National Toxicology Program
Toxicity Report Series
Number 39

NTP Technical Report
on Toxicity Studies of

Cadmium Oxide

(CAS No. 1306-19-0)

Administered by Inhalation
to F344/N Rats and B6C3F$_1$ Mice

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NIH Publication 95-3388
March 1995

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

Note to the Reader

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- the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health;
- the National Center for Toxicological Research (NCTR) of the Food and Drug Administration; and
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The studies described in this toxicity study report were performed under the direction of NIEHS and were conducted in compliance with NTP laboratory health and safety requirements. These studies met or exceeded all applicable federal, state, and local health and safety regulations. Animal care and use were in accord and compliance with the Public Health Service Policy on Humane Care and Use of Animals.

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This NTP report on the toxicity studies of cadmium oxide is based primarily on 2-week studies conducted in November and December 1988 and on 13-week studies that began in August 1989 and ended in November 1989 at Battelle Pacific Northwest Laboratories, Richland, WA.

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PEER REVIEW

The draft report on the toxicity studies of cadmium oxide was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the toxicity study report presents the experimental results and conclusions fully and clearly. The comments of the reviewers were received and reviewed prior to the finalization of this document. Changes have been made such that the concerns of the reviewers have been addressed to the extent possible.

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ABSTRACT

Cadmium Oxide

CdO

Molecular Formula	CdO
CAS Number	1306-19-0
Molecular Weight	128.41

Three thousand tons of cadmium are imported or produced annually in the United States, and approximately 90% of this is cadmium oxide. Cadmium oxide is used in batteries, electroplating baths, pigments, plastics, synthetic products, and a variety of other materials. Cadmium oxide was nominated for study by the National Cancer Institute because of its widespread use and to obtain toxicity and carcinogenicity information. This report describes toxicity studies of cadmium oxide aerosol in F344/N rats and B6C3F$_1$ mice, including sperm motility and vaginal cytology evaluations, and developmental toxicity studies of cadmium oxide aerosol in Sprague-Dawley rats and Swiss (CD-1®) mice. Genetic toxicology studies were done in *Salmonella typhimurium* and B6C3F$_1$ mice erythrocytes.

Cadmium oxide has been evaluated by other investigators for long-term carcinogenic effects, and recently the International Agency for Research on Cancer (IARC) evaluated cadmium and cadmium compounds for carcinogenic risks to humans. IARC (1993) classified cadmium and cadmium compounds as human carcinogens (Group I chemicals).

Genetic Toxicity

Cadmium oxide was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without exogenous metabolic activation, and did not induce micronuclei in erythrocytes of mice exposed by inhalation for 13 weeks.

Developmental Toxicity Studies

For these studies, sperm-positive Sprague-Dawley rats and Swiss (CD-1®) mice were exposed to 0, 0.05, 0.5, or 2 mg/m^3 cadmium oxide 6 hours per day, 7 days per week, on gestation Day 4 through 19 (rats) or gestation Day 4 through 17 (mice). Maternal toxicity was observed in Sprague-Dawley

rats exposed to 2 mg/m^3 cadmium oxide for 16 days and included body weights lower than those of the controls and clinical signs of toxicity (dyspnea and hypoactivity). There was no evidence of embryolethality in rats at any exposure level. However, in rats exposed to 2 mg/m^3, developmental toxicity was evidenced by lower fetal weights and a significant increase in the incidence of reduced skeletal ossifications.

Maternal toxicity was also observed in Swiss (CD-1®) mice exposed to 2 mg/m^3 cadmium oxide for 14 days. Clinical signs were dyspnea, hypoactivity, lower body weight, and a lower pregnancy rate (30% vs. 97% in the control group). The total number of resorptions per litter was increased at the 2 mg/m^3 level. Developmental toxicity was evidenced by lower fetal weights in the 0.5 and 2 mg/m^3 groups and an increase in the incidence of reduced sternebral ossification in the 2 mg/m^3 group.

Toxicity Studies

Male and female F344/N rats and B6C3F$_1$ mice were exposed to cadmium oxide aerosol (MMAD=1.1-1.6 µm) for 6 hours per day, 5 days per week, for 2 or 13 weeks. Exposure levels were 0.1 to 10 mg/m^3 for the 2-week studies and 0.025 to 1 mg/m^3 for the 13-week studies. The current Occupational Safety and Health Administration (OSHA) standards for cadmium, based on the results of these and other studies, are 2.5 µg/m^3 for the action level (AL) and 5 µg/m^3 for the permissible exposure limit (PEL) (29 CFR § 1910.1027). The AL and PEL are calculated as an 8-hour, time-weighted average exposure.

In the 2-week studies, all rats and mice at the highest exposure level (10 mg/m^3) died from respiratory toxicity characterized by inflammation, necrosis, and fibrosis of the lung. Toxicity to the nasal cavity and tracheobronchial lymph nodes was also observed in the 10 mg/m^3 groups. At the lower exposure levels, treatment-related toxic lesions were not life threatening, and all body weights were within 10% of controls.

In the 13-week studies, all rats and mice (with the exception of one control mouse) survived to the end of the studies. The final mean body weight of rats in the highest exposure groups (1 mg/m^3) was 93% of the control value (Table 1). For all other exposed rat and mouse groups, final mean body weights corresponded to those of the respective controls.

For rats and mice in the 13-week studies, the major toxicity was to the respiratory system. Treatment-related lesions were observed in the lung, tracheobronchial lymph node, larynx, and nose

(Tables 1 and 2). The no-observed-adverse-effect level (NOAEL) in the lungs was 0.025 mg/m^3 for rats. A NOAEL was not found in the lungs or larynx of mice or in the larynx of rats. At the 0.025 and 0.05 mg/m^3 levels in mice, lung lesions were minimal and not considered life threatening. A NOAEL in the nasal cavity was 0.05 mg/m^3 for rats and mice. Reproductive toxicity was observed in the 1 mg/m^3 groups of rats and was evidenced by a reduced number of spermatids per testis and an increase in the length of the estrous cycle (Table 1). Reproductive toxicity was not observed at any exposure level in mice (Table 2).

TABLE 1 Selected Parameters for F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)					
	0	**0.025**	**0.05**	**0.1**	**0.25**	**1**
MALE						
Final body weight[2]						
(percentage of controls)		97	100	96	102	93
Respiratory System						
Lung						
Cadmium concentration						
(µg/g lung)	0.05	NM[3]	NM	19.1*	29.4**	39.5**
Weight						
(absolute and relative)	)	)[4]	)	↑[5]	↑[5]	↑[5]
Histopathologic findings						
alveolar histiocytic infiltrate	)	)	+ (1.0)	+ (2.0)	+ (3.0)	+ (3.0)
alveolar epithelial hyperplasia	)	)	+ (1.0)	+ (1.0)	+ (2.0)	+ (2.1)
inflammation	)	)	)	)	+ (2.6)	+ (4.0)
fibrosis	)	)	)	+ (1.0)	+ (2.0)	+ (2.7)
Mediastinal lymph node						
inflammation	)	)	)	+ (1.3)	+ (3.2)	+ (3.3)
Larynx						
epithelial degeneration	)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)
Nose						
Olfactory epithelium						
degeneration	)	)	)	)	+ (1.0)	+ (3.0)
respiratory metaplasia	)	)	)	)	)	+ (1.3)
squamous metaplasia	)	)	)	)	)	+ (1.9)
Respiratory epithelium						
inflammation	)	)	)	)	+ (1.0)	+ (2.6)
degeneration	)	)	)	)	)	+ (1.5)
Kidney						
Cadmium concentration						
(µg/g kidney)	0.02	NM	NM	3.1*	5.5**	15.2**
Weight, right kidney (relative)	)	)	)	↑[5]	↑[5]	↑[5]
Urinalysis parameters	)	)	)	)	)	)
Reproductive System						
Testis/epididymis weight	)	)	NM	)	NM	)
Spermatid count	)	)	NM	)	NM	↓[6]
Sperm motility	)	)	NM	)	NM	)

TABLE 1 Selected Parameters for F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide (continued)

	Concentration (mg/m^3)					
	0	**0.025**	**0.05**	**0.1**	**0.25**	**1**
FEMALE						
Final body weight[2]						
(percentage of controls)		100	97	103	99	93
Respiratory System						
Lung						
Weight						
(absolute and relative)	)	)	)	↑ [5]	↑ [5]	↑ [5]
Histopathologic findings						
alveolar histiocytic infiltrate	)	)	+ (1.0)	+ (2.1)	+ (3.0)	+ (3.0)
alveolar epithelial hyperplasia	)	)	+ (1.0)	+ (1.0)	+ (2.0)	+ (2.1)
inflammation	)	)	)	)	+ (1.6)	+
(3.5)	fibrosis	)	)	)	+ (1.0)	+ (2.0)
+ (2.1)						
Mediastinal lymph node						
inflammation	)	)	+ (1.0)	+ (1.5)	+ (3.6)	+ (4.0)
Larynx						
epithelial degeneration	)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)
Nose						
Olfactory epithelium						
degeneration	)	)	)	)	+ (1.0)	+ (2.8)
respiratory metaplasia	)	)	)	)	+ (1.0)	+ (1.0)
squamous metaplasia	)	)	)	)	)	+ (1.4)
Respiratory epithelium						
inflammation	)	)	)	+ (1.0)	+ (1.6)	+ (1.8)
Kidney						
Weight, right kidney (relative)	)	)	)	)	↑ [5]	↑ [5]
Urinalysis parameters	)	)	)	)	↑ [7]	↑ [7]
Reproductive System						
Estrous cycle length	)	)	NM	)	NM	↑ [8]

[1] For each control and exposure group, statistical analyses were performed on the mean value for 10 rats (organ weights), 7 to 10 rats (urinalysis parameters), 9 to 10 rats (reproductive parameters), or 4 to 5 rats (tissue cadmium concentrations). For histopathologic findings, average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

[2] (Exposure group mean/control group mean) x 100.

[3] NM = not measured at this exposure level.

[4]) = No lesions present (histopathology) or not significantly different from the control group (organ weights and urinalysis and reproductive parameters).

[5] Organ weights significantly greater than the control group.

[6] Spermatid count significantly lower than in the control group.

[7] Aspartate aminotransferase levels (mU/mg creatinine) significantly greater than in the control group.

[8] Estrous cycle significantly longer than in the control group.

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.

** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE 2 Selected Parameters for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)					
	0	**0.025**	**0.05**	**0.1**	**0.25**	**1**
MALE						
Final body weight[2]						
(percentage of controls)		106	106	105	106	102
Respiratory System						
Lung						
Weight						
(absolute and relative)	)	) [3]	↑ [4]	↑	↑	↑
Histopathologic findings						
alveolar epithelial hyperplasia	)	+ (1.0)	+ (1.0)	+ (1.8)	+ (1.7)	+ (2.0)
inflammation	)	)	)	+ (3.0)	+ (2.2)	+ (2.7)
fibrosis	)	)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)
Tracheobronchial lymph node						
hyperplasia	)	)	+ (1.0)	+ (2.3)	+ (2.4)	+ (2.7)
Larynx						
squamous metaplasia	)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.1)
Nose						
Olfactory epithelium						
degeneration	)	)	)	+ (1.0)	+ (1.7)	+ (2.0)
respiratory metaplasia	)	)	)	)	+ (1.0)	+ (1.5)
squamous metaplasia	)	)	)	)	)	+ (1.0)
Respiratory epithelium						
hyaline droplets	)	)	)	)	+ (1.0)	+ (1.0)
Kidney						
Weight, right kidney (absolute)	)	↑	↑	↑	↑	↑
Reproductive System						
Testis/epididymis weight	)	)	NM [5]	)	NM	)
Spermatid count	)	)	NM	)	NM	)
Sperm motility	)	)	NM	)	NM	)

TABLE 2 Selected Parameters for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Cadmium Oxide (continued)

	Concentration (mg/m^3)					
	0	**0.025**	**0.05**	**0.1**	**0.25**	**1**
FEMALE						
Final body weight[2]						
(percentage of controls)		105	110	103	99	103
Respiratory System						
Lung						
Weight						
(absolute)	)	)	↑	↑	↑	↑
Histopathologic findings						
alveolar histiocytic infiltrate	)	+ (1.0)	+ (1.0)	+ (2.0)	+ (2.0)	+ (3.0)
alveolar epithelial hyperplasia	)	)	)	+ (1.4)	+ (2.0)	+ (2.0)
inflammation	)	)	)	+ (2.3)	+ (2.1)	+ (2.9)
fibrosis	)	)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)
Tracheobronchial lymph node						
hyperplasia	)	)	+ (1.0)	+ (1.5)	+ (2.0)	+ (2.4)
Larynx						
squamous metaplasia	)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)
Nose						
Olfactory epithelium						
degeneration	)	)	)	+ (1.0)	+ (1.0)	+ (2.0)
respiratory metaplasia	)	)	)	)	)	+ (1.0)
squamous metaplasia	)	)	)	)	)	)
Respiratory epithelium						
hyaline droplets	)	)	)	)	+ (1.0)	+ (1.0)
Kidney						
Weight, right kidney (absolute)	)	↑	↑	↑	↑	↑
Reproductive System						
Estrous cycle length	)	)	NM	)	NM	)

[1] For each control and exposure group, statistical analyses were performed on the mean value for 9 to 10 mice (organ weights and reproductive parameters). For histopathologic findings, average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

[2] (Exposure group mean/control group mean) x 100.

[3]) = No lesions present (histopathology) or not significantly different from the control group (organ weights and reproductive parameters).

[4] Organ weights significantly greater than the control group.

[5] NM = not measured at this exposure level.

INTRODUCTION

Physical and Chemical Properties, Production, Use, and Exposure

In elemental form, cadmium is a white-silver metal morphologically arranged as closely packed, hexagonal crystals (Weast, 1980). The only valence state for cadmium is Cd^{2+}. Major cadmium-containing compounds include cadmium acetate, cadmium chloride, cadmium nitrate, cadmium oxide, cadmium sulfate, and cadmium sulfide.

Cadmium oxide, the focus of the present inhalation studies, is produced by the reaction of cadmium metal vapor with air, by the thermal decomposition of cadmium nitrate or cadmium carbonate, or by the oxidation of molten cadmium (Schulte-Schrepping and Piscator, 1985; Herron, 1992).

Atomic absorption spectroscopy, with a sensitivity in the nanogram per milliliter range, is the most widely used method for quantifying cadmium in various media (Friberg *et al.*, 1974, 1979). This method measures total cadmium without discerning the forms of cadmium present.

Cadmium is widely distributed in the environment at relatively low concentrations except where it has been concentrated anthropogenically. Nonpolluted natural waters contain less than 1 µg cadmium per liter, and seawater reportedly contains 0.04 to 0.30 µg cadmium per liter (Friberg *et al.*, 1974, 1979). Soils generally contain less than 1 mg cadmium per kilogram, but in certain rice-growing areas of Japan, topsoil contained between 1 and 69 mg cadmium per kilogram. Sewage sludge from some U.S. cities contained up to 100 mg/kg cadmium, which could be available for plant uptake, depending on soil conditions. In air over the United States, 0.006 to 0.036 $\mu g/m^3$ cadmium was reported (Friberg *et al.*, 1974, 1979).

In 1991, the United States produced and imported a total of 3,238 tons of cadmium (Bureau of Mines, 1993). In 1984, the United States produced approximately 771 tons of cadmium sulfide and approximately 1,500 tons of other cadmium compounds, of which 90% was cadmium oxide (Plunkert, 1984). Cadmium is used in batteries, 50%; coating and plating, 15%; pigments, 18%, plastics and synthetic products, 12%; and alloys and other materials, 5% (Bureau of Mines, 1993).

Cadmium oxide is used as a starting material for polyvinyl chloride heat stabilizers and for other inorganic cadmium compounds (Herron, 1992; IARC, 1993). It is also a catalyst for oxidation-

reduction reactions, dehydrogenation, cleavage, polymerization, production of saturated alcohols, and hydrogenation of unsaturated fatty acids. It is a mixed catalyst for production of methanol from carbon monoxide and water. Other uses include heat-resistant enamels, metal coatings for plastics, heat-resistant plastics, and selenium ruby glass. Cadmium oxide combined with an alkali-metal cyanide is the salt mixture used in cadmium electroplating baths. Because of its temperature resistance, highly purified cadmium oxide is used, in addition to silver oxide, as a depolarizer in silver-zinc storage batteries. Cadmium oxide has also been used as a nematocide, vermicide, and ascaricide in swine (Herron, 1992; IARC, 1993).

The National Institute for Occupational Safety and Health (NIOSH) estimates that approximately 1.5 million workers may be exposed to cadmium, a nonessential element (NIOSH, 1981). Approximately 15,000 workers are potentially exposed to cadmium oxide (NIOSH, 1994). Workers are exposed by inhaling finely ground particulates or by inhaling cadmium oxide fumes generated by heating or welding cadmium-containing materials.

Standards for occupational exposures to all forms of cadmium and cadmium compounds have been established by the Occupational Safety and Health Act (1993) for all industries (29 CFR § 1910.1027). The action level (AL) for cadmium and cadmium compounds is 2.5 µg cadmium per cubic meter of air, calculated as an 8-hour, time-weighted average (TWA). The permissible exposure level (PEL) for cadmium and cadmium compounds is 5 $\mu g/m^3$, calculated as an 8-hour TWA.

Exposure to cadmium also occurs via food, water, and air. It has been estimated that a 70 kg person in Europe or the United States with a diet containing normal amounts of cadmium ingests between 25 and 60 µg cadmium daily (Friberg *et al.*, 1974, 1979). Considerably higher dietary intake may occur among persons consuming fruits, vegetables, meat, or shellfish from cadmium-contaminated areas. The U.S. Fish and Wildlife Service (USFWS) monitors concentrations of metals in freshwater fish. The USFWS found that fish samples from 109 stations nationwide contained an average of 0.03 µg cadmium per gram wet weight (Schmitt and Brumbaugh, 1990).

Cigarette smoking is another major source of cadmium exposure. Each cigarette may contain 1 to 2 µg of cadmium, and inhalation is a highly efficient mode for cadmium entry into the body (Friberg *et al.*, 1974, 1979). Fishbein (1984) and Ryan *et al.* (1982) estimate that a person inhales 0.1 to 0.2 µg of cadmium from each cigarette.

Absorption, Disposition, Metabolism, and Excretion

Comparative data are not available on the absorption of cadmium oxide following administration by different routes. However, 30 days after a single exposure to cadmium chloride ($^{115m}CdCl_2$), male rats retained 2.3% of a 50 µCi oral dose, 41.1% of a 4-hour 1,800 µg Cd/m^3 inhalation exposure, 91.4% of a 20 µCi intravenous dose, and 93.1% of a 40 µCi intraperitoneal dose (Moore *et al.*, 1973). In monkeys, a single oral dose of 1.7 mg cadmium ($^{115m}CdCl_2$) per kilogram body weight had a half-life of 1.7 years (Nordberg, 1972). In male CBA mice, the half-life of cadmium chloride was 2 to 4 years after a subcutaneous injection of 0.25 mg/kg $^{109}CdCl_2$ followed by daily injections of 0.25 mg/kg nonradioactive cadmium for 25 weeks (Nordberg, 1972).

Weigel *et al.* (1984) reported that male Wistar rats administered cadmium oxide in feed (2.8 or 7.15 ppm) for 60 days accumulated cadmium in the liver, kidney, lung, and spleen. Tissues and fluids examined included the lung, liver, spleen, kidney, bone, blood, feces, hair, muscle, testes, and urine. Excretion of cadmium was primarily in the feces (Weigel *et al.*, 1984). Similar distribution and excretion patterns were observed following oral administration of a single dose of 50 µCi ^{115m}Cd as cadmium nitrate to male albino rats (Kanwar *et al.*, 1980).

Fishbein (1984) and Ryan *et al.* (1982) estimate that humans absorb approximately 5% of ingested cadmium and approximately 10% to 40% of inhaled cadmium. The main organs that accumulate cadmium in humans are the kidneys, liver, lungs, and pancreas (Cherry, 1981). The kidneys are particularly affected in long-term, low-level cadmium exposures because of the long biological half-life of cadmium (greater than 10 years) in this organ (Friberg *et al.*, 1974, 1979).

After ingestion or inhalation, cadmium is transported to the liver via high molecular weight proteins in the blood (Nordberg *et al.*, 1971). In the liver, cadmium stimulates synthesis of metallothionein, a protein of low molecular weight (approximately 7,000), which has been extensively studied in mammals and is also known to exist in a number of nonmammalian species (Nordberg and Kojima, 1979; Dunnick and Fowler, 1987). Metallothionein binds the cadmium, and this complex is released from the liver and moves via the blood to the kidney.

In the kidney, the cadmium-metallothionein complex is filtered by the glomerulus and is reabsorbed by the proximal tubule cells (Friberg *et al.* 1974, 1979; Cherian and Shaikh, 1975; Nordberg *et al.*, 1975; Cherian *et al.*, 1976; Fowler and Nordberg, 1978; Squibb *et al.*, 1979, 1982, 1984; Cherian, 1983; Nordberg, 1984; Squibb and Fowler, 1984; Dudley *et al.*, 1985). Elemental cadmium is released at low

pH in the lysosomes during proteolysis of the reabsorbed metallothionein. The free cadmium induces the production of renal metallothionein which subsequently binds the metal (Dorian *et al.*, 1992).

The cadmium-metallothionein complex was found in the serum of experimental animals and humans following exposure to cadmium, depending on the dose administered and the duration of exposure (Shaikh and Hirayama, 1979; Chang *et al.*, 1980; Garvey and Chang, 1981). A number of authors have shown that metallothionein has a turnover rate similar to that of other cytosolic proteins (Nordberg and Kojima, 1979). Feldman *et al.* (1978) calculated a half-life of approximately 3.5 days for the metallothionein-cadmium complex. In long-term exposures (6 months), cadmium-metallothionein turnover results in distribution of cadmium into other cellular compartments of the kidney, but not the liver (Ridlington *et al.*, 1981; Lehman-McKeeman *et al.*, 1988; Kershaw and Klaassen, 1992). Cadmium stored in the kidney is excreted in the urine, and cadmium may also be excreted in bile, feces, saliva, and sweat (Friberg *et al.*, 1974, 1979).

Long-term pulmonary clearance and translocation of cadmium are not based on chemical solubility (USEPA, 1984). Oberdoerster *et al.* (1979) compared the pulmonary clearance of water-insoluble cadmium oxide (930 µg/m^3; MMAD=0.46 µm) with that of water-soluble cadmium chloride (760 µg/m^3; MMAD=0.38 µm). In this inhalation study, after a 45-minute exposure of male Wistar rats to cadmium oxide or cadmium chloride, both compounds had a lung half-life of 70 days. Initial clearance of cadmium oxide was greater, which the authors attributed to mucociliary clearance. The authors hypothesized that both compounds bind to metallothionein in the lungs and are then cleared at the same rate (Oberdoerster *et al.*, 1979).

Intratracheally instilled cadmium oxide (^{109}CdO; 15µg; particle size = approximately 1.0 µm) had a half-life of 4 hours in the lungs of male Wistar SPF rats (Hadley *et al.*, 1980). After 24 hours, the distribution of cadmium was 58% in the liver, 24% in the lungs, 3% in the kidneys, and 0.2% in the testes. After 2 weeks, the distribution of cadmium was 57% in the liver, 18% in the lungs, 8% in the kidneys, and 0.2% in the testes. Less than 10% of the cadmium was excreted during the first 2 weeks.

Larsson *et al.* (1981) found low levels of cadmium in human milk. Cadmium was also excreted in the milk of rodents after subcutaneous injection of ^{109}Cd (Lucis *et al.*, 1972). These findings indicate the potential for exposure of offspring to cadmium during lactation.

Toxicity

Data explaining the mechanism and effects of cadmium toxicity (in various organs and systems) have been reviewed by a number of authors, including Friberg *et al.* (1974, 1979), Friberg *et al.* (1975), Nriagu (1981), and Dunnick and Fowler, (1987). Cadmium toxicity may be manifested by lung damage, renal dysfunction, hepatic injury, bone defects, hypertension, reproductive toxicity, and teratogenicity. The Agency for Toxic Substances and Disease Registry (ATSDR, 1993) has prepared a toxicologic profile for cadmium which summarizes the human health effects from cadmium exposure.

RESPIRATORY TOXICITY

Acute cadmium toxicity in rodents after respiratory exposure is characterized by lung edema, damage of alveolar type I cells, proliferation of alveolar type II cells, and fibrosis (Friberg *et al.*, 1974, 1979). In man, symptoms of cadmium toxicity include coughing, shortness of breath, irritation of the upper respiratory system, and loss of olfaction. Yellow rings on the teeth have also been reported (Friberg *et al.*, 1974, 1979).

To determine whether the physico-chemical form of cadmium compounds affects their pulmonary toxicity, Oberdörster *et al.* (1987) compared the pulmonary toxicities of water-insoluble cadmium compounds (cadmium oxide dust, cadmium oxide fume, and cadmium sulfide) with the pulmonary toxicity of the water-soluble cadmium chloride. Groups of male Long Evans rats were exposed by inhalation to approximately 2 $\mu g/m^3$ cadmium oxide dust (MMAD=0.51 µm), cadmium oxide fumes (MMAD=0.40 µm), or cadmium sulfide (MMAD=0.45 µm) for 60 minutes. Other groups of male Long Evans rats were instilled with 10, 30, or 100 µg cadmium chloride in the lungs. In all groups, cadmium treatment induced an acute inflammatory response characterized by decreased cell viability and number of lavaged macrophages and increased numbers of polymorphonuclear leukocytes, lymphocytes, and macrophages. The authors concluded that pulmonary effects of cadmium compounds cannot be predicted from their water solubility (Oberdörster *et al.*, 1987).

KIDNEY TOXICITY

The amount of cadmium that reaches the kidney depends on the metal speciation and the rate and route of administration (Foulkes, 1990). Symptoms of cadmium nephrotoxicity (primarily in studies with cadmium chloride) include tubular proteinuria, decreased capacity for concentrating urine, glucosuria, calciuria, and microglobulinuria (Garvey and Chang, 1981).

Renal toxicity was observed at a concentration of 10 µg cadmium per gram wet kidney when cadmium-metallothionein was administered to male Sprague-Dawley rats (Wang *et al.*, 1993). When cadmium chloride was administered to male Sprague-Dawley rats, the amount of stored cadmium needed to induce toxicity was 200 µg/g wet kidney (Goyer *et al.*, 1984, 1989).

The metallothionein-cadmium complex plays a major role in cadmium nephrotoxicity by acting as a vehicle for selectively delivering cadmium ions to the proximal tubule cell lysosomes (Maitani *et al.*, 1988). During lysosomal degradation of the metallothionein-cadmium complex, elemental cadmium is released and is subsequently bound by induced renal metallothionein. High concentrations of free cadmium appear to disrupt the normal formation of primary and secondary (mature) lysosomes prior to the induction of renal metallothionein (Squibb *et al.*, 1979, 1982, 1984; Squibb and Fowler, 1984). Marked inhibition of lysosomal protease activity follows and tubular proteinuria develops, causing renal tubule cell injury that may lead to interstitial nephropathy (Squibb *et al.*, 1979, 1982, 1984; Squibb and Fowler, 1984).

In vivo measurements of cadmium in the renal cortex have shown that about 10% of workers with a cadmium tissue level of 200 mg/kg and about 50% of people with a cadmium tissue level of 300 mg/kg have renal tubule proteinuria (IPCS, 1992). In experimental animals, proteinuria was eliminated by zinc induction of metallothionein in the kidney before cadmium exposure (Squibb *et al.*, 1979, 1982, 1984; Squibb and Fowler, 1984). Metallothionein produced prior to cadmium exposure complexes cadmium ions in the kidney and liver, apparently reducing their intracellular bioavailability and toxicity (Squibb *et al.*, 1984).

LIVER TOXICITY

Liver damage in male rats has been shown after administration of water-soluble forms of cadmium. Faeder *et al.* (1977) administered subcutaneous injections of 0.5 to 0.75 mg/kg cadmium chloride to male Wistar rats 3 days per week for 8 weeks. Changes included elevated plasma enzyme levels and microscopic changes in the liver, including dilation of the rough endoplasmic reticulum and prominent connective tissue bundles. Dudley *et al.* (1985) administered subcutaneous injections of 0.5 mg/kg cadmium chloride to male Sprague-Dawley rats 6 days per week for up to 26 weeks. Elevated plasma enzyme levels, liver enlargement, and liver cell injury characterized by parenchymal cell swelling and necrosis were observed. Acute exposure of male Sprague-Dawley rats to cadmium chloride (a single intravenous injection of 3.9 mg/kg) also induced liver damage (Dudley *et al.*, 1982).

Liver damage in rats was prevented by zinc or cadmium induction of metallothionein in the liver before exposure of the rats to hepatotoxic doses of cadmium (Goering and Klaassen, 1984a,b). In addition, Goering and Klaassen (1984c) found that in male Sprague-Dawley rats, a 4 mg/kg intravenous injection of cadmium was hepatotoxic to adults, while an intravenous injection of 4, 5, or 6 mg/kg cadmium produced no liver toxicity in 10-day-old pups. The authors proposed that the high concentration of metallothionein in the livers of immature rats (10 to 20 times higher than levels present in adult rat livers) conferred tolerance to cadmium-induced hepatotoxicity.

Other studies have shown that C3H/HeJ mice are more sensitive to cadmium-induced liver damage than are DBA/2J mice. Hepatic endothelial cells isolated from C3H/HeJ mice were more sensitive to cadmium toxicity than hepatic endothelial cells from DBA mice (Liu *et al.*, 1992). The mechanism that accounts for this genetic variation in endothelial cell response to cadmium is unknown, but does not appear to be related to the cellular disposition of cadmium nor to a defect in the metabolism of metallothionein.

BONE TOXICITY

Friberg *et al.* (1974, 1979) and Nogawa (1981), after reviewing data from a number of sources, concluded that the ingestion of cadmium played the most important role in the development of itai-itai (ouch-ouch) disease among the human population of Toyama City, Japan in 1955. Studies showed that cadmium levels were particularly high in rice, a staple food for this population. The cadmium source was believed to be contaminated water from the Kamioka mine upstream from Toyama City.

Symptoms of itai-itai disease include femoral pain and lumbago, painful sites throughout the body, and a ducklike gait (Friberg *et al.*, 1974, 1979; Nogawa, 1981). Pathologic changes include osteomalacia and osteoporosis, which are most prevalent in postmenopausal women. Other pathologic changes include kidney tubule atrophy and degeneration. As the condition progresses, bone fractures are common. Patients show normochromic anemia, increased granulocyte count, decreased lymphocyte count, and high urinary cadmium levels. Vitamin D is sometimes effective in treating severe cases (Friberg *et al.*, 1974, 1979; Nogawa, 1981).

Pathologic mechanisms leading to itai-itai disease are not fully understood. It is suggested that the etiology of this disease in women is a combination of low dietary calcium, mobilization of calcium from the skeleton during pregnancy, and cadmium-induced calciuria (Katsuta *et al.*, 1993). Cadmium-induced calciuria has also been observed in cadmium-exposed workers who also developed radiological signs of osteomalacia (Kazantzis, 1979). Fowler *et al.* (1987) demonstrated that cadmium-induced calciuria is not due to altered kidney cell membrane transport of calcium, but rather seems to be related to the binding of calcium to the increased number of excreted proteins following tubular proteinuria. Ninety percent of itai-itai disease occurs in postmenopausal women, and ovariectomy in Sprague-Dawley rats enhanced cadmium chloride-induced nephrotoxicity and hepatotoxicity (Katsuta *et al.*, 1993). Further studies using similar model systems might help explain the disease.

CARDIOVASCULAR TOXICITY

Cadmium acetate and cadmium chloride reportedly caused hypertension in rats (Kopp *et al.*, 1982; Nishiyama *et al.*, 1986), although hypertension was not reported in rats exposed to cadmium oxide (Barański *et al.*, 1983).

Kopp *et al.* (1982) administered cadmium acetate to Long Evans rats in drinking water at doses of 0.01 to 50 ppm for 18 months. Exposures of 10 to 20 µg cadmium per kilogram body weight per day (0.5 ppm level) increased average systolic blood pressure while exposures to higher concentrations of cadmium lowered blood pressure. These authors hypothesized that low-level exposures to cadmium, comparable to dietary exposures for the average American, are more toxic to the cardiovascular system than high-level exposures because a threshold amount of cadmium is needed to stimulate metallothionein synthesis. These authors further suggest that cadmium cardiovascular toxicity may be due to functional and biochemical lesions in cardiovascular tissues.

IMMUNOLOGIC TOXICITY

Malavé and de Ruffino (1984) showed that, depending on the dose, cadmium may increase or decrease immunologic response to foreign antigens. These authors exposed male C57BL/6 mice, in drinking water, to 50, 200, or 300 ppm cadmium chloride for 3 to 11 weeks. Exposures of 50 to 200 ppm increased the antibody response to sheep erythrocytes, whereas exposures of 300 ppm decreased the antibody response to sheep erythrocytes. Further work is needed to understand the effects of cadmium on the immune system.

REPRODUCTIVE SYSTEM TOXICITY

Reproductive system toxicity of cadmium compounds in male rodents was first noted by Pařízek and Záhoř in 1956. Other studies have shown that water-soluble cadmium compounds given orally or intravenously cause toxicity to the male rat reproductive system (Phelps and Laskey, 1989; Wahba and Waalkes *et al.*, 1990; Hew *et al.*, 1993a,b). Laskey *et al.*, 1984 observed decreased testis, seminal vesicle, and epididymal weights and decreased sperm concentrations and serum testosterone levels in Sprague-Dawley rats receiving a single subcutaneous injection of 16 or 33 µmol/kg cadmium chloride. Young Sprague-Dawley rats (2-5 weeks old) were apparently more resistant to cadmium-induced testicular damage than Sprague-Dawley rats 6 weeks of age or older (Wong and Klaassen, 1980). In addition, the animal strain seems to affect the susceptibility of rats and mice to cadmium-induced testicular damage (Gunn *et al.*, 1965).

Studies by Lee and Dixon (1973) suggest that cadmium exposure affects spermiogenic cells by inhibiting zinc utilization and deoxyribonucleic acid synthesis. Dwivedi (1983) suggests that cadmium produces sterility by inhibiting spermatozoan choline acetyltransferase, by decreasing acetylcholine synthesis, and by impairing spermatozoan motility. In addition, Laskey and Phelps (1991) found that cadmium chloride depressed *in vitro* production of testosterone by stimulated Leydig cells.

Kutzman (1984) administered cadmium chloride aerosols (MMAD=0.66-0.73 µm) to F344/N rats at levels of 0, 0.3, 1, or 2 mg/m^3 for 62 days, 6 hours per day, 5 days per week. Rats in the 2 mg/m^3 groups died during the first 45 exposure days. Six days following the last exposure, treated males were mated with untreated females and treated females were mated with untreated males. In the 1 mg/m^3 groups, there were no differences in total viable embryos, fetal death, or preimplantation loss.

Exposure of female rats to cadmium oxide aerosol before and during mating had no effect on fertility (Barański, 1984). In this experiment, female Wistar rats were exposed to cadmium oxide aerosol (MMAD<0.65 µm) at concentrations of 0.02 or 0.16 mg/m^3 5 days per week, 5 hours daily, for

5 months, and then for a maximum of 3 weeks during mating with untreated male rats. For each exposure group, the number of rats becoming pregnant was compared to the number of pregnancies in the control group using the Fisher exact probability test. In studies of workers exposed to cadmium oxide fumes for longer than a year (exposure concentrations not available), no testicular endocrine effects or depressed reproductive function were apparent (Mason, 1990; Gennart *et al.*, 1992). In the study by Mason (1990), testicular endocrine effects were assessed by measuring the levels of serum testosterone, luteinizing hormone, and follicular-stimulating hormone. Gennart *et al.* (1992) evaluated the fertility of male workers by studying the birth experiences of their wives through data collected by a questionnaire and analyzed by a logistic regression model.

A single subcutaneous injection of 5 or 10 mg/kg cadmium chloride inhibited ovulation in female golden hamsters, and the effect was reversible with time (Saksena and Salmonsen, 1983). In addition, a subcutaneous injection of 0.5 mg cadmium chloride per 100 g body weight induced pathologic changes in the uterus and ovary of rats (Jenny *et al.*, 1979).

TERATOGENICITY

Teratogenic effects of cadmium were first reported by Ferm and Carpenter, 1968. Following a single intravenous injection of cadmium sulfate (2 mg/kg) into pregnant golden hamsters, embryos had cleft palate, anophthalmia, exencephaly, limb defects, and rib fusions. A literature review suggests that cadmium chloride, cadmium acetate, cadmium sulfate, and elemental cadmium (subcutaneous, intravenous, or intraperitoneal administration) may induce limb defects, cleft palate, and delayed ossification in rats, mice, and hamsters (Degraeve, 1981). Oral administration of cadmium chloride (20-80 mg/kg/day) to pregnant COBS albino rats from gestation day 6 through 19 caused fetal heart and kidney abnormalities (Scharpf *et al.*, 1972). Limb defects were observed in Long-Evans rat embryos after administration of 18.4 or 61.3 mg/kg cadmium by oral gavage to pregnant females from gestation day 6 through 15 (Machemar and Lorke, 1981). There is little experimental evidence for the teratogenicity of cadmium oxide, cadmium sulfide, or cadmium nitrate. Pretreatment of female mice with cadmium reduces the teratogenicity of cadmium, probably by increasing metallothionein levels (Layton and Ferm, 1980). Teratogenicity of cadmium in humans has not been shown (Ferm and Carpenter, 1968).

CARCINOGENICITY

Animal Studies

Experimental carcinogenicity studies with cadmium have been summarized by the International Agency for Research on Cancer (IARC, 1976, 1993) and the U.S. Environmental Protection Agency (USEPA, 1984). These studies have shown that intramuscular or subcutaneous injection of cadmium (as cadmium powder, cadmium oxide, cadmium chloride, cadmium sulfide, or cadmium sulfate) into rodents caused local tumors. In addition, elemental cadmium, cadmium chloride, and cadmium sulfate induced tumors distal to the injection site, such as interstitial cell tumors in the testes. Poirier *et al.* (1983) found that a single subcutaneous dose of cadmium chloride (0.02 or 0.04 mmol/kg) administered to male Wistar rats increased the incidence of pancreatic islet cell tumors and testis interstitial cell tumors, in addition to inducing fibrosarcomas at the injection site.

A single intratracheal instillation of 25, 50, or 75 µg cadmium oxide into male F344 rats induced mammary gland tumors, but not lung tumors (Sanders and Mahaffey, 1984).

After inhalation exposure to cadmium oxide (30-90 $\mu g/m^3$), treatment-related lung neoplasms were observed in male Wistar rats but not in female Han:NMRI mice or male or female Syrian golden hamsters (Aufderheide *et al.*, 1989; Heinrich *et al.*, 1989; Thiedemann *et al.*, 1989; Glaser *et al.*, 1990; Takenaka *et al.*, 1990). In these studies, rats were exposed for 22 hours per day, 7 days per week, for up to 18 months, and hamsters and mice were exposed for 8 or 19 hours per day, 5 days per week, for up to 16 months. Heinrich *et al.* (1989) and Glaser *et al.* (1990) reported an MMAD of 0.2 to 0.6 µm for cadmium oxide (versus an MMAD of 1.1 to 1.6 µm in the present 13-week NTP studies). In the above studies, the carcinogenic effects of inhaling other forms of cadmium, including cadmium chloride, cadmium sulfate, and cadmium sulfide were also evaluated. As with cadmium oxide, a carcinogenic response to these chemicals was seen in rats but not in mice or hamsters.

Takenaka *et al.* (1983) exposed male Wistar rats to cadmium chloride aerosol (MMAD=0.55 µm) at cadmium concentrations of 12.5, 25, or 50 $\mu g/m^3$ for 23 hours per day, 7 days per week, for 18 months. The control group was housed in filtered air for the same time period. Thirteen months after the last exposure, exposed rats had dose-related increases in the incidence of lung tumors, including adenocarcinomas, squamous cell carcinomas, and mucoepidermoid carcinomas.

Several early oral administration studies of cadmium were summarized by the USEPA (1984), but these studies were considered inadequate tests of carcinogenicity. In one such study, cadmium

chloride administered in feed for 2 years to male and female Wistar rats at cadmium levels of 3, 10, or 50 ppm produced no evidence of carcinogenicity (Löser, 1980). Body weights were reduced in males and females in the 50 ppm groups. The USEPA (1984) concluded that "while it is possible that cadmium is not at all carcinogenic by ingestion because of very limited absorption, the negative animal evidence can only set an upper limit on the carcinogenic potency of ingested cadmium, which in the rat appears to be almost two orders of magnitude less than for inhalation." In a more recent study in which cadmium chloride was administered to male Wistar rats in feed at 0, 25, 50, 100, or 200 mg/kg, there was some evidence for proliferative lesions of the prostate gland after 77 weeks of exposure, but these lesions were only significant when the data from the exposed groups were pooled (Waalkes and Rehm, 1992).

Epidemiology Studies

The International Agency for Research on Cancer (IARC, 1993) recently convened a group of experts to review the epidemiologic association between cadmium exposure and lung and prostate cancer in humans. They concluded that there was an association between exposure to cadmium and lung cancer, but that there was not sufficient evidence for an increase in prostate cancer due to cadmium exposure.

Thun *et al.* (1985) showed that in U.S. cadmium smelter workers there was an increase in lung cancer, but no new cases of prostate cancer were reported. Stayner *et al.* (1992), analyzing the data from these workers, reported that the risk for lung cancer is increased after exposure to cadmium fumes at 100 µg/m^3 for 45 years. Lamm *et al.* (1992), analyzing the same data, noted that the risk of lung cancer was related to the period of hire and may have been related to exposure to arsenic and cigarette particulates.

Inskip *et al.* (1982) noted no significant risk of lung cancer in female residents of Shipman, a small English village, where oral exposure to cadmium was thought to occur due to soil contamination. In a review of epidemiology data, Doll (1992) showed that exposure to cadmium was most often related to lung cancer when the exposure level was high and that exposure to the low levels of cadmium in foods was not associated with an increase in lung cancer. Average cadmium consumption is estimated to be 13 to 16 µg per day, lower than the level of exposure reported in cadmium smelter plants (Kowal *et al.*, 1979; Louekari *et al.*, 1991).

Before 1947, lung and prostate cancer were associated with cadmium exposure, and occupational exposure limits were higher than current limits. However, in recent studies, workers exposed to

cadmium showed no increase in prostate cancer, and the relative risk of lung cancer was increased only after approximately 20 years of exposure to cadmium at levels of greater than 10 $\mu g/m^3$ (Ades and Kazantzis, 1988). Sorahan and Waterhouse (1983) studied workers exposed to cadmium at a zinc-lead-cadmium smelter in England and concluded that there was no new evidence of lung or prostate cancer in these workers Among workers exposed to cadmium for an average of 11 years, Armstrong and Kazantzis (1983) reported a marginal increase in the incidence of lung cancer but no increase in number of deaths due to prostate cancer.

GENETIC TOXICITY

Cadmium oxide was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without S9 metabolic activation (Mortelmans *et al.*, 1986). Although no additional mutagenicity data are available for cadmium oxide, there are numerous reports regarding the mutagenic activity of cadmium chloride. Early investigations into the mutagenicity of cadmium in bacteria, using standard protocols, gave mostly negative results (Kanematsu *et al.*, 1980; De Flora *et al.*, 1984; Rossman *et al.*, 1984; Arlauskas *et al.*, 1985; Marzin and Phi, 1985). However, when preincubation exposure was carried out in sterile, distilled, deionized water instead of sodium phosphate buffer, which is normally used, cadmium chloride was mutagenic in *S. typhimurium* strain TA97 (Pagano and Zeiger, 1992). The authors suggested that heavy metals such as cadmium may be inactivated by interaction with standard media components or with elements in the active or passive cellular transport processes. This would explain the apparent insensitivity of the *Salmonella* assay to divalent metals.

Denizeau and Marion (1989) showed that the nucleus is a target organelle for heavy metals. These authors measured the nuclear uptake of cadmium in freshly isolated rat hepatocytes using [^{3}H]-thymidine (a measure of DNA repair synthesis), mass spectroscopy, and atomic absorption spectroscopy. After a 20-hour cadmium exposure, the *in situ* DNA binding ratio was 0.98 ± 0.23 ng cadmium per microgram DNA.

Cadmium chloride was reportedly mutagenic at the HGPRT locus in hamster V-79 cells (Kanematsu *et al.*, 1990) and at the tk^+/tk^- locus in L5178Y mouse lymphoma cells (McGregor *et al.*, 1988). Numerous studies showed that DNA single strand breaks and DNA-protein crosslinks were induced by cadmium in cultured mammalian cells (for a review of these data, see Waalkes *et al.*, 1992). Results of *in vitro* mammalian cell investigations indicated that generation of free radicals was a primary mechanism for cadmium-induced DNA damage (Burkart and Ogorek, 1986; Ochi *et al.*, 1987; Biggart and Murphy, 1988; Snyder, 1988).

Results of mammalian chromosomal aberration studies with cadmium are mixed, possibly because of differences in cell type and dose. Positive results were reported in Chinese hamster ovary cells treated with 30 µM or more cadmium (5.5 µg/mL) for 36 hours (Deaven and Campbell, 1980). Also, a significant dose-related increase in chromosomal aberrations was seen in Chinese hamster ovary cells treated for 17 to 24 hours with 1.0 to 3.0 µM cadmium sulfate (Armstrong *et al.*, 1992; Bean *et al.*, 1992). However, no clastogenicity was observed in mouse FM3A cells treated with up to 32.2 µM cadmium (5.9 µg/mL) for 24 or 48 hours (Umeda and Nishimura, 1979) or in Chinese hamster ovary cells treated with 28 µM cadmium for 20 hours (Yamada *et al.*, 1993).

Significantly increased chromosomal aberrations were also noted in peripheral lymphocytes of individuals living in a cadmium-contaminated area of China compared to a control group living nearby in an uncontaminated location (Tang *et al.*, 1990). Increased chromosomal aberration frequencies were directly correlated to increased urinary cadmium levels. The authors suggested that cadmium acted alone, not synergistically with another contaminant.

Yamada *et al.* (1993) investigated the co-clastogenic activity of cadmium chloride in Chinese hamster ovary cells and SV40 transformed XP20SSV xeroderma pigmentosum cells. The frequencies of chromosomal aberrations induced by known clastogens (mitomycin C, cisplatin, and methyl methanesulfonate) were enhanced by posttreatment of cells with cadmium. The authors concluded that the enhancement was not due to the additive effect of two clastogens (cadmium alone did not induce chromosomal aberrations at the doses employed) but rather was due to the inhibition of DNA excision repair by cadmium ions, also proposed by Nocentini (1987). The action of cadmium is specific, as evidenced by the fact that the frequencies of chromosomal aberrations induced by clastogens operating through different mechanisms (bleomycin and actinomycin D) were not enhanced by posttreatment with cadmium.

Cadmium chloride is a suspected spindle poison and induced aneuploidy in germ cells of female *Drosophila melanogaster* (Osgood *et al.*, 1991) and a small dose-related increase ($P \leq 0.05$) in hyperploidy in spermatocytes of male mice (Miller and Adler, 1992). Hyperploidy in secondary spermatocytes is a measure of nondisjunction in primary spermatocytes.

Somewhat conflicting results are presented in the literature regarding induction of aneuploidy in mammalian oocytes. Hyperploidy was reported in oocytes of mice and golden hamsters treated with cadmium chloride (Watanabe *et al.*, 1977, 1979; Watanabe and Endo, 1982), but Mailhes *et al.* (1988) found no effect of cadmium chloride in mouse oocytes. These differences may have been due to

protocol variations, such as differences in the time of administration of human chorionic gonadotrophin.

Other aneuploidy assays performed with cadmium chloride have also given negative results. For example, cadmium chloride was not active in the *in vitro* porcine brain tubulin assembly assay designed to detect potential aneuploidogens (Brunner *et al.*, 1991), nor did it induce mitotic chromosome malsegregation in *Saccharomyces cerevisiae* (Whittaker *et al.*, 1989; Albertini, 1990).

The data appear to support the conclusion that cadmium, when investigated under conditions appropriate for its detection, is mutagenic and induces chromosomal damage, directly or indirectly, through interference with DNA repair or spindle function.

Study Rationale and Design

Cadmium oxide was nominated by the National Cancer Institute for toxicity studies because of widespread occupational exposure to cadmium oxide and because at the time there were no adequate inhalation toxicity or carcinogenicity studies of this form of cadmium. Inhalation studies have now demonstrated that cadmium oxide is carcinogenic, causing lung neoplasms in male rats (Aufderheide *et al.*, 1989; Heinrich *et al.*, 1989; Glaser *et al.*, 1990; Thiedemann *et al.*, 1989), and IARC (1993) has classified cadmium and cadmium compounds as human carcinogens.

Two-week and 13-week inhalation studies of cadmium oxide were conducted in F344/N rats and B6C3F$_1$ mice. Toxicologic characterization included assessments of gross and histopathologic organ toxicity (especially upper respiratory tract toxicity), clinical pathology, reproductive and developmental toxicity, and genetic toxicity.

MATERIALS AND METHODS

Procurement and Characterization of Cadmium Oxide

Cadmium oxide was obtained in a single lot (Lot 110383) from Johnson Matthey Aesar Group (Seabrook, NH) and used as received for all of the studies. Chemical analyses performed by Midwest Research Institute (Kansas City, MO) included X-ray diffraction, elemental analysis, spark source mass spectrometry, water analysis, and chelometric titration. X-ray diffraction analysis confirmed that the test material was cubic, face-centered crystals of cadmium oxide. Scanning electron microscopy indicated that approximately 99% of the particles were less than or equal to 1 micron in size. The results of elemental analyses for cadmium (inductively coupled plasma atomic emission spectroscopy) agreed with theoretical values for cadmium oxide. Weight loss on drying indicated less than 0.2% water. Only low concentrations of impurities were found by spark source mass spectroscopy. The impurity present at the highest concentration (400 ppm) was chlorine, while all other impurities detected totaled less than 263 ppm. Chelometric titration with 0.02 M EDTA indicated a purity of 99.4% ± 0.6%.

Because literature references indicate that cadmium oxide is stable at normal storage temperatures when kept dry and not exposed to acids or ammonium salts (IARC, 1976; Sax, 1984), no accelerated stability studies were performed on the bulk chemical. At the study laboratory, the bulk chemical was stored in sealed containers protected from light at approximately 25 ° C.

On receipt at the study laboratory, the identity of the bulk chemical was confirmed by elemental analyses (Galbraith Laboratories, Incorporated; Knoxville, TN). For both the 2-week and 13-week studies, the bulk chemical purity was evaluated with chelometric titration before the start of the studies and again after the studies ended; no significant change in purity was observed.

Aerosol Generation System

Animals were exposed and maintained in whole-body inhalation chambers (Hazleton 2000) developed at Battelle Pacific Northwest Laboratories (Richland, WA) and produced by Harford System Division of Lab Products, Incorporated (Aberdeen, MD). The total volume of the chamber was 2.3 m^3 with an active mixing volume of 1.7 m^3, the remainder being the inlet and exhaust volumes where animals were not placed. Chemical concentration, airflow, vacuum, temperature, and relative humidity were controlled and monitored using an automated system. The overall design of the exposure system is shown in Figure 1.

The cadmium oxide aerosol generation and delivery system (Figure 2) was composed of three basic components: a Model 2 Wright Dust Feed Mechanism, an aerosol charge neutralizer, and an aerosol distribution system. The Wright Dust Feed Mechanism (BGI Incorporated, Watham, MA) provides relatively high concentrations of dry dust. In a ventilated hood containing a high efficiency particulate (HEPA) filter, bulk cadmium oxide under constant pressure (2,500 psi) was packed into a reservoir cup using a dedicated press. During aerosol generation, the cup rotated about a fixed scraper blade, dispersing the packed cadmium oxide into fine particles. Filtered, dry, compressed air passed through a groove at the outer edge of the scraper and radially along the blade, entraining the dust. The dust and air then exited the dust feed mechanism through an axial hole.

The aerosol then flowed through a piece of plastic duct with two 10 mCi [^{63}Ni]-plated foils suspended in the center. The diameter of the duct and the activity of the foils were matched to provide sufficient time for the aerosol to reach Boltzmann equilibrium at the system flow rate. The aerosol was passed through this charge neutralizer system to remove excess static charges acquired by the particles due to the scraping action of the dust feed mechanism; the excess static charge was removed to prevent reduction of aerosol delivery efficiency or alteration of deposition patterns in the respiratory tract of exposed animals.

A primary distribution line conveyed the aerosol to the two chambers with the highest cadmium oxide concentrations. An Air-Vac® pump (Air-Vac Engineering, Inc., Milford, CT) siphoned aerosol into a secondary distribution line, where the aerosol was diluted with HEPA- and charcoal-filtered air and delivered to the chambers with lower cadmium oxide concentrations. Excess cadmium oxide was distributed in each line to allow for independent adjustment of the concentration in each exposure chamber. At each chamber location, an Air-Vac® pump siphoned aerosol from the distribution line into the chamber inlet. Unused aerosol was removed from the distribution lines by an HEPA filter.

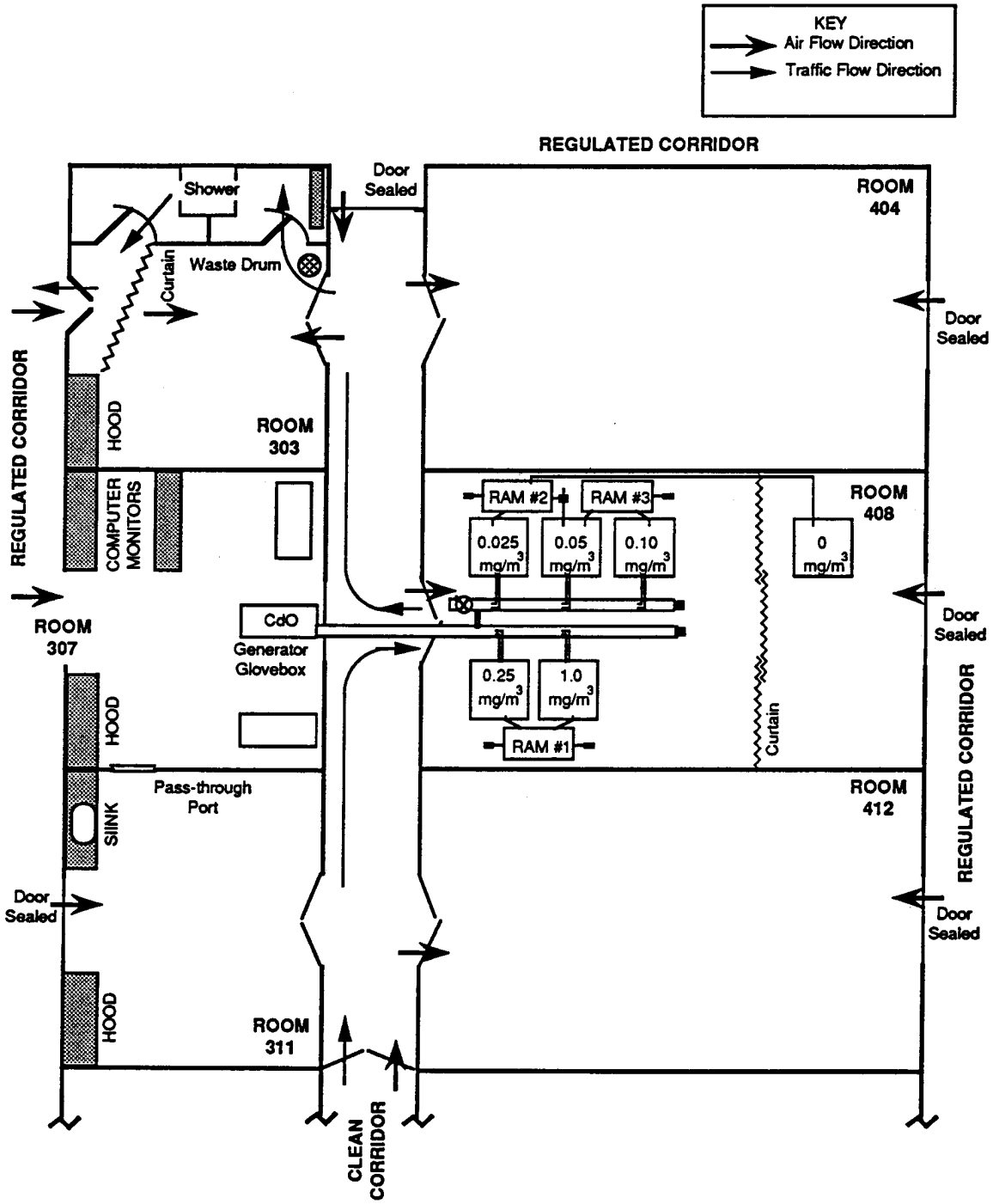


FIGURE 1 Overall Design of the Exposure System in the 13-Week Inhalation Studies of Cadmium Oxide

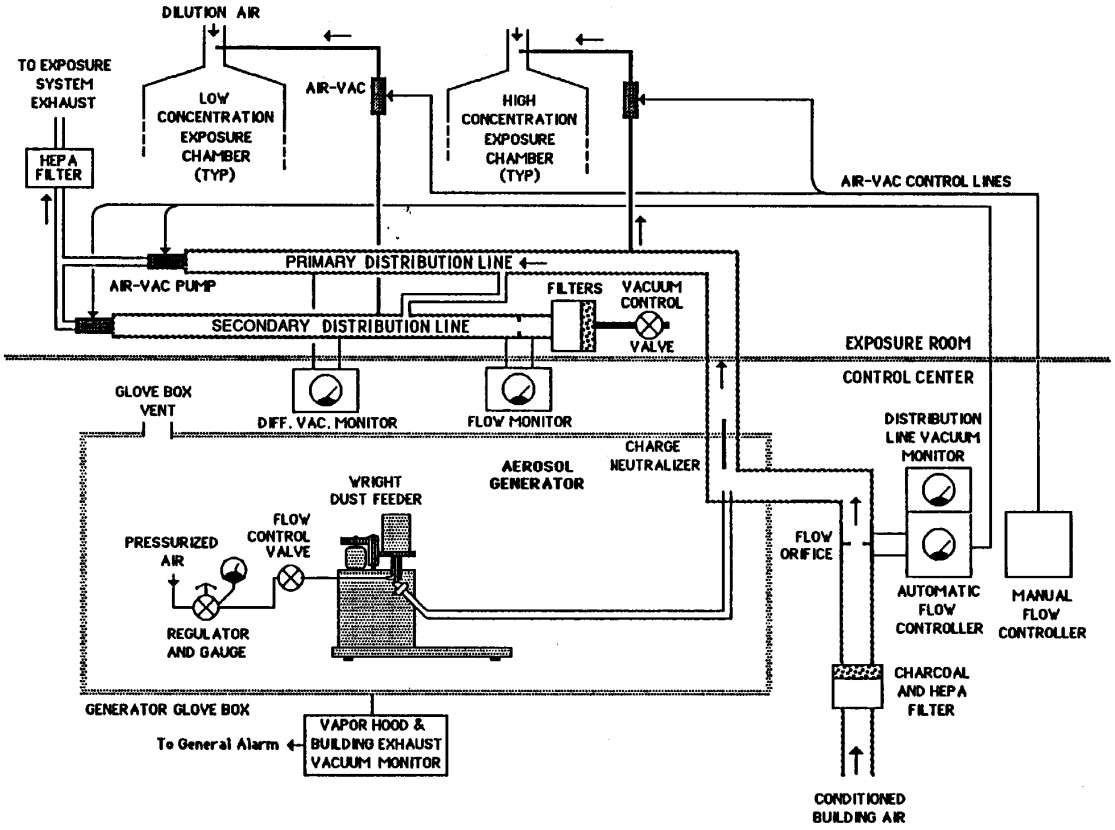
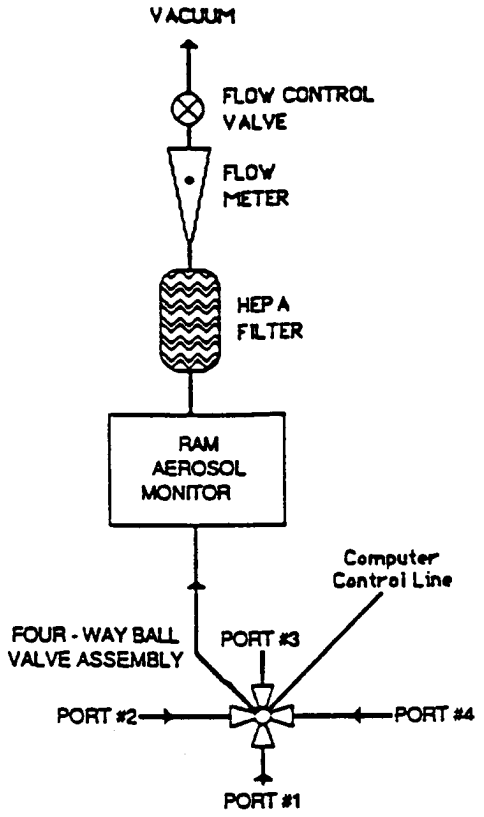


FIGURE 2 Aerosol Generation and Delivery System in the 13-Week Inhalation Studies of Cadmium Oxide

Concentration Monitoring

Cadmium oxide aerosol concentrations were monitored with three on-line real-time aerosol monitors (RAM-1; MIE, Inc., Bedford, MA) which consist of a diode emitting pulsed light and a silicon detector. The RAM-1 detects particles from 0.1 to 20 µm in diameter. The sampling system consisted of a valve which multiplexed each RAM-1 to two exposure chambers and either the control chamber, the room, or a HEPA filter (Figure 3). Aerosol flowed through sample lines designed to reduce aerosol particle losses due to settling or impaction and then passed into the RAM-1. Results were automatically recorded by an automated data acquisition and control system. An HP-85B computer controlled the selection of the correct sample stream and the acquisition of data from each RAM-1. Monitoring of each chamber was performed at least every 30 minutes. Calibration of each RAM-1 was performed approximately twice weekly by correlating the voltages measured by the RAM-1 with cadmium oxide concentrations determined by off-line analysis of exposure chamber filter samples using flame atomic absorption spectroscopy; the atomic absorption spectrophotometer was calibrated with serially diluted solutions of a cadmium oxide standard. Accuracy of RAM-1 calibration was assessed by examining the ratio of the chamber concentration measured by the RAM-1 to that determined by the analysis of filter samples. Averaged over all chambers during the 13-week study, 97% of the values for the ratio of RAM-1:filter were between 0.8 and 1.2.



RAM \ Port	1	2	3	4
1	1.0 mg/m³	0.25 mg/m³	Not Used (HEPA)	Not Used (HEPA)
2	0.025 mg/m³	Room / *0.05 mg/m³	0.0 mg/m³	Not Used (HEPA)
3	0.10 mg/m³	0.05 mg/m³	Not Used (HEPA)	Not Used (HEPA)

* 0.05 mg chamber monitored at this RAM/PORT during calibration procedures only.

FIGURE 3 Schematic Diagram of the Exposure Chamber On-Line Monitoring System in the 13-Week Inhalation Studies of Cadmium Oxide

Mean exposure unit concentrations of cadmium oxide during the 2-week and 13-week studies were calculated from daily monitoring data (Table 3). For the 2-week studies, the mean concentrations in all exposure chambers were between 93% and 98% of target concentrations, with relative standard deviations ranging from 10% to 23%. For the 13-week studies, the mean concentrations in all exposure chambers except the 0.05 mg/m^3 chamber were between 96% and 104% of the target concentrations, with relative standard deviations ranging from 7% to 14%. In the 0.05 mg/m^3 chamber, the relative standard deviation of 32% was outside the acceptable range of 20%, primarily due to a single concentration excursion of 0.44 mg/m^3. At least 85% of all individual concentration measurements for the 2-week studies and 84% of all individual concentration measurements for the 13-week studies were within 20% of the target concentrations.

In the 2-week studies, several excursions in exposure concentrations occurred in the 0.1, 1, and 3 mg/m^3 chambers due to failures of the Wright Dust Feed Mechanism. The exposure periods were extended on the days of feed mechanism failure to compensate for time required for repairs. No more than 15% of the concentration measurements in a single chamber were out of range during the 2-week studies. The feed mechanism was replaced before the start of the 13-week studies and no subsequent failures occurred. Brief (5 to 7 minute) increases in exposure concentrations occasionally occurred in the 13-week studies, possibly due to sudden releases of accumulated cadmium oxide in the distribution lines and Air-Vac® pumps. While the concentration spikes were of short duration, the magnitude (worst case 0.44 mg/m^3 in the 0.05 mg/m^3 chamber) was sufficient to have a considerable effect on the relative standard deviation.

TABLE 3 Mean Chamber Concentrations of Cadmium Oxide in the 2-Week and 13-Week Inhalation Studies in F344/N Rats and B6C3F$_1$ Mice

Target Concentration (mg/m^3)	Mean ± SD	Target ± RSD[1]	Maximum	Minimum	Samples within Range[2] (%)
2-Week Studies[3]					
0	<LOD[4]	)	0.029[5]	<LOD	)
0.1	0.093 ± 0.021	93 ± 23	0.189	0.003	85
0.3	0.285 ± 0.055	95 ± 19	0.396	0.014	89
1	0.964 ± 0.219	96 ± 23	1.930	0.014	89
3	2.84 ± 0.588	95 ± 21	3.650	0.010	91
10	9.82 ± 0.938	98 ± 10	11.7	4.91	98
13-Week Studies[6]					
RATS					
0	<LOD	)	<LOQ[7]	<LOD	)
0.025	0.024 ± 0.003	96 ± 14	0.059	0.009	88
0.05	0.052 ± 0.016	103 ± 32	0.438	0.035	84
0.10	0.101 ± 0.008	101 ± 8	0.159	0.067	88
0.25	0.242 ± 0.027	97 ± 11	0.478	0.133	89
1.0	1.03 ± 0.075	104 ± 7	1.28	0.750	91
MICE					
0	<LOD	)	<LOQ	<LOD	)
0.025	0.024 ± 0.003	96 ± 14	0.059	0.009	88
0.05	0.052 ± 0.016	103 ± 32	0.438	0.035	86
0.10	0.101 ± 0.008	101 ± 8	0.159	0.067	89
0.25	0.241 ± 0.027	96 ± 11	0.478	0.133	90
1.0	1.04 ± 0.075	104 ± 7	1.28	0.750	92

[1] Mean concentration ± relative standard deviation as a percent of target concentration.
[2] Samples within 20% of the target concentration were considered to be in range.
[3] For the 2-week studies, rats and mice were exposed in the same chambers; the date of first exposure for rats was 29 November 1988 and for mice was 30 November 1988.
[4] LOD = limit of detection. For the 2-week studies, LOD = 0.003 mg/m^3; for the 13-week studies, LOD = 0.002 mg/m^3.
[5] Limit of quantitation (LOQ) not established for 2-week studies.
[6] For the 13-week studies, rats and mice were exposed in the same chambers. Differences in mean chamber concentrations of cadmium oxide and percent of samples within range are due to differences in starting exposure dates for rats and mice. For rats, the dates of first exposure were 14 August 1989 (males) and 15 August 1989 (females). For mice, the dates of first exposure were 16 August 1989 (males) and 17 August 1989 (females).
[7] For the 0 and 0.025 mg/m^3 chambers, LOQ = 0.005 mg/m^3; for the 0.05 and 0.10 mg/m^3 chambers, LOQ = 0.01 mg/m^3; for the 0.25 and 1.0 mg/m^3 chambers, LOQ = 0.05 mg/m^3.

Chamber Characterization

PARTICLE SIZE DISTRIBUTION

The mass median aerodynamic diameters (MMADs) of the aerosol particles in each exposure chamber were measured before the studies began and then once during the 2-week studies and monthly during the 13-week studies. Cascade impactor samples (Mercer-style seven-stage impactor; In-Tox Products, Albuquerque, NM) were taken from each exposure chamber, and the impactor stages were analyzed for cadmium content with inductively coupled plasma-mass spectroscopy

(ICP-MS). The relative mass collected on each stage was analyzed by NEWCAS probit analysis (Hill *et al.*, 1977). For the 2-week studies, the mean MMAD was 1.5 µm, with a geometric standard deviation of 1.6 to 1.8. For the 13-week studies, the mean MMADs ranged from 1.1 to 1.6 µm, with geometric standard deviations of 1.7 to 1.8. The MMADs in all studies were within the acceptable range of 1 to 3 µm for particle size and less than 2 µm for geometric standard deviation (Table 4).

TABLE 4 Particle Size Distribution of Cadmium Oxide in the 13-Week Inhalation Studies in F344/N Rats and B6C3F$_1$ Mice

	Chamber Concentration									
	0.025 mg/m^3		0.05 mg/m^3		0.10 mg/m^3		0.25 mg/mg^3		1.0 mg/m^3	
Date	MMAD[1]	GSD[2]	MMAD	GSD	MMAD	GSD	MMAD	GSD	MMAD	GSD
Prestart	1.1	1.9	1.3	1.9	1.2	2.0	1.4	1.9	1.6	1.8
August 1989	1.1	2.0	1.0	1.8	1.1	1.8	1.3	1.7	1.6	1.6
September 1989	1.1	1.7	1.2	1.6	1.2	1.7	1.5	1.7	1.6	1.6
October 1989	1.2	1.6	1.2	1.7	1.1	1.7	1.4	1.7	1.5	1.7
Mean	1.1	1.8	1.2	1.8	1.2	1.8	1.4	1.8	1.6	1.7
Standard Deviation	0.1		0.1		0.1		0.1		0.0	

[1] MMAD=mass median aerodynamic diameter, given in µm.
[2] GSD=geometric standard deviation, given in µm.

CONCENTRATION UNIFORMITY

For the 2-week and 13-week studies, the uniformity of aerosol concentration throughout each exposure chamber was measured before the studies began and once during the studies. Samples were taken at each RAM-1 and at chamber ports in the front and back of each animal chamber. An extension tube was fitted to the sample lines of each RAM-1 to allow sampling from all chamber ports. The variation in overall uniformity of concentration was within the specified limits for all studies of less than 5% relative standard deviation.

CONCENTRATION BUILDUP AND DECAY

During the 2-week and 13-week studies, the time following the start of exposure for the cadmium oxide concentration to reach 90% of the final stable concentration in the chamber (T_{90}) and the time following the termination of generation for the aerosol concentration to decay to 10% of the stable concentration (T_{10}) were determined.

For the 2-week studies with animals present in the chambers, T_{90} ranged from 11 to 17 minutes, while T_{10} ranged from 9 to 13 minutes. A T_{90} of 12 minutes was chosen for the 2-week studies. For the 13-week studies with animals present in the chambers, T_{90} ranged from 10 to 14 minutes, while T_{10} ranged from 6 to 9 minutes. A somewhat longer T_{90} of 20 minutes was chosen for the 13-week studies to ensure that the animals were exposed to the target concentrations for a full 6-hour period.

STABILITY STUDIES

The stability of cadmium oxide in the exposure chambers, aerosol distribution line, and generator reservoir was confirmed by X-ray diffraction analysis and ICP-MS in the 2-week studies and by X-ray diffraction analysis, X-ray fluorescence spectroscopy, and proton-induced X-ray emission spectroscopy in the 13-week studies. Samples were collected from the 0.1 and 10 mg/m^3 chambers in the 2-week studies and from the 0.025 and 1 mg/m^3 chambers in the 13-week studies. Small amounts of cadmium hydroxide and cadmium carbonate were identified in all samples in the 13-week studies except those from the 0.025 mg/m^3 chambers; cadmium carbonate was also detected in the bulk cadmium oxide samples. The presence of these impurities was attributed to the exposure of bulk cadmium oxide to atmospheric water and carbon dioxide during storage. Small amounts of elemental impurities detected in the cadmium oxide samples were attributed to contact of the compound with the materials used in construction of the exposure generation system. Overall, no significant degradation of cadmium oxide was detected during the 2-week or 13-week studies.

Toxicity Study Designs

BASE STUDIES

Male and female F344/N rats and $B6C3F_1$ mice used for the 2-week and 13-week studies were obtained from Simonsen Laboratories (Gilroy, CA). Rats and mice were approximately 4 weeks old at receipt, were quarantined for 12 to 15 days, and were approximately 6 weeks old when the studies began. Blood samples were collected from sentinel rats and mice 3 weeks after receipt for the 2-week and 13-week studies and from sentinel rats and control mice at the end of the 13-week studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b); all results were negative. Additional details concerning the study design are provided in Table 5.

During the 2-week studies, groups of five rats and five mice of each sex were exposed to 0, 0.1, 0.3, 1, 3, or 10 mg/m^3 cadmium oxide aerosol through whole-body exposure for 6 hours plus T_{90} per day, 5 days per week, except weekends and holidays, for 12 exposure days, with at least two consecutive exposure days before sacrifice. In the 13-week base studies, groups of 10 rats and 10 mice of each

sex were exposed to 0, 0.025, 0.05, 0.1, 0.25, or 1 mg/m^3 cadmium oxide aerosol through whole-body exposure for 6 hours plus T_{90} per day, 5 days per week, except weekends and holidays, for 13 weeks, with at least two consecutive exposure days before sacrifice.

For all studies, rats and mice were housed in individual cages within the exposure chambers. City water (Richland, WA) was available *ad libitum* and NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form was available *ad libitum* except during the daily exposure periods. Animal rooms were maintained with 12 hours of fluorescent light per day.

Complete necropsies were performed on all animals in the 2-week and 13-week base studies. The heart, right kidney, liver, lungs, spleen, right testis, and thymus of each animal were weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathologic examination of gross lesions and selected tissues was performed on all animals in the 2-week studies; complete histopathologic examinations were performed on all animals in the control and 1 mg/m^3 in the 13-week base studies. Gross lesions and selected organs of rats and mice in lower exposure groups in the 13-week studies were examined. Tissues examined microscopically are listed in Table 5.

Upon completion of the laboratory pathologist's histologic evaluation, 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. Results were reviewed and evaluated by the NTP Pathology Working Group (PWG); 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).

SUPPLEMENTAL EVALUATIONS

Supplemental evaluations included clinical pathology, blood pressure measurements, cadmium tissue distribution, sperm motility and vaginal cytology, and developmental toxicity. A summary of the developmental toxicity studies is given in Appendix C.

Clinical Pathology

Blood for hematology and clinical chemistry evaluations was taken from supplemental rats on Days 4 and 24 and from base-study rats at the end of the study. Additionally, bone marrow samples were collected from base-study rats at the end of the 13-week study for hematologic evaluations. Urine samples were collected from base-study rats during Week 12 for urinalysis; additional urine samples were collected 2 days after the first samples were collected for evaluation of the urine-concentrating ability of the rats.

For the hematology and clinical chemistry evaluations, rats were anesthetized with a CO_2:room air gas mixture (70:30) and blood samples were drawn from the retroorbital plexus. Blood for hematology was placed in tubes containing potassium EDTA. Blood for clinical chemistry evaluations was placed in tubes with separator gel but no anticoagulant and was allowed to clot at room temperature; the samples were then centrifuged and the serum was removed. All hematologic and clinical chemistry analyses were performed on the day of sample collection.

Hematology determinations were performed with an Ortho ELT-8/ds hematology analyzer (Ortho Instruments, Westwood, MA). The parameters that were evaluated are listed in Table 5. Hematocrit was measured manually with a Damon/IEC MB microcentrifuge and Damon/IEC capillary reader (International Equipment Company, Needham Heights, MA). Differential leukocyte counts were determined by light microscopy from blood smears stained with Wright-Giemsa. Smears made from blood samples stained with new methylene blue were examined microscopically with a Miller disc for the quantitative determination of reticulocytes.

Bone marrow was collected from the right femur of five male and five female base-study rats from the 0, 0.1, 0.25, and 1 mg/m^3 groups at the end of the 13-week studies. Marrow cells were flushed from the femur with Hank's balanced salt solution containing EDTA and 5% bovine serum albumin but no magnesium or calcium. After lysis of the red cells, the nucleated cell concentration of the bone marrow was determined with a Coulter Counter Model Z_H (Coulter Electronics, Inc., Hialeah, FL). Cellularity, cell distribution characteristics, megakaryocyte concentrations, and cytology of

marrow cells were determined microscopically from marrow smears stained with Wright-Giemsa. In addition, marrow samples were stained with Prussian blue, counterstained with safranin, and then examined microscopically to detect iron.

Clinical chemistry variables were measured with an Abbott VP (Abbott Laboratories, Abbott Park, IL) or a Roche Cobas Fara chemistry analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ). The parameters that were evaluated are listed in Table 5. Reagents for assay of sorbitol dehydrogenase activity and bile acid concentration were obtained from Sigma Chemical Company (St. Louis, MO); reagents for the other endpoints were obtained from the equipment manufacturer.

Base-study rats from all exposure groups were placed in metabolism cages for a 14-hour, overnight urine collection immediately after exposure during Week 12. Urine was collected in sample tubes immersed in ice. During the collection period, rats had access to water but not feed. After urine volume and appearance were recorded, the urine samples were centrifuged and the sediment was stained with Sedi-Stain (Clay Adams, Parsippany, NJ) and examined microscopically. The specific gravity was determined for all exposure groups with an American Optical refractometer (American Optical, Buffalo, NY) calibrated against double-distilled water. The following urinalysis variables were measured for the 0, 0.1, 0.25, and 1 mg/m^3 groups with a Roche Cobas Fara chemistry analyzer: urine creatinine, glucose, and protein concentrations and alkaline phosphatase, aspartate aminotransferase, and *N*-acetyl-β-D-glucosaminidase activities. Two days after the first urinalysis, rats in the 0, 0.1, 0.25, and 1 mg/m^3 groups were fasted for 14 hours overnight after exposure to cadmium oxide. On the following morning, the bladders of the rats were manually expressed and the rats were then placed in metabolism cages for a 4-hour period of urine collection. The ability of the rats to concentrate urine was assessed by quantifying the volume and the specific gravity of the urine samples.

Blood Pressure Measurements

In the 13-week base study, the indirect systolic blood pressure of six male and six female base-study rats from each of the 0, 0.1, 0.25, and 1 mg/m^3 groups was measured before the study began, during Weeks 5 and 9, and at the end of the study. The same rats were used for blood pressure evaluations throughout the studies and were selected before the start of the studies based on their ability to acclimate to the restraining system. Blood pressure measurements during Weeks 5 and 9 and at the end of the study were taken immediately following exposure, after a minimum of two consecutive exposure days. The rats were restrained in tubes in a heated (approximately 30° C) chamber, and the

blood pressures were measured with indirect tail-cuff devices (IITC, Inc., Woodland Hills, CA and Buxco Electronics, Sharon, CT).

Cadmium Tissue Distribution Study

A cadmium tissue distribution study was performed on supplemental male rats exposed to 0, 0.1, 0.25, or 1 mg/m^3 cadmium oxide for up to 13 weeks. Blood, lung, and kidney samples were collected from five male rats per exposure group at Days 3, 9, and 30 and at the end of the study.

Rats were anesthetized with a CO_2:room air gas (70:30) mixture. Blood was collected by cardiac puncture, placed in vacuum tubes containing EDTA, and stored in sealed vials at −20° C until analysis. Lungs (without attached trachea and mainstem bronchi) and kidneys (without adrenal glands) were frozen in liquid nitrogen and stored at −20° C until analysis. Samples of blood and tissue, each weighing 0.5 to 2.0 g, were placed in Teflon®-lined Parr Bomb Model 4749 acid digestion vessels and 2 to 3 mL of nitric acid were added. After cooling, the digested samples were analyzed for cadmium with a Model 5100 Graphite Furnace Atomic Absorption Spectrophotometer with Zeeman effect background correction (Perkin-Elmer, Norwalk, CT). Cadmium concentrations were determined by comparing the instrument response to the digested tissues to the instrument response to spiked tissue standards (Dill *et al.*, 1994). The limit of quantitation (LOQ) for cadmium oxide in lung was 0.12 µg/g; the LOQ for cadmium in whole blood was 0.0025 µg/g and in kidney was 0.012 µg/g.

Sperm Motility and Vaginal Cytology

At the end of the 13-week base studies, vaginal cytology and sperm motility evaluations were performed on 10 rats and 10 mice per sex from the 0, 0.025, 0.1, and 1 mg/m^3 groups. Methods were those outlined in the National Toxicology Program's Sperm Motility Vaginal Cytology Evaluation protocol (NTP, 1987). Briefly, for the 12 days before sacrifice, the vaginal vaults of 10 females of each species per exposure group were lavaged, and the aspirated lavage fluid and cells were stained with toluidine blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (*i.e.*, diestrus, proestrus, estrus, and metestrus).

Sperm motility was evaluated at necropsy in the following manner. The left testis and epididymis were weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body and weighed. Test yolk (rats) or Tyrode's buffer (mice) was applied to slides, and a small

incision was made in the cauda. Then sperm efluxing from the incision were dispersed in the buffer on the slides and the numbers of motile and nonmotile spermatozoa were counted for five microscopic fields per slide by two observers.

Following completion of sperm motility estimates, each left cauda was placed in phosphate-buffered saline solution. Caudae were finely minced and the tissue was incubated and then heat fixed. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

TABLE 5 Experimental Design and Materials and Methods in the 2-Week and 13-Week Inhalation Studies of Cadmium Oxide

2-Week Studies	13-Week Studies
EXPERIMENTAL DESIGN	
Study Laboratory Battelle Pacific Northwest Laboratories (Richland, WA)	Same as 2-week studies
Strain and Species F344/N rats B6C3F$_1$ mice	Same as 2-week studies
Animal Source Simonsen Laboratories (Gilroy, CA)	Same as 2-week studies
Size of Study Groups Five male and five female animals per exposure group	Base Studies: 10 male and 10 female animals per exposure group Clinical Pathology Study: 10 male and 10 female rats per exposure group Cadmium Tissue Distribution Study: 20 male rats per exposure group
Route of Administration Whole-body inhalation	Same as 2-week studies
Exposure Concentrations/Duration 0, 0.1, 0.3, 1, 3, or 10 mg/m^3 daily, 6 hours plus 12 minutes per day, 5 days per week, for 2 weeks (12 exposure days)	0, 0.025, 0.05, 0.1, 0.25, or 1 mg/m^3 daily, 6 hours plus 20 minutes per day, 5 days per week, for 13 weeks
Date of First Exposure Rats: 29 November 1988 Mice: 30 November 1988	Rats: 14 August 1989 (males), 15 August 1989 (females) Mice: 16 August 1989 (males), 17 August 1989 (females)
Date of Last Exposure Rats: 14 December 1988 Mice: 15 December 1988	Rats: 13 November 1989 (males), 14 November 1989 (females) Mice: 15 November 1989 (males), 16 November 1989 (females)
Date of Necropsy Rats: 15 December 1988 Mice: 16 December 1988	Rats: 14 November 1989 (males), 15 November 1989 (females) Mice: 16 November 1989 (males), 17 November 1989 (females)
Type and Frequency of Observation Animals were observed twice daily and were weighed on Days 1 and 8 and at necropsy. Clinical observations were recorded daily.	Animals were observed twice daily for mortality and signs of toxicity and were weighed on Day 1, weekly thereafter, and on the day of necropsy. Clinical observations were recorded weekly.
Necropsy Complete necropsies were performed on all animals. The following organs were weighed: heart, right kidney, liver, lungs, spleen, right testis, and thymus.	Complete necropsies were performed on all base-study animals. The following organs were weighed: heart, right kidney, liver, lungs, spleen, right testis, and thymus.

TABLE 5 Experimental Design and Materials and Methods in the 2-Week and 13-Week Inhalation Studies of Cadmium Oxide (continued)

2-Week Studies	13-Week Studies
EXPERIMENTAL DESIGN (continued)	
Histologic Examination	
The following tissues were histopathologically examined in all animals: gallbladder (mice only), gross lesions, heart, kidneys, liver, lungs, tracheobronchial lymph nodes, and nasal cavity and turbinates (three sections).	Histopathologic evaluations were performed on all animals in the 0 and 1 mg/m^3 groups. The following tissues were examined: adrenal glands, brain (3 sections), clitoral glands, esophagus, eyes (if grossly abnormal), femur and marrow, gallbladder (mice only), gross lesions and tissue masses, heart, kidneys, large intestine (cecum, colon, rectum), larynx, liver, lungs, lymph nodes (bronchial, mandibular, mediastinal, mesenteric, and tracheobronchial), mammary gland, nasal cavity and turbinates (3 sections), ovaries, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial glands, prostate gland, salivary glands, seminal vesicle, small intestine (duodenum, jejunum, ileum), spinal cord/sciatic nerve (if neurologic signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thigh muscle, thymus, thyroid gland, trachea, urinary bladder, and uterus. The following organs were examined in the lower exposure groups: larynx, lungs, lymph nodes (mediastinal, mesenteric, and tracheobronchial for rats and tracheobronchial for mice), and nasal cavity.
Supplemental Evaluations	
Clinical Pathology Study	Blood for hematology and clinical chemistry evaluations was collected from supplemental rats on Days 4 and 24; blood and bone marrow samples were collected from base-study rats at the end of the study. Urine samples were collected from base-study rats during Week 12. Hematology parameters included hematocrit (Hct), hemoglobin (Hgb) concentration, erythrocyte (RBC) count, reticulocyte count, nucleated erythrocyte count, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count, leukocyte (WBC) count and differential, and total cellularity. Clinical chemistry parameters included urea nitrogen (UN), creatinine, unbound iron-binding capacity, iron, total iron-binding capacity, total protein, albumin, globulin, albumin/globulin ratio, alanine aminotransferase, alkaline phosphatase, creatine kinase (CK), sorbitol dehydrogenase (SDH), and bile acids. Urinalysis parameters included creatinine, glucose, glucose/creatinine ratio, protein, protein/creatinine ratio, alkaline phosphatase, aspartate aminotransferase, *N*-acetyl-β-D-glucosaminidase, volume, and specific gravity.
Cadmium Tissue Distribution Study	Blood, lung, and kidney samples were collected on Days 3, 9, 30, and at the end of the study from supplemental male rats administered 0, 0.1, 0.25, or 1 mg/m^3 cadmium oxide. All samples were analyzed for cadmium.

TABLE 5 Experimental Design and Materials and Methods in the 2-Week and 13-Week Inhalation Studies of Cadmium Oxide (continued)

2-Week Studies	13-Week Studies
EXPERIMENTAL DESIGN (continued)	
Blood Pressure Measurements	The indirect systolic blood pressure of six male and six female base-study rats from each of the 0.1, 0.25, and 1 mg/m^3 groups was measured before the start of the study, during Weeks 5 and 9, and at the end of the study.
Sperm Motility and Vaginal Cytology Evaluations	Sperm motility and vaginal cytology evaluations were performed on base-study animals in the 0, 0.025, 0.1, and 1 mg/m^3 groups at the end of the 13-week studies. Male rats and mice were evaluated for necropsy body and reproductive tissue weights, spermatozoal data, and spermatogenesis. Females were evaluated for necropsy body weight, estrous cycle length, and the percent of cycle spent in the various stages.
ANIMAL MAINTENANCE	
Time Held Before Study Rats: 13 days Mice: 14 days	Rats: 12 days (males), 13 days (females) Mice: 14 days (males), 15 days (females)
Age When Study Began 6 weeks	Same as 2-week studies
Age When Killed 8 weeks	19 weeks
Method of Animal Distribution Animals were weighed and were randomized with a computer program.	Same as 2-week studies
Diet NIH-07 Open Formula Diet (Zeigler Bros., Inc., Gardners, PA) in pellet form, available *ad libitum* except during exposure periods, and softened water (City of Richland), available *ad libitum*.	Same as 2-week studies
Animal Room Environment Rats and mice were housed in individual cages in the exposure chambers. The temperature was maintained at 72° to 78° F with 40% to 70% relative humidity and 12 to 18 air changes per hour. Fluorescent light was provided for 12 hours per day.	Same as 2-week studies

Genetic Toxicity Studies

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Mortelmans *et al*. (1986). Cadmium oxide was sent to the laboratory as a coded aliquot and was incubated with the *S. typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37 ° C.

Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37 ° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of cadmium oxide; the high dose was limited by toxicity.

PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented in MacGregor *et al*. (1990). Blood samples were obtained from B6C3F$_1$ mice at the end of the 13-week study. Smears were immediately prepared, fixed in absolute methanol, stained with acridine orange (a chromatin-specific fluorescent dye), and coded. The slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of five male and five female mice per exposure concentration. The criteria of Schmid (1976) were used to define micronuclei.

Statistical Methods

ANALYSIS OF CONTINUOUS VARIABLES

Two approaches were employed during the 13-week studies to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed with the parametric multiple comparisons procedures of Williams (1971, 1972) and Dunnett (1955). Clinical pathology, spermatid, epididymal spermatozoal data, and cadmium tissue concentrations were analyzed with the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose response (Dunnett's or Dunn's test). Trend-sensitive tests were used when Jonckheere's test was significant at a P-value less than 0.1. For indirect systolic blood pressure measurements, a one-way analysis of variance test (Weter *et al.*, 1985) was used to assess dose-response and time-response trends.

Before analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel. Implausible values, extreme values from animals that were suspected of being sick due to causes other than treatment, and values that the study laboratory indicated as being inadequate due to technical problems were eliminated from the analysis.

ANALYSIS OF VAGINAL CYTOLOGY DATA

Because the data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across dose levels.

ANALYSIS OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

A positive response in the *S. typhimurium* assay is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that was not dose-related, not reproducible, or not of sufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

ANALYSIS OF PERIPHERAL BLOOD MICRONUCLEUS DATA

The results were tabulated as the mean of the pooled results from all animals within a treatment group, plus or minus the standard error of the mean. The frequency of micronucleated cells among normochromatic erythrocytes was analyzed by a statistical software package that tested for increasing trend over exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposure group and the control group (Margolin *et al.*, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial was considered positive if the trend test P-value was less than or equal to 0.025 or the P-value for any single exposure group was less than or equal to 0.025 divided by the number of exposure groups. A final call of positive for micronucleus induction is preferably based on reproducible positive trials (as noted above). Ultimately, the final call was determined by the scientific staff after considering the results of statistical analyses, reproducibility of any effects observed, and the magnitudes of those effects.

Quality Assurance

The animal studies of cadmium oxide were performed in compliance with United States Food and Drug Administration Good Laboratory Practices regulations (21 CFR, Part 58). The Quality Assurance Unit of Battelle Pacific Northwest Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

RESULTS

2-Week Inhalation Study in F344/N Rats

All rats in the highest exposure group (10 mg/m^3) died by Day 6 of the study; no other deaths occurred (Table 6). The final mean body weights and mean body weight gains of male and female rats in the 3 mg/m^3 groups were lower than those of the control groups. The mean body weight gain of males in the 1 mg/m^3 group was also lower than that of the control group. Clinical signs of toxicity were noted in males and females in the three highest exposure groups (1, 3, and 10 mg/m^3) by Day 5 of the study and included hypoactivity, dehydration, ruffled fur, dyspnea, abnormal posture, and nasal discharge. By the second week of the study, rats in the two lowest exposure groups (0.1 and 0.3 mg/m^3) also displayed ruffled fur.

TABLE 6 Survival and Body Weights of F344/N Rats in the 2-Week Inhalation Study of Cadmium Oxide

Concentration (mg/m^3)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]
		Initial	Final	Change[2]	
MALE					
0	5/5	129	195	66	
0.1	5/5	126	194	68	100
0.3	5/5	128	198	70	101
1	5/5	130	189	59	97
3	5/5	129	181	52	93
10	0/5[4]	129	)	)	)
FEMALE					
0	5/5	98	136	38	
0.1	5/5	98	132	34	97
0.3	5/5	97	136	39	100
1	5/5	97	137	40	101
3	5/5	95	124	29	91
10	0/5[5]	98	)	)	)

[1] Number surviving at 2 weeks/number of animals per exposure group.
[2] Mean weight change.
[3] (Exposure group mean/control group mean) x 100.
[4] Day of death: 3, 4, 4, 4, 6.
[5] Day of death: 4, 4, 4, 4, 5.

Male and female rats exposed to cadmium oxide for 2 weeks had exposure-related increases in absolute and relative lung weights, and the absolute and relative lung weights were significantly greater than the controls at the 1 and 3 mg/m^3 exposure concentrations for males and females and at the 0.3 mg/m^3 exposure concentration for males (Table A1). Relative liver weights of males in all exposed groups and females in the 1 and 3 mg/m^3 groups, relative right kidney weights of females in all exposed groups, and relative heart weights of males and females in the 0.3 mg/m^3 and higher groups were greater than those of the controls (Table A1).

There were no gross lesions attributed to the 2-week cadmium oxide exposures. Treatment-related histopathologic lesions occurred in the lungs, tracheobronchial lymph nodes, and nasal passages of male and female rats administered 0.1 to 10 mg/m^3 cadmium oxide aerosol (Table 7).

At all exposure concentrations, treatment-related lesions were present in the lungs. These lesions, in the 0.1 and 0.3 mg/m^3 exposure groups, were limited to an increase in the number of inflammatory cells (primarily macrophages) within alveolar lumen throughout the lungs. Surrounding many alveolar ducts and extending into adjacent alveolar septa were focal areas of inflammation. Associated with the focal inflammation, there was minimal fibrosis in alveolar septa in the 0.3, 1, and 3 mg/m^3 groups. At exposure concentrations of 0.3 mg/m^3 and higher, the histiocytic infiltrates and focal inflammation increased in severity. Necrosis of the epithelium lining alveolar ducts was present at exposure concentrations of 0.3 mg/m^3 and higher. In the 10 mg/m^3 groups, all rats died by Day 6 and had marked necrosis of alveolar duct epithelium; a fibrin exudate was in the alveolar spaces and sometimes adhered to the surface of the alveolar ducts.

Inflammation in the tracheobronchial lymph nodes was present in males in the 1 mg/m^3 and higher groups and in females in all exposure groups. Inflammation was characterized by an accumulation of macrophages (histiocytosis) within the sinusoids. Frequently the cytoplasm of these macrophages contained basophilic-staining cellular debris.

Treatment-related lesions in the nasal passages were present at exposure concentrations of 1 mg/m^3 and higher and were most severe in the olfactory mucosa. Degeneration of the olfactory epithelium was typically more prominent in the posterior section of the nasal cavity and was seen primarily on the medial surface of the ethmoid turbinates adjacent to the nasal septum. Olfactory epithelium degeneration occurred at other sites in some rats, including the dorsal meatus in Level II. Morphologic features of olfactory epithelium degeneration ranged from a slight decrease in thickness and disorganization of the usual stratified arrangement of nuclei to necrosis with focal erosions of

the mucosal surface. With the exception of one male and one female rat in the 1 mg/m^3 groups, treatment-related lesions in the respiratory epithelium of the nasal passages were limited to the two highest exposure groups. Histopathologic findings in the respiratory epithelium included hyperplasia, squamous metaplasia, and inflammation. Hyperplasia consisted of a minimal to mild increase in the number of respiratory epithelial cells at the tips of nasal and maxillary turbinates. In rats primarily from the highest exposure group, there was minimal squamous metaplasia of the respiratory epithelium in the same location where hyperplasia occurred. Squamous metaplasia was characterized by a slight flattening of cells of the respiratory epithelium at the tips of the turbinate; keratinization of the superficial cell layers did not occur. Inflammation consisted of a minimal to mild infiltration of neutrophils in the submucosa of the lateral walls and nasal septum; a minimal cellular exudate was sometimes present in the lumen of the nasal passages.

Based on mortality at the 10 mg/m^3 level and the organ weight differences, lower body weight gains, clinical signs of toxicity, and the severity of the microscopic findings at the 3 and 10 mg/m^3 levels, the concentrations selected for the 13-week study in rats were 0, 0.025, 0.05, 0.1, 0.25, and 1 mg/m^3.

TABLE 7 Selected Histopathologic Lesions for Male and Female F344/N Rats in the 2-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)					
	0	0.1	0.3	1	3	10
MALE						
Lung						
Alveolar histiocytic infiltrate	0/5	5/5 (2.0)	5/5 (2.0)	5/5 (3.0)	5/5 (3.8)	5/5 (4.0)
Focal inflammation/fibrosis	0/5	5/5 (1.0)	5/5 (2.0)	5/5 (3.0)	5/5 (2.8)	5/5 (4.0)
Necrosis	0/5	0/5	5/5 (2.0)	5/5 (3.0)	5/5 (3.0)	5/5 (4.0)
Tracheobronchial lymph node						
Inflammation	0/5	0/3	0/5	5/5 (1.4)	5/5 (1.4)	3/4 (2.3)
Nose						
Olfactory epithelium						
Degeneration	0/5	0/5	0/5	2/5 (1.0)	5/5 (2.0)	5/5 (2.2)
Respiratory epithelium						
Hyperplasia	0/5	0/5	0/5	0/5	5/5 (1.0)	2/5 (1.0)
Squamous metaplasia	0/5	0/5	0/5	1/5 (1.0)	0/5	5/5 (1.0)
Inflammation	0/5	0/5	0/5	1/5 (1.0)	5/5 (1.4)	3/5 (1.7)
FEMALE						
Lung						
Alveolar histiocytic infiltrate	0/5	5/5 (2.0)	5/5 (2.2)	5/5 (3.0)	5/5 (4.0)	5/5 (4.0)
Focal inflammation/fibrosis	0/5	3/5 (1.0)	5/5 (2.0)	5/5 (3.0)	5/5 (3.0)	5/5 (4.0)
Necrosis	0/5	0/5	5/5 (2.0)	5/5 (3.0)	5/5 (3.0)	5/5 (4.0)
Tracheobronchial lymph node						
Inflammation	0/4	1/5 (2.0)	1/5 (1.0)	3/5 (1.0)	5/5 (1.6)	3/5 (1.0)
Nose						
Olfactory epithelium						
Degeneration	0/5	0/5	0/5	4/5 (1.3)	4/5 (2.3)	4/4 (3.0)
Respiratory epithelium						
Hyperplasia	0/5	0/5	0/5	1/5 (1.0)	4/5 (1.3)	3/4 (1.0)
Squamous metaplasia	0/5	0/5	0/5	0/5	4/5 (1.5)	4/4 (1.5)
Inflammation	0/5	0/5	0/5	0/5	4/5 (2.3)	3/4 (1.0)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked. All rats in the 10 mg/m^3 groups died between Day 2 and Day 7.

13-Week Inhalation Study in F344/N Rats

All rats survived until the end of the study (Table 8). The final mean body weights and mean body weight gains of male and female rats in the highest exposure groups (1 mg/m^3) were notably lower than those of the control groups (Table 8 and Figure 4). Clinical signs of toxicity included nasal discharge in males and females; in females, the frequency of this sign increased with increasing exposure concentration.

TABLE 8 Survival and Body Weights of F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide

Concentration (mg/m^3)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]
		Initial	Final	Change[2]	
MALE					
0	10/10	104	328	224	
0.025	10/10	101	319	218	97
0.05	10/10	106	327	221	100
0.1	10/10	103	313	210	96
0.25	10/10	109	333	224	102
1	10/10	101	305	204	93
FEMALE					
0	10/10	94	189	95	
0.025	10/10	92	189	97	100
0.05	10/10	92	183	91	97
0.1	10/10	92	195	103	103
0.25	10/10	94	187	93	99
1	10/10	95	177	82	93

[1] Number surviving at 13 weeks/number of animals per exposure group.
[2] Mean weight change.
[3] (Exposure group mean/control group mean) x 100.

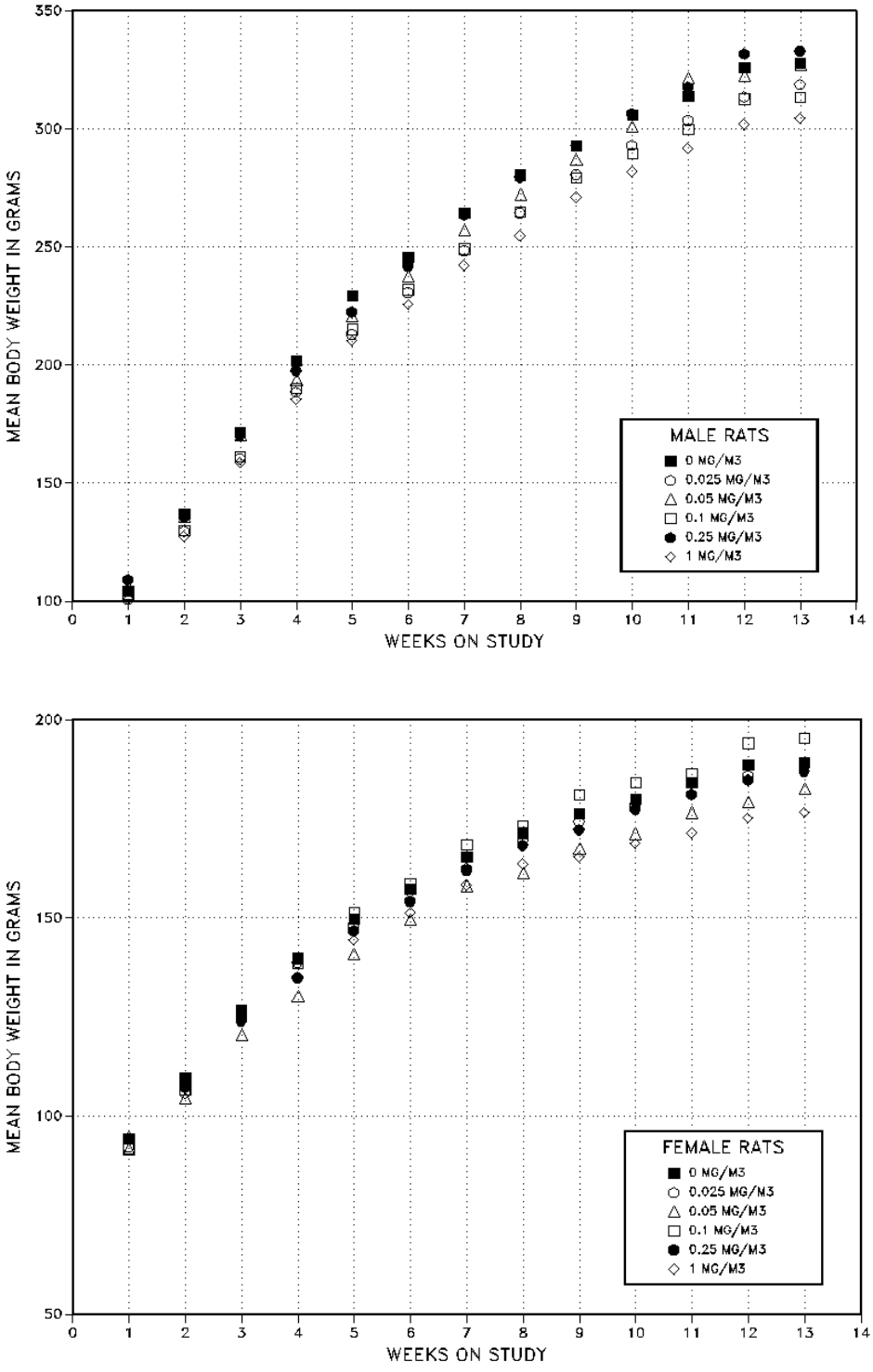


FIGURE 4 Body Weights of F344/N Rats Administered Cadmium Oxide by Inhalation for 13 Weeks

Data for hematology, clinical chemistry, and urinalysis parameters are listed in Appendix B. In general, changes in hematology and clinical chemistry parameters in rats were minor, sporadic, and not considered significant. A minimal decrease in erythrocyte (RBC) size (microcytosis), evidenced by mean cell volume (MCV) values less than the control values, occurred in most male and female exposure groups at Day 24. These findings are compatible with an ineffective erythropoiesis in which the bone marrow releases smaller than normal erythrocytes to the peripheral blood. This effect was transient, and by the end of the study, MCV values and RBC size were similar in exposed and control rats.

Reticulocyte numbers were greater in exposed females (0.025 mg/m^3 and higher groups) than in the controls at Day 24, indicating a bone marrow response. However, the absolute reticulocyte numbers for the exposed female rats were within an acceptable physiologic range. There were no consistent changes indicating anemia.

Leukocyte (WBC) counts less than the control values occurred at Days 4 and 24 in males and females in the 0.25 and 1 mg/m^3 groups. However, WBC counts were normal by Week 13. This difference was related to a decrease in lymphocyte numbers and would be consistent with a stress related change.

Total protein and globulin concentrations less than the controls occurred in male and female rats in various exposure groups at Days 4 and 24. Most of the lower total protein concentration could be accounted for by lower globulin concentrations. The decrease in protein was transient and was resolved by Week 13.

Other changes in hematology, clinical chemistry, and urinalysis parameters in rats were not considered biologically significant.

Significant differences in organ weights from control values occurred in the three highest exposure groups (0.1, 0.25, and 1 mg/m^3; Tables 9 and A2). At these exposure levels in males, the relative right kidney and liver weights were significantly greater than the controls. In males and females, absolute and relative lung weights increased in an exposure-related manner and were significantly greater than in the controls for rats exposed to 0.1, 0.25, or 1 mg/m^3 cadmium oxide. Relative right kidney weights and absolute and relative thymus weights were significantly greater than control values in females in the 0.25 and 1 mg/m^3 groups. The absolute liver weight of males in the 0.25 mg/m^3 group was significantly greater than the control values. At the 1 mg/m^3 exposure level, relative spleen

weights of males and females and absolute and relative thymus weights of males were significantly greater than in the controls.

There were no treatment-related microscopic changes in the kidney or liver. It was determined that thymus weights were artificially increased, particularly in the two highest exposure groups, because portions of the hyperplastic tracheal and mediastinal lymph nodes were attached.

TABLE 9 Selected Organ Weights for F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)					
	0	0.025	0.05	0.1	0.25	1
MALE						
n	10	10	10	10	10	10
Necropsy body wt	334 ± 5	331 ± 7	338 ± 5	322 ± 6	345 ± 7	314 ± 5
Right kidney						
Absolute	1.13 ± 0.021	1.12 ± 0.036	1.16 ± 0.026	1.15 ± 0.022	1.22 ± 0.033	1.15 ± 0.015
Relative	3.38 ± 0.06	3.37 ± 0.07	3.43 ± 0.04	3.57 ± 0.02*	3.55 ± 0.04*	3.66 ± 0.04**
Liver						
Absolute	12.2 ± 0.274	12.8 ± 0.374	12.7 ± 0.373	12.5 ± 0.342	13.9 ± 0.391**	12.0 ± 0.363
Relative	36.6 ± 0.79	38.7 ± 0.35	37.6 ± 0.83	38.7 ± 0.40*	40.2 ± 0.63**	38.0 ± 0.69**
Lungs						
Absolute	1.54 ± 0.043	1.55 ± 0.050	1.56 ± 0.029	1.72 ± 0.035*	2.28 ± 0.065**	2.54 ± 0.078**
Relative	4.60 ± 0.10	4.69 ± 0.10	4.61 ± 0.09	5.36 ± 0.11**	6.62 ± 0.11**	8.08 ± 0.19**
Spleen						
Absolute	0.648 ± 0.011	0.652 ± 0.016	0.642 ± 0.014	0.633 ± 0.014	0.689 ± 0.020	0.639 ± 0.017
Relative	1.94 ± 0.02	1.97 ± 0.02	1.90 ± 0.03	1.96 ± 0.02	2.00 ± 0.03	2.03 ± 0.05*
Thymus						
Absolute	0.411 ± 0.021	0.382 ± 0.017	0.394 ± 0.013	0.382 ± 0.012	0.422 ± 0.014	0.495 ± 0.012**
Relative	1.23 ± 0.05	1.16 ± 0.05	1.17 ± 0.04	1.18 ± 0.03	1.23 ± 0.03	1.58 ± 0.04**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	193 ± 3	193 ± 5	186 ± 5	199 ± 3	191 ± 3	183 ± 4
Right kidney						
Absolute	0.680 ± 0.008	0.711 ± 0.008	0.684 ± 0.023	0.707 ± 0.010	0.715 ± 0.007	0.719 ± 0.017
Relative	3.53 ± 0.03	3.71 ± 0.08	3.67 ± 0.06	3.55 ± 0.05	3.75 ± 0.06**	3.93 ± 0.04**
Liver						
Absolute	6.37 ± 0.155	6.50 ± 0.153	6.04 ± 0.236	7.02 ± 0.271	6.29 ± 0.138	6.15 ± 0.187
Relative	33.0 ± 0.50	33.8 ± 0.51	32.4 ± 0.79	35.2 ± 1.26	32.9 ± 0.55	33.6 ± 0.54
Lungs						
Absolute	1.10 ± 0.032	1.08 ± 0.019	1.08 ± 0.036	1.32 ± 0.023**	1.45 ± 0.027**	1.67 ± 0.049**
Relative	5.68 ± 0.15	5.61 ± 0.13	5.82 ± 0.13	6.62 ± 0.09**	7.58 ± 0.11**	9.12 ± 0.17**
Spleen						
Absolute	0.394 ± 0.011	0.405 ± 0.012	0.384 ± 0.010	0.439 ± 0.023	0.410 ± 0.009	0.415 ± 0.008
Relative	2.04 ± 0.04	2.11 ± 0.06	2.07 ± 0.03	2.21 ± 0.11	2.15 ± 0.04	2.27 ± 0.04*
Thymus						
Absolute	0.283 ± 0.013	0.293 ± 0.010[2]	0.291 ± 0.016	0.299 ± 0.009	0.342 ± 0.010**	0.339 ± 0.018**
Relative	1.47 ± 0.07	1.52 ± 0.03[2]	1.56 ± 0.06	1.50 ± 0.03	1.79 ± 0.06**	1.84 ± 0.07**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).
[2] n=9.
* Significantly different ($P \leq 0.05$) from the control group by Williams' test.
** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

There were no biologically significant effects of cadmium oxide exposure on blood pressure measurements at any time point (Table 10). There were statistically significant differences in females in the 0.25 and 1 mg/m^3 groups at Week 13. However, these differences were considered to be anomalies for the following reasons: the 0.25 and 1 mg/m^3 group means (120 to 130 mm) were within the normal range, the control value (102 mm) was on the low end of the normal range, a dose-response relationship was not present, and the data for females at the initial time point varied considerably from one group to another. Using a one-way analysis of variance test (Weter *et al.*, 1985), a significant difference among female rats was found at the initial time point. When the data were standardized by the initial time point data, there were no significant differences in dose-response or time-response for blood pressure measurements.

TABLE 10 Indirect Systolic Blood Pressure Measurements for F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)			
	0	0.1	0.25	1
MALE				
n	4	6	6	6
Initial	121.0 ± 4.7	132.4 ± 6.5[2]	129.8 ± 9.7[2]	124.5 ± 11.7[3]
Week 5	110.2 ± 6.4[2]	122.5 ± 10.4	130.8 ± 15.3[2]	119.8 ± 8.1
Week 9	107.3 ± 6.7	102.0 ± 3.8[4]	129.2 ± 3.0	113.3 ± 12.3[3]
Week 13	128.5 ± 3.3[5]	125.3 ± 3.6	149.7 ± 8.4	127.7 ± 7.5
				P=0.9877[6]
				P=0.2064[7]
FEMALE				
n	5	6	5	6
Initial	117.4 ± 7.2	124.5 ± 6.7	130.8 ± 4.9	102.2 ± 4.6[2]
Week 5	93.5 ± 5.9[3]	125.2 ± 11.5[2]	120.0 ± 7.8	115.6 ± 6.5[2]
Week 9	103.3 ± 10.3[3]	119.3 ± 11.5	118.3 ± 7.0[5]	119.0 ± 6.2
Week 13	102.2 ± 3.4	108.0 ± 6.5	128.6 ± 7.0*	122.3 ± 6.7*
				P=0.2087[6]
				P=0.6496[7]

[1] Data are given as mean ± standard error (mm).
[2] n=5.
[3] n=4.
[4] n=3.
[5] n=6.
[6] Significance of indirect systolic blood pressure dose response tested by a one-way analysis of variance (Weter *et al.*, 1985).
[7] Significance of indirect systolic blood pressure time response tested by a one-way analysis of variance (Weter *et al.*, 1985).
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.

Cadmium accumulation increased with exposure concentration at all time points, but the increases were not proportional to the increases in exposure concentration. Cadmium lung concentration did not achieve steady state over the course of the study (Table 11). Cadmium concentrations increased with increasing exposure concentration in the kidney at all time points (Table 12) and in the blood at Day 9, Day 30, and Week 13 (Table 13). Concentrations of cadmium in the lung and kidney were significantly greater than those of the controls for all exposure groups at every time point. Cadmium concentrations in the blood of rats exposed to 1 mg/m^3 cadmium oxide were significantly greater than the controls after just three days of exposure and remained so throughout the study. For rats in the 0.25 mg/m^3 group, cadmium concentrations in the blood were significantly greater than in the controls after 9 days of exposure and throughout the study. Further details on tissue levels of cadmium are reported by Dill *et al.* (1994).

TABLE 11 Lung Weight and Lung Burden of Elemental Cadmium in Male F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)			
	0	0.1	0.25	1
n	5	5	5	5
Day 3				
Absolute lung weight (g)	0.57 ± 0.005	0.68 ± 0.056*[2]	0.68 ± 0.041*	0.76 ± 0.016**
µg Cd/lung	)[3]	1.1 ± 0.047*[2]	2.2 ± 0.087**[2]	3.7 ± 0.17**
µg Cd/g lung	)	1.7 ± 0.17*[2]	3.2 ± 0.25**[2]	4.9 ± 0.27**
µg Cd/g control lung	)	2.0 ± 0.086*[2]	3.9 ± 0.15**[2]	6.5 ± 0.30**
Day 9				
Absolute lung weight (g)	0.74 ± 0.072	0.93 ± 0.12	1.0 ± 0.13	0.99 ± 0.070
µg Cd/lung	)	3.9 ± 0.27**	5.9 ± 0.21**	10.5 ± 0.29**
µg Cd/g lung	)	4.4 ± 0.41**	6.2 ± 0.68**	10.8 ± 0.72**
µg Cd/g control lung	)	5.3 ± 0.36**	8.0 ± 0.28**	14.1 ± 0.39**
Day 30				
Absolute lung weight (g)	0.77 ± 0.057	1.1 ± 0.13*	1.1 ± 0.050*	1.4 ± 0.062**
µg Cd/lung	)	7.6 ± 0.18**	13.7 ± 0.43**	27.3 ± 1.1**
µg Cd/g lung	)	7.3 ± 0.83**	13.1 ± 0.56**	19.3 ± 0.72**
µg Cd/g control lung	)	9.8 ± 0.23**	17.8 ± 0.56**	35.5 ± 1.5**
Week 13				
Absolute lung weight (g)	1.1 ± 0.022	1.5 ± 0.071**	1.8 ± 0.078**	2.2 ± 0.053**
µg Cd/lung	)	25.6 ± 1.5*[2]	45.7 ± 1.0**	75.1 ± 3.8**
µg Cd/g lung	)	16.7 ± 1.6*[2]	25.7 ± 1.2**	34.5 ± 2.3**
µg Cd/g control lung	)	22.7 ± 1.3*[2]	40.5 ± 0.92**	66.5 ± 3.4**

[1] Data are given as mean ± standard deviation.
[2] n=4.
[3] Results were below 0.1 µg Cd (the limit of quantitation).
* Significantly different ($P \leq 0.05$) from the control group by Williams' test (lung weight) or Shirley's test (lung burden parameters).
** Significantly different ($P \leq 0.01$) from the control group by Williams' test (lung weight) or Shirley's test (lung burden parameters).

TABLE 12 Kidney Weight and Kidney Burden of Elemental Cadmium in Male F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)			
	0	0.1	0.25	1
n	5	5	5	5
Day 3				
Absolute kidney weight (g)	1.1 ± 0.034	0.99 ± 0.047	1.1 ± 0.021	0.99 ± 0.034
µg Cd/kidney	0.015 ± 0.004	0.030 ± 0.004	0.061 ± 0.001**	0.20 ± 0.005**
µg Cd/g kidney	0.013 ± 0.003	0.030 ± 0.003*	0.057 ± 0.002**	0.20 ± 0.006**
µg Cd/g control kidney	0.014 ± 0.003	0.027 ± 0.004	0.055 ± 0.001**	0.18 ± 0.004**
Day 9				
Absolute kidney weight (g)	1.4 ± 0.065	1.2 ± 0.065	1.2 ± 0.051*	1.2 ± 0.049
µg Cd/kidney	0.035 ± 0.011	0.28 ± 0.026**	0.51 ± 0.040**	1.3 ± 0.084**
µg Cd/g kidney	0.026 ± 0.009	0.23 ± 0.016**	0.45 ± 0.055**	1.1 ± 0.076**
µg Cd/g control kidney	0.025 ± 0.008	0.20 ± 0.019**	0.37 ± 0.029**	0.97 ± 0.048**
Day 30				
Absolute kidney weight (g)	1.6 ± 0.11	1.7 ± 0.063	1.8 ± 0.091	1.6 ± 0.22
µg Cd/kidney	0.020 ± 0.002	1.5 ± 0.094**	3.1 ± 0.13**	7.1 ± 0.86**
µg Cd/g kidney	) [2]	0.86 ± 0.035**	1.8 ± 0.63**	4.6 ± 0.24**
µg Cd/g control kidney	)	0.92 ± 0.059**	1.9 ± 0.082**	4.5 ± 0.54**
Week 13				
Absolute kidney weight (g)	2.2 ± 0.11	2.4 ± 0.072 [3]	2.2 ± 0.073	2.2 ± 0.044 [3]
µg Cd/kidney	0.033 ± 0.004	7.3 ± 0.53* [3]	12.3 ± 0.49**	33.9 ± 1.2** [3]
µg Cd/g kidney	0.015 ± 0.002	3.1 ± 0.21* [3]	5.5 ± 0.061**	15.2 ± 0.84** [3]
µg Cd/g control kidney	0.015 ± 0.002	3.4 ± 0.25* [3]	5.7 ± 0.23**	15.6 ± 0.56** [3]

[1] Data are given as mean ± standard deviation.
[2] Results were below 0.012 µg Cd/g kidney (the limit of quantitation).
[3] n=4.
* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test (kidney weight) or Shirley's test (kidney burden parameters).
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE 13 Elemental Cadmium Concentrations in the Blood of Male F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)			
	0	0.1	0.25	1
n	5	5	5	5
Day 3	) [2]	) [2]	) [2,3]	0.0036 ± 0.0004*
Day 9	) [2]	) [2]	0.0036 ± 0.0009*	0.0039 ± 0.0004**
Day 30	) [2]	0.0025 ± 0.0003	0.0042 ± 0.0003**	0.0111 ± 0.0007**
Week 13	0.003 ± 0.0017	0.0037 ± 0.0007	0.0050 ± 0.0005*	0.0225 ± 0.0038**

[1] Data are given as mean ± standard error.
[2] Results were below 0.0025 µg Cd/g blood (the limit of quantitation).
[3] n=4.
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

At necropsy the only treatment-related gross lesions in rats were enlargement and paleness of the tracheobronchial and mediastinal lymph nodes. These changes were in male and female rats from the 0.25 and 1 mg/m^3 groups and in a few female rats from the 0.05 and 0.1 mg/m^3 groups. Histopathologic lesions occurred in the lung; tracheobronchial, mediastinal, and mesenteric lymph nodes; larynx; and nasal passages (Table 14).

In the lungs, treatment-related microscopic lesions were present in all exposed rats except those in the 0.025 mg/m^3 group. Histopathologic findings, including alveolar histiocytic (macrophage) infiltrates, inflammation, and fibrosis, were similar to those seen in rats in the 2-week study. In the 0.25 and 1 mg/m^3 groups, focal inflammation (Plate 1) and fibrosis (Plate 2) in the interstitium around alveolar ducts and terminal bronchioles was slightly more severe but similar to the inflammation and fibrosis seen in these areas in 2-week study rats. Fibrosis ranged in severity from minimal to moderate, based on the increase in thickness of the alveolar septa, and focal inflammation was of similar severity. Although histologic stains demonstrated increased collagen in the interstitium of alveoli and alveolar ducts of exposed rats, much of the increase in thickness of the alveolar septa was a result of the focal inflammatory cell infiltration at these sites. In the 0.1 mg/m^3 groups, a few lymphocytes were sometimes associated with the minimal fibrosis in the alveolar interstitium but the prominent foci of inflammation seen at higher exposure concentrations were not observed. At the end of the 13-week study, necrosis of alveolar epithelium (observed in the 2-week study) was not apparent, but a dose-related increase in hyperplasia of the type II epithelium (Plate 1) was evident at exposure concentrations of 0.05 mg/m^3 and greater.

Inflammation in the lymph nodes consisted of an accumulation of macrophages in the sinusoids. Mild diffuse lymphoid hyperplasia in the paracortex was also present. Focal aggregates of macrophages frequently formed small granulomas throughout the lymph nodes (Plates 3 and 4). Although the tracheobronchial and mediastinal nodes were most often affected, similar lesions were increased in incidence in the mesenteric lymph nodes of rats from the higher exposure concentrations.

Minimal degeneration of the laryngeal epithelium was present in all exposed male and female rats. In the larynx, the one to two layers of cuboidal to columnar, ciliated epithelium typically seen in control rats were slightly increased in thickness (three to four

TABLE 14 Incidence and Severity of Selected Lesions in F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)					
	0	0.025	0.05	0.1	0.25	1
MALE						
Lung						
alveolar histiocytic infiltrate	0/10	0/10	10/10 (1.0)	10/10 (2.0)	10/10 (3.0)	10/10 (3.0)
alveolar epithelial hyperplasia	0/10	0/10	10/10 (1.0)	10/10 (1.0)	10/10 (2.0)	10/10 (2.1)
inflammation	0/10	0/10	0/10	0/10	10/10 (2.6)	10/10 (4.0)
fibrosis	0/10	0/10	0/10	10/10 (1.0)	10/10 (2.0)	10/10 (2.7)
Tracheobronchial lymph node						
inflammation	0/9	0/7	0/5	3/7 (1.0)	9/9 (3.0)	10/10 (3.1)
Mediastinal lymph node						
inflammation	0/9	0/9	0/9	6/10 (1.3)	10/10 (3.2)	8/10 (3.3)
Mesenteric lymph node						
inflammation	0/10	0/9	0/10	1/10 (1.0)	8/10 (1.4)	10/10 (2.9)
Larynx						
epithelial degeneration	0/10	3/10 (1.0)	4/10 (1.0)	10/10 (1.0)	8/10 (1.0)	10/10 (1.0)
Nose						
Olfactory epithelium						
degeneration	0/10	0/10	0/10	0/10	1/10 (1.0)	10/10 (3.0)
respiratory metaplasia	0/10	0/10	0/10	0/10	0/10	4/10 (1.3)
squamous metaplasia	0/10	0/10	0/10	0/10	0/10	9/10 (1.9)
Respiratory epithelium						
inflammation	0/10	0/10	0/10	0/10	7/10 (1.0)	9/10 (2.6)
degeneration	0/10	0/10	0/10	0/10	0/10	2/10 (1.5)
Nasopharyngeal duct epithelium						
hypertrophy	0/10	0/10	0/10	0/10	0/10	9/10 (1.0)
FEMALE						
Lung						
alveolar histiocytic infiltrate	0/10	0/10	10/10 (1.0)	10/10 (2.1)	10/10 (3.0)	10/10 (3.0)
alveolar epithelial hyperplasia	0/10	0/10	10/10 (1.0)	10/10 (1.0)	10/10 (2.0)	10/10 (2.1)
inflammation	0/10	0/10	0/10	0/10	10/10 (1.6)	10/10 (3.5)
fibrosis	0/10	0/10	0/10	10/10 (1.0)	10/10 (2.0)	10/10 (2.1)
Tracheobronchial lymph node						
inflammation	0/7	0/4	0/8	6/8 (1.2)	6/9 (2.8)	10/10 (3.5)
Mediastinal lymph node						
inflammation	0/8	0/8	1/10 (1.0)	8/9 (1.5)	9/9 (3.6)	9/10 (4.0)
Mesenteric lymph node						
inflammation	3/10 (2.7)	3/10 (2.0)	4/10 (1.3)	5/10 (1.4)	9/10 (1.7)	10/10 (2.6)
Larynx						
epithelial degeneration	0/10	10/10 (1.0)	9/10 (1.0)	10/10 (1.0)	10/10 (1.0)	10/10 (1.0)
Nose						
Olfactory epithelium						
degeneration	0/10	0/10	0/10	0/10	2/10 (1.0)	10/10 (2.8)
respiratory metaplasia	0/10	0/10	0/10	0/10	1/10 (1.0)	2/10 (1.0)
squamous metaplasia	0/10	0/10	0/10	0/10	0/10	9/10 (1.4)
Respiratory epithelium						
inflammation	0/10	0/10	0/10	3/10 (1.0)	10/10 (1.6)	10/10 (1.8)
Nasopharyngeal duct epithelium						
hypertrophy	0/10	0/10	0/10	0/10	0/10	10/10 (1.0)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

cell layers) in exposed rats. Many of the ciliated cells were replaced by rounded or slightly flattened epithelial cells.

Lesions in the nasal passages were generally limited to the olfactory epithelium and occurred primarily in male and female rats from the 1 mg/m^3 groups. Treatment-related changes in the olfactory epithelium included degeneration with squamous or respiratory metaplasia. Minimal degeneration of the olfactory epithelium consisted of slightly decreased thickness of the nuclear layers and reduction in the thickness of the apical cytoplasm of the sustentacular cells. More severe degeneration was characterized by a loss of the orderly arrangement of the olfactory cell nuclei and atrophy of olfactory nerve axons in the adjacent lamina propria. Evidence of regeneration in the olfactory mucosa was observed in some areas of degeneration and consisted of focal proliferations of cells arranged in cluster or rosette formations (Plate 5). Focal areas of squamous or respiratory metaplasia were also present (Plate 6). In the anterior portion of the nasal passages, inflammation was present in the respiratory mucosa and consisted of an infiltrate of lymphocytes and macrophages in the lateral wall and lateral portions of the nasal turbinates. Minimal fibrosis was present in the areas of the nasal respiratory mucosa where inflammation was more severe. There was occasionally a slight increase in height (hypertrophy) of the respiratory epithelial cells in the areas of chronic inflammation; hypertrophy of the respiratory epithelium was also seen in the cells lining the nasopharyngeal duct.

In males in the 1 mg/m^3 group, spermatid heads per gram of testis, spermatid heads per testis, and spermatid count were significantly lower than those of control males (Table C1). There were no treatment-related microscopic changes in the testis or epididymis. In females, there was a significantly greater estrous cycle length than the controls at the 1 mg/m^3 exposure level (Table C2), but no treatment-related histologic changes in the reproductive organs.

Developmental Toxicity Study in Sprague-Dawley Rats

To assess the maternal and developmental toxicity of cadmium oxide, a study was performed in mated Sprague-Dawley rats exposed to 0, 0.05, 0.5, or 2 mg/m^3 cadmium oxide aerosol through whole-body exposure on gestation Days 4 through 19 (Appendix C). The same aerosol generation system and lot of cadmium oxide was used in this study as was used in the base studies.

One female rat in the highest exposure group (2 mg/m^3) died on gestation Day 17 of the study; no other deaths occurred. Clinical signs of toxicity included dyspnea in all exposure groups and hypoactivity in most rats in the 2 mg/m^3 group. Statistically significant differences in maternal and embryo/fetal parameters were limited to rats in the 2 mg/m^3 group. At this exposure level, the mean body weight and maternal weight change of pregnant females were significantly less than the controls by the end of the study. In addition, absolute and relative liver weights and absolute kidney weights were significantly less than control values. Relative gravid uterine weights and relative kidney weights were significantly greater than the control values, probably due to decreased maternal body weight. Embryolethality was not present at any exposure level, and most embryo/fetal parameters were not affected by cadmium oxide exposure. Body weights of male and female fetuses in the 2 mg/m^3 group were significantly lower than those of the control fetuses. The only significant fetal variations noted were exposure-related increases in the incidence of reduced ossifications of the pelvis and of the sternebrae; the differences were significant in the 2 mg/m^3 group for both parameters. The complete methods and results of the developmental toxicity study in rats are presented in Appendix C.

2-Week Inhalation Study in B6C3F$_1$ Mice

All male and female mice in the highest exposure groups (10 mg/m^3) died by Day 7 of the study (Table 15). These deaths were attributed to severe respiratory toxicity; no other deaths occurred. The final mean body weights and mean body weight gains of female mice in the 1 mg/m^3 group and male and female mice in the 3 mg/m^3 groups were lower than those of the control groups. In addition, the mean body weight gain of female mice in the 0.3 mg/m^3 group was lower than that of the control mice. Clinical signs of toxicity occurred in males and females in the 1, 3, and 10 mg/m^3 groups by Day 2 of the study, and included hypoactivity, dehydration, abnormal posture, rapid breathing, ataxia, nasal discharge, and ruffled fur. By Day 5 of the study, mice in the 0.3 mg/m^3 groups also displayed ruffled fur.

TABLE 15 Survival and Body Weights of B6C3F$_1$ Mice in the 2-Week Inhalation Study of Cadmium Oxide

Concentration (mg/m^3)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]
		Initial	Final	Change[2]	
MALE					
0	5/5	24.2	27.8	3.6	
0.1	5/5	24.6	28.6	4.0	103
0.3	5/5	24.2	27.4	3.2	99
1	5/5	24.3	28.2	3.9	101
3	5/5	24.3	26.4	2.1	95
10	0/5[4]	24.3	)	)	)
FEMALE					
0	5/5	20.2	24.5	4.3	
0.1	5/5	19.9	24.1	4.2	98
0.3	5/5	20.3	23.4	3.1	96
1	5/5	19.9	22.7	2.8	93
3	5/5	19.9	22.9	3.0	93
10	0/5[5]	19.5	)	)	)

[1] Number surviving at 2 weeks/number of animals per exposure group.
[2] Mean weight change.
[3] (Exposure group mean/control group mean) x 100.
[4] Day of death: 3, 4, 4, 4, 4.
[5] Day of death: 5, 5, 6, 6, 7.

In the 2-week study in mice, the only notable organ weight differences were exposure-related increases in absolute and relative lung weights in males and females; the differences were significant in mice in the 0.3 mg/m^3 and higher exposure groups (Table A3).

Treatment-related gross lesions attributed to the 2-week cadmium oxide exposures were limited to enlargement (2 mm × 3 mm) of the tracheobronchial lymph nodes in male and female mice. This was seen most consistently in females in the 1 and 3 mg/m^3 groups. Treatment-related histopathologic lesions occurred in the lung, tracheobronchial lymph nodes, and nasal passages of male and female mice administered 0.1 to 10 mg/m^3 cadmium oxide aerosol (Table 16).

Histopathologic lesions in the lung included an alveolar histiocytic (macrophage) infiltration, focal inflammation, and necrosis of alveolar duct epithelium. At the lowest exposure concentration (0.1 mg/m^3), only a minimal to mild histiocytic infiltration in alveolar spaces was evident. At higher exposure concentrations, the severity of the histiocytic infiltrate increased and focal interstitial inflammation occurred around alveolar ducts. Fibrosis was associated with the focal inflammation and was present within the alveolar septa and around alveolar ducts in the 1 and 3 mg/m^3 groups. In mice that died during the first week of the study (10 mg/m^3 groups), focal inflammation and fibrosis did not occur, but an inflammatory cell exudate consisting primarily of neutrophils was present within alveolar spaces. Necrosis of alveolar duct epithelium occurred in one male and one female mouse from the 0.3 mg/m^3 groups; necrosis of the alveolar duct epithelium was more severe in mice from the 1, 3, and 10 mg/m^3 groups.

A diffuse, minimal to moderate lymphoid hyperplasia was present in the paracortex of the tracheobronchial lymph nodes. There was also a mild increase in the number of macrophages in the sinusoids of the hyperplastic lymph nodes.

Degeneration of the olfactory epithelium was present at exposure concentrations of 1 mg/m^3 and greater. The severity of this lesion was minimal to mild in the 1 mg/m^3 groups and moderate at higher concentrations. Degeneration was present in the olfactory epithelium lining the dorsal meatus in Level II as well as on the nasal septum and medial surface of the ethmoid turbinates in the posterior nasal section (Level III). Morphologic features included a decrease in the usual thickness of the olfactory epithelium and loss of the normal arrangement of the olfactory nuclear layers. Necrotic cellular debris was sometimes present on the epithelial surface of the mucosa.

Based on mortality in the 10 mg/m^3 groups and the organ weight differences, decreased body weights, clinical signs of toxicity, and severity of the microscopic findings in the 3 and 10 mg/m^3 groups, the concentrations selected for the 13-week study in mice were 0, 0.025, 0.05, 0.1, 0.25, and 1 mg/m^3.

TABLE 16 Selected Histopathologic Lesions for Male and Female B6C3F$_1$ Mice in the 2-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)					
	0	0.1	0.3	1	3	10
MALE						
Lung						
Alveolar histiocytic infiltrate[2]	0/5	5/5 (1.2)	5/5 (2.0)	5/5 (3.0)	5/5 (3.0)	0/5
Focal inflammation/fibrosis[2]	0/5	0/5	5/5	5/5	5/5	0/5
Necrosis	0/5	0/5	1/5 (1.0)	5/5 (2.0)	5/5 (2.2)	5/5 (3.0)
Inflammation, acute	0/5	0/5	0/5	0/5	0/5	5/5 (4.0)
Tracheobronchial lymph node						
Hyperplasia	0/5	3/5 (1.0)	5/5 (1.0)	5/5 (2.0)	5/5 (2.0)	1/5 (3.0)
Nose						
Olfactory epithelium						
Degeneration	0/5	0/5	0/5	5/5 (1.4)	5/5 (3.0)	3/5 (3.0)
FEMALE						
Lung						
Alveolar histiocytic infiltrate	0/5	5/5 (1.0)	5/5 (1.8)	5/5 (3.0)	5/5 (3.0)	0/5
Focal inflammation/fibrosis	0/5	0/5	5/5	5/5	5/5	0/5
Necrosis	0/5	0/5	1/5 (1.0)	5/5 (2.0)	5/5 (2.0)	5/5 (3.0)
Inflammation, acute	0/5	0/5	0/5	0/5	0/5	5/5 (4.0)
Tracheobronchial lymph node						
Hyperplasia	0/5	4/5 (1.0)	4/5 (1.0)	4/4 (1.5)	5/5 (1.8)	0/5
Nose						
Olfactory epithelium						
Degeneration	0/5	0/5	0/5	5/5 (2.0)	5/5 (3.0)	5/5 (3.0)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked. All mice in the 10 mg/m^3 groups died between Day 2 and Day 8.

[2] Alveolar histiocytic infiltrate and focal inflammation/fibrosis are two of the major components of the treatment-related lesion originally diagnosed as "inflammation, granulomatous." For consistency of terminology between the 2-week and 13-week studies and for comparison between rats and mice, these two components of "inflammation, granulomatous" have been listed separately above. A separate severity grade was not assigned to the focal inflammation/fibrosis component.

13-Week Inhalation Study in B6C3F$_1$ Mice

One male mouse in the control group died during Week 13 of the study; no other deaths occurred (Table 17). The final mean body weights and mean body weight gains of exposed mice were similar to or slightly greater than those of the control mice (Table 17 and Figure 5). No clinical signs of toxicity considered to be related to cadmium oxide exposure were observed in male or female mice during the study.

TABLE 17 Survival and Body Weights of B6C3F$_1$ Mice in the 13-Week Inhalation Study of Cadmium Oxide

Concentration (mg/m^3)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]
		Initial	Final	Change[2]	
MALE					
0	9/10[4]	23.4	32.4[5]	9.0	
0.025	10/10	23.7	34.4	10.6	106
0.05	10/10	23.5	34.4	11.0	106
0.1	10/10	23.4	34.2	10.8	105
0.25	10/10	23.4	34.2	10.9	106
1	10/10	23.4	33.1	9.7	102
FEMALE					
0	10/10	19.7	28.6	8.9	
0.025	10/10	19.7	30.0	10.4	105
0.05	10/10	20.4	31.4	11.1	110
0.1	10/10	19.6	29.4	9.8	103
0.25	10/10	19.2	28.4	9.2	99
1	10/10	19.9	29.4	9.5	103

[1] Number surviving at 13 weeks/number of animals per exposure group.
[2] Mean weight change.
[3] (Exposure group mean/control group mean) x 100.
[4] Week of death: 13.
[5] n=10; the single death in this exposure group occurred after final mean body weights were determined.

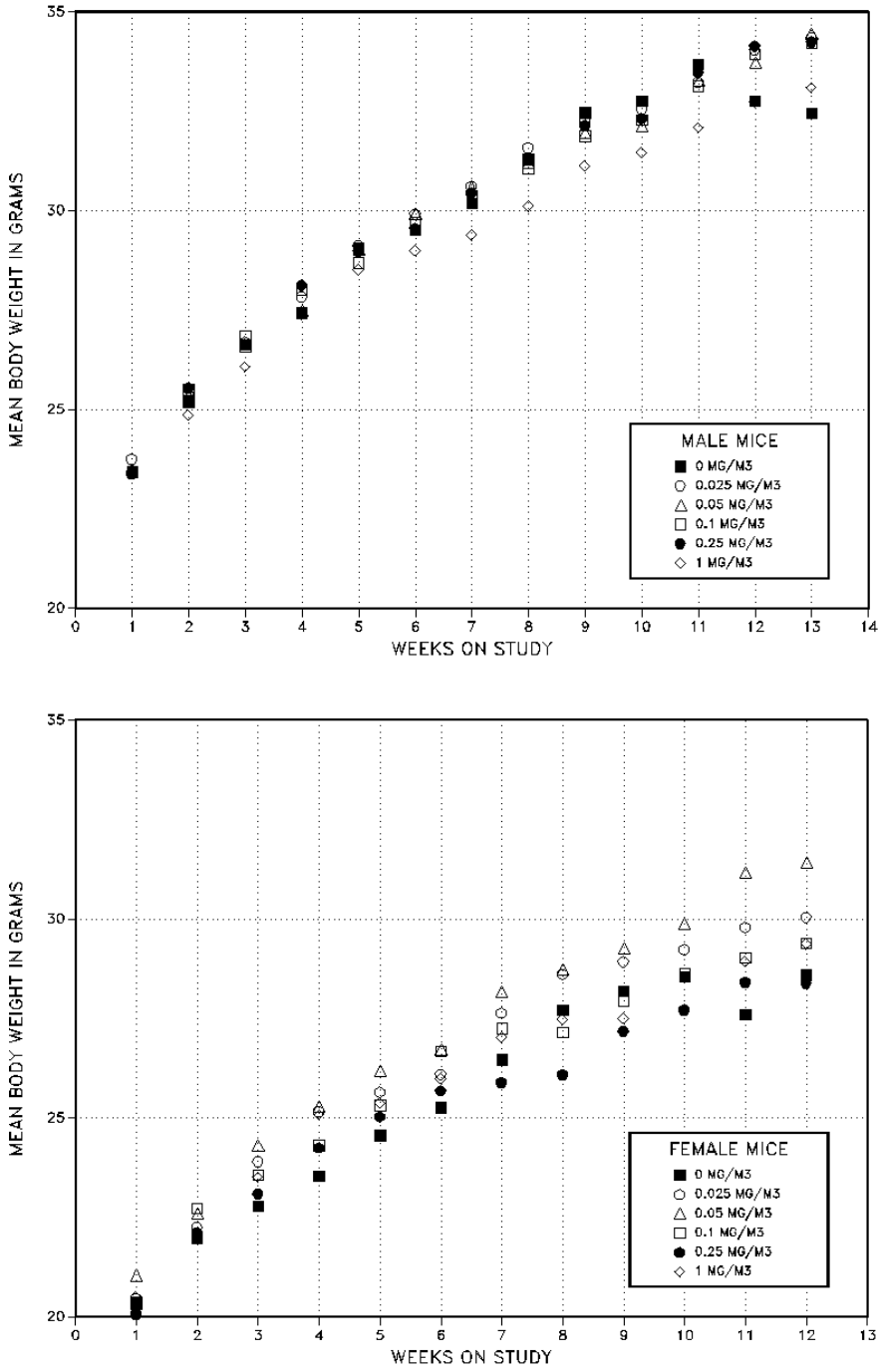


FIGURE 5 Body Weights of B6C3F$_1$ Mice Administered Cadmium Oxide by Inhalation for 13 Weeks

For mice in the 13-week study, significant differences in organ weights occurred at all exposure levels (Tables 18 and A4). The absolute and relative lung weights of males and females increased in an exposure-related manner, and most absolute and relative lung weights were significantly greater than the control values. Absolute and relative kidney and thymus weights in males and kidney, liver, and spleen weights in females were greater than in the controls for all groups exposed to cadmium oxide; most of these differences were significant. Absolute and relative spleen weights were also significantly greater than the controls in males in the three highest exposure groups (0.1, 0.25, and 1 mg/m^3). Absolute liver weights were significantly greater than control values in males exposed to 0.25 or 1 mg/m^3 cadmium oxide, and the relative liver weight of males in the 1 mg/m^3 group was also significantly greater than the control values. For females in the 1 mg/m^3 group, absolute and relative thymus weights were significantly greater than in the controls. There were no treatment-related microscopic changes in the liver, kidney, spleen, or thymus.

TABLE 18 Selected Organ Weights for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)					
	0	0.025	0.05	0.1	0.25	1
MALE						
n	10	10	10	10	10	10
Necropsy body wt	34.0 ± 1.0	35.4 ± 0.7	34.8 ± 0.6	34.4 ± 0.5	35.1 ± 0.7	34.0 ± 0.7
Right kidney						
Absolute	0.303 ± 0.008	0.334 ± 0.007**	0.340 ± 0.007**	0.344 ± 0.008**	0.364 ± 0.007**	0.352 ± 0.006**
Relative	8.98 ± 0.28	9.46 ± 0.17	9.82 ± 0.32*	9.99 ± 0.21**	10.4 ± 0.23**	10.4 ± 0.19**
Liver						
Absolute	1.56 ± 0.065	1.60 ± 0.035	1.68 ± 0.051	1.64 ± 0.054	1.73 ± 0.051*	1.76 ± 0.040**
Relative	46.0 ± 1.40	45.4 ± 1.33	48.4 ± 1.06	47.6 ± 1.18	49.2 ± 1.24	51.9 ± 0.92**
Lungs						
Absolute	0.218 ± 0.010	0.246 ± 0.014	0.254 ± 0.008*	0.334 ± 0.009**	0.372 ± 0.008**	0.452 ± 0.009**
Relative	6.43 ± 0.28	6.94 ± 0.31	7.32 ± 0.24*	9.70 ± 0.26**	10.6 ± 0.29**	13.3 ± 0.22**
Spleen						
Absolute	0.073 ± 0.003	0.071 ± 0.003	0.074 ± 0.002	0.091 ± 0.002**	0.104 ± 0.004**	0.103 ± 0.004**
Relative	2.19 ± 0.15	2.01 ± 0.06	2.13 ± 0.04	2.64 ± 0.07**	2.97 ± 0.11**	3.03 ± 0.10**
Thymus						
Absolute	0.040 ± 0.005	0.049 ± 0.002*	0.050 ± 0.003*	0.049 ± 0.003*	0.054 ± 0.002**	0.051 ± 0.003**
Relative	1.14 ± 0.13	1.40 ± 0.05*	1.43 ± 0.08*	1.41 ± 0.08*	1.54 ± 0.06**	1.49 ± 0.08**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	29.1 ± 0.6	31.4 ± 0.9	32.2 ± 0.9*	30.0 ± 0.5	28.8 ± 0.6	30.6 ± 0.7
Right kidney						
Absolute	0.214 ± 0.009	0.231 ± 0.005*	0.251 ± 0.005**	0.234 ± 0.004**	0.238 ± 0.006**	0.241 ± 0.005**
Relative	7.38 ± 0.30	7.39 ± 0.20	7.83 ± 0.19	7.82 ± 0.11	8.28 ± 0.15*	7.88 ± 0.16*
Liver						
Absolute	1.36 ± 0.025	1.50 ± 0.055*	1.71 ± 0.050**	1.47 ± 0.042**	1.51 ± 0.059**	1.54 ± 0.048**
Relative	47.1 ± 1.23	47.9 ± 1.29	53.1 ± 0.93*	48.9 ± 0.83*	52.3 ± 1.11*	50.4 ± 1.33*
Lungs						
Absolute	0.213 ± 0.013	0.241 ± 0.013	0.258 ± 0.007*	0.308 ± 0.009**	0.363 ± 0.014**	0.470 ± 0.014**
Relative	7.40 ± 0.57	7.69 ± 0.40	8.05 ± 0.24	10.3 ± 0.27**	12.6 ± 0.31**	15.4 ± 0.40**
Spleen						
Absolute	0.086 ± 0.005	0.101 ± 0.004[2]	0.114 ± 0.006*	0.116 ± 0.006*	0.152 ± 0.016**	0.152 ± 0.008**
Relative	2.97 ± 0.18	3.23 ± 0.09[2]	3.55 ± 0.16	3.87 ± 0.19*	5.29 ± 0.57**	4.97 ± 0.24**
Thymus						
Absolute	0.056 ± 0.002	0.063 ± 0.002	0.061 ± 0.004	0.056 ± 0.002	0.056 ± 0.002	0.071 ± 0.003**
Relative	1.92 ± 0.06	2.01 ± 0.05	1.90 ± 0.10	1.87 ± 0.07	1.94 ± 0.05	2.33 ± 0.07**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] n=9.

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

At necropsy the only treatment-related gross lesions in mice were enlargement of the tracheobronchial lymph nodes and pale grey, mottled lungs. These changes were observed in most male and female mice from the 0.25 and 1 mg/m^3 groups. Treatment-related histopathologic lesions occurred in the lungs, tracheobronchial lymph nodes, larynx, and nasal passages (Table 19).

In the lungs, treatment-related lesions were present at all exposure concentrations. In the two lowest exposure groups (0.025 and 0.05 mg/m^3), the most prominent histopathologic finding consisted of a minimal increase in alveolar histiocytes within alveolar spaces. In male mice, minimal alveolar (type II cell) hyperplasia was also present. At exposure concentrations of 0.1 mg/m^3 and greater, the alveolar histiocytic infiltrates and alveolar epithelial hyperplasia increased in severity (Plate 7). In addition, focal areas of inflammation consisting of a mixture of neutrophils and macrophages within alveolar spaces occurred at the three highest exposure concentrations (Plate 8). In these foci of inflammation there was cellular debris and an extracellular, eosinophilic granular material. A few basophilic to black particles, possibly cadmium oxide, were seen within the cytoplasm of some alveolar macrophages. Fibrosis in the lung was characterized by a minimal thickening of alveolar ducts and alveolar duct septa. Fibrosis did not increase in severity with increasing dose. Special histologic stains demonstrated only a slight increase in collagen within the alveolar septa of exposed mice compared to the controls.

A dose-related increase in the incidence and severity of hyperplasia occurred in the tracheobronchial lymph nodes. There was a slight increase in the prominence and number of germinal centers in lymph nodes, but the most consistent change was a diffuse lymphoid hyperplasia in the paracortical and medullary portions of these lymph nodes (Plates 9 and 10).

Squamous metaplasia of the laryngeal respiratory mucosa was a minimal change present at all exposure concentrations. This lesion was characterized by an increase in thickness of the laryngeal epithelium at the base of the epiglottis and replacement of the normal cuboidal and ciliated epithelial cells with a more rounded or flattened epithelium. Keratinization of the mucosa was not a feature of this change.

Lesions in the nasal passages were generally limited to the olfactory epithelium and occurred primarily in male and female mice from the 0.25 and 1 mg/m^3 groups. Degeneration of the olfactory epithelium was the most prominent change and occurred in the dorsal meatus of the anterior portion of the nose as well as on the nasal septum and medial aspect of the ethmoid turbinates in the posterior portion of the nose (Plates 11 and 12). Degeneration was characterized by a disorganization of the normal

arrangement of the olfactory nuclear layers and a decreased thickness of the olfactory epithelium. In some mice, a mild glandular dilatation and hyperplasia of the epithelium of Bowman's gland was present. In mice with mild olfactory epithelium degeneration, a decrease in diameter of the nerve fibers (axonal atrophy) and respiratory and/or squamous metaplasia also occurred. Hyaline droplet accumulation was seen in the respiratory epithelium of male and female mice in the 1 mg/m^3 groups. This minimal change was characterized by the presence of eosinophilic protein droplets in the cytoplasm of the respiratory epithelial cells, primarily in the middle portion (Level II) of the nasal passages.

TABLE 19 Incidence and Severity of Selected Lesions in B6C3F$_1$ Mice in the 13-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)											
	0		0.025		0.05		0.1		0.25		1	
MALE												
Lung												
alveolar histiocytic infiltrate	0/10		9/10	(1.1)	10/10	(1.0)	10/10	(2.0)	10/10	(2.0)	10/10	(3.0)
alveolar epithelial hyperplasia	0/10		1/10	(1.0)	10/10	(1.0)	10/10	(1.8)	10/10	(1.7)	10/10	(2.0)
inflammation	0/10		0/10		0/10		8/10	(3.0)	10/10	(2.2)	10/10	(2.7)
fibrosis	0/10		0/10		2/10	(1.0)	10/10	(1.0)	10/10	(1.0)	10/10	(1.0)
Tracheobronchial lymph node												
hyperplasia	0/6		0/8		4/9	(1.0)	9/9	(2.3)	8/10	(2.4)	9/10	(2.7)
Larynx												
squamous metaplasia	0/9		10/10	(1.0)	10/10	(1.0)	10/10	(1.0)	10/10	(1.0)	9/10	(1.1)
Nose												
Olfactory epithelium												
degeneration	0/10		0/10		0/10		4/10	(1.0)	10/10	(1.7)	10/10	(2.0)
respiratory metaplasia	0/10		0/10		0/10		0/10		2/10	(1.0)	8/10	(1.5)
squamous metaplasia	0/10		0/10		0/10		0/10		0/10		2/10	(1.0)
Respiratory epithelium												
hyaline droplets	0/10		0/10		0/10		0/10		2/10	(1.0)	10/10	(1.0)
FEMALE												
Lung												
alveolar histiocytic infiltrate	0/10		9/10	(1.0)	10/10	(1.0)	10/10	(2.0)	10/10	(2.0)	10/10	(3.0)
alveolar epithelial hyperplasia	0/10		0/10		0/10		10/10	(1.4)	10/10	(2.0)	10/10	(2.0)
inflammation	0/10		0/10		0/10		6/10	(2.3)	8/10	(2.1)	10/10	
(2.9)	fibrosis	0/10		0/10		1/10	(1.0)	10/10	(1.0)	10/10	(1.0)	
10/10	(1.0)											
Tracheobronchial lymph node												
hyperplasia	0/6		0/6		2/9	(1.0)	8/9	(1.5)	9/10	(2.0)	10/10	(2.4)
Larynx												
squamous metaplasia	0/10		10/10	(1.0)	10/10	(1.0)	10/10	(1.0)	10/10	(1.0)	10/10	(1.0)
Nose												
Olfactory epithelium												
degeneration	0/10		0/10		0/10		1/10	(1.0)	10/10	(1.0)	10/10	(2.0)
respiratory metaplasia	0/10		0/10		0/10		0/10		0/10		8/10	(1.0)
squamous metaplasia	0/10		0/10		0/10		0/10		0/10		0/10	
Respiratory epithelium												
hyaline droplets	0/10		0/10		0/10		0/10		2/10	(1.0)	10/10	(1.0)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

Sperm motility and vaginal cytology evaluations were performed on base-study mice exposed to 0, 0.025, 0.1, or 1 mg/m^3 cadmium oxide for 13 weeks. No significant differences occurred in males or females (Tables C3 and C4).

Developmental Toxicity Study in Swiss (CD-1®) Mice

To assess the maternal and developmental toxicity of cadmium oxide, a study was conducted in mated Swiss (CD-1®) mice exposed to 0, 0.05, 0.5, or 2 mg/m^3 cadmium oxide aerosol through whole-body exposure on gestation Days 4 through 17 (Appendix C). The same aerosol generation system and lot of cadmium oxide was used in this study as was used in the base studies.

Five females in the highest exposure group (2 mg/m^3) were sacrificed moribund before the end of the study; no other deaths occurred. The number of mice becoming pregnant was significantly less than in the controls at the 0.5 and 2 mg/m^3 exposure levels. Clinical signs of toxicity included dyspnea and hypoactivity in all mice in the 2 mg/m^3 group and in most mice in the 0.5 mg/m^3 group. Dyspnea also occurred in some mice in the 0.05 mg/m^3 group. Statistically significant differences in maternal parameters were limited to mice in the 2 mg/m^3 group and included significantly lower mean body weight, maternal weight change, absolute and relative gravid uterine weights, and absolute liver weight and a significantly greater relative kidney weight than the control values. Most statistically significant differences in embryo/fetal parameters also occurred in the 2 mg/m^3 group. At this exposure level, the total number of resorptions per litter was significantly greater than in the controls, and fetal body weights (male and female) and the percentage of live male fetuses per litter were significantly less than in the controls. At the 0.5 mg/m^3 level, fetal body weights (male and female) were also significantly less than the control values. The only significant fetal variation noted was an exposure-related increase in the incidence of reduced ossifications of the sternebrae; the difference was significant in the 2 mg/m^3 group. The complete methods and results of the developmental toxicity study in mice are presented in Appendix C.

Genetic Toxicity

Cadmium oxide (3.3 to 3333 µg/plate) was tested for mutagenic activity in four strains of *Salmonella typhimurium* using a preincubation protocol with and without Aroclor-induced liver S9 metabolic activation enzymes; no mutagenic activity was noted (Table D1; Mortelmans *et al.*, 1986). In addition, inhalation exposure to cadmium oxide (0.025-1 mg/m^3) for 13 weeks did not result in increased frequencies of micronucleated erythrocytes in peripheral blood of male or female $B6C3F_1$ mice (Table D2).

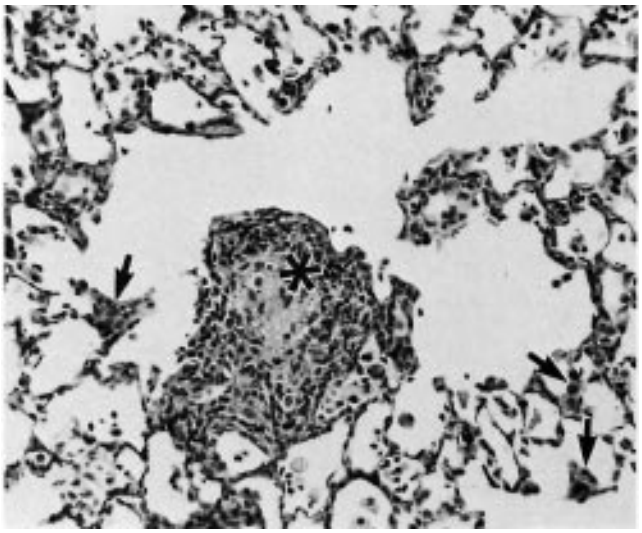


PLATE 1
Pulmonary acinus region of lung from a female F344/N rat exposed to 1mg/m^3 cadmium oxide by inhalation for 13 weeks. Interstitium of the alveolar duct has a focal inflammatory cell infiltrate (*) consisting primarily of lymphocytes which surround a paler staining aggregate of macrophages. Macrophages are also present within the adjacent alveolar spaces and there is a minimal hyperplasia of alveolar type II cells (arrows). H&E, 125x.

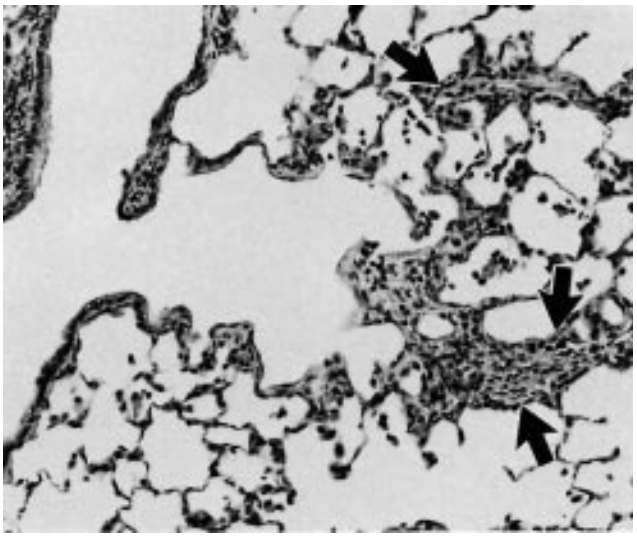


PLATE 2
Pulmonary acinus region of lung from a female F344/N rat exposed to 1mg/m^3 cadmium oxide by inhalation for 13 weeks. Interstitium of alveolar ducts is thickened by fibrosis (arrows) and an inflammatory cell infiltrate. Macrophages are also present within the adjacent alveolar spaces. H&E, 125x.

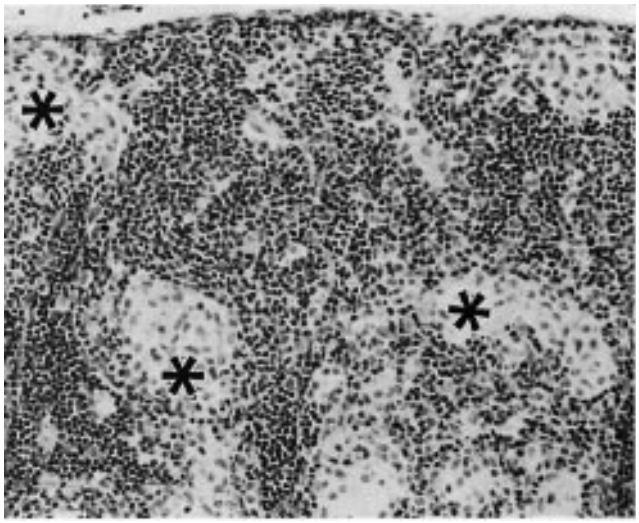


PLATE 3
Tracheobronchial lymph node from a female F344/N rat exposed to 1mg/m^3 cadmium oxide by inhalation for 13 weeks. Throughout the lymph node are multiple foci of inflammation (granulomas) consisting of aggregates of pale staining macrophages (*). Compare with lymph node from control rat in Plate 4. H&E, 150x.

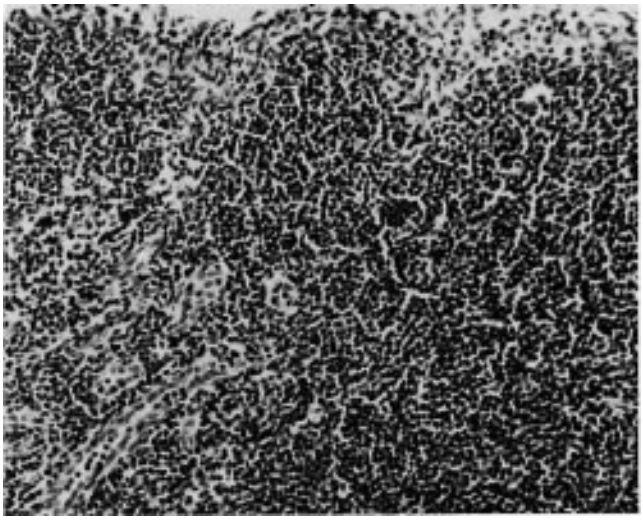


PLATE 4
Tracheobronchial lymph node from a control female F344/N rat for comparison with inflammatory changes in lymph node of cadmium-exposied rat shown in Plate 3. H&E, 150x.

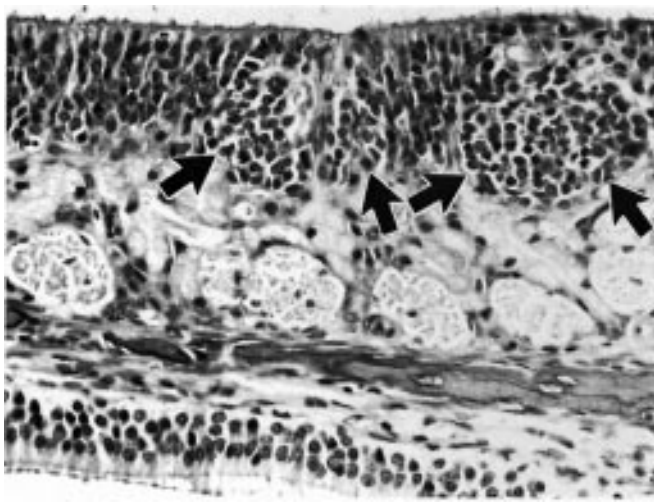


PLATE 5

Olfactory nasal turbinate from a male F344/N rat exposed to 1 mg/m^3 cadmium oxide by inhalation for 13 weeks. Within the mucosa are nodular foci (arrows) of regenerating cells in the olfactory epithelium. H&E, 300x.

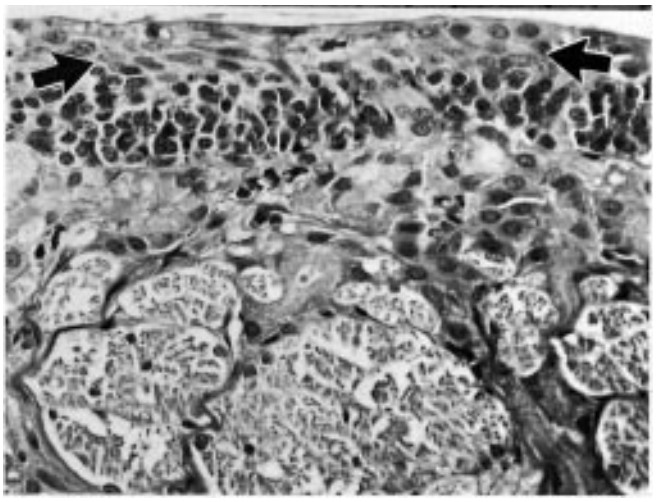


PLATE 6

Olfactory nasal turbinate from a male F344/N rat exposed to 1 mg/m^3 cadmium oxide by inhalation for 13 weeks. Changes in the olfactory mucosa include squamous metaplasia (arrows) and degeneration characterized by a decreased number of olfactory epithelial cells. H&E, 300x.

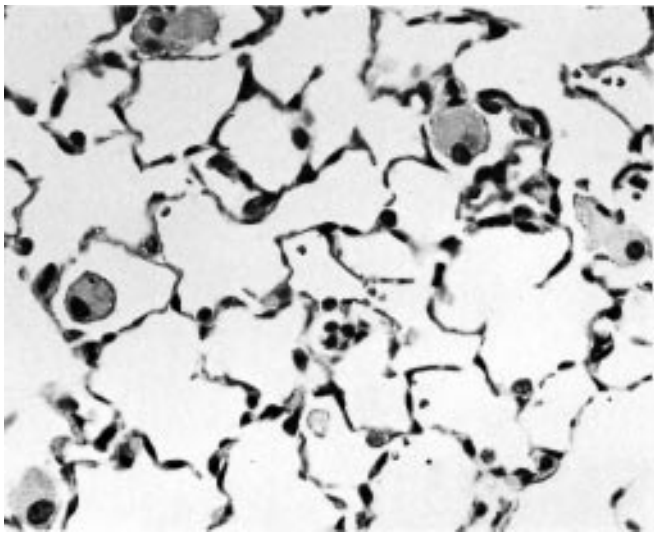


PLATE 7

Lung from a male B6C3F$_1$ mouse exposed to 0.1 mg/m^3 cadmium oxide by inhalation for 13 weeks. Mild alveolar histiocytic (macrophage) infiltration is present in alveolar spaces. H&E, 300x.

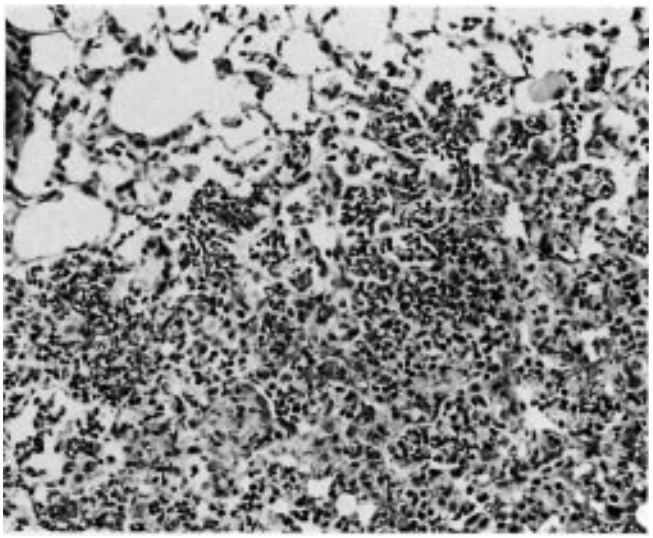


PLATE 7

Lung from a male B6C3F$_1$ mouse exposed to 0.1 mg/m^3 cadmium oxide for 13 weeks. Focal inflammatory cell exudate present in alveolar duct and alveolar spaces consists primarily of neutrophils and macrophages. H&E, 150x.

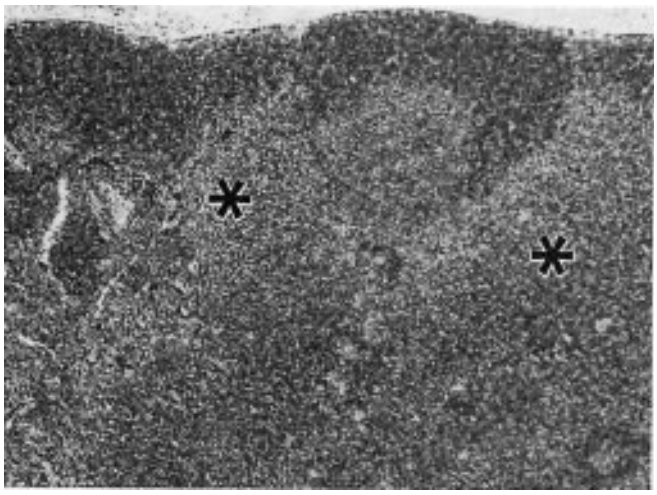


PLATE 9

Tracheobronchial lymph node from a female B6C3F$_1$ mouse exposed to 1 mg/m^3 cadmium oxide by inhalation for 13 weeks. Lymphoid hyperplasia, primarily in the paracortex (*) of this markedly enlarged node, is prominent compared to the typical size and morphology of the tracheobronchial lymph node from a control mouse shown in Plate 10. H&E, 60x.

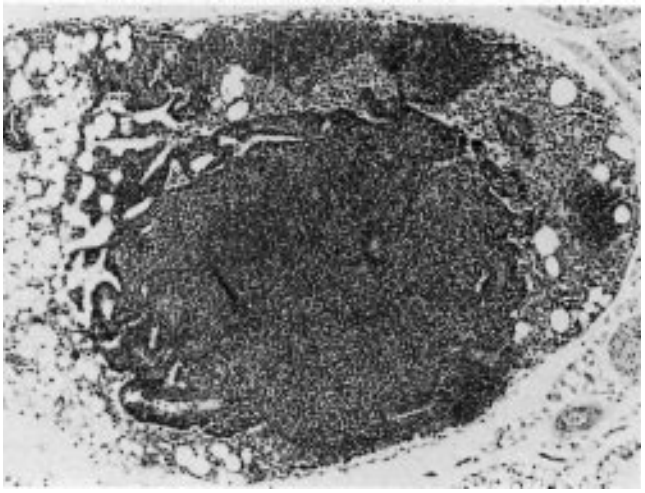


PLATE 10

Tracheobronchial lymph node from a control female B6C3F$_1$ mouse for comparison with Plate 9. Note the smaller overall size and absence of hyperplastic response compared to lymph node from mouse exposed to cadmium oxide. H&E, 60x.

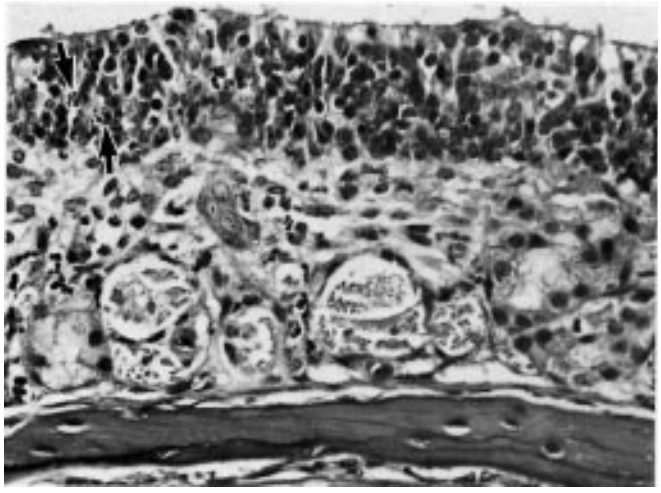


PLATE 11

Nasal turbinate from a male B6C3F$_1$ mouse exposed to 1 mg/m^3 cadmium oxide by inhalation for 13 weeks. Degeneration in the olfactory mucosa is characterized by disorganization of the normal arrangement of the olfactory nuclear layers and single cell necrosis (arrows). H&E, 300x.

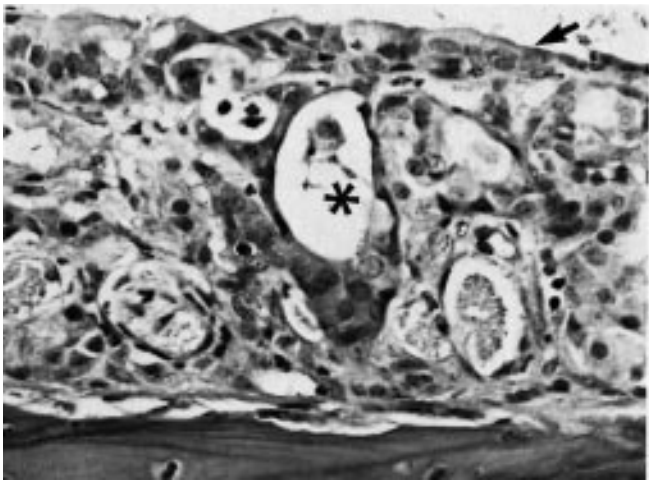


PLATE 12

Nasal turbinate from a male B6C3F$_1$ mouse exposed to 1 mg/m^3 cadmium oxide by inhalation for 13 weeks. Compared to Plate 11, the degeneration in the olfactory mucosa is more severe, with marked thinning of the olfactory epithelial layers, squamous metaplasia (arrow), and dilation of Bowman's glands (*). H&E, 300x.

DISCUSSION

In these inhalation studies of cadmium oxide, the major toxicity to rats and mice occurred in the respiratory system, as expected, based on previous rodent inhalation studies of cadmium (Elinder, 1986; Oberdörster, 1986).

Respiratory Toxicity

In the 2-week studies, inhalation exposure of F344/N rats or B6C3F$_1$ mice to cadmium oxide at levels of 10 mg/m^3 was fatal after 3 to 7 days of exposure, and this mortality was attributed to severe respiratory toxicity. The severity of lung toxicity increased with increasing exposure level (from 0.1 to 10 mg/m^3) and was characterized by necrosis and inflammation of the alveolar ducts and adjacent alveoli. Degeneration of the nasal olfactory epithelium was seen at levels of 1 mg/m^3 and greater in rats and mice, and hyperplasia, squamous metaplasia, and inflammation of the respiratory epithelium was observed at levels of 1 mg/m^3 and greater in rats.

In the 13-week studies, there were no treatment-related effects on the survival of rats or mice. In rats in the 1 mg/m^3 groups (the highest exposure level), final mean body weights were lower than those of controls. Respiratory toxicity was observed in rats and mice and was characterized by increased lung weights and treatment-related histopathologic lesions of the lung, larynx, and nasal cavity (Table 20). Lung lesions in rats and mice consisted of macrophage infiltrates, hyperplasia, inflammation, and fibrosis. Fibrosis was more prominent in rats than in mice. The no-observed-adverse-effect level (NOAEL) for lung toxicity in rats was 0.025 mg/m^3, but in the mouse a NOAEL was not reached (Tables 20 and 21). There was no difference in lung toxicity between males and females.

Cadmium oxide respiratory toxicity has previously been reported in other studies. Elinder (1986) and Oberdörster (1986) have summarized the results of earlier cadmium inhalation studies which show that cadmium oxide and other forms of cadmium cause toxic lesions in the lung. Inhalation exposure of female Wistar rats to cadmium oxide at 25 to 50 µg/m^3 for 24 hours per day for up to 90 days produced emphysematous areas and cell proliferation in the bronchi and bronchioli (Prigge, 1978). Inhalation exposure of rats to cadmium chloride at 10 mg/m^3 for 10 days gave rise to granulation tissue and localized fibrosis in the lung (Snider *et al.*, 1973). The mechanism underlying the acute cadmium toxicity in the lungs is not known, but Elinder (1986) hypothesized that it involves damage to alveolar type I cells.

There are little data in humans from which to obtain comparison dose-response relationships for inhalation exposure to cadmium oxide (Elinder, 1986). In humans, an exposure to cadmium at an estimated level of 1 mg/m^3 reduced pulmonary function as measured by curved expiratory flow volume and respiratory impedance (Sakurai *et al.*, 1982). Other studies in humans also show respiratory toxicity or emphysema after exposure to cadmium at estimated levels of 0.1 to 0.4 mg/m^3 for 2 years (Lane and Campbell, 1954) or to cadmium oxide at an estimated level of 0.2 mg/m^3 for 6 years (Smith *et al.*, 1957). Based on the amount of cadmium found in the lungs of two fatal human cases, Elinder (1986) estimated the lethal dose to be 5 mg/kg lung (wet weight).

In other experimental inhalation carcinogenicity studies, cadmium oxide caused lung neoplasms in male Wistar rats at exposure concentrations of 30 or 90 µg cadmium per cubic meter, but no treatment-related lung neoplasms were found in the female Han:NMRI mouse or in the Syrian hamster (Aufderheide *et al.*, 1989; Heinrich et al., 1989; Thiedemann *et al.*, 1989; Glaser *et al.*, 1990; and Takenaka *et al.*, 1990). Toxic lesions of the lung were observed in rats, mice, and hamsters and were characterized by alveolar lipoproteinosis, interstitial fibrosis, and hyperplasia (mice); bronchiolar-alveolar hyperplasia, thickening of septa, and proliferation of connective tissue (hamsters); and necrosis of type I pneumocytes, proliferation of epithelial cells, and focal alveolar inflammation (rats). These results suggest that factors other than toxicity, such as genetic susceptibility, species differences in metabolism, or anatomic differences in the respiratory system, might contribute to metal carcinogenicity in the lungs of rodents (Gross *et al.*, 1982; Proctor, 1989).

In the present 13-week studies, treatment-related lesions were observed in the olfactory and respiratory epithelium in rats and mice exposed to 1 mg/m^3 and in the nasopharyngeal duct epithelium of rats exposed to 0.25 mg/m^3 and greater (Tables 20 and 21). The nasal toxicity reported in this study is characteristic of inhalation exposure to metal compounds (Dunnick *et al.*, 1989) and is most likely due to direct exposure of the nasal cavity to the metal compound, not to systemic exposure. In other inhalation studies conducted at the Fraunhofer Institute, toxicity of cadmium oxide (90 µg/m^3) to the nasal cavities of male Wistar rats or female Han:NMRI mice was not reported (Aufderheide *et al.*, 1989; Heinrich *et al.*, 1989; Thiedemann *et al.*, 1989; Glaser *et al.*, 1990; Takenaka *et al.*, 1990).

In the present 13-week studies, cadmium oxide was toxic to the larynx in rats and mice, and effects were seen at all exposure levels in mice (Tables 20 and 21). The larynx is a common site for lesions in rodents exposed by inhalation to various chemicals and pharmaceuticals, and characteristic lesions include metaplasia, erosion, ulceration, and inflammation (Gopinath *et al.*, 1987). For example,

inhalation studies with cobalt sulfate also resulted in larynx lesions characterized by polyps, necrosis, and inflammation in rats and inflammation, necrosis, and squamous metaplasia in mice (NTP, 1991). The minimal squamous metaplasia in mice was morphologically similar to the epithelial degeneration that occurred in the larynx of rats. Although there was slight flattening of epithelium with squamous metaplasia in mice, there was no cellular or nuclear atypia suggestive of a preneoplastic lesion.

Kidney Toxicity

Cadmium is toxic to the kidney of rodents exposed to water soluble salts of cadmium administered by subcutaneous or intravenous injection (Kjellström, 1986). In these 13-week inhalation studies of cadmium oxide, kidney weights were elevated in rats exposed to 0.25 and 1 mg/m^3 and in mice at all exposure levels, but there were no corresponding histopathologic lesions (Tables 20 and 21). Cadmium concentrations in the kidney were below the concentration reported by Goyer *et al.* (1984, 1989) to be toxic to the rodent kidney (200 µg per gram of kidney).

Cadmium has a biological half-life of over 10 years in the kidney, and long-term inhalation exposure to cadmium could result in accumulation with resultant kidney damage (IPCS, 1992). Studies have shown renal function impairment in workers inhaling cadmium for long periods of time (Järup *et al.*, 1993; and Staessen and Lauwerys, 1993); although, in these studies it was not possible to identify forms of cadmium exposure or to quantitate amounts of exposure. Workplace exposure to cadmium is measured in part by urinalysis because urinary cadmium concentrations primarily reflect cadmium concentrations in the kidneys. OSHA has recommended that control measures be implemented when the levels of urinary cadmium exceed 5 µg/g creatinine (Lauwerys and Hoet, 1993).

Cardiovascular Toxicity

Studies with water-soluble forms of cadmium have reported some hypertensive effects in rodents (Kopp, *et al.*, 1982; Nishiyama *et al.*, 1986), but no effects considered biologically significant were observed in rats in the present 13-week study of cadmium oxide.

Reproductive Toxicity

In rats at the highest exposure level (1 mg/m^3), there was a reduced number of spermatids per testis and an increase in the length of the estrous cycle (Table 20). However, there were no histopathologic lesions indicative of toxicity to the reproductive system, suggesting that reproductive effects at the highest exposure level in rats may be related to other effects of cadmium, such as hormonal changes.

TABLE 20 Selected Parameters for F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)					
	0	**0.025**	**0.05**	**0.1**	**0.25**	**1**
MALE						
Final body weight[2]						
(percentage of controls)		97	100	96	102	93
Respiratory System						
Lung						
Cadmium concentration (µg/g lung)	0.05	NM[3]	NM	19.1*	29.4**	39.5**
Weight (absolute and relative)	)	)[4]	)	↑[5]	↑[5]	↑[5]
Histopathologic findings						
alveolar histiocytic infiltrate	)	)	+ (1.0)	+ (2.0)	+ (3.0)	+ (3.0)
alveolar epithelial hyperplasia	)	)	+ (1.0)	+ (1.0)	+ (2.0)	+ (2.1)
inflammation	)	)	)	)	+ (2.6)	+ (4.0)
fibrosis	)	)	)	+ (1.0)	+ (2.0)	+ (2.7)
Mediastinal lymph node						
inflammation	)	)	)	+ (1.3)	+ (3.2)	+ (3.3)
Larynx						
epithelial degeneration	)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)
Nose						
Olfactory epithelium						
degeneration	)	)	)	)	+ (1.0)	+ (3.0)
respiratory metaplasia	)	)	)	)	)	+ (1.3)
squamous metaplasia	)	)	)	)	)	+ (1.9)
Respiratory epithelium						
inflammation	)	)	)	)	+ (1.0)	+ (2.6)
degeneration	)	)	)	)	)	+ (1.5)
Kidney						
Cadmium concentration (µg/g kidney)	0.02	NM	NM	3.1*	5.5**	15.2**
Weight, right kidney (relative)	)	)	)	↑[5]	↑[5]	↑[5]
Urinalysis parameters	)	)	)	)	)	)
Reproductive System						
Testis/epididymis weight	)	)	NM	)	NM	)
Spermatid count	)	)	NM	)	NM	↓[6]
Sperm motility	)	)	NM	)	NM	)

TABLE 20 Selected Parameters for F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide (continued)

	Concentration (mg/m^3)					
	0	0.025	0.05	0.1	0.25	1
FEMALE						
Final body weight[2]						
(percentage of controls)		100	97	103	99	93
Respiratory System						
Lung						
Weight						
(absolute and relative)	)	)	)	↑ [5]	↑ [5]	↑ [5]
Histopathologic findings						
alveolar histiocytic infiltrate	)	)	+ (1.0)	+ (2.1)	+ (3.0)	+ (3.0)
alveolar epithelial hyperplasia	)	)	+ (1.0)	+ (1.0)	+ (2.0)	+ (2.1)
inflammation	)	)	)	)	+ (1.6)	+
(3.5)	fibrosis	)	)	)	+ (1.0)	+ (2.0)
+ (2.1)						
Mediastinal lymph node						
inflammation	)	)	+ (1.0)	+ (1.5)	+ (3.6)	+ (4.0)
Larynx						
epithelial degeneration	)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)
Nose						
Olfactory epithelium						
degeneration	)	)	)	)	+ (1.0)	+ (2.8)
respiratory metaplasia	)	)	)	)	+ (1.0)	+ (1.0)
squamous metaplasia	)	)	)	)	)	+ (1.4)
Respiratory epithelium						
inflammation	)	)	)	+ (1.0)	+ (1.6)	+ (1.8)
Kidney						
Weight, right kidney (relative)	)	)	)	)	↑ [5]	↑ [5]
Urinalysis parameters	)	)	)	)	↑ [7]	↑ [7]
Reproductive System						
Estrous cycle length	)	)	NM	)	NM	↑ [8]

[1] For each control and exposure group, statistical analyses were performed on the mean value for 10 rats (organ weights), 7 to 10 rats (urinalysis parameters), 9 to 10 rats (reproductive parameters), or 4 to 5 rats (tissue cadmium concentrations). For histopathologic findings, average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

[2] (Exposure group mean/control group mean) x 100.

[3] NM = not measured at this exposure level.

[4]) = No lesions present (histopathology) or not significantly different from the control group (organ weights and urinalysis and reproductive parameters).

[5] Organ weights significantly greater than the control group.

[6] Spermatid count significantly lower than in the control group.

[7] Aspartate aminotransferase levels (mU/mg creatinine) significantly greater than in the control group.

[8] Estrous cycle significantly longer than in the control group.

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.

** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE 21 Selected Parameters for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Cadmium Oxide[1]

	Concentration (mg/m^3)					
	0	**0.025**	**0.05**	**0.1**	**0.25**	**1**
MALE						
Final body weight[2]						
(percentage of controls)		106	106	105	106	102
Respiratory System						
Lung						
Weight						
(absolute and relative)	)	) [3]	↑ [4]	↑	↑	↑
Histopathologic findings						
alveolar epithelial hyperplasia	)	+ (1.0)	+ (1.0)	+ (1.8)	+ (1.7)	+ (2.0)
inflammation	)	)	)	+ (3.0)	+ (2.2)	+ (2.7)
fibrosis	)	)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)
Tracheobronchial lymph node						
hyperplasia	)	)	+ (1.0)	+ (2.3)	+ (2.4)	+ (2.7)
Larynx						
squamous metaplasia	)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.1)
Nose						
Olfactory epithelium						
degeneration	)	)	)	+ (1.0)	+ (1.7)	+ (2.0)
respiratory metaplasia	)	)	)	)	+ (1.0)	+ (1.5)
squamous metaplasia	)	)	)	)	)	+ (1.0)
Respiratory epithelium						
hyaline droplets	)	)	)	)	+ (1.0)	+ (1.0)
Kidney						
Weight, right kidney (absolute)	)	↑	↑	↑	↑	↑
Reproductive System						
Testis/epididymis weight	)	)	NM [5]	)	NM	)
Spermatid count	)	)	NM	)	NM	)
Sperm motility	)	)	NM	)	NM	)
FEMALE						
Final body weight[2]						
(percentage of controls)		105	110	103	99 103	
Respiratory System						
Lung						
Weight						
(absolute)	)	)	↑	↑	↑	↑
Histopathologic findings						
alveolar histiocytic infiltrate	)	+ (1.0)	+ (1.0)	+ (2.0)	+ (2.0)	+ (3.0)
alveolar epithelial hyperplasia	)	)	)	+ (1.4)	+ (2.0)	+ (2.0)
inflammation	)	)	)	+ (2.3)	+ (2.1)	+ (2.9)
fibrosis	)	)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)
Tracheobronchial lymph node						
hyperplasia	)	)	+ (1.0)	+ (1.5)	+ (2.0)	+ (2.4)
Larynx						
squamous metaplasia	)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)	+ (1.0)

TABLE 21 Selected Parameters for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Cadmium Oxide (continued)

	Concentration (mg/m^3)					
	0	0.025	0.05	0.1	0.25	1
FEMALE (continued)						
Respiratory System (continued)						
Nose						
Olfactory epithelium						
degeneration	)	)	)	+ (1.0)	+ (1.0)	+ (2.0)
respiratory metaplasia	)	)	)	)	)	+ (1.0)
squamous metaplasia	)	)	)	)	)	)
Respiratory epithelium						
hyaline droplets	)	)	)	)	+ (1.0)	+ (1.0)
Kidney						
Weight, right kidney (absolute)	)	↑	↑	↑	↑	↑
Reproductive System						
Estrous cycle length	)	)	NM	)	NM	)

[1] For each control and exposure group, statistical analyses were performed on the mean value for 9 to 10 mice (organ weights and reproductive parameters). For histopathologic findings, average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.
[2] (Exposure group mean/control group mean) x 100.
[3]) = No lesions present (histopathology) or not significantly different from the control group (organ weights and reproductive parameters).
[4] Organ weights significantly greater than the control group.
[5] NM = not measured at this exposure level.

Developmental Toxicity

In developmental toxicity studies in Sprague-Dawley rats (exposures of 0.05-2 mg/m^3), maternal toxicity was observed in the 2 mg/m^3 group and included body weights lower than those of the controls and clinical signs of toxicity (dyspnea and hypoactivity). There was no evidence of embryolethality at any exposure level. However, in the 2 mg/m^3 group, developmental toxicity was evidenced by lower fetal weights and a significant increase in the incidence of reduced skeleton ossifications.

Developmental toxicity studies in Swiss (CD-1®) mice (same exposure levels as in rats) also showed maternal toxicity at the 2 mg/m^3 exposure level, evidenced by dyspnea, hypoactivity, lower body weight, and a decreased pregnancy rate (30% vs. 97% in the control group). The total number of resorptions per litter was increased in the 2 mg/m^3 group, and developmental toxicity was evidenced by a decrease in fetal weights in the 0.5 and 2 mg/m^3 groups and an increase in the incidence of reduced sternebral ossification in the 2 mg/m^3 group. In feed restrictive studies conducted to evaluate the effect of decreased body weight on reproductive function, it was shown that up to a 20%

decrease in body weight had no effect on most of the reproductive parameters in male and female Swiss (CD-1®) mice (Chapin *et al.*, 1993). These results suggest that the reproductive effects observed in this study are in part a result of cadmium toxicity and that body weight effects alone cannot account for this toxicity.

Summary

Treatment-related respiratory tract lesions were found in the lungs, nose, and larynx of F344/N rats and $B6C3F_1$ mice exposed by inhalation to cadmium oxide for 13 weeks. The no-observed-adverse-effect level (NOAEL) in the lungs was 0.025 mg/m^3 for rats. A NOAEL was not found in the lungs or larynx of mice or in the larynx of rats. At the 0.025 and 0.05 mg/m^3 levels in mice, lung lesions were minimal and not considered life threatening. Nasal lesions were observed in rats and mice exposed to 0.25 or 1 mg/m^3 and in a few rats and mice exposed to 0.1 mg/m^3. The NOAEL in the nasal cavity was 0.05 mg/m^3 for rats and mice. Reproductive system toxicity was observed in rats in the 1 mg/m^3 groups and was evidenced by a reduced number of spermatids per testis and an increase in the length of the estrous cycle. Reproductive system toxicity was not observed at any exposure level in mice. Developmental toxicity studies in Sprague-Dawley rats and Swiss (CD-1®) mice showed a reduction in fetal weights in rats at 2 mg/m^3 and in mice at 0.5 and 2 mg/m^3.

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APPENDIX A

Organ Weights and Organ-Weight-to-Body-Weight Ratios

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 2-Week Inhalation Study of Cadmium Oxide[1]

	0 mg/m^3	0.1 mg/m^3	0.3 mg/m^3	1 mg/m^3	3 mg/m^3	10 mg/m^3
MALE						
n	5	5	5	5	5	0
Necropsy body wt	195 ± 7	194 ± 5	198 ± 3	189 ± 9	181 ± 3	)[2]
Heart						
Absolute	0.648 ± 0.023	0.646 ± 0.009	0.706 ± 0.007	0.698 ± 0.027	0.690 ± 0.015	)
Relative	3.32 ± 0.04	3.33 ± 0.06	3.57 ± 0.05**	3.69 ± 0.07**	3.81 ± 0.05**	)
Right kidney						
Absolute	0.816 ± 0.032	0.822 ± 0.030	0.840 ± 0.008	0.796 ± 0.043	0.814 ± 0.033	)
Relative	4.18 ± 0.09	4.23 ± 0.09	4.25 ± 0.06	4.20 ± 0.08	4.49 ± 0.13	)
Liver						
Absolute	8.764 ± 0.453	9.990 ± 0.432	10.338 ± 0.306	10.756 ± 0.639*	9.792 ± 0.448*	)
Relative	44.82 ± 0.95	51.33 ± 1.09**	52.29 ± 1.55**	56.69 ± 1.53**	53.94 ± 1.57**	)
Lungs						
Absolute	1.430 ± 0.089	1.352 ± 0.043	1.776 ± 0.140*	1.698 ± 0.081*	2.004 ± 0.089**	)
Relative	7.34 ± 0.45	6.96 ± 0.15	8.99 ± 0.73*	8.97 ± 0.24*	11.04 ± 0.30**	)
Spleen						
Absolute	0.444 ± 0.027	0.458 ± 0.015	0.474 ± 0.006	0.456 ± 0.024	0.418 ± 0.017	)
Relative	2.27 ± 0.06	2.36 ± 0.06	2.40 ± 0.04	2.41 ± 0.05	2.30 ± 0.06	)
Right testis						
Absolute	1.094 ± 0.054	1.032 ± 0.024	1.092 ± 0.025	1.039 ± 0.029	1.053 ± 0.024	)
Relative	5.60 ± 0.21	5.31 ± 0.08	5.53 ± 0.15	5.52 ± 0.23	5.81 ± 0.09	)
Thymus						
Absolute	0.455 ± 0.015	0.435 ± 0.015	0.489 ± 0.015	0.419 ± 0.028	0.440 ± 0.027	)
Relative	2.33 ± 0.02	2.25 ± 0.12	2.47 ± 0.08	2.22 ± 0.13	2.43 ± 0.12	)
FEMALE						
n	5	5	5	5	5	0
Necropsy body wt	136 ± 2	132 ± 2	136 ± 3	137 ± 5	124 ± 2	)
Heart						
Absolute	0.478 ± 0.012	0.490 ± 0.016	0.524 ± 0.012	0.548 ± 0.020*	0.514 ± 0.013	)
Relative	3.51 ± 0.09	3.71 ± 0.12	3.86 ± 0.08*	4.00 ± 0.07**	4.15 ± 0.12**	)
Right kidney						
Absolute	0.592 ± 0.016	0.634 ± 0.019	0.632 ± 0.022	0.636 ± 0.024	0.584 ± 0.008	)
Relative	4.34 ± 0.05	4.80 ± 0.11**	4.65 ± 0.07*	4.64 ± 0.06*	4.71 ± 0.06**	)
Liver						
Absolute	5.742 ± 0.170	5.770 ± 0.292	5.802 ± 0.211	6.986 ± 0.161**	6.198 ± 0.182	)
Relative	42.23 ± 1.65	43.66 ± 1.92	42.73 ± 1.02	51.04 ± 0.71**	50.10 ± 1.99**	)
Lungs						
Absolute	1.108 ± 0.116	1.058 ± 0.062	1.270 ± 0.055	1.462 ± 0.060**	1.550 ± 0.067**	)
Relative	8.14 ± 0.84	8.02 ± 0.50	9.37 ± 0.42	10.66 ± 0.20**	12.52 ± 0.58**	)
Spleen						
Absolute	0.340 ± 0.013	0.342 ± 0.014	0.352 ± 0.011	0.362 ± 0.011	0.336 ± 0.007	)
Relative	2.50 ± 0.09	2.59 ± 0.10	2.59 ± 0.06	2.64 ± 0.07	2.71 ± 0.07	)
Thymus						
Absolute	0.364 ± 0.018	0.383 ± 0.017	0.417 ± 0.016	0.472 ± 0.033**	0.379 ± 0.018	)
Relative	2.68 ± 0.17	2.90 ± 0.12	3.07 ± 0.09	3.46 ± 0.28*	3.06 ± 0.14	)

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] All rats in the 10 mg/m^3 groups died between Day 2 and Day 7.

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

	0 mg/m^3	0.025 mg/m^3	0.05 mg/m^3	0.1 mg/m^3	0.25 mg/m^3	1 mg/m^3
MALE						
n	10	10	10	10	10	10
Necropsy body wt	334 ± 5	331 ± 7	338 ± 5	322 ± 6	345 ± 7	314 ± 5
Heart						
Absolute	0.974 ± 0.017	0.940 ± 0.019	0.982 ± 0.014	0.943 ± 0.017	0.980 ± 0.024	0.942 ± 0.013
Relative	2.92 ± 0.04	2.84 ± 0.03	2.90 ± 0.04	2.93 ± 0.03	2.84 ± 0.03	3.00 ± 0.03
Right kidney						
Absolute	1.130 ± 0.021	1.115 ± 0.036	1.163 ± 0.026	1.152 ± 0.022	1.223 ± 0.033	1.149 ± 0.015
Relative	3.38 ± 0.06	3.37 ± 0.07	3.43 ± 0.04	3.57 ± 0.02*	3.55 ± 0.04*	3.66 ± 0.04**
Liver						
Absolute	12.201 ± 0.274	12.814 ± 0.374	12.728 ± 0.373	12.497 ± 0.342	13.869 ± 0.391**	11.970 ± 0.363
Relative	36.55 ± 0.79	38.69 ± 0.35	37.59 ± 0.83	38.72 ± 0.40*	40.22 ± 0.63**	38.02 ± 0.69**
Lungs						
Absolute	1.539 ± 0.043	1.551 ± 0.050	1.559 ± 0.029	1.724 ± 0.035*	2.282 ± 0.065**	2.543 ± 0.078**
Relative	4.60 ± 0.10	4.69 ± 0.10	4.61 ± 0.09	5.36 ± 0.11**	6.62 ± 0.11**	8.08 ± 0.19**
Spleen						
Absolute	0.648 ± 0.011	0.652 ± 0.016	0.642 ± 0.014	0.633 ± 0.014	0.689 ± 0.020	0.639 ± 0.017
Relative	1.94 ± 0.02	1.97 ± 0.02	1.90 ± 0.03	1.96 ± 0.02	2.00 ± 0.03	2.03 ± 0.05*
Right testis						
Absolute	1.412 ± 0.025	1.358 ± 0.026	1.366 ± 0.026	1.384 ± 0.027	1.418 ± 0.027	1.342 ± 0.022
Relative	4.23 ± 0.08	4.11 ± 0.06	4.04 ± 0.08	4.30 ± 0.04	4.12 ± 0.05	4.27 ± 0.05
Thymus						
Absolute	0.411 ± 0.021	0.382 ± 0.017	0.394 ± 0.013	0.382 ± 0.012	0.422 ± 0.014	0.495 ± 0.012**
Relative	1.23 ± 0.05	1.16 ± 0.05	1.17 ± 0.04	1.18 ± 0.03	1.23 ± 0.03	1.58 ± 0.04**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	193 ± 3	193 ± 5	186 ± 5	199 ± 3	191 ± 3	183 ± 4
Heart						
Absolute	0.649 ± 0.018	0.650 ± 0.009	0.631 ± 0.016	0.665 ± 0.007	0.648 ± 0.007	0.631 ± 0.016
Relative	3.36 ± 0.06	3.38 ± 0.05	3.39 ± 0.04	3.34 ± 0.04	3.39 ± 0.03	3.44 ± 0.04
Right kidney						
Absolute	0.680 ± 0.008	0.711 ± 0.008	0.684 ± 0.023	0.707 ± 0.010	0.715 ± 0.007	0.719 ± 0.017
Relative	3.53 ± 0.03	3.71 ± 0.08	3.67 ± 0.06	3.55 ± 0.05	3.75 ± 0.06**	3.93 ± 0.04**
Liver						
Absolute	6.371 ± 0.155	6.500 ± 0.153	6.038 ± 0.236	7.015 ± 0.271	6.292 ± 0.138	6.149 ± 0.187
Relative	33.01 ± 0.50	33.79 ± 0.51	32.42 ± 0.79	35.22 ± 1.26	32.92 ± 0.55	33.55 ± 0.54
Lungs						
Absolute	1.095 ± 0.032	1.076 ± 0.019	1.082 ± 0.036	1.318 ± 0.023**	1.448 ± 0.027**	1.670 ± 0.049**
Relative	5.68 ± 0.15	5.61 ± 0.13	5.82 ± 0.13	6.62 ± 0.09**	7.58 ± 0.11**	9.12 ± 0.17**
Spleen						
Absolute	0.394 ± 0.011	0.405 ± 0.012	0.384 ± 0.010	0.439 ± 0.023	0.410 ± 0.009	0.415 ± 0.008
Relative	2.04 ± 0.04	2.11 ± 0.06	2.07 ± 0.03	2.21 ± 0.11	2.15 ± 0.04	2.27 ± 0.04*
Thymus						
Absolute	0.283 ± 0.013	0.293 ± 0.010[2]	0.291 ± 0.016	0.299 ± 0.009	0.342 ± 0.010**	0.339 ± 0.018**
Relative	1.47 ± 0.07	1.52 ± 0.03[2]	1.56 ± 0.06	1.50 ± 0.03	1.79 ± 0.06**	1.84 ± 0.07**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] n=9.

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

TABLE A3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 2-Week Study of Cadmium Oxide[1]

	0 mg/m^3	0.1 mg/m^3	0.3 mg/m^3	1 mg/m^3	3 mg/m^3	10 mg/m^3
MALE						
n	5	5	5	5	5	0
Necropsy body wt	27.8 ± 0.3	28.6 ± 0.1	27.4 ± 0.6	28.2 ± 0.5	26.4 ± 0.4	)[2]
Heart						
Absolute	0.156 ± 0.007	0.146 ± 0.010	0.132 ± 0.007	0.156 ± 0.010	0.142 ± 0.006	)
Relative	5.61 ± 0.30	5.10 ± 0.34	4.81 ± 0.18	5.51 ± 0.25	5.38 ± 0.20	)
Right kidney						
Absolute	0.278 ± 0.008	0.294 ± 0.009	0.302 ± 0.012	0.300 ± 0.016	0.284 ± 0.007	)
Relative	9.98 ± 0.19	10.27 ± 0.30	11.03 ± 0.33	10.63 ± 0.55	10.76 ± 0.22	)
Liver						
Absolute	1.732 ± 0.019	1.764 ± 0.053	1.738 ± 0.070	1.878 ± 0.083	1.742 ± 0.075	)
Relative	62.24 ± 0.84	61.61 ± 1.62	63.46 ± 1.72	66.44 ± 2.00	65.91 ± 2.00	)
Lungs						
Absolute	0.212 ± 0.006	0.240 ± 0.007	0.258 ± 0.009**	0.310 ± 0.015**	0.368 ± 0.012**	)
Relative	7.62 ± 0.24	8.38 ± 0.22	9.42 ± 0.15**	10.98 ± 0.46**	13.94 ± 0.37**	)
Spleen						
Absolute	0.070 ± 0.000	0.076 ± 0.002	0.072 ± 0.004	0.072 ± 0.002	0.072 ± 0.004	)
Relative	2.52 ± 0.03	2.66 ± 0.09	2.63 ± 0.13	2.55 ± 0.07	2.73 ± 0.13	)
Right testis						
Absolute	0.114 ± 0.002	0.103 ± 0.005	0.111 ± 0.003	0.106 ± 0.005	0.111 ± 0.005	)
Relative	4.09 ± 0.09	3.61 ± 0.16	4.05 ± 0.09	3.76 ± 0.20	4.20 ± 0.22	)
Thymus						
Absolute	0.050 ± 0.005	0.054 ± 0.005	0.048 ± 0.004	0.053 ± 0.004	0.045 ± 0.003	)
Relative	1.81 ± 0.18	1.90 ± 0.19	1.76 ± 0.17	1.88 ± 0.18	1.71 ± 0.11	)
FEMALE						
n	5	5	5	5	5	0
Necropsy body wt	24.5 ± 0.4	24.1 ± 0.2	23.4 ± 0.3	22.7 ± 0.7**	22.9 ± 0.3**	)
Heart						
Absolute	0.132 ± 0.008	0.130 ± 0.003	0.120 ± 0.003	0.116 ± 0.004	0.118 ± 0.006	)
Relative	5.42 ± 0.41	5.40 ± 0.09	5.12 ± 0.12	5.11 ± 0.09	5.15 ± 0.27	)
Right kidney						
Absolute	0.202 ± 0.006	0.216 ± 0.007	0.212 ± 0.007	0.212 ± 0.007	0.216 ± 0.005	)
Relative	8.25 ± 0.20	8.98 ± 0.25*	9.05 ± 0.25*	9.36 ± 0.26**	9.42 ± 0.20**	)
Liver						
Absolute	1.580 ± 0.033	1.522 ± 0.022	1.434 ± 0.060	1.420 ± 0.052	1.452 ± 0.042	)
Relative	64.55 ± 1.05	63.27 ± 0.90	61.20 ± 2.25	62.63 ± 1.54	63.29 ± 1.62	)
Lungs						
Absolute	0.206 ± 0.007	0.222 ± 0.008	0.260 ± 0.004**	0.300 ± 0.008**	0.354 ± 0.007**	)
Relative	8.42 ± 0.28	9.22 ± 0.28*	11.10 ± 0.12**	13.25 ± 0.35**	15.43 ± 0.20**	)
Spleen						
Absolute	0.090 ± 0.004	0.092 ± 0.004	0.088 ± 0.002	0.090 ± 0.004	0.096 ± 0.005	)
Relative	3.67 ± 0.16	3.82 ± 0.14	3.76 ± 0.09	3.96 ± 0.11	4.18 ± 0.21	)
Thymus						
Absolute	0.078 ± 0.004	0.081 ± 0.002	0.076 ± 0.007	0.077 ± 0.010	0.083 ± 0.005	)
Relative	3.18 ± 0.14	3.35 ± 0.09	3.26 ± 0.31	3.44 ± 0.47	3.63 ± 0.21	)

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] All rats in the 10 mg/m^3 groups died between Day 2 and Day 7.

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

TABLE A4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Study of Cadmium Oxide[1]

	0 mg/m^3	0.025 mg/m^3	0.05 mg/m^3	0.1 mg/m^3	0.25 mg/m^3	1 mg/m^3
MALE						
n	9	10	10	10	10	10
Necropsy body wt	34.0 ± 1.0	35.4 ± 0.7	34.8 ± 0.6	34.4 ± 0.5	35.1 ± 0.7	34.0 ± 0.7
Heart						
Absolute	0.154 ± 0.005	0.159 ± 0.004	0.156 ± 0.002	0.148 ± 0.002	0.162 ± 0.004	0.157 ± 0.004
Relative	4.58 ± 0.21	4.50 ± 0.12	4.49 ± 0.07	4.30 ± 0.05	4.62 ± 0.11	4.62 ± 0.11
Right kidney						
Absolute	0.303 ± 0.008	0.334 ± 0.007**	0.340 ± 0.007**	0.344 ± 0.008**	0.364 ± 0.007**	0.352 ± 0.006**
Relative	8.98 ± 0.28	9.46 ± 0.17	9.82 ± 0.32*	9.99 ± 0.21**	10.39 ± 0.23**	10.38 ± 0.19**
Liver						
Absolute	1.561 ± 0.065	1.601 ± 0.035	1.684 ± 0.051	1.641 ± 0.054	1.727 ± 0.051*	1.763 ± 0.040**
Relative	46.00 ± 1.40	45.42 ± 1.33	48.42 ± 1.06	47.59 ± 1.18	49.24 ± 1.24	51.90 ± 0.92**
Lungs						
Absolute	0.218 ± 0.010	0.246 ± 0.014	0.254 ± 0.008*	0.334 ± 0.009**	0.372 ± 0.008**	0.452 ± 0.009**
Relative	6.43 ± 0.28	6.94 ± 0.31	7.32 ± 0.24*	9.70 ± 0.26**	10.63 ± 0.29**	13.32 ± 0.22**
Spleen						
Absolute	0.073 ± 0.003	0.071 ± 0.003	0.074 ± 0.002	0.091 ± 0.002**	0.104 ± 0.004**	0.103 ± 0.004**
Relative	2.19 ± 0.15	2.01 ± 0.06	2.13 ± 0.04	2.64 ± 0.07**	2.97 ± 0.11**	3.03 ± 0.10**
Right testis						
Absolute	0.122 ± 0.003	0.122 ± 0.003	0.122 ± 0.003	0.124 ± 0.002	0.126 ± 0.003	0.124 ± 0.002
Relative	3.59 ± 0.08	3.45 ± 0.11	3.50 ± 0.08	3.59 ± 0.07	3.61 ± 0.10	3.65 ± 0.07
Thymus						
Absolute	0.040 ± 0.005	0.049 ± 0.002*	0.050 ± 0.003*	0.049 ± 0.003*	0.054 ± 0.002**	0.051 ± 0.003**
Relative	1.14 ± 0.13	1.40 ± 0.05*	1.43 ± 0.08*	1.41 ± 0.08*	1.54 ± 0.06**	1.49 ± 0.08**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	29.1 ± 0.6	31.4 ± 0.9	32.2 ± 0.9*	30.0 ± 0.5	28.8 ± 0.6	30.6 ± 0.7
Heart						
Absolute	0.137 ± 0.005	0.137 ± 0.003	0.140 ± 0.002	0.133 ± 0.002	0.132 ± 0.003	0.136 ± 0.003
Relative	4.74 ± 0.24	4.38 ± 0.13	4.37 ± 0.11	4.44 ± 0.06	4.59 ± 0.04	4.45 ± 0.12
Right kidney						
Absolute	0.214 ± 0.009	0.231 ± 0.005*	0.251 ± 0.005**	0.234 ± 0.004**	0.238 ± 0.006**	0.241 ± 0.005**
Relative	7.38 ± 0.30	7.39 ± 0.20	7.83 ± 0.19	7.82 ± 0.11	8.28 ± 0.15*	7.88 ± 0.16*
Liver						
Absolute	1.364 ± 0.025	1.502 ± 0.055*	1.708 ± 0.050**	1.466 ± 0.042**	1.509 ± 0.059**	1.543 ± 0.048**
Relative	47.08 ± 1.23	47.86 ± 1.29	53.09 ± 0.93*	48.89 ± 0.83*	52.30 ± 1.11*	50.41 ± 1.33*
Lungs						
Absolute	0.213 ± 0.013	0.241 ± 0.013	0.258 ± 0.007*	0.308 ± 0.009**	0.363 ± 0.014**	0.470 ± 0.014**
Relative	7.40 ± 0.57	7.69 ± 0.40	8.05 ± 0.24	10.29 ± 0.27**	12.59 ± 0.31**	15.37 ± 0.40**
Spleen						
Absolute	0.086 ± 0.005	0.101 ± 0.004[2]	0.114 ± 0.006*	0.116 ± 0.006*	0.152 ± 0.016**	0.152 ± 0.008**
Relative	2.97 ± 0.18	3.23 ± 0.09[2]	3.55 ± 0.16	3.87 ± 0.19*	5.29 ± 0.57**	4.97 ± 0.24**
Thymus						
Absolute	0.056 ± 0.002	0.063 ± 0.002	0.061 ± 0.004	0.056 ± 0.002	0.056 ± 0.002	0.071 ± 0.003**
Relative	1.92 ± 0.06	2.01 ± 0.05	1.90 ± 0.10	1.87 ± 0.07	1.94 ± 0.05	2.33 ± 0.07**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] n=9.

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

APPENDIX B

Hematology, Clinical Chemistry, and Urinalysis Results

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

	0 mg/m^3	0.025 mg/m^3	0.05 mg/m^3	0.1 mg/m^3	0.25 mg/m^3	1 mg/m^3
MALE						
n						
Day 4	10	10	9	10	10	10
Day 24	10	10	10	10	10	10
Week 13	10	10	10	10	10	10
Hematocrit (automated) (%)						
Day 4	42.1 ± 0.4	42.5 ± 0.4	42.3 ± 0.4	42.0 ± 0.5	41.6 ± 0.4	42.1 ± 0.4
Day 24	46.8 ± 0.2	45.3 ± 0.4*	45.1 ± 0.4**	45.5 ± 0.3**	45.4 ± 0.4**	45.2 ± 0.3**
Week 13	45.7 ± 0.5	45.1 ± 0.5	46.1 ± 0.5	45.8 ± 0.3	45.7 ± 0.3	45.6 ± 0.4
Hematocrit (manual) (%)						
Day 4	42.7 ± 0.4	42.7 ± 0.5	43.2 ± 0.5	43.0 ± 0.4	42.8 ± 0.5	43.5 ± 0.4
Day 24	46.8 ± 0.3	45.5 ± 0.5	45.0 ± 0.4**	45.7 ± 0.3	45.5 ± 0.4	45.6 ± 0.3
Week 13	44.3 ± 0.4	44.5 ± 0.4	44.7 ± 0.7	44.3 ± 0.4	44.7 ± 0.4	44.2 ± 0.6
Hemoglobin (g/dL)						
Day 4	13.4 ± 0.1	13.5 ± 0.1	13.5 ± 0.1	13.5 ± 0.2	13.2 ± 0.2	13.4 ± 0.1
Day 24	15.0 ± 0.1	14.5 ± 0.1*	14.5 ± 0.1*	14.6 ± 0.2*	14.6 ± 0.1*	14.5 ± 0.1**
Week 13	14.3 ± 0.1	14.1 ± 0.2	14.3 ± 0.2	14.3 ± 0.1	14.3 ± 0.1	14.3 ± 0.2
Erythrocytes (10^6/µL)						
Day 4	7.13 ± 0.09	7.28 ± 0.09	7.14 ± 0.10	7.20 ± 0.13	7.12 ± 0.12	7.19 ± 0.10
Day 24	8.10 ± 0.05	8.05 ± 0.09	7.93 ± 0.07	8.09 ± 0.08	8.08 ± 0.07	8.17 ± 0.06
Week 13	8.59 ± 0.07	8.53 ± 0.08	8.70 ± 0.11	8.67 ± 0.06	8.66 ± 0.06	8.75 ± 0.08
Reticulocytes (10^6/µL)						
Day 4	0.44 ± 0.03	0.44 ± 0.02	0.46 ± 0.02	0.47 ± 0.02	0.51 ± 0.02	0.53 ± 0.01*
Day 24	0.24 ± 0.01	0.22 ± 0.02	0.24 ± 0.01	0.23 ± 0.02	0.21 ± 0.01	0.23 ± 0.01
Week 13	0.21 ± 0.01	0.21 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.00
Nucleated erythrocytes (10^3/µL)						
Day 4	0.13 ± 0.02	0.12 ± 0.04	0.12 ± 0.03	0.10 ± 0.03	0.14 ± 0.03	0.22 ± 0.04
Day 24	0.03 ± 0.02	0.05 ± 0.03	0.03 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.02
Week 13	0.05 ± 0.02	0.04 ± 0.02	0.07 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.07 ± 0.03
Mean cell volume (fL)						
Day 4	59.0 ± 0.3	58.3 ± 0.3	59.3 ± 0.4	58.4 ± 0.5	58.5 ± 0.4	58.7 ± 0.4
Day 24	57.7 ± 0.2	56.3 ± 0.2**	56.7 ± 0.3**	56.3 ± 0.3**	56.1 ± 0.3**	55.5 ± 0.4**
Week 13	53.2 ± 0.2	52.7 ± 0.2	52.9 ± 0.2	52.8 ± 0.2	52.7 ± 0.2	52.1 ± 0.2**
Mean cell hemoglobin (pg)						
Day 4	18.8 ± 0.1	18.6 ± 0.1	18.9 ± 0.1	18.7 ± 0.1	18.6 ± 0.1	18.7 ± 0.2
Day 24	18.5 ± 0.1	18.1 ± 0.1**	18.3 ± 0.1*	18.1 ± 0.1**	18.1 ± 0.1**	17.8 ± 0.1**
Week 13	16.6 ± 0.1	16.5 ± 0.1	16.5 ± 0.1	16.5 ± 0.1	16.5 ± 0.1	16.3 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	31.8 ± 0.1	31.9 ± 0.1	31.8 ± 0.1	32.0 ± 0.1	31.8 ± 0.1	31.9 ± 0.1
Day 24	32.1 ± 0.1	32.1 ± 0.1	32.2 ± 0.1	32.1 ± 0.1	32.2 ± 0.1	32.1 ± 0.2
Week 13	31.2 ± 0.1	31.3 ± 0.1	31.1 ± 0.1	31.1 ± 0.1	31.3 ± 0.1	31.3 ± 0.1
Platelets (10^3/µL)						
Day 4	813.1 ± 19.1	766.4 ± 7.5	797.7 ± 19.2	762.0 ± 13.5	798.4 ± 26.0	814.9 ± 15.6
Day 24	636.1 ± 14.7	656.4 ± 10.9	637.3 ± 12.3	666.5 ± 12.8	627.3 ± 15.6	644.2 ± 8.5
Week 13	496.7 ± 9.7	484.0 ± 12.5	477.0 ± 15.1	454.9 ± 14.3	480.3 ± 16.0	480.8 ± 9.6
Leukocytes (10^3/µL)						
Day 4	7.61 ± 0.21	8.52 ± 0.22	7.27 ± 0.27	7.59 ± 0.33	6.64 ± 0.25*	6.33 ± 0.30*
Day 24	10.10 ± 0.47	9.34 ± 0.34	8.66 ± 0.34*	9.20 ± 0.16*	8.78 ± 0.22*	8.27 ± 0.31**
Week 13	3.87 ± 0.31	3.89 ± 0.37	3.56 ± 0.45	4.13 ± 0.33	4.23 ± 0.34	4.93 ± 0.45
Segmented neutrophils (10^3/µL)						
Day 4	1.17 ± 0.09	1.02 ± 0.09	0.99 ± 0.09	1.12 ± 0.11	1.01 ± 0.07	1.04 ± 0.10
Day 24	1.22 ± 0.15	1.02 ± 0.08	1.05 ± 0.07	1.26 ± 0.12	1.36 ± 0.13	1.37 ± 0.20
Week 13	0.67 ± 0.09	0.73 ± 0.08	0.97 ± 0.12*	1.04 ± 0.10**	0.91 ± 0.09*	1.22 ± 0.16**

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide (continued)

	0 mg/m^3	0.025 mg/m^3	0.05 mg/m^3	0.1 mg/m^3	0.25 mg/m^3	1 mg/m^3
MALE (continued)						
Lymphocytes (10^3/µL)						
Day 4	6.41 ± 0.21	7.47 ± 0.24	6.26 ± 0.26	6.47 ± 0.30	5.63 ± 0.21	5.28 ± 0.26*
Day 24	8.82 ± 0.41	8.29 ± 0.33	7.53 ± 0.33*	7.91 ± 0.19*	7.38 ± 0.20**	6.85 ± 0.30**
Week 13	3.18 ± 0.24	3.13 ± 0.33	2.56 ± 0.35	3.05 ± 0.28	3.29 ± 0.28	3.69 ± 0.36
Monocytes (10^3/µL)						
Day 4	0.02 ± 0.01	0.04 ± 0.02	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Day 24	0.03 ± 0.02	0.00 ± 0.00	0.04 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Week 13	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Eosinophils (10^3/µL)						
Day 4	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Day 24	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.05 ± 0.02
Week 13	0.01 ± 0.00	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Total bone marrow cellularity (10^6/femur)						
Week 13	117.1 ± 6.5[2]	)[3]	)	111.0 ± 4.8[2]	124.4 ± 6.6[2]	118.7 ± 3.3[2]
FEMALE						
n						
Day 4	10	10	10	10	10	10
Day 24	10	10	9	10	10	10
Week 13	10	10	10	10	10	10
Hematocrit (automated) (%)						
Day 4	45.3 ± 0.5	45.0 ± 0.6	45.2 ± 0.6	45.1 ± 0.4	44.0 ± 0.6	43.8 ± 0.2*
Day 24	49.2 ± 0.5	49.7 ± 0.3	49.1 ± 0.3	49.2 ± 0.4	48.2 ± 0.3	47.6 ± 0.3**
Week 13	43.2 ± 0.7	44.3 ± 0.3	44.0 ± 0.4	43.4 ± 0.9	44.3 ± 0.5	44.2 ± 0.5
Hematocrit (manual) (%)						
Day 4	45.2 ± 0.5	45.1 ± 0.4	45.8 ± 0.6	45.5 ± 0.4	44.3 ± 0.5	44.0 ± 0.3
Day 24	47.5 ± 0.5	47.9 ± 0.3	47.9 ± 0.3	47.3 ± 0.4	47.2 ± 0.5	46.8 ± 0.3
Week 13	41.3 ± 0.6	43.2 ± 0.3*	43.0 ± 0.5*	42.3 ± 0.9*	43.2 ± 0.4*	43.4 ± 0.7*
Hemoglobin (g/dL)						
Day 4	14.4 ± 0.2	14.5 ± 0.2	14.4 ± 0.2	14.7 ± 0.2	14.1 ± 0.2	14.1 ± 0.1
Day 24	15.5 ± 0.2	15.8 ± 0.1	15.5 ± 0.1	15.6 ± 0.1	15.3 ± 0.1	15.1 ± 0.1
Week 13	13.9 ± 0.2	14.1 ± 0.1	14.0 ± 0.1	13.9 ± 0.3	14.1 ± 0.2	14.0 ± 0.2
Erythrocytes (10^6/µL)						
Day 4	7.79 ± 0.10	7.79 ± 0.15	7.58 ± 0.12	7.76 ± 0.09	7.45 ± 0.13	7.45 ± 0.07*
Day 24	8.30 ± 0.08	8.49 ± 0.08	8.29 ± 0.06	8.54 ± 0.06	8.29 ± 0.05	8.31 ± 0.09
Week 13	7.79 ± 0.12	7.91 ± 0.05	7.88 ± 0.07	7.77 ± 0.16	7.95 ± 0.09	8.03 ± 0.10
Reticulocytes (10^6/µL)						
Day 4	0.36 ± 0.02	0.37 ± 0.02	0.35 ± 0.02	0.35 ± 0.02	0.41 ± 0.02	0.42 ± 0.01*
Day 24	0.14 ± 0.01	0.15 ± 0.01	0.19 ± 0.01**	0.19 ± 0.01**	0.23 ± 0.01**	0.21 ± 0.01**
Week 13	0.12 ± 0.02	0.15 ± 0.01	0.14 ± 0.01	0.12 ± 0.02	0.13 ± 0.01	0.14 ± 0.02
Nucleated erythrocytes (10^3/µL)						
Day 4	0.16 ± 0.04	0.17 ± 0.03	0.14 ± 0.04	0.15 ± 0.04	0.20 ± 0.04	0.09 ± 0.02
Day 24	0.06 ± 0.04	0.05 ± 0.03	0.08 ± 0.03	0.01 ± 0.01	0.04 ± 0.02	0.04 ± 0.02
Week 13	0.03 ± 0.01	0.04 ± 0.01	0.06 ± 0.02	0.09 ± 0.03	0.10 ± 0.03	0.07 ± 0.02
Mean cell volume (fL)						
Day 4	58.1 ± 0.3	57.8 ± 0.3	59.6 ± 0.4**	58.1 ± 0.3	59.1 ± 0.4	58.9 ± 0.4
Day 24	59.2 ± 0.3	58.6 ± 0.5	59.1 ± 0.2	57.6 ± 0.4**	58.0 ± 0.2**	57.3 ± 0.5**
Week 13	55.5 ± 0.3	55.9 ± 0.2	55.9 ± 0.2	56.0 ± 0.2	55.8 ± 0.1	55.0 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	18.5 ± 0.1	18.6 ± 0.1	19.0 ± 0.1**	18.9 ± 0.1*	19.0 ± 0.1*	19.0 ± 0.1*
Day 24	18.6 ± 0.1	18.7 ± 0.2	18.7 ± 0.1	18.3 ± 0.1	18.4 ± 0.1	18.2 ± 0.1*
Week 13	17.8 ± 0.1	17.8 ± 0.1	17.8 ± 0.1	17.9 ± 0.1	17.7 ± 0.1	17.5 ± 0.1**

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide (continued)

	0 mg/m^3	0.025 mg/m^3	0.05 mg/m^3	0.1 mg/m^3	0.25 mg/m^3	1 mg/m^3
FEMALE (continued)						
Mean cell hemoglobin concentration (g/dL)						
Day 4	31.9 ± 0.2	32.2 ± 0.1	31.9 ± 0.1	32.5 ± 0.1**	32.1 ± 0.1*	32.2 ± 0.1
Day 24	31.4 ± 0.2	31.9 ± 0.1*	31.6 ± 0.1	31.8 ± 0.2	31.7 ± 0.1	31.7 ± 0.1
Week 13	32.2 ± 0.2	31.8 ± 0.1	31.9 ± 0.1	31.9 ± 0.2	31.8 ± 0.1	31.8 ± 0.1
Platelets (10^3/µL)						
Day 4	779.9 ± 16.2	733.7 ± 25.8	762.2 ± 12.6	763.6 ± 20.0	774.3 ± 24.7	820.2 ± 20.1
Day 24	604.3 ± 10.4	612.5 ± 12.5	621.9 ± 10.5	630.7 ± 10.8	643.2 ± 13.4*	631.0 ± 10.9*
Week 13	521.2 ± 15.6	533.1 ± 8.7	524.2 ± 12.5	574.5 ± 60.0	521.9 ± 8.1	523.5 ± 6.5
Leukocytes (10^3/µL)						
Day 4	9.79 ± 0.40	9.55 ± 0.32	9.92 ± 0.41	8.56 ± 0.37*	7.56 ± 0.27**	7.95 ± 0.42**
Day 24	12.85 ± 0.40	12.90 ± 0.20	12.13 ± 0.35	12.54 ± 0.46	11.52 ± 0.47*	10.18 ± 0.32**
Week 13	4.37 ± 0.86	5.64 ± 0.84	4.73 ± 0.68	4.81 ± 0.91	5.21 ± 0.72	5.40 ± 1.04
Segmented neutrophils (10^3/µL)						
Day 4	0.89 ± 0.13	1.06 ± 0.11	1.31 ± 0.13	0.84 ± 0.07	0.91 ± 0.06	1.08 ± 0.07
Day 24	1.15 ± 0.15	1.51 ± 0.16	1.36 ± 0.19	1.51 ± 0.18	1.20 ± 0.10	1.01 ± 0.12
Week 13	0.95 ± 0.22	1.01 ± 0.19	1.04 ± 0.15	1.00 ± 0.25	0.95 ± 0.13	1.08 ± 0.17
Lymphocytes (10^3/µL)						
Day 4	8.86 ± 0.40	8.40 ± 0.29	8.60 ± 0.39	7.69 ± 0.36	6.63 ± 0.32**	6.88 ± 0.46**
Day 24	11.59 ± 0.26	11.31 ± 0.16	10.72 ± 0.31*	10.96 ± 0.30	10.19 ± 0.43**	9.02 ± 0.40**
Week 13	3.36 ± 0.69	4.58 ± 0.67	3.63 ± 0.55	3.72 ± 0.79	4.23 ± 0.63	4.25 ± 0.88
Monocytes (10^3/µL)						
Day 4	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
Day 24	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
Week 13	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.01
Eosinophils (10^3/µL)						
Day 4	0.03 ± 0.02	0.10 ± 0.03	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.02	0.00 ± 0.00
Day 24	0.10 ± 0.04	0.08 ± 0.04	0.04 ± 0.02	0.06 ± 0.03	0.12 ± 0.03	0.14 ± 0.05
Week 13	0.05 ± 0.02	0.04 ± 0.01	0.06 ± 0.02	0.08 ± 0.02	0.03 ± 0.02	0.03 ± 0.03
Total bone marrow cellularity (10^6/femur)						
Week 13	76.9 ± 3.1[2]	)	)	80.4 ± 4.5[2]	78.9 ± 5.4[2]	83.1 ± 4.4[2]

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[2] n=5.
[3] Not measured for this exposure group.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

TABLE B2 Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

	0 mg/m^3	0.025 mg/m^3	0.05 mg/m^3	0.1 mg/m^3	0.25 mg/m^3	1 mg/m^3
MALE						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	13.4 ± 0.3	14.0 ± 0.6	12.8 ± 0.5	12.5 ± 0.3	12.6 ± 0.3	13.5 ± 0.3
Day 24	14.6 ± 0.4	13.3 ± 0.5	14.1 ± 0.4	13.5 ± 0.3	13.9 ± 0.3	14.3 ± 0.6
Week 13	20.4 ± 1.1	21.4 ± 0.8	19.7 ± 0.7	20.8 ± 0.7	21.9 ± 0.9	20.1 ± 0.6
Creatinine (mg/dL)						
Day 4	0.61 ± 0.01	0.61 ± 0.01	0.61 ± 0.01	0.61 ± 0.01	0.61 ± 0.01	0.64 ± 0.02
Day 24	0.62 ± 0.01	0.65 ± 0.02	0.70 ± 0.02*	0.68 ± 0.01	0.64 ± 0.02	0.67 ± 0.02
Week 13	0.78 ± 0.02	0.79 ± 0.01	0.76 ± 0.02	0.77 ± 0.02	0.76 ± 0.03	0.73 ± 0.02
Unbound iron-binding capacity (µg/dL)						
Week 13	486.9 ± 8.1	)[2]	)	489.9 ± 3.1	502.0 ± 7.0	493.5 ± 9.2
Iron (µg/dL)						
Week 13	162.3 ± 2.8	)	)	163.2 ± 5.3	170.5 ± 5.7	180.4 ± 6.5
Total iron-binding capacity (µg/dL)						
Week 13	649.2 ± 7.2	)	)	653.1 ± 6.3	672.5 ± 5.8*	673.9 ± 9.6*
Total protein (g/dL)						
Day 4	5.9 ± 0.1	5.9 ± 0.0	5.7 ± 0.1	5.6 ± 0.0**	5.9 ± 0.1	5.8 ± 0.1
Day 24	6.5 ± 0.1	6.3 ± 0.1*	6.2 ± 0.1**	6.4 ± 0.1*	6.2 ± 0.1**	6.3 ± 0.0**
Week 13	7.1 ± 0.1	7.2 ± 0.1	7.0 ± 0.1	6.9 ± 0.1	7.2 ± 0.1	6.9 ± 0.1
Albumin (g/dL)						
Day 4	4.6 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.0	4.8 ± 0.1*	4.7 ± 0.1
Day 24	4.7 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.9 ± 0.1
Week 13	4.9 ± 0.1	5.0 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	4.6 ± 0.1
Globulin (g/dL)						
Day 4	1.3 ± 0.0	1.4 ± 0.0	1.2 ± 0.0*	1.1 ± 0.0**	1.2 ± 0.1**	1.1 ± 0.1**
Day 24	1.8 ± 0.1	1.8 ± 0.0	1.7 ± 0.0	1.8 ± 0.1	1.4 ± 0.1**	1.4 ± 0.1**
Week 13	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.3 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	40 ± 1	36 ± 1*	41 ± 1	39 ± 1	38 ± 1	40 ± 0
Day 24	35 ± 1	35 ± 1	34 ± 1	34 ± 2	32 ± 1	36 ± 1
Week 13	59 ± 3	55 ± 3	58 ± 4	53 ± 2[3]	55 ± 3	57 ± 2
Alkaline phosphatase (IU/L)						
Day 4	746 ± 24	734 ± 23	738 ± 15	711 ± 24	713 ± 19	692 ± 18
Day 24	517 ± 16	508 ± 11	495 ± 10	472 ± 11*	471 ± 12*	485 ± 9
Week 13	378 ± 14	393 ± 9	365 ± 13	369 ± 8	397 ± 18	388 ± 9
Creatine kinase (IU/L)						
Day 4	364 ± 24	261 ± 16**	296 ± 33	289 ± 23	268 ± 18*	311 ± 16
Day 24	276 ± 23	285 ± 22	242 ± 16	217 ± 17	227 ± 14	255 ± 21
Week 13	99 ± 8	97 ± 8	100 ± 8	109 ± 17[3]	114 ± 14	124 ± 16
Sorbitol dehydrogenase (IU/L)						
Day 4	13 ± 0	13 ± 0	14 ± 1*	14 ± 0*	14 ± 0*	14 ± 0**
Day 24	16 ± 0	16 ± 1	17 ± 0	16 ± 1	16 ± 0	16 ± 1
Week 13	22 ± 2	23 ± 2	22 ± 2	19 ± 1[3]	21 ± 2	21 ± 2
Bile acids (µmol/L)						
Day 4	20.7 ± 1.6	20.9 ± 1.4	23.5 ± 1.5	18.4 ± 1.1	18.2 ± 0.9	22.7 ± 2.0
Day 24	18.7 ± 0.9	17.1 ± 1.4	15.6 ± 0.8	16.1 ± 0.5	17.3 ± 0.7	18.2 ± 0.6
Week 13	17.6 ± 0.2[3]	19.2 ± 0.8	17.3 ± 0.8	17.7 ± 1.2	19.1 ± 0.9	22.5 ± 1.9*

TABLE B2 Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide (continued)

	0 mg/m^3	0.025 mg/m^3	0.05 mg/m^3	0.1 mg/m^3	0.25 mg/m^3	1 mg/m^3
FEMALE						
n						
Day 4	10	10	10	10	10	10
Day 24	10	10	9	10	10	10
Week 13	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	16.2 ± 0.5	15.8 ± 0.8	15.9 ± 0.5	15.8 ± 0.5	13.2 ± 0.4**	13.2 ± 0.4**
Day 24	15.1 ± 0.6	14.8 ± 0.4	15.6 ± 0.6	14.5 ± 0.3	14.6 ± 0.5	13.9 ± 0.3
Week 13	23.0 ± 0.8	23.5 ± 0.8	22.2 ± 1.0	23.1 ± 0.8	21.2 ± 1.0	21.1 ± 0.9
Creatinine (mg/dL)						
Day 4	0.58 ± 0.02	0.56 ± 0.02	0.52 ± 0.01	0.53 ± 0.02	0.60 ± 0.00	0.57 ± 0.02
Day 24	0.70 ± 0.02	0.67 ± 0.02	0.67 ± 0.02	0.67 ± 0.02	0.62 ± 0.01**	0.63 ± 0.02**
Week 13	0.74 ± 0.02	0.70 ± 0.02	0.73 ± 0.03	0.74 ± 0.02	0.73 ± 0.02	0.69 ± 0.03
Unbound iron-binding capacity (µg/dL)						
Week 13	289.8 ± 15.7	)	)	339.7 ± 5.9*	331.8 ± 9.4	360.9 ± 9.6**
Iron (µg/dL)						
Week 13	283.3 ± 10.1	)	)	236.2 ± 13.6*	251.5 ± 9.1*	237.1 ± 10.8**
Total iron-binding capacity (µg/dL)						
Week 13	573.1 ± 8.1	)	)	575.9 ± 13.5	583.3 ± 8.9	598.0 ± 9.8
Total protein (g/dL)						
Day 4	6.0 ± 0.1	5.9 ± 0.0*	5.9 ± 0.1	5.9 ± 0.1*	5.6 ± 0.1**	5.5 ± 0.0**
Day 24	6.4 ± 0.1	6.2 ± 0.1	6.2 ± 0.1*	6.1 ± 0.0**	6.1 ± 0.1**	6.1 ± 0.1**
Week 13	7.0 ± 0.1	6.8 ± 0.1	6.9 ± 0.1	6.9 ± 0.2	6.8 ± 0.1	6.7 ± 0.1
Albumin (g/dL)						
Day 4	4.5 ± 0.1	4.3 ± 0.1*	4.3 ± 0.1	4.3 ± 0.1**	4.3 ± 0.1**	4.2 ± 0.0**
Day 24	4.4 ± 0.1	4.3 ± 0.0	4.5 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	4.3 ± 0.1
Week 13	4.8 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	4.7 ± 0.2	4.8 ± 0.1	4.7 ± 0.1
Globulin (g/dL)						
Day 4	1.5 ± 0.0	1.5 ± 0.0	1.6 ± 0.0	1.6 ± 0.1	1.3 ± 0.0**	1.3 ± 0.0**
Day 24	2.0 ± 0.1	2.0 ± 0.1	1.7 ± 0.1**	1.6 ± 0.1**	1.8 ± 0.0**	1.8 ± 0.1*
Week 13	2.2 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.0 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	38 ± 1	38 ± 1	35 ± 1	35 ± 1	35 ± 1	35 ± 1
Day 24	32 ± 1	34 ± 1	33 ± 1	31 ± 1	32 ± 1	33 ± 1
Week 13	47 ± 3	50 ± 6	42 ± 3	47 ± 4	46 ± 2	50 ± 2
Alkaline phosphatase (IU/L)						
Day 4	689 ± 18	654 ± 28	704 ± 15	612 ± 35	674 ± 24	660 ± 17
Day 24	424 ± 11	420 ± 10	413 ± 7	425 ± 7	426 ± 9	380 ± 10*
Week 13	359 ± 12	371 ± 10	374 ± 9	384 ± 24	374 ± 18	375 ± 13
Creatine kinase (IU/L)						
Day 4	435 ± 97	316 ± 32	307 ± 22	355 ± 28	313 ± 48	303 ± 21
Day 24	282 ± 20	271 ± 19	264 ± 40	236 ± 18	234 ± 10	301 ± 27
Week 13	109 ± 13	110 ± 11	98 ± 16	121 ± 20	117 ± 16	147 ± 22
Sorbitol dehydrogenase (IU/L)						
Day 4	15 ± 1	15 ± 0	15 ± 1	15 ± 1	14 ± 0*	14 ± 0**
Day 24	22 ± 1	21 ± 1	21 ± 1	21 ± 1	22 ± 1	21 ± 1
Week 13	17 ± 1	19 ± 2	16 ± 1	20 ± 2	18 ± 0	20 ± 1*
Bile acids (µmol/L)						
Day 4	17.7 ± 1.3[3]	22.1 ± 4.0	20.6 ± 2.2	19.3 ± 1.8	21.5 ± 1.9	19.3 ± 1.2
Day 24	12.1 ± 1.0	14.3 ± 0.9	19.0 ± 2.3*	12.3 ± 1.0	13.9 ± 1.4	15.9 ± 0.8*
Week 13	16.4 ± 1.9	24.0 ± 5.5	21.5 ± 2.2	15.7 ± 1.5	26.7 ± 4.6	22.3 ± 2.5

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[2] Not measured for this exposure group.
[3] n=9.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

TABLE B3 Urinalysis Data for F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

	0 mg/m^3	0.025 mg/m^3	0.05 mg/m^3	0.1 mg/m^3	0.25 mg/m^3	1 mg/m^3
MALE						
n	10	10	10	10	10	10
Creatinine (mg/dL)	69.00 ± 3.73	)[2]	)	70.00 ± 7.59	80.50 ± 7.11	72.50 ± 7.83
Glucose (µg/mg creatinine)	138 ± 3	)	)	140 ± 3	135 ± 3	162 ± 8*
Protein (µg/mg creatinine)	1243 ± 38	)	)	1319 ± 74	1229 ± 72	1348 ± 97
Alkaline phosphatase (mU/mg creatinine)	274 ± 15	)	)	261 ± 12	275 ± 16	288 ± 14
Aspartate aminotransferase (mU/mg creatinine)	31 ± 3	)	)	29 ± 1	29 ± 1	32 ± 2
N-acetyl-β-D-glucosaminidase (mU/mg creatinine)	14 ± 1	)	)	13 ± 1	13 ± 1	14 ± 1
Volume (mL/16 hr)	9.1 ± 0.7	8.3 ± 0.9	9.3 ± 1.8	10.0 ± 1.3	9.5 ± 1.0	9.4 ± 1.7
Concentrated volume (mL)	1.6 ± 0.3	)	)	1.8 ± 0.2	2.1 ± 0.2	1.8 ± 0.3
Specific gravity	1.019 ± 0.001	1.022 ± 0.002	1.022 ± 0.002	1.020 ± 0.002	1.021 ± 0.002	1.021 ± 0.002
Concentrated specific gravity	1.015 ± 0.002[3]	)	)	1.017 ± 0.002	1.016 ± 0.002	1.025 ± 0.004*[3]
FEMALE						
n	10	10	10	10	10	10
Creatinine (mg/dL)	52.60 ± 7.46	)	)	40.90 ± 6.99	40.60 ± 3.49	49.30 ± 4.89
Glucose (µg/mg creatinine)	117.0 ± 3.5	)	)	109 ± 4	105 ± 4	113 ± 3
Protein (µg/mg creatinine)	239 ± 8	)	)	247 ± 11	256 ± 6	251 ± 9
Alkaline phosphatase (mU/mg creatinine)	152 ± 8	)	)	164 ± 15	158 ± 10	185 ± 12
Aspartate aminotransferase (mU/mg creatinine)	7 ± 0[3]	)	)	9 ± 1	9 ± 1*	9 ± 1*
N-acetyl-β-D-glucosaminidase (mU/mg creatinine)	11 ± 0	)	)	10 ± 0	11 ± 1	12 ± 0
Volume (mL/16 hr)	8.6 ± 1.3	9.1 ± 0.7	11.0 ± 1.5	12.9 ± 2.4	10.2 ± 1.1	7.7 ± 1.3
Concentrated volume (mL)	0.9 ± 0.3	)	)	0.7 ± 0.1	0.9 ± 0.2	0.6 ± 0.2
Specific gravity	1.017 ± 0.002	1.015 ± 0.001	1.013 ± 0.001	1.014 ± 0.002	1.014 ± 0.001	1.018 ± 0.002
Concentrated specific gravity	1.020 ± 0.002[4]	)	)	1.023 ± 0.001[5]	1.019 ± 0.002[5]	1.023 ± 0.002[4]

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[2] Not measured for this exposure group.
[3] n=9.
[4] n=7.
[5] n=8.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.

APPENDIX C

Reproductive Tissue Evaluations, Estrous Cycle Characterization, and Developmental Toxicity Studies

TABLE C1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

Study Parameters	0 mg/m^3	0.025 mg/m^3	0.1 mg/m^3	1 mg/m^3
n	10	10	10	10
Weights (g)				
Necropsy body weight	334 ± 5	331 ± 7	322 ± 6	314 ± 5
Left epididymis	0.272 ± 0.005	0.258 ± 0.003	0.265 ± 0.009	0.255 ± 0.005
Left cauda epididymis	0.163 ± 0.003	0.165 ± 0.004	0.162 ± 0.003	0.164 ± 0.004
Left testis	1.44 ± 0.02	1.40 ± 0.02	1.38 ± 0.03	1.38 ± 0.02
Spermatid measurements				
Spermatid heads (10^7/g testis)	12.65 ± 0.24	12.90 ± 0.34	12.31 ± 0.51	10.50 ± 0.40**
Spermatid heads (10^7/testis)	18.16 ± 0.43	17.97 ± 0.47	17.04 ± 0.79	14.42 ± 0.46**
Spermatid count (mean/10^{-4} mL suspension)	90.80 ± 2.13	89.83 ± 2.33	85.18 ± 3.93	72.10 ± 2.31**
Epididymal spermatozoal measurements				
Motility (%)	95.99 ± 0.44	95.44 ± 0.54	95.84 ± 0.31	95.57 ± 0.69
Concentration (10^6/g cauda epididymal tissue)	915 ± 58	999 ± 63	927 ± 34	817 ± 57

[1] Data presented as mean ± standard error. Differences from the control group for necropsy body weights are not significant by Dunnett's test; differences from the control group for epididymal, cauda epididymal, and testicular weights and epididymal spermatozoal measurements are not significant by Dunn's test.

** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE C2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Inhalation Study of Cadmium Oxide[1]

Study Parameters	0 mg/m^3	0.025 mg/m^3	0.1 mg/m^3	1 mg/m^3
n	10	9	10	10
Necropsy body weight (g)	193 ± 3	193 ± 5[2]	199 ± 3	183 ± 4
Estrous cycle length (days)	4.75 ± 0.08	4.56 ± 0.18[3]	4.85 ± 0.13	5.45 ± 0.33*
Estrous stages (% of cycle)				
Diestrus	36.7	36.7	38.3	40.8
Proestrus	13.3	11.7	15.0	10.8
Estrus	29.2	29.2	27.5	27.5
Metestrus	20.8	22.5	18.3	20.0
Uncertain diagnoses	0.0	0.0	0.8	0.8

[1] Necropsy body weight and estrous cycle length presented as mean ± standard error. Differences from the control group for necropsy body weights are not significant by Dunnett's test. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.

[2] n=10.

[3] Estrous cycle longer than 12 days or unclear in 1 of 10 animals.

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.

TABLE C3 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Inhalation Study of Cadmium Oxide[1]

Study Parameters	0 mg/m^3	0.025 mg/m^3	0.1 mg/m^3	1 mg/m^3
n	9	9	10	10
Weights (g)				
Necropsy body weight	34.0 ± 1.0	35.4 ± 0.7[2]	34.4 ± 0.5	34.0 ± 0.7
Left epididymis	0.028 ± 0.001	0.029 ± 0.001	0.029 ± 0.001	0.028 ± 0.001
Left cauda epididymis	0.016 ± 0.001	0.018 ± 0.001	0.017 ± 0.001	0.016 ± 0.001
Left testis	0.116 ± 0.003	0.115 ± 0.004	0.120 ± 0.002	0.117 ± 0.001
Spermatid measurements				
Spermatid heads (10^7/g testis)	18.83 ± 0.62	18.86 ± 1.49	17.46 ± 0.86	19.64 ± 0.55
Spermatid heads (10^7/testis)	2.19 ± 0.10	2.09 ± 0.14[2]	2.10 ± 0.11	2.29 ± 0.06
Spermatid count (mean/10^{-4} mL suspension)	68.53 ± 3.18	65.15 ± 4.48[2]	65.60 ± 3.60	71.68 ± 1.83
Epididymal spermatozoal measurements				
Motility (%)	90.82 ± 1.90	92.14 ± 1.00	93.14 ± 0.90	90.68 ± 1.14
Concentration (10^6/g cauda epididymal tissue)	1299 ± 173	1469 ± 127	1550 ± 90	1408 ± 126

[1] Data presented as mean ± standard error. Differences from the control group for necropsy body weights are not significant by Dunnett's test; differences from the control group for epididymal, cauda epididymal, and testicular weights and spermatid and epididymal spermatozoal measurements are not significant by Dunn's test.
[2] n=10.

TABLE C4 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Inhalation Study of Cadmium Oxide[1]

Study Parameters	0 mg/m^3	0.025 mg/m^3	0.1 mg/m^3	1 mg/m^3
n	10	10	9	10
Necropsy body weight (g)	29.1 ± 0.6	31.4 ± 0.9	30.0 ± 0.5[2]	30.6 ± 0.7
Estrous cycle length (days)	4.10 ± 0.07	4.10 ± 0.07	4.00 ± 0.00[3]	4.15 ± 0.11
Estrous stages (% of cycle)				
Diestrus	28.3	25.0	34.2	30.8
Proestrus	16.7	20.0	19.2	22.5
Estrus	33.3	32.5	26.7	29.2
Metestrus	21.7	22.5	20.0	17.5

[1] Necropsy body weight and estrous cycle length presented as mean ± standard error. Differences from the control group for necropsy body weights are not significant by Dunnett's test; differences from the control group for estrous cycle lengths are not significant by Dunn's test. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.
[2] n=10.
[3] Estrous cycle longer than 12 days or unclear in 1 of 10 animals.

DEVELOPMENTAL TOXICITY STUDIES

Materials and Methods

DEVELOPMENTAL TOXICITY STUDIES

To assess the maternal and developmental toxicity of cadmium oxide, studies were performed in female Sprague-Dawley rats and Swiss (CD-1®) mice. Initially, range-finding studies were conducted in rats and mice to determine the exposure concentrations to be used for the developmental toxicity studies. For the range-finding and developmental toxicity studies, male and female Sprague-Dawley rats and Swiss (CD-1®) mice were obtained from Charles River Laboratories (Raleigh, NC). Rats and mice were approximately 7 to 8 weeks old at receipt and were quarantined for 32 to 33 days before the start of the studies. Blood samples were collected from rats and mice of each sex 3 weeks after receipt and from control and exposed females at the end of the studies; sera were analyzed for antibody titers to rodent viruses and all results were negative. Additional details concerning the study design are presented in Table 1.

For the range-finding studies, two to three females were housed overnight with each male. On the first day of vaginal plug or sperm detection (gestation Day 0), positively mated females were assigned to exposure groups by weight. Breeding was conducted for 2 consecutive nights to obtain 7 positively mated female rats and 8 positively mated female mice per exposure group. Females were exposed to cadmium oxide aerosol through whole-body exposure at concentrations of 0, 0.1, 0.3, 1, 3, or 10 mg/m^3 for 6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 12 exposure days (rats, gestation Days 4-19) or 11 exposure days (mice, gestation Days 4-17).

For the developmental toxicity studies, two to three females were housed overnight with each male. On the first day of vaginal plug or sperm detection (gestation Day 0), positively mated females were assigned to exposure groups by weight. Breeding was conducted for 3 consecutive nights to obtain 32 positively mated female rats and 33 positively mated female mice per exposure group. Based on the results of the range-finding studies, females in the developmental toxicity studies were exposed to cadmium oxide aerosol through whole-body exposure at concentrations of 0, 0.05, 0.5, or 2 mg/m^3 for 6 hours plus T_{90} (16 minutes) per day, 7 days per week for 16 exposure days (rats, gestation Days 4-19) or 14 exposure days (mice, gestation Days 4-17).

For all studies, female rats and mice were housed individually in cages within the exposure chambers. Drinking water was available *ad libitum*, and NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form was available *ad libitum* except during the daily exposure periods. Rats and mice were observed twice daily for mortality/morbidity and clinical signs of toxicity. Rats were weighed on gestation Days 0, 4, 6, 10, 14, 17, and at

necropsy (gestation Day 20); mice were weighed on gestation Days 0, 4, 6, 9, 12, 15, and at necropsy (gestation Day 18).

For all studies, females were killed 1 day after the final day of exposure, weighed, and examined grossly for signs of toxicity. Maternal livers, kidneys, and uteri were weighed, and the corpora lutea, implantation sites, resorptions, and live and dead fetuses were counted. Extra-gestational weight change was calculated by subtracting the gravid uterine weight from the maternal body weight. Uteri with no visible implantation sites were stained with ammonium sulfide to detect very early resorptions. Placentas were examined and discarded unless abnormal. Live fetuses were weighed and examined for gross defects and then killed and sexed. Half of the fetuses from each litter as well as fetuses with gross external abnormalities were examined for visceral defects using methods adapted from Staples (1974). The other half of the fetuses were decapitated; heads were fixed in Bouin's fixative, sectioned, and examined for soft-tissue craniofacial defects. All carcasses were double stained with Alcian Blue and Alizarin Red S and examined for skeletal malformations.

TABLE 1 Experimental Design and Materials and Methods in the Range-Finding and Developmental Toxicity Studies of Cadmium Oxide

Range-Finding Studies	Developmental Toxicity Studies
EXPERIMENTAL DESIGN	
Study Laboratory	
Battelle Pacific Northwest Laboratories (Richland, WA)	Same as 2-week studies
Strain and Species	
Sprague-Dawley rats Swiss (CD-1®) mice	Same as 2-week studies
Animal Source	
Charles River Laboratories (Raleigh, NC)	Same as 2-week studies
Size of Study Groups	
7 positively mated female rats per exposure group 8 positively mated female mice per exposure group	32 positively mated female rats per exposure group 33 positively mated female mice per exposure group
Route of Administration	
Whole-body inhalation	Same as 2-week studies
Exposure Concentrations/Duration	
0, 0.1, 0.3, 1, 3, or 10 mg/m^3 daily, 6 hours plus 12 minutes per day, 5 days per week, for 12 exposure days (rats, gestation Days 4-19) or 11 exposure days (mice, gestation Days 4-17)	0, 0.05, 0.5, or 2 mg/m^3 daily, 6 hours plus 16 minutes per day, 7 days per week, for 16 exposure days (rats, gestation Days 4-19) or 14 exposure days (mice, gestation Days 4-17)
Date of First Exposure	
Rats: 28 November 1988 (gestation group A) 29 November 1988 (gestation group B) Mice: 28 November 1988 (gestation group A) 29 November 1988 (gestation group B)	Rats: 28 January 1989 (gestation group A) 29 January 1989 (gestation group B) 30 January 1989 (gestation group C) Mice: 24 January 1989 (gestation group A) 25 January 1989 (gestation group B) 26 January 1989 (gestation group C)
Date of Last Exposure	
Rats: 13 December 1988 (gestation group A) 14 December 1988 (gestation group B) Mice: 12 December 1988 (gestation group A) 13 December 1988 (gestation group B)	Rats: 12 February 1989 (gestation group A) 13 February 1989 (gestation group B) 14 February 1989 (gestation group C) Mice: 6 February 1989 (gestation group A) 7 February 1989 (gestation group B) 8 February 1989 (gestation group C)
Date of Necropsy	
Rats: 14 December 1988 (gestation group A) 15 December 1988 (gestation group B) Mice: 12 December 1988 (gestation group A) 13 December 1988 (gestation group B)	Rats: 13 February 1989 (gestation group A) 14 February 1989 (gestation group B) 15 February 1989 (gestation group C) Mice: 7 February 1989 (gestation group A) 8 February 1989 (gestation group B) 9 February 1989 (gestation group C)
Type and Frequency of Observation	
Clinical observations were recorded twice daily. Rats were weighed on gestation Days 0, 4, 6, 10, 14, 17, and on the day of necropsy. Mice were weighed on gestation Days 0, 4, 6, 9, 12, 15 and on the day of necropsy.	Clinical observations were recorded twice daily. Rats were weighed on gestation Days 0, 4, 6, 10, 14, 17, and on the day of necropsy. Mice were weighed on gestation Days 0, 4, 6, 9, 12, 15 and on the day of necropsy.

TABLE 1 Experimental Design and Materials and Methods in the Range-Finding and Developmental Toxicity Studies of Cadmium Oxide (continued)

Range-Finding Studies	Developmental Toxicity Studies
EXPERIMENTAL DESIGN (continued)	
Maternal and Fetal Evaluations	
At necropsy, dams were weighed and examined for gross tissue abnormalities. Maternal liver, kidney, and uterine weights were recorded. The number, position, and status of implants were recorded. Placentas were examined, and ovarian corpora lutea were counted. Fetuses were weighed, examined for gross defects, and sexed. Fetal lungs were weighed.	At necropsy, dams were weighed and examined for gross tissue abnormalities. Maternal liver, kidney, and uterine weights were recorded. The number, position, and status of uterine implants were recorded. Placentas were examined, and ovarian corpora lutea were counted. Fetuses were weighed, examined for gross defects, and sexed. Fresh visceral examinations were performed on 50% of the fetuses. Fetal heads from 50% of the fetuses were fixed in Bouin's fixative, and carcasses from all fetuses were stained for visualization of skeletal abnormalities.
ANIMAL MAINTENANCE	
Time Held Before Study	
Rats: 32 days Mice: 33 days	Rats and Mice: 28 days
Age When Study Began	
Rats: approximately 13 weeks Mice: approximately 12 weeks	Rats and Mice: 14 weeks
Age at Necropsy	
Rats: approximately 15 weeks Mice: approximately 14 weeks	Rats and Mice: 17 weeks
Method of Animal Distribution	
Animals were weighed and were randomized with a computer program.	Same as 2-week studies
Diet	
NIH-07 Open Formula Diet (Zeigler Bros., Inc., Gardners, PA) in pellet form, available *ad libitum* except during exposure periods, and water (City of Richland), available *ad libitum*.	Same as 2-week studies
Animal Room Environment	
Rats and mice were housed in individual cages in the exposure chambers. The temperature was maintained at 72° to 78° F with 40% to 70% relative humidity and 12 to 18 air changes per hour. Fluorescent light was provided for 12 hours per day.	Same as 2-week studies

STATISTICAL METHODS

For the range-finding studies, a non-parametric analysis of variance (ANOVA), based on multiple comparisons when appropriate, was performed on the data. For the developmental toxicity studies, exposure-related trends in pregnancy indices were determined by the Cochran-Armitage test (Armitage, 1971). Each exposed group was compared to the control group with a chi-square test (Conover, 1971). The pregnancy index for an exposure group was defined as the number of females found pregnant in that group at the end of the study divided by the number of females that were sperm-positive or plug-positive in that group before the start of the study. Organ and body weight data, which have approximately normal distributions, were analyzed with the parametric multiple comparisons procedures of Williams

(1971, 1972) and Dunnett (1955). Exposure group means for data with skewed distributions were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose response (Dunnett's or Dunn's test). Trend-sensitive tests were used when Jonckheere's test was significant at a P-value less than 0.1. The significance of the dose-response trend for extra-gestational weight change was tested with the SAS® General Linear Models Procedure (SAS, 1985).

Before analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel. Implausible values, extreme values from animals that were suspected of being sick due to causes other than treatment, and values that the study laboratory indicated as being inadequate due to technical problems were eliminated from the analysis.

For fetal malformations and variations, the arc sine transformation of each proportional incidence was analyzed against the class variable, "treatment", using a one-way analysis of variance test. A Tukey's *t*-test (two-tailed) was used to assess statistically significant differences between control and exposed groups. If appropriate, the dose-response relationship was determined by an orthogonal trend test (Winer, 1971). The litter was used as the basis for analysis of fetal variables.

Results

RANGE-FINDING STUDY IN SPRAGUE-DAWLEY RATS

All female rats in the highest exposure group (10 mg/m^3) died by gestation Day 6 of the study. No other rats died, except for one rat in the 3 mg/m^3 group that was injured. In each exposed group with survivors, at least six rats were confirmed pregnant; five were confirmed pregnant in the control group. Generally, clinical signs of toxicity were limited to rats in the 1, 3, and 10 mg/m^3 groups and included hypoactivity, dyspnea, nasal discharge, and ruffled fur; signs of toxicity increased in incidence and severity with increasing exposure concentration. For all groups with survivors, the mean body weights, gravid uterine weights, and absolute and relative liver and kidney weights of dams were not significantly different from those of the control group throughout the study. However, for the 3 mg/m^3 group, the extra-gestational weight change was significantly less than in the controls by the end of the study.

DEVELOPMENTAL TOXICITY STUDY IN SPRAGUE-DAWLEY RATS

In the developmental toxicity study of cadmium oxide in rats, one female rat in the highest exposure group (2 mg/m^3) died on gestation Day 17 of the study; no other deaths occurred. In each exposed group, 28 to 31 rats were confirmed pregnant; 26 were confirmed pregnant in the control group. Clinical signs of toxicity included dyspnea in all exposed groups; the incidence, duration, and severity of this sign increased in an exposure-related manner. In addition, hypoactivity was noted in most rats in the highest exposure group. The mean body weight and maternal weight change of pregnant females exposed to 2 mg/m^3 cadmium oxide were significantly lower than those of the control group (Table 2). In addition, for rats in the 2 mg/m^3 group, absolute and relative liver weights and absolute kidney weight were significantly less than in the controls, while relative uterine and kidney weights were significantly greater than in the controls (Table 2).

Gestational exposure of rats to cadmium oxide did not affect the number of implantations per dam, litters with resorptions, or resorptions per litter (Table 3). In addition, no statistically significant differences in fetal mortality, the number of live fetuses per litter, or sex ratios were noted between the control and exposed groups. However, the mean body weights of male and female fetuses exposed to 2 mg/m^3 cadmium oxide were significantly lower than those of the controls (Table 3). Gestational exposure to cadmium oxide did not significantly increase the incidence of total fetal malformations or the mean percent of malformed fetuses per litter (Table 4). In addition, there were no statistically significant differences between the control and exposed groups in the overall incidence of fetal variations or the mean percent of fetuses per litter with variations. However, the mean percent of fetuses per litter with reduced ossifications of the pelvis and sternebrae increased in an exposure-related manner, with both parameters being significantly greater at the 2 mg/m^3 exposure level than in the controls (Table 4).

RANGE-FINDING STUDY IN SWISS (CD-1®) MICE

All mice in the highest exposure group (10 mg/m^3) died or were killed moribund by gestation Day 11 of the study. In the 3 mg/m^3 group, two mice died on gestation Day 10 and one mouse died on gestation Day 17. In each exposure group (eight sperm-positive mice per group) with survivors, three to eight mice were confirmed pregnant; four were confirmed pregnant in the control group. Generally, clinical signs of toxicity were limited to mice in the 1, 3, and 10 mg/m^3 groups and included dyspnea, hypoactivity, ruffled fur, abnormal posture, and dehydration; signs of toxicity increased in incidence and severity with increasing exposure concentration. For all groups with survivors, the mean body weights, absolute liver weights, and absolute and relative kidney weights of dams were similar to those of the control group at the end of the study. However, for dams in the 3 mg/m^3 group, gravid uterine weight and the extra-gestational weight change were significantly less than the controls by the end of the study. The relative liver weight for mice in the 1 mg/m^3 group was significantly greater than in the controls, but this difference in relative liver weights was probably due to a statistical variation caused by the small number of pregnant females in this group.

DEVELOPMENTAL TOXICITY STUDY IN SWISS (CD-1®) MICE

In the developmental toxicity study of cadmium oxide in mice, five mice in the highest exposure group (2 mg/m^3) were sacrificed moribund before the end of the study; no other deaths occurred. Gestational exposure to 0.05 mg/m^3 cadmium oxide did not affect the number of mice becoming pregnant; however, the number of pregnant mice was significantly less in the 0.5 and 2 mg/m^3 groups than in the controls (Table 5). Two dams in the control group were not examined for maternal or embryo/fetal parameters due to premature delivery on gestation Day 18. Clinical signs of toxicity included dyspnea and hypoactivity in all mice in the 2 mg/m^3 group and in most mice in the 0.5 mg/m^3 group; dyspnea increased in incidence, duration, and severity with increasing exposure concentration. Dyspnea also occurred in some mice in the 0.05 mg/m^3 group. The mean body weight and maternal weight change of pregnant females exposed to 2 mg/m^3 cadmium oxide were significantly lower than those of the control group by the end of the study (Table 5). In addition, absolute and relative gravid uterine weights and absolute liver weight were significantly lower than in the controls for females in the 2 mg/m^3 group. The relative kidney weight of females in the 2 mg/m^3 group was significantly greater than the control values at the end of the study (Table 5).

No statistically significant differences in implantations per dam, litters with resorptions, fetal mortality, or live fetuses per litter were noted between the control and exposed groups (Table 6). However, the total incidence of resorptions per litter was significantly greater in the 2 mg/m^3 group than in the controls, even though early and late resorptions per litter were not significantly greater than in the controls for mice in this group (Table 6). The mean body weights of male and female fetuses in the 0.5 and 2 mg/m^3 groups were significantly less than the controls; in addition, the percentage of live male fetuses per litter was significantly less at the 2 mg/m^3 exposure level than in the controls (Table 6). Total fetal malformations and the mean percent of malformed fetuses per litter noted for exposed groups were not significantly greater than those of the control group (Table 7). In addition, there were no statistically significant differences between the control and exposed groups in the overall incidence of fetal variations; however, the mean percent of fetuses per litter with variations increased with increasing exposure concentration. The mean percent of fetuses per litter with reduced ossification of the sternebrae also increased in an exposure-related manner, with significantly more occurrences in the 2 mg/m^3 group than in the controls (Table 7).

TABLE 2 Maternal Toxicity in Sprague-Dawley Rats Exposed to Cadmium Oxide Through Inhalation on Gestation Days 4 to 19[1]

	0 mg/m^3	0.05 mg/m^3	0.5 mg/m^3	2 mg/m^3
Pregnancy index[2]	26/32 (81%)	28/32 (88%)	29/32 (91%)	31/32 (97%)*
Number examined	26	28	29	30
Maternal body weight (g)				
Gestation Day 0	269 ± 4	268 ± 3	270 ± 3	269 ± 3
Gestation Day 20	401 ± 5	403 ± 5	401 ± 5	347 ± 6**
Maternal weight change (g)				
(gestation Days 0-20)	133 ± 3	135 ± 3	131 ± 3	78 ± 5**
Extra-gestational weight change (g)	57.9 ± 12.9	54.3 ± 12.3	53.1 ± 13.8	7.1 ± 20.6▲
Gravid uterine weight				
Absolute (g)	74.667 ± 2.801	80.248 ± 2.823	78.196 ± 2.707	70.789 ± 1.723
Relative (% body weight)	185.61 ± 6.19	198.78 ± 6.46	194.92 ± 6.50	203.85 ± 3.82*
Maternal liver weight				
Absolute (g)	15.639 ± 0.292	15.419 ± 0.242	16.264 ± 0.220	12.807 ± 0.281**
Relative (% body weight)	38.96 ± 0.53	38.28 ± 0.33	40.56 ± 0.39	36.82 ± 0.41**
Maternal kidney weight				
Absolute (g)	2.232 ± 0.039	2.236 ± 0.037	2.198 ± 0.031	2.026 ± 0.027**
Relative (% body weight)	5.57 ± 0.10	5.57 ± 0.11	5.48 ± 0.06	5.86 ± 0.09*

[1] Maternal body weights, weight changes, and absolute and relative organ weights are given as mean ± standard deviation.
[2] Number of pregnant females/number of sperm-positive females (percent pregnant).
* Significantly different ($P \leq 0.05$) from the control group by a chi-square test (pregnancy index; Conover, 1971) or by Williams' test (all other parameters).
** Significantly different ($P \leq 0.01$) from the control group by Williams' test.
▲ Significant exposure-related trend ($P \leq 0.05$) identified with the SAS® General Linear Models Procedure.

TABLE 3 Developmental Toxicity in Sprague-Dawley Rats Following Maternal Exposure to Cadmium Oxide Through Inhalation on Gestation Days 4 to 19

	0 mg/m^3	0.05 mg/m^3	0.5 mg/m^3	2 mg/m^3
Number of dams/litters examined	26	28	29	30
Implantations per dam[1]	14.12 ± 0.51	15.36 ± 0.50	14.76 ± 0.55	15.27 ± 0.33
Litters with resorptions	17	18	13	18
Resorptions per litter[1]				
Early	0.885 ± 0.169	0.786 ± 0.173	0.517 ± 0.146	0.767 ± 0.157
Late	0.077 ± 0.053	0.107 ± 0.079	0.172 ± 0.071	0.033 ± 0.033
Total	0.962 ± 0.171	0.893 ± 0.173	0.690 ± 0.173	0.800 ± 0.155
Dead fetuses per litter[1]	0	0	0	0
Live fetuses per litter[1]	13.15 ± 0.53	14.46 ± 0.55	14.07 ± 0.53	14.47 ± 0.38
Average fetal body weight per litter[1] (g)				
Live male fetuses	3.83 ± 0.05	3.76 ± 0.05	3.70 ± 0.05	3.20 ± 0.06**
Live female fetuses	3.64 ± 0.06	3.52 ± 0.05	3.52 ± 0.06	3.01 ± 0.06**
Live male fetuses per litter[1] (%)	50.1 ± 3.3	47.2 ± 2.9	50.5 ± 2.9	47.2 ± 2.3

[1] Data are given as mean ± standard deviation.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE 4 Morphologic Abnormalities Observed in Live Sprague-Dawley Rat Fetuses Following Maternal Exposure to Cadmium Oxide Through Inhalation on Gestation Days 4 to 19

	0 mg/m^3	0.05 mg/m^3	0.5 mg/m^3	2 mg/m^3
Total live fetuses examined	342	405	408	434
Total litters examined	26	28	29	30
Malformations				
Fetuses with malformations	1 (0.3%)	2 (0.5%)	0 (0.0%)	1 (0.2%)
Litters with malformations	1 (3.8%)	2 (7.1%)	0 (0.0%)	1 (3.3%)
Malformed fetuses per litter[1] (%)	0.3 ± 1.5	0.5 ± 1.8	0	0.2 ± 1.1
Variations				
Reduced ossifications per litter[1,2] (%)				
Pelvis[3]	2.4 ± 5.5	2.3 ± 5.2	3.4 ± 7.3	12.0 ± 19.6*
Sternebrae[3]	4.4 ± 7.0	7.5 ± 10.5	8.4 ± 8.4	24.7 ± 32.1*
Fetuses with variations	50 (14.6%)	88 (21.7%)	84 (20.6%)	123 (28.3%)
Litters with variations	22 (84.6%)	21 (75.0%)	27 (93.1%)	19 (63.3%)
Fetuses with variations per litter[1] (%)	14.3 ± 12.2	21.2 ± 20.1	20.6 ± 12.3	27.8 ± 32.6

[1] Data are given as mean ± standard deviation.
[2] Reduced ossifications occurred in other skeletal components, but only the significantly reduced ossifications are given here.
[3] Significantly correlated ($P \leq 0.05$) with exposure concentration by an orthogonal trend test after arc sin transformation.
* Significantly different ($P \leq 0.05$) from the control group by Tukey's *t*-test after arc sin transformation.

TABLE 5 Maternal Toxicity in Swiss (CD-1®) Mice Exposed to Cadmium Oxide Through Inhalation on Gestation Days 4 to 17[1]

	0 mg/m³	0.05 mg/m³	0.5 mg/m³	2 mg/m³
Pregnancy index[2]	32/33 (97%)	32/33 (97%)	23/33 (70%)**	10/33 (30%)**
Number examined	30[3]	32	23	6[4]
Maternal body weight (g)				
Gestation Day 0	28.0 ± 0.4	28.1 ± 0.4	28.3 ± 0.4	28.5 ± 0.9
Gestation Day 18	55.5 ± 0.8	56.4 ± 0.9	56.0 ± 0.9	43.3 ± 4.0**
Maternal weight change (g)				
(gestation Days 0-18)	27.5 ± 0.6	28.3 ± 0.7	27.7 ± 0.7	14.8 ± 3.4**
Extra-gestational weight change	7.0 ± 1.4	7.7 ± 1.5	7.5 ± 2.2	1.6 ± 1.4▲
Gravid uterine weight				
Absolute (g)	20.465 ± 0.527	20.595 ± 0.609	20.226 ± 0.509	13.282 ± 3.000**
Relative (% body weight)	367.54 ± 5.33	363.29 ± 6.21	360.30 ± 5.22	282.36 ± 57.52**
Maternal liver weight				
Absolute (g)	2.733 ± 0.057	2.818 ± 0.054	2.936 ± 0.063	2.225 ± 0.206**
Relative (% body weight)	49.32 ± 0.86	50.15 ± 0.81	52.37 ± 0.58*	51.52 ± 1.46
Maternal kidney weight				
Absolute (g)	0.439 ± 0.010	0.466 ± 0.008	0.469 ± 0.011	0.398 ± 0.019
Relative (% body weight)	7.91 ± 0.15	8.29 ± 0.13	8.37 ± 0.15	9.48 ± 0.66**

[1] Maternal body weights, weight changes, and absolute and relative organ weights are given as mean ± standard deviation.
[2] Number of pregnant females/number of sperm-positive females (percent pregnant).
[3] Two pregnant females were excluded from the study due to premature delivery on gestation Day 18.
[4] Four pregnant females were found moribund prior to the end of the study.
* Significantly different ($P \leq 0.05$) from the control group by Williams' test.
** Significantly different ($P \leq 0.01$) from the control group by a chi-square test (pregnancy index; Conover, 1971), by Dunnett's test (maternal body weight and weight change and absolute organ weights), or by Williams' test (relative organ weights).
▲ Significant exposure-related trend ($P \leq 0.05$) identified with the SAS® General Linear Models Procedure.

TABLE 6 Developmental Toxicity in Swiss (CD-1®) Mice Following Maternal Exposure to Cadmium Oxide Through Inhalation on Gestation Days 4 to 17

	0 mg/m³	0.05 mg/m³	0.5 mg/m³	2 mg/m³
Number of dams/litters examined	30	32	23	6
Implantations per dam[1]	12.53 ± 0.31	12.78 ± 0.37	13.35 ± 0.36	13.67 ± 1.12
Litters with resorptions	15	16	12	6
Resorptions per litter[1]				
Early	0.300 ± 0.098	0.375 ± 0.108	0.348 ± 0.102	2.667 ± 1.892
Late	0.267 ± 0.082	0.313 ± 0.122	0.348 ± 0.119	1.667 ± 1.085
Total	0.567 ± 0.133	0.688 ± 0.145	0.696 ± 0.159	4.333 ± 1.874**
Dead fetuses per litter[1]	0.033 ± 0.033	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Live fetuses per litter[1]	11.93 ± 0.33	12.13 ± 0.41	12.65 ± 0.42	9.33 ± 2.49
Average fetal body weight per litter[1] (g)				
Live male fetuses	1.389 ± 0.015	1.386 ± 0.013	1.265 ± 0.018**	0.985 ± 0.104**[2]
Live female fetuses	1.328 ± 0.014	1.328 ± 0.015	1.224 ± 0.018**	0.931 ± 0.082**[2]
Live male fetuses per litter[1] (%)	53.1 ± 2.3	57.7 ± 3.0	49.3 ± 3.7	38.9 ± 1.5*[2]

[1] Data are given as mean ± standard deviation.
[2] n=5; one of the six pregnant females had 100% resorptions.
* Significantly different (P≤0.05) from the control group by Dunn's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE 7 Morphologic Abnormalities Observed in Live Swiss (CD-1®) Mice Fetuses Following Maternal Exposure to Cadmium Oxide Through Inhalation on Gestation Days 4 to 17

	0 mg/m³	0.05 mg/m³	0.5 mg/m³	2 mg/m³
Total live fetuses examined	358	387	291	56
Total litters examined	30	32	23	5
Malformations				
Fetuses with malformations	6 (2.0%)	7 (2.1%)	8 (2.7%)	1 (1.8%)
Litters with malformations	6 (20.0%)	7 (21.9%)	7 (30.4%)	1 (20.0%)
Malformed fetuses per litter[1] (%)	1.7 ± 3.5	1.7 ± 3.3	2.7 ± 4.4	1.7 ± 3.7
Variations				
Reduced ossifications per litter[1,2] (%)				
Sternebrae[3]	6.0 ± 8.7	7.1 ± 14.0	11.1 ± 5.4	65.8 ± 34.0*
Fetuses with variations	89 (24.9%)	109 (28.2%)	113 (38.8%)	40 (71.4%)
Litters with variations	29 (96.7%)	26 (81.3%)	22 (95.7%)	5 (100.0%)
Fetuses with variations per litter[1,3] (%)	24.9 ± 18.8	29.8 ± 26.8	39.1 ± 25.4	73.1 ± 28.1

[1] Data are given as mean ± standard deviation.
[2] Reduced ossifications occurred in other skeletal components, but only the significantly reduced ossifications are given here.
[3] Significantly correlated (P≤0.05) with exposure concentration by an orthogonal trend test after arc sin transformation.
* Significantly different (P≤0.05) from the control group by Tukey's *t*-test after arc sin transformation.

APPENDIX D

Genetic Toxicology

TABLE D1 Mutagenicity of Cadmium Oxide in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9	+10% hamster S9	+10% rat S9
TA100	0.0	152 ± 10.9	171 ± 5.2	181 ± 4.0
	3.3	137 ± 2.3		
	10.0	132 ± 4.2		
	33.0	137 ± 6.0	175 ± 10.3	186 ± 4.3
	100.0	156 ± 4.7	176 ± 0.9	176 ± 11.9
	333.0	172 ± 8.4	182 ± 11.6	182 ± 6.1
	1000.0		180 ± 10.1	165 ± 8.7
	3333.0		169 ± 7.3	167 ± 2.4
Trial summary		Negative	Negative	Negative
Positive control[3]		1117 ± 50.1	1813 ± 103.0	1706 ± 73.7
TA1535	0.0	21 ± 3.2	30 ± 1.8	41 ± 2.4
	3.3	24 ± 3.3	36 ± 1.2	36 ± 4.7
	10.0	23 ± 2.6	42 ± 1.5	37 ± 2.5
	33.0	25 ± 1.7	43 ± 1.7	37 ± 2.5
	100.0	23 ± 1.2	38 ± 4.0	28 ± 1.0
	333.0	20 ± 1.9	22 ± 0.3	18 ± 2.7
Trial summary		Negative	Negative	Negative
Positive control		1161 ± 30.8	164 ± 4.1	214 ± 18.7
TA1537	0.0	15 ± 2.3	31 ± 3.3	32 ± 2.3
	3.3	13 ± 0.7	35 ± 3.0	23 ± 2.4
	10.0	11 ± 2.3	35 ± 0.9	18 ± 1.2
	33.0	11 ± 2.6	32 ± 0.9	17 ± 1.5
	100.0	11 ± 1.5	23 ± 3.8	21 ± 1.7
	333.0	10 ± 1.2	19 ± 0.3	20 ± 2.3
Trial summary		Negative	Negative	Negative
Positive control		322 ± 80.9	387 ± 32.4	529 ± 28.2
TA98	0.0	27 ± 1.0	24 ± 2.2	28 ± 1.8
	33.0	24 ± 1.5	22 ± 2.4	25 ± 2.9
	100.0	28 ± 2.7	24 ± 3.6	28 ± 1.5
	333.0	29 ± 1.5	25 ± 3.5	27 ± 4.5
	1000.0	26 ± 7.0	13 ± 1.5	18 ± 4.2
	3333.0	26 ± 3.5	22 ± 2.9	28 ± 1.7
Trial summary		Negative	Negative	Negative
Positive control		188 ± 34.3	1015 ± 158.5	1043 ± 108.4

[1] Study performed at Case Western Reserve University. The detailed protocol and these data are presented in Mortelmans *et al*. (1992); 0 µg/plate is the solvent control.

[2] Revertants are presented as mean ± standard error from three plates.

[3] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for trials with metabolic activation with all strains was 2-aminoanthracene.

TABLE D2 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Cadmium Oxide by Inhalation for 13 Weeks[1]

Concentration (mg/m^3)	Micronucleated NCEs/1,000 NCEs[2]
MALE	
Air	3.5 ± 0.4
0.025	2.8 ± 0.3
0.050	3.7 ± 0.6
0.100	3.1 ± 0.9
0.250	3.1 ± 0.4
1.000	4.3 ± 0.6
FEMALE	
Air	2.1 ± 0.2
0.025	2.1 ± 0.4
0.050	2.2 ± 0.3
0.100	2.1 ± 0.3
0.250	2.7 ± 0.4
1.000	2.7 ± 0.3

[1] A detailed description of the protocol is found in MacGregor *et al.* (1990). NCEs = normochromatic erythrocytes. Data are presented as mean ± standard error.

[2] Two thousand normochromatic erythrocytes were scored per animal.

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Toxicity Report Number	Chemical	Route of Exposure	Publication Number
1	Hexachloro-1,3-butadiene	Dosed Feed	91-3120
2	*n*-Hexane	Inhalation	91-3121
3	Acetone	Drinking Water	91-3122
4	1,2-Dichloroethane	Drinking Water, Gavage	91-3123
5	Cobalt Sulfate Heptahydrate	Inhalation	91-3124
6	Pentachlorobenzene	Dosed Feed	91-3125
7	1,2,4,5-Tetrachlorobenzene	Dosed Feed	91-3126
8	D & C Yellow No. 11	Dosed Feed	91-3127
9	*o*-Cresol *m*-Cresol *p*-Cresol	Dosed Feed	92-3128
10	Ethylbenzene	Inhalation	92-3129
11	Antimony Potassium Tartrate	Drinking Water, I.P. Inject.	92-3130
12	Castor Oil	Dosed Feed	92-3131
13	Trinitrofluorenone	Dermal, Dosed Feed	92-3132
14	*p*-Chloro-α,α,α-Trifluorotoluene	Gavage (corn oil, a-CD)	92-3133
15	*t*-Butyl Perbenzoate	Gavage	92-3134
16	Glyphosate	Dosed Feed	92-3135
17	Black Newsprint Ink	Dermal	92-3340
18	Methyl Ethyl Ketone Peroxide	Dermal	92-3341
19	Formic Acid	Inhalation	92-3342
20	Diethanolamine	Drinking Water, Dermal	92-3343
21	2-Hydroxy-4-Methoxybenzophenone	Dosed Feed, Drinking Water	92-3344
22	N, N-Dimethylformamide	Inhalation	93-3345
23	*o*-Nitrotoluene *m*-Nitrotoluene *p*-Nitrotoluene	Dosed Feed	92-3346
24	1,6-Hexanediamine	Inhalation	93-3347
25	Glutaraldehyde	Inhalation	93-3348
26	Ethylene Glycol Ethers	Drinking Water	93-3349
27	Riddelliine	Gavage	94-3350
28	Tetrachlorophthalic Anhydride	Gavage	93-3351
29	Cupric Sulfate	Drinking Water, Dosed Feed	93-3352
31	Isoprene	Inhalation	95-3354

NTP TECHNICAL REPORTS ON TOXICITY STUDIES
PRINTED AS OF MARCH 1995 (continued)

Toxicity Report Number	Chemical	Route of Exposure	Publication Number
32	Methylene Bis(thiocyanate)	Gavage	94-3381
33	2-Chloronitrobenzene 4-Chloronitrobenzene	Inhalation	93-3382
35	Chemical Mixture of 25 Groundwater Contaminants	Drinking Water	93-3384
36	Pesticide / Fertilizer Mixtures	Drinking Water	93-3385
37	Sodium Cyanide	Drinking Water	94-3386
38	Sodium Selenate Sodium Selenite	Drinking Water	94-3387
40	β-Bromo-β-nitrostyrene	Gavage	94-3389