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

NTP Technical Report on the Toxicology Studies of Cobalt Metal (CASRN 7440-48-4) in F344/N Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Cobalt Metal in F344/NTac Rats and B6C3F1/N Mice (Inhalation Studies)

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NTP Technical Report on the Toxicology Studies of Cobalt Metal (CASRN 7440-48-4) in F344/N Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Cobalt Metal in F344/NTac Rats and B6C3F1/N Mice (Inhalation Studies)

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Errata

Errors were identified in the *NTP Technical Report on Cobalt Metal* (Technical Report 581). Descriptions of cobalt exposure concentrations in the report were unclear regarding the form of cobalt used for the calculations.

In the Introduction on pages 4, 6, 7, and 8, and in the Discussion on page 113, the text was revised to clarify the form of cobalt used to calculate the exposure concentrations. The text on page 6 was also revised to clarify the effects on specific study animals. Text on pages 6 and 7 was revised to accurately reflect the study durations of chronic studies. This information was added to the HTML and PDF versions of this report. [July 3, 2023]

The cobalt sulfate heptahydrate nomenclature corrections are part of a broader issue affecting multiple peer-reviewed publications and NTP reports, as described below.

In the late 1980s and early 1990s, the National Toxicology Program (NTP) commissioned Battelle Pacific Northwest Laboratories to design and carry out prechronic (16- and 90-day) and chronic (2-year) inhalation toxicology and carcinogenicity studies with cobalt sulfate heptahydrate.[1-4] The chemical, procured in one lot for all NTP studies, was crystalline cobalt sulfate heptahydrate, with a purity of approximately 99%.

The test material was aerosolized from an aqueous solution via compressed air nebulizer and dried prior to distribution to the exposure chambers. It was noted that generation of the aerosol from an aqueous solution, followed by drying, produced cobalt sulfate aerosol particles with fewer waters of hydration than the bulk chemical. The extent of hydration of the exposure atmosphere was evaluated in each study, with a more extensive analysis prior to the chronic study, when it was confirmed that the exposure chamber atmosphere consisted of the hexahydrate form.

Chamber aerosol concentrations were determined using real-time aerosol monitors and calibrated using filter grab samples collected from the chambers. The conversion of analyzed filter cobalt concentrations to chamber concentrations was based on the molecular weight ratio of cobalt sulfate:cobalt (155.00:58.993). Thus, in the prechronic and chronic studies, the units for the exposure concentrations were mg cobalt sulfate/m^3 air. The basis for the exposure concentrations (i.e., as mg cobalt sulfate/m^3) was presented in the laboratory report describing the inhalation exposure system and atmosphere as well as in the methods sections of the publications on the prechronic studies.[1; 3] However, when preparing Appendix F of NTP Technical Report 471 for the chronic studies, the detailed description of the expression of exposure concentrations was not included. Therefore, the identification of the test material as cobalt sulfate heptahydrate or the description of the primary species delivered to the chambers as cobalt sulfate hexahydrate may lead the reader to conclude that the exposure concentrations were based on the heptahydrate or hexahydrate form, respectively. This may further lead a reader to conclude that the chamber concentrations were based on a hydrated form of the chemical, which would underestimate the amount of cobalt in the atmosphere. The subsequent manuscript[4] explicitly stated that the exposure concentrations were based on the hexahydrate form, and an erratum was issued to correct this error.

A study of cobalt metal was also conducted as part of the NTP cobalt research program.[5] A comparison of the chronic toxicity and carcinogenicity findings in the two NTP cobalt studies (i.e., cobalt sulfate heptahydrate and cobalt metal) is presented in Behl et al. 2015.[6] The exposure

metric used for this comparison was mg cobalt/m^3. The exposure concentrations in the cobalt sulfate heptahydrate study were calculated by multiplying the reported concentrations of cobalt sulfate by the molecular weight ratio of cobalt:cobalt sulfate (58.993:155.00). This calculation produced cobalt concentrations of 0.114, 0.38, and 1.14 mg cobalt/mg^3 in the 0.3, 1.0, and 3.0 mg cobalt sulfate/m^3 groups, respectively, as reported in Behl et al. 2015.[6]

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Foreword

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

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

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

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

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

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Tables

Figures

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

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Explanation of Levels of Evidence of Carcinogenic Activity

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

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

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

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been

adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as "were also related" to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as "may have been" related to chemical exposure.

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

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

Peer Review

The members of the Peer Review Panel who evaluated the draft *NTP Technical Report on the Toxicology Studies of Cobalt Metal (CASRN 7440-48-4) in F344/N Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Cobalt Metal in F344/NTAC Rats and B6C3F1/N Mice (Inhalation Studies)* on October 29, 2013, are listed below. Panel members served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers had five major responsibilities in reviewing the NTP studies:

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

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Abstract

Widespread exposure to cobalt metal dust occurs occupationally through the production of alloys, in the manufacture of cobalt salts, and in nuclear technology. It is an effective catalyst for many organic reactions, particularly in hydrotreating catalysts, which have molybdenum and cobalt sulfides as active components. Concerns have been raised about the occurrence of occupational disease, i.e., hard metal disease, associated with exposure to cobalt and its compounds, including cobalt metal-tungsten carbide. Cobalt metal is also widely dispersed in low concentrations in the environment and the general population may be exposed by breathing air, drinking water, or skin contact with soil, water, cobalt alloys, or other substances that contain cobalt. In addition, cobalt metal is an essential trace element as a component of cyanocobalamin (vitamin B_{12}). Cobalt metal dust was nominated for toxicology and carcinogenesis studies by the United Auto Workers and the Cobalt Development Institute based on the widespread occupational exposure and limited availability of data on chronic toxicity and carcinogenic potential of inhaled insoluble cobalt compounds, particularly cobalt metal dust. Inhalation was selected as the route of exposure because this is the most common route of exposure to cobalt metal dust in occupational settings in humans. Male and female F344/N or F344/NTac rats and B6C3F1/N mice were exposed to cobalt metal by inhalation for 2 weeks, 3 months, or 2 years (F344/NTac rats). In addition, genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and mouse peripheral blood erythrocytes.

Two-week Study in Rats

Groups of five male and five female rats were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 2.5, 5, 10, 20, or 40 mg/m^3, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 16 days. Additional groups of five female rats were exposed to the same concentrations for 16 days for tissue burden studies. All rats exposed to 40 mg/m^3 and all male and three female rats exposed to 20 mg/m^3 died before the end of the study. Mean body weights of males exposed to 10 mg/m^3 and of females exposed to 10 or 20 mg/m^3 were significantly decreased. Females exposed to 20 mg/m^3 lost weight during the study. Exposure-related clinical findings included abnormal breathing, lethargy, and thinness in male rats exposed to 20 or 40 mg/m^3 and in females exposed to 40 mg/m^3. Dark lungs were observed at necropsy in all rats exposed to 40 mg/m^3 and most rats exposed to 20 mg/m^3 that died early. Absolute lung weights of females exposed to 10 or 20 mg/m^3 and the relative lung weights of both sexes exposed to 10 mg/m^3 and females exposed to 20 mg/m^3 were significantly greater than those of the chamber controls. Absolute and relative liver weights of males exposed to 2.5 mg/m^3 or greater and absolute liver weights of females exposed to 5 mg/m^3 or greater were significantly less than those of the chamber controls. The relative liver weight of 20 mg/m^3 females was significantly greater than that of the chamber controls. Absolute kidney weights of males exposed to 10 mg/m^3 and females exposed to 20 mg/m^3 were significantly less than those of the chamber controls. The absolute testis weight of the 10 mg/m^3 group was significantly less than that of the chamber controls. Increased incidences of nonneoplastic lesions of the lung occurred in exposed male and female rats and included hemorrhage, acute inflammation, alveolar epithelium hyperplasia, histiocytic cellular infiltration of the alveolus, cytoplasmic vacuolization of bronchiolar epithelium, necrosis of the bronchiolar epithelium, and interstitial fibrosis of the alveolar epithelium. Increased incidences of nonneoplastic lesions of the nose occurred in exposed male and female rats and included olfactory epithelium necrosis, olfactory epithelium atrophy, respiratory epithelium necrosis, and respiratory epithelium squamous metaplasia. Tissue

concentrations of cobalt increased with increasing exposure concentration in all tissues examined.

Two-week Study in Mice

Groups of five male and five female mice were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 2.5, 5, 10, 20, or 40 mg/m^3, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 17 days. Additional groups of five female mice were exposed to the same concentrations for 17 days for tissue burden studies. Three male and three female mice exposed to 40 mg/m^3 died before the end of the study. Final mean body weights were significantly decreased in male and female mice exposed to 20 or 40 mg/m^3, and mean body weight gains of 20 and 40 mg/m^3 males and all exposed groups of females were significantly less than those of the chamber controls. Females exposed to 20 mg/m^3 and males and females exposed to 40 mg/m^3 lost weight during the study. Exposure-related clinical findings included abnormal breathing, lethargy, and thinness in male mice exposed to 20 or 40 mg/m^3 and females exposed to 10 mg/m^3 or greater. At necropsy, tan lungs were observed in most males and females exposed to 20 or 40 mg/m^3. Lung weights of both sexes exposed to 10 mg/m^3 or greater were significantly greater than those of the chamber controls. Liver weights of exposed male and female mice were significantly less than those of the chamber controls (except relative weight at 40 mg/m^3). Increased incidences of nonneoplastic lesions of the lung occurred in exposed male and female mice and included alveolar histiocytic cellular infiltration, cytoplasmic vacuolization of the bronchiolar epithelium, alveolar/bronchiolar epithelium karyomegaly, interstitial fibrosis, and acute inflammation. Increased incidences of nonneoplastic lesions of the nose occurred in exposed groups of male and female mice and included acute inflammation, olfactory epithelium atrophy, olfactory epithelium necrosis, cytoplasmic vacuolization of the respiratory epithelium, and squamous metaplasia of the respiratory epithelium. Tissue concentrations of cobalt increased with increasing exposure concentration in all tissues examined.

Three-month Study in Rats

Groups of 10 male and 10 female rats were exposed to particulate aerosols of cobalt metal by inhalation at concentrations of 0, 0.625, 1.25, 2.5, or 5 mg/m^3, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. Additional groups of 10 male rats (clinical pathology study) and 32 to 36 female rats (special study) were exposed to the same concentrations for 14 weeks. All male and female rats survived to the end of the study. Final mean body weights of males and females exposed to 5 mg/m^3 were significantly less than those of the chamber controls, and the mean body weight gain of 5 mg/m^3 males was significantly less than that of the chamber controls. At necropsy, pale foci were noted in the lungs of most exposed male and female rats. In male rats, exposure concentration-related increases in the hemoglobin concentration, erythrocyte count, hematocrit value, and manual packed cell volume occurred in the 2.5 and 5 mg/m^3 groups on days 3 and 23 and in all exposed groups by week 14; at week 14, female rats also had increases in these parameters. Exposure concentration-related decreases in cholesterol concentrations were observed at all three time points in male and female rats. While this change was not always observed in the lower exposure groups, decreases were consistently observed in the 2.5 and 5 mg/m^3 groups of both sexes on day 23 and at week 14. In addition, glucose concentration was decreased in males exposed to 1.25 mg/m^3 or greater at week 14. Lung weights of all exposed groups of males and females were significantly greater than those of the chamber controls. Sperm motility was significantly decreased in male rats exposed to cobalt, suggesting a potential for cobalt metal to be a reproductive toxicant in male rats.

In the lung, chronic active inflammation and alveolar proteinosis occurred in all exposed males and females, and bronchiole epithelium hyperplasia occurred in all males and females exposed to 1.25 mg/m^3 or greater. In the nose, incidences of olfactory epithelium degeneration and respiratory epithelium hyperplasia were significantly increased in males and females exposed to 2.5 or 5 mg/m^3. The incidences of olfactory epithelium hyperplasia were significantly increased in 2.5 and 5 mg/m^3 males and in 5 mg/m^3 females. Significantly increased incidences of turbinate atrophy occurred in 2.5 mg/m^3 females and 5 mg/m^3 males and females. Tissue concentrations of cobalt increased with increasing exposure concentration in all tissues examined.

Three-month Study in Mice

Groups of 10 male and 10 female mice were exposed to particulate aerosols of cobalt metal by inhalation at concentrations of 0, 0.625, 1.25, 2.5, 5, or 10 mg/m^3, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. Additional groups of 32 to 36 female mice (special study) were exposed to the same concentrations for 14 weeks. One 2.5 mg/m^3 female mouse was accidentally killed during the first week of the study; all other mice survived to the end of the study. The mean body weights of males and females exposed to 10 mg/m^3 were significantly less than those of the chamber controls. Abnormal breathing was noted in approximately 50% of males and females exposed to 10 mg/m^3. At necropsy, tan lungs were noted in mice exposed to 5 or 10 mg/m^3. Lung weights of males exposed to 2.5 mg/m^3 or greater and females exposed to 5 or 10 mg/m^3 were significantly greater than those of the chamber controls. Liver weights of males exposed to 10 mg/m^3 and females exposed to 2.5 mg/m^3 or greater were significantly less than those of the chamber controls. Kidney weights of males and females exposed to 5 or 10 mg/m^3 were significantly less than those of the chamber controls. Testes weights of males exposed to 5 or 10 mg/m^3 were significantly less than those of the chamber controls. Exposure concentration-related decreases in reproductive tissue weights, spermatid and epididymal spermatozoa counts, and sperm motility in combination with histopathologic findings in both the testis and epididymis indicate that cobalt metal is likely to be a reproductive toxicant in male mice.

In the lung, alveolar histiocytic cellular infiltration and bronchiole epithelium cytoplasmic vacuolization occurred in the lung of all exposed male and female mice. Bronchiole epithelium hyperplasia occurred in all mice exposed to 2.5 mg/m^3 or greater. Alveolar proteinosis and alveolar/bronchiolar epithelium karyomegaly occurred in all males and females exposed to 5 or 10 mg/m^3. The incidences of hemorrhage were significantly increased in 5 mg/m^3 females and in 5 and 10 mg/m^3 males. In the nose, the incidences of olfactory epithelium degeneration were significantly increased in males and females exposed to 1.25 mg/m^3 or greater. Incidences of respiratory epithelium degeneration were significantly increased in males exposed to 1.25 mg/m^3 or greater and females exposed to 2.5 mg/m^3 or greater. Incidences of respiratory epithelium squamous metaplasia were significantly increased in males and females exposed to 2.5 mg/m^3 or greater, and incidences of turbinate atrophy and chronic active inflammation were significantly increased in the 5 and 10 mg/m^3 groups of males and females. The incidences of squamous metaplasia were significantly increased in the larynx of all exposed groups of males and females. Tissue concentrations of cobalt increased with increasing exposure concentration in all tissues examined.

Two-year Study in Rats

Groups of 50 male and 50 female rats were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 1.25, 2.5, or 5 mg/m^3, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for up to 105 weeks. Additional groups of 35 lung burden study female rats were exposed to the same concentrations of cobalt metal for up to 105 weeks. Survival of female rats exposed to 2.5 mg/m^3 was significantly less than that of the chamber control group. Mean body weights of 2.5 and 5 mg/m^3 males were at least 10% less than those of the chamber control group after weeks 99 and 12, respectively, and those of 2.5 and 5 mg/m^3 females were at least 10% less after weeks 57 and 21, respectively. Exposure-related clinical findings included abnormal breathing and thinness in male and female rats.

In the lung, the incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and alveolar/bronchiolar adenoma or carcinoma (combined) occurred with positive trends in male and female rats and with the exception of the incidence of alveolar/bronchiolar adenoma in 1.25 mg/m^3 females, the incidences were significantly greater than those in the chamber controls. The incidences of multiple alveolar/bronchiolar adenoma and carcinoma generally increased with increasing exposure concentration, and the incidences of multiple carcinoma were significantly increased in all exposed groups of males and in 5 mg/m^3 females. The incidences of cystic keratinizing epithelioma were increased in exposed groups of female rats; cystic keratinizing epithelioma also occurred in two exposed males. One female rat exposed to 5 mg/m^3 had a squamous cell carcinoma. The incidences of alveolar epithelium hyperplasia, alveolar proteinosis, chronic active inflammation, and bronchiole epithelium hyperplasia in all exposed groups were significantly greater than those in the chamber control groups.

There was a higher frequency and different spectrum of point mutations within hot spot regions of *Kras, Egfr*, and *Tp53* genes within alveolar/bronchiolar carcinomas from cobalt metal-exposed male and female rats compared to spontaneous alveolar/bronchiolar carcinomas. *Kras* mutations and G→T transversions were most frequent in rats chronically exposed to cobalt metal.

A spectrum of nonneoplastic lesions occurred in the nose of exposed male and female rats including chronic active and suppurative inflammation, respiratory metaplasia, atrophy, hyperplasia, basal cell hyperplasia, and necrosis of the olfactory epithelium; hyperplasia, squamous metaplasia, and necrosis of the respiratory epithelium; and atrophy of the turbinate.

In the adrenal medulla, incidences of benign pheochromocytoma, malignant pheochromocytoma, and benign or malignant pheochromocytoma (combined) occurred with positive trends in male and female rats, and with the exception of the incidence of malignant pheochromocytoma in 2.5 mg/m^3 females, the incidences in rats exposed to 2.5 or 5 mg/m^3 were significantly greater than those in the chamber controls. The incidences of bilateral benign and malignant pheochromocytoma were significantly increased in the 5 mg/m^3 groups. Incidences of hyperplasia were significantly increased in female rats exposed to 1.25 or 2.5 mg/m^3.

The incidences of carcinoma and adenoma or carcinoma (combined) of the pancreatic islets occurred with positive trends in male rats. The incidences of adenoma in 2.5 mg/m^3 males and of adenoma or carcinoma (combined) in males exposed to 2.5 or 5 mg/m^3 were significantly greater than those in the chamber controls. Incidences of neoplasms of the pancreatic islets in 5 mg/m^3 females were slightly increased.

The incidences of mononuclear cell leukemia were significantly increased in all exposed groups of female rats.

In the combined standard and extended (step-section) evaluations of the kidney, the incidence of renal tubule adenoma or carcinoma (combined) was increased in male rats exposed to 5 mg/m^3.

The incidence of infarct in the testes was significantly increased in male rats exposed to 5 mg/m^3.

Cobalt concentrations in the lung increased with increasing exposure concentration.

Two-year Study in Mice

Groups of 50 male and 50 female mice were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 1.25, 2.5, or 5 mg/m^3, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for up to 105 weeks. Additional groups of 35 lung burden study female mice were exposed to the same concentrations of cobalt metal for up to 105 weeks. Survival of males exposed to 2.5 or 5 mg/m^3 was significantly less than that of the chamber control group. Mean body weights of 5 mg/m^3 males and females were at least 10% less than those of the chamber control groups after weeks 85 and 21, respectively. Abnormal breathing and thinness were noted in exposed male and female mice.

In the lung, incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) occurred with positive trends in male and female mice, and the incidences were all significantly greater than those in the chamber controls. The incidences of alveolar/bronchiolar adenoma were significantly increased in 2.5 mg/m^3 males and in 5 mg/m^3 females. The incidences of multiple alveolar/bronchiolar carcinoma were significantly increased in all exposed groups of males and females.

The incidences of alveolar/bronchiolar epithelium hyperplasia and cytoplasmic vacuolization, alveolar epithelium hyperplasia, proteinosis, and alveolus infiltration cellular histiocyte were significantly increased in all exposed groups of males and females. The incidences of bronchiole epithelium hyperplasia were significantly increased in males exposed to 5 mg/m^3 and females exposed to 2.5 or 5 mg/m^3. The incidence of bronchiole epithelium erosion was significantly increased in males exposed to 2.5 mg/m^3. The incidences of suppurative inflammation were significantly increased in males exposed to 2.5 or 5 mg/m^3 and females exposed to 5 mg/m^3.

There was a higher frequency and different spectrum of point mutations within hot spot regions of *Kras, Egfr*, and *Tp53* genes within alveolar/bronchiolar carcinomas from cobalt metal-exposed male and female mice compared to spontaneous alveolar/bronchiolar carcinomas. *Kras* mutations and G→T transversions were most frequent in mice chronically exposed to cobalt metal.

In all groups of exposed male and female mice, significant increases occurred in nasal lesions including suppurative inflammation; olfactory epithelium atrophy, hyperplasia, and respiratory metaplasia; cytoplasmic vacuolization and squamous metaplasia of the respiratory epithelium; and atrophy of the turbinate. The incidences of atypical respiratory metaplasia of the olfactory epithelium and hyaline droplet accumulation of the respiratory epithelium were significantly increased in 1.25 and 2.5 mg/m^3 males and females.

The incidences of respiratory epithelium squamous metaplasia and cytoplasmic vacuolization of the larynx in all exposed groups of males and females were significantly greater than those in the

chamber control groups. The incidences of squamous epithelium hyperplasia were significantly increased in all exposed groups of females and in males exposed to 5 mg/m^3. In the trachea, the incidences of epithelium cytoplasmic vacuolization were significantly increased in all exposed groups of males and females.

The incidence of germinal epithelium degeneration in the testes was significantly increased in male mice exposed to 5 mg/m^3.

Cobalt concentrations in the lung increased with increasing exposure concentration.

Genetic Toxicology

Cobalt metal was mutagenic in *S. typhimurium* strain TA98 in the absence of exogenous metabolic activation (S9); no activity was seen in the presence of S9. Cobalt metal induced a small increase in mutant colonies in strain TA100 in the absence of S9, and no mutagenic activity was seen with S9. No mutagenic activity was detected in *E. coli* strain WP2 *uvrA*/pKM101 with or without S9. Results of peripheral blood erythrocyte micronucleus tests in male and female mice in the 3-month study were negative.

Conclusions

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity* of cobalt metal in male F344/NTac rats based on increased incidences of alveolar/bronchiolar adenoma and carcinoma in the lung, including multiples, and on increased incidences of benign and malignant pheochromocytoma of the adrenal medulla, including bilateral neoplasms (see Explanation of Levels of Evidence of Carcinogenic Activity; a summary of the Peer Review Panel comments and the public discussion on this Technical Report appears in Appendix O). The increased incidences of pancreatic islet adenoma or carcinoma (combined) were considered related to exposure. The occurrences of cystic keratinizing epithelioma of the lung and of renal tubule adenoma or carcinoma (combined) may have been related to exposure. There was *clear evidence of carcinogenic activity* of cobalt metal in female F344/NTac rats based on increased incidences of alveolar/bronchiolar adenoma and carcinoma in the lung, including multiples, and on increased incidences of benign and malignant pheochromocytoma of the adrenal medulla, including bilateral neoplasms. The occurrences of squamous cell neoplasms of the lung (predominantly cystic keratinizing epithelioma), and of mononuclear cell leukemia were considered related to exposure. The occurrences of pancreatic islet carcinoma may have been related to exposure. There was *clear evidence of carcinogenic activity* of cobalt metal in male and female B6C3F1/N mice based on increased incidences of alveolar/bronchiolar neoplasms of the lung (predominantly carcinoma), including multiple carcinoma.

Exposure to cobalt metal resulted in increased incidences of nonneoplastic lesions of the lung and nose in male and female rats, the testes in the male rats and mice, the adrenal medulla in female rats, and the lung, nose, larynx, and trachea in male and female mice.

Synonyms: Cobalt element; super cobalt

Trade name: Aquacat

Summary of the Two-year Carcinogenesis and Genetic Toxicology Studies of Cobalt Metal

	Male F344/NTac Rats	Female F344/NTac Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in Air	0, 1.25, 2.5, or 5 mg/m^3	0, 1.25, 2.5, or 5 mg/m^3	0, 1.25, 2.5, or 5 mg/m^3	0, 1.25, 2.5, or 5 mg/m^3
Body Weights	2.5 and 5 mg/m^3 groups at least 10% less than the chamber control group after weeks 99 and 12, respectively	2.5 and 5 mg/m^3 groups at least 10% less than the chamber control group after weeks 57 and 21, respectively	5 mg/m^3 group at least 10% less than chamber control group after week 85	5 mg/m^3 group at least 10% less than the chamber control group after week 21
Survival Rates	17/50, 20/50, 16/50, 16/50	35/50, 26/50, 24/50, 25/50	39/50, 31/50, 29/50, 25/50	36/50, 36/50, 27/50, 26/50
Nonneoplastic Effects	Lung: alveolar epithelium, hyperplasia (3/50, 47/50, 49/50, 49/50); alveolus, proteinosis (0/50, 48/50, 49/50, 49/50); inflammation, chronic active (22/50, 50/50, 50/50, 50/50); bronchiole, epithelium, hyperplasia (0/50, 44/50, 47/50, 50/50) Nose: inflammation, chronic active (28/48, 35/47, 40/45, 49/50); inflammation, suppurative (9/48, 12/47, 24/45, 46/50); olfactory epithelium, metaplasia, respiratory (12/48, 26/47, 37/45, 50/50); olfactory epithelium, atrophy (2/48, 21/47, 34/45, 29/50); olfactory epithelium, hyperplasia (0/48, 1/47, 2/45, 7/50); olfactory epithelium, hyperplasia, basal cell (0/48, 1/47, 0/45, 13/50); olfactory epithelium, necrosis (0/48, 1/47, 5/45, 5/50); respiratory epithelium,	Lung: alveolar epithelium, hyperplasia (9/50, 49/50, 50/50, 49/50); alveolus, proteinosis (0/50, 50/50, 50/50, 50/50); inflammation, chronic active (20/50, 50/50, 50/50, 50/50); bronchiole, epithelium, hyperplasia (0/50, 47/50, 46/50, 48/50) Nose: inflammation, chronic active (22/50, 42/50, 39/49, 50/50); inflammation, suppurative (6/50, 4/50, 4/49, 42/50); olfactory epithelium, metaplasia, respiratory (6/50, 18/50, 24/49, 47/50); olfactory epithelium, atrophy (0/50, 22/50, 35/49, 35/50): olfactory epithelium, hyperplasia (0/50, 0/50, 3/49, 5/50); olfactory epithelium, hyperplasia, basal cell (0/50, 0/50,	Lung: alveolar/bronchiolar epithelium, hyperplasia (0/50, 46/49, 49/50, 50/50); alveolar/bronchiolar epithelium, vacuolization cytoplasmic (0/50, 49/49, 47/50, 48/50); alveolar epithelium, hyperplasia (4/50, 29/49, 24/50, 43/50); bronchiole, epithelium, hyperplasia (4/50, 7/49, 9/50, 11/50); bronchiole, epithelium, erosion (0/50, 4/49, 10/50, 2/50); proteinosis (2/50, 46/49, 49/50, 50/50); alveolus, infiltration cellular, histiocyte (10/50, 49/49, 48/50, 48/50); inflammation, suppurative (1/50, 2/49, 6/50, 16/50) Nose: inflammation, suppurative (16/50, 32/49, 49/50, 50/50); olfactory epithelium, atrophy (3/50, 46/49, 42/50, 31/50); olfactory epithelium, hyperplasia (0/50, 25/49, 17/50, 8/50); olfactory epithelium,	Lung: alveolar/bronchiolar epithelium, hyperplasia (0/49, 49/50, 49/50, 50/50); alveolar/bronchiolar epithelium, vacuolization cytoplasmic (0/49, 48/50, 49/50, 48/50); alveolar epithelium, hyperplasia (2/49, 27/50, 26/50, 41/50); bronchiole, epithelium, hyperplasia (0/49, 3/50, 12/50, 26/50); proteinosis (0/49, 45/50, 50/50, 50/50); alveolus, infiltration cellular, histiocyte (10/49, 49/50, 50/50, 49/50); inflammation, suppurative (0/49, 3/50, 2/50, 15/50) Nose: inflammation, suppurative (3/50, 47/50, 50/50, 50/50); olfactory epithelium, atrophy (4/50, 44/50, 39/50, 24/50); olfactory epithelium, hyperplasia (1/50, 22/50, 16/50, 8/50); olfactory epithelium, metaplasia, respiratory (1/50, 26/50, 44/50, 50/50); olfactory epithelium, respiratory metaplasia, atypical (0/50, 18/50, 14/50,

	Male F344/NTac Rats	Female F344/NTac Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
	hyperplasia (20/48, 35/47, 45/45, 50/50); respiratory epithelium, metaplasia, squamous (0/48, 1/47, 11/45, 35/50); respiratory epithelium, necrosis (1/48, 4/47, 5/45, 13/50); turbinate, atrophy (1/48, 35/47, 35/45, 41/50) Testes: infarct (1/50, 0/50, 2/50, 12/50)	1/49, 19/50); respiratory epithelium, hyperplasia (15/50, 43/50, 48/49, 49/50); respiratory epithelium, metaplasia, squamous (2/50, 0/50, 3/49, 45/50); respiratory epithelium, necrosis (1/50, 1/50, 1/49, 15/50); turbinate, atrophy (1/50, 38/50, 27/49, 45/50) Adrenal medulla: hyperplasia (12/50, 27/50, 27/50, 10/50)	metaplasia, respiratory (5/50, 24/49, 44/50, 50/50); olfactory epithelium, respiratory metaplasia, atypical (0/50, 14/49, 9/50, 1/50); respiratory epithelium, accumulation, hyaline droplet (13/50, 29/49, 29/50, 7/50); respiratory epithelium, vacuolization cytoplasmic (0/50, 41/49, 39/50, 37/50); respiratory epithelium, metaplasia, squamous (3/50, 45/49, 35/50, 33/50); turbinate, atrophy (3/50, 25/49, 49/50, 50/50) Larynx: respiratory epithelium, metaplasia, squamous (7/48, 47/47, 49/49, 49/50); respiratory epithelium, vacuolization cytoplasmic (0/48, 20/47, 24/49, 32/50); squamous epithelium, hyperplasia (2/48, 5/47, 5/49, 8/50) Trachea: epithelium, vacuolization cytoplasmic (0/48, 14/47, 31/48, 37/50) Testes: germinal epithelium, degeneration (9/50, 14/49, 8/50, 21/50)	1/50); respiratory epithelium, accumulation, hyaline droplet (12/50, 38/50, 40/50, 10/50); respiratory epithelium, vacuolization cytoplasmic (0/50, 40/50, 47/50, 47/50); respiratory epithelium, metaplasia, squamous (0/50, 49/50, 49/50, 50/50); turbinate, atrophy (0/50, 44/50, 50/50, 50/50) Larynx: respiratory epithelium, metaplasia, squamous (2/47, 49/50, 50/50, 47/47); respiratory epithelium, vacuolization cytoplasmic (0/47, 24/50, 31/50, 34/47); squamous epithelium, hyperplasia (2/47, 13/50, 21/50, 21/47) Trachea: epithelium, vacuolization cytoplasmic (0/48, 26/50, 37/48, 39/49)
Neoplastic Effects	Lung: alveolar/bronchiolar adenoma (2/50, 10/50, 10/50, 14/50); alveolar/bronchiolar carcinoma (0/50, 16/50, 34/50, 36/50); alveolar/bronchiolar adenoma or	Lung: alveolar/bronchiolar adenoma (2/50, 7/50, 9/50, 13/50); alveolar/bronchiolar carcinoma (0/50, 9/50, 17/50, 30/50); alveolar/bronchiolar adenoma or	Lung: alveolar/bronchiolar adenoma (7/50, 11/49, 15/50, 3/50); alveolar/bronchiolar carcinoma (11/50, 38/49, 42/50, 46/50); alveolar/bronchiolar adenoma or carcinoma	Lung: alveolar/bronchiolar adenoma (3/49, 9/50, 8/50, 10/50); alveolar/bronchiolar carcinoma (5/49, 25/50, 38/50, 43/50); alveolar/bronchiolar adenoma or carcinoma

	Male F344/NTac Rats	Female F344/NTac Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
	carcinoma (2/50, 25/50, 39/50, 44/50) Adrenal medulla: benign pheochromocytoma (15/50, 23/50, 37/50, 34/50); malignant pheochromocytoma (2/50, 2/50, 9/50, 16/50); benign or malignant pheochromocytoma (17/50, 23/50, 38/50, 41/50) Pancreatic islets: adenoma or carcinoma (2/50, 2/50, 10/48, 9/49)	carcinoma (2/50, 15/50, 20/50, 38/50); cystic keratinizing epithelioma (0/50, 4/50, 1/50, 2/50) Adrenal medulla: benign pheochromocytoma (6/50, 12/50, 22/50, 36/50); malignant pheochromocytoma (0/50, 2/50, 3/50, 11/50); benign or malignant pheochromocytoma (6/50, 13/50, 23/50, 40/50) Mononuclear cell leukemia: (16/50, 29/50, 28/50, 27/50)	(16/50, 41/49, 43/50, 47/50)	(8/49, 30/50, 41/50, 45/50)
Equivocal Findings	Lung: cystic keratinizing epithelioma (0/50, 1/50, 0/50, 1/50) Kidney: adenoma or carcinoma (standard evaluation - 0/50, 1/50, 0/50, 4/50; standard and extended evaluations combined - 3/50, 1/50, 1/50, 7/50)	Pancreatic islets: carcinoma (1/50, 0/50, 0/50, 3/50)	None	None
Level of Evidence of Carcinogenic Activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence
Genetic Toxicology				
Bacterial gene mutations:		Positive in *S. typhimurium* strain TA98 without S9 and negative in TA98 with S9; equivocal in strain TA100 without S9, and negative with S9; negative in *E. coli* strain WP2 *uvrA*/pKM101 with and without S9		
Micronucleated erythrocytes				
Mouse peripheral blood in vivo:		Negative		

Introduction

Chemical and Physical Properties

Cobalt is a brittle, hard, silver-gray transition metal with ferromagnetic properties. Heating cobalt metal causes oxidation to the mixed oxide, Co (II, III) oxide (Co_3O_4); above 900°C, Co (II) oxide (CoO) is the end product. Cobalt metal combines with sulfur, phosphorus, and carbon when heated[1]. It readily concentrates under oxidizing conditions with manganese oxides[2]. Cobalt salts have a distinctive brilliant blue color. Cobalt metal exists in two allotropic forms, hexagonal and cubic, both of which are stable in air and water at room temperature[3].

Production and Use

Cobalt metal is widely used in the production of cemented tungsten-cobalt (hard metal) and as an alloying element in superalloys, magnetic and hard-facing alloys, cobalt-containing high-strength steels, electrodeposited alloys, and other alloys with special properties[1; 2]. Extra fine cobalt metal powder is an important raw material used in production of cemented carbides, diamond tools, and metal welding and spraying components[4]. The major uses of cemented carbide-coated tools are metal-cutting operations and mining and quarrying[5]. It is also used in the manufacture of cobalt salts, and in nuclear medicine, where the isotope ^{60}Co is used as a gamma-ray source[3]. Cobalt is an effective catalyst for many organic reactions, particularly in hydrotreating catalysts, which have molybdenum and cobalt sulfides as active components. It is also used as a target material in electrical x-ray generators[1; 6-10] and in several other military and industrial applications[9].

Although the United States is the world's largest consumer of cobalt[11], it does not have well-established primary cobalt mining facilities. In 2006, the aggregated national production volume was between 100 and 500 million pounds[12], and in 2010 the aggregated national production volume reported to EPA was 23,384,002 pounds[13]. Comparatively, United States cobalt production between 1964 and 1971 ranged from 690,000 to 1,215,000 pounds[14]. Import volumes (metric tons) of cobalt into the United States have remained fairly steady during the past years: 10,700 in 2008; 7,680 in 2009; 11,100 in 2010; 10,600 in 2011; and an estimated 11,000 in 2012[15].

Environmental and Human Exposure

Occupational exposure to cobalt occurring during the production of cobalt powder is a major concern due to the occurrence of hard metal disease and is primarily via inhalation of dusts, fumes, or mists containing cobalt, targeting the skin and the respiratory tract, during the production, processing, and use of hard metal[9]. The USEPA's 2006 IUR records estimated the number of workers likely exposed to cobalt via industrial manufacturing, processing, and use to be ≥1,000 in 1 to 99 worksites[12].

In the environment, cobalt is the 33rd most abundant element, composing approximately 0.0025% of the weight of the earth's crust and is present naturally in the soil in varying concentrations ranging from approximately 1 to 40 ppm with an average level of 7 ppm. It occurs naturally in the groundwater and sediments and is mainly derived from erosion of volcanic rocks in the mountains. Exposure of the general population is mainly through breathing

air, drinking water, or skin contact with soil, water, cobalt alloys, or other substances that contain cobalt[9].

Cobalt is also an essential trace element because it is an integral component of cyanocobalamin (vitamin B_{12}), the only metal-containing vitamin. Vitamin B_{12} is found in meat and dairy products. Vitamin B_{12} acts as a coenzyme in many enzymatic reactions, most notably in a methyl transfer reaction that converts homocysteine to methionine. It also acts as a coenzyme in a reaction that converts l-methylmalonylcoenzyme A (CoA) to succinyl-CoA[16]. Vitamin B_{12} is also a part of some enzymes involved in hematopoiesis, and a deficiency can lead to pernicious anemia[17]. Conditions such as iron deficiency anemia can lead to increased absorption of cobalt from the gastrointestinal tract, and simultaneous administration of cobalt and iron can reduce the amount of cobalt absorbed[18; 19].

Regulatory Status

Cobalt is included in the Unregulated Contaminant Monitory Rule contaminant list; its minimum reporting level is 1 µg/L for the reporting period of January 1, 2013, to December 31, 2015[20]. Total cobalt is also listed as a hazardous constituent for municipal solid waste landfills[21], and is on the groundwater monitoring list[22]. Cobalt became regulated under the toxic chemical release reporting: community right-to-know on January 1, 1987[23]. The American Conference of Governmental Industrial Hygienists (ACGIH)[24] has given cobalt a classification of A3, confirmed animal carcinogen with unknown relevance to humans, and established an 8-hour time-weighted average (TWA) of 0.02 mg/m^3 for occupational exposure. Occupational Safety and Health Administration (OSHA)[25] has promulgated an 8-hour permissible exposure limit of 0.1 mg/m^3, and the National Institute for Occupational Safety and Health recommends an 8-hour TWA of 0.05 mg/m^3 [26]. The Minimal Risk Level (MRL) is based on the no-observed-adverse-effect level of 0.0053 mg cobalt/m^3 for decreased respiratory function in exposed workers[27]. A MRL of 1×10^{-4} mg/kg per day has been derived for chronic-duration inhalation exposure (> 365 days) to cobalt[9].

Absorption, Distribution, Metabolism, Excretion, and Toxicokinetics

Experimental Animals

There are numerous studies showing that cobalt is absorbed rapidly following inhalation exposure in animals and distributed to various tissues with significant levels in the lungs[28-35]. Following inhalation exposure of rats to 0.0004 to 0.2 ppm (0.001 to 0.5 mg/m^3) pure cobalt 24 hours per day for 3 months, a dose-dependent distribution and accumulation of cobalt was reported in the thyroid gland, spleen, liver, kidney, and lung[35]. In SD-Jcl rats exposed to 0.880 ppm (2.12 mg/m^3) cobalt aerosol 5 hours/day for 4 days, the average cobalt content of the lung and blood 2 hours after the last exposure was 6.42 µg/g and 28.94 µg/L, respectively. The values 28 days after exposure were 0.09 µg/g (1.5 nmol/g) and 0.40 µg/L (6.8 nM), respectively, for lung and blood. The clearance of cobalt in both blood and lung was biphasic with half-lives in the lung of 52.8 and 156 hours and in the blood of 52.8 and 172.8 hours, for the first and second phases, respectively[33]. In miniature swine following inhalation exposure to 0.04 to

0.41 ppm (0.1 to 1.0 mg/m^3) pure cobalt powder 6 hours/day, 5 days/week for 3 months, cobalt was excreted mostly by the kidney[31; 36].

Cobalt levels in rat urine 24 hours following intratracheal instillation of a tungsten carbide-cobalt mixture were approximately threefold higher compared to instillation of cobalt powder at the same dose[37]. It was later confirmed that this was not due to higher bioavailability but due to rapid urinary excretion following exposure to the tungsten carbide-cobalt mixture[38]. The mean lung cobalt concentration in rats given cobalt was two times more than that of rats given a tungsten carbide-cobalt mixture at 48 hours following exposure; by day 7, mean levels had decreased significantly to almost the same level in all exposed rats[38].

The chemical form of a cobalt compound can affect the absorption of cobalt following oral exposure. In rats following oral exposure to cobalt chloride, the absorption was 13% to 34%, whereas the absorption following administration of insoluble cobalt oxide was in the range of 1% to 3%[19; 30; 34; 39-42]. Although no species difference was observed for absorption of cobalt oxide[40], absorption of soluble cobalt compounds was greater in rats (13% to 34%) than in cows (1% to 2%) and guinea pigs (4% to 5%)[19; 30; 34; 39-42]. Absorption was 3- to 15-fold greater in younger animals than in adults[43]. The absorbed cobalt was distributed primarily to the liver; appreciable levels were also found in the kidney, heart, stomach, and intestines[39]. In studies where rats were exposed orally to cobalt sulfate or cobalt chloride for longer terms, significant increases in cobalt concentration were reported in the myocardium, muscle and serum, liver, kidney, brain, and testes[41; 44-47]. Fecal excretion of cobalt is the primary route of elimination in animals following oral exposure and varies depending on the dose and the type of cobalt given; no difference in elimination has been noted between species[30; 40; 48-51].

Humans

Workers exposed to cobalt dust and fumes in the production of cobalt powder had mean concentrations of 5 to 48 µg/L in blood and 19 to 438 µg/L in urine compared to blood and urine concentrations in the range from 0.1 to 2 µg/L in nonoccupationally exposed persons[52]. Following inhalation exposure to insoluble cobalt compounds such as cobalt metal and cobalt oxide, three-phase elimination kinetics were observed in humans. The half-life for the first phase, likely representing mucociliary clearance in the tracheobronchial region, was approximately 2 to 44 hours[53; 54]. The second phase with a half-life of approximately 10 to 78 days may represent macrophage-mediated clearance of cobalt particles from the lung[54; 55]. The third phase clearance with a half-life on the order of years may represent long-term clearance from the lung[40; 54-56]. Using control-aerosol experiments in humans, it has been shown that about 40% of the initial lung burden of inhaled cobalt oxide was retained for a period of 6 months after exposure[57]. About 33% of the initial lung burden was found in the urine with 28% in feces 6 months after exposure.

The absorption of cobalt in humans following oral exposure varied (18% to 97% of the dose) depending on the type and dose of the cobalt compound and other nutritional status. Fecal excretion of cobalt is the primary route of elimination in humans following oral exposure and varies depending on the dose and the type of cobalt given (3% to 99%)[30; 40; 48-51; 58; 59].

Following dermal exposure to hard metal dust (approximately 5% to 15% cobalt metal, 85% to 95% tungsten carbide) for 90 minutes, urinary levels of cobalt increased by about an order of

magnitude compared to preexposure samples, demonstrating that dermal absorption occurs[60]. Cobalt was detected in the fingernails of volunteers who placed their fingers in a cobalt dust solution 10 minutes/day for 7 days[61].

Toxicity

The major targets of cobalt toxicity in animals include the respiratory, cardiovascular, hematopoietic, and immune systems. Other targets for cobalt toxicity in animals include the endocrine system, the nervous system, the liver, and the kidney.

Respiratory System

Inhalation studies of metallic cobalt aerosols and cobalt salts have identified respiratory tract hyperplasia, pulmonary fibrosis, alveolar septa thickening with collagen, elastic tissue, fibroblasts, pleuritis, firm dust lesions, and emphysema as sensitive effects of cobalt on respiratory tissues[26; 31; 33; 36; 62-67]. Exposure of rats and mice to cobalt sulfate heptahydrate by inhalation (up to 30 mg *of cobalt sulfate*/m^3) for 13 weeks showed degeneration of the olfactory epithelium, squamous metaplasia of the respiratory epithelium, and inflammation in the nose; inflammation, necrosis, and inflammatory polyps (rats) of the larynx; metaplasia of the trachea (mice); and fibrosis, histiocytic infiltrates, bronchiolar epithelial regeneration, and epithelial hyperplasia in the alveoli of the lung[66; 68].[a] The most sensitive tissue was found to be the larynx.

Following inhalation exposure to cobalt-containing particles, the primary target of toxicity in humans is the respiratory tract. Occupational exposure of humans to cobalt metal or cobalt-containing hard metal primarily affects the respiratory system, including decreased pulmonary function, asthma, interstitial lung disease, wheezing, and dyspnea; these effects were reported at occupational exposure levels ranging from 0.015 to 0.13 mg cobalt/m^3 [27; 69-92]. These effects have been noted in workers employed in cobalt metal refineries, as well as hard metal workers, diamond polishers, and ceramic dish painters.

Cardiovascular System

Cardiomyopathies appear to be another primary effect of cobalt-induced toxicity in both animals and humans[9]. Rats exposed to cobalt sulfate heptahydrate for 13 weeks showed marginal increases in the severity of cardiomyopathy; no such findings were reported in mice[66; 68]. Studies suggest that cardiomyopathies may occur by impairment of oxidation of pyruvate or fatty acids[93] or by stimulation of carotid body chemoreceptors, thereby mimicking the action of hypoxia[94-96].

In the 1960s, several breweries added cobalt salts to beer to stabilize foam; this resulted in exposures of 0.04 to 0.14 mg/kg[9]. Soon after this, an epidemic of "beer-drinkers' cardiomyopathy" occurred in people who drank 8 to 25 pints/day, resulting in severe cardiovascular system effects, including cardiomyopathy and death.

This cardiomyopathy was evidenced by fragmentation and degeneration of myofibers, enlargement of the heart, and aggregates of abnormal mitochondria[9; 97-99]. The mitochondrial changes are indicative of disturbances in energy production or utilization possibly related to

[a]ERRATUM: An error was identified in the *NTP Technical Report on Cobalt Metal* (Technical Report 581). Text clarifying the form of cobalt used to calculate the exposure concentration was added in the HTML and PDF versions of this report; the new information is italicized. [July 3, 2022]

cobalt effects on lipoic acid. Cobalt irreversibly chelates lipoic acids under aerobic conditions[100]. Lipoic acid is a required cofactor for oxidative decarboxylation of pyruvate to acetyl CoA and of α-ketoglutarate to succinate[101].

Other observations in people who drank the cobalt-treated beer were gastrointestinal effects (nausea, vomiting) and hepatic necrosis[102-104]. The epidemic ceased when the addition of cobalt salts to beer was discontinued.

Hematopoietic System

Exposure to cobalt also affects the hematopoietic system by increasing levels of erythrocytes and hemoglobin in both humans and animals[9]. Palmes et al.[105] demonstrated increased levels of hemoglobin in rats and guinea pigs, but not dogs, exposed to cobalt hydrocarbonyl by inhalation. Polycythemia has been reported in rats, but not mice, exposed to airborne cobalt sulfate heptahydrate[66; 68]. Other studies corroborate the above findings of increases in hemoglobin and erythrocyte levels and decreases in blood phospholipids, cholesterol, and β-lipoproteins in rats exposed to metallic cobalt aerosol via inhalation[35]. Of particular note is an 8-week study in rats that reported dose- and time-related increases in erythrocyte number following oral administration of cobalt chloride[106].

The most sensitive endpoint following oral exposure to cobalt in humans appears to be an increase in erythrocyte numbers (polycythemia)[9]. This effect has been noted in both normal subjects and in patients who were anemic as a result of being anephric.

Immune System

Cobalt may exert its effects through interactions with the immune system, mainly resulting in contact sensitization. In guinea pigs, nickel and cobalt sensitization appear to be interrelated and mutually enhancing, though cross-reactivity has not been reported to occur[107].

The most commonly observed effect following dermal exposure is dermatitis, as demonstrated by a large number of human studies. Patch tests and intradermal injections demonstrate that the dermatitis is probably caused by an allergic reaction to cobalt, with the cobalt ion functioning as a hapten[84; 85; 108-115]. Exposure to inhaled cobalt chloride aerosols can precipitate an asthmatic attack in sensitized individuals, suggesting cobalt sensitization is one mechanism by which cobalt-induced asthma may be produced[84]. IgE and IgA antibodies specific to cobalt have been reported in humans[84; 85; 115]. There is evidence that cobalt sensitivity in humans may be regulated by T-lymphocytes[116]. A human helper T-lymphocyte cell line specific for cobalt ($CoCl_2$) has been established[117]. Cobalt may also interact directly with immunologic proteins, such as antibodies or Fc receptors, resulting in immunosensitization[118].

Endocrine System

A study in female mice exposed to 26 mg cobalt/kg per day in drinking water for up to 45 days produced histopathologic changes to the thyroid[119]. Cobalt significantly stimulated serum testosterone in mice treated orally with 23 mg cobalt chloride/kg, although no dose-response relationship was present[120]. There have also been reports of increased incidences of

pheochromocytoma, a tumor of the adrenal medulla, in female rats exposed to *3.0 mg cobalt sulfate/m³(*1.14 mg cobalt/m³*)* for 2 years[121; 122].[b]

Nervous System

In a study in OFA Sprague Dawley rats, exposure-related delays in hearing, swimming ability, and development of muscle strength and locomotor system were reported in the offspring of dams that were exposed to 0, 25, 50, or 100 mg/kg body weight cobalt sulfate by gavage throughout gestation[123]. Occupational exposure to cobalt metal in humans has been reported to cause several effects on the nervous system, including memory loss (Wechsler Memory Scale-Revised), nerve deafness, and decreased visual acuity[124; 125]. However, these studies had small numbers of subjects (n=38 or 1), and exposure characterization was not reported.

Liver

No histologic effects on the liver were found in pigs exposed to cobalt metal dust up to 1.0 mg/m³ for 3 months[31]. Hyperemia of the liver and cytoplasmic changes in hepatocytes (clumpy cytoplasm located along the cell membrane) were noted in rats following oral administration of a single dose of 68.2 mg cobalt fluoride/kg or a single dose of 157.3 mg cobalt oxide/kg[126]. Increased liver weight (17%) was found in rats exposed to 10 mg cobalt chloride/kg per day for 5 months[127]. No morphologic or enzymatic changes were noted in the livers of rats exposed to 2.5 to 30.2 mg cobalt chloride/kg by gavage or to cobalt chloride in the drinking water for 3 to 7 months[128-130]. However, in previous NTP studies, rats and mice exposed by inhalation to *cobalt sulfate heptahydrate (*~~50 or~~ *up to* 200 mg cobalt sulfate~~ heptahydrate~~/m³*)* ~~or greater~~ for 16 days demonstrated *lesions in the liver, including* necrosis and congestion *(rats only)* ~~in the liver~~[66; 68].[c]

Kidney

Significant increases were noted in the relative kidney weight in male rats exposed by inhalation to cobalt sulfate heptahydrate (0.3 to 30 mg/m³ *of cobalt sulfate*) for 13 weeks[66; 68]. No effects were observed upon histologic examination of the kidneys in rats or mice following exposure to cobalt sulfate heptahydrate at *concentrations of* up to 200 mg *cobalt sulfate*/m³ for 16 days, up to 30 mg *cobalt sulfate*/m³ for 13 weeks, or up to 3.0 mg *cobalt sulfate*/m³ for *2 years*[66; 68; 121; 122]. No histologic effects on the kidney were found in pigs exposed to up to 1.0 mg cobalt/m³ for 3 months[31].[d]

[b]ERRATUM: An error was identified in the NTP Technical Report on Cobalt Metal (Technical Report 581). Text clarifying the form of cobalt used to calculate the exposure concentration was added in the HTML and PDF versions of this report; the new information is italicized. [July 3, 2022]

[c]ERRATUM: Errors were identified in the NTP Technical Report on Cobalt Metal (Technical Report 581). Text clarifying the form of cobalt used to calculate the exposure concentration, the effects on specific study animals, and the number of studies evaluated was added in the HTML and PDF versions of this report; the new information is italicized. [July 3, 2022]

[d]ERRATUM: Errors were identified in the NTP Technical Report on Cobalt Metal (Technical Report 581). Text clarifying the form of cobalt used to calculate the exposure concentration was added in the HTML and PDF versions of this report; the new information is italicized. [July 3, 2022]

Reproductive and Developmental Toxicity

Experimental Animals

Exposure to cobalt-containing aerosols has been shown to affect reproductive endpoints in rats and mice. A decrease in sperm motility in mice was found following exposure to *cobalt sulfate heptahydrate at concentrations of* 3 to 30 mg cobalt sulfate ~~heptahydrate~~/m^3 for 13 weeks, and a significant increase in the length of the estrous cycle was reported in female mice exposed to *cobalt sulfate heptahydrate at concentrations of* 30 mg *cobalt sulfate*/m^3 for 13 weeks[66; 68]. Testicular atrophy was reported in rats exposed by inhalation to 50 mg cobalt sulfate ~~heptahydrate~~/m^3, 6 hours per day for 16 days, but not in rats exposed to 30 mg/m^3 for 13 weeks. Testicular atrophy was, however, observed in mice exposed to 30 mg/m^3 per day for 13 weeks[66; 68]. No testicular effects were observed in rats or mice exposed to cobalt sulfate heptahydrate *at concentrations of* up to 3.0 mg *cobalt sulfate*/m^3 for *2 years*[121; 122]. These findings are consistent with studies following oral exposure to soluble cobalt chloride for 2 to 3 months in the diet or drinking water. These studies reported testicular degeneration and atrophy in rats exposed to cobalt chloride in the diet[128; 131-133] and in mice exposed to cobalt chloride for 3 weeks in drinking water[134; 135].[e]

No studies were found in the literature regarding developmental effects in animals following inhalation exposure to cobalt. In a perinatal study design where rat dams were administered 0, 12, 24, or 48 mg/kg of cobalt chloride via oral gavage from gestation day 14 through lactation day 21, offspring displayed stunted growth (all cobalt dose levels) and decreased survival (24 and 48 mg/kg)[136]. No external malformations were observed in the pups. No measurements were collected on the dams, although the authors stated that toxic signs were previously observed in male and female rats administered 24 or 48 mg/kg. The effects on the offspring occurred at levels that also caused maternal toxicity (reduced body weight and food consumption and altered hematological measurements); no teratogenic effects were noted. These authors also reported that rabbits exposed to 20, 100, or 200 mg/kg cobalt sulfate from gestation days 6 to 20 exhibited excessive dose-related maternal toxicity (death and total resorptions). Fetuses in the 20 mg/kg group (only cobalt group available for examination) evaluated at term displayed apparent delays in ossification and increases in percentage of fetuses with retarded body weight. Szakmáry et al.[123] reported that exposure of pregnant rats to cobalt sulfate did not result in changes in fetal death rates, maternal body weight gain, average litter size, or average fetal or placental weights; however, a dose-related trend was seen for the percent of fetuses with retarded body weights. Increased incidences of axial skeletal anomalies were also observed in rat fetuses exposed to 25 to 100 mg/kg cobalt sulfate[123]. In contrast, no effects on fetal growth or survival were found following exposure of rats to cobalt chloride during gestation days 6 to 15[137]. However, the difference in results between the two studies may be explained, at least in part, by exposure concentration, with cobalt sulfate exposure being almost double that of cobalt chloride. There are also reports of preimplantation losses following administration of cobalt in male mice[138].

Exposure of mice to cobalt chloride during gestation days 8 to 12 was reported to have no effect on fetal growth or mortality[139]. There were no changes in litter size, postimplantation losses, or

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average fetal or placental weights in a study that exposed pregnant mice to 50 mg cobalt sulfate/kg body weight per day; there was an increase in the percent of fetuses with retarded body weights[123]. These authors also reported that rabbits exposed to 20 mg/kg cobalt sulfate showed nearly complete maternal lethality and complete fetal loss. In addition, there were significant increases in mortality and fetal resorption, as well as an increase in fetuses with retarded body weight.

Humans

No reports were found in the literature on the reproductive effects in humans following inhalation or oral exposure to cobalt. Pregnant women were treated with cobalt chloride to raise hematocrit and hemoglobin levels that are often depressed during pregnancy. Doses up to 0.6 mg cobalt/kg per day for 3 months were given during the final trimester[129]. No developmental effects were reported in the infants, although examination was limited only to the reporting of obvious birth defects.

Carcinogenicity

Experimental Animals

In 2-year inhalation studies of soluble cobalt sulfate heptahydrate, pronounced effects on the respiratory tract, including hyperplasia, inflammation, fibrosis, metaplasia, and increased incidences of cancer in rats and mice were reported[121; 122]. Increased incidences of alveolar/bronchiolar neoplasms occurred in male rats exposed to 3.0 mg *cobalt sulfate*/m^3 and in female rats exposed to 1 or 3 mg *cobalt sulfate*/m^3.[f] Neoplasms occurred in both sexes with significantly positive trends. Male and female mice exposed to 3 mg/m^3 showed increases in alveolar/bronchiolar neoplasms; neoplasms in the lung occurred with significantly positive trends. The findings also demonstrated an increased incidence of pheochromocytoma, a neoplasm of the adrenal medulla, in female rats exposed to 3.0 mg/m^3 for 2 years without any other endocrine effects. The NTP concluded that under the conditions of the 2-year studies, there was some evidence of carcinogenic activity of cobalt sulfate heptahydrate in male F344/N rats based on increased incidences of alveolar/ bronchiolar neoplasms. Marginal increases in the incidences of pheochromocytomas of the adrenal medulla may have been related to exposure to cobalt sulfate heptahydrate. There was clear evidence of carcinogenic activity in female F344/N rats based on increased incidences of alveolar/bronchiolar neoplasms and pheochromocytomas of the adrenal medulla in groups exposed to cobalt sulfate heptahydrate. There was clear evidence of carcinogenic activity of cobalt sulfate heptahydrate in male and female B6C3F1 mice based on increased incidences of alveolar/bronchiolar neoplasms. Molecular analyses of lung neoplasms were also performed in the study; findings showed an exposure concentration-dependent increase in *Kras* mutations, thereby suggesting a mechanism of tumorigenesis.

Parenteral exposure to cobalt has also been found to induce neoplasms in rodents[140-145]. One study reported sarcomas in rats at the site of injection of cobalt salts or cobalt metal powder[1]. In another study, rats of an unspecified strain were given a single injection of 0.28 mg cobalt metal

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powder in fowl serum into the thigh muscle[142; 143]. Within 2 weeks, atypical myoblasts were observed[143], and between 5 and 12 months, malignant neoplasms developed at the injection site in 17 of 30 rats; 11 of the neoplasms were rhabdomyosarcomas[142]. Similar neoplastic responses to injections of cobalt sulfide and cobalt oxide were noted in an unspecified strain of rats but not in mice[140]. Shabaan et al.[145] noted fibrosarcomas in male Wistar rats 8 months to 1 year after administration of 40 mg cobalt chloride/kg per day by subcutaneous injection for 10 days.

Humans

Few epidemiological studies of cancer risk in cobalt metal-exposed workers exist[146]. A high incidence of pulmonary cancer was found in English cobalt metal miners; however, the etiology was not known[65]. Epidemiological studies of cobalt metal miners in the United States, Canada, Zaire, and other countries found no association between cobalt metal and neoplasm rates; however, cobalt metal was the cause of hard metal respiratory disease[147]. In a mortality study of a cohort of 1,143 workers in an electrochemical plant producing cobalt metal and sodium (110 were engaged in cobalt metal production) for at least a year during 1950 to 1980, an increased number of deaths from lung cancers were observed in those producing cobalt metal; however, smoking may have been a factor[148]. Confounding by nickel and arsenic exposures and the limited size of the exposed population were identified as some limitations[146]. The follow-up (1981 to 1988) did not support the proposed relationship between lung cancer and cobalt metal exposure[149].

The 12th Report on Carcinogens lists cobalt sulfate and cobalt-tungsten carbide powders and hard metals as reasonably anticipated to be human carcinogens based on sufficient evidence of carcinogenicity from studies in experimental animals[150]. IARC[1] has listed cobalt compounds as possibly carcinogenic to humans (Group 2B) based on sufficient evidence for cobalt metal and cobalt oxides and limited evidence for cobalt chloride and cobalt sulfate. However, to date, there are no chronic studies reported on cobalt metal dust in rodents.

Genetic Toxicity

There is a paucity of data on the genetic toxicity of metallic cobalt, likely due to the assumption that its biological activity is mediated by its ionic forms. However, it has been shown that at least some of the biological activities of the metal cannot be attributed to its ionic forms[151]. The focus of this review is on the genetic toxicity of cobalt metal and not on its ionic or hard metal forms. For reviews of cobalt and cobalt compound toxicities, see De Boeck et al.[152;] Lison et al.[153;] Simonsen et al.[154].

Two studies published in 1997 showed that cobalt powder causes DNA damage in cultured mammalian cells. In one study, non-cytotoxic doses of cobalt powder were added to cultured human lymphocytes and a dose-dependent increase in DNA single strand breaks was noted at concentrations of 4.5 μg/mL and above[155]. Sodium formate, a hydroxyl radical scavenger, was shown to lower the levels of cobalt-induced DNA damage in this study. The second study examined induction of DNA strand breaks and micronuclei in human lymphocytes exposed in culture to pure cobalt powder (up to 12 μg/mL)[156]. The authors reported dose-related increases in DNA migration (using the comet assay) and dose-related increases in micronucleated lymphocytes (using the cytokinesis block method). Another study from the same laboratory, focused on measuring the genetic toxicity of cobalt combined with various metallic carbide

particles, found induction of DNA strand breaks by cobalt alone to be so variable that results from the testing of the combination materials could not be clearly evaluated[157]. However, induction of micronucleated lymphocytes by cobalt alone or in combination with tungsten carbide, chromium carbide, niobium carbide, and molybdenum carbide occurred in a dose-related fashion[157]; none of the four individual carbides induced micronuclei in the absence of cobalt.

Several lines of investigation suggest that cobalt interferes with DNA repair processes (for a review, see Hartwig et al.[158]). One way that proteins interact with DNA is through zinc-finger domains. Proteins in the base excision repair (BER) and nucleotide excision repair (NER) pathways, as well as transcription factors such as p53, contain zinc-finger domains. Biochemical studies have shown that cobalt can take the place of zinc in such domains; for example, cobalt will complex within the bacterial BER enzyme, formamidopyrimidine-DNA glycosylase[159], and a peptide containing the zinc-finger domain of XPA will accept cobalt[160]. The substitution of cobalt for zinc appears to have negative consequences, as cobalt impaired the ability of XPA purified from mouse cells to bind DNA specifically through its zinc-finger domain[161]. In the NER pathway, XPA plays a critical role in the initial detection of DNA that has been distorted by various chemical modifications that are known to induce mutations, such as oxidized bases and bulky adducts. In another approach, De Boeck et al.[162] investigated the interference of cobalt with the repair of mutagen-induced DNA damage using the Comet assay. Cultured human lymphocytes were exposed to MMS alone, or to MMS followed by a non-genotoxic dose of 1.2 μg/mL cobalt metal. In the presence of cobalt, there was an increase in the persistence of MMS-induced DNA damage, suggesting that cobalt inhibited the repair of MMS-induced DNA lesions.

Taken together, results from these in vitro studies suggest that cobalt particles can affect the integrity of DNA by producing activated oxygen species and/or by inhibiting DNA repair pathways.

To assess the genotoxic effects of cobalt dust on workers from cobalt refineries, where the average dust concentration was 20 mg/m^3, 35 workers were examined for lymphocyte DNA damage using the comet assay and for lymphocyte micronucleus frequencies. No significant effects were observed for either of these endpoints in exposed workers compared to matched controls[163].

Study Rationale

Cobalt metal dust was nominated for toxicology and carcinogenesis studies by the United Auto Workers and the Cobalt Development Institute based on the widespread occupational exposure and the occurrence of occupational disease, i.e., hard metal disease, associated with exposure to cobalt and its compounds, including cobalt metal-tungsten carbide. The carcinogenicity of a soluble cobalt compound, cobalt sulfate heptahydrate, in experimental animals exposed by inhalation was previously assessed by NTP[122]. Limited data were available to assess the chronic toxicity and carcinogenic potential of inhaled insoluble cobalt compounds, particularly cobalt metal dust. Inhalation was selected as the route of exposure because this is the most common route of exposure to cobalt metal dust in occupational settings in humans.

Materials and Methods

Procurement and Characterization of Cobalt Metal

Cobalt metal was produced by OMG Kokkola Chemicals Oy (Kokkola, Finland) and was provided by the Cobalt Development Institute via PEL Technologies in one lot (P32 3040-1) that was used in the 2-week, 3-month, and 2-year studies. Identity and purity analyses were performed by the study laboratory at Battelle Toxicology Northwest [Richland, WA; inductively coupled plasma/atomic emission spectroscopy (ICP/AES) analysis] and by the analytical chemistry laboratories at Pacific Northwest National Laboratory [Richland, WA; X-ray diffraction (XRD) and proton-induced X-ray emission (PIXE) analyses], AMIA Laboratories (The Woodlands, TX; XRD using Rietveld analysis), H&M Analytical Services, Inc. (Allentown, NJ; XRD with and without Rietveld analysis), Elemental Analysis, Inc. (Lexington, KY; PIXE), and Galbraith Laboratories (Knoxville, TN; coulometry for total carbon) (Appendix L). Reports on analyses performed in support of the cobalt metal studies are on file at the National Institute of Environmental Health Sciences.

Lot P32 3040-1 of the chemical, a silver-gray powder, was identified as cobalt metal by the analytical chemistry laboratories using XRD. XRD patterns were consistent with library reference patterns for cubic and hexagonal phases of cobalt. The purity of lot P32 3040-1 was determined by the analytical chemistry laboratories by determination of the carbon content using combustion/coulometric analysis by induction furnace with a carbon dioxide coulometer and by PIXE analyses to determine the presence of cobalt metal and trace element impurities with atomic numbers from 11 (sodium) to 53 (iodine) or 92 (uranium). The study laboratory quantitated the purity of the bulk chemical using ICP/AES. The carbon content was determined to be 0.09% ± 0.01%. PIXE analysis indicated trace elements of aluminum, sulfur, calcium, chromium, and iron. Chromium was consistently current at approximately 84 ppm; the other impurities were below the minimum detection limits. ICP/AES analysis indicated a purity of 98.2% ± 0.6% relative to a National Institute of Standards and Technology standard reference material [(SRM); cobalt SRM 3113, Gaithersburg, MD]. The overall purity of cobalt metal was determined to be greater than 98%.

To ensure stability, the bulk chemical was stored at room temperature in safety-coated amber glass containers with Teflon®-lined caps under a nitrogen headspace. Periodic reanalyses of the bulk chemical were performed by the study laboratory using ICP/AES; no degradation of the bulk chemical was detected.

Aerosol Generation and Exposure System

During the 2-week studies, an auger feed device was used to meter cobalt metal into a Trost jet mill for aerosolization and particle size reduction. For the 3-month and 2-year studies, the generation system used a linear feed device to meter cobalt metal into the jet mill. Initial particle size reduction was accomplished within the Trost jet mill. From the jet mill, aerosol was directed to the main distribution line where it was diluted with humidified air then conveyed from the exposure control center to the exposure room where it passed through a cyclone separator to further reduce particle size. On exiting the cyclone, the aerosol-laden air was directed to either of two smaller branch lines. From the branch lines, aerosol was delivered to each exposure chamber

by a sampling tube. The flow through the sampling tube was induced by a stainless steel ejector pump. The aerosol then entered the chamber inlet duct where it was further diluted with conditioned chamber air to achieve the desired exposure concentration.

The study laboratory designed the inhalation exposure chambers so that uniform aerosol concentrations could be maintained throughout the chambers with the catch pans in place. The total volume of the chamber was 2.3 m^3 with an active mixing volume of 1.7 m^3. Tests showed that aerosol concentration could be reliably maintained homogenous within 8% throughout the chambers, provided the aerosol was uniformly mixed before passing through the chamber inlet and provided the test material did not react to a significant extent with animals, animal excrement, or the chamber interior[164].

Aerosol Concentration Monitoring

Summaries of the chamber aerosol concentrations are given in Table L-1 through Table L-3. The concentration of cobalt metal in the exposure chambers and room air was monitored using three real-time aerosol monitors (RAMs). Each RAM was calibrated by constructing a response curve using the measured RAM voltages (voltage readings were corrected by subtracting the RAM zero-offset voltage from measured RAM voltages) and cobalt metal concentrations that were determined by analyzing tandem Teflon®-coated, glass-fiber filters collected daily from the exposure chambers. Cobalt was extracted from the filters and analyzed using ICP/AES. The ICP/AES instrument was calibrated against serially diluted NIST-traceable 10 mg/mL spectrometric standards of cobalt and the internal standard yttrium.

Chamber Atmosphere Characterization

Particle size distribution was determined once prior to the 3-month and 2-year studies, once during the 2-week studies, twice during the 3-month studies, and monthly during the 2-year studies. Impactor samples were taken from each exposure chamber using a Mercer-style seven-stage impactor and the stages (glass coverslips lightly coated with silicone to prevent particle bounce) were analyzed using ICP/AES after cobalt was extracted from the slides. The relative mass collected on each stage was analyzed by the CASPACT impactor analysis program developed at Battelle based on probit analysis[165]. The resulting estimates of the mass median aerodynamic particle diameter and the geometric standard deviation of each set of samples are given in Table L-4 through Table L-7.

Buildup and decay rates for chamber aerosol concentrations were determined with (all studies) and without (3-month and 2-year studies) animals present in the chambers. At a chamber airflow rate of 15 air changes per hour, the theoretical value for the time to achieve 90% of the target concentration after the beginning of aerosol generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after aerosol generation was terminated (T_{10}) was approximately 9.4 minutes. A T_{90} value of 12 minutes was selected for all studies.

The uniformity of aerosol concentration in the inhalation exposure chambers without animals was evaluated before the 3-month and 2-year studies began; in addition, concentration uniformity with animals present in the chambers was measured once during the 2-week and 3-month studies and every 3 to 4 months during the 2-year studies. Chamber concentration uniformity was maintained throughout the studies. The persistence of cobalt metal in the

exposure chambers after aerosol delivery ended was determined by monitoring the concentration overnight in the 40 mg/m^3 rat and mouse chambers in the 2-week studies, the 5 mg/m^3 rat and 10 mg/m^3 mouse chambers in the 3-month studies, and the 5 mg/m^3 rat and mouse chambers in the 2-year studies, with and without (except for the 2-week studies) animals present in the chambers. The average cobalt metal concentration decreased to 1% of the target concentration within 15 (2-week studies), 17 to 18 (3-month studies), or 19 (2-year studies) minutes.

Stability studies of the test material in the generation and exposure system were performed before and during the studies by the study laboratory and the analytical chemistry laboratories. In these studies, XRD analyses consistently indicated two primary phases of cobalt in the samples, cubic and hexagonal, and minimal detectable concentrations of cobalt oxides. Low and acceptable levels of trace element inorganic impurities were detected in these stability samples using PIXE and ICP/AES assays.

Animal Source

Male and female F344/N rats and B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc. (Germantown, NY) for the 2-week and 3-month studies. For the 2-year studies, male and female F344/NTac rats were obtained from the commercial colony at Taconic Farms, Inc., and B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc. The rationale for change of rat strain from F344/N to F344/NTac was a programmatic decision. For many years, NTP used the inbred F344/N rat for its toxicity and carcinogenicity studies. Over a period of time, the F344/N rat exhibited sporadic seizures and idiopathic chylothorax, and consistently high rates of mononuclear cell leukemia and testicular neoplasia. Because of these issues in the F344/N rat and NTP's desire to find a more fecund rat model that could be used in both reproductive and carcinogenesis studies for comparative purposes, a change in the rat model was explored. Following a workshop in 2005, the F344 rat from the Taconic commercial colony (F344/NTac) was used for a few NTP studies to allow NTP to evaluate different rat models. The F344/NTac rat was used in four subchronic and two chronic studies (cobalt metal and bromodichloroacetic acid) between 2005 and 2006[166]. The current cobalt metal study is the first of the NTP 2-year studies using the F344/NTac rat to be reported.

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.

Two-week Studies

On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 12 days and were 5 to 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. At the end of the studies, serologic analyses were performed

on five male and five female sentinel rats and mice housed in the control chamber using the protocols of the NTP Sentinel Animal Program (Appendix N).

Groups of five male and five female rats and mice were exposed to cobalt metal particulate aerosol by inhalation at concentrations of 0, 2.5, 5, 10, 20, or 40 mg/m^3, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 16 (rats) or 17 (mice) days. Additional groups of five female rats and mice were exposed to the same concentrations for 16 (rats) or 17 (mice) days for tissue burden studies. Feed and water were available ad libitum, except feed was withheld during exposure periods and urine collection. Rats and mice were housed individually. Clinical findings were recorded once daily and at terminal kill for core study rats and mice. The core study animals were weighed on days 1, 5, and 12 and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

As part of the tissue burden studies, surviving core study rats were placed in metabolism cages on day 12 for a 16-hour urine collection. Urine samples were collected over ice. Volume was measured, and creatine concentration was determined. Remaining samples were stored at approximately −70°C until analyzed for cobalt metal concentration using ICP/AES following open digestion in a heat block. The parameters measured are listed in Table 1.

For tissue burden studies, blood was collected from the retroorbital sinus of all surviving core study rats and mice and two female tissue burden study rats and mice per group on the last day of exposure and from three female tissue burden study rats and mice per group 3 weeks postexposure. Blood was divided between a collection tube containing EDTA and a serum tube without anticoagulant. Blood and serum samples were stored at approximately −70°C for analysis of cobalt metal concentration. Following blood collection, the right femur, heart, right kidney, liver (right lateral and caudate lobes), right lung lobe, and right testis were collected from core study rats and mice and weighed. In addition, whole liver, whole lung, and left lung plus mainstem bronchi were weighed. For tissue burden study rats and mice, the lungs with mainstem bronchi were removed and weighed and the right and left lung lobes were collected and weighed individually. Samples were stored in plastic containers at approximately −70°C until analyzed for cobalt metal. For analyses, blood, serum, femur (femurs were boiled in water, extraneous tissue scraped off, and dried for at least 48 hours before digestion), heart, kidney, and testis samples were prepared in acid-leached Parr bomb digestion liners (Parr Instrument Co., Moline, IL) using an Imperial II radiant heat oven (Lab-line Instruments, Inc., Melrose Park, IL), and liver and lung samples were prepared in microwave digestion liners and a microwave sample preparation station (CEM Corp., Matthews, NC) with internal standard (10 µg yttrium; 0.1 µg gallium) and HNO_3:HCl (1:1). Digests were quantitatively transferred to plastic containers and diluted to an appropriate volume with deionized water before analysis using ICP/AES (IRIS Intrepid ICP/AES spectrometer, Thermo Elemental, Franklin, MA) (kidney, liver, lung) or ICP/mass spectrometry (MS) (Agilent 7500cc ICP/MS, Agilent Technologies, Palo Alto, CA) (blood, serum, femur, heart, and testis).

Necropsies were performed on all core study rats and mice. The heart, left kidney, liver, lung, left testis, thymus, and thyroid gland were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathologic examinations were performed on core study chamber control animals, 20 mg/m^3 rats, and 40 mg/m^3 rats and mice. The left kidney, left and median liver lobes, and

thyroid gland of rats and mice, the brain of rats, and the urinary bladder of male rats were examined to a no-effect level; the left lobe of the lung and the nose were examined in all core study groups. Table 1 lists the tissues and organs examined.

Three-month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to cobalt metal and to determine the appropriate exposure concentrations to be used in the 2-year studies.

On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 12 or 13 days and were 5 to 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. Serologic analyses were performed during the studies using the protocols of the NTP Sentinel Animal Program (Appendix N).

Groups of 10 male and 10 female rats and mice were exposed to particulate aerosols of cobalt metal by inhalation at concentrations of 0, 0.625, 1.25, 2.5, 5, or 10 (mice only) mg/m^3, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 14 weeks. Additional groups of 10 male rats (clinical pathology study) and 32 to 36 female rats and mice (special study) were exposed to the same concentrations for 14 weeks. Feed was available ad libitum except during exposure periods; water was available ad libitum. Rats and mice were housed individually. Clinical findings were recorded weekly beginning day 9 (male rats) or 10 and at the end of the studies. Core study animals were weighed initially, weekly beginning day 9 (male rats) or 10, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Blood was collected from the retroorbital sinus of male clinical pathology rats and 10 female special study rats on days 3 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats) analyses. Blood for hematology was placed in tubes containing potassium EDTA, and blood for clinical chemistry was placed in tubes containing a separator gel. Hematology analyses were performed on an Abbott Cell-Dyn 3700 analyzer (Abbott Diagnostics Systems, Abbott Park, IL), except manual hematocrit determinations were performed using a microcentrifuge (Hereaus Holding Gmbh., Hanau, Germany) and a Damon/IEC capillary reader (International Equipment Co., Needhum Heights, MA). Platelet, leukocyte, and erythrocyte morphology and nucleated erythrocytes were assessed using smears stained with a Romanowsky-type aqueous stain in a Wescor 7100 aerospray slide stainer (Wescor, Inc., Logan, UT). Reticulocytes were stained with new methylene blue and counted using the Miller disc method[167]. Samples for clinical chemistry were centrifuged, and parameters were measured using a Roche Hitachi 912 system (Roche Diagnostic Corp., Indianapolis, IN). Table 1 lists the clinical pathology parameters measured.

Lungs and blood (retroorbital sinus) were collected from three special study female rats and mice per exposure group on days 5, 12, 26, 40, 61, and 89 and on days 7, 14, 28, and 42 postexposure. On days 26 and 40, livers were also collected. Liver and lungs were weighed. Blood, liver (right lateral and caudate lobes), and lungs were analyzed for cobalt metal concentrations as described for the 2-week studies, except all samples for analysis were placed in microwave sample digestion vessels.

On days 26 and 40, the remaining liver samples from special study rats and mice were collected and stored at approximately −70°C until analyses for cytochrome P450 activities. Microsomal suspensions were prepared using the Pearce Method[168]. The concentration of protein in each suspension was determined using the microtiter plate method of the Coomassie® Plus Protein Assay (Pierce Chemical Co., Rockford, IL) with bovine serum albumin as the standard. Acetanilide-4-hydroxylase (A4H), 7-ethoxyresorufin-*O*-deethylase (EROD), and 7-pentoxyresorufin-*O*-deethylase (PROD) were determined in microsomal proteins isolated from frozen liver samples according to established procedures.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on rats exposed to 0, 1.25, 2.5, or 5 mg/m^3 and mice exposed to 0, 2.5, 5, or 10 mg/m^3. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal kill, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study and special study animals. The heart, right kidney, liver, lung, right testis, thymus, and thyroid gland (rats) of core study animals were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed by the study laboratory pathologist on core study 0, 5 (rats), and 10 (mice) mg/m^3 groups of rats and mice; tissues were examined to a no-effect level in the remaining groups. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman[169] and Boorman et al.[170].

Two-year Studies

Study Design

Groups of 50 male and 50 female rats and mice were exposed to cobalt metal by inhalation of particulate aerosol at concentrations of 0, 1.25, 2.5, or 5 mg/m^3, 6 hours plus T_{90} (12 minutes) per day, 5 days per week for up to 105 weeks. Additional groups of 35 lung burden study female rats and mice were exposed to the same concentrations of cobalt metal for up to 79 weeks.

Rats were 3 to 4 weeks old on receipt, and mice were 4 to 5 weeks old. The animals were quarantined for 12 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats were approximately 5 to 6 weeks old and mice approximately 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix N).

Rats and mice were housed individually. Feed and water were available ad libitum, except feed was withheld during animal exposures. Chambers and racks were rotated weekly. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix M.

Clinical Examinations and Pathology

All animals were observed twice daily. Core study animal body weights were recorded on day 1, weekly for the first 13 weeks, every 4 weeks through week 93, every 2 weeks thereafter, and at terminal kill. Clinical findings were recorded every 4 weeks through week 93, then every 2 weeks, and at terminal kill.

Complete necropsies and microscopic examinations were performed on all core study rats and mice; selected necropsies were performed on lung burden study animals. At necropsy, organs and tissues were examined for grossly visible lesions, and all (core study) major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1. For extended evaluation of renal proliferative lesions in male rats, kidneys were step sectioned at 1 mm intervals, and three to eight additional sections were obtained from each kidney.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy; the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included larynx, lung, and nose of rats and mice; adrenal medulla and pancreas of rats; kidney and testis of male rats; and spleen of female rats.

The quality assessment report and the reviewed slides were submitted to the NTP PWG coordinator who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman[169] and Boorman et al.[170]. For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell et al.[171].

Mutation Analysis of Alveolar/bronchiolar Carcinomas

After histopathology examination, formalin-fixed, paraffin-embedded (FFPE) blocks from alveolar/bronchiolar carcinomas that arose either spontaneously (in chamber controls) or due to cobalt metal exposure were selected from rats and mice for mutation analysis of commonly altered genes in lung cancer (*Kras*, *Egfr* and *Tp53*). DNA was extracted from the FFPE tissues and subjected to a seminested polymerase chain reaction (PCR) to amplify hot spot regions of *Kras*, *Egfr* and *Tp53.* The lyophilized PCR products were sequenced, and the resulting electropherograms were compared to identify mutations in alveolar/bronchiolar carcinomas that arose spontaneously or due to exposure to cobalt metal. The results are presented in Appendix K.

Lung Burden Study

Five female lung burden rats and mice per group were randomly selected and sent to necropsy immediately after exposure on days 1, 2, 3, 4, 184, 366, and 548. The lungs were removed, weighed, and stored at approximately −70°C until analyzed for cobalt metal concentration using an ICP/AES method similar to that described for the 2-week studies.

Table 1. Experimental Design and Materials and Methods in the Inhalation Studies of Cobalt Metal

Two-week Studies	Three-month Studies	Two-year Studies
Study Laboratory		
Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)	Battelle Toxicology Northwest (Richland, WA)
Strain and Species		
F344/N rats B6C3F1/N mice	F344/N rats B6C3F1/N mice	F344/NTac rats B6C3F1/N mice
Animal Source		
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies		
12 days	12 or 13 (male rats) days	12 days
Average Age When Studies Began		
5 to 6 weeks	5 to 6 weeks	5 to 6 weeks
Date of First Exposure		
August 2, 2004	Rats: March 7 (females) or 8 (males), 2005 Mice: March 7, 2005	Rats: May 8, 2006 Mice: May 15, 2006
Duration of Exposure		
6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 16 (rats) or 17 (mice) days	6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 14 weeks	6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 105 weeks
Date of Last Exposure		
Rats: August 17, 2004 Mice: August 18, 2004	Rats: June 6 (female) or 7 (males), 2005 Mice: June 8 (males) or 9 (females), 2005	Rats: May 5–8, 2008 Mice: May 15, 2008
Necropsy Dates		
Rats: August 17, 2004 Mice: August 18, 2004	Rats: June 7 (females) or 8 (males), 2005 Mice: June 9 (males) or 10 (females), 2005	Rats: May 5–8, 2008 Mice: May 12–16, 2008
Average Age at Necropsy		
8 weeks	19 to 20 weeks	Rats: 109 to 110 weeks Mice: 109 to 111 weeks
Size of Study Groups		
5 males and 5 females (core study) 5 females (tissue burden study)	Rats: 10 males and 10 females (core study) 10 males (clinical pathology) 32 to 36 females (special study) Mice: 10 males and 10 females (core study) 32 to 36 females (special study)	50 males and 50 females (core study) 35 females (lung burden study)

Two-week Studies	Three-month Studies	Two-year Studies
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage		
1	1	1
Method of Animal Identification		
Tail tattoo	Tail tattoo	Tail tattoo
Diet		
Irradiated NTP-2000 wafer diet (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, except during exposure periods and urine collection, changed weekly	Irradiated NTP-2000 wafer diet (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, except during exposure periods, changed weekly	Same as 3-month studies
Water		
Tap water (Richland, WA municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available ad libitum	Same as 2-week studies	Same as 2-week studies
Cages		
Stainless steel wire bottom (Lab Products, Inc., Seaford, DE), changed weekly, rotated daily	Stainless steel wire bottom (Lab Products, Inc., Seaford, DE), changed weekly, rotated weekly in chambers	Same as 3-month studies
Cageboard		
Untreated paper cage pan liner (Techboard, Shepherd Specialty Papers, Kalamazoo, MI), changed daily	Same as 2-week studies	Same as 2-week studies
Chamber Air Supply Filters		
Single HEPA (open stock), charcoal (RSE, Inc., New Baltimore, MI), Purafil (Environmental Systems, Lynnwood, WA), all new at study start	Same as 2-week studies, except new at beginning of 2-week studies and changed as needed	Same as 2-week studies, except HEPA filter changed annually
Chambers		
Stainless steel, excreta pan at each of six levels (Lab Products, Inc., Seaford, DE); chambers changed weekly; excreta pans changed daily	Same as 2-week	Same as 2-week studies

Two-week Studies	Three-month Studies	Two-year Studies
Chamber Environment		
Temperature: 75° ± 3°F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15 ± 2/hour	Temperature: 72° ± 3°F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15 ± 2/hour	Temperature: 72° ± 3°F Relative humidity: 55% ± 15% Room fluorescent light: 12 hours/day Chamber air changes: 15 ± 2/hour
Exposure Concentrations		
0, 2.5, 5, 10, 20, or 40 mg/m^3	Rats: 0, 0.625, 1.25, 2.5, or 5 mg/m^3 Mice: 0, 0.625, 1.25, 2.5, 5, or 10 mg/m^3	0, 1.25, 2.5, or 5 mg/m^3
Type and Frequency of Observation		
Observed once daily; core study animals were weighed on days 1, 5, and 12 and at the end of the studies; clinical findings were recorded once daily and at terminal kill.	Observed twice daily; core study animals were weighed initially, on day 9 (male rats) or 10, weekly thereafter, and at the end of the studies; clinical findings were recorded on day 9 (male rats) or 10, weekly thereafter, and at the end of the studies.	Observed twice daily; core study animals weighed initially, weekly for the first 13 weeks, every 4 weeks through week 93, every 2 weeks thereafter, and at terminal kill; clinical findings were recorded initially and every 4 weeks through week 93, every 2 weeks thereafter, and at terminal kill.
Method of Kill		
Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy		
Necropsies were performed on all core study rats and mice. Following blood collection, the right femur, heart, right kidney, liver (right lateral and caudate lobes), right lung lobe, and right testis were collected from core study rats and mice and weighed. In addition, whole liver, whole lung and left lung plus mainstem bronchi were weighed. For tissue burden study rats and mice, the lungs with mainstem bronchi were removed and weighed and the right and left lung lobes were collected and weighed individually. Samples were stored in plastic containers at approximately −70°C until analyzed for cobalt metal.	Necropsies were performed on core study and special study rats and mice. For core study animals, organs weighed were heart, right kidney, liver, lung, right testis, thymus, and thyroid gland (rats).	Necropsies were performed on all animals.
Clinical Pathology		

Two-week Studies	Three-month Studies	Two-year Studies
None	Blood was collected from the retroorbital sinus of clinical pathology male rats and 10 special study female rats on days 3 and 23 and from core study animals at the end of the studies for hematology and clinical chemistry (rats). ***Hematology:*** hematocrit; packed cell volume; hemoglobin; erythrocyte, reticulocyte, and platelet counts; total nucleated cells; Howell-Jolly bodies (mice); mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, glucose, total protein, albumin, globulin, cholesterol, triglyceride, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile salts	None
Histopathology		
Histopathology was performed on core study 0, 20 (rats), and 40 (rats and mice) mg/m^3 animals. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: left kidney, liver (left and median lobes), and thyroid gland (rats and mice), brain (rats), and urinary bladder (male rats). The left lung and nose were examined in all core study animals.	Complete histopathology was performed on core study 0 (rats and mice), 5 (rats), and 10 (mice) mg/m^3 animals. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (bronchial, mandibular, mesenteric, and mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathology was performed on all core study rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (bronchial, mandibular, mesenteric, and mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.

Two-week Studies	Three-month Studies	Two-year Studies
Sperm Motility and Vaginal Cytology		
None	At the end of the studies, spermatid and sperm samples were collected from male animals in the 0, 1.25 (rats), 2.5, 5, or 10 (mice) mg/m^3 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. Vaginal samples were collected for 12 days during the last 2 weeks of the study from females exposed to 0, 1.25 (rats), 2.5, 5, or 10 (mice) mg/m^3.	None
Tissue Burden Studies		
Urine was collected from core study rats for 16 hours beginning day 12; volume and creatinine and cobalt metal concentrations were determined. Blood was collected from the retroorbital sinus of core study rats and mice and two female tissue burden study rats and mice per group on the last day of exposure and from three female tissue burden study rats and mice per group 3 weeks postexposure; blood and serum were analyzed for cobalt metal concentration. Following blood collection, the right femur, heart, right kidney, liver (right lateral and caudate lobes), right lung lobe, and right testis were collected from core study animals and weighed. In addition, whole liver, whole lung, and left lung plus mainstem bronchi were removed and weighed and the right and left lung lobes were collected and weighed individually. Tissues were analyzed for cobalt metal concentration.	Lungs and blood (retroorbital sinus) were collected from three special study female rats and mice per exposure group on days 5, 12, 26, 40, 61, and 89 and on days 7, 14, 28, and 42 postexposure. Liver (right lateral and caudate lobes) was also collected on days 26 and 40. Liver and lungs were weighed; blood, liver, and lungs were analyzed for cobalt metal concentration.	On days 1, 2, 3, 4, 184, 366, and 548, lungs were removed from five female lung burden study rats and mice per group, weighed, and analyzed for cobalt metal concentration.

Two-week Studies	Three-month Studies	Two-year Studies
Cytochrome P450 Activities		
None	On days 26 and 40, liver samples from special study rats and mice not used for the tissue burden studies were collected and acetanilide-4-hydroxylase, 7-ethoxyresorufin-O-deethylase, and 7-pentoxyresorufin-O-deethylase activities were determined.	None
Mutation Analysis of Alveolar/bronchiolar Carcinomas		
None	None	DNA was extracted from the formalin-fixed, paraffin-embedded, rat and mouse alveolar/bronchiolar carcinomas. The samples were subjected to semi-nested PCR to amplify hot spot regions of *Kras*, *Egfr*, and *Tp53*. The lyophilized polymerase chain reaction products were sequenced, and the resulting electropherograms were compared to identify mutations in alveolar/bronchiolar carcinomas that either arose spontaneously or due to exposure to cobalt metal.

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier[172] and is presented in the form of graphs. Animals found dead of other than natural causes were censored; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's[173] method for testing two groups for equality and Tarone's[174] life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Table A-1, Table A-7, Table B-1, Table B-7, Table C-1, Table C-4, Table D-1, and Table D-4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Table A-2, Table B-2, Table C-2, and Table D-2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Table A-2,

Table B-2, Table C-2, and Table D-2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal kill.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test[175-177] was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal kill; if the animal died prior to terminal kill and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time[175]. Unless otherwise specified, a value of k = 3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier[175] following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344/N rats and B6C3F1 mice[178]. Bailer and Portier[175] showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams[179].

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

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett[180] and Williams[181; 182]. Hematology; clinical chemistry; urine, blood, serum, and tissue cobalt metal concentrations; cytochrome P450 activities; spermatid and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[183] (as modified by Williams[184]) and Dunn[185]. Jonckheere's test[186] 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[187] were examined by NTP personnel,

and implausible values were eliminated from the analysis. Proportions of regular cycling females in each dosed group were compared to the control group using the Fisher exact test[188]. Tests for extended periods of estrus, diestrus, metestrus, and proestrus, as well as skipped estrus and skipped diestrus, were constructed based on a Markov chain model proposed by Girard and Sager[189]. For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within each stage as well as for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical control database must be generally similar. Significant factors affecting the background incidences of neoplasms at a variety of sites are diet, sex, strain/stock, and route of exposure. The NTP historical control database contains all 2-year studies for each species, sex, and strain/stock with histopathology findings in control animals completed within the most recent 5-year period[190-192]. In general, the historical control database for a given study includes studies using the same route of administration, and the overall incidences of neoplasms in controls for all routes of administration are included for comparison, including the current mouse study. The current study is the only inhalation study in F344/NTac rats in the historical control database; therefore only historical control incidences for all routes and all vehicles are used for F344/NTac rats in this Technical Report.

Quality Assurance Methods

The 2-week, 3-month, and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58[193]). In addition, as records from the 3-month and 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assessment contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of cobalt metal was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division[194; 195]. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term in vitro and in vivo genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity[196] and the somatic mutation theory of cancer[197; 198]. However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites[199]. A positive response in the *Salmonella* test was shown to be the most predictive in vitro indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens)[200; 201]. Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute in vivo bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test[202; 203]. However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies[204]. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of in vivo genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

Results

Data Availability

The National Toxicology Program (NTP) evaluated all study data. Data relevant for evaluating toxicological findings are presented here. All study data are available in the NTP Chemical Effects in Biological Systems (CEBS) database: https://doi.org/10.22427/NTP-DATA-TR-581.

Rats

Two-week Study

The exposure concentrations for the 2-week study were estimated based on exposures of previously studied cobalt sulfate heptahydrate[66]. All rats exposed to 40 mg/m^3 and all male and three female rats exposed to 20 mg/m^3 died before the end of the study; the majority of deaths occurred by study day 7 (Table 2). Final mean body weights were significantly decreased in male and female rats exposed to 10 mg/m^3 and were 20% and 12% less than those of the chamber control groups, respectively. In addition, the final mean body weight was significantly decreased in female rats exposed to 20 mg/m^3 and was 45% less than that of the chamber controls. Mean body weight gains of 10 mg/m^3 males and females and 20 mg/m^3 females were significantly less than those of the chamber controls. Females exposed to 20 mg/m^3 lost weight during the study. Exposure-related clinical findings included abnormal breathing, lethargy, and thinness in male rats exposed to 20 or 40 mg/m^3, and in females exposed to 40 mg/m^3. Dark lungs were observed at necropsy in all early-death rats of both sexes exposed to 40 mg/m^3 and most rats exposed to 20 mg/m^3. Pale lungs were noted in two females exposed to 20 mg/m^3, four males exposed to 10 mg/m^3, and one male exposed to 5 mg/m^3.

Table 2. Survival and Body Weights of Rats in the Two-week Inhalation Study of Cobalt Metal[a]

Concentration (mg/m^3)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	5/5	102 ± 2	144 ± 3	41 ± 2	
2.5	5/5	102 ± 2	144 ± 2	42 ± 2	100
5	5/5	102 ± 3	140 ± 4	38 ± 2	97
10	5/5	100 ± 3	155 ± 6**	14 ± 4**	80
20	0/5[c]	103 ± 3	–	–	–
40	0/5[d]	101 ± 3	–	–	–
Female					
0	5/5	88 ± 4	112 ± 4	24 ± 2	
2.5	5/5	88 ± 2	112 ± 2	24 ± 1	100
5	5/5	86 ± 3	107 ± 3	21 ± 1	96
10	5/5	87 ± 4	98 ± 4**	11 ± 1**	88
20	2/5[e]	86 ± 3	61 ± 5**	−23 ± 0**	55

Concentration (mg/m^3)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
40	0/5[f]	86 ± 3	–	–	–

**Significantly different ($P \leq 0.01$) from the chamber control group by Williams' test.
[a]Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[b]Number of animals surviving at 2 weeks/number initially in group.
[c]Days of deaths: 5, 5, 5, 9, 13.
[d]Days of deaths: 5, 6, 6, 7, 7.
[e]Days of deaths: 5, 7, 13.
[f]Days of deaths: 5, 6, 6, 6, 7.

Absolute lung weights of females exposed to 10 or 20 mg/m^3 and the relative lung weights of both sexes exposed to 10 mg/m^3 and females exposed to 20 mg/m^3 were significantly greater than those of the chamber controls (Table 3 and Table G-1). Absolute and relative liver weights of males exposed to 2.5 mg/m^3 or greater and absolute liver weights of females exposed to 5 mg/m^3 or greater were significantly less than those of the chamber controls. The relative liver weight of 20 mg/m^3 females was significantly greater than that of the chamber controls. Absolute kidney and thymus weights of males exposed to 10 mg/m^3 and females exposed to 20 mg/m^3.

Table 3. Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Two-week Inhalation Study of Cobalt Metal[a]

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Male						
n	5	5	5	5	0	0
Necropsy body wt	144 ± 3	144 ± 2	140 ± 4	115 ± 6**	–	–
L. Kidney						
Absolute	0.61 ± 0.02	0.61 ± 0.01	0.58 ± 0.01	0.52 ± 0.02**	–	–
Relative	4.25 ± 0.06	4.26 ± 0.08	4.12 ± 0.09	4.57 ± 0.10*	–	–
Liver						
Absolute	5.84 ± 0.16	5.10 ± 0.09**	5.08 ± 0.15**	4.29 ± 0.24**	–	–
Relative	40.61 ± 0.46	35.40 ± 0.28**	36.35 ± 0.63**	37.43 ± 0.86**	–	–
Lung						
Absolute	1.14 ± 0.10	1.16 ± 0.08	1.19 ± 0.04	1.28 ± 0.12	–	–
Relative	7.91 ± 0.61	8.07 ± 0.55	8.49 ± 0.30	11.13 f± 0.50**	–	–
L. Testis						
Absolute	0.886 ± 0.040	0.928 ± 0.017	0.852 ± 0.035	0.590 ± 0.088**	–	–
Relative	6.165 ± 0.246	6.446 ± 0.155	6.103 ± 0.248	5.053 ± 0.502	–	–
Thymus						
Absolute	0.374 ± 0.013	0.358 ± 0.025	0.358 ± 0.007	0.284 ± 0.008**	–	–

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Relative	2.605 ± 0.054	2.485 ± 0.161	2.560 ± 0.023	2.498 ± 0.112	–	–
Female						
n	5	5	5	5	2	0
Necropsy body wt	112 ± 4	112 ± 2	107 ± 3	98 ± 4**	61 ± 5**	–
L. Kidney						
Absolute	0.52 ± 0.02	0.50 ± 0.01	0.50 ± 0.02	0.46 ± 0.01*	0.35 ± 0.00**	–
Relative	4.66 ± 0.11	4.46 ± 0.05	4.63 ± 0.08	4.74 ± 0.12	5.75 ± 0.42**	–
Liver						
Absolute	4.07 ± 0.16	3.77 ± 0.05	3.61 ± 0.13**	3.44 ± 0.05**	2.57 ± 0.06**	–
Relative	36.37 ± 0.49	33.59 ± 0.16	33.78 ± 1.08	35.17 ± 1.00	42.15 ± 2.12**	–
Lung						
Absolute	0.86 ± 0.04	0.83 ± 0.01	0.91 ± 0.04	1.03 ± 0.06*	1.01 ± 0.04*	–
Relative	7.71 ± 0.36	7.44 ± 0.07	8.49 ± 0.34	10.54 ± 0.69**	16.54 ± 0.56**	–
Thymus						
Absolute	0.317 ± 0.016	0.324 ± 0.011	0.352 ± 0.022	0.289 ± 0.011	0.064 ± 0.016**	–
Relative	2.842 ± 0.167	2.895 ± 0.126	3.289 ± 0.201	2.948 ± 0.092	1.024 ± 0.178**	–

*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). No data are available for 20 mg/m^3 males or 40 mg/m^3 males or females due to 100% mortality and the relative thymus weights of females exposed to 20 mg/m^3 were significantly less than those of the chamber controls. Absolute testis weight of the 10 mg/m^{3}group was significantly less than that of the chamber controls; no histopathologic assessment was performed.

Increased incidences of nonneoplastic lesions of the lung occurred in exposed male and female rats, and the lesions were generally of minimal to mild severity (Table 4). The incidences of minimal to moderate hemorrhage were significantly increased in male rats exposed to 20 or 40 mg/m^3 and females exposed to 40 mg/m^3. Hemorrhage was observed in rats that died or were killed moribund and consisted of erythrocytes within the alveoli. Incidences of minimal to mild acute inflammation were significantly increased in males exposed to 20 or 40 mg/m^3 and females exposed to 40 mg/m^3. Inflammation consisted of proteinaceous fluid (edema) and infiltrates of neutrophils within the alveolar septa and spaces. Incidences of minimal to mild alveolar epithelium hyperplasia were increased in male and female rats exposed to 20 or 40 mg/m^3; the incidence in 40 mg/m^3 males was significantly increased. Hyperplasia consisted of focal, variably sized proliferation of the cuboidal to polygonal epithelial (Type II) cells along the alveolar septa; cell nuclei varied from round to pleomorphic. Incidences of alveolus histiocytic cellular infiltration were generally significantly increased in males exposed to 5 mg/m^3 or greater and in females exposed to 20 or 40 mg/m^3. Histiocyte infiltration consisted of increased numbers of foamy histiocytes (macrophages) within alveolar spaces. Incidences of cytoplasmic vacuolization of bronchiolar epithelium were significantly increased in 2.5, 5, or 10 mg/m^3 males and females. Affected bronchiolar epithelial cells were enlarged and had a foamy appearance due

to the accumulation of poorly demarcated cytoplasmic vacuoles. Incidences of necrosis of the bronchiolar epithelium were increased in males exposed to 20 or 40 mg/m^3 and in females exposed to 10 mg/m^3 or greater. Necrosis consisted of segmental to complete loss of the epithelium in terminal bronchioles; in some cases the remaining epithelial cells appeared hypereosinophilic. Minimal to moderate randomly scattered areas of interstitial fibrosis of the alveolar septa were associated with areas of inflammation and alveolar epithelium hyperplasia in males and females exposed to 10 or 20 mg/m^3. Fibrosis consisted of irregular expansion of the interstitium of the alveolar septa by variable amounts of collagen fibers.

Increased incidences of nonneoplastic lesions of the nose occurred in exposed male and female rats (Table 4). Incidences of minimal to moderate olfactory epithelium necrosis were significantly increased in most exposed groups of rats. Necrosis consisted of vacuolization and disorganization of the olfactory epithelium lining the dorsal meatus in Levels II and III nasal sections and the medial surface of ethmoturbinates in Level III; in some cases there was partial or complete loss of the epithelium. The incidences of olfactory epithelium atrophy were significantly increased in all groups of rats exposed to 2.5, 5, or 10 mg/m^3 and in females exposed to 20 mg/m^3. Atrophy accompanied necrosis and appeared as decreased numbers of layers and density of olfactory epithelial cells in the areas adjacent to necrosis. The remaining olfactory cells were often disorganized with clear spaces between rows of neuronal and sustentacular epithelial cells. The incidences of respiratory epithelium necrosis were significantly increased in 40 mg/m^3 males and females exposed to 20 or 40 mg/m^3. Necrosis was a subtle lesion characterized by focal degeneration and loss of the mucosal epithelium; in some cases, a decrease in the height (atrophy) of the epithelium resulted. In some areas, a single layer of flat epithelial cells covered areas of degenerated epithelium and was considered an attempt at epithelial regeneration. In a few other areas, a thin layer of squamous epithelium replaced the olfactory and respiratory epithelia and was diagnosed as respiratory epithelium squamous metaplasia.

Table 4. Incidences of Selected Nonneoplastic Lesions of the Respiratory System in Rats in the Two-week Inhalation Study of Cobalt Metal

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Male						
Lung[a]	5	5	5	5	5	5
Hemorrhage[b]	0	0	0	1 (1.0)[c]	5** (1.2)	5** (3.0)
Inflammation, Acute	0	0	0	0	4* (1.3)	5** (2.0)
Alveolar Epithelium, Hyperplasia	0	0	0	0	3 (1.7)	5** (1.4)
Alveolus, Infiltration Cellular, Histiocyte	0	0	4* (1.0)	3 (1.3)	5** (2.0)	5** (1.2)
Bronchiole, Epithelium, Vacuolization Cytoplasmic	0	5** (1.2)	5** (1.6)	5**(2.0)	1 (2.0)	0
Bronchiole, Epithelium, Necrosis	0	0	0	0	2 (1.0)	3 (1.0)

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Interstitium, Fibrosis	0	0	0	5** (1.2)	2 (3.0)	0
Nose	5	5	5	5	5	5
Olfactory Epithelium, Necrosis	0	3 (1.0)	4* (1.3)	4* (1.0)	4* (2.8)	5** (3.0)
Olfactory Epithelium, Atrophy	0	5** (1.6)	5** (1.8)	5** (2.4)	3 (1.7)	3 (1.7)
Respiratory Epithelium, Necrosis	0	0	0	1 (1.0)	3 (1.3)	5** (1.4)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	1 (1.0)	2 (1.0)	1 (1.0)
Female						
Lung	5	5	5	5	5	5
Hemorrhage	0	0	0	0	3 (2.0)	5** (2.8)
Inflammation, Acute	0	0	0	0	2 (1.0)	5** (1.4)
Alveolar Epithelium, Hyperplasia	0	0	0	0	2 (1.0)	2 (1.0)
Alveolus, Infiltration Cellular, Histiocyte	0	0	0	0	5** (2.0)	5** (1.8)
Bronchiole, Epithelium, Vacuolization Cytoplasmic	0	4* (1.0)	5** (1.0)	5** (1.8)	3 (1.7)	0
Bronchiole, Epithelium, Necrosis	0	1 (1.0)	1 (1.0)	4* (1.0)	3 (1.0)	3 (1.0)
Interstitium, Fibrosis	0	0	0	4* (1.0)	3 (3.0)	0
Nose	5	5	5	5	5	5
Olfactory Epithelium, Necrosis	0	5** (1.0)	3 (1.0)	5** (1.0)	5** (2.0)	5** (3.0)
Olfactory Epithelium, Atrophy	0	5** (1.8)	5** (2.0)	5** (2.0)	4* (2.8)	1 (2.0)
Respiratory Epithelium, Necrosis	0	0	0	0	5** (1.4)	5** (1.4)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	0	1 (1.0)	0

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

Tissue Burden Studies

Tissue weights and concentrations were determined in male and female rats at terminal kill and in additional female rats held for 3 weeks postexposure (Appendix I). Data were generated on

male rats exposed to 10 mg/m^3 or less due to mortality at 20 mg/m^3. In females, data were generated on all exposure groups; however, a relatively small number of samples (n = 1 to 3) was available in 20 mg/m^3 females due to decreased survival.

Male and female rat lung weights increased with increasing exposure concentration at terminal kill and in females held for the 3-week recovery period (Table I-1); these increases were significant at higher exposure concentrations in females. In general, kidney, liver, heart, and femur weights decreased with increasing exposure concentration in males and females; some of these decreases were significant at higher exposure concentrations. In males exposed to 10 mg/m^3, testis weights were decreased in comparison to chamber controls. Because of the significant changes in female lung weights, lung burdens rather than concentrations were evaluated for toxicokinetic parameters.

At terminal kill, cobalt concentrations and burdens increased with increasing exposure concentration in all tissues examined (Table I-1). In general, normalized burdens did not increase with increasing exposure concentration, with the exception of the liver in males and females. Cobalt concentrations in tissues decreased in the order of lung > liver > kidney > femur > heart > serum > blood ~ testes (males). Cobalt burdens in the tissues of male and female rats decreased in the order of liver > lung > kidney > heart > femur ~ testes (males). These data indicate that the tissues examined tended to accumulate cobalt at concentrations greater than could be found in blood and serum, that cobalt was distributed to extra-pulmonary tissues, and that more cobalt accumulated in the liver than in the lung, particularly at the higher concentrations. At 3 weeks postexposure in female rats, cobalt concentrations were markedly reduced in blood, serum, and lung.

Kinetic analysis of data from female rats exposed to 20 mg/m^3 or less indicated elimination half-lives of 9.2 to 11.1 days (blood), 2.8 to 3.4 days (serum; 10 and 20 mg/m^3 only, due to undetectable serum concentrations of cobalt at lower exposure concentrations at 3 weeks postexposure) and 4.2 to 5.6 days (lung) (Table I-2). Lung cobalt deposition rates and predicted steady-state lung cobalt burdens generally increased less than proportionally across exposure concentrations except when comparing 10 and 20 mg/m^3.

In general, the volume of urine collected from male and female rats during the 16-hour collection period after exposure on day 12 decreased with increasing exposure concentration (Table I-3). Increased creatinine concentrations were observed in both sexes in the higher exposure concentration groups. Urinary cobalt concentration increased with increasing exposure concentration in both sexes. When normalized to creatinine, cobalt concentrations increased approximately in proportion to exposure concentration. Total cobalt excreted increased with exposure at lower concentrations before decreasing at higher concentrations.

Exposure Concentration Selection Rationale: Based on significant mortality in male and female rats exposed to 20 and 40 mg/m^3 and body weight reductions in the 10 mg/m^3 groups coupled with reduced urine volumes with concomitant increases in urine creatinine at the end of the 2-week study, 5 mg/m^3 was selected as the highest exposure concentration for the 3-month inhalation study in rats. The lesions in the nose were minimal in the 5 mg/m^3 group and were not considered sufficiently severe to preclude the use of this concentration. When exposure concentrations for the 3-month studies are different for two species, NTP has elected to have one less exposure concentration for one species as opposed to adding an extra chamber to

accommodate the differences in species. Hence, only four concentrations were used in the 3-month rat studies.

Three-month Study

All male and female rats survived to the end of the study (Table 5). The final mean body weights of males and females exposed to 5 mg/m^3 were significantly less than those of the chamber controls, and the mean body weight gain of 5 mg/m^3 males was significantly less than that of the chamber controls (Table 5 and Figure 1). There were no clinical signs related to cobalt metal exposure. At necropsy, pale foci were noted in the lungs of most exposed male and female rats. Based on reports in the literature describing the ability of cobalt to decrease hepatic cytochrome P450 levels and activity[9], these enzymes were assayed in the current study. On days 26 and 40, microsomal suspensions of liver samples from special study female rats not used for tissue burden studies were prepared and acetanilide-4-hydroxylase (A4H), 7-ethoxyresorufin-*O*-deethylase (EROD), and 7-pentoxyresorufin-*O*-deethylase (PROD) activities were determined (Table J-1). There were no consistent trends in A4H, EROD, or PROD activities relative to exposure concentrations at either time point.

Table 5. Survival and Body Weights of Rats in the Three-month Inhalation Study of Cobalt Metal[a]

Concentration (mg/m^3)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	107 ± 2	319 ± 5	212 ± 4	
0.625	10/10	107 ± 3	336 ± 6	229 ± 4	105
1.25	10/10	107 ± 2	327 ± 7	220 ± 6	102
2.5	10/10	107 ± 3	326 ± 6	220 ± 5	102
5	10/10	107 ± 3	297 ± 5*	190 ± 4**	93
Female					
0	10/10	88 ± 3	201 ± 3	113 ± 4	
0.625	10/10	88 ± 3	205 ± 4	117 ± 5	102
1.25	10/10	89 ± 3	198 ± 4	109 ± 2	98
2.5	10/10	88 ± 2	199 ± 4	111 ± 3	99
5	10/10	87 ± 2	187 ± 3*	100 ± 4	93

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

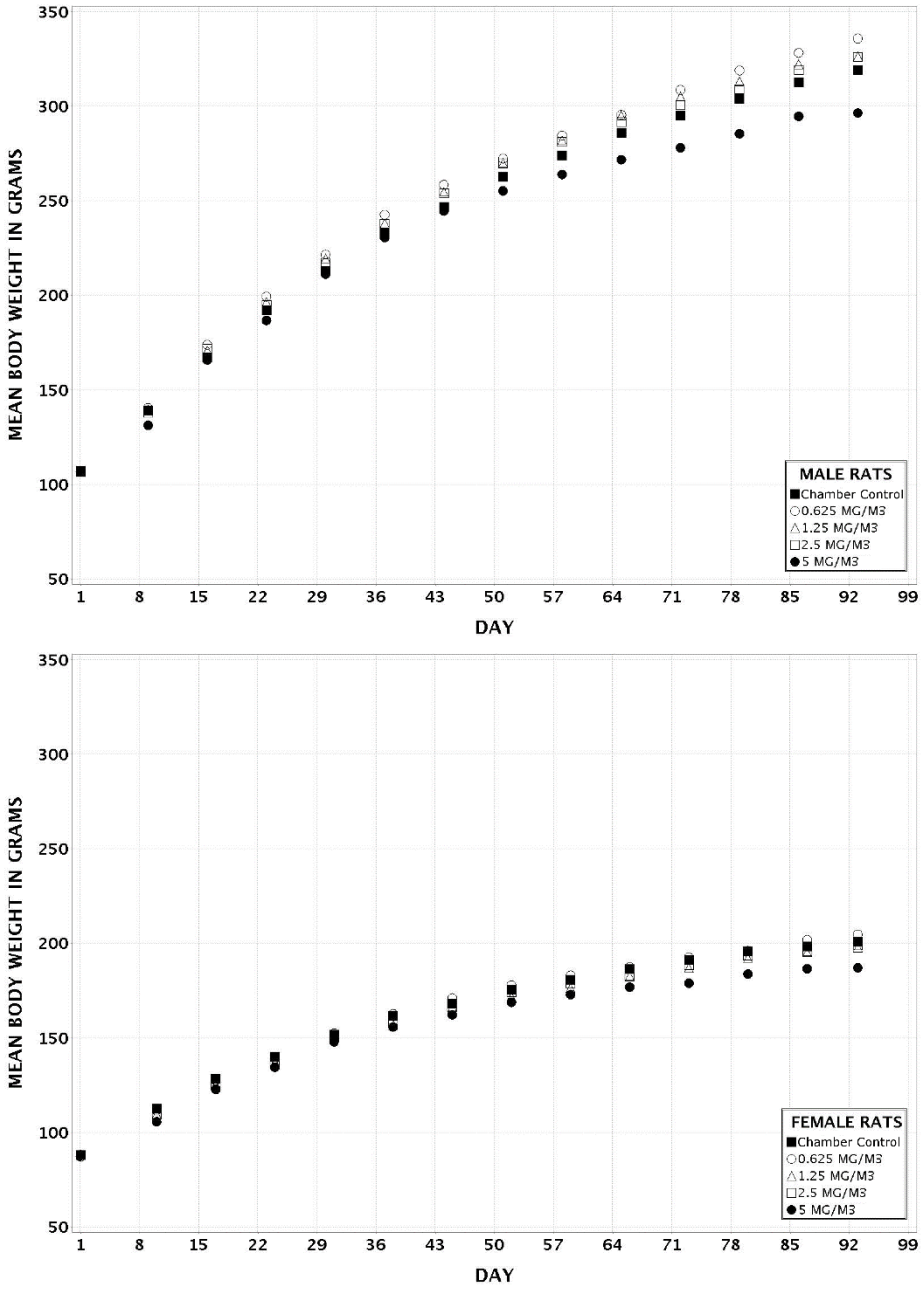


Figure 1. Growth Curves for Rats Exposed to Cobalt Metal by Inhalation for Three Months

The hematology and clinical chemistry data for rats are presented in Table 6 and Table F-1. Erythrocytosis characterized by exposure concentration-related increases in the hemoglobin concentration, erythrocyte count, hematocrit value, and manual packed cell volume occurred in males exposed to 2.5 and 5 mg/m^3 on days 3 and 23 and all exposed groups by week 14; at week 14, female rats also had increases in these parameters. Animals in the lower exposure groups demonstrated these increases but less consistently. In addition, reticulocyte counts were increased in 5 mg/m^3 males at all three time points, while in the female rats, reticulocyte counts were increased in all exposed groups on day 23 and in the 2.5 and 5 mg/m^3 groups at week 14. At week 14, platelet counts were mildly (<18%) decreased in all of the exposed groups of

females and in males exposed to 1.25 mg/m^3 or greater. These platelet count changes may represent an altered peripheral distribution or decreased production. All other hematology changes observed were considered within biological variability and not toxicologically relevant.

At week 14, total protein concentrations were mildly decreased (<10%) in 2.5 and 5 mg/m^3 females, as well as 5 mg/m^3 males. This change was paired with mild decreases in albumin and globulin concentrations in 5 mg/m^3 females and a mild decrease in globulin concentration in 5 mg/m^3 males. These changes are most likely related to altered food intake compared to concurrent chamber controls and are supported by the mild decreases (7%) in body weights of 5 mg/m^3 males and females. Exposure concentration-dependent decreases in cholesterol concentrations were observed at all three time points in both males and females. While this change was not always observed with the lower exposure concentrations, these decreases were consistently observed in the 2.5 and 5 mg/m^3 groups of both sexes on day 23 and at week 14. In addition, glucose concentration was decreased in 1.25 mg/m^3 or greater males at week 14. All other biochemical changes were transient or inconsistent and not considered toxicologically relevant.

Absolute and relative lung weights of all exposed groups of males and females were significantly greater than those of the chamber controls (Table G-2). The increased lung weights are related to the histopathologic changes observed in the lungs.

Table 6. Selected Clinical Pathology Data for Rats in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male					
Hematology					
n					
Day 3	10	10	10	10	10
Day 23	10	10	10	9	10
Week 14	10	10	10	10	10
Hematocrit (spun) (%)					
Day 3	46.5 ± 0.2	46.4 ± 0.2	47.4 ± 0.2*	47.7 ± 0.3**	51.2 ± 0.4**
Day 23	47.9 ± 0.4	48.5 ± 0.4	49.9 ± 0.5**	51.1 ± 0.2**	53.3 ± 0.4**
Week 14	49.6 ± 0.4	51.6 ± 0.4**	59.2 ± 0.4**	61.8 ± 0.2**	63.9 ± 0.3**
Packed cell volume (mL/dL)					
Day 3	45.1 ± 0.2	44.5 ± 0.3	45.7 ± 0.3	46.1 ± 0.4*	49.7 ± 0.4**
Day 23	46.8 ± 0.4	47.3 ± 0.4	49.0 ± 0.6**	49.7 ± 0.2**	51.5 ± 0.5**
Week 14	49.6 ± 0.5	51.7 ± 0.4**	58.3 ± 0.4**	60.7 ± 0.1**	62.9 ± 0.3**
Hemoglobin (g/dL)					
Day 3	13.7 ± 0.1	13.8 ± 0.1	14.1 ± 0.1**	14.2 ± 0.1**	15.5 ± 0.1**
Day 23	15.0 ± 0.1	14.9 ± 0.1	15.4 ± 0.2	15.8 ± 0.1**	16.1 ± 0.2**

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Week 14	15.6 ± 0.1	16.3 ± 0.1**	18.7 ± 0.1**	19.7 ± 0.1**	20.3 ± 0.1**
Erythrocytes (10^6/μL)					
Day 3	7.30 ± 0.06	7.27 ± 0.09	7.46 ± 0.05	7.57 ± 0.08*	8.22 ± 0.08**
Day 23	7.97 ± 0.09	8.05 ± 0.09	8.36 ± 0.11*	8.44 ± 0.06**	9.27 ± 0.14**
Week 14	9.19 ± 0.10	9.67 ± 0.09**	11.20 ± 0.06**	11.80 ± 0.06**	11.90 ± 0.08**
Reticulocytes (10^3/μL)					
Day 3	612.0 ± 42.3	562.3 ± 24.5[b]	613.0 ± 30.8	622.1 ± 25.6	873.8 ± 53.4**
Day 23	262.9 ± 13.9	261.3 ± 17.8	280.9 ± 14.3	291.6 ± 18.6	339.6 ± 25.1*
Week 14	219.8 ± 16.0	191.7 ± 19.9	189.3 ± 13.1	272.7 ± 18.7	360.0 ± 35.9*
Platelets (10^3/μL)					
Day 3	899.7 ± 14.0	883.5 ± 23.9	939.4 ± 14.4	903.8 ± 22.8	1,123.7 ± 34.2**
Day 23	740.8 ± 16.2	712.0 ± 16.3	732.1 ± 22.7	682.0 ± 44.5	796.7 ± 12.8
Week 14	682.5 ± 27.7	646.8 ± 8.7	611.2 ± 13.8*	548.2 ± 22.3**	573.2 ± 15.3**
Clinical Chemistry					
n	10	10	10	10	10
Total protein (g/dL)					
Day 3	6.1 ± 0.1	5.9 ± 0.0	6.0 ± 0.0	6.1 ± 0.1	6.3 ± 0.1*
Day 23	6.5 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.4 ± 0.1
Week 14	7.4 ± 0.1	7.3 ± 0.0	7.4 ± 0.1	7.3 ± 0.1	7.1 ± 0.1**
Albumin (g/dL)					
Day 3	4.3 ± 0.0	4.1 ± 0.0**	4.2 ± 0.0	4.3 ± 0.0	4.3 ± 0.0
Day 23	4.6 ± 0.1	4.5 ± 0.0	4.5 ± 0.1	4.5 ± 0.0	4.4 ± 0.0
Week 14	4.9 ± 0.1	4.8 ± 0.0	4.8 ± 0.0	4.8 ± 0.0	4.8 ± 0.0
Globulin (g/dL)					
Day 3	1.8 ± 0.0	1.7 ± 0.0	1.8 ± 0.0	1.9 ± 0.1	2.0 ± 0.0**
Day 23	2.0 ± 0.0	2.0 ± 0.0	2.1 ± 0.1	2.0 ± 0.0	2.0 ± 0.0
Week 14	2.5 ± 0.0	2.5 ± 0.0	2.6 ± 0.0	2.5 ± 0.0	2.3 ± 0.0**
Cholesterol (mg/dL)					
Day 3	95 ± 2	87 ± 1*	84 ± 1**	79 ± 2**	81 ± 2**
Day 23	79 ± 1	76 ± 2	70 ± 1**	73 ± 2**	63 ± 1**
Week 14	91 ± 1	91 ± 1	88 ± 2	79 ± 2**	67 ± 1**
Glucose (mg/dL)					
Day 3	135 ± 2	138 ± 1	134 ± 2	136 ± 1	129 ± 4
Day 23	132 ± 3	132 ± 4	143 ± 9	139 ± 8	120 ± 2
Week 14	126 ± 3	128 ± 3	118 ± 5*	111 ± 3**	104 ± 4**

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Female					
n	10	10	10	10	10
Hematology					
Hematocrit (spun) (%)					
Day 3	49.0 ± 0.6	47.8 ± 0.7	47.6 ± 0.5	48.9 ± 0.3	49.9 ± 0.5
Day 23	51.6 ± 0.5	50.9 ± 0.3	51.0 ± 0.3	52.0 ± 0.2	53.3 ± 1.9**
Week 14	48.3 ± 0.5	48.7 ± 0.4	52.6 ± 0.6**	57.3 ± 0.5**	59.5 ± 0.3**
Packed cell volume (mL/dL)					
Day 3	47.6 ± 0.6	46.7 ± 0.6	46.5 ± 0.5	47.6 ± 0.3	48.3 ± 0.5
Day 23	51.3 ± 0.5	50.2 ± 0.3	50.5 ± 0.3	51.0 ± 0.3	51.7 ± 1.7
Week 14	49.1 ± 0.5	49.5 ± 0.4	53.1 ± 0.5**	57.1 ± 0.5**	60.0 ± 0.4**
Hemoglobin (g/dL)					
Day 3	14.8 ± 0.2	14.4 ± 0.2	14.5 ± 0.2	14.7 ± 0.1	15.2 ± 0.2
Day 23	16.1 ± 0.1	15.8 ± 0.1	16.0 ± 0.1	16.2 ± 0.1	16.4 ± 0.6*
Week 14	15.5 ± 0.2	15.8 ± 0.1*	16.9 ± 0.2**	18.3 ± 0.1**	19.1 ± 0.1**
Erythrocytes (10^6/µL)					
Day 3	7.79 ± 0.12	7.62 ± 0.13	7.65 ± 0.09	7.77 ± 0.08	8.07 ± 0.12
Day 23	8.49 ± 0.09	8.24 ± 0.07	8.43 ± 0.10	8.56 ± 0.05	8.75 ± 0.31*
Week 14	8.55 ± 0.09	8.69 ± 0.06	9.30 ± 0.10**	10.05± 0.07**	10.47 ± 0.09 **
Reticulocytes (10^3/µL)					
Day 3	489.3 ± 22.4	497.7 ± 30.8	544.1 ± 28.1	511.9 ± 25.5	520.0 ± 23.6
Day 23	184.1 ± 11.1	244.2 ± 9.9**	212.9 ± 12.4*	276.7 ± 18.9**	386.6 ± 20.4**
Week 14	202.5 ± 13.0	224.5 ± 13.0	222.2 ± 8.8	246.5 ± 10.8*	316.7 ± 25.0**
Platelets (10^3/µL)					
Day 3	905.6 ± 27.8	841.4 ± 22.5	914.7 ± 18.8	912.4 ± 20.1	875.0 ± 27.4
Day 23	783.1 ± 20.5	799.2 ± 13.2	763.0 ± 25.7	800.7 ± 21.5	807.9 ± 22.2
Week 14	702.1 ± 8.0	660.2 ± 14.7*	646.6 ± 17.3**	575.3 ± 16.7**[b]	608.0 ± 16.8**
Clinical Chemistry					
Total protein (g/dL)					
Day 3	6.2 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.0
Day 23	6.5 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.5 ± 0.1
Week 14	7.6 ± 0.1	7.5 ± 0.1	7.4 ± 0.1	7.4 ± 0.1*	7.0 ± 0.1**
Albumin (g/dL)					
Day 3	4.6 ± 0.1	4.4 ± 0.0	4.4 ± 0.1	4.5 ± 0.0	4.4 ± 0.0

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Day 23	4.7 ± 0.0	4.7 ± 0.0	4.7 ± 0.0	4.7 ± 0.0	4.7 ± 0.1
Week 14	5.3 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.0	5.0 ± 0.1**
Globulin (g/dL)					
Day 3	1.6 ± 0.0	1.6 ± 0.0	1.7 ± 0.1	1.6 ± 0.0	1.8 ± 0.0**
Day 23	1.8 ± 0.0	1.8 ± 0.0	1.9 ± 0.1	1.9 ± 0.0	1.9 ± 0.1
Week 14	2.3 ± 0.0	2.3 ± 0.0	2.3 ± 0.1	2.3 ± 0.0	2.1 ± 0.1**
Cholesterol (mg/dL)					
Day 3	98 ± 2	95 ± 2	91 ± 2*	91 ± 2*	93 ± 2
Day 23	102 ± 3	97 ± 2	97 ± 2	93 ± 3*	89 ± 3**
Week 14	102 ± 3	100 ± 2	92 ± 3*	91 ± 3**	76 ± 2**

*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.
[b]n = 9.

Sperm motility was significantly decreased in males exposed to 1.25, 2.5, or 5 mg/m^3, and the decrease in the 5 mg/m^3 group was approximately 8% (Table 7 and Table H-1). The Markov transition matrix analyses of estrous cyclicity indicated that females in the 5 mg/m^3 group had a significantly higher probability of extended diestrus than the chamber control females (Table H-2 and Table H-3; Figure H-1). The toxicologic significance of this subtle alteration in the estrous cycle is unclear and there were no cobalt-related histopathologic findings observed in the female reproductive organs.

In the lung, a spectrum of nonneoplastic lesions was observed that included chronic active inflammation, alveolus proteinosis, bronchiole epithelium hyperplasia, and alveolar epithelium hyperplasia (Table 8). Minimal to mild chronic active inflammation and generally minimal to mild alveolus proteinosis occurred in all exposed males and females, and minimal to mild bronchiole epithelium hyperplasia occurred in all males and females exposed to 1.25 mg/m^3 or greater. The severities of these lesions generally increased with increasing exposure concentration. Chronic active inflammation consisted of a mixture of macrophages, neutrophils, and lymphocytes in the alveolar spaces and was sometimes associated with minimal hyperplasia of the alveolar epithelium and minimal fibrosis of the alveolar interstitium. At higher exposure concentrations, the inflammatory cells were more diffusely distributed throughout the lung. However, at the lower exposure concentrations, the inflammatory cells tended to occur in focal, subpleural aggregates. The pale foci noted grossly at necropsy correlated with subpleural inflammation. There were also perivascular and peribronchiolar infiltrates of macrophages, neutrophils, and lymphocytes with extension into the adjacent alveoli. Some males and females exposed to 5 mg/m^3 had minimal infiltrates of inflammatory cells in the walls of bronchioles with minimal proliferation of fibrovascular tissue into the lumen. Alveolar proteinosis consisted of clumps of dense amorphous eosinophilic material or less dense, more dispersed accumulations of proteinaceous debris within the alveolar spaces. Alveoli also contained increased numbers of macrophages that contained eosinophilic material similar to that in the alveolar spaces. Hyperplasia of bronchiolar epithelium was characterized by proliferation of the epithelial cells

lining terminal bronchioles and alveolar ducts. The hyperplastic cells were cuboidal with increased cytoplasm that sometimes contained poorly defined cytoplasmic vacuoles.

Table 7. Summary of Reproductive Tissue Evaluations for Male Rats in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
n	10	10	10	10
Weights (g)				
Necropsy body wt	319 ± 5	327 ± 7	326 ± 6	297 ± 5*
L. Cauda epididymis	0.1741 ± 0.0054	0.1775 ± 0.0043	0.1853 ± 0.0075	0.1688 ± 0.0038
L. Epididymis	0.4850 ± 0.0095	0.4999 ± 0.0117	0.4926 ± 0.0146	0.4846 ± 0.0116
L. Testis	1.3700 ± 0.0179	1.3680 ± 0.0147	1.3778 ± 0.0205	1.3947 ± 0.0124
Spermatid measurements				
Spermatid heads (10^6/testis)	174.00 ± 10.16	180.00 ± 10.06	176.50 ± 5.81	172.50 ± 5.94
Spermatid heads (10^6/g testis)	141.3 ± 8.7	146.5 ± 8.2	142.5 ± 3.7	139.9 ± 4.8
Epididymal spermatozoal measurements				
Sperm motility (%)	88.8 ± 0.8	86.0 ± 1.1*	83.8 ± 1.3**	81.9 ± 1.3**
Sperm (10^6/cauda epididymis)	104.52 ± 3.78	98.40 ± 3.13	102.27 ± 3.04	94.15 ± 3.18
Sperm (10^6/g cauda epididymis)	602.3 ± 21.6	556.4 ± 19.3	564.1 ± 37.0	559.4 ± 19.6

*Significantly different ($P \leq 0.05$) from the chamber control group by Dunnett's test (body weight) or Shirley's test (sperm motility).
**$P \leq 0.01$.
[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, sperm/cauda epididymis, and sperm/g cauda epididymis).

Table 8. Incidences of Selected Nonneoplastic Lesions of the Respiratory System in Rats in the Three-month Inhalation Study of Cobalt Metal

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male					
Lung[a]	10	10	10	10	10
Inflammation, Chronic Active[b]	0	10** (1.9)[c]	10** (1.9)	10** (1.5)	10** (2.4)
Alveolus, Proteinosis	0	10** (1.8)	10** (2.2)	10** (2.2)	10** (2.7)
Bronchiole, Epithelium, Hyperplasia	0	0	10** (1.0)	10** (1.4)	10** (2.2)
Alveolar Epithelium, Hyperplasia	0	0	0	0	2 (1.5)
Nose	10	10	10	10	10

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Olfactory Epithelium, Degeneration	0	0	2 (1.0)	9** (1.0)	10** (2.5)
Olfactory Epithelium, Hyperplasia	0	0	2 (1.0)	6** (1.2)	10** (1.7)
Respiratory Epithelium, Hyperplasia	0	0	3 (1.0)	9** (1.0)	10** (1.8)
Turbinate, Atrophy	0	0	0	3 (1.0)	9** (1.0)
Female					
Lung	10	10	10	10	10
Inflammation, Chronic Active	2 (1.0)	10** (1.9)	10** (1.5)	10** (1.6)	10** (2.4)
Alveolus, Proteinosis	0	10** (1.8)	10** (1.9)	10** (1.9)	10** (2.1)
Bronchiole, Epithelium, Hyperplasia	0	0	10** (1.0)	10** (1.3)	10** (2.0)
Alveolar Epithelium, Hyperplasia	0	0	0	0	1 (1.0)
Nose	10	10	10	10	10
Olfactory Epithelium, Degeneration	0	0	5* (1.0)	10** (1.0)	10** (2.5)
Olfactory Epithelium, Hyperplasia	0	0	0	3 (1.0)	10** (2.2)
Respiratory Epithelium, Hyperplasia	0	1 (1.0)	0	9** (1.0)	10** (1.8)
Turbinate, Atrophy	0	0	0	4* (1.0)	6** (1.0)

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

Minimal alveolar epithelium hyperplasia occurred in low numbers of males and females exposed to 5 mg/m^3, but the increased incidences of this lesion were not statistically significant (Table 8). Alveolar epithelium hyperplasia was characterized by randomly scattered, focal, irregular proliferations of cuboidal or sometimes ciliated alveolar epithelial cells. Alveolar epithelial hyperplasia is frequently observed in inhalation studies with particulates and may be considered

a reparative response and sometimes occurs as a component of inflammatory changes in the lung.

In the nose, a spectrum of nonneoplastic lesions were observed that included olfactory epithelium degeneration and hyperplasia, respiratory epithelium hyperplasia, and turbinate atrophy. The incidences of olfactory epithelium degeneration and minimal to mild respiratory epithelium hyperplasia were significantly increased in males and females exposed to 2.5 or 5 mg/m^3; the incidence of olfactory epithelium degeneration was also significantly increased in 1.25 mg/m^3 females (Table 8). In addition, the incidences of olfactory epithelium hyperplasia were significantly increased in 2.5 mg/m^3 males and 5 mg/m^3 males and females. Degeneration of the olfactory epithelium was a focal or multifocal lesion that variably involved the epithelium lining the dorsal meatus, ethmoid turbinates, and nasal septa. In affected sites, there was vacuolization and disorganization of the epithelium with variable individual cell necrosis and/or loss of epithelial cells. Olfactory epithelium hyperplasia was characterized by clusters or nests of cells proliferating within or just adjacent to the olfactory epithelium, sometimes with extension into the lamina propria around glandular ducts. The proliferating cells sometimes formed rosettes and had scant cytoplasm and large, round to oval nuclei. Increased incidences of minimal turbinate atrophy occurred in males and females exposed to 2.5 or 5 mg/m^3; the increases were statistically significant in 2.5 mg/m^3 females and 5 mg/m^3 males and females. Respiratory epithelium hyperplasia involved the turbinates and/or lateral walls of the Level I and II nasal sections. Hyperplasia appeared as increased numbers of cells in the respiratory or transitional epithelium resulting in crowding of the cells or an increase in the number of cell layers. The epithelial cells were squamous to cuboidal, usually not ciliated, and some cells were hypertrophied. Turbinate atrophy occurred in all three sections of the nose and was characterized by short and blunt turbinates that had attenuation and/or loss of turbinate bone and interstitial tissue in the lamina propria including the glands, vessels, nerve bundles, and connective tissue, and as a result, the nasal passages appeared wider than normal.

Tissue Burden Studies

Lung and liver weights and lung, blood, and liver cobalt concentrations were determined in female rats (Table I-4). Lung weights were increased in all exposed groups starting on day 40 (5 mg/m^3) or day 61 (2.5 mg/m^3 or less) and remained greater than those in the chamber controls throughout the exposure and postexposure periods. Because of the significant changes in lung weights with exposure concentration, lung cobalt burdens rather than lung cobalt concentrations were evaluated for toxicokinetic parameters.

Liver weights of exposed groups of females were either decreased or similar to chamber controls at each time point (Table I-4).

Lung cobalt concentrations and burdens increased with increasing exposure concentration and were significantly increased over chamber controls with all exposure concentrations at all time points. By day 26, the concentrations and burdens of cobalt in the lung of all exposed groups appeared to reach steady state and did not change significantly through the end of exposure (day 89) before decreasing rapidly during the first week of the postexposure period and then more slowly until the end of the postexposure period (Table I-4). Lung cobalt concentrations in chamber control animals were at or below the limit of detection (LOD) at all time points. Lung cobalt burden data normalized to exposure concentration indicated increases in burden that were proportional to exposure concentration.

During the 3-month exposure, blood cobalt concentrations in chamber control animals were at or below the LOD at all time points and concentrations in the exposed groups generally increased in proportion to exposure concentration at all time points (Table I-4). Within each exposure concentration, blood cobalt concentrations appeared to be at or near steady state starting from the earliest time point and continuing throughout the exposure period. However, during the recovery period, blood cobalt concentrations fell very rapidly; the largest declines occurred during the first week postexposure. Accordingly, because of the extensive elimination of cobalt from the blood, it was not possible to demonstrate dose proportionality from blood concentration data collected during the recovery period. In addition, it was not possible to fit the blood data to a two-compartment model due to the lack of early sampling times; however, it appears that there were both rapid and slow clearance phases from the blood.

Liver cobalt concentrations in the chamber control group were at or below the LOD, and concentrations and burdens in the exposed groups increased with increasing exposure concentration at both time points (days 26 and 40) (Table I-4). Cobalt concentrations and burdens in the liver of exposed animals were generally lower on day 40 compared to day 26. The normalized liver cobalt burdens were similar across the exposed groups at both time points. At both time points liver cobalt burdens were similar to and in some cases greater than the corresponding lung cobalt burdens.

Pulmonary clearance of cobalt during the recovery period showed a well-defined two-phase elimination profile (Table I-5). The rapid phase exhibited half-lives ranging from 1.8 to 2.6 days and was followed by a slower lung clearance phase with half-lives of 19 to 23 days. A two-compartment clearance model could not be fit to the lung cobalt burden data collected during the 3-month study due to the lack of data collected prior to 5 days of exposure, but a one-compartment model provided an adequate fit to these data (Table I-6). The results indicated that half-lives ranged from 4.7 to 9.0 days.

Exposure Concentration Selection Rationale: There were no significant effects on survival or body weight in the 5 mg/m^3 groups in the 3-month study. In addition, increases in lung weights, and incidences of nonneoplastic lesions in the nose and lung, and alterations of erythroid parameters were not considered sufficiently severe to preclude exposure at this concentration. Hence, 5 mg/m^3 was selected as the highest exposure concentration for the 2-year inhalation study in rats.

Two-year Study

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 9 and in the Kaplan-Meier survival curves (Figure 2). Survival of female rats exposed to 2.5 mg/m^3 was significantly less than that of the chamber control group.

Body Weights and Clinical Findings

Mean body weights of 2.5 and 5 mg/m^3 males were at least 10% less than those of the chamber control group after weeks 99 and 12, respectively, and those of 2.5 and 5 mg/m^3 females were at least 10% less after weeks 57 and 21, respectively (Figure 3; Table 10 and Table 11). Exposure-related clinical findings included abnormal breathing and thinness in male and female rats.

Table 9. Survival of Rats in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	0	1	0
Moribund	28	28	27	32
Natural deaths	5	2	6	2
Animals surviving to study termination	17	20	16	16
Percent probability of survival at end of study[b]	34	40	33	32
Mean survival (days)[c]	663	670	677	669
Survival analysis[d]	P = 0.808	P = 0.796N	P = 0.988N	P = 1.000
Female				
Animals initially in study	50	50	50	50
Moribund	11	20	19	24
Natural deaths	4	4	7	1
Animals surviving to study termination	35	26	24	25[e]
Percent probability of survival at end of study	70	52	48	50
Mean survival (days)	688	685	663	672
Survival analysis	P = 0.135	P = 0.112	P = 0.038	P = 0.060

[a]Censored from survival analyses.
[b]Kaplan-Meier determinations.
[c]Mean of all deaths (uncensored, censored, and terminal kill).
[d]The result of the life table trend test[174] is in the chamber control column, and the results of the life table pairwise comparisons[173] with the chamber controls are in the exposed group columns. A lower mortality in an exposure group is indicated by N.
[e]Includes one animal that died during the last week of the study.

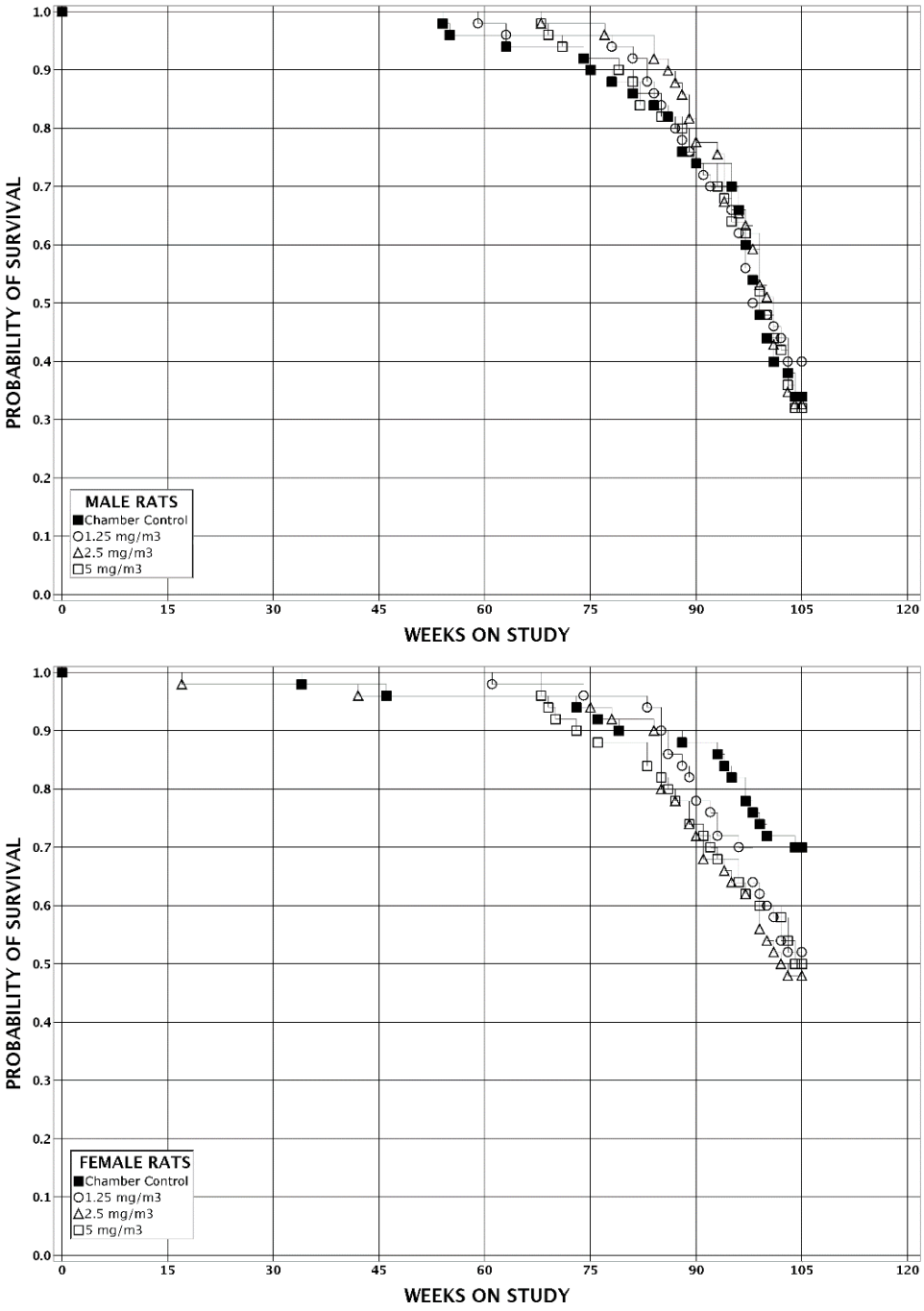


Figure 2. Kaplan-Meier Survival Curves for Rats Exposed to Cobalt Metal by Inhalation for Two Years

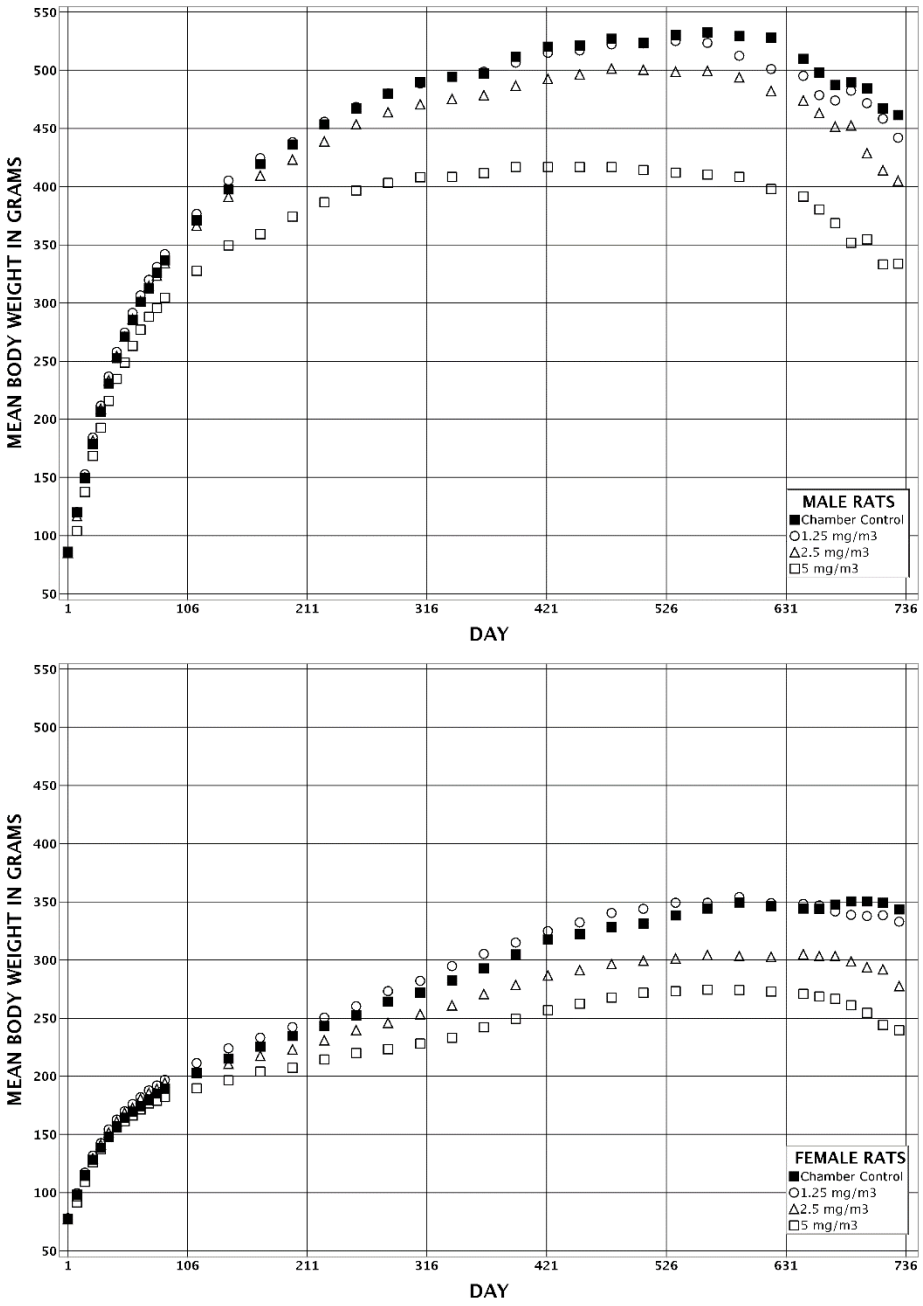


Figure 3. Growth Curves for Rats Exposed to Cobalt Metal by Inhalation for Two Years

Table 10. Mean Body Weights and Survival of Male Rats in the Two-year Inhalation Study of Cobalt Metal

Day	Chamber Control		1.25 mg/m^3			2.5 mg/m^3			5 mg/m^3		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	86	50	86	100	50	85	99	50	86	99	50
9	120	50	120	100	50	117	97	50	104	87	50
16	150	50	153	102	50	150	100	50	138	92	50
23	179	50	184	103	50	182	102	50	169	95	50
30	207	50	212	103	50	209	101	50	193	93	50
37	231	50	237	103	50	234	101	50	216	94	50
44	253	50	258	102	50	254	101	50	235	93	50
51	271	50	275	101	50	271	100	50	249	92	50
58	286	50	292	102	50	286	100	50	263	92	50
65	301	50	307	102	50	302	100	50	277	92	50
72	313	50	320	102	50	315	101	50	288	92	50
79	326	50	331	102	50	324	99	50	296	91	50
86	337	50	342	102	50	334	99	50	305	90	50
114	371	50	377	102	50	367	99	50	328	88	50
142	398	50	405	102	50	391	98	50	350	88	50
170	420	50	425	101	50	410	98	50	359	86	50
198	436	50	438	100	50	423	97	50	374	86	50
226	454	50	456	101	50	439	97	50	387	85	50
254	467	50	469	100	50	454	97	50	397	85	50
282	480	50	480	100	50	464	97	50	403	84	50
310	490	50	489	100	50	471	96	50	408	83	50
338	495	50	495	100	50	475	96	50	409	83	50
366	498	50	499	100	50	479	96	50	412	83	50
394	512	48	507	99	50	487	95	50	417	82	50
422	520	48	515	99	49	493	95	50	417	80	50
450	522	47	517	99	48	497	95	50	417	80	50
478	527	47	522	99	48	501	95	49	417	79	48
506	524	47	523	100	48	500	96	49	415	79	47
534	531	45	525	99	48	499	94	49	412	78	46
562	533	43	524	98	46	499	94	47	410	77	44
590	530	42	513	97	43	494	93	45	409	77	41
618	528	38	501	95	38	482	91	40	398	75	39
646	510	37	495	97	35	474	93	37	392	77	35
660	498	37	479	96	35	463	93	33	381	76	32
674	488	33	474	97	30	452	93	32	369	76	32
688	490	25	482	99	25	453	92	27	352	72	30
702	485	21	472	97	24	429	89	25	355	73	22
716	467	20	459	98	21	414	89	19	333	71	20
Mean for Weeks											
1–13	235	–	240	102	–	236	100	–	217	92	–
14–52	446	–	448	101	–	433	97	–	379	85	–
53–103	510	–	500	98	–	476	93	–	394	77	–

Table 11. Mean Body Weights and Survival of Female Rats in the Two-year Inhalation Study of Cobalt Metal

Day	Chamber Control		1.25 mg/m^3			2.5 mg/m^3			5 mg/m^3		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors
1	77	50	78	102	50	77	100	50	78	101	50
9	98	50	100	101	50	97	99	50	92	93	50
16	115	50	117	102	50	115	100	50	110	95	50
23	128	50	132	103	50	130	101	50	126	98	50
30	139	50	143	103	50	141	102	50	138	99	50
37	148	50	154	104	50	152	103	50	148	100	50
44	157	50	163	103	50	161	103	50	156	99	50
51	164	50	170	103	50	169	103	50	161	98	50
58	170	50	176	104	50	173	102	50	167	98	50
65	175	50	182	104	50	181	103	50	172	98	50
72	180	50	188	105	50	186	103	50	177	99	50
79	185	50	192	104	50	189	102	50	179	97	50
86	190	50	197	104	50	195	103	50	182	96	50
114	203	50	211	104	50	203	100	50	190	93	50
142	215	50	224	104	50	211	98	49	197	91	50
170	226	50	233	103	50	218	96	49	204	90	50
198	235	50	242	103	50	223	95	49	208	88	50
226	243	50	251	103	50	231	95	49	215	88	50
254	252	49	260	103	50	240	95	49	220	87	50
282	264	49	273	103	50	246	93	49	224	85	50
310	272	49	282	104	50	256	93	48	229	84	50
338	283	48	295	104	50	261	92	48	233	83	50
366	293	48	305	104	50	271	92	48	242	83	50
394	305	48	315	103	50	279	91	48	249	82	50
422	318	48	325	102	49	287	90	48	257	81	50
450	322	48	332	103	49	291	90	48	263	81	50
478	328	48	340	104	49	296	90	48	268	82	47
506	331	48	344	104	49	299	90	48	272	82	45
534	338	46	349	103	48	302	89	47	273	81	44
562	344	45	349	101	48	304	88	46	275	80	44
590	350	45	354	101	46	303	87	42	274	79	41
618	346	44	349	101	41	303	87	37	273	79	38
646	344	43	348	101	36	305	89	34	271	79	34
660	344	42	347	101	36	303	88	33	269	78	34
674	348	39	342	98	35	303	87	32	267	77	31
688	350	38	339	97	32	299	85	29	261	75	31
702	351	36	338	96	29	294	84	26	255	73	30
716	349	36	338	97	26	292	84	24	244	70	29
Mean for Weeks											
1–13	148	–	153	103	–	151	102	–	145	98	–
14–52	244	–	252	103	–	232	95	–	213	88	–
53–103	335	–	338	101	–	296	88	–	263	79	–

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of mononuclear cell leukemia and neoplasms and/or nonneoplastic lesions of the lung, nose, adrenal medulla, pancreatic islets, kidney, liver, and testes. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Lung: The incidences of alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, and alveolar/ bronchiolar adenoma or carcinoma (combined) occurred with positive trends in male and female rats and with the exception of the incidence of alveolar/bronchiolar adenoma in 1.25 mg/m^3 females, the incidences were significantly greater than those in the chamber controls (Table 12, Table A-3, and Table B-3). In addition, the incidences of these neoplasms in all exposed groups exceeded the historical control ranges for all routes of administration. Incidences of multiple alveolar/bronchiolar adenoma generally increased with increasing exposure concentrations in males and females. Significantly increased incidences of multiple alveolar/bronchiolar carcinoma occurred in all exposed groups of males and in females exposed to 5 mg/m^3 (Table 12, Table A-1, and Table B-1). Increased incidences of cystic keratinizing epithelioma occurred in exposed groups of female rats; however, the increases were not statistically significant. In male rats, single incidences of cystic keratinizing epithelioma occurred in the 1.25 and 5 mg/m^3 exposure groups. One female rat exposed to 5 mg/m^3 had a squamous cell carcinoma (Table 12 and Table B-1). Cystic keratinizing epithelioma and squamous cell carcinoma have not been observed in the lung of 100 historical controls for all routes of administration (Table 12).

Alveolar/bronchiolar adenomas were discrete, expansile, densely cellular masses that compressed the surrounding lung parenchyma (Figure 7). They were composed of relatively well differentiated, uniform, cuboidal to columnar cells supported by a fine fibrovascular stroma and arranged in solid nests or papillary fronds that projected into alveolar spaces. Alveolar/bronchiolar carcinomas were larger, irregular, poorly circumscribed, unencapsulated, expansile, locally invasive masses that effaced the lung parenchyma (Figure 8). They were composed of poorly differentiated, moderately to markedly pleomorphic (anaplastic) cuboidal, columnar, or polygonal cells with pleomorphic nuclei; occasionally, cells had mitotic figures. The cells were arranged in single to multiple layers, formed irregular papillary or acinar structures and/or solid sheets and were supported by fibrovascular stroma. Some carcinomas had areas of squamous differentiation, and many contained extensive areas of necrosis, desmoplastic tissue, and inflammation (Figure 9). Other carcinomas had a core of dense fibrous tissue with embedded islands of malignant cells arranged in irregular cords, clusters, and acini. In several animals, metastases were observed in other tissues. Cystic keratinizing epitheliomas were well circumscribed, unencapsulated, irregularly expansive masses that effaced the lung parenchyma. The epitheliomas consisted of an irregular wall of well differentiated squamous epithelium surrounding a core of concentrically arranged keratin (Figure 10). Invariably the walls of these neoplasms had areas that lacked orderly maturation with foci of basal cell disorganization. The outer portion of the lesion grew by expansion into the adjacent lung, but evidence of invasion was not observed. The squamous cell carcinoma was an infiltrative mass that obliterated the normal lung architecture. The neoplastic cells formed swirling clusters, often around laminated

keratin, separated by small to moderate amounts of fibrous stroma. The cells were polygonal, variable in size and shape, and contained small to moderate amounts of eosinophilic cytoplasm.

Point mutations in *Kras* (31%), *Egfr* (17%), and *Tp53* (23%) were noted in the alveolar/bronchiolar carcinomas in rats chronically exposed to cobalt metal dust. Because there were no spontaneous alveolar/bronchiolar carcinomas in the F344/NTac rats in the concurrent cobalt metal study, spontaneous alveolar/bronchiolar carcinomas were evaluated from F344 rat vehicle control groups in previous NTP chronic bioassays. None of these mutations were noted in the controls.

The incidences of alveolar epithelium hyperplasia, alveolar proteinosis, chronic active inflammation, and bronchiole epithelium hyperplasia in all exposed groups of male and female rats were significantly greater than those in the chamber control groups (Table 12, Table A-7, and Table B-7). The severities of these lesions generally increased with increasing exposure concentration. This spectrum of nonneoplastic lesions invariably occurred together and presented as a complex mix of changes, and at times it was difficult to separate the individual components. Alveolar epithelium hyperplasia was a multifocal and sometimes focally extensive, discrete, randomly distributed but frequently subpleural lesion characterized by proliferation of flat to cuboidal to low columnar epithelial cells (presumed to be Type II pneumocytes).

Table 12. Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Rats in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Number Examined Microscopically	50	50	50	50
Alveolar Epithelium, Hyperplasia[a]	3 (1.0)[b]	47** (2.8)	49** (3.3)	49** (3.6)
Alveolus, Proteinosis	0	48** (2.6)	49** (2.9)	49** (3.1)
Inflammation, Chronic Active	22 (1.1)	50** (3.0)	50** (2.9)	50** (2.9)
Bronchiole, Epithelium, Hyperplasia	0	44** (1.5)	47** (2.7)	50** (3.7)
Alveolar/bronchiolar Adenoma, Multiple	1	3	2	6
Alveolar/bronchiolar Adenoma (includes multiple)[c]				
Overall rate[d]	2/50 (4%)	10/50 (20%)	10/50 (20%)	14/50 (28%)
Adjusted rate[e]	5.0%	24.1%	23.3%	32.5%
Terminal rate[f]	1/17 (6%)	6/20 (30%)	2/16 (13%)	4/16 (25%)
First incidence (days)	611	577	535	478
Poly-3 test[g]	P = 0.011	P = 0.015	P = 0.018	P < 0.001
Alveolar/bronchiolar Carcinoma, Multiple	0	6*	14**	30**
Alveolar/bronchiolar Carcinoma (includes multiple)[h]				
Overall rate	0/50 (0%)	16/50 (32%)	34/50 (68%)	36/50 (72%)
Adjusted rate	0.0%	38.2%	76.8%	80.6%
Terminal rate	0/17 (0%)	7/20 (35%)	16/16 (100%)	14/16 (88%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
First incidence (days)	–[i]	580	472	552
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Alveolar/bronchiolar Adenoma or Carcinoma[j]				
Overall rate	2/50 (4%)	25/50 (50%)	39/50 (78%)	44/50 (88%)
Adjusted rate	5.0%	58.0%	84.6%	93.6%
Terminal rate	1/17 (6%)	13/20 (65%)	16/16 (100%)	16/16 (100%)
First incidence (days)	611	577	472	478
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Cystic Keratinizing Epithelioma[h]	0	1	0	1
Female				
Number Examined Microscopically	50	50	50	50
Alveolar Epithelium, Hyperplasia	9 (1.1)	49** (2.8)	50** (2.7)	49** (3.4)
Alveolus, Proteinosis	0	50** (2.7)	50** (2.7)	50** (2.9)
Inflammation, Chronic Active	20 (1.0)	50** (3.0)	50** (2.9)	50** (2.9)
Bronchiole, Epithelium, Hyperplasia	0	47** (1.5)	46** (2.1)	48** (3.8)
Alveolar/bronchiolar Adenoma, Multiple	0	1	3	4
Alveolar/bronchiolar Adenoma (includes multiple)[j]				
Overall rate	2/50 (4%)	7/50 (14%)	9/50 (18%)	13/50 (26%)
Adjusted rate	4.5%	16.2%	22.1%	30.9%
Terminal rate	1/35 (3%)	5/26 (19%)	6/24 (25%)	8/25 (32%)
First incidence (days)	698	590	587	579
Poly-3 test	P = 0.002	P = 0.072	P = 0.016	P < 0.001
Alveolar/bronchiolar Carcinoma, Multiple		0	4	3
Alveolar/bronchiolar Carcinoma (includes multiple)[h]				
Overall rate	0/50 (0%)	9/50 (18%)	17/50 (34%)	30/50 (60%)
Adjusted rate	0.0%	21.3%	42.0%	69.2%
Terminal rate	0/35 (0%)	9/26 (35%)	14/24 (58%)	20/25 (80%)
First incidence (days)	–	730 (T)	690	471
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Alveolar/bronchiolar Adenoma or Carcinoma (combined)[j]				
Overall rate	2/50 (4%)	15/50 (30%)	20/50 (40%)	38/50 (76%)
Adjusted rate	4.5%	34.7%	48.5%	86.2%
Terminal rate	1/35 (3%)	13/26 (50%)	14/24 (58%)	25/25 (100%)
First incidence (days)	698	590	587	471

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Cystic Keratinizing Epithelioma[h]	0	4	1	2
Squamous Cell Carcinoma[h]	0	0	0	1

*Significantly different (P ≤ 0.05) from the chamber control group by the Poly-3 test.
**P ≤ 0.01.
(T) Terminal kill.
[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[c]Historical control incidence for 2-year studies (all routes): 5/100.
[d]Number of animals with neoplasm per number of animals with lung examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[h]Historical control incidence: 0/100.
[i]Not applicable; no neoplasms in animal group.
[j]Historical control incidence: 2/100.

lining the alveolar septa; however, the underlying alveolar architecture was generally maintained (Figure 11 and Figure 12). The interstitium of the alveolar septa was also variably expanded by increased amounts of collagen. Alveolar proteinosis was characterized by accumulations of brightly eosinophilic, wispy to globular, homogeneous, proteinaceous material filling the alveolar spaces (Figure 13); this proteinaceous material frequently contained acicular cholesterol crystals or cleft-like spaces. These lesions were invariably accompanied by chronic active inflammation which consisted of complex mixtures of predominantly macrophages and lymphocytes mixed with lesser numbers of neutrophils within the alveolar spaces and septa and low numbers of multinucleated giant cells (Figure 14); clear cleft-like spaces (cholesterol clefts) were frequently present among the inflammatory cells. Also associated with areas of chronic active inflammation, there were frequently variable proliferation of the alveolar epithelial (Type II) cells and variable alveolar septal interstitial fibrosis. Frequently, there were large numbers of inflammatory cell infiltrates, mostly macrophages, accumulated around the alveolar/bronchiolar neoplasms. The alveolar macrophages were frequently engorged with an intensely eosinophilic material similar to that in the alveolar spaces, and many macrophages also contained acicular cholesterol crystals. Multifocal accumulations of plump foamy macrophages within the alveolar spaces were considered a component of the inflammation changes. Bronchiole epithelium hyperplasia was characterized by proliferation and disorganized crowding of ciliated, cuboidal columnar to pleomorphic epithelial cells lining terminal bronchioles with extension onto adjacent alveolar septa (Figure 15). There was often minimal to mildly increased amounts of collagen in the interstitium of the bronchiolar wall.

Nose: A spectrum of nonneoplastic lesions occurred with positive trends in male and female rats, and the incidences were often significantly greater than those in the chamber controls (Table 13, Table A-7, and Table B-7). For some lesions, the severities increased with increasing exposure concentration. Chronic active inflammation was most prominent in Levels I and II and less often in Level III nasal section. Chronic active inflammation consisted of infiltrates of mostly lymphocytes, plasma cells, neutrophils, and fewer macrophages within the lamina propria and overlying epithelium accompanied by cellular debris in the nasal passages. Suppurative

inflammation occurred primarily in Level II and consisted of accumulations of nondegenerate and degenerate neutrophils mixed with eosinophilic proteinaceous material, occasional macrophages, cellular debris, and sometimes colonies of coccobacilli and foreign material within the nasal passages and adjacent epithelium and lamina propria of the nasal turbinates (Figure 16). More pronounced suppurative inflammation was often accompanied by florid hyperplasia of the adjacent epithelium.

Respiratory metaplasia and/or atrophy of the olfactory epithelium occurred in the dorsal meatuses of Level II and sometimes Level III. When the predominant change in the affected segment was replacement of the olfactory epithelium by respiratory type columnar epithelial cells, olfactory epithelium respiratory metaplasia was diagnosed (Figure 17). When the olfactory epithelium was attenuated due to loss of olfactory epithelial cells, olfactory epithelium atrophy was diagnosed (Figure 18). Olfactory epithelium hyperplasia mostly occurred in rats exposed to 5 mg/m^3 and consisted of small, focal, intraepithelial proliferations of epithelial cells that formed clusters or rosettes that sometimes extended into the lamina propria (Figure 18). Olfactory epithelium basal cell hyperplasia was invariably associated with olfactory epithelium hyperplasia and consisted of disorganized proliferation and crowding of the basal olfactory epithelial cells. Necrosis of the olfactory epithelium was a minimal to mild lesion mostly affecting male rats and a few females and was associated with inflammatory lesions. In sites of necrosis, the epithelium was effaced and replaced by cellular and karyorrhectic debris.

Respiratory epithelium hyperplasia occurred in the epithelium lining the tips of the nasoturbinates, maxilloturbinates, and the septa in Levels I and II. In affected sites, the epithelium was thickened by increased numbers of cuboidal to ciliated columnar epithelial cells crowded in multiple layers sometimes forming undulations with invaginations into the underlying lamina propria (Figure 19). This lesion was most prominent in areas of suppurative inflammation. Respiratory epithelium squamous metaplasia was most common at the tips of the nasoturbinates and along the lateral walls of Level I and less often Level II. In affected sites, flattened squamous epithelium of variable thickness replaced the ciliated columnar epithelium normally present in this location (Figure 19). Necrosis of the respiratory epithelium was associated with the inflammatory lesions and primarily affected rats in the 5 mg/m^3 groups. In areas of necrosis, the epithelium was effaced and replaced by cellular and karyorrhectic debris (Figure 20); necrosis would sometimes extend into the submucosa and sinuses.

Turbinate atrophy was a minimal to mild change that primarily affected the naso- and maxilloturbinates in Levels I and II and occasionally Level III. Affected turbinates were short, thin, and blunted due to attenuation of the turbinate bone and loss of structures in the lamina propria, including the glands, vessels, nerve bundles, and connective tissue. As a result, the nasal passages appeared wider than normal (Figure 21). The nasal septum was sometimes similarly affected and had a noticeable decrease in width.

Table 13. Incidences of Nonneoplastic Lesions of the Nose in Rats in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Number Examined Microscopically	48	47	45	50
Inflammation, Chronic Active[a]	28 (1.2)[b]	35* (1.3)	40** (1.7)	49** (2.6)
Inflammation, Suppurative	9 (1.0)	12 (1.7)	24** (2.2)	46** (2.6)
Olfactory Epithelium, Metaplasia, Respiratory	12 (1.1)	26** (1.7)	37** (1.5)	50** (2.2)
Olfactory Epithelium, Atrophy	2 (1.0)	21** (1.0)	34** (1.0)	29** (1.2)
Olfactory Epithelium, Hyperplasia	0	1 (1.0)	2 (1.5)	7** (1.1)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	1 (1.0)	0	13** (1.0)
Olfactory Epithelium, Necrosis	0	1 (1.0)	5* (1.6)	5* (1.8)
Respiratory Epithelium, Hyperplasia	20 (1.3)	35** (1.2)	45** (1.7)	50** (2.2)
Respiratory Epithelium, Metaplasia, Squamous	0	1 (1.0)	11** (1.2)	35** (1.3)
Respiratory Epithelium, Necrosis	1 (1.0)	4 (1.8)	5 (1.4)	13** (1.6)
Turbinate, Atrophy	1 (1.0)	35** (1.0)	35** (1.0)	41** (1.0)
Female				
Number Examined Microscopically	50	50	49	50
Inflammation, Chronic Active	22 (1.3)	42** (1.1)	39** (1.1)	50** (2.4)
Inflammation, Suppurative	6 (1.2)	4 (1.3)	4 (1.0)	42** (2.2)
Olfactory Epithelium, Metaplasia, Respiratory	6 (1.0)	18** (1.3)	24** (1.2)	47** (2.1)
Olfactory Epithelium, Atrophy	0	22** (1.1)	35** (1.0)	35** (1.2)
Olfactory Epithelium, Hyperplasia	0	0	3 (1.0)	5* (1.0)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	0	1 (1.0)	19** (1.0)
Olfactory Epithelium, Necrosis	0	2 (1.5)	0	1 (3.0)
Respiratory Epithelium, Hyperplasia	15 (1.2)	43** (1.0)	48** (1.0)	49** (2.1)
Respiratory Epithelium, Metaplasia, Squamous	2 (1.0)	0	3 (1.0)	45** (2.0)
Respiratory Epithelium, Necrosis	1 (3.0)	1 (2.0)	1 (1.0)	15** (1.6)
Turbinate, Atrophy	1 (1.0)	38** (1.0)	27** (1.0)	45** (1.0)

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

Adrenal Medulla: The incidences of benign pheochromocytoma, malignant pheochromocytoma, and benign or malignant pheochromocytoma (combined) occurred with positive trends in male and female rats and with the exception of the incidence of malignant pheochromocytoma in 2.5 mg/m^3 females, the incidences in rats exposed to 2.5 or 5 mg/m^3 were significantly greater than those in the chamber controls and exceeded the historical control incidences for all routes of administration (Table 14, Table A-4, and Table B-4). The incidences of bilateral benign pheochromocytoma were significantly increased in all exposed groups of males and in 2.5 and 5 mg/m^3 females, and the incidences of bilateral malignant pheochromocytoma were significantly increased in male and female rats exposed to 5 mg/m^3.

Benign pheochromocytoma occurred as variably sized, well-demarcated, expansile proliferations of medullary cells that formed large trabeculae or solid clusters separated by delicate fibrous stroma and/or sinusoids (Figure 22). The cells were polygonal to spindyloid and moderately uniform in size and shape.

Table 14. Incidences of Neoplasms and Nonneoplastic Lesions of the Adrenal Medulla in Rats in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Number Examined Microscopically	50	50	50	50
Hyperplasia[a]	19 (2.3)[b]	21 (2.5)	9* (3.0)	9** (2.4)
Benign Pheochromocytoma, Bilateral	4	13*	22**	21**
Benign Pheochromocytoma (includes bilateral)[c]				
Overall rate[d]	15/50 (30%)	23/50 (46%)	37/50 (74%)	34/50 (68%)
Adjusted rate[e]	35.8%	54.3%	81.2%	76.4%
Terminal rate[f]	3/17 (18%)	12/20 60%)	15/16 (94%)	14/16 (88%)
First incidence (days)	519	583	582	572
Poly-3 test[g]	P < 0.001	P = 0.059	P < 0.001	P < 0.001
Malignant Pheochromocytoma, Bilateral	0	0	0	7**
Malignant Pheochromocytoma (includes bilateral)[h]				
Overall rate	2/50 (4%)	2/50 (4%)	9/50 (18%)	16/50 (32%)
Adjusted rate	5.0%	5.0%	21.4%	39.1%
Terminal rate	0/17 (0%)	2/20 (10%)	3/16 (19%)	9/16 (56%)
First incidence (days)	668	729 (T)	628	646
Poly-3 test	P < 0.001	P = 0.693N	P = 0.030	P < 0.001
Benign or Malignant Pheochromocytoma[i]				
Overall rate	17/50 (34%)	23/50 (46%)	38/50 (76%)	41/50 (82%)
Adjusted rate	40.2%	54.3%	82.7%	90.7%
Terminal rate	3/17 (18%)	12/20 (60%)	15/16 (94%)	16/16 (100%)
First incidence (days)	519	583	582	572

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Poly-3 test	P < 0.001	P = 0.130	P < 0.001	P < 0.001
Female				
Number Examined Microscopically	50	50	50	50
Hyperplasia	12 (1.8)	27** (2.0)	27** (2.3)	10 (2.8)
Benign Pheochromocytoma, Bilateral	2	4	8*	19**
Benign Pheochromocytoma (includes bilateral)[j]				
Overall rate	6/50 (12%)	12/50 (24%)	22/50 (44%)	36/50 (72%)
Adjusted rate	13.6%	27.2%	52.1%	80.6%
Terminal rate	6/35 (17%)	5/26 (19%)	13/24 (54%)	21/25 (84%)
First incidence (days)	730 (T)	598	590	579
Poly-3 test	P < 0.001	P = 0.091	P < 0.001	P < 0.001
Malignant Pheochromocytoma, Bilateral	0	1	1	4*
Malignant Pheochromocytoma (includes bilateral)[k]				
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	11/50 (22%)
Adjusted rate	0.0%	4.7%	7.5%	27.0%
Terminal rate	0/35 (0%)	2/26 (8%)	2/24 (8%)	9/25 (36%)
First incidence (days)	–[l]	730 (T)	715	712
Poly-3 test	P < 0.001	P = 0.228	P = 0.102	P < 0.001
Benign or Malignant Pheochromocytoma[m]				
Overall rate	6/50 (12%)	13/50 (26%)	23/50 (46%)	40/50 (80%)
Adjusted rate	13.6%	29.4%	54.5%	89.4%
Terminal rate	6/35 (17%)	6/26 (23%)	14/24 (58%)	24/25 (96%)
First incidence (days)	730 (T)	598	590	579
Poly-3 test	P < 0.001	P = 0.058	P < 0.001	P < 0.001

*Significantly different ($P \leq 0.05$) from the chamber control group by the Poly-3 test.
**$P \leq 0.01$.
(T) Terminal kill.
[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[c]Historical control incidence for 2-year studies (all routes): 25/100.
[d]Number of animals with neoplasm per number of animals with adrenal medulla examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in an exposure group is indicated by N.
[h]Historical control incidence: 2/100.
[i]Historical control incidence: 27/100.
[j]Historical control incidence: 7/100.
[k]Historical control incidence: 1/100.
[l]Not applicable; no neoplasms in animal group.
[m]Historical control incidence: 8/100.

Malignant pheochromocytomas were generally larger, irregular, poorly demarcated invasive masses that effaced the adrenal gland extending through the capsule into the periadrenal tissue; the neoplastic cells were poorly differentiated and pleomorphic (Figure 23). In some animals, malignant pheochromocytomas metastasized to other organs. The incidences of medullary hyperplasia in the adrenal gland were significantly increased in female rats exposed to 1.25 or 2.5 mg/m^3 (Table 14 and Table B-7); incidences of this lesion were significantly decreased in male rats exposed to 2.5 or 5 mg/m^3 (Table 14 and Table A-7). Hyperplasia occurred as focally discrete proliferations of medullary epithelial cells that blended with, but did not compress, the surrounding medullary parenchyma. The cells were generally smaller and more basophilic than the surrounding normal medullary epithelial cells.

Pancreatic Islets: The incidences of carcinoma and adenoma or carcinoma (combined) occurred with positive trends in male rats, and the incidences of adenoma, carcinoma, and adenoma or carcinoma (combined) generally exceeded the historical control incidences for all routes of administration (Table 15, Table A-1, Table A-2, and Table A-5). The incidences of adenoma in 2.5 mg/m^3 males and of adenoma or carcinoma (combined) in males exposed to 2.5 or 5 mg/m^3 were significantly greater than those in the chamber controls. Incidences of adenoma, carcinoma, and adenoma or carcinoma (combined) in 5 mg/m^3 females were slightly increased; the increases were not statistically significant but did exceed the historical control incidences for all routes of administration (Table 15, Table B-1, Table B-2, and Table B-6). Adenomas were well circumscribed, expansile masses that compressed the acini. The neoplastic cells were well differentiated with minimal to mild cellular atypia and slightly altered growth patterns (Figure 24). Carcinomas were poorly circumscribed, unencapsulated, irregular, expansile, and invasive masses that effaced the parenchyma (Figure 25). Carcinomas had a heterogeneous growth pattern with cells that were moderately to markedly pleomorphic.

Table 15. Incidences of Neoplasms of the Pancreatic Islets in Rats in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Adenoma[a]				
Overall rate[b]	0/50 (0%)	1/50 (2%)	6/48 (13%)	3/49 (6%)
Adjusted rate[c]	0.0%	2.5%	15.1%	7.7%
Terminal rate[d]	0/17 (0%)	0/20 (0%)	1/16 (6%)	3/16 (19%)
First incidence (days)	–[f]	684	618	729 (T)
Poly-3 test[e]	P = 0.052	P = 0.504	P = 0.015	P = 0.116
Carcinoma[g]				
Overall rate	2/50 (4%)	1/50 (2%)	5/48 (10%)	6/49 (12%)
Adjusted rate	5.0%	2.5%	12.6%	15.1%
Terminal rate	0/17 (0%)	0/20 (0%)	3/16 (19%)	2/16 (13%)
First incidence (days)	675	675	618	679
Poly-3 test	P=0.021	P=0.496N	P=0.213	P=0.129
Adenoma or Carcinoma (combined)[g]				

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Overall rate	2/50 (4%)	2/50 (4%)	10/48 (21%)	9/49 (18%)
Adjusted rate	5.0%	4.9%	24.7%	22.6%
Terminal rate	0/17 (0%)	0/20 (0%)	3/16 (19%)	5/16 (31%)
First incidence (days)	675	675	618	679
Poly-3 test	P = 0.002	P = 0.689N	P = 0.013	P = 0.022
Female				
Number Examined Microscopically	50	50	50	50
Adenoma[h]	0	0	0	1 (2%)
Carcinoma[h]	1 (2%)	0	0	3 (6%)
Adenoma or Carcinoma[g]				
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.2%	0.0%	0.0%	7.2%
Terminal rate	0/35 (0%)	0/26 (0%)	0/24 (0%)	1/25 (4%)
First incidence (days)	234	–	–	506
Poly-3 test	P = 0.060	P = 0.512N	P = 0.523N	P = 0.279

(T) Terminal kill.
[a]Historical control incidence for 2-year studies (all routes): 0/100.
[b]Number of animals with neoplasm per number of animals with pancreatic islets examined microscopically.
[c]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[d]Observed incidence at terminal kill.
[e]Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in an exposure group is indicated by **N**.
[f]Not applicable; no neoplasms in animal group.
[g]Historical control incidence for all routes: 2/100.
[h]Historical control incidence for all routes: 1/100.

Kidney: In the standard evaluation of the kidney, the incidences of renal tubule adenoma, carcinoma, and adenoma or carcinoma (combined) were slightly increased in male rats exposed to 5 mg/m^3 (Table 16, Table A-1, and Table A-2). Although not statistically significant, the incidences in this group exceeded the historical control incidences for all routes of administration (Table 16 and Table A-6). In the standard evaluation, a single section of each kidney is routinely examined microscopically. Because the incidences of renal tubule neoplasms in the standard evaluation suggested the possibility of a treatment-related carcinogenic effect, an extended evaluation of the kidney was performed in male rats to explore this possibility. For the extended evaluation, kidneys of male rats were step-sectioned at 1 mm intervals to obtain three to four additional sections from each kidney, and these sections were examined microscopically. In the extended evaluation, additional renal tubule adenomas and renal tubule hyperplasias were identified but no additional renal tubule carcinomas (Table 16); a renal tubule oncocytoma was identified in one male exposed to 2.5 mg/m^3. In the combined standard and extended evaluations, the incidences of renal tubule hyperplasia in the exposed groups were similar to that in the chamber controls. The incidence of renal tubule adenoma in the 5 mg/m^3 group was greater than that in the chamber control group, but the increase was not statistically significant. The incidences of renal tubule carcinomas were unchanged.

Renal tubule adenomas were small, well circumscribed proliferations of renal tubule epithelial cells with a cross-sectional area greater than five times that of a single, normal renal tubule. The cells were well differentiated and uniform in size and shape and formed poorly defined papillary structures or solid clusters of cells (Figure 26). Renal tubule carcinomas were larger, expansive, and invasive masses that effaced and replaced much of the renal parenchyma (Figure 27).

Table 16. Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Male Rats in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Single Sections (Standard Evaluation)				
Number Examined Microscopically	50	50	50	50
Renal Tubule, Hyperplasia[a]	0	0	0	0
Renal Tubule, Adenoma, Multiple	0	0	0	1
Renal Tubule, Adenoma (includes multiple)[b]	0	1	0	3
Renal Tubule, Carcinoma[c]	0	0	0	2
Renal Tubule, Adenoma or Carcinoma[b]	0	1	0	4
Step Sections (Extended Evaluation)				
Number Examined Microscopically	50	50	50	50
Renal Tubule, Hyperplasia	5	3	5	4
Renal Tubule, Adenoma	3	1	1	3
Renal Tubule, Carcinoma	0	0	0	2
Renal Tubule, Adenoma or Carcinoma	3	1	1	5
Renal Tubule, Oncocytoma	0	0	1	0
Single Sections and Step Sections (Combined)				
Number Examined Microscopically	50	50	50	50
Renal Tubule, Hyperplasia	5	3	5	4
Renal Tubule, Adenoma (includes multiple)	3	1	1	6
Renal Tubule, Carcinoma	0	0	0	2
Renal Tubule, Adenoma or Carcinoma				
Overall rate[d]	3/50 (6%)	1/50 (2%)	1/50 (2%)	7/50 (14%)
Adjusted rate[e]	7.5%	2.5%	2.4%	17.4%
Terminal rate[f]	0/17 (0%)	1/20 (5%)	1/16 (6%)	4/16 (25%)
First incidence (days)	678	729 (T)	729 (T)	691
Poly-3 test[g]	P = 0.023	P = 0.302N	P = 0.294N	P = 0.158

(T) Terminal kill.
[a]Number of animals with lesion.
[b]Historical control incidence for 2-year studies (all routes): 1/100.
[c]Historical control incidence: 0/100.
[d]Number of animals with neoplasm per number of animals with kidney examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A lower incidence in an exposure group is indicated by N.

Mononuclear Cell Leukemia: The incidences of mononuclear cell leukemia were significantly increased in all exposed groups of female rats and exceeded the historical control incidence for all routes of administration (Table 17, Table B-1, Table B-2, and Table B-6).

Table 17. Incidences of Mononuclear Cell Leukemia in Female Rats in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
All Organs: Mononuclear Cell Leukemia[a]				
Overall rate[b]	16/50 (32%)	29/50 (58%)	28/50 (56%)	27/50 (54%)
Adjusted rate[c]	35.7%	62.4%	60.5%	58.9%
Terminal rate[d]	12/35 (34%)	15/26 (58%)	12/24 (50%)	13/25 (52%)
First incidence (days)	663	590	117	473
Poly-3 test[e]	P = 0.118	P = 0.007	P = 0.013	P = 0.019

[a]Historical control incidence for 2-year studies (all routes): 35/100.
[b]Number of animals with mononuclear cell leukemia per number of animals necropsied.
[c]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[d]Observed incidence at terminal kill.
[e]Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.

Liver: The incidences of basophilic focus occurred with positive trends in male and female rats and in all exposed groups of males (chamber control, 5/50; 1.25 mg/m^3, 17/50; 2.5 mg/m^3, 17/50; 5 mg/m^3, 19/50) and in females exposed to 5 mg/m^3 (16/50, 20/50, 22/50, 33/50), and the incidences were significantly greater than those in the chamber control groups (Table A-7 and Table B-7). Basophilic foci occur spontaneously in rats, and the incidences are sometimes increased with exposure to chemicals. They are considered putative preneoplastic lesions; however, the incidences of hepatocellular neoplasms were not increased in male or female rats exposed to cobalt metal (Table A-1 and Table B-1).

Testes: The incidence of infarct was significantly increased in male rats exposed to 5 mg/m^3 (1/50, 0/50, 2/50, 12/50) (Table A-4). Infarcts were mostly unilateral, and in affected testes, there was complete effacement of the parenchyma due to necrosis with loss of differential staining (tissue was diffusely hypereosinophilic) and cellular detail. Multifocal intratubular mineralization was current in a few of the affected testes.

Tissue Burden Studies

Lung weights and lung cobalt burdens were determined in female rats (Table I-7). Lung weights increased in all exposed groups; however, increases in lung weights occurred earlier in the study (day 184) in the 2.5 and 5 mg/m^3 groups than in the 1.25 mg/m^3 group (day 366). Because of the significant changes in lung weights with increasing exposure concentration, lung cobalt burdens rather than lung cobalt concentrations were evaluated for toxicokinetic parameters.

Cobalt concentrations and burdens in the lung increased with increasing exposure concentration and were significantly increased in all exposed groups of female rats at all time points compared to those in the chamber control group (Table I-7). Cobalt concentrations in the chamber control

group were at or below the limit of detection (LOD) at all time points except day 548 [one animal had a lung cobalt concentration exceeding the LOD but less than the experimental limit of quantitation (ELOQ)]. By day 184, lung cobalt concentrations for all exposed groups appeared to reach steady state and did not change significantly through day 548; lung cobalt burdens increased rapidly by day 4, but by day 184 the rate of increase slowed as lung burdens asymptotically approached steady state. Analysis of normalized lung cobalt burdens revealed no tendency toward disproportionate changes and no biologically significant differences in normalized burdens with increasing exposure concentration.

The lung cobalt burden data from the exposure phases of the 3-month and 2-year studies were modeled using a two-compartment model; these data show that steady state was clearly reached at 2.5 and 5 mg/m^3 but not at 1.25 mg/m^3 (Table I-1). Rapid clearance phase half-lives were between 1.53 days and 2.94 days (Table I-8), while slow clearance phase half-lives were 789 days, 167 days, and 83 days for 1.25 mg/m^3, 2.5 mg/m^3, and 5 mg/m^3, respectively. The apparent lack of achievement of steady state and long half-life at 1.25 mg/m^3 are likely spurious findings due to uncertainty in the model. Cobalt deposition rates were 1.4, 2.1, and 5.6 µg cobalt/day during the rapid clearance phase and 0.018, 0.078, and 0.29 µg cobalt/day during the slow clearance phase at 1.25, 2.5, and 5 mg/m^3, respectively. Steady-state lung cobalt burdens including both the rapid and slow clearance phases ($L_{SSa} + L_{SSb}$) were approximately 25.4, 27.8, and 46.8 µg cobalt/lung in animals exposed to 1.25, 2.5, and 5 mg/m^3, respectively. The fractions of deposition in the slow clearance phase (F_B) for the exposed groups were quite low, increasing from 0.012 to 0.049 as exposure concentrations increased, corresponding to total slow phase lung cobalt clearances of 1.2% to 4.9%; clearances of total deposited cobalt during the rapid clearance phase ranged from 98.8% to 95.1% [$(1-F_B) \times 100$] with increasing exposure concentration.

Mice

Two-week Study

The exposure concentrations for the 2-week study were estimated based on exposures of previously studied cobalt sulfate heptahydrate[66]. Three male and three female mice exposed to 40 mg/m^3 died before the end of the study (Table 18). Final mean body weights were significantly decreased in male and female mice exposed to 20 or 40 mg/m^3 by 9% and 27% (males) or 16% and 38% (females), respectively, compared to the chamber control groups. Mean body weight gains of 20 and 40 mg/m^3 males and all exposed groups of females were significantly less than those of the chamber controls. Females exposed to 20 mg/m^3 and males and females exposed to 40 mg/m^3 lost weight during the study. Exposure-related clinical findings included abnormal breathing, lethargy, and thinness in males exposed to 20 or 40 mg/m^3 and females exposed to 10 mg/m^3 or greater. At necropsy, tan lungs were observed in most males and females exposed to 20 or 40 mg/m^3. Dark lung lobes were observed in one early-death male.

Absolute lung weights of both sexes exposed to 5 mg/m^3 or greater and relative lung weights of males exposed to 10 mg/m^3 or greater and of females exposed to 5 mg/m^3 or greater were significantly increased compared to the chamber controls (Table 19 and Table G-3). Absolute liver weights of all exposed groups of males and females and the relative liver weights of both sexes exposed to 2.5, 5, 10, or 20 mg/m^3 were significantly less than those of the chamber

controls. Absolute testis weight of the 40 mg/m^3 group was significantly less than that of the chamber controls.

Table 18. Survival and Body Weights of Mice in the Two-week Inhalation Study of Cobalt Metal[a]

Concentration (mg/m^3)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	5/5	23.4 ± 0.3	25.7 ± 0.5	2.3 ± 0.3	
2.5	5/5	23.5 ± 0.3	25.0 ± 0.5	1.5 ± 0.2	97
5	5/5	23.6 ± 0.3	25.9 ± 0.3	2.2 ± 0.4	101
10	5/5	23.8 ± 0.3	25.3 ± 0.5	1.5 ± 0.2	98
20	5/5	23.1 ± 0.4	23.4 ± 0.4**	0.2 ± 0.4**	91
40	2/5[c]	23.0 ± 0.4	18.9 ± 1.1**	−4.7 ± 1.7**	73
Female					
0	5/5	19.1 ± 0.3	20.8 ± 0.1	1.7 ± 0.3	
2.5	5/5	19.8 ± 0.5	20.3 ± 0.5	0.5 ± 0.2*	98
5	5/5	19.8 ± 0.5	20.1 ± 0.5	0.3 ± 0.4*	97
10	5/5	19.4 ± 0.4	20.0 ± 0.6	0.6 ± 0.4*	96
20	5/5	19.0 ± 0.3	17.4 ± 0.4**	−1.6 ± 0.2**	84
40	2/5[d]	18.9 ± 0.2	13.0 ± 1.6**	−6.1 ± 1.1**	62

*Significantly different ($P \leq 0.05$) from the chamber control group by Williams' test.
**$P \leq 0.01$.
[a]Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[b]Number of animals surviving at 17 days/number initially in group.
[c]Days of deaths: 5, 5, 8.
[d]Days of deaths: 6, 7, 9.

Table 19. Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Two-week Inhalation Study of Cobalt Metal[a]

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 m/m^3
n	5	5	5	5	5	2
Male						
Necropsy body wt	25.7 ± 0.5	25.0 ± 0.5	25.9 ± 0.3	25.3 ± 0.5	23.4 ± 0.4**	18.9 ± 1.1**
Liver						
Absolute	1.13 ± 0.04	0.98 ± 0.04**	0.98 ± 0.04**	0.99 ± 0.02**	0.89 ± 0.02**	0.83 ± 0.01**
Relative	43.88 ± 0.80	39.18 ± 1.35*	37.67 ± 1.09**	39.32 ± 0.91*	37.93 ± 0.51**	44.20 ± 2.99
Lung						

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 m/m^3
Absolute	0.18 ± 0.01	0.21 ± 0.01	0.23 ± 0.01*	0.24 ± 0.01**	0.29 ± 0.01**	0.36 ± 0.05**
Relative	7.08 ± 0.15	8.33 ± 0.32	8.73 ± 0.33	9.61 ± 0.62*	12.62 ± 0.51**	19.31 ± 3.73**
L. Testis						
Absolute	0.098 ± 0.002	0.104 ± 0.001	0.099 ± 0.004	0.084 ± 0.009	0.089 ± 0.003	0.070 ± 0.002**
Relative	3.834 ± 0.074	4.180 ± 0.114	3.812 ± 0.149	3.322 ± 0.311	3.807 ± 0.088	3.731 ± 0.314
Female						
Necropsy body wt	20.8 ± 0.1	20.3 ± 0.5	20.1 ± 0.5	20.0 ± 0.6	17.4 ± 0.4**	13.0 ± 1.6**
Liver						
Absolute	0.93 ± 0.03	0.81 ± 0.02**	0.80 ± 0.03**	0.75 ± 0.03**	0.69 ± 0.03**	0.61 ± 0.06**
Relative	44.56 ± 1.13	40.09 ± 0.31**	39.75 ± 0.82**	37.40 ± 1.12**	39.73 ± 0.70**	46.88 ± 1.36
Lung						
Absolute	0.19 ± 0.01	0.19 ± 0.00	0.22 ± 0.01*	0.23 ± 0.01**	0.29 ± 0.01**	0.33 ± 0.02**
Relative	9.34 ± 0.38	9.49 ± 0.37	11.14 ± 0.24*	11.77 ± 0.59**	16.80 ± 0.58**	25.67 ± 1.53**

*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).

Increased incidences of nonneoplastic lesions of the lung occurred in exposed male and female mice (Table 20). The incidences of minimal to moderate alveolar histiocytic cellular infiltration were significantly increased in males and females exposed to 5 mg/m^3 or greater and consisted of varying numbers of histiocytes (macrophages) within the alveolar spaces and septa. The incidences of minimal to mild cytoplasmic vacuolization of the bronchiolar epithelium were significantly increased in males exposed to 2.5 or 10 mg/m^3 and females exposed to 5 mg/m^3. The lesion was characterized by the presence of poorly defined clear spaces within the cytoplasm of bronchiolar epithelial cells. The incidences of minimal to mild alveolar/bronchiolar epithelium karyomegaly were significantly increased in males and females exposed to 5 mg/m^3 or greater. Karyomegaly consisted of scattered, hypertrophied epithelial cells with single large and sometimes multiple, atypical nuclei in the terminal bronchioles, alveolar ducts, and alveoli. The incidences of minimal to moderate interstitial fibrosis were increased in all groups of mice exposed to 10 mg/m^3 or greater. Fibrosis consisted of multifocal to coalescing areas in which the alveolar architecture was effaced or obscured by fibroblasts and/or collagen within which were accumulations of histiocytes. Minimal to mild acute inflammation occurred in 40 mg/m^3 males and females that died before the end of the study. Minimal acute inflammation was also observed in some females exposed to 5, 10, or 20 mg/m^3. Acute inflammation consisted of perivascular and, to a lesser extent, peribronchiolar edema mixed with infiltrates of neutrophils.

Increased incidences of nonneoplastic lesions of the nose occurred in exposed groups of male and female mice (Table 20). The incidences of minimal to moderate acute inflammation were significantly increased in males exposed to 10 mg/m^3 or greater and females exposed to 5 mg/m^3 or greater. There was no increase in severity.

Table 20. Incidences of Selected Nonneoplastic Lesions of the Respiratory System in Mice in the Two-week Inhalation Study of Cobalt Metal

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Male						
Lung[a]	5	5	5	5	5	5
Alveolus, Infiltration Cellular, Histiocyte[b]	0	2 (1.0)[c]	5** (1.0)	5** (1.4)	5** (2.4)	5** (2.0)
Bronchiole, Epithelium, Vacuolization, Cytoplasmic	0	4* (1.0)	3 (1.0)	5** (1.6)	3 (1.0)	3 (1.3)
Alveolar/bronchiolar Epithelium, Karyomegaly	0	0	4* (1.0)	5** (1.0)	5** (1.8)	4* (1.5)
Interstitium, Fibrosis	0	0	0	3 (1.0)	5** (2.2)	3 (2.7)
Inflammation, Acute	0	0	0	0	0	3 (1.7)
Nose	5	5	5	5	5	5
Inflammation, Acute	0	0	1 (1.0)	5** (2.4)	5** (1.6)	5** (1.8)
Olfactory Epithelium, Atrophy	0	5** (1.0)	5** (1.0)	5** (1.8)	5** (1.8)	4** (2.0)
Olfactory Epithelium, Necrosis	0	2 (1.0)	3 (1.0)	0	5** (1.2)	5** (1.4)
Respiratory Epithelium, Vacuolization Cytoplasmic	0	4* (1.0)	5** (1.0)	4* (1.0)	5** (1.2)	5** (1.2)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	4* (1.0)	4* (1.0)	2 (1.0)
Female						
Lung	5	5	5	5	5	5
Alveolus, Infiltration Cellular, Histiocyte	0	2 (1.0)	5** (1.4)	5** (1.6)	5** (2.6)	5** (2.4)
Bronchiole, Epithelium, Vacuolization, Cytoplasmic	0	2 (1.0)	4* (1.0)	3 (1.7)	2 (1.0)	1 (1.0)
Alveolar/bronchiolar Epithelium, Karyomegaly	0	3 (1.0)	4* (1.0)	5** (1.2)	4* (1.3)	4* (1.0)
Interstitium, Fibrosis	0	0	0	2 (1.0)	5** (2.8)	2 (3.5)
Inflammation, Acute	0	0	2 (1.0)	1 (1.0)	3 (1.0)	2 (1.5)
Nose	5	5	5	5	5	5
Acute Inflammation	0	0	5** (2.0)	5** (2.6)	5** (2.4)	5** (2.2)
Olfactory Epithelium, Atrophy	0	5** (1.4)	5** (1.6)	5** (1.8)	5** (2.2)	3 (2.0)
Olfactory Epithelium, Necrosis	0	3 (1.0)	5** (1.0)	2 (1.5)	4* (1.5)	3 (1.7)
Respiratory Epithelium, Vacuolization Cytoplasmic	0	5** (1.0)	5** (1.0)	5** (1.2)	4* (1.0)	4* (1.0)

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	1 (1.0)	3 (1.0)	1 (1.0)

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

with increasing exposure concentration. Acute inflammation occurred in the respiratory and olfactory regions of the nasal cavity and was characterized by accumulation of proteinaceous fluid, mucus, and neutrophilic cell debris in the nasal passages. Occasionally, there were low numbers of neutrophils within the mucosa and lamina propria. The incidences of minimal to mild olfactory epithelium atrophy were significantly increased in all exposed groups except the 40 mg/m^3 females. Atrophy often accompanied necrosis and was characterized by loss of olfactory epithelial cells with decreased height of the epithelium. The remaining olfactory cells were often disorganized, flattened to cuboidal, or respiratory-type ciliated columnar and replaced the olfactory epithelial cells. In some areas, there were clusters or nests of sensory-type cells within the remaining olfactory epithelium; this was interpreted as a regenerative response. The incidences of minimal to mild olfactory epithelium necrosis were significantly increased in males exposed to 20 or 40 mg/m^3 and females exposed to 5 or 20 mg/m^3. Necrosis was characterized by segmental vacuolization, disorganization, and loss of epithelial cells in the dorsal meatus of the Level II nasal section and the lateral and ventral aspects of the ethmoid turbinates in Level III with full-thickness sloughing of the epithelium in some areas. The incidences of minimal cytoplasmic vacuolization of the respiratory epithelium were significantly increased in all exposed groups of males and females. Cytoplasmic vacuolization was characterized by the presence of poorly defined clear spaces within the cytoplasm of respiratory epithelial cells in Levels I and II. The incidences of minimal squamous metaplasia of the respiratory epithelium were significantly increased in males exposed to 10 or 20 mg/m^3. Squamous epithelial metaplasia involved lateral walls, turbinates, and ventral septa in Levels I and II. In affected sites, the normal single layer of tall columnar, ciliated epithelial cells was replaced by one to three layers of flattened (squamous) epithelial cells that lacked cilia.

Tissue Burden Studies

Tissue weights and concentrations were determined in male and female mice at terminal kill and in additional female mice held for 3 weeks after the exposure. Data were generated on male and female mice in all exposure groups; however, relatively small numbers of samples (n = 1 to 2) were available in 40 mg/m^3 females due to decreased survival.

Male and female mouse lung weights increased with increasing exposure concentration, reaching weights that were up to 1.5- to 2-fold greater than those of the chamber controls at terminal kill (Table I-9). In female mice that were held for the 3-week recovery period, lung weights of exposed groups recovered such that they were similar to those of the chamber controls at the end of the recovery period. In both males and females, treatment-related decreases in the weights of all other tissues occurred. Because of the significant changes in lung weight, lung cobalt burdens rather than lung concentrations were evaluated for toxicokinetic parameters.

At terminal kill, cobalt concentrations and burdens increased with exposure concentration in all tissues examined (Table I-9). Cobalt concentrations in tissues decreased in the order of lung > liver > kidney > serum > heart approximately equal to femur > blood > testes (males). Tissue cobalt burdens in male mouse tissues decreased in the order of lung > liver > kidney > heart > femur > testes. With the exception of testes, all tissues examined represented sites where cobalt could accumulate at concentrations greater than observed in the blood or serum. Mice of both sexes accumulated large amounts of cobalt in the liver. While lung cobalt burdens were generally higher than liver cobalt burdens at exposures of 20 mg/m^3, liver and lung burdens were similar in females exposed to 20 mg/m^3 or less, and liver burdens were greater than lung burdens in 40 mg/m^3 males and females. Normalized tissue burdens generally remained the same or decreased with increasing exposure concentration.

Kinetic analysis of data from female mice exposed to 20 mg/m^3 or less indicated elimination half-lives of 4.1 to 7.3 days (blood), 2.9 to 3.7 days (serum), or 5.5 to 6.6 days (lung) (Table I-10); in general, half-lives decreased with increasing exposure concentration. Lung cobalt deposition rates and predicted steady-state lung cobalt burdens increased in proportion to exposure concentrations of 2.5 and 5 mg/m^3, but the increases were less than proportional at greater exposure concentrations.

Exposure Concentration Selection Rationale: Significant mortality was observed in male and female mice exposed to 40 mg/m^3 in the 2-week study. There were significant decreases in body weights in 20 mg/m^3 males (9%) and females (16%). Significantly increased incidences of alveolar infiltration (histiocytic) were observed in the lung of males and females exposed to 5 mg/m^3 or greater. However, the average severity grade was minimal in the 5 and 10 mg/m^3 groups, and the increases in lung weights in the 10 mg/m^3 groups were not considered sufficiently severe to preclude the use of this concentration. Hence, 10 mg/m^3 was selected as the highest exposure concentration for the 3-month inhalation study in mice.

Three-month Study

One 2.5 mg/m^3 female mouse was accidentally killed during the first week of the study; all other mice survived to the end of the study (Table 21). The final mean body weights and mean body weight gains of males and females exposed to 10 mg/m^3 were significantly less than those of the chamber controls (Table 21 and Figure 4). Abnormal breathing was noted in approximately 50% of males and females exposed to 10 mg/m^3. At necropsy, tan lungs were noted in mice exposed to 5 or 10 mg/m^3.

On days 26 and 40, microsomal suspensions of liver samples from special study female mice not used for tissue burden studies were prepared and assayed for A4H, EROD, and PROD activities (Table J-2). There were no consistent trends in A4H, EROD, or PROD activities relative to exposure concentrations at either time point or from day 26 to day 40. A4H activities were significantly increased in the 5 and 10 mg/m^3 groups on day 40. EROD activities were significantly increased in the 5 and 10 mg/m^3 groups at both time points.

Similar to effects noted in rats, statistically significant increases were observed in hemoglobin concentration and erythrocyte count of 10 mg/m^3 males and in the erythrocyte count of 10 mg/m^3 females at 14 weeks (Table F-2). These changes in comparison to the rats, however, were minimal (<5%).

Table 21. Survival and Body Weights of Mice in the Three-month Inhalation Study of Cobalt Metal[a]

Concentration (mg/m^3)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	23.7 ± 0.2	37.7 ± 0.8	14.0 ± 0.7	
0.625	10/10	23.7 ± 0.3	38.2 ± 0.6	14.5 ± 0.4	101
1.25	10/10	23.7 ± 0.2	37.9 ± 0.8	14.2 ± 0.8	101
2.5	10/10	23.8 ± 0.2	37.0 ± 0.5	13.3 ± 0.4	98
5	10/10	23.7 ± 0.2	37.0 ± 0.9	13.4 ± 0.8	98
10	10/10	23.8 ± 0.2	32.5 ± 0.5**	8.7 ± 0.5**	86
Female					
0	10/10	20.5 ± 0.3	30.9 ± 1.0	10.4 ± 1.1	
0.625	10/10	20.0 ± 0.3	31.6 ± 1.1	11.6 ± 1.3	102
1.25	10/10	20.2 ± 0.4	31.4 ± 0.9	11.2 ± 0.7	102
2.5	9/10[c]	19.8 ± 0.2	30.1 ± 0.7	10.1 ± 0.7	97
5	10/10	20.1 ± 0.3	29.0 ± 1.1	8.9 ± 1.0	94
10	10/10	19.8 ± 0.2	26.8 ± 1.0**	7.0 ± 1.0*	87

*Significantly different ($P \leq 0.05$) from the chamber control group by Williams' test.
**$P \leq 0.01$.
[a]Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[b]Number of animals surviving at 14 weeks/number initially in group.
[c]Week of death: 1.

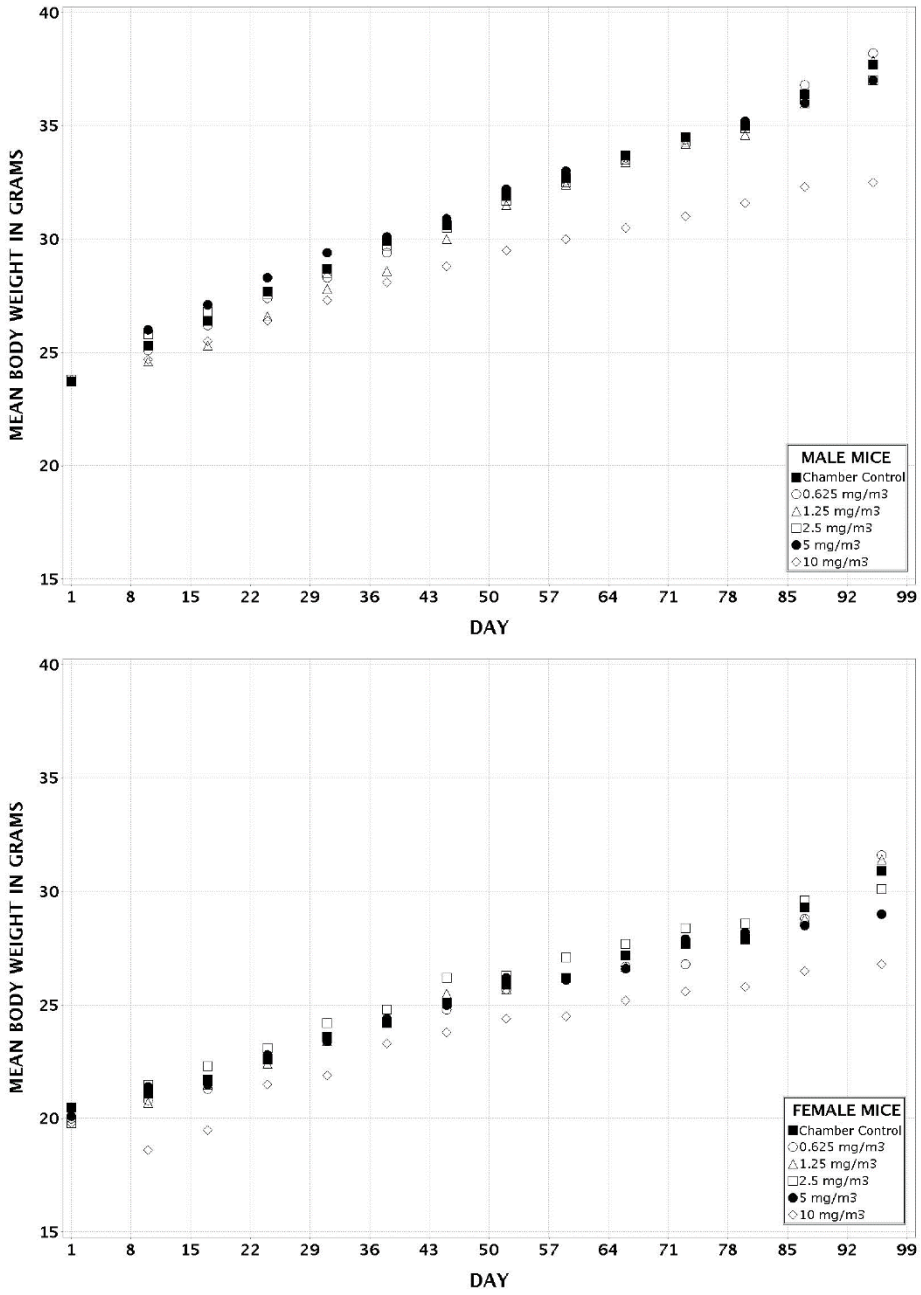


Figure 4. Growth Curves for Mice Exposed to Cobalt Metal by Inhalation for Three Months

Absolute and relative lung weights of males exposed to 2.5 mg/m^3 or greater and females exposed to 5 or 10 mg/m^3 were significantly greater than those of the chamber controls (Table 22 and Table G-4). Absolute and relative liver weights of males exposed to 10 mg/m^3 and females exposed to 2.5 mg/m^3 or greater were significantly less than those of the chamber controls. Absolute and relative kidney weights of males and females exposed to 5 or 10 mg/m^3 were significantly less than those of the chamber controls. Absolute and relative testes weights of males exposed to 5 or 10 mg/m^3 were significantly less than those of the chamber controls.

Table 22. Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
Male						
n	10	10	10	10	10	10
Necropsy body wt	37.7 ± 0.8	38.2 ± 0.6	37.6 ± 0.8	37.0 ± 0.5	37.0 ± 0.9	32.5 ± 0.5**
R. Kidney						
Absolute	0.31 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.00	0.29 ± 0.01**	0.23 ± 0.01**
Relative	8.360 ± 0.192	8.441 ± 0.122	8.333 ± 0.237	8.507 ± 0.047	7.714 ± 0.145**	7.176 ± 0.131**
Liver						
Absolute	1.48 ± 0.04	1.53 ± 0.04	1.51 ± 0.07	1.49 ± 0.04	1.42 ± 0.05	1.15 ± 0.03**
Relative	39.217 ± 0.586	40.049 ± 0.671	39.753 ± 1.032	40.301 ± 0.698	38.159 ± 0.723	35.457 ± 0.668**
Lung						
Absolute	0.20 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.23 ± 0.01*	0.27 ± 0.01**	0.30 ± 0.01**
Relative	5.416 ± 0.116	6.051 ± 0.235	5.737 ± 0.147	6.234 ± 0.088**	7.436 ± 0.262**	9.142 ± 0.177**
R. Testis						
Absolute	0.118 ± 0.002	0.119 ± 0.002	0.114 ± 0.002	0.114 ± 0.002	0.104 ± 0.003**	0.033 ± 0.001**
Relative	3.136 ± 0.058	3.131 ± 0.037	3.019 ± 0.078	3.073 ± 0.056	2.825 ± 0.082**	1.004 ± 0.025**
Female						
n	10	10	10	9	10	10
Necropsy body wt	30.9 ± 1.0	31.6 ± 1.1	31.4 ± 0.9	30.1 ± 0.7	29.0 ± 1.1	26.8 ± 1.0**
R. Kidney						
Absolute	0.21 ± 0.01	0.22 ± 0.00	0.21 ± 0.01	0.20 ± 0.00	0.17 ± 0.00**	0.16 ± 0.00**
Relative	6.887 ± 0.184	6.849 ± 0.155	6.661 ± 0.126	6.689 ± 0.254	6.031 ± 0.132**	6.142 ± 0.185**
Liver						
Absolute	1.46 ± 0.06	1.51 ± 0.07	1.46 ± 0.05	1.30 ± 0.03*	1.16 ± 0.04**	1.01 ± 0.03**
Relative	47.051 ± 0.808	47.552 ± 0.952	46.455 ± 1.046	43.092 ± 0.773**	39.831 ± 0.459**	38.045 ± 1.246**
Lung						
Absolute	0.21 ± 0.01	0.22 ± 0.00	0.23 ± 0.01	0.23 ± 0.01	0.28 ± 0.01**	0.33 ± 0.01**
Relative	6.904 ± 0.227	6.884 ± 0.176	7.300 ± 0.274	7.555 ± 0.184	9.787 ± 0.241**	12.602 ± 0.487**

*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).

Males exposed to 10 mg/m^3 exhibited significant decreases in the weights of the cauda epididymis, epididymis, and testis; testis weight was also significantly decreased in the 5 mg/m^3 group (Table 23 and Table H-4). Spermatids per testis were significantly decreased in 5 and 10 mg/m^3 males, and spermatids per gram testis were significantly decreased in 10 mg/m^3 males.

Sperm motility and total sperm per epididymis and per gram epididymis were significantly decreased in 5 and 10 mg/m^3 males; sperm motility was also significantly decreased in 2.5 mg/m^3 males. Findings in the 10 mg/m^3 males were associated with histopathologic changes. In female mice, the estrous cycle was significantly longer in the 10 mg/m^3 group (Table H-5). The Markov transition matrix analyses indicated no significant differences in estrous cyclicity between the exposed and chamber control groups of females (Table H-5 and Table H-6; Figure H-2).

Table 23. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
n	10	10	10	10
Weights (g)				
Necropsy body wt	37.7 ± 0.8	37.0 ± 0.5	37.0 ± 0.9	32.5 ± 0.5**
L. Cauda epididymis	0.0217 ± 0.0014	0.0210 ± 0.0008	0.0231 ± 0.0018	0.0168 ± 0.0006*
L. Epididymis	0.0603 ± 0.0022	0.0578 ± 0.0019	0.0614 ± 0.0035	0.0429 ± 0.0021**
L. Testis	0.1185 ± 0.0017	0.1132 ± 0.0023	0.1027 ± 0.0036**	0.0316 ± 0.0014**
Spermatid measurements				
Spermatid heads (10^6/testis)	22.34 ± 0.84	22.22 ± 0.65	18.90 ± 1.20*	0.53 ± 0.10**
Spermatid heads (10^6/g testis)	210.84 ± 6.85	227.74 ± 7.16	205.67 ± 7.43	24.27 ± 4.78**
Epididymal spermatozoal measurements				
Sperm motility (%)	86.0 ± 1.1	82.0 ± 0.8*	82.2 ± 1.1*	2.6 ± 1.2**
Sperm (10^6/cauda epididymis)	11.55 ± 0.39	10.53 ± 0.43	9.62 ± 0.49**	0.71 ± 0.06**
Sperm (10^6/g cauda epididymis)	551.1 ± 37.9	505.9 ± 23.3	439.9 ± 40.3*	43.4 ± 3.7**

*Significantly different ($P \leq 0.05$) from the chamber control group by Dunnett's test (cauda epididymis weight) or Shirley's test (spermatid and epididymal spermatozoal measurements).
**Significantly different ($P \leq 0.01$) from the chamber control group by Williams' test (body and tissue weights) or Shirley's test (spermatid and epididymal spermatozoal measurements).
[a]Data are presented as mean ± standard error.

Increased incidences of nonneoplastic lesions of the lung in exposed male and female mice included alveolus infiltration cellular, histiocyte; alveolus proteinosis; alveolar/bronchiolar epithelium karyomegaly; bronchiole epithelium hyperplasia and cytoplasmic vacuolization; and hemorrhage; the severities of the lesions increased with increasing exposure concentration (Table 24). Alveolar histiocytic cellular infiltration occurred in the lung of every exposed male and female mouse and was characterized by the presence of low to moderate numbers of histiocytes (macrophages) within alveolar spaces and septa. In some alveoli, the histiocytes were swollen and contained phagocytosed erythrocytes and/or eosinophilic material similar to that in the alveolar spaces. In mice exposed to 2.5 mg/m^3 or greater, the intracytoplasmic material appeared golden brown to grey. Minimal to mild alveolar proteinosis occurred in all males and females exposed to 5 or 10 mg/m^3 and was characterized by the accumulation of variable amounts of homogenous globular, granular, or fibrillar eosinophilic material within the alveolar spaces. Alveolar/bronchiolar epithelium karyomegaly occurred in all mice exposed to 5 or

10 mg/m^3 and was characterized by the presence of scattered, hypertrophied cells with single large and, sometimes multiple, atypical nuclei in the epithelium of the terminal bronchioles, alveolar ducts, and immediately adjacent alveoli. Minimal to moderate bronchiole epithelium hyperplasia occurred in every mouse exposed to 2.5 mg/m^3 or greater and was characterized by proliferation and hypertrophy of the epithelial cells lining terminal bronchioles with involvement of the alveolar ducts and adjacent alveolar septa in more severe cases. At affected sites, there was piling up of the pleomorphic cells in three to eight disorganized layers. The proliferating epithelial cells generally contained clear cytoplasmic vacuoles; this change was diagnosed as minimal to marked bronchiole epithelium cytoplasmic vacuolization, and it occurred in every exposed mouse. Several males and females exposed to 5 or 10 mg/m^3 had minimal hemorrhage characterized by low numbers of red blood cells within a few alveolar spaces.

In the nose, increased incidences of nonneoplastic lesions in exposed male and female mice included chronic active inflammation, olfactory epithelium degeneration and hyperplasia, respiratory epithelium degeneration, and squamous metaplasia, and turbinate atrophy; the severities of these lesions generally increased with increasing exposure concentration (Table 24).

Incidences of chronic active inflammation were significantly increased in the 5 and 10 mg/m^3 groups of males and females. Chronic active inflammation was observed in all three nasal sections of the nasal cavity and consisted of infiltrates of neutrophils, lymphocytes, macrophages, and plasma cells within the lamina propria and to a lesser extent the epithelium of the turbinates and nasal septum.

In some cases, the nasal passages contained proteinaceous fluid mixed with variable numbers of degenerate neutrophils, macrophages, and cellular debris. Incidences of olfactory epithelium degeneration were significantly increased in males and females exposed to 1.25 mg/m^3 or greater. Degeneration was a focal or multifocal lesion that variably involved the epithelium lining the dorsal meatus, turbinates, or nasal septa. In affected sites, there was disorganization of the epithelium with vacuolization, individual cell death, and/or loss of epithelial cells and decrease in the height of the epithelium and in some cases a decrease in the size of the olfactory nerve bundles in the lamina propria. Scattered incidences of olfactory epithelium hyperplasia occurred primarily in exposed groups of males; the lesion was characterized by clusters or nests of proliferating cells within or just adjacent to the olfactory epithelium and sometimes with extension into the lamina propria around glandular ducts. The cells sometimes formed rosettes and had scant cytoplasm and large, round to oval nuclei. Incidences of minimal to mild respiratory epithelium degeneration were significantly increased in males exposed to 1.25 mg/m^3 or greater and females exposed to 2.5 mg/m^3 or greater. Respiratory epithelium degeneration involved the turbinates and/or lateral walls of the Level I and II nasal sections. In affected sites, the epithelium appeared disorganized and there was variable epithelial cell vacuolation with loss of cilia, individual cell death, and loss of the epithelial cells. Incidences of minimal to mild respiratory epithelium squamous metaplasia were significantly increased in males and females exposed to 2.5 mg/m^3 or greater; the lesion involved the transitional epithelium of the lateral wall and the lateral surface and tips of the nasoturbinates and was often associated with proteinaceous fluid in the nasal passages. In affected sites, the normally ciliated, columnar epithelium was replaced by one or two layers of squamous epithelium. Incidences of turbinate atrophy were significantly increased in the 5 and 10 mg/m^3 groups of males and females. Turbinate atrophy occurred in all three histologic sections of the nose. Microscopically, turbinate atrophy appeared as more space in the nasal passages because the individual turbinates were short, blunt, and/or

narrow due to attenuation and/or loss of turbinate bone and other structures (glands, vessels, nerve bundles, and interstitial tissue) in the lamina propria.

Table 24. Incidences of Selected Nonneoplastic Lesions of the Respiratory System in Mice in the Three-month Inhalation Study of Cobalt Metal

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
Male						
Lung[a]	10	10	10	10	10	10
Alveolus, Infiltration Cellular, Histiocyte[b]	0	10** (1.0)[c]	10** (1.0)	10** (1.0)	10** (2.0)	10** (3.0)
Bronchiole, Epithelium, Hyperplasia	0	0	0	10** (1.0)	10** (1.9)	10** (3.0)
Bronchiole, Epithelium, Vacuolization Cytoplasmic	0	10** (1.0)	10** (1.0)	10** (1.5)	10** (2.7)	10** (3.9)
Alveolus, Proteinosis	0	0	0	0	10** (1.0)[c]	10** (2.0)
Alveolar/bronchiolar, Epithelium, Karyomegaly	0	0	0	0	10** (1.0)	10** (3.0)
Hemorrhage	0	1 (1.0)	0	1 (1.0)	7** (1.1)	6** (1.0)
Nose	10	10	10	10	10	10
Inflammation, Chronic Active	0	0	0	0	8** (1.4)	10** (2.5)
Olfactory Epithelium, Degeneration	0	2 (1.0)	10** (1.0)	10** (1.0)	10** (2.0)	10** (3.0)
Olfactory Epithelium, Hyperplasia	0	0	1 (1.0)	5* (1.0)	2 (1.0)	3 (1.3)
Respiratory Epithelium, Degeneration	0	0	6** (1.0)	9** (1.0)	10** (1.9)	10** (2.0)
Respiratory Epithelium, Metaplasia, Squamous	0	0	2 (1.0)	5* (1.0)	10** (1.3)	10** (1.9)
Turbinate, Atrophy	0	0	0	0	8** (2.1)	10** (3.0)
Larynx	10	10	10	10	10	10
Metaplasia, Squamous	0	10** (1.8)	10** (1.8)	10** (1.9)	10** (1.9)	10** (2.1)
Female						
Lung	10	10	10	10	10	10
Alveolus, Infiltration Cellular, Histiocyte	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (2.1)	10** (3.0)
Bronchiole, Epithelium, Hyperplasia	0	0	0	10** (1.0)	10** (1.9)	10** (3.0)
Bronchiole, Epithelium, Vacuolization Cytoplasmic	0	10** (1.0)	10** (1.0)	10** (1.1)	10** (2.6)	10** (3.9)

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
Alveolus, Proteinosis	0	0	0	0	10** (1.0)	10** (1.8)
Alveolar/bronchiolar, Epithelium, Karyomegaly	0	0	0	0	10** (1.7)	10** (3.0)
Hemorrhage	0	0	0	0	8** (1.0)	2 (1.0)
Nose	10	10	10	10	10	10
Inflammation, Chronic Active	0	0	0	1 (1.0)	10** (2.5)	10** (2.4)
Olfactory Epithelium, Degeneration	0	1 (1.0)	7** (1.0)	9** (1.0)	10** (2.5)	10** (2.9)
Olfactory Epithelium, Hyperplasia	0	0	0	3 (1.0)	0	0
Respiratory Epithelium, Degeneration	0	0	1 (1.0)	8** (1.0)	10** (1.9)	10** (1.9)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	9** (1.0)	10** (2.0)	10** (2.0)
Turbinate, Atrophy	0	0	0	0	10** (2.2)	10** (2.9)
Larynx	10	10	10	10	10	10
Metaplasia, Squamous	0	10** (1.3)	10** (1.4)	10** (1.6)	10** (1.8)	10** (2.2)

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

The incidences of squamous metaplasia were significantly increased in the larynx of all exposed groups of males and females, and severities of the lesion increased with increasing exposure concentration (Table 24). Squamous metaplasia occurred in the epithelium overlying the submucosal glands at the base of the epiglottis and was characterized by replacement of ciliated columnar epithelium with one to a few layers of flattened, nonciliated, squamous epithelial cells.

The incidence of marked germinal epithelium degeneration in the testes was significantly increased in males exposed to 10 mg/m^3 (Table 25). Testes diagnosed with germinal epithelium degeneration had greatly decreased overall cross-sectional diameter compared to chamber control testes. All or most seminiferous tubules had irregular to somewhat flattened outlines with markedly decreased or completely absent germinal epithelium, vacuolated Sertoli cells, and aspermia; intraluminal clumps of sloughed germinal cells and amorphous mineralized debris were current in the lumina of the seminiferous tubules.

In the epididymis, the incidences of exfoliated germ cells, hypospermia, cytoplasmic vacuolization, and atrophy were significantly increased in males exposed to 10 mg/m^3 (Table 25). Mild to moderate germ cell exfoliation was characterized by sloughed,

Table 25. Incidences of Selected Nonneoplastic Lesions of the Genital System in Male Mice in the Three-month Inhalation Study of Cobalt Metal

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
Testes[a]	10	10	10	10	10	10
Germinal Epithelium, Degeneration[b]	2 (1.0)[c]	0	0	0	1 (1.0)	10** (4.0)
Epididymis	10	10	10	9	10	10
Exfoliated Germ Cell	0	0	0	0	0	10** (2.7)
Hypospermia	0	0	0	0	0	10** (2.9)
Vacuolization Cytoplasmic	0	0	0	0	0	9** (1.0)
Atrophy	0	0	0	0	0	10** (1.0)

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

degenerate germinal epithelial cells (spermatocytes, spermatogonia, and spermatids), as well as amorphous debris in the body and tail of the epididymal duct. Hypospermia, most likely the consequence of loss of the germinal epithelium, was characterized by mild to moderate decreases in the numbers of mature spermatozoa in the body and tail of the epididymal ducts. Cytoplasmic vacuolization of duct epithelium was a subtle finding of minimal severity noted predominantly in the tail of the epididymis and characterized by increased numbers of ductal epithelial cells that were swollen by a single, large, clear cytoplasmic vacuole. Atrophy was a subtle change noted in the head region of the epididymal duct. In chamber control males, the epididymal duct was lined by densely packed, tall, columnar epithelial cells that appeared pseudostratified due to the occurrence of the basal nuclei at different levels in the cells. In animals exposed to 10 mg/m^3, the epithelial cells lining the head were not as tall and the basal nuclei were less numerous and less crowded and generally spaced evenly along the basement membrane in a single layer.

Tissue Burden Studies

Lung and liver weights and lung, blood, and liver cobalt concentrations were determined in female mice (Table I-11). During the exposure period, lung weights of the 5 and 10 mg/m^3 groups were significantly greater than those of the chamber controls starting on study day 12 and generally remained elevated compared to the chamber controls until the end of the postexposure period. Increased lung weights were occasionally observed at 2.5 mg/m^3. Because of the significant changes in lung weights with exposure concentration, lung cobalt burdens rather than lung cobalt concentrations were evaluated for toxicokinetic parameters.

Lung cobalt concentrations and burdens increased with increasing exposure concentration and were increased over chamber controls. Lung cobalt concentrations in chamber control animals were near or below the LOD at all time points. By day 40, lung cobalt concentrations in all exposed groups appeared to be approaching steady state and did not change significantly through the end of exposure (day 89) before steadily decreasing during the recovery period (Table I-11). Lung cobalt burdens increased rapidly within the first 5 to 26 days, but by days 12 to 40, the rate

of increase slowed as lung burdens asymptotically approached steady state with the higher concentrations taking longer to approach steady state. During the recovery period, lung cobalt burdens decreased very rapidly during the first week, after which lung clearance of cobalt slowed significantly. Normalized lung cobalt burdens tended to increase with exposure concentration up to 5 mg/m^3 but were lower in animals exposed to 10 mg/m^3 than in animals exposed to 5 mg/m^3, indicating a lack of a nonproportional accumulation at 10 mg/m^3.

Blood cobalt concentrations in the chamber control animals were at or below the LOD at all time points (Table I-11). During the 3-month exposure, blood cobalt concentrations generally increased in proportion to exposure concentration at all time points and were increased over chamber controls in all groups at all exposure time points and remained elevated through the later postexposure time points. Within each exposure concentration, blood cobalt concentrations appeared to be at or near steady state by study day 12. However during the recovery period, blood cobalt concentrations fell very rapidly to concentrations that were near or below the LOD in an exposure concentration-related manner. Accordingly, because of the rapid and extensive elimination of cobalt from the blood, it was not possible to demonstrate dose proportionality from blood concentration data collected during the recovery period.

Liver weights of the 5 and 10 mg/m^3 groups were significantly less than that of the chamber control group on day 26; similar, although not statistically significant decreased liver weights in these exposed groups were observed on day 40 (Table I-11). Liver cobalt concentrations in chamber control animals were at or below the LOD at both time points. During the 3-month exposure, liver cobalt concentrations and burdens generally increased with exposure concentration and were increased compared to the chamber controls at both time points. Liver cobalt concentrations and total liver cobalt burdens for exposed animals were higher at all exposure concentrations on day 26 compared to day 40 (except for cobalt concentration in animals exposed to 10 mg/m^3).

Pulmonary clearance of cobalt during the recovery period showed a well-defined two-phase elimination profile (Table I-12). The rapid phase exhibited half-lives ranging from 1.4 to 3.2 days and was followed by a slower lung clearance phase with half-lives of 27 to 39 days; there was no clear relationship to exposure concentration in either phase. A two-compartment clearance model could not be fit to the lung cobalt burden data collected during the 3-month study due to the lack of data collected prior to 5 days of exposure, however a one-compartment model provided an adequate fit to these data (Table I-13). The results indicated that half-lives ranged from 2.4 to 17 days (increased with increasing exposure concentration) for animals exposed to 5 mg/m^3 or less. The half-life in animals exposed to 10 mg/m^3 was 122 days, but the standard errors for the clearance rate constant and subsequently the calculated half-life were high (>80%) making these data unreliable.

Exposure Concentration Selection Rationale: Based on reductions in body weights and moderate severity of nose and lung lesions in the 10 mg/m^3 groups in the 3-month study, this exposure concentration was considered too high to be used in the 2-year study. Hence, 5 mg/m^3 was selected as the highest exposure concentration for the 2-year inhalation study in mice.

Two-year Study

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 26 and in the Kaplan-Meier survival curves (Figure 5). Survival of males exposed to 2.5 or 5 mg/m^3 was significantly less than that of the chamber control group.

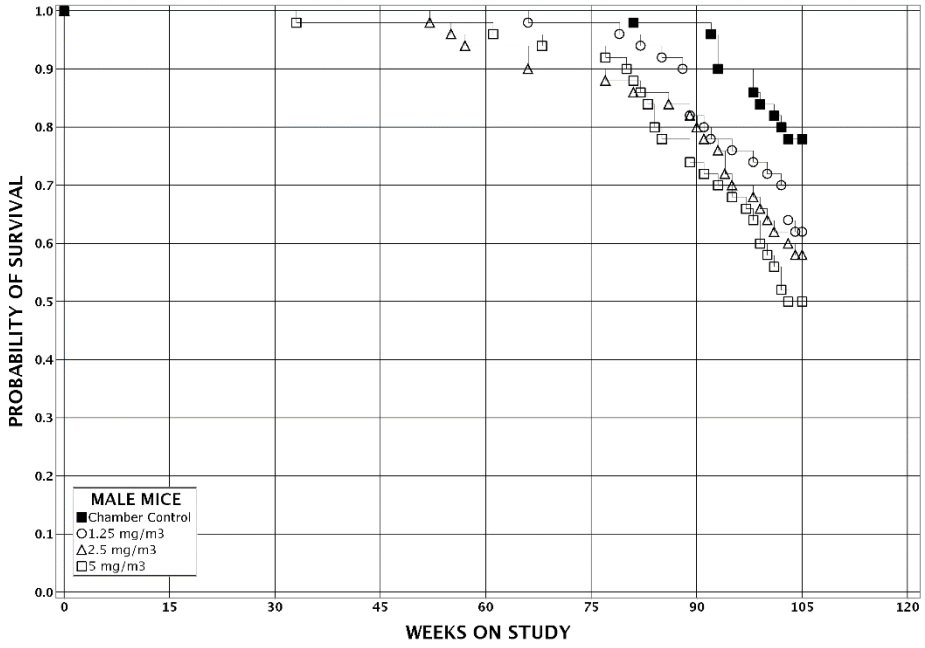
Body Weights and Clinical Findings

Mean body weights of 5 mg/m^3 males and females were at least 10% less than those of the chamber control groups after weeks 85 and 21, respectively (Table 27, Table 28 and Figure 6). Abnormal breathing and thinness were noted in exposed male and female mice.

Table 26. Survival of Mice in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Animals initially in study	50	50	50	50
Moribund	5	13	12	17
Natural deaths	6	6	9	8
Animals surviving to study termination	39	31	29[c]	25[d]
Percent probability of survival at end of study[a]	78	62	58	50
Mean survival (days)[b]	715	695	672	668
Survival analysis[e]	P = 0.004	P = 0.102	P = 0.035	P = 0.003
Female				
Animals initially in study	50	50	50	50
Moribund	9	12	19	21
Natural deaths	5	2	4	3
Animals surviving to study termination	36	36[f]	27	26
Percent probability of survival at end of study	72	70	54	52
Mean survival (days)	686	695	680	668
Survival analysis	P = 0.019	P = 1.000	P = 0.110	P = 0.061

[a]Kaplan-Meier determinations.
[b]Mean of all deaths (uncensored, censored, and terminal kill).
[c]Includes one animal that died during the last week of the study.
[d]Includes three animals that died during the last week of the study.
[e]The result of the life table trend test[174] is in the chamber control column, and the results of the life table pairwise comparisons[173] with the chamber controls are in the exposed group columns.
[f]Includes two animals that died during the last week of the study.



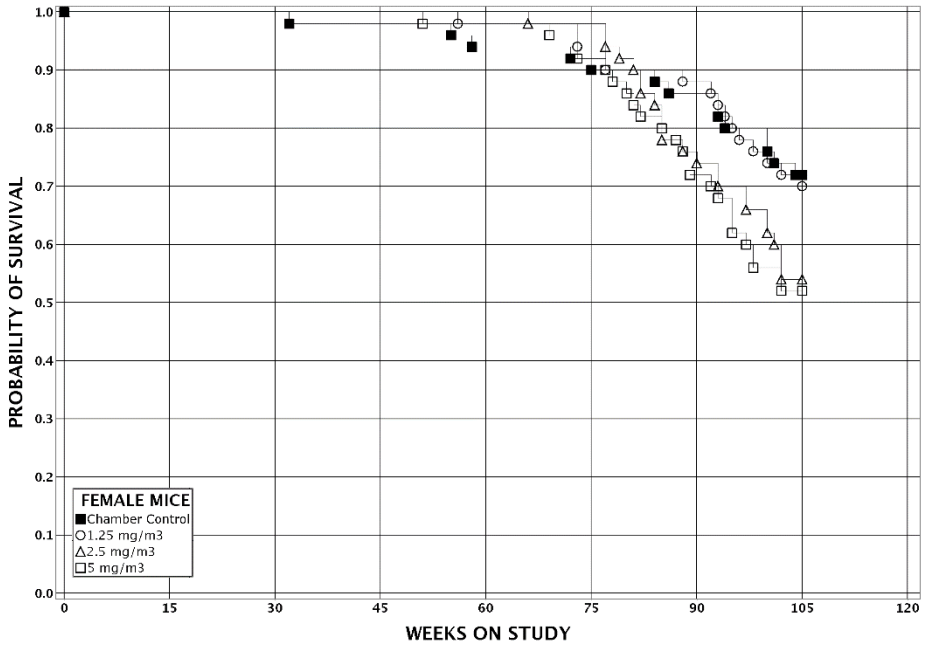


Figure 5. Kaplan-Meier Survival Curves for Mice Exposed to Cobalt Metal by Inhalation for Two Years

Table 27. Mean Body Weights and Survival of Male Mice in the Two-year Inhalation Study of Cobalt Metal

Day	Chamber Control		1.25 mg/m^3			2.5 mg/m^3			5 mg/m^3		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	23.6	50	23.5	99	50	23.1	98	50	23.2	98	50
8	25.4	50	25.1	99	50	24.9	98	50	24.9	98	50
15	26.5	50	26.0	98	50	25.5	96	50	26.1	98	50
22	27.7	50	27.1	98	50	27.0	97	50	26.9	97	50
29	28.8	50	28.3	98	50	28.2	98	50	28.2	98	50
36	30.1	50	29.5	98	50	29.3	98	50	29.2	97	50
43	31.1	50	30.5	98	50	30.1	97	50	30.0	96	50
50	32.4	50	31.8	98	50	31.2	96	50	30.8	95	50
57	33.2	50	32.9	99	50	32.3	98	50	31.9	96	50
64	34.3	50	34.0	99	50	33.3	97	50	33.0	96	50
71	35.5	50	35.1	99	50	34.1	96	50	33.6	95	50
78	36.4	50	35.9	99	50	35.0	96	50	34.1	94	50
85	37.4	50	37.1	99	50	35.9	96	50	35.0	94	50
113	40.8	50	41.1	101	50	39.6	97	50	37.7	93	50
141	43.5	50	44.0	101	50	42.7	98	50	40.0	92	50
169	45.7	50	45.8	100	50	45.0	99	50	42.3	93	50
197	47.4	50	47.8	101	50	47.1	100	50	44.7	94	50
225	49.1	50	49.0	100	50	48.6	99	50	45.5	93	50
253	50.0	50	50.0	100	50	50.2	100	50	47.5	95	49
281	51.1	50	50.8	100	50	50.9	100	50	48.1	94	49
309	51.5	50	51.6	100	50	51.8	101	50	49.1	95	49
337	52.1	50	52.2	100	50	52.3	101	50	49.7	96	49
365	52.9	50	52.8	100	50	52.9	100	49	50.3	95	49
393	53.3	50	53.4	100	50	53.3	100	48	50.9	96	49
421	53.6	50	53.5	100	50	54.2	101	47	51.3	96	49
449	53.5	50	53.6	100	50	54.0	101	47	51.5	96	48
477	53.8	50	53.9	100	49	54.5	101	45	51.4	96	47
505	54.0	50	53.5	99	49	54.3	101	45	51.1	95	47
533	54.2	50	53.4	99	49	54.5	101	44	49.9	92	47
561	54.3	49	52.8	97	48	54.1	101	44	49.5	91	45
589	53.2	49	51.8	97	46	52.6	99	43	48.8	92	40
617	53.6	49	52.2	98	43	51.5	96	42	47.6	89	38
645	53.4	48	53.1	100	39	51.3	96	39	46.3	87	36
659	53.8	45	52.4	98	39	50.4	94	36	44.2	82	35
673	52.7	45	52.3	99	38	50.0	95	35	42.5	81	34
687	52.4	43	52.0	99	37	49.0	94	34	40.9	78	32
701	51.8	42	51.8	100	36	47.5	92	32	40.1	77	29
715	51.2	40	51.8	101	34	47.0	92	31	39.5	77	26
Mean for Weeks											
1–13	31.0	–	30.5	99	–	30.0	97	–	29.8	96	–
14–52	47.9	–	48.0	100	–	47.6	99	–	45.0	94	–
53–103	53.2	–	52.8	99	–	51.9	98	–	47.2	89	–

Table 28. Mean Body Weights and Survival of Female Mice in the Two-year Inhalation Study of Cobalt Metal

Day	Chamber Control		1.25 mg/m^3			2.5 mg/m^3			5 mg/m^3		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt.(g)	Wt. (% of Controls)	No. of Survivors
1	19.8	50	19.7	99	50	19.6	99	50	19.6	99	50
8	21.3	50	21.0	99	50	21.1	99	50	21.0	99	50
15	22.1	50	21.6	98	50	21.9	99	50	21.8	99	50
22	23.2	50	22.6	97	50	22.9	99	50	23.0	99	50
29	23.9	50	23.7	99	50	23.9	100	50	23.5	98	50
36	24.9	50	24.6	99	50	25.0	100	50	24.2	97	50
43	25.6	50	25.7	100	50	25.8	101	50	25.0	98	50
50	26.2	50	26.7	102	50	26.9	103	50	26.0	99	50
57	27.3	50	27.7	102	50	28.0	103	50	26.9	98	50
64	28.6	50	28.7	100	50	29.1	102	50	27.4	96	50
71	29.1	50	29.9	103	50	30.3	104	50	28.5	98	50
78	29.7	50	31.0	104	50	31.3	106	50	29.2	98	50
85	30.6	50	32.3	105	50	32.6	106	50	29.5	96	50
113	34.6	50	37.0	107	50	37.0	107	50	32.2	93	50
141	38.6	50	40.8	106	50	40.9	106	50	35.0	91	50
169	41.8	50	43.4	104	50	43.8	105	50	37.6	90	50
197	44.3	50	46.2	104	50	46.3	105	50	39.7	90	50
225	47.4	49	48.6	103	50	48.0	101	50	41.0	87	50
253	49.7	49	50.8	102	50	50.4	101	50	43.1	87	50
281	53.2	49	53.1	100	50	51.9	98	50	44.1	83	50
309	56.5	49	55.5	98	50	53.9	95	50	45.1	80	50
337	59.1	49	57.5	97	50	56.0	95	50	46.7	79	50
365	60.4	49	59.2	98	50	57.7	96	50	47.7	79	49
393	62.6	48	61.1	98	49	59.2	95	50	48.8	78	49
421	64.6	47	62.4	97	49	60.7	94	50	50.1	78	49
449	65.6	47	62.5	95	49	61.1	93	50	50.3	77	49
477	66.7	47	62.9	94	49	61.2	92	49	49.7	75	49
505	65.9	46	62.9	95	48	61.0	93	49	49.7	75	47
533	66.3	45	62.8	95	47	60.9	92	48	50.1	76	45
561	65.1	45	64.3	99	45	61.1	94	46	49.6	76	43
589	64.9	44	62.6	96	45	60.5	93	42	48.5	75	41
617	63.2	43	61.9	98	44	60.8	96	38	46.8	74	38
645	62.0	43	60.0	97	43	58.2	94	37	47.5	77	34
659	61.9	40	60.2	97	41	58.3	94	35	44.7	72	34
673	60.7	40	60.7	100	39	56.3	93	35	44.8	74	30
687	59.1	40	59.5	101	38	56.2	95	33	43.8	74	28
701	59.1	37	58.6	99	37	56.0	95	31	42.0	71	28
715	58.5	37	57.3	98	36	56.3	96	27	40.6	70	26
Mean for Weeks											
1–13	25.6	–	25.6	101	–	26.0	102	–	25.0	98	–
14–52	47.2	–	48.1	102	–	47.6	101	–	40.5	87	–
53–103	62.9	–	61.2	97	–	59.1	94	–	47.2	75	–

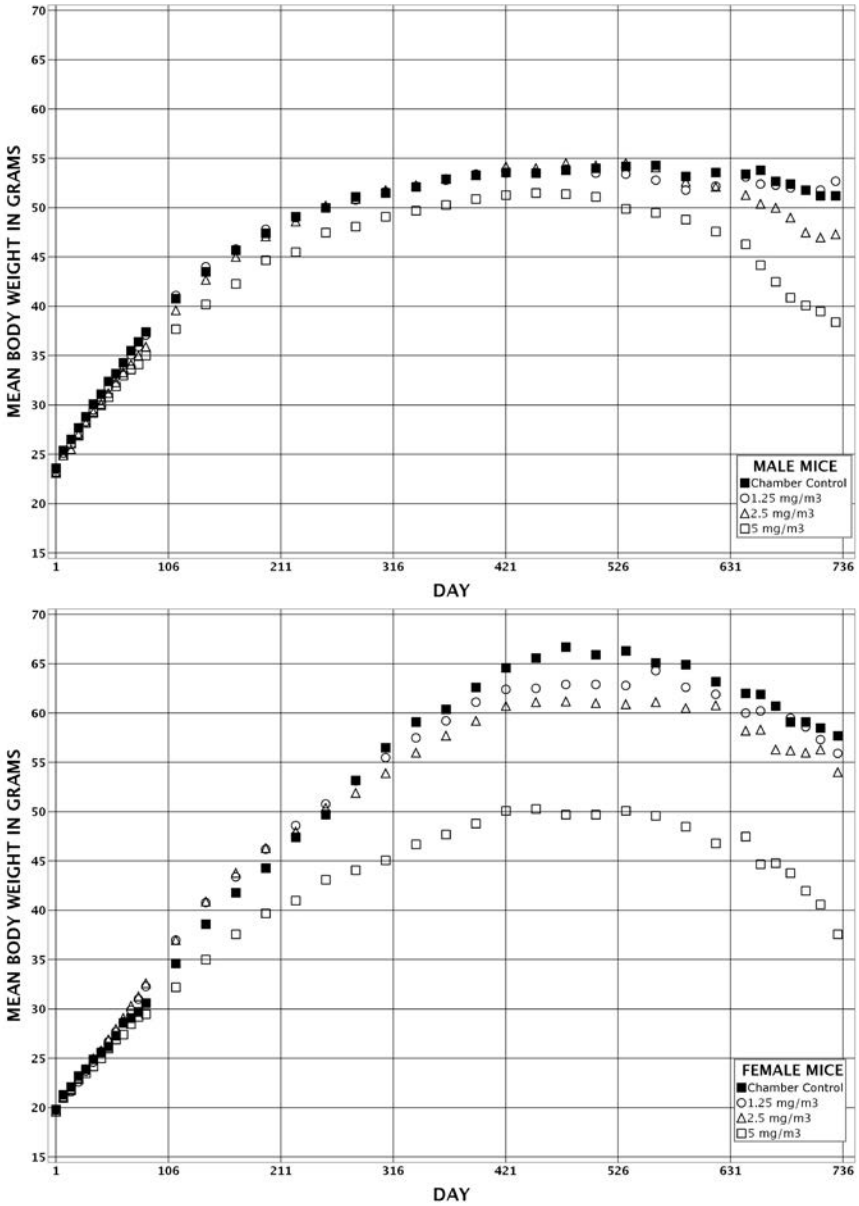


Figure 6. Growth Curves for Mice Exposed to Cobalt Metal by Inhalation for Two Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the lung, nose, larynx, trachea, and testes. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Lung: The incidences of alveolar/bronchiolar adenoma in males exposed to 2.5 mg/m^3 and females exposed to 5 mg/m^3 and the incidences of alveolar/bronchiolar carcinoma and alveolar adenoma or carcinoma (combined) in all exposed groups of male and female mice were significantly greater than those in the chamber controls (Table 29, Table C-1, Table C-2,

Table D-1, and Table D-2). The incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) occurred with positive trends and exceeded the historical control ranges for inhalation studies and all routes of administration (Table 29, Table C-3, and Table D-3). In addition, significantly increased incidences of multiple alveolar/bronchiolar carcinoma occurred in all exposed groups of males and females.

Alveolar/bronchiolar adenomas were small, generally nodular masses that distorted the alveolar architecture and compressed the adjacent parenchyma. Most adenomas consisted of interlacing papillary fronds and folds which projected into alveolar spaces and were lined by well differentiated cuboidal to low columnar epithelium supported by scant fibrovascular stroma (Figure 28). Other more solid appearing adenomas were composed of closely packed nests or cords of polygonal to cuboidal, lightly eosinophilic cells that completely filled the alveolar spaces. Alveolar/bronchiolar carcinomas were discrete to locally infiltrative, compressive, nodular to irregularly shaped masses that effaced the alveolar parenchyma and ranged in diameter from 1 to over 10 millimeters. Smaller carcinomas were relatively well differentiated neoplasms that were morphologically similar to adenomas but were distinguished by their slightly greater cellular pleomorphism and architectural disorganization. Larger carcinomas were clearly malignant neoplasms which were composed of poorly differentiated, pleomorphic anaplastic cells with a variety of growth patterns ranging from complex papillary, tubular, and/or glandular, and less commonly, solid sheets (Figure 29, Figure 30, and Figure 31). Many lungs that had carcinomas also had multiple, small nests of neoplastic cells randomly scattered throughout the lung parenchyma and occasionally on the pleura. Among the exposed mice with lung carcinomas, metastases occurred in various other organs including the liver, kidney, heart, nose, trachea, pancreas, cecum, adrenal cortex/medulla, coagulating gland, epididymis, testes, lymph nodes, thymus, skin, and skeletal muscle.

Point mutations in *Kras* (67%)*, Egfr* (17%), and *Tp53* (19%) were noted in the alveolar/bronchiolar carcinomas in mice chronically exposed to cobalt metal dust, and none were found in spontaneously arising alveolar/bronchiolar carcinomas in chamber control mice. The results for the molecular analyses are presented in Appendix K.

A spectrum of nonneoplastic lesions occurred in the lungs of male and female mice (Table 29, Table C-4, and Table D-4). The incidences of alveolar/bronchiolar epithelium hyperplasia and cytoplasmic vacuolization, alveolar epithelium hyperplasia, proteinosis, and alveolus infiltration cellular histiocyte occurred with positive trends in male and female mice and were significantly increased in all exposed groups of males and females. The incidences of bronchiole epithelium hyperplasia occurred with positive trends in males and females and were significantly increased in males exposed to 5 mg/m^3 and females exposed to 2.5 or 5 mg/m^3. The incidence of bronchiole epithelium erosion was significantly increased in males exposed to 2.5 mg/m^3. The incidences of suppurative inflammation were significantly increased in males exposed to 2.5 or 5 mg/m^3 and females exposed to 5 mg/m^3. In general, the severities of these nonneoplastic lesions increased with increasing exposure concentration.

Nonneoplastic lesions invariably occurred together and presented as a complex mixture of lesions that at times were difficult to separate as individual lesions. Alveolar/bronchiolar epithelium hyperplasia and alveolar/bronchiolar epithelium cytoplasmic vacuolization were changes that occurred in the epithelium of the periacinar region of the lung which encompassed the terminal bronchioles, associated alveolar ducts, and immediately adjacent alveoli. The

primary change throughout the epithelium was cytoplasmic vacuolization of the epithelium of terminal bronchioles and associated alveolar ducts and immediately adjacent alveoli that was also observed to a lesser extent in the larger bronchioles and bronchi. Cells with cytoplasmic vacuolization were swollen, cuboidal to irregularly polygonal, and had finely vacuolated to diffusely clear cytoplasm with small hyperchromic nuclei (Figure 32); generally, there was a decrease or absence of the surface cilia and apical blebbing that are characteristic of the normal ciliated epithelium and mouse Clara cells. Focally to multifocally, the epithelium of the terminal bronchioles appeared thickened by disorganized proliferation and piling up of vacuolated, cuboidal to polygonal epithelial cells that frequently extended into the alveolar ducts and the adjacent alveoli; these changes were diagnosed as alveolar/bronchiolar epithelium hyperplasia (Figure 32). Hyperplasia of the alveolar epithelium occurred as focal, discrete, irregular, noncompressive proliferations of alveolar epithelial (Type II) cells distributed randomly within the parenchyma or adjacent to the terminal bronchioles with preservation of the underlying alveolar architecture. These foci consisted of several contiguous alveolar septa lined by uniformly small cuboidal cells with small, hyperchromatic nuclei (Figure 33); occasional karyomegalic cells were present. Hyperplasia of the bronchiole epithelium was characterized by proliferation of cuboidal to low columnar bronchiolar epithelial cells as few to multiple papillary structures that were supported by scant fibrous stroma and which projected into the lumens of the terminal bronchioles (Figure 34). Erosion of the bronchiole epithelium consisted of minimal, focal denudation of bronchiolar epithelial cells with associated minimal necrosis.

Table 29. Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Number Examined Microscopically	50	49	50	50
Alveolar/bronchiolar Epithelium, Hyperplasia[a]	0	46** (1.0)[b]	49** (1.6)	50** (2.3)
Alveolar/bronchiolar Epithelium, Vacuolization Cytoplasmic	0	49** (1.1)	47** (1.9)	48** (3.1)
Alveolar Epithelium, Hyperplasia	4 (2.3)	29** (1.7)	24** (1.8)	43** (2.0)
Bronchiole, Epithelium, Hyperplasia	4 (2.5)	7 (1.3)	9 (1.3)	11* (1.5)
Bronchiole, Epithelium, Erosion	0	4 (1.0)	10** (1.3)	2 (1.0)
Proteinosis	2 (1.0)	46** (1.7)	49** (3.1)	50** (3.9)
Alveolus, Infiltration Cellular, Histiocyte	10 (1.8)	49** (1.8)	48** (2.5)	48** (3.1)
Inflammation, Suppurative	1 (1.0)	2 (2.0)	6* (1.5)	16** (2.3)
Alveolar/bronchiolar Adenoma, Multiple	0	1	1	0
Alveolar/bronchiolar Adenoma (includes multiple)[c]				
Overall rate[d]	7/50 (14%)	11/49 (22%)	15/50 (30%)	3/50 (6%)
Adjusted rate[e]	14.7%	24.5%	35.9%	7.3%

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Terminal rate[f]	5/39 (13%)	7/31 (23%)	14/29 (48%)	2/25 (8%)
First incidence (days)	684	571	660	571
Poly-3 test[g]	P = 0.254N	P = 0.176	P = 0.016	P = 0.226N
Alveolar/bronchiolar Carcinoma, Multiple	3	18**	24**	36**
Alveolar/bronchiolar Carcinoma (includes multiple)[h]				
Overall rate	11/50 (22%)	38/49 (78%)	42/50 (84%)	46/50 (92%)
Adjusted rate	22.8%	79.4%	87.6%	93.8%
Terminal rate	8/39 (21%)	24/31 (77%)	25/29 (86%)	22/25 (88%)
First incidence (days)	561	551	382	425
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Alveolar/bronchiolar Adenoma or Carcinoma[i]				
Overall rate	16/50 (32%)	41/49 (84%)	43/50 (86%)	47/50 (94%)
Adjusted rate	33.0%	85.0%	89.7%	95.9%
Terminal rate	11/39 (28%)	26/31 (84%)	26/29 (90%)	23/25 (92%)
First incidence (days)	561	551	382	425
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Female				
Number Examined Microscopically	49	50	50	50
Alveolar/bronchiolar Epithelium, Hyperplasia	0	49** (1.1)	49** (1.9)	50** (2.7)
Alveolar/bronchiolar Epithelium, Vacuolization Cytoplasmic	0	48** (1.1)	49** (1.9)	48** (3.5)
Alveolar Epithelium, Hyperplasia	2 (2.5)	27** (1.6)	26** (1.4)	41** (1.4)
Bronchiole, Epithelium, Hyperplasia	0	3 (1.0)	12** (1.1)	26** (1.2)
Bronchiole, Epithelium, Erosion	0	0	4 (1.0)	3 (1.0)
Proteinosis	0	45** (1.4)	50** (2.6)	50** (3.9)
Alveolus, Infiltration Cellular, Histiocyte	10 (1.7)	49** (1.6)	50** (2.5)	49** (3.1)
Inflammation, Suppurative	0	3 (1.3)	2 (1.0)	15** (1.7)
Alveolar/bronchiolar Adenoma, Multiple	0	1	0	1
Alveolar/bronchiolar Adenoma (includes multiple)[j]				
Overall rate	3/49 (6%)	9/50 (18%)	8/50 (16%)	10/50 (20%)
Adjusted rate	6.9%	19.9%	18.9%	24.5%
Terminal rate	3/36 (8%)	7/35 (20%)	6/27 (22%)	6/26 (23%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
First incidence (days)	731 (T)	505	626	593
Poly-3 test	P = 0.037	P = 0.067	P = 0.087	P = 0.024
Alveolar/bronchiolar Carcinoma, Multiple	1	7*	20**	24**
Alveolar/bronchiolar Carcinoma (includes multiple)[k]				
Overall rate	5/49 (10%)	25/50 (50%)	38/50 (76%)	43/50 (86%)
Adjusted rate	11.3%	53.8%	78.9%	87.7%
Terminal rate	3/36 (8%)	18/35 (51%)	19/27 (70%)	21/26 (81%)
First incidence (days)	583	537	457	478
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Alveolar/bronchiolar Adenoma or Carcinoma[l]				
Overall rate	8/49 (16%)	30/50 (60%)	41/50 (82%)	45/50 (90%)
Adjusted rate	18.0%	63.7%	84.6%	91.6%
Terminal rate	6/36 (17%)	22/35 (63%)	21/27 (78%)	22/26 (85%)
First incidence (days)	583	505	457	478
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001

*Significantly different ($P \leq 0.05$) from the chamber control group by the Poly-3 test.
**$P \leq 0.01$.
(T) Terminal kill.
[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[c]Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 39/300 (13.0% ± 4.2%), range 8%–20%; all routes: 145/950 (15.3% ± 6.2%), range 2%–26%.
[d]Number of animals with neoplasm per number of animals with lung examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[h]Historical incidence for inhalation studies: 59/300 (19.7% ± 3.4%), range 16%–24%; all routes: 132/950 (13.9% ± 7.1%), range 4%–24%.
[i]Historical incidence for inhalation studies: 90/300 (30.0% ± 5.5%), range 26%–40%; all routes: 263/950 (27.7% ± 5.7%), range 16%–40%.
[j]Historical incidence for inhalation studies: 16/299 (5.4% ± 3.7%), range 2%–12%; all routes: 54/949 (5.7% ± 3.6%), range 0%–12%.
[k]Historical incidence for inhalation studies: 13/299 (4.4% ± 4.3%), range 0%–10%; all routes: 38/949 (4.0% ± 3.6%), range 0%–14%.
[l]Historical incidence for inhalation studies: 28/299 (9.4% ± 4.8%), range 2%–16%; all routes: 90/949 (9.5% ± 4.8%), range 2%–22%.

Alveoli contained a complex mixture of proteinaceous material (diagnosed as proteinosis) and inflammatory cells. Alveolar proteinosis in mice was morphologically similar to proteinosis in rats and was characterized by accumulation of variable amounts of brightly eosinophilic material within alveolar spaces and ducts with extension into the lumens of the bronchioles in the more extreme cases. The character of this material ranged from pale eosinophilic, flocculent to amorphous aggregates to brightly eosinophilic, dense, round to irregular clumps free within

alveolar spaces or alveolar macrophages. In the more severe cases of proteinosis, there were single or aggregated slender, elongated, acicular to rectangular fractile, crystalline or spicule-like structures free within the alveoli or alveolar macrophages (Figure 35). Almost diffusely mixed with this proteinaceous material were increased numbers of histiocytes/macrophages that occurred as small, scattered aggregates to massive accumulations that on occasion completely occluded the alveoli in large regions of the lung. The histiocyte/macrophage infiltrates were mixtures of small to swollen macrophages to multinucleated giant cells. Many were distended with proteinaceous material or, especially in the lower exposure concentration groups, had cytoplasm that was lightly eosinophilic to gray, finely granular or foamy (amphophilic). Large histiocytes/macrophages and multinucleated giant cells contained the refractile, crystalline/specular-like structures. Accumulations of small histiocyte/macrophage infiltrates aggregated adjacent to alveolar/bronchiolar neoplasms (especially larger carcinomas). Also considered a component of this lesion were multifocal accumulations of plump foamy histiocytes/macrophages within the alveolar spaces; such cells were more frequent in the lower exposure concentration groups. Together, these histiocyte/macrophage infiltrates were diagnosed as histiocytic cellular infiltration of the alveolus (Figure 35). Mixed with the alveolar proteinaceous material and histiocyte/macrophage infiltrates were areas of prominent neutrophil accumulation that were diagnosed as suppurative inflammation. This occurred primarily in the male and female mice exposed to 5 mg/m^3 and consisted of variably sized, localized accumulations of neutrophils and necrotic debris within alveoli. In areas of intense neutrophil accumulation, the alveolar septa were sometimes necrotic or even completely effaced; peribronchiolar edema, intraalveolar hemorrhage, and bacteria were occasionally observed in association with suppurative inflammation.

Nose: A spectrum of nonneoplastic lesions occurred in exposed groups of males and females and the incidences of these lesions were generally significantly greater than those in the chamber control groups (Table 30, Table C-4, and Table D-4). For some lesions, the severities increased with increasing exposure concentration.

Suppurative inflammation in the nasal cavity of mice was morphologically similar to suppurative inflammation in the rats. It occurred primarily in the Level II nasal section and consisted of accumulations of neutrophils, proteinaceous fluid, and cellular debris in the nasal passages at all levels of the nose and was occasionally associated with fragments of plant material. Neutrophils sometimes infiltrated the nasal epithelium and lamina propria, and occasionally, the inflammatory process extended into the nasolacrimal duct, maxillary sinuses, and vomeronasal organ.

Atrophy of the olfactory epithelium was of minimal to mild severity and occurred in the epithelium of the dorsal meatus in Levels II and III and the ethmoturbinates of Level III. In general, olfactory epithelial atrophy in mice was morphologically similar to olfactory epithelial atrophy in the rats and was characterized by focal to diffuse hypocellularity and disorganization of the epithelium, often with increased extent of clear intercellular spaces with or without an overall decrease in height of the epithelium. There were variable decreases in the size and number of the nerve bundles and submucosal glands in the adjacent lamina propria. Hyperplasia of the olfactory epithelium was of minimal to mild severity and consisted of scattered focal proliferation of basal olfactory epithelial cells that extended through the basal lamina into the adjacent lamina propria often associated with Bowman’s gland ducts. The cells were sometimes

clustered in small intraepithelial nests or extended into the lamina propria around the Bowman's gland ducts.

Table 30. Incidences of Nonneoplastic Lesions of the Nose, Larynx, and Trachea in Mice in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male				
Nose[a]	50	49	50	50
Inflammation, Suppurative[b]	16 (1.1)[c]	32** (1.9)	49** (2.7)	50** (3.1)
Olfactory Epithelium, Atrophy	3 (1.0)	46** (1.2)	42** (1.2)	31** (1.2)
Olfactory Epithelium, Hyperplasia	0	25** (1.2)	17** (1.0)	8** (1.1)
Olfactory Epithelium, Metaplasia, Respiratory	5 (1.4)	24** (1.3)	44** (2.3)	50** (3.1)
Olfactory Epithelium, Respiratory Metaplasia, Atypical	0	14** (2.0)	9** (1.1)	1 (1.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	13 (1.2)	29** (1.1)	29** (1.1)	7 (1.0)
Respiratory Epithelium, Vacuolization Cytoplasmic	0	41** (1.2)	39** (1.2)	37** (1.4)
Respiratory Epithelium, Metaplasia, Squamous	3 (1.0)	45** (1.0)	35** (1.1)	33** (1.2)
Turbinate, Atrophy	3 (1.3)	25** (1.3)	49** (2.1)	50** (3.3)
Larynx	48	47	49	50
Respiratory Epithelium, Metaplasia, Squamous	7 (1.0)	47** (1.0)	49** (1.0)	49** (1.0)
Respiratory Epithelium, Vacuolization Cytoplasmic	0	20** (1.0)	24** (1.0)	32** (1.1)
Squamous Epithelium, Hyperplasia	2 (1.0)	5 (1.0)	5 (1.0)	8* (1.0)
Trachea	48	47	48	50
Epithelium, Vacuolization Cytoplasmic	0	14** (1.4)	31** (1.6)	37** (1.4)
Female				
Nose	50	50	50	50
Inflammation, Suppurative	3 (1.0)	47** (2.3)	50** (3.1)	50** (3.3)
Olfactory Epithelium, Atrophy	4 (1.0)	44** (1.2)	39** (1.2)	24** (1.2)
Olfactory Epithelium, Hyperplasia	1 (1.0)	22** (1.1)	16** (1.0)	8* (1.0)
Olfactory Epithelium, Metaplasia, Respiratory	1 (1.0)	26** (1.8)	44** (2.7)	50** (3.3)
Olfactory Epithelium, Respiratory Metaplasia, Atypical	0	18** (1.6)	14** (1.5)	1 (1.0)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Respiratory Epithelium, Accumulation, Hyaline Droplet	12 (1.0)	38** (1.1)	40** (1.2)	10 (1.0)
Respiratory Epithelium, Vacuolization Cytoplasmic	0	40** (1.0)	47** (1.1)	47** (1.1)
Respiratory Epithelium, Metaplasia, Squamous	0	49** (1.2)	49** (1.4)	50** (1.5)
Turbinate, Atrophy	0	44** (2.2)	50** (2.9)	50** (3.4)
Larynx	47	50	50	47
Respiratory Epithelium, Metaplasia, Squamous	2 (1.0)	49** (1.0)	50** (1.0)	47** (1.1)
Respiratory Epithelium, Vacuolization Cytoplasmic	0	24** (1.0)	31** (1.0)	34** (1.0)
Squamous Epithelium, Hyperplasia	2 (1.0)	13** (1.1)	21** (1.0)	21** (1.0)
Squamous Epithelium, Erosion	1 (1.0)	2 (1.0)	7* (1.0)	4 (1.0)
Trachea	48	50	48	49
Epithelium, Vacuolization Cytoplasmic	0	26** (1.4)	37** (1.6)	39** (1.8)

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

Respiratory metaplasia of the olfactory epithelium was of minimal to mild severity and observed more frequently in the dorsal meatus of Level II and on the nasal septum and ethmoturbinates in Level III. These lesions consisted of replacement of olfactory epithelium by ciliated, cuboidal to tall columnar, respiratory-type epithelial cells. The metaplastic epithelium occurred as crypt-like folds and invaginations and extended into the ducts of the submucosal Bowman's glands. In 1.25 and 2.5 mg/m^3 animals of both sexes, there were focal, highly site-specific, often bilaterally symmetrical, exophytic lesions located on the dorsal surface of the dorsal scroll. These focal lesions seemingly arose in areas that resembled respiratory metaplasia. The lesions were slightly to prominently elevated above the surface of the ethmoturbinates and in extreme cases, formed synechia with opposing dorsal turbinates and the roof of the dorsal meatus. Morphology ranged from small, single, or few rosette- or gland-like structures, to larger more complex formations of glands lined by flattened to ciliated respiratory type epithelium (Figure 36). These unusual lesions were diagnosed as olfactory epithelium atypical respiratory metaplasia.

Hyaline droplet accumulation in the respiratory epithelium occurred with minimal severity and involved the respiratory epithelium adjacent to the junction with squamous epithelium lining the ventral aspects of the nasal passages in Levels I and II, adjacent to the incisive duct in Level II, and overlying the vomeronasal organ. Hyaline droplet accumulation consisted of intracytoplasmic, homogenous, eosinophilic, globular material in the cytoplasm of the respiratory epithelial cells.

Cytoplasmic vacuolization of the respiratory epithelium primarily occurred with minimal to mild severity and affected the respiratory epithelium of the dorsal to mid-septum and/or the dorsal meatus of Level I and occasionally Level II (lateral walls and metaplastic region of the dorsal meatus). The morphology of cytoplasmic vacuolization was similar to that observed in the bronchiolar epithelium. In affected sites, the normally tall, pseudostratified, ciliated, columnar epithelium was replaced by plump, variably ciliated, cuboidal to polygonal epithelial cells that had finely vacuolated to diffusely clear cytoplasm.

Squamous metaplasia of the respiratory epithelium of minimal to mild severity was generally similar to this lesion in the rat study. It occurred in the Level I and to a lesser extent, Level II sections along the dorsal to mid-septum and on the tips of the nasoturbinates and maxilloturbinates. Squamous metaplasia was characterized by replacement of the normally current single layer of ciliated columnar respiratory epithelium by nonkeratinized, flat, squamous epithelial cells.

Turbinate atrophy was a minimal to moderate change that was similar to this lesion in the rat study. It was characterized by often prominent attenuation of the bone and structures of the lamina propria including the glands, vessels, nerve bundles, interstitial stroma on the naso-, maxilla-, and ethmoturbinates and the medial septum at Levels I, II, III. This resulted in narrowing, shortening, distortion, and sometimes loss of the turbinates, as well as occasional adhesions of turbinate remnants to each other, the nasal septum, or the lateral walls. The nasal septum was often buckled, bent, and sometimes perforated.

Larynx: The incidences of respiratory epithelium squamous metaplasia and cytoplasmic vacuolization in all exposed groups of males and females were significantly greater than those in the chamber controls (Table 30, Table C-4, and Table D-4). The incidences of squamous epithelium hyperplasia were significantly increased in all exposed groups of females and in males exposed to 5 mg/m^3. The incidence of squamous epithelium erosion was significantly increased in females exposed to 2.5 mg/m^3. All of these laryngeal lesions were of minimal severity.

Respiratory epithelium squamous metaplasia involved the epithelium at the base of the epiglottis overlying the medial submucosal glands and consisted of one to a few layers of flattened, non-ciliated, low-cuboidal to squamous epithelial cells replacing the ciliated, tall, columnar epithelium that normally occurs in this location. Respiratory epithelium cytoplasmic vacuolization was a subtle focal to diffuse change that occurred in the epithelium lining the dorsal aspects and lateral walls of Levels II and III laryngeal sections and was morphologically similar to cytoplasmic vacuolization observed in the bronchiolar epithelium. The ciliated columnar epithelial cells normally seen in the sites were shorter (cuboidal), in general had lost their cilia, and had slightly vacuolated to clear cytoplasm. Squamous epithelium hyperplasia was a focal change most common in the epithelium along the medial aspects and tips of the vocal processes of the arytenoid cartilages and consisted of increased layers of lining epithelial cells from the normal two to three cell layers to four to six cell layers. Erosion was characterized by small focal areas of epithelial necrosis and loss of the superficial epithelium in areas of hyperplastic squamous epithelium.

Trachea: The incidences of minimal to mild epithelium cytoplasmic vacuolization were significantly increased in all exposed groups of males and females (Table 30, Table C-4, and

Table D-4). Cytoplasmic vacuolization in the epithelium lining the trachea and the submucosal tracheal glands was morphologically similar to that observed in the bronchiolar and laryngeal epithelia.

Testes: The incidence of minimal to mild germinal epithelium degeneration in male mice exposed to 5 mg/m^3 was significantly greater than that in the chamber controls (chamber control, 9/50; 1.25 mg/m^3, 14/49; 2.5 mg/m^3, 8/50; 5 mg/m^3, 21/50; Table C-4). Germinal epithelium hyperplasia was generally a minimal to mild lesion usually affecting one to a few scattered seminiferous tubules. Affected tubules were characterized by partial to complete absence of spermatogenic cells often with concurrent swelling of the Sertoli cells with resultant hypocellularity and decreased height of the germinal epithelium. The lumens were generally empty but sometimes contained few spermatozoa, sloughed germinal epithelial cells, or cellular debris.

Tissue Burden Studies

Lung weights of female mice were significantly increased starting on day 4 in groups exposed to 2.5 or 5 mg/m^3 and continuing until day 548 (Table I-14). At 1.25 mg/m^3, lung weights were increased on days 366 and 548; because of these increases in lung weights, lung cobalt burdens rather than lung cobalt concentrations were evaluated for toxicokinetic parameters.

Cobalt concentrations and burdens in the lung increased with increasing exposure concentration and were significantly increased in all exposed groups of female mice at all time points compared to those in the chamber control group (Table I-14). Cobalt concentrations in the chamber control group were at or below the LOD at all time points. By day 184, lung cobalt concentrations for all exposed groups appeared to reach steady state and did not change significantly through day 548. Lung cobalt burdens increased rapidly by day 4, but by day 184, the rate of increase slowed as lung burdens asymptotically approached steady state. Analysis of lung cobalt burdens normalized to exposure concentration indicated that there were proportional increases between the 1.25 and 2.5 mg/m^3 groups, but nonproportional increases were observed between the 2.5 and 5 mg/m^3 groups. At the earlier time points, normalized lung cobalt burdens were lower in animals exposed to 5 mg/m^3 than in those exposed to 2.5 mg/m^3; however the opposite was true at the longer exposure durations, where normalized cobalt burdens were greater than proportional relative to the 2.5 mg/m^3 group.

The lung cobalt burden data from the exposure phases of the 3-month and 2-year studies were modeled using a two-compartment model (Figure I-2). Rapid clearance phase half-lives were 1.2, 1.1, and 5.2 days, respectively, for the 1.25, 2.5, and 5 mg/m^3 groups, indicating a slightly longer half-life in animals exposed to 5 mg/m^3 (Table I-15). Cobalt deposition rates for the rapid clearance phase were 0.87, 1.84, and 1.18 µg cobalt/day at 1.25, 2.5, and 5 mg/m^3, respectively. Slow clearance phase half-lives revealed the opposite trend, with half-lives of 409, 172, and 118 days with increasing exposure concentration. Cobalt deposition rates for the slow clearance phase were 0.027, 0.075, and 0.25 µg cobalt/day. The overall theoretical steady-state lung cobalt burdens, including both the rapid and slow clearance phases ($L_{SSa} + L_{SSb}$), were approximately 17.8, 21.4, and 51.8 µg cobalt/lung in the 1.25, 2.5, and 5 mg/m^3 groups, respectively; these data support the achievement of steady state in the 2.5 and 5 mg/m^3 groups but not in the 1.25 mg/m^3 group. The fractions of deposition in the slow clearance phase (F_B) for the exposed groups were quite low, increasing from 0.031 to 0.176 as exposure concentration increased, corresponding to total slow phase lung cobalt clearances of 3.1% to 17.6%; clearances of total deposited cobalt

during the rapid clearance phase ranged from 96.9% to 82.4% [$(1-F_B) \times 100$] with increasing exposure concentration.

Genetic Toxicology

Results of the bacterial mutagenicity tests conducted with cobalt metal (the same lot of chemical that was used in the 2-year studies) are presented in Table E-1 and Table E-2. Cobalt metal (100 to 5,000 µg/plate) gave an equivocal response in *Salmonella typhimurium* strain TA100 in the absence of S9 activation mix; with 10% rat liver S9, doses up to 7,500 µg/plate did not induce an increase in mutant colonies in TA100. In *S. typhimurium* strain TA98 without S9, cobalt metal (100 to 3,500 µg/plate) was mutagenic, although the responses observed were weak and not well correlated with dose level; with S9, no mutagenic activity was observed. In *Escherichia coli* strain WP2 *uvrA*/pKM101, doses of cobalt metal up to 450 µg/plate were not associated with mutagenic activity, with or without S9. No increases in the frequencies of micronucleated normochromatic erythrocytes were observed in peripheral blood of male or female mice exposed to cobalt metal (0.625 to 10 mg/m^3) for 3 months by inhalation (Table E-3). No significant alterations in the percentages of reticulocytes (polychromatic erythrocytes) were seen in male or female mice, suggesting that exposure to cobalt metal under these conditions did not cause bone marrow toxicity.

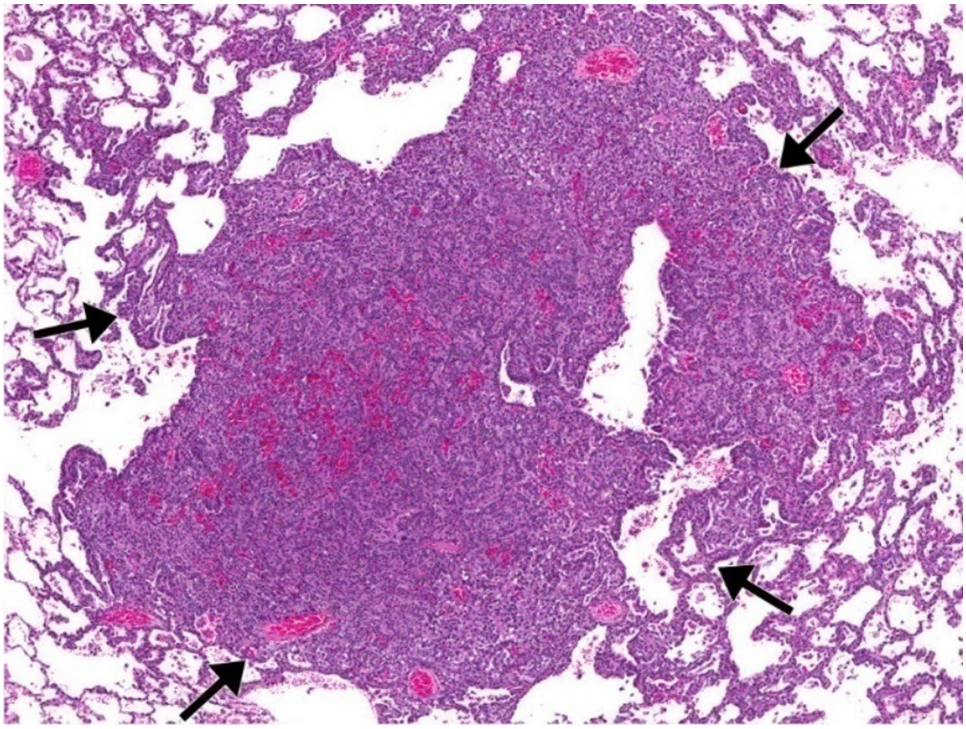


Figure 7. Alveolar/Bronchiolar Adenoma in the Lung of a F344/NTac Female Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The adenoma is distinctly demarcated from the surrounding alveolar parenchyma (arrows).

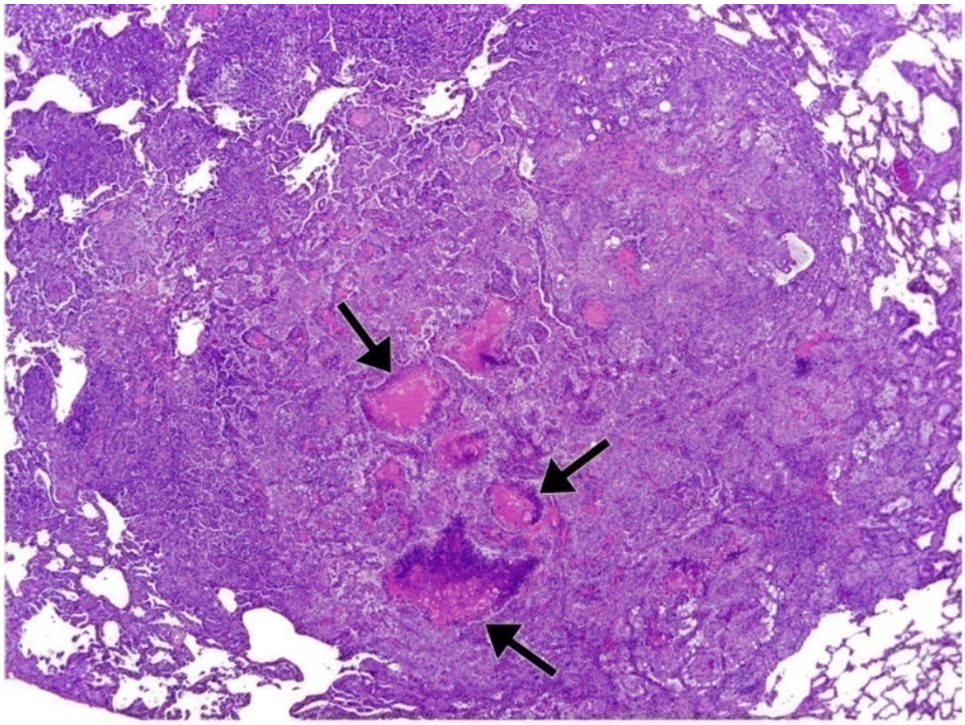


Figure 8. Alveolar/Bronchiolar Carcinoma in the Lung of a F344/NTac Female Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The carcinoma is highly invasive and has effaced the lung parenchyma. Note several areas of necrosis (arrows) within the carcinoma.

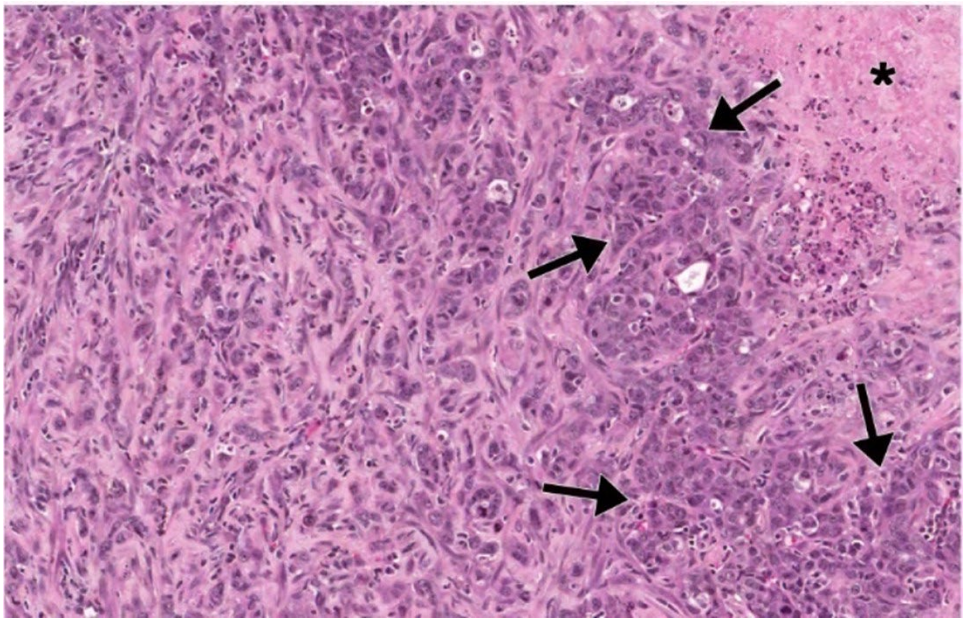


Figure 9. Sarcomatous Type Alveolar/Bronchiolar Carcinoma in the Lung of a F344/NTac Female Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The carcinoma is composed mostly of anaplastic spindyloid cells that surround islands of neoplastic epithelial cells (arrows). Note an area of necrosis in the upper right corner of the plate (asterisk).

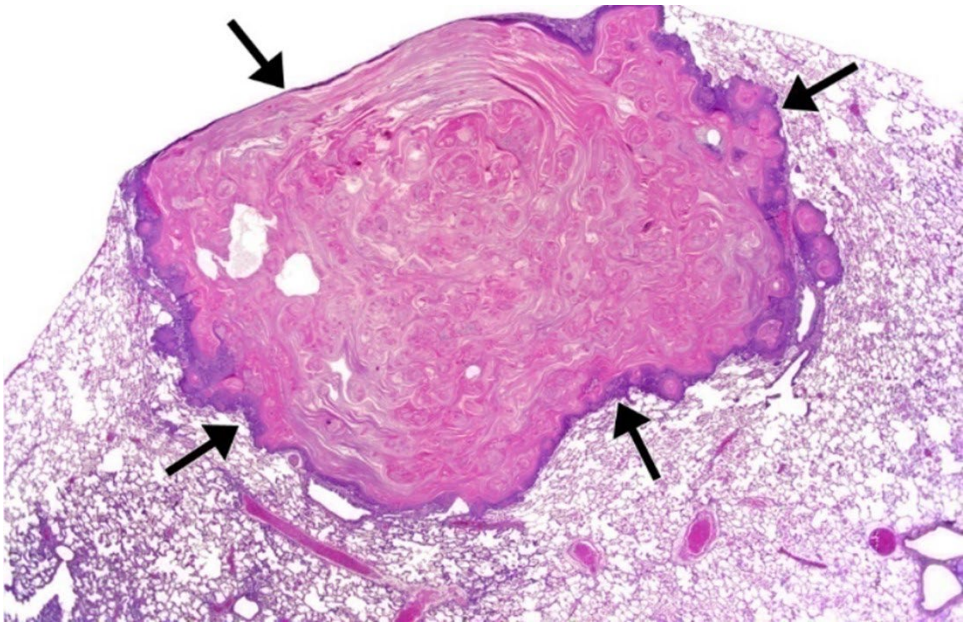


Figure 10. Cystic Keratinizing Epithelioma in the Lung of a Male F344/NTac Rat Exposed to 1.25 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

This neoplasm is characterized by a central mass of concentrically arranged keratin surrounded by wall of squamous epithelium (arrows).

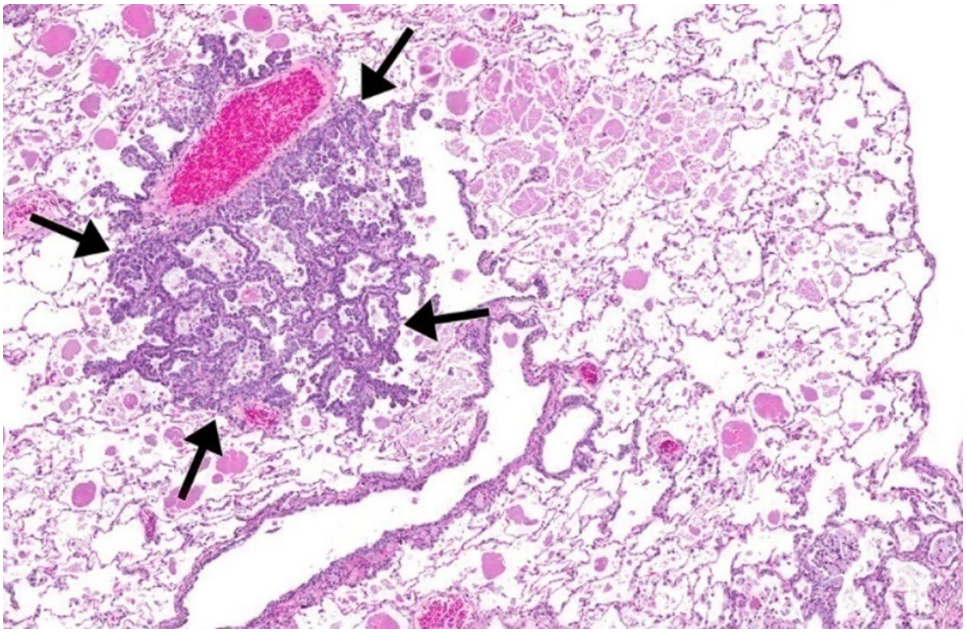


Figure 11. Discrete, Focal Alveolar Epithelial Hyperplasia (Arrows) in the Lung of a Female F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

Alveolar epithelial hyperplasia is characterized by proliferation of uniformly cuboidal alveolar epithelial (Type II) cells along the alveolar septa. The architecture of the alveolar parenchyma is generally maintained; however, the proliferation cells are forming papillary structures that project into the alveolar spaces. Note macrophages within the alveolar spaces.

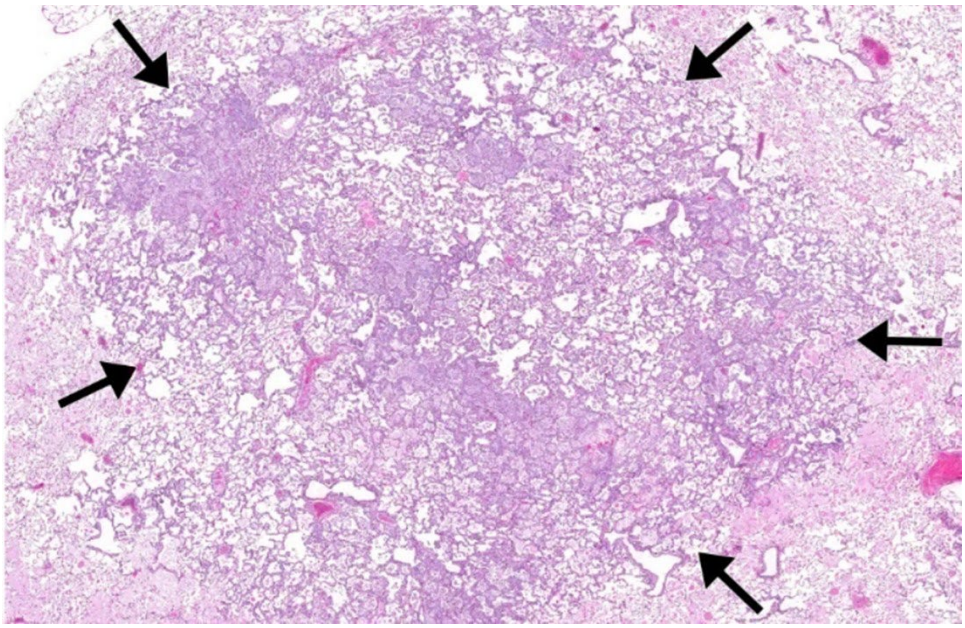


Figure 12. Focally Extensive Area of Alveolar Epithelial Hyperplasia (Arrows) in the Lung of a Female F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

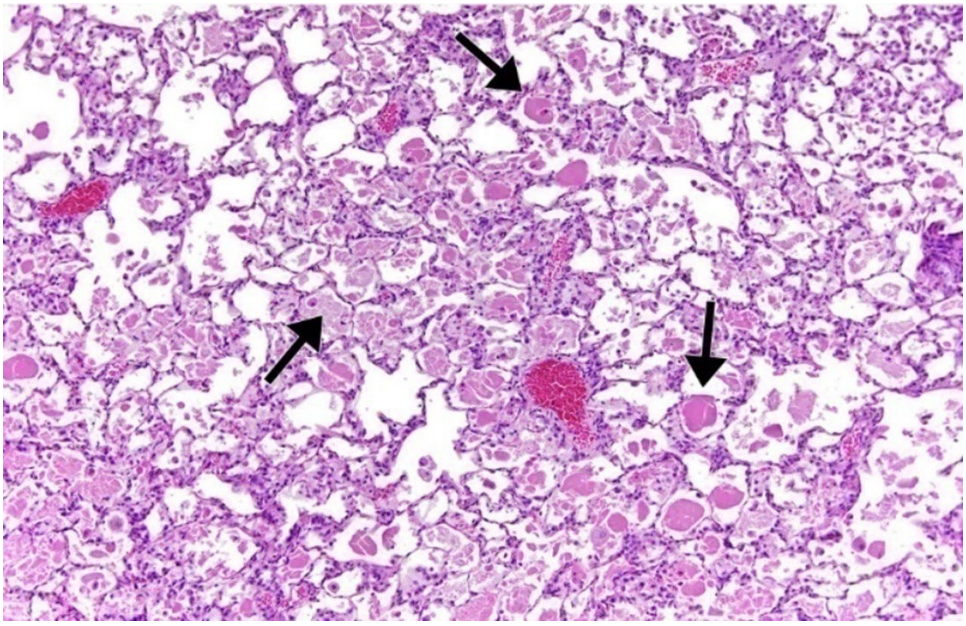


Figure 13. Alveolar Proteinosis in the Lung of a Male F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

Note the homogenously eosinophilic protein material (arrows) within the alveolar spaces some of which stains brightly eosinophilic.

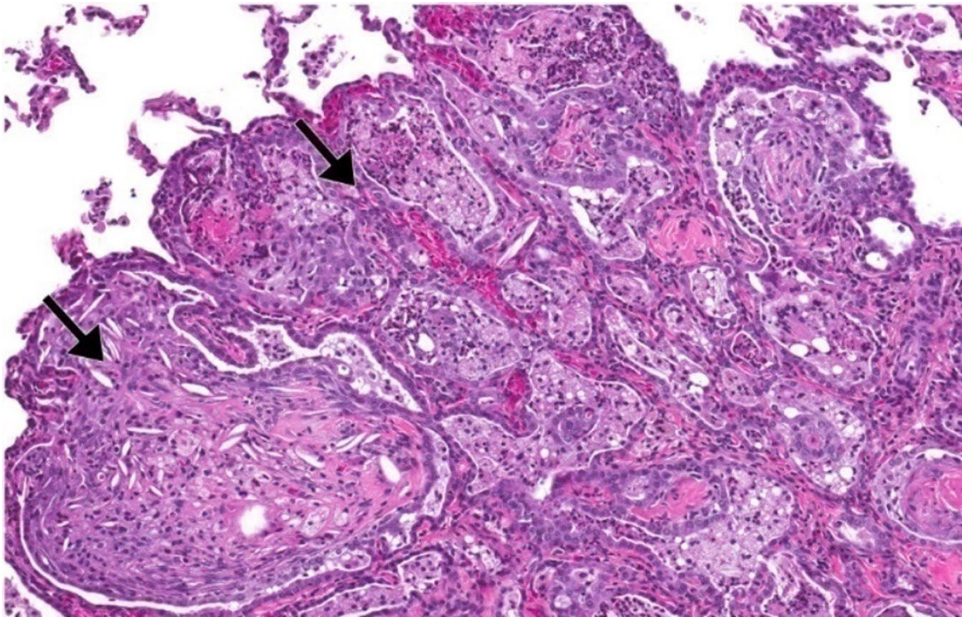


Figure 14. An Area of Chronic Active Inflammation (Arrows) in the Lung of a Male F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The alveoli are filled with mostly macrophages, mixed with lesser numbers of neutrophils and degenerate cellular debris (arrows). Note clear angular cleft-like spaces (cholesterol clefts) among the inflammatory cells and debris. Proliferating alveolar epithelial cells line the alveoli.

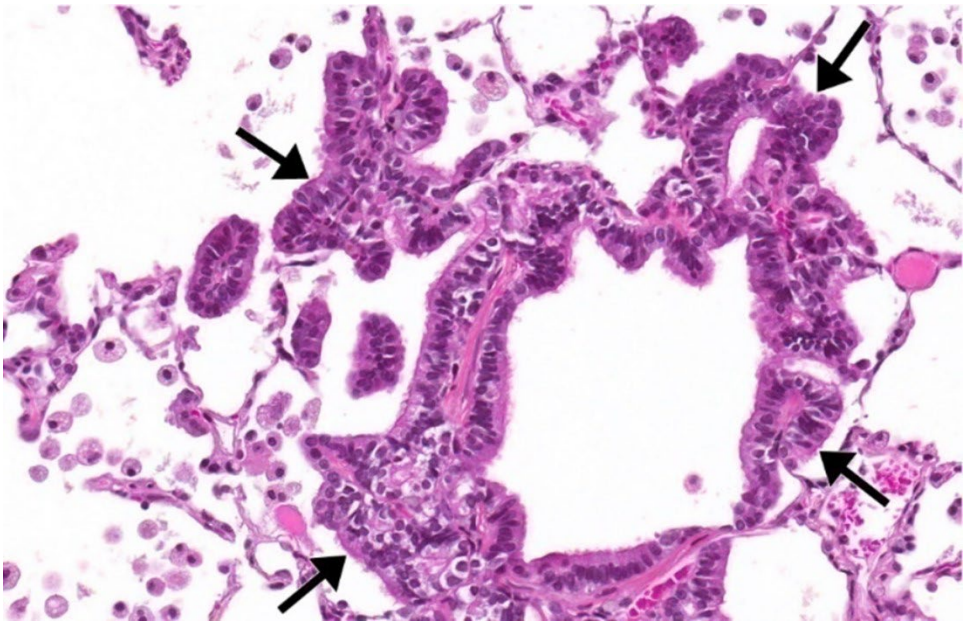


Figure 15. Bronchiolar Epithelial Hyperplasia in the Lung of a Female F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

Increased numbers of disorganized cuboidal to columnar epithelial cells (arrows), some of which are ciliated, line the terminal bronchiole. The proliferating cells have extended to line alveoli immediately adjacent to the bronchiole. Note macrophages within the alveoli.

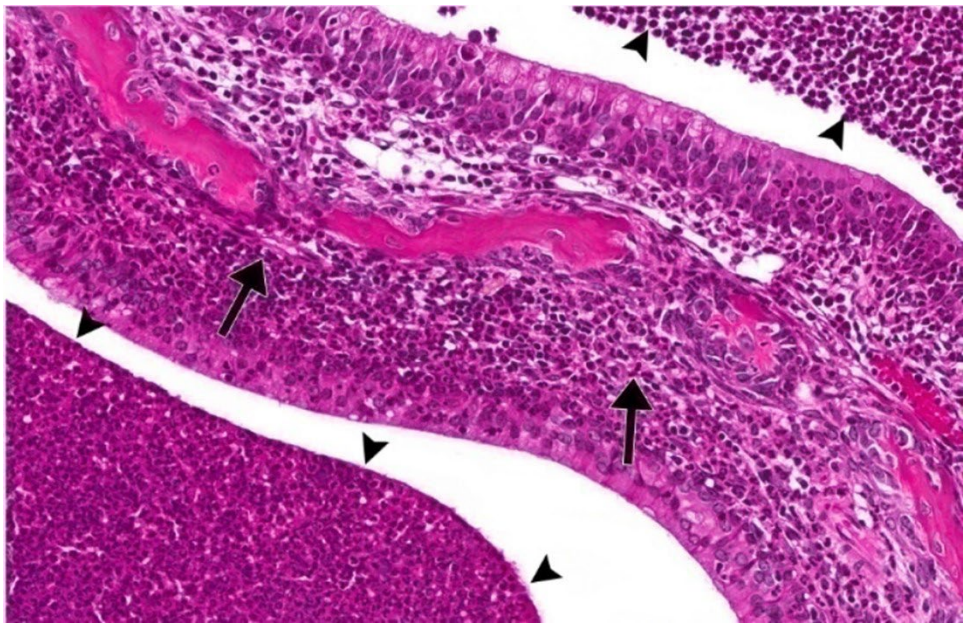


Figure 16. Suppurative Inflammation in a Nasal Maxilloturbinate in the Level II Section in the Nose of a Female F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

There are numerous neutrophils throughout the submucosal tissue of the turbinate (long arrows) and a purely neutrophilic exudate in the nasal passages (arrowheads).

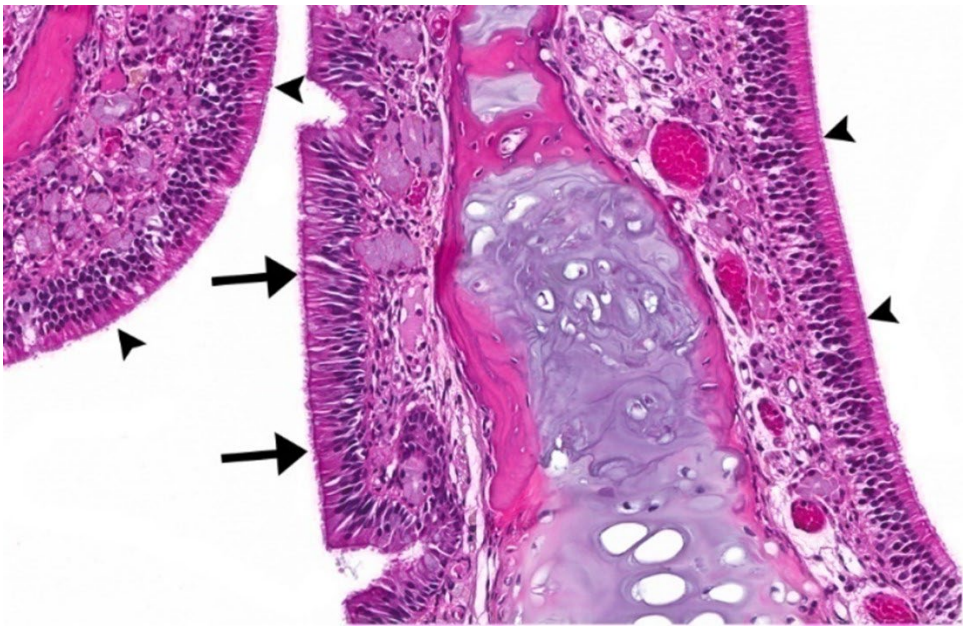


Figure 17. Respiratory Epithelial Metaplasia of the Olfactory Epithelium of an Ethmoid Turbinate in the Nose of a Female F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The normal olfactory epithelium (arrowheads) is replaced by tall ciliated columnar epithelial cells (arrows) similar to respiratory epithelium that lines the naso- and maxilloturbinates.

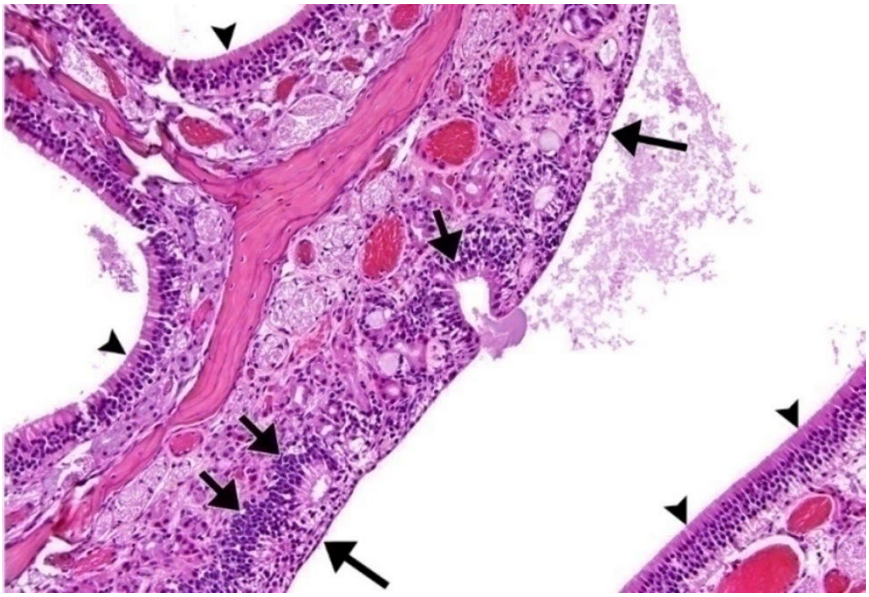


Figure 18. Olfactory Epithelial Atrophy and Olfactory Epithelial Hyperplasia in the Ethmoid Turbinate in the Nose of a Male F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

In contrast to the normal olfactory epithelium (arrowheads), the atrophic epithelium is attenuated (long arrows). Note hyperplastic olfactory epithelium forming rosette-like structures along the lower margins of the atrophic epithelium (short arrows).

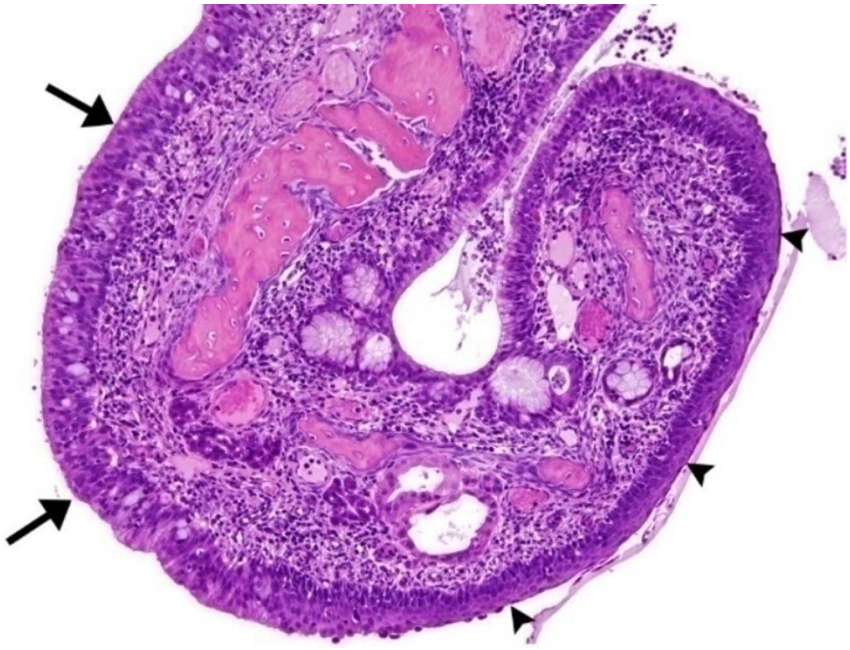


Figure 19. Respiratory Epithelial Hyperplasia and Squamous Metaplasia in a Maxilloturbinate in the Nose of a Male F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The normal single layer of tall ciliated columnar epithelial cells is replaced by multiple disorganized layers of proliferating epithelial cells (arrows). Squamous metaplasia is characterized by replacement of the normal respiratory epithelium by flattened squamous epithelial cells (arrowheads). There is chronic active inflammation throughout the submucosal tissue.

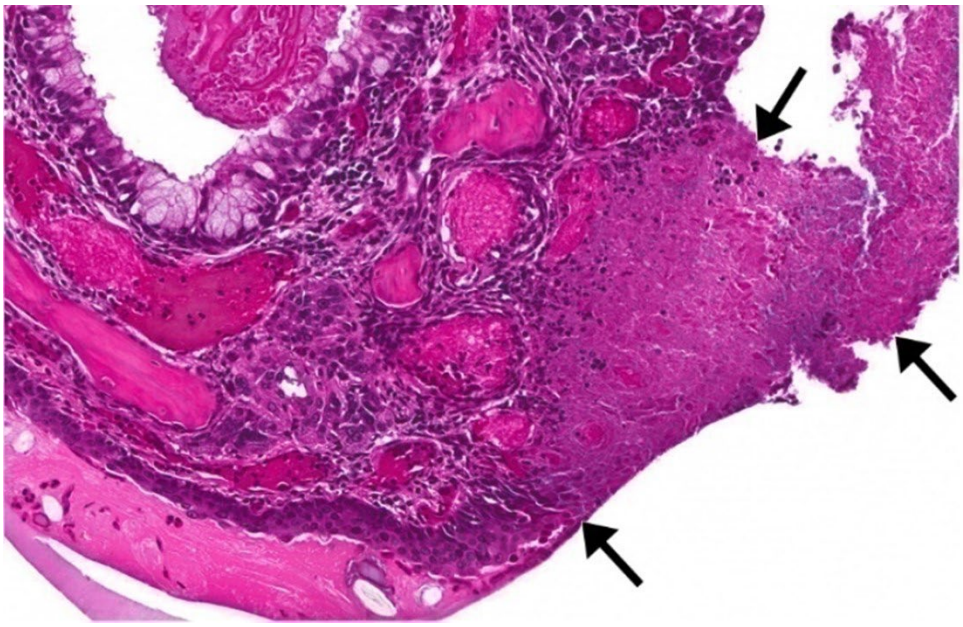


Figure 20. Necrosis of the Respiratory Epithelium in a Maxilloturbinate in the Nose of a Female F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The epithelium is replaced by a coagulum of necrotic epithelial cells and cellular debris (arrows).

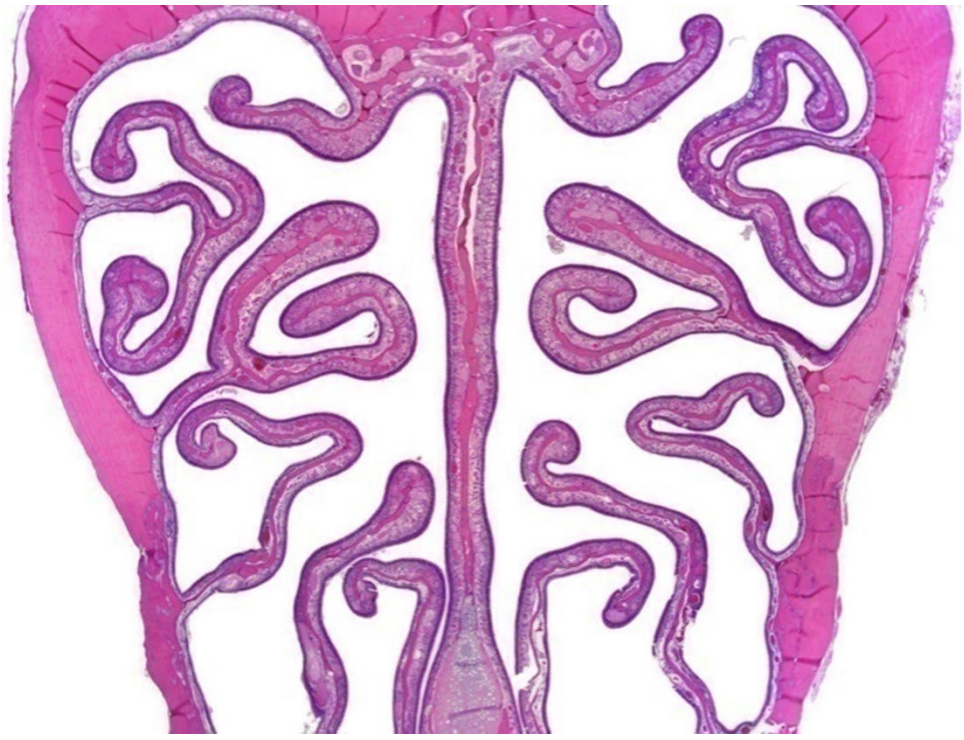


Figure 21. Atrophy of the Ethmoid Turbinates in the Nose of a Male F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The turbinate scrolls are short, thin, and somewhat blunted, and there is increased space within the nasal passages.

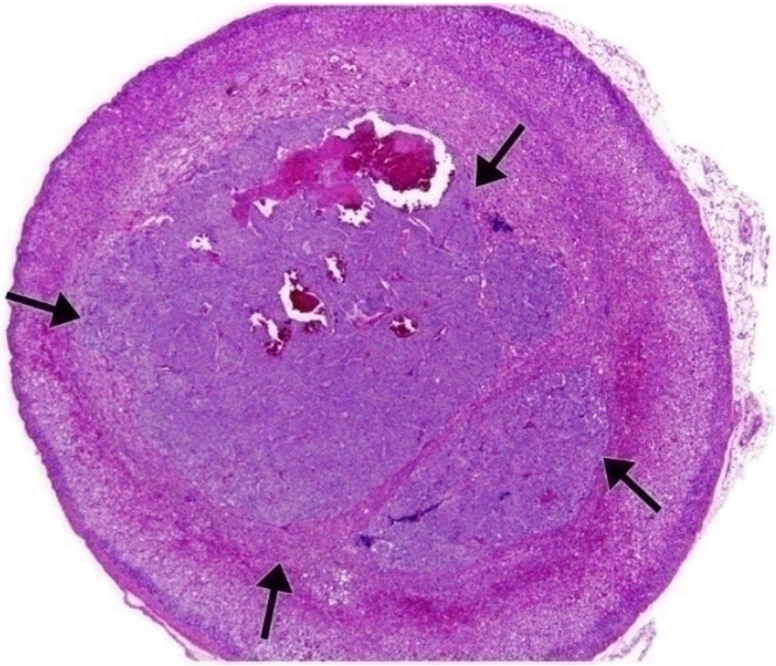


Figure 22. Benign Pheochromocytoma in the Adrenal Medulla of a Male F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The neoplasm is well-circumscribed and expansive but still within the confines of the adrenal medulla (arrows).

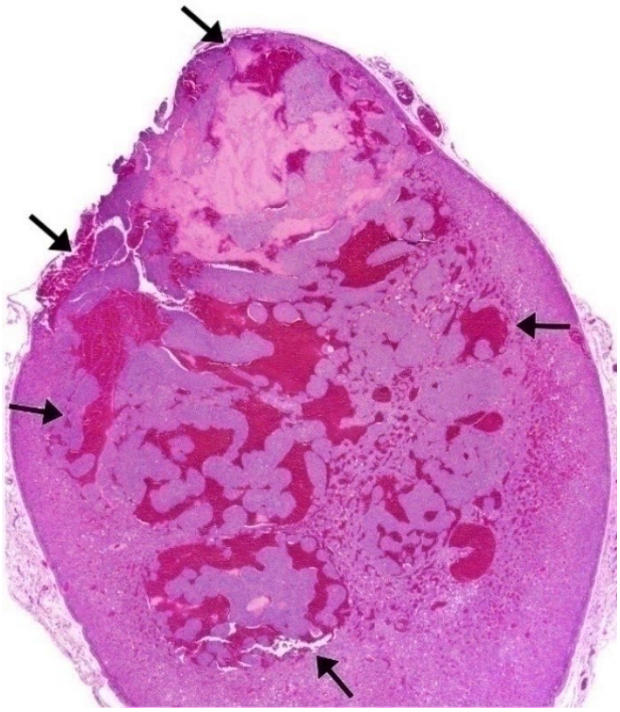


Figure 23. Malignant Pheochromocytoma in the Adrenal Medulla of a Male F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The neoplasm (arrows) is expansive, has replaced the adrenal medulla, and has invaded into and through the adrenal cortex.

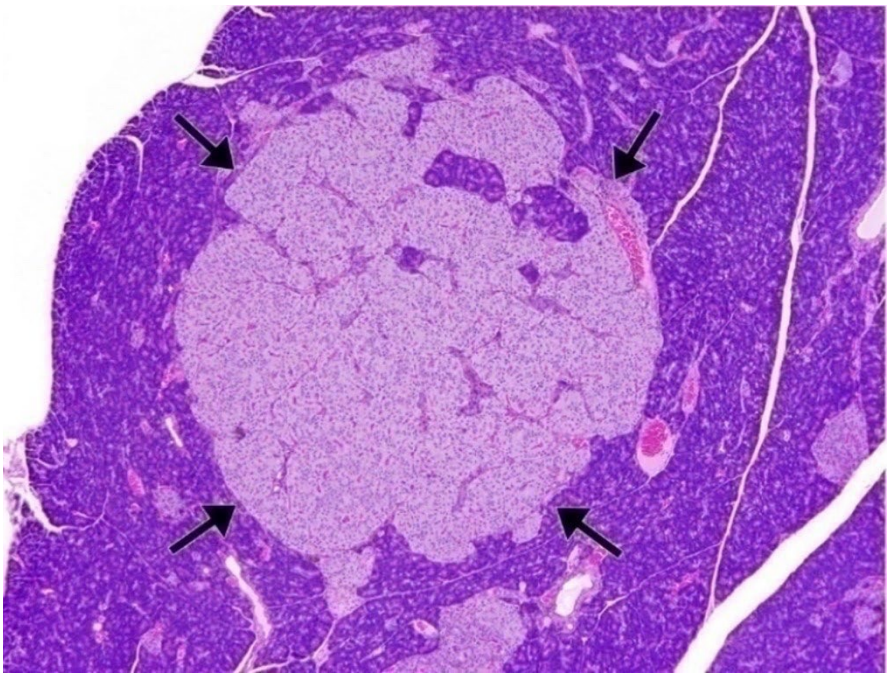


Figure 24. Islet Cell Adenoma in the Pancreas of a Male F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The adenoma (arrows) is well-circumscribed and expansive and composed of uniformly normal islet cells partially separated into variably sized packets by a delicate fibrovascular stroma.

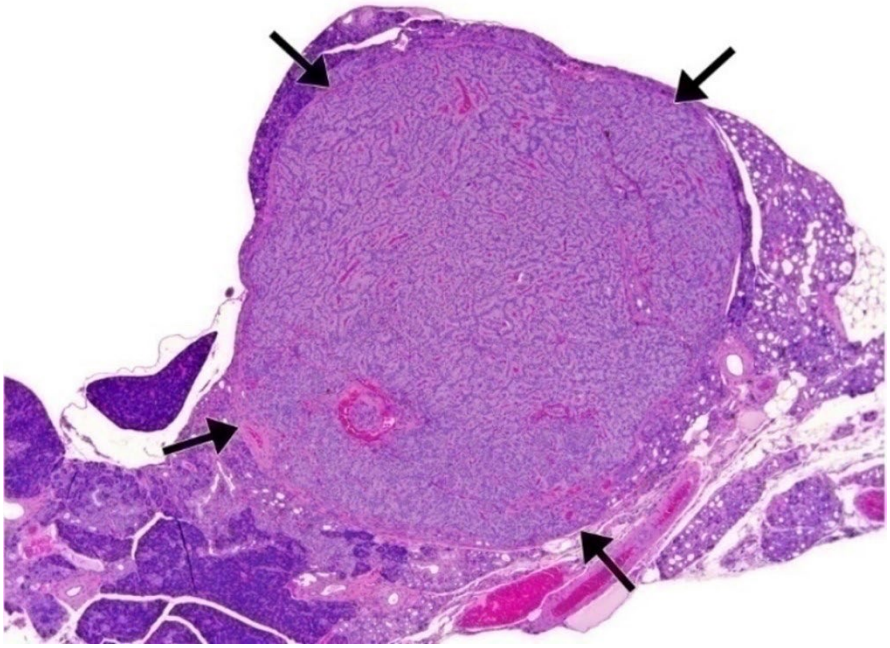


Figure 25. Islet Cell Carcinoma in the Pancreas of a Male F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The carcinoma (arrows) has almost completely replaced the pancreas and invaded adjacent tissue.

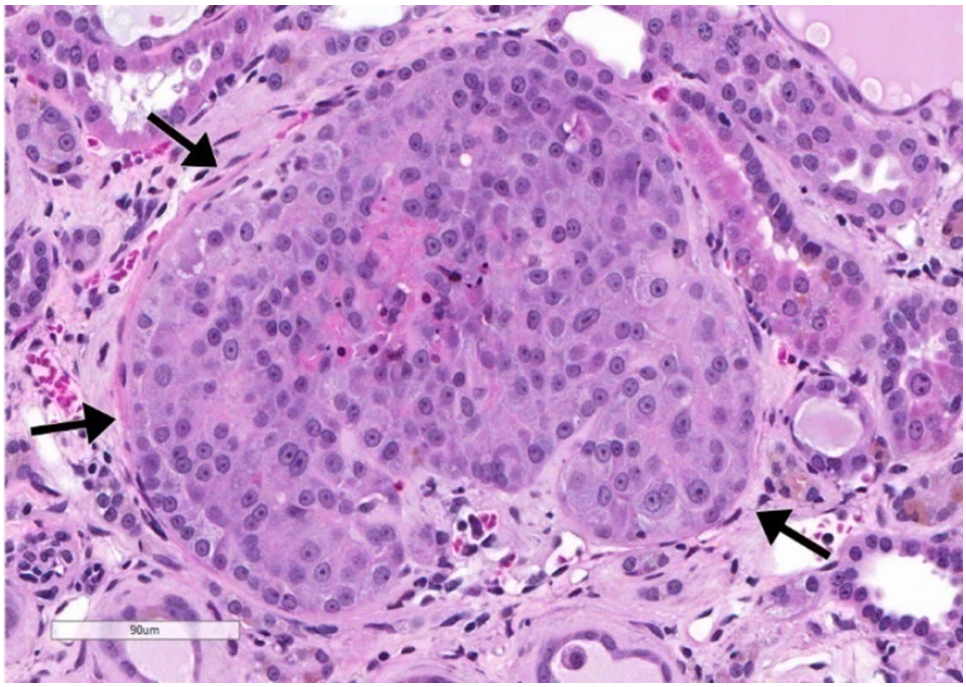


Figure 26. Renal Tubule Adenoma in the Kidney of a Male F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The adenoma (arrows) is well-circumscribed and consists of relatively uniform large cells with a granular to glassy cytoplasm.

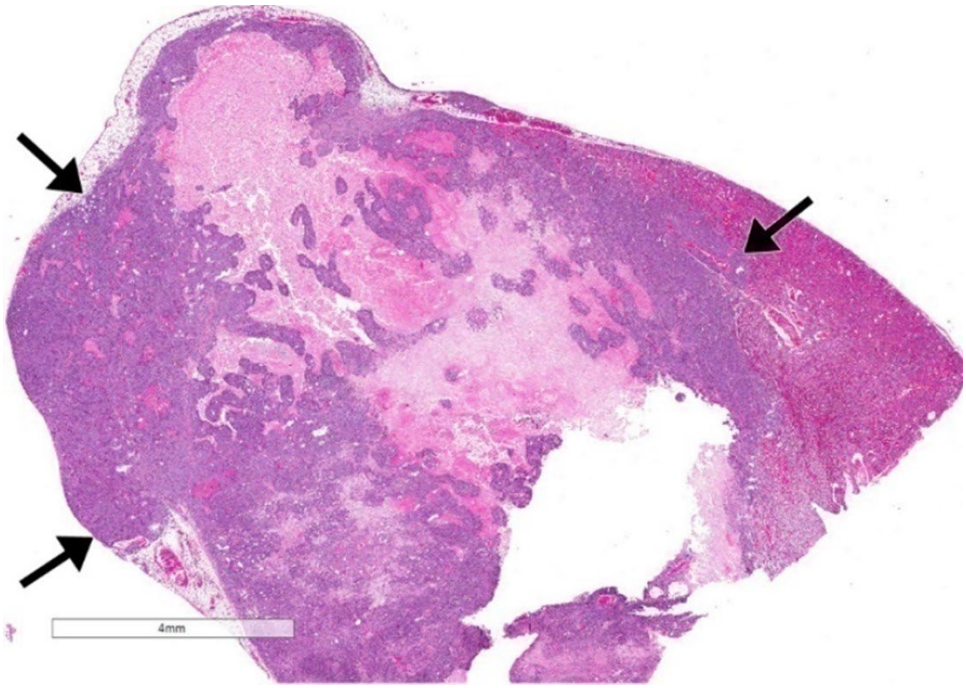


Figure 27. Renal Tubule Carcinoma in the Kidney of a Male F344/NTac Rat Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The carcinoma (arrows) is invasive and has replaced much of the renal parenchyma.

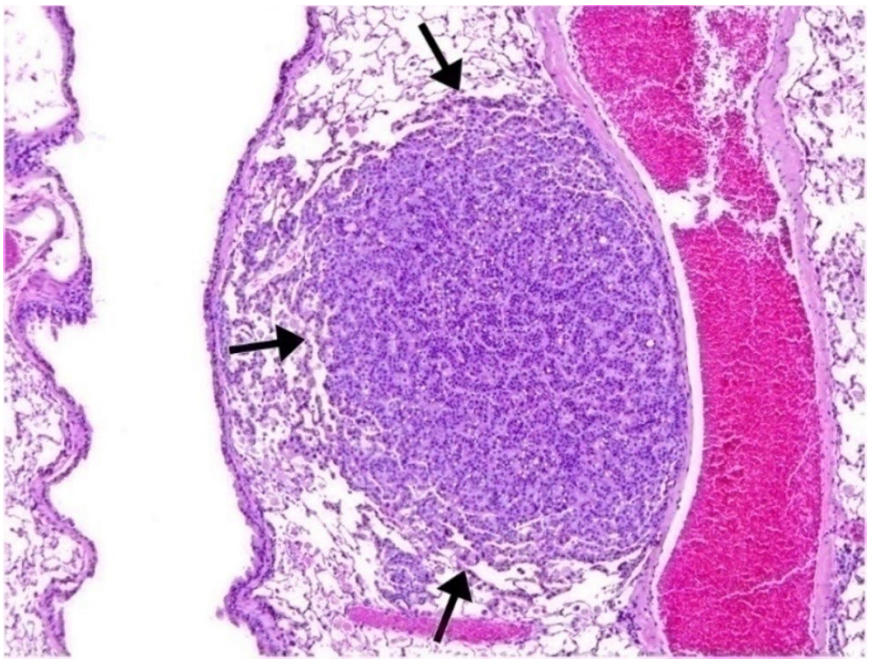


Figure 28. Low Magnification of an Alveolar/Bronchiolar Adenoma in the Lung of a Male B6C3F1/N Mouse Exposed to 2.5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The adenoma (arrows) has distinctly demarcated from the surrounding alveolar parenchyma and is composed of uniformly cuboidal cells arranged as papillary structures.

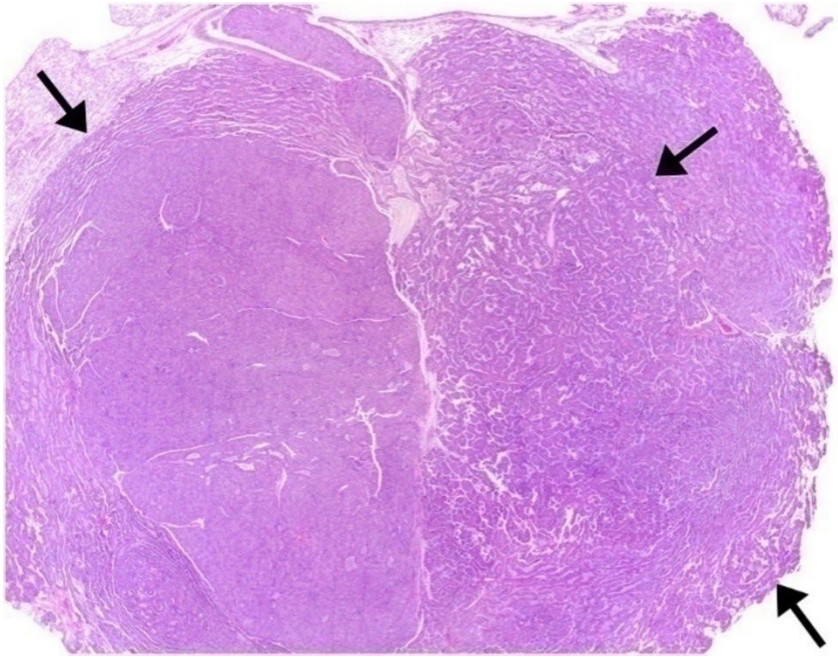


Figure 29. Low Magnification of an Alveolar/Bronchiolar Carcinoma in the Lung of a Male B6C3F1/N Mouse Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The carcinoma (arrows) has invaded and almost completely effaced the lung lobe. Note the pleomorphic appearance of the neoplasm.

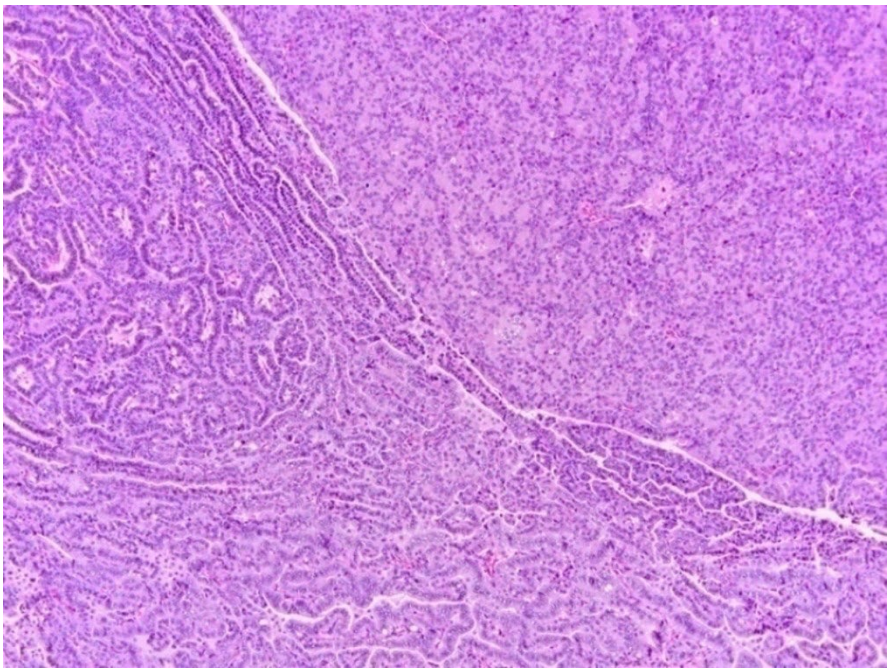


Figure 30. Higher Magnification of Figure 29 (H&E)

Note the pleomorphic arrangement of the neoplastic cells that form poorly and well-defined papillary structures composed of cuboidal to columnar cells in one area of the neoplasm and in a solid sheet in another area.

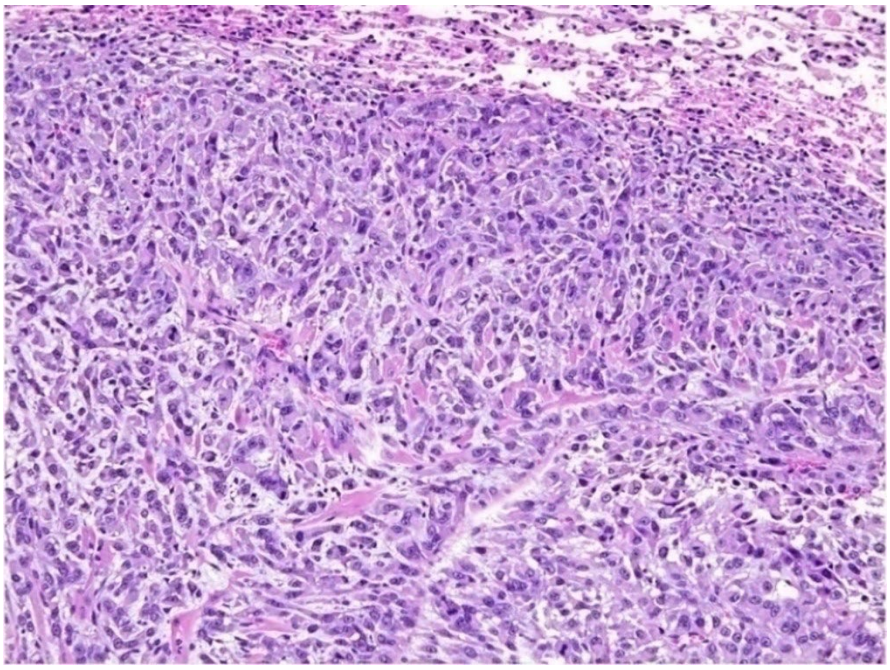


Figure 31. Alveolar/Bronchiolar Carcinoma in the Lung of a Female B6C3F1/N Mouse Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

Note the highly anaplastic nature of this carcinoma with marked variation in cellular and size and shape, pleomorphic sometimes bizarre and multilobulated nuclei and numerous mitotic figures.

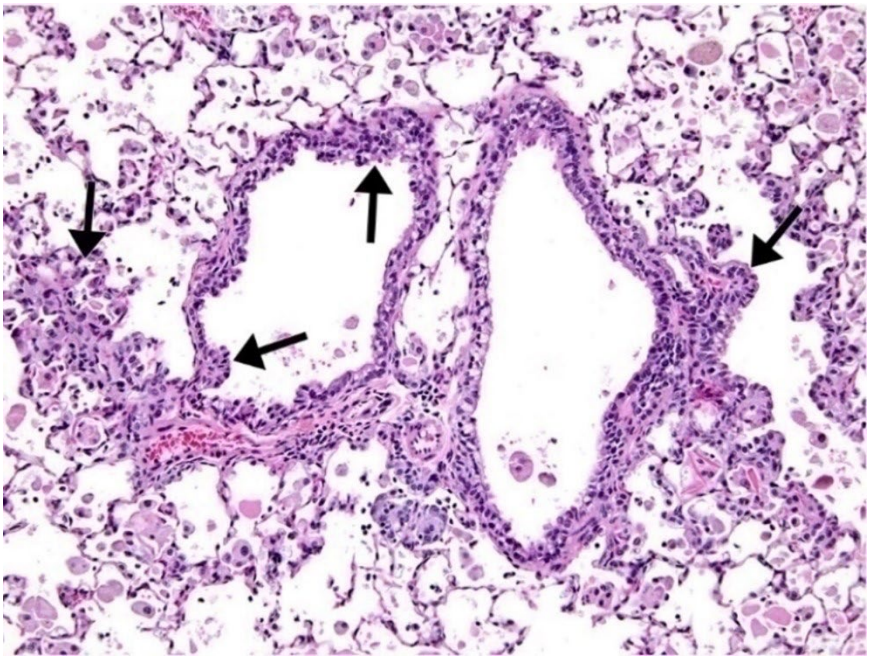


Figure 32. Alveolar/Bronchiolar Epithelial Hyperplasia in the Lung of a Female B6C3F1/N Mouse Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

There is disorganized proliferation and piling up of the epithelial cells with extension of the proliferating cells along the immediately adjacent alveolar septae (arrows). Note numerous macrophages and proteinaceous material within the alveoli.

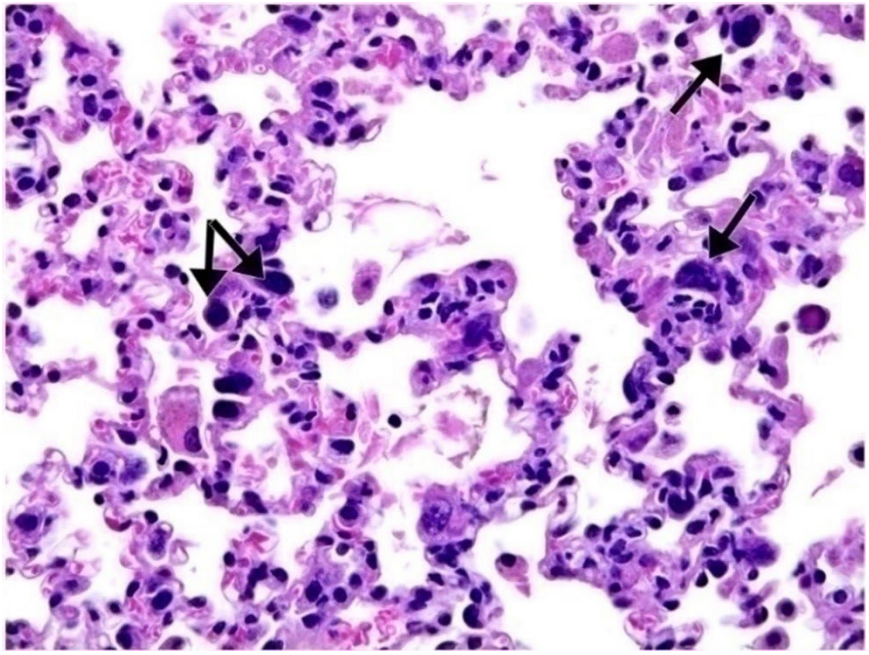


Figure 33. Focal Alveolar Epithelial Hyperplasia in the Lung of a Male B6C3F1/N Mouse Exposed to 2.5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The alveolar septa are lined by cuboidal to polygonal alveolar epithelial (Type II) cells; the alveolar architecture is generally maintained. Note epithelial cells with large (karyomegaly) pleomorphic nuclei (arrows).

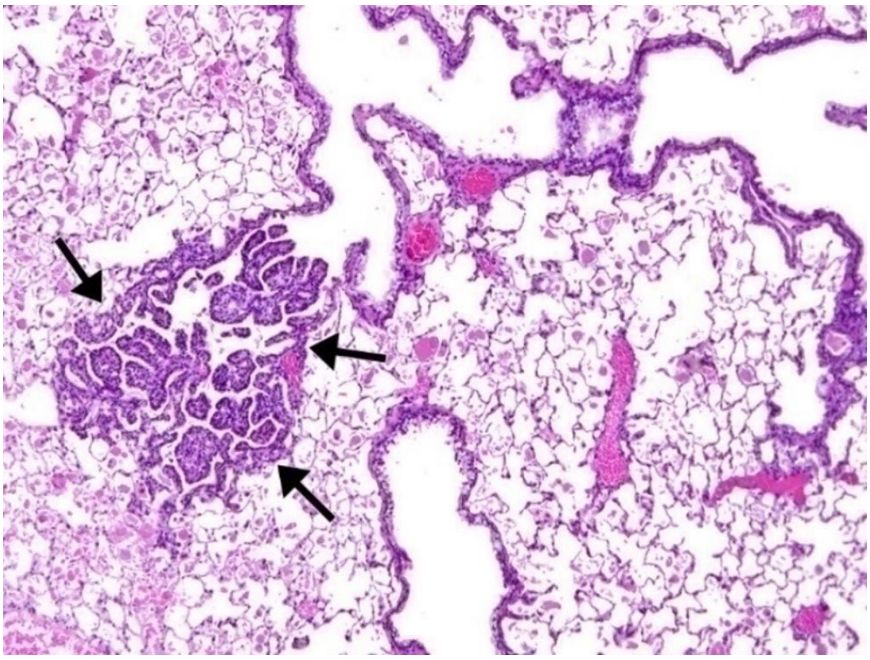


Figure 34. Focal Bronchiolar Epithelial Hyperplasia in a Terminal Bronchiole (Arrows) of a Male B6C3F1/N Mouse Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

The hyperplastic epithelium (arrows) forms multiple papillary structures that project into the lumen and are lined by cuboidal to low columnar epithelial cells and supported by scant amounts of fibrovascular stroma.

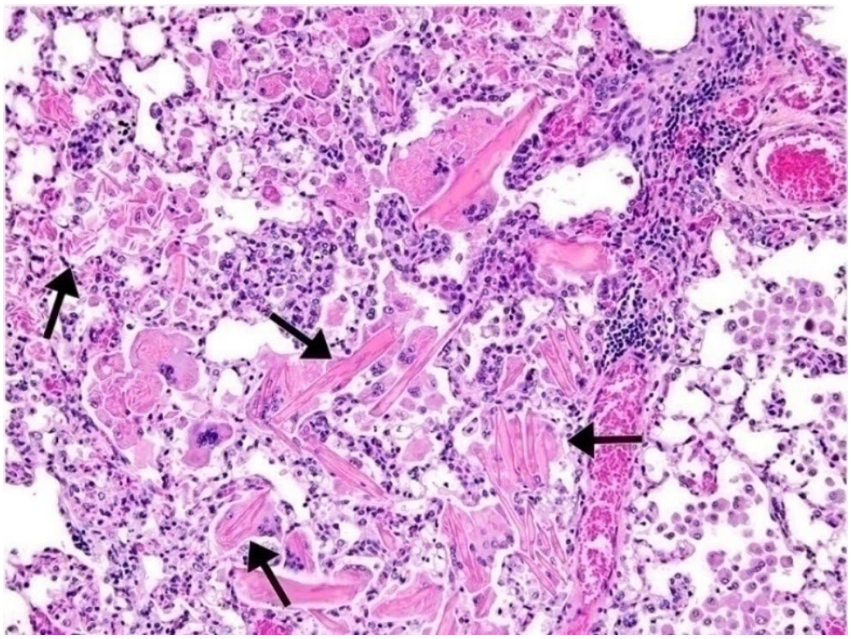


Figure 35. Alveolar Histiocytic Infiltrates in the Lung of a Male B6C3F1/N Mouse Exposed to 5 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

Alveoli contain numerous macrophages many of which are multinucleated and swollen with protein and slender needle-like crystalline material (arrows). Note larger crystals free within the alveoli. Note low numbers of neutrophils and lymphocytes within the alveoli.

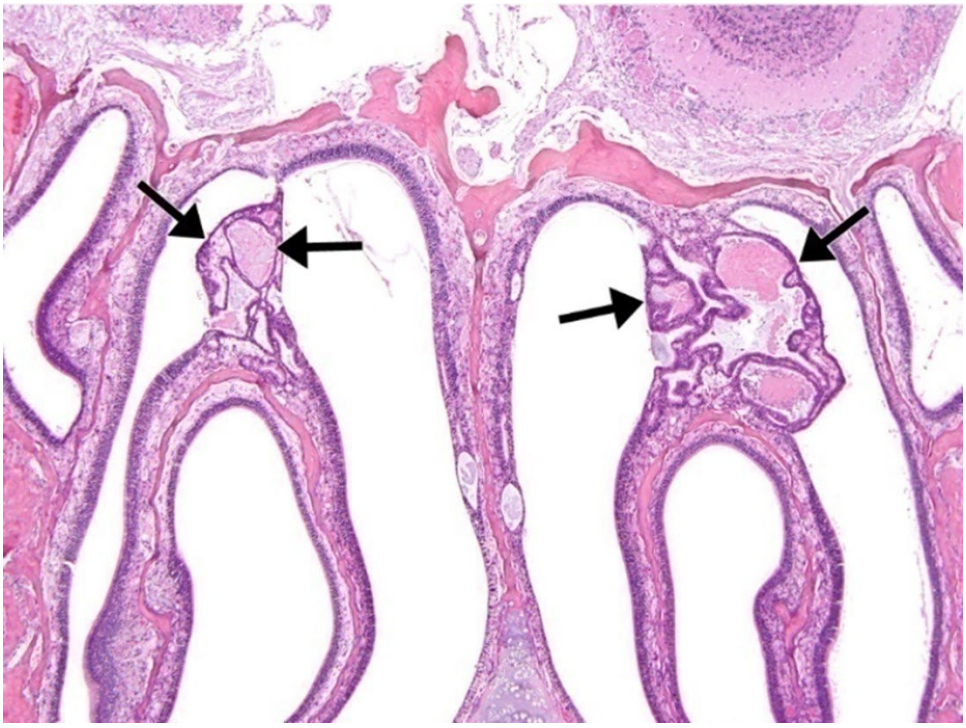


Figure 36. Bilateral Atypical Respiratory Epithelial Metaplasia in the Olfactory Epithelium of a Male B6C3F1/N Mouse Exposed to 1.25 mg/m^3 Cobalt Metal by Inhalation for Two Years (H&E)

There is exophytic proliferation of tall ciliated columnar epithelial cells from the dorsal surface of the dorsal scrolls of the ethmoid turbinates with synechia formation between the turbinate scroll and the epithelium lining the dorsal meatus (arrows).

Discussion

Cobalt metal was nominated by the International Union of United Auto Workers and the Cobalt Development Institute for toxicology and carcinogenesis studies based on widespread occupational exposure, the occurrence of hard metal disease, and limited toxicity data. The NTP previously assessed the toxicity and carcinogenicity of a soluble cobalt compound, cobalt sulfate heptahydrate, in experimental animals exposed by inhalation[66; 68; 122]. Findings from those studies showed lung cancer in rats and mice, thereby raising concerns regarding the carcinogenic potential of cobalt metal dust. Hence, NTP conducted studies on cobalt metal to obtain its toxicity and carcinogenicity profile and to compare and contrast the toxicity of insoluble cobalt metal with a soluble cobalt salt. This Technical Report presents the findings and conclusions of the 2-week, 3-month, and 2-year inhalation studies of rats and mice exposed to cobalt metal.

The exposure concentrations for the 2-week studies were estimated based on exposures of previously studied cobalt sulfate heptahydrate[66]. In the current study, there was significant mortality in male and female rats exposed to 20 and 40 mg/m^3 and body weight reductions in the 10 mg/m^3 groups coupled with reduced urine volumes and concomitant increases in urine creatinine at the end of 2 weeks. Hence, 5 mg/m^3 was selected as the highest exposure concentration for rats in the 3-month studies. In mice, there was significant mortality in males and females exposed to 40 mg/m^3 and significant decreases in body weights in the 20 mg/m^3 groups. Based on this, 10 mg/m^3 was selected as the highest exposure concentration for mice in the 3-month study. Because the exposure concentrations in the mouse study were different from those in the rat study, only four concentrations were used in the rat study in order to optimize the number of inhalation chambers used in the studies.

In the 3-month studies, there was no exposure-related mortality in rats or mice. Consistent with the results of the 2-week studies, the respiratory tract was the primary site of toxicity in both rats and mice. In the lung, nonneoplastic lesions including chronic active lung inflammation, minimal to mild alveolar proteinosis, and minimal to moderate bronchiole epithelium hyperplasia generally occurred in both males and females. In the nose, there were indications of olfactory epithelium degeneration (rats and mice) and minimal to mild respiratory epithelium hyperplasia (rats) in males and females. There were also exposure-related lesions noted in the larynx and trachea of male and female mice. These findings are comparable with the previously conducted inhalation study on cobalt sulfate heptahydrate[66; 68].

Findings from the clinical pathology assessments conducted in the 3-month studies indicated erythrocytosis, as defined by an increase in the red blood cell mass and hemoglobin concentration. There were exposure concentration-related changes on days 3 and 23 and at 14 weeks in male rats and at 14 weeks in female rats with the red blood cell count reaching greater than or equal to 22% of the concurrent chamber control values in the high concentration groups (5 mg/m^3). The differences in the time and magnitude of erythron changes due to cobalt metal exposure between the male and female rats is likely due, in part, to differences in sex hormones as testosterone is a known stimulator of erythropoiesis and estrogen a known suppressor[205]. Minimal hematological effects were seen in the mice. The reason for the species differences in the erythrocytic response to cobalt is not known but has been previously reported for cobalt sulfate heptahydrate[66].

Cobalt exposure has long been known to cause an erythrocytosis (independent of oxygen tension) through stimulation of erythropoietin[206], the mechanisms of which have been recently explained. It is now known that cobalt's effects are due to its ability to stabilize hypoxia-inducible factor-1α (HIF-1α), a subunit of hypoxia-inducible factor-1 (HIF-1) a major regulator of oxygen homeostasis. There are a multitude of genes targeted by HIF-1 including erythropoietin, vascular endothelial growth factor, glucose transporter-1 (GLUT-1), and those related to various glycolytic enzymes[207]. During normoxia, ubiquitin- and proteosome-dependent pathways utilize specific proline hydroxylases to continually degrade HIF-1α. By replacing the iron-binding site on these proline hydroxylases, cobalt renders them inactive, thereby indirectly stabilizing HIF-1α[208; 209]. There is also evidence that cobalt directly binds to HIF-1α thereby preventing its degradation[209].

Other clinical chemistry indicators including glucose, cholesterol, and triglycerides were assessed in special study rats based on reports in the literature which suggest that cobalt administration affects triglyceride and cholesterol blood levels in rodents[68; 210]. Findings from the current studies indicated significant exposure-related decreases in cholesterol concentrations in both male and female rats, as well as significant decreases in glucose concentrations in male rats. Decreases in cholesterol and glucose concentrations are not uncommon findings in toxicology studies and usually represent a combination of decreased food intake and/or altered lipid and carbohydrate metabolism, the mechanisms of which are typically not known. Various studies have revealed several mechanisms by which cobalt may alter lipid and glucose metabolism. As previously mentioned, cobalt stabilizes HIF-1α which has the potential to activate genes involved in energy metabolism including those of glycolysis. In addition, short-term administration of cobalt to diabetic rats was shown to have a glycemia-lowering effect that may be mediated by reductions in systemic glucose production (modulation of glycogen metabolism and gluconeogenesis), increased tissue glucose uptake by induction of GLUT-1, or a combination of these two mechanisms[211-213]. Administration of cobalt has also been shown to improve insulin sensitivity and glucose tolerance, as well as alter the ratio of HDL to LDL cholesterol in the blood of obese rats and mice by activation of heme-oxygenase-1 and increased secretion of adiponectin with AMK-activated protein kinase activation[214-216]. In the current study, similar exposure-dependent effects were noted in the cobalt metal exposed rats and mice.

Cobalt metal exposure for 3 months had effects on the male reproductive system as indicated by a significant decrease in sperm motility in male rats and mice accompanied by marked germinal epithelium degeneration in the testes of mice. Cobalt has been shown to affect the motility of human sperm in vitro, suggesting a potential direct effect on the spermatozoa[217]. The observed cobalt-related effects in the testes and epididymides of mice are consistent with those that have been previously reported, however the mode of action is unknown. Nevertheless, similar findings are also observed with cadmium chloride, and this finding is believed to be the result of cadmium chloride-induced testicular ischemia resulting in testicular degeneration and atrophy[218; 219]. Cobalt has been shown to induce hypoxia, resulting in ischemia in a variety of in vitro and in vivo models[220]. Therefore, it is possible that cobalt-related effects on the testis and subsequently on the epididymis were the result of localized hypoxia. In female rats, although there appeared to be a higher probability of extended diestrus in the 5 mg/m^3 group the toxicologic significance of this subtle alteration is unclear.

In the current 2-year studies, the survival of female rats was decreased in the top two exposure groups (2.5 and 5 mg/m^3) with a statistically significant reduction in the 2.5 mg/m^3 group. Mean

body weights of both male and female rats were significantly decreased in the 2.5 and 5 mg/m^3 groups. In mice, the survival of the males exposed to 2.5 or 5 mg/m^3 was significantly less than that of the concurrent chamber controls. Mean body weights were reduced by greater than 10% in both males and females in the 5 mg/m^3 group.

As with the 3-month studies, the respiratory tract was the major target for toxicity in the 2-year studies with the lung as the primary site for carcinogenicity in both rats and mice following cobalt metal exposure. In rats, there were exposure concentration-related significant increases in the incidences of alveolar/bronchiolar adenoma and carcinoma including multiple adenomas and carcinomas, thereby leading to clear evidence of carcinogenicity in male and female rats. In general, the alveolar/bronchiolar adenomas that occurred in rats and mice were morphologically similar to those that occur spontaneously. However, the alveolar/bronchiolar carcinomas tended to be larger, more invasive, pleomorphic masses that sometimes replaced entire lung lobes. In rats, several of the carcinomas had a prominent sarcomatous or desmoplastic component which is uncommon in spontaneous alveolar/bronchiolar carcinomas but not uncommon in inhalation studies with particulates[122; 221; 222]. In male mice, there was clear evidence of carcinogenicity based on statistically significant increases in the incidences of alveolar/bronchiolar carcinoma including multiple carcinomas. In female mice, there was clear evidence of carcinogenicity based on significantly increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) including multiple carcinomas. In rats and mice, the incidences of hyperplasia in the alveolar and bronchiolar epithelia were significantly increased. In the lung, such hyperplasia is considered a preneoplastic lesion and can progress to neoplasia. In female mice, there was clear evidence of carcinogenicity based on significantly increased incidences of alveolar/bronchiolar neoplasms of the lung (predominantly carcinoma), including multiple carcinomas.

In mice, the lung neoplasms metastasized to other regions such as the cecum, liver, pancreas, heart, adrenal gland, testes, thymus, skin, skeletal muscle, nose, trachea, and kidney as indicated by the presence of neoplasms in these tissues that were morphologically similar to those in the lung.

In rats, exposure to cobalt metal induced cystic keratinizing epitheliomas (CKE) in the lungs with higher occurrences in females compared to males. CKEs are rarely observed spontaneous neoplasms in rats from NTP 2-year inhalation studies and are rarely found in other species[223-225]. CKEs have been induced in the lungs of rats following intratracheal instillation or chronic inhalation exposure to various particulate compounds[223; 225-232]. Significantly increased incidences of CKEs were observed in female Sprague Dawley rats following exposure to polychlorinated biphenyls (PCB), dioxin, dioxin-like compounds, and mixtures of dioxin-like compounds by gavage for up to two years[233-239]. CKEs are considered part of a spectrum of lesions that form a continuum considered to progress from squamous metaplasia to keratin cysts to CKE to squamous cell carcinoma. In the current study, few incidences of squamous metaplasia occurred in female rats, and squamous cysts were not observed, although minimal squamous differentiation was observed in some alveolar/bronchiolar carcinomas and a single incidence of a squamous cell carcinoma was observed in a female in the high concentration 5 mg/m^3 group. The occurrence of CKEs was considered related to exposure in females based on the rarity of these neoplasms in NTP inhalation studies and the increase over the historical control range for all routes of administration.

Prolonged exposure to cobalt metal appeared to cause progressive injury to the lungs and nose in both male and female rats as indicated by the increasing incidences and severities of the nonneoplastic lesions relative to the 2-week and 3-month studies. In the 2-year studies, neoplasms that occurred in the lung were accompanied by a spectrum of generally similar inflammatory and nonneoplastic proliferative lesions of the respiratory tract. These lesions generally included epithelial hyperplasia, metaplasia, necrosis, and inflammation in the nose and bronchiolar and alveolar epithelial hyperplasia and inflammation and proteinosis of the lung in male and female rats and mice. A similar spectrum of inflammatory, fibrotic, and proliferative lesions were reported in the respiratory tract of female rats and mice in a previous NTP study on cobalt sulfate heptahydrate[122].

In rats, another major target organ for carcinogenicity was the adrenal medulla as indicated by exposure concentration-related increased incidences of benign and malignant pheochromocytomas, including bilateral, in both males and females. The results of several NTP inhalation studies with particulate compounds suggest that there may be an association between the occurrence of benign and malignant alveolar/bronchiolar neoplasms and variably extensive chronic pulmonary nonneoplastic lesions of the lung and significantly increased incidences of hyperplasias and benign and malignant pheochromocytomas of the adrenal medulla in exposed male and female rats[122; 221; 222; 240-243]. With varying degrees of statistical significance, in some but not all studies, this relationship appeared to be associated with the severities of lung fibrosis and inflammation[244]. In NTP studies, the mechanism(s) of this association between lung lesions and pheochromocytoma in rats is not understood. However, reduced gas exchange induced by extensive space-occupying neoplasms and nonneoplastic lung lesions such as fibrosis and chronic inflammation may lead to systemic hypoxemia that chronically stimulates catecholamine secretion from the adrenal medulla. This chronic hypersecretory activity may lead to medullary hyperplasia and subsequent neoplasia [244]. In the present studies, abnormal breathing was observed in some animals, however clinical signs of cyanosis were not noted.

Cobalt metal also induced pancreatic islet neoplasms following inhalation exposure in male rats. There were significant increases in the incidences of pancreatic islet adenoma or carcinoma (combined) in the 2.5 and 5 mg/m^3 groups and the instances exceeded the historical control range for all routes of administration. Based on the low spontaneous background rate, and significantly increased incidences, it was concluded that there was some evidence of carcinogenicity in the pancreatic islets of male rats. In female rats, there was an increase in the incidence of pancreatic islet carcinoma in the 5 mg/m^3 group relative to concurrent chamber controls and historical controls (all routes). However, the absence of statistically significant trends or pairwise comparisons led to the conclusion of equivocal evidence of pancreatic islet carcinoma in female rats. This is the first time that the pancreas has been reported as a target organ of carcinogenicity in NTP inhalation studies.

In female rats, there were statistically significant increases in the incidences of mononuclear cell leukemia at all exposure concentrations compared to the chamber controls; however, no clear exposure-concentration relationship was seen. Although mononuclear cell leukemia is a common spontaneous neoplasm in F344 rats, the increased incidences in females in the current study were considered related to cobalt exposure.

Increased incidences of renal tubule adenoma or carcinoma (combined) occurred in male rats exposed to cobalt metal compared to the chamber control group, but the increases were not

statistically significant. Since the standard evaluation of the kidney of males suggested the possibility of an exposure-related carcinogenic effect, an extended evaluation was performed by step-sectioning the kidney; however, the findings were not significantly different from the original evaluation. Although there was an overall positive trend in the incidences of renal tubule adenoma or carcinoma (combined) in male rats, no pairwise tests were significant, there was no exposure-concentration response, and three adenomas occurred in chamber control males. In addition, no supporting nonneoplastic findings occurred. However, because these lesions are relatively rare, NTP concluded that they may have been related to cobalt exposure.

Based on reports in the literature, the heart is one of the major target organs following occupational exposure to cobalt in both humans and animals[9]. Occupational exposure of humans to cobalt-containing dust, either as cobalt metal or as hard metal, is believed to result in cardiomyopathy; however, in the majority of these and other reported occupational studies, coexposure to other substances was common[9; 97]. However, there was no indication of cardiomyopathy in the current studies.

Toxicokinetic studies were conducted as part of the 2-week, 3-month, and 2-year studies in rats and mice to inform study design, as well as to provide data on pulmonary retention and clearance and systemic distribution. In the 2-week studies, tissues from males and females were weighed and examined for cobalt concentrations and burdens at terminal kill. In females, cobalt was measured in the blood, serum, and lung of additional groups held for 3 weeks after exposure so that clearance from tissues could be evaluated. In the 3-month and 2-year studies, lung weights, lung cobalt concentrations, and lung cobalt burdens were determined in females. In the 3-month studies, the toxicokinetic study included time points during the exposure as well as during a postexposure period. In the 2-year studies, lung cobalt measurements were determined over the first 18 months of the studies. In the 3-month studies, blood cobalt concentrations and liver weights, cobalt concentrations, and cobalt burdens were also determined in females. In all three studies, exposure-related changes in tissue weights, especially exposure-related increases in lung weights which demonstrated greater increases, earlier onsets, and/or greater persistence during the postexposure period in rats and mice (2-week and 3-month studies), prompted the use of tissue cobalt burden (rather than concentration) for determination of cobalt deposition and clearance.

Cobalt concentrations and burdens were increased in all studies in all tissues examined, indicating systemic exposure of rats and mice to cobalt. In particular, liver cobalt concentrations in the 2-week and 3-month studies approached or even exceeded lung cobalt concentrations, particularly at higher exposure concentrations. Normalized lung burden data generally indicated that there were no biologically significant disproportionate changes in cobalt deposition or clearance with increasing exposure concentration; these data are consistent with the modeling data discussed below.

Lung deposition and clearance data from the 2-week studies were used to estimate lung clearance. As there were only two time points, the data were fit to a one-compartment model. Data from the 2-week studies were used to select time points for the 3-month studies (exposure and postexposure phases). Due to a lack of sufficiently early time points during the exposure phase, the data generated during the exposure phase were fit to a one-compartment model, while a two-compartment model was utilized for the postexposure phase. Time points for the 2-year lung deposition and clearance studies were selected using the 3-month data and included early

time points (days 1, 2, 3, and 4). Following completion of these studies, the lung deposition and clearance data from the exposure phases of the 3-month and 2-year studies were fit to a two-compartment model. Modeling of the lung data generally indicated both rapid (approximately 1 to 5 days for all studies) and slow (longer with increasing exposure duration) clearance phases; the two-phase clearance from the lung probably contributed to the apparent two-phase elimination from the blood. Steady state was reached by the midpoint of the 3-month study in each species. In the 2-year studies, the times required to reach steady state were relatively long (12 to 18 months), due to the slow clearance half-lives. This phenomenon is consistent with previous studies[30; 32; 90] and may be caused by binding of dissolved cobalt to tissues or movement of particles to the interstitium and the formation of foci of macrophages on the alveolar wall[245]. In the 2-year studies, slow phase half-lives increased significantly with decreasing exposure concentration. When comparing the rapid and slow clearance phases, the majority (>95% in rats and >82% in mice) of the deposited cobalt was cleared with a very short half-life (approximately 1 to 5 days across studies), while the remainder was cleared more slowly. When comparing steady state lung cobalt burdens, it was apparent that burdens were similar in rats and mice, with maximum and steady state lung cobalt burdens (rapid + slow) of approximately 50 µg per lung at 5 mg/m^3 in both species. These burdens were compared to those that would be required to cause lung overload, which occurs as a result of excessive volume of insoluble particles relative to that of the alveolar macrophage pool. Clearance by macrophages slows when particle volume is 6% of the macrophage pool volume and then ceases when particle volume is 60% of the macrophage pool volume, resulting in dramatically increased lung burdens and very long clearance half-lives[246-249]. Overload was originally studied in F344 rats and assumes a density of one; however, for the current studies, the ratio of mouse to rat lung weight at 18 months of the chronic study and the use of the density of the cobalt test article (approximately 8.81 g/cm^3) allowed for evaluation of overload specific to rats and mice exposed to cobalt metal. Based on these assumptions, 13.2 mg (rats) or 2.1 mg (mice) per lung would be required to cause overload. These values are 264 (rats) or 42 (mice) times the maximum cobalt lung burdens observed in the 2-year studies, indicating that overload was not approached in these studies.

Multiple lines of evidence, including the rapid clearance of cobalt from the lung and blood, the low lung cobalt burdens, the absence of particle overload, the systemic distribution and elimination of cobalt, and the observed toxicity/carcinogenicity to extrapulmonary sites are consistent with relatively soluble cobalt particles rather than insoluble particles[30; 32; 33]. Cobalt has been reported to be insoluble in aqueous environments but able to be solubilized by strong mineral acids[33; 250]. In vivo studies by Rae[251] show that macrophages were able to dissolve a significant amount of cobalt, despite toxicity to the cell. Based on this evidence, alveolar macrophages likely contributed to the solubilizaton and systemic absorption of cobalt via the lung in the current studies. Furthermore, studies by Stopford et al.[252] using artificial fluids to mimic ingestion and inhalation indicate that lysosomes are likely responsible for dissolving cobalt taken up by macrophages and that any cobalt ingested via grooming or mucocilliary clearance would be solubilized by gastric juices. Because dissolution of cobalt results in toxicity to the macrophages, it is likely that the clearance of cobalt is due primarily to the dissolution and absorption of cobalt, rather than alveolar macrophage mediated clearance of intact particles via mucocilliary clearance. However, gastrointestinal absorption and systemic distribution following grooming or mucocilliary clearance may have also contributed to the tissue distribution of cobalt.

The mechanisms of cobalt-induced carcinogenesis are not well understood, although the genotoxicity of cobalt compounds has been established in a variety of test systems (reviewed in IARC[1]). In the current study, to identify a potential mode of action through which cobalt metal may be inducing its carcinogenic effects, mutation analysis was conducted on the most commonly altered cancer genes in human lung cancer: *Kras*, *Egfr*, and *Tp53* in lung neoplasms from F344/NTac rats and B6C3F1/N mice exposed to cobalt metal. Chemical-specific genetic mutations have been previously demonstrated with tobacco smoke-induced lung cancer (C:G→A:T), ultraviolet light-induced melanoma (C:G→T:A), aflatoxin-induced hepatocellular carcinoma (C:G→A:T), and aristolochic acid-induced urothelial carcinoma (A:T→T:A)[253]. In addition, several cancers also harbor mutations in genes that may have several functions, such as tumor suppressor genes, oncogenes, DNA repair genes, apoptosis genes, and growth factor genes. Several of these mutations are "driver" mutations that are specific to each cancer type. In human lung cancer, the most common driver mutations occur in *KRAS*, *EGFR*, and *TP53* genes[254; 255]. In the current study, hot spot regions in these genes from alveolar/bronchiolar carcinomas from rats and mice chronically exposed to cobalt metal were evaluated.

Findings showed that *Kras* mutations were more frequent than *Tp53* and *Egfr* mutations within the alveolar/bronchiolar carcinomas from F344/NTac rats and B6C3F1/N mice chronically exposed to cobalt metal. Mutations in *KRAS* are considered to be an early event, whereas mutations within *TP53* are thought to be a late event in the pathogenesis of lung cancer[256; 257]. *KRAS* and *EGFR* mutations are considered to be mutually exclusive in human lung cancer[258], but unexpectedly, in the current study 38% (3/8) of rats and 25% (3/12) of mice that harbored *Egfr* mutations also had *Kras* mutations. The significance of the presence of the independent occurrence of mutations in some alveolar/bronchiolar mutations is unclear but may be related to the repeated exposure to cobalt metal over the 2-year period or due to sampling of genetically heterogeneous tumors from the same lung sample. Alternatively, it may reflect the many pathways that still lead to cancer.

Mutations within codon 12 of *Kras* were observed in both spontaneous alveolar/bronchiolar carcinomas [27% (34/124)[259]] and alveolar/bronchiolar carcinomas from cobalt metal-exposed mice [67% (46/69)]. However, alveolar/bronchiolar carcinomas from cobalt metal-exposed mice had predominantly G→T transversions [80% (24/30)], whereas the spontaneous carcinomas had G→A transitions [70% (14/20)] in codon 12. The G→T transversions were also the most predominant mutations in alveolar/bronchiolar carcinomas from mice chronically exposed to cobalt sulfate heptahydrate aerosols[122], as well as other chemicals such as ozone, ethylene oxide, and cumene (Appendix K, Table K-17). This suggests that these chemicals target guanine or cytosine bases suggesting that these chemicals induce mutations at multiple sites and tissues by a common mechanism. G→T transversions are one of the more common *Kras* mutations in human lung cancer[260]. G→T *Kras* mutations were reported to correlate with 8-hydroxydeoxyguanine adducts that result from oxidative stress. In the current study, these transversion mutations were seen almost exclusively in murine alveolar/bronchiolar carcinomas from cobalt exposure but not in spontaneous alveolar/bronchiolar carcinomas.

The results of the NTP bacterial mutagenicity assays lend support to the possibility that cobalt metal induces tumorigenesis by increasing oxidative stress. In bacterial mutagenicity assays conducted by NTP, positive results were seen in *Salmonella typhimurium* strain TA98 and equivocal results were seen in strain TA100 in the absence of S9 metabolizing enzymes; results for both strains were negative with the addition of S9 mix. The *Escherichia coli* WP2

uvrA/pKM101 strain gave negative results in the absence or presence of S9 mix. These observations are of interest considering that cobalt is known to produce reactive oxygen species that could lead to increases in 8-hydroxydeoxyguanine adducts, and the mutations identified in the cancer-related genes sequenced from cobalt metal-induced mouse lung tumors predominately occurred at G:C base pairs. Strain T98 detects a -1 frameshift that disrupts a dinucleotide run of $(CG)_4$ residues; strain TA100 detects reverse mutations at a codon for proline (GGG) in *hisG46*, and the *E. coli* WP2 *uvrA*/pKM101 strain detects reverse mutations at the *trpE* ochre (TAA) codon. Taken together, the degree to which cobalt metal was mutagenic in the three strains correlated with the ability of each strain to detect mutational events at G:C base pairs. In support of this observation, sequencing of the *supF* tRNA mutational reporter gene in bacteria exposed to cobalt chloride showed that almost all mutational events (base substitutions and frameshifts) occurred at G:C base pairs[261]. Cobalt metal-induced mutagenicity was not apparent with addition of S9 mix in any bacterial strain that was tested by NTP. Although the composition of S9 mix has not been fully characterized, it contains microsomal and cytosolic enzymes, and could, therefore, contain radical scavenging enzymes such as glutathione peroxidase, glutathione reductase, glutathione-S-transferase, catalase, and superoxide dismutase. The presence of these enzymes in S9 mix may have ameliorated the mutagenic effects of cobalt. Alternatively (or additionally), the absence of cobalt-induced mutagenic activity in the presence of S9 mix might have been due to binding of cobalt to S9 proteins.

The NTP has conducted two 2-year inhalation studies, one on cobalt sulfate heptahydrate[122] and the current study on cobalt metal. Both compounds induced alveolar/bronchiolar adenomas and carcinomas and nonneoplastic respiratory tract lesions in rats and mice and pheochromocytomas in rats. As there are no tissue burden data available for the cobalt sulfate heptahydrate study, exposure concentrations of cobalt have been used for comparison. When the exposure concentrations *in the* ~~of~~ cobalt sulfate heptahydrate *study, which were based on cobalt sulfate,* are normalized to cobalt metal using the molecular weight ratio of cobalt:cobalt sulfate ~~heptahydrate~~, the highest concentration of cobalt sulfate ~~heptahydrate~~ (3.0 mg/m^3) results in an exposure concentration of cobalt (1.14 mg/m^3) that is similar to the lowest exposure concentration (1.25 mg/m^3) of cobalt metal in the current study.[g] At the similar cobalt exposure concentration, there were more neoplasms in each sex-species group with cobalt metal, with the exception of female rats; however, significantly increased incidences of lung neoplasms were observed in all four sex-species groups with both cobalt sulfate heptahydrate and cobalt metal. For pheochromocytomas, the responses at the similar cobalt concentration were also similar. Increased incidences of pancreatic islet neoplasms in male and female rats and mononuclear cell leukemia in female rats exposed to cobalt metal indicated systemic carcinogenicity; however, incidences of these neoplasms were increased at concentrations greater than those used in the cobalt sulfate heptahydrate studies. There were also some differences in the affected tissues, types, incidences, and severities of nonneoplastic respiratory tract lesions between the two studies at the similar exposure concentration. Overall, there was significant toxicity to the respiratory tract in both studies at the similar cobalt exposure concentration, and comparisons of data between the two studies suggest that cobalt is toxic and carcinogenic, at least in the respiratory tract, at a similar exposure concentration.

[g]ERRATUM: Errors were identified in the NTP Technical Report on Cobalt Metal (Technical Report 581). Text clarifying the form of cobalt used to calculate the exposure concentration was added in the HTML and PDF versions of this report; the new information is italicized. [July 3, 2022]

Conclusions

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity* of cobalt metal in male F344/NTac rats based on increased incidences of alveolar/bronchiolar adenoma and carcinoma in the lung, including multiples, and on increased incidences of benign and malignant pheochromocytoma of the adrenal medulla, including bilateral neoplasms.[h] The increased incidences of pancreatic islet adenoma or carcinoma (combined) were considered related to exposure. The occurrences of cystic keratinizing epithelioma of the lung and of renal tubule adenoma or carcinoma (combined) may have been related to exposure. There was *clear evidence of carcinogenic activity* of cobalt metal in female F344/NTac rats based on increased incidences of alveolar/bronchiolar adenoma and carcinoma in the lung, including multiples, and on increased incidences of benign and malignant pheochromocytoma of the adrenal medulla, including bilateral neoplasms. The occurrences of squamous cell neoplasms of the lung (predominantly cystic keratinizing epithelioma), and of mononuclear cell leukemia were considered related to exposure. The occurrences of pancreatic islet carcinoma may have been related to exposure. There was *clear evidence of carcinogenic activity* of cobalt metal in male and female B6C3F1/N mice based on increased incidences of alveolar/bronchiolar neoplasms of the lung (predominantly carcinoma), including multiple carcinoma.

Exposure to cobalt metal resulted in increased incidences of nonneoplastic lesions of the lung and nose in male and female rats, the testes in male rats and mice, the adrenal medulla in female rats, and the lung, nose, larynx, and trachea in male and female mice.

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

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Appendix A. Summary of Lesions in Male Rats in the Two-year Inhalation Study of Cobalt Metal

Tables

Table A-1. Summary of the Incidence of Neoplasms in Male Rats in the Two-year Inhalation Study of Cobalt Metal[a]

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	–	1	–
Moribund	28	28	27	32
Natural deaths	5	2	6	2
Survivors				
Terminal kill	17	20	16	16
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(48)	(48)
Intestine large, cecum	(49)	(50)	(49)	(50)
Intestine large, colon	(50)	(50)	(49)	(50)
Carcinoma	–	–	1 (2%)	–
Intestine large, rectum	(50)	(49)	(49)	(49)
Intestine small, duodenum	(50)	(50)	(49)	(50)
Intestine small, ileum	(48)	(50)	(47)	(50)
Intestine small, jejunum	(48)	(49)	(48)	(49)
Liver	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (2%)
Carcinoma, metastatic, kidney	–	–	–	1 (2%)
Hemangiosarcoma	–	–	–	1 (2%)
Hepatocellular adenoma	–	1 (2%)	–	1 (2%)
Mesentery	(18)	(4)	(9)	(3)
Lipoma	–	–	1 (11%)	–
Pancreas	(50)	(50)	(49)	(50)
Carcinoma, metastatic, kidney	–	–	–	1 (2%)
Mixed tumor malignant	1 (2%)	–	–	–
Acinus, adenoma		1 (2%)	–	–
Salivary glands	(50)	(50)	(50)	(50)
Adenoma, tubular	1 (2%)	–	–	–
Schwannoma malignant	–	–	1 (2%)	–
Stomach, forestomach	(50)	(50)	(50)	(50)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Squamous cell papilloma	–	1 (2%)	–	–
Stomach, glandular	(50)	(50)	(49)	(50)
Tooth	(0)	(2)	(0)	(0)
Cardiovascular System				
Blood vessel	(1)	(1)	(0)	(0)
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic	–	–	1 (2%)	2 (4%)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	2 (4%)	–	1 (2%)
Pheochromocytoma malignant, metastatic, adrenal medulla	–	–	–	1 (2%)
Schwannoma malignant	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	11 (22%)	10 (20%)	15 (30%)	13 (26%)
Pheochromocytoma malignant	2 (4%)	2 (4%)	9 (18%)	9 (18%)
Bilateral, pheochromocytoma benign	4 (8%)	13 (26%)	22 (44%)	21 (42%)
Bilateral, pheochromocytoma malignant	–	–	–	7 (14%)
Islets, pancreatic	(50)	(50)	(48)	(49)
Adenoma	–	1 (2%)	6 (13%)	3 (6%)
Carcinoma	2 (4%)	1 (2%)	5 (10%)	6 (12%)
Parathyroid gland	(45)	(45)	(47)	(46)
Pituitary gland	(50)	(50)	(49)	(49)
Pars distalis, adenoma	27 (54%)	32 (64%)	31 (63%)	24 (49%)
Pars intermedia, adenoma	–	1 (2%)	–	–
Thyroid gland	(49)	(50)	(50)	(49)
Bilateral, C-cell, adenoma	–	–	–	1 (2%)
C-cell, adenoma	4 (8%)	8 (16%)	8 (16%)	6 (12%)
C-cell, carcinoma	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Follicular cell, carcinoma	2 (4%)	2 (4%)	–	–
General Body System				
Peritoneum	(0)	(0)	(0)	(1)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Penis	(0)	(1)	(0)	(0)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Preputial gland	(50)	(50)	(49)	(48)
Adenoma	1 (2%)	1 (2%)	–	–
Carcinoma	2 (4%)	–	1 (2%)	3 (6%)
Prostate gland	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	–	–	–
Pheochromocytoma malignant, metastatic, adrenal medulla	–	–	1 (2%)	–
Seminal vesicle	(50)	(50)	(50)	(50)
Carcinoma, metastatic, kidney	–	–	–	1 (2%)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	23 (46%)	21 (42%)	17 (34%)	12 (24%)
Interstitial cell, adenoma	12 (24%)	18 (36%)	17 (34%)	17 (34%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(12)	(9)	(5)	(9)
Renal, pheochromocytoma malignant, metastatic, adrenal medulla	–	–	–	1 (11%)
Lymph node, bronchial	(27)	(25)	(21)	(20)
Lymph node, mandibular	(48)	(49)	(50)	(48)
Carcinoma, metastatic, Zymbal's gland	–	1 (2%)	–	–
Lymph node, mediastinal	(46)	(48)	(46)	(48)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	3 (6%)
Lymph node, mesenteric	(49)	(50)	(49)	(49)
Spleen	(50)	(50)	(50)	(50)
Thymus	(46)	(44)	(47)	(46)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	2 (4%)
Integumentary System				
Mammary gland	(31)	(32)	(30)	(31)
Carcinoma	1 (3%)	–	–	–
Fibroadenoma	1 (3%)	1 (3%)	–	1 (3%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	2 (4%)	–	1 (2%)	3 (6%)
Basal cell carcinoma	–	–	1 (2%)	–
Hematoma	1 (2%)	–	–	–
Keratoacanthoma	4 (8%)	6 (12%)	7 (14%)	2 (4%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Squamous cell carcinoma	1 (2%)	–	–	–
Squamous cell papilloma	–	1 (2%)	–	–
Trichoepithelioma	2 (4%)	–	–	1 (2%)
Lip, squamous cell papilloma	–	1 (2%)	–	–
Pinna, neural crest tumor	–	1 (2%)	–	–
Sebaceous gland, adenoma	–	–	1 (2%)	–
Subcutaneous tissue, fibroma	2 (4%)	3 (6%)	3 (6%)	–
Subcutaneous tissue, fibrous histiocytoma	–	–	–	2 (4%)
Subcutaneous tissue, lipoma	2 (4%)	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (2%)
Skeletal muscle	(1)	(0)	(0)	(4)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	2 (50%)
Carcinoma, metastatic, kidney	–	–	–	1 (25%)
Fibrous histiocytoma, metastatic, skin	–	–	–	1 (25%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant	1 (2%)	–	–	–
Carcinoma, metastatic, Zymbal's gland	–	1 (2%)	–	–
Oligodendroglioma malignant	–	2 (4%)	–	–
Spinal cord	(1)	(0)	(0)	(0)
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	7 (14%)	8 (16%)	8 (16%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)	3 (6%)	2 (4%)	6 (12%)
Alveolar/bronchiolar carcinoma	–	10 (20%)	20 (40%)	6 (12%)
Alveolar/bronchiolar carcinoma, multiple	–	6 (12%)	14 (28%)	30 (60%)
Carcinoma, metastatic, kidney	–	–	–	1 (2%)
Carcinoma, metastatic, Zymbal's gland	–	1 (2%)	–	1 (2%)
Cystic keratinizing epithelioma	–	1 (2%)	–	1 (2%)
Pheochromocytoma malignant, metastatic, adrenal medulla	1 (2%)	–	–	1 (2%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Squamous cell carcinoma, metastatic, skin	1 (2%)	–	–	–
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	–	2 (4%)	5 (10%)	3 (6%)
Mediastinum, carcinoma, metastatic, kidney	–	–	–	1 (2%)
Mediastinum, carcinoma, metastatic, uncertain primary site	–	–	–	1 (2%)
Nose	(48)	(47)	(45)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(1)	(5)	(0)	(0)
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(4)	(3)	(0)	(1)
Adenoma	1 (25%)	–	–	–
Carcinoma	3 (75%)	3 (100%)	–	1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (2%)	1 (2%)
Renal tubule, adenoma	–	1 (2%)	–	2 (4%)
Renal tubule, adenoma, multiple	–	–	–	1 (2%)
Renal tubule, carcinoma	–	–	–	2 (4%)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	–	–	1 (2%)	–
Leukemia mononuclear	21 (42%)	25 (50%)	22 (44%)	22 (44%)
Mesothelioma malignant	2 (4%)	3 (6%)	–	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	50	49	50
Total primary neoplasms	141	191	218	216
Total animals with benign neoplasms	47	49	49	47
Total benign neoplasms	101	133	139	123
Total animals with malignant neoplasms	31	37	42	48
Total malignant neoplasms	40	57	79	93
Total animals with metastatic neoplasms	2	4	6	12

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Total metastatic neoplasms	2	7	8	28
Total animals with malignant neoplasms of uncertain primary site	–	–	–	1
Total animals with uncertain neoplasms-benign or malignant	–	1	–	–
Total uncertain neoplasms	–	1	–	–

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

Table A-2. Statistical Analysis of Primary Neoplasms in Male Rats in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	15/50 (30%)	23/50 (46%)	37/50 (74%)	34/50 (68%)
Adjusted rate[b]	35.8%	54.3%	81.2%	76.4%
Terminal rate[c]	3/17 (18%)	12/20 (60%)	15/16 (94%)	14/16 (88%)
First incidence (days)	519	583	582	572
Poly-3 test[d]	P < 0.001	P = 0.059	P < 0.001	P < 0.001
Adrenal Medulla: Malignant Pheochromocytoma				
Overall rate	2/50 (4%)	2/50 (4%)	9/50 (18%)	16/50 (32%)
Adjusted rate	5.0%	5.0%	21.4%	39.1%
Terminal rate	0/17 (0%)	2/20 (10%)	3/16 (19%)	9/16 (56%)
First incidence (days)	668	729 (T)	628	646
Poly-3 test	P < 0.001	P = 0.693N	P = 0.030	P < 0.001
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	17/50 (34%)	23/50 (46%)	38/50 (76%)	41/50 (82%)
Adjusted rate	40.2%	54.3%	82.7%	90.7%
Terminal rate	3/17 (18%)	12/20 (60%)	15/16 (94%)	16/16 (100%)
First incidence (days)	519	583	582	572
Poly-3 test	P < 0.001	P = 0.130	P < 0.001	P < 0.001
Kidney (Renal Tubule): Adenoma (Single Sections)				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	2.5%	0.0%	7.5%
Terminal rate	0/17 (0%)	1/20 (5%)	0/16 (0%)	2/16 (13%)
First incidence (days)	–[e]	729 (T)	–	696
Poly-3 test	P = 0.061	P = 0.503	–[f]	P = 0.120
Kidney (Renal Tubule): Adenoma (Step Sections)				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	7.5%	2.5%	2.4%	7.5%
Terminal rate	0/17 (0%)	1/20 (5%)	1/16 (6%)	1/16 (6%)
First incidence (days)	678	729 (T)	729 (T)	691
Poly-3 test	P = 0.424	P = 0.302N	P = 0.294N	P = 0.660N
Kidney (Renal Tubule): Adenoma (Single and Step Sections)				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	6/50 (12%)
Adjusted rate	7.5%	2.5%	2.4%	14.9%
Terminal rate	0/17 (0%)	1/20 (5%)	1/16 (6%)	3/16 (19%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
First incidence (days)	678	729 (T)	729 (T)	691
Poly-3 test	P = 0.056	P = 0.302N	P = 0.294N	P = 0.244
Kidney (Renal Tubule): Adenoma or Carcinoma (Single Sections)				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	2.5%	0.0%	10.0%
Terminal rate	0/17 (0%)	1/20 (5%)	0/16 (0%)	3/16 (19%)
First incidence (days)	–	729 (T)	–	696
Poly-3 test	P = 0.018	P = 0.503	–	P = 0.061
Kidney (Renal Tubule): Adenoma or Carcinoma (Step Sections)				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	5/50 (10%)
Adjusted rate	7.5%	2.5%	2.4%	12.4%
Terminal rate	0/17 (0%)	1/20 (5%)	1/16 (6%)	2/16 (13%)
First incidence (days)	678	729 (T)	729 (T)	691
Poly-3 test	P = 0.123	P = 0.302N	P = 0.294N	P = 0.361
Kidney (Renal Tubule): Adenoma or Carcinoma (Single and Step Sections)				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	7/50 (14%)
Adjusted rate	7.5%	2.5%	2.4%	17.4%
Terminal rate	0/17 (0%)	1/20 (5%)	1/16 (6%)	4/16 (25%)
First incidence (days)	678	729 (T)	729 (T)	691
Poly-3 test	P = 0.023	P = 0.302N	P = 0.294N	P = 0.158
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/50 (4%)	10/50 (20%)	10/50 (20%)	14/50 (28%)
Adjusted rate	5.0%	24.1%	23.3%	32.5%
Terminal rate	1/17 (6%)	6/20 (30%)	2/16 (13%)	4/16 (25%)
First incidence (days)	611	577	535	478
Poly-3 test	P = 0.011	P = 0.015	P = 0.018	P < 0.001
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	0/50 (0%)	16/50 (32%)	34/50 (68%)	36/50 (72%)
Adjusted rate	0.0%	38.2%	76.8%	80.6%
Terminal rate	0/17 (0%)	7/20 (35%)	16/16 (100%)	14/16 (88%)
First incidence (days)	–	580	472	552
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	2/50 (4%)	25/50 (50%)	39/50 (50%)	44/50 (88%)
Adjusted rate	5.0%	58.0%	84.6%	93.6%

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Terminal rate	1/17 (6%)	13/20 (65%)	16/16 (100%)	16/16 (100%)
First incidence (days)	611	577	472	478
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Pancreatic Islets: Adenoma				
Overall rate	0/50 (0%)	1/50 (2%)	6/48 (13%)	3/49 (6%)
Adjusted rate	0.0%	2.5%	15.1%	7.7%
Terminal rate	0/17 (0%)	0/20 (0%)	1/16 (6%)	3/16 (19%)
First incidence (days)	–	684	618	729 (T)
Poly-3 test	P = 0.052	P = 0.504	P = 0.015	P = 0.116
Pancreatic Islets: Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	5/48 (10%)	6/49 (12%)
Adjusted rate	5.0%	2.5%	12.6%	15.1%
Terminal rate	0/17 (0%)	0/20 (0%)	3/16 (19%)	2/16 (13%)
First incidence (days)	675	675	618	679
Poly-3 test	P = 0.021	P = 0.496N	P = 0.213	P = 0.129
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	10/48 (21%)	9/49 (18%)
Adjusted rate	5.0%	4.9%	24.7%	22.6%
Terminal rate	0/17 (0%)	0/20 (0%)	3/16 (19%)	5/16 (31%)
First incidence (days)	675	675	618	679
Poly-3 test	P = 0.002	P = 0.689N	P = 0.013	P = 0.022
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	27/50 (54%)	32/50 (64%)	31/49 (63%)	24/49 (49%)
Adjusted rate	60.5%	67.7%	68.2%	55.1%
Terminal rate	8/17 (47%)	10/20 (50%)	10/15 (67%)	6/15 (40%)
First incidence (days)	441	413	472	470
Poly-3 test	P = 0.219N	P = 0.305	P = 0.289	P = 0.377N
Preputial Gland: Carcinoma				
Overall rate	2/50 (4%)	0/50 (0%)	1/49 (2%)	3/48 (6%)
Adjusted rate	5.0%	0.0%	2.5%	7.8%
Terminal rate	0/17 (0%)	0/20 (0%)	0/16 (0%)	1/15 (7%)
First incidence (days)	562	–	598	572
Poly-3 test	P = 0.155	P = 0.237N	P = 0.497N	P = 0.483
Preputial Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/49 (2%)	3/48 (6%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Adjusted rate	7.5%	2.5%	2.5%	7.8%
Terminal rate	1/17 (6%)	1/20 (5%)	0/16 (0%)	1/15 (7%)
First incidence (days)	562	729 (T)	598	572
Poly-3 test	P = 0.406	P = 0.305N	P = 0.301N	P = 0.646
Skin: Keratoacanthoma				
Overall rate	4/50 (8%)	6/50 (12%)	7/50 (14%)	2/50 (4%)
Adjusted rate	9.9%	14.7%	16.4%	5.0%
Terminal rate	3/17 (18%)	3/20 (15%)	0/16 (0%)	1/16 (6%)
First incidence (days)	376	580	582	652
Poly-3 test	P = 0.212N	P = 0.376	P = 0.292	P = 0.337N
Skin: Basal Cell Adenoma				
Overall rate	2/50 (4%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	5.0%	0.0%	2.4%	7.5%
Terminal rate	1/17 (6%)	0/20 (0%)	0/16 (0%)	1/16 (6%)
First incidence (days)	542	–	705	689
Poly-3 test	P = 0.165	P = 0.236N	P = 0.492N	P = 0.502
Skin: Trichoepithelioma or Basal Cell Adenoma				
Overall rate	4/50 (8%)	0/50 (0%)	1/50 (2%)	4/50 (8%)
Adjusted rate	9.9%	0.0%	2.4%	9.9%
Terminal rate	1/17 (6%)	0/20 (0%)	0/16 (0%)	1/16 (6%)
First incidence (days)	542	–	705	689
Poly-3 test	P = 0.248	P = 0.060N	P = 0.172N	P = 0.643
Skin: Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	4/50 (8%)	0/50 (0%)	2/50 (4%)	4/50 (8%)
Adjusted rate	9.9%	0.0%	4.8%	9.9%
Terminal rate	1/17 (6%)	0/20 (0%)	0/16 (0%)	1/16 (6%)
First incidence (days)	542	–	652	689
Poly-3 test	P = 0.229	P = 0.060N	P = 0.326N	P = 0.643
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	4/50 (8%)	8/50 (16%)	7/50 (14%)	2/50 (4%)
Adjusted rate	9.9%	19.3%	16.4%	5.0%
Terminal rate	3/17 (18%)	3/20 (15%)	0/16 (0%)	1/16 (6%)
First incidence (days)	376	580	582	652
Poly-3 test	P = 0.120N	P = 0.186	P = 0.292	P = 0.337N

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	5/50 (10%)	8/50 (16%)	7/50 (14%)	2/50 (4%)
Adjusted rate	12.4%	19.3%	16.4%	5.0%
Terminal rate	3/17 (18%)	3/20 (15%)	0/16 (0%)	1/16 (6%)
First incidence (days)	376	580	582	652
Poly-3 test	P = 0.086N	P = 0.290	P = 0.418	P = 0.215N
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma				
Overall rate	8/50 (16%)	8/50 (16%)	8/50 (16%)	6/50 (12%)
Adjusted rate	19.4%	19.3%	18.6%	14.8%
Terminal rate	3/17 (18%)	3/20 (15%)	0/16 (0%)	2/16 (13%)
First incidence (days)	376	580	582	652
Poly-3 test	P = 0.322N	P = 0.605N	P = 0.574N	P = 0.398N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	0/50 (0%)
Adjusted rate	5.0%	7.5%	7.2%	0.0%
Terminal rate	0/17 (0%)	2/20 (10%)	1/16 (6%)	0/16 (0%)
First incidence (days)	682	674	628	–
Poly-3 test	P = 0.137N	P = 0.506	P = 0.519	P = 0.236N
Skin (Subcutaneous Tissue): Fibrous Histiocytoma or Fibroma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted rate	5.0%	7.5%	7.2%	4.9%
Terminal rate	0/17 (0%)	2/20 (10%)	1/16 (6%)	0/16 (0%)
First incidence (days)	682	674	628	516
Poly-3 test	P = 0.487N	P = 0.506	P = 0.519	P = 0.683N
Testes: Adenoma				
Overall rate	35/50 (70%)	39/50 (78%)	34/50 (68%)	29/50 (58%)
Adjusted rate	78.6%	87.3%	76.3%	65.2%
Terminal rate	15/17(88%)	19/20 (95%)	15/16 (94%)	10/16 (63%)
First incidence (days)	441	540	548	552
Poly-3 test	P = 0.008N	P = 0.176	P = 0.499N	P = 0.103N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	4/49 (8%)	8/50 (16%)	8/50 (16%)	7/49 (14%)
Adjusted rate	10.2%	19.5%	18.9%	17.5%
Terminal rate	2/17 (12%)	5/20 (25%)	2/16 (13%)	4/16 (25%)
First incidence (days)	613	562	548	618

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Poly-3 test	P = 0.379	P = 0.196	P = 0.213	P = 0.271
Thyroid Gland (C-Cell): Carcinoma				
Overall rate	1/49 (2%)	2/50 (4%)	3/50 (6%)	1/49 (2%)
Adjusted rate	2.6%	5.0%	7.3%	2.5%
Terminal rate	0/17 (0%)	2/20 (10%)	2/16 (13%)	0/16 (0%)
First incidence (days)	670	729 (T)	618	717
Poly-3 test	P = 0.545N	P = 0.512	P = 0.328	P = 0.757N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	5/49 (10%)	10/50 (20%)	11/50 (22%)	8/49 (16%)
Adjusted rate	12.7%	24.4%	25.8%	20.0%
Terminal rate	2/17 (12%)	7/20 (35%)	4/16 (25%)	4/16 (25%)
First incidence (days)	613	562	548	618
Poly-3 test	P = 0.411	P = 0.143	P = 0.110	P = 0.284
Zymbal's Gland: Carcinoma				
Overall rate	3/50 (6%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	7.4%	7.4%	0.0%	2.5%
Terminal rate	1/17 (6%)	1/20 (5%)	0/16 (0%)	0/16 (0%)
First incidence (days)	383	603	–	717
Poly-3 test	P = 0.102N	P = 0.662	P = 0.117N	P = 0.311N
Zymbal's Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	9.7%	7.4%	0.0%	2.5%
Terminal rate	1/17 (6%)	1/20 (5%)	0/16 (0%)	0/16 (0%)
First incidence (days)	383	603	–	717
Poly-3 test	P = 0.063N	P = 0.508N	P = 0.060N	P = 0.186N
All Organs: Malignant Mesothelioma				
Overall rate	2/50 (4%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	5.0%	7.4%	0.0%	2.5%
Terminal rate	1/17 (6%)	1/20 (5%)	0/16 (0%)	1/16 (6%)
First incidence (days)	441	663	–	729 (T)
Poly-3 test	P = 0.163N	P = 0.503	P = 0.233N	P = 0.503N
All Organs: Mononuclear Cell Leukemia				
Overall rate	21/50 (42%)	25/50 (50%)	22/50 (44%)	22/50 (44%)
Adjusted rate	48.9%	58.0%	50.2%	47.9%
Terminal rate	7/17 (41%)	15/20 (75%)	7/16 (44%)	4/16 (25%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
First incidence (days)	519	540	582	478
Poly-3 test	P = 0.296N	P = 0.257	P = 0.541	P = 0.547N
All Organs: Benign Neoplasms				
Overall rate	47/50 (94%)	49/50 (98%)	49/50 (98%)	47/50 (94%)
Adjusted rate	97.4%	98.9%	98.8%	96.9%
Terminal rate	17/17 (100%)	20/20 (100%)	16/16 (100%)	16/16 (100%)
First incidence (days)	376	413	472	470
Poly-3 test	P = 0.436N	P = 0.613	P = 0.627	P = 0.719N
All Organs: Malignant Neoplasms				
Overall rate	31/50 (62%)	37/50 (74%)	42/50 (84%)	48/50 (96%)
Adjusted rate	66.3%	82.4%	89.7%	97.4%
Terminal rate	7/17 (41%)	18/20 (90%)	16/16 (100%)	15/16 (94%)
First incidence (days)	383	540	472	478
Poly-3 test	P < 0.001	P = 0.051	P = 0.003	P < 0.001
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	50/50 (100%)	49/50 (98%)	50/50 (100%)
Adjusted rate	100.0%	100.0%	98.8%	100.0%
Terminal rate	17/17 (100%)	20/20 (100%)	16/16 (100%)	16/16 (100%)
First incidence (days)	376	413	472	470
Poly-3 test	P = 0.776N	–	P = 0.761N	–

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

Table A-3. Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Male F344/NTac Rats[a]

	Adenoma	Carcinoma	Adenoma or Carcinoma
Overall Historical Incidence: All Routes			
Total (%)	5/100 (5.0%)	0/100	5/100 (5.0%)
Mean ± standard deviation	5.0% ± 1.4%	–	5.0% ± 1.4%
Range	4%–6%	–	4%–6%

[a]Data as of June 2013.

Table A-4. Historical Incidence of Pheochromocytoma of the Adrenal Medulla in Control Male F344/NTac Rats[a]

	Benign	Malignant	Benign or Malignant
Overall Historical Incidence: All Routes			
Total (%)	25/100 (25.0%)	2/100 (2.0%)	27/100 (27.0%)
Mean ± standard deviation	25.0% ± 7.1%	2.0% ± 2.8%	27.0% ± 9.9%
Range	20%–30%	0%–4%	20%–34%

[a]Data as of June 2013.

Table A-5. Historical Incidence of Pancreatic Islet Neoplasms in Control Male F344/NTac Rats[a]

	Adenoma	Carcinoma	Adenoma or Carcinoma
Overall Historical Incidence: All Routes			
Total (%)	0/100	2/100 (2.0%)	2/100 (2.0%)
Mean ± standard deviation	–	2.0% ± 2.8%	2.0% ± 2.8%
Range	–	0%–4%	0%–4%

[a]Data as of June 2013.

Table A-6. Historical Incidence of Renal Tubule Neoplasms in Control Male F344/NTac Rats[a]

	Adenoma	Carcinoma	Adenoma or Carcinoma
Overall Historical Incidence: All Routes			
Total (%)	1/100 (1.0%)	0/100	1/100 (1.0%)
Mean ± standard deviation	1.0% ± 1.41%	–	1.0% ± 1.41%
Range	0%–2%	–	0%–2%

[a]Data as of June 2013.

Table A-7. Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Two-year Inhalation Study of Cobalt Metal[a]

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	–	1	–
Moribund	28	28	27	32
Natural deaths	5	2	6	2
Survivors				
Terminal kill	17	20	16	16
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(48)	(48)
Inflammation, suppurative	1 (2%)	–	–	–
Intestine large, cecum	(49)	(50)	(49)	(50)
Inflammation, granulomatous	–	1 (2%)	–	–
Epithelium, hyperplasia	–	–	1 (2%)	–
Intestine large, colon	(50)	(50)	(49)	(50)
Epithelium, hyperplasia	–	–	1 (2%)	–
Intestine large, rectum	(50)	(49)	(49)	(49)
Intestine small, duodenum	(50)	(50)	(49)	(50)
Inflammation, chronic active	–	–	5 (10%)	–
Necrosis	–	–	1 (2%)	1 (2%)
Intestine small, ileum	(48)	(50)	(47)	(50)
Inflammation, suppurative	–	–	1 (2%)	–
Epithelium, hyperplasia	–	–	1 (2%)	–
Intestine small, jejunum	(48)	(49)	(48)	(49)
Epithelium, hyperplasia	–	–	1 (2%)	–
Liver	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	–	–	2 (4%)
Basophilic focus	5 (10%)	17 (34%)	17 (34%)	19 (38%)
Clear cell focus	9 (18%)	11 (22%)	7 (14%)	9 (18%)
Degeneration, cystic	–	5 (10%)	–	1 (2%)
Eosinophilic focus	3 (6%)	2 (4%)	–	1 (2%)
Fatty change	2 (4%)	4 (8%)	3 (6%)	4 (8%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Hepatodiaphragmatic nodule	4 (8%)	9 (18%)	11 (22%)	9 (18%)
Inflammation, chronic active	1 (2%)	–	–	1 (2%)
Mixed cell focus	–	1 (2%)	1 (2%)	1 (2%)
Necrosis	5 (10%)	6 (12%)	4 (8%)	2 (4%)
Regeneration	–	–	1 (2%)	–
Thrombosis	1 (2%)	–	–	–
Bile duct, hyperplasia	1 (2%)	–	–	–
Mesentery	(18)	(4)	(9)	(3)
Infiltration cellular	–	–	1 (11%)	–
Artery, inflammation, chronic active	–	–	–	1 (33%)
Artery, thrombosis	1 (6%)	–	–	–
Fat, necrosis	14 (78%)	4 (100%)	8 (89%)	1 (33%)
Pancreas	(50)	(50)	(49)	(50)
Acinus, atrophy	23 (46%)	35 (70%)	28 (57%)	30 (60%)
Acinus, hyperplasia	1 (2%)	–	–	–
Artery, mineralization	–	–	–	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Degeneration	–	–	–	1 (2%)
Stomach, forestomac[h]	(50)	(50)	(50)	(50)
Edema	2 (4%)	–	–	–
Hyperplasia, squamous	10 (20%)	14 (28%)	13 (26%)	11 (22%)
Inflammation, chronic active	2 (4%)	4 (8%)	2 (4%)	1 (2%)
Mineralization	2 (4%)	–	–	–
Ulcer	4 (8%)	4 (8%)	10 (20%)	2 (4%)
Stomach, glandular	(50)	(50)	(49)	(50)
Edema	1 (2%)	–	–	–
Erosion	–	1 (2%)	2 (4%)	–
Inflammation, chronic active	–	1 (2%)	1 (2%)	1 (2%)
Mineralization	1 (2%)	–	–	–
Ulcer	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Tooth	(0)	(2)	(0)	(0)
Inflammation, chronic active	–	2 (100%)	–	–
Cardiovascular System				
Blood vessel	(1)	(1)	(0)	(0)
Aorta, mineralization	1 (100%)	–	–	–

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	45 (90%)	44 (88%)	46 (92%)	39 (78%)
Inflammation, suppurative	–	1 (2%)	2 (4%)	–
Thrombosis	4 (8%)	2 (4%)	2 (4%)	–
Artery, inflammation, chronic active	–	1 (2%)	–	1 (2%)
Atrium, congestion	–	1 (2%)	1 (2%)	–
Epicardium, hyperplasia	–	1 (2%)	–	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	3 (6%)	–	2 (4%)	3 (6%)
Atrophy	–	1 (2%)	–	–
Degeneration	1 (2%)	–	–	–
Hyperplasia	35 (70%)	24(48%)	23 (46%)	29 (58%)
Necrosis	–	1 (2%)	1 (2%)	–
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	19 (38%)	21(42%)	9 (18%)	9 (18%)
Necrosis	–	1 (2%)	–	–
Islets, pancreatic	(50)	(50)	(48)	(49)
Hyperplasia	–	1 (2%)	1 (2%)	3 (6%)
Parathyroid gland	(45)	(45)	(47)	(46)
Pituitary gland	(50)	(50)	(49)	(49)
Angiectasis	1 (2%)	–	–	1 (2%)
Cyst	1 (2%)	–	–	–
Hemorrhage	–	1 (2%)	1 (2%)	–
Pars distalis, hyperplasia	18 (36%)	12 (24%)	14 (29%)	22 (45%)
Thyroid gland	(49)	(50)	(50)	(49)
Hemorrhage	1 (2%)	–	–	–
C-cell, hyperplasia	7 (14%)	8 (16%)	5 (10%)	9 (18%)
Follicle, cyst	–	–	–	1 (2%)
General Body System				
Peritoneum	(0)	(0)	(0)	(1)
Inflammation, chronic active	–	–	–	1 (100%)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Cyst	1 (2%)	–	–	–

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Penis	(0)	(1)	(0)	(0)
Concretion	–	1 (100%)	–	–
Preputial gland	(50)	(50)	(49)	(48)
Atrophy	–	–	–	1 (2%)
Ectasia	3 (6%)	1 (2%)	1 (2%)	–
Inflammation, chronic active	5 (10%)	4 (8%)	3 (6%)	3 (6%)
Prostate gland	(50)	(50)	(50)	(50)
Fibrosis	–	–	1 (2%)	–
Hyperplasia	1 (2%)	6 (12%)	1 (2%)	1 (2%)
Inflammation, chronic active	36 (72%)	39 (78%)	42 (84%)	28 (56%)
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation, chronic active	1 (2%)	1 (2%)	–	1 (2%)
Testes	(50)	(50)	(50)	(50)
Atrophy	35 (70%)	34 (68%)	36 (72%)	39 (78%)
Infarct	1 (2%)	–	2 (4%)	12 (24%)
Arteriole, inflammation, chronic active	2 (4%)	–	1 (2%)	–
Interstitial cell, hyperplasia	7 (14%)	10 (20%)	12 (24%)	12 (24%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Necrosis	–	–	1 (2%)	–
Lymph node	(12)	(9)	(5)	(9)
Iliac, ectasia	1 (8%)	–	–	–
Iliac, lumbar, ectasia	–	1 (11%)	–	–
Iliac, renal, ectasia	–	1 (11%)	–	–
Lumbar, hyperplasia	–	1 (11%)	–	–
Pancreatic, ectasia	1 (8%)	–	–	1 (11%)
Pancreatic, infiltration cellular, histiocyte	–	–	1 (20%)	–
Pancreatic, necrosis	–	1 (11%)	–	–
Renal, ectasia	2 (17%)	3 (33%)	1 (20%)	1 (11%)
Renal, hemorrhage	–	–	–	1 (11%)
Lymph node, bronchial	(27)	(25)	(21)	(20)
Lymph node, mandibular	(48)	(49)	(50)	(48)
Atrophy	1 (2%)	–	–	–
Congestion	–	1 (2%)	–	–
Ectasia	1 (2%)	2 (4%)	1 (2%)	2 (4%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Hyperplasia	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Necrosis	1 (2%)	1 (2%)	–	–
Lymph node, mediastinal	(46)	(48)	(46)	(48)
Hyperplasia, lymphoid	–	–	1 (2%)	–
Lymph node, mesenteric	(49)	(50)	(49)	(49)
Hemorrhage	1 (2%)	–	–	–
Infiltration cellular, histiocyte	–	–	–	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Congestion	–	1 (2%)	–	–
Fibrosis	8 (16%)	8 (16%)	7 (14%)	4 (8%)
Hematopoietic cell proliferation	3 (6%)	2 (4%)	–	3 (6%)
Hemorrhage	1 (2%)	1 (2%)	–	–
Necrosis	3 (6%)	2 (4%)	1 (2%)	6 (12%)
Stromal hyperplasia	–	–	1 (2%)	–
Capsule, angiectasis	1 (2%)	–	–	–
Thymus	(46)	(44)	(47)	(46)
Integumentary System				
Mammary gland	(31)	(32)	(30)	(31)
Galactocele	2 (6%)	1 (3%)	1 (3%)	–
Hyperplasia	2 (6%)	1 (3%)	1 (3%)	–
Inflammation, chronic active	1 (3%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	–	–	1 (2%)
Hemorrhage	–	1 (2%)	–	–
Hyperplasia, squamous	2 (4%)	2 (4%)	5 (10%)	1 (2%)
Inflammation	7 (14%)	5 (10%)	9 (18%)	4 (8%)
Inflammation, granulomatous	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, fibrosis	–	1 (2%)	–	–
Cranium, fracture	–	–	1 (2%)	–
Cranium, inflammation, chronic active	–	1 (2%)	–	–
Maxilla, inflammation, chronic active	–	–	–	1 (2%)
Vertebra, degeneration	–	1 (2%)	–	–
Skeletal muscle	(1)	(0)	(0)	(4)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	15 (30%)	14 (28%)	18 (36%)	7 (14%)
Edema	2 (4%)	–	–	1 (2%)
Hemorrhage	2 (4%)	3 (6%)	4 (8%)	3 (6%)
Infiltration cellular, mononuclear cell	1 (2%)	–	–	1 (2%)
Metaplasia, osseous	1 (2%)	–	–	–
Meninges, inflammation, suppurative	1 (2%)	–	–	–
Spinal cord	(1)	(0)	(0)	(0)
Infiltration cellular	1 (100%)	–	–	–
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Autolysis	–	–	1 (2%)	–
Foreign body	3 (6%)	–	2 (4%)	–
Hyperkeratosis	–	–	–	1 (2%)
Inflammation	28 (56%)	18 (36%)	18 (36%)	16 (32%)
Metaplasia, squamous	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Ulcer	1 (2%)	–	–	–
Respiratory epithelium, hyperplasia	–	–	–	1 (2%)
Squamous epithelium, hyperplasia	–	–	–	1 (2%)
Lung	(50)	(50)	(50)	(50)
Inflammation, suppurative	–	1 (2%)	1 (2%)	–
Inflammation, chronic active	22 (44%)	50 (100%)	50 (100%)	50 (100%)
Metaplasia, osseous	3 (6%)	3 (6%)	–	–
Mineralization	1 (2%)	–	–	–
Thrombosis	2 (4%)	–	–	–
Alveolar epithelium, hyperplasia	3 (6%)	47 (94%)	49 (98%)	49 (98%)
Alveolus, proteinosis	–	48 (96%)	49 (98%)	49 (98%)
Artery, mediastinum, inflammation, chronic active	–	1 (2%)	–	–
Bronchiole, epithelium, hyperplasia	–	44 (88%)	47 (94%)	50 (100%)
Mediastinum, inflammation, suppurative	–	–	1 (2%)	–
Mediastinum, metaplasia, osseous	–	1 (2%)	–	–
Nose	(48)	(47)	(45)	(50)
Foreign body	5 (10%)	2 (4%)	4 (9%)	5 (10%)
Hemorrhage	–	–	1 (2%)	–

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Inflammation, suppurative	9 (19%)	12 (26%)	24 (53%)	46 (92%)
Inflammation, chronic active	28 (58%)	35 (74%)	40 (89%)	49 (98%)
Thrombosis	7 (15%)	2 (4%)	4 (9%)	2 (4%)
Olfactory epithelium, accumulation, hyaline droplet	2 (4%)	4 (9%)	1 (2%)	2 (4%)
Olfactory epithelium, atrophy	2 (4%)	21 (45%)	34 (76%)	29 (58%)
Olfactory epithelium, hyperplasia	–	1 (2%)	2 (4%)	7 (14%)
Olfactory epithelium, hyperplasia, basal cell	–	1 (2%)	–	13 (26%)
Olfactory epithelium, metaplasia, respiratory	12 (25%)	26 (55%)	37 (82%)	50 (100%)
Olfactory epithelium, necrosis	–	1 (2%)	5 (11%)	5 (10%)
Respiratory epithelium, hyperplasia	20 (42%)	35 (74%)	45 (100%)	50 (100%)
Respiratory epithelium, metaplasia, squamous	–	1 (2%)	11 (24%)	35 (70%)
Respiratory epithelium, necrosis	1 (2%)	4 (9%)	5 (11%)	13 (26%)
Turbinate, atrophy	1 (2%)	35 (74%)	35 (78%)	41 (82%)
Turbinate, hyperostosis	2 (4%)	–	–	–
Trachea	(50)	(50)	(50)	(50)
Inflammation, suppurative	–	–	–	1 (2%)
Metaplasia, squamous	–	–	–	1 (2%)
Special Senses System				
Ear	(1)	(5)	(0)	(0)
Inflammation, chronic active	1 (100%)	5 (100%)	–	–
Eye	(50)	(50)	(50)	(50)
Cataract	3 (6%)	9 (18%)	9 (18%)	7 (14%)
Inflammation, suppurative	1 (2%)	–	–	–
Metaplasia, osseous	31 (62%)	36 (72%)	33 (66%)	31 (62%)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(4)	(3)	(0)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	–	1 (2%)	4 (8%)	1 (2%)
Congestion	–	–	1 (2%)	–
Cyst	–	3 (6%)	–	1 (2%)
Fibrosis	–	–	–	1 (2%)
Infarct	2 (4%)	–	–	1 (2%)
Metaplasia, osseous	–	–	–	1 (2%)
Nephropathy	49 (98%)	49 (98%)	50 (100%)	50 (100%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Thrombosis	–	1 (2%)	–	–
Artery, inflammation, chronic active	–	–	–	1 (2%)
Pelvis, inflammation, suppurative	–	–	1 (2%)	–
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)	–	–	–
Inflammation		–	2 (4%)	1 (2%)
Inflammation, granulomatous	2 (4%)	–	–	–
Transitional epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	–

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

Appendix B. Summary of Lesions in Female Rats in the Two-year Inhalation Study of Cobalt Metal

Tables

Table B-1. Summary of the Incidence of Neoplasms in Female Rats in the Two-year Inhalation Study of Cobalt Metal[a]

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	11	20	19	24
Natural deaths	4	4	7	1
Survivors				
Died last week of study	–	–	–	1
Terminal kill	35	26	24	24
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(49)	(48)
Intestine large, cecum	(50)	(50)	(49)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Intestine large, colon	(50)	(50)	(49)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Intestine large, rectum	(50)	(50)	(50)	(49)
Intestine small, duodenum	(48)	(50)	(50)	(50)
Intestine small, ileum	(49)	(50)	(48)	(50)
Carcinoma	1 (2%)	–	–	–
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Fibrous histiocytoma, metastatic, skin	1 (2%)	–	–	–
Intestine small, jejunum	(50)	(50)	(47)	(50)
Carcinoma	1 (2%)	–	–	–
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Fibrous histiocytoma, metastatic, skin	1 (2%)	1 (2%)	–	–
Hepatocellular adenoma	–	1 (2%)	–	–
Hepatocellular carcinoma	–	1 (2%)	–	–
Mesentery	(13)	(15)	(8)	(9)
Carcinoma, metastatic, islets, pancreatic	1 (8%)	–	–	–
Pancreas	(50)	(50)	(50)	(50)
Acinus, carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Salivary glands	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	1 (2%)	–	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)	–	–	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(1)	(0)	(1)	(0)
Squamous cell carcinoma	–	–	1 (100%)	–
Squamous cell papilloma	1 (100%)	–	–	–
Tooth	(1)	(0)	(2)	(2)
Cardiovascular System				
Blood vessel	(0)	(1)	(0)	(0)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (100%)	–	–
Aorta, alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (100%)	–	–
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Fibrous histiocytoma, metastatic, skin	1 (2%)	–	–	–
Pheochromocytoma malignant, metastatic, adrenal medulla	–	–	–	1 (2%)
Schwannoma malignant	–	–	1 (2%)	–
Pericardium, rhabdomyosarcoma, metastatic, uncertain primary site	–	–	–	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	4 (8%)	8 (16%)	14 (28%)	17 (34%)
Pheochromocytoma malignant	–	1 (2%)	2 (4%)	7 (14%)
Bilateral, fibrous histiocytoma, metastatic, skin	1 (2%)	–	–	–
Bilateral, pheochromocytoma benign	2 (4%)	4 (8%)	8 (16%)	19 (38%)
Bilateral, pheochromocytoma malignant	–	1 (2%)	1 (2%)	4 (8%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	–	–	–	1 (2%)
Carcinoma	1 (2%)	–	–	3 (6%)
Parathyroid gland	(42)	(45)	(38)	(45)
Pituitary gland	(50)	(50)	(49)	(50)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Pars distalis, adenoma	29 (58%)	23 (46%)	26 (53%)	22 (44%)
Pars distalis, carcinoma	–	3 (6%)	1 (2%)	1 (2%)
Thyroid gland	(50)	(49)	(49)	(50)
Bilateral, C-cell, adenoma	1 (2%)	–	2 (4%)	–
C-cell, adenoma	7 (14%)	6 (12%)	2 (4%)	2 (4%)
C-cell, carcinoma	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Follicular cell, adenoma	–	–	1 (2%)	1 (2%)
Follicular cell, carcinoma	1 (2%)	–	–	–
General Body System				
Peritoneum	(1)	(1)	(0)	(0)
Carcinoma, metastatic, islets, pancreatic	1 (100%)	–	–	–
Fibrous histiocytoma, metastatic, skin	–	1 (100%)	–	–
Genital System				
Clitoral gland	(49)	(47)	(47)	(46)
Adenoma	2 (4%)	4 (9%)	6 (13%)	2 (4%)
Carcinoma	4 (8%)	5 (11%)	3 (6%)	4 (9%)
Bilateral, adenoma	–	1 (2%)	–	–
Ovary	(50)	(50)	(50)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Cystadenocarcinoma	–	1 (2%)	–	–
Granulosa cell tumor malignant	–	–	1 (2%)	–
Thecoma benign	–	–	–	1 (2%)
Uterus	(50)	(50)	(50)	(50)
Carcinoma	–	1 (2%)	1 (2%)	–
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Hemangiosarcoma	1 (2%)	–	–	–
Leiomyoma	1 (2%)	1 (2%)	–	–
Polyp stromal	10 (20%)	7 (14%)	6 (12%)	10 (20%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(4)	(3)	(7)	(5)
Deep cervical, fibrous histiocytoma, metastatic, skin	1 (25%)	–	–	–
Lumbar, renal, carcinoma, metastatic, islets, pancreatic	1 (25%)	–	–	–
Lymph node, bronchial	(26)	(18)	(22)	(17)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (6%)
Fibrous histiocytoma, metastatic, skin	1 (4%)	–	–	–
Lymph node, mandibular	(48)	(48)	(47)	(47)
Fibrous histiocytoma, metastatic, skin	1 (2%)	–	–	–
Lymph node, mediastinal	(42)	(43)	(48)	(48)
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Spleen	(50)	(50)	(50)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Fibrous histiocytoma, metastatic, skin	1 (2%)	–	–	–
Thymus	(47)	(46)	(46)	(47)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenocarcinoma	–	–	1 (2%)	–
Adenoma	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Carcinoma	2 (4%)	5 (10%)	–	3 (6%)
Fibroadenoma	17 (34%)	21 (42%)	15 (30%)	5 (10%)
Fibroadenoma, multiple	1 (2%)	1 (2%)	–	–
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)	–	–	–
Keratoacanthoma	–	–	2 (4%)	1 (2%)
Papilloma	–	1 (2%)	–	–
Squamous cell papilloma	1 (2%)	–	1 (2%)	–
Subcutaneous tissue, fibroma	2 (4%)	1 (2%)	–	1 (2%)
Subcutaneous tissue, fibrous histiocytoma	1 (2%)	2 (4%)	–	–
Subcutaneous tissue, lipoma	1 (2%)	–	–	–
Subcutaneous tissue, schwannoma malignant	1 (2%)	–	–	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(1)	(1)	(0)	(1)
Carcinoma, metastatic, islets, pancreatic	1 (100%)	–	–	–
Lipoma	–	1 (100%)	–	–

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Schwannoma malignant, metastatic, skin	–	–	–	1 (100%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland	–	3 (6%)	1 (2%)	1 (2%)
Glioma malignant	–	–	1 (2%)	–
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	6 (12%)	6 (12%)	9 (18%)
Alveolar/bronchiolar adenoma, multiple	–	1 (2%)	3 (6%)	4 (8%)
Alveolar/bronchiolar carcinoma	–	5 (10%)	14 (28%)	12 (24%)
Alveolar/bronchiolar carcinoma, multiple	–	4 (8%)	3 (6%)	18 (36%)
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Cystic keratinizing epithelioma	–	4 (8%)	1 (2%)	2 (4%)
Fibrous histiocytoma, metastatic, skin	1 (2%)	–	–	–
Pheochromocytoma malignant, metastatic, adrenal medulla	–	–	–	1 (2%)
Squamous cell carcinoma	–	–	–	1 (2%)
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	–	2 (4%)	–	1 (2%)
Mediastinum, fibrous histiocytoma, metastatic, skin	1 (2%)	–	–	–
Nose	(50)	(50)	(49)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(4)	(4)	(0)	(3)
Eye	(50)	(50)	(49)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal’s gland	(0)	(1)	(0)	(0)
Adenoma	–	1 (100%)	–	–
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Transitional epithelium, papilloma	–	1 (2%)	–	–
Urinary bladder	(50)	(50)	(50)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)	–	–	–
Schwannoma malignant, metastatic, skin	–	–	–	1 (2%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	16 (32%)	29 (58%)	28 (56%)	27 (54%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	50	50	49
Total primary neoplasms	114	155	153	181
Total animals with benign neoplasms	44	44	43	43
Total benign neoplasms	84	95	94	99
Total animals with malignant neoplasms	25	42	42	48
Total malignant neoplasms	30	60	59	82
Total animals with metastatic neoplasms	2	5	1	6
Total metastatic neoplasms	30	9	1	9
Total animals with malignant neoplasms of uncertain primary site	–	–	–	1

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

Table B-2. Statistical Analysis of Primary Neoplasms in Female Rats in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	6/50 (12%)	12/50 (24%)	22/50 (44%)	36/50 (72%)
Adjusted rate[b]	13.6%	27.2%	52.1%	80.6%
Terminal rate[c]	6/35 (17%)	5/26 (19%)	13/24 (54%)	21/25 (84%)
First incidence (days)	730 (T)	598	590	579
Poly-3 test[d]	P < 0.001	P = 0.091	P < 0.001	P < 0.001
Adrenal Medulla: Malignant Pheochromocytoma				
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	11/50 (22%)
Adjusted rate	0.0%	4.7%	7.5%	27.0%
Terminal rate	0/35 (0%)	2/26 (8%)	2/24 (8%)	9/25 (36%)
First incidence (days)	–[e]	730 (T)	715	712
Poly-3 test	P < 0.001	P = 0.228	P = 0.102	P < 0.001
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	6/50 (12%)	13/50 (26%)	23/50 (46%)	40/50 (80%)
Adjusted rate	13.6%	29.4%	54.5%	89.4%
Terminal rate	6/35 (17%)	6/26 (23%)	14/24 (58%)	24/25 (96%)
First incidence (days)	730 (T)	598	590	579
Poly-3 test	P < 0.001	P = 0.058	P < 0.001	P < 0.001
Clitoral Gland: Adenoma				
Overall rate	2/49 (4%)	5/47 (11%)	6/47 (13%)	2/46 (4%)
Adjusted rate	4.5%	12.3%	16.1%	5.4%
Terminal rate	1/35 (3%)	1/23 (4%)	6/22 (27%)	1/22 (5%)
First incidence (days)	674	593	730 (T)	691
Poly-3 test	P = 0.517N	P = 0.181	P = 0.083	P = 0.631
Clitoral Gland: Carcinoma				
Overall rate	4/49 (8%)	5/47 (11%)	3/47 (6%)	4/46 (9%)
Adjusted rate	9.1%	12.5%	8.0%	10.6%
Terminal rate	3/35 (9%)	1/23 (4%)	1/22 (5%)	2/22 (9%)
First incidence (days)	722	646	618	478
Poly-3 test	P = 0.524N	P = 0.438	P = 0.585N	P = 0.558
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	6/49 (12%)	10/47 (21%)	9/47 (19%)	6/46 (13%)
Adjusted rate	13.5%	24.3%	23.9%	15.8%
Terminal rate	4/35 (11%)	2/23 (9%)	7/22 (32%)	3/22 (14%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
First incidence (days)	674	593	618	478
Poly-3 test	P = 0.480N	P = 0.159	P = 0.179	P = 0.511
Lung: Cystic Keratinizing Epithelioma				
Overall rate	0/50 (0%)	4/50 (8%)	1/50 (2%)	2/50 (4%)
Adjusted rate	0.0%	9.5%	2.5%	4.9%
Terminal rate	0/35 (0%)	4/26 (15%)	1/24 (4%)	1/25 (4%)
First incidence (days)	–	730 (T)	730 (T)	603
Poly-3 test	P = 0.582	P = 0.055	P = 0.481	P = 0.222
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/50 (4%)	7/50 (14%)	9/50 (18%)	13/50 (26%)
Adjusted rate	4.5%	16.2%	22.1%	30.9%
Terminal rate	1/35 (3%)	5/26 (19%)	6/24 (25%)	8/25 (32%)
First incidence (days)	698	590	587	579
Poly-3 test	P = 0.002	P = 0.072	P = 0.016	P < 0.001
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	0/50 (0%)	9/50 (18%)	17/50 (34%)	30/50 (60%)
Adjusted rate	0.0%	21.3%	42.0%	69.2%
Terminal rate	0/35 (0%)	9/26 (35%)	14/24 (58%)	20/25 (80%)
First incidence (days)	–	730 (T)	690	471
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	2/50 (4%)	15/50 (30%)	20/50 (40%)	38/50 (76%)
Adjusted rate	4.5%	34.7%	48.5%	86.2%
Terminal rate	1/35 (3%)	13/26 (50%)	14/24 (58%)	25/25 (100%)
First incidence (days)	698	590	587	471
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Mammary Gland: Fibroadenoma				
Overall rate	18/50 (36%)	22/50 (44%)	15/50 (30%)	5/50 (10%)
Adjusted rate	39.3%	49.5%	36.0%	12.3%
Terminal rate	14/35 (40%)	16/26 (62%)	9/24 (38%)	5/25 (20%)
First incidence (days)	316	516	590	730 (T)
Poly-3 test	P < 0.001N	P = 0.220	P = 0.459N	P = 0.003N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	19/50 (38%)	23/50 (46%)	16/50 (32%)	6/50 (12%)
Adjusted rate	41.5%	51.3%	38.4%	14.7%

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Terminal rate	15/35 (43%)	16/26 (62%)	10/24 (42%)	6/25 (24%)
First incidence (days)	316	516	590	730 (T)
Poly-3 test	P < 0.001N	P = 0.231	P = 0.467N	P = 0.005N
Mammary Gland: Carcinoma				
Overall rate	2/50 (4%)	5/50 (10%)	1/50 (2%)	3/50 (6%)
Adjusted rate	4.5%	11.7%	2.5%	7.4%
Terminal rate	1/35 (3%)	3/26 (12%)	1/24 (4%)	3/25 (12%)
First incidence (days)	698	578	730 (T)	730 (T)
Poly-3 test	P = 0.446N	P = 0.203	P = 0.534N	P = 0.462
Mammary Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	7/50 (14%)	1/50 (2%)	4/50 (8%)
Adjusted rate	6.8%	16.2%	2.5%	9.8%
Terminal rate	2/35 (6%)	4/26 (15%)	1/24 (4%)	4/25 (16%)
First incidence (days)	698	578	730 (T)	730 (T)
Poly-3 test	P = 0.345N	P = 0.147	P = 0.341N	P = 0.455
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	20/50 (40%)	26/50 (52%)	16/50 (32%)	9/50 (18%)
Adjusted rate	43.6%	57.2%	38.4%	22.1%
Terminal rate	15/35 (43%)	17/26 (65%)	10/24 (42%)	9/25 (36%)
First incidence (days)	316	516	590	730 (T)
Poly-3 test	P < 0.001N	P = 0.131	P = 0.389N	P = 0.026N
Pancreatic Islets: Carcinoma				
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.2%	0.0%	0.0%	7.2%
Terminal rate	0/35 (0%)	0/26 (0%)	0/24 (0%)	1/25 (4%)
First incidence (days)	234	–	–	506
Poly-3 test	P = 0.060	P = 0.512N	P = 0.523N	P = 0.279
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.2%	0.0%	0.0%	7.2%
Terminal rate	0/35 (0%)	0/26 (0%)	0/24 (0%)	1/25 (4%)
First incidence (days)	234	–	–	506
Poly-3 test	P = 0.060	P = 0.512N	P = 0.523N	P = 0.279
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	29/50 (58%)	23/50 (46%)	26/49 (53%)	22/50 (44%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Adjusted rate	61.5%	51.6%	59.9%	50.1%
Terminal rate	20/35 (57%)	14/26 (54%)	15/24 (63%)	11/25 (44%)
First incidence (days)	511	593	291	506
Poly-3 test	P = 0.287N	P = 0.223N	P = 0.525N	P = 0.183N
Pituitary Gland (Pars Distalis): Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	1/49 (2%)	1/50 (2%)
Adjusted rate	0.0%	6.9%	2.5%	2.5%
Terminal rate	0/35 (0%)	1/26 (4%)	1/24 (4%)	1/25 (4%)
First incidence (days)	–	578	730 (T)	730 (T)
Poly-3 test	P = 0.497N	P = 0.115	P = 0.478	P = 0.484
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	29/50 (58%)	26/50 (52%)	27/49 (55%)	23/50 (46%)
Adjusted rate	61.5%	57.1%	62.2%	52.3%
Terminal rate	20/35 (57%)	15/26 (58%)	16/24 (67%)	12/25 (48%)
First incidence (days)	511	578	291	506
Poly-3 test	P = 0.279N	P = 0.412N	P = 0.559	P = 0.247N
Skin: Papilloma, Squamous Cell Papilloma, or Keratoacanthoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.3%	2.4%	7.5%	2.5%
Terminal rate	1/35 (3%)	1/26 (4%)	3/24 (13%)	1/25 (4%)
First incidence (days)	730 (T)	730 (T)	730 (T)	730 (T)
Poly-3 test	P = 0.469	P = 0.752	P = 0.272	P = 0.743
Skin: Papilloma, Squamous Cell Papilloma, Keratoacanthoma, or Basal Cell Adenoma				
Overall rate	2/50 (4%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.5%	2.4%	7.5%	2.5%
Terminal rate	2/35 (6%)	1/26 (4%)	3/24 (13%)	1/25 (4%)
First incidence (days)	730 (T)	730 (T)	730 (T)	730 (T)
Poly-3 test	P = 0.570N	P = 0.514N	P = 0.457	P = 0.528N
Skin (Subcutaneous Tissue): Fibroma or Fibrous Histiocytoma				
Overall rate	3/50 (6%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	6.6%	7.0%	0.0%	2.5%
Terminal rate	1/35 (3%)	1/26 (4%)	0/24 (0%)	1/25 (4%)
First incidence (days)	316	422	–	730 (T)
Poly-3 test	P = 0.118N	P = 0.641	P = 0.141N	P = 0.344N
Thyroid Gland (C-Cell): Adenoma				

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Overall rate	8/50 (16%)	6/49 (12%)	4/49 (8%)	2/50 (4%)
Adjusted rate	18.1%	14.5%	10.2%	4.8%
Terminal rate	7/35 (20%)	5/25 (20%)	4/24 (17%)	1/25 (4%)
First incidence (days)	674	684	730 (T)	506
Poly-3 test	P = 0.036N	P = 0.437N	P = 0.237N	P = 0.056N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	9/50 (18%)	8/49 (16%)	5/49 (10%)	3/50 (6%)
Adjusted rate	20.3%	19.3%	12.7%	7.2%
Terminal rate	8/35 (23%)	7/25 (28%)	5/24 (21%)	1/25 (4%)
First incidence (days)	674	684	730 (T)	506
Poly-3 test	P = 0.035N	P = 0.560N	P = 0.262N	P = 0.072N
Uterus: Stromal Polyp				
Overall rate	10/50 (20%)	7/50 (14%)	6/50 (12%)	10/50 (20%)
Adjusted rate	22.3%	16.2%	14.5%	24.1%
Terminal rate	7/35 (20%)	4/26 (15%)	3/24 (13%)	6/25 (24%)
First incidence (days)	646	624	541	646
Poly-3 test	P = 0.377	P = 0.326N	P = 0.253N	P = 0.524
All Organs: Mononuclear Cell Leukemia				
Overall rate	16/50 (32%)	29/50 (58%)	28/50 (56%)	27/50 (54%)
Adjusted rate	35.7%	62.4%	60.5%	58.9%
Terminal rate	12/35 (34%)	15/26 (58%)	12/24 (50%)	13/25 (52%)
First incidence (days)	663	590	117	473
Poly-3 test	P = 0.118	P = 0.007	P = 0.013	P = 0.019
All Organs: Benign Neoplasms				
Overall rate	44/50 (88%)	44/50 (88%)	43/50 (86%)	43/50 (86%)
Adjusted rate	89.7%	91.4%	92.5%	92.6%
Terminal rate	30/35 (86%)	24/26 (92%)	24/24 (100%)	24/25 (96%)
First incidence (days)	316	516	291	506
Poly-3 test	P = 0.373	P = 0.525	P = 0.454	P = 0.447
All Organs: Malignant Neoplasms				
Overall rate	25/50 (50%)	42/50 (84%)	42/50 (84%)	49/50 (98%)
Adjusted rate	52.5%	86.6%	89.0%	98.7%
Terminal rate	16/35 (46%)	23/26 (89%)	22/24 (92%)	25/25 (100%)
First incidence (days)	234	422	117	471
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rate	96.0%	100.0%	100.0%	100.0%
Terminal rate	33/35 (94%)	26/26 (100%)	24/24 (100%)	25/25 (100%)
First incidence (days)	234	422	117	471
Poly-3 test	P = 0.185	P = 0.237	P = 0.237	P = 0.237

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

Table B-3. Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female F344/NTac Rats[a]

	Adenoma	Carcinoma	Adenoma or Carcinoma
Overall Historical Incidence: All Routes			
Total (%)	2/100 (2.0%)	0/100	2/100 (2.0%)
Mean ± standard deviation	2.0% ± 2.8%	–	2.0% ± 2.8%
Range	0%–4%	–	0%–4%

[a]Data as of June 2013.

Table B-4. Historical Incidence of Pheochromocytoma of the Adrenal Medulla in Control Female F344/NTac Rats[a]

	Benign	Malignant	Benign or Malignant
Overall Historical Incidence: All Routes			
Total (%)	7/100 (7.0%)	1/100 (1.0%)	8/100 (8.0%)
Mean ± standard deviation	7.0% ± 7.1%	1.0% ± 1.4%	8.0% ± 5.7%
Range	2%–12%	0%–2%	4%–12%

[a]Data as of June 2013.

Table B-5. Historical Incidence of Pancreatic Islet Neoplasms in Control Female F344/NTac Rats[a]

	Adenoma	Carcinoma	Adenoma or Carcinoma
Overall Historical Incidence: All Routes			
Total (%)	1/100 (1.0%)	1/100 (1.0%)	2/100 (2.0%)
Mean ± standard deviation	1.0% ± 1.4%	1.0% ± 1.4%	2.0% ± 0.0%
Range	0%–2%	0%–2%	2%

[a]Data as of June 2013.

Table B-6. Historical Incidence of Mononuclear Cell Leukemia in Control Female F344/NTac Rats[a]

	Incidence in Controls
Overall Historical Incidence: All Routes	
Total (%)	35/100 (35.0%)
Mean ± standard deviation	35.0% ± 4.2%
Range	32%–38%

[a]Data as of June 2013.

Table B-7. Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Two-year Inhalation Study of Cobalt Metal[a]

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	11	20	19	24
Natural deaths	4	4	7	1
Survivors				
Died last week of study	–	–	–	1
Terminal kill	35	26	24	24
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(49)	(48)
Intestine large, cecum	(50)	(50)	(49)	(50)
Hemorrhage	–	–	1 (2%)	–
Inflammation, chronic active	–	–	–	1 (2%)
Intestine large, colon	(50)	(50)	(49)	(50)
Mucosa, hyperplasia	–	2 (4%)	–	–
Intestine large, rectum	(50)	(50)	(50)	(49)
Intestine small, duodenum	(48)	(50)	(50)	(50)
Infiltration cellular, chronic active	–	1 (2%)	–	–
Intestine small, ileum	(49)	(50)	(48)	(50)
Intestine small, jejunum	(50)	(50)	(47)	(50)
Congestion	–	–	1 (2%)	–
Hemorrhage	–	–	1 (2%)	–
Inflammation, suppurative	–	–	1 (2%)	–
Necrosis	–	–	1 (2%)	–
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)	–	1 (2%)
Atrophy	1 (2%)	–	–	–
Basophilic focus	16 (32%)	20 (40%)	22 (44%)	33 (66%)
Clear cell focus	12 (24%)	6 (12%)	3 (6%)	6 (12%)
Eosinophilic focus	–	1 (2%)	2 (4%)	2 (4%)
Fatty change	13 (26%)	9 (18%)	6 (12%)	3 (6%)
Fibrosis	–	1 (2%)	–	–

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Hepatodiaphragmatic nodule	6 (12%)	11 (22%)	12 (24%)	15 (30%)
Inflammation, chronic active	1 (2%)	–	–	2 (4%)
Mixed cell focus	1 (2%)	4 (8%)	2 (4%)	–
Necrosis	6 (12%)	3 (6%)	2 (4%)	2 (4%)
Regeneration	2 (4%)	3 (6%)	5 (10%)	4(8%)
Bile duct, cyst	–	–	1 (2%)	–
Serosa, thrombosis	1 (2%)	–	–	–
Mesentery	(13)	(15)	(8)	(9)
Fibrosis	–	1 (7%)	–	–
Fat, necrosis	12 (92%)	14 (93%)	8 (100%)	9 (100%)
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus	–	1 (2%)	–	–
Acinus, atrophy	18 (36%)	23 (46%)	27 (54%)	32 (64%)
Acinus, hyperplasia	–	–	1 (2%)	–
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	–	1 (2%)	–
Inflammation, chronic active	–	–	1 (2%)	–
Duct, hyperplasia	–	1 (2%)	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	–	–	1 (2%)	1 (2%)
Erosion	–	–	1 (2%)	–
Fibrosis	–	1 (2%)	–	–
Hyperplasia, squamous	5 (10%)	10 (20%)	2 (4%)	3 (6%)
Inflammation, chronic active	–	1 (2%)	1 (2%)	–
Ulcer	1 (2%)	5 (10%)	2 (4%)	2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Edema	2 (4%)	–	–	–
Erosion	1 (2%)	–	1 (2%)	–
Inflammation, suppurative	–	–	–	1 (2%)
Necrosis	–	–	–	1 (2%)
Thrombosis	–	–	1 (2%)	–
Ulcer	–	3 (6%)	1 (2%)	1 (2%)
Tongue	(1)	(0)	(1)	(0)
Tooth	(1)	(0)	(2)	(2)
Inflammation, chronic active	1 (100%)	–	2 (100%)	2 (100%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Cardiovascular System				
Blood vessel	(0)	(1)	(0)	(0)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	41 (82%)	34 (68%)	42 (84%)	35 (70%)
Thrombosis	2 (4%)	4 (8%)	2 (4%)	–
Artery, inflammation, chronic active	1 (2%)	–	1 (2%)	–
Atrium, congestion	1 (2%)	–	–	–
Pericardium, hyperplasia	–	–	–	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	5 (10%)	–	3 (6%)	4 (8%)
Atrophy	1 (2%)	–	1 (2%)	–
Degeneration	3 (6%)	1 (2%)	3 (6%)	3 (6%)
Hemorrhage	1 (2%)	–	–	–
Hyperplasia	25 (50%)	27 (54%)	28 (56%)	28 (56%)
Necrosis	1 (2%)	–	1 (2%)	2 (4%)
Adrenal medulla	(50)	(50)	(50)	(50)
Degeneration, cystic	1 (2%)	–	–	–
Hyperplasia	12 (24%)	27 (54%)	27 (54%)	10 (20%)
Infiltration cellular, mononuclear cell	1 (2%)	–	–	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)	–	1 (2%)
Parathyroid gland	(42)	(45)	(38)	(45)
Pituitary gland	(50)	(50)	(49)	(50)
Angiectasis	–	–	1 (2%)	2 (4%)
Cyst	–	–	–	1 (2%)
Pars distalis, hyperplasia	19 (38%)	20 (40%)	18 (37%)	19 (38%)
Thyroid gland	(50)	(49)	(49)	(50)
Hemorrhage	–	–	–	1 (2%)
C-cell, hyperplasia	9 (18%)	9 (18%)	8 (16%)	6 (12%)
Follicular cell, hyperplasia	1 (2%)	–	–	–
General Body System				
Peritoneum	(1)	(1)	(0)	(0)
Genital System				
Clitoral gland	(49)	(47)	(47)	(46)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Cyst	–	–	–	1 (2%)
Hyperplasia	2 (4%)	1 (2%)	1 (2%)	–
Inflammation, suppurative	2 (4%)	–	–	1 (2%)
Inflammation, chronic active	–	4 (9%)	–	2 (4%)
Ovary	(50)	(50)	(50)	(50)
Cyst	5 (10%)	6 (12%)	7 (14%)	5 (10%)
Inflammation, suppurative	–	–	1 (2%)	–
Inflammation, chronic active	1 (2%)	–	–	–
Necrosis	–	1 (2%)	–	–
Uterus	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	–	–	–
Cyst	1 (2%)	–	–	–
Inflammation, suppurative	1 (2%)	1 (2%)	–	–
Inflammation, chronic active	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Necrosis	2 (4%)	–	–	–
Thrombosis	–	–	1 (2%)	–
Endometrium, hyperplasia, cystic	4 (8%)	2 (4%)	1 (2%)	4 (8%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Fibrosis	–	–	–	1 (2%)
Lymph node	(4)	(3)	(7)	(5)
Deep cervical, infiltration cellular	–	1 (33%)	–	–
Iliac, ectasia	–	1 (33%)	–	1 (20%)
Inguinal, fibrosis	–	–	–	1 (20%)
Renal, ectasia	1 (25%)	–	1 (14%)	–
Lymph node, bronchial	(26)	(18)	(22)	(17)
Lymph node, mandibular	(48)	(48)	(47)	(47)
Atrophy	1 (2%)	–	–	–
Ectasia	–	–	1 (2%)	–
Fibrosis	–	1 (2%)	–	–
Necrosis	–	1 (2%)	–	–
Lymph node, mediastinal	(42)	(43)	(48)	(48)
Atrophy	1 (2%)	–	–	–
Ectasia	1 (2%)	1 (2%)	–	–
Hyperplasia	–	–	–	1 (2%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Infiltration cellular, histiocyte	–	–	–	1 (2%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia	–	1 (2%)	–	–
Fibrosis	–	–	–	1 (2%)
Infiltration cellular, histiocyte	1 (2%)	–	–	–
Spleen	(50)	(50)	(50)	(50)
Fibrosis	–	1 (2%)	5 (10%)	3 (6%)
Hematopoietic cell proliferation	2 (4%)	5 (10%)	3 (6%)	2 (4%)
Hemorrhage	–	–	3 (6%)	–
Infiltration cellular, histiocyte	1 (2%)	–	–	–
Necrosis	–	1 (2%)	–	2 (4%)
Thymus	(47)	(46)	(46)	(47)
Thrombosis	–	1 (2%)	–	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	1 (2%)	1 (2%)	4 (8%)	1 (2%)
Hyperplasia	3 (6%)	2 (4%)	1 (2%)	5 (10%)
Inflammation, chronic active	–	1 (2%)	–	–
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	1 (2%)	–	–
Hyperkeratosis	1 (2%)	–	–	–
Hyperplasia, squamous	1 (2%)	1 (2%)	–	2 (4%)
Inflammation	–	1 (2%)	1 (2%)	–
Ulcer	–	–	–	1 (2%)
Subcutaneous tissue, inflammation	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fracture	1 (2%)	–	–	–
Femur, hyperostosis	4 (8%)	2 (4%)	5 (10%)	2 (4%)
Maxilla, inflammation, chronic active	2 (4%)	–	–	1 (2%)
Skeletal muscle	(1)	(1)	(0)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	16 (32%)	14 (28%)	14 (28%)	17 (34%)
Hemorrhage	–	2 (4%)	–	3 (6%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Infiltration cellular, mononuclear cell	1 (2%)	–	–	–
Necrosis	–	–	1 (2%)	–
Respiratory System				
Larynx	(50)	(50)	(50)	(50)
Foreign body	1 (2%)	3 (6%)	6 (12%)	2 (4%)
Inflammation	22 (44%)	23 (46%)	14 (28%)	13 (26%)
Metaplasia, squamous	4 (8%)	4 (8%)	3 (6%)	5 (10%)
Respiratory epithelium, hyperplasia	–	4 (8%)	–	1 (2%)
Lung	(50)	(50)	(50)	(50)
Hemorrhage	–	–	–	1 (2%)
Inflammation, suppurative	–	–	–	1 (2%)
Inflammation, chronic active	20 (40%)	50 (100%)	50 (100%)	50 (100%)
Necrosis	–	–	–	1 (2%)
Pigmentation	–	–	–	1 (2%)
Alveolar epithelium, hyperplasia	9 (18%)	49 (98%)	50 (100%)	49 (98%)
Alveolar epithelium, metaplasia, squamous	–	2 (4%)	1 (2%)	–
Alveolus, proteinosis	–	50 (100%)	50 (100%)	50 (100%)
Bronchiole, epithelium, hyperplasia	–	47 (94%)	46 (92%)	48 (96%)
Interstitium, fibrosis	1 (2%)	–	–	–
Mediastinum, inflammation	1 (2%)	–	–	–
Nose	(50)	(50)	(49)	(50)
Foreign body	5 (10%)	3 (6%)	–	–
Inflammation	–	–	1 (2%)	–
Inflammation, suppurative	6 (12%)	4 (8%)	4 (8%)	42 (84%)
Inflammation, chronic active	22 (44%)	42 (84%)	39 (80%)	50 (100%)
Thrombosis	1 (2%)	4 (8%)	6 (12%)	3 (6%)
Olfactory epithelium, accumulation, hyaline droplet	8 (16%)	2 (4%)	–	–
Olfactory epithelium, atrophy	–	22 (44%)	35 (71%)	35 (70%)
Olfactory epithelium, hyperplasia	–	–	3 (6%)	5 (10%)
Olfactory epithelium, hyperplasia, basal cell	–	–	1 (2%)	19 (38%)
Olfactory epithelium, metaplasia, respiratory	6 (12%)	18 (36%)	24 (49%)	47 (94%)
Olfactory epithelium, necrosis	–	2 (4%)	–	1 (2%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Respiratory epithelium, accumulation, hyaline droplet	1 (2%)	–	–	–
Respiratory epithelium, hyperplasia	15 (30%)	43 (86%)	48 (98%)	49 (98%)
Respiratory epithelium, metaplasia, squamous	2 (4%)	–	3 (6%)	45 (90%)
Respiratory epithelium, necrosis	1 (2%)	1 (2%)	1 (2%)	15 (30%)
Turbinate, atrophy	1 (2%)	38 (76%)	27 (55%)	45 (90%)
Turbinate, hyperostosis	3 (6%)	2 (4%)	5 (10%)	2 (4%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(4)	(4)	(0)	(3)
Inflammation, chronic active	4 (100%)	4 (100%)	–	3 (100%)
Eye	(50)	(50)	(49)	(50)
Cataract	8 (16%)	10 (20%)	11 (22%)	3 (6%)
Hemorrhage	–	1 (2%)	–	–
Metaplasia, osseous	24 (48%)	19 (38%)	26 (53%)	13 (26%)
Cornea, inflammation, chronic active	–	1 (2%)	–	–
Harderian gland	(50)	(50)	(50)	(50)
Atrophy	–	1 (2%)	–	–
Hyperplasia	–	–	1 (2%)	–
Inflammation, chronic active	3 (6%)	–	–	2 (4%)
Zymbal's gland	(0)	(1)	(0)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	–	1 (2%)	–	1 (2%)
Infarct	–	1 (2%)	–	2 (4%)
Mineralization	–	–	1 (2%)	–
Necrosis, focal	1 (2%)	–	–	–
Nephropathy	48 (96%)	48 (96%)	47 (94%)	48 (96%)
Renal tubule, hyperplasia	–	2 (4%)	–	–
Renal tubule, necrosis	–	–	1 (2%)	–
Urinary bladder	(50)	(50)	(50)	(50)

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

Appendix C. Summary of Lesions in Male Mice in the Two-year Inhalation Study of Cobalt Metal

Tables

Table C-1. Summary of the Incidence of Neoplasms in Male Mice in the Two-year Inhalation Study of Cobalt Metal[a]

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	5	13	12	17
Natural deaths	6	6	9	8
Survivors				
Died last week of study	–	–	1	3
Terminal kill	39	31	28	22
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(43)	(37)	(34)	(35)
Intestine large, cecum	(45)	(45)	(44)	(45)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (2%)
Intestine large, colon	(47)	(45)	(45)	(46)
Intestine large, rectum	(44)	(44)	(45)	(48)
Intestine small, duodenum	(45)	(44)	(44)	(44)
Adenoma	1 (2%)	–	–	–
Intestine small, ileum	(45)	(45)	(43)	(44)
Adenoma	1 (2%)	–	–	–
Intestine small, jejunum	(45)	(45)	(43)	(44)
Liver	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	1 (2%)
Cholangiocarcinoma	–	1 (2%)	–	1 (2%)
Hemangiosarcoma	3 (6%)	1 (2%)	3 (6%)	–
Hemangiosarcoma, multiple	1 (2%)	1 (2%)	–	–
Hepatoblastoma	2 (4%)	2 (4%)	–	–
Hepatocellular adenoma	15 (30%)	9 (18%)	13 (26%)	11 (22%)
Hepatocellular adenoma, multiple	13 (26%)	16 (32%)	8 (16%)	1 (2%)
Hepatocellular carcinoma	15 (30%)	15 (30%)	11 (22%)	8 (16%)
Hepatocellular carcinoma, multiple	10 (20%)	6 (12%)	3 (6%)	1 (2%)
Sarcoma, metastatic, mesentery	–	1 (2%)	–	–
Mesentery	(5)	(5)	(5)	(3)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Sarcoma	–	1 (20%)	–	–
Pancreas	(50)	(47)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (2%)
Carcinoma, metastatic, islets, pancreatic	–	1 (2%)	–	–
Salivary glands	(50)	(49)	(50)	(50)
Stomach, forestomach	(50)	(48)	(50)	(50)
Sarcoma, metastatic, mesentery	–	1 (2%)	–	–
Stomach, glandular	(48)	(46)	(48)	(49)
Sarcoma, metastatic, mesentery	–	1 (2%)	–	–
Tooth	(8)	(2)	(2)	(1)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	1 (2%)	2 (4%)
Hemangiosarcoma	1 (2%)	–	1 (2%)	–
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (2%)	–
Hepatocellular carcinoma, metastatic, liver	1 (2%)	–	–	–
Bilateral, subcapsular, adenoma	1 (2%)	–	–	–
Subcapsular, adenoma	–	4 (8%)	1 (2%)	1 (2%)
Adrenal medulla	(50)	(49)	(50)	(48)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	–
Islets, pancreatic	(49)	(47)	(50)	(50)
Adenoma	–	1 (2%)	–	–
Carcinoma	–	2 (4%)	–	–
Parathyroid gland	(29)	(25)	(26)	(27)
Pituitary gland	(47)	(49)	(48)	(48)
Thyroid gland	(49)	(49)	(50)	(49)
General Body System				
Tissue NOS	(0)	(0)	(0)	(1)
Genital System				
Coagulating gland	(0)	(1)	(0)	(1)
Adenoma	–	1 (100%)	–	–

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (100%)
Epididymis	(50)	(49)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	1 (2%)
Hemangiosarcoma	–	1 (2%)	–	–
Penis	(0)	(0)	(0)	(1)
Preputial gland	(49)	(50)	(49)	(50)
Prostate	(50)	(49)	(48)	(50)
Seminal vesicle	(48)	(48)	(49)	(50)
Testes	(50)	(49)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	1 (2%)
Interstitial cell, adenoma	1 (2%)	–	–	–
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(49)
Hemangiosarcoma	1 (2%)	1 (2%)	2 (4%)	–
Lymph node	(2)	(0)	(1)	(1)
Lymph node, bronchial	(26)	(32)	(20)	(24)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	2 (6%)	1 (5%)	2 (8%)
Hepatocellular carcinoma, metastatic, liver	–	1 (3%)	–	–
Lymph node, mandibular	(37)	(23)	(27)	(37)
Lymph node, mediastinal	(34)	(34)	(36)	(44)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	2 (6%)	3 (8%)	–
Hepatocellular carcinoma, metastatic, liver	–	1 (3%)	1 (3%)	–
Lymph node, mesenteric	(47)	(44)	(44)	(42)
Hemangiosarcoma	–	–	1 (2%)	–
Spleen	(50)	(48)	(49)	(48)
Hemangioma	–	1 (2%)	–	1 (2%)
Hemangiosarcoma	3 (6%)	–	4 (8%)	3 (6%)
Thymus	(42)	(44)	(40)	(38)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (3%)	1 (3%)
Hemangiosarcoma	–	–	1 (3%)	–

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Hepatocellular carcinoma, metastatic, liver	–	1 (2%)	–	–
Integumentary System				
Skin	(50)	(50)	(50)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	–
Subcutaneous tissue, fibrous histiocytoma	–	1 (2%)	–	–
Subcutaneous tissue, hemangiosarcoma	–	–	1 (2%)	–
Subcutaneous tissue, neural crest tumor	1 (2%)	–	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(49)	(50)	(50)
Skeletal muscle	(0)	(3)	(3)	(1)
Head, alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (33%)	–
Thoracic, alveolar/bronchiolar carcinoma, metastatic, lung	–	3 (100%)	3 (100%)	1 (100%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(1)	(0)	(0)	(1)
Spinal cord	(1)	(0)	(0)	(1)
Respiratory System				
Larynx	(48)	(47)	(49)	(50)
Lung	(50)	(49)	(50)	(50)
Alveolar/bronchiolar adenoma	7 (14%)	10 (20%)	14 (28%)	3 (6%)
Alveolar/bronchiolar adenoma, multiple	–	1 (2%)	1 (2%)	–
Alveolar/bronchiolar carcinoma	8 (16%)	20 (41%)	18 (36%)	10 (20%)
Alveolar/bronchiolar carcinoma, multiple	3 (6%)	18 (37%)	24 (48%)	36 (72%)
Carcinoma, metastatic, islets, pancreatic	–	1 (2%)	–	–
Cholangiocarcinoma, metastatic, liver	–	–	–	1 (2%)
Hepatoblastoma, metastatic, liver	–	2 (4%)	–	–
Hepatocellular carcinoma, metastatic, liver	9 (18%)	6 (12%)	7 (14%)	1 (2%)
Nose	(50)	(49)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (2%)
Glands, olfactory epithelium, adenoma	–	1 (2%)	–	–
Trachea	(48)	(47)	(48)	(50)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (2%)	–
Special Senses System				
Eye	(47)	(46)	(43)	(45)
Harderian gland	(49)	(48)	(48)	(50)
Adenoma	6 (12%)	3 (6%)	4 (8%)	4 (8%)
Carcinoma	2 (4%)	1 (2%)	–	–
Zymbal's gland	(0)	(1)	(0)	(0)
Urinary System				
Kidney	(50)	(49)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	3 (6%)	1 (2%)	2 (4%)
Hepatocellular carcinoma, metastatic, liver	–	1 (2%)	–	–
Urinary bladder	(48)	(48)	(50)	(47)
Hemangiosarcoma	–	–	1 (2%)	–
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	–	–	–
Lymphoma malignant	4 (8%)	1 (2%)	1 (2%)	3 (6%)
Neoplasm Summary				
Total animals with primary neoplasmsc	45	49	50	49
Total primary neoplasms	100	119	113	83
Total animals with benign neoplasms	35	32	34	19
Total benign neoplasms	45	47	41	21
Total animals with malignant neoplasms	36	46	46	48
Total malignant neoplasms	54	72	71	62
Total animals with metastatic neoplasms	9	13	11	5
Total metastatic neoplasms	10	33	21	17
Total animals with uncertain neoplasms-benign or malignant	1	–	1	–
Total uncertain neoplasms	1	–	1	–

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

Table C-2. Statistical Analysis of Primary Neoplasms in Male Mice in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Adrenal Cortex: Adenoma				
Overall rate[a]	1/50 (2%)	4/49 (8%)	1/50 (2%)	1/50 (2%)
Adjusted rate[b]	2.1%	9.1%	2.4%	2.5%
Terminal rate[c]	1/39 (3%)	3/31 (10%)	1/29 (3%)	1/25 (4%)
First incidence (days)	729 (T)	715	729 (T)	729 (T)
Poly-3 test[d]	P = 0.444N	P = 0.157	P = 0.730	P = 0.724
Harderian Gland: Adenoma				
Overall rate	6/50 (12%)	3/50 (6%)	4/50 (8%)	4/50 (8%)
Adjusted rate	12.6%	6.8%	9.6%	9.7%
Terminal rate	5/39 (13%)	3/31 (10%)	3/29 (10%)	2/25 (8%)
First incidence (days)	651	729 (T)	706	635
Poly-3 test	P = 0.454N	P = 0.281N	P = 0.460N	P = 0.465N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	8/50 (16%)	4/50 (8%)	4/50 (8%)	4/50 (8%)
Adjusted rate	16.6%	9.0%	9.6%	9.7%
Terminal rate	6/39 (15%)	4/31 (13%)	3/29 (10%)	2/25 (8%)
First incidence (days)	646	729 (T)	706	635
Poly-3 test	P = 0.224N	P = 0.220N	P = 0.255N	P = 0.260N
Liver: Hemangiosarcoma				
Overall rate	4/50 (8%)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted rate	8.4%	4.5%	7.1%	0.0%
Terminal rate	2/39 (5%)	1/31 (3%)	0/29 (0%)	0/25 (0%)
First incidence (days)	682	611	597	–[e]
Poly-3 test	P = 0.083N	P = 0.369N	P = 0.563N	P = 0.084N
Liver: Hepatocellular Adenoma				
Overall rate	28/50 (56%)	25/50 (50%)	21/50 (42%)	12/50 (24%)
Adjusted rate	57.5%	56.0%	46.7%	29.1%
Terminal rate	22/39 (56%)	20/31 (65%)	14/29 (48%)	8/25 (32%)
First incidence (days)	646	684	361	635
Poly-3 test	P = 0.002N	P = 0.527N	P = 0.199N	P = 0.005N
Liver: Hepatocellular Carcinoma				
Overall rate	25/50 (50%)	21/50 (42%)	14/50 (28%)	9/50 (18%)
Adjusted rate	50.0%	43.6%	31.5%	21.9%

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Terminal rate	14/39 (36%)	9/31 (29%)	5/29 (17%)	6/25 (24%)
First incidence (days)	561	457	394	649
Poly-3 test	P = 0.002N	P = 0.334N	P = 0.052N	P = 0.004N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	38/50 (76%)	38/50 (76%)	32/50 (64%)	18/50 (36%)
Adjusted rate	76.0%	78.7%	68.4%	43.1%
Terminal rate	27/39 (69%)	23/31 (74%)	19/29 (66%)	11/25 (44%)
First incidence (days)	561	457	361	635
Poly-3 test	P < 0.001N	P = 0.469	P = 0.269N	P < 0.001N
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	27/50 (54%)	22/50 (44%)	14/50 (28%)	9/50 (18%)
Adjusted rate	54.0%	45.7%	31.5%	21.9%
Terminal rate	16/39 (41%)	10/31 (32%)	5/29 (17%)	6/25 (24%)
First incidence (days)	561	457	394	649
Poly-3 test	P < 0.001N	P = 0.268N	P = 0.021N	P < 0.001N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	39/50 (78%)	39/50 (78%)	32/50 (64%)	18/50 (36%)
Adjusted rate	78.0%	80.8%	68.4%	43.1%
Terminal rate	28/39 (72%)	24/31 (77%)	19/29 (66%)	11/25 (44%)
First incidence (days)	561	457	361	635
Poly-3 test	P < 0.001N	P = 0.464	P = 0.197N	P < 0.001N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	7/50 (14%)	11/49 (22%)	15/50 (30%)	3/50 (6%)
Adjusted rate	14.7%	24.5%	35.9%	7.3%
Terminal rate	5/39 (13%)	7/31 (23%)	14/29 (48%)	2/25 (8%)
First incidence (days)	684	571	660	571
Poly-3 test	P = 0.254N	P = 0.176	P = 0.016*	P = 0.226N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	11/50 (22%)	38/49 (78%)	42/50 (84%)	46/50 (92%)
Adjusted rate	22.8%	79.4%	87.6%	93.8%
Terminal rate	8/39 (21%)	24/31 (77%)	25/29 (86%)	22/25 (88%)
First incidence (days)	561	551	382	425
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	16/50 (32%)	41/49 (84%)	43/50 (86%)	47/50 (94%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Adjusted rate	33.0%	85.0%	89.7%	95.9%
Terminal rate	11/39 (28%)	26/31 (84%)	26/29 (90%)	23/25 (92%)
First incidence (days)	561	551	382	425
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	0/49 (0%)	3/47 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	0.0%	7.0%	0.0%	0.0%
Terminal rate	0/39 (0%)	0/31 (0%)	0/29 (0%)	0/25 (0%)
First incidence (days)	–	589	–	–
Poly-3 test	P = 0.357N	P = 0.104	–[f]	–
Spleen: Hemangiosarcoma				
Overall rate	3/50 (6%)	0/48 (0%)	4/49 (8%)	3/48 (6%)
Adjusted rate	6.3%	0.0%	9.6%	7.7%
Terminal rate	2/39 (5%)	0/31 (0%)	1/29 (3%)	2/25 (8%)
First incidence (days)	708	–	652	649
Poly-3 test	P = 0.292	P = 0.137N	P = 0.431	P = 0.568
All Organs: Hemangiosarcoma				
Overall rate	7/50 (14%)	3/50 (6%)	7/50 (14%)	3/50 (6%)
Adjusted rate	14.7%	6.7%	16.4%	7.3%
Terminal rate	5/39 (13%)	2/31 (7%)	2/29 (7%)	2/25 (8%)
First incidence (days)	682	611	597	649
Poly-3 test	P = 0.291N	P = 0.186N	P = 0.526	P = 0.228N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	7/50 (14%)	3/50 (6%)	7/50 (14%)	4/50 (8%)
Adjusted rate	14.7%	6.7%	16.4%	9.8%
Terminal rate	5/39 (13%)	2/31 (7%)	2/29 (7%)	3/25 (12%)
First incidence (days)	682	611	597	649
Poly-3 test	P = 0.428N	P = 0.186N	P = 0.526	P = 0.357N
All Organs: Malignant Lymphoma				
Overall rate	4/50 (8%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	8.3%	2.3%	2.4%	7.2%
Terminal rate	1/39 (3%)	1/31 (3%)	0/29 (0%)	1/25 (4%)
First incidence (days)	646	729 (T)	627	227
Poly-3 test	P = 0.551N	P = 0.207N	P = 0.226N	P = 0.578N

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
All Organs: Benign Neoplasms				
Overall rate	35/50 (70%)	32/50 (64%)	34/50 (68%)	19/50 (38%)
Adjusted rate	71.8%	70.3%	75.1%	45.2%
Terminal rate	28/39 (72%)	24/31 (77%)	25/29 (86%)	13/25 (52%)
First incidence (days)	646	571	361	571
Poly-3 test	P = 0.004N	P = 0.528N	P = 0.446	P = 0.006N
All Organs: Malignant Neoplasms				
Overall rate	36/50 (72%)	46/50 (92%)	46/50 (92%)	48/50 (96%)
Adjusted rate	72.0%	92.0%	93.7%	96.0%
Terminal rate	25/39 (64%)	27/31 (87%)	26/29 (90%)	23/25 (92%)
First incidence (days)	561	457	382	227
Poly-3 test	P < 0.001	P = 0.008	P = 0.004	P < 0.001
All Organs: Benign or Malignant Neoplasms				
Overall rate	45/50 (90%)	49/50 (98%)	50/50 (100%)	49/50 (98%)
Adjusted rate	90.0%	98.0%	100.0%	98.0%
Terminal rate	34/39 (87%)	30/31 (97%)	29/29 (100%)	24/25 (96%)
First incidence (days)	561	457	361	227
Poly-3 test	P = 0.050	P = 0.102	P = 0.031	P = 0.102

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

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

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Inhalation Studies			
1-Bromopropane (July 2003)	6/50	8/50	13/50
CIMSTAR 3800 (May 2008)	5/50	8/50	13/50
Cobalt metal (May 2006)	7/50	11/50	16/50
Diethylamine (August 2003)	4/50	12/50	15/50
Tetralin (June 2003)	10/50	11/50	20/50
Vinylidene chloride (June 2005)	7/50	9/50	13/50
Total (%)	39/300 (13.0%)	59/300 (19.7%)	90/300 (30.0%)
Mean ± standard deviation	13.0% ± 4.2%	19.7% ± 3.4%	30.0% ± 5.5%
Range	8%–20%	16%–24%	26%–40%
Overall Historical Incidence: All Routes			
Total (%)	145/950 (15.3%)	132/950 (13.9%)	263/950 (27.7%)
Mean ± standard deviation	15.3% ± 6.2%	13.9% ± 7.1%	27.7% ± 5.7%
Range	2%–26%	4%–24%	16%–40%

[a]Data as of May 2013.

Table C-4. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Two-year Inhalation Study of Cobalt Metal[a]

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	5	13	12	17
Natural deaths	6	6	9	8
Survivors				
Died last week of study	–	–	1	3
Terminal kill	39	31	28	22
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Arteriole, inflammation, acute	–	–	1 (2%)	–
Gallbladder	(43)	(37)	(34)	(35)
Degeneration, hyaline	–	–	1 (3%)	–
Intestine large, cecum	(45)	(45)	(44)	(45)
Intestine large, colon	(47)	(45)	(45)	(46)
Intestine large, rectum	(44)	(44)	(45)	(48)
Intestine small, duodenum	(45)	(44)	(44)	(44)
Inflammation, chronic active	1 (2%)	–	–	–
Ulcer	–	–	1 (2%)	–
Intestine small, ileum	(45)	(45)	(43)	(44)
Inflammation, acute	–	1 (2%)	–	–
Inflammation, chronic active	–	–	1 (2%)	–
Intestine small, jejunum	(45)	(45)	(43)	(44)
Liver	(50)	(50)	(50)	(50)
Angiectasis	–	1 (2%)	–	1 (2%)
Basophilic focus	4 (8%)	4 (8%)	6 (12%)	4 (8%)
Clear cell focus	17 (34%)	17 (34%)	8 (16%)	–
Eosinophilic focus	6 (12%)	5 (10%)	4 (8%)	2 (4%)
Fatty change	–	–	1 (2%)	–
Hemorrhage	–	–	1 (2%)	–
Hepatodiaphragmatic nodule	–	3 (6%)	–	3 (6%)
Inflammation, chronic	–	–	1 (2%)	–

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Mixed cell focus	1 (2%)	–	–	–
Necrosis	2 (4%)	3 (6%)	1 (2%)	1 (2%)
Tension lipidosis	2 (4%)	–	2 (4%)	–
Mesentery	(5)	(5)	(5)	(3)
Fat, necrosis	5 (100%)	4 (80%)	5 (100%)	3 (100%)
Pancreas	(50)	(47)	(50)	(50)
Atrophy	–	–	1 (2%)	1 (2%)
Salivary glands	(50)	(49)	(50)	(50)
Inflammation, chronic	–	1 (2%)	–	–
Stomach, forestomach	(50)	(48)	(50)	(50)
Hyperplasia, squamous	1 (2%)	2 (4%)	2 (4%)	4 (8%)
Inflammation, chronic active	–	1 (2%)	–	3 (6%)
Ulcer	1 (2%)	5 (10%)	3 (6%)	1 (2%)
Stomach, glandular	(48)	(46)	(48)	(49)
Necrosis	1 (2%)	1 (2%)	1 (2%)	–
Ulcer	–	1 (2%)	–	1 (2%)
Arteriole, inflammation, acute	–	–	1 (2%)	–
Tooth	(8)	(2)	(2)	(1)
Dysplasia	8 (100%)	1 (50%)	2 (100%)	1 (100%)
Inflammation, chronic active	–	1 (50%)	–	–
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	13 (26%)	5 (10%)	12 (24%)	8 (16%)
Hemorrhage	–	–	–	1 (2%)
Inflammation, suppurative	–	1 (2%)	–	–
Mineralization	1 (2%)	1 (2%)	–	–
Necrosis	–	–	1 (2%)	–
Thrombosis	1 (2%)	–	–	–
Pericardium, inflammation, chronic	–	–	–	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Hyperplasia	6 (12%)	10 (20%)	2 (4%)	1 (2%)
Hypertrophy	18 (36%)	9 (18%)	12 (24%)	2 (4%)
Necrosis	1 (2%)	–	–	–
Adrenal medulla	(50)	(49)	(50)	(48)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Hyperplasia	2 (4%)	2 (4%)	–	–
Islets, pancreatic	(49)	(47)	(50)	(50)
Hyperplasia	2 (4%)	3 (6%)	–	2 (4%)
Parathyroid gland	(29)	(25)	(26)	(27)
Pituitary gland	(47)	(49)	(48)	(48)
Pars distalis, hyperplasia	1 (2%)	2 (4%)	1 (2%)	–
Thyroid gland	(49)	(49)	(50)	(49)
General Body System				
Tissue NOS	(0)	(0)	(0)	(1)
Genital System				
Coagulating gland	(0)	(1)	(0)	(1)
Epididymis	(50)	(49)	(50)	(50)
Granuloma sperm	2 (4%)	–	1 (2%)	–
Penis	(0)	(0)	(0)	(1)
Inflammation, acute	–	–	–	1 (100%)
Preputial gland	(49)	(50)	(49)	(50)
Ectasia	1 (2%)	1 (2%)	–	–
Inflammation, chronic active	1 (2%)	1 (2%)	–	–
Prostate gland	(50)	(49)	(48)	(50)
Inflammation, acute	–	1 (2%)	–	–
Seminal vesicle	(48)	(48)	(49)	(50)
Congestion	–	1 (2%)	–	–
Inflammation, chronic active	–	1 (2%)	–	1 (2%)
Testes	(50)	(49)	(50)	(50)
Germinal epithelium, degeneration	9 (18%)	14 (29%)	8 (16%)	21 (42%)
Interstitial cell, hyperplasia	–	–	–	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(49)
Angiectasis	–	–	–	1 (2%)
Thrombosis	–	–	–	1 (2%)
Lymph node	(2)	(0)	(1)	(1)
Renal, hemorrhage	1 (50%)	–	–	–
Lymph node, bronchial	(26)	(32)	(20)	(24)
Lymph node, mandibular	(37)	(23)	(27)	(37)
Lymph node, mediastinal	(34)	(34)	(36)	(44)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Hyperplasia, lymphoid	–	–	–	1 (2%)
Lymph node, mesenteric	(47)	(44)	(44)	(42)
Hemorrhage	1 (2%)	1 (2%)	–	–
Hyperplasia, lymphoid	–	–	1 (2%)	–
Arteriole, inflammation, chronic active	–	–	–	1 (2%)
Spleen	(50)	(48)	(49)	(48)
Hematopoietic cell proliferation	5 (10%)	–	–	–
Infarct	–	1 (2%)	–	–
Thymus	(42)	(44)	(40)	(38)
Integumentary System				
Skin	(50)	(50)	(50)	(49)
Inflammation, chronic active	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Metaplasia, osseous	–	–	1 (2%)	–
Subcutaneous tissue, edema	1 (2%)	–	–	–
Musculoskeletal System				
Bone	(50)	(49)	(50)	(50)
Skeletal muscle	(0)	(3)	(3)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Infiltration cellular, histiocyte	–	1 (2%)	–	–
Necrosis	–	1 (2%)	–	–
Peripheral nerve	(1)	(0)	(0)	(1)
Degeneration	1 (100%)	–	–	–
Spinal cord	(1)	(0)	(0)	(1)
Respiratory System				
Larynx	(48)	(47)	(49)	(50)
Inflammation, suppurative	7 (15%)	2 (4%)	2 (4%)	4 (8%)
Inflammation, chronic	1 (2%)	1 (2%)	–	–
Arteriole, inflammation, acute	–	–	1 (2%)	–
Respiratory epithelium, erosion	–	–	–	1 (2%)
Respiratory epithelium, metaplasia, squamous	7 (15%)	47 (100%)	49 (100%)	49 (98%)
Respiratory epithelium, vacuolization cytoplasmic	–	20 (43%)	24 (49%)	32 (64%)
Squamous epithelium, erosion	1 (2%)	3 (6%)	–	1 (2%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Squamous epithelium, hyperplasia	2 (4%)	5 (11%)	5 (10%)	8 (16%)
Lung	(50)	(49)	(50)	(50)
Inflammation, suppurative	1 (2%)	2 (4%)	6 (12%)	16 (32%)
Proteinosis	2 (4%)	46 (94%)	49 (98%)	50 (100%)
Alveolar/bronchiolar epithelium, hyperplasia	–	46 (94%)	49 (98%)	50 (100%)
Alveolar/bronchiolar epithelium, vacuolization cytoplasmic	–	49 (100%)	47 (94%)	48 (96%)
Alveolar epithelium, hyperplasia	4 (8%)	29 (59%)	24 (48%)	43 (86%)
Alveolus, infiltration cellular, histiocyte	10 (20%)	49 (100%)	48 (96%)	48 (96%)
Bronchiole, epithelium, erosion	–	4 (8%)	10 (20%)	2 (4%)
Bronchiole, epithelium, hyperplasia	4 (8%)	7 (14%)	9 (18%)	11 (22%)
Nose	(50)	(49)	(50)	(50)
Inflammation, suppurative	16 (32%)	32 (65%)	49 (98%)	50 (100%)
Olfactory epithelium, atrophy	3 (6%)	46 (94%)	42 (84%)	31 (62%)
Olfactory epithelium, hyperplasia	–	25 (51%)	17 (34%)	8 (16%)
Olfactory epithelium, metaplasia, respiratory	5 (10%)	24 (49%)	44 (88%)	50 (100%)
Olfactory epithelium, respiratory metaplasia, atypical	–	14 (29%)	9 (18%)	1 (2%)
Respiratory epithelium, accumulation, hyaline droplet	13 (26%)	29 (59%)	29 (58%)	7 (14%)
Respiratory epithelium, erosion	–	1 (2%)	–	–
Respiratory epithelium, hyperplasia	44 (88%)	41 (84%)	36 (72%)	19 (38%)
Respiratory epithelium, metaplasia, squamous	3 (6%)	45 (92%)	35 (70%)	33 (66%)
Respiratory epithelium, vacuolization cytoplasmic	–	41 (84%)	39 (78%)	37 (74%)
Squamous epithelium, erosion	1 (2%)	1 (2%)	2 (4%)	–
Turbinate, atrophy	3 (6%)	25 (51%)	49 (98%)	50 (100%)
Trachea	(48)	(47)	(48)	(50)
Inflammation, suppurative	–	–	–	1 (2%)
Epithelium, vacuolization cytoplasmic	–	14 (30%)	31 (65%)	37 (74%)
Special Senses System				
Eye	(47)	(46)	(43)	(45)
Cataract	1 (2%)	–	–	1 (2%)
Cornea, inflammation, chronic active	4 (9%)	1 (2%)	1 (2%)	–

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Harderian gland	(49)	(48)	(48)	(50)
Hyperplasia	1 (2%)	–	–	2 (4%)
Zymbal's gland	(0)	(1)	(0)	(0)
Inflammation, suppurative	–	1 (100%)	–	–
Urinary System				
Kidney	(50)	(49)	(49)	(50)
Cyst	1 (2%)	1 (2%)	–	–
Infarct	2 (4%)	4 (8%)	1 (2%)	3 (6%)
Nephropathy	46 (92%)	44 (90%)	42 (86%)	37 (74%)
Thrombosis	–	–	1 (2%)	–
Arteriole, inflammation, chronic active	–	–	–	1 (2%)
Capsule, hemorrhage	1 (2%)	–	–	–
Pelvis, inflammation, suppurative	1 (2%)	–	–	–
Renal tubule, hyperplasia	–	1 (2%)	1 (2%)	1 (2%)
Renal tubule, necrosis	–	–	1 (2%)	–
Urinary bladder	(48)	(48)	(50)	(47)
Hemorrhage	–	–	–	1 (2%)
Inflammation, chronic active	1 (2%)	–	–	–
Arteriole, inflammation, chronic active	–	–	–	1 (2%)
Transitional epithelium, hyperplasia	1 (2%)	–	1 (2%)	–

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

Appendix D. Summary of Lesions in Female Mice in the Two-year Inhalation Study of Cobalt Metal

Tables

Table D-1. Summary of the Incidence of Neoplasms in Female Mice in the Two-year Inhalation Study of Cobalt Metal[a]

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	12	19	21
Natural deaths	5	2	4	3
Survivors				
Died last week of study	–	2	–	–
Terminal kill	36	34	27	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(44)	(41)	(38)	(46)
Intestine large, cecum	(46)	(47)	(49)	(48)
Intestine large, colon	(46)	(48)	(49)	(49)
Intestine large, rectum	(46)	(48)	(48)	(49)
Intestine small, duodenum	(45)	(47)	(48)	(49)
Intestine small, ileum	(45)	(46)	(49)	(49)
Carcinoma	1 (2%)	–	–	–
Intestine small, jejunum	(45)	(47)	(49)	(49)
Carcinoma	–	1 (2%)	–	–
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)	1 (2%)	–	–
Hepatocellular adenoma	9 (18%)	13 (26%)	6 (12%)	2 (4%)
Hepatocellular adenoma, multiple	10 (20%)	5 (10%)	4 (8%)	
Hepatocellular carcinoma	6 (12%)	4 (8%)	5 (10%)	2 (4%)
Hepatocellular carcinoma, multiple	4 (8%)	3 (6%)	–	–
Hepatocholangiocarcinoma	–	1 (2%)	–	–
Mast cell tumor malignant	1 (2%)	–	–	–
Mesentery	(18)	(13)	(10)	(8)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (13%)
Sarcoma	–	1 (8%)	–	–
Pancreas	(50)	(50)	(50)	(49)
Sarcoma, metastatic, skin	1 (2%)	–	–	–

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)	–	–	–
Stomach, glandular	(48)	(50)	(49)	(49)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)	–	–	2 (4%)
Hemangiosarcoma	–	–	1 (2%)	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(48)
Subcapsular, carcinoma	–	–	2 (4%)	–
Adrenal medulla	(50)	(50)	(49)	(48)
Pheochromocytoma benign	1 (2%)	1 (2%)	–	–
Islets, pancreatic	(50)	(50)	(50)	(49)
Parathyroid gland	(28)	(28)	(24)	(32)
Pituitary gland	(47)	(50)	(48)	(49)
Pars distalis, adenoma	3 (6%)	4 (8%)	1 (2%)	–
Pars intermedia, adenoma	–	1 (2%)	–	–
Thyroid gland	(50)	(50)	(50)	(49)
Follicular cell, adenoma	1 (2%)	–	–	1 (2%)
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(43)	(44)	(41)	(43)
Ovary	(48)	(50)	(50)	(50)
Cystadenoma	1 (2%)	4 (8%)	–	3 (6%)
Hemangiosarcoma	–	1 (2%)	1 (2%)	–
Osteosarcoma, metastatic, bone	–	–	1 (2%)	–
Yolk sac carcinoma	1 (2%)	–	–	–
Uterus	(49)	(50)	(50)	(50)
Hemangiosarcoma	–	2 (4%)	–	–
Polyp stromal	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Endometrium, carcinoma	–	1 (2%)	–	–
Vagina	(0)	(0)	(1)	(0)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Mast cell tumor malignant	1 (2%)	–	–	–
Lymph node	(11)	(8)	(7)	(1)
Lumbar, carcinoma, metastatic, kidney	1 (9%)	–	–	–
Lymph node, bronchial	(22)	(33)	(34)	(22)
Alveolar/bronchiolar carcinoma, metastatic, lung	2 (9%)	1 (3%)	1 (3%)	1 (5%)
Lymph node, mandibular	(40)	(41)	(38)	(31)
Mast cell tumor malignant	1 (3%)	–	–	–
Lymph node, mediastinal	(42)	(39)	(44)	(46)
Alveolar/bronchiolar carcinoma, metastatic, lung	2 (5%)	1 (3%)	3 (7%)	3 (7%)
Mast cell tumor malignant	1 (2%)	–	–	–
Lymph node, mesenteric	(49)	(49)	(47)	(47)
Sarcoma, metastatic, skin	1 (2%)	1 (2%)	–	–
Spleen	(49)	(49)	(48)	(49)
Hemangioma	–	1 (2%)	–	–
Hemangiosarcoma	2 (4%)	–	1 (2%)	–
Mast cell tumor malignant	1 (2%)	–	–	–
Thymus	(46)	(46)	(46)	(46)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)	–	–	2 (4%)
Neoplasm NOS	1 (2%)	–	–	–
Osteosarcoma, metastatic, bone	–	–	1 (2%)	–
Integumentary System				
Mammary gland	(48)	(50)	(50)	(50)
Carcinoma	1 (2%)	–	1 (2%)	–
Skin	(50)	(50)	(49)	(50)
Subcutaneous tissue, hemangioma	–	–	–	1 (2%)
Subcutaneous tissue, hemangiosarcoma	–	2 (4%)	–	–
Subcutaneous tissue, neural crest tumor	1 (2%)	–	–	–
Subcutaneous tissue, sarcoma	2 (4%)	2 (4%)	2 (4%)	–
Subcutaneous tissue, sarcoma, multiple	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Osteosarcoma	–	–	1 (2%)	–
Vertebra, schwannoma malignant, metastatic, peripheral nerve	–	–	1 (2%)	–
Skeletal muscle	(0)	(2)	(1)	(0)
Thoracic, alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (50%)	1 (100%)	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(0)	(0)	(1)	(0)
Schwannoma malignant	–	–	1 (100%)	–
Respiratory System				
Larynx	(47)	(50)	(50)	(47)
Lung	(49)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	8 (16%)	8 (16%)	9 (18%)
Alveolar/bronchiolar adenoma, multiple	–	1 (2%)	–	1 (2%)
Alveolar/bronchiolar carcinoma	4 (8%)	18 (36%)	18 (36%)	19 (38%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)	7 (14%)	20 (40%)	24 (48%)
Hepatocellular carcinoma, metastatic, liver	5 (10%)	2 (4%)	1 (2%)	–
Osteosarcoma, metastatic, bone	–	–	1 (2%)	–
Nose	(50)	(50)	(50)	(50)
Pleura	(0)	(1)	(0)	(1)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (100%)	–	1 (100%)
Trachea	(48)	(50)	(48)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (2%)	–
Special Senses System				
Eye	(46)	(46)	(48)	(48)
Carcinoma, metastatic, Harderian gland	–	1 (2%)	–	–
Harderian gland	(49)	(49)	(49)	(50)
Adenoma	3 (6%)	5 (10%)	4 (8%)	3 (6%)
Carcinoma	–	2 (4%)	–	–
Zymbal's gland	(0)	(1)	(1)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	2 (4%)
Renal tubule, adenoma	–	–	–	1 (2%)
Transitional epithelium, carcinoma	1 (2%)	–	–	–
Urinary bladder	(49)	(49)	(48)	(49)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	–	1 (2%)	1 (2%)
Lymphoma malignant	14 (28%)	14 (28%)	12 (24%)	4 (8%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	40	47	49	47
Total primary neoplasms	81	105	90	72
Total animals with benign neoplasms	29	31	20	17
Total benign neoplasms	34	44	24	22
Total animals with malignant neoplasms	31	41	46	44
Total malignant neoplasms	45	61	66	50
Total animals with metastatic neoplasms	8	5	8	4
Total metastatic neoplasms	14	8	11	12
Total animals with uncertain neoplasms-benign or malignant	2	–	–	–
Total uncertain neoplasms	2	–	–	–

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

Table D-2. Statistical Analysis of Primary Neoplasms in Female Mice in the Two-year Inhalation Study of Cobalt Metal

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Harderian Gland: Adenoma				
Overall rate[a]	3/50 (6%)	5/50 (10%)	4/50 (8%)	3/50 (6%)
Adjusted rate[b]	6.8%	11.2%	9.3%	7.4%
Terminal rate[c]	2/36 (6%)	4/35 (11%)	1/27 (4%)	1/26 (4%)
First incidence (days)	697	712	562	618
Poly-3 test[d]	P = 0.535N	P = 0.363	P = 0.487	P = 0.626
Harderian Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	7/50 (14%)	4/50 (8%)	3/50 (6%)
Adjusted rate	6.8%	15.7%	9.3%	7.4%
Terminal rate	2/36 (6%)	5/35 (14%)	1/27 (4%)	1/26 (4%)
First incidence (days)	697	671	562	618
Poly-3 test	P = 0.438N	P = 0.164	P = 0.487	P = 0.626
Liver: Hepatocellular Adenoma				
Overall rate	19/50 (38%)	18/50 (36%)	10/50 (20%)	2/50 (4%)
Adjusted rate	42.6%	40.5%	23.7%	5.0%
Terminal rate	16/36 (44%)	17/35 (49%)	7/27 (26%)	2/26 (8%)
First incidence (days)	499	712	646	731 (T)
Poly-3 test	P < 0.001N	P = 0.505N	P = 0.046N	P < 0.001N
Liver: Hepatocellular Carcinoma				
Overall rate	10/50 (20%)	7/50 (14%)	5/50 (10%)	2/50 (4%)
Adjusted rate	22.2%	15.7%	11.9%	4.9%
Terminal rate	7/36 (19%)	6/35 (17%)	3/27 (11%)	0/26 (0%)
First incidence (days)	499	661	646	506
Poly-3 test	P = 0.013N	P = 0.302N	P = 0.162N	P = 0.020N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	25/50 (50%)	21/50 (42%)	13/50 (26%)	4/50 (8%)
Adjusted rate	55.3%	46.9%	30.7%	9.8%
Terminal rate	20/36 (56%)	19/35 (54%)	9/27 (33%)	2/26 (8%)
First incidence (days)	499	661	646	506
Poly-3 test	P < 0.001N	P = 0.277N	P = 0.014N	P < 0.001N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	3/49 (6%)	9/50 (18%)	8/50 (16%)	10/50 (20%)
Adjusted rate	6.9%	19.9%	18.9%	24.5%
Terminal rate	3/36 (8%)	7/35 (20%)	6/27 (22%)	6/26 (23%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
First incidence (days)	731 (T)	505	626	593
Poly-3 test	P = 0.037	P = 0.067	P = 0.087	P = 0.024
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	5/49 (10%)	25/50 (50%)	38/50 (76%)	43/50 (86%)
Adjusted rate	11.3%	53.8%	78.9%	87.7%
Terminal rate	3/36 (8%)	18/35 (51%)	19/27 (70%)	21/26 (81%)
First incidence (days)	583	537	457	478
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	8/49 (16%)	30/50 (60%)	41/50 (82%)	45/50 (90%)
Adjusted rate	18.0%	63.7%	84.6%	91.6%
Terminal rate	6/36 (17%)	22/35 (63%)	21/27 (78%)	22/26 (85%)
First incidence (days)	583	505	457	478
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Ovary: Cystadenoma				
Overall rate	1/48 (2%)	4/50 (8%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.3%	8.9%	0.0%	7.4%
Terminal rate	1/35 (3%)	3/35 (9%)	0/27 (0%)	2/26 (8%)
First incidence (days)	731 (T)	614	–[e]	618
Poly-3 test	P = 0.374	P = 0.194	P = 0.505N	P = 0.285
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	3/47 (6%)	4/50 (8%)	1/48 (2%)	0/49 (0%)
Adjusted rate	7.2%	9.0%	2.5%	0.0%
Terminal rate	2/34 (6%)	3/35 (9%)	0/27 (0%)	0/25 (0%)
First incidence (days)	649	671	646	–
Poly-3 test	P = 0.052N	P = 0.534	P = 0.321N	P = 0.132N
Skin (Subcutaneous Tissue): Sarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	4.5%	6.6%	4.8%	0.0%
Terminal rate	1/36 (3%)	0/35 (0%)	0/27 (0%)	0/26 (0%)
First incidence (days)	649	506	626	–
Poly-3 test	P = 0.163N	P = 0.517	P = 0.677	P = 0.260N
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	6/50 (12%)	3/50 (6%)	0/50 (0%)
Adjusted rate	6.9%	13.5%	7.2%	0.0%

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Terminal rate	2/36 (6%)	5/35 (14%)	2/27 (7%)	0/26 (0%)
First incidence (days)	725	712	709	–
Poly-3 test	P = 0.070N	P = 0.249	P = 0.639	P = 0.136N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	3/50 (6%)	7/50 (14%)	3/50 (6%)	1/50 (2%)
Adjusted rate	6.9%	15.6%	7.2%	2.5%
Terminal rate	2/36 (6%)	5/35 (14%)	2/27 (7%)	0/26 (0%)
First incidence (days)	725	649	709	660
Poly-3 test	P = 0.140N	P = 0.165	P = 0.639	P = 0.336N
All Organs: Malignant Lymphoma				
Overall rate	14/50 (28%)	14/50 (28%)	12/50 (24%)	4/50 (8%)
Adjusted rate	31.7%	29.8%	28.0%	10.0%
Terminal rate	11/36 (31%)	8/35 (23%)	8/27 (30%)	3/26 (12%)
First incidence (days)	697	391	537	673
Poly-3 test	P = 0.011N	P = 0.513N	P = 0.444N	P = 0.013N
All Organs: Benign Neoplasms				
Overall rate	29/50 (58%)	31/50 (62%)	20/50 (40%)	17/50 (34%)
Adjusted rate	63.0%	66.1%	45.8%	40.5%
Terminal rate	23/36 (64%)	24/35 (69%)	14/27 (52%)	10/26 (39%)
First incidence (days)	223	505	562	593
Poly-3 test	P = 0.005N	P = 0.460	P = 0.070N	P = 0.024N
All Organs: Malignant Neoplasms				
Overall rate	31/50 (62%)	41/50 (82%)	46/50 (92%)	44/50 (88%)
Adjusted rate	66.1%	82.0%	92.0%	89.8%
Terminal rate	21/36 (58%)	26/35 (74%)	23/27 (85%)	22/26 (85%)
First incidence (days)	499	391	457	478
Poly-3 test	P = 0.002	P = 0.057	P < 0.001	P = 0.004
All Organs: Benign or Malignant Neoplasms				
Overall rate	40/50 (80%)	47/50 (94%)	49/50 (98%)	47/50 (94%)
Adjusted rate	83.6%	94.0%	98.0%	95.7%
Terminal rate	29/36 (81%)	32/35 (91%)	26/27 (96%)	24/26 (92%)
First incidence (days)	223	391	457	478
Poly-3 test	P = 0.026	P = 0.088	P = 0.012	P = 0.045

(T) Terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, and pituitary gland; for other tissues, denominator is number of animals necropsied.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.

[c]Observed incidence at terminal kill.
[d]Beneath the chamber control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the chamber controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e]Not applicable; no neoplasms in animal group.

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

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Inhalation Studies			
1-Bromopropane (July 2003)	1/50	0/50	1/50
CIMSTAR 3800 (May 2008)	1/50	4/50	4/50
Cobalt metal (May 2006)	3/49	5/49	8/49
Diethylamine (August 2003)	2/50	3/50	5/50
Tetralin (June 2003)	6/50	0/50	6/50
Vinylidene chloride (June 2005)	3/50	1/50	4/50
Total (%)	16/299 (5.4%)	13/299 (4.4%)	28/299 (9.4%)
Mean ± standard deviation	5.4% ± 3.7%	4.4% ± 4.3%	9.4% ± 4.8%
Range	2%–12%	0%–10%	2%–16%
Overall Historical Incidence: All Routes			
Total (%)	54/949 (5.7%)	38/949 (4.0%)	90/949 (9.5%)
Mean ± standard deviation	5.7% ± 3.6%	4.0% ± 3.6%	9.5% ± 4.8%
Range	0%–12%	0%–14%	2%–22%

[a]Data as of May 2013.

Table D-4. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Two-year Inhalation Study of Cobalt Metal[a]

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	12	19	21
Natural deaths	5	2	4	3
Survivors				
Died last week of study	–	2	–	–
Terminal kill	36	34	27	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(44)	(41)	(38)	(46)
Intestine large, cecum	(46)	(47)	(49)	(48)
Hemorrhage	1 (2%)	–	–	–
Intestine large, colon	(46)	(48)	(49)	(49)
Intestine large, rectum	(46)	(48)	(48)	(49)
Intestine small, duodenum	(45)	(47)	(48)	(49)
Inflammation, acute	–	1 (2%)	–	–
Necrosis	–	1 (2%)	–	–
Intestine small, ileum	(45)	(46)	(49)	(49)
Intestine small, jejunum	(45)	(47)	(49)	(49)
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	–	–	–
Basophilic focus	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Clear cell focus	1 (2%)	1 (2%)	1 (2%)	–
Eosinophilic focus	3 (6%)	2 (4%)	4 (8%)	–
Fatty change	–	–	1 (2%)	–
Hematopoietic cell proliferation	1 (2%)	–	–	–
Hepatodiaphragmatic nodule	–	–	1 (2%)	1 (2%)
Mixed cell focus	–	1 (2%)	1 (2%)	–
Necrosis	6 (12%)	3 (6%)	2 (4%)	1 (2%)
Tension lipidosis	5 (10%)	5 (10%)	3 (6%)	2 (4%)
Vacuolization cytoplasmic	1 (2%)	1 (2%)	–	1 (2%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Bile duct, cyst	–	–	–	1 (2%)
Mesentery	(18)	(13)	(10)	(8)
Congestion	–	–	1 (10%)	–
Inflammation, chronic active	–	1 (8%)	–	–
Fat, hemorrhage	1 (6%)	–	–	–
Fat, necrosis	18 (100%)	11 (85%)	10 (100%)	8 (100%)
Pancreas	(50)	(50)	(50)	(49)
Atrophy	1 (2%)	–	2 (4%)	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia, squamous	–	–	3 (6%)	1 (2%)
Metaplasia, hepatocyte	–	–	–	1 (2%)
Ulcer	2 (4%)	2 (4%)	1 (2%)	–
Stomach, glandular	(48)	(50)	(49)	(49)
Metaplasia, hepatocyte	–	–	1 (2%)	–
Ulcer	1 (2%)	2 (4%)	–	–
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	6 (12%)	3 (6%)	11 (22%)	9 (18%)
Inflammation, suppurative	–	–	–	1 (2%)
Mineralization	1 (2%)	1 (2%)	–	–
Necrosis	–	1 (2%)	–	1 (2%)
Thrombosis	1 (2%)	–	–	1 (2%)
Capillary, hyperplasia	1 (2%)	–	–	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(48)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Hypertrophy	–	2 (4%)	–	–
Adrenal medulla	(50)	(50)	(49)	(48)
Hyperplasia	2 (4%)	1 (2%)	–	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(49)
Hyperplasia	–	2 (4%)	–	1 (2%)
Parathyroid gland	(28)	(28)	(24)	(32)
Pituitary gland	(47)	(50)	(48)	(49)
Pars distalis, angiectasis	2 (4%)	1 (2%)	–	–

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Pars distalis, hyperplasia	12 (26%)	5 (10%)	6 (13%)	5 (10%)
Thyroid gland	(50)	(50)	(50)	(49)
Follicular cell, hyperplasia	–	1 (2%)	–	–
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(43)	(44)	(41)	(43)
Ovary	(48)	(50)	(50)	(50)
Angiectasis	–	–	–	1 (2%)
Cyst	8 (17%)	2 (4%)	11 (22%)	10 (20%)
Inflammation, suppurative	–	–	–	1 (2%)
Thrombosis	1 (2%)	–	1 (2%)	–
Uterus	(49)	(50)	(50)	(50)
Angiectasis	–	4 (8%)	–	–
Inflammation, chronic active	2 (4%)	–	1 (2%)	–
Thrombosis	–	2 (4%)	–	1 (2%)
Endometrium, hyperplasia, cystic	41 (84%)	38 (76%)	40 (80%)	34 (68%)
Vagina	(0)	(0)	(1)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(11)	(8)	(7)	(1)
Iliac, ectasia	3 (27%)	–	1 (14%)	–
Lumbar, ectasia	–	1 (13%)	–	–
Renal, ectasia	2 (18%)	2 (25%)	–	–
Renal, erythrophagocytosis	–	1 (13%)	–	–
Renal, hyperplasia, lymphoid	1 (9%)	–	–	–
Lymph node, bronchial	(22)	(33)	(34)	(22)
Inflammation, suppurative	–	–	–	1 (5%)
Lymph node, mandibular	(40)	(41)	(38)	(31)
Hyperplasia, lymphoid	–	1 (2%)	–	–
Lymph node, mediastinal	(42)	(39)	(44)	(46)
Hyperplasia, lymphoid	1 (2%)	–	–	1 (2%)
Inflammation, suppurative	–	–	–	1 (2%)
Lymph node, mesenteric	(49)	(49)	(47)	(47)
Ectasia	–	1 (2%)	–	–

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Hyperplasia, lymphoid	1 (2%)	1 (2%)	–	–
Spleen	(49)	(49)	(48)	(49)
Hematopoietic cell proliferation	5 (10%)	4 (8%)	4 (8%)	1 (2%)
Hyperplasia, lymphoid	–	1 (2%)	1 (2%)	–
Necrosis	1 (2%)	–	–	–
Thymus	(46)	(46)	(46)	(46)
Hyperplasia, lymphoid	–	–	–	1 (2%)
Integumentary System				
Mammary gland	(48)	(50)	(50)	(50)
Hyperplasia	1 (2%)	–	1 (2%)	–
Skin	(50)	(50)	(49)	(50)
Inflammation, chronic active	2 (4%)	1 (2%)	2 (4%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis	1 (2%)	–	–	–
Skeletal muscle	(0)	(2)	(1)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Meninges, infiltration cellular, mononuclear cell	–	2 (4%)	–	–
Peripheral nerve	(0)	(0)	(1)	(0)
Respiratory System				
Larynx	(47)	(50)	(50)	(47)
Inflammation, suppurative	–	1 (2%)	–	2 (4%)
Inflammation, chronic	–	–	1 (2%)	–
Inflammation, chronic active	–	1 (2%)	–	1 (2%)
Respiratory epithelium, metaplasia, squamous	2 (4%)	49 (98%)	50 (100%)	47 (100%)
Respiratory epithelium, vacuolization cytoplasmic	–	24 (48%)	31 (62%)	34 (72%)
Squamous epithelium, erosion	1 (2%)	2 (4%)	7 (14%)	4 (9%)
Squamous epithelium, hyperplasia	2 (4%)	13 (26%)	21 (42%)	21 (45%)
Lung	(49)	(50)	(50)	(50)
Infiltration cellular, histiocyte	–	–	1 (2%)	–
Inflammation, suppurative	–	3 (6%)	2 (4%)	15 (30%)
Proteinosis	–	45 (90%)	50 (100%)	50 (100%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Thrombosis	–	–	1 (2%)	–
Alveolar/bronchiolar epithelium, hyperplasia	–	49 (98%)	49 (98%)	50 (100%)
Alveolar/bronchiolar epithelium, vacuolization cytoplasmic	–	48 (96%)	49 (98%)	48 (96%)
Alveolar epithelium, hyperplasia	2 (4%)	27 (54%)	26 (52%)	41 (82%)
Alveolar epithelium, metaplasia, squamous	–	–	–	1 (2%)
Alveolus, infiltration cellular, histiocyte	10 (20%)	49 (98%)	50 (100%)	49 (98%)
Bronchiole, epithelium, erosion	–	–	4 (8%)	3 (6%)
Bronchiole, epithelium, hyperplasia	–	3 (6%)	12 (24%)	26 (52%)
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative	3 (6%)	47 (94%)	50 (100%)	50 (100%)
Olfactory epithelium, atrophy	4 (8%)	44 (88%)	39 (78%)	24 (48%)
Olfactory epithelium, hyperplasia	1 (2%)	22 (44%)	16 (32%)	8 (16%)
Olfactory epithelium, metaplasia, respiratory	1 (2%)	26 (52%)	44 (88%)	50 (100%)
Olfactory epithelium, metaplasia, squamous	–	–	–	1 (2%)
Olfactory epithelium, respiratory metaplasia, atypical	–	18 (36%)	14 (28%)	1 (2%)
Respiratory epithelium, accumulation, hyaline droplet	12 (24%)	38 (76%)	40 (80%)	10 (20%)
Respiratory epithelium, hyperplasia	43 (86%)	40 (80%)	40 (80%)	9 (18%)
Respiratory epithelium, hyperplasia, histiocytic	1 (2%)	–	–	–
Respiratory epithelium, metaplasia, squamous	–	49 (98%)	49 (98%)	50 (100%)
Respiratory epithelium, vacuolization cytoplasmic	–	40 (80%)	47 (94%)	47 (94%)
Squamous epithelium, erosion	–	7 (14%)	1 (2%)	5 (10%)
Turbinate, atrophy	–	44 (88%)	50 (100%)	50 (100%)
Pleura	(0)	(1)	(0)	(1)
Trachea	(48)	(50)	(48)	(49)
Inflammation, suppurative	–	1 (2%)	–	1 (2%)
Epithelium, vacuolization cytoplasmic	–	26 (52%)	37 (77%)	39 (80%)

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Special Senses System				
Eye	(46)	(46)	(48)	(48)
Phthisis bulbi	–	2 (4%)	–	–
Cornea, inflammation, chronic active	–	2 (4%)	1 (2%)	–
Harderian gland	(49)	(49)	(49)	(50)
Hyperplasia	4 (8%)	1 (2%)	1 (2%)	–
Zymbal's gland	(0)	(1)	(1)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Amyloid deposition	1 (2%)	–	–	–
Cyst	1 (2%)	–	–	–
Infarct	–	2 (4%)	2 (4%)	1 (2%)
Nephropathy	35 (70%)	31 (62%)	20 (40%)	19 (38%)
Renal tubule, hyperplasia	1 (2%)	–	–	–
Renal tubule, necrosis	1 (2%)	1 (2%)	–	–
Urinary bladder	(49)	(49)	(48)	(49)

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

Appendix E. Genetic Toxicology

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Tables

E.1. Bacterial Mutagenicity Test Protocol

Testing procedures were modified from those reported by Zeiger et al.[262]. Coded samples of cobalt metal [the same chemical lot (P32 3040-1) that was used in the 2-week, 3-month, and 2-year studies] were incubated with the *Salmonella typhimurium* (TA98, TA100) and *Escherichia coli* (WP2 *uvrA*/pKM101) tester strains either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat liver) for 20 minutes at 37°C. Top agar supplemented with L-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following 2 days incubation at 37°C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of five doses of cobalt metal. The highest dose was limited by toxicity. All trials were repeated.

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

E.2. Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor et al.[263]. At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs; mature erythrocytes) in each of five animals per exposure group. In addition, the percentage of circulating polychromatic erythrocytes (PCEs; reticulocytes) in 1,000 total erythrocytes per animal was scored for each exposure group as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposed group and the control group. In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single exposed group is less than or equal to 0.025 divided by the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month study were accepted without repeat tests, because additional test data could not be obtained.

Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

E.3. Evaluation Protocol

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

E.4. Results

Results of the bacterial mutagenicity tests conducted with cobalt metal are presented in Table E-1 and Table E-2. Cobalt metal (100 to 5,000 µg/plate) gave an equivocal response in *S. typhimurium* strain TA100 in the absence of S9 activation mix; with 10% rat liver S9, doses up to 7,500 µg/plate did not induce an increase in mutant colonies in TA100. In *S. typhimurium* strain TA98 without S9, cobalt metal (100 to 3,500 µg/plate) was mutagenic, although the responses observed were weak and not well correlated with dose level; with S9, no mutagenic activity was observed. In *E. coli* strain WP2 *uvrA*/pKM101, doses of cobalt metal up to 450 µg/plate were not associated with mutagenic activity, with or without S9. No increases in the frequencies of NCEs were observed in peripheral blood of male or female mice exposed to cobalt metal (0.625 to 10 mg/m^3) for 3 months by inhalation (Table E-3). No significant alterations in the percentages of reticulocytes were seen in male or female mice, suggesting that exposure to cobalt metal under these conditions did not cause bone marrow toxicity.

Table E-1. Mutagenicity of Cobalt Metal in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9	With 10% Rat S9
TA100							
	0	78 ± 2	85 ± 7	57 ± 5	96 ± 4	84 ± 8	
	100	81 ± 2	77 ± 12	85 ± 5			
	500	97 ± 3	146 ± 10	107 ± 1	101 ± 5	83 ± 1	
	1,000	91 ± 4	165 ± 4	133 ± 4	97 ± 2	92 ± 10[b]	
	2,500	86 ± 4	128 ± 11	118 ± 3	80 ± 9	80 ± 6[b]	
	5,000	84 ± 5	25 ± 16	104 ± 1	85 ± 7	44 ± 16[b]	
	7,500				96 ± 4	55 ± 9[b]	
Trial summary		Negative	Equivocal	Equivocal	Negative	Negative	
Positive control[c]		304 ± 1	400 ± 62	206 ± 2	511 ± 15	949 ± 38	
TA98							
	0	35 ± 3	22 ± 3	19 ± 2	34 ± 2	28 ± 2	29 ± 2
	100	24 ± 4	43 ± 7	66 ± 5			
	500	54 ± 3	47 ± 7	33 ± 3	22 ± 2	37 ± 6	18 ± 1
	1,000	70 ± 5			20 ± 1	20 ± 1[b]	12 ± 1
	1,500		52 ± 8	12 ± 1			
	2,500	18 ± 6	11 ± 1	10 ± 0	26 ± 2	16 ± 5[b]	11 ± 2
	3,500		2 ± 1	0 ± 0			
	5,000	Toxic			21 ± 1	12 ± 4[b]	12 ± 2
	7,500				15 ± 2	5 ± 2[b]	2 ± 1
Trial summary		Equivocal	Positive	Positive	Negative	Negative	Negative
Positive control		517 ± 30	254 ± 20	461 ± 18	353 ± 28	751 ± 72	474 ± 44

Table E-2. Mutagenicity of Cobalt Metal in Bacterial Tester Strains (*Escherichia coli*)

Strain	Dose (µg/plate)	Without S9	Without S9	Without S9	Without S9
***Escherichia coli* WP2 *uvrA*/pKM101 (analogous to TA102)**					
	0	201 ± 7	197 ± 9	272 ± 4	163 ± 13
	5			283 ± 13	
	25	148 ± 9	238 ± 6	297 ± 9	
	50	90 ± 8	184 ± 16	268 ± 1	237 ± 5
	75			243 ± 3	
	100	134 ± 9	42 ± 1	224 ± 2	206 ± 22
	150	0 ± 0	0 ± 0		235 ± 4
	200	0 ± 0	0 ± 0		
	300				Toxic
	450				Toxic
	Trial summary	Negative	Negative	Negative	Negative
	Positive control	1,237 ± 56	1,531 ± 43	1,981 ± 23	1,485 ± 205
		With 10% Rat S9	**With 10% Rat S9**	**With 10% Rat S9**	**With 10% Rat S9**
	0	233 ± 4	250 ± 10	291 ± 2	220 ± 37
	25			319 ± 9	
	50	58 ± 6	238 ± 9	278 ± 10	239 ± 6
	75			276 ± 10	
	100	41 ± 3	175 ± 4	255 ± 3	130 ± 7
	150	16 ± 4	73 ± 6	261 ± 4	220 ± 18
	300	1 ± 1	4 ± 1		Toxic
	450	1 ± 1	1 ± 0		Toxic
	Trial summary	Negative	Negative	Negative	Negative
	Positive control	855 ± 5	886 ± 9	1,172 ± 36	1,017 ± 151

[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 control.
[b]Precipitate on plate.
[c]The positive controls in the absence of metabolic activation were sodium azide (TA100), 2-nitrofluorene (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

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

	Dose (mg/m^3)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Air[d]	0	5	2.40 ± 0.33		2.56 ± 0.17
Cobalt metal	0.625	5	2.40 ± 0.33	0.5000	3.04 ± 0.29
	1.25	5	2.30 ± 0.37	0.5581	2.64 ± 0.14
	2.5	5	3.10 ± 0.19	0.1723	2.70 ± 0.15
	5	5	2.80 ± 0.34	0.2893	2.34 ± 0.02
	10	5	2.80 ± 0.37	0.2893	2.64 ± 0.07
			P = 0.236[e]		
Female					
Air	0	5	2.50 ± 0.35		2.62 ± 0.18
Cobalt metal	0.625	5	2.60 ± 0.29	0.4442	2.64 ± 0.29
	1.25	5	2.00 ± 0.22	0.7722	2.54 ± 0.16
	2.5	5	2.80 ± 0.30	0.3399	2.32 ± 0.15
	5	5	2.00 ± 0.32	0.7722	2.30 ± 0.11
	10	5	2.30 ± 0.34	0.6137	2.22 ± 0.20
			P = 0.664		

[a]Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor et al.[263]. NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the chamber control group; exposed group values are significant at $P \leq 0.005$.
[d]Chamber control.
[e]Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at $P \leq 0.025$.

Appendix F. Clinical Pathology Results

Tables

Table F-1. Hematology and Clinical Chemistry Data for Rats in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Male					
Hematology					
n					
Day 3	10	10	10	10	10
Day 23	10	10	10	9	10
Week 14	10	10	10	10	10
Hematocrit (spun) (%)					
Day 3	46.5 ± 0.2	46.4 ± 0.2	47.4 ± 0.2*	47.7 ± 0.3**	51.2 ± 0.4**
Day 23	47.9 ± 0.4	48.5 ± 0.4	49.9 ± 0.5**	51.1 ± 0.2**	53.3 ± 0.4**
Week 14	49.6 ± 0.4	51.6 ± 0.4**	59.2 ± 0.4**	61.8 ± 0.2**	63.9 ± 0.3**
Packed cell volume (mL/dL)					
Day 3	45.1 ± 0.2	44.5 ± 0.3	45.7 ± 0.3	46.1 ± 0.4*	49.7 ± 0.4**
Day 23	46.8 ± 0.4	47.3 ± 0.4	49.0 ± 0.6**	49.7 ± 0.2**	51.5 ± 0.5**
Week 14	49.6 ± 0.5	51.7 ± 0.4**	58.3 ± 0.4**	60.7 ± 0.1**	62.9 ± 0.3**
Hemoglobin (g/dL)					
Day 3	13.7 ± 0.1	13.8 ± 0.1	14.1 ± 0.1**	14.2 ± 0.1**	15.5 ± 0.1**
Day 23	15.0 ± 0.1	14.9 ± 0.1	15.4 ± 0.2	15.8 ± 0.1**	16.1 ± 0.2**
Week 14	15.6 ± 0.1	16.3 ± 0.1**	18.7 ± 0.1**	19.7 ± 0.1**	20.3 ± 0.1**
Erythrocytes (10^6/µL)					
Day 3	7.30 ± 0.06	7.27 ± 0.09	7.46 ± 0.05	7.57 ± 0.08*	8.22 ± 0.08**
Day 23	7.97 ± 0.09	8.05 ± 0.09	8.36 ± 0.11*	8.44 ± 0.06**	9.27 ± 0.14**
Week 14	9.19 ± 0.10	9.67 ± 0.09**	11.20 ± 0.06**	11.80 ± 0.06**	11.90 ± 0.08**
Reticulocytes (10^3/µL)					
Day 3	612.0 ± 42.3	562.3 ± 24.5[b]	613.0 ± 30.8	622.1 ± 25.6	873.8 ± 53.4**
Day 23	262.9 ± 13.9	261.3 ± 17.8	280.9 ± 14.3	291.6 ± 18.6	339.6 ± 25.1*
Week 14	219.8 ± 16.0	191.7 ± 19.9	189.3 ± 13.1	272.7 ± 18.7	360.0 ± 35.9*
Nucleated erythrocytes/100 leukocytes					
Day 3	2.0 ± 0.3	1.6 ± 0.3	2.6 ± 0.6	2.4 ± 0.5	1.0 ± 0.3
Day 23	0.6 ± 0.2	0.3 ± 0.2	0.6 ± 0.2	0.3 ± 0.2	0.4 ± 0.2
Week 14	0.3 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.2
Total nucleated cells (10^3/µL)					
Day 3	8.4 ± 0.4	9.5 ± 0.3	8.9 ± 0.4	8.8 ± 0.7	8.2 ± 0.3
Day 23	7.0 ± 0.6	6.8 ± 0.3	7.4 ± 0.6	7.7 ± 0.6	6.5 ± 0.4
Week 14	7.3 ± 0.3	7.0 ± 0.4	7.2 ± 0.5	7.0 ± 0.4	7.3 ± 0.2

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Mean cell volume (fL)					
Day 3	61.8 ± 0.3	61.2 ± 0.5	61.3 ± 0.2	61.0 ± 0.4	60.5 ± 0.4*
Day 23	58.8 ± 0.2	58.7 ± 0.2	58.7 ± 0.4	58.9 ± 0.3	55.7 ± 0.7**
Week 14	54.0 ± 0.3	53.5 ± 0.2	52.1 ± 0.2**	51.4 ± 0.2**	52.9 ± 0.2**
Mean cell hemoglobin (pg)					
Day 3	18.8 ± 0.1	19.0 ± 0.1	18.9 ± 0.1	18.8 ± 0.1	18.9 ± 0.1
Day 23	18.8 ± 0.1	18.5 ± 0.1	18.5 ± 0.1	18.7 ± 0.1	17.4 ± 0.2**
Week 14	17.0 ± 0.1	16.9 ± 0.0	16.7 ± 0.0**	16.7 ± 0.1*	17.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)					
Day 3	30.4 ± 0.1	31.1 ± 0.2**	30.9 ± 0.2**	30.9 ± 0.2*	31.2 ± 0.1**
Day 23	31.9 ± 0.1	31.5 ± 0.1	31.5 ± 0.2	31.7 ± 0.1	31.3 ± 0.1**
Week 14	31.6 ± 0.1	31.6 ± 0.1	32.1 ± 0.1**	32.5 ± 0.2**	32.3 ± 0.1**
Platelets (10^3/µL)					
Day 3	899.7 ± 14.0	883.5 ± 23.9	939.4 ± 14.4	903.8 ± 22.8	1,123.7 ± 34.2**
Day 23	740.8 ± 16.2	712.0 ± 16.3	732.1 ± 22.7	682.0 ± 44.5	796.7 ± 12.8
Week 14	682.5 ± 27.7	646.8 ± 8.7	611.2 ± 13.8*	548.2 ± 22.3**	573.2 ± 15.3**
Leukocytes (10^3/µL)					
Day 3	8.22 ± 0.34	9.38 ± 0.28	8.63 ± 0.37	8.57 ± 0.64	8.10 ± 0.32
Day 23	6.93 ± 0.58	6.77 ± 0.32	7.31 ± 0.58	7.66 ± 0.57	6.44 ± 0.44
Week 14	7.25 ± 0.34	6.98 ± 0.42	7.19 ± 0.48	6.96 ± 0.39	7.27 ± 0.24
Segmented neutrophils (10^3/µL)					
Day 3	0.86 ± 0.03	1.02 ± 0.06	1.01 ± 0.04	0.98 ± 0.05	0.98 ± 0.09
Day 23	0.83 ± 0.04	0.96 ± 0.03*	1.02 ± 0.03**	1.19 ± 0.04**	1.13 ± 0.08**
Week 14	1.36 ± 0.06	1.54 ± 0.07	1.52 ± 0.09	1.48 ± 0.05	1.41 ± 0.05
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
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	7.04 ± 0.33	7.99 ± 0.26	7.40 ± 0.36	7.34 ± 0.58	6.88 ± 0.25
Day 23	5.92 ± 0.55	5.68 ± 0.31	6.10 ± 0.56	6.28 ± 0.56	5.13 ± 0.35
Week 14	5.46 ± 0.33	5.08 ± 0.38	5.18 ± 0.45	5.15 ± 0.39	5.61 ± 0.20
Monocytes (10^3/µL)					
Day 3	0.23 ± 0.03	0.26 ± 0.04	0.12 ± 0.04	0.16 ± 0.05	0.15 ± 0.05
Day 23	0.12 ± 0.04	0.07 ± 0.02	0.12 ± 0.03	0.11 ± 0.04	0.13 ± 0.04

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Week 14	0.30 ± 0.07	0.23 ± 0.06	0.36 ± 0.07	0.17 ± 0.06	0.12 ± 0.05
Basophils (10^3/µL)					
Day 3	0.008 ± 0.002	0.007 ± 0.002	0.006 ± 0.002	0.005 ± 0.002	0.010 ± 0.001
Day 23	0.010 ± 0.003	0.007 ± 0.002	0.009 ± 0.003	0.008 ± 0.003	0.008 ± 0.004
Week 14	0.009 ± 0.003	0.005 ± 0.002	0.010 ± 0.005	0.003 ± 0.002	0.002 ± 0.001
Eosinophils (10^3/µL)					
Day 3	0.08 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Day 23	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.05 ± 0.01
Week 14	0.12 ± 0.01	0.13 ± 0.02	0.11 ± 0.02	0.16 ± 0.02	0.13 ± 0.02
Clinical Chemistry					
n	10	10	10	10	10
Urea nitrogen (mg/dL)					
Day 3	8.0 ± 0.4	8.0 ± 0.5	8.1 ± 0.4	8.9 ± 0.4	16.5 ± 1.1**
Day 23	11.0 ± 0.5	10.9 ± 0.4	9.7 ± 0.4	9.9 ± 0.5	8.6 ± 0.4**
Week 14	13.4 ± 0.3	13.9 ± 0.3	14.5 ± 0.5	14.1 ± 0.6	13.2 ± 0.5
Creatinine (mg/dL)					
Day 3	0.23 ± 0.02	0.25 ± 0.02	0.26 ± 0.02	0.25 ± 0.02	0.22 ± 0.01
Day 23	0.28 ± 0.01	0.26 ± 0.02	0.30 ± 0.00	0.32 ± 0.01	0.30 ± 0.00
Week 14	0.34 ± 0.02	0.38 ± 0.01	0.36 ± 0.02	0.37 ± 0.02	0.42 ± 0.01**
Glucose (mg/dL)					
Day 3	135 ± 2	138 ± 1	134 ± 2	136 ± 1	129 ± 4
Day 23	132 ± 3	132 ± 4	143 ± 9	139 ± 8	120 ± 2
Week 14	126 ± 3	128 ± 3	118 ± 5*	111 ± 3**	104 ± 4**
Total protein (g/dL)					
Day 3	6.1 ± 0.1	5.9 ± 0.0	6.0 ± 0.0	6.1 ± 0.1	6.3 ± 0.1*
Day 23	6.5 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.4 ± 0.1
Week 14	7.4 ± 0.1	7.3 ± 0.0	7.4 ± 0.1	7.3 ± 0.1	7.1 ± 0.1**
Albumin (g/dL)					
Day 3	4.3 ± 0.0	4.1 ± 0.0**	4.2 ± 0.0	4.3 ± 0.0	4.3 ± 0.0
Day 23	4.6 ± 0.1	4.5 ± 0.0	4.5 ± 0.1	4.5 ± 0.0	4.4 ± 0.0
Week 14	4.9 ± 0.1	4.8 ± 0.0	4.8 ± 0.0	4.8 ± 0.0	4.8 ± 0.0
Globulin (g/dL)					
Day 3	1.8 ± 0.0	1.7 ± 0.0	1.8 ± 0.0	1.9 ± 0.1	2.0 ± 0.0**
Day 23	2.0 ± 0.0	2.0 ± 0.0	2.1 ± 0.1	2.0 ± 0.0	2.0 ± 0.0
Week 14	2.5 ± 0.0	2.5 ± 0.0	2.6 ± 0.0	2.5 ± 0.0	2.3 ± 0.0**

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Albumin to globulin ratio					
Day 3	2.4 ± 0.3	2.4 ± 0.0	2.3 ± 0.0	2.3 ± 0.1	2.1 ± 0.0**
Day 23	2.3 ± 0.1	2.3 ± 0.0	2.2 ± 0.1	2.2 ± 0.0	2.3 ± 0.0
Week 14	1.9 ± 0.0	2.0 ± 0.0	1.9 ± 0.0	1.9 ± 0.0	2.1 ± 0.0
Cholesterol (mg/dL)					
Day 3	95 ± 2	87 ± 1*	84 ± 1**	79 ± 2**	81 ± 2**
Day 23	79 ± 1	76 ± 2	70 ± 1**	73 ± 2**	63 ± 1**
Week 14	91 ± 1	91 ± 1	88 ± 2	79 ± 2**	67 ± 1**
Triglycerides (mg/dL)					
Day 3	52 ± 3	53 ± 3	57 ± 3	61 ± 4	95 ± 6**
Day 23	61 ± 5	63 ± 8	54 ± 5	69 ± 5	56 ± 4
Week 14	112 ± 7	122 ± 8	137 ± 10	141 ± 8	130 ± 20
Alanine aminotransferase (IU/L)					
Day 3	55 ± 1	52 ± 1	50 ± 1*	44 ± 1**	39 ± 1**
Day 23	47 ± 3	41 ± 1	41 ± 1	45 ± 1	40 ± 1
Week 14	88 ± 7	99 ± 8	106 ± 8	97 ± 6	77 ± 2
Alkaline phosphatase (IU/L)					
Day 3	579 ± 6	586 ± 8	601 ± 6	609 ± 10	518 ± 12
Day 23	387 ± 7	392 ± 9	410 ± 8	415 ± 10	458 ± 9**
Week 14	236 ± 8	238 ± 5	238 ± 4	249 ± 10	235 ± 5
Creatine kinase (IU/L)					
Day 3	402 ± 29	521 ± 55	482 ± 29	467 ± 30	480 ± 65
Day 23	378 ± 113	418 ± 70	569 ± 98	630 ± 114	467 ± 54
Week 14	224 ± 47	202 ± 33	266 ± 44	231 ± 25	254 ± 44
Sorbitol dehydrogenase (IU/L)					
Day 3	15 ± 0	13 ± 1*	14 ± 1	14 ± 0	12 ± 1**
Day 23	15 ± 2	13 ± 1	13 ± 0	14 ± 1	14 ± 1
Week 14	23 ± 1	27 ± 1	28 ± 1*	26 ± 1	24 ± 1
Bile salts (μmol/L)					
Day 3	5.3 ± 0.4	5.7 ± 1.3	5.3 ± 0.3	5.6 ± 0.6	7.3 ± 0.8
Day 23	4.2 ± 0.4	3.9 ± 0.2	5.3 ± 1.2	4.6 ± 0.6	4.1 ± 0.2
Week 14	3.2 ± 0.1	3.4 ± 0.2	3.3 ± 0.3	3.6 ± 0.4	5.2 ± 1.2
Female					
n	10	10	10	10	10
Hematology					

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Hematocrit (spun) (%)					
Day 3	49.0 ± 0.6	47.8 ± 0.7	47.6 ± 0.5	48.9 ± 0.3	49.9 ± 0.5
Day 23	51.6 ± 0.5	50.9 ± 0.3	51.0 ± 0.3	52.0 ± 0.2	53.3 ± 1.9**
Week 14	48.3 ± 0.5	48.7 ± 0.4	52.6 ± 0.6**	57.3 ± 0.5**	59.5 ± 0.3**
Packed cell volume (mL/dL)					
Day 3	47.6 ± 0.6	46.7 ± 0.6	46.5 ± 0.5	47.6 ± 0.3	48.3 ± 0.5
Day 23	51.3 ± 0.5	50.2 ± 0.3	50.5 ± 0.3	51.0 ± 0.3	51.7 ± 1.7
Week 14	49.1 ± 0.5	49.5 ± 0.4	53.1 ± 0.5**	57.1 ± 0.5**	60.0 ± 0.4**
Hemoglobin (g/dL)					
Day 3	14.8 ± 0.2	14.4 ± 0.2	14.5 ± 0.2	14.7 ± 0.1	15.2 ± 0.2
Day 23	16.1 ± 0.1	15.8 ± 0.1	16.0 ± 0.1	16.2 ± 0.1	16.4 ± 0.6*
Week 14	15.5 ± 0.2	15.8 ± 0.1*	16.9 ± 0.2**	18.3 ± 0.1**	19.1 ± 0.1**
Erythrocytes (10^6/µL)					
Day 3	7.79 ± 0.12	7.62 ± 0.13	7.65 ± 0.09	7.77 ± 0.08	8.07 ± 0.12
Day 23	8.49 ± 0.09	8.24 ± 0.07	8.43 ± 0.10	8.56 ± 0.05	8.75 ± 0.31*
Week 14	8.55 ± 0.09	8.69 ± 0.06	9.30 ± 0.10**	10.05 ± 0.07**	10.47 ± 0.09**
Reticulocytes (10^3/µL)					
Day 3	489.3 ± 22.4	497.7 ± 30.8	544.1 ± 28.1	511.9 ± 25.5	520.0 ± 23.6
Day 23	184.1 ± 11.1	244.2 ± 9.9**	212.9 ± 12.4*	276.7 ± 18.9**	386.6 ± 20.4**
Week 14	202.5 ± 13.0	224.5 ± 13.0	222.2 ± 8.8	246.5 ± 10.8*	316.7 ± 25.0**
Nucleated erythrocytes/100 leukocytes					
Day 3	1.3 ± 0.4	1.6 ± 0.3	1.3 ± 0.7	2.5 ± 0.3	1.5 ± 0.3
Day 23	0.6 ± 0.2	0.9 ± 0.3	0.7 ± 0.3	0.6 ± 0.2	1.2 ± 0.4
Week 14	0.4 ± 0.2	0.2 ± 0.2	0.6 ± 0.3	0.2 ± 0.2	0.3 ± 0.2
Total nucleated cells (10^3/µL)					
Day 3	11.7 ± 0.7	9.2 ± 0.4*	10.0 ± 0.5	9.6 ± 0.4	9.1 ± 0.6*
Day 23	12.7 ± 0.5	12.8 ± 0.3	12.7 ± 0.4	11.3 ± 0.5	10.5 ± 0.4**
Week 14	6.2 ± 0.3	6.3 ± 0.3	7.0 ± 0.3	6.8 ± 0.3	6.6 ± 0.2
Mean cell volume (fL)					
Day 3	61.1 ± 0.3	61.4 ± 0.4	60.7 ± 0.3	61.3 ± 0.2	59.8 ± 0.4
Day 23	60.4 ± 0.3	60.9 ± 0.3	59.9 ± 0.4	59.5 ± 0.4	59.2 ± 0.3*
Week 14	57.4 ± 0.2	56.9 ± 0.2	57.2 ± 0.2	56.8 ± 0.3	57.2 ± 0.2
Mean cell hemoglobin (pg)					
Day 3	18.9 ± 0.1	18.9 ± 0.1	18.9 ± 0.1	19.0 ± 0.1	18.8 ± 0.1
Day 23	19.0 ± 0.1	19.2 ± 0.1	19.0 ± 0.1	19.0 ± 0.1	18.7 ± 0.1

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Week 14	18.1 ± 0.1	18.2 ± 0.1	18.1 ± 0.1	18.2 ± 0.1	18.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)					
Day 3	31.0 ± 0.1	30.7 ± 0.2	31.2 ± 0.2	31.0 ± 0.1	31.5 ± 0.2
Day 23	31.5 ± 0.1	31.5 ± 0.1	31.8 ± 0.1	31.9 ± 0.2	31.7 ± 0.2
Week 14	31.6 ± 0.1	32.0 ± 0.1*	31.8 ± 0.1	32.1 ± 0.2	31.9 ± 0.1
Platelets (10^3/µL)					
Day 3	905.6 ± 27.8	841.4 ± 22.5	914.7 ± 18.8	912.4 ± 20.1	875.0 ± 27.4
Day 23	783.1 ± 20.5	799.2 ± 13.2	763.0 ± 25.7	800.7 ± 21.5	807.9 ± 22.2
Week 14	702.1 ± 8.0	660.2 ± 14.7*	646.6 ± 17.3**	575.3 ± 16.7**[b]	608.0 ± 16.8**
Leukocytes (10^3/µL)					
Day 3	11.57 ± 0.66	9.09 ± 0.45*	9.85 ± 0.52	9.41 ± 0.38	9.00 ± 0.57*
Day 23	12.66 ± 0.52	12.72 ± 0.30	12.61 ± 0.44	11.21 ± 0.50	10.37 ± 0.40**
Week 14	6.16 ± 0.32	6.26 ± 0.29	6.93 ± 0.35	6.80 ± 0.30	6.58 ± 0.21
Segmented neutrophils (10^3/µL)					
Day 3	0.93 ± 0.06	0.88 ± 0.07	0.98 ± 0.05	1.06 ± 0.03	0.98 ± 0.09
Day 23	1.16 ± 0.07	1.28 ± 0.09	1.37 ± 0.10	1.07 ± 0.05	1.25 ± 0.13
Week 14	1.00 ± 0.07	1.20 ± 0.09	1.32 ± 0.10	1.25 ± 0.06	1.17 ± 0.04
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
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	10.33 ± 0.61	7.93 ± 0.45*	8.56 ± 0.48	8.10 ± 0.36	7.80 ± 0.54*
Day 23	11.18 ± 0.52	11.12 ± 0.32	10.89 ± 0.43	9.84 ± 0.44	8.82 ± 0.30**
Week 14	4.83 ± 0.26	4.64 ± 0.20	5.36 ± 0.28	5.17 ± 0.24	4.94 ± 0.22
Monocytes (10^3/µL)					
Day 3	0.16 ± 0.03	0.14 ± 0.03	0.18 ± 0.05	0.11 ± 0.03	0.09 ± 0.02
Day 23	0.14 ± 0.03	0.15 ± 0.03	0.17 ± 0.03	0.15 ± 0.03	0.15 ± 0.03
Week 14	0.24 ± 0.07	0.32 ± 0.06	0.14 ± 0.05	0.25 ± 0.08	0.34 ± 0.05
Basophils (10^3/µL)					
Day 3	0.016 ± 0.004	0.012 ± 0.004	0.017 ± 0.004	0.014 ± 0.003	0.011 ± 0.003
Day 23	0.012 ± 0.003	0.024 ± 0.002*	0.022 ± 0.003	0.015 ± 0.003	0.012 ± 0.004
Week 14	0.006 ± 0.003	0.006 ± 0.002	0.002 ± 0.001	0.007 ± 0.003	0.008 ± 0.002
Eosinophils (10^3/µL)					
Day 3	0.13 ± 0.02	0.13 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.02

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Day 23	0.17 ± 0.02	0.15 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
Week 14	0.09 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Clinical Chemistry					
Urea nitrogen (mg/dL)					
Day 3	10.7 ± 0.6	8.7 ± 0.6*	8.7 ± 0.5	9.3 ± 0.4	10.8 ± 0.2
Day 23	11.8 ± 0.4	12.5 ± 0.8	12.4 ± 0.4	10.3 ± 0.3*	10.1 ± 0.4*
Week 14	15.0 ± 0.4	16.7 ± 0.4	15.0 ± 0.4	15.6 ± 0.6	13.5 ± 0.5
Creatinine (mg/dL)					
Day 3	0.25 ± 0.02	0.24 ± 0.02	0.23 ± 0.02	0.23 ± 0.02	0.22 ± 0.01
Day 23	0.26 ± 0.02	0.28 ± 0.01	0.28 ± 0.02	0.29 ± 0.01	0.29 ± 0.02
Week 14	0.39 ± 0.01	0.37 ± 0.02	0.37 ± 0.02	0.39 ± 0.01	0.41 ± 0.01
Glucose (mg/dL)					
Day 3	137 ± 3	136 ± 3	139 ± 2	136 ± 1	125 ± 3
Day 23	140 ± 4	141 ± 2	147 ± 7	141 ± 3	132 ± 5
Week 14	130 ± 7	133 ± 7	131 ± 11	112 ± 3	123 ± 10
Total protein (g/dL)					
Day 3	6.2 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	6.2 ± 0.0
Day 23	6.5 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.5 ± 0.1
Week 14	7.6 ± 0.1	7.5 ± 0.1	7.4 ± 0.1	7.4 ± 0.1*	7.0 ± 0.1**
Albumin (g/dL)					
Day 3	4.6 ± 0.1	4.4 ± 0.0	4.4 ± 0.1	4.5 ± 0.0	4.4 ± 0.0
Day 23	4.7 ± 0.0	4.7 ± 0.0	4.7 ± 0.0	4.7 ± 0.0	4.7 ± 0.1
Week 14	5.3 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.0	5.0 ± 0.1**
Globulin (g/dL)					
Day 3	1.6 ± 0.0	1.6 ± 0.0	1.7 ± 0.1	1.6 ± 0.0	1.8 ± 0.0**
Day 23	1.8 ± 0.0	1.8 ± 0.0	1.9 ± 0.1	1.9 ± 0.0	1.9 ± 0.1
Week 14	2.3 ± 0.0	2.3 ± 0.0	2.3 ± 0.1	2.3 ± 0.0	2.1 ± 0.1**
Albumin to globulin ratio					
Day 3	2.8 ± 0.0	2.8 ± 0.0	2.6 ± 0.1	2.7 ± 0.0	2.5 ± 0.0**
Day 23	2.6 ± 0.0	2.6 ± 0.0	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1
Week 14	2.3 ± 0.0	2.3 ± 0.0	2.3 ± 0.0	2.3 ± 0.0	2.4 ± 0.0
Cholesterol (mg/dL)					
Day 3	98 ± 2	95 ± 2	91 ± 2*	91 ± 2*	93 ± 2
Day 23	102 ± 3	97 ± 2	97 ± 2	93 ± 3*	89 ± 3**
Week 14	102 ± 3	100 ± 2	92 ± 3*	91 ± 3**	76 ± 2**

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Triglycerides (mg/dL)					
Day 3	63 ± 4	60 ± 5	59 ± 4	63 ± 4	78 ± 7
Day 23	79 ± 6	79 ± 4	89 ± 10	67 ± 6	80 ± 7
Week 14	98 ± 10	134 ± 15	91 ± 9	93 ± 8	71 ± 3
Alanine aminotransferase (IU/L)					
Day 3	49 ± 2	49 ± 1	48 ± 1	46 ± 1	43 ± 1*
Day 23	39 ± 2	38 ± 1	40 ± 1	39 ± 1	37 ± 1
Week 14	68 ± 6	75 ± 6	65 ± 6	65 ± 5	58 ± 6
Alkaline phosphatase (IU/L)					
Day 3	506 ± 13	539 ± 9	521 ± 10	530 ± 10	487 ± 15
Day 23	336 ± 10	359 ± 7	332 ± 10	354 ± 9	348 ± 13
Week 14	203 ± 7	216 ± 7	208 ± 5	225 ± 6	202 ± 7
Creatine kinase (IU/L)					
Day 3	538 ± 60	375 ± 36*	519 ± 74	367 ± 18	421 ± 39
Day 23	431 ± 67	323 ± 42	411 ± 65	340 ± 39	393 ± 45
Week 14	189 ± 15	209 ± 25	172 ± 18	195 ± 20	225 ± 32
Sorbitol dehydrogenase (IU/L)					
Day 3	15 ± 1	14 ± 0	14 ± 1	14 ± 0	13 ± 0**
Day 23	17 ± 1	18 ± 0	19 ± 1*	17 ± 1	16 ± 1
Week 14	19 ± 1	20 ± 1	19 ± 1	20 ± 1	18 ± 1
Bile salts (µmol/L)					
Day 3	5.3 ± 0.6	5.8 ± 0.8	5.5 ± 0.4	5.0 ± 0.3	6.1 ± 0.7
Day 23	4.4 ± 1.0	4.0 ± 0.5	5.6 ± 1.3	4.7 ± 0.6	4.9 ± 0.6
Week 14	4.8 ± 0.3	5.4 ± 0.5	5.4 ± 0.8	4.6 ± 0.3	10.6 ± 2.5

*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.
[b]n = 9.

Table F-2. Hematology Data for Mice in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
Male						
n	10	10	10	10	10	10
Hematocrit (spun) (%)	50.1 ± 0.4	50.6 ± 0.4	50.7 ± 0.4	51.0 ± 0.3	50.7 ± 0.3	51.3 ± 0.5
Packed cell volume (%)	51.3 ± 0.5	51.8 ± 0.3	51.9 ± 0.5	52.3 ± 0.3	51.8 ± 0.3	52.0 ± 0.5
Hemoglobin (g/dL)	15.7 ± 0.1	15.9 ± 0.1	15.9 ± 0.1	16.0 ± 0.1	16.0 ± 0.1	16.2 ± 0.1**
Erythrocytes (106/µL)	10.51 ± 0.08	10.59 ± 0.05	10.54 ± 0.08	10.61 ± 0.07	10.63 ± 0.07	10.91 ± 0.14*
Reticulocytes (103/µL)	238.60 ± 13.00	211.70 ± 16.50	227.70 ± 21.50	254.00 ± 15.30	192.90 ± 23.40	230.50 ± 16.30
Nucleated erythrocytes/ 100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total nucleated erythrocytes (10^3/µL)	2.9 ± 0.3	3.4 ± 0.3	3.3 ± 0.4*	2.8 ± 0.3	3.5 ± 0.3	3.3 ± 0.2
Howell-Jolly bodies (% erythrocytes)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Mean cell volume (fL)	48.8 ± 0.2	48.9 ± 0.3	49.2 ± 0.2	49.4 ± 0.2	48.7 ± 0.1	47.6 ± 0.2
Mean cell hemoglobin (pg)	14.9 ± 0.1	15.0 ± 0.0	15.1 ± 0.1	15.1 ± 0.1	15.0 ± 0.1	14.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)	30.5 ± 0.1	30.6 ± 0.1	30.6 ± 0.1	30.5 ± 0.1	30.8 ± 0.1	31.0 ± 0.1**
Platelets (10^3/µL)	903.7 ± 19.5	929.8 ± 18.5	883.7 ± 13.6	919.5 ± 11.7	894.7 ± 10.0	834.2 ± 11.6**
Leukocytes (10^3/µL)	2.85 ± 0.27	3.38 ± 0.25	3.33 ± 0.36	2.76 ± 0.32	3.46 ± 0.33	3.33 ± 0.23
Segmented neutrophils (10^3/µL)	0.34 ± 0.04	0.42 ± 0.04	0.37 ± 0.06	0.36 ± 0.05	0.59 ± 0.09*	0.49 ± 0.03*
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	2.44 ± 0.24	2.86 ± 0.22	2.88 ± 0.34	2.27 ± 0.27	2.74 ± 0.31	2.74 ± 0.22
Monocytes (10^3/µL)	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.08 ± 0.02	0.07 ± 0.02	0.03 ± 0.01
Basophils (10^3/µL)	0.013 ± 0.002	0.020 ± 0.005	0.014 ± 0.003	0.024 ± 0.003	0.019 ± 0.003	0.019 ± 0.005
Eosinophils (10^3/µL)	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
Female						
n	10	10	10	9	10	10
Hematocrit spun (%)	49.8 ± 0.7	50.2 ± 0.5	50.2 ± 0.4	50.2 ± 0.6	50.2 ± 0.3	51.2 ± 0.5
Packed cell volume (%)	50.9 ± 0.6	51.6 ± 0.4	51.8 ± 0.4	51.4 ± 0.6	51.1 ± 0.3	52.3 ± 0.4
Hemoglobin (g/dL)	15.7 ± 0.2	15.9 ± 0.1	16.0 ± 0.1	15.9 ± 0.2	15.9 ± 0.1	16.1 ± 0.1
Erythrocytes (10^6/µL)	10.12 ± 0.15	10.24 ± 0.08	10.25 ± 0.07	10.24 ± 0.11	10.37 ± 0.06	10.60 ± 0.09**
Reticulocytes (10^3/µL)	290.00 ± 19.00	289.10 ± 24.90	250.00 ± 15.30	259.40 ± 16.30	260.30 ± 13.20	281.70 ± 21.20
Nucleated erythrocytes/100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total nucleated erythrocytes (10^3/µL)	2.8 ± 0.3	3.0 ± 0.4	3.2 ± 0.3	3.3 ± 0.3	3.6 ± 0.2	3.3 ± 0.2
Howell-Jolly bodies (% erythrocytes)	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Mean cell volume (fL)	50.3 ± 0.2	50.4 ± 0.1	50.6 ± 0.1	50.1 ± 0.1	49.3 ± 0.2**	49.4 ± 0.2**
Mean cell hemoglobin (pg)	15.5 ± 0.1	15.5 ± 0.0	15.6 ± 0.1	15.5 ± 0.1	15.3 ± 0.1*	15.2 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	30.8 ± 0.1	30.8 ± 0.1	30.8 ± 0.1	30.9 ± 0.1	31.0 ± 0.1	30.8 ± 0.1
Platelets (10^3/µL)	755.2 ± 19.5	826.6 ± 7.4**	819.1 ± 9.9*	811.1 ± 16.4	801.1 ± 15.0	722.7 ± 35.7
Leukocytes (10^3/µL)	2.75 ± 0.30	2.98 ± 0.39	3.16 ± 0.29	3.29 ± 0.27	3.57 ± 0.21	3.30 ± 0.23
Segmented neutrophils (10^3/µL)	0.33 ± 0.04	0.38 ± 0.08	0.32 ± 0.03	0.41 ± 0.06	0.58 ± 0.06**	0.52 ± 0.05**
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	2.33 ± 0.26	2.52 ± 0.31	2.74 ± 0.28	2.79 ± 0.23	2.87 ± 0.17	2.65 ± 0.23
Monocytes (10^3/µL)	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Basophils (10^3/µL)	0.014 ± 0.003	0.010 ± 0.002	0.018 ± 0.002	0.017 ± 0.002	0.019 ± 0.002	0.024 ± 0.004*
Eosinophils (10^3/µL)	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.06 ± 0.01*	0.07 ± 0.01*

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

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

Tables

Table G-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Two-week Inhalation Study of Cobalt Metal[a]

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Male						
n	5	5	5	5	0	0
Necropsy body wt	144 ± 3	144 ± 2	140 ± 4	115 ± 6**	–	–
Heart						
Absolute	0.55 ± 0.01	0.54 ± 0.01	0.53 ± 0.01	0.47 ± 0.01**	–	–
Relative	3.84 ± 0.07	3.73 ± 0.02	3.79 ± 0.14	4.11 ± 0.17	–	–
L. Kidney						
Absolute	0.61 ± 0.02	0.61 ± 0.01	0.58 ± 0.01	0.52 ± 0.02**	–	–
Relative	4.25 ± 0.06	4.26 ± 0.08	4.12 ± 0.09	4.57 ± 0.10*	–	–
Liver						
Absolute	5.84 ± 0.16	5.10 ± 0.09**	5.08 ± 0.15**	4.29 ± 0.24**	–	–
Relative	40.61 ± 0.46	35.40 ± 0.28**	36.35 ± 0.63**	37.43 ± 0.86**	–	–
Lung						
Absolute	1.14 ± 0.10	1.16 ± 0.08	1.19 ± 0.04	1.28 ± 0.12	–	–
Relative	7.91 ± 0.61	8.07 ± 0.55	8.49 ± 0.30	11.13 ± 0.50**	–	–
L. Testis						
Absolute	0.886 ± 0.040	0.928 ± 0.017	0.852 ± 0.035	0.590 ± 0.088**	–	–
Relative	6.165 ± 0.246	6.446 ± 0.155	6.103 ± 0.248	5.053 ± 0.502	–	–
Thymus						
Absolute	0.374 ± 0.013	0.358 ± 0.025	0.358 ± 0.007	0.284 ± 0.008**	–	–
Relative	2.605 ± 0.054	2.485 ± 0.161	2.560 ± 0.023	2.498 ± 0.112	–	–
Thyroid gland						
Absolute	0.017 ± 0.002	0.019 ± 0.002	0.016 ± 0.001	0.015 ± 0.001	–	–
Relative	0.115 ± 0.014	0.131 ± 0.011	0.115 ± 0.010	0.130 ± 0.008	–	–
Female						
n	5	5	5	5	2	0
Necropsy body wt	112 ± 4	112 ± 2	107 ± 3	98 ± 4**	61 ± 5**	–
Heart						
Absolute	0.42 ± 0.01	0.47 ± 0.01*	0.44 ± 0.01	0.42 ± 0.01	0.34 ± 0.00**	–
Relative	3.75 ± 0.06	4.17 ± 0.07**	4.15 ± 0.06**	4.25 ± 0.10**	5.59 ± 0.41**	–
L. Kidney						
Absolute	0.52 ± 0.02	0.50 ± 0.01	0.50 ± 0.02	0.46 ± 0.01*	0.35 ± 0.00**	–
Relative	4.66 ± 0.11	4.46 ± 0.05	4.63 ± 0.08	4.74 ± 0.12	5.75 ± 0.42**	–
Liver						
Absolute	4.07 ± 0.16	3.77 ± 0.05	3.61 ± 0.13**	3.44 ± 0.05**	2.57 ± 0.06**	–
Relative	36.37 ± 0.49	33.59 ± 0.16	33.78 ± 1.08	35.17 ± 1.00	42.15 ± 2.12**	–

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Lung						
Absolute	0.86 ± 0.04	0.83 ± 0.01	0.91 ± 0.04	1.03 ± 0.06*	1.01 ± 0.04*	–
Relative	7.71 ± 0.36	7.44 ± 0.07	8.49 ± 0.34	10.54 ± 0.69**	16.54 ± 0.56**	–
Thymus						
Absolute	0.317 ± 0.016	0.324 ± 0.011	0.352 ± 0.022	0.289 ± 0.011	0.064 ± 0.016**	–
Relative	2.842 ± 0.167	2.895 ± 0.126	3.289 ± 0.201	2.948 ± 0.092	1.024 ± 0.178**	–
Thyroid gland						
Absolute	0.011 ± 0.002	0.017 ± 0.002	0.019 ± 0.003	0.014 ± 0.003	0.017 ± 0.005	–
Relative	0.099 ± 0.012	0.151 ± 0.018	0.174 ± 0.024	0.140 ± 0.026	0.266 ± 0.054**	–

*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). No data available for 20 mg/m^3 males or 40 mg/m^3 males or females due to 100% mortality.

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

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
n	10	10	10	10	10
Male					
Necropsy body wt	319 ± 5	336 ± 6	327 ± 7	326 ± 6	297 ± 5*
Heart					
Absolute	0.86 ± 0.02	0.89 ± 0.02	0.86 ± 0.02	0.86 ± 0.02	0.81 ± 0.01*
Relative	2.707 ± 0.036	2.650 ± 0.035	2.624 ± 0.040	2.636 ± 0.020	2.717 ± 0.038
R. Kidney					
Absolute	0.95 ± 0.01	1.00 ± 0.02	0.98 ± 0.02	0.99 ± 0.02	0.91 ± 0.02
Relative	2.988 ± 0.037	2.970 ± 0.036	3.014 ± 0.021	3.044 ± 0.041	3.074 ± 0.036
Liver					
Absolute	9.97 ± 0.19	10.89 ± 0.33	10.08 ± 0.31	10.22 ± 0.20	9.07 ± 0.18*
Relative	31.232 ± 0.479	32.362 ± 0.443	30.808 ± 0.467	31.374 ± 0.401	30.596 ± 0.389
Lung					
Absolute	1.63 ± 0.03	1.99 ± 0.05**	2.14 ± 0.08**	2.04 ± 0.06**	2.05 ± 0.05**
Relative	5.129 ± 0.111	5.932 ± 0.146**	6.549 ± 0.174**	6.259 ± 0.115**	6.915 ± 0.138**
R. Testis					
Absolute	1.292 ± 0.014	1.325 ± 0.023	1.311 ± 0.015	1.314 ± 0.016	1.319 ± 0.020
Relative	4.052 ± 0.051	3.949 ± 0.065	4.025 ± 0.061	4.039 ± 0.060	4.462 ± 0.117**
Thymus					
Absolute	0.322 ± 0.024	0.335 ± 0.018	0.328 ± 0.016	0.294 ± 0.019	0.277 ± 0.013
Relative	1.004 ± 0.063	0.995 ± 0.045	1.008 ± 0.053	0.905 ± 0.064	0.935 ± 0.041
Thyroid gland					
Absolute	0.019 ± 0.001	0.019 ± 0.002	0.020 ± 0.002	0.024 ± 0.002	0.021 ± 0.001
Relative	0.060 ± 0.002	0.057 ± 0.005	0.060 ± 0.006	0.072 ± 0.006	0.070 ± 0.004
Female					
Necropsy body wt	201 ± 3	205 ± 4	198 ± 4	199 ± 4	187 ± 3*
Heart					
Absolute	0.64 ± 0.01	0.62 ± 0.01	0.61 ± 0.01	0.63 ± 0.01	0.60 ± 0.01
Relative	3.198 ± 0.071	3.049 ± 0.030	3.090 ± 0.052	3.156 ± 0.042	3.197 ± 0.033
R. Kidney					
Absolute	0.64 ± 0.01	0.63 ± 0.02	0.66 ± 0.02	0.67 ± 0.02	0.67 ± 0.01
Relative	3.184 ± 0.036	3.068 ± 0.082	3.343 ± 0.052	3.349 ± 0.047	3.589 ± 0.055**
Liver					
Absolute	6.06 ± 0.09	6.37 ± 0.18	6.03 ± 0.21	6.06 ± 0.19	5.80 ± 0.12
Relative	30.171 ± 0.262	31.073 ± 0.519	30.400 ± 0.557	30.444 ± 0.511	31.012 ± 0.288

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Lung					
Absolute	1.05 ± 0.02	1.31 ± 0.02**	1.33 ± 0.05**	1.32 ± 0.03**	1.36 ± 0.02**
Relative	5.237 ± 0.087	6.388 ± 0.107**	6.687 ± 0.149**	6.650 ± 0.131**	7.298 ± 0.111**
Thymus					
Absolute	0.347 ± 0.013	0.363 ± 0.011	0.337 ± 0.009	0.354 ± 0.014	0.318 ± 0.009
Relative	1.725 ± 0.053	1.775 ± 0.059	1.715 ± 0.071	1.785 ± 0.069	1.704 ± 0.045
Thyroid gland					
Absolute	0.021 ± 0.001	0.018 ± 0.002	0.019 ± 0.001	0.019 ± 0.001	0.021 ± 0.001
Relative	0.103 ± 0.007	0.090 ± 0.008	0.097 ± 0.006	0.096 ± 0.007	0.109 ± 0.005

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

Table G-3. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Two-week Inhalation Study of Cobalt Metal[a]

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
n	5	5	5	5	5	2
Male						
Necropsy body wt	25.7 ± 0.5	25.0 ± 0.5	25.9 ± 0.3	25.3 ± 0.5	23.4 ± 0.4**	18.9 ± 1.1**
Heart						
Absolute	0.12 ± 0.01	0.13 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.11 ± 0.00	0.11 ± 0.01*
Relative	4.83 ± 0.16	5.14 ± 0.21	4.79 ± 0.12	4.68 ± 0.20	4.79 ± 0.11	5.57 ± 0.05
L. Kidney						
Absolute	0.21 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	0.18 ± 0.02	0.16 ± 0.01**	0.13 ± 0.00**
Relative	8.02 ± 0.20	8.24 ± 0.45	7.64 ± 0.23	7.02 ± 0.52	7.02 ± 0.25	6.92 ± 0.39
Liver						
Absolute	1.13 ± 0.04	0.98 ± 0.04**	0.98 ± 0.04**	0.99 ± 0.02**	0.89 ± 0.02**	0.83 ± 0.01**
Relative	43.88 ± 0.80	39.18 ± 1.35*	37.67 ± 1.09**	39.32 ± 0.91*	37.93 ± 0.51**	44.20 ± 2.99
Lung						
Absolute	0.18 ± 0.01	0.21 ± 0.01	0.23 ± 0.01*	0.24 ± 0.01**	0.29 ± 0.01**	0.36 ± 0.05**
Relative	7.08 ± 0.15	8.33 ± 0.32	8.73 ± 0.33	9.61 ± 0.62*	12.62 ± 0.51**	19.31 ± 3.73**
L. Testis						
Absolute	0.098 ± 0.002	0.104 ± 0.001	0.099 ± 0.004	0.084 ± 0.009	0.089 ± 0.003	0.070 ± 0.002**
Relative	3.834 ± 0.074	4.180 ± 0.114	3.812 ± 0.149	3.322 ± 0.311	3.807 ± 0.088	3.731 ± 0.314
Thymus						
Absolute	0.049 ± 0.004	0.053 ± 0.003	0.046 ± 0.008	0.046 ± 0.004	0.045 ± 0.002	0.025 ± 0.014*
Relative	1.906 ± 0.148	2.137 ± 0.136	1.789 ± 0.300	1.851 ± 0.210	1.910 ± 0.074	1.264 ± 0.646
Thyroid gland						
Absolute	0.004 ± 0.000	0.004 ± 0.001	0.003 ± 0.000	0.003 ± 0.000	0.003 ± 0.001	0.003 ± 0.001
Relative	0.141 ± 0.011	0.146 ± 0.023	0.131 ± 0.015	0.118 ± 0.017	0.144 ± 0.020	0.132 ± 0.019
Female						
Necropsy body wt	20.8 ± 0.1	20.3 ± 0.5	20.1 ± 0.5	20.0 ± 0.6	17.4 ± 0.4**	13.0 ± 1.6**
Heart						
Absolute	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.10 ± 0.00*	0.10 ± 0.01**	0.10 ± 0.01*
Relative	5.39 ± 0.09	5.32 ± 0.10	5.48 ± 0.15	5.02 ± 0.16	5.51 ± 0.23	7.49 ± 1.28**
L. Kidney						
Absolute	0.14 ± 0.00	0.14 ± 0.01	0.14 ± 0.01	0.12 ± 0.00	0.11 ± 0.01**	0.10 ± 0.01**
Relative	6.74 ± 0.16	6.69 ± 0.23	6.86 ± 0.29	6.23 ± 0.23	6.31 ± 0.27	7.74 ± 0.15
Liver						
Absolute	0.93 ± 0.03	0.81 ± 0.02**	0.80 ± 0.03**	0.75 ± 0.03**	0.69 ± 0.03**	0.61 ± 0.06**
Relative	44.56 ± 1.13	40.09 ± 0.31**	39.75 ± 0.82**	37.40 ± 1.12**	39.73 ± 0.70**	46.88 ± 1.36

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Lung						
Absolute	0.19 ± 0.01	0.19 ± 0.00	0.22 ± 0.01*	0.23 ± 0.01**	0.29 ± 0.01**	0.33 ± 0.02**
Relative	9.34 ± 0.38	9.49 ± 0.37	11.14 ± 0.24*	11.77 ± 0.59**	16.80 ± 0.58**	25.67 ± 1.53**
Thymus						
Absolute	0.078 ± 0.000	0.075 ± 0.003	0.074 ± 0.003	0.073 ± 0.004	0.059 ± 0.006**	0.016 ± 0.008**
Relative	3.735 ± 0.024	3.699 ± 0.199	3.658 ± 0.118	3.668 ± 0.182	3.361 ± 0.353	1.144 ± 0.442**
Thyroid gland						
Absolute	0.003 ± 0.001	0.004 ± 0.001	0.003 ± 0.000	0.004 ± 0.000	0.003 ± 0.001	0.002 ± 0.001
Relative	0.126 ± 0.025	0.208 ± 0.037	0.168 ± 0.017	0.202 ± 0.025	0.162 ± 0.036	0.147 ± 0.060

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

Table G-4. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
Male						
n	10	10	10	10	10	10
Necropsy body wt	37.7 ± 0.8	38.2 ± 0.6	37.9 ± 0.8	37.0 ± 0.5	37.0 ± 0.9	32.5 ± 0.5**
Heart						
Absolute	0.16 ± 0.00	0.16 ± 0.00	0.16 ± 0.01	0.16 ± 0.00	0.15 ± 0.00	0.14 ± 0.00**
Relative	4.233 ± 0.095	4.219 ± 0.067	4.224 ± 0.056	4.187 ± 0.063	4.029 ± 0.053	4.385 ± 0.157
R. Kidney						
Absolute	0.31 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.00	0.29 ± 0.01**	0.23 ± 0.01**
Relative	8.360 ± 0.192	8.441 ± 0.122	8.333 ± 0.237	8.507 ± 0.047	7.714 ± 0.145**	7.176 ± 0.131**
Liver						
Absolute	1.48 ± 0.04	1.53 ± 0.04	1.51 ± 0.07	1.49 ± 0.04	1.42 ± 0.05	1.15 ± 0.03**
Relative	39.217 ± 0.586	40.049 ± 0.671	39.753 ± 1.032	40.301 ± 0.698	38.159 ± 0.723	35.457 ± 0.668**
Lung						
Absolute	0.20 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.23 ± 0.01*	0.27 ± 0.01**	0.30 ± 0.01**
Relative	5.416 ± 0.116	6.051 ± 0.235	5.737 ± 0.147	6.234 ± 0.088**	7.436 ± 0.262**	9.142 ± 0.177**
R. Testis						
Absolute	0.118 ± 0.002	0.119 ± 0.002	0.114 ± 0.002	0.114 ± 0.002	0.104 ± 0.003**	0.033 ± 0.001**
Relative	3.136 ± 0.058	3.131 ± 0.037	3.019 ± 0.078	3.073 ± 0.056	2.825 ± 0.082**	1.004 ± 0.025**
Thymus						
Absolute	0.043 ± 0.002	0.046 ± 0.003	0.051 ± 0.003	0.048 ± 0.002	0.048 ± 0.003	0.049 ± 0.002
Relative	1.136 ± 0.054	1.190 ± 0.078	1.348 ± 0.060	1.290 ± 0.057	1.298 ± 0.081	1.507 ± 0.048**
Female						
n	10	10	10	9	10	10
Necropsy body wt	30.9 ± 1.0	31.6 ± 1.1	31.4 ± 0.9	30.1 ± 0.7	29.0 ± 1.1	26.8 ± 1.0**
Heart						
Absolute	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.13 ± 0.00	0.13 ± 0.00*	0.13 ± 0.00**
Relative	4.561 ± 0.124	4.549 ± 0.092	4.472 ± 0.101	4.477 ± 0.082	4.475 ± 0.133	4.753 ± 0.195
R. Kidney						
Absolute	0.21 ± 0.01	0.22 ± 0.00	0.21 ± 0.01	0.20 ± 0.00	0.17 ± 0.00**	0.16 ± 0.00**
Relative	6.887 ± 0.184	6.849 ± 0.155	6.661 ± 0.126	6.689 ± 0.254	6.031 ± 0.132**	6.142 ± 0.185**
Liver						
Absolute	1.46 ± 0.06	1.51 ± 0.07	1.46 ± 0.05	1.30 ± 0.03*	1.16 ± 0.04**	1.01 ± 0.03**
Relative	47.051 ± 0.808	47.552 ± 0.952	46.455 ± 1.046	43.092 ± 0.773**	39.831 ± 0.459**	38.045 ± 1.246**
Lung						
Absolute	0.21 ± 0.01	0.22 ± 0.00	0.23 ± 0.01	0.23 ± 0.01	0.28 ± 0.01**	0.33 ± 0.01**
Relative	6.904 ± 0.227	6.884 ± 0.176	7.300 ± 0.274	7.555 ± 0.184	9.787 ± 0.241**	12.602 ± 0.487**

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
Thymus						
Absolute	0.060 ± 0.004	0.064 ± 0.004	0.068 ± 0.005	0.062 ± 0.003	0.066 ± 0.004	0.064 ± 0.003
Relative	1.960 ± 0.106	2.029 ± 0.100	2.163 ± 0.113	2.084 ± 0.105	2.257 ± 0.110	2.431 ± 0.155**

*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).

Appendix H. Reproductive Tissue Evaluations and Estrous Cycle Characterization

Tables

Figures

Table H-1. Summary of Reproductive Tissue Evaluations for Male Rats in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
n	10	10	10	10
Weights (g)				
Necropsy body wt	319 ± 5	327 ± 7	326 ± 6	297 ± 5*
L. Cauda epididymis	0.1741 ± 0.0054	0.1775 ± 0.0043	0.1853 ± 0.0075	0.1688 ± 0.0038
L. Epididymis	0.4850 ± 0.0095	0.4999 ± 0.0117	0.4926 ± 0.0146	0.4846 ± 0.0116
L. Testis	1.3700 ± 0.0179	1.3680 ± 0.0147	1.3778 ± 0.0205	1.3947 ± 0.0124
Spermatid measurements				
Spermatid heads (10^6/testis)	174.00 ± 10.16	180.00 ± 10.06	176.50 ± 5.81	172.50 ± 5.94
Spermatid heads (10^6/g testis)	141.3 ± 8.7	146.5 ± 8.2	142.5 ± 3.7	139.9 ± 4.8
Epididymal spermatozoal measurements				
Sperm motility (%)	88.8 ± 0.8	86.0 ± 1.1*	83.8 ± 1.3**	81.9 ± 1.3**
Sperm (10^6/cauda epididymis)	104.52 ± 3.78	98.40 ± 3.13	102.27 ± 3.04	94.15 ± 3.18
Sperm (10^6/g cauda epididymis)	602.3 ± 21.6	556.4 ± 19.3	564.1 ± 37.0	559.4 ± 19.6

*Significantly different ($P \leq 0.05$) from the chamber control group by Dunnett's test (body weight) or Shirley's test (sperm motility).
**$P \leq 0.01$.
[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, sperm/cauda epididymis, and sperm/g cauda epididymis).

Table H-2. Estrous Cycle Characterization for Female Rats in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	201 ± 3	198 ± 4	199 ± 4	187 ± 3*
Proportion of regular cycling females[b]	10/10	10/10	10/10	10/10
Estrous cycle length (days)	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.1 ± 0.05
Estrous stages (% of cycle)				
Diestrus	50.0	52.5	45.0	60.8
Proestrus	19.2	19.2	19.2	15.8
Estrus	22.5	21.7	20.8	23.3
Metestrus	8.3	6.7	15.0	0.0

*Significantly different ($P \leq 0.05$) from the chamber control group by Dunnett's test.
[a]Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the chamber control group are not significant by Dunn's test (estrous cycle length). By multivariate analysis of variance, exposed females do not differ significantly from the chamber control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices among all groups and between the chamber control group and each exposed group indicated a significantly higher probability of extended diestrus in the 5 mg/m^3 group compared to the chamber control group.
[b]Number of females with a regular cycle/number of females cycling.

Table H-3. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Rats Exposed to Cobalt Metal by Inhalation for Three Months

Stage	Comparison	P Value	Trend[a]
Overall Tests	Overall	0.01	
Overall Tests	1.25 mg/m^3 vs. chamber controls	0.944	–
Overall Tests	2.5 mg/m^3 vs. chamber controls	0.163	N
Overall Tests	5 mg/m^3 vs. chamber controls	0.001	–
Extended Estrus	Overall	0.407	
Extended Estrus	1.25 mg/m^3 vs. chamber controls	0.601	N
Extended Estrus	2.5 mg/m^3 vs. chamber controls	0.601	N
Extended Estrus	5 mg/m^3 vs. chamber controls	0.128	–
Extended Diestrus	Overall	0.06	
Extended Diestrus	1.25 mg/m^3 vs. chamber controls	0.785	–
Extended Diestrus	2.5 mg/m^3 vs. chamber controls	0.267	N
Extended Diestrus	5 mg/m^3 vs. chamber controls	0.011	–
Extended Metestrus	Overall	1	
Extended Metestrus	1.25 mg/m^3 vs. chamber controls	1	–
Extended Metestrus	2.5 mg/m^3 vs. chamber controls	1	–
Extended Metestrus	5 mg/m^3 vs. chamber controls	1	–
Extended Proestrus	Overall	1	
Extended Proestrus	1.25 mg/m^3 vs. chamber controls	1	–
Extended Proestrus	2.5 mg/m^3 vs. chamber controls	1	–
Extended Proestrus	5 mg/m^3 vs. chamber controls	1	–
Skipped Estrus	Overall	1	
Skipped Estrus	1.25 mg/m^3 vs. chamber controls	1	–
Skipped Estrus	2.5 mg/m^3 vs. chamber controls	1	–
Skipped Estrus	5 mg/m^3 vs. chamber controls	1	–
Skipped Diestrus	Overall	1	
Skipped Diestrus	1.25 mg/m^3 vs. chamber controls	1	–
Skipped Diestrus	2.5 mg/m^3 vs. chamber controls	1	–
Skipped Diestrus	5 mg/m^3 vs. chamber controls	1	–
Summary of Significant Groups			
Overall Tests	5 mg/m^3 vs. chamber controls	0.001	–
Extended Diestrus	5 mg/m^3 vs. Chamber Controls	0.011	–

[a]N means that the exposed group had a lower probability of transitioning to the relevant abnormal state (extended estrus, extended diestrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the chamber control group.

Table H-4. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
n	10	10	10	10
Weights (g)				
Necropsy body wt	37.7 ± 0.8	37.0 ± 0.5	37.0 ± 0.9	32.5 ± 0.5**
L. Cauda epididymis	0.0217 ± 0.0014	0.0210 ± 0.0008	0.0231 ± 0.0018	0.0168 ± 0.0006*
L. Epididymis	0.0603 ± 0.0022	0.0578 ± 0.0019	0.0614 ± 0.0035	0.0429 ± 0.0021**
L. Testis	0.1185 ± 0.0017	0.1132 ± 0.0023	0.1027 ± 0.0036**	0.0316 ± 0.0014**
Spermatid measurements				
Spermatid heads (10^6/testis)	22.34 ± 0.84	22.22 ± 0.65	18.90 ± 1.20*	0.53 ± 0.10**
Spermatid heads (10^6/g testis)	210.84 ± 6.85	227.74 ± 7.16	205.67 ± 7.43	24.27 ± 4.78**
Epididymal spermatozoal measurements				
Sperm motility (%)	86.0 ± 1.1	82.0 ± 0.8*	82.2 ± 1.1*	2.6 ± 1.2**
Sperm (10^6/cauda epididymis)	11.55 ± 0.39	10.53 ± 0.43	9.62 ± 0.49**	0.71 ± 0.06**
Sperm (10^6/g cauda epididymis)	551.1 ± 37.9	505.9 ± 23.3	439.9 ± 40.3*	43.4 ± 3.7**

*Significantly different ($P \leq 0.05$) from the chamber control group by Dunnett's test (cauda epididymis weight) or Shirley's test (spermatid and epididymal spermatozoal measurements).
**Significantly different ($P \leq 0.01$) from the chamber control group by Williams' test (body and tissue weights) or Shirley's test (spermatid and epididymal spermatozoal measurements).
[a]Data are presented as mean ± standard error.

Table H-5. Estrous Cycle Characterization for Female Mice in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
Number weighed at necropsy	10	9	10	10
Necropsy body wt (g)	30.9 ± 1.0	30.1 ± 0.7	29.0 ± 1.1	26.8 ± 1.0**
Proportion of regular cycling females[b]	9/10	9/9	9/10	6/10
Estrous cycle length (days)	4.1 ± 0.05	4.0 ± 0.00	4.1 ± 0.13	4.9 ± 0.36*
Estrous stages (% of cycle)				
Diestrus	28.3	25.9	29.2	30.0
Proestrus	0.0	0.0	0.0	0.0
Estrus	47.5	50.0	48.3	49.2
Metestrus	24.2	24.1	22.5	20.8

*Significantly different ($P \leq 0.05$) from the chamber control group by Dunn's test.
**Significantly different ($P \leq 0.01$) from the chamber control group by Williams' test.
[a]Necropsy body weights and estrous cycle length data are presented as mean ± standard error. By multivariate analysis of variance, exposed females do not differ significantly from the chamber control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices among all groups and between the chamber control group and each exposed group indicated no significant differences in estrous cyclicity of the exposed and chamber control groups.
[b]Number of females with a regular cycle/number of females cycling.

Table H-6. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Mice Exposed to Cobalt Metal by Inhalation for Three Months

Stage	Comparison	P Value	Trend[a]
Overall Tests	Overall	<0.001	
Overall Tests	2.5 mg/m^3 vs. chamber controls	0.627	N
Overall Tests	5 mg/m^3 vs. chamber controls	0.107	–
Overall Tests	10 mg/m^3 vs. chamber controls	<0.001	–
Extended Estrus	Overall	0.932	
Extended Estrus	2.5 mg/m^3 vs. chamber controls	1	–
Extended Estrus	5 mg/m^3 vs. chamber controls	0.596	–
Extended Estrus	10 mg/m^3 vs. chamber controls	0.663	–
Extended Diestrus	Overall	0.812	
Extended Diestrus	2.5 mg/m^3 vs. chamber controls	0.627	N
Extended Diestrus	5 mg/m^3 vs. chamber controls	1	N
Extended Diestrus	10 mg/m^3 vs. chamber controls	0.361	–
Extended Metestrus	Overall	1	
Extended Metestrus	2.5 mg/m^3 vs. chamber controls	1	–
Extended Metestrus	5 mg/m^3 vs. chamber controls	1	–
Extended Metestrus	10 mg/m^3 vs. chamber controls	1	–
Extended Proestrus	Overall	1	
Extended Proestrus	2.5 mg/m^3 vs. chamber controls	1	–
Extended Proestrus	5 mg/m^3 vs. chamber controls	1	–
Extended Proestrus	10 mg/m^3 vs. chamber controls	1	–
Skipped Estrus	Overall	1	
Skipped Estrus	2.5 mg/m^3 vs. chamber controls	1	–
Skipped Estrus	5 mg/m^3 vs. chamber controls	1	–
Skipped Estrus	10 mg/m^3 vs. chamber controls	1	–
Skipped Diestrus	Overall	1	
Skipped Diestrus	2.5 mg/m^3 vs. chamber controls	1	–
Skipped Diestrus	5 mg/m^3 vs. chamber controls	1	–
Skipped Diestrus	10 mg/m^3 vs. chamber controls	1	–
Summary of Significant Groups			
Overall Tests	10 mg/m^3 vs. Chamber Controls	<0.001	

[a]N means that the exposed group had a lower probability of transitioning to the relevant abnormal state (extended estrus, extended diestrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the chamber control group.

Dose (mg/m^3)																		
0						E	M	D	D	P	E	M	D	D	E	E	M	
0						E	D	D	D	P	E	D	D	D	P	E	D	
0			D	D	P	E	M	D	D	P	E	D	D	D				
0			D	D	P	E	M	D	D	P	E	D	D	D				
0					P	E	M	D	D	P	E	M	D	D	P	E		
0						E	M	D	D	P	E	D	D	D	P	E	M	
0				D	P	E	D	D	D	P	E	M	D	D	P			
0					P	E	D	D	D	P	E	D	D	D	P	E		
0		D	D	D	P	E	D	D	D	P	E	D	D					
0					P	E	D	D	D	P	E	D	D	D	P	E		
1.25			D	D	P	E	D	D	D	P	E	D	D	D				
1.25					P	E	M	D	D	P	E	M	D	D	P	E		
1.25						E	D	D	D	P	E	D	D	D	P	E	D	
1.25						E	D	D	D	P	E	D	D	D	P	E	D	
1.25			D	D	P	E	D	D	D	P	E	D	D	D				
1.25					P	E	M	D	D	P	E	D	D	D	P	E		
1.25					P	E	D	D	D	P	E	D	D	D	P	E		
1.25						E	D	D	D	P	E	D	D	D	P	E	D	
1.25		D	D	D	P	E	M	D	D	P	E	M	D					
1.25		M	D	D	P	E	M	D	D	P	E	M	D					
2.5		D	D	D	P	E	D	D	D	P	E	M	D					
2.5						E	M	D	D	P	E	D	D	D	P	E	M	
2.5						E	D	D	D	P	E	D	D	D	P	E	D	
2.5						E	M	D	D	P	E	M	D	D	P	E	M	
2.5					P	E	M	D	D	P	E	M	D	D	P	E		
2.5				D	P	E	M	D	D	P	E	M	D	D	P			
2.5		M	D	D	P	E	M	D	D	P	E	M	D					
2.5		M	D	D	P	E	M	D	D	P	E	M	D					
2.5						E	M	D	D	P	E	D	D	D	P	E	D	
2.5				D	P	E	M	D	D	P	E	D	D	D	P			
5.0					P	E	D	D	D	P	E	D	D	D	E	E		
5.0						E	D	D	D	P	E	D	D	D	E	E	D	
5.0			D	D	P	E	D	D	D	P	E	D	D	D				
5.0				D	P	E	D	D	D	P	E	D	D	D	P			
5.0			D	D	D	P	E	D	D	D	E	E	D	D				
5.0		D	D	D	P	E	D	D	D	P	E	D	D					
5.0		D	D	D	P	E	D	D	D	P	E	D	D					
5.0						E	D	D	D	P	E	D	D	D	P	E	D	
5.0						E	D	D	D	P	E	D	D	D	P	E	D	
5.0					E	D	D	D	D	P	E	D	D	D	P	E		

Figure H-1. Vaginal Cytology Plots for Female Rats in the Three-month Inhalation Study of Cobalt Metal

D = diestrus, P = proestrus, E = estrus, M = metestrus.

Dose (mg/m^3)																				
0					M	D	E	E	M	D	E	E	M	D	E	E				
0							E	E	M	D	E	E	M	D	E	E	M	D		
0						E	E	M	D	D	E	E	M	D	E	E	M			
0					M	D	E	E	M	D	E	E	M	D	E	E				
0								E	M	D	E	E	M	D	D	D	D	D	D	
0							E	E	M	D	E	E	M	D	E	E	M	D		
0						D	E	E	M	D	E	E	M	D	E	E	M			
0							E	E	M	D	E	E	M	D	E	E	M	D		
0					M	D	E	E	M	D	E	E	M	D	E	E				
0							E	E	M	D	E	E	M	D	E	E	M	D		
2.5					M	D	E	E	M	D	E	E	M	D	E	E				
2.5						D	E	E	M	D	E	E	M	D	E	E	M			
2.5					M	D	E	E	M	D	E	E	M	D	E	E				
2.5							E	E	M	D	E	E	M	D	E	E	M	D		
2.5						D	E	E	M	D	E	E	M	D	E	E	M			
2.5							E	E	D	D	E	E	M	D	E	E	M	D		
2.5							E	E	M	D	E	E	M	D	E	E	M	D		
2.5							E	E	M	D	E	E	M	D	E	E	M	D		
2.5					M	D	E	E	M	D	E	E	M	D	E	E				
5.0								E	M	D	E	E	M	D	E	E	M	D	E	
5.0								E	M	D	E	E	M	D	E	E	M	D	E	
5.0				M	D	E	E	M	D	D	E	E	M	D	D					
5.0					D	D	E	E	M	D	E	E	M	D	E	E				
5.0								E	M	D	E	E	M	D	E	E	M	D	E	
5.0							E	E	M	D	E	E	M	D	E	E	M	D		
5.0							E	E	M	D	E	E	M	D	E	E	M	D		
5.0							E	E	M	D	E	E	E	M	D	E	E	E		
5.0						D	E	E	M	D	E	E	M	D	D	D	D			
5.0							E	E	M	D	E	E	M	D	E	E	M	D		
10.0							E	E	D	D	E	E	M	D	E	E	M	D		
10.0						E	E	M	D	D	E	E	M	D	E	E	M			
10.0					D	E	E	M	D	D	E	E	M	D	E	E				
10.0							E	E	M	D	E	E	E	M	D	E	E	M		
10.0								E	M	D	E	E	E	M	D	E	E	M	D	
10.0			D	E	E	M	D	D	D	D	E	E	M	D						
10.0						D	E	E	M	D	E	E	M	D	E	E	M			
10.0		D	D	E	E	M	D	D	D	D	E	E	M							
10.0					M	D	E	E	M	D	E	E	E	E	M	D				
10.0					E	E	E	E	M	D	E	E	M	D	E	E				

Figure H-2. Vaginal Cytology Plots for Female Mice in the Three-month Inhalation Study of Cobalt Metal

D = diestrus, E = estrus, M = metestrus.

Appendix I. Tissue Burden Results

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I.1. Lung Deposition and Clearance Equations Used in the Two-week Inhalation Studies of Cobalt Metal

Lung clearance rates were calculated using postexposure data and Equation (1):

Equation (1): $A_{(t)} = A_0(e^{-kt})$

where $A_{(t)}$ is the lung burden (µg cobalt) at time t (t = 21 days postexposure), A_0 is the lung burden at t = 0 days postexposure, and k is the lung clearance rate constant (fraction cleared per day).

Lung clearance half-lives in days ($t_{½}$) were calculated from Equation (2), where ln2 is the Napierian logarithm of 2:

Equation (2): $t_{1/2} = \ln 2/k$

Deposition rates were calculated from lung cobalt burdens using Equation (3). The lung cobalt burden and time at terminal kill and the calculated lung clearance rate constant were used to solve for the deposition rate α (µg/day).

Equation (3): $A_{(t)} = (\alpha/k)(1-e^{-kt})$

In Equation (3), $A_{(t)}$ is the lung burden (µg cobalt) at time t [t = 16 (rats) or 17 (mice) days on study]; α is the amount of cobalt deposited (µg/day); and k is the first-order clearance rate constant derived from Equation (1). Steady-state or equilibrium lung burdens (A_e, µg cobalt) were calculated according to Equation (4):

Equation (4): $A_e = \alpha/k$

Blood elimination rates and half-lives were calculated as described for lung cobalt burden, except blood cobalt concentration data were used.

I.2. Lung Deposition and Clearance Equations Used in the Three-month and Two-year Inhalation Studies of Cobalt Metal

Lung burdens during the recovery period initially decreased rapidly, followed by a slower clearance phase. The pattern of decrease fit a two-compartment, biexponential clearance model of the form shown in Equation (5):

Equation (5): $L_{(t)} = Ae^{-at} + Be^{-bt}$

where $L_{(t)}$ is the retained lung burden (µg cobalt/lung) at any recovery time point t (days); A and B are the lung burdens (µg cobalt/lung) at t = 0 days after exposure was terminated postexposure in the rapid and slow clearance compartments, respectively; and a and b are the lung clearance rate constants (in $days^{-1}$) in the rapid and slow clearance compartments, respectively. This model was fitted to lung cobalt burdens normalized to exposure concentrations at 7, 14, 28, and 42 days after termination of exposures in the animals sampled. An exposure concentration term was included in Equation (5) to account for this normalization, and model output parameters A and B were subsequently converted to appropriate values by multiplying by exposure concentration.

Half-lives for the rapid and slow clearance phases were calculated using Equation (6):

Equation (6): $t_{½\ (rapid\ phase)} = ln2/a$ or $t_{½\ (slow\ phase)} = ln2/b$

where $t_{½}$ is the rapid or slow phase lung clearance half-life (in days), ln2 is the Napierian logarithm of 2, and a and b are the rapid phase and slow phase clearance rate constants, respectively, defined in Equation (5).

Given the nature of lung clearance observed with the recovery data, it was expected that a reasonable model for lung cobalt burden during exposure would incorporate a constant deposition rate and a two-compartment, biexponential clearance rate. Attempts were made to fit such a model to lung burden data collected during exposure. However, attempts to fit this model to the data were unsuccessful. The model fit to the data was poor, and standard errors for the parameter estimates were excessively large.

The inability of the two-compartment model to fit these data was possibly due to the fact that the data were inadequate to fully define the lung cobalt burden versus time curve, especially during the early part of the study. The rapid lung clearance half-lives calculated from the recovery period were on the order of 1 to 3 days. In examining plots of lung burden versus time during exposure, it was clear that lung burdens rapidly approached steady state. Within the first 5 days of exposure, lung burdens increased rapidly and were already approaching steady-state values. This was consistent with the rapid clearance half-life observed during the recovery period. Generally, after the first 5 to 40 days of exposure, lung burden values continued to increase slowly for the remainder of the exposure period as they asymptotically approached steady state over time. However, given that no data were collected until 5 days of exposure, there were insufficient data to define the early part of the lung burden versus the time curve during this period when lung burdens were rapidly changing. Thus, a possible explanation for the inability to fit the two-compartment model to lung burden data during exposure was the lack of data during the very early part of the study (<5 days).

However, a model that assumes a constant cobalt deposition rate and a one-compartment, monoexponential clearance rate provided a reasonable fit to the data collected during exposure. This model is defined by Equations (7), (8), and (9):

Equation (7): $L_{(t)} = (D/k)(1–e^{–kt})$

Equation (8): $t_{½} = ln2/k$

Equation (9): $L_{ss} = D/k$

where $L_{(t)}$ is the retained lung burden (µg cobalt/lung) at any time t (days) during exposure; D is the deposition rate of cobalt in the lungs (µg cobalt/lung per day); k is the lung clearance rate constant ($days^{–1}$); $t_{½}$ is the clearance half-life in days; ln2 is the Napierian logarithm of 2; and L_{ss} is the predicted steady-state lung burden (µg cobalt/lung).

This one-compartment model does not adequately define the two-phase lung clearance process that was seen during the recovery period. This model predicts only one clearance half-life, and it is expected that this half-life would be intermediate between the true rapid and slow clearance half-lives. However, it was found that this model did generally fit the data adequately to provide a reasonably precise estimate for the cobalt deposition rate, which is important in predicting the

dose of cobalt to the lungs arising from the inhalation process. While this model does not adequately describe the true clearance process, it provides reasonable predictive power for determining the lung dose. The two-compartment model provides an adequate description of the lung clearance process during the recovery period. Thus, between the two models used for data collected during and after exposure, reasonable estimates of both deposition and clearance parameters were obtained.

Because the 3-month study postexposure lung cobalt burden data fit the model described by Equation (5) reasonably well, the model chosen for the 2-year studies also included both rapid and slow lung clearance phases. However, lung burden data collected during the 2-year studies were all collected during the in-life parts of the studies because there was not a recovery period. Accordingly, the model used for the 2-year studies had to account for deposition rates as well as clearance rates, because both deposition and clearance were occurring during the in-life part of the study.

The model used for the 2-year studies assumed zero-order (constant) deposition and first-order (with respect to lung burden) clearance rates and included rapid and slow clearance phases. This model is described by Equation (10):

Equation (10): $L_{(t)} = A/k_a(1–e^{–k_a t}) + B/k_b(1–e^{–k_b t})$

Equation (10) is essentially the sum of two processes that incorporate zero-order deposition and first-order clearance. A and B represent the deposition rates (µg cobalt/day) in the rapid and slow phases, respectively, and k_a and k_b represent the lung clearance rate constants ($days^{–1}$) in the rapid and slow clearance phases, respectively.

This model was fit to the lung cobalt burden data collected during the in-life parts of the 3-month and 2-year studies using data collected in both studies from the 1.25, 2.5, and 5 mg/m^3 exposure groups. The model was fit to the data from each exposure group using SAS PROC NLIN (SAS Institute Inc., Cary, NC). This fit provided direct estimates of A, k_a, B, and k_b along with their asymptotic standard errors. These values were used to calculate the following quantities along with their approximate standard errors using propagation of error techniques:

- Fraction of deposition in the slow clearance phase: $F_B = B/(A + B)$
- Half-life of the rapid clearance phase: $t_{½a} = \ln 2/k_a$ (days)
- Half-life of the slow clearance phase: $t_{½b} = \ln 2/k_b$ (days)
- Theoretical steady-state lung burden for the rapid phase: $L_{SSa} = A/k_a$ (µg cobalt/lung)
- Theoretical steady-state lung burden for the slow phase: $L_{SSb} = B/k_b$ (µg cobalt/lung)

Due to the potential for more uncertainty with relatively higher lung cobalt burdens, several weighting schemes were investigated, including unweighted, 1/mean, and 1/variance. Review of the results suggested that 1/mean was the best choice, so all results presented are from model fits using 1/mean weighting.

Table I-1. Tissue Weights, Cobalt Concentrations, and Cobalt Burdens for Rats in the Two-week Inhalation Study of Cobalt Metal[a]

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3
Male					
n	5	5	5	5	0[b]
Blood					
µg Co/g blood					
Day 16	0	0.14 ± 0.01	0.27 ± 0.02	0.45 ± 0.02	–
Serum					
µg Co/g serum					
Day 16	0	0.28 ± 0.01	0.48 ± 0.02	0.72 ± 0.04	–
Right femur					
Absolute right femur wt (g)					
Day 16	0.142 ± 0.008	0.143 ± 0.004	0.135 ± 0.003	0.116 ± 0.005*	–
µg Co/g right femur					
Day 16	0	0.58 ± 0.04	1.08 ± 0.09	2.24 ± 0.13	–
µg Co/right femur					
Day 16	0	0.082 ± 0.006	0.146 ± 0.014	0.253 ± 0.009	–
µg Co/right femur per mg Co/m^3					
Day 16	NA	0.033 ± 0.002	0.029 ± 0.003	0.025 ± 0.001	–
Heart					
Absolute heart wt (g)					
Day 16	0.551 ± 0.013	0.540 ± 0.007	0.527 ± 0.014	0.468 ± 0.014**	–
µg Co/g heart					
Day 16	0	0.48 ± 0.03	0.73 ± 0.06	1.07 ± 0.06	–
µg Co/heart					
Day 16	0	0.258 ± 0.014	0.384 ± 0.034	0.498 ± 0.024	–
µg Co/heart per mg Co/m^3					
Day 16	NA	0.103 ± 0.006	0.077 ± 0.007	0.050 ± 0.002	–
Right kidney					
Absolute right kidney wt (g)					
Day 16	0.586 ± 0.017	0.579 ± 0.008	0.566 ± 0.016	0.519 ± 0.018	–
µg Co/g right kidney					
Day 16	0	2.04 ± 0.02	3.26 ± 0.18	5.90 ± 0.38	–
µg Co/right kidney					
Day 16	0	1.177 ± 0.018	1.838 ± 0.116	3.031 ± 0.146	–

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3
µg Co/right kidney per mg Co/m^3					
Day 16	NA	0.471 ± 0.007	0.368 ± 0.023	0.303 ± 0.015	–
Liver					
Absolute liver wt (g)					
Day 16	5.834 ± 0.164	5.102 ± 0.094*	5.081 ± 0.156*	4.292 ± 0.238**	–
µg Co/g liver					
Day 16	0	2.69 ± 0.09	5.14 ± 0.46	14.00 ± 1.58	–
µg Co/liver					
Day 16	0	13.638 ± 0.335	25.956 ± 2.189	58.632 ± 4.098	–
µg Co/liver per mg Co/m^3					
Day 16	NA	5.455 ± 0.134	5.191 ± 0.438	5.863 ± 0.410	–
Total lung[c]					
Absolute total lung wt (g)					
Day 16	1.140 ± 0.096	1.167 ± 0.085	1.186 ± 0.043	1.285 ± 0.116	–
µg Co/total lung					
Day 16	0	10.806 ± 0.619	20.077 ± 0.675	23.518 ± 1.647	–
µg Co/total lung per mg Co/m^3					
Day 16	NA	4.322 ± 0.247	4.015 ± 0.135	2.352 ± 0.165	–
Right lung[d]					
Absolute right lung wt (g)					
Day 16	0.573 ± 0.047	0.570 ± 0.055	0.578 ± 0.020	0.636 ± 0.063	–
µg Co/g right lung					
Day 16	0	9.53 ± 1.07	16.95 ± 0.38	18.44 ± 0.70	–
Right testis					
Absolute right testis wt (g)					
Day 16	0.850 ± 0.040	0.870 ± 0.016	0.831 ± 0.033	0.563 ± 0.089	–
µg Co/g right testis					
Day 16	0	0.09 ± 0.00	0.20 ± 0.02	0.48 ± 0.06	–
µg Co/right testis					
Day 16	0	0.086 ± 0.003	0.170 ± 0.014	0.245 ± 0.011	–
µg Co/right testis per mg Co/m^3					
Day 16	NA	0.034 ± 0.001	0.034 ± 0.003	0.024 ± 0.001	–
Female					
n					

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3
Day 16	5	5	5	5	2
Week 3 PE	3	3	3	3	1[e]
Blood					
µg Co/g blood					
Day 16	0[f]	0.13 ± 0.01[f]	0.25 ± 0.02[f]	0.48 ± 0.02[f]	1.06 ± 0.17[g]
Week 3 PE	0	0.02 ± 0.00	0.06 ± 0.01	0.12 ± 0.01	0.22
Serum					
µg Co/g serum					
Day 16	0[f]	0.25 ± 0.01[f]	0.44 ± 0.04[f]	0.76 ± 0.04[f]	1.88 ± 0.21[g]
Week 3 PE	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01
Right femur					
Absolute right femur wt (g)					
Day 16	0.105 ± 0.006	0.115 ± 0.004	0.108 ± 0.003	0.100 ± 0.006	0.070 ± 0.005
µg Co/g right femur					
Day 16	0	0.69 ± 0.06	1.30 ± 0.11	2.40 ± 0.24	6.09 ± 0.72
µg Co/right femur					
Day 16	0	0.080 ± 0.005	0.139 ± 0.007	0.235 ± 0.012	0.418 ± 0.018
µg Co/right femur per mg Co/m^3					
Day 16	NA	0.032 ± 0.002	0.028 ± 0.001	0.023 ± 0.001	0.021 ± 0.001
Heart					
Absolute heart wt (g)					
Day 16	0.420 ± 0.013	0.469 ± 0.013	0.445 ± 0.013	0.416 ± 0.006	0.341 ± 0.004
µg Co/g heart					
Day 16	0	0.44 ± 0.03	0.76 ± 0.04	1.03 ± 0.03	1.81 ± 0.19
µg Co/heart					
Day 16	0	0.206 ± 0.008	0.339 ± 0.015	0.427 ± 0.014	0.613 ± 0.068
µg Co/heart per mg Co/m^3					
Day 16	NA	0.083 ± 0.003	0.068 ± 0.003	0.043 ± 0.001	0.031 ± 0.003
Right kidney					
Absolute right kidney wt (g)					
Day 16	0.499 ± 0.017	0.477 ± 0.008	0.465 ± 0.017	0.448 ± 0.008*	0.356 ± 0.003**
µg Co/g right kidney					
Day 16	0	1.78 ± 0.07	3.01 ± 0.21	5.74 ± 0.23	13.87 ± 0.51
µg Co/right kidney					
Day 16	0	0.844 ± 0.025	1.382 ± 0.059	2.556 ± 0.111	4.896 ± 0.145

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3
µg Co/right kidney per mg Co/m^3					
Day 16	NA	0.338 ± 0.010	0.276 ± 0.012	0.256 ± 0.011	0.245 ± 0.007
Liver					
Absolute liver wt (g)					
Day 16	4.073 ± 0.163	3.768 ± 0.046	3.613 ± 0.125*	3.440 ± 0.049**	2.567 ± 0.061**
µg Co/g liver					
Day 16	0	2.15 ± 0.13	4.81 ± 0.43	15.93 ± 1.59	59.02 ± 9.05
µg Co/liver					
Day 16	0	8.064 ± 0.403	17.223 ± 1.292	54.542 ± 5.203	150.785 ± 19.614
µg Co/liver per mg Co/m^3					
Day 16	NA	3.226 ± 0.161	3.445 ± 0.258	5.454 ± 0.520	7.539 ± 0.981
Total lung[c]					
Absolute total lung wt (g)					
Day 16	0.850 ± 0.032[f]	0.891 ± 0.051[f]	0.943 ± 0.034[f]	1.004 ± 0.042*[f]	0.996 ± 0.026*[g]
Week 3 PE	0.90 ± 0.02	0.96 ± 0.07	1.24 ± 0.10*	1.17 ± 0.04*	1.41
µg Co/total lung					
Day 16	0[f]	9.713 ± 0.431[f]	17.289 ± 0.805[f]	20.710 ± 1.070[f]	49.842 ± 11.212[g]
Week 3 PE	0	0.605 ± 0.011	1.288 ± 0.113	1.278 ± 0.112	1.514
µg Co/total lung per mg Co/m^3					
Day 16	NA[f]	3.885 ± 0.173[f]	3.458 ± 0.161[f]	2.071 ± 0.107[f]	2.492 ± 0.561[g]
Week 3 PE	NA	0.242 ± 0.004	0.258 ± 0.023	0.128 ± 0.011	0.076
Left lung[h]					
Absolute left lung wt (g)					
Day 16	0.237 ± 0.048[i]	0.333 ± 0.061[i]	0.299 ± 0.009[i]	0.275 ± 0.003[i]	0.299[e]
Week 3 PE	0.24 ± 0.01	0.28 ± 0.02	0.38 ± 0.04**	0.35 ± 0.00**	0.40
µg Co/g left lung					
Day 16	0[i]	9.89 ± 2.35[i]	19.04 ± 0.32[i]	21.87 ± 2.07[i]	35.68[e]
Week 3 PE	0	0.62 ± 0.01	0.93 ± 0.17	1.09 ± 0.10	0.96
µg Co/left lung					
Day 16	0[i]	3.150 ± 0.178[i]	5.698 ± 0.267[i]	6.000 ± 0.514[i]	10.669[e]
Week 3 PE	0	0.171 ± 0.011	0.335 ± 0.027	0.377 ± 0.032	0.386
µg Co/left lung per mg Co/m^3					
Day 16	NA[i]	1.260 ± 0.071[i]	1.140 ± 0.053[i]	0.600 ± 0.051[i]	0.533[e]
Week 3 PE	NA	0.069 ± 0.004	0.067 ± 0.005	0.038 ± 0.003	0.019
Right lung[d]					

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3
Absolute right lung wt (g)					
Day 16	0.404 ± 0.018[f]	0.446 ± 0.036[f]	0.471 ± 0.025[f]	0.519 ± 0.022**[f]	0.539 ± 0.018**[g]
Week 3 PE	0.44 ± 0.01	0.48 ± 0.03	0.63 ± 0.05**	0.60 ± 0.01**	0.77
µg Co/g right lung					
Day 16	0[f]	11.06 ± 0.66[f]	18.43 ± 0.92[f]	20.82 ± 1.31[f]	49.55 ± 9.81[f]
Week 3 PE	0	0.64 ± 0.04	1.06 ± 0.16	1.09 ± 0.10	1.07
µg Co/right lung					
Day 16	0[i]	5.501 ± 0.323[i]	10.283 ± 0.593[i]	11.304 ± 1.402[i]	17.765[e]
Week 3 PE	0	0.302 ± 0.006	0.652 ± 0.057	0.661 ± 0.059	0.823
µg Co/right lung per mg Co/m^3					
Day 16	NA[i]	2.200 ± 0.129[i]	2.057 ± 0.119[i]	1.130 ± 0.140[i]	0.888[e]
Week 3 PE	NA	0.121 ± 0.002	0.130 ± 0.011	0.066 ± 0.006	0.041

*Significantly different ($P \leq 0.05$) from the chamber control group by Williams' or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. All values except absolute tissue weights are control corrected. Statistical tests were performed only on absolute tissue weight data. All 40 mg/m^3 rats died before the end of the study. NA = not applicable; PE = postexposure.
[b]All 20 mg/m^3 male rats died before the end of the study; no data are available for this group.
[c]Total lung cobalt burden was calculated using the weight of the total lung with mainstem bronchi at collection and cobalt concentration measured in the right lung.
[d]Right lung cobalt burden was calculated using the right lung weight and the concentration of cobalt measured in the right lung.
[e]n = 1; No standard error was calculated; less than two measurements were available.
[f]n = 7.
[g]n = 3.
[h]Left lung cobalt burden was calculated using the left lung weight and the concentration of cobalt measured in the left lung.
[i]n = 2.

Table I-2. Deposition and Clearance Parameter Estimates for Female Rats in the Two-week Inhalation Study of Cobalt Metal[a]

Tissue	Exposure Concentration (mg/m^3)	k (days^{-1})	$t_{1/2}$(days)	α (µg Co/day)	A_e (µg Co)
Blood	2.5	0.07	9.45	NA	NA
	5	0.06	11.09	NA	NA
	10	0.07	10.35	NA	NA
	20	0.08	9.15	NA	NA
Serum	2.5	–[b]	NA	NA	NA
	5	–	NA	NA	NA
	10	0.21	3.35	NA	NA
	20	0.25	2.77	NA	NA
Lung[c]	2.5	0.13	5.24	1.46	11.06
	5	0.12	5.61	2.48	20.07
	10	0.13	5.23	3.12	23.54
	20	0.17	4.17	8.91	53.59

[a]Statistical analyses of these data were not performed due to the limited number of time points and data points within some time points. k = first-order clearance rate constant; $t_{½}$ = clearance half-life; α=lung deposition rate; A_e = steady-state lung burden; NA = not applicable.
[b]Parameters not calculated when average concentration for a time point is zero.
[c]Total lung cobalt burden was calculated using the weight of the total lung with mainstem bronchi at collection and cobalt concentration measured in the right lung.

Table I-3. Urine Volume, Creatinine and Cobalt Concentrations, and Cobalt Burdens for Rats on Day 12 in the Two-week Inhalation Study of Cobalt Metal[a]

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3
Male					
n	5	5	5	5	1[b]
Volume (mL/16 hours)	19.54 ± 2.70	17.88 ± 1.06	17.42 ± 0.43	5.88 ± 1.47**	0.40
Creatinine (mg/dL)	13.0 ± 1.8	13.0 ± 0.8	13.4 ± 1.0	23.4 ± 2.0**	46.1
µg Co/mL (control corrected)	NA	3.23 ± 0.33	5.98 ± 0.21	19.61 ± 2.97	71.27
µg Co/mg creatinine	0.2 ± 0.1	25.0 ± 1.8**	45.4 ± 3.0**	81.8 ± 6.6**	154.6
µg Co/16 hours (control corrected)	NA	56.87 ± 4.24	104.13 ± 4.58	100.18 ± 8.95	28.51
µg Co/16 hours per mg Co/m^3 (control corrected)	NA	22.721 ± 1.691	20.817 ± 0.918	9.990 ± 0.897	1.407
Female					
n	5	5	5	5	2
Volume (mL/16 hours)	15.74 ± 3.09	17.90 ± 2.68	10.98 ± 1.89	5.62 ± 2.23	0.25 ± 0.05*
Creatinine (mg/dL)	12.9 ± 2.1	11.4 ± 1.3	16.8 ± 2.7	25.0 ± 4.3	50.4 ± 24.2*
µg Co/mL (control corrected)	NA	2.51 ± 0.22	6.76 ± 1.10	20.21 ± 4.72	70.27 ± 22.35
µg Co/mg creatinine	0.4 ± 0.1	22.7 ± 1.1**	40.8 ± 2.5**	76.9 ± 6.6**	153.6 ± 29.4**
µg Co/16 hours (control corrected)	NA	42.71 ± 1.86	66.22 ± 3.06	77.85 ± 5.27	18.68 ± 9.10
µg Co/16 hours per mg Co/m^3 (control corrected)	NA	17.111 ± 0.785	13.213 ± 0.621	7.749 ± 0.536	0.906 ± 0.455

*Significantly different ($P \leq 0.05$) from the chamber control group by Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Statistical tests were performed only on data that were not control corrected. All 40 mg/m^3 rats died before day 12; no data are available for this group. NA = not applicable.
[b]No standard error was calculated for data in this exposed group; less than two measurements were available.

Table I-4. Tissue Weights, Cobalt Concentrations, and Cobalt Burdens for Female Rats in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
n	3	3	3	3	3
Blood					
µg Co/g blood					
Day 5	0.004 ± 0.000	0.066 ± 0.006*	0.109 ± 0.021**	0.344 ± 0.071**	0.745 ± 0.116**
Day 12	0.004 ± 0.000	0.065 ± 0.006*	0.109 ± 0.014**	0.235 ± 0.035**	0.512 ± 0.009**
Day 26	0.004 ± 0.000	0.060 ± 0.002*	0.096 ± 0.011**	0.230 ± 0.003**	0.520 ± 0.036**
Day 40	0.004 ± 0.000	0.053 ± 0.014*	0.115 ± 0.020**	0.199 ± 0.026**	0.479 ± 0.046**
Day 61	0.004 ± 0.000	0.047 ± 0.011*	0.116 ± 0.010**	0.213 ± 0.013**	0.440 ± 0.027**
Day 89	0.004 ± 0.000	0.037 ± 0.012*	0.097 ± 0.013**	0.164 ± 0.024**	0.401 ± 0.012**
PE day 7	0.004 ± 0.000	0.025 ± 0.000*	0.025 ± 0.000*	0.048 ± 0.012**	0.142 ± 0.013**
PE day 14	0.004 ± 0.000	0.018 ± 0.007	0.025 ± 0.000*	0.038 ± 0.013**	0.119 ± 0.010**
PE day 28	0.004 ± 0.000	0.011 ± 0.007	0.025 ± 0.000*	0.025 ± 0.000*	0.067 ± 0.003**
PE day 42	0.004 ± 0.000	0.004 ± 0.000	0.018 ± 0.007	0.025 ± 0.000*	0.025 ± 0.000*
µg Co/g blood per mg Co/m^3					
Day 5	–	0.106 ± 0.009	0.087 ± 0.017	0.138 ± 0.029	0.149 ± 0.023
Day 12	–	0.104 ± 0.010	0.087 ± 0.011	0.094 ± 0.014	0.102 ± 0.002
Day 26	–	0.095 ± 0.004	0.077 ± 0.009	0.092 ± 0.001	0.104 ± 0.007
Day 40	–	0.085 ± 0.022	0.092 ± 0.016	0.079 ± 0.011	0.096 ± 0.009
Day 61	–	0.076 ± 0.018	0.093 ± 0.008	0.085 ± 0.005	0.088 ± 0.005
Day 89	–	0.059 ± 0.019	0.078 ± 0.010	0.066 ± 0.009	0.080 ± 0.002
PE day 7	–	0.040 ± 0.000	0.020 ± 0.000	0.019 ± 0.005	0.028 ± 0.003
PE day 14	–	0.029 ± 0.011	0.020 ± 0.000	0.015 ± 0.005	0.024 ± 0.002
PE day 28	–	0.017 ± 0.011	0.020 ± 0.000	0.010 ± 0.000	0.013 ± 0.001
PE day 42	–	0.006 ± 0.000	0.014 ± 0.006	0.010 ± 0.000	0.005 ± 0.000
Liver					
Absolute liver wt (g)					
Day 26	5.601 ± 0.170	4.917 ± 0.119	5.342 ± 0.163	5.324 ± 0.111	4.188 ± 0.281*
Day 40	5.810 ± 0.134	5.261 ± 0.039	5.689 ± 0.388	5.843 ± 0.320	5.194 ± 0.358
µg Co/g liver					
Day 26	0.025 ± 0.000	0.801 ± 0.069*	1.360 ± 0.146**	2.975 ± 0.103**	6.762 ± 0.100**
Day 40	0.025 ± 0.000	0.666 ± 0.128*	1.322 ± 0.268*	2.070 ± 0.084**	4.893 ± 0.462**
µg Co/liver					
Day 26	0.140 ± 0.004	3.937 ± 0.333*	7.279 ± 0.886**	15.833 ± 0.551**	28.273 ± 1.584**
Day 40	0.145 ± 0.003	3.503 ± 0.671*	7.324 ± 1.093**	12.139 ± 1.079**	25.095 ± 0.995**

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
µg Co per liver Co/m^3					
Day 26	–	6.299 ± 0.533	5.823 ± 0.709	6.333 ± 0.220	5.655 ± 0.317
Day 40	–	5.605 ± 1.074	5.859 ± 0.874	4.856 ± 0.432	5.019 ± 0.199
Total lung[b]					
Absolute total lung wt (g)					
Day 5	0.957 ± 0.072	0.626 ± 0.057*	0.768 ± 0.029	0.711 ± 0.097	0.809 ± 0.094
Day 12	0.826 ± 0.030	0.944 ± 0.170	0.723 ± 0.052	0.763 ± 0.031	0.953 ± 0.020
Day 26	1.028 ± 0.134	0.741 ± 0.019	0.862 ± 0.058	0.965 ± 0.021	0.957 ± 0.077
Day 40	0.841 ± 0.012	0.817 ± 0.014	0.959 ± 0.075	1.111 ± 0.064	1.206 ± 0.075*
Day 61	0.849 ± 0.027	1.046 ± 0.019*	1.093 ± 0.038*	1.129 ± 0.056*	1.291 ± 0.078**
Day 89	0.816 ± 0.034	1.233 ± 0.014	1.224 ± 0.095	1.322 ± 0.040**	1.318 ± 0.006**
PE day 7	0.755 ± 0.059	1.176 ± 0.034	1.295 ± 0.022**	1.247 ± 0.031	1.206 ± 0.065
PE day 14	0.961 ± 0.101	1.369 ± 0.152	1.312 ± 0.093	1.165 ± 0.131	1.565 ± 0.292
PE day 28	0.904 ± 0.069	1.010 ± 0.057	1.065 ± 0.047	1.203 ± 0.109*	1.181 ± 0.076*
PE day 42	0.842 ± 0.062	1.151 ± 0.074*	0.993 ± 0.030	0.948 ± 0.030	1.451 ± 0.344
µg Co/g total lung					
Day 5	0.040 ± 0.000	2.407 ± 0.107*	3.829 ± 0.349**	7.433 ± 0.773**	12.976 ± 1.766**
Day 12	0.040 ± 0.000	2.407 ± 0.377*	6.435 ± 0.725**	12.663 ± 1.047**	16.101 ± 0.689**
Day 26	0.040 ± 0.000	3.235 ± 0.034*	6.456 ± 0.314**	11.423 ± 0.814**	21.581 ± 0.734**[c]
Day 40	0.040 ± 0.000	3.135 ± 0.117*	6.114 ± 0.245**	12.214 ± 0.180**	21.263 ± 0.520**
Day 61	0.040 ± 0.000	2.682 ± 0.152*	5.978 ± 0.303**	11.146 ± 0.301**	22.517 ± 1.509**
Day 89	0.040 ± 0.000	2.741 ± 0.131*	6.018 ± 0.184**	12.108 ± 0.417**	21.882 ± 0.806**
PE day 7	0.040 ± 0.000	0.601 ± 0.011*	1.216 ± 0.018**	2.496 ± 0.015**	6.067 ± 0.065**
PE day 14	0.040 ± 0.000	0.339 ± 0.035*	0.769 ± 0.061**	1.613 ± 0.121**	2.705 ± 0.344**
PE day 28	0.040 ± 0.000	0.277 ± 0.010*	0.499 ± 0.020**	0.852 ± 0.080**	1.603 ± 0.227**
PE day 42	0.040 ± 0.000	0.100 ± 0.000*	0.372 ± 0.004**	0.670 ± 0.023**	0.959 ± 0.195**
µg Co/total lung					
Day 5	0.038 ± 0.003	1.497 ± 0.082*	2.923 ± 0.173**	5.132 ± 0.107**	10.304 ± 1.063**
Day 12	0.033 ± 0.001	2.144 ± 0.024*	4.591 ± 0.334**	9.607 ± 0.454**	15.337 ± 0.723**
Day 26	0.041 ± 0.005	2.397 ± 0.086*	5.530 ± 0.125**	10.993 ± 0.592**	20.545 ± 3.555**[c]
Day 40	0.034 ± 0.000	2.559 ± 0.051*	5.888 ± 0.648**	13.543 ± 0.593**	25.710 ± 2.135**
Day 61	0.034 ± 0.001	2.809 ± 0.194*	6.512 ± 0.146**	12.603 ± 0.859**	28.860 ± 0.770**
Day 89	0.033 ± 0.001	3.379 ± 0.162*	7.345 ± 0.453**	15.996 ± 0.544**	28.857 ± 1.184**
PE day 7	0.030 ± 0.002	0.706 ± 0.014*	1.576 ± 0.045**	3.114 ± 0.095**	7.325 ± 0.471**
PE day 14	0.038 ± 0.004	0.453 ± 0.011*	0.999 ± 0.022**	1.856 ± 0.135**	4.042 ± 0.227**

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
PE day 28	0.036 ± 0.003	0.279 ± 0.012*	0.531 ± 0.026**	1.007 ± 0.013**	1.864 ± 0.164**
PE day 42	0.034 ± 0.002	0.115 ± 0.007*	0.370 ± 0.015**	0.634 ± 0.021**	1.261 ± 0.019**
µg Co/total lung per mg Co/m^3)					
Day 5	–	2.395 ± 0.132	2.338 ± 0.139	2.053 ± 0.043	2.061 ± 0.213
Day 12	–	3.430 ± 0.038	3.673 ± 0.267	3.843 ± 0.182	3.067 ± 0.145
Day 26	–	3.835 ± 0.138	4.424 ± 0.100	4.397 ± 0.237	4.109 ± 0.711[c]
Day 40	–	4.094 ± 0.082	4.710 ± 0.519	5.417 ± 0.237	5.142 ± 0.427
Day 61	–	4.494 ± 0.310	5.210 ± 0.116	5.041 ± 0.343	5.772 ± 0.154
Day 89	–	5.406 ± 0.259	5.876 ± 0.363	6.399 ± 0.218	5.771 ± 0.237
PE day 7	–	1.130 ± 0.022	1.260 ± 0.036	1.246 ± 0.038	1.465 ± 0.094
PE day 14	–	0.726 ± 0.017	0.799 ± 0.017	0.742 ± 0.054	0.808 ± 0.045
PE day 28	–	0.446 ± 0.019	0.425 ± 0.021	0.403 ± 0.005	0.373 ± 0.033
PE day 42	–	0.184 ± 0.012	0.296 ± 0.012	0.254 ± 0.009	0.252 ± 0.004

*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. Values below the limit of detection (LOD) or below the experimental limit of quantitation (ELOQ) were replaced with ½ the LOD or ELOQ value. Statistical tests were performed only on data that were not normalized. PE = postexposure.
[b]Total lung cobalt burden was calculated using the weight of the total lung with mainstem bronchi at collection and cobalt concentration measured in the right lung.
[c]n = 2.

Table I-5. Lung Deposition and Clearance Parameter Estimates for Female Rats During the Recovery Period in the Three-month Inhalation Study of Cobalt Metal (Two-Compartment Model)[a]

Parameter	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
A (µg Co/total lung)	2.69 ± 0.15	5.78 ± 0.39	13.0 ± 0.69	23.4 ± 2.03
A (days^{-1})	0.39 ± 0.04	0.37 ± 0.04	0.38 ± 0.03	0.27 ± 0.03
$t_{½\,(rapid)}$ (days)	1.78 ± 0.02	1.90 ± 0.02	1.81 ± 0.01	2.59 ± 0.04
B (µg Co/total lung)	0.65 ± 0.05	1.45 ± 0.15	2.86 ± 0.23	4.95 ± 0.95
b (days^{-1})	0.030 ± 0.003	0.034 ± 0.003	0.036 ± 0.003	0.033 ± 0.006
$t_{½\,(slow)}$ (days)	23.5 ± 0.2	20.6 ± 0.2	19.1 ± 0.1	20.7 ± 0.6

[a]Data are presented as mean ± standard error. A = lung burden in the rapid clearance compartment at t = 0 days postexposure; a = rapid phase lung clearance rate constant; $t_{½(rapid)}$ = rapid phase lung clearance rate constant; B = lung burden in the slow clearance compartment at t = 0 days postexposure; b = slow phase lung clearance rate constant; $t_{½(slow)}$ = slow phase lung clearance rate constant.

Table I-6. Lung Deposition and Clearance Parameter Estimates for Female Rats During the Exposure Period in the Three-month Inhalation Study of Cobalt Metal (One-Compartment Model)[a]

Parameter	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
D (µg Co/total lung per day)	0.40 ± 0.04	0.72 ± 0.07	1.28 ± 0.10	2.06 ± 0.20
k (days^{-1})	0.15 ± 0.02	0.12 ± 0.01	0.10 ± 0.01	0.08 ± 0.01
$t_{½}$ (days)	4.65 ± 0.07	5.94 ± 0.08	7.23 ± 0.07	8.99 ± 0.16
L_{ss} (µg Co/total lung)	2.7 ± 0.1	6.2 ± 0.2	13.3 ± 0.5	26.8 ± 1.5

[a]Data are presented as mean ± standard error. D = deposition rate; k = lung clearance rate constant; $t_{½}$ = lung clearance half-life; L_{ss} = steady-state lung burden.

Table I-7. Lung Weights, Cobalt Concentrations, and Cobalt Burdens for Female Rats in the Two-year Inhalation Study of Cobalt Metal[a]

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
n	5	5	5	5
Absolute total lung wt (g)[b]				
Day 1	0.509 ± 0.023	0.632 ± 0.070	0.497 ± 0.027	0.465 ± 0.012
Day 2	0.552 ± 0.037	0.523 ± 0.021	0.488 ± 0.017	0.539 ± 0.016
Day 3	0.539 ± 0.035	0.567 ± 0.018	0.616 ± 0.074	0.647 ± 0.027
Day 4	0.786 ± 0.091	0.877 ± 0.041	0.688 ± 0.025	0.693 ± 0.018
Day 184	1.125 ± 0.237	1.468 ± 0.064	1.682 ± 0.075*	1.693 ± 0.030*
Day 366	1.039 ± 0.025	1.801 ± 0.064**	2.103 ± 0.066**	2.333 ± 0.070**
Day 548	1.279 ± 0.039	2.678 ± 0.187**	2.691 ± 0.145*[c]	3.815 ± 0.323**
µg Co/g total lung				
Day 1	0.040 ± 0.000	2.797 ± 0.393**	6.028 ± 0.400**	16.152 ± 0.466**
Day 2	0.040 ± 0.000	4.603 ± 0.219**	8.987 ± 0.045**	12.662 ± 0.865**
Day 3	0.040 ± 0.000	5.474 ± 0.237**	8.177 ± 0.735**	13.511 ± 0.853**
Day 4	0.040 ± 0.000	3.804 ± 0.232**	7.338 ± 0.393**	18.406 ± 1.266**
Day 184	0.040 ± 0.000	5.685 ± 0.126**	11.238 ± 0.592**	21.670 ± 0.606**
Day 366	0.040 ± 0.000	5.251 ± 0.058**	10.748 ± 0.351**	18.491 ± 0.401**
Day 548	0.052 ± 0.012	5.113 ± 0.400**	10.108 ± 0.532**[c]	13.248 ± 0.567**
µg Co/total lung				
Day 1	0.020 ± 0.001	1.663 ± 0.081**	2.962 ± 0.114**	7.505 ± 0.253**
Day 2	0.022 ± 0.001	2.404 ± 0.136**	4.382 ± 0.154**	6.792 ± 0.379**
Day 3	0.022 ± 0.001	3.103 ± 0.165**	4.829 ± 0.147**	8.705 ± 0.522**
Day 4	0.031 ± 0.004	3.306 ± 0.137**	5.018 ± 0.124**	12.780 ± 1.038**
Day 184	0.045 ± 0.009	8.327 ± 0.279**	18.758 ± 0.484**	36.678 ± 1.061**
Day 366	0.042 ± 0.001	9.454 ± 0.309**	22.578 ± 0.923**	43.068 ± 0.985**
Day 548	0.067 ± 0.016	13.419 ± 0.454**	26.998 ± 0.677**[c]	49.954 ± 2.876**

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
µg Co/total lung per mg Co/m^3)				
Day 1	–	1.330 ± 0.065	1.185 ± 0.046	1.501 ± 0.051
Day 2	–	1.923 ± 0.109	1.753 ± 0.062	1.358 ± 0.076
Day 3	–	2.482 ± 0.132	1.932 ± 0.059	1.741 ± 0.104
Day 4	–	2.645 ± 0.110	2.007 ± 0.050	2.556 ± 0.208
Day 184	–	6.662 ± 0.223	7.503 ± 0.194	7.336 ± 0.212
Day 366	–	7.563 ± 0.247	9.031 ± 0.369	8.614 ± 0.197
Day 548	–	10.735 ± 0.363	10.799 ± 0.271[c]	9.991 ± 0.575

*Significantly different ($P \leq 0.05$) from the chamber control group by Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Values below the limit of detection (LOD) or below the experimental limit of quantitation (ELOQ) were replaced with ½ the LOD or ELOQ value. Statistical tests were performed only on data that were not normalized.
[b]Total lung cobalt burden was calculated using the weight of the total lung with mainstem bronchi at collection and cobalt concentration measured in the right lung.
[c]n = 4.

Table I-8. Lung Deposition and Clearance Parameter Estimates for Female Rats During the Exposure Periods in the Three-month and Two-year Inhalation Studies of Cobalt Metal (Two-Compartment Model)[a]

Parameter	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
A (µg Co/day)	1.449 ± 0.112	2.128 ± 0.158	5.555 ± 0.832
k_a (days^{-1})	0.293 ± 0.035	0.236 ± 0.032	0.453 ± 0.114
B (µg Co/day)	0.0179 ± 0.0043	0.0778 ± 0.0156	0.2885 ± 0.0530
k_b (days^{-1})	0.00088 ± 0.00097	0.00414 ± 0.00110	0.00837 ± 0.00156
F_B	0.012 ± 0.003	0.035 ± 0.007	0.049 ± 0.008
$t_{½a}$ (days)	2.37 ± 0.28	2.94 ± 0.39	1.53 ± 0.38
L_{SSa} (µg Co/total lung)	4.95 ± 0.30	9.02 ± 0.75	12.25 ± 1.55
$t_{½b}$ (days)	789 ± 874	167 ± 45	83 ± 15
L_{SSb} (µg Co/total lung)	20.4 ± 18.0	18.8 ± 1.8	34.5 ± 2.1

[a]Data are presented as mean ± standard error. A = deposition rate in the rapid phase; k_a = rapid phase clearance rate constant; B = deposition rate in the slow phase; k_b = slow phase clearance rate constant; F_B = fraction of deposition in the slow clearance phase; $t_{½a}$ = half-life of the rapid clearance phase; L_{SSa} = theoretical steady-state lung burden for the rapid phase; $t_{½b}$ = half-life of the slow clearance phase; L_{SSb} = theoretical steady-state lung burden for the slow phase.

Table I-9. Tissue Weights, Cobalt Concentrations, and Cobalt Burdens for Mice in the Two-week Inhalation Study of Cobalt Metal[a]

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Male						
n	5	5	5	5	5	2
Blood						
µg Co/g blood						
Day 17	0[b]	0.40 ± 0.04	0.74 ± 0.06	0.65 ± 0.03	1.44 ± 0.22	2.53 ± 0.13
Serum						
µg Co/g serum						
Day 17	0	0.63 ± 0.06	1.38 ± 0.12	1.31 ± 0.04	2.72 ± 0.38	4.94 ± 0.03
Right femur						
Absolute right femur wt (g)						
Day 17	0.025 ± 0.001	0.026 ± 0.001	0.025 ± 0.001	0.025 ± 0.001	0.024 ± 0.001	0.021 ± 0.001
µg Co/g right femur						
Day 17	0	0.42 ± 0.10	0.56 ± 0.06	1.13 ± 0.15	1.65 ± 0.23	2.78 ± 0.24
µg Co/right femur						
Day 17	0	0.011 ± 0.002	0.014 ± 0.001	0.029 ± 0.003	0.039 ± 0.005	0.057 ± 0.007
µg Co/right femur per mg Co/m^3						
Day 17	NA	0.004 ± 0.001	0.003 ± 0.000	0.003 ± 0.000	0.002 ± 0.000	0.001 ± 0.000
Heart						
Absolute heart wt (g)						
Day 17	0.125 ± 0.005	0.127 ± 0.003	0.124 ± 0.003	0.120 ± 0.004	0.110 ± 0.003*	0.106 ± 0.007*
µg Co/g heart						
Day 17	0	0.39 ± 0.09	0.98 ± 0.20	0.95 ± 0.13	1.65 ± 0.21	6.45 ± 2.77
µg Co/heart						
Day 17	0	0.050 ± 0.011	0.123 ± 0.028	0.112 ± 0.014	0.178 ± 0.018	0.700 ± 0.340
µg Co/heart per mg Co/m^3						
Day 17	NA	0.020 ± 0.004	0.025 ± 0.006	0.011 ± 0.001	0.009 ± 0.001	0.017 ± 0.008
Right kidney						

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Absolute right kidney wt (g)						
Day 17	0.223 ± 0.009	0.216 ± 0.011	0.197 ± 0.005	0.199 ± 0.007	0.171 ± 0.003**	0.150 ± 0.003**
µg Co/g right kidney						
Day 17	0	0.91 ± 0.06	1.86 ± 0.12	2.02 ± 0.06	3.82 ± 0.35	4.97 ± 1.76
µg Co/right kidney						
Day 17	0	0.197 ± 0.019	0.360 ± 0.019	0.396 ± 0.018	0.642 ± 0.058	0.737 ± 0.279
µg Co/right kidney per mg Co/m^3						
Day 17	NA	0.079 ± 0.007	0.072 ± 0.004	0.040 ± 0.002	0.032 ± 0.003	0.018 ± 0.007
Liver						
Absolute liver wt (g)						
Day 17	1.127 ± 0.037	0.978 ± 0.035*	0.976 ± 0.037*	0.993 ± 0.018*	0.888 ± 0.019**	0.830 ± 0.013**
µg Co/g liver						
Day 17	0	1.72 ± 0.13	4.79 ± 0.70	4.55 ± 0.41	12.84 ± 2.07	33.73 ± 2.79
µg Co/liver						
Day 17	0	1.669 ± 0.138	4.566 ± 0.512	4.516 ± 0.437	11.370 ± 1.836	28.007 ± 2.751
µg Co/liver per mg Co/m^3						
Day 17	NA	0.668 ± 0.055	0.913 ± 0.102	0.452 ± 0.044	0.568 ± 0.092	0.700 ± 0.069
Total lung[c]						
Absolute total lung wt (g)						
Day 17	0.182 ± 0.007	0.210 ± 0.009*	0.225 ± 0.009**	0.244 ± 0.014**	0.295 ± 0.007**	0.360 ± 0.050**
µg Co/total lung						
Day 17	0	5.587 ± 0.473	10.451 ± 0.448	16.403 ± 0.980	20.356 ± 0.666	28.931 ± 11.814
µg Co/total lung per mg Co/m^3						
Day 17	NA	2.235 ± 0.189	2.090 ± 0.090	1.640 ± 0.098	1.018 ± 0.033	0.723 ± 0.295
Right lung[d]						
Absolute right lung wt (g)						
Day 17	0.078 ± 0.006	0.097 ± 0.002	0.110 ± 0.003	0.117 ± 0.004	0.152 ± 0.005	0.199 ± 0.031**
µg Co/g right lung						

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Day 17	0	26.49 ± 1.37	46.36 ± 67.26	67.26 ± 1.13	69.14 ± 2.93	86.50 ± 44.86
µg Co/right lung						
Day 17	–	0.942 ± 0.048	0.988 ± 0.048	0.730 ± 0.151	0.442	–[e]
µg Co/right lung per mg Co/m^3						
Day 17	NA	0.523 ± 0.012	0.590 ± 0.006	0.455 ± 0.002	0.309	–
Right testis						
Absolute right testis wt (g)						
Day 17	0.098 ± 0.004	0.100 ± 0.002	0.098 ± 0.002	0.097 ± 0.002	0.088 ± 0.002*	0.076 ± 0.005*
µg Co/g right testis						
Day 17	0	0.06 ± 0.01	0.15 ± 0.01	0.34 ± 0.08	0.58 ± 0.06	1.20 ± 0.03
µg Co/right testis						
Day 17	0	0.005 ± 0.001	0.014 ± 0.001	0.033 ± 0.009	0.051 ± 0.005	0.090 ± 0.008
µg Co/right testis per mg Co/m^3						
Day 17	NA	0.002 ± 0.000	0.003 ± 0.000	0.003 ± 0.001	0.003 ± 0.000	0.002 ± 0.000
Female						
n						
Day 17	5	5	5	5	5	2
Week 3 PE	3	3	3	3	3	1[b]
Blood						
µg Co/g blood						
Day 17	0[g]	0.47 ± 0.06[f]	0.78 ± 0.08[f]	0.97 ± 0.10[f]	2.01 ± 0.13[g]	2.52 ± 0.88
Week 3 PE	0	0.06 ± 0.02	0.07 ± 0.05	0.05 ± 0.01	0.05 ± 0.01	0.13
Serum						
µg Co/g serum						
Day 17	0[g]	0.99 ± 0.13[f]	1.52 ± 0.18[f]	1.83 ± 0.20[f]	3.92 ± 0.28[g]	5.10 ± 1.57
Week 3 PE	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.07
Right femur						
Absolute right femur wt (g)						

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Day 17	0.024 ± 0.001	0.025 ± 0.000	0.025 ± 0.001	0.023 ± 0.001	0.020 ± 0.001*	0.018 ± 0.001*
µg Co/g right femur						
Day 17	0	0.71 ± 0.05	1.24 ± 0.10	1.80 ± 0.14	3.03 ± 0.29	3.92 ± 1.07
µg Co/right femur						
Day 17	0	0.019 ± 0.001	0.030 ± 0.002	0.041 ± 0.003	0.058 ± 0.009	0.065 ± 0.016
µg Co/right femur per mg Co/m^3						
Day 17	NA	0.007 ± 0.001	0.006 ± 0.000	0.004 ± 0.000	0.003 ± 0.000	0.002 ± 0.000
Heart						
Absolute heart wt (g)						
Day 17	0.111 ± 0.002	0.110 ± 0.003	0.112 ± 0.001	0.100 ± 0.002	0.097 ± 0.005	0.099 ± 0.004
µg Co/g heart						
Day 17	0	0.70 ± 0.12	0.99 ± 0.08	1.33 ± 0.12	2.53 ± 0.13	3.26 ± 1.11
µg Co/heart						
Day 17	0	0.076 ± 0.012	0.111 ± 0.009	0.131 ± 0.009	0.243 ± 0.012	0.316 ± 0.097
µg Co/heart per mg Co/m^3						
Day 17	NA	0.030 ± 0.005	0.02 ± 0.002	0.013 ± 0.001	0.012 ± 0.001	0.008 ± 0.002
Right kidney						
Absolute right kidney wt (g)						
Day 17	0.151 ± 0.002	0.147 ± 0.004	0.142 ± 0.005	0.132 ± 0.004**	0.113 ± 0.003**	0.098 ± 0.006**
µg Co/g right kidney						
Day 17	0	1.44 ± 0.15	2.08 ± 0.17	2.76 ± 0.16	5.45 ± 0.32	8.63 ± 0.69
µg Co/right kidney						
Day 17	0	0.213 ± 0.028	0.291 ± 0.017	0.355 ± 0.015	0.600 ± 0.028	0.826 ± 0.117
µg Co/right kidney per mg Co/m^3						
Day 17	NA	0.085 ± 0.011	0.058 ± 0.003	0.035 ± 0.001	0.030 ± 0.001	0.021 ± 0.003
Liver						
Absolute liver wt (g)						
Day 17	0.928 ± 0.026	0.814 ± 0.021*	0.799 ± 0.029*	0.748 ± 0.030**	0.692 ± 0.026**	0.605 ± 0.057**

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
µg Co/g liver						
Day 17	0	2.74 ± 0.28	4.94 ± 0.63	7.21 ± 0.74	21.98 ± 1.62	35.83 ± 5.10
µg Co/liver						
Day 17	0	2.213 ± 0.229	3.884 ± 0.426	5.329 ± 0.474	15.155 ± 1.123	21.941 ± 5.131
µg Co/liver per mg Co/m^3						
Day 17	NA	0.885 ± 0.092	0.777 ± 0.085	0.533 ± 0.047	0.758 ± 0.056	0.549 ± 0.128
Total lung[c]						
Absolute total lung wt (g)						
Day 17	0.189 ± 0.007[e]	0.194 ± 0.004[e]	0.223 ± 0.006**[f]	0.234 ± 0.006**[f]	0.290 ± 0.010**[g]	0.329 ± 0.020**
Week 3 PE	0.18 ± 0.01	0.16 ± 0.01	0.19 ± 0.02	0.18 ± 0.01	0.24 ± 0.03	0.20
µg Co/total lung						
Day 17	0[f]	4.511 ± 0.159[f]	9.630 ± 0.327[f]	14.495 ± 0.855[f]	16.303 ± 0.843[g]	8.205 ± 4.106
Week 3 PE	0	0.483 ± 0.029	0.947 ± 0.112	1.484 ± 0.187	1.123 ± 0.144	1.927
µg Co/total lung per mg Co/m^3						
Day 17	0[f]	1.804 ± 0.064[f]	1.926 ± 0.065[f]	1.450 ± 0.085[f]	0.815 ± 0.042[g]	0.205 ± 0.103
Week 3 PE	0	0.193 ± 0.012	0.189 ± 0.022	0.148 ± 0.019	0.056 ± 0.007	0.048
Left lung[g]						
Absolute left lung wt (g)						
Day 17	0.103 ± 0.006[i]	0.088 ± 0.004[i]	0.091 ± 0.006[i]	0.099 ± 0.002[i]	0.113[b]	–
Week 3 PE	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.07 ± 0.01*	0.06
µg Co/g left lung						
Day 17	NA[h]	14.88 ± 0.34[i]	32.72 ± 2.34[i]	45.98 ± 1.16[i]	54.72[b]	–
Week 3 PE	NA	3.05 ± 0.06	5.66 ± 0.49	8.00 ± 0.20	5.75 ± 1.06	9.71
µg Co/left lung						
Day 17	NA[h]	1.308 ± 0.030[i]	2.949 ± 0.032[i]	4.550 ± 0.024[i]	6.183[b]	–
Week 3 PE	NA	0.123 ± 0.004	0.246 ± 0.030	0.360 ± 0.017	0.365 ± 0.028	0.566
µg Co/left lung per mg Co/m^3						
Day 17	NA[h]	0.523 ± 0.012[i]	0.590 ± 0.006[i]	0.455 ± 0.002[i]	0.309[b]	–

	Chamber Control	2.5 mg/m^3	5 mg/m^3	10 mg/m^3	20 mg/m^3	40 mg/m^3
Week 3 PE	NA	0.049 ± 0.002	0.049 ± 0.006	0.036 ± 0.002	0.018 ± 0.001	0.014
Right lung[d]						
Absolute right lung wt (g)						
Day 17	0.090 ± 0.005[f]	0.098 ± 0.002[f]	0.112± 0.003**[f]	0.120 ± 0.004**[f]	0.159 ± 002**[g]	0.186 ± 0.006
Week 3 PE	0.09 ± 0.00	0.08 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.12 ± 0.01*	0.11
µg Co/g right lung						
Day 17	NA[f]	23.28 ± 0.86[f]	43.24 ± 1.18[f]	61.99 ± 3.66[f]	56.24 ± 2.78[g]	24.28 ± 11.05
Week 3 PE	NA	3.10 ± 0.03	5.04 ± 0.15	8.06 ± 0.48	4.99 ± 1.14	9.68
µg Co/right lung						
Day 17	NA[h]	2.356 ± 0.120i	4.939 ± 0.241[i]	7.303 ± 1.512[i]	8.848[b]	NA
Week 3 PE	NA	0.253 ± 0.014	0.473 ± 0.036	0.751 ± 0.053	0.553 ± 0.086	1.017
µg Co/right lung per mg Co/m^3						
Day 17	NA[i]	0.942 ± 0.048[i]	0.988 ± 0.048[i]	0.730 ± 0.151[h]	0.442[b]	NA
Week 3 PE	NA	0.101 ± 0.005	0.095 ± 0.007	0.075 ± 0.005	0.028 ± 0.004	0.025

*Significantly different ($P \leq 0.05$) from the chamber control group by Dunnett's or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. All values except absolute tissue weights are control corrected. Statistical tests were performed only on absolute tissue weight data.
NA = not applicable; PE = postexposure.
[b]n = 1; No standard error was calculated; less than two measurements were available.
[c]Total lung cobalt burden was calculated using the weight of the total lung with mainstem bronchi at collection and cobalt concentration measured in the right lung.
[d]Right lung cobalt burden was calculated using the right lung weight and the concentration of cobalt measured in the right lung.
[e]No data are available.
[f]n = 7.
[g]n =6.
[h]Left lung cobalt burden was calculated using the left lung weight and the concentration of cobalt measured in the left lung.
[i]n = 2.

Table I-10. Deposition and Clearance Parameter Estimates for Female Mice in the Two-week Inhalation Study of Cobalt Metal[a]

Tissue	Exposure Concentration (mg/m^3)	k ($days^{-1}$)	$t_{½}$ (days)	α (µg Co/day)	A_e (µg Co)
Blood	2.5	0.10	7.26	NA	NA
	5	0.11	6.19	NA	NA
	10	0.14	4.81	NA	NA
	20	0.17	4.11	NA	NA
Serum	2.5	0.19	3.71	NA	NA
	5	0.24	2.89	NA	NA
	10	0.22	3.22	NA	NA
	20	0.22	3.17	NA	NA
Lung[b]	2.5	0.11	6.57	0.57	5.44
	5	0.11	6.30	1.25	11.41
	10	0.11	6.40	1.87	17.26
	20	0.13	5.46	2.34	18.45

[a]Statistical analyses of these data were not performed due to the limited number of time points and data points within some time points. k = first order clearance rate constant; $t_{1/2}$ = clearance half-life; α = lung deposition rate; A_e = steady-state lung burden; NA = not applicable.
[b]Total lung cobalt burden was calculated using the weight of total lung with mainstem bronchi at collection and cobalt concentration measured in the right lung.

Table I-11. Tissue Weights, Cobalt Concentrations, and Cobalt Burdens for Female Mice in the Three-month Inhalation Study of Cobalt Metal[a]

Parameter	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
n	3	3	3	3	3	3
Blood						
µg Co/g blood						
Day 5	0.009 ± 0.000	0.056 ± 0.015*	0.154 ± 0.030**	0.412 ± 0.087**	0.857 ± 0.114**	1.073 ± 0.029**
Day 12	0.009 ± 0.000	0.134 ± 0.012*	0.250 ± 0.045**	0.352 ± 0.025**	0.949 ± 0.078**	1.540 ± 0.097**
Day 26	0.009 ± 0.000	0.077 ± 0.010*	0.154 ± 0.011**	0.421 ± 0.023**	0.874 ± 0.113**	1.634 ± 0.169**
Day 40	0.009 ± 0.000	0.155 ± 0.014*	0.263 ± 0.036**	0.520 ± 0.026**	0.941 ± 0.119**	1.619 ± 0.053**
Day 61	0.009 ± 0.000	0.111 ± 0.028*	0.188 ± 0.008**	0.359 ± 0.046**	0.916 ± 0.071**	2.576 ± 0.944**
Day 89	0.009 ± 0.000	0.093 ± 0.013*	0.155 ± 0.007**	0.308 ± 0.008**	0.660 ± 0.035**	1.257 ± 0.088**
PE day 7	0.009 ± 0.000	0.009 ± 0.000	0.014 ± 0.005	0.025 ± 0.000*	0.063 ± 0.004**	0.115 ± 0.002**
PE day 14	0.009 ± 0.000	0.009 ± 0.000	0.009 ± 0.000	0.009 ± 0.000	0.025 ± 0.000**	0.069 ± 0.008**
PE day 28	0.009 ± 0.000	0.009 ± 0.000	0.009 ± 0.000	0.009 ± 0.000	0.009 ± 0.000	0.025 ± 0.000**
PE day 42	0.009 ± 0.000	0.009 ± 0.000	0.009 ± 0.000	0.009 ± 0.000	0.009 ± 0.000	0.009 ± 0.000
µg Co/g blood per mg Co/m^3						
Day 5	–	0.089 ± 0.025	0.123 ± 0.024	0.165 ± 0.035	0.171 ± 0.023	0.107 ± 0.003
Day 12	–	0.214 ± 0.019	0.200 ± 0.036	0.141 ± 0.010	0.190 ± 0.016	0.154 ± 0.010
Day 26	–	0.123 ± 0.016	0.123 ± 0.009	0.168 ± 0.009	0.175 ± 0.023	0.163 ± 0.017
Day 40	–	0.248 ± 0.023	0.211 ± 0.029	0.208 ± 0.010	0.188 ± 0.024	0.162 ± 0.005
Day 61	–	0.178 ± 0.045	0.151 ± 0.006	0.144 ± 0.019	0.183 ± 0.014	0.258 ± 0.094
Day 89	–	0.149 ± 0.020	0.124 ± 0.006	0.123 ± 0.003	0.132 ± 0.007	0.126 ± 0.009
PE day 7	–	0.014 ± 0.000	0.011 ± 0.004	0.010 ± 0.000	0.013 ± 0.001	0.011 ± 0.000
PE day 14	–	0.014 ± 0.000	0.007 ± 0.000	0.004 ± 0.000	0.005 ± 0.000	0.007 ± 0.001
PE day 28	–	0.014 ± 0.000	0.007 ± 0.000	0.004 ± 0.000	0.002 ± 0.000	0.003 ± 0.000

Parameter	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
PE day 42	–	0.014 ± 0.000	0.007 ± 0.000	0.004 ± 0.000	0.002 ± 0.000	0.001 ± 0.000
Liver						
Absolute liver wt (g)						
Day 26	0.969 ± 0.055`	0.970 ± 0.024	0.902 ± 0.019	0.873 ± 0.056	0.813 ± 0.031*	0.754 ± 0.022*
Day 40	1.011 ± 0.011	1.046 ± 0.021	1.082 ± 0.008	0.989 ± 0.032	0.880 ± 0.052	0.802 ± 0.019
µg Co/g liver						
Day 26	0.220 ± 0.000	0.500 ± 0.000*	0.956 ± 0.240**	2.494 ± 0.247**	5.768 ± 0.593**	12.002 ± 1.095**
Day 40	0.220 ± 0.000[b]	0.722 ± 0.222	1.808 ± 0.289*	3.307 ± 0.101**	6.676 ± 1.189**	11.583 ± 0.434**
µg Co/liver						
Day 26	0.213 ± 0.012	0.485 ± 0.012*	0.866 ± 0.220*	2.157 ± 0.149**	4.699 ± 0.538**	9.065 ± 0.943**
Day 40	0.222 ± 0.004[b]	0.747 ± 0.214	1.952 ± 0.297*	3.263 ± 0.022**	5.963 ± 1.311**	9.310 ± 0.560**
µg Co/liver per mg Co/m^3						
Day 26	–	0.776 ± 0.019	0.693 ± 0.176	0.863 ± 0.060	0.940 ± 0.108	0.906 ± 0.094
Day 40	–	1.195 ± 0.343	1.561 ± 0.237	1.305 ± 0.009	1.193 ± 0.262	0.931 ± 0.056
Total lung[c]						
Absolute total lung wt (g)						
Day 5	0.144 ± 0.006	0.138 ± 0.012	0.151 ± 0.007	0.158 ± 0.007	0.167 ± 0.011	0.178 ± 0.023
Day 12	0.144 ± 0.005	0.158 ± 0.010	0.168 ± 0.015	0.177 ± 0.012*	0.200 ± 0.006**	0.238 ± 0.023**
Day 26	0.145 ± 0.006	0.157 ± 0.002	0.163 ± 0.009	0.169 ± 0.008	0.203 ± 0.014**	0.251 ± 0.015**
Day 40	0.168 ± 0.008	0.165 ± 0.007	0.183 ± 0.006	0.191 ± 0.002*	0.247 ± 0.015**	0.259 ± 0.006**
Day 61	0.188 ± 0.023	0.170 ± 0.002	0.227 ± 0.025	0.189 ± 0.003	0.255 ± 0.005*	0.279 ± 0.015*
Day 89	0.189 ± 0.022	0.187 ± 0.006	0.197 ± 0.002	0.206 ± 0.003	0.260 ± 0.010*	0.300 ± 0.014**
PE day 7	0.174 ± 0.015	0.178 ± 0.013	0.203 ± 0.019	0.201 ± 0.019	0.237 ± 0.014*	0.289 ± 0.014**
PE day 14	0.176 ± 0.011	0.186 ± 0.009	0.190 ± 0.006	0.183 ± 0.004	0.209 ± 0.009*	0.249 ± 0.016**
PE day 28	0.174 ± 0.008	0.192 ± 0.013	0.168 ± 0.006	0.184 ± 0.010	0.184 ± 0.007	0.209 ± 0.006

Parameter	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
PE day 42	0.169 ± 0.002	0.169 ± 0.005	0.181 ± 0.007	0.178 ± 0.002*	0.196 ± 0.005**	0.213 ± 0.014**
µg Co/g total lung						
Day 5	0.200 ± 0.000	5.920 ± 0.456*	10.292 ± 0.874**	20.877 ± 1.371**	28.959 ± 2.874**	15.697 ± 2.720**
Day 12	0.200 ± 0.000	6.770 ± 0.817*	12.310 ± 1.396**	22.624 ± 1.973**	46.133 ± 3.142**	47.334 ± 19.444**
Day 26	0.200 ± 0.000[b]	5.505 ± 0.313	12.957 ± 1.029*	27.070 ± 0.926**	72.553 ± 3.071**	109.153 ± 3.027**
Day 40	0.200 ± 0.000	7.038 ± 0.531*	16.986 ± 0.344**	28.659 ± 1.896**	85.895 ± 5.226**	141.694 ± 2.904**
Day 61	0.200 ± 0.000	6.517 ± 0.412*	12.891 ± 1.662**	32.787 ± 2.120**	77.234 ± 6.030**	143.304 ± 6.747**
Day 89	0.200 ± 0.000	6.944 ± 0.431*	18.504 ± 1.996**	39.129 ± 0.956**	108.420 ± 3.413**	141.003 ± 5.456**
PE day 7	0.200 ± 0.000	2.342 ± 0.041*	6.392 ± 0.619**	17.275 ± 2.533**	44.664 ± 1.910**	65.663 ± 3.483**
PE day 14	0.200 ± 0.000	1.565 ± 0.160*	5.147 ± 0.114**	15.226 ± 0.822**	42.632 ± 3.321**	48.445 ± 4.201**
PE day 28	0.200 ± 0.000	0.670 ± 0.170*	3.548 ± 0.174**	9.758 ± 0.484**	30.466 ± 0.878**	42.285 ± 1.482**
PE day 42	0.200 ± 0.000	0.500 ± 0.000*	2.762 ± 0.027**	7.583 ± 0.446**	24.409 ± 1.234**	31.551 ± 2.958**
µg Co/total lung						
Day 5	0.029 ± 0.001	0.809 ± 0.050*	1.539 ± 0.072**	3.273 ± 0.100**	4.791 ± 0.286**	2.671 ± 0.206**
Day 12	0.029 ± 0.001	1.051 ± 0.054*	2.031 ± 0.080**	3.996 ± 0.460**	9.219 ± 0.537**	10.431 ± 3.867**
Day 26	0.030 ± 0.001[b]	0.864 ± 0.044	2.094 ± 0.060*	4.561 ± 0.054**	14.723 ± 1.257**	27.397 ± 1.454**
Day 40	0.034 ± 0.002	1.154 ± 0.071*	3.110 ± 0.141**	5.479 ± 0.334**	21.033 ± 0.324**	36.623 ± 0.529**
Day 61	0.038 ± 0.005	1.104 ± 0.060*	2.849 ± 0.109**	6.193 ± 0.388**	19.777 ± 1.877**	39.990 ± 2.606**
Day 89	0.038 ± 0.004	1.293 ± 0.067*	3.644 ± 0.413**	8.070 ± 0.150**	28.293 ± 1.946**	42.263 ± 1.680**
PE day 7	0.035 ± 0.003	0.417 ± 0.033*	1.274 ± 0.012**	3.384 ± 0.327**	10.517 ± 0.251**	18.903 ± 0.681**
PE day 14	0.035 ± 0.002	0.290 ± 0.030*	0.977 ± 0.051**	2.798 ± 0.207**	8.857 ± 0.430**	11.943 ± 0.455**
PE day 28	0.035 ± 0.002	0.126 ± 0.026*	0.598 ± 0.050**	1.801 ± 0.150**	5.594 ± 0.064**	8.864 ± 0.515**
PE day 42	0.034 ± 0.000	0.085 ± 0.003*	0.499 ± 0.015**	1.347 ± 0.079**	4.765 ± 0.137**	6.678 ± 0.517**
µg Co/total lung per mg Co/m^3						
Day 5	–	1.294 ± 0.080	1.231 ± 0.058	1.309 ± 0.040	0.958 ± 0.057	0.267 ± 0.021

Parameter	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
Day 12	–	1.682 ± 0.086	1.625 ± 0.064	1.599 ± 0.184	1.844 ± 0.107	1.043 ± 0.387
Day 26	–	1.382 ± 0.070	1.675 ± 0.048	1.824 ± 0.022	2.945 ± 0.251	2.740 ± 0.145
Day 40	–	1.847 ± 0.114	2.488 ± 0.112	2.191 ± 0.134	4.207 ± 0.065	3.662 ± 0.053
Day 61	–	1.767 ± 0.096	2.279 ± 0.087	2.477 ± 0.155	3.955 ± 0.375	3.999 ± 0.261
Day 89	–	2.069 ± 0.106	2.915 ± 0.330	3.228 ± 0.060	5.659 ± 0.389	4.226 ± 0.168
PE day 7	–	0.667 ± 0.052	1.019 ± 0.010	1.354 ± 0.131	2.103 ± 0.050	1.890 ± 0.068
PE day 14	–	0.464 ± 0.048	0.782 ± 0.041	1.119 ± 0.083	1.771 ± 0.086	1.194 ± 0.046
PE day 28	–	0.201 ± 0.042	0.478 ± 0.040	0.721 ± 0.060	1.119 ± 0.013	0.886 ± 0.051
PE day 42	–	0.135 ± 0.004	0.399 ± 0.012	0.539 ± 0.032	0.953 ± 0.027	0.668 ± 0.052

*Significantly different ($P \leq 0.05$) from the chamber control group by Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Values below the limit of detection (LOD) or below the experimental limit of quantitation (ELOQ) were replaced with ½ the LOD or ELOQ value. Statistical tests were performed only on data that were not normalized. PE = postexposure.
[b]n = 2.
[c]Total lung cobalt burden was calculated using the weight of the total lung with mainstem bronchi at collection and cobalt concentration measured in the right lung.

Table I-12. Lung Deposition and Clearance Parameter Estimates for Female Mice During the Recovery Period in the Three-month Inhalation Study of Cobalt Metal (Two-Compartment Model)[a]

Parameter	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
A (µg Co/total lung)	0.947 ± 0.124	2.302 ± 0.341	4.283 ± 0.736	16.746 ± 1.876	28.342 ± 3.014
A (days^{-1})	0.271 ± 0.060	0.268 ± 0.073	0.509 ± 0.454	0.381 ± 0.119	0.215 ± 0.042
$t_{½\,(rapid)}$ (days)	2.556 ± 0.127	2.592 ± 0.194	1.361 ± 1.080	1.818 ± 0.177	3.225 ± 0.123
B (µg Co/total lung)	0.324 ± 0.070	1.103 ± 0.222	3.776 ± 0.458	10.933 ± 1.141	13.811 ± 2.331
b (days^{-1})	0.022 ± 0.006	0.020 ± 0.006	0.026 ± 0.004	0.021 ± 0.003	0.018 ± 0.005
$t_{½\,(slow)}$ (days)	30.88 ± 2.56	34.66 ± 3.11	27.08 ± 0.64	33.01 ± 0.82	39.33 ± 2.80

[a]Data are presented as mean ± standard error. A = lung burden in the rapid clearance compartment at t = 0 days postexposure; a = rapid phase lung clearance rate constant; $t_{½(rapid)}$ = rapid phase lung clearance rate constant; B = lung burden in the slow clearance compartment at t = 0 days postexposure; b = slow phase lung clearance rate constant; $t_{½(slow)}$ = slow phase lung clearance rate constant.

Table I-13. Lung Deposition and Clearance Parameter Estimates for Female Mice During the Exposure Period in the Three-month Inhalation Study of Cobalt Metal (One-Compartment Model)[a]

Parameter	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
D (µg Co/total lung per day)	0.301 ± 0.062	0.381 ± 0.061	0.737 ± 0.129	0.971 ± 0.076	0.710 ± 0.094
k (days^{-1})	0.287 ± 0.065	0.141 ± 0.027	0.132 ± 0.028	0.041 ± 0.006	0.006 ± 0.005
$t_{½}$ (days)	2.412 ± 0.122	4.903 ± 0.183	5.249 ± 0.235	16.996 ± 0.321	121.975 ± 104.527
L_{ss} (µg Co/total lung)	1.048 ± 0.045	2.691 ± 0.162	5.578 ± 0.375	23.810 ± 1.853	124.948 ± 103.207

[a]Data are presented as mean ± standard error. D = deposition rate; k = lung clearance rate constant; $t_{½}$ = lung clearance half-life; L_{ss} = steady-state lung burden.

Table I-14. Lung Weights, Cobalt Concentrations, and Cobalt Burdens for Female Mice in the Two-year Inhalation Study of Cobalt Metal[a]

	Chamber Control	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
n	5	5	5	5
Absolute total lung wt (g)[b]				
Day 1	0.130 ± 0.005	0.134 ± 0.002	0.133 ± 0.004	0.130 ± 0.002
Day 2	0.139 ± 0.003	0.130 ± 0.004	0.139 ± 0.004	0.139 ± 0.003
Day 3	0.132 ± 0.006	0.140 ± 0.002	0.136 ± 0.005	0.146 ± 0.006
Day 4	0.130 ± 0.004	0.138 ± 0.007	0.156 ± 0.002**	0.163 ± 0.007**
Day 184	0.180 ± 0.015	0.175 ± 0.005	0.208 ± 0.006*	0.283 ± 0.005**
Day 366	0.170 ± 0.003	0.218 ± 0.004**	0.249 ± 0.004**	0.418 ± 0.005**
Day 548	0.208 ± 0.015	0.279 ± 0.024[c]	0.380 ± 0.077**	0.508 ± 0.029**
µg Co/g total lung				
Day 1	0.200 ± 0.000[c]	5.527 ± 0.184*[c]	11.246 ± 0.403**	21.869 ± 0.910**
Day 2	0.200 ± 0.000	7.797 ± 0.396**	14.953 ± 0.393**	19.443 ± 1.488**
Day 3	0.200 ± 0.000	9.994 ± 0.340**	18.467 ± 0.502**	25.041 ± 0.392**
Day 4	0.200 ± 0.000	11.009 ± 0.391**	20.554 ± 0.638**	26.425 ± 0.767**
Day 184	0.200 ± 0.000	32.370 ± 2.691**	58.677 ± 2.508**	120.993 ± 6.170**
Day 366	0.200 ± 0.000	44.922 ± 2.798**	82.778 ± 8.031**	126.628 ± 4.668**
Day 548	0.200 ± 0.000	38.918 ± 0.471**[c]	53.982 ± 9.362**	93.214 ± 3.957**
µg Co/total lung				
Day 1	0.026 ± 0.001[c]	0.743 ± 0.017*[c]	1.499 ± 0.084**	2.839 ± 0.085**
Day 2	0.028 ± 0.001	1.020 ± 0.072**	2.075 ± 0.081**	2.698 ± 0.191**
Day 3	0.026 ± 0.001	1.399 ± 0.057**	2.512 ± 0.157**	3.654 ± 0.144**
Day 4	0.026 ± 0.001	1.524 ± 0.118**	3.191 ± 0.056**	4.303 ± 0.143**
Day 184	0.036 ± 0.003	5.663 ± 0.475**	12.134 ± 0.285**	34.246 ± 1.933**
Day 366	0.034 ± 0.001	9.834 ± 0.698**	20.538 ± 1.833**	52.896 ± 1.698**
Day 548	0.042 ± 0.003	10.883 ± 0.995*[c]	17.680 ± 1.790**	47.218 ± 2.652**
µg Co/total lung per mg Co/m^3				
Day 1	–	0.595 ± 0.014[c]	0.600 ± 0.033	0.568 ± 0.017
Day 2	–	0.816 ± 0.058	0.830 ± 0.032	0.540 ± 0.038
Day 3	–	1.119 ± 0.046	1.005 ± 0.063	0.731 ± 0.029
Day 4	–	1.220 ± 0.095	1.276 ± 0.022	0.861 ± 0.029
Day 184	–	4.530 ± 0.380	4.854 ± 0.114	6.849 ± 0.387
Day 366	–	7.867 ± 0.559	8.215 ± 0.733	10.579 ± 0.340
Day 548	–	8.706 ± 0.796[c]	7.072 ± 0.716	9.444 ± 0.530

*Significantly different ($P \leq 0.05$) from the chamber control group by Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Values below the limit of detection (LOD) or below the experimental limit of quantitation (ELOQ) were replaced with ½ the LOD or ELOQ value. Statistical tests were performed only on data that were not normalized.
[b]Total lung cobalt burden was calculated using the weight of the total lung with mainstem bronchi at collection and cobalt concentration measured in the right lung.
[c]n = 4.

Table I-15. Lung Deposition and Clearance Parameter Estimates for Female Mice During the Exposure Periods in the Three-month and Two-year Inhalation Studies of Cobalt Metal (Two-Compartment Model)[a]

Parameter	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
A (µg Co/day)	0.870 ± 0.161	1.842 ± 0.431	1.175 ± 0.147
k_a (days^{-1})	0.559 ± 0.146	0.652 ± 0.214	0.132 ± 0.040
B (µg Co/day)	0.0274 ± 0.0034	0.0750 ± 0.0103	0.2516 ± 0.0499
k_b (days^{-1})	0.00169 ± 0.00060	0.00404 ± 0.00083	0.00586 ± 0.00119
F_B	0.031 ± 0.005	0.039 ± 0.008	0.176 ± 0.033
$t_{½a}$ (days)	1.24 ± 0.32	1.06 ± 0.35	5.24 ± 1.57
L_{SSa} (µg Co/total lung)	1.56 ± 0.16	2.82 ± 0.37	8.89 ± 2.20
$t_{½b}$ (days)	409 ± 145	172 ± 35	118 ± 24
L_{SSb} (µg Co/total lung)	16.2 ± 3.9	18.6 ± 1.7	42.9 ± 2.5

[a]Data are presented as mean ± standard error. A = deposition rate in the rapid phase; k_a = rapid phase clearance rate constant; B = deposition rate in the slow phase; k_b = slow phase clearance rate constant; F_B = fraction of deposition in the slow clearance phase; $t_{½a}$ = half-life of the rapid clearance phase; L_{SSa} = theoretical steady-state lung burden for the rapid phase; $t_{½b}$ = half-life of the slow clearance phase; L_{SSb} = theoretical steady-state lung burden for the slow phase.

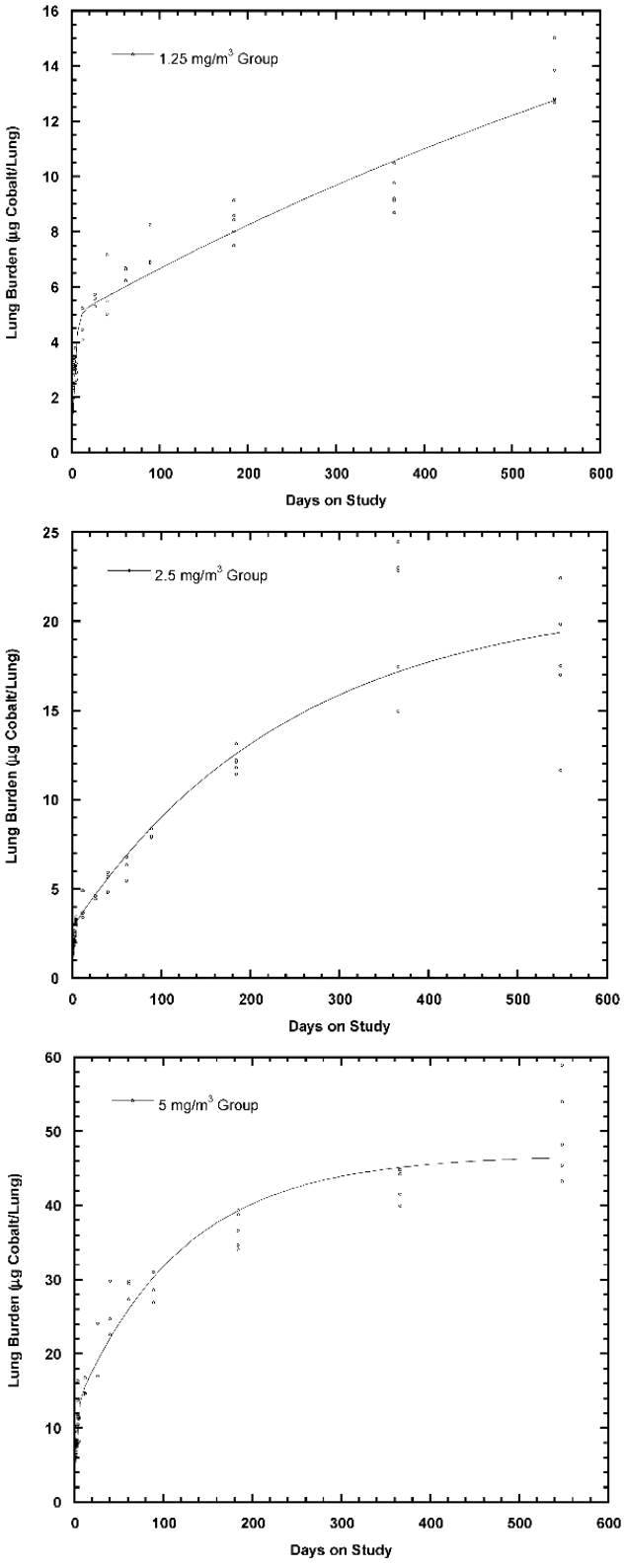

Figure I-1. Lung Cobalt Burdens in 1.25 (top), 2.5 (middle), and 5 (bottom) mg/m^3 Female Rats in the Three-month and Two-year Inhalation Studies of Cobalt Metal

The lines represent the fit of the lung deposition and clearance model to the data.

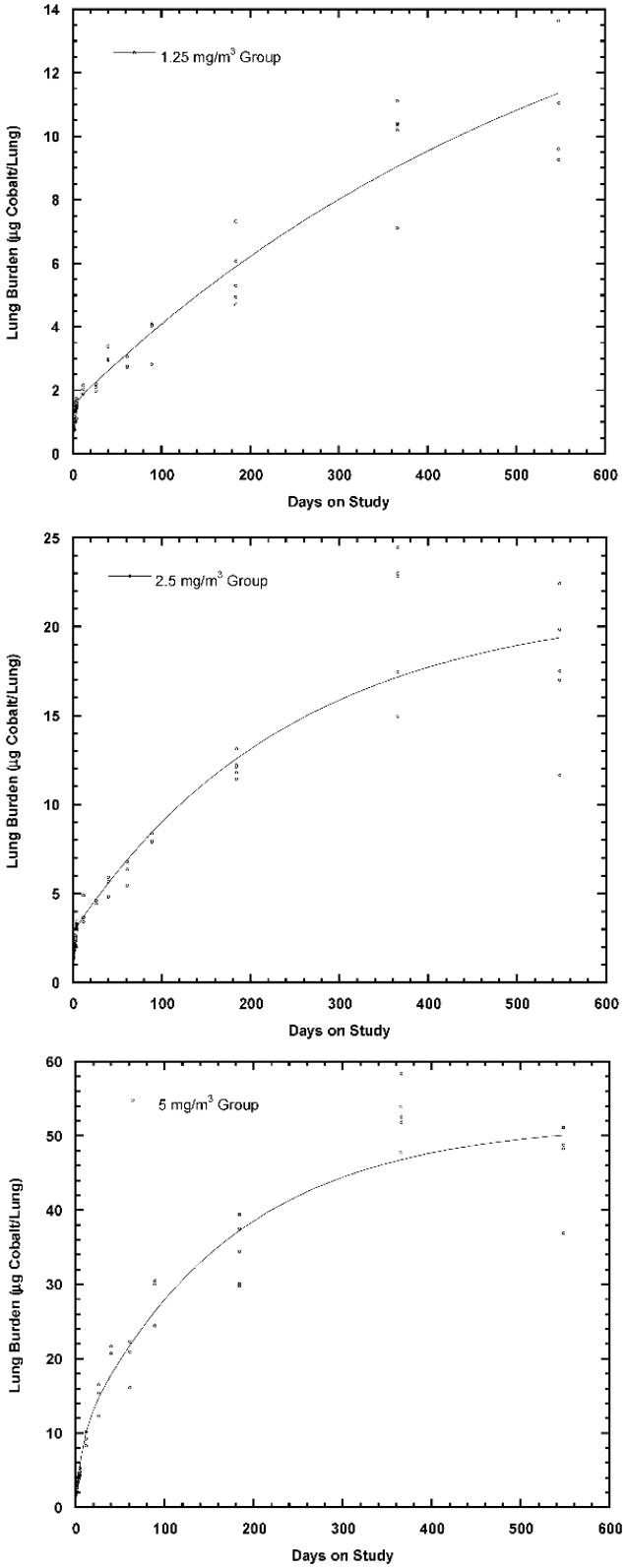

Figure I-2. Lung Cobalt Burdens in 1.25 (top), 2.5 (middle), and 5 (bottom) mg/m^3 Female Mice in the Three-month and Two-year Inhalation Studies of Cobalt Metal

The lines represent the fit of the lung deposition and clearance model to the data.

Appendix J. Liver Cytochrome P450 Data

Tables

Table J-1. Liver Cytochrome P450 Data for Female Rats in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3
n	3	3	3	3	3
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)					
Day 26	0.571 ± 0.022	0.707 ± 0.022	0.730 ± 0.042*	0.615 ± 0.039	0.635 ± 0.009
Day 40	0.615 ± 0.035	0.703 ± 0.048	0.650 ± 0.050	0.592 ± 0.047	0.637 ± 0.027
7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein)					
Day 26	44.185 ± 2.751	41.774 ± 1.129	36.922 ± 1.376*	36.933 ± 0.636*	36.119 ± 2.635*
Day 40	34.291 ± 1.548	37.698 ± 4.281	36.055 ± 2.482	32.852 ± 2.045	38.460 ± 3.395
7-Pentoxyresorufin-*O*-deethylase (PROD) (pmol/minute per mg microsomal protein)					
Day 26	4.766 ± 0.122	4.918 ± 0.094	4.489 ± 0.214	4.089 ± 0.161*	3.899 ± 0.247*
Day 40	3.905 ± 0.154	4.334 ± 0.311	4.283 ± 0.071	3.721 ± 0.230	3.747 ± 0.241

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

Table J-2. Liver Cytochrome P450 Data for Special Study Female Mice in the Three-month Inhalation Study of Cobalt Metal[a]

	Chamber Control	0.625 mg/m^3	1.25 mg/m^3	2.5 mg/m^3	5 mg/m^3	10 mg/m^3
n	3	3	3	3	3	3
Acetanilide-4-hydroxylase (A4H) (nmol/minute per mg microsomal protein)						
Day 26	0.515 ± 0.018	0.434 ± 0.017	0.521 ± 0.043	0.525 ± 0.016	0.752 ± 0.081	0.659 ± 0.014
Day 40	0.483 ± 0.031	0.557 ± 0.041	0.515 ± 0.025	0.526 ± 0.052	0.656 ± 0.044**	0.736 ± 0.070**
7-Ethoxyresorufin-*O*-deethylase (EROD) (pmol/minute per mg microsomal protein)						
Day 26	52.375 ± 4.334	53.046 ± 0.746	63.475 ± 6.343	58.552 ± 3.113	65.036 ± 0.191*	70.237 ± 4.834*
Day 40	52.914 ± 3.989	53.358 ± 0.936	60.768 ± 2.310	58.398 ± 1.156	67.890 ± 2.157**	73.859 ± 7.616*
7-Pentoxyresorufin-*O*-deethylase (PROD) (pmol/minute per mg microsomal protein)						
Day 26	9.992 ± 0.740	10.329 ± 0.517	10.349 ± 0.676	9.577 ± 0.800	11.013 ± 0.651	9.737 ± 0.337
Day 40	10.212 ± 0.388	10.150 ± 0.404	11.274 ± 0.558	10.234 ± 0.252	10.539 ± 0.492	9.148 ± 0.503

*Significantly different ($P \leq 0.05$) from the chamber control group by Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error.

Appendix K. Analysis of *Kras*, *Egfr*, and *Tp53* Mutations in F344/NTac Rat and B6C3F1/N Mouse Alveolar/Bronchiolar Carcinomas Resulting from Chronic Inhalation Exposure to Cobalt Metal

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K.1. Introduction

Spontaneous incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in control B6C3F1/N mice range from 8.4% (female) to 26.2% (male) (all routes, all vehicles, NTP historical control database, May 2011), and the incidences of these lung neoplasms after exposure to certain chemicals can be considerably higher in 2-year NTP studies. The incidences of spontaneous alveolar/bronchiolar adenoma or carcinoma (combined) in control F344/N rats are considerably lower [2.3% females, 3.6% males (all routes, all vehicles, NTP historical control database, May 2011)] than in the B6C3F1/N mouse. In the concurrent 2-year cobalt metal studies, there were significantly increased incidences of alveolar/bronchiolar neoplasms in both B6C3F1/N mice and F344/NTac rats exposed by inhalation compared to those in the chamber controls. The rodent lung neoplasms that arise either spontaneously or from chronic exposure to chemicals morphologically resemble non-small cell lung cancer in humans[264].

The carcinogenic process involves the alteration of four broad categories of cancer-associated genes: proto-oncogenes, tumor suppressor genes, apoptosis genes, and repair genes[265]. At least 80 genetic alterations in cancer genes, a dozen of which are considered "driver" mutations, are involved in the cancer process. Proto-oncogene and tumor suppressor gene mutation analysis has revealed mechanistic insights in chemical carcinogenesis. Mutations may be due to direct interaction of the chemical or its metabolite with DNA, or indirectly via altering DNA replicative or repair (epigenetic) processes. A change in the frequency and spectrum of cancer gene mutations reveals chemical-specific pathways that either override or include promotion of endogenous events. Interpretation of these data is complicated because mutation profiles are dependent on species, strain, gender, tumor differentiation, dose of carcinogen, as well as dosing regimen. Our main objective was to assess whether mutation frequencies and spectra in alveolar/bronchiolar carcinomas in treated rats and mice differed from published literature on spontaneous rodent and human lung cancers.

Lung cancer is a complex disease with variable clinical presentations and behavior. Genome sequencing of lung cancers in humans has identified several "driver" mutations that may play an important role in lung carcinogenesis. These cancer genes include *KRAS, EGFR, TP53, EML4-ALK, HER2* (or *ERBB2*), *BRAF, PIK3CA, PTEN, STK11, FGFR1, DDR2, AKT1, MAP2K1,* and *MET*[266]. These genes encode proteins that are critical for cellular proliferation and survival, as well as cellular transformation and tumorigenesis. We performed extensive mutation analyses on the most commonly altered cancer genes in human lung cancer (*KRAS*, *EGFR*, and *TP53*) in alveolar/bronchiolar carcinomas from F344/NTac rats and B6C3F1/N mice exposed to various concentrations of cobalt metal by inhalation for 2 years.

In human non-small cell lung cancer, the incidence of *KRAS* mutations is approximately 26% (67/254)[267]. Of these, 86% of the mutations arise within codon 12, and 14% occur in codon 13. Point mutations in codons 12, 13, and 61 of *KRAS* are activating mutations that result in constitutive activation of the KRAS protein, making it refractory to the inhibitory GTPase activating proteins, which results in stimulus-independent, persistent activation of downstream effectors, in particular the RAF-MEK-ERK cascade. Constitutive activation of this kinase cascade results in promotion of cellular proliferation and transformation[268; 269].

The incidence of *EGFR* mutations in the non-small cell lung cancer subtype of adenocarcinoma in humans is about 9% (22/254) with the majority (70%) of the mutations located within codons

19 and 21[267]. EGFR is a transmembrane receptor, which upon ligand binding, dimerizes and activates the cytosolic kinase domain of the receptor tyrosine kinase resulting in activation of signaling pathways that support cancer development and progression. These signaling pathways include the PI3K pathway, which when activated leads to AKT activation and apoptosis inhibition, and the GRB2 and SOS pathways, which lead to activation of P21RAS, resulting in cell cycle progression[270].

The incidence of *TP53* mutations in human non-small cell lung cancer is approximately 50%; the frequency of this mutation is relatively increased in smokers[271]. *TP53* is regarded as a master regulator gene that is frequently altered in a wide range of cancers, and as a tumor suppressor, its critical roles in cell cycle control, apoptosis, and DNA repair are compromised when the gene is mutated. Tobacco-associated lung cancer has a high frequency of transversions (e.g., purine to pyrimidine or pyrimidine to purine) in *TP53*, whereas lung cancers in humans who have never smoked are associated with transitions (e.g., pyrimidine to pyrimidine or purine to purine), especially in women[272; 273]. In contrast to *KRAS* and *EGFR* mutations, which are predominantly noted only in the non-small cell lung cancer subtype of adenocarcinoma, mutations in *TP53* are observed equally in both squamous cell carcinoma and adenocarcinoma subtypes of non-small cell lung cancer[274].

K.2. Materials and Methods

K.2.1. Animals and Tissue Sampling

Alveolar/bronchiolar carcinoma tissue with adjacent nontumor tissue was obtained from F344/NTac rats and B6C3F1/N mice exposed to cobalt metal by inhalation for 2 years; spontaneous alveolar/bronchiolar carcinomas were obtained from chamber control mice at terminal kill. There were no spontaneous alveolar/bronchiolar carcinomas in the F344/NTac rats in the concurrent cobalt metal study. Hence, the spontaneous alveolar/bronchiolar carcinomas (n=10) were sourced from F344 rat vehicle control groups in various NTP chronic bioassays. These studies include sodium azide (VM55), elmiron (VM17), ginseng (VM46), *tert*-butyl alcohol (VM10) (all gavage, water vehicle); probenecid (VF23), isoeugenol (VM3, VM33) (all gavage, corn oil vehicle); trimethylolpropane triacrylate (VM63) (topical application in acetone vehicle); lauric acid diethanolamine condensate (VF163) (topical application in ethanol vehicle); and ethylbenzene (VM460) (inhalation of filtered air). These spontaneous alveolar/bronchiolar carcinoma samples were sourced from eight male and two female F344 rats. The mouse tumors that were selected for mutation analysis were generally greater than 5 mm in diameter, whereas the rat tumors were smaller and scattered throughout the pulmonary parenchyma. Normal lungs without tumors from age-matched chamber control rats and mice were also collected for histologic evaluation and molecular analysis. After formalin fixation, the tissues were transferred to 70% ethanol and were routinely processed in graded alcohols, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for microscopic analysis. The formalin fixed paraffin embedded (FFPE) tissues were used for mutation analysis. The alveolar/bronchiolar carcinomas were selected for molecular biology analysis based on their overall size and viability (minimal to no necrosis/hemorrhage microscopically) in order to maximize the amount and quality of DNA obtained from FFPE sections. DNA quality was measured using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington DE) to calculate the 260/280 nm absorbance ratio, and DNA samples with a purity range of 1.7 to 2.0 were used

for analysis. Samples falling outside of this range were reisolated from FFPE sections until a suitable purity measure was obtained or discarded.

K.2.2. DNA Extraction, Polymerase Chain Reaction, Autosequencing, and Mutation Analysis

Alveolar/bronchiolar carcinoma samples (48 from F344/NTac rats and 69 from B6C3F1/N mice) representing all cobalt metal exposed groups and spontaneous alveolar/bronchiolar carcinomas (10 each from chamber control rats and mice) were evaluated for hot spot mutations within specific codons or exons in *Kras*, *Egfr*, and *Tp53* genes that are relevant in human lung cancer. FFPE sections at 10 μm thickness were collected on glass slides. The neoplasms (single or multiple from the same lung) were dissected with a sharp microtome blade and collected into screw top tubes. In some cases (especially in rats) where the alveolar/bronchiolar carcinomas had a miliary distribution and were scattered throughout the pulmonary parenchyma, the entire lung section was used for DNA isolation. DNA was isolated from these FFPE dissected neoplasm tissue sections with the DNeasy® Tissue Kit (Qiagen, Valencia, CA). Amplification reactions were carried out by semi-nested polymerase chain reaction (PCR) using primer sets designed for *Kras* (exons 1 and 2), *Tp53* (exons 5 through 8), and *Egfr* (exons 18 through 21) (Table K-1 and Table K-2). Controls lacking DNA were run with all sets of reactions. PCR products were purified using a QIAquick® Gel Extraction Kit (Qiagen). The purified products were cycled with Terminal Ready Reaction Mix-Big Dye® (Perkin-Elmer, Foster City, CA), and the extension products were purified with the DyeEx® 2.0 SpinKit (Qiagen). The lyophilized PCR products were sequenced with an automatic sequencer (Perkin-Elmer ABI Model 3100). The resulting electropherograms were compared to identify mutations in alveolar/bronchiolar carcinomas that either arise spontaneously or due to exposure to cobalt metal[275].

K.2.3. Statistics

To test for significance of exposure concentration-related trends in the incidences of mutations, a one-sided Cochran-Armitage test was conducted. A Fisher exact test was conducted to test for significant differences in the number of mutations between the control and various exposure groups.

K.3. Results

K.3.1. Rats

The incidence of *Kras* mutations in lung alveolar/bronchiolar carcinomas from F344/NTac rats exposed to cobalt metal by inhalation for 2 years was 31% (15/48) (Table K-3, supplemented by Table K-4 and Table K-5). The majority of *Kras* mutations in the rat were within codon 12 [29% (14/48)], followed by codon 13 [2% (1/48)]. Surprisingly there were no mutations within codon 61. The most common codon 12 mutations in rat alveolar/bronchiolar carcinomas from all groups exposed to cobalt metal were G→T transversions [57% (8/14)] and G→A transitions [43% (6/14)]. The frequencies of *Kras* mutations were 14% (2/14), 35% (6/17), and 41% (7/17) in alveolar/bronchiolar carcinomas from the 1.25, 2.5, and 5 mg/m^3 groups, respectively. *Kras* mutations were not detected in spontaneous alveolar/bronchiolar carcinomas from chamber control rats in this study.

The incidence of *Egfr* mutations in alveolar/bronchiolar carcinomas from F344/NTac rats exposed to cobalt metal by inhalation for 2 years was 17% (8/48) (Table K-3, supplemented by Table K-4 and Table K-5). The majority of *Egfr* mutations in alveolar/bronchiolar carcinomas from rats exposed to cobalt metal for 2 years were present in exon 20 (codons 768, 770, 771, 785, 790, 791, and 806) [13% (6/48)], followed by exon 21 (codons 845 and 875) [4% (2/48)] and exon 19 (codon 750) [2% (1/48)]. The frequencies of *Egfr* mutations in alveolar/bronchiolar carcinomas from rats exposed to cobalt metal were 14% (2/14), 18% (3/17), and 18% (3/17) from the 1.25, 2.5, and 5 mg/m^3 groups, respectively. A majority of *Egfr* mutations in rat alveolar/bronchiolar carcinomas were transitions such as G→A [50% (5/10)] or C→T [30% (3/10)]. *Egfr* mutations were not detected in spontaneous alveolar/bronchiolar carcinomas from chamber control rats in this study.

The incidence of *Tp53* mutations in alveolar/bronchiolar carcinomas from F344/NTac rats exposed to cobalt metal for 2 years was 23% (11/48) (Table K-7, supplemented by Table K-8 and Table K-9). The majority of *Tp53* mutations in alveolar/bronchiolar carcinomas from exposed rats were present within exon 6 (codons 203, 212, 242, and 247) [10% (5/48)], followed by exon 7 (codons 266, 277, and 278) [6% (3/48)] and exon 8 (codons 314 and 321) [6% (3/48)]. The frequencies of *Tp53* mutations were 21% (3/14), 35% (6/17), and 12% (2/17) in the alveolar/bronchiolar carcinomas from the 1.25, 2.5, and 5 mg/m^3 groups, respectively. The majority of *Tp53* mutations in rats were transitions [85% (11/13)] such as C→T [38% (5/13)] or G→A [31% (4/13)]. *Tp53* mutations were not observed in spontaneous alveolar/bronchiolar carcinomas from chamber control rats in this study.

K.3.2. Mice

The incidence of *Kras* mutations in lung alveolar/bronchiolar carcinomas from B6C3F1/N mice exposed to cobalt metal for 2 years was 67% (46/69) (Table K-10; supplemented by Table K-11 and Table K-12). The majority of *Kras* mutations were localized within codon 12 [43% (30/69)], followed by codons 61 [20% (14/69)] and 13 [6% (4/69)]. The most common codon 12 mutations in alveolar/bronchiolar carcinomas from all groups exposed to cobalt metal were G→T transversions (G<u>G</u>T→G<u>T</u>T or <u>T</u>GT) [80% (24/30)] and G→A transitions (G<u>G</u>T→G<u>A</u>T) [17% (5/30)]. The frequencies of *Kras* mutations were 69% (11/16), 48% (11/23), and 80% (24/30) in alveolar/bronchiolar carcinomas from the 1.25, 2.5, and 5 mg/m^3 groups, respectively. *Kras* mutations were not detected in spontaneous alveolar/bronchiolar carcinomas in this study. However, according to historical control data, *Kras* mutation incidence in spontaneous alveolar/bronchiolar carcinomas is 27% (34/124), and these mutations are localized within codon 12 [16% (20/124)], followed by codons 61 [6% (8/124)] and 13 [5% (6/124)][275]. The most common mutation in codon 12 is a G→A transition (G<u>G</u>T→G<u>A</u>T) [70% (14/20)].

The incidence of *Egfr* mutations in alveolar/bronchiolar carcinomas from B6C3F1/N mice exposed to cobalt metal by inhalation was relatively low. The incidence of *Egfr* mutations in mouse alveolar/bronchiolar carcinomas due to exposure to cobalt metal for 2 years was 17% (12/69) (Table K-13, supplemented by Table K-11 and Table K-12). All regions of DNA that were queried for mutations (exons 18 through 21) encode the tyrosine kinase domain. The majority of *Egfr* mutations within alveolar/bronchiolar carcinomas from groups exposed to cobalt metal were present within exon 20 (codons 780, 791, 801, 812, and 817) [9% (6/69)], followed by exons 21 (codons 846, 861, 870, and 873) [6% (4/69)], 18 (codon 721) and 19 (codon 735) [each 1% (1/69)]. The frequencies of *Egfr* mutations in mouse alveolar/bronchiolar

carcinomas exposed to cobalt metal were 13% (2/16), 30% (7/23), and 10% (3/30) from the 1.25, 2.5, and 5 mg/m^3 groups, respectively. A majority of *Egfr* mutations in mouse alveolar/bronchiolar carcinomas were transition mutations such as G→A [42% (5/12)] or C→T [17% (2/12)]. *Egfr* mutations were not detected in spontaneous alveolar/bronchiolar carcinomas from chamber control mice in this study.

The incidence of *Tp53* mutations in alveolar/bronchiolar carcinomas from B6C3F1/N mice exposed to cobalt metal for 2 years was 19% (13/69) (Table K-14, supplemented by Table K-15 and Table K-16). The majority of *Tp53* mutations in alveolar/bronchiolar carcinomas from mice exposed to cobalt metal was found within exon 5 (codons 155, 156, 158, and 179) [10% (7/69)], followed by exons 6 (codons 194 and 212) [4% (3/69)] and 7 (codons 230, 232, 239, and 257) [6% (4/69)]. The majority of mutations were transversions [60% (9/15)], most of which were G→C. The frequencies of *Tp53* mutations were 19% (3/16), 13% (3/23), and 20% (6/30) in alveolar/bronchiolar carcinomas from the 1.25, 2.5, and 5 mg/m^3 groups, respectively. *Tp53* mutations were not detected in spontaneous alveolar/bronchiolar carcinomas from chamber control mice in this study.

According to the historical control database (all routes, all vehicles, NTP historical control database, May 2011), male B6C3F1 mice have a higher incidence (12.5%) of spontaneous alveolar/bronchiolar carcinomas than B6C3F1 female mice (3.7%). However, there were no significant differences in the number or type of mutations evaluated in *Kras, Egfr*, or *Tp53* genes between male and female B6C3F1/N mice chronically exposed to cobalt metal by inhalation.

K.4. Discussion

Kras mutations were more frequent than *Egfr* and *Tp53* mutations within the lung alveolar/bronchiolar carcinomas from F344/NTac rats and B6C3F1/N mice exposed to cobalt metal for 2 years in the concurrent studies. It is interesting to note that in mice, *Kras* mutations were observed in both spontaneous alveolar/bronchiolar carcinomas [27% (34/124); Hong et al.[275]] as well as in alveolar/bronchiolar carcinomas from mice exposed to cobalt metal [67% (46/69)]. However, alveolar/bronchiolar carcinomas from mice exposed to cobalt metal had predominantly G→T transversions [80% (24/30)], whereas the spontaneous alveolar/bronchiolar carcinomas had G→A transitions [70% (14/20)] in codon 12. Not surprisingly, G→T transversions were also the most predominant [67% (6/9)] mutations in alveolar/bronchiolar carcinomas from mice exposed to cobalt sulfate heptahydrate aerosols for 2 years (Table K-17; NTP[122]). Incidentally, G→T transversions are one of the most common *Kras* mutations in human lung cancer[260]. G→T *Kras* mutations appear to correlate with 8-hydroxydeoxyguanine adducts that result from oxidative stress. G→T transversions in codon 12 of *Kras* were also noted previously in several lung neoplasms in mice exposed to various other chemicals such as tobacco-specific nitrosamines, aflatoxin B1, vanadium pentoxide, ozone, cumene, ethylene oxide, and transplacental AZT exposure[276-279]. In the current study, it is not surprising that these mutations were seen almost exclusively in alveolar/bronchiolar carcinomas from mice exposed to cobalt metal and not in spontaneous alveolar/bronchiolar carcinomas. It has been demonstrated that cobalt can induce hypoxia and upregulate HIF-1α signaling, thereby modulating inflammatory responses and inducing oxidative stress[154]. Thus, the G→T transversions that were observed in mice, primarily within codon 12 of *Kras*, may be attributable to oxidative stress resulting from chronic cobalt metal exposure.

EGFR, together with KRAS, plays an important role in initiating and maintaining the MAPK signaling cascade and other signaling pathways that are relevant to cancer. *Kras* mutations in non-small cell lung cancer are observed more frequently in smokers, while *Egfr* mutations in lung cancers are most frequently observed in women and people who have never smoked[267]. The incidence of *Egfr* mutations in alveolar/bronchiolar carcinomas from rats and mice exposed to cobalt metal was lower than the incidence of *Kras* mutations. In contrast to the *Kras* mutations which are predominantly transversions, *Egfr* mutations in both rat and mouse alveolar/bronchiolar carcinomas were G→A or C→T transitions. This is the first study in which *Egfr* mutations were examined in the context of chemically induced rodent lung neoplasms. Mutations within *Egfr* and *Kras* are mutually exclusive in human lung and colon cancers, and the treatment modalities depend on *Egfr* and *Kras* mutation status of the respective neoplasm. Surprisingly, in alveolar/bronchiolar carcinomas from rodents exposed to cobalt metal, 38% (3/8) of rats and 25% (3/12) of mice that harbored *Egfr* mutations also had *Kras* mutations.

Mutations in *Tp53* in chemically induced rodent models are considered a late event, especially in neoplasms that are initiated by mutations in *Kras*[256]. The incidences of *Tp53* mutations resulting from exposure to cobalt metal for 2 years were similar in rats and mice, but the nature of the mutations was different; *Tp53* mutations in rats were predominantly transitions, whereas in mice, they were predominantly transversions. Interestingly, in humans, *Tp53* transversions are commonly observed in non-small cell lung cancer from smokers, while *Tp53* transitions are often noted in non-small cell lung cancer from people who have never smoked. The significance of this differential mutation spectrum in rats and mice and its relationship to human lung cancer needs to be further explored.

In summary, there was a significantly higher incidence of *Kras* mutations, accompanied by a lower incidence of *Egfr* and *Tp53* mutations, in alveolar/bronchiolar carcinomas from rats and mice exposed to cobalt metal for 2 years. Several of the observed mutations in *Kras* are comparable to mutations observed in other NTP studies in which a significant increase in the incidence of lung neoplasms in response to chronic chemical exposure was evident. In addition, these mutations arise within the hotspot regions of *Kras, Egfr*, and *Tp53* genes, and are thus comparable to the mutations observed in human non-small cell lung cancer.

Table K-1. Primers Used to Amplify the Hot Spot Regions of Rat *Kras*, *Tp53*, and *Egfr* Genes

Exon	Codon	Primer	Strand	Sequence
1	*Kras*-12-13	RK12F25927	Sense	5′-ACTTGTGGTAGTTGGAGC-3′
		RK12R26069	Antisense	5′-CTGCCACCCTTTACAAATTG-3′
		RK12R26034	Antisense	5′-GCAGCATTTACCTCTATCGT-3′
2	*Kras*-61	RK61F14325	Sense	5′-ATCCAGACTGTGTTTCTACC-3′
		RK61R13986	Antisense	5′-CAGGAATTCTACATACTTGACAC-3′
		RK61R14035	Antisense	5′-TGCAGGCCTAACAACTAGC-3′
5	*Tp53*-124-184	Rp53Ex5OF1366	Sense	5′-CCTAGTTGGCTTGTCCG-3′
		Rp53Ex5OR1671	Antisense	5′-AGCAAGAATAAGTCAGAGGC-3′
		Rp53Ex5IF1382	Sense	5′-CGCTGACCTTTGATTCTTTCTCC-3′
		Rp53Ex5IR1639	Antisense	5′-GACAACCAGTTCTAAACCCCACAG-3′
6	*Tp53*-185-259	Rp53Ex6OF1620	Sense	5′-TGGGGTTAGAACTGGTTG-3′
		Rp53Ex6OR1963	Antisense	5′-GAACAAAAACAGGCCGAG-3′
		Rp53Ex6IF1645	Sense	5′-TCTCCCGGCCTCTGACTTATTC-3′
		Rp53Ex6IR1927	Antisense	5′-CAGCCCAACCTGGCACAC-3′
7	*Tp53*-260-304	Rp53Ex7OF2101	Sense	5′-AGCTCCAGATAGGACAAG-3′
		Rp53Ex7OR2434	Antisense	5′-TGGGCAGTGCTATGGAAG-3′
		Rp53Ex7IF2166	Sense	5′-AGCTTTCTTACTGCCTTGTG-3′
		Rp53Ex7IR2402	Antisense	5′-TGACTTTGGGGTGAAGCTG-3′
8	*Tp53*-305-329	Rp53Ex8OF2333	Sense	5′-GGAGTGCAAAGAGAGGTG-3′
		Rp53Ex8OR2602	Antisense	5′-TGCGCTCTGACGATAATG-3′
		Rp53Ex8IF2386	Sense	5′-GCTTCACCCCAAAGTCAC-3′
		Rp53Ex8IR2549	Antisense	5′-GCGTTTTGTGTCCTAGACTTAG-3′
18	*Egfr*-689-729	REgfr18F144544	Sense	5′-ACACATTGCTCCTTTGATCAC-3′
		REgfr18R144922	Antisense	5′-AACACGAGTTCCTACATAAACC-3′
		REgfr18R145009	Antisense	5′-CACTCCCAAGTTTATGCCTC-3′
19	*Egfr*-730-762	REgfr19F146377	Sense	5′-ACAAGGCAACATGCTGCTG-3′
		REgfr19R146775	Antisense	5′-TGACTTTACTCTTCCCTCCCC-3′
		REgfr19R146605	Antisense	5′-ACACAAACTAAGGAAGCAAGAC-3′
20	*Egfr*-763-824	REgfr20F150643	Sense	5′-ACATGTGTTGTCCTTACC-3′
		REgfr20R150924	Antisense	5′-ATTCATCCTGCTTCTGAAACC-3′
		REgfr20R150905	Antisense	5′-CCTGCTATTGGCTCTTTG-3′
21	*Egfr*-825-876	REgfr21F162620	Sense	5′-TCACTCCCTCACTGAAGC-3′
		REgfr21R162881	Antisense	5′-CTACAGCTGACACATAGG-3′
		REgfr21R162852	Antisense	5′-GGGCTGTCAGGAAAATGC-3′

Table K-2. Primers Used to Amplify the Hot Spot Regions of Mouse *Kras*, *TP53*, and *Egfr* Genes

Exon	Codon	Primer	Strand	Sequence
1	*Kras*-12-13	K12AOS	Sense	5′-TTATTGTAAGGCCTGCTGAA-3′
		K12AOA	Antisense	5′-GCAGCGTTACCTCTATCGTA-3′
		K12AIS	Sense	5′-ATGACTGAGTATAAACTTGT-3′
		K12AIA	Antisense	5′-TCGTACTCATCCACAAAGTG-3′
2	*Kras*-61	K61OS	Sense	5′-TTCTCAGGACTCCTACAGGA-3′
		K61OA	Antisense	5′-ACCCACCTATAATGGTGAAT-3′
		APK61IS	Sense	5′-TACAGGAAACAAGTAGTAATTGATGGAGAA-3′
		APK61IA	Antisense	5′-ATAATGGTGAATATCTTCAAATGATTTAGT-3′
5-6	*Tp53*-123-221	Mp53F1407	Sense	5′-TCCCCACCTTGACACCTG-3′
		Mp53R1885	Antisense	5′-GTCTCTAAGACGCACAAACC-3′
		Mp53F1453	Sense	5′-GTTCTCTCTCCTCTCTTCCAG-3′
7	*Tp53*-222-258	Mp7FO	Sense	5′-GGTCACCTGTAGTGAGGTAG-3′
		Mp7RO	Antisense	5′-GGAACAGAAACAGGCAGAAG-3′
		Mp7FI	Sense	5′-TGTAGTGAGGTAGGGAGCGAC-3′
		Mp7RI	Antisense	5′-AAGCTGGGGAAGAAACAGGC-3′
8	*Tp53*-259-303	Mp8FO	Sense	5′-GTTTACACACAGTCAGGATGG-3′
		Mp8RO	Antisense	5′-TGTGGAAGGAGAGAGCAAG-3′
		Mp8FI	Sense	5′-AGCTTTCTTACTGCCTTGTGC-3′
		Mp8RI	Antisense	5′-TGAAGCTCAACAGGCTCCTC-3′
18	*Egfr*-688-728	Egfr18F138824	Sense	5′-CCACTGCTCCTTTGAACAC-3′
		Egfr18F138836	Sense	5′-TGAACACATTGCTCCTTTGAAC-3′
		Egfr18R139212	Antisense	5′-TGGAGAGCACAGCAAACAC-3′
19	*Egfr*-729-761	Egfr19F140636	Sense	5′-GAGCTTGATAGCTAAGAACCTC-3′
		Egfr19F140659	Sense	5′-GAAATATGAAGAGTCCCAGCAC-3′
		Egfr19R141071	Antisense	5′-CCAGAATACTTCCAAACAGTCC-3′
20	*Egfr*-762-823	Egfr20F144483	Sense	5′-TCC TTTTAACATGCAACATCCC-3′
		Egfr20F144598	Sense	5′-GGGGGGGCATTTCATTTTAC-3′
		Egfr20R145114	Antisense	5′-CAGACACACACACCTATCATC-3′
21	*Egfr*-824-875	Egfr21F151895	Sense	5′-TGTCTTGTCATTCATGCCAG-3′
		Egfr21F151978	Sense	5′-TGTTGAGCAGCCTAGAGATTC-3′
		Egfr21R152444	Antisense	5′-TCCTCCTTACTACTCCCACC-3′

Table K-3. *Kras* Mutations in Alveolar/Bronchiolar Carcinomas from Male and Female F344/NTac Rats in the Two-year Inhalation Study of Cobalt Metal

Cobalt Metal Concentration	Mutation Frequency	Codon 12 (GGT)				Codon 13 (GGC)	Codon 61 (CAA)
		(GAT)	(TGT)	(GTT)	(TTT)	(CGC)	
0 mg/m3#	0/10 (0%)**	0	0	0	0	0	0
1.25 mg/m3	2/14 (14%)	1	0	1	0	0	0
2.5 mg/m3	6/17 (35%)*	4	1	0	0	1	0
5 mg/m3	7/17 (41%)*	1	2	3	1	0	0
All exposed groups combined	15/48 (31%)*	6	3	4	1	1	0

*Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact test.
**Significant trend ($P \leq 0.001$) by the Cochran-Armitage trend test.
#There were no spontaneous alveolar/bronchiolar carcinomas in the cobalt metal study. Hence, the spontaneous alveolar/bronchiolar carcinomas (n = 10) were sourced from vehicle or chamber control groups in various NTP chronic bioassays. These spontaneous alveolar/bronchiolar samples were sourced from eight male and two female F344/N rats.

Table K-4. *Kras* and *Egfr* Mutations in Alveolar/Bronchiolar Carcinomas from Male F344/NTac Rats in the Two-year Inhalation Study of Cobalt Metal

Sample/ Animal #	Cobalt Conc. (mg/m^3)	*Kras*			*Egfr*			
		Codon 12	Codon 13	Codon 61	Exon 18	Exon 19	Exon 20	Exon 21
1/M55	0	none	none	none	none	none	none	none
2/M46	0	none	none	none	none	none	none	none
3/M17	0	none	none	none	none	none	none	none
4/M10	0	none	none	none	none	none	none	none
5/M03	0	none	none	none	none	none	none	none
6/M33	0	none	none	none	none	none	none	none
7/M63	0	none	none	none	none	none	none	Codon 837 CGT→CGC (Arg→Arg)
8/M46	0	none	none	none	none	none	none	Codon 863 CTT→CTA (Leu→Leu)
9/M215	1.25	none	none	none	none	none	none	none
10/M230	1.25	none	none	none	none	none	none	Codon 875 GGC→GGT (Gly→Gly)
11/M249	1.25	none	none	none	none	none	none	none
12/M202	1.25	none	none	none	none	none	none	none
13/M226	1.25	none	none	none	none	none	none	none
14/M233	1.25	none	none	none	none	none	none	none
15/M241	1.25	none	none	none	none	none	none	none

Sample/ Animal #	Cobalt Conc. (mg/m^3)	*Kras*			*Egfr*			
		Codon 12	**Codon 13**	**Codon 61**	**Exon 18**	**Exon 19**	**Exon 20**	**Exon 21**
16/M247	1.25	none	none	none	none	none	none	none
17/M248	1.25	none	none	none	none	none	none	Codon 845 CTG→CAG (Leu→Gln)
18/M406	2.5	none	none	none	none	none	none	none
19/M408	2.5	none	GGC→CGC (Gly→Arg)	none	none	none	Codon 791 ACA→ATA (Thr→Ile) Codon 806 CAT→TAT (His→Tyr)	none
20/M413	2.5	none	none	none	none	none	none	none
21/M425	2.5	none	none	none	none	none	none	none
22/M438	2.5	none	none	none	none	none	none	none
23/M448	2.5	GGT→TGT (Gly→Cys)	none	none	none	none	none	none
24/M417	2.5	GGT→GAT (Gly→Asp)	none	none	none	none	none	none
25/M418	2.5	GGT→GAT (Gly→Asp)	none	none	none	none	none	none
26/M411	5	GGT→TGT (Gly→Cys)	none	none	none	none	none	none
27/M641	5	none	none	none	none	none	none	none
28/M649	5	none	none	none	none	none	none	none
29/M605	5	none	none	none	none	none	none	none
30/M608	5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
31/M611	5	none	none	none	none	none	none	none
32/M615	5	none	none	none	none	none	none	Codon 875 GGC→AGC (Gly→Ser)
33/M616	5	none	none	none	none	none	none	none
34/M618	5	none	none	none	none	none	none	none

Table K-5. *Kras* and *Egfr* Mutations in Alveolar/Bronchiolar Carcinomas from Female F344/NTac Rats in the Two-year Inhalation Study of Cobalt Metal

Sample/ Animal #	Cobalt Conc. (mg/m^3)	*Kras*			*Egfr*			
		Codon 12	Codon 13	Codon 61	Exon 18	Exon 19	Exon 20	Exon 21
1/F224	0	none	none	none	none	none	none	none
2/F163	0	none	none	none	none	none	none	none
3/F314	1.25	GGT→GTT (Gly→Val)	none	none	none	none	none	none
4/F337	1.25	none	none	none	none	none	none	none
5/F338	1.25	none	none	none	none	none	none	none
6/F342	1.25	none	none	none	none	none	Codon 790 ATT→ACT (Ile→Thr)	none
7/F348	1.25	GGT→GAT (Gly→Asp)	none	none	none	none	none	Codon 844 GTA→GTG (Val→Val)
8/F504	2.5	none	none	none	none	none	none	none
9/F505	2.5	GGT→GAT (Gly→Asp)	none	none	none	none	none	Codon 838 GAC→GAT (Asp→Asp)
10/F513	2.5	none	none	none	none	none	Codon 770 GTG→ATG (Val→Met)	none
11/F514	2.5	GGT→GAT (Gly→Asp)	none	none	none	none	none	none
12/F519	2.5	none	none	none	none	none	none	none
13/F520	2.5	none	none	none	none	Codon 750 GAA→AAA (Glu→Lys)	Codon 771 GAC→AAC (Asp→Asn)	none
14/F539	2.5	none	none	none	none	none	none	none
15/F543	2.5	none	none	none	none	none	none	none
16/F548	2.5	none	none	none	none	none	none	none
17/F707	5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
18/F723	5	GGT→GAT (Gly→Asp)	none	none	none	none	Codon 768 GCC→ACC (Ala→Thr)	none
19/F728	5	none	none	none	none	none	none	none
20/F746	5	none	none	none	none	none	none	none
22/F703	5	GGT→TGT (Gly→Cys)	none	none	none	none	none	none

Sample/ Animal #	Cobalt Conc. (mg/m^3)	*Kras*			*Egfr*			
		Codon 12	Codon 13	Codon 61	Exon 18	Exon 19	Exon 20	Exon 21
21/F701	5	GGT→GTT (Gly→Val)	none	none	none	none	Codon 785 TCC→TTC (Ser→Phe)	none
23/F711	5	none	none	none	none	none	none	none
24/F717	5	GGT→TTT (Gly→Phe)	none	none	none	none	none	none

Table K-6. *Egfr* Mutations in Alveolar/Bronchiolar Carcinomas from Male and Female F344/NTac Rats in the Two-year Inhalation Study of Cobalt Metal[a]

Cobalt Metal Concentration	Mutation Frequency	Exon 18	Exon 19	Exon 20	Exon 21
0 $mg/m^{3\#}$	0/10 (0%)	0	0	0	0
1.25 mg/m^3	2/14 (14%)	0	0	1	1
2.5 mg/m^3	3/17 (18%)	0	1[b]	3[b]	0
5 mg/m^3	3/17 (18%)	0	0	2	1
All exposed groups combined	8/48 (17%)	0	1[b]	6[b]	2

[#]There were no spontaneous alveolar/bronchiolar carcinomas in the cobalt metal study. Hence, the spontaneous alveolar/bronchiolar carcinomas (n = 10) were sourced from vehicle or chamber control groups in various NTP chronic bioassays. These spontaneous alveolar/bronchiolar samples were sourced from eight male and two female F344/N rats.
[a]Silent mutations are not included.
[b]Same animal with multiple mutations.

Table K-7. *Tp53* Mutations in Alveolar/Bronchiolar Carcinomas from Male and Female F344/NTac Rats in the Two-year Inhalation Study of Cobalt Metal[a]

Cobalt Metal Concentration	Mutation Frequency	Exon 5	Exon 6	Exon 7	Exon 8
0 $mg/m^{3\#}$	0/10 (0%)	0	0	0	0
1.25 mg/m^3	3/14 (21%)	1	1[b]	1[b]	1
2.5 mg/m^3	6/17 (35%)*	0	4[b]	1[b]	2
5 mg/m^3	2/17 (12%)	1	0	1	0
All exposed groups combined	11/48 (23%)	2	5[b]	3[b]	3

*Significantly different ($P \leq 0.05$) from the chamber control group by the Fisher exact test.
[#]There were no spontaneous alveolar/bronchiolar carcinomas in the cobalt metal study. Hence, the spontaneous alveolar/bronchiolar carcinomas (n = 10) were sourced from vehicle or chamber control groups in various NTP chronic bioassays. These spontaneous alveolar/bronchiolar samples were sourced from eight male and two female F344/N rats.
[a]Silent mutations are not included.
[b]Same animal with double mutations.

Table K-8. *Tp53* Mutations in Alveolar/Bronchiolar Carcinomas from Male F344/NTac Rats in the Two-year Inhalation Study of Cobalt Metal

Sample/Animal #	Cobalt Metal Concentration (mg/m^3)	Exon 5	Exon 6	Exon 7	Exon 8
1/M55	0	none	none	none	none
2/M46	0	none	none	none	none
3/M17	0	none	none	none	none
4/M10	0	none	none	none	none
5/M03	0	none	Codon 205 GAC→GAT (Asp→Asp)	none	none
6/M33	0	none	none	none	none
7/M63	0	none	none	none	none
8/M46	0	none	none	none	none
9/M215	1.25	Codon 134 CAG→TAG (Gln→Stop)	none	none	none
10/M230	1.25	Codon 160 ATC→ATT (Ile→Ile)	none	none	none
11/M249	1.25	none	none	none	none
12/M202	1.25	none	none	none	none
13/M226	1.25	none	none	none	none
14/M233	1.25	none	none	none	none
15/M241	1.25	none	none	none	none
16/M247	1.25	none	none	none	none
17/M248	1.25	none	none	none	none
18/M406	2.5	none	Codon 247 CGG→CAG (Arg→Gln)	none	none
19/M408	2.5	none	none	none	Codon 321 CTC→TTC (Leu→Phe)
20/M413	2.5	none	Codon 206 (GAC→GAT (Asp→Asp) Codon 242 GGG→GAG (Gly→Gln)	none	none
21/M425	2.5	none	none	none	none
22/M438	2.5	none	none	none	none
23/M448	2.5	none	none	none	none

Sample/Animal #	Cobalt Metal Concentration (mg/m^3)	Exon 5	Exon 6	Exon 7	Exon 8
24/M417	2.5	none	none	none	none
25/M418	2.5	none	none	none	none
26/M411	5	none	none	none	none
27/M641	5	none	none	none	none
28/M649	5	none	Codon 258 TCC→TCT (Ser→Ser)	none	none
29/M605	5	none	Codon 242 GGG→GGA (Gly→Gly)	Codon 277 GGG→GAG (Gly→Glu)	none
30/M608	5	none	none	none	none
31/M611	5	none	none	none	none
32/M615	5	none	none	none	none
33/M616	5	none	none	none	none
34/M618	5	Codon 135 CTG→CGG (Leu→Arg)	none	none	none

Table K-9. *Tp53* Mutations in Alveolar/Bronchiolar Carcinomas from Female F344/NTac Rats in the Two-year Inhalation Study of Cobalt Metal

Sample/Animal #	Cobalt Metal Concentration (mg/m^3)	Exon 5	Exon 6	Exon 7	Exon 8
1/F224	0	none	none	none	none
2/F163	0	none	none	none	none
3/F314	1.25	none	Codon 203 TAT→CAT (Tyr→His)	Codon 278 AGA→GGA (Arg→Gly)	none
4/F337	1.25	none	none	none	none
5/F338	1.25	none	none	none	none
6/F342	1.25	none	none	none	Codon 314 CCC→CTC (Pro→Leu)
7/F348	1.25	none	none	none	none
8/F504	2.5	none	Codon 242 GGG→AGG (Gly→Arg)	Codon 266 GAC→TAC (Asp→Tyr)	none
9/F505	2.5	none	none	none	none
10/F513	2.5	none	none	none	Codon 314 CCC→CTC (Pro→Leu)
11/F514	2.5	none	Codon 212 CAC→TAC (His→Tyr)	none	none
12/F519	2.5	none	none	none	none
13/F520	2.5	none	none	none	none
14/F539	2.5	none	none	none	none
15/F543	2.5	none	none	none	none
16/F548	2.5	none	none	none	none
17/F707	5	none	none	none	none
18/F723	5	none	none	none	none
19/F728	5	none	none	none	none
20/F746	5	none	none	none	none
21/F701	5	none	none	none	none
22/F703	5	none	none	none	none
23/F711	5	none	none	none	none
24/F717	5	none	none	none	none

Table K-10. *Kras* Mutations in Alveolar/Bronchiolar Carcinomas from Male and Female B6C3F1/N Mice in the Two-year Inhalation Study of Cobalt Metal

Cobalt Metal Concentration	Mutation Frequency	Codon 12 (GGT)				Codon 13 (GGC)	Codon 61 (CAA)		
		(GAT)	(TGT)	(GTT)	(CGT)	(CGC)	(CGA)	(CAT)	(CAC)
Historical Control[a]	34/124 (27%)	14	5	1	0	6	3	4	1
0 mg/m^3	0/10 (0%)[###]	0	0	0	0	0	0	0	0
1.25 mg/m^3	11/16 (69%)***	1	0	7	0	0	1	2	0
2.5 mg/m^3	11/23 (48%)**	2	1	5	0	0	1	0	2
5 mg/m^3	24/30 (80%)***	2	0	11[b]	1[b]	4	3[b]	2	3
All exposed groups combined	46/69 (67%)***	5	1	23[b]	1[b]	4	5[b]	4	5

**Significantly different ($P \leq 0.01$) from the chamber control group by the Fischer's exact test.
***$P \leq 0.001$ by one-sided Fischer exact test for single or combined exposure groups or a one-sided Cochran-Armitage trend test for the chamber control group.
###Significant trend ($P \leq 0.001$) by the Cochran-Armitage trend test.
[a]Spontaneous lung neoplasms from control B6C3F1 mice[275].
[b]Same animal with double mutations.

Table K-11. *Kras* and *Egfr* Mutations in Alveolar/Bronchiolar Carcinomas from Male B6C3F1/N Mice in the Two-year Inhalation Study of Cobalt Metal

Sample/ Animal #	Cobalt Conc. (mg/m^3)	*Kras*			*Egfr*			
		Codon 12	Codon 13	Codon 61	Exon 18	Exon 19	Exon 20	Exon 21
1/M42	0	none	none	none	none	none	none	none
2/M12	0	none	none	none	none	none	none	none
3/M30	0	none	none	none	none	none	none	none
4/M36	0	none	none	none	none	none	none	none
5/M21	0	none	none	none	none	none	none	none
6/M24	0	none	none	none	none	none	none	none
7/M201	1.25	none	none	none	none	none	Codon 807 CAC→CAT (His→His)	none
8/M203	1.25	GGT→GTT (Gly→Val)	none	none	none	none	none	none
9/M209	1.25	GGT→GTT (Gly→Val)	none	none	none	none	none	none
10/M211	1.25	none	none	none	none	none	none	none
11/M216	1.25	none	none	none	Codon 721 GGT→AGT (Gly→Ser)	none	none	none
12/M219	1.25	GGT→GAT (Gly→Asp)	none	none	none	none	none	Codon 846 CTG→CAG (Leu→Gln)
13/M204	1.25	none	none	CAA→CAT (Gln→His)	none	none	none	none
14/M217	1.25	GGT→GTT (Gly→Val)	none	none	none	none	none	none
15/M218	1.25	none	none	CAA→CGA (Gln→Arg)	none	none	none	none
16/M401	2.5	none	none	none	none	none	none	Codon 870 GAA→AAA (Glu→Lys)
17/M403	2.5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
18/M405	2.5	none	none	none	none	Codon 735 CCA→CTA (Pro→Leu)	none	none
19/M407	2.5	none	none	none	none	none	Codon 812 GGC→GAC (Gly→Asp)	none
20/M409	2.5	none	none	none	none	none	none	none
21/M404	2.5	none	none	none	none	none	none	none
22/M411	2.5	GGT→GTT (Gly→Val)	none	none	none	none	none	none

Sample/ Animal #	Cobalt Conc. (mg/m^3)	*Kras*			*Egfr*			
		Codon 12	Codon 13	Codon 61	Exon 18	Exon 19	Exon 20	Exon 21
23/M413	2.5	GGT→GTT (Gly→Val)	none	CAA→CGA (Gln→Arg)	none	none	none	none
24/M417	2.5	GGT→TGT (Gly→Cys)	none	none	none	none	none	none
25/M418	2.5	none	none	none	none	none	none	none
26/M618	5	none	none	CAA→CAT (Gln→His)	none	none	none	none
27/M627	5	none	none	CAA→CAT (Gln→His)	none	none	none	none
28/M629	5	none	none	CAACAC (Gln→His)				
29/M606	5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
30/M648	5	none	GGC→CGC (Gly→Arg)	none	none	none	none	none
31/M632	5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
32/M601	5	none	none	none	none	none	none	none
33/M602	5	none	none	none	none	none	none	Codon 861 GCC→ACC (Ala→Thr)
34/M605	5	GGT→GTT (Gly→Val)	none	CAA→CGA (Gln→Arg)	none	none	Codon 791 ATT→GTT (Ile→Val)	none
35/M609	5	none	GGC→CGC (Gly→Arg)	none	none	none	Codon 804 GTC→GTA (Val→Val)	none
36/M611	5	GGT→GAT (Gly→Asp)	none	none	none	none	none	none
37/M604	5	none	none	none	none	none	none	none
38/M607	5	none	none	CAA→CAC (Gln→His)	none	none	none	none
39/M608-12a	5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
40/M608-13a	5	GGT→GTT (Gly→Val)	none	none	none	none	none	none

[a]Same animal with different blocks.

Table K-12. *Kras* and *Egfr* Mutations in Alveolar/Bronchiolar Carcinomas from Female B6C3F1/N Mice in the Two-year Inhalation Study of Cobalt Metal

Sample/ Animal #	Cobalt Conc. (mg/m^3)	*Kras*			*Egfr*			
		Codon 12	Codon 13	Codon 61	Exon 18	Exon 19	Exon 20	Exon 21
1/F145	0	none	none	none	none	none	none	none
2/F125	0	none	none	none	none	none	none	none
3/F106	0	none	none	none	none	none	none	none
4/F131	0	none	none	none	none	none	none	Codon 832 GAT→GAC (Asp→Asp) Codon 859 GGG→GGA (Gly→Gly)
5/F308	1.25	none	none	none	none	none	none	none
6/F318	1.25	GGT→GTT (Gly→Val)	none	none	none	none	none	none
7/F322	1.25	none	none	none	none	none	none	none
8/F306	1.25	GGT→GTT (Gly→Val)	none	none	none	none	none	none
9/F307	1.25	none	none	CAA→CAT (Gln→His)	none	none	none	none
10/F321	1.25	GGT→GTT (Gly→Val)	none	none	none	none	none	none
11/F326	1.25	GGT→GTT (Gly→Val)	none	none	none	none	none	none
12/F502	2.5	GGT→GAT (Gly→Asp)	none	none	none	none	none	none
13/F506	2.5	none	none	none	none	none	Codon 780 CTG→CAG (Leu→Gln)	none
14/F508	2.5	none	none	none	none	none	Codon 817 CTC→TTC (Leu→Phe)	none
15/F509	2.5	none	none	none	none	none	none	none
16/F511	2.5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
17/F512	2.5	none	none	none	none	none	Codon 801 CTG→CAG (Leu→Gln)	none
18/F517	2.5	GGT→GAT (Gly→Asp)	none	none	none	none	none	Codon 873 GCC→ACC (Ala→Thr)
19/F504	2.5	none	none	none	none	none	none	none

Sample/ Animal #	Cobalt Conc. (mg/m^3)	*Kras*			*Egfr*			
		Codon 12	**Codon 13**	**Codon 61**	**Exon 18**	**Exon 19**	**Exon 20**	**Exon 21**
20/F505	2.5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
21/F507	2.5	none	none	CAA→CAC (Gln→His)	none	none	none	none
22/F514	2.5	none	none	none	none	none	none	none
23/F515	2.5	none	none	CAA→CAC (Gln→His)	none	none	none	none
24/F517	2.5	none	none	none	none	none	none	none
25/F746	5	none	GGC→CGC (Gly→Arg)	none	none	none	none	none
26/F717	5	none	GGC→CGC (Gly→Arg)	none	none	none	none	none
27/F720	5	none	none	none	none	none	none	none
28/F732	5	none	none	CAA→CAC (Gln→His)	none	none	none	none
29/F718	5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
30/F714	5	GGT→GAT (Gly→Asp)	none	none	none	none	none	none
31/F723	5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
32/F710	5	GGT→CGT (Gly→Arg)	none	CAA→CGA (Gln→Arg)	none	none	none	none
33/F729	5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
34/F747	5	none	none	none	none	none	none	none
35/F711	5	none	none	none	none	none	Codon 780 CTG→CAG (Leu→Gln)	none
36/F712	5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
37/F713	5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
38/F715	5	GGT→GTT (Gly→Val)	none	none	none	none	none	none
39/F710	5	none	none	CAA→CGA (Gln→Arg)	none	none	none	none

Table K-13. *Egfr* Mutations in Alveolar/Bronchiolar Carcinomas from Male and Female B6C3F1/N Mice in the Two-year Inhalation Study of Cobalt Metal[a]

Cobalt Metal Concentration	Mutation Frequency	Exon 18	Exon 19	Exon 20	Exon 21
0 mg/m^3	0/10 (0%)	0	0	0	0
1.25 mg/m^3	2/16 (13%)	1	0	0	1
2.5 mg/m^3	7/23 (30%)	0	1	4	2
5 mg/m^3	3/30 (10%)	0	0	2	1
All exposed groups combined	12/69 (17%)	1	1	6	4

[a]Silent mutations are not included.

Table K-14. *Tp53* Mutations in Alveolar/Bronchiolar Carcinomas from Male and Female B6C3F1/N Mice in the Two-year Inhalation Study of Cobalt Metal[a]

Cobalt Metal Concentration	Mutation Frequency	Exon 5	Exon 6	Exon 7
0 mg/m^3	0/10 (0%)	0	0	0
1.25 mg/m^3	3/16 (19%)	1	0	2
2.5 mg/m^3	3/23 (13%)	2[b]	1	1[b]
5 mg/m^3	6/30 (20%)	4	2	1
All exposed groups combined	13/69 (19%)	7[b]	3	4[b]

[a]Silent mutations are not included. No mutations were detected in exon 8.
[b]Same animal with double mutations.

Table K-15. *Tp53* Mutations in Alveolar/Bronchiolar Carcinomas from Male B6C3F1/N Mice in the Two-year Inhalation Study of Cobalt Metal

Sample/Animal #	Cobalt Metal Concentration (mg/m^3)	Exon 5	Exon 6	Exon 7	Exon 8
1/M42	0	none	none	none	none
2/M12	0	none	none	none	none
3/M30	0	none	none	none	none
4/M36	0	none	none	none	none
5/M21	0	none	none	none	none
6/M24	0	none	none	none	none
7/M201	1.25	none	Codon 203 CTG→CTA (Leu→Leu)	none	none
8/M203	1.25	none	none	none	none
9/M209	1.25	none	none	Codon 230 CAC→TAC (His→Tyr)	none
10/M211	1.25	none	none	none	none
11/M216	1.25	Codon 156 GCC→CCC (Ala→Pro)	none	none	none
12/M219	1.25	none	none	none	none
13/M204	1.25	none	none	none	none
14/M217	1.25	none	none	none	none
15/M218	1.25	none	none	none	none
16/M401	2.5	none	none	none	none
17/M403	2.5	none	none	none	none
18/M405	2.5	Codon 179 TGC→TAC (Cys→Tyr)	none	none	none
19/M407	2.5	none	none	none	none
20/M409	2.5	none	Codon 194 GTG→GAG (Val→Glu)	none	none
21/M404	2.5	none	none	none	none
22/M411	2.5	none	none	none	none
23/M413	2.5	Codon 156 GCC→CCC (Ala→Pro)	none	Codon 232 AAG→ACG (Lys→Thr)	none
24/M417	2.5	none	none	none	none

Sample/Animal #	Cobalt Metal Concentration (mg/m^3)	Exon 5	Exon 6	Exon 7	Exon 8
25/M418	2.5	none	none	Codon 231 TAC→TAT (Tyr→Tyr)	none
26/M618	5	none	none	none	none
27/M627	5	none	none	none	none
28/M629	5	none	Codon 212 AGC→GGC (Ser→Gly)	none	none
29/M606	5	none	none	none	none
30/M648	5	none	none	none	none
31/M632	5	none	none	none	none
32/M601	5	none	Codon 192 ATC→ATT (Ile→Ile)	none	none
33/M602	5	none	none	none	none
34/M605	5	Codon 155 CGC→CCC (Arg→Pro)	none	none	none
35/M609	5	Codon 155 CGC→CCC (Arg→Pro)	none	none	none
36/M611	5	none	none	none	none
37/M604	5	none	none	none	none
38/M607	5	none	none	none	none
39/M608-12a	5	none	none	none	none
40/M608-13a	5	none	none	none	none

[a]Same animal with different blocks.

Table K-16. *Tp53* Mutations in Alveolar/Bronchiolar Carcinomas from Female B6C3F1/N Mice in the Two-year Inhalation Study of Cobalt Metal

Sample/Animal #	Cobalt Metal Concentration (mg/m^3)	Exon 5	Exon 6	Exon 7	Exon 8
1/F145	0	none	none	none	none
2/F125	0	none	none	none	none
3/F106	0	none	none	none	none
4/F131	0	none	none	none	none
5/F308	1.25	none	none	Codon 257 TCC→TTC (Ser→Phe)	none
6/F318	1.25	none	none	none	none
7/F322	1.25	none	none	none	none
8/F306	1.25	none	none	none	none
9/F307	1.25	none	none	none	none
10/F321	1.25	none	none	none	none
11/F326	1.25	none	none	none	none
12/F502	2.5	none	none	none	none
13/F506	2.5	none	none	none	none
14/F508	2.5	none	none	none	none
15/F509	2.5	none	none	none	none
16/F511	2.5	none	none	none	none
17/F512	2.5	none	none	none	none
18/F517	2.5	none	none	none	none
19/F504	2.5	none	none	none	none
20/F505	2.5	none	none	none	none
21/F507	2.5	none	none	none	none
22/F514	2.5	none	none	none	none
23/F515	2.5	none	none	none	none
24/F517	2.5	none	none	none	none
25/F746	5	none	none	none	none
26/F717	5	none	none	none	none
27/F720	5	none	none	none	none
28/F732	5	none	Codon 212 AGC→GGC (Ser→Gly)	none	none
29/F718	5	none	none	none	none
30/F714	5	none	none	none	none

Sample/Animal #	Cobalt Metal Concentration (mg/m^3)	Exon 5	Exon 6	Exon 7	Exon 8
31/F723	5	none	none	none	none
32/F710	5	none	none	none	none
33/F729	5	none	none	none	none
34/F747	5	none	none	none	none
35/F711	5	Codon 161 AAG→AAA (Lys→Lys) Codon 179 TGC→TTC (Cys→Gly)	none	none	none
36/F712	5	Codon 158 GCC→ACC (Ala→Thr) Codon 179 TGC→GGC (Cys→Gly)	none	none	none
37/F713	5	none	none	none	none
38/F715	5	none	none	Codon 239 TGC→TGG (Cys→Trp)	none
39/F710	5	none	none	none	none

Table K-17. Summary of *Kras* Mutations in Alveolar/Bronchiolar Neoplasms from Male and Female B6C3F1/N Mice in Selected Two-year NTP Studies

Chemical (Study Type)	Mutations	Codon 12 GGT					Codon 13 GGC					Codon 61 CAA				
		GAT	TGT	GTT	CGT	Other	CGC	GAC	CGA	CAT	Other	CAC	CTA	CGA	CAT	CAC
Historical controls																
	34/124 (27%)	14	5	1	0	–	6	0	0	0	–	0	0	3	4	1
Concurrent chamber controls (inhalation)																
	0/10 (0%)	0	0	0	0	–	0	0	0	0	–	0	0	0	0	0
Cobalt metal (inhalation)[a]																
	46/69 (67%)	5	1	23	1	–	4	0	0	0	–	0	0	5	4	5
Cobalt sulfate heptahydrate (inhalation)																
	9/26 (35%)	2	1	5	0	–	1	0	0	0	–	0	0	0	0	0
Ozone (inhalation)																
	19/26 (73%)	3	2	5	0	–	0	1	0	0	–	0	8	0	0	0
1,3,Butadiene (inhalation)																
	20/24 (83%)	1	2	0	0	–	8	0	0	0	–	0	6	3	0	0
Methylene chloride (inhalation)																
	11/54 (20%)	1	1	1	0	–	1	0	0	0	–	4	1	1	1	0
Ethylene oxide (inhalation)[a]																
	23/23 (100%)	2	0	21	0	–	0	0	0	0	AGC	0	0	1	0	0
Cumene (inhalation)																
	45/52 (87%)	6	5	11	3	–	4	0	13	0	–	2	1	0	0	0
2,2-Bis(bromomethyl-1,3-propanediol (feed)																
	29/51 (57%)	20	1	7	0	–	1	0	0	0	–	0	0	0	0	0
Tetranitromethane (inhalation)[a]																
	14/26 (54%)	13	0	0	0	–	2	0	0	0	–	0	0	0	0	0
Isoprene (inhalation)																
	11/11 (100%)	0	0	0	0	–	1	0	0	0	–	0	10	0	0	0
Chloroprene (inhalation)																
	37/46 (80%)	5	0	2	1	ATT CTT	2	0	0	0	–	0	22	3	0	0

[a]If the same neoplasm had two point mutations, it was counted as one.

Appendix L. Chemical Characterization and Generation of Chamber Concentrations

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L.1. Procurement and Characterization of Cobalt Metal

Cobalt metal was produced by OMG Kokkola Chemicals Oy (Kokkola, Finland) and was provided by the Cobalt Development Institute via PEL Technologies in one lot (P32 3040-1) that was used in the 2-week, 3-month, and 2-year studies. Identity and purity analyses were performed by the study laboratory at Battelle Toxicology Northwest [Richland, WA; inductively coupled plasma/atomic emission spectroscopy (ICP/AES) analysis] and by the analytical chemistry laboratories at Pacific Northwest National Laboratory [Richland, WA; X-ray diffraction (XRD) and proton-induced X-ray emission (PIXE) analyses], AMIA Laboratories (The Woodlands, TX; XRD using Rietveld analysis), H&M Analytical Services, Inc. (Allentown, NJ; XRD with and without Rietveld analysis), Elemental Analysis, Inc. (Lexington, KY; PIXE), and Galbraith Laboratories (Knoxville, TN; coulometry for total carbon). Reports on analyses performed in support of the cobalt metal studies are on file at the National Institute of Environmental Health Sciences.

Lot P32 3040-1 of the chemical, a silver-gray powder, was identified as cobalt metal by the analytical chemistry laboratories using XRD. XRD patterns were consistent with library reference patterns (Joint Center for Powder Diffraction Studies/International Centre for Diffraction Data) for cubic and hexagonal phases of cobalt; Rietveld analysis indicated two crystalline forms of cobalt metal, cubic at 13.9% and hexagonal at 85.9%, and cobalt oxide at 0.2%. A representative XRD pattern is presented in Figure L-1.

The purity of lot P32 3040-1 was determined by the analytical chemistry laboratories by determination of the carbon content using combustion/coulometric analysis by induction furnace (Leco Corporation, St. Joseph, MI) with a carbon dioxide coulometer (Coulometrics, Inc., Wheat Ridge, CO) and by PIXE analyses using system A to determine the presence of cobalt metal and trace element impurities with atomic numbers from 11 (sodium) to 53 (iodine) or 92 (uranium). The study laboratory quantitated the purity of the bulk chemical using ICP/AES by system B.

A) The PIXE systems included the use of purchased elemental standards, a 2.5 MeV H^+ ion beam with an incident angle of 45° and an exit angle of 39°, a 160 µm thick graphite disk attenuator, a 0.05 µC charge, and a beam current of 0.2 nA.

B) For ICP/AES, samples were dissolved in trace metal grade concentrated HCl:HNO_3 (1:1) and analyzed for cobalt (230.786 nm) and yttrium (internal standard) (224.306 nm). Analyses were performed on a Thermo Elemental IRIS Intrepid Inductively Coupled Plasma-Atomic Emission Spectrometer (Thermo Elemental, Franklin, MA), and the results were normalized against those of cobalt reference standards obtained from the National Institute of Standards and Technology (NIST).

For lot P32 3040-1, the carbon content was 0.09% ± 0.01%. PIXE analysis by system A indicated trace elements of aluminum, sulfur, calcium, chromium, and iron. Chromium was consistently current at approximately 84 ppm; the other impurities were below the minimum detection limits. ICP/AES analysis by system B indicated a purity of 98.2% ± 0.6% relative to a NIST standard reference material [(SRM); cobalt SRM 3113, Gaithersburg, MD]. The overall purity of cobalt metal was determined to be greater than 98%.

To ensure stability, the bulk chemical was stored at room temperature in safety-coated amber glass containers with Teflon®-lined caps under a nitrogen headspace. Periodic reanalyses of the bulk chemical were performed by the study laboratory using ICP/AES by system B; no degradation of the bulk chemical was detected.

L.2. Aerosol Generation and Exposure System

Schematic diagrams of the cobalt metal generation and distribution systems used during the 2-week studies and the 3-month and 2-year studies are shown in Figure L-2 and Figure L-3, respectively. During the 2-week studies, an auger feed device (Tuf-Flex, Model 102 powder feeder; Schenk AccuRate, Inc., Whitewater, WI) was used to meter cobalt metal into a Trost jet mill (Garlock, Inc., Newtown, PA) for aerosolization and particle size reduction. For the 3-month and 2-year studies, the generation system used a linear feed device (Figure L-4) designed and built by Battelle to meter cobalt metal into the jet mill. The linear feed device consisted of a slide bar, a body, a delivery tube, and a test article reservoir. A motor-driven brush (not shown in Figure L-3 and Figure L-4), mounted above and extending into the reservoir, gently rotated and continually stirred the cobalt metal held within the reservoir; this action aided the metering port filling process.

The compressed air driven slide bar slid back and forth during generation. As the slide bar moved to the filling position, the metering port on the shuttle bar was aligned with the reservoir opening and was filled with a small metered amount of test article. A stainless steel screen at the bottom of the metering port held the material within the port. A slight vacuum was applied to the metering port to assist with filling. As the slide bar moved to the dispersing position, the metering port was aligned with a compressed air port in the body. A puff of air from the port dispersed the test article from the metering port. The output of the linear feeder was regulated by adjusting the shuttle bar cadence.

Initial particle size reduction was accomplished within the Trost jet mill. Opposing nitrogen and air gas streams drove the jet mill. All components of the generation system were housed within a glove box located within the exposure control center.

From the jet mill, aerosol was directed to the main distribution line where it was diluted with humidified air then conveyed from the exposure control center to the exposure room where it passed through a cyclone separator to further reduce particle size. On exiting the cyclone, the aerosol-laden air was directed to either of two smaller branch lines. The main distribution and branch lines were made of stainless steel, bonded and grounded to prevent the buildup of electrostatic charge. The distribution line pressure was continuously monitored and maintained slightly negative to the exposure room.

From the branch line, aerosol was delivered to each exposure chamber by a sampling tube. The flow through the sampling tube was induced by a stainless steel ejector pump designed and fabricated at Battelle. The flow rate and configuration of the ejector pumps were chosen to optimize the efficiency of the delivery system. The aerosol then entered the chamber inlet duct where it was further diluted with conditioned chamber air to achieve the desired exposure concentration.

During exposure periods, there was a small excess of aerosol in each branch line over that needed to maintain chamber concentrations. This additional aerosol was available for making adjustments to the chamber aerosol delivery flow rates and was controlled using house vacuum regulated by a filter-protected flow meter. A second flow control system was available during off-exposure periods. This system consisted of a vacuum transducer pump (Air-Vac Engineering Company, Inc., Seymour, CT) of higher flow capacity positioned in parallel with each branch line flow meter control assembly that became operational only during critical shut-down periods. This backup pump was intended to create sufficient vacuum in the branch line to overcome the negative pressure in the chambers and prevent the flow of aerosol-laden air from the branch line to the chambers as the air supply to each chamber ejector pump was shut off. A high-efficiency particulate air (HEPA) filter was placed before the endline flow control assembly of each branch to remove aerosol from the airstream prior to exhausting from the room.

The study laboratory designed the inhalation exposure chambers (Lab Products, Inc., Seaford, DE) so that uniform aerosol concentrations could be maintained throughout the chambers with the catch pans in place. The total volume of the chamber was 2.3 m^3 with an active mixing volume of 1.7 m^3. There were three levels of caging, each level split into two tiers that were offset from each other and from the chamber walls. Drawer-like stainless steel cage units composed of individual animal cages were suspended in the space above each tier. Stainless steel catch pans for the collection of urine and feces were suspended below each cage unit.

Incoming air that contained a uniform mixture of test chemical was diverted so that it flowed vertically along the inner surfaces of the chambers. Eddies were formed at each tier as the aerosol flowed past the catch pans. Stagnant zones that would normally exist above each pair of catch pans were cleared by exhaust flow through the space between the tiers. Aerosol reaching the lowest level was deflected across the bottom tiers by metal strips in the space between the catch pan and the wall. Tests showed that aerosol concentration could be reliably maintained homogenous within 8% throughout the chambers, provided the aerosol was uniformly mixed before passing through the chamber inlet and provided the test material did not react to a significant extent with animals, animal excrement, or the chamber interior[164].

L.3. Aerosol Concentration Monitoring

Summaries of the chamber aerosol concentrations are given in Table L-1 through Table L-3. The concentration of cobalt metal in the exposure chambers and room air was monitored using three real-time aerosol monitors (RAMs) (Model RAM-1; MIE, Inc., Bedford, MA). The monitors were connected to the chambers by a sampling system designed by Battelle incorporating a valve that multiplexed each RAM to a 0 mg/m^3 chamber or the room, a HEPA-filtered room air blank, and two exposure chambers. The output (voltage) of the RAM was recorded by a program designed by Battelle (Battelle Exposure Data Acquisition and Control) to select the correct sample stream and acquire a raw voltage signal from each RAM. Equations for the calibration curves resided within the program and were used to convert the measured RAM voltages to exposure chamber concentrations. Concentration control limits within the program were compared to each measured concentration and, if limits were exceeded, an audible alarm was triggered or, in extreme cases, exposure was terminated.

Each RAM was calibrated by constructing a response curve using the measured RAM voltages (voltage readings were corrected by subtracting the RAM zero-offset voltage from measured RAM voltages) and cobalt metal concentrations that were determined by analyzing tandem Pallflex TX40HI20WW (Pall Corporation, Ann Arbor, MI) Teflon®-coated, glass-fiber filters collected daily from the exposure chambers. Cobalt was extracted from the filters with 1:1 $HCl:HNO_3$ and analyzed using ICP/AES by system B.

The ICP/AES instrument was calibrated against serially diluted NIST-traceable 10 mg/mL spectrometric standards of cobalt and the internal standard yttrium. Quality control standards and a reagent blank were analyzed after calibration, after approximately every tenth sample, and at the end of the analysis to determine accuracy and calibration drift during analysis.

L.4. Chamber Atmosphere Characterization

Particle size distribution was determined once prior to the 3-month and 2-year studies, once during the 2-week studies, twice during the 3-month studies, and monthly during the 2-year studies. Impactor samples were taken from each exposure chamber using a Mercer-style seven-stage impactor (In-Tox Products, Moriarty, NM) and the stages (glass coverslips lightly coated with silicone to prevent particle bounce) were analyzed using ICP/AES by system B after cobalt was extracted from the slides with 1:1 $HCl:HNO_3$. The relative mass collected on each stage was analyzed by the CASPACT impactor analysis program developed at Battelle based on probit analysis[165]. The resulting estimates of the mass median aerodynamic particle diameter and the geometric standard deviation of each set of samples are given in Table L-4 through Table L-7. All samples were within the 1 to 3 μm range required by the protocol.

Buildup and decay rates for chamber aerosol 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 aerosol generation (T_{90}) and the time for the chamber concentration to decay to 10% of the target concentration after aerosol generation was terminated (T_{10}) was approximately 9.4 minutes. For rats and mice in the 2-week studies, T_{90} values ranged from 9 to 11 minutes with animals present; T_{10} values ranged from 7 to 10 minutes. For rats and mice in the 3-month studies, T_{90} values ranged from 10 to 14 minutes without animals present and from 11 to 14 minutes with animals present; T_{10} values ranged from 8 to 10 minutes without animals and were 9 minutes with animals present. For rats in the 2-year studies, T_{90} values ranged from 10 to 13 minutes without animals present and from 9 to 10 minutes with animals present; T_{10} values ranged from 7 to 10 minutes without animals present and from 9 to 10 minutes with animals present. For mice, T_{90} values ranged from 10 to 13 minutes without animals present and from 11 to 13 minutes with animals present; T_{10} values ranged from 6 to 9 minutes without animals present and from 9 to 10 minutes with animals present. A T_{90} value of 12 minutes was selected for all studies.

The uniformity of aerosol concentration in the inhalation exposure chambers without animals was evaluated before the 3-month and 2-year studies began; in addition, concentration uniformity with animals present in the chambers was measured once during the 2-week and 3-month studies and every 3 to 4 months during the 2-year studies. Aerosol concentrations were measured using the on-line monitor with the stream-selection valve fixed in one position to allow continuous monitoring from a single input line. Concentrations were measured at 12 chamber

sample ports; one in front and one in back for each of six possible cage unit positions per chamber. Chamber concentration uniformity was maintained throughout the studies.

The persistence of cobalt metal in the exposure chambers after aerosol delivery ended was determined by monitoring the concentration overnight in the 40 mg/m^3 rat and mouse chambers in the 2-week studies, the 5 mg/m^3 rat and 10 mg/m^3 mouse chambers in the 3-month studies, and the 5 mg/m^3 rat and mouse chambers in the 2-year studies, with and without (except for the 2-week studies) animals present in the chambers. The average cobalt metal concentration decreased to 1% of the target concentration within 15 (2-week studies), 17 to 18 (3-month studies), or 19 (2-year studies) minutes.

Stability studies of the test material in the generation and exposure system were performed before and during the studies by the study laboratory and the analytical chemistry laboratories. Before the start of each study, a cobalt powder sample was taken from the aerosol distribution line using a Gore-Tex lined polyester filter bag (Sturtevant, Inc., Hanover, MA). Before (except for the 2-week studies) and during each study, microporous filters were collected from the 2.5 and 40 mg/m^3 (2-week studies), 0.625, 5, and 10 (mice only) mg/m^3 (3-month studies), or 1.25 and 5 mg/m^3 (2-year studies) exposure chambers and the aerosol distribution line. All microporous filters were obtained from Pall Corporation; samples for all XRD and ICP/AES analyses were collected on 25 mm A/E glass-fiber filters (1 µm pore size:330 µm thickness) and those for PIXE analyses were collected on 25 mm Zefluor™ polytetrafluoroethylene filters (1 µm pore size:165 µm thickness) for the 3-month studies or 25 mm GH Polypro filters for the 2-year studies. On each sample collection day, a sample of the bulk cobalt metal was collected before filling the reservoir, and a sample of the test article from the generator reservoir was collected at the end of the generation day; additional test material was added to the generator each day. In-system test article stability was assessed using multiple but similar XRD systems as well as PIXE by system A and ICP/AES by system B. The XRD systems included a Siemens D5000$^{\Theta/\Theta}$ (Siemens, Munich, Germany) or Philips PW1800 or 3020 (Panalytical, Inc., Westborough, PA) diffractometer using Cu radiation at 40 to 50 KV/30 to 300 mA. Angular ranges varied from 15°, 20°, 30°, or 35° to 65°, 70°, 78°, 80°, or 90°; stepsizes were 0.01°, 0.02°, or 0.05°, and counting times were 1.2, 4.8, 10, 70, or 100 seconds.

For the 2-week studies, XRD analyses of the bulk chemical, filter samples, and material from the generator reservoir indicated two primary crystal forms of cobalt, cubic and hexagonal, with no detection of known cobalt oxides. XRD with Rietveld analysis of samples from the distribution line, bulk chemical, and generator reservoir indicated the presence of two crystal forms of cobalt, approximately 15% to 18% cubic and 78% to 79% hexagonal, and approximately 4% to 5% cubic cobalt oxide. Due to the unexpected presence of significant amounts of cobalt oxide in these samples, they were reanalyzed using XRD with standards containing cobalt metal as well as cobalt oxide (CoO) added at 0%, 1%, or 2% by weight. No cobalt oxide was detected in the distribution line or bulk chemical samples; cobalt oxide was detected in the generator reservoir sample, but it was less than 1% relative to the cobalt oxide standard. Bulk chemical, generator reservoir, and filter samples were analyzed using ICP/AES by system B to determine if inorganic impurities were introduced into the test atmosphere by the exposure generation system. Samples were analyzed for aluminum, arsenic, beryllium, cadmium, chromium, cobalt, copper, iron, manganese, molybdenum, nickel, lead, and tin. All measured trace element impurities were present at less than 0.05% by weight relative to cobalt.

Before the 3-month studies, XRD analyses indicated two crystal forms of cobalt (cubic and hexagonal) in all analyzed samples. Cobalt oxides were not detected and were less than 1% in the powder and filter samples relative to cobalt oxide standards. XRD coupled with Rietveld analysis of similar samples collected during the 3-month studies with animals in the exposure chambers detected cobalt oxide (Co_3O_4) at approximately 1.6% and 1.7%, respectively, with an uncertainty of approximately ± 2%. Comparison of the XRD patterns of these samples relative to those of cobalt oxide standards analyzed at the same time indicated less than 1% CoO and less than 1% Co_3O_4. No cobalt oxides were detected in the filter samples.

Before the 3-month studies, PIXE analysis for trace elements using system A indicated no inorganic impurities in the distribution line or exposure chamber filter samples or in the generator reservoir bulk samples. In similar PIXE analyses conducted during the 3-month studies with animals in the exposure chambers, no inorganic impurities were detected in the bulk chemical samples from the generator reservoir. Relative to cobalt concentrations in the same samples, chromium was detected in the aerosol distribution line and the 0.625 and 5 mg/m^3 exposure chamber filter samples at levels of 0.13%, 0.21%, and 0.11%, respectively, and iron was detected in the 0.625 mg/m^3 chamber filter sample at a level of 0.25%.

Before the 2-year studies, XRD with Rietveld analysis indicated the presence of two crystal forms of cobalt in all of the analyzed samples; cubic at 14% to 25% and hexagonal at 74% to 84%. Cobalt oxide (Co_3O_4) was detected at 1% to 2% in the bulk and generator samples and at 2% to 3% in the chamber and distribution line filter samples (with an uncertainty of approximately ± 2%). Comparison of the XRD patterns of these samples to those of cobalt oxide standards analyzed at the same time indicated less than 1% CoO and less than 1% Co_3O_4. Powder (bulk and generator reservoir) and filter (exposure chamber and distribution line) samples were collected for PIXE analyses for trace element impurities using system A; analyses detected chromium in three filter samples (distribution line, 5 mg/m^3 rat chamber, and 1.25 mg/m^3 mouse chamber) and chlorine in one filter sample (1.25 mg/m^3 mouse chamber) at less than 0.1% relative to cobalt. An additional set of powder (bulk, generator reservoir, and distribution line) samples were collected and similarly analyzed by PIXE; chlorine was detected in all three of these powder samples and silicon was detected in the powder from the distribution line at less than 0.1% relative to cobalt.

At the beginning of the 2-year studies, XRD with Rietveld analysis indicated the presence of two crystal forms of cobalt in all of the analyzed samples, cubic at approximately 27% to 31% and hexagonal at 66% to 72%. Cobalt oxide (Co_3O_4) was detected at approximately 2% and 3% (with an uncertainty of approximately ± 3%) in the bulk and generator reservoir samples and chamber and distribution line filter samples, respectively. Comparison of the XRD patterns of these samples to those of cobalt oxide standards analyzed at the same time indicated less than 1% CoO and less than 1% Co_3O_4. PIXE analysis for trace element impurities by system A indicated the presence of silicon in the generator reservoir powder samples at 0.4% relative to cobalt. The bulk and generator reservoir samples were reanalyzed using ICP/AES by system B following microwave acid digestion in HNO_3/HCl/HF. The following element impurities were detected at less than 0.1% relative to cobalt: silicon in the bulk and generator powder samples, chlorine in all powder and filter samples, vanadium in the 5 mg/m^3 mouse chamber filter sample, and potassium and chromium in the 1.25 mg/m^3 rat chamber filter sample.

XRD reanalyses of test chemical stability in the exposure system were performed at 1year and at the end of the 2-year studies. Two crystal forms of cobalt were present in all analyzed samples, cubic at 21% to 24% and hexagonal at 73% to 78%. Cobalt oxide (Co_3O_4) was detected in the bulk and generator samples at approximately 2% to 3% and in the chamber and distribution line filter samples at approximately 2% to 5% with an uncertainty of approximately ± 2%; comparison of the XRD patterns of these samples to those of cobalt oxide standards analyzed at the same time indicated less than 1% CoO and less than 1% Co_3O_4.

Table L-1. Summary of Chamber Concentrations in the Two-week Inhalation Studies of Cobalt Metal

Target Concentration (mg/m^3)	Total Number of Readings	Average Concentration[a] (mg/m^3)
Rat Chambers		
2.5	134	2.5 ± 0.2
5	131	4.9 ± 0.4
10	134	9.7 ± 1.1
20	131	19.7 ± 2.0
40	57	40.1 ± 3.4
Mouse Chambers		
2.5	145	2.5 ± 0.2
5	141	4.9 ± 0.4
10	145	9.7 ± 1.3
20	141	19.6 ± 2.0
40	145	40.1 ± 4.2

[a]Mean ± standard deviation.

Table L-2. Summary of Chamber Concentrations in the Three-month Inhalation Studies of Cobalt Metal

Target Concentration (mg/m^3)	Total Number of Readings	Average Concentration[a] (mg/m^3)
Rat Chambers		
0.625	760	0.61 ± 0.04
1.25	750	1.23 ± 0.07
2.5	760	2.5 ± 0.1
5	749	5.0 ± 0.3
Mouse Chambers		
0.625	782	0.61 ± 0.04
1.25	772	1.23 ± 0.07
2.5	782	2.5 ± 0.1
5	771	5.0 ± 0.3
10	782	10.0 ± 0.5

[a]Mean ± standard deviation.

Table L-3. Summary of Chamber Concentrations in the Two-year Inhalation Studies of Cobalt Metal

Target Concentration (mg/m^3)	Total Number of Readings	Average Concentration[a] (mg/m^3)
Rat Chambers		
1.25	5,644	1.24 ± 0.07
2.5	5,647	2.50 ± 0.10
5	5,707	5.01 ± 0.18
Mouse Chambers		
1.25	5,723	1.24 ± 0.06
2.5	5,719	2.49 ± 0.11
5	5,664	5.01 ± 0.20

[a]Mean ± standard deviation.

Table L-4. Summary of Aerosol Size Measurements for the Rat and Mouse Exposure Chambers in the Two-week Inhalation Studies of Cobalt Metal

Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
2.5	1.86	1.76
5	1.79	1.73
10	1.92	1.74
20	1.94	1.77
40	1.92	1.79

Table L-5. Summary of Aerosol Size Measurements for the Rat and Mouse Exposure Chambers in the Three-month Inhalation Studies of Cobalt Metal

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
March 2005	0.625	1.69	1.94
	1.25	1.73	1.84
	2.5	1.84	1.76
	5	1.86	1.76
	10[a]	2.00	1.79
April 2005	0.625	1.61	1.97
	1.25	1.71	1.83
	2.5	1.76	1.75
	5	1.77	1.74
	10	1.98	1.80
May 2005	0.625	1.66	1.97
	1.25	1.72	1.81
	2.5	1.96	1.78
	5	1.90	1.78
	10	1.91	1.77

[a]Mice only.

Table L-6. Summary of Aerosol Size Measurements for the Rat Exposure Chambers in the Two-year Inhalation Study of Cobalt Metal

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
May 2006	1.25	1.7	1.7
	2.5	1.8	1.7
	5	2.0	1.7
June 2006	1.25	1.6	1.7
	2.5	1.7	1.7
	5	1.7	1.7
July 2006	1.25	1.6	1.9
	2.5	1.7	1.7
	5	1.8	1.7
August 2006	1.25	1.7	1.7
	2.5	1.9	1.7
	5	1.8	1.7
September 2006	1.25	1.7	1.7
	2.5	1.8	1.7
	5	1.9	1.7
October 2006	1.25	1.7	1.7
	2.5	2.0	1.7
	5	1.8	1.7
November 2006	1.25	1.7	1.7
	2.5	1.8	1.6
	5	1.9	1.7
December 2006	1.25	1.6	1.7
	2.5	1.9	1.7
	5	1.8	1.7
January 2007	1.25	1.7	1.7
	2.5	1.9	1.7
	5	1.7	1.7
February 2007	1.25	1.5	1.8
	2.5	1.7	1.7
	5	1.6	1.7
March 2007	1.25	1.7	1.7
	2.5	2.0	1.8
	5	1.8	1.8

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
April 2007	1.25	1.5	1.8
	2.5	1.8	1.7
	5	1.7	1.7
May 2007	1.25	1.6	1.7
	2.5	1.8	1.7
	5	1.7	1.7
June 2007	1.25	1.6	1.7
	2.5	1.7	1.7
	5	1.7	1.7
July 2007	1.25	1.5	1.7
	2.5	1.8	1.7
	5	1.7	1.7
August 2007	1.25	1.7	1.7
	2.5	1.7	1.7
	5	1.8	1.7
September 2007	1.25	1.6	1.8
	2.5	1.7	1.7
	5	1.7	1.7
October 2007	1.25	1.7	1.7
	2.5	1.8	1.8
	5	1.8	1.7
November 2007	1.25	1.6	1.8
	2.5	1.7	1.7
	5	1.6	1.8
December 2007	1.25	1.6	1.8
	2.5	1.6	1.7
	5	1.8	1.7
January 2008	1.25	1.5	1.8
	2.5	1.7	1.7
	5	1.7	1.7
February 2008	1.25	1.4	1.8
	2.5	1.7	1.7
	5	1.7	1.7

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
March 2008	1.25	1.5	1.7
	2.5	1.7	1.7
	5	1.7	1.7
April 2008	1.25	1.6	1.9
	2.5	1.7	1.7
	5	1.8	1.8
May 2008	1.25	1.6	1.7
	2.5	1.7	1.7
	5	1.8	1.7
Range	1.25	1.4–1.7	1.7–1.9
	2.5	1.6–2.0	1.6–1.8
	5	1.6–2.0	1.7–1.8

Table L-7. Summary of Aerosol Size Measurements for the Mouse Exposure Chambers in the Two-year Inhalation Study of Cobalt Metal

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
May 2006	1.25	1.8	1.7
	2.5	2.1	1.7
	5	2.0	1.7
June 2006	1.25	1.7	1.7
	2.5	1.7	1.7
	5	1.7	1.7
July 2006	1.25	1.7	1.7
	2.5	1.8	1.7
	5	2.0	1.7
August 2006	1.25	1.7	1.7
	2.5	1.8	1.7
	5	1.9	1.7
September 2006	1.25	1.7	1.7
	2.5	1.9	1.6
	5	2.0	1.7
October 2006	1.25	1.7	1.7
	2.5	1.8	1.7
	5	2.0	1.7
November 2006	1.25	1.6	1.7
	2.5	1.8	1.7
	5	2.0	1.6
December 2006	1.25	1.7	1.7
	2.5	1.6	1.7
	5	1.7	1.7
January 2007	1.25	1.7	1.7
	2.5	1.7	1.7
	5	1.9	1.7
February 2007	1.25	1.7	1.7
	2.5	1.9	1.7
	5	2.0	1.7
March 2007	1.25	1.7	1.7
	2.5	2.0	1.7
	5	1.9	1.7

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
April 2007	1.25	1.5	1.7
	2.5	1.8	1.7
	5	1.7	1.7
May 2007	1.25	1.6	1.7
	2.5	1.8	1.8
	5	1.8	1.7
June 2007	1.25	1.7	1.7
	2.5	1.8	1.7
	5	1.9	1.7
July 2007	1.25	1.7	1.7
	2.5	1.9	1.7
	5	1.9	1.8
August 2007	1.25	1.7	1.7
	2.5	1.7	1.7
	5	1.8	1.7
September 2007	1.25	1.6	1.8
	2.5	1.7	1.8
	5	1.7	1.7
October 2007	1.25	1.6	1.8
	2.5	1.9	1.7
	5	1.8	1.8
November 2007	1.25	1.7	1.7
	2.5	1.7	1.7
	5	1.8	1.7
December 2007	1.25	1.6	1.9
	2.5	1.8	1.7
	5	1.9	1.7
January 2008	1.25	1.6	1.8
	2.5	1.7	1.7
	5	1.7	1.7
February 2008	1.25	1.5	1.8
	2.5	1.7	1.8
	5	1.8	1.8

Date of Test	Target Concentration (mg/m^3)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation
March 2008	1.25	1.7	1.8
	2.5	1.8	1.8
	5	1.9	1.7
April 2008	1.25	1.5	1.8
	2.5	1.7	1.7
	5	1.8	1.7
May 2008	1.25	1.7	1.9
	2.5	1.7	1.7
	5	1.7	1.8
Range	1.25	1.5–1.8	1.7–1.9
	2.5	1.6–2.1	1.6–1.8
	5	1.7–2.0	1.6–1.8

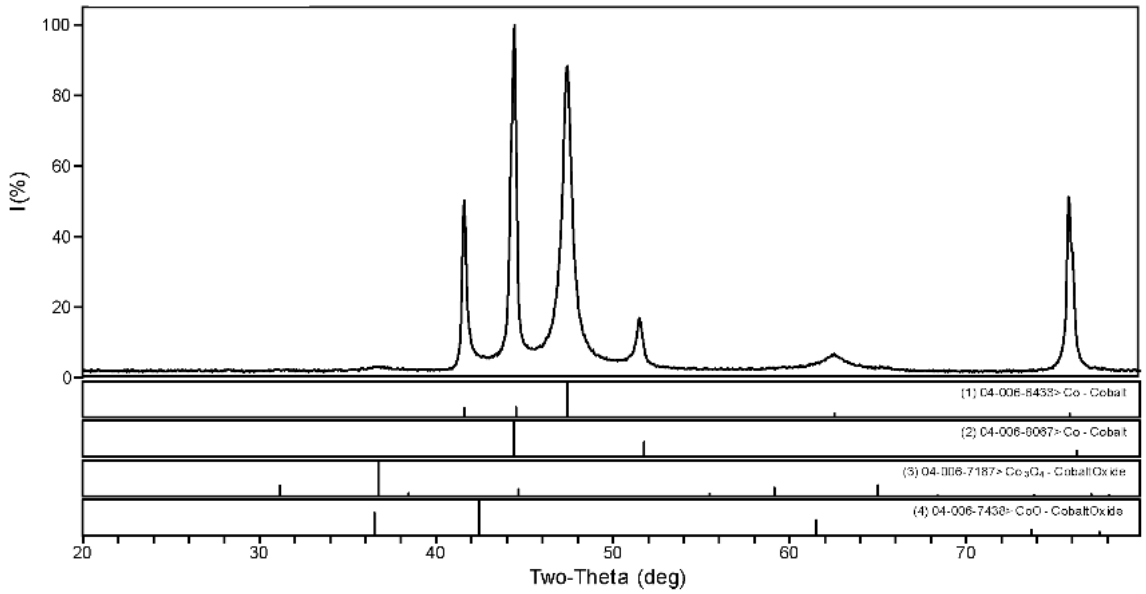

Figure L-1. X-ray Diffraction Pattern of Cobalt Metal

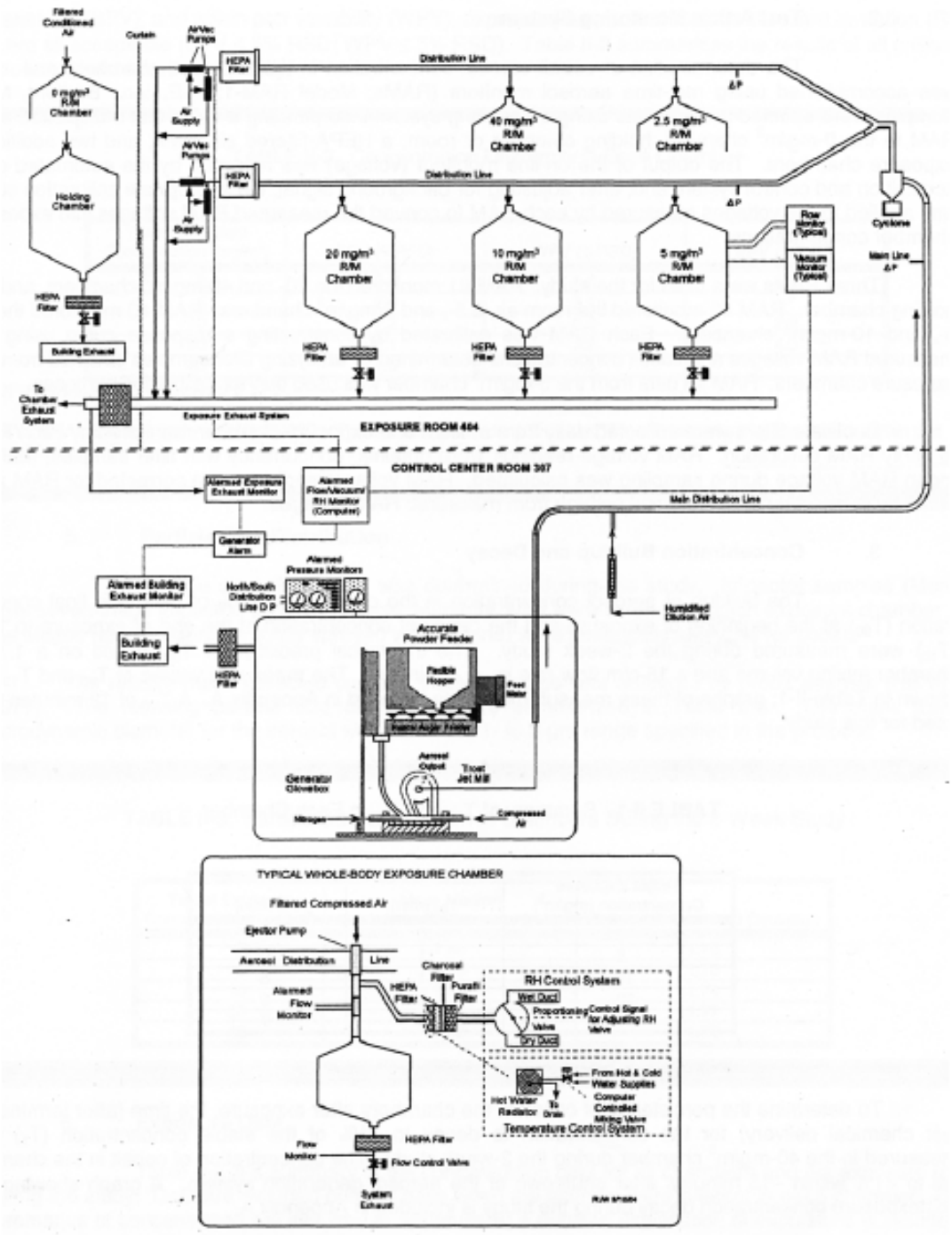

Figure L-2. Schematic of the Aerosol Generation and Delivery System in the Two-week Inhalation Studies of Cobalt Metal

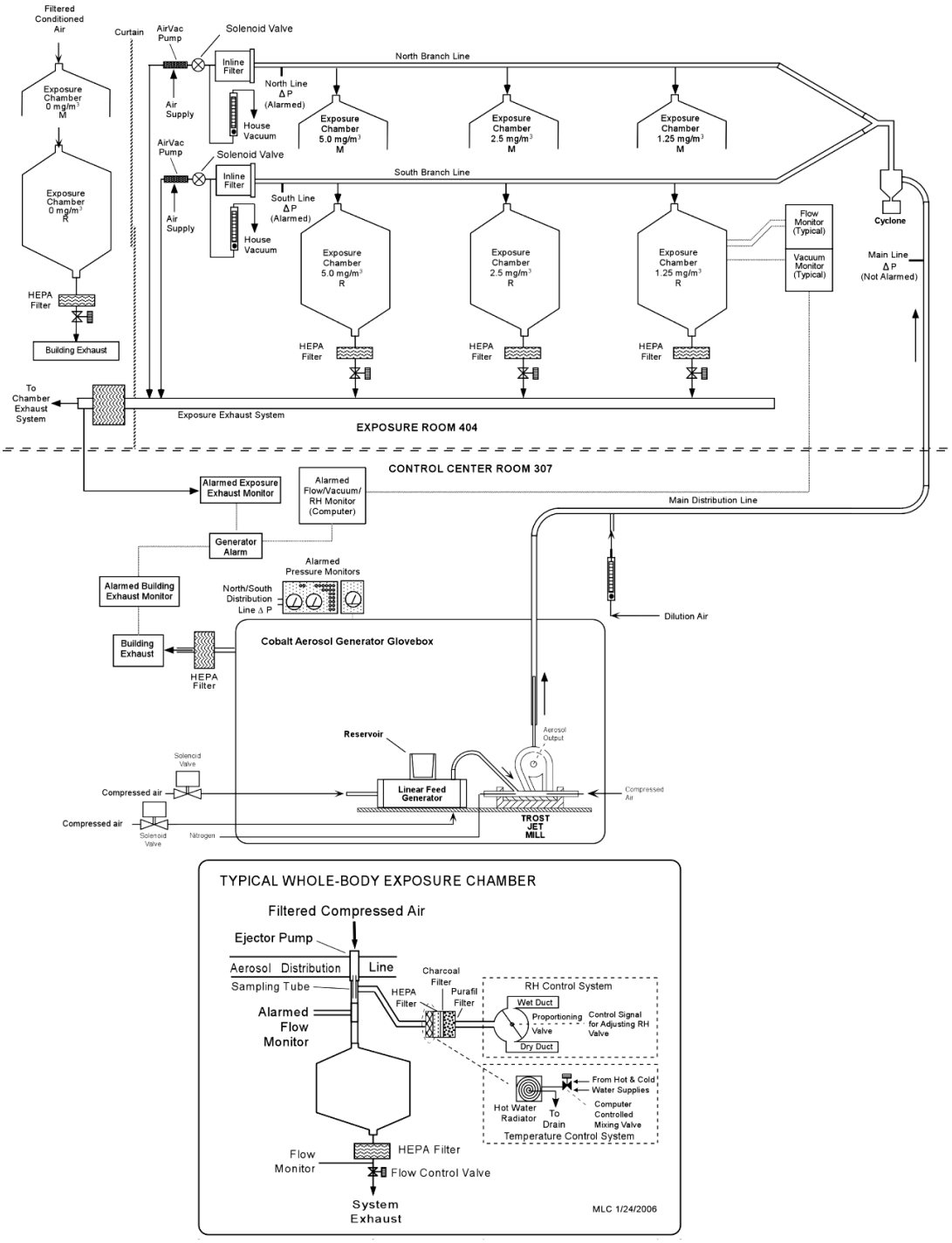

Figure L-3. Schematic of the Aerosol Generation and Delivery System in the Three-month and Two-year Inhalation Studies of Cobalt Metal

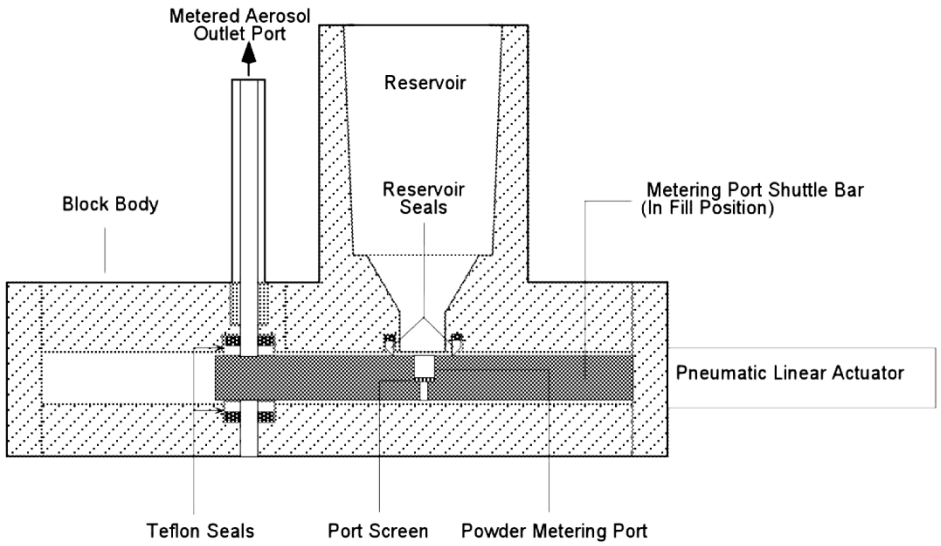

Figure L-4. Schematic of the Linear Feed Generator in the Fill Position Used in the Three-month and Two-year Inhalation Studies of Cobalt Metal

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

Tables

Table M-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 M-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

	Amount	Source
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a]Per kg of finished product.

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

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.6 ± 0.61	13.5–15.9	24
Crude fat (% by weight)	8.2 ± 0.31	7.7–8.9	24
Crude fiber (% by weight)	9.1 ± 0.57	8.1–10.3	24
Ash (% by weight)	4.9 ± 0.22	4.4–5.2	24
Amino Acids (% of total diet)			
Arginine	0.783 ± 0.70	0.67–0.97	22
Cystine	0.220 ± 0.024	0.15–0.25	22
Glycine	0.701 ± 0.041	0.62–0.80	22
Histidine	0.352 ± 0.077	0.27–0.68	22
Isoleucine	0.546 ± 0.044	0.43–0.66	22
Leucine	1.095 ± 0.067	0.96–1.24	22
Lysine	0.711 ± 0.114	0.31–0.86	22
Methionine	0.409 ± 0.046	0.26–0.49	22
Phenylalanine	0.628 ± 0.040	0.54–0.72	22
Threonine	0.505 ± 0.043	0.43–0.61	22
Tryptophan	0.150 ± 0.028	0.11–0.20	22
Tyrosine	0.401 ± 0.061	0.28–0.54	22
Valine	0.665 ± 0.043	0.55–0.73	22
Essential Fatty Acids (% of total diet)			
Linoleic	3.95 ± 0.259	3.49–4.55	22
Linolenic	0.30 ± 0.032	0.21–0.35	22
Vitamins			
Vitamin A (IU/kg)	3,569 ± 52	2,340–4,780	24
Vitamin D (IU/kg)	1,000[a]	–	–

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
α-Tocopherol (ppm)	80.6 ± 22.03	27.0–124.0	22
Thiamine (ppm)[b]	7.2 ± 1.12	5.1–9.3	24
Riboflavin (ppm)	7.6 ± 2.89	4.20–17.50	22
Niacin (ppm)	78.9 ± 9.08	66.4–98.2	22
Pantothenic acid (ppm)	26.9 ± 12.63	17.4–81.0	22
Pyridoxine (ppm)[b]	9.54 ± 1.99	6.44–13.7	22
Folic acid (ppm)	1.62 ± 0.48	1.15–3.27	22
Biotin (ppm)	0.32 ± 0.10	0.20–0.704	22
Vitamin B_{12} (ppb)	53.6 ± 39.6	18.3–174.0	22
Choline (ppm)[b]	2,846 ± 485	1,820–3,790	22
Minerals			
Calcium (%)	0.932 ± 0.055	0.808–1.030	24
Phosphorus (%)	0.538 ± 0.030	0.471–0.592	24
Potassium (%)	0.666 ± 0.030	0.626–0.733	22
Chloride (%)	0.386 ± 0.039	0.300–0.474	22
Sodium (%)	0.189 ± 0.016	0.160–0.222	22
Magnesium (%)	0.216 ± 0.062	0.185–0.490	22
Sulfur (%)	0.170 ± 0.029	0.116–0.209	14
Iron (ppm)	186 ± 39.2	135–311	22
Manganese (ppm)	51.4 ± 10.28	21.0–73.1	22
Zinc (ppm)	53.4 ± 8.46	43.3–78.5	22
Copper (ppm)	7.01 ± 2.562	3.21–16.3	22
Iodine (ppm)	0.503 ± 0.206	0.158–0.972	22
Chromium (ppm)	0.694 ± 0.276	0.330–1.380	22
Cobalt (ppm)	0.256 ± 0.164	0.098–0.864	22

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

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

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.24 ± 0.054	0.16–0.40	24
Cadmium (ppm)	0.06 ± 0.010	0.04–0.08	24
Lead (ppm)	0.10 ± 0.020	0.08–0.16	24
Mercury (ppm)	<0.02	–	24
Selenium (ppm)	0.33 ± 0.248	0.16–1.02	24

	Mean ± Standard Deviation[b]	Range	Number of Samples
Aflatoxins (ppb)	<5.00	–	24
Nitrate nitrogen (ppm)[c]	15.32 ± 7.22	5.09–36.8	24
Nitrite nitrogen (ppm)[c]	0.80 ± 0.68	0.30–3.04	24
BHA (ppm)[d]	1.17 ± 0.82	1.0–5.0	24
BHT (ppm)[d]	1.17 ± 0.82	1.0–5.0	24
Aerobic plate count (CFU/g)	10 ± 0	10	24
Coliform (MPN/g)	3.0 ± 0.0	3.0	24
Escherichia coli (MPN/g)	<10	–	24
Salmonella (MPN/g)	Negative	–	24
Total nitrosamines (ppb)[e]	7.3 ± 6.10	2.0–28.0	24
N-Nitrosodimethylamine (ppb)[e]	2.5 ± 2.15	1.0–10.3	24
N-Nitrosopyrrolidine (ppb)[e]	4.7 ± 4.69	1.0–17.7	24
Pesticides (ppm)			
α-BHC	<0.01	–	24
β-BHC	<0.02	–	24
γ-BHC	<0.01	–	24
δ-BHC	<0.01	–	24
Heptachlor	<0.01	–	24
Aldrin	<0.01	–	24
Heptachlor epoxide	<0.01	–	24
DDE	<0.01	–	24
DDD	<0.01	–	24
DDT	<0.01	–	24
HCB	<0.01	–	24
Mirex	<0.01	–	24
Methoxychlor	<0.05	–	24
Dieldrin	<0.01	–	24
Endrin	<0.01	–	24
Telodrin	<0.01	–	24
Chlordane	<0.05	–	24
Toxaphene	<0.10	–	24
Estimated PCBs	<0.20	–	24
Ronnel	<0.01	–	24
Ethion	<0.02	–	24
Trithion	<0.05	–	24

	Mean ± Standard Deviation[b]	Range	Number of Samples
Diazinon	<0.10	–	24
Methyl chlorpyrifos	0.058 ± 0.038	0.02–0.139	24
Methyl parathion	<0.02	–	24
Ethyl parathion	<0.02	–	24
Malathion	0.116 ± 0.137	0.020–0.581	24
Endosulfan I	<0.01	–	24
Endosulfan II	<0.01	–	24
Endosulfan sulfate	<0.03	–	24

[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 N. Sentinel Animal Program

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Tables

N.1. Methods

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

Blood samples were collected, allowed to clot, and the serum was separated. Additionally, fecal samples were collected and tested for *Helicobacter* species. All samples were processed appropriately and tested in-house during week 2 of the study or sent to BioReliance Corporation (Rockville, MD) (end of the 3-month studies and at 6, 12 and 18 months of the 2-year studies) or the Research Animal Diagnostic Laboratory (RADIL), University of Missouri (Columbia, MO) (end of 2-year studies) for determination of the presence of pathogens. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Blood was collected from five animals per sex except at the following collection time points:

Two-week study (rats): Start of study collections–four males and five females

Two-year study (rats): 6, 12, 18 month collections–0 males and 10 females

Fecal samples were collected from five male and five female mice.

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

Method and Test	Time of Collection
Rats	
Two-week Study	
In-House Antibody Testing	
Mycoplasma pulmonis	Study termination
PVM (Pneumonia virus of mice)	Study termination
RCV/SDA (Rat coronavirus/sialodacryoadenitis virus)	Study termination
RPV (Rat parvovirus)	Study termination
Sendai	Study termination
Three-month Study	
In-House Antibody Testing	
M. pulmonis	2 weeks
PVM	2 weeks
RCV/SDA	2 weeks
RPV	2 weeks

Method and Test	Time of Collection
Sendai	2 weeks
ELISA	
PVM	Study termination
RCV/SDA	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination
Two-year Study	
In-House Antibody Testing	
M. pulmonis	2 weeks
PVM	2 weeks
RCV/SDA	2 weeks
RPV	2 weeks
Sendai	2 weeks
ELISA	
Mycoplasma arthritidis	18 months
M. pulmonis	18 months
PVM	6, 12, and 18 months
RCV/SDA	6, 12, and 18 months
Sendai	6, 12, and 18 months
Immunofluorescence Assay	
KRV (Kilham's rat virus)	Study termination
Multiplex Fluorescent Immunoassay	
M. pulmonis	Study termination
Parvo NS-1	Study termination
Parvovirus	6, 12, and 18 months
PVM	Study termination
RCV/SDA	Study termination
RMV (Rat minute virus)	Study termination
RPV	Study termination
RTV (Rat theilovirus)	Study termination
Sendai	Study termination
TMEV GDVII (Theiler's murine encephalomyelitis virus– mouse poliovirus, strain GDVII)	Study termination
H-1 (Toolan's H-1)	Study termination

Mice

Method and Test	Time of Collection
Two-week Study	
In-House Antibody Testing	
MHV (Mouse hepatitis virus)	Study termination
MPV (Mouse parvovirus)	Study termination
M. pulmonis	Study termination
PVM	Study termination
Sendai	Study termination
TMEV GDVII	Study termination
Three-month Study	
In-House Antibody Testing	
MHV	2 weeks
MPV	2 weeks
M. pulmonis	2 weeks
PVM	2 weeks
Sendai	2 weeks
TMEV GDVII	2 weeks
ELISA	
Ectromelia virus	Study termination
EDIM (epizootic diarrhea of infant mice)	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
MAd-FL (Mouse adenovirus)	Study termination
MHV	Study termination
MMV VP2 (Mouse minute virus viral protein 2)	Study termination
MPV VP2 (Mouse parvovirus viral protein 2)	Study termination
PVM	Study termination
Reovirus	Study termination
Sendai	Study termination
TMEV GDVII	Study termination
Immunofluorescence Assay	
Ectromelia virus	Study termination
EDIM	Study termination
Two-year Study	
In-House Antibody Testing	
GDVII	2 weeks
MHV	2 weeks
MPV	2 weeks

Method and Test	Time of Collection
M. pulmonis	2 weeks
PVM	2 weeks
Sendai	2 weeks
ELISA	
Ectromelia virus	6, 12, and 18 months
EDIM	6, 12, and 18 months
LCM	6, 12, and 18 months
MAd-1	6, 12, and 18 months
MHV	6, 12, and 18 months
MMV VP2	6, 12, and 18 months
MPV VP2	6, 12, and 18 months
PVM	6, 12, and 18 months
Reovirus	6, 12, and 18 months
Sendai	6, 12, and 18 months
TMEV GDVII	6, 12, and 18 months
Immunofluorescence Assay	
EDIM	6 months and study termination
LCM	6 months
MHV	18 months
PVM	6 months
Reovirus	6 and 18 months
TMEV GDVII	6 months
Multiplex Fluorescent Immunoassay	
Ectromelia virus	Study termination
EDIM	Study termination
LCM	Study termination
MHV	Study termination
MMV	Study termination
Mouse norovirus	Study termination
MPV	Study termination
M. pulmonis	Study termination
Parvo NS-1	Study termination
PVM	Study termination
Reovirus	Study termination
Sendai	Study termination
TMEV GDVII	Study termination

Method and Test	Time of Collection
Polymerase Chain Reaction	
Helicobacter species	18 months

N.2. Results

All test results were negative.

Appendix O. Summary of Peer Review Panel Comments

On October 29, 2013, the draft Technical Report on the toxicology and carcinogenesis studies of cobalt metal received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. M. Behl, NIEHS, introduced the studies on cobalt metal. The United Auto Workers and the Cobalt Development Institute nominated cobalt metal for toxicology and carcinogenesis studies, with support from OSHA and NIOSH. The nomination was based on widespread occupational exposure and occurrence of hard metal disease associated with exposure to cobalt and its compounds. Dr. Behl presented results of mutagenicity studies and described the nonneoplastic and neoplastic lesions observed in the studies.

The proposed conclusions for the 2-year inhalation studies were clear evidence of carcinogenic activity of cobalt metal in male and female F344/NTac rats and male and female B6C3F1/N mice.

NTP contract pathologist Dr. A.R. Pandiri of Experimental Pathology Laboratories, Inc., described the molecular analyses of *Kras*, *Egfr*, and *Tp53* mutations in rat and mouse alveolar/bronchiolar carcinomas in these studies. He presented data demonstrating that mutations within *Kras* were higher than within *Egfr* and *Tp53* genes in both rat and mouse alveolar/bronchiolar carcinomas resulting from chronic inhalation exposure to cobalt metal.

Dr. Cullen noted receipt and distribution to the panel of written comments from Dr. S. Verberckmoes of Umicore S.A. and Dr. R. Danzeisen of the Cobalt Development Institute. Dr. Cullen opened the floor for oral public comments.

Dr. Danzeisen commented on the draft Technical Report by telephone. Dr. Danzeisen noted that the Cobalt Development Institute nominated cobalt metal for NTP testing. She anticipated that NTP studies on cobalt metal would lead to an industry self-classification of cobalt metal by the inhalation route, pending the outcome of the Peer Review Panel's deliberations. She felt that the study was well designed and conducted, but the particle size used was very small compared to that in typical human exposure scenarios. She said the high concentration used was relatively high as reflected in the early reductions in body weights. There was no no-observed-adverse-effect level, which made it more difficult to extrapolate the findings for risk assessment. Her group agreed with the NTP conclusion that there was clear evidence of carcinogenicity, but suggested limiting the conclusions to indicate that the evidence was by inhalation exposure and in the respiratory tract. The NTP findings were in line with her group's thinking and evidence from past human epidemiologic studies that cobalt causes cancer in the lung by causing local inflammation leading to reparative mechanisms. She noted that the systemic cancers were seen only in rats and not in mice, and are not relevant for humans and human risk assessment. She remarked that the systemic cobalt levels were highest in the liver, at times even exceeding lung levels, but the liver had no neoplasms. The cobalt levels achieved in the tissues seemed disconnected with adverse effects, particularly neoplasms. She found this supported her group's hypothesis that there is local inflammation leading to reparative mechanisms, hyperplasia, and subsequently cancer. She stressed that the Cobalt Development Institute has strong evidence that

cobalt is not a mutagen and agrees with the NTP review that oxidative stress causes interaction with the DNA.

Dr. Gordon, the first primary reviewer, indicated that the NTP's cobalt metal particle inhalation studies were very well designed and conducted and yielded important information regarding the carcinogenicity of a less soluble form of cobalt that complements the previous NTP studies with the soluble form. He said considerable data suggest that the soluble and insoluble forms can have differences in long-term toxicity or potency; therefore, he commended the testing of cobalt metal in the NTP bioassay program. He found the choices of exposure concentrations to be generally acceptable, but an additional low concentration would have been appropriate for both the rat and mouse 2-year studies. On the basis of some of the 3-month endpoints (e.g., larynx), there may have been sufficient data to justify using a lower exposure-concentration, which would enhance the relevance of the exposure concentrations. The multiple alveolar/bronchiolar carcinomas with exposure-concentration response in rats and mice, and the statistical significance of the tumors, provide additional evidence of the carcinogenicity of the particles in the lung. He indicated that if the concentration was lower, a clearer exposure concentration-related response might have been seen. He suggested particle size be addressed earlier in the report. He noted that a stainless steel jet mill was used to break up the cobalt metal into respirable particle sizes; the report should explain the resulting chromium contamination of the bulk chemical and note contamination was minimal. The potential for cobalt metal to be carcinogenic is strengthened by the similar lung tumors seen with the soluble form of cobalt. He inquired about a potential miscalculation in the normalization of the exposure concentration of cobalt sulfate heptahydrate to elemental cobalt. He noted that the cobalt metal particles might have been more potent than the soluble form, which would support the mode of action suggested by the public commenter. He agreed with the conclusion of clear evidence in the lung. He noted that the other conclusions, except for the cystic keratinizing epitheliomas, may not warrant the higher ratings and should perhaps be lowered.

Dr. Cory-Slechta, the second primary reviewer, noted that the studies were very well designed and conducted. Given that cobalt can be taken up by the nasal mucosa and into the brain, she said there should have been analysis of brain tissue.

Dr. Regan, the third primary reviewer, said the studies were well designed and had no interpretation differences at the clear evidence of carcinogenicity level. Regarding the equivocal evidence of carcinogenicity in the renal tubule adenomas and carcinomas, she asked about the lack of preneoplastic lesions in the kidneys. She asked what triggered the extended evaluation of the kidneys. She asked whether there was any evidence of the amphophilic-vacuolar carcinomas that have been found to be spontaneous; if so, then that should be taken into account. Regarding the pancreatic islet tumors, she said there was an increase in incidence compared to the historical control data, but the historical control data were not appropriate for this particular study because of the strain used. Thus, she proposed that the pancreatic islet tumors were ranked too high but agreed with the conclusions for all of the other tumor types.

Dr. Zacharewski, the fourth primary reviewer, asked whether the Kras, Egfr, and Tp53 mutations mapped to any specific consequences in terms of the activity of those proteins subsequent to the mutation. He also inquired whether any additional studies could have been done to demonstrate that the mutation actually had functional significance to the protein itself. He asked whether

there was any correlation between a mutation and a tumor outcome in terms of aggressiveness, metastatic ability, etc.

Dr. Barlow asked why no mutations were found in the concurrent controls, despite a robust response in the historical animals. He also noted that there was a well-known and direct mechanism for the development of adrenal medullary tumors and asked for comment from the study pathologist.

Dr. Parker endorsed more large-scale sequencing efforts to allow for more accurate identification of genetic mutations and assessment of other types of mutations, such as indels, as opposed to just point mutations.

Dr. Cullen asked if cardiomyopathy was observed in the study. Dr. Behl replied that there was no evidence of cardiomyopathy in these studies. She said that cardiomyopathy was observed in a female rat in the 13-week cobalt sulfate heptahydrate study.

Responding to Dr. Gordon's review, Dr. Behl agreed with his comments about nonneoplastic lesions in the larynx. She explained that when NTP has different exposure concentrations in two species in studies involving inhalation chambers, it has elected to go with one less concentration rather than adding an additional group. Hence, a lower concentration was not used. She noted that the particle size used was consistent with the rat respirable range (see pages 29, 30, and 275). Regarding the dosing calculation Dr. Gordon had questioned, she explained that the exposure concentration in the cobalt sulfate heptahydrate study was based on the mass percentage of cobalt in anhydrous cobalt sulfate. Dr. Gordon asked for better justification for the conclusion related to cystic keratinizing epitheliomas, suggesting perhaps that it should have been equivocal. Dr. Behl explained the basis for the some evidence call in the females. Because cystic keratinizing epitheliomas are rare and are part of a continuum of lung lesions, their occurrence was included as a chemical-related effect in the conclusions. Dr. R.A. Herbert, NIEHS, added that in the nonneoplastic lesions, there was some evidence of squamous cell hyperplasia within the lung and evidence of a progression from nonneoplastic lesions to benign lesions to carcinomas, leading to the some evidence conclusion.

Responding to Dr. Cory-Slechta's comment, Dr. Behl said the brain tissue was examined, and there was no evidence of neoplasms. Dr. Cory-Slechta said neoplasms might not have been expected, although there were likely nonneoplastic lesions, such as white matter injury. Dr. Herbert said there was no evidence of nonneoplastic or neoplastic lesions. Dr. D.E. Malarkey, NIEHS, said NTP is very interested in improving evaluation of the brain, having recently invoked a new method for its analysis.

Dr. Herbert responded first to Dr. Regan's question about what triggers an extended review in the kidney. He said the renal tubule adenomas are usually small tumors, and an extended review is triggered if there is an indication from the data that there could be an effect. Dr. Regan asked if there was a specific level used. Dr. Herbert said there was not. Dr. Barlow asked how often the extended review yields additional results that affect the conclusions. Dr. Herbert did not have data on that issue at the time. Dr. Regan asked whether any amphophilic-vacuolated renal tubule neoplasms were observed in the male rat study. Dr. Herbert said none were seen in this study and indicated that NTP has not traditionally made a distinction between the amphophilic-vacuolated type and other types of renal tubular neoplasms in studies. He noted that one publication indicates such tumors are spontaneous, but the toxicologic pathology community does not

generally accept this distinction. Dr. Regan mentioned that there are other publications on the topic. She asked how NTP could know that such a tumor type did not occur, if NTP does not distinguish that tumor type. Dr. Herbert indicated he had looked at all of the tumors, and that no amphophilic-vacuolated renal tubular neoplasms occurred in the study. Dr. Behl responded to Dr. Regan's comments about pancreatic islet tumors in the females and said the call equivocal evidence of carcinogenicity was primarily based on the increase in malignant neoplasms at the 5 mg/m^3 dose, as well as supporting evidence from the males (e.g., significant trend and pairwise comparisons in the top two exposure concentrations).

Dr. Pandiri responded to Dr. Zacharewski's comments. Dr. Pandiri said the selection of the hot spot exons in all three genes was based on extensive literature review of human lung cancer as well as rodent models of chemical-induced pulmonary carcinogenesis. Dr. Pandiri indicated that immunohistochemistry could be used to demonstrate the alterations in protein expression within the molecular pathways associated with the mutated genes.

Regarding Dr. Barlow's question about why no Kras mutations were observed in the spontaneous alveolar/bronchiolar carcinomas from the concurrent chamber controls, Dr. Pandiri speculated that there were in fact mutations present, but perhaps not in the exons examined.

In response to Dr. Parker's question about the primary focus being on point mutations, Dr. Pandiri said point mutations account for the majority of genetic changes seen in some of the well-known carcinogenesis studies in the literature. Dr. Pandiri agreed that massive parallel sequencing of the cancer genes in tumor tissue is a more powerful tool for detecting mutations and differentiating chemical-induced tumors from spontaneous tumors. He also informed the committee that NTP is currently running a pilot project using exome sequencing and RNA-seq technologies for evaluating chemically induced and spontaneous hepatocellular carcinomas from previous NTP chronic bioassays.

Dr. Malarkey responded to Dr. Gordon's comments regarding the cystic keratinizing epitheliomas and indicated that they are very rare in most species, so it is a significant finding when present. Even though they are benign, this tumor type would be considered in the levels of evidence of carcinogenic activity, especially because it can progress to a malignant tumor. Dr. Malarkey also responded to Dr. Barlow's question concerning how extended reviews of the kidneys are triggered and how often the extended reviews yielded additional results that affect the conclusions. He noted that when the response is weak, follow-up serial sections might confirm a finding.

Dr. Cory-Slechta moved to accept the conclusions as written, and Dr. Gordon seconded. The Peer Review Panel voted unanimously to accept the conclusions on cobalt metal as written in the draft report.

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