NTP REPORT ON THE

TOXICITY STUDIES OF COBALT SULFATE HEPTAHYDRATE IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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ADDENDUM

Additional information has been added to the *NTP Report on the Toxicity Studies of Cobalt Sulfate Heptahydrate in F344/N Rats and B6C3F1 Mice (Inhalation Studies)* (TOX-05) [July 3, 2023].

Addendum to the Report

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 (Bucher et al. 1990; Bucher et al. 1999; NTP 1991; NTP 1998). 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 (Bucher et al. 1990; NTP 1991). 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 (Bucher et al. 1999) 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 (NTP 2014). 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). 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).

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FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Toxicity Study Report were performed under the direction of the NIEHS and were conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals. Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure.

Anyone who is aware of related ongoing or published studies not mentioned in this report, or of any errors in this report is encouraged to make this information known to the NTP. Comments and questions should be directed to Dr. J.R. Bucher, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709(919-541-4532).

These NTP Toxicity Study Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Toxicity Study Report are available without charge while supplies last from the NTP Public Information Office, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3991).

TOXICITY STUDIES OF

COBALT SULFATE HEPTAHYDRATE

(CAS NO. 10026-24-1)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

John R. Bucher, Ph.D., Study Scientist

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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$CoSO_4 \cdot 7H_2O$

COBALT SULFATE HEPTAHYDRATE

CAS No. 10026-24-1

Molecular weight 281

ABSTRACT

Toxicology studies of cobalt sulfate heptahydrate (99% pure) were conducted by exposing groups of F344/N rats and $B6C3F_1$ mice of each sex to a cobalt sulfate heptahydrate aerosol 6 hours per day, 5 days per week, for 16 days or 13 weeks.

In 16-day studies, all rats and mice exposed at the top concentration of 200 mg cobalt sulfate/m^3 died (5 animals per group); partial survival was seen in the 50 mg/m^3 exposure groups. Degeneration of the olfactory epithelium and necrotizing inflammation occurred in the nose of all rats and mice that died and in animals exposed to 50 mg/m^3. Necrotizing inflammation was observed in the larynx and trachea of rats and mice at concentrations as low as 5 mg/m^3, and a similar lesion was present in the bronchi at exposure concentrations of 50 mg/m^3 or higher. Regenerative and inflammatory lesions, including peribronchial and septal fibrosis in the lung, were found in rats and mice exposed to 50 mg/m^3.

In 13-week studies, all rats, all female mice, and all but 2 male mice exposed at the top concentration survived to the end of the studies (target exposure concentrations of 0, 0.3, 1, 3, 10, and 30 mg/m^3, 10 animals per group). Rats and mice exposed to 30 mg/m^3 lost weight during the first exposure week and then gained weight at the same rate as controls. Lung weights were increased over those of controls in rats exposed at concentrations as low as 1 mg/m^3 and in mice exposed to 10 mg/m^3 or more. Polycythemia was observed in rats exposed to cobalt sulfate but not in mice. Sperm motility was decreased in mice exposed at 3 mg/m^3 or at higher concentrations (lower concentrations were not evaluated), and increased numbers of abnormal sperm were found in mice exposed to 30 mg/m^3. Testis and epididymal weights were decreased in mice exposed to 30 mg/m^3. Cobalt content in the urine of rats increased with increasing atmospheric cobalt exposure.

Lesions seen in the respiratory tract in 13-week studies in rats and mice included degeneration of the olfactory epithelium, squamous metaplasia of the respiratory epithelium, and inflammation in the nose; inflammation, necrosis, squamous metaplasia, ulcers (rats), and inflammatory polyps (rats) of the larynx; squamous metaplasia of the trachea (mice); and histiocytic infiltrates, bronchiolar regeneration, peribronchiolar and septal fibrosis, and epithelial hyperplasia in the alveoli of the lung. The most sensitive tissue was the larynx, with squamous metaplasia observed in rats and mice at the lowest exposure concentration of 0.3 mg/m^3. Thus, a no-observed-adverse-effect level was not reached in these studies.

CONTRIBUTORS

The NTP Report on the Toxicity Studies of Cobalt Sulfate Heptahydrate is based on the 16-day and 13-week studies that began in June 1986 at Battelle Pacific Northwest Laboratories (Richland, WA).

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on cobalt sulfate heptahydrate on June 27, 1989, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have four major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, and (d) to judge the significance of the experimental results by scientific criteria.

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SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICITY STUDIES OF COBALT SULFATE HEPTAHYDRATE

On June 27, 1989, the draft Technical Report on the toxicity studies of cobalt sulfate heptahydrate received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.R. Bucher, NIEHS, introduced the short-term toxicity studies of cobalt sulfate heptahydrate by reviewing the rationale, experimental design, and results. Cobalt sulfate aerosols were administered by whole body inhalation exposure to groups of F344/N rats and B6C3F$_1$ mice of each sex for 16 days or 13 weeks.

Dr. Garman, a principal reviewer, said that the draft report was well prepared. In particular, he thought that the histopathology portion of the Results section was commendable for the detailed description of the localization of the lesions at the suborgan level and for the high quality photomicrographs of the lesions. He suggested changes to the discussion which emphasized the unique susceptibility to chemical injury of the area of the larynx affected by cobalt. He said that, in view of the irritation observed, the aerosol used needed to be better characterized in the Report, especially its pH. Dr. Bucher noted that the aerosol was a dry aerosol and that the pH was concentration dependent and not as acidic as predicted; there was thus less concern that effects seen were due to acidity.

Dr. Klaassen, a second principal reviewer, said that the observation that cobalt activates heme oxygenase should be corrected to indicate that cobalt induces the enzyme.

Dr. Mirer stated that cobalt is an extremely important compound from an industrial health point of view and that the lowest concentration tested in these studies is only slightly above the allowed occupational health limit. He said that there was some indication that respiratory effects of cobalt in humans may have allergic aspects. Dr. Mirer asked whether the National Toxicology Program planned to do long-term studies; Dr. Bucher said that no decision had been made on further studies. Dr. R. Griesemer, NIEHS, said that the Panel's advice would be helpful in setting priorities. Dr. Scala said that, seeing no objections, the Panel would accept the Report with the clarifications noted.

$CoSO_4 \cdot 7H_2O$

COBALT SULFATE HEPTAHYDRATE

CAS No. 10026-24-1

Molecular weight 281

I. INTRODUCTION

Physical Properties, Production, Uses, and Exposure

Cobalt sulfate is a reddish, crystalline, water-soluble powder. It is usually produced as cobaltous (Co^{2+}) sulfate but can also exist in the cobaltic (Co^{3+}) form with a formula of $Co_2(SO_4)_3 \cdot 18H_2O$. The heptahydrate salt is reported to have a structure of $[Co(H_2O)_6] \cdot [H_2SO_5]$ (Merck, 1983). Cobaltous salts are stable to autoxidation in air or in solution (Smith and Carson, 1981).

The production of cobalt sulfate in the United States in 1983 was estimated to be 450,000 pounds (204,000 kg) (J.V. Gandhi, Hall Chemical Co., personal communication to P. Jackson Schad, NTP, September 21, 1983). Cobalt sulfate has been widely used in the electroplating and electrochemical industries. It is used as a coloring agent for ceramics and as a drying agent in inks, paints, varnishes, and linoleum. Cobalt sulfate may be added to animal feed as a mineral supplement and has been used as a top dressing on pasture lands (De Bie and Doyen, 1962).

Cobalt is an essential trace element because it is an integral part of vitamin B_{12}. The human body burden is approximately 1.1 mg, and the daily intake is about 0.3 mg, primarily via food (Hammond and Beliles, 1980). Cobalt is found in urban air (0.5-60 ng/m^3) (Morgan et al., 1970) and has been identified in trace amounts in natural waters; values in excess of 10 µg/liter are rare (NRC, 1977). Cobalt has been identified in chemical waste dumps (Barrett, 1983).

In the 1960's, several breweries added cobalt sulfate to beer at a level of about 1 ppm to counteract the antifoaming activity of detergent residues left on poorly rinsed glasses (Morin and Daniel, 1967). Soon after this, an epidemic of "beer-drinkers' cardiomyopathy" occurred, and cobalt was identified as the causative agent. The addition of cobalt salts to beer was discontinued, and the epidemic ceased. Doses of cobalt chloride of up to 200-300 mg per day were given by mouth to patients as treatment for various types of anemia in the 1950's (Finch, 1980). This practice has largely stopped because of associated toxicity (gastrointestinal upset, goiter, cardiomyopathies) and the development of less hazardous therapies.

It has been estimated that approximately 1,000 workers worldwide are routinely exposed to soluble cobalt salts (J.M. Johnston, Cobalt Development Institute, personal communication to J.R. Bucher, NTP, May 5, 1988). Occupational exposure to cobalt occurs principally in refining processes, in the production of alloys, and in the tungsten carbide hard metal industry (Kazantzis, 1981). Exposure under these conditions is primarily dermal or via inhalation of cobalt metal dusts or fumes, often in combination with other elements such as nickel, arsenic, or tungsten; adverse respiratory effects (pneumoconiosis) have been reported at cobalt concentrations between 0.1 and 2 mg/m^3 (Domingo, 1989). The threshold limit value/time-weighted average for cobalt metal, dust, or fumes is 0.05 mg/m^3 (ACGIH, 1988).

Absorption, Distribution, and Excretion

The absorption of cobalt salts after oral administration is variable and is influenced by the nature of the salt, the size of the dose, and the presence of food in the gastrointestinal tract (Murdock, 1959; Smith et al., 1972). Clearance of inhaled soluble cobalt salts from the lung has not been studied but is expected to be rapid (Kerfoot et al., 1975). Several processes could contribute to this effect. The water-soluble salts dissolve directly, and certain insoluble salts and cobalt metal powder appear to have an appreciable solubility in protein-containing fluids (Harding, 1950). Clearance by phagocytic alveolar macrophages may also occur (Kerfoot et al., 1975).

Cobalt is distributed to all tissues after administration by the oral or inhalation routes or by injection (Smith and Carson, 1981). Tissue retention is not marked, but higher concentrations have been noted in the liver, kidney, spleen, and heart than in other organs (Domingo et al., 1984a,b; Llobet et al., 1986). In an unspecified strain of rabbits administered 0.25 mg/kg cobalt sulfate per day orally or by injection for 2 months, some accumulation of cobalt occurred in the liver, small intestine, lung, blood, kidney, and stomach (Kichina, 1974). Excretion is primarily via the urine and secondarily via the feces. The cobalt content of bile collected for 2 hours after intravenous administration of [^{57}Co]cobalt chloride to Sprague Dawley rats totaled about 2%-5% of the dose over a thirtyfold dose range (0.03-1 mg/kg of Co^{2+}) (Gregus and Klaassen, 1986).

Toxic Effects

Exposure to cobalt results in a wide spectrum of toxicities in mammals. The ionic radius of cobalt is between that of Mg^{2+} and Ca^{2+}, so it can replace or mimic these ions and also may influence reactions normally involving Fe^{2+}, Zn^{2+}, Cu^{2+}, or Mn^{2+} (Jennette, 1981). For example, cobalt can bind to Ca^{2+}-binding protein in or near microtubules (Phillips, 1980) and has been shown to block Ca^{2+} channels in squid axons (Baker et al., 1973). Cobalt promotes aberrant microtubule assembly (Buttlaire et al., 1980) and can alter the activity of metaloenzymes such as carboxypeptidase (Jennette, 1981). Cobalt also inhibits the activity of DNA polymerase I from *Micrococcus luteus* (Korman et al., 1978). Cobalt binds to sulfhydryl groups, including those of glutathione and cysteine, and through its binding to lipoic acid inhibits pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, effectively stopping oxidative metabolism (Dingle et al., 1962). A 250 µmol/kg (approximately 60 mg/kg) dose of cobalt chloride heptahydrate administered by subcutaneous injection to male Sprague Dawley rats caused a rapid increase in biliary excretion of both reduced and oxidized glutathione, but total hepatic glutathione tended to increase after cobalt exposure (Stelzer and Klaassen, 1985).

A dose of 60 mg cobalt/kg body weight given to rats was found to inhibit heme synthesis in the liver (De Matteis and Gibb, 1977). This apparently results from the formation of cobalt protoporphyrin by ferrochelatase and the feedback depression of the abnormal protoporphyrin on δ-aminolevulinic acid synthetase activity (Sinclair et al., 1982). In addition, cobalt induces the enzyme heme oxygenase (Maines and Kappas, 1976), and the combined effect of these actions is to rapidly deplete the cytochrome P450 concentrations in the liver. Other cytochromes appear to be less affected (Tephly and Hibbeln, 1971).

In contrast to its actions on heme synthesis in the liver, cobalt administration promotes polycythemia. This effect is more pronounced in humans than in rodents (Smith and Carson, 1981) and is the basis for the use of cobalt chloride to treat anemia. The oral administration of 10 mg/kg of cobalt given as cobalt chloride to male rats, five times per week for 150 days, resulted in an increase in the erythrocyte count, hematocrit value, and hemoglobin concentration of the blood; however, the mean corpuscular volume and hemoglobin concentration per cell were unchanged, indicating a simple polycythemic effect (Murdock, 1959). This response is mediated by an increase in circulating erythropoietin, postulated to be a secondary response to a central nervous system effect of cobalt which results in respiratory alkalosis. Alkalosis increases the affinity of heme for oxygen, which is interpreted by tissue "sensors" as hypoxia (Miller et al., 1974).

A second effect of cobalt administration on the blood is an increase in triglycerides, cholesterol, and free fatty acids (Taylor and Marks, 1978). This may be caused by inhibition of tissue lipoprotein lipase, resulting in the failure to clear very low-density lipoprotein (Taylor and Marks, 1978), and perhaps by stimulation of lipoprotein synthesis by the liver (Eaton, 1972).

A single injection of 35 mg/kg cobalt chloride caused degranulation and disintegration of the α cells of the pancreatic islets of rabbits (Telib, 1972). This was followed by degranulation of the β cells.

Although exposure to cobalt affects a wide variety of enzymatic processes, the acute toxicity of cobalt is not as great as might be expected. The oral LD_{50} for anhydrous cobalt sulfate is 420 mg/kg in male and female Wistar rats (Spiejers et al., 1982).

Krasovskii and Fridlyand (1971) administered 0.5 or 2.5 mg/kg cobalt chloride by gavage to rats six times per week for 7 months. They found polycythemia and a suppression of leukocyte function. Myocardial histologic changes were seen in 26/30 rats given 26 mg/kg cobalt sulfate by gavage once per day for 8 weeks (Grice et al., 1969). This study is representative of a large number of animal studies designed to examine beer-drinkers' cardiomyopathy (cited in Smith and Carson, 1981). Overall, these studies indicated that rather large doses of cobalt could mimic the cardiomyopathy caused by cobalt-treated beer but that cobalt probably acted synergistically in humans with thiamine deficiency and an insufficient intake of sulfur-containing amino acids (Smith and Carson, 1981).

A second effect of cobalt observed in victims of beer-drinkers' cardiomyopathy was goiter or hypothyroidism (Taylor and Marks, 1978). Thyroid function tests, including uptake of [^{131}I]iodide, were also depressed in patients receiving 0.17-3.9 mg/kg cobalt per day for treatment of anemia (Paley et al., 1958). It has been proposed that cobalt interferes with binding of inorganic iodide to tyrosine in the thyroid gland. Effects of cobalt on other species are not as clear. Deficits in thyroid function have been shown in 1-day-old chicks and guinea pigs but not in young chicks, rats, mice, or rabbits given cobalt (Sederholm et al., 1968).

A variety of cobalt dusts and aerosols have been administered to animals via inhalation. Results of these studies indicate that lung compliance is decreased and that electrical properties of the heart are affected as in beer-drinkers' cardiomyopathy (Kerfoot et al., 1975; Smith, 1980). In general, evidence of the same toxicity elicited by cobalt administered orally has been found after inhalation of cobalt. These effects have been seen after exposure of rats to atmosphere containing 0.05 or 0.5 mg/m^3 cobalt for 3 months (Popov, 1977). In addition, specific pulmonary effects in male rabbits exposed to 0.5 mg/m^3 cobalt (as cobalt chloride) by inhalation for 6 hours per day, 5 days per week for 4-6 weeks, included a change in the growth pattern of alveolar type II cells, resulting in clusters of cells projecting into the alveolar lumen, and changes in oxidative metabolism of lung macrophages (Johansson et al., 1984, 1986).

Cobalt can elicit hypersensitivity reactions. Such reactions have been observed in patients who received prosthetic implants made of a cobalt alloy and in industrial workers exposed to cobalt dusts (Smith and Carson, 1981). Erythema and edema in the ears and paws of rats resulted from the administration of 5 mg cobalt sulfate by injection (Jasmin, 1974).

In National Toxicology Program (NTP) studies (unpublished, available upon request), cobalt sulfate elicited contact hypersensitivity. Female Hartley guinea pigs received dermal applications of 100 µl of an aqueous 6% solution once per day for 14 days. A dose-related increase in contact hypersensitivity, as measured by retention of labeled inflammatory cells in the skin, was observed upon challenge application of solutions of 0.3%, 1%, or 3% aqueous cobalt sulfate to a site distant from the induction site 7 days after the last induction dose.

Genetic Toxicology

In unpublished NTP studies, cobalt sulfate heptahydrate was found to be mutagenic in *Salmonella typhimurium* strain TA100 in the presence

and absence of rat or hamster liver S9. No mutagenic activity was observed in strains TA1535 or TA98 with or without activation.

Carcinogenicity

Sarcomas have been observed at the site of injection of cobalt salts or cobalt metal powder. Heath (1956, 1960) gave rats a single injection of 0.28 g cobalt metal powder in fowl serum into the thigh muscle. Within 2 weeks, atypical myoblasts were observed (Heath, 1960), and between 5 and 12 months, malignant neoplasms developed at the injection site in 17/30 rats; 11 were rhabdomyosarcomas (Heath, 1956). Gilman (1962) reported a similar neoplastic response to injections of cobalt sulfide and cobalt oxide in rats but saw no neoplasms in mice. These materials are relatively insoluble, and Abbracchio et al. (1982) attributed the carcinogenic response to cobalt sulfide to the surface charge of the metal sulfide particles. They determined that amorphous cobalt sulfide particles, which have a natural surface charge of neutral or positive, were only phagocytized to a very limited extent by Chinese hamster ovary (CHO) cells and did not transform Syrian hamster embryo (SHE) cells. However, crystalline cobalt sulfide particles, which have a negative surface charge, were extensively phagocytized by CHO cells and did transform SHE cells. These authors suggested that intracellular solubilization of relatively insoluble cobalt salts would favor cellular transformation. Heath and Webb (1967) determined that, in primary rhabdomyosarcomas induced by intramuscular injection of metalic cobalt, cobalt is bound intracellularly, with 70%-90% found in the nucleus. Further fractionation studies demonstrated that 50% of the nuclear cobalt is bound in the nucleolus (Webb et al., 1972).

There is only one report of the formation of neoplasms after injection of a soluble cobalt salt. Shabaan et al. (1977) observed fibrosarcomas in 14/40 male Wistar rats 8 months to 1 year after administration of 40 mg/kg cobalt chloride by subcutaneous injection once per day for 10 days. Four of these neoplasms were not at the site of injection.

Epidemiology

Cobalt has been used in hundreds of patients as part of an alloy with chromium and molybdenum in prosthetic implants. During the first 14 years of its use for this purpose, no fibrosarcomas were identified in the recipients (McKee, 1971). Certain of these patients had high urinary cobalt levels, in the range of 30 µg/liter, and some had necrosis of bone and muscle surrounding the implant (Jones et al., 1975).

Most inhalation of cobalt is by workers in the refining and alloy production industries (NIOSH, 1981). The dusts may be in the form of the metal, its alloys, or its salts, but most often the oxide form is present. Consequently, no epidemiologic studies exist on exposure to pure cobalt metal or to cobalt sulfate. There is no evidence that cobalt dusts are carcinogenic in humans, but exposure appears to cause pulmonary fibrosis, splenic enlargement, dermatitis, and losses of appetite and sense of smell (Dorsit et al., 1970). Cobalt is used in the cemented tungsten carbide industry and is thought to be responsible for pulmonary "hard metal disease," consisting of upper respiratory tract irritation, pneumonitis, and pulmonary fibrosis (NIOSH, 1981).

Reproductive Effects

Cobalt has not been shown to cause significant teratogenic or reproductive effects in humans (Smith and Carson, 1981). No effects were noted in the babies of women who had taken cobalt chloride to counter anemia while pregnant (Jacobziner and Raybin, 1961). However, cobalt has been shown to cross the placenta; cobalt chloride and nitrite salt solutions induced fetal cleft palates when injected alone into mouse dams but inhibited cleft formation caused by cortisone or phenytoin (Kasirsky et al., 1969; Mitala et al., 1978). Sprague Dawley rats maintained on diets containing 265 ppm cobalt for 98 days showed degenerative changes in the testis; these changes were considered secondary to hypoxia (Mollenhaur et al., 1985). Paternain et al. (1988) reported that doses of up to 100 mg/kg cobalt chloride administered by gavage to pregnant Sprague Dawley rats once per day on days

6-15 of gestation did not result in significant evidence of fetotoxicity or teratogenicity.

Cobalt sulfate was nominated by the National Cancer Institute for study based on a lack of information on the toxicity of soluble cobalt salts. The more common cobaltous form and the inhalation route were selected for study to mimic worker exposure.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF COBALT SULFATE HEPTAHYDRATE

Cobalt sulfate heptahydrate was obtained in one lot (lot no. 412092) from Curtin Matheson Scientific, Inc. (Kansas City, MO). Analyses (infrared, ultraviolet and visible spectroscopy, Karl Fisher water analysis) by the National Toxicology Program (NTP) confirmed the identity; purity was approximately 99%. The impurity with the highest concentration (140 ppm) was nickel. Periodic infrared and visible spectroscopic analyses of the chemical at the study laboratory indicated no degradation over the course of the studies.

GENERATION AND MEASUREMENT OF CHAMBER ATMOSPHERIC CONCENTRATIONS

Aerosol Generation System

Cobalt sulfate heptahydrate aerosol was generated from an aqueous solution by nebulization using dried compressed air. The aerosol was heated to about 26° C to dry the particles partially and then was passed into a Nalgene® settling tank to eliminate large particles and water droplets. Further drying was accomplished by heating the aerosol to 45° C as it left the tank. The cobalt sulfate heptahydrate/air stream entered the distribution tube and was injected into each chamber (Hazleton 2000, Lab Products, Inc.) with air multiplier pumps. The aerosol was diluted to the desired concentration with air from the chamber air-conditioning system.

The pH of aqueous solutions of cobalt sulfate heptahydrate was measured at three concentrations. Solutions of 0.01, 0.1, and 1 M were found to have pH values of 6.3, 6.2, and 5.2, respectively.

Aerosol Concentration Monitoring

Throughout this Report, atmospheric concentrations are expressed in milligrams of cobalt sulfate per cubic meter of air rather than in milligrams of the heptahydrate. Three real-time aerosol monitors (Model RAM-1, GCA Environmental Instuments) were used to determine the concentration of the aerosol in the exposure chambers once every 20 minutes throughout the exposure period. The monitors were calibrated through the use of filter grab samples. Samples collected on filter paper were analyzed for cobalt by inductively coupled plasma analysis after extraction with dilute nitric acid. Daily mean exposures for 16-day and 13-week studies are given in Tables 1 and 2. Although the mean chamber concentrations achieved during the 16-day studies were quite close to the target concentrations, the variations about the means were much larger than desired. Variations were much smaller in the 13-week studies, due primarily to a change in the aerosol dilution system. Cascade impactor samples were taken to determine aerosol size distribution. The mass median aerodynamic diameter of the aerosol for all exposures ranged from 0.83 to 1.10 μm. Cobalt sulfate hydration in the aerosol distribution line was determined by ultraviolet/visible spectroscopy. During the 16-day studies, the extent of hydration was determined by thermal gravimetric analysis and ultraviolet/visible spectroscopy. Hydration ranged from 6.1 to 6.2 (water to cobalt mole ratio) for two samples taken during the 16-day studies, compared with 6.8 for the bulk chemical; hydration ratios of 7.66 and 7.67 were determined for two samples taken during the 13-week studies.

TABLE 1. MEAN CHAMBER CONCENTRATIONS OF COBALT SULFATE HEPTAHYDRATE IN THE SIXTEEN-DAY INHALATION STUDIES

Target Concentration (mg/m^3)	Determined Concentration (a) (mg/m^3)	Maximum Concentration (mg/m^3)	Minimum Concentration (mg/m^3)	Percent of Samples in Range (b)
0	(c)			
0.1	0.093 ± 0.038	0.25	0.020	36
0.5	0.50 ± 0.123	1.08	0.034	43
5	4.73 ± 0.80	7.21	0.34	58
50	50.1 ± 7.57	77.6	0.21	68
200	199 ± 23.3	236	85.8	57

(a) Mean ± standard deviation for approximately 230 determinations
(b) Within 10% of target concentration
(c) Less than the detectable value of 0.005 mg/m^3 for 98% of samples; occasionally higher values were found but were determined to be due to instrumental baseline drift.

TABLE 2. MEAN CHAMBER CONCENTRATIONS OF COBALT SULFATE HEPTAHYDRATE IN THE THIRTEEN-WEEK INHALATION STUDIES

Target Concentration (mg/m^3)	Determined Concentration (a) (mg/m^3)	Maximum Concentration (mg/m^3)	Minimum Concentration (mg/m^3)	Percent of Samples in Range (b)
0	(c)			
0.3	0.300 ± 0.029	0.672	0.095	81
1	0.990 ± 0.087	1.83	0.156	90
3	2.93 ± 0.275	3.79	0.22	88
10	9.95 ± 0.579	13.2	6.53	93
30	30.0 ± 1.64	36.3	21.2	94

(a) Mean ± standard deviation for approximately 880 determinations
(b) Within 10% of target concentration
(c) Less than the detectable value of 0.005 mg/m^3 for 88% of samples; occasionally higher values were found but were determined to be due to instrumental baseline drift.

Chamber Characterization

The uniformity of the aerosol concentration in each exposure chamber, with and without animals present, was measured once before the beginning and once during each study with a RAM-1 monitor. The between-port variability, expressed as percent relative deviation, was less than 4.4% for all measurements.

The build-up and bleed-off times were similar for all chamber concentrations. The time to reach 90% of the target chamber concentration (T_{90}) as well as the time to bleed off to 10% of the target concentration after the generator was discontinued (T_{10}) were both approximately 8 minutes.

The stability of the chemical in the aerosol distribution line and chamber was evaluated by derivative spectrophotometry in the range of 20-800 nm. The similar spectral features of all samples was indicative of the stability of the cobalt sulfate heptahydrate aerosol.

SIXTEEN-DAY STUDY DESIGN

Groups of five rats and five mice of each sex were exposed to air containing cobalt sulfate heptahydrate at concentrations of 0 (chamber controls), 0.1, 0.5, 5, 50, or 200 mg/m^3 (calculated on the basis of the anhydrous salt) 6 hours (plus T_{90}) per day, for 12 exposures over 16 days. A necropsy was performed on all animals. Histologic

examinations were performed on controls and animals that were exposed to 50 or 200 mg/m^3 and on male mice exposed to 5 mg/m^3. The organs examined are listed in Table 3.

THIRTEEN-WEEK STUDY DESIGN

Groups of 10 rats and 10 mice of each sex were exposed to air containing cobalt sulfate heptahydrate at concentrations of 0 (chamber controls), 0.3, 1, 3, 10, or 30 mg/m^3 (calculated on the basis of the anhydrous salt) 6 hours (plus T_{90}) per day, 5 days per week for 13 weeks. Sperm morphology and vaginal cytology evaluations were performed for rats and mice exposed to 0, 3, 10, or 30 mg/m^3.

Male and female F344/N rats and B6C3F$_1$ (C57BL/6N, female × C3H/HeN MTV$^-$, male) mice used in these studies were produced under strict barrier conditions at Taconic Farms, Inc. Animals were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Rats and mice were shipped to the study laboratory at 4 weeks of age, quarantined at the study laboratory for 2 weeks, and placed on study at 6 (rats) or 7 (mice) weeks of age.

Clinical Examinations, Supplemental Studies, and Pathology

Details of clinical examinations and pathology procedures are outlined in Table 3. Animals surviving to the end of the studies were humanely killed with carbon dioxide. The brain, heart, right kidney, liver, lung, right testis, and thymus were weighed. Hematologic analyses were performed on blood obtained from the retroorbital (rats) or supraorbital sinus (mice). Analyses included leukocyte, lymphocyte, segmented neutrophil, monocyte, basophil, eosinophil, erythrocyte, reticulocyte, and platelet counts; hemoglobin concentration; mean corpuscular hemoglobin; mean corpuscular hemoglobin concentration; and mean cell volume. All data except those for reticulocyte and differential counts were obtained by using an Ortho ELT-8 Hematology Analyzer. Serum chemistry analyses, urinalyses, and thyroid function tests were performed for rats only. Serum chemistry analyses included glucose, cholesterol, and triglyceride concentrations; total creatine kinase activity; and quantitation of the three isoenzymes of creatine kinase. Thyroid function tests were performed by radioimmunoassay methods and included determination of triiodothyronine (T_3), thyroxin (T_4) (Tri-Tab and Tetra-Tab kits, NML Organon Teknika Corp.), and thyrotropin (TSH) concentrations. Urinalyses determinations included volume, appearance, and specific gravity; urinary cobalt content was determined by inductively coupled plasma analysis.

A necropsy was performed on all animals. In some instances, a particular organ was lost or autolyzed; thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals placed on study. Organs and tissues were examined for gross lesions. Tissues were preserved in 10% neutral buffered formalin and routinely processed for preparation of histologic sections for microscopic examination. Additional sections of the larynx were prepared to allow evaluation of the laryngeal epithelium of the base of the epiglottis. Tissues and groups examined are listed in Table 3.

Upon completion of the histologic evaluation by the laboratory pathologist, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed, and the results were reviewed and evaluated by the NTP Pathology Working Group (PWG). Tissues evaluated by the PWG included the nasal cavity, larynx, lung, and mandibular lymph nodes for both rats and mice and the testis for mice. The final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman et al. (1985).

TABLE 3. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE SIXTEEN-DAY AND THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE

Sixteen-Day Studies	Thirteen-Week Studies
Strain and Species F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice
Animal Source Taconic Farms (Germantown, NY)	Taconic Farms (Germantown, NY)
Study Laboratory Battelle Pacific Northwest Laboratories	Battelle Pacific Northwest Laboratories
Size of Study Groups 5 males and 5 females of each species, individually caged	10 males and 10 females of each species, individually caged
Concentrations 0, 0.1, 0.5, 5, 50, or 200 mg/m^3 cobalt sulfate heptahydrate (calculated as the anhydrous salt) in deionized water as an aerosol	0, 0.3, 1, 3, 10, or 30 mg/m^3 cobalt sulfate heptahydrate (calculated as the anhydrous salt) in deionized water as an aerosol
Method of Animal Distribution Animals distributed to weight classes and then assigned to cages and groups by a table of random numbers	Same as 16-d studies
Diet NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum	Same as 16-d studies
Animal Room Environment Temp--69.2°-77.3° F; fluorescent light 12 h/d	Temp--71°-78.7° F; fluorescent light 12 h/d
Time Held Before Study Rats--24 d; mice--25 d	Rats--13 d; mice--14 d
Age When Placed on Study 8 wk	Rats--6 wk; mice--7 wk
Duration of Dosing 6 h/d for 12 exposures over 16 d	6 h/d, 5 d/wk for 13 wk
Age When Killed 10 wk	Rats--19 wk; mice--19-20 wk
Type and Frequency of Observation Observed 2 or 3 × d; weighed initially, on d 8, and at necropsy	Observed 2 × d; weighed initially and 1 × wk thereafter
Necropsy and Histologic Examinations Necropsy performed on all animals; the following tissues examined histologically for all controls, all 50 and 200 mg/m^3 animals, and 5 mg/m^3 male mice: adrenal glands, brain, bronchial lymph nodes, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or ovaries uterus, esophagus, eyes (if grossly abnormal), gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx, liver, lungs and mainstem bronchi, mammary gland, mandibular and mesenteric lymph nodes, mediastinal lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pharynx, pituitary gland, preputial or clitoral gland (rats), rectum, salivary glands, skin, spinal cord (if neurologic signs present), spleen, sternebrae including marrow, stomach, thymus, thyroid gland, trachea, and urinary bladder. Tissues examined for lower dose rat groups include brain for all males, lung and ovaries for all females, and heart, larynx, lymph nodes, nose, thymus, and trachea for 5 mg/m^3 males and females; liver and testes for 0.5 mg/m^3	Necropsy performed on all animals; the same tissues were examined for the control and high dose groups as were examined for the 16-d studies. Tissues examined in all other groups (except 0.3 mg/m^3 male mice) include gross lesions, larynx, lungs, and nose; larynx and lungs only examined for 0.3 mg/m^3 male mice. Mediastinal lymph nodes, spleen, testes, thymus, and trachea examined for 10 mg/m^3 male mice; mediastinal lymph nodes and trachea examined for 10 mg/m^3 female mice; and mediastinal lymph nodes examined for 3 mg/m^3 female mice. A transverse section was made through the larynx of rats, caudal to the thyroid cartilage; 4-6 step-sections were prepared to ensure that the base of the epiglottis was present for examination. Hematologic and serum chemical analyses, thyroid function tests, and urinalyses performed; sperm morphology and vaginal cytology evaluated

TABLE 3. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE SIXTEEN-DAY AND THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE (Continued)

Sixteen-Day Studies	Thirteen-Week Studies
Necropsy and Histologic Examinations (Continued) males; and larynx, mediastinal and tracheobronchial lymph nodes, and trachea for 0.5 mg/m^3 females. Tissues examined for lower dose mice include liver for all males; brain, larynx, lungs, thymus, trachea, and tracheobronchiol lymph nodes for 5 mg/m^3 females; larynx and trachea for 0.5 mg/m^3 mice; and brain for 0.5 mg/m^3 males. Organ weights obtained at necropsy	

STATISTICAL METHODS

The analysis of organ weight, serum chemistry, hematologic, urinalysis, and male reproductive system data was carried out by using the nonparametric multiple comparison procedures of Dunn (1964) or Shirley (1977) to assess the significance of pairwise comparisons between dosed and chamber control groups. Jonckheere's test (Jonckheere, 1954) was used to evaluate the significance of dose-response trends and to determine whether Dunn's or Shirley's test was more appropriate for pairwise comparisons.

The proportion of time spent in each stage of the estrous cycle was compared by using the Wilks criterion statistic (Wilks, 1932) of the multivariate analysis of variance procedure, which was performed after an arc sine transformation of the data.

QUALITY ASSURANCE

The studies of cobalt sulfate heptahydrate were performed in compliance with Good Laboratory Practices and regulations (21 CFR 58). The Quality Assurance Unit of Battelle Pacific Northwest Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the conduct of the studies. The operations of the Quality Assurance Unit were monitored by the NTP, including a site visit during the period of study performance.

III. RESULTS

RATS

Sixteen-Day Studies

All rats exposed to 200 mg/m^3 and 2/5 male rats exposed to 50 mg/m^3 died before the end of the studies (Table 4). Rats exposed to 50 mg/m^3 lost weight; final mean body weights at other concentrations were similar to those of controls. Clinical signs in rats exposed to 50 mg/m^3 (after two exposures) or 200 mg/m^3 (after one exposure) included hypoactivity, chromodacryorrhea, hypothermia, rapid and shallow breathing, and reduced body tone. These clinical signs progressively worsened with subsequent days of exposure. Gross observations in animals dying before the end of the studies and in rats killed after exposure to 5 or 50 mg/m^3 included red discoloration and increased firmness in the lungs. The absolute lung weight and lung weight to body weight ratios were significantly increased for male and female rats at 50 mg/m^3. Absolute thymus weights and thymus weight to body weight ratios were markedly decreased (one-fourth to one-half those of controls) for male and female rats at 50 mg/m^3.

Lesions attributed to cobalt sulfate exposure were seen at all levels of the respiratory tract. At the two highest exposure concentrations, inflammation and necrosis of the respiratory epithelium were seen in the larynx, trachea, bronchioles, and the respiratory turbinates of the nose. Degeneration of the olfactory epithelium was also present. In the 50 mg/m^3 groups, hyperplasia and squamous metaplasia in the epithelium of the respiratory turbinates and hyperplasia (acanthosis) of the squamous epithelium of the larynx occurred in rats that survived at least 9 days or were killed at the end of the 16-day exposure period. Inflammation in the nose at 50 mg/m^3 consisted of a serous exudate in the lumen of the nasal cavity.

In the lungs, edema and hemorrhage into alveolar spaces were seen at the 200 mg/m^3 exposure concentration. At the 50 mg/m^3 exposure concentration, inflammation and histiocytic (macrophage) infiltration in the lungs were present. Fibrosis around bronchioles and mild-to-moderate ectasia (dilatation) of bronchioles were also present at this concentration.

Other lesions observed in exposed rats that died during the exposure period consisted of lymphoid necrosis in the thymus and congestion of vessels in the brain/meninges. At the highest concentration, centrilobular congestion and necrosis were present in the liver of both male and female rats. Atrophy of the testis, characterized by a decreased number of cells in the seminiferous tubules and atypical germinal epithelial cells in the epididymal ducts, was observed in rats exposed to 50 mg/m^3.

Cardiomyopathy of minimal severity, characterized by mononuclear inflammatory cell infiltrates, hyalinized myocardial fibers, and/or fibrosis in the myocardium, was observed primarily in animals that died but was also seen in 2/5 male controls and thus was not clearly compound related.

Thirteen-Week Studies

All rats lived to the end of the studies (Table 5). Mean body weights of male rats exposed to 30 mg/m^3 were lower than those of controls throughout the study (Figure 1). The final mean body weight of male rats exposed to 30 mg/m^3 was 14% lower than that of controls. Mean body weights of exposed female rats were not related to the cobalt sulfate exposure. Compound-related clinical signs included ruffled fur in rats and hunched posture in male rats exposed to 30 mg/m^3.

The absolute lung weights and/or the lung weight to body weight ratio were significantly increased for male rats exposed to 0.3 mg/m^3 or more and for female rats exposed to 1 mg/m^3 or more (Table 6). Relative kidney weights were increased in male rats at all exposure concentrations.

TABLE 4. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE SIXTEEN-DAY INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE

Concentration (mg/m^3)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)
MALE					
0	5/5	190 ± 3	242 ± 3	+52 ± 2	
0.1	5/5	191 ± 4	250 ± 4	+59 ± 5	103
0.5	5/5	190 ± 5	252 ± 8	+62 ± 4	104
5	5/5	184 ± 6	234 ± 9	+50 ± 3	97
50	(d) 3/5	190 ± 5	128 ± 9	−61 ± 6	53
200	(e) 0/5	190 ± 2	(f)	(f)	(f)
FEMALE					
0	5/5	128 ± 1	155 ± 3	+27 ± 3	
0.1	5/5	131 ± 2	164 ± 5	+33 ± 3	106
0.5	5/5	129 ± 1	158 ± 2	+29 ± 2	102
5	5/5	131 ± 2	157 ± 3	+26 ± 2	101
50	5/5	130 ± 2	120 ± 11	−10 ± 9	77
200	(g) 0/5	133 ± 2	(f)	(f)	(f)

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Day of death: 9,11
(e) Day of death: 2,2,2,3,3
(f) No data are reported due to 100% mortality in this group.
(g) Day of death: 2,2,3,5,5

TABLE 5. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE

Concentration (mg/m^3)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)
MALE					
0	10/10	130 ± 3	352 ± 9	+222 ± 7	
0.3	10/10	130 ± 3	352 ± 9	+222 ± 9	100
1	10/10	131 ± 3	347 ± 6	+216 ± 5	99
3	10/10	131 ± 3	349 ± 11	+218 ± 9	99
10	10/10	131 ± 3	350 ± 9	+219 ± 7	99
30	10/10	130 ± 2	302 ± 9	+172 ± 10	86
FEMALE					
0	10/10	109 ± 2	204 ± 5	+95 ± 4	
0.3	10/10	104 ± 2	189 ± 5	+85 ± 4	93
1	10/10	107 ± 3	196 ± 6	+89 ± 5	96
3	10/10	109 ± 3	210 ± 5	+101 ± 3	103
10	10/10	109 ± 2	205 ± 4	+96 ± 3	100
30	10/10	110 ± 2	191 ± 3	+81 ± 2	94

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean

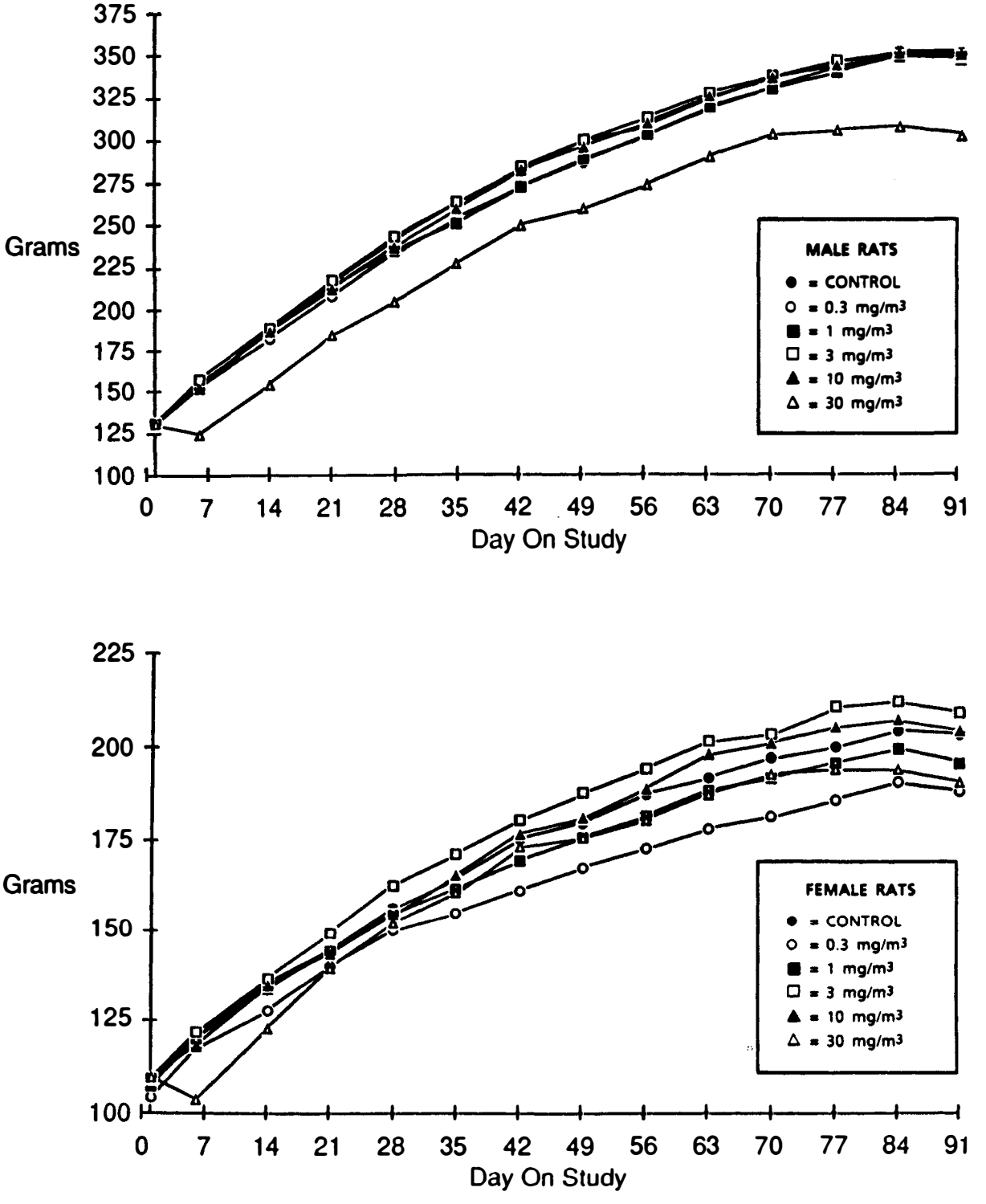

FIGURE 1. GROWTH CURVES FOR RATS EXPOSED TO COBALT SULFATE HEPTAHYDRATE BY INHALATION FOR THIRTEEN WEEKS

TABLE 6. SELECTED ORGAN WEIGHTS OF RATS IN THE THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE (a)

Organ	Control	0.3 mg/m^3	1 mg/m^3	3 mg/m^3	10 mg/m^3	30 mg/m^3
MALE						
Body weight (grams)	331 ± 8.0	330 ± 8.9	325 ± 6.2	328 ± 10.4	327 ± 8.4	**282 ± 8.8
Kidney						
Absolute	1,093 ± 36	1,150 ± 37	1,140 ± 27	1,145 ± 35	1,145 ± 39	1,042 ± 35
Relative	3.3 ± 0.06	*3.5 ± 0.04	*3.5 ± 0.03	*3.5 ± 0.07	*3.5 ± 0.05	**3.7 ± 0.06
Lung						
Absolute	1,364 ± 45	1,451 ± 33	*1,506 ± 53	**1,690 ± 79	**1,951 ± 78	**2,008 ± 75
Relative	4.1 ± 0.11	*4.4 ± 0.11	**4.6 ± 0.11	**5.1 ± 0.12	**6.0 ± 0.11	**7.1 ± 0.11
FEMALE						
Body weight (grams)	188 ± 4.7	173 ± 4.8	181 ± 5.9	196 ± 4.8	191 ± 4.2	175 ± 2.8
Kidney						
Absolute	668 ± 25	617 ± 14	646 ± 19	691 ± 19	666 ± 22	673 ± 19
Relative	3.5 ± 0.09	3.6 ± 0.07	3.6 ± 0.07	3.5 ± 0.05	3.5 ± 0.06	3.8 ± 0.08
Lung						
Absolute	935 ± 28	904 ± 18	*1,035 ± 24	**1,282 ± 38	**1,344 ± 40	**1,573 ± 47
Relative	5.0 ± 0.10	5.2 ± 0.10	**5.7 ± 0.11	**6.6 ± 0.13	**7.0 ± 0.16	**9.0 ± 0.21

(a) Mean ± standard error in milligrams (absolute) or milligrams per gram (relative) for groups of 10 animals; P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
*P<0.05
**P<0.01

Polycythemia, seen at 10 and 30 mg/m^3 for female rats and at 3 mg/m^3 and at higher concentrations for male rats, was indicated by significant increases in erythrocytes, in the mean hemoglobin concentration, and in the hematocrit value (Table A1). The reticulocyte count was significantly increased in female rats exposed to 30 mg/m^3. The platelet count was significantly decreased in rats exposed to 10 or 30 mg/m^3. No significant changes were found in the leukocyte or differential counts.

Mean serum cholesterol values were significantly decreased for males exposed to 10 or 30 mg/m^3 and for females exposed to 30 mg/m^3 (Table A1). No consistent dose-related effects were seen on the glucose concentration; total creatine kinase activity, or the percentage of each of three creatine kinase isoenzymes present (Table A2); or serum triglyceride concentration. The triiodothyronine (T_3) concentration was significantly lower for females exposed to 10 and 30 mg/m^3 and the thyrotropin (TSH) concentration was significantly lower for males at 30 mg/m^3, but the thyroxin (T_4) and TSH concentrations for female rats and the T_3 and total and free T_4 concentrations for male rats were not consistently dose related.

Granular casts were observed in the urine from many exposed male rats (3-7 animals per group of 10), whereas none was observed in the urine from controls. A dose-related increase was seen in the number of epithelial cells in the urine from males that were exposed to 3 mg/m^3 or more. Urine volumes collected over 16 hours were variable and not statistically different from those for controls except in the 30 mg/m^3 females, which averaged approximately two-thirds that of controls.

The amount of cobalt excreted in urine over 16 hours varied from 2.5 µg at 0.3 mg/m^3 to 105 µg at 30 mg/m^3 for males and from 2.0 µg at 0.3 mg/m^3 to 67 µg at 30 mg/m^3 for females (Table A4). The amount of cobalt excreted in the urine of rats exposed to 0.3 mg/m^3 was approximately 10 times that excreted by controls.

No statistically significant effects on sperm motility, sperm counts, or the incidence of abnormal sperm were observed in exposed rats. The average estrous cycle of females exposed to 30 mg/m^3 was longer (but not significantly) than that of controls (Table A3).

Compound-related lesions were limited to the respiratory tract of rats of each sex exposed to cobalt sulfate. Lesions were concentration related and similar in incidence and severity in males and females (Table 7). In the nose, hyperplasia and squamous metaplasia of the respiratory epithelium were seen primarily at the two highest exposure concentrations. This was most prominent at the tips of the naso- and maxilloturbinates and on the lateral wall of the nasal cavity in the most anterior section of the nose. Degeneration of the olfactory epithelium was characterized by a thinning of the olfactory epithelial cell layer in the dorsal meatus and also on the nasal septum in the ethmoid region (degeneration was slightly more prominent in males).

At the higher exposure concentrations, inflammatory polyps were seen in the larynx of most rats (Figures 2 and 3). Polyps were consistently located at the base of the epiglottis and extended into the lumen of the larynx. These polyps had a fibrovascular stroma, which was covered by a well-differentiated squamous epithelium. Focal areas of necrosis and ulceration were frequently present in the epithelium of the polyp. Chronic inflammation and mineralization were prominent in the stroma of the polyp. At the lower concentrations at which polyps did not occur, squamous metaplasia of the laryngeal respiratory epithelium and chronic inflammation in the stroma persisted. At 0.3 mg/m^3, the severity of the metaplasia and inflammation was minimal to mild.

Regeneration of bronchiolar epithelium with dilatation (ectasia) of bronchioles was observed in the lung of rats exposed to 30 mg/m^3; distension or disruption of alveolar septa (emphysema) was also present. Fibrosis was present around bronchioles and within alveolar septae. Histiocytic infiltration, characterized by intra-alveolar accumulation of macrophages and infiltration of alveolar septae with inflammatory cells (subacute inflammation) also occurred at this exposure concentration. At lower concentrations, only intra-alveolar histiocytic infiltrates and subacute inflammation were present. Lymphoid hyperplasia was present in the mediastinal lymph nodes of exposed rats, but the incidence was not concentration related. Cardiomyopathy was seen in 3/10 control and 3/10 male rats exposed to 30 mg/m^3; the severity was marginally increased in the exposed group (minimal-mild vs. minimal). Cardiomyopathy of minimal severity was seen in 1/10 female rats exposed to 30 mg/m^3.

MICE

Sixteen-Day Studies

All mice exposed to 200 mg/m^3 and 4/5 males and 1/5 females exposed to 50 mg/m^3 died before the end of the studies (Table 8). Clinical signs in mice exposed to 50 or 200 mg/m^3 included hypoactivity, chromodacryorrhea, hypothermia, rapid and shallow breathing, and reduced body tone. Clinical signs progressively worsened with increased numbers of exposures. Mice exposed to 50 mg/m^3 lost weight; final mean body weights at other exposure concentrations were similar to those of controls.

Exposure-related lesions observed at necropsy in mice from the three highest exposure groups consisted of gray discoloration of the lungs and fluid in the larynx and trachea. The absolute lung weight and lung weight to body weight ratios were significantly increased for male and female mice exposed to 50 mg/m^3. Absolute thymus weights and thymus weight to body weight ratios were markedly decreased (less than one-half those of controls) for male and female mice exposed to 50 mg/m^3.

Lesions attributed to cobalt sulfate exposure were seen at all levels of the respiratory tract in mice. At the three highest concentrations, inflammation and necrosis of the respiratory epithelium were seen in the larynx, trachea, bronchioles, and respiratory turbinates of the nose. Degeneration of the olfactory epithelium was also present. In the 50 mg/m^3 group, mice that survived more than 1 week or were killed at the end of the 16-day exposure period had hyperplasia (acanthosis) of the squamous epithelium

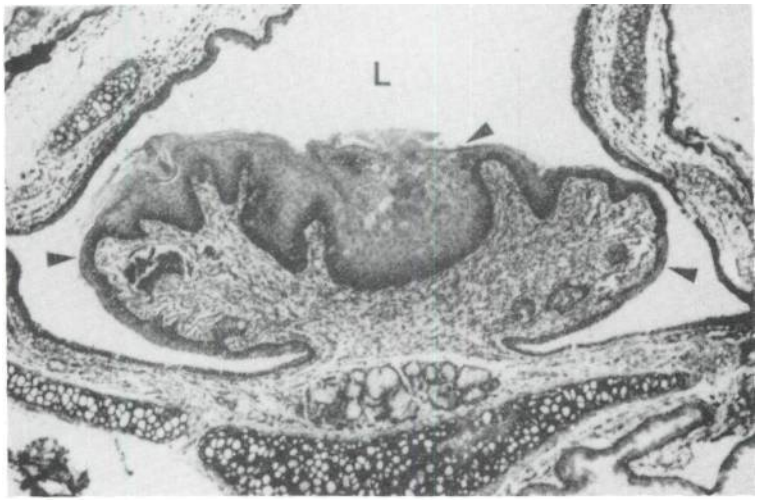

Figure 2. Transverse section through the base of the epiglottis of a male rat exposed to 10 mg/m^3 cobalt sulfate heptahydrate for 13 weeks. Large inflammatory polyp (arrows) arising from the dorsal surface of the epiglottis has extended into the lumen (L) of the larynx (hematoxylin and eosin, 30 x).

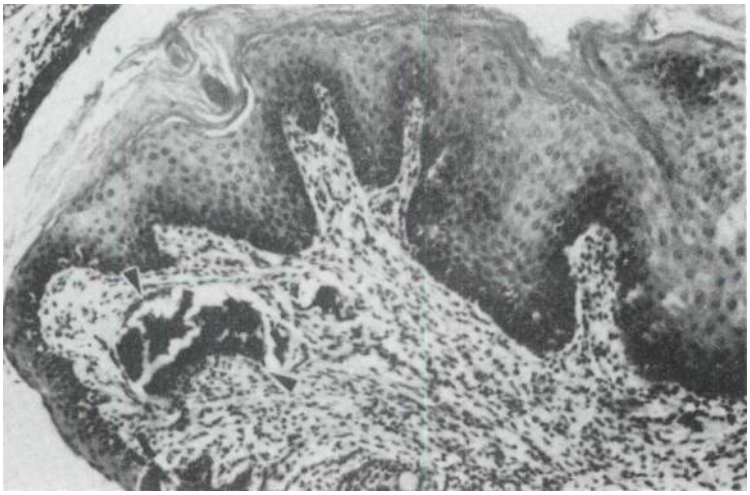

Figure 3. Detail of polyp from Figure 2 shows well-differentiated, hyperplastic epithelium with keratinitation. The fibrous stroma contains inflammatory cells and a focus of mineralization (arrows) (hematoxylin andeosin. 100 x).

TABLE 7. NUMBERS OF RATS WITH SELECTED LESIONS IN THE THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE (a)

Site/Lesion	Control	0.3 mg/m^3	1 mg/m^3	3 mg/m^3	10 mg/m^3	30 mg/m^3
MALE						
Nose						
Acute inflammation	0	0	0	0	0	3
Olfactory epithelium degeneration	0	0	0	0	**7	**10
Respiratory epithelium hyperplasia	0	0	1	1	2	*5
Respiratory epithelium squamous metaplasia	0	0	0	1	*5	**9
Larynx (step sections)						
Mineralization	0	(b) 0	0	2	**10	**10
Chronic inflammation	0	(b) 2	**8	**9	**9	**9
Suppurative inflammation	0	(b) 0	0	0	*4	2
Ulcer	0	(b) 0	0	0	**7	**7
Necrosis	0	(b) 1	0	0	**10	**10
Inflammatory polyp	0	(b) 0	0	2	**10	**8
Squamous metaplasia	0	**(b) 9	**10	**10	**10	**10
Lung						
Histiocytic infiltrates	1	0	*6	**10	**10	**10
Inflammation, subacute	0	0	1	*5	**10	**10
Fibrosis	0	0	0	0	1	**10
Bronchiolar epithelium regeneration	0	0	0	0	0	**7
Bronchiolar ectasia	0	0	0	0	**8	**10
Alveolar emphysema	0	0	0	0	1	2
Alveolar epithelium hyperplasia	0	0	0	3	**6	**6
FEMALE						
Nose						
Olfactory epithelium degeneration	0	0	0	0	**6	**10
Respiratory epithelial hyperplasia	0	0	0	3	**9	**9
Respiratory epithelial squamous metaplasia	0	0	0	1	3	**6
Larynx (step sections)						
Mineralization	0	(c) 0	0	1	**8	**10
Chronic inflammation	1	(c) 2	**7	**10	**10	**10
Ulcer	0	(c) 0	0	0	3	**6
Necrosis	0	(c) 0	0	2	**9	**10
Inflammatory polyp	0	(c) 0	0	1	**10	**9
Squamous metaplasia	1	**(c) 7	**10	**10	**10	**10
Lung						
Histiocytic infiltrates	0	3	**10	**10	**10	**10
Inflammation, subacute	0	0	2	**9	**10	**10
Fibrosis	0	0	0	1	*4	*5
Bronchiolar epithelium regeneration	0	0	0	0	0	*5
Bronchiolar ectasia	0	0	0	2	**8	**10
Alveolar emphysema	0	0	0	1	2	**7
Alveolar epithelium hyperplasia	0	0	0	3	1	1

(a) Ten rats were examined in each group unless otherwise specified.
(b) Nine rats were examined.
(c) Eight rats were examined.
*P<0.05 by Fisher exact test
**P<0.01 by Fisher exact test

TABLE 8. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE SIXTEEN-DAY INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE

Concentration (mg/m^3)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)
MALE					
0	5/5	26.2 ± 0.5	28.4 ± 0.8	+2.2 ± 0.5	
0.1	5/5	27.0 ± 0.3	29.0 ± 1.0	+2.0 ± 0.8	102.1
0.5	5/5	27.0 ± 0.4	29.1 ± 0.7	+2.1 ± 0.4	102.5
5	5/5	27.3 ± 0.2	29.7 ± 0.7	+2.4 ± 0.6	104.6
50	(d) 1/5	26.8 ± 0.6	19.0 ± 0.0	−7.4 ± 0.0	66.9
200	(e) 0/5	27.0 ± 0.5	(f)	(f)	(f)
FEMALE					
0	5/5	21.2 ± 0.4	24.2 ± 0.3	+3.0 ± 0.3	
0.1	5/5	21.6 ± 0.2	24.3 ± 0.2	+2.7 ± 0.3	100.4
0.5	5/5	22.1 ± 0.4	24.9 ± 0.5	+2.8 ± 0.2	102.9
5	5/5	21.8 ± 0.5	24.0 ± 0.9	+2.2 ± 0.5	99.2
50	(g) 4/5	21.9 ± 0.3	19.4 ± 0.4	−2.7 ± 0.3	80.2
200	(h) 0/5	21.2 ± 0.5	(f)	(f)	(f)

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Day of death: 5,6,10,12
(e) Day of death: 2,2,2,3,3
(f) No data are reported due to 100% mortality in this group.
(g) Day of death: 8
(h) Day of death: 2,3,3,3,3

in the larynx and regeneration of the bronchiolar epithelium in the lung. Also at the 50 mg/m^3 exposure concentration, an inflammatory response in the lung was characterized by fibrosis around bronchioles and infiltration of histiocytes into alveolar spaces.

Other lesions observed in exposed mice that died during the exposure period consisted of lymphoid depletion and necrosis in the thymus and congestion of vessels in the brain/meninges. In the liver, necrosis of hepatocytes was present in all mice that died during the exposure period; minimal necrosis was present in the liver of one male mouse (50 mg/m^3) that was killed at the end of the study.

Thirteen-Week Studies

Two of 10 males exposed to 30 mg/m^3 died before the end of the studies (Table 9). Mean body weights of mice exposed to 30 mg/m^3 and females exposed to 10 mg/m^3 were lower than those of controls throughout the studies (Figure 4). The final mean body weight of mice at 30 mg/m^3 was 14% lower than that of the controls for males and 22% lower for females. No observed clinical signs appeared to be related to cobalt sulfate exposure, with the exception of rapid breathing and skin discoloration in one high exposure concentration male mouse that died during week 11. The absolute lung weight and the lung weight to body weight ratios were significantly increased in the 10 and 30 mg/m^3 exposure groups, and the absolute testis weight and the testis weight to body weight ratio were significantly decreased for males exposed to 30 mg/m^3 (Table 10). No consistent or dose-related hematologic effects were observed (Table A5).

The epididymal weight was significantly lower than that of controls for male mice exposed to 30 mg/m^3. The number of abnormal sperm in mice

TABLE 9. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE

Concentration (mg/m^3)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)
MALE					
0	10/10	26.2 ± 0.5	36.8 ± 1.5	+10.6 ± 1.1	
0.3	10/10	25.8 ± 0.6	36.1 ± 1.2	+10.4 ± 0.7	98.1
1	10/10	25.9 ± 0.6	38.5 ± 1.2	+12.6 ± 0.8	104.6
3	10/10	25.3 ± 0.4	35.0 ± 0.9	+9.8 ± 0.7	95.1
10	10/10	25.4 ± 0.3	35.0 ± 0.9	+9.6 ± 0.8	95.1
30	(d) 8/10	25.3 ± 0.4	31.7 ± 0.8	+6.5 ± 0.8	86.1
FEMALE					
0	10/10	21.7 ± 0.5	32.8 ± 1.3	+11.2 ± 0.9	
0.3	10/10	21.4 ± 0.3	32.6 ± 1.2	+11.2 ± 1.0	99.4
1	10/10	21.1 ± 0.3	33.8 ± 1.2	+12.6 ± 0.9	103.0
3	10/10	21.2 ± 0.3	32.8 ± 0.8	+11.6 ± 0.6	100.0
10	10/10	21.0 ± 0.4	30.8 ± 0.8	+9.6 ± 0.6	93.9
30	10/10	21.3 ± 0.3	25.7 ± 0.6	+4.4 ± 0.5	78.4

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Week of death: 2,12

TABLE 10. SELECTED ORGAN WEIGHTS OF MICE IN THE THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE (a)

Organ	Control	0.3 mg/m^3	1 mg/m^3	3 mg/m^3	10 mg/m^3	30 mg/m^3
MALE						
Body weight (grams)	37.5 ± 1.54	37.1 ± 1.28	39.9 ± 1.28	35.7 ± 0.88	35.8 ± 0.98	**(b) 32.5 ± 0.81
Lung						
Absolute	181 ± 4.3	179 ± 9.6	186 ± 6.5	187 ± 4.2	**213 ± 4.5	**(b) 321 ± 6.7
Relative	4.9 ± 0.13	4.8 ± 0.18	4.7 ± 0.08	5.2 ± 0.09	**6.0 ± 0.15	**(b) 9.9 ± 0.32
Testis						
Absolute	(c) 120 ± 1.9	125 ± 2.7	123 ± 2.3	120 ± 2.4	121 ± 2.1	**57 ± 6.8
Relative	(c) 3.3 ± 0.11	3.4 ± 0.07	3.1 ± 0.09	3.4 ± 0.10	3.4 ± 0.05	**1.7 ± 0.19
FEMALE						
Body weight (grams)	33.2 ± 1.31	33.8 ± 1.25	34.7 ± 1.33	33.3 ± 0.94	31.6 ± 0.74	**26.1 ± 0.59
Lung						
Absolute	194 ± 9.0	192 ± 4.2	187 ± 4.7	198 ± 4.7	**232 ± 7.3	**327 ± 5.8
Relative	5.9 ± 0.28	5.8 ± 0.26	5.4 ± 0.12	6.0 ± 0.22	**7.3 ± 0.11	**12.6 ± 0.40

(a) Mean ± standard error in milligrams (absolute) or milligrams per gram (relative) for groups of 10 animals unless otherwise specified; P values vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
(b) Eight animals were weighed.
(c) Nine animals were weighed.
*$P<0.05$
**$P<0.01$

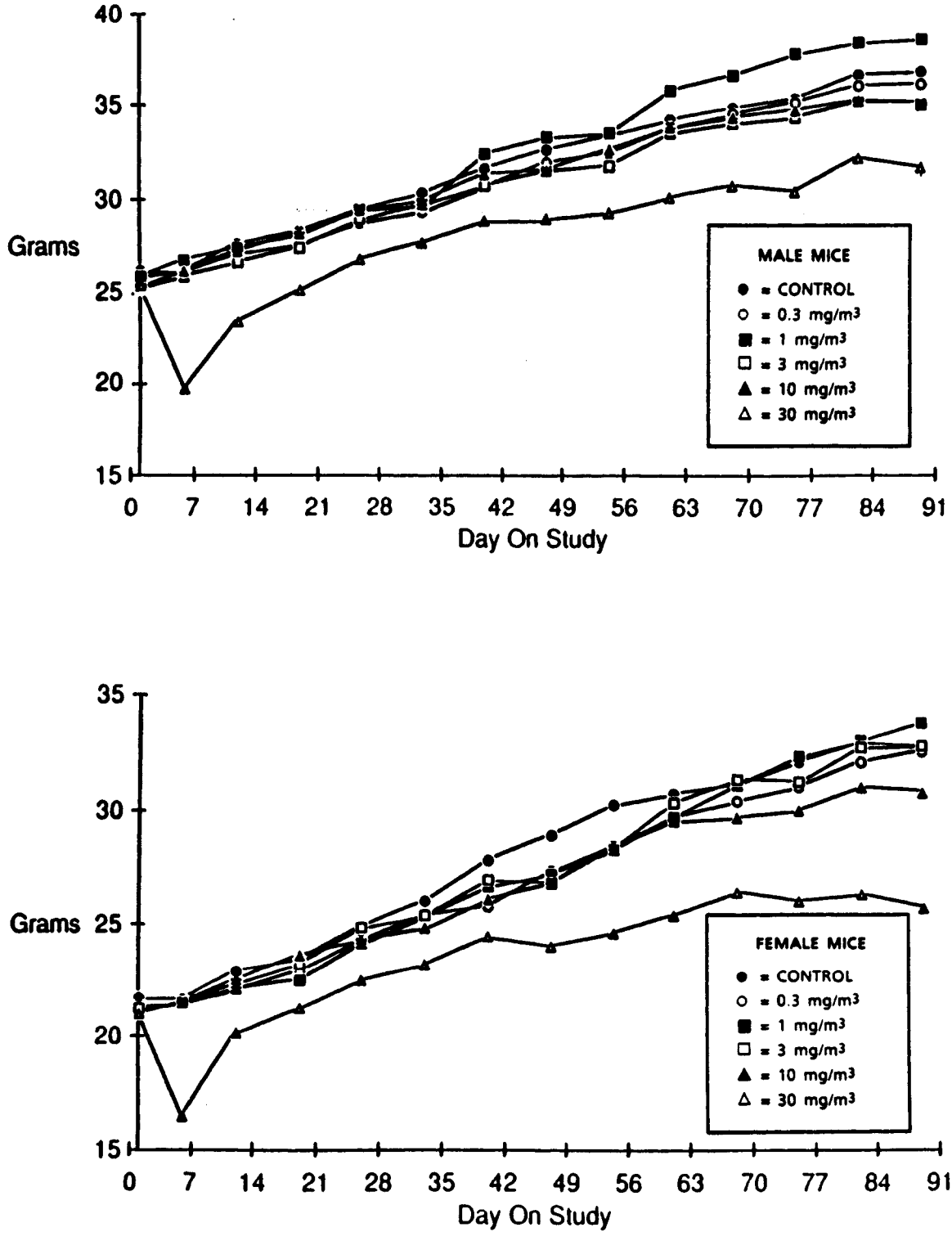

FIGURE 4. GROWTH CURVES FOR MICE EXPOSED TO COBALT SULFATE HEPTAHYDRATE BY INHALATION FOR THIRTEEN WEEKS

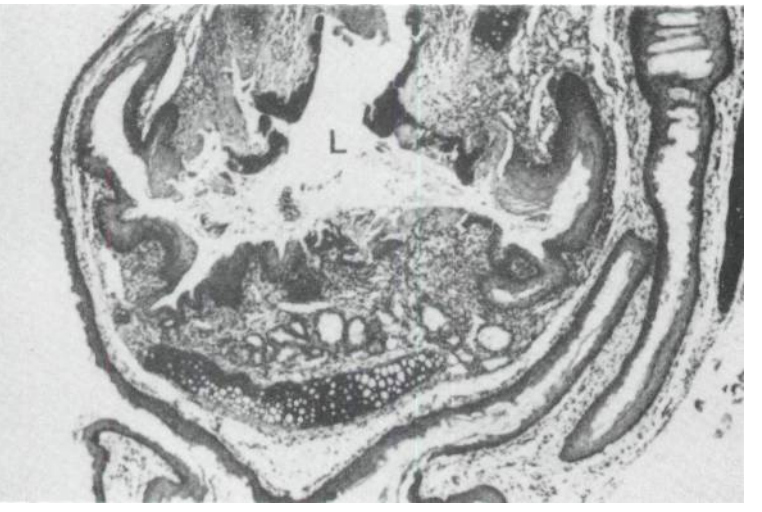

Figure 5. Transverse section through the base of the epiglottis of a female mouse exposed to 30 mg/m^3 cobalt sulfate heptahydrate for 13 weeks. Lumen of larynx contains keratin and cell debris from necrotic laryngeal epithelium (hematoxylin and eosin, 30 x).

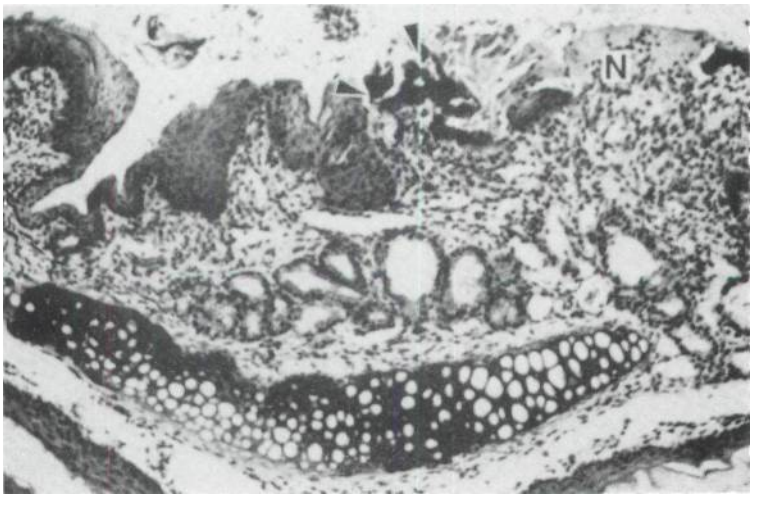

Figure 6. Detail of base of epiglottis from Figure 5 shows squamous metaplasia of laryngeal epithelium with pale area of epithelial necrosis (N) and densely stained area of mineralization (arrows) in the mucosa (hematoxylin and eosin, 100 x).

exposed to 30 mg/m^3 was significantly increased, and sperm motility was significantly reduced in mice exposed to 3, 10, or 30 mg/m^3 (Table A6). Data were not collected on mice exposed at lower concentrations. The estrous cycle was significantly longer in mice exposed to 30 mg/m^3.

Compound-related microscopic lesions were generally limited to the respiratory tract of mice of each sex. Lesions were concentration related and similar in incidence and severity in males and females (Table 11). In the nose, degeneration of olfactory epithelium, squamous metaplasia of the respiratory epithelium, and an acute inflammatory cell exudate in the nasal cavity were seen primarily at the two highest exposure concentrations.

At the highest exposure concentration, necrosis, inflammation, and squamous metaplasia of the laryngeal epithelium were present in most mice (see Figures 5 and 6). Some foci of necrosis in the laryngeal epithelium extended through the basement membrane into the underlying lamina propria. Squamous metaplasia of the the respiratory epithelium in the trachea also occurred in mice in this exposure group. At exposure concentrations below 30 mg/m^3, only inflammation and squamous metaplasia were observed.

In the lung of mice exposed to 10 or 30 mg/m^3, there was regeneration of bronchiolar epithelium and hyperplasia of the alveolar epithelium. Infiltration of histiocytes (macrophages) into the alveolar spaces was also present. Chronic inflammation occurred primarily at the highest exposure concentration and consisted of fibrosis around bronchioles and in alveolar septae along with an inflammatory cell infiltrate. At the lower concentration, only a minimal increase in macrophages (histiocytic infiltrate) was seen in the alveoli.

Lymphoid hyperplasia was present in the mediastinal lymph nodes of mice at the 30 mg/m^3 exposure concentration.

At the highest exposure concentration, atrophy of the testis was observed, which consisted of a loss of germinal epithelium in the seminiferous tubules; more severely affected testes also contained foci of mineralization.

IV. DISCUSSION AND CONCLUSIONS

In 16-day studies, exposure to 200 mg/m^3 cobalt sulfate heptahydrate as an aerosol resulted in deaths of all rats and mice of each sex within the first 5 days on study. Several male rats and male and female mice exposed to 50 mg/m^3 also died somewhat later. These relatively short periods of exposure to 50 or 200 mg/m^3 cobalt sulfate heptahydrate resulted in necrotizing inflammation in the upper respiratory tract (nares, larynx, and trachea) as well as in the bronchiolar epithelium of the lung. Only edema and hemorrhage were seen in the alveolar portion of the lung in animals dying early in the exposure period. Animals that survived beyond 1 week developed an inflammatory response in the lungs characterized by infiltration of macrophages and fibrosis around bronchioles. Necrotizing inflammatory lesions in the airways were less common in animals that survived; in these animals, metaplasia of the respiratory epithelium to a squamous epithelial cell with acanthosis or hyperplasia, fibrosis, and histiocyte infiltration was commonly seen. These necrotizing and regenerative responses are similar to and, in fact, are characteristic of the response of the respiratory system to a variety of inhaled irritant chemicals and particles (Gopinath et al., 1987). The mean aerodynamic diameter of the cobalt sulfate heptahydrate aerosol particles was approximately 1 µm, well within the size range of particles shown to deposit at all levels of the respiratory tract of the rat (Raabe, 1980).

Lesions observed in other organs in rats and mice in the 16-day studies, including congestion and lymphoid necrosis in the thymus, congestion and necrosis in the liver, and congestion of vessels in the brain, are typical changes associated with an agonal or stressed condition in moribund or early-death animals. Similarly,

TABLE 11. NUMBERS OF MICE WITH SELECTED LESIONS IN THE THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE (a)

Site/Lesion	Control	0.3 mg/m^3	1 mg/m^3	3 mg/m^3	10 mg/m^3	30 mg/m^3
MALE						
Nose						
Acute inflammation	0	--	0	1	**10	**9
Olfactory epithelium degeneration	0	--	0	0	**9	**8
Respiratory epithelium squamous metaplasia	0	--	0	0	**8	**8
Larynx						
Inflammation	0	0	0	(b) 0	1	**9
Necrosis	0	0	0	(b) 0	0	3
Squamous metaplasia	0	**7	**10	*(b) 5	**9	**10
Trachea						
Squamous metaplasia	0	--	--	--	0	2
Lung						
Histiocytic infiltrates	0	**10	**9	**10	**10	**10
Chronic inflammation	0	0	0	0	1	**10
Bronchiolar epithelium regeneration	0	0	0	0	0	**10
Alveolar epithelium hyperplasia	0	0	0	0	3	**8
Mediastinal lymph nodes						
Hyperplasia	0	--	--	--	(c) 0	**(b) 6
Testis						
Atrophy	0	--	--	--	0	**9
Mineralization	0	--	--	--	0	*4
FEMALE						
Nose						
Acute inflammation	0	0	1	*4	**10	**10
Olfactory epithelium degeneration	0	0	0	1	**10	**10
Respiratory epithelium squamous metaplasia	0	0	0	1	**9	**9
Larynx						
Inflammation	0	0	0	(b) 0	**6	**(b) 8
Necrosis	0	0	0	(b) 0	0	**(b) 6
Squamous metaplasia	0	**8	**8	**(b) 8	**9	**(b) 9
Trachea						
Squamous metaplasia	0	--	--	--	0	3
Lung						
Histiocytic infiltrates	0	0	**9	**10	**10	**10
Chronic inflammation	0	0	0	0	*5	**10
Bronchiolar epithelium regeneration	0	0	0	0	0	**10
Alveolar epithelium hyperplasia	0	0	0	0	**10	**10
Mediastinal lymph nodes						
Hyperplasia	0	--	--	(d) 0	(e) 1	**7

(a) Ten mice were examined in each group unless otherwise specified; -- indicates tissue not examined.
(b) Nine mice were examined.
(c) Seven mice were examined.
(d) Five mice were examined.
(e) Six mice were examined.
*P<0.05 by Fisher exact test
**P<0.01 by Fisher exact test

testicular atrophy was seen only in rats from the 50 mg/m^3 group, animals that had a marked weight loss during the study. Cardiomyopathy, which is a well-documented toxic effect of cobalt sulfate ingestion in humans and rats (Grice et al., 1969; Smith and Carson, 1981), was present in rats in the top two exposure groups. However, the heart lesions were not severe, and the morphology of this lesion is typical of the degenerative cardiomyopathy that commonly occurs in F344 rats. This lesion was also noted in 2/5 control males in this study. Thus, evidence for a cardiotoxic effect of cobalt sulfate heptahydrate was equivocal in these studies. No adverse effects in the heart of mice were noted.

The selection of 30 mg/m^3 as the top exposure concentration for both rats and mice in the 13-week studies was based on the deaths of animals exposed to 50 mg/m^3 in the 16-day studies and consideration of the severity of the respiratory tract lesions at 5 mg/m^3, the next lower exposure concentration. The selection appeared appropriate, as only two male mice exposed to 30 mg/m^3 died during the 13-week studies. Both male and female rats and mice initially lost weight at this concentration, but a normal rate of weight gain resumed after the first week of exposure, and only in female mice did it appear that weight gain might be significantly slowed by exposure to 30 mg/m^3 cobalt sulfate heptahydrate.

The respiratory tract was clearly the major target of toxicity of inhaled cobalt sulfate in the 13-week studies. Quite similar degenerative, inflammatory, and regenerative changes were present from the nasal cavity to the alveoli in rats and mice of each sex. The differences in the susceptibility of the various components of the respiratory system were fairly consistent in rats and mice. The trachea showed metaplastic changes only in mice exposed to 30 mg/m^3, but squamous metaplasia of the larynx was seen in both rats and mice at concentrations as low as 0.3 mg/m^3, the lowest exposure concentration studied. In rats exposed to 3 mg/m^3 cobalt sulfate heptahydrate or more, quite remarkable inflammatory polyps were found, typically arising caudal to the base of the epiglottis in the larynx. These exophytic masses occupied up to half the laryngeal lumen and consisted of a hyperplastic squamous epithelium, with abundant vascular stroma. The larynx is a common site for lesions in rodents exposed by inhalation to various chemicals and pharmaceuticals; erosion, ulceration, and an inflammatory exudate are frequently observed (Gopinath et al., 1987). What is unusual about the lesion caused by cobalt sulfate inhalation is the apparent organization and vascularization of the inflammatory exudate and the squamous metaplasia of these fibrous masses. The pathogenesis of somewhat similar intraluminal fibrotic projections produced in the trachea and bronchioles of mice exposed to methyl isocyanate has been described by Boorman et al. (1987). It is proposed that these types of lesions arise from an area where the respiratory epithelium has been destroyed. A relatively slow reepithelialization must occur from the margin of the lesion, giving time for migration of fibroblasts into the exudate and further organization of the lesion (Basset et al., 1986). Klonne et al. (1987) observed polypoid protrusions in the larynx of F344 rats exposed by inhalation to aerosols of an aqueous silane solution. These protrusions arose as part of a granulomatous reaction in response to embedded silane particles and may differ somewhat in pathogenesis from those observed with cobalt sulfate. Nevertheless, they developed in the same region of the larynx as did the inflammatory polyps induced by cobalt sulfate, i.e., the ventral floor of the larynx in the posterior epiglottal region.

In hematologic analyses of blood taken at the end of the 13-week studies, rats showed a pronounced polycythemia in males at exposure concentrations as low as 3 mg/m^3 and in females exposed at 10 mg/m^3 or at higher concentrations. This appeared to be a simple erythrocytosis, as most other formed elements were within normal ranges. Reticulocytes were increased only in high exposure concentration female rats. These changes are consistent with the well-characterized cobalt-induced polycythemia, which appears to be due to an increase in circulating erythropoietin (Taylor and Marks, 1978). No consistent significant hematologic effects were seen in mice. Species differences in the polycythemic response to cobalt have previously been reported (Smith and Carson, 1981).

In contrast to the reports of hyperlipemia after cobalt administration to rats, rabbits, or humans

(Gross et al., 1955; Taylor and Marks, 1978), serum cholesterol levels after 13 weeks of exposure in the 10 and 30 mg/m^3 groups of male rats and in the 30 mg/m^3 group of females were lower than in the controls and triglyceride levels were unchanged. It is possible that this may represent an adaptation to an earlier hyperlipidemia, as a 3-week regimen of intermittent cobalt chloride injections to rats is used as a model system to study forms of endogenous lipemia involving high concentrations of very low-density lipoproteins (Eaton, 1972). However, the duration or persistence of this effect is not clear from these earlier studies.

There have been many reports of goiter as a side effect of cobalt therapy for anemia in humans (reviewed by Smith and Carson, 1981), and this effect appears to be due to inhibition of uptake of iodine by the thyroid gland. Thyroid function as indicated by serum triiodothyronine (T_3), thyroxin (T_4), and thyrotropin (TSH) concentrations did not appear to be consistently affected in rats in the current studies. These results support the opinion expressed by Sederholm et al. (1968) that effects of cobalt on the thyroid gland have not been clearly demonstrated in studies with rats, mice, or rabbits.

As in the 16-day studies, cardiomyopathy appeared slightly more severe in male rats in the high exposure group compared with the controls, but the incidences were the same in the 13-week studies. Minimal cardiomyopathy was seen in one 30 mg/m^3 female rat and in no controls. Myocardial injury can also be indirectly assessed by measuring the activity in the serum of specific isozymes of creatine kinase (CK-2 and CK-3), which are released from damaged cardiac muscle cells (Boyd, 1983). In the current study, total serum creatine kinase activity was highly variable in the 30 mg/m^3 group of female rats and appeared to be slightly increased in females (but not in males), although not statistically so. The CK-3 form of the enzyme appeared to be slightly increased in the high exposure concentration female rats, but the amount of CK-2 isozyme present was decreased. Thus, the data did not suggest a cardiotoxic effect.

The motility of sperm appeared to be lower in exposed mice, and the number of abnormal sperm was increased, especially at the highest exposure concentration, at which clear testicular atrophy occurred. The magnitude and number of these effects would suggest that these changes represent a direct toxic effect of cobalt on the reproductive system; the site of action remains to be determined. Rats appeared much less susceptible to the testicular toxicity of cobalt than did mice.

There was no indication microscopically of an increase in kidney lesions in rats or mice in the 13-week studies; however, granular casts were observed in the urine from a number of male rats in all exposure groups, and a concentration-related increase in the number of epithelial cells sloughed into the urine was seen in exposed male rats. Both observations are suggestive of a minimal nephrotoxic effect.

The excretion of absorbed cobalt is primarily via the urine. Urinary cobalt excretion was measured in male and female rats and found to exhibit a concentration-dependent pattern, although the magnitude of the difference in urinary cobalt from one group to the next was not as large as was the difference in the atmospheric concentrations. Urinary cobalt concentrations have been measured in workers exposed to cobalt in the cobalt-tungsten carbide "hard metal" industry (Ichikawa et al., 1985). Concentrations as high as 0.39 µg/ml have been found in the urine of workers in certain high exposure areas. By comparison, the urine cobalt concentrations in the current rat studies ranged from 0.11 to 7.79 µg/ml in the various groups. The value obtained in male rats exposed to 1 mg/m^3 was 0.39 µg/ml, the same as that cited in the Ichikawa et al. study. Exposure to cobalt in the hard metal industry is probably to the cobalt metal powder rather than to the sulfate; but in simple terms of cobalt exposure, it is possible that current worker exposure is higher than that shown to cause laryngeal inflammation and squamous metaplasia in rodents. According to the National Institute for Occupational Safety and Health (1977), the symptoms most commonly reported after occupational exposure to cobalt in the cemented tungsten carbide industry include upper respiratory tract irritation, exertional dyspnea, and diffuse interstitial pneumonitis and fibrosis.

There are few comparable animal inhalation studies with cobalt in the literature. Kerfoot et al. (1975) exposed miniature swine to 0.1 or 1 mg/m^3 cobalt powder for 6 hours per day, 5 days per week for 3 months. They reported decreased lung compliance and microscopic evidence of interstitial fibrosis at both exposure concentrations. Johansson et al. (1984) reported alveolar type II cell hyperplasia in rabbits after 4- to 6-week exposures to cobalt chloride (0.4-0.6 mg cobalt/m^3, 6 hours per day, 5 days per week). This observation suggests that type I cells had been damaged and the type II cell proliferation was an effort to repair the injury. Apparently no other part of the respiratory system was examined in these studies.

In summary, exposure of rats and mice to aerosols of cobalt sulfate heptahydrate resulted primarily in severe necrotizing injury to the respiratory tract. The larynx appeared to be the most sensitive tissue, showing metaplastic and inflammatory lesions after exposure at concentrations as low as 0.3 mg/m^3 cobalt sulfate heptahydrate (equivalent to 0.11 mg cobalt/m^3). A no-observed-adverse-effect concentration could not be determined from these studies.

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APPENDIX

RESULTS OF SUPPLEMENTAL ANALYSES IN THE THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE

TABLE A1. SELECTED HEMATOLOGIC AND SERUM CHEMISTRY DATA FOR RATS IN THE THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE (a)

Analysis	Control	0.3 mg/m^3	1 mg/m^3	3 mg/m^3	10 mg/m^3	30 mg/m^3
MALE						
Hemoglobin (g/dl)	14.9 ± 0.19	14.5 ± 0.18	15.1 ± 0.06	*14.7 ± 0.80	**17.1 ± 0.16	**19.6 ± 0.15
Mean corpuscular hemoglobin (pg)	16.3 ± 0.03	*16.1 ± 0.07	16.2 ± 0.04	*15.3 ± 0.80	**15.9 ± 0.04	*16.2 ± 0.07
Mean corpuscular hemoglobin concentration (g/dl)	32.4 ± 0.09	32.3 ± 0.10	32.2 ± 0.09	30.7 ± 1.57	*32.9 ± 0.12	**33.1 ± 0.12
Mean cell volume (μ^3)	50.1 ± 0.18	49.7 ± 0.21	50.4 ± 0.22	49.4 ± 0.31	**48.2 ± 0.13	**49.2 ± 0.33
Platelets ($10^3/\mu l$)	543 ± 8.7	528 ± 11.7	538 ± 10.5	525 ± 8.8	**434 ± 25.7	**382 ± 9.4
Erythrocytes ($10^6/\mu l$)	9.2 ± 0.12	9.0 ± 0.09	9.3 ± 0.04	**9.6 ± 0.07	**10.8 ± 0.10	**12.1 ± 0.12
Reticulocytes ($10^6/\mu l$)	0.08 ± 0.013	0.08 ± 0.015	0.11 ± 0.010	0.10 ± 0.009	0.07 ± 0.011	0.08 ± 0.013
Hematocrit (percent)	46.0 ± 0.56	44.9 ± 0.48	46.8 ± 0.20	*47.8 ± 0.44	**52.1 ± 0.48	**59.4 ± 0.47
Cholesterol (mg/dl)	72.3 ± 2.48	67.4 ± 2.60	71.3 ± 5.65	65.4 ± 2.64	**61.9 ± 2.93	**51.4 ± 2.33
Triiodothyronine (ng/dl)	68.5 ± 5.00	62.1 ± 3.95	76.8 ± 5.87	67.6 ± 3.89	74.4 ± 5.49	64.3 ± 4.40
Free thyroxin (ng/dl)	1.8 ± 0.08	1.8 ± 0.07	1.9 ± 0.12	*2.0 ± 0.06	1.9 ± 0.06	2.0 ± 0.11
Total thyroxin (μg/dl)	3.85 ± 0.115	3.72 ± 0.135	4.28 ± 0.285	4.17 ± 0.129	4.06 ± 0.136	4.10 ± 0.252
Thyrotropin (ng/ml)	187 ± 45.5	93 ± 36.7	202 ± 54.4	143 ± 36.0	195 ± 54.7	*56 ± 27.8
FEMALE						
Hemoglobin (g/dl)	15.2 ± 0.20	*15.6 ± 0.10	15.1 ± 0.22	15.5 ± 0.22	**16.8 ± 0.10	**19.4 ± 0.11
Mean corpuscular hemoglobin (pg)	17.3 ± 0.07	17.2 ± 0.06	17.2 ± 0.04	17.3 ± 0.03	17.2 ± 0.04	17.4 ± 0.05
Mean corpuscular hemoglobin concentration (g/dl)	32.9 ± 0.08	32.6 ± 0.10	32.9 ± 0.14	32.9 ± 0.13	33.2 ± 0.21	*33.4 ± 0.14
Mean cell volume (μ^3)	52.4 ± 0.16	52.5 ± 0.17	52.4 ± 0.22	52.4 ± 0.27	*51.6 ± 0.27	52.0 ± 0.21
Platelets ($10^3/\mu l$)	643 ± 16.3	619 ± 8.8	638 ± 20.3	614 ± 8.4	**569 ± 14.4	**512 ± 10.8
Erythrocytes ($10^6/\mu l$)	8.8 ± 0.10	*9.1 ± 0.07	8.8 ± 0.14	9.0 ± 0.11	**9.8 ± 0.05	**11.2 ± 0.05
Reticulocytes ($10^6/\mu l$)	0.08 ± 0.013	0.09 ± 0.009	0.08 ± 0.011	0.08 ± 0.011	0.09 ± 0.010	*0.16 ± 0.028
Hematocrit (percent)	46.2 ± 0.59	*47.9 ± 0.35	46.1 ± 0.75	47.2 ± 0.78	**50.6 ± 0.43	**58.1 ± 0.43
Cholesterol (mg/dl)	123.4 ± 4.62	118.4 ± 4.43	*107.5 ± 3.72	114.3 ± 6.12	113.9 ± 5.08	**91.3 ± 5.50
Triiodothyronine (ng/dl)	96.4 ± 5.56	80.2 ± 4.55	85.2 ± 4.87	88.9 ± 4.35	*79.7 ± 5.45	**60.1 ± 4.27
Free thyroxin (ng/dl)	1.2 ± 0.10	1.2 ± 0.07	1.3 ± 0.09	1.2 ± 0.08	1.1 ± 0.08	1.2 ± 0.07
Total thyroxin (μg/dl)	3.44 ± 0.180	2.96 ± 0.169	3.31 ± 0.220	3.39 ± 0.135	2.96 ± 0.175	*3.01 ± 0.336
Thyrotropin (ng/ml)	134 ± 17.4	195 ± 30.7	171 ± 19.6	128 ± 17.7	143 ± 32.9	107 ± 22.8

(a) Mean ± standard error for groups of 10 animals; P values are vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
*P<0.05
**P<0.01

TABLE A2. CREATINE KINASE ACTIVITY FOR RATS IN THE THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE (a)

	Control	0.3 mg/m³	1 mg/m³	3 mg/m³	10 mg/m³	30 mg/m³
MALE						
Total activity	279 ± 30	321 ± 58	347 ± 32	349 ± 22	(b) 278 ± 13	284 ± 25
Isozymes (percent)						
CK-1	46.0 ± 4.03	45.4 ± 4.74	41.2 ± 2.98	43.9 ± 3.99	41.7 ± 4.88	47.9 ± 4.91
CK-2	31.9 ± 5.16	26.4 ± 5.20	37.0 ± 4.12	37.1 ± 3.09	27.4 ± 4.04	19.4 ± 3.61
CK-3	22.4 ± 3.15	28.2 ± 5.33	21.8 ± 5.23	19.0 ± 4.39	30.9 ± 4.84	32.8 ± 4.46
FEMALE						
Total activity	347 ± 48	367 ± 47	335 ± 54	248 ± 18	347 ± 41	(c) 398 ± 43
Isozymes (percent)						
CK-1	35.9 ± 2.53	34.9 ± 4.10	35.0 ± 3.72	43.2 ± 4.30	32.8 ± 1.70	34.1 ± 3.42
CK-2	29.4 ± 3.18	19.1 ± 4.94	17.0 ± 2.76	26.6 ± 4.15	27.3 ± 4.45	*14.6 ± 4.10
CK-3	34.7 ± 4.00	46.0 ± 6.32	48.0 ± 4.00	30.1 ± 2.91	39.9 ± 5.26	51.3 ± 4.53

(a) Mean ± standard error in international units/liter for total activity or in percent for isozymes CK-1, CK-2, and CK-3; 10 animals examined per group unless otherwise indicated.
(b) Represents nine animals; one extreme value of 725 eliminated from analysis. The inclusion of this animal would result in a mean and standard error of 323 ± 46.
(c) Represents nine animals; one extreme value of 1,455 eliminated from analysis. The inclusion of this animal would result in a mean and standard error of 504 ± 112.
*$P<0.05$ by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977)

TABLE A3. REPRODUCTIVE SYSTEM DATA FOR RATS IN THE THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE

	Control	3 mg/m³	10 mg/m³	30 mg/m³
MALE (a)				
Sperm count ($\times 10^6$)	494 ± 33	441 ± 17	461 ± 29	476 ± 29
Sperm motility (percent)	85.3 ± 1.44	86.8 ± 0.85	85.1 ± 0.73	81.3 ± 1.91
Abnormal sperm (percent)	0.74 ± 0.123	0.62 ± 0.113	0.80 ± 0.103	0.86 ± 0.143
FEMALE (b)				
Estrous stage (percent) (c)				
Proestrus	18.6	18.6	15.7	15.7
Estrus	28.6	25.7	22.9	24.3
Metestrus	18.6	21.4	25.7	20.0
Diestrus	34.3	34.3	35.7	40.0
Cycle length (days)	4.60 ± 0.16	(d) 4.78 ± 0.22	4.90 ± 0.18	(d) 5.00 ± 0.17

(a) Mean ± standard error for groups of 10 animals; no significant differences vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977).
(b) Mean for groups of 10 animals unless otherwise specified
(c) No significant differences in the proportion of time spent in the different estrous-cycle stages were observed by the Wilks criterion (Wilks, 1932).
(d) Estrous cycle longer than 7 days or unclear in 1/10 animals; data presented are for the other 9 animals.

TABLE A4. COBALT CONTENT IN URINE OF RATS IN THE THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE (a)

	Control	0.3 mg/m^3	1 mg/m^3	3 mg/m^3	10 mg/m^3	30 mg/m^3
Male	0.22 ± 0.03	2.51 ± 0.23	5.21 ± 0.34	33.4 ± 5.15	42.6 ± 7.6	105 ± 11.8
Female	0.17 ± 0.05	1.99 ± 0.47	2.36 ± 0.28	18.1 ± 1.23	21.4 ± 1.64	66.9 ± 4.0

(a) Micrograms excreted per 16 hours; mean ± standard error for groups of 10 animals; $P<0.01$ for all dose groups vs. the controls by Dunnett's test (Dunnett, 1955) performed using a log transformation of the individual values.

TABLE A5. SELECTED HEMATOLOGIC DATA FOR MICE IN THE THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE (a)

Analysis	Control	0.3 mg/m^3	1 mg/m^3	3 mg/m^3	10 mg/m^3	30 mg/m^3
MALE						
Number examined	10	10	10	10	10	8
Hemoglobin (g/dl)	16.0 ± 0.10	15.7 ± 0.22	16.2 ± 0.11	16.0 ± 0.16	15.7 ± 0.28	16.1 ± 0.20
Platelets (10^3/µl)	1,002 ± 12	984 ± 16	*953 ± 16	971 ± 11	976 ± 21	950 ± 29
Erythrocytes (10^6/µl)	10.0 ± 0.10	9.8 ± 0.17	10.1 ± 0.09	10.1 ± 0.12	9.8 ± 0.17	10.3 ± 0.16
Hematocrit (percent)	47.3 ± 0.33	46.3 ± 0.65	47.8 ± 0.29	47.3 ± 0.41	46.6 ± 0.69	48.1 ± 0.54
FEMALE						
Number examined	10	10	10	10	10	10
Hemoglobin (g/dl)	16.1 ± 0.19	*15.5 ± 0.19	15.7 ± 0.21	15.5 ± 0.21	**15.2 ± 0.19	*15.6 ± 0.20
Platelets (10^3/µl)	880 ± 14	896 ± 10	878 ± 17	865 ± 16	852 ± 12	*831 ± 17
Erythrocytes (10^6/µl)	10.0 ± 0.13	9.6 ± 0.12	9.8 ± 0.15	9.7 ± 0.14	*9.5 ± 0.11	9.8 ± 0.11
Hematocrit (percent)	48.0 ± 0.62	*46.4 ± 0.57	47.2 ± 0.61	*46.3 ± 0.63	**45.5 ± 0.62	*46.8 ± 0.58

(a) Mean ± standard error
*$P<0.05$ vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977)
**$P<0.01$ vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977)

TABLE A6. REPRODUCTIVE SYSTEM DATA FOR MICE IN THE THIRTEEN-WEEK INHALATION STUDIES OF COBALT SULFATE HEPTAHYDRATE (a)

	Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
MALE				
Caudal weight (mg)	0.015 ± 0.001	0.016 ± 0.001	0.017 ± 0.001	0.014 ± 0.001
Right epididymal weight (mg)	0.042 ± 0.002	0.043 ± 0.001	0.045 ± 0.001	**0.034 ± 0.001
Sperm count (× 10^6)	1,074 ± 151	1,342 ± 140	1,136 ± 86	(b) 776 ± 194
Sperm motility (percent)	87.0 ± 0.76	**78.6 ± 2.44	**75.6 ± 2.25	**(b) 46.6 ± 7.76
Abnormal sperm (percent)	(c) 1.29 ± 0.164	1.38 ± 0.113	0.98 ± 0.105	**(b) 3.80 ± 0.626
FEMALE (d)				
Estrous stage (percent)				
Proestrus	24.3	18.6	27.1	17.1
Estrus	25.7	25.7	28.6	32.9
Metestrus	21.4	27.1	21.4	22.9
Diestrus	28.6	28.6	22.9	25.7
NC (e)	0.0	0.0	0.0	1.4
Cycle length (days) (f)	4.20 ± 0.20	4.11 ± 0.11	4.20 ± 0.13	***5.00 ± 0.24

(a) Mean ± standard error for groups of 10 animals unless otherwise indicated.
(b) Eight animals were examined.
(c) Nine animals were examined.
(d) Dose-related differences occurred in the relative frequency of time spent in different stages of the estrous stages (Wilks, 1932), P = 0.03.
(e) NC = not clear or no cells observed
(f) Estrous cycle longer than 7 days or unclear in 1/10 animals
**P < 0.01 vs. the controls by Dunn's test (Dunn, 1964) or Shirley's test (Shirley, 1977)
***P < 0.01 vs. the controls by Dunnett's test (Dunnett, 1980)