 ± 0.1**	–
Cholesterol (mg/dL)						
Day 3	114 ± 5	114 ± 4	111 ± 4	111 ± 5	109 ± 4	81 ± 5**
Day 23	102 ± 5	104 ± 5	109 ± 4	105 ± 6	87 ± 6	–
Week 14	98 ± 5	110 ± 5	102 ± 3	99 ± 5	80 ± 4	–
Triglycerides (mg/dL)						
Day 3	36 ± 2	33 ± 1	32 ± 3	29 ± 3	29 ± 2	31 ± 1
Day 23	32 ± 1	35 ± 3	40 ± 3	34 ± 3	34 ± 4	–
Week 14	37 ± 3	43 ± 2	43 ± 2	73 ± 9**	59 ± 8**	–
Alanine aminotransferase (IU/L)						
Day 3	57 ± 2	49 ± 3	44 ± 2**	44 ± 2**	35 ± 2**	39 ± 3**
Day 23	42 ± 4	36 ± 1	40 ± 3	45 ± 2	49 ± 4	–
Week 14	53 ± 3	54 ± 2	64 ± 6	66 ± 4	88 ± 6**	–
Alkaline phosphatase (IU/L)						
Day 3	245 ± 9	222 ± 7	218 ± 9*	197 ± 6**	158 ± 7**	134 ± 6**
Day 23	124 ± 5	121 ± 5	148 ± 8*	156 ± 8**	152 ± 4**	–
Week 14	127 ± 6	146 ± 6*	176 ± 23*	184 ± 9**	220 ± 17**	–
Creatine kinase (IU/L)						
Day 3	540 ± 74	586 ± 113	651 ± 143	428 ± 81	476 ± 69	417 ± 50
Day 23	370 ± 123	269 ± 35	373 ± 51	316 ± 44	465 ± 52**	–
Week 14	188 ± 20	200 ± 26	212 ± 20	258 ± 43	449 ± 123*	–

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm	7.0 ppm
Sorbitol dehydrogenase (IU/L)						
Day 3	10 ± 1	10 ± 1	11 ± 1	10 ± 1	10 ± 1	14 ± 1*
Day 23	14 ± 3	11 ± 0	11 ± 1	12 ± 1	12 ± 1	–
Week 14	12 ± 0	11 ± 1	12 ± 1	11 ± 1	11 ± 1	–
Bile acids (µmol/L)						
Day 3	19.4 ± 4.5	20.3 ± 5.6	13.9 ± 4.1	9.1 ± 1.5	7.8 ± 0.8*	10.2 ± 1.4
Day 23	9.6 ± 1.6	11.9 ± 1.3	9.4 ± 2.5	13.8 ± 4.2	12.7 ± 2.6	–
Week 14	12.5 ± 4.8	18.4 ± 4.0	43.4 ± 13.9**	25.5 ± 3.4**	24.2 ± 3.8**	–

*Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data. Due to 100% mortality in clinical pathology and core study rats exposed to 7.0 ppm, no data are available for these groups at day 23 or week 14.

Table B-2. Hematology Data for Mice in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm
Male					
n	10	10	10	10	5
Hematology					
Hematocrit (spun) (%)	50.2 ± 0.4	50.3 ± 0.3	50.3 ± 0.4	49.0 ± 0.3*	47.5 ± 0.9*
Packed cell volume (%)	49.8 ± 0.4	50.3 ± 0.5	49.7 ± 0.3	47.9 ± 0.3**	46.4 ± 1.0**
Hemoglobin (g/dL)	16.0 ± 0.1	16.4 ± 0.1	16.1 ± 0.1	15.6 ± 0.1*	15.0 ± 0.3*
Erythrocytes (10^6/μL)	10.38 ± 0.07	10.66 ± 0.12	10.52 ± 0.08	10.04 ± 0.09*	9.72 ± 0.14**
Reticulocytes (10^3/μL)	256 ± 9	257 ± 14	258 ± 9	224 ± 10	228 ± 28
Nucleated erythrocytes/100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Howell-Jolly bodies (% erythrocytes)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Mean cell volume (fL)	47.9 ± 0.2	47.2 ± 0.1	47.2 ± 0.2*	47.8 ± 0.2	47.7 ± 0.5
Mean cell hemoglobin (pg)	15.5 ± 0.1	15.4 ± 0.1	15.3 ± 0.1	15.5 ± 0.1	15.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.2 ± 0.1	32.6 ± 0.1	32.5 ± 0.2	32.5 ± 0.1	32.3 ± 0.1
Platelets (10^3/μL)	992 ± 11	911 ± 34*	931 ± 16	978 ± 18	1,090 ± 112
Leukocytes (10^3/μL)	2.32 ± 0.19	3.32 ± 0.21**	3.08 ± 0.12**	4.61 ± 0.42**	6.15 ± 1.22**
Segmented neutrophils (10^3/μL)	0.30 ± 0.04	0.42 ± 0.02	0.41 ± 0.03	0.87 ± 0.08**	2.90 ± 1.14**
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	1.91 ± 0.15	2.82 ± 0.19**	2.57 ± 0.13**	3.57 ± 0.35**	2.83 ± 0.21**
Monocytes (10^3/μL)	0.06 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.22 ± 0.10
Basophils (10^3/μL)	0.018 ± 0.002	0.018 ± 0.002	0.020 ± 0.004	0.017 ± 0.004	0.008 ± 0.002
Eosinophils (10^3/μL)	0.02 ± 0.00	0.02 ± 0.01	0.05 ± 0.01	0.12 ± 0.02**	0.19 ± 0.06**
Female					
n	10	10	10	10	6
Hematology					
Hematocrit (spun) (%)	50.7 ± 0.6	50.2 ± 0.3	48.8 ± 0.4**	49.3 ± 0.7*	46.0 ± 1.0**[b]
Packed cell volume (%)	50.1 ± 0.6	50.2 ± 0.3	48.6 ± 0.4**	48.7 ± 0.6*	47.1 ± 0.4**
Hemoglobin (g/dL)	16.5 ± 0.2	16.3 ± 0.1	15.8 ± 0.1**	15.9 ± 0.2**	15.2 ± 0.2**
Erythrocytes (10^6/μL)	10.33 ± 0.08	10.44 ± 0.05	10.10 ± 0.07	10.16 ± 0.11	9.70 ± 0.11**
Reticulocytes (10^3/μL)	285 ± 23	279 ± 16	299 ± 16	276 ± 13	243 ± 13
Nucleated erythrocytes/100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm
Howell-Jolly bodies (% erythrocytes)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Mean cell volume (fL)	48.4 ± 0.3	48.1 ± 0.2	48.1 ± 0.1	48.0 ± 0.2	48.5 ± 0.3
Mean cell hemoglobin (pg)	15.9 ± 0.1	15.7 ± 0.1*	15.7 ± 0.0*	15.7 ± 0.1**	15.6 ± 0.1*
Mean cell hemoglobin concentration (g/dL)	32.9 ± 0.1	32.5 ± 0.1	32.6 ± 0.1	32.7 ± 0.1	32.2 ± 0.2*
Platelets (10^3/μL)	846 ± 55	835 ± 18	895 ± 20	890 ± 14	883 ± 100
Leukocytes (10^3/μL)	3.80 ± 0.51	2.78 ± 0.18	3.37 ± 0.34	4.01 ± 0.36	5.31 ± 0.36*
Segmented neutrophils (10^3/μL)	0.69 ± 0.23	0.47 ± 0.08	0.61 ± 0.06	0.84 ± 0.12	2.14 ± 0.31**
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	3.03 ± 0.32	2.27 ± 0.14	2.65 ± 0.30	3.04 ± 0.24	2.94 ± 0.37
Monocytes (10^3/μL)	0.03 ± 0.01	0.02 ± 0.00	0.06 ± 0.02	0.04 ± 0.02	0.04 ± 0.01
Basophils (10^3/μL)	0.015 ± 0.004	0.014 ± 0.006	0.019 ± 0.003	0.021 ± 0.003	0.002 ± 0.002
Eosinophils (10^3/μL)	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.07 ± 0.01*	0.19 ± 0.05**

*Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data. Due to 100% mortality in mice exposed to 7.0 ppm, no data are available for this group.
[b]n = 5.

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

Tables

Table C-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm
Male					
n	10	10	10	10	3
Necropsy body wt	409 ± 4	377 ± 6**	350 ± 5**	309 ± 9**	222 ± 15**
Heart					
Absolute	1.37 ± 0.03	1.30 ± 0.03*	1.23 ± 0.02**	1.12 ± 0.03**	0.92 ± 0.04**
Relative	3.36 ± 0.05	3.44 ± 0.05	3.50 ± 0.05	3.63 ± 0.07**	4.18 ± 0.21**
R. Kidney					
Absolute	1.31 ± 0.03	1.21 ± 0.03*	1.15 ± 0.03**	1.05 ± 0.03**	0.82 ± 0.03**
Relative	3.21 ± 0.07	3.21 ± 0.06	3.28 ± 0.07	3.40 ± 0.04*	3.71 ± 0.18**
Liver					
Absolute	12.17 ± 0.25	11.11 ± 0.32*	10.53 ± 0.33**	9.68 ± 0.45**	6.93 ± 0.19**
Relative	29.82 ± 0.64	29.45 ± 0.71	30.05 ± 0.77	31.20 ± 0.78	31.44 ± 2.07
Lung					
Absolute	2.16 ± 0.10	2.02 ± 0.07	2.02 ± 0.14	2.17 ± 0.10	2.00 ± 0.14
Relative	5.31 ± 0.27	5.36 ± 0.19	5.75 ± 0.38	7.07 ± 0.40**	9.06 ± 0.75**
R. Testis					
Absolute	2.014 ± 0.033	1.956 ± 0.052	1.772 ± 0.118*	1.744 ± 0.054*	1.607 ± 0.041**
Relative	4.932 ± 0.077	5.192 ± 0.129	5.059 ± 0.329	5.649 ± 0.155*	7.274 ± 0.321**
Thymus					
Absolute	0.348 ± 0.014	0.314 ± 0.019	0.298 ± 0.012*	0.241 ± 0.017**	0.154 ± 0.009**
Relative	0.851 ± 0.034	0.834 ± 0.055	0.851 ± 0.033	0.779 ± 0.051	0.704 ± 0.089
Female					
n	10	10	10	10	8
Necropsy body wt	239 ± 5	238 ± 5	230 ± 3	214 ± 5**	205 ± 6**
Heart					
Absolute	0.89 ± 0.02	0.88 ± 0.02	0.90 ± 0.04	0.82 ± 0.02	0.86 ± 0.02
Relative	3.73 ± 0.06	3.71 ± 0.06	3.93 ± 0.18	3.85 ± 0.08	4.23 ± 0.13**
R. Kidney					
Absolute	0.83 ± 0.02	0.83 ± 0.02	0.81 ± 0.02	0.73 ± 0.01**	0.72 ± 0.02**
Relative	3.47 ± 0.08	3.49 ± 0.05	3.51 ± 0.08	3.42 ± 0.06	3.52 ± 0.05
Liver					
Absolute	6.98 ± 0.15	7.13 ± 0.28	6.79 ± 0.32	6.74 ± 0.24	7.06 ± 0.26
Relative	29.19 ± 0.43	29.88 ± 0.65	29.53 ± 1.31	31.54 ± 0.66	34.54 ± 0.80**

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm
Lung					
Absolute	1.78 ± 0.07	1.66 ± 0.03	1.67 ± 0.07	1.79 ± 0.08	1.75 ± 0.07
Relative	7.42 ± 0.26	7.01 ± 0.14	7.30 ± 0.34	8.46 ± 0.48	8.61 ± 0.44
Thymus					
Absolute	0.274 ± 0.015	0.226 ± 0.013*	0.246 ± 0.010*	0.173 ± 0.013**	0.154 ± 0.009**
Relative	1.139 ± 0.051	0.951 ± 0.052	1.068 ± 0.036	0.802 ± 0.052**	0.754 ± 0.039**

*Significantly different ($P \leq 0.05$) from the chamber control group by Williams' test.
**$P \leq 0.01$.
[a]Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). Due to 100% mortality in rats exposed to 7.0 ppm, no data are available for this group.

Table C-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm
Male					
n	10	10	10	10	5
Necropsy body wt	35.6 ± 0.9	29.5 ± 0.4**	27.2 ± 0.4**	25.8 ± 0.3**	21.6 ± 0.8**
Heart					
Absolute	0.15 ± 0.00	0.13 ± 0.00**	0.13 ± 0.00**	0.13 ± 0.00**	0.12 ± 0.01**
Relative	4.31 ± 0.10	4.41 ± 0.11	4.60 ± 0.04*	4.88 ± 0.08**	5.57 ± 0.20**
R. Kidney					
Absolute	0.29 ± 0.01	0.23 ± 0.00**	0.21 ± 0.00**	0.21 ± 0.00**	0.19 ± 0.00**
Relative	8.15 ± 0.15	7.74 ± 0.17	7.86 ± 0.10	8.02 ± 0.19	8.92 ± 0.17*
Liver					
Absolute	1.41 ± 0.05	1.07 ± 0.03**	1.09 ± 0.02**	1.03 ± 0.04**	0.97 ± 0.05**
Relative	39.55 ± 1.02	36.28 ± 1.03	40.14 ± 1.12	39.91 ± 1.64	44.96 ± 2.04*
Lung					
Absolute	0.20 ± 0.01	0.19 ± 0.00	0.19 ± 0.00	0.18 ± 0.00*	0.19 ± 0.01
Relative	5.65 ± 0.19	6.55 ± 0.16**	7.03 ± 0.18**	7.08 ± 0.10**	8.64 ± 0.29**
R. Testis					
Absolute	0.110 ± 0.002	0.105 ± 0.002*	0.104 ± 0.001*	0.105 ± 0.002*	0.084 ± 0.002**
Relative	3.108 ± 0.071	3.563 ± 0.069**	3.810 ± 0.069**	4.060 ± 0.057**	3.903 ± 0.104**
Thymus					
Absolute	0.041 ± 0.002	0.028 ± 0.003**	0.026 ± 0.002**	0.028 ± 0.002**	0.020 ± 0.005**
Relative	1.159 ± 0.041	0.933 ± 0.084	0.955 ± 0.064	1.089 ± 0.078	0.917 ± 0.199
Female					
n	10	10	10	10	6
Necropsy body wt	29.8 ± 0.9	25.5 ± 0.3**	23.3 ± 0.3**	22.8 ± 0.4**	20.7 ± 0.8**
Heart					
Absolute	0.14 ± 0.00	0.12 ± 0.00**	0.12 ± 0.00**	0.11 ± 0.00**	0.13 ± 0.01**
Relative	4.65 ± 0.10	4.72 ± 0.10	5.03 ± 0.08	4.98 ± 0.15	6.38 ± 0.62**
R. Kidney					
Absolute	0.20 ± 0.01	0.17 ± 0.01**	0.16 ± 0.01**	0.17 ± 0.00**	0.17 ± 0.01**
Relative	6.76 ± 0.23	6.76 ± 0.21	7.00 ± 0.16	7.49 ± 0.19*	8.39 ± 0.07**
Liver					
Absolute	1.31 ± 0.04	1.12 ± 0.02**	1.06 ± 0.03**	1.01 ± 0.02**	1.03 ± 0.05**
Relative	43.97 ± 0.85	43.96 ± 1.04	45.62 ± 0.85	44.54 ± 0.96	49.88 ± 1.74**

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm	3.5 ppm
Lung					
Absolute	0.21 ± 0.00	0.21 ± 0.01	0.19 ± 0.00*	0.19 ± 0.00**	0.21 ± 0.00
Relative	7.07 ± 0.22	8.05 ± 0.20**	8.35 ± 0.13**	8.32 ± 0.18**	10.31 ± 0.38**
Thymus					
Absolute	0.056 ± 0.003	0.043 ± 0.002**	0.038 ± 0.003**	0.040 ± 0.002**	0.030 ± 0.004**
Relative	1.898 ± 0.085	1.692 ± 0.091	1.641 ± 0.124	1.749 ± 0.088	1.433 ± 0.151*

*Significantly different ($P \leq 0.05$) from the chamber control group by Williams' or Dunnett's test.
**$P \leq 0.01$.
[a]Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error). Due to 100% mortality in mice exposed to 7.0 ppm, no data are available for this group.

Appendix D. Reproductive Tissue Evaluations

Tables

Table D-1. Summary of Reproductive Tissue Evaluations for Male Rats in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	409 ± 4	377 ± 6**	350 ± 5**	309 ± 9**
L. Cauda epididymis	0.2555 ± 0.0092	0.2697 ± 0.0109	0.2140 ± 0.0103**	0.1956 ± 0.0072**
L. Epididymis	0.6692 ± 0.0100	0.6559 ± 0.0197	0.5721 ± 0.0254**	0.5405 ± 0.0133**
L. Testis	2.0070 ± 0.0294	1.9794 ± 0.0590	1.7603 ± 0.1211*	1.7549 ± 0.0316*
Spermatid measurements				
Spermatid heads (10^6/testis)	236.31 ± 24.29	295.95 ± 23.57	183.59 ± 30.05	280.25 ± 17.80
Spermatid heads (10^6/g testis)	118.57 ± 13.03	148.74 ± 10.06	98.55 ± 14.70	160.29 ± 11.14
Epididymal spermatozoal measurements				
Sperm motility (%)	83.1 ± 1.5	75.2 ± 1.5**	67.1 ± 8.1**	65.6 ± 2.5**
Sperm (10^6/cauda epididymis)	81.19 ± 2.44	87.24 ± 6.47	69.88 ± 10.56	66.68 ± 5.57
Sperm (10^6/g cauda epididymis)	321 ± 13	325 ± 22	314 ± 48	340 ± 25

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

Table D-2. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Inhalation Study of *o*-Phthalaldehyde[a]

	Chamber Control	0.44 ppm	0.88 ppm	1.75 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	35.6 ± 0.9	29.5 ± 0.4**	27.2 ± 0.4**	25.8 ± 0.3**
L. Cauda epididymis	0.0157 ± 0.0013	0.0140 ± 0.0010	0.0143 ± 0.0012	0.0147 ± 0.0014
L. Epididymis	0.0443 ± 0.0021	0.0420 ± 0.0030	0.0359 ± 0.0025	0.0413 ± 0.0025
L. Testis	0.1080 ± 0.0023	0.1055 ± 0.0015	0.1013 ± 0.0019	0.1029 ± 0.0021
Spermatid measurements				
Spermatid heads (10^6/testis)	21.65 ± 2.10	22.26 ± 2.68	23.88 ± 1.28	18.73 ± 1.61
Spermatid heads (10^6/g testis)	199.4 ± 17.2	210.0 ± 24.0	236.1 ± 12.5	182.7 ± 15.8
Epididymal spermatozoal measurements				
Sperm motility (%)	74.7 ± 8.4	69.5 ± 7.8*	68.8 ± 7.7**	41.8 ± 9.6**
Sperm (10^6/cauda epididymis)	8.52 ± 0.48	7.79 ± 0.44	6.89 ± 0.90	7.26 ± 0.48
Sperm (10^6/g cauda epididymis)	573 ± 53	571 ± 41	510 ± 67	542 ± 68

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

Appendix E. Genetic Toxicology

Tables

Table E-1. Mutagenicity of *o*-Phthalaldehyde in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100					
	0	98 ± 1	104 ± 4	116 ± 7	145 ± 4
	0.5		100 ± 11		
	1		108 ± 3		
	5	124 ± 2	110 ± 8		
	10	147 ± 8	155 ± 5	139 ± 5	
	25	191 ± 15	224 ± 2	129 ± 3	
	50	58 ± 6		127 ± 3	140 ± 2
	100	0 ± 0		152 ± 3	157 ± 2
	200			162 ± 15	136 ± 3
	300				62 ± 8
	400				1 ± 1
Trial summary		Positive	Positive	Negative	Negative
Positive control[b]		443 ± 15	599 ± 29	771 ± 34	680 ± 15
TA98					
	0	40 ± 4	37 ± 1	41 ± 2	46 ± 4
	0.5	40 ± 4			
	1	47 ± 3			
	5	55 ± 1	40 ± 1		
	10	53 ± 0	39 ± 1	36 ± 3	62 ± 3
	25	19 ± 3	31 ± 5	37 ± 3	49 ± 2
	50		43 ± 4	32 ± 2	61 ± 3
	100		0 ± 0	27 ± 1	55 ± 4
	200			28 ± 2	25 ± 4
Trial summary		Negative	Negative	Negative	Negative
Positive control		560 ± 36	564 ± 11	1,061 ± 146	861 ± 71
***Escherichia coli* WP2 *uvrA*/pKM101**					
	0	176 ± 15	228 ± 20	205 ± 8	194 ± 22
	0.5		230 ± 6		
	1		254 ± 20		
	5	175 ± 12	244 ± 7		
	10	200 ± 5	285 ± 8	248 ± 8	194 ± 14

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
	25	207 ± 4	77 ± 7	241 ± 15	194 ± 25
	50	252 ± 8		245 ± 13	198 ± 5
	100	81 ± 13		251 ± 8	224 ± 28
	200			264 ± 14	223 ± 9
Trial summary		Negative	Negative	Negative	Negative
Positive control		799 ± 52	667 ± 29	641 ± 25	913 ± 64

[a]Study was performed at SITEK Research Laboratories. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent (dimethyl sulfoxide) control.
[b]The positive controls in the absence of metabolic activation were sodium azide (TA100), 2-nitrofluorine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table E-2. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Rats Following Exposure to *o*-Phthalaldehyde by Inhalation for Three Months[a]

Concentration (ppm)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[d]	PCEs[b] (%)	P Value[c]
Male							
Air[e]	5	0.72 ± 0.08		0.10 ± 0.02		0.818 ± 0.05	
o-Phthalaldehyde							
0.44	5	0.71 ± 0.10	0.5195	0.09 ± 0.01	0.7466	0.988 ± 0.09	0.102
0.88	5	0.77 ± 0.06	0.4043	0.08 ± 0.02	0.8261	0.990 ± 0.06	0.120
1.75	5	0.90 ± 0.09	0.0916	0.08 ± 0.01	0.8567	0.898 ± 0.03	0.124
3.5	2	0.93 ± 0.02	–[f]	0.08 ± 0.03	–	1.249 ± 0.18	–
		P = 0.043[g]		P = 0.905[g]		P = 0.526[g]	
Female							
Air	5	0.53 ± 0.05		0.13 ± 0.03		0.811 ± 0.09	
o-Phthalaldehyde							
0.44	5	0.65 ± 0.12	0.2322	0.12 ± 0.03	1.0000	0.784 ± 0.05	1.000
0.88	5	0.63 ± 0.09	0.2785	0.08 ± 0.01	1.0000	0.837 ± 0.08	1.000
1.75	5	0.75 ± 0.13	0.0915	0.07 ± 0.01	1.0000	0.838 ± 0.11	1.000
3.5	5	0.77 ± 0.11	0.0729	0.06 ± 0.01	1.0000	0.729 ± 0.08	0.663
		P = 0.046[g]		P = 0.989[h]		P = 0.442[g]	

[a]Study was performed at ILS, Inc. The detailed protocol is presented by Witt et al.[53]. PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the chamber control group; exposed group values are significant at $P \leq 0.025$ by William's test.
[d]Pairwise comparison with the chamber control group; exposed group values are significant at $P \leq 0.025$ by William's test (males) or Dunn's test (females).
[e]Chamber control.
[f]Small sample size precludes statistical analysis.
[g]Exposure concentration-related trend; significant at $P \leq 0.025$ by linear regression.
[h]Exposure concentration-related trend; significant at $P \leq 0.025$ by Jonckheere's test.

Table E-3. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Exposure to *o*-Phthalaldehyde by Inhalation for Three Months[a]

Concentration (ppm)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[d]	PCEs[b] (%)	P Value[c]
Male							
Air[e]	5	2.31 ± 0.17		1.40 ± 0.02		1.413 ± 0.03	
o-Phthalaldehyde							
0.44	5	2.02 ± 0.15	0.6297	1.40 ± 0.01	1.0000	1.251 ± 0.01	0.1137
0.88	5	2.16 ± 0.21	0.7169	1.35 ± 0.03	1.0000	1.446 ± 0.05	1.0000
1.75	5	2.25 ± 0.10	0.6720	1.29 ± 0.04	1.0000	1.378 ± 0.05	1.0000
3.5	5	3.24 ± 0.61	0.0289	1.44 ± 0.13	1.0000	1.521 ± 0.21	1.0000
		P = 0.005[f]		P = 0.992[g]		P = 0.151[g]	
Female							
Air	5	2.39 ± 0.19		0.98 ± 0.02		1.400 ± 0.16	
o-Phthalaldehyde							
0.44	5	2.06 ± 0.17	0.9680	0.99 ± 0.02	1.0000	1.323 ± 0.04	1.0000
0.88	5	1.85 ± 0.08	0.9873	1.02 ± 0.02	0.3124	1.398 ± 0.06	1.0000
1.75	5	1.88 ± 0.13	0.9918	0.98 ± 0.02	1.0000	1.460 ± 0.04	1.0000
3.5	5	1.79 ± 0.12	0.9942	0.98 ± 0.03	1.0000	1.920 ± 0.11	0.0351
		P=0.989[f]		P=0.424[g]		P=0.001[g]	

[a]Study was performed at ILS, Inc. The detailed protocol is presented by Witt et al.[53]. PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the chamber control group; exposed group values are significant at $P \leq 0.025$ by William's test..
[d]Pairwise comparison with the chamber control group; exposed group values are significant at $P \leq 0.025$ by Dunn's test.
[e]Chamber control.
[f]Exposure concentration-related trend; significant at $P \leq 0.025$ by linear regression.
[g]Exposure concentration-related trend; significant at $P \leq 0.025$ by Jonckheere's test.

Appendix F. Chemical Characterization and Generation of Chamber Concentrations

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F.1. Procurement and Characterization of *o*-Phthalaldehyde

o-Phthalaldehyde was obtained from MP Biomedicals, LLC (Solon, OH), in one lot (8674J) that was used during the 3-month studies. Identity and purity analyses were conducted by the study laboratory at Battelle Toxicology Northwest (Richland, WA) and by the analytical chemistry laboratory at Chemir Analytical Services (Maryland Heights, MO). Reports on analyses performed in support of the *o*-phthalaldehyde studies are on file at the National Institute of Environmental Health Sciences.

Lot 8674J of the chemical, a pale-yellow, coarse, crystalline material, was identified as *o*-phthalaldehyde by the study laboratory using infrared (IR) spectroscopy and by the analytical chemistry laboratory using Fourier transform IR and proton nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with the structure of *o*-phthalaldehyde. Representative IR and NMR spectra are presented in Figure F-1 and Figure F-2.

The analytical chemistry laboratory determined the moisture content of lot 8674J using Karl Fischer titration and elemental analyses for carbon, hydrogen, nitrogen, and sulfur using inductively coupled plasma/optical emission spectroscopy. The study laboratory determined the purity of lot 8674J directly as well as relative to a commercial *o*-phthalaldehyde standard using gas chromatography (GC) with flame ionization detection (FID), and GC with mass spectrometry (MS) for identification of impurities.

For lot 8674J, Karl Fischer titration indicated approximately 0.12% water; elemental analyses for carbon and hydrogen were in agreement with the theoretical values for *o*-phthalaldehyde. GC/FID by system A (Table F-1) indicated one major peak that was 99% of the total peak area and three impurities that were each over 0.1% of the total peak area, with a combined total of approximately 1% of the total peak area. Two of the impurities were tentatively identified as toluene and phthalide by comparison of GC retention times to a chromatogram obtained from a standard solution containing possible impurities or degradation products that included toluene, phthalide, benzaldehyde, phthalan, *N*-hydroxyphthalimide, naphthalene, isophthalaldehyde, and terepthaldicarboxaldehyde. The third peak was not identified. GC/MS by system B indicated that the phthalide peak also contained phthalic acid, which eluted at the same retention time. Different GC columns of varying polarity with FID were used but failed to resolve these two compounds. To ensure the absence of certain degradation products, GC/FID by system A was used to determine the presence of acetonitrile, GC/MS by system B was used for chloroform, and high-performance liquid chromatography with ultraviolet detection (HPLC/UV) was used for 2-carboxybenzaldehyde. Acetonitrile and chloroform were less than 0.1%, and 2-carboxybenzaldehyde was approximately 0.4% by weight. The overall purity of lot 8674J was determined to be greater than 99%. The purity relative to the commercial standard was greater than 99.7%.

To ensure stability, the test chemical was stored refrigerated in the original sealed plastic containers. Periodic reanalyses of the bulk chemical were performed during the 3-month studies using GC/FID by system A, GC/MS by system B, and HPLC/UV and no degradation of the test chemical occurred.

F.2. Vapor Generation and Exposure System

A diagram of the vapor generation and delivery system used in the 3-month studies is shown in Figure F-3. *o*-Phthalaldehyde vapor was generated from a flask heated with a heating mantle, purged by a heated nitrogen flow entering above the flask area, blended with heated dilution air to obtain the vapor concentration desired, and transported into a distribution manifold located above the generator. Vapor concentration was determined by the reservoir temperature, nitrogen flow rate, and dilution air flow rate. Pressure in the distribution manifold was fixed to ensure constant flows through the manifold and into the chambers.

Due to the high boiling point of *o*-phthalaldehyde, all vapor transport lines and the on-line GC transport sample line of the 7.0 ppm chambers were heated above the minimum temperature needed to transport vapor without condensation. Individual Teflon®-delivery lines carried the vapor from the distribution manifold to three-way exposure valves at the chamber inlets. The exposure valves diverted vapor from the metering valves to exposure chamber exhaust until the generation system stabilized and exposure could proceed; an additional 60 minutes was added to the prestart stabilization time to purge residual toluene present in the test chemical to less than 1% before exposures began. To initiate exposure, the chamber exposure valves were rotated to allow the vapor to flow to each chamber exposure duct where it was diluted with conditioned chamber air to achieve the desired exposure concentration.

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

F.3. Vapor Concentration Monitoring

Summaries of the chamber vapor concentrations are given in Table F-2. Concentrations of *o*-phthalaldehyde in exposure chambers were monitored by an on-line GC/FID by system C (Table F-1). Samples were drawn from all exposure and control chambers approximately every 20 minutes during each exposure period using Hasteloy-C stream-select and gas-sampling valves (VALCO Instruments Company, Houston, TX) in a separate, heated oven. The sampling lines composing the sample loop were made from Teflon® tubing and were connected to the exposure chamber relative humidity sampling lines near the gas chromatograph. A vacuum regulator maintained a constant vacuum in the sample loop to compensate for variations in sample line pressure. An in-line flow meter between the vacuum regulator and the gas chromatograph allowed digital measurement of sample flow.

The on-line gas chromatograph was checked throughout the day for instrument drift against an on-line standard vapor of *o*-phthalaldehyde in nitrogen supplied by a standard generator (Kin-Tek; Precision Calibration Systems, La Marque, TX). The on-line gas chromatograph was checked before the start of each exposure day and after every tenth sample throughout the exposure period. The on-line gas chromatograph was calibrated prior to the start of the study and at least monthly by a comparison of chamber concentration data to data from grab samples that

were collected with adsorbent gas sampling tubes containing silica gel (ORBO-52™; Supelco, Bellefonte, PA), extracted with acetonitrile containing α-terpineol as an internal standard, and analyzed using an off-line GC/FID system (system D). Originally, the grab samples were located within the whole body exposure chambers; however, after animals were transferred into the chambers, ammonia produced by the action of fecal bacteria on urine[37] caused inaccurate measurements of exposure concentration; sampling tubes were moved within the inlet lines. Additional samples were collected from the on-line standard generator to bracket concentrations found in the exposure chambers. The off-line gas chromatograph was calibrated with gravimetrically prepared standards of *o*-phthalaldehyde and the internal standard (α-terpineol) in acrylonitrile.

F.4. Chamber Atmosphere Characterization

Buildup and decay rates for chamber vapor concentrations were determined with and without animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after vapor generation was terminated (T_{10}) was approximately 9.2 minutes. For rats and mice in the 3-month studies, T_{90} values ranged from 15 to 21 minutes without animals present and from 24 to 35 minutes with animals; T_{10} values ranged from 10 to 18 minutes without animals present and from 14 to 39 minutes with animals. A T_{90} value of 17 minutes was used for these studies.

The uniformity of vapor concentration in the inhalation exposure chambers without animals was evaluated before each of the studies began; in addition, concentration uniformity with animals present in the chambers was measured once during the 3-month studies. The vapor concentration was measured using the on-line GC/FID system (system C, Table F-1). The automatic 12-port sample valve was disabled to allow continuous monitoring from a single input line. Samples were collected from 12 positions in each chamber. Chamber concentration uniformity was maintained throughout the studies.

The persistence of *o*-phthalaldehyde in the chambers after vapor delivery ended was determined by monitoring the vapor concentration overnight in the 7.0 ppm chamber without and with animals present in the chambers. The concentration decreased to 1% of the target concentration within 128 minutes without animals present and 279 minutes with animals present.

Stability studies of the test chemical were performed by the study laboratory on the distribution line and 7.0 and 0.44 ppm exposure chambers without animals present and on the 3.5 and 0.44 ppm exposure chambers with animals present. Samples were collected with two adsorbent tubes in series [ORBO-52™ (silica gel) and ORBO-32™ (activated coconut charcoal); Supelco, Bellefone, PA] during the first and last hours of generation, extracted with acetonitrile (internal standard, α-terpineol) and analyzed using GC/FID by system A. Samples were also collected from the generator reservoir. The presence of chloroform, acetonitrile, and 2-carboxybenzaldehyde in the exposure atmosphere was investigated using samples collected on ORBO-52™ tubes by GC/FID by system A (acetonitrile), system B (chloroform), or HPLC/UV (2-carboxybenzaldehyde). These studies indicated that the *o*-phthalaldehyde test chemical was stable for 1 day.

Table F-1. Gas Chromatography Systems Used in the Inhalation Studies of *o*-Phthalaldehyde[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	DB 624 Megabore, 30 m × 530 µm, 3.0 µm film (J&W Scientific, Folsom, CA)	Helium at 12 psi	35°C for 5 minutes, then 8°C/minute to 240°C, held for 2 minutes
System B			
Mass spectrometry Electron impact with selected ion monitoring	RTX®-5, 30 m × 320 µm, 1.0 µm film (Restek, Bellefonte, PA)	Helium at 6 psi head pressure	38°C for 5 minutes, then 8°C/minute to 320°C, held for 2 minutes
System C			
Flame ionization	DB 5, 15 m × 530 µm, 1.5 µm film (J&W Scientific)	Nitrogen at 25 mL/minute	Isothermal at 140°C
System D			
Flame ionization	DB 624 Megabore, 30 m × 530 µm, 3.0 µm film (J&W Scientific)	Helium at 12 psi head pressure	65°C for 1 minute, then 15°C/minute to 210°C, held for 2 minutes

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

Table F-2. Summary of Chamber Concentrations in the Three-month Inhalation Studies of *o*-Phthalaldehyde

	Target Concentration (ppm)	Total Number of Readings	Average Concentration[a] (ppm)
Rat Chambers			
	0.44	989	0.43 ± 0.04
	0.88	982	0.86 ± 0.07
	1.75	990	1.69 ± 0.14
	3.5	1,013	3.4 ± 0.4
	7.0	127	7.1 ± 0.5
Mouse Chambers			
	0.44	1,020	0.43 ± 0.04
	0.88	1,013	0.86 ± 0.07
	1.75	1,021	1.69 ± 0.13
	3.5	1,044	3.4 ± 0.4
	7.0	68	6.9 ± 0.6

[a]Mean ± standard deviation.

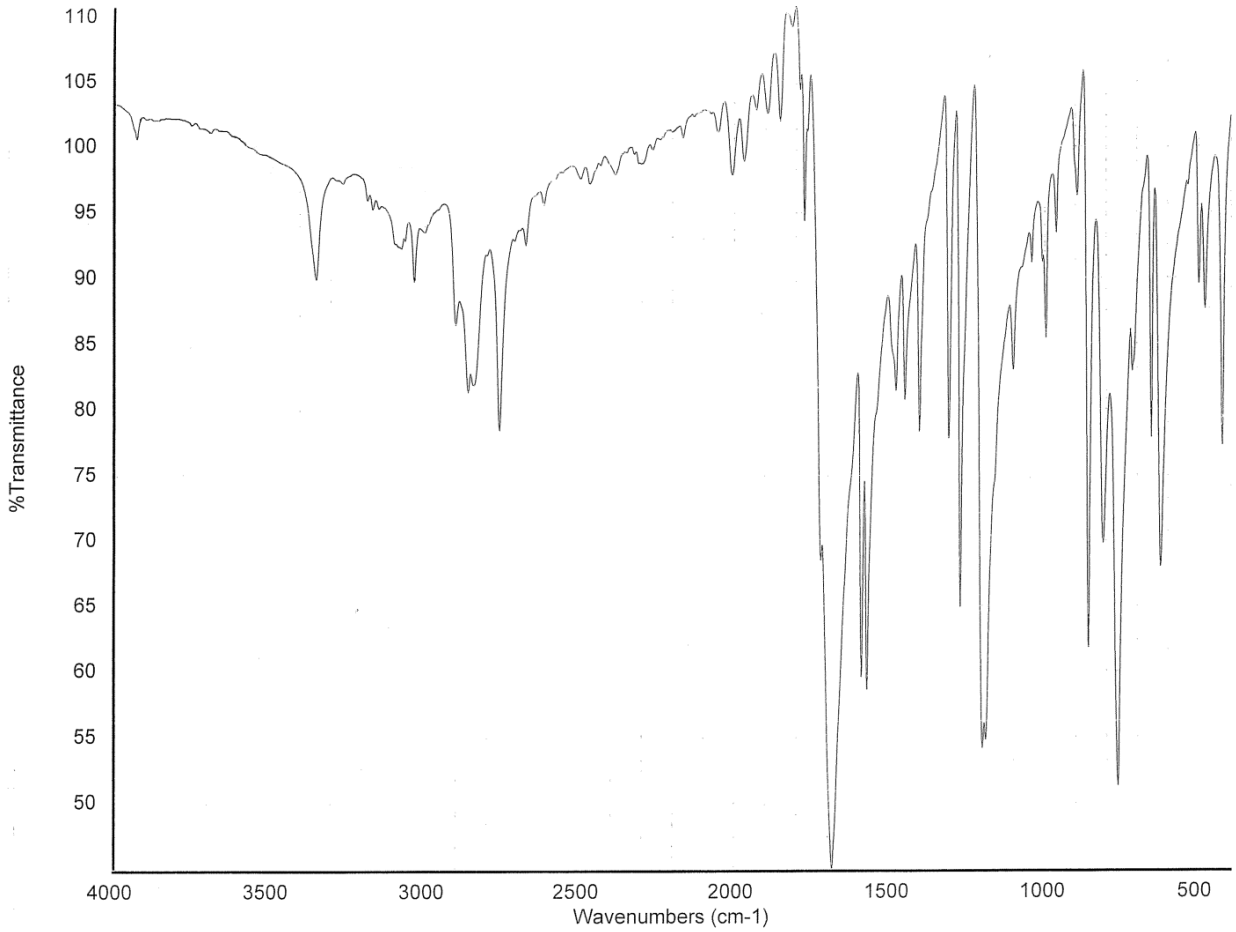

Figure F-1. Infrared Absorption Spectrum of *o*-Phthalaldehyde

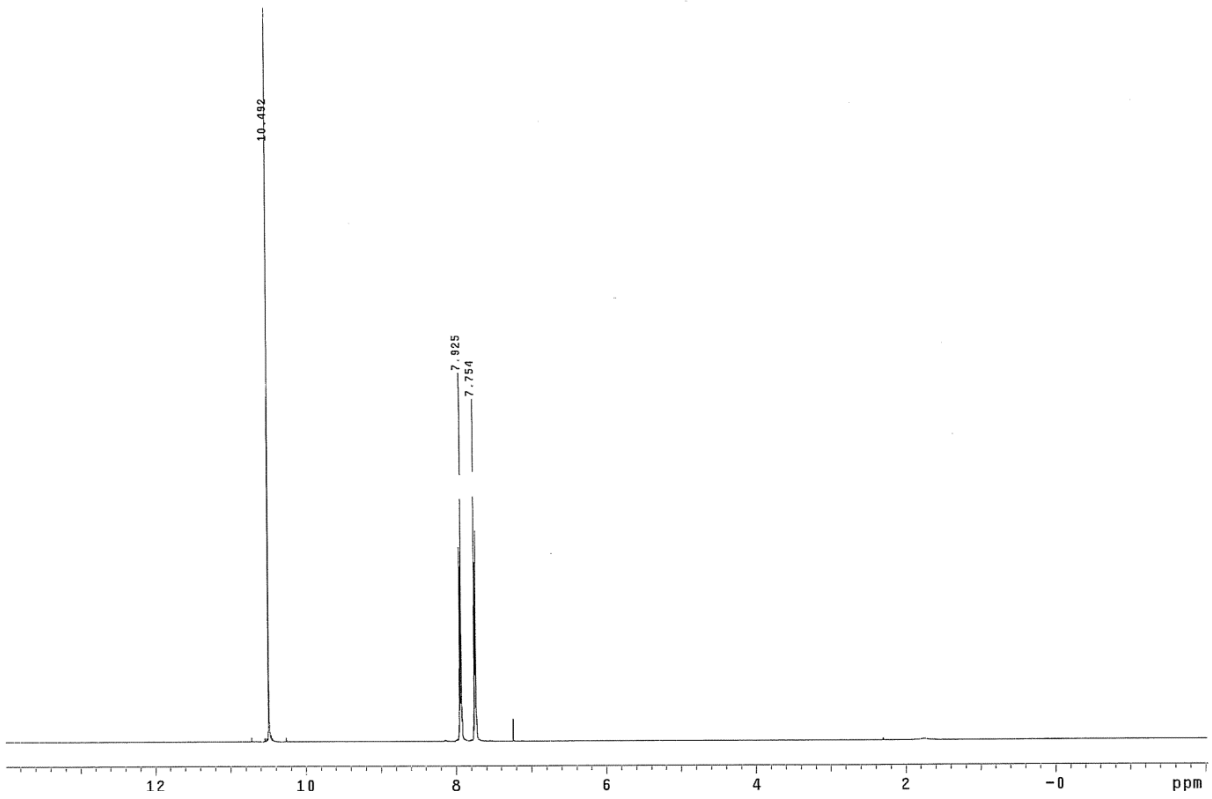

Figure F-2. Proton Nuclear Magnetic Resonance Spectrum of *o*-Phthalaldehyde

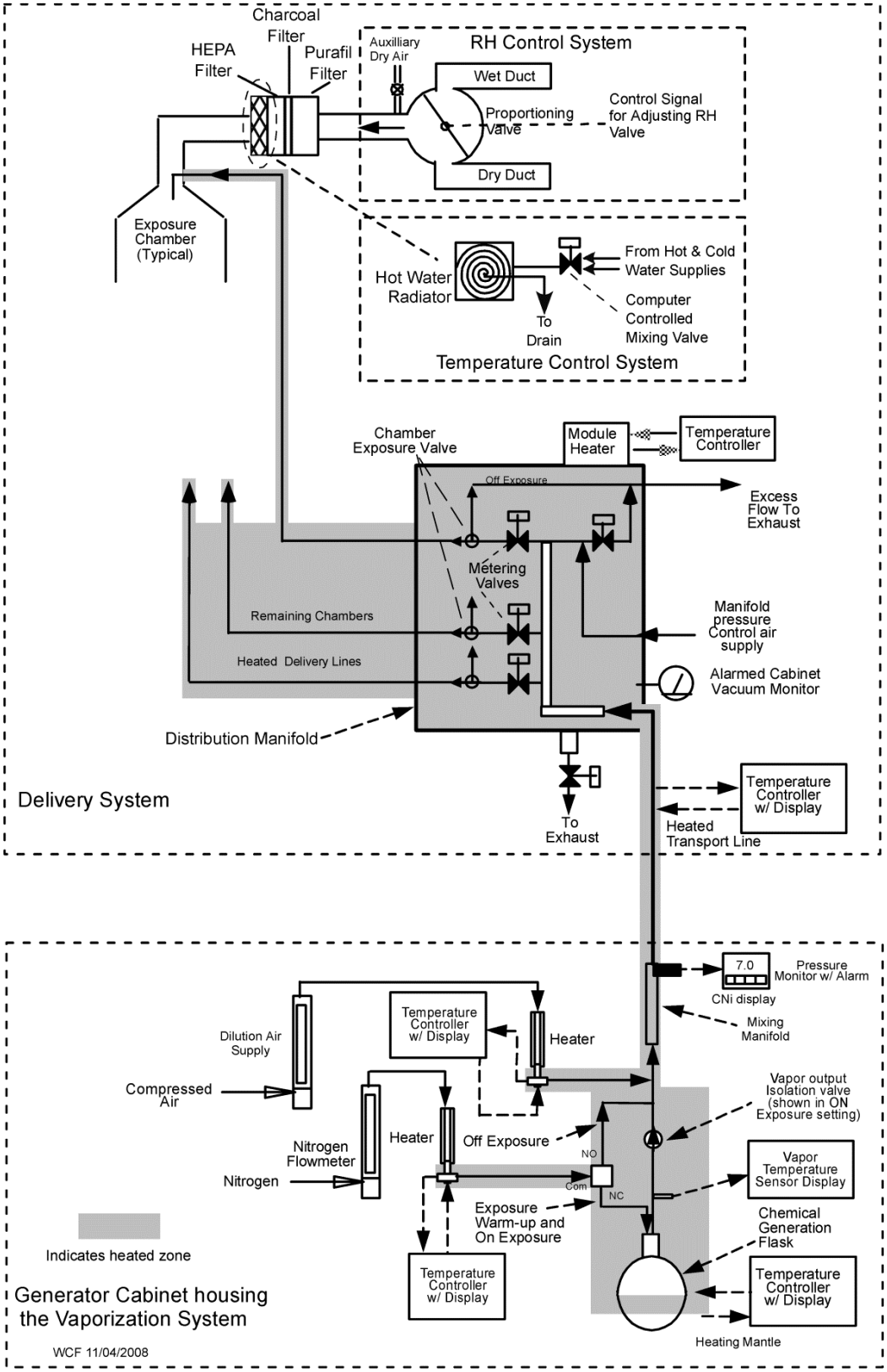

Figure F-3. Schematic of the Vapor Generation and Delivery System in the Inhalation Studies of *o*-Phthalaldehyde

Appendix G. Ingredients, Nutrient Composition, and Contaminant Levels In NTP 2000 Rat and Mouse Ration

Tables

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

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

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

Table G-2. Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

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

[a]Per kg of finished product.

Table G-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.3 ± 1.07	13.7–15.9	4
Crude fat (% by weight)	8.2 ± 0.33	7.8–8.5	4
Crude fiber (% by weight)	9.1 ± 0.32	8.8–9.4	4
Ash (% by weight)	5.1 ± 0.15	4.9–5.2	4
Amino Acids (% of total diet)			
Arginine	0.786 ± 0.070	0.67–0.97	23
Cystine	0.220 ± 0.024	0.15–0.25	23
Glycine	0.700 ± 0.040	0.62–0.80	23
Histidine	0.351 ± 0.076	0.27–0.68	23
Isoleucine	0.546 ± 0.044	0.43–0.66	23
Leucine	1.095 ± 0.066	0.96–1.24	23
Lysine	0.705 ± 0.116	0.31–0.86	23
Methionine	0.409 ± 0.045	0.26–0.49	23
Phenylalanine	0.628 ± 0.039	0.54–0.72	23
Threonine	0.506 ± 0.042	0.43–0.61	23
Tryptophan	0.150 ± 0.028	0.11–0.20	23
Tyrosine	0.405 ± 0.063	0.28–0.54	23
Valine	0.664 ± 0.042	0.55–0.73	23
Essential Fatty Acids (% of total diet)			
Linoleic	3.96 ± 0.254	3.49–4.55	23
Linolenic	0.30 ± 0.031	0.21–0.35	23
Vitamins			
Vitamin A (IU/kg)	4,120 ± 152	2,770–5,720	4
Vitamin D (IU/kg)	1,000[a]	–	–
α-Tocopherol (ppm)	80.3 ± 21.56	27.0–124.0	23
Thiamine (ppm)[b]	6.7 ± 0.56	6.2–7.5	4
Riboflavin (ppm)	7.7 ± 2.87	4.20–17.50	23
Niacin (ppm)	79.2 ± 8.97	66.4–98.2	23
Pantothenic acid (ppm)	27 ± 12.35	17.4–81.0	23
Pyridoxine (ppm)[b]	9.54 ± 1.94	6.44–13.7	23
Folic acid (ppm)	1.61 ± 0.47	1.15–3.27	23
Biotin (ppm)	0.32 ± 0.10	0.20–0.704	23
Vitamin B_{12} (ppb)	53.4 ± 38.7	18.3–174.0	23
Choline (ppm)[b]	2,773 ± 590	1,160–3,790	23

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.911 ± 0.043	0.870–0.969	4
Phosphorus (%)	0.546 ± 0.047	0.490–0.606	4
Potassium (%)	0.667 ± 0.030	0.626–0.733	23
Chloride (%)	0.385 ± 0.038	0.300–0.474	23
Sodium (%)	0.189 ± 0.016	0.160–0.222	23
Magnesium (%)	0.216 ± 0.062	0.185–0.490	23
Sulfur (%)	0.170 ± 0.029	0.116–0.209	14
Iron (ppm)	187 ± 38.6	135–311	23
Manganese (ppm)	51.0 ± 10.19	21.0–73.1	23
Zinc (ppm)	53.6 ± 8.34	43.3–78.5	23
Copper (ppm)	7.1 ± 2.540	3.21–16.3	23
Iodine (ppm)	0.503 ± 0.201	0.158–0.972	23
Chromium (ppm)	0.696 ± 0.269	0.330–1.380	23
Cobalt (ppm)	0.248 ± 0.163	0.094–0.864	23

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

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

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.20 ± 0.049	0.155–0.25	–
Cadmium (ppm)	0.06 ± 0.006	0.06–0.07	4
Lead (ppm)	0.09 ± 0.007	0.08–0.10	4
Mercury (ppm)	<0.02	–	4
Selenium (ppm)	0.19 ± 0.046	0.14–0.25	4
Aflatoxins (ppb)	<5.00	–	4
Nitrate nitrogen (ppm)[c]	17.02 ± 7.84	10.0–26.5	4
Nitrite nitrogen (ppm)[c]	<0.061	–	4
BHA (ppm)[d]	<1	–	4
BHT (ppm)[d]	<1	–	4
Aerobic plate count (CFU/g)	10 ± 0	10	4
Coliform (MPN/g)	3.0 ± 0	3.0	4
Escherichia coli (MPN/g)	<10	–	4
Salmonella (MPN/g)	Negative	–	4
Total nitrosoamines (ppb)[e]	9.3 ± 2.53	5.6–11.3	4
N-Nitrosodimethylamine (ppb)[e]	1.0 ± 0.10	0.9–1.1	4
N-Nitrosopyrrolidine (ppb)[e]	8.8 ± 2.13	5.6–10.2	4
Pesticides (ppm)			
α-BHC	<0.01	–	4
β-BHC	<0.02	–	4
γ-BHC	<0.01	–	4
δ-BHC	<0.01	–	4
Heptachlor	<0.01	–	4
Aldrin	<0.01	–	4
Heptachlor epoxide	<0.01	–	4
DDE	<0.01	–	4
DDD	<0.01	–	4
DDT	<0.01	–	4
HCB	<0.01	–	4
Mirex	<0.01	–	4
Methoxychlor	<0.05	–	4
Dieldrin	<0.01	–	4
Endrin	<0.01	–	4

	Mean ± Standard Deviation[b]	Range	Number of Samples
Telodrin	<0.01	–	4
Chlordane	<0.05	–	4
Toxaphene	<0.10	–	4
Estimated PCBs	<0.20	–	4
Ronnel	<0.01	–	4
Ethion	<0.02	–	4
Trithion	<0.05	–	4
Diazinon	<0.10	–	4
Methyl chlorpyrifos	0.157 ± 0.038	0.010 – 0.180	4
Methyl parathion	<0.02	–	4
Ethyl parathion	<0.02	–	4
Malathion	0.058 ± 0.020	0.032 – 0.079	4
Endosulfan I	<0.01	–	4
Endosulfan II	<0.01	–	4
Endosulfan sulfate	<0.03	–	4

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

Appendix H. Sentinel Animal Program

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Tables

H.1. Methods

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

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

Blood was collected from five male and five female rats and mice per time point during the 3-month studies.

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

Method/Test	Time of Collection
Rats	
ELISA	
Mycoplasma pulmonis	2 weeks
Pneumonia virus of mice (PVM)	2 weeks
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	2 weeks
Rat parvovirus	2 weeks
Sendai	2 weeks
Multiplex Fluorescent Immunoassay	
Kilham rat virus	Study termination
M. pulmonis	Study termination
Parvo NS-1	Study termination
PVM	Study termination
RCV/SDA	Study termination
Rat minute virus	Study termination
Rat parvovirus	Study termination
Rat Theiler's murine encephalomyelitis virus (TMEV)-like virus	Study termination
Sendai	Study termination
TMEV	Study termination

Method/Test	Time of Collection
Toolan's H-1 virus	Study termination
Mice	
ELISA	
Mouse hepatitis virus (MHV)	2 weeks
Mouse parvovirus	2 weeks
M. pulmonis	2 weeks
PVM	2 weeks
Sendai	2 weeks
TMEV	2 weeks
Multiplex Fluorescent Immunoassay	
Ectromelia virus	Study termination
Epizootic diarrhea of infant mice	Study termination
Lymphocytic choriomeningitis virus	Study termination
M. pulmonis	Study termination
MHV	Study termination
Mouse norovirus	Study termination
Parvo NS-1	Study termination
Mouse parvovirus	Study termination
Minute virus of mice	Study termination
PVM	Study termination
Reovirus	Study termination
TMEV	Study termination
Sendai	Study termination

H.2. Results

All test results were negative.

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ISSN 2378-8992