EVIDENCE ON DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF CADMIUM

Reproductive and Cancer Hazard Assessment Section (RCHAS)
Office of Environmental Health Hazard Assessment (OEHHA)
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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq.*) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that "a chemical is known to the state to cause cancer or reproductive toxicity … if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principals to cause cancer or reproductive toxicity." The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency. The "state's qualified experts" regarding findings of reproductive toxicity are identified as members of the Developmental and Reproductive Toxicant Identification Committee of the Office of Environmental Health Hazard Assessment's Science Advisory Board (22 CCR 12301).

During a public meeting held in Sacramento, California, on May 12,1995 the Committee selected cadmium as a candidate for evaluation and requested that OEHHA staff prepare a review of the scientific evidence relevant to the reproductive toxicity of this agent. This draft document, which was released to the Committee and the public on October 4, 1996, responds to that request. While this hazard identification document does not provide dose-response evaluation, exposure assessment, or determination of allowable or safe exposure levels, the document does provide information which may be useful in such appraisals.

A public meeting of the Committee will be held December 4, 1996, in Sacramento, California. Following discussion and Committee deliberation, the Committee will determine whether cadmium "has been clearly shown through scientifically valid testing according to generally accepted principles" to cause reproductive toxicity

Contents

List of Tables

1. EXECUTIVE SUMMARY

Cadmium is a silver-white lustrous metal with an atomic weight of 112.41. It occurs in nature as cadmium oxide (CdO), cadmium carbonate ($CdCO_3$), cadmium chloride ($CdCl_2$), cadmium sulfide (CdS), and cadmium sulfate ($CdSO_4$). Unless specifically stated otherwise, "cadmium" in this document will refer both to elemental cadmium and cadmium-containing compounds.

Cadmium metal is used in NiCd batteries, metal plating, pigments, plastics, and alloys. In addition to occupational exposures related to these uses, exposures to cadmium are mainly through cigarette smoke and food. Several food crops, including potatoes, root crops, leafy vegetables, and fruits, are known to take up and concentrate cadmium from the soil. Cadmium can be absorbed by the oral and inhalation routes of exposure. Absorption and retention of cadmium are known to be influenced by species, age, pregnancy, and by the adequacy of other components of the diet.

The purpose of this document is to review the evidence concerning the developmental and reproductive toxicity of cadmium. The principal other toxic endpoints of cadmium are considered to be lung damage, renal dysfunction, hepatic injury, bone deficiencies, hypertension, and cancer. Cadmium is listed as a carcinogen under Proposition 65, and has also been identified as a toxic air contaminant by the California Air Resources Board.

Data are available pertaining to the developmental toxicity, female reproductive toxicity, and male reproductive toxicity of cadmium. Human studies have reported on all of these endpoints, and their results do not contradict the available animal studies. Specifically, epidemiological reports have evaluated whether cadmium exposure is associated with reduced birthweight, premature birth, stillbirth, spontaneous abortion, birth defects, effects on learning and behavior in offspring, and effects on male reproductive parameters. Elevated Cd levels in humans, as measured in maternal blood or infant hair, have been significantly correlated with adverse effects such as pre-term labor or a low birthweight for gestation age. Fertility of human males has been studied to a very limited degree. Fertility has not been demonstrated to be compromised with Cd exposure, although cadmium blood concentrations have been correlated with adverse effects on several sperm parameters. It is very difficult in these studies to isolate the effects of Cd from potentially confounding exposures such as Pb or cigarette smoking, and the available epidmiological studies on developmental and reproductive endpoints should not be considered conclusive.

Exposure of pregnant rodents to cadmium by the oral or inhalation routes has been shown to cause adverse effects in offspring, including reductions in fetal or birth weights, retarded ossification, reduced viability, and behavioral alterations. Death of the developing organism has been reported in studies on experimental animals following maternal exposure by the inhalation, injection, feed, or gavage routes of exposure. Malformations have been reported with Cd exposure, but are not a consistent finding with relevant routes of exposure. Growth deficits, evidenced as reduced birthweight and

reduced fetal weight, are consistently reported in inhalation and oral studies of Cd exposure.

Fertility deficits have been reported in studies where both females and males, or males alone were exposed to cadmium. When females alone were cadmium-exposed, decreased fertility was observed only if the animals were restricted to a diet deficient in vitamins and minerals. Cadmium treatment by the oral or inhalation routes has been associated in rats, but not in mice, with lengthened estrus cycles.

In male experimental animals, under certain conditions of dose and exposure period, cadmium has been shown to affect testes weight, cause histopathological lesions of the testes, result in reduced sperm counts and impaired sperm motility, and to adversely affect fertility. Histopathological changes have been observed in the testes following single oral doses, or with chronic exposure to cadmium. Higher doses and longer exposure periods were associated with more severe effects.

Since the body of information on the effects of exposure to cadmium via inhalation and oral exposures is extensive, the data from studies by injection, not typically a route for human exposure, are reviewed in an appendix. There are a substantial number of studies in which the developmental and reproductive effects in animal models of cadmium injection have been studied. In rat, mouse, and hamster, a highly consistent picture of fetal death or resorptions, malformations, and reduced fetal or birth weight has been observed. Effects on the placenta have been frequently observed. In several studies, degeneration or necrosis of the placenta occur at the same doses as fetal death. More subtle alterations occur at lower doses. The pre-implantation embryo has been found to be relatively insensitive to maternal Cd injection. However, injection shortly before implantation would take place has been observed to block implantation.

2. INTRODUCTION

2.1 Chemical structure and main physical characteristics

Cadmium is a silver-white, lustrous metal with an atomic weight of 112.41 found primarily in the +2 oxidation state. It makes up 1-2 ppm of the earth's crust. It often co-occurs with zinc, lead and copper. Cadmium occurs in nature as cadmium oxide (CdO), cadmium carbonate ($CdCO_3$), cadmium chloride ($CdCl_2$), cadmium sulfide (CdS) and cadmium sulfate ($CdSO_4$) (ATSDR, 1993).

2.2 Regulatory history

Cadmium is listed as a carcinogen under Proposition 65 (22 CCR 12000) and has been identified as a toxic air contaminant by the California Air Resources Board (1986). It has been identified as a human carcinogen (group 1) by the International Agency for Research on Cancer (IARC, 1993) and a probable human carcinogen (group B1) by the US Environmental Protection Agency (US EPA, 1992). The Occupational Safety and Health Administration (OSHA) has set an 8 hour Time Weighted Average of 0.1 mg/m^3 for cadmium fumes and 0.2 mg/m^3 for cadmium dust. The federal drinking water standard set by US EPA for cadmium is 5 μg/L (NLM, 1996); this standard has been adopted by the state of California. The Food and Drug Administration has set a separate level for bottled water of 10 μg/L (ATSDR, 1993). Disposal of cadmium-containing waste is covered under the Resource Conservation and Recovery Act and the Clean Water Act.

Cadmium was ranked second of 164 developmental and reproductive toxicant (DART) candidates compiled for consideration under Proposition 65 by the Office of Environmental Health Hazard Assessment (OEHHA) (Donald *et al*., 1992). This ranking was based on production, use, and exposure data, inclusion on published lists of reproductive toxicants, and nomination by experts in reproductive toxicity. Cadmium was also one of 14 high priority agents chosen by a Delphi committee of experts organized by OEHHA to prioritize candidate DARTs.

2.3 Exposure information

Cadmium is used primarily in nickel-cadmium batteries (35%) and metal plating (30%). It is also used in pigments, plastics and alloys (ATSDR, 1993). According to the 1988 US Toxic Release Inventory (TRI), there are 13 facilities which manufacture or process cadmium in California (with "maximum amounts on site" ranging from 1,000 to 99,000 lb. per facility) (ATSDR, 1993). Releases reported by the California TRI varied over the last 5 years from 500 to 30,000 lb. (though up to 50% appears to be recycled rather than

released). The 1983 National Occupational Exposure Survey estimated 88,968 employees worked in cadmium-related industries (14,541 women) (NIOSH, 1966).

The two main sources of non-occupational exposure to cadmium are cigarette smoking and the diet. The amount of cadmium absorbed is highly dependent on the route of exposure. Inhalation (including smoking and breathing polluted air) results in 20-50% absorption. Ingestion, on the other hand, usually results in only 2-6% absorption (Elinder, 1985). Plants tend to bioaccumulate cadmium from the soil, leading to high levels in certain vegetables. Average cadmium concentrations reported for some vegetables and fruits are:

- Potatoes 0.042 ppm
- Leafy vegetables 0.033 ppm
- Grain and cereal products 0.024 ppm
- Root Vegetables 0.016 ppm
- Garden fruits 0.017 ppm (ATSDR, 1993)

High levels of exposure may also occur via the consumption of organ meats (specifically liver and kidney). In beef cattle, cadmium levels ranged from 0.05-1.13 ppm (wet weight) in liver and 0.2-1.6 ppm in kidney (Elinder, 1985). Tobacco plants also have relatively high levels of cadmium, and smoking one pack of cigarettes per day gives an average exposure of 1-3 μg cadmium/day. One study estimated that smoking accounts for 50% of a smoker's cadmium body burden by age 50 (Elinder, 1985).

Cadmium in the environment is relatively mobile for a heavy metal. When released to the air by combustion, it tends to adsorb to small (<10 μm) particles which are within the respirable range (Keitz, 1980 as cited in ATSDR). Atmospheric concentrations range between 0.005-0.5 $\mu g/m^3$ (Elinder, 1985). It can be transported in water as a hydrate ion, but tends to adsorb to sediments quickly. Cadmium is found in some soils naturally as well as through deposition from anthropogenic sources. Cadmium contamination of topsoil can lead to bioaccumulation in plants followed by biomagnification to herbivores. Biomagnification is not thought to occur in nature at higher trophic levels due to low absorption by ingestion. Additionally, mammals tend to store their cadmium body-burden in their liver and kidneys, rather than in muscle tissue (ATSDR, 1993). However, the "plant to herbivore" biomagnification remains important in relation to human consumption of organ meats.

2.4 Pharmacokinetics

2.4.1 Absorption and retention

Cadmium can be absorbed by the oral, dermal, and inhalation routes of exposure. Absorption and retention of Cd are known to be influenced by species, age, pregnancy, and by the adequacy of other components of the diet.

Estimates of absorption by the oral route are complicated by retention of Cd within the GI tract, and by the gradual excretion of Cd via feces. In humans, an average of 2.7% to 6% retention of Cd ingested in a single meal has been reported from several studies (McLellan *et al*., 1978; Newton *et al*., 1984; Rahola *et al*., 1973). In non-pregnant women, oral absorption of Cd is influenced by body iron stores (Flanagan *et al*., 1978). Absorption of ingested Cd was found to be 2.3 % in subjects with adequate iron stores, as indicated by serum ferritin levels, and 8.9% in subjects with low iron stores.

In rats and mice, oral retention and absorption appear to be somewhat lower than in humans. Depending upon conditions, 0.3% to 4.2% retention has been observed (Anderson *et al*., 1988 Engstrom and Nordberg, 1979; Moore *et al*., 1973; Muller *et al*., 1986; Sasser and Jarboe, 1977; Schafer *et al*., 1986; Schafer *et al*., 1990). Retention of Cd is influenced by protein, fiber, and other components of the diet (Schafer *et al*., 1986). Iron deficiency in the diet substantially increases the retention of Cd (Flanagan *et al*., 1978; Schafer *et al*., 1990). In intact animals, the fraction of Cd which was retained generally increased with dose (Anderson *et al*., 1988; Engstrom and Nordberg, 1979; Lehman and Klassen, 1986). Actual absorption (whole body minus GI tract) has been found to range from 0.087% to 0.60% (Bhattacharyya *et al*., 1986, Sasser and Jarboe, 1977).

Retention and absorption of orally administered Cd were found to be increased 2-3 fold in the pregnant or lactating mouse over the non-pregnant mouse (Bhattacharya *et al*., 1981, 1982). In the neonatal rat, absorption and retention of oral Cd were found to be comparatively high. (Sasser and Jarboe, 1977). Absorption of Cd by neonates has been reported to be as high as 6 - 12%.

Dermal absorption in non-pregnant animals can be significant. On guinea pig skin, Cd solutions from 0.005 to 0.239 M were absorbed at about 1-2% efficiency over 5 hours (Skog and Wahlberg, 1964). Absorption has also been shown from aqueous solutions and ointments on rabbit and mouse (Kimura and Otaki, 1972).

By inhalation, retention and absorption varies by particle size, with smaller particles being more readily retained and absorbed. Estimates of absorption are complicated by retention of Cd in lung tissue, and by mucociliary clearance. In several species of experimental animals, about 5-20% of inhaled Cd was found to be retained in the lungs (Barrett *et al*., 1947; Moore *et al*., 1973). Inhalation also resulted in considerable transfer to the GI tract, due to mucociliary clearance (Moore *et al*., 1973; Henderson *et al*., 1979).

Absorption of inhaled Cd into the blood and other organs occurred with a rapid component (hours) and a slow component (days). Large amounts of Cd remained in the lungs for weeks after single exposures (Henderson *et al*., 1979; Rusch *et al*., 1986) or months after multiple exposures (Glaser *et al*., 1986).

2.4.2 Distribution

The distribution of Cd in human tissues has been studied in several chronic environmental and occupational exposure situations. In general, the highest concentrations were found in kidney, followed by liver. Blood-Cd concentrations were much lower, and appeared to be primarily related to more recent Cd exposures (reviewed in Elinder, 1985; Adamsson *et al*., 1979; Ghezzi *et al*., 1985; Gross *et al*., 1976; Hansen *et al*., 1985; Radisch *et al*., 1987; Roels *et al*., 1981; 1989). In human males having a wide range of Cd exposures, testicular Cd levels were only a small percentage of the concentrations found in the liver and kidneys (Smith *et al*., 1960; Wisniewska-Knypl *et al*., 1971). Similarly, ovarian Cd levels have been found to be low compared to the concentrations found in the liver and kidneys of women in the same age-range (Gross *et al*., 1976; Varga *et al*., 1993).

In animal models, following a single inhalation exposure, the Cd concentration in the lungs drops rapidly over a period of days, but remains higher than that in any other organ for several weeks or longer (Rusch *et al*.; 1986; Henderson *et al*.; 1979). Following oral exposure to Cd, high concentrations remain in the GI tract (Anderson *et al*.; 1988; Bhattacharyya *et al*.; 1986; Flanagan *et al*.; 1978; Lehman and Klassen; 1986; Muller *et al*.; 1986). Cd concentrations in the GI tract are typically similar to, or lower than, those found in liver and kidney. With some exceptions, other tissues have considerably lower concentrations than do liver and kidney. Blood typically has much lower concentrations than liver and kidney. Over time, Cd redistributes to the kidney (Anderson *et al*., 1988; Barrett *et al*., 1947; Engstrom and Nordberg, 1979; Flanagan *et al*., 1978; Henderson *et al*., 1979; Kimura and Otaki, 1972; Lehman and Klaassen, 1986; Muller *et al*., 1986; Rusch *et al*., 1986).

2.4.3 Metabolism

Cd reversibly binds to proteins and other biological molecules. Cd is stable in the +2 valence, which does not undergo oxidation or reduction in biological systems. In animal models, fractionation of cells has found that Cd is bound to both soluble and insoluble components. Soluble components include high molecular weight proteins and metallothionein (Flanagan *et al*., 1978; Glaser *et al*., 1986; Goon and Klaassen, 1989; Lehman and Klaassen, 1986; Nordberg *et al*., 1971). Metallothioneins (MT) are able to tightly bind up to 7 bivalent metal ions in metal-thiolate clusters. They are present in many tissues, especially liver, kidney, pancreas and intestine. The major low-molecular weight Cd and Zn-binding protein in testes is not MT in mice, rats, monkeys or humans (Deagan and Whanger, 1985; Kaur *et al*., 1993; Ohta *et al*., 1988; Waalkes and Perantoni, 1986; Waalkes *et al*., 1984a, 1984b, 1988a, 1988b). In MT-containing tissues, exposure to a number of stimuli increases the levels of MT mRNA and protein. These

stimuli include several metals (Cd, Zn, Mg, Cu, etc.), glucocorticoids, several organic toxicants, radiation, and inflammation.

Which cellular components Cd is bound to has been found to be dose and time dependent. Trace amounts of injected Cd tend to be bound mainly to metallothionein. Higher doses, however, are initially found bound mainly to higher molecular weight proteins (Goon and Klaassen, 1989; Lehman and Klaassen, 1986). Over time, less Cd is bound to higher molecular weight proteins, having shifted to metallothioneins. When administered by injection over a longer period of time, most of a dose of Cd is found bound to metallothionein (Nordberg *et al*., 1971). This shift in binding is due to the time required for the induction of metallothionein. Following a single injection of Cd, metallothionein-I and II in liver were slightly increased by 6 hours, and rapidly increased from 6 to 72 hours (Lehman-McKeeman and Klaassen, 1987).

The possible role of MTs in protecting against Cd-induced toxicity is not entirely clear (Kagi and Schaffer, 1988; Waalkes and Goering, 1990; Webb, 1987a, 1987b). Alleviation of developmental toxicity has been shown when Cd exposure by injection was preceded by an MT-inducing pretreatment (Ferm and Layton, 1979; Layton and Ferm, 1980; Naruse and Hayashi, 1989). Also suggestive are results with recently developed strains of mice in which the main MT genes have been "knocked out" (Masters *et al*., 1994; Michalska and Choo, 1993). These animals appeared to survive and reproduce normally, but were markedly more sensitive than normal mice to Cd-induced toxicity.

2.4.4 Excretion

Cadmium is primarily excreted in the feces and urine. The rate of excretion of Cd which has entered the circulation and internal tissues is relatively slow. In humans most of the Cd appearing in feces is never absorbed, and results from relatively recent oral or inhalation exposure (Adamsson *et al*., 1979; Kjellstrom *et al*., 1978; McKenzie-Parnell *et al*., 1988; Rahola *et al*., 1973). Urinary excretion has been studied extensively, and been found to relate primarily to cumulative Cd exposure. Levels around 2-20 μg/L in urine have been observed; these are somewhat higher than blood-Cd levels of the same subjects. With a high kidney burden of around 200 ppm, kidney dysfunction results in proteinuria and excretion of elevated levels of Cd (Ghezzi *et al*., 1985; Roels *et al*., 1981, 1989).

In humans, elimination of Cd appears to be multiphasic, with different body compartments having different rates (reviewed in Friberg *et al*., 1992; Kostial, 1986; Probst, 1979). Estimates of Cd's biological half-life vary greatly. Some studies have found it to be between 93 and 202 days (Flanagan *et al*., 1978; McLellan *et al*., 1978), another found a minimum of several hundred days (Rahola *et al*., 1973), while another found between 10 and 33 years (Ellis *et al*., 1979). Most studies have attempted to find the half-life for a "slow component", and those studies of longer duration tend to find longer half-lives.

The biological half-life in rats has been reported to be 206, 200, 252, and 173 days for Cd administered by the oral, inhalation, and by iv and ip injection routes, respectively (Moore *et al*., 1973).

2.5 Non-DART toxicities

The toxicity of Cd has been discussed in a recent National Toxicology Program Technical Report (NTP, 1995), a Priority Substances List Assessment Report (Environment Canada, 1994), and in an Agency for Toxic Substances and Disease Registry document (ATSDR, 1993). As reviewed by the NTP (1995), the primary toxic manifestations of Cd exposure are lung damage, renal dysfunction, hepatic injury, bone defects, hypertension, reproductive toxicity, and teratogenicity. Cd has been classified as a human carcinogen (group 1) by IARC (1993), and as a probable human carcinogen by US EPA (1992) and by Environment Canada (1994), and is listed as a carcinogen under Proposition 65.

Severe irritation of gastrointestinal epithelium and liver damage have been reported after high levels of oral exposure in humans. Liver histopathological changes, including necrosis, have been reported in animals given high oral doses of Cd. There are some reports of elevated blood pressure in experimental animals resulting from exposure to Cd acetate or Cd chloride, but the association has not been well supported. The NTP (1995) study found no biologically significant hypertensive effects in rats in a 13-week inhalation study of Cd oxide.

Occupational exposure to Cd has consistently been associated with adverse effects on lung function. Symptoms of Cd toxicity in humans include coughing, shortness of breath, irritation of the upper respiratory tract, and loss of olfaction. Severity can progress to emphysema-like lung dysfunction, and eventual death (ATSDR, 1993; Smith *et al*., 1960). In the NTP (1995) study rats and mice were exposed to Cd oxide by inhalation for 2 or 13 weeks. All animals exposed to a concentration of 10 mg/m^3 died within 3 to 7 days of exposure; death resulted from severe respiratory toxicity. Less severe lung toxicity was observed at lower, non-lethal doses, with increasing severity related to increasing dose. These findings were considered to be consistent with previous reports.

Kidney dysfunction is one of the manifestations of "Itai-Itai" disease, a condition for which severe chronic Cd poisoning has been strongly implicated as a causative factor (ATSDR, 1993). Following World War II, "Itai-Itai" disease was endemic among post-menopausal women in certain areas of Japan. Apart from effects on the kidneys, the disease is characterized by osteomalacia and osteoporosis. Kidney damage has also been reported in humans having occupational exposure to Cd, as well as in experimental animals. Tubular proteinuria develops after prolonged exposure, and is related to total Cd accumulation in the kidney. A "critical concentration" for kidney toxicity of 200 ppm

is frequently cited (ATSDR, 1993). This concentration has been used by US EPA in developing the reference dose (RfD) for chronic oral exposure to Cd (5×10^{-4} mg/kg/day, US EPA, 1994). Kidney weights were elevated in rats and mice exposed to Cd oxide vapor; but no effect on this endpoint was found in rats inhaling concentrations of 0.1 mg/m^3 or less (NTP, 1995). No histopathological lesions of the kidneys were observed in this study at any dose, in either species.

3. DEVELOPMENTAL TOXICITY

3.1 Human Data

Epidemiological investigations have evaluated the possibility of associations between prenatal exposure to Cd and reduced birthweight, premature birth, stillbirth, birth defects, and effects on learning and behavior. The nature of workplace, environmental, and consumer exposure to Cd make it difficult to isolate the effects of Cd on human populations from other, potentially confounding, factors. In the workplace and the environment, Cd exposure generally occurs in combination with exposure to other known and suspected reproductive toxicants, such as lead. Since Cd is also a component of cigarette smoke, it is difficult to properly control for confounding effects of smoking in study populations which include smokers. Finally, in exploring the reproductive toxicity of Cd in communities with high environmental levels, it may be difficult to distinguish between developmental, and male or female reproductive toxicity, since both parents are likely to be exposed.

3.1.1 Birthweight

A population of 106 non-smoking, pregnant women residing near a smelter were compared to 55 women living at a distance from the smelter in the former Yugoslavia (Loiacono *et al*., 1992). Socioeconomic and clinical variables were similar between the two groups. Placental Cd levels were elevated about 1.5-fold in women living near the smelter ($p<0.0001$). Lead concentrations in maternal blood, cord blood, and placenta were elevated 3-4-fold in women living near the smelter. There were no significant differences between the two groups in birthweight or in gestational age at birth. There were no associations between placental levels of Cd or Pb and birthweight or gestational age at birth.

A retrospective cohort study was conducted on workers in a United Kingdom Ni-Cd battery factory (Berlin *et al*., 1992). Birthweights of 157 children of 137 factory workers were compared to 109 children of mothers who were not known to be occupationally exposed to Cd. No measurements of Cd exposure levels or biological concentrations were made. Birthweight was not associated with occupation ($p=0.95$), but was significantly associated with maternal height, weight, and smoking ($p<0.05$).

A group of 100 mothers and neonates in a Polish hospital were studied for effects of smoking and blood Cd levels on birthweight (Sikorski *et al*., 1988). Birthweight was significantly reduced among offspring of women who smoked more than 6 cigarettes/day. Smoking habits were categorized into 3 groups: 0 cigarettes/day, 0-6 cigarettes/day, and >6 cigarettes/day. Concentrations of Cd in maternal and cord blood increased with

increased smoking, but no significant association was shown between birthweight and blood Cd level.

A significant reduction in birthweight was observed for infants of smokers as compared to non-smokers in a Cleveland, Ohio hospital (Kuhnert *et al*., 1987). Among the cohort of 125 non-smokers, no association was found between birthweight and maternal blood Cd level. Among the cohort of 77 smokers, however, there was a significant ($p<0.05$) association between maternal blood Cd and birthweight. This association remained after several clinical variables and thiocyanate, as an indicator of recent smoking, were entered into the regression analysis.

Other studies (Frery *et al*., 1993; Bonithon-Kopp *et al*., 1986a; Huel *et al*., 1981) have found elevated maternal or newborn Cd levels to be associated with reduced birthweight. An association between reduced birthweight and Cd levels in the hair of newborns was observed in 102 infants in an obstetrical care unit in Paris (Frery *et al*., 1993). This relationship was significant ($r = -0.49$, $p<0.01$) only when placental calcification was present. Statistical significance was maintained even when smoking habits and gestational age were taken into account. In the absence of placental calcification, no association was demonstrable between birthweight and Cd levels in newborn hair, maternal hair, or placental tissue.

Two studies have reported a significant association between Cd levels in infant hair and reduced birthweight for study populations in eastern France (Bonithon-Kopp *et al*., 1986; Huel *et al*., 1981). Maternal smoking was not mentioned in the study of Bonithon-Kopp and coworkers (1986a), and was not controlled for in the study of Huel and coworkers (1981). No association was found between birthweight and Pb levels in infant hair in either study. Nor was birthweight-class associated with parental social class or educational level (Bonithon-Kopp *et al*., 1986a). Another study conducted in this region of France found a 250 gram reduction in the birthweights of infants whose mothers were occupationally exposed to Cd (Huel *et al*., 1984). This difference was not statistically significant, although it remained after adjustments for infant sex and gestational age, as well as for maternal height and weight. In the exposed group of 26 mothers having occupational exposure to Cd, maternal and neonatal hair Cd levels were about 2.4-fold higher than those of the control group. Hair Pb levels were also elevated in this group by 1.3 to 2.2-fold. Exposed and control groups did not differ significantly in maternal age, height, weight, parity, or smoking history.

Two other reports of the relationship between maternal Cd exposure and birthweight were available for review only in foreign languages with English summaries (Cresta *et al*., 1989; Tsvetkova, 1970). A non-significant elevation of urinary Cd was found for mothers of low birthweight infants among a sample of 84 pregnancies in Italy (Cresta *et al*., 1989). This finding was said to be independent of maternal smoking habits. Compared to 20 controls, a significant reduction in birthweight was reported for infants of 106 Russian women who were occupationally exposed to Cd (Tsvetkova, 1970). Exposures were said to be in the range of 0.03 - 35 mg/m^3. As these studies could not be reviewed in detail, little weight can be put on their reported findings.

Blood Cd levels of 136 women living in contaminated areas of Poland were significantly higher than those of 269 women living in relatively uncontaminated regions (Laudanski *et al*., 1991). The average Cd levels for these groups were 2.9 and 2.5 μg/L, respectively (a significant difference at $p = 0.03$). Blood Pb concentrations were also elevated in the "exposed" group, but this was not a significant difference. The frequency of smoking was higher in the contaminated areas (16% as compared to 8% for controls), but the socioeconomic status of women in the two areas was similar. The mean weight of pre-term newborns was 15% lower in the contaminated than in the uncontaminated regions ($p = 0.049$). There were no significant differences between the two areas in the frequency of miscarriage, stillbirth, or pre-term labor.

3.1.2 Stillbirth

Stillborn infants were found to have extremely high Cd and Pb levels in their bones, as compared to adults killed in road accidents in the United Kingdom (Bryce-Smith *et al*., 1977). In a sample of 26 ribs and 42 vertebrae from stillborn infants, the average Cd level was about 10-fold higher than that of the adults used for comparison. Bone Pb levels were similarly elevated in the stillborn infants, but Cd and Pb were correlated only weakly ($r = 0.24$). While this association of bone Cd levels and stillbirth is interesting, considerable further study would be needed to ascertain whether or not the relationship was causal. For example, further comparison with live-born infants could provide information as to whether the high levels found in stillborn infants were unusual for that age group.

3.1.3 Malformations

An association between primary cleft palate and environmental levels of Cd and cobalt (Co) was suggested by a study of 110 affected children in eastern Europe (Cesany *et al*., 1991). In eastern France, no association was found between malformation frequency and Cd levels in maternal or newborn hair (Huel *et al*., 1981).

3.1.4 Behavior

Statistically significant correlations between deficits in behavior and learning in school children and the Cd and Pb contents of their hair have been reported (Bonithon-Kopp *et al*., 1986a; Pihl and Parkes, 1977; Pihl, 1979). Reductions in several psychometric scores were associated with elevated levels of Cd in newborn and maternal hair, and with hair Pb levels at 6 years of age (Bonithon-Kopp *et al*., 1986a). Pihl and Parkes (1977) demonstrated that levels of Cd and lead in hair had a positive correlation ($r = + 0.53$ $p < 0.001$) with each other. An evaluation of psychomotor development of 6-year old children revealed a statistically significant association between motor and perceptual abilities and *in utero* exposure to Cd and lead. Correlation of Cd with lead exposure in these studies confounds determination of the role of Cd alone.

3.2 Developmental Toxicity in Animals; Prenatal Endpoints and Postnatal Growth and Viability

3.2.1 Inhalation exposure: rats and mice

An NTP Technical Report (NTP, 1995) describes inhalation developmental toxicity studies in Sprague-Dawley rats and Swiss (CD-1) mice. Pregnant animals were exposed to 0, 0.05, 0.5 or 2.0 mg Cd oxide /m^3 air for 6 hours/day, 7 days/week on gestation days 4 - 19 (rats) or 4 - 17 (mice). In both species, maternal toxicity was evidenced at the high dose of 2.0 mg/m^3. This toxicity consisted of significant reductions of maternal body weight, as well as dyspnea and hypoactivity. Embryolethality was not observed in rats at any concentration, but there was a significant increase in resorptions in mice exposed to 2 mg Cd oxide/m^3 air. There was also a significant decrease in the ratio of pregnant mice to sperm-positive mice at 0.5 and 2.0 mg/m^3. Fetal weights were significantly decreased at 2.0 mg/m^3 in rats and mice and at 0.5 mg/m^3 in mice. Ossification reductions were seen in both species at the high concentration of Cd oxide.

Maternal weight gain was significantly depressed in pregnant Wistar rats exposed to $CdCl_2$ aerosols containing 0.2, 0.4, or 0.6 mg Cd/m^3 throughout gestation (Prigge, 1978). Fetal weights were significantly decreased only at the highest concentration. More severe effects were seen in this same strain of rat, when exposed to Cd oxide aerosol for 5 months prior to mating as well as during mating and gestation (Baranski, 1984, 1985). At the highest concentration of 1.0 mg Cd/m^3, over 50% of the maternal animals died, preventing meaningful interpretation of fetal data in that group. Fetal viability did not differ between controls and the groups exposed to 0.02 or 0.16 mg/m^3, but viability was extremely low for all three groups (mean number of live fetuses in the control group = 5.4 ± 4.0). Statistical analysis of fetal weight data was not presented, but sufficient information was included to determine that fetal weights were significantly reduced from control values in the 0.16 mg/m^3 group. Other effects stated to be associated with Cd exposure were increased frequencies of subcutaneous edema and retarded ossification.

Some animals in the Baranski reports were allowed to deliver their litters for evaluation of postnatal growth and behavior. Birthweight and viability at birth and postnatal days 21 and 60 were not affected by treatment, but the viability index (survival to postnatal day 4) was significantly decreased in the 0.16 mg/m^3 group (Baranski, 1984, 1985). Postnatal weight-gain was also decreased in this group.

3.2.2 Oral exposure

3.2.2.1 Drinking water, rats

Neither litter size nor postnatal viability have been shown to be affected by administration of Cd in drinking water to pregnant rats (Ahokas *et al*., 1980; Ali *et al*., 1986; Baranski, 1987; Hastings *et al*., 1978; Kelman *et al*., 1978; Sasser *et al*., 1985; Saxena *et al*., 1986; Sorell and Graziano, 1990; Sowa *et al*., 1982; Sowa and Steibert, 1985). Cd concentrations used in these studies ranged from 1 ppm to nearly 200 ppm. In some cases Cd was administered only during gestation or part of gestation (Ahokas *et al*., 1980; Ali *et al*., 1986; Baranski, 1987; Kelman *et al*., 1978; Sasser *et al*., 1985; Saxena *et al*., 1986; Sorell and Graziano, 1990; Sowa and Steibert, 1985). In other studies, Cd exposure commenced sometime prior to mating (Hastings *et al*., 1978), or protocols differing in duration and timing of treatment were compared (Sowa *et al*., 1982).

No gross malformations were described in any of the studies cited above. Reduced numbers of renal nephrons, however, were found in the offspring of female Wistar rats given 50 ppm Cd in drinking water for 5 months prior to mating and throughout gestation (Steibert *et al*., 1984). The functional significance of this finding is unclear; histoenzymatic studies revealed changes only in the maternal, and not the fetal, kidneys.

Statistically significant reductions in birthweights or fetal weights have been found at Cd concentrations of 50 ppm or more in drinking water in many studies (Ahokas *et al*., 1980; Baranski, 1987; Hastings *et al*., 1978; Sorell and Graziano, 1990; Steibert *et al*., 1984). One study reported no significant effect on fetal weight following gestational exposure to 50 ppm in the dams drinking water (Sowa and Steibert, 1985), while another reported no effect at a concentration of 100 ppm in drinking water (Sowa *et al*., 1982). Other studies have reported decreases in fetal weight or birthweight and/or postnatal weight gain following gestational exposure to Cd in drinking water at concentrations less than 50 ppm. A concentration of 8.4 μg Cd/ml in drinking water, determined by the authors to give a dose of 1.21 mg/kg bw/day, led to significantly decreased birthweights in exposed pups (Ali *et al*., 1986). A lower concentration of 4.2 μg/ml (for an average dose of 0.706 mg/kg) had no effect on birthweight. When female rats were given 17.2 or 34.4 μg Cd/ml in drinking water 90 days prior to mating and throughout gestation, birthweights and postnatal weight gain were significantly decreased (Cooper *et al*., 1978). No effects on birthweight or weight gain were seen at lower concentrations of 4.3 or 8.6 μg/ml.

The reductions in offspring weight described above often occurred at Cd concentrations which were also associated with evidence of maternal toxicity. Significant reductions in the consumption of drinking water which contains Cd has been a consistent finding, even at concentrations as low as 1 ppm (Ahokas *et al*., 1980; Baranski, 1987; Sorell and Graziano, 1990; Sowa and Steibert, 1985; Steibert *et al*., 1984). In these same studies, significant reductions in maternal weight gain have been found with Cd concentrations in drinking water of 10 ppm or higher. Ali and coworkers (1986) found a non-significant

decrease in maternal water consumption and no change in maternal weight gain with 4.2 or 8.4 μg Cd/ml in drinking water.

Despite their co-occurrence, a simple causal relation between the effects of Cd on maternal feed consumption or weight gain and reductions in fetal or birth weight has not been supported by more detailed analyses. Sorell and Graziano (1990) found reductions in both fetal and maternal weights following maternal consumption of drinking water containing Cd at 50 or 100 ppm. Multiple regression analysis, however, did not support a complete dependence of fetal weight on maternal weight. In the study by Ahokas and coworkers (1980), maternal feed consumption, feed efficiency, total weight gain, and net weight gain were all significantly reduced with exposure to 10 or 100 ppm Cd in drinking water. A pair-fed control group, matched to the high-dose Cd dams, showed total and net weight gains and feed efficiency which were significantly increased over those of the treated animals. While maternal variables for the pair-fed animals did not reach parity with controls fed ad lib, fetal weights did not differ between these groups. These results indicated that reduced feed consumption alone, while leading to reductions in maternal weight gain and feed efficiency, was not responsible for the effects of Cd on the offspring.

Alterations in the renal concentrations of MT, Cd, Zn, Cu, and Fe in maternal animals have also been reported (Sasser *et al*., 1985; Steibert *et al*., 1984). Maternal serum Zn and Fe concentrations, and ceruloplasmin activity were significantly decreased by exposure to 50 ppm Cd in drinking water throughout gestation (Sowa and Steibert, 1985). The toxicological significance of these findings is unclear, but such alterations could be important in the pathogenesis of Cd-induced effects on fetal growth. The potential importance of Zn, in particular, is supported by the results of Ahokas and coworkers (1980). The effects on fetuses and maternal animals of exposure to 100 ppm Cd in drinking water have been described above. An additional group of pregnant animals were given 100 ppm Cd in drinking water, supplemented with 5 ppm Zn. Zinc supplementation significantly improved maternal feed consumption and feed efficiency, as well as total and net weight gain. Fetal weights for this group were no different from controls

3.2.2.2 Drinking water, mice

In mice, the effects of maternal exposure to Cd in drinking water were similar to those observed in rats. In a series of studies by Webster (1978, 1979a and 1979b), pregnant Quackenbrush mice were given Cd in drinking water throughout gestation. Litter sizes were not affected by Cd exposure at concentrations as high as 40 ppm. Mean fetal weights were significantly reduced compared to controls, in a dose-dependent manner at Cd concentrations of 10, 20, and 40 ppm. Neither visceral nor skeletal evaluations were performed in these studies, but significant changes in hematological measures supported the visual impression that fetuses were anemic. Supplementation of Cd-treated animals with iron at least partially alleviated both the hematological effects and the adverse effects on growth caused by Cd. Supplementation of Cd-treated animals with zinc, copper, or selenium had no such effect.

Litter size, and viability at birth and on postnatal days 4 and 10 did not differ between Cd-treated and control Wistar mice (Xu *et al*., 1993a). Both paternal and maternal animals were given Cd in drinking water (0, 30, or 75 ppm) for 2 months prior to and during mating. Exposure of the females continued through pregnancy and lactation. Number of litters and litter size were similarly unaffected in Quackenbrush mice under a protocol similar to the Xu study (Webster, 1988). Drinking water contained Cd at concentrations of 0, 0.0015 ppm, 0.24 ppm, or 40 ppm. The two higher concentrations are estimated to have resulted in doses of approximately 0.06 and 7.0 mg Cd/kg body weight/day, respectively. Mean body weights of term fetuses in the high-dose group were significantly lower than controls, and these fetuses were described as appearing anemic. Otherwise, all fetuses appeared normal externally.

While Xu and coworkers (1993a) made no mention of maternal toxicity, Webster (1978, 1988) noted that consumption of drinking water was reduced to about 70% of control levels in female mice given water containing 40 ppm Cd. It is unlikely that reduced water consumption was responsible for the decreased fetal growth seen with this treatment, however, as fetal growth, but not water consumption, was restored by concurrent iron supplementation. Food intake was not affected by the concentration of Cd in the drinking water. Maternal gestational weight gain was found to taper off during the last 3 - 4 days of pregnancy, but this was not tested statistically. Effects were observed on hematological variables in the dams, but only to an extent far less severe than observed in their fetuses (Webster, 1978, 1979a).

3.2.2.3 Feed: rats and mice

Machemer and Lorke (1981) limited Cd exposure of rats to gestation days 6-15. When given in the feed at a concentrations of up to 100 ppm as Cd chloride, there were no observed adverse effects on development. Maternal animals in this study showed no observable adverse effects of Cd chloride in their feed at concentrations of up to 30 ppm. At the highest concentration of 100 ppm Cd chloride (corresponding to a dose of 22 mg Cd chloride/kg body weight), maternal weight gain was significantly reduced during the treatment period, but reached control levels by the end of gestation.

At a higher concentration of 200 ppm Cd given in the feed throughout gestation, maternal food consumption and weight gain were significantly depressed (Pond and Walker, 1975). No abnormalities or changes in viability were observed, but pup birthweights were significantly reduced. Addition of excess calcium to the Cd-containing diet significantly reduced the amount of Cd measured in pup tissues, but did not alter the Cd-induced reduction in birthweight.

Whelton and coworkers (1988) subjected female CF1 mice to 6 consecutive, 42-day rounds of gestation-lactation. Animals were given diets containing 0.25, 5.0, or 50.0 ppm Cd throughout the study. Diets containing the same cadmium concentration were also formulated to be either sufficient or deficient in the vitamin, mineral, and fat content. The deficient diet was formulated with reduced concentrations of fat, calcium, thiamine

HCl, riboflavin, and vitamins A and D. With a sufficient diet, no changes in fertility occurred with the addition of cadmium although litter size was significantly reduced at the highest concentration of 50 ppm. Birthweights were also reduced at this concentration, as were pup weights at weaning. Cd had an effect on pup growth which was independent of dietary sufficiency.

3.2.2.4 Gavage: rats and mice

No adverse effects were observed on the fetuses of Wistar rats given Cd by gavage at doses of 0.04, 0.4, or 4 mg/kg (Baranski *et al*., 1983). Treatment was given daily, 5 days/week for 5 weeks prior to mating, and then during the mating and gestation periods. Cd given by gavage on gestation days 6-15 had no observable adverse effects on development at doses of 1.8 or 6.1 mg/kg (Machemer and Lorke, 1981). Cd given to Sprague-Dawley rats on gestation days 6 - 18 at doses of 10, 25, or 50 mg/kg did not affect litter size or resorption frequency (Simmons *et al*., 1984; Wardell *et al*., 1982). Resorption frequency was not affected at a dose of 25 mg/kg in another study using the same rat strain, route, and days of treatment (Cornwall *et al*., 1984). Similarly, viability on postnatal day 1 was not affected by Cd given at doses of 10 or 25 mg/kg (Simmons *et al*., 1984). Viability was unchanged in fetuses of Charles River COBS rats given 20 or 40 mg/kg $CdCl_2$ in combination with NaCl (40 or 80 mg/kg, respectively) on each of gestation days 6 - 19 (Scharpf *et al*., 1972). Reductions in fetal viability were seen at higher doses of $CdCl_2$ (60 and 80 mg/kg), but interpretation of these changes is complicated by significant maternal mortality.

Sutou and coworkers (1980b) gave Cd to male and female Sprague-Dawley rats prior to mating, and to females throughout gestation. The route of exposure was stated to be oral, and while the method of administration was not specified, the context suggests that it was gavage. No adverse fetal effects were reported at doses of 0.1 or 1.0 mg/kg. At the high dose of 10 mg/kg, there was a significant decrease in the number of total implants, and in the number of live fetuses. There was also a significant increase in the number of resorbed fetuses. Fetal body weights, body length, and tail length were also all significantly reduced at this dose. Placental weights were significantly increased over control levels, a change attributed to adaptive hypertrophy.

There was no apparent change in fetal weight among offspring of Charles River COBS rats given 20, 40, or 60 mg/kg $CdCl_2$ in combination with NaCl (40, 80 or 120 mg/kg, respectively) on each of gestation days 6 - 19 (Scharpf *et al*., 1972). A decrease in fetal weight observed at a higher dose of 80 mg/kg (in combination with 160 mg/kg of NaCl) was associated with maternal mortality in excess of 27%. In contrast, with exposure to a dose of 18.4 mg Cd/kg on gestation days 6-15, fetal and placental weights were significantly decreased (Machemer and Lorke, 1981). At a dose of 50 mg/kg, given on gestation days 6-18, adverse effects on fetal growth were evidenced by statistically significant reductions in mean fetal weights and lengths, as well as in mean tibial length (Wardell *et al*., 1982). A dose of 25 mg Cd/kg body weight on each of gestation days 6-18 did not affect fetal weights on gestation day 19 (Simmons *et al*., 1984), but did result in significantly depressed pup weights ($p<0.01$) on postnatal days 1, 5, 10, and 20.

The frequency of malformations was reported to be significantly increased with Cd exposure in some studies (Machemer and Lorke, 1981; Baranski *et al*., 1982; Scharpf *et al*., 1972), but not in others under similar treatment conditions (Wardell *et al*., 1982; Sutou *et al*., 1980b). External and/or internal anomalies were reported in fetuses of Charles River COBS rats given 20, 40, 60, or 80 mg/kg $CdCl_2$ in combination with NaCl (40, 80, 120, or 160 mg/kg, respectively) on each of gestation days 6 - 19 (Scharpf *et al*., 1972). The external anomalies consisted of edema and translucent skin; heart and kidney defects were the principal internal anomalies observed. There was no clear dose-response relationship for these effects, and maternal mortality was significant at the 2 highest doses (12 and 28%, respectively). Reduced ossification of skeletal elements was reported in this study to have occurred in a dose-related manner. Baranski *et al*. (1982) reported significantly increased frequencies of subcutaneous hemorrhages and hydropericardium at Cd doses of 20 and 40 mg/kg; anomalies such as sirenomelia and amelia were reported following the high dose of 40 mg/kg (Baranski, 1985). Hydropericardium also occurred at a significantly increased frequency at a dose of 4 mg/kg. The frequency of delayed ossification was significantly increased in all treated groups (2, 4, 8, 12, or 20 mg/kg), but there was no clear relationship to dose. When 10 mg Cd/kg body weight was given both before and during gestation, there were no significant increases in the frequencies of external or visceral malformations, but there were significant delays in ossification of certain skeletal elements (Sutou *et al*., 1980b).

High doses of Cd (40 mg/kg or greater) given by gavage have had such extreme adverse effects on maternal animals that any coincident developmental toxicity cannot be interpreted. Nearly 50% of maternal animals died from a dosing regimen of 40 mg/kg/day (Baranski *et al*., 1982); maternal mortality reached 60% with a Cd dose of 61.3 mg/kg/day (Machemer and Lorke, 1981). Wardell *et al*., (1982) mentioned maternal mortality occurring with gavage doses of 75 or 100 mg Cd/kg body weight in rats, but no details were given. Four out of 9 dams died as a result of repeated gavage exposure to a dose of 50 mg Cd/kg bw (Simmons *et al*., 1984). Scharpf and coworkers (1972) did not observe maternal mortality at doses of 20 or 40 mg $CdCl_2$/kg. At higher doses of 60 and 80 mg/kg, maternal mortality was 12 and 28%, respectively. Interestingly, co-administration of Cd with sodium nitrilotriacetate (NTA) eliminated the maternal mortality associated with Cd exposure alone, the combination resulting in reduced Cd contents and Cd/Zn ratios in maternal liver, kidney, and muscle. Fetal Cd content and Cd/Zn ratio were unchanged by co-administration of NTA, and fetuses still displayed increased frequencies of internal abnormalities.

Lower doses of Cd given by gavage also produced evidence of maternal toxicity. Significant reductions in weight gain during the treatment period were found at Cd doses of 6.1, 18.4, and 61.3 mg/kg (Machemer and Lorke, 1981), but total gestational weight gain was not affected at the lowest dose. Gestational weight gain was significantly reduced, in a dose-dependent progression, in Wistar rats given Cd by gavage at doses of 2, 4, 8, 12, 20, and 40 mg/kg (Baranski *et al*., 1982). Body weight gain, feed and water consumption, and feed efficiency were all reduced following oral dosing with 10 mg Cd/kg body weight given prior to mating and throughout gestation (Sutou *et al*., 1980a).

It is not clear from the paper, however, whether these changes were statistically significant. Clinical symptoms of Cd toxicity observed in maternal animals consisted of depilation, excessive salivation, bleached incisors, roughened fur, constipation or diarrhea, vaginal hemorrhaging, and stomach ulcers (Machemer and Lorke, 1981; Sutou *et al*., 1980a). Sutou and coworkers (1980a) did not measure hematological variables in pregnant females, but nonpregnant females given Cd at a dose of 10 mg/kg/day showed significant decreases in hemoglobin content and hematocrit.

A screening study conducted in ICR/SIM mice included administration of Cd on gestation days 8 - 12 at a gavage dose of 340 mg/kg bw/day (Seidenberg *et al*., 1986). Corn oil was used as the vehicle in this study, in contrast to other gavage studies which generally used water. Compared to controls, the number of live pups on postnatal day one was unchanged, but the number of dead pups was significantly increased. Mean birth weight was also significantly lower in the exposed than in the control group. None of the dams died at this dose of Cd.

3.3 Developmental Toxicity in Animals; Behavior

Developmental-neurotoxicity studies of Cd have focused on locomotor activity, aspects of learning, and the development of certain reflexes.

3.3.1 Locomotor activity

The nature of Cd's effects on locomotor activity depends both upon the developmental stage at the time of treatment and the developmental stage at the time activity is evaluated.

Prenatal exposure to Cd at doses of 0.7 or 1.2 mg/kg/day in drinking water led to significantly increased motor activity at preweaning ages (Ali *et al*., 1986). Postweaning locomotor activity following prenatal Cd exposure was significantly decreased when measured at time points between 5 and 18 weeks of postnatal age (Ali *et al*., 1986; Baranski, 1984, 1985 and 1986; Baranski *et al*., 1983; Cooper *et al*., 1977; Hastings *et al*., 1978). Effective doses/concentrations ranged from 0.7 - 5.0 mg/kg/day in drinking water, from 0.4 - 4.0 mg/kg/day by gavage, and from 0.02 to 0.16 mg/m^3 by inhalation. In the study by Cooper and coworkers (1977), an approximate dose of 2.75 mg Cd/kg/day in drinking water was associated with decreased locomotor activity at postweaning timepoints, but a higher dose of 5.5 mg/kg/day was associated with increased pup locomotor activity. At this higher dose, given prior to and throughout gestation, pup birth weight and lactational weight gain were depressed, as were pup Cu and Fe contents. Cd contents of treated groups did not differ from controls.

With Cd exposure restricted to the neonatal period, the pattern of effects on locomotor behavior is slightly different. Rastogi and coworkers (1977) reported a significant increase in spontaneous locomotor activity on postnatal day 30 following administration of 0.1 or 1.0 mg Cd/kg bw/day by gavage to rat pups on each of postnatal days 1 - 30. Repeated gavage dosing with 0.25 mg Cd/kg bw on postnatal days 6-15 caused a

significant increase in locomotor activity measured on postnatal day 45 (Smith *et al*., 1982). A single, subcutaneous injection of 4 mg Cd/kg body weight on postnatal day 5 produced a biphasic effect on activity levels of rat pups (Ruppert *et al*., 1985). Treated pups were significantly less active than controls on days 13 to 15, and more active than controls on days 20 and 21. A peak in activity levels seen in control pups on days 15 to 16 was not evident in the Cd-exposed group, nor did the latter group habituate normally to the test maze. The delayed onset of metal-induced hyperactivity may have reflected the time-course of damage to the nervous system; brain weights of treated animals being more affected at 19 days post-dosing than at 4 days post-dosing.

3.3.2 Learning

Pre- or postnatal exposure to Cd by the oral, inhalation or injection route has been shown to affect various measures of learning. Avoidance learning in rats was altered by prenatal exposure to Cd (Ali *et al*., 1986; Baranski, 1983, 1986). Effects on this endpoint were seen after maternal consumption of Cd in drinking water at concentrations as low as 4.2 μg/ml (Ali *et al*., 1986; Baranski, 1986), or after maternal inhalation of 0.02 or 0.16 mg Cd oxide/m^3 air (Baranski, 1983). Performance on a conditioned-reflex response test was impaired in 3-month old rats prenatally exposed to Cd oxide at concentrations of 0.02 or 0.16 mg/m^3 (Baranski, 1985). Acquisition of a conditioned escape response on postnatal day 39 was significantly delayed in the offspring of rats given $CdCl_2$ by subcutaneous injection at doses of 0, 0.2, 0.62, and 2.0 mg/kg, on each of gestation days 7 - 15 (Lehotzky *et al*., 1990). The endpoint measured, "number of seconds spent in immobility in a 5-minute swim stress situation following the administration of 5 mg/kg of d-amphetamine", was increased in a statistically-significant and dose-dependent manner. In a different study, acquisition of a cued spatial discrimination task was not affected in 130-day old rats after prenatal exposure to 2.4 mg Cd/kg/day in drinking water (Hastings *et al.*, 1978).

Administration of a single subcutaneous injection of 1-2 mg Cd chloride on postnatal day 5 or 6 altered the normal social behavior of male rats tested at 150 days of age (Holloway *et al*., 1988). Treated animals were unable to recognize a familiar conspecific. The authors concluded that Cd exposure in infancy had long lasting effects on a type of learning and memory task comparable to visual recognition and memory tests performed in humans and nonhuman primates.

3.3.3 Reflex development

Development of some, but not other, reflexes has been adversely affected by prenatal exposure to Cd. Rat pups tested on postnatal days 5-13 showed decrements in the negative geotaxis reflex after prenatal exposure to 0.16 mg/m^3 of Cd oxide (Baranski, 1985). Development of the cliff aversion response and of swimming behavior were significantly delayed in rat pups prenatally exposed to doses of 0.7 or 1.2 mg Cd/kg maternal body weight/day (Ali *et al*., 1986). The maturation of surface- and air-righting reflexes, however, as well as visual placing were not affected in these same pups.

3.4 Other Relevant Data

3.4.1 Transfer and distribution of Cd in offspring

3.4.1.1. Cd distribution

Bhattacharyya (1983) reviewed the transport of Cd from the mother to the fetus during gestation and to the pup during lactation. The major steps in this pathway are: 1) absorption by the maternal GI tract, 2) transfer from the maternal blood to the fetus via the placenta; or transfer from maternal blood to milk via the mammary gland, 3) distribution in the fetus; or absorption by the neonate from milk, followed by distribution; and 4) elimination. Pregnant rats and mice show an increase of 1.5 to 2-fold in GI absorption of Cd after gestation day 15 through delivery. Very little Cd is actually transferred to the fetus, however. In both humans and animals, Cd present in the maternal circulation is rapidly sequestered into the placenta, liver, and kidneys. Cd is also taken up and retained by mammary tissues of rats and mice. Transfer to milk does occur, but only a fraction of the cadmium measurable in maternal tissues appears in the milk. Neonatal animals are able to absorb far more of an orally administered dose of Cd than are older juveniles (4-23% and 0.59-1.3%, respectively). The majority of Cd absorbed by offspring is localized to the GI tract, from where it may be gradually eliminated via the feces.

3.4.1.2 Absorption and distribution in the maternal animal

Absorption and distribution of Cd, as well as other metals, is considerably different during pregnancy and lactation than in the non-pregnant state (Bhattacharyya *et al*., 1981, 1982; Chan and Cherian, 1993a). Pregnant $B6CF_1$/Anl mice retained twice as much of the ^{109}Cd provided in drinking water than did non-pregnant controls (Bhattacharyya *et al*., 1982). Lactating mice, as well as those exposed during both gestation and lactation, retained an even greater proportion of the ingested dose.

Cd given by subcutaneous injection to female rats prior to mating and pregnancy was preferentially sequestered in the liver (Chan and Cherian, 1993a). Subsequent pregnancy apparently mobilized hepatic stores of Cd which was then available for transfer to the placenta and maternal kidney. Quantitatively, the concentration of Cd in the liver decreased by 40% during pregnancy, while that in the kidney increased by 60%.

Single, tracer doses of ^{109}Cd were given by gavage on different days to pregnant or lactating rats, and organ Cd content was assayed at 72 hours post-dosing (Bhattacharyya *et al*., 1981). Significant increases, as compared to nonpregnant controls, occurred in Cd retained in the duodenum, mammary tissue, and kidneys. A similar pattern of distribution was seen in mice given ^{109}Cd in drinking water throughout gestation and/or lactation (Bhattacharyya *et al*., 1982). Female rats given 50 ppm Cd in drinking water on gestation days 6 - 20 showed significant elevations over controls in the Cd content of blood, kidney, liver, pancreas, lung, thymus, heart, and placenta; bone also showed a significant increase at the higher Cd concentration of 100 ppm (Sorell and Graziano,

1990). The Cd contents of the GI tract and mammary tissue were not assayed in this study.

3.4.1.3 Transfer across the placenta

Cadmium and Pb levels were measured in samples of placenta, maternal blood, and cord blood from women residing in Upper Silesia, one of the most polluted regions in Poland (Baranowska, 1995). Only traces of Cd were measured in cord blood (1.13 ng/ml), compared to average levels in maternal blood and placenta (4.9 ng/ml and 0.11 μg/g, respectively). In contrast, the average Pb levels in cord blood (38.31 ng/ml) represented a far higher proportion of the concentrations found in placenta or maternal blood (0.50 μg/g and 72.50 ng/ml, respectively). The author concluded from these findings that the human placenta is a better barrier against Cd than it is against Pb. The same conclusion was reached by Schramel and coworkers (1988), who found no correlation between Cd levels in maternal and cord blood, and only a weak correlation between the Cd levels in maternal blood and the placenta. In the same samples, Pb levels in maternal blood were positively correlated with Pb levels in placenta and cord blood. A comparison of Cd levels in maternal blood, cord blood, and placenta demonstrated that the human placenta acts as a barrier to the transfer of Cd to the fetus (Kuhnert *et al*., 1982). The authors caution that this should not be taken to infer that Cd is not hazardous to the fetus, as indirect effects on placental function could occur.

Webster (1988) studied distribution of ^{109}Cd in maternal and fetal tissues following its administration to pregnant females in drinking water. The concentrations given were calculated to provide widely ranging doses of 0.0004, 0.60, or 7.0 mg/kg/day. On gestation day 18, all maternal and fetal tissues tested contained measurable levels of ^{109}Cd. Maternal tissues varied greatly in Cd content, with chorioallantoic placenta showing the highest levels. Accumulation of Cd in the placenta was directly proportional to the administered dose. Furthermore, at the two higher doses, less than 1% of the total tissue-bound Cd was localized to the fetuses rather than the dam. Similar findings were reported by Whelton and coworkers (1993d), who identified low or nondetectable levels of ^{109}Cd in newborn pups of treated CF1 mice. Similarly the amount of Cd bound to fetal liver following maternal exposure via inhalation represented only about 1% of maternal levels (Prigge, 1978).

Fetal Cd levels have not been found to be proportional to the maternally administered dose (Webster, 1988). Furthermore, the extent and severity of adverse effects on offspring can be related to the dose of Cd administered to the maternal animal, but not to the fetal Cd-content (Baranski *et al*., 1982). Placental tissue, on the other hand, selectively accumulates Cd following oral dosing of pregnant animals, but proportionately little Cd crosses the placenta to accumulate in fetal tissues (Ahokas *et al*., 1980; Sorell and Graziano, 1990; Webster, 1988). These results support the contention that the developmental effects of Cd may be, at least in part, mediated by indirect effects on the dam or the placenta.

3.4.1.4 Transfer during lactation, and distribution in the neonate

While Cd accumulated in the dam's body prior to mating and pregnancy did not readily transfer to pups during lactation (Whelton *et al*., 1993d), Cd ingested by the mother during gestation can accumulate in mammary tissues, and later be secreted in the milk (Baranski, 1986; Lucis *et al*., 1972). Baranski (1986) did not find Cd in the gut of term rat fetuses exposed via their dams' drinking water during gestation. In 2-week old offspring of similarly-treated females, Cd was localized to the intestinal wall, with no other consistent differences from controls. Localization of Cd to the GI-tract of 3-week old, lactationally-exposed pups was also noted by Whelton and coworkers (1993d). By 16 weeks of age, pups of treated dams showed significantly elevated Cd concentrations in all organs tested (Baranksi, 1986). Since after 2 weeks of lactation, Cd levels were similar in milk from treated and control dams, it would appear that most of the Cd was transferred prior to that time. Somewhat different results were obtained by Lucis and coworkers (1972), who used single subcutaneous injections of ^{109}Cd to pregnant or lactating rats in order to trace the transfer of Cd in the milk. ^{109}Cd accumulated in maternal mammary tissues, and levels remained fairly stable during, and even subsequent to, the lactation period. Similarly, the concentration of ^{109}Cd in milk remained at consistent and low levels throughout lactation. In the pups, ^{109}Cd was found to accumulate primarily in the intestine.

At birth, Cd content did not differ between control and treated pups of Wistar mice exposed to Cd in drinking water at levels of 0, 30, or 75 ppm (Xu *et al*., 1993a). Treatment of the dams was continued through lactation, and by postnatal day 20 Cd content was significantly higher in pups of treated dams, in a dose-dependent manner. Other studies of Cd transfer during gestation and lactation have had similar results (Bhattacharyya *et al*., 1982; Whelton *et al*., 1993d). In the study of Whelton and coworkers (1993d), CF1 mice were given Cd in both feed and water throughout gestation and lactation. While pups were born with negligible amounts of Cd bound to their tissues, pup-Cd levels approximately tripled with each week of the lactation period. Additional experiments indicated that transfer of ^{109}Cd to nursing pups was increased by 30% formultiparous, rather than primiparous, dams. While the results of these studies indicate that transfer of Cd to the pups took place via milk, it is also possible that older pups may have been directly consuming their dams' treated feed and/or drinking water. However, the authors claim to have controlled for this possibility, and the direct route of pup exposure would not have been available in the study of Bhattacharyya and coworkers (1981). In this case, nursing pups evaluated 72 hours following administration of a gavage dose of ^{109}Cd to their dams, were found to contain approximately 2.5% of the total dose.

Secretion of Cd in human milk has been found to be very low (Radisch *et al*., 1987). The average concentrations of Cd in human milk were 0.07 ug/L in non-smokers and 0.16 ug/L in smokers. These concentrations were 13% and 10%, respectively, of the concentrations in maternal blood, and far below the levels of exposure for formula-fed infants.

3.4.1.5 Effects of Cd exposure on the distribution of other trace elements

Several studies have reported evidence of reduced iron content, such as anemia, in term fetuses or newborn rats following prenatal exposure to Cd (Baranski, 1986; Cooper *et al*., 1977; Hastings *et al*., 1978; Murthy *et al*., 1986; Sutou *et al*., 1980b; Webster, 1978, 1979a, 1979b; 1988). Webster (1978, 1979a, 1979b) found that both the anemia and the growth retarding effects of prenatal exposure to Cd could be at least partially alleviated by concurrent iron supplementation. Webster concluded from these findings, that Cd causes fetal anemia, which in turn causes fetal growth retardation. As fetuses were more severely anemic than their dams, he further concluded that Cd interferes specifically with placental transport of iron.

Statistically significant effects were observed on specific organ levels of zinc, copper, iron and calcium in young rats prenatally exposed to Cd at an approximate dose of 1.6 mg/kg/day (Murthy *et al*., 1986). No consistent pattern of effects emerged, excepting a reduction in liver copper content measured on postnatal days 1, 21, and 90. Hastings and coworkers (1978) reported no differences between Cd-treated and control pups in Cd, zinc, or copper content. In contrast, other studies have reported significant reductions in the zinc contents of placenta and the whole fetus or fetal liver when pregnant rats were given Cd at 100 ppm in drinking water (Ahokas *et al*., 1980; Sorell and Graziano, 1990; Sowa *et al*., 1982). Furthermore, zinc supplementation of maternal animals partially alleviated the decreased fetal weights associated with Cd treatment (Ahokas *et al*., 1980). The authors suggest that Cd competitively interacts with dietary zinc in a manner leading to zinc deficiency, in turn causing the observed decreases in feed efficiency, resulting in decreased fetal growth.

The interaction between Cd and other trace metals may be mediated by the effects of Cd on metallothionein. Sowa and coworkers (1982) found significant effects of Cd exposure on both maternal and fetal hepatic metallothionein levels. In the dams this was a significant increase; in fetuses it was a significant decrease.

3.4.2 Potential mechanisms causing the developmental neurotoxicity of Cd

The mechanism(s) by which Cd affects behavior is not known, but studies have been done on certain aspects of this question. Abnormalities of brain histopathology and biochemistry have been identified in developing experimental animals exposed to Cd at doses known to affect behavior (Gupta *et al*., 1991; Rastogi *et al*., 1977; Webster and Valois, 1981). None of these changes, however, have a demonstrated causal relationship with the effects of Cd on behavior. It has also been suggested that the effects of Cd on locomotor behavior in rats could result from generalized malnutrition, or from deficits in a specific nutrient such as iron (Cooper *et al*., 1978).

Food restricted pregnant dams lost about 22% of their initial body weight and gave birth to pups which were significantly lighter than controls (Smart and Dobbing, 1971). However, even this extent of general nutritional deprivation during the prenatal period had little effect on behavioral parameters measured in adult offspring, unless combined

with continued deprivation of dams during lactation (Smart and Dobbing, 1971; Smart *et al*., 1973). There is also evidence for the converse: prenatal exposure to Cd, at levels producing significant effects on postnatal locomotor behavior, was not necessarily associated with effects on maternal gestational weight-gain (Hastings *et al*., 1978). Similarly, in the study by Rastogi and coworkers (1977), a dose of 0.1 mg Cd/kg body weight/day was associated with hyperactivity but not with a significant decrease in pup body weight.

Suckling is altered in the acute toxic phase of Cd or other heavy metals, but the magnitude of resulting growth reduction has not been directly related to the magnitude of concurrent hyperactivity (Ruppert *et al*., 1985). Severe food deprivation of female rats during lactation has been shown to affect locomotor activity, as well as other behavioral measures (Altman *et al*., 1971; Smart and Dobbing, 1971; Smart *et al*., 1973). This regimen, however, did not mimic the hyperactivity observed with postnatal, preweaning exposure to Cd (Rastogi *et al*., 1977).

Specific nutrient deficiencies resulting from Cd exposure remain a possible mechanism. Iron levels have been found to be significantly reduced in rat pups showing significant behavioral effects of prenatal exposure to Cd, in the absence of significant increases in tissue Cd levels (Cooper *et al*., 1977; Hastings *et al*., 1978). It should be noted, however, that in one study (Cooper *et al*., 1977) reduced iron was associated with increased locomotor activity, while in the other study (Hastings *et al*., 1978) reduced iron content was associated with depressed locomotor activity.

3.5 Integrative Evaluation

The developmental toxicity of Cd has been studied in animal models and in humans. Experimental animals have been exposed to Cd by the injection, inhalation, and oral routes. Epidemiological investigations have evaluated the possibility of associations between prenatal exposure to Cd and reduced birthweight, premature birth, birth defects, and effects on learning and behavior. The most consistently observed effects of oral or inhalation exposure to Cd on the development of experimental animals are reductions in fetal or birthweight, followed by alterations in locomotor behavior. Retarded ossification of skeletal elements has also been found. Other effects, such as reduced viability, and major and minor malformations have been observed under some combinations of route and dose. In humans, some studies have reported Cd exposure to be correlated with reduced birthweight, others have correlated elevated levels of Cd in children's hair with behavioral and psychomotor deficits. While interpretation of the human epidemiological studies is complicated by the difficulty of controlling for confounding exposures, particularly cigarette smoke and Pb, the overall evidence from human studies is consistent with that from experimental animals.

Death of the developing organism has been reported in studies on experimental animals following maternal exposure by the inhalation, injection, feed, or gavage routes of exposure. With Cd exposure by inhalation, one study found the frequency of resorbed

fetuses to be significantly increased in mice, but not in rats. Another study, conducted in rats, found no effect of inhaled Cd on viability at birth, but survival to postnatal day 4 was significantly reduced. Other studies conducted in rats or mice by the inhalation route did not report effects on viability. Death of the developing organism has been a frequent finding following injection of Cd into pregnant animals, particularly when given during the later part of gestation, at higher doses, and/or with repeated injections over multiple days. As reviewed in the Appendix on injection studies of developmental and reproductive toxicity of cadmium, extensive placental damage has also been a frequent result of injections of Cd into pregnant animals. The nature of this damage has ranged from reduced blood flow and effects on nutrient transfer, to necrosis, hemorrhage, and/ blood clotting. There are data consistent with the interpretation that the extreme placental damage observed in injection studies is causally related to fetal death. With one exception, gross placental pathology has not been described in studies conducted by the inhalation or oral routes. Significantly increased placental weights were reported in one study following Cd exposure by gavage; effects on offspring at the same dose included an increased frequency of resorptions, and decreased frequencies of implantations and live fetuses. In other studies conducted by the gavage route, significant reductions in fetal viability have been reported, but only at doses where excessive levels of maternal death render the fetal data of limited value. Placental damage was not mentioned in a long-term feeding study conducted in mice, nor did excessive maternal mortality occur, but litter size was reduced.

Malformations have been reported with Cd exposure under some circumstances, in both humans and animals, but are not a consistent finding with relevant routes of exposure. In rats, mice, and hamsters given Cd by injection, malformations have been observed. Injection studies typically involve a single treatment, or treatment on a restricted number of days; hence, the specific defects observed are highly dependent upon the day of treatment. Injection of Cd on multiple days, or during the later parts of gestation, tended to be associated with fetal death rather than malformations. Major malformations have not been reported in animal studies using the inhalation, drinking water, or feed routes of exposure. Effects observed with Cd exposure by these routes have included: increased frequencies of subcutaneous edema, retarded ossification, and a reduced number of renal nephrons. With the gavage route of exposure to Cd, malformations have been reported to be significantly increased in some studies, but not in others using similar treatment conditions. Defects reported include edema and translucent skin, subcutaneous hemorrhages, hyperpericardium, and retarded ossification. One report mentioned sirenomelia and amelia, but no quantitative data were presented and the effective dose also caused 50% mortality among maternal animals.

Growth deficits, evidenced as reduced birthweight in humans and animals, and reduced fetal weights in animals are the most consistently reported developmental effects associated with exposure to Cd. Interpretation of the human epidemiological studies is complicated by the difficulty of controlling for confounding exposures, such as lead or cigarette smoke. Cd is a component of cigarette smoke, making it difficult to properly control for confounding effects of smoking in populations which include smokers. This has been a particular problem for some of the studies reporting reduced birthweights in

association with Cd exposure. Other studies have found no association between Cd exposure and reduced birthweight, even when significant negative correlations between smoking and birthweight were identified in the same study populations. In animal studies, reductions in fetal weights or birthweights have been reported following exposure to Cd by inhalation, in drinking water or feed, or by gavage. Fetal weight reductions reported in some studies occurred at concentrations or doses also affecting the maternal animal. Despite overlap between the dose-response curves for developmental and maternal toxicity, there is no evidence that the reductions in offspring weight are due to decrements in maternal weight gain or feed or water intake. To the contrary, results of pair-feeding experiments support the independence of these responses.

Functional deficits in behavior and psychomotor deficits have been correlated with elevated levels of Cd in children's hair. However, significant correlations between Cd and Pb levels in the same samples obscures assessment of the contribution of Cd alone. Pre- and/or postnatal Cd exposure of developing test animals has consistently been shown to have demonstrable effects on the development of locomotor activity. For exposures limited to the prenatal period, effective doses/concentrations ranged from 0.7 - 5.0 mg/kg/day in drinking water, from 0.4 - 4.0 mg/kg/day by gavage, and from 0.02 to 0.16 mg/m^3 by inhalation. Whether the effect is manifested as an increase or as a decrease in activity level appears to depend upon: 1) the administered dose, and 2) the developmental stage at the time of evaluation.

Overall, the most consistent effects on development, when Cd exposure is by a relevant route of exposure, are on growth parameters, followed by locomotor behavior. Other effects, such as fetal death and major and minor malformations have also been reported under some circumstances of route and dose of Cd. In particular, retarded ossification of skeletal elements have been observed following oral or inhalation Cd exposure.

Table 1. Animal studies of the developmental effects (viability and growth) of Cd by inhalation

Reference	Study Design	Reported effects[(1)]
Baranski, 1984, 1985	rat, 4-5 months plus gestation, 5 hr/d, 5 d/wk 0, 0.02, 0.16, 1.0 mg/m^3	50% maternal death at 1.0 mg/m^3. No change in maternal weight gain at any exposure. Fetal weight reduced at 0.16 mg/m^3. No change in litter size at any exposure. Some animals allowed to deliver: no change in birthweight, or viability on pnd 21 or 60. At 0.16 mg/m^3, decreased viability on pnd 4 and decreased postnatal weight gain.
NTP, 1995	mouse, gestation days 4-17, 6 hr/d 0, 0.05, 0.5, 2.0 mg/m^3	Maternal death increased at 2.0 mg/m^3 (0/33 control, 5/33 treated). Maternal weight gain reduced at 2.0 mg/m^3. Resorption frequency and reduced

		ossification increased at 2.0 mg/m^3. Fetal weight reduced at 0.5 and 2.0 mg/m^3. No increase in malformations at any exposure.
NTP, 1995	rat, gestation days 4-19, 6 hr/d 0, 0.05, 0.5, 2.0 mg/m^3	Fetal weight and maternal weight gain reduced at 2.0 mg/m^3. Reduced ossification at the high concentration. No increase in maternal death, resorptions, or malformations at any exposure.
Prigge, 1978	rat, gestation days 1-21, 24 hr/d 0, 0.2, 0.4, 0.6 mg/m^3	Reduced maternal weight gain at 0.2, 0.4, 0.6 mg/m^3. Reduced fetal weight at 0.6.

(1) Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

Table 2. Animal studies of developmental effects (viability and growth) of Cd by oral routes

Reference	Study Design	Reported effects[(1)]
Food		
Machemer and Lorke, 1981	rat, in feed, gestation days 6-15 0, 30, 100 ppm	Transitory reduction in maternal weight gain at 100 ppm. No effects on implantation, viability, litter size, fetal weight, malformations or ossification of skeletal elements.
Pond and Walker, 1975	rat, in feed, gestation 0, 200 ppm	Birthweight and maternal weight gain reduced at 200 ppm. No changes in litter size or increase in malformations
Whelton *et al*., 1988	mice, in feed, throughout 6, 42 day rounds of gestation-lactation 0.25, 5.0, 50.0 ppm	No significant effect on maternal survival. At 50 ppm, 15% decrease in litter size at birth, and 25% decrease in pre-weaning weight gain.
Gavage		
Baranski *et al*., 1982	rat, gavage, gestation days 7-16 0, 2, 4, 8, 12, 20, 40 mg/kg/d	Maternal death at 40 mg/kg, maternal weight gain reduced at all doses. Fetal weight reduced at 8, 12, 20, 40 mg/kg/d. Early and late resorptions increased at 40 mg/kg/d. Malformations increased at 20 and 40 mg/kg. Delayed ossification at all doses.
Baranski, 1983	rat, gavage, 5 weeks plus gestation, 5d/wk 0, 0.04, 0.4, 4 mg/kg/d	No effect on maternal or fetal viability, or on fetal weight.
Baranski *et al*., 1985	rat, gavage, gestation days 7-16 2, 12, 40 mg/kg/d	Maternal death at 40 mg/kg/d. Decreased maternal weight gain at all doses. Decreased fetal weights at 12 and 40 mg/kg/d. Decreased viability at 40 mg/kg/d. Major malformations at 40 mg/kg/d. Retarded ossification at all doses.
Cornwall *et al*., 1984	rat, gavage, gestation days 6-18 0, 25 mg/kg/d	No effect on resorption frequency or fetal weight.

[(1)] Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

Table 2 (continued). Animal studies of developmental effects (viability and growth) of Cd by oral routes

Reference	Study Design	Reported effects[(1)]
Machemer and Lorke, 1981	rat, gavage, gestation days 6-15 0, 1.8, 6.1, 18.4, 61.3 mg/kg/d	Maternal weight gain reduced at 6.1, 18.4, 61.3 mg/kg/d. Malformations increased at 18.4, 61.3 mg/kg/d. Fetal weight decreased at 18.4 mg/kg/d. 60% maternal mortality at 61.3 mg/kg/d, no pregnancies at this dose.
Scharpf *et al*., 1972	rat, gavage, gestation days 6-19 0, 20, 40, 60, 80 mg/kg/d	Maternal death increased at 60 and 80 mg/kg/d. Fetal viability not affected at 20 or 40 mg/kg/d, decreased at 60 or 80 mg/kg/d. Decreased fetal weight only at 80 mg/kg/d. External and internal anomalies increased at all doses.
Seidenberg *et al*., 1986	mice, gavage, gestation days 8 - 12 340 mg/kg	No maternal death. No change in the number of live pups on pnd 1, but an increase in the number of dead pups on the same day. Decreased birth weights.
Simmons *et al*., 1984	rat, gavage, gestation days 6-18 0, 10, 25, 50 mg/kg/d	Fetal weight not decreased at any dose on gd19. Some litters from the 25 mg/kg/d group allowed to deliver; pup weights were decreased on pnd 1, 5, 10, 20. No effects on litter size, resorption frequency, or implantation frequency. Maternal mortality: 4/9 at 50 mg/kg/d. Two litters surviving this dose had reduced viability on pnd 1.
Sutuo *et al*., 1980a, 1980b	rat, gavage, males and females 6-9 weeks prior to mating, plus females during gestation 0, 0.1, 1.0, 10 mg/kg/d	Decreased implants and live fetuses, and increased resorptions at 10 mg/kg/d. Fetal weight and maternal weight gain decreased at 10 mg/kg/d. Increased placental weights.

[(1)] Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

Table 2 (continued). Animal studies of developmental effects (viability and growth) of Cd by oral routes

Reference	Study Design	Reported effects[(1)]
WATER		
Wardell *et al*., 1982	rat, gavage, gestation days 6-18 0, 25, 50, 75, 100 mg/kg/d	Maternal deaths increased at 75 and 100 mg/kg/d. All fetuses resorbed at these doses. Fetal weight, length, and tibial length decreased at 50, 75 mg/kg/d. Malformations not increased at any dose.
Ahokas *et al*., 1980	rat, water, gestation days 1-21 0, 1, 10, 100 ppm	Fetal weight decreased at 100 ppm. No change in litter size at any exposure. Maternal weight reduced at 10, 100 ppm.
Ali *et al*., 1986	rat, water, gestation 0, 4.2, 8.4 ppm	Birthweight decreased at 8.4 ppm. No changes in maternal weight gain, litter size, or malformation frequency at any exposure level.
Baranski, 1987	rat, water, gestation days 1-20 0, 60, 180 ppm	Fetal weight reduced at 60, 180 ppm. Maternal weight gain reduced at 60, 180 ppm. No change in litter size, fetal death, resorption frequency, or the frequency of preimplantation loss at any exposure.
Cooper *et al*., 1978	rat, water, 90 d plus gestation 0, 4.3, 8.6, 17.2, 34.4 ppm	No effect on birthweight or postnatal growth at 4.3 or 8.6 ppm. At 17.2 and 34.4 ppm, birthweights and postnatal weight gain were reduced.
Hastings *et al*., 1978	rat, water, 90 d. plus gestation 0, 17.2 ppm	Birthweight decreased at 17.2 ppm. No change in maternal weight gain or litter size at any exposure.
Kelman *et al*, 1978	rat, water, gestation 0, 10, 25 ppm	No changes in litter size or malformation frequency
Sasser *et al*., 1985	rat, water, gestation 0, 50, 100 ppm	No maternal death, no change in maternal weight gain. No effect on litter size, no malformations, no effect on fetal weights.

[(1)] Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

Table 2 (continued). Animal studies of developmental effects (viability and growth) of Cd by oral routes

Reference	Study Design	Reported effects[(1)]
Saxena *et al*., 1986	rat, water, gestation 0, 100 ppm	Fetal weight and maternal weight gain decreased at 100 ppm. No change in litter size or malformation frequency at any exposure.
Sorell and Graziano, 1990	rat, water, gestation days 6-20 0, 5, 50, 100 ppm	Fetal weight decreased at 100 ppm. Maternal weight gain reduced at 50, 100 ppm. No change in litter size or malformation frequency at any exposure.
Sowa and Steibert, 1985	rat, water, gestation days 1-20 0, 50 ppm	Maternal weight gain reduced at 50 ppm. No change in litter size, fetal weight, or malformation frequency at any exposure.
Sowa *et al*., 1982	rat, water, 30 d; 30d + gestation; gestation 0, 100 ppm	No change in fetal weight or litter size at any exposure.
Steibert *et al*., 1984	rat, water, 5 months plus gestation 0, 50 ppm	Fetal weight and maternal weight gain reduced at 50 ppm. Reduced numbers of renal nephrons in offspring.
Webster, 1978	mouse, water, gestation 0, 10, 20, 40 ppm	Fetal weight decreased at 10, 40 ppm. Maternal weight gain said to be decreased at 40 ppm, but not clear if this was statistically significant. No change in litter size at any exposure.
Webster, 1979a, 1979b	mouse, water, gestation 0, 40 ppm	Fetal weight decreased at 40 ppm. No change in litter size at any exposure.
Webster, 1988	mouse, water, 1 month plus gestation 0, 0.0015, 0.24, 40 ppm	Fetal weight decreased at 40 ppm. No change in litter size at any exposure. All fetuses externally normal, excepting for anemic appearance of the high concentration group.
Xu *et al*., 1993a	mouse, water, males and females 2 months, plus gestation and lactation for females 0, 30, 75 ppm	No change in birthweight, litter size, or postnatal viability at any exposure.

[(1)] Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

4. FEMALE REPRODUCTIVE TOXICITY

4.1 Human data

Samples of maternal and newborn hair were analyzed for cadmium and lead content in a cohort of women (110 mothers and infants) from a small town in eastern France (Huel *et al*., 1981). This region which was presumed to represent a range of environmental exposures, since several chemical and metallurgical factories were present. The upper and lower 17% groups differed by more than a factor of 10 in cadmium content (maternal hair less than or equal to 0.13 ppm to greater than or equal to 1.23 ppm, newborn hair less than or equal to 0.15 ppm to greater than or equal to 1.95 ppm). Cadmium levels in the hair of newborns were significantly correlated with lead levels in the same sample, and with cadmium levels in maternal hair. A significant association was found between pre-term birth and lead, but not cadmium, levels in maternal and infant hair. Cadmium content of infant hair was significantly correlated with a finding of small-for-dates. No relationship was identified between maternal smoking habits during pregnancy, as a potential confounder, and the trace metal content of hair samples.

No association was found between blood cadmium levels and threatened spontaneous abortion, anemia, or "toxemia" (defined by the authors as preeclampsia plus either proteinuria or edema) for a cohort of 71 pregnant women living in a small town near a copper smelter in Bulgaria (Tabacova *et al*., 1994). Maternal blood levels of lead and cadmium, and urinary arsenic were measured. Average blood cadmium levels were around 0.2-0.3 μg/L, with a range of less than 0.1 μg/L to 1.67 μg/L. Smoking during pregnancy was associated with higher blood cadmium and lead levels. Higher individual cadmium levels were significantly correlated with higher individual lead levels.

A study by Laudanski *et al*. (1991) examined two groups of women from a rural region of eastern Poland. One group lived and worked in areas with relatively high levels of cadmium and lead in the soil ("contaminated area," n = 136), while another group lived and worked in areas with relatively low levels of cadmium and lead in the soil ("control area," n = 264). No measurements of soil cadmium or lead concentrations were reported. Blood concentrations of cadmium of women in the contaminated area were elevated over those from the control area (2.9 +/- 1.2 μg/L vs. 2.5 +/- 1.4 μg/L, $p = 0.03$). Blood concentrations of lead were also elevated (67.5 +/- 65.3 μg/L vs. 62.1 +/- 33.6 μg/L), but this was not statistically significant. Alcohol consumption, smoking, socioeconomic status, general health, and age distribution were similar between the two groups. Women from the contaminated areas had a lower percentage of more than three pregnancies than women from the control areas (39% vs. 52%, $p < 0.01$). There were no significant differences between the two groups in the frequency of miscarriage, stillbirth, or pre-term labor. There was, however, a weak but statistically significant correlation of blood Cd levels with number of pre-term labors ($r = 0.17$, $p < 0.05$). The blood Cd concentrations reported in both the contaminated and uncontaminated areas were several fold higher

than those typically reported from general populations in Western Europe or the U.S. (e.g. Alessio *et al*., 1984; Berglund *et al*., 1994; Bonithon Kopp *et al*., 1986a; Buchet *et al*., 1978; Janousek *et al*., 1994; Lauwerys *et al*., 1978).

4.2 Animal Data

While many studies have reported on parameters which are related to certain aspects of female reproduction, not all provide data which could be used to calculate "female fertility" or "female fecundity" indices (Moore *et al*., 1995). Hence the discussion of "fertility" below is restricted to studies which provide data on the number of successfully mated females for whom pregnancy was confirmed, and/or the number of females cohabited who displayed evidence of having successfully mated (i.e. a copulatory plug, or a sperm-positive vaginal smear). In many of the developmental toxicity studies of Cd, females were treated prior to mating, as well as during the mating period and throughout gestation. Typically, these studies present data on litter size and fetal weights for pregnant females, but give no indication as to the frequency of mating or the frequency of pregnancy among mated females. To avoid confusion between these aspects of female reproduction, studies which report only on outcomes for pregnant females are discussed below under the heading "litter size".

4.2.1 Fertility

Adverse effects on fertility have been reported from oral exposure of rats or mice to Cd (Schroeder and Mitchener, 1971; Sutuo *et al*., 1980a, 1980b; Wills *et al*., 1981). Schroeder and Mitchener (1971) gave cadmium to male and female Charles River mice at 10 ppm in drinking water for multiple generations. When 3 out of 5 pairs in the F2B generation failed to breed, the experiment was discontinued. In the study of Sutuo and coworkers (1980a, 1980b), cadmium was given to male and female Sprague-Dawley rats prior to and during mating, and to females throughout gestation. The route of exposure was stated to be oral, and while the method of administration was not specified, the context suggests that it was gavage. The doses given were 0.1, 1.0, or 10 mg Cd as $CdCl_2$/kg/day. Males and females were caged together for mating 6 days per week, for a maximum of 3 weeks, with a weekly change of partners if necessary. Females exposed to the highest dose of Cd had reduced body weight gain before and during gestation, and decreased food and water intake. Of the females which successfully mated, all were pregnant. Within this group, however, there were significant decreases in the number of total implants, and in the number of live fetuses per litter. Only 5/13 high-dose group females mated successfully, as evidenced by the presence of sperm in vaginal smears. There was also an increase in the mean length of time to mating from 3.6 days in the controls to 9.0 days in the high-dose group. Among other possibilities, failure to achieve a sperm-positive mating could have resulted from male infertility, abnormalities of male and/or female mating behavior, or possible alterations in the estrus cycle.

Wills and coworkers (1981) conducted a multigeneration study of cadmium in rats. Cadmium was given in the diet at concentrations of 80 (basal diet), 100 and 125 μg/kg, providing doses of approximately 4.4, 5.5, and 6.9 μg/kg body weight/day. The numbers of litters produced per female mated was significantly reduced in the high-dose group. This decrease was progressive in succeeding generations, from the F_2 through the F_4. There was also a trend for the mean weight of pups at weaning to decrease with increasing dose, but this never reached statistical significance. The decrease in numbers of litters produced, combined with the lack of effect on other pregnancy or fetal parameters, was interpreted as indicating a primary effect of cadmium on fertility. As the design of this study, as well as the others cited above, involved treating both males and females, it is not possible to determine whether one or both sexes were affected. In contrast to the results of Wills and coworkers (1981), no decreases in fertility were observed in a two-generation reproductive toxicity study of Sprague-Dawley rats given cadmium in drinking water at concentrations of 0, 0.1, 1.0, and 5.0 ppm (Laskey *et al*., 1980).

In attempting to produce an animal model of Itai-Itai disease, female rats were given 50 or 200 ppm $CdCl_2$ in their drinking water throughout 3 rounds of mating, gestation, and lactation (Takizawa *et al*., 1981). Although animals in the 200 ppm group gained less weight than controls, all treated animals were able to produce a litter in each of the 60-day breeding rounds. No details such as time-to-mating or litter size were provided in the paper. Using a somewhat similar protocol, Whelton and coworkers (1988) subjected female CF1 mice to 6 consecutive, 42-day rounds of gestation-lactation. Animals were given diets containing 0.25, 5.0, or 50.0 ppm Cd. Diets containing the same cadmium concentration were also formulated to be either sufficient or deficient in the vitamin, mineral, and fat content. The deficient diet was formulated with reduced concentrations of fat, calcium, thiamine HCl, riboflavin, and vitamins A and D. With a sufficient diet, no changes in fertility or litter size occurred with the addition of cadmium. With an insufficient diet containing 50 ppm cadmium, fertility was decreased by 45%, as compared to a 12% drop for the deficient diet alone. The design of this study was similar to a continuous breeding protocol, although breeding was conducted in discrete rounds with a mating period of 5 days. This is different from the more usual procedure in which male-female pairs are kept together throughout the study for determination of inter-litter intervals during a set period of time. The former procedure would have been less sensitive to any changes in the estrus cycle.

Female rats were exposed via inhalation to cadmium oxide at concentrations of 0.02 or 0.16 mg Cd/m^3 for 5 hours/day, 5 days/week for 5 months, or to 1 mg Cd/m^3 air for 4 months prior to mating (Baranski, 1985). Exposure was continued during mating and throughout gestation. Fertility was stated to be reduced in this group, although no data are presented in the paper. Over half the animals died in the highest exposure group, confounding interpretation of any observed reproductive changes among survivors in this group. There were no differences in litter size between controls and the lower-concentration groups, although litter sizes were quite small for all of these groups (average of 5.4 live fetuses per litter in the control group). Using a similar protocol, Baranski (1984) found no differences in fertility or litter size between controls and

female rats exposed to 0.02 or 0.16 mg Cd/m^3. In this study, however, the proportions of pregnant females out of those classified as "inseminated" was low in controls and in the two treated groups (54%, 44%, and 41%, respectively).

Although no data were presented, the percentages of inseminated and pregnant females were said to be unaffected in female rats given cadmium chloride 5 days/week for 5 weeks prior to mating, as well as during mating and gestation (Baranski *et al*., 1983). Treatment was by gavage at doses of 0.04, 0.4, or 4 mg Cd/kg/day. Viability of treated females was also said to be unchanged, although "some" deaths occurred in the high-dose group. There were also no effects of treatment on the average numbers of implantations, corpora lutea, live fetuses, or resorptions.

4.2.2 Litter size

Many studies have demonstrated no change in litter size with maternal exposure to cadmium by the oral route. This has been the case whether cadmium was given prior to and during gestation (Hastings *et al*., 1978; Pond and Yen, 1983; Sowa *et al*., 1982; Webster, 1988), or during the gestation period alone (Ahokas *et al*., 1980; Ali *et al*., 1986; Baranski, 1985, 1986, and 1987; Kelman *et al*., 1978; Pond and Walker, 1975; Saxena *et al*., 1986; Simmons *et al*., 1984; Sowa *et al*., 1982; Sowa and Steibert, 1985; Webster, 1978, 1979a, 1979b). The doses and concentrations of Cd which have been tested range from: 0.04 - 50 mg/kg bw/day by gavage, 0.25 - 200 ppm in the diet, and 0.0015 - 180 ppm in the drinking water. Decreased numbers of viable fetuses/litter were reported by Scharpf and coworkers (1972) when $CdCl_2$ was given by gavage at doses of 60 or 80 mg/kg bw on gestation days 6 - 19. Maternal viability was also severely compromised at these doses. In this same study, lower doses of 20 or 40 mg $CdCl_2$/kg had no adverse effects on maternal or fetal viability. As described in the preceding section, Baranski (1984, 1985) reported no changes in litter size with Cd exposure by inhalation. In the 1985 paper, information on oral exposure of rats via gavage to 2, 12 or 40 mg/kg on days 7-16 of gestation was also presented. At 40 mg/kg exposure, the mean number of live pups per litter was 5.0 compared to 11.4 in controls. No specific data on maternal toxicity were provided, but it appears that the highest exposure used caused maternal systemic toxicity, while the lower exposures did not.

4.2.3 Other litter endpoints

An inhalation study of female Fischer-344 rats treated prior to mating, and then mated to untreated males, failed to report on whether all the females actually mated, or if any of the females which mated were not pregnant (Kutzman *et al*., 1986). Beginning at 10 weeks of age female and male Fischer-344 rats were exposed to $CdCl_2$ aerosol at concentrations of 0.0, 0.3, 1.0, or 2.0 mg Cd/m^3 for 6 hours/day, 5 days/week, for a total of 62 exposure days. All animals exposed to the highest concentration died within 45 days, although females were somewhat more resistant than males. Six days after the end of the exposure period, treated females were caged for mating with untreated males, for a maximum mating period of 7 days. Pregnant animals were evaluated on gestation day 19, and there were no differences between treated and control groups in the number of viable fetuses, early or late deaths, or corpora lutea. Similarly, in the study of Sutuo and coworkers (1980a, 1980b), there were significant decreases in the number of total implants, as well as in the number of live fetuses per litter, at an oral dose of 10 mg Cd/kg bw.

Female Sprague-Dawley rats and Swiss (CD-1) mice were exposed to Cd in a developmental toxicity study (NTP, 1995). A significant decrease in the ratio of pregnant mice to sperm-positive mice at 0.5 and 2.0 mg cadmium oxide/m^3 air was observed, while a significant increase in the ratio of pregnant rats to sperm-positive rats was observed at 2.0 mg cadmium oxide/m^3. Exposure to Cd commenced only after mating had been confirmed, on gestation days 4 - 19 (rats) or 4 - 17 (mice). Hence change in the fecundity indices could not have resulted from treatment-related effects occurring at the time of mating. Neither litter size nor the average number of implantation sites per dam differed between treatment groups for either species. No data on corpora lutea were presented.

4.2.4 Pathology of Reproductive Organs

Ovarian weights of pregnant rats were significantly reduced following oral exposure to doses of 0.1 and 10 mg, but not 1.0, Cd/kg bw/day for 6 weeks prior to mating, as well as during mating and gestation (Sutuo *et al*., 1980a, 1980b). Thymus, liver, and adrenal weights were also significantly decreased in these animals, as were the total carcass weights.

No adverse effects were seen in non-pregnant female rats given $CdCl_2$ by gavage at doses of 25, 51, 107 or 225 mg/kg for 1 day (Borzelleca *et al*., 1989). In the same study, non-pregnant females exposed to the same levels for 10 consecutive days had dose-dependent increases in mortality and reductions in weight-gain of surviving animals, but ovarian weights were not affected. Absolute and/or relative liver, spleen, thymus, and kidney weights were depressed in a dose-dependent manner in the latter group, and there were focal necrotic changes in renal tubular epithelium as well as tubular degeneration.

Borzelleca and coworkers (1989) also studied the toxicity of cadmium chloride given to rats in drinking water at concentrations of 13 to 323 mg/L for 10 consecutive days. There were no significant effects on ovarian weight, or the weights of any other organs in females.

Female rats exposed for 13 weeks to cadmium oxide aerosol at a concentration of 1 mg/m^3 showed no treatment-related histological changes in the reproductive organs. (NTP, 1995).

The ovaries and uteri of 2 female beagle dogs were normal in weight, and gross and histological pathology after 3 months of a diet with cadmium chloride added to concentrations of 0, 1, 3, 10, or 30 ppm (Loeser and Lorke, 1977).

4.2.5 Estrus Cycle

There was a significant increase in the length of the estrus cycle in female rats exposed for 13 weeks to cadmium oxide aerosol at a concentration of 1 mg/m^3 (NTP, 1995). The estrus cycle of female mice was not affected by this same treatment. Neither species showed lengthened estrus cycles after 13 weeks inhalation exposure to lower concentrations of 0.025, 0.05, 0.1, or 0.25 mg/m^3. For both species, body weights at the end of the study were unchanged from control values, except for rats exposed to the highest concentration. In that group, body weights were 93% of the control values. The primary toxic effects were on the respiratory tract, with a reported NOAEL for lung effects in rats of 0.025 mg/m^3, and no reported NOAEL for this endpoint in mice. All treated animals survived the study period.

The mean duration of the estrus cycle was significantly increased in female rats given 40 mg Cd/kg bw orally, or exposed by inhalation to 1 mg Cd/m^3 for at least 6 weeks (Baranski and Sitarek, 1987). By the oral route, cadmium had no effect on the estrus cycle at lower doses of 0.04, 0.4, or 4.0 mg/kg/day. After 8 or 14 weeks of exposure, animals in the high-dose group had a mean estrus cycle length which was double that of the control animals, and approximately 30% of the animals in that group went into persistent diestrus. Mortality was significantly increased in this group, while weight-gain was significantly decreased.

Following 13-14 weeks of inhalation exposure to a concentration of 1 mg Cd/m^3, estrus cycles were significantly prolonged, along with significant decreases in weight gain and increases in mortality (Baranski and Sitarek, 1987). After 20 weeks, females exposed to lower concentrations of 0.02 and 0.16 mg Cd/m^3 had estrus cycles which were significantly longer than at the commencement of the study. This was also seen in the 0.16 mg/m^3 group by 13-14 weeks of exposure. The estrus cycles in all groups, including the controls, lengthened with time, but those of the treated groups lengthened more dramatically. Thus, there seems to be both a dose and time effect on estrus cycle length. Mortality and weight-gain were not significantly affected in the 2 lower concentration groups.

4.3 Other relevant data

Cadmium has been identified in the female reproductive organs of humans (Varga *et al*., 1993) and experimental animals (Bhattacharyya *et al*., 1981; Webster, 1988). An age-dependent increase was found in the cadmium-content of human ovaries, with a peak at around 65 years of age at 0.24 ppm (Varga *et al*., 1993).

Female mice treated once with trace amounts of ^{109}Cd by gavage during pregnancy or lactation showed concentrations of Cd in the reproductive tract which ranged from 1% to 12% those in the liver or kidney (Bhattacharyya *et al*., 1981). Female mice treated with trace, 0.25 or 40 ppm Cd (labeled with ^{109}Cd) in water for 1 month and throughout gestation showed relatively low concentrations in reproductive organs. Ovaries, uterus, and vagina had <1% to 10% of the concentrations found in liver and kidney (Webster, 1988).

Cadmium exposure has been shown to affect the tissue concentrations of other metals, such as Zn, Fe, and Cu, in female rats (Baranski, 1987; Pond and Walker, 1975, Sowa and Steibert 1985; Steibert *et al*., 1984; Takizawa *et al*., 1981; Yuen *et al*., 1994). In female rats given 50 or 200 ppm $CdCl_2$ in drinking water throughout 3 consecutive rounds of mating, gestation, and lactation, bone-Zn content decreased significantly as Cd intake increased (Takizawa *et al*., 1981). Additionally, the receptors for steroid and thyroid hormones are zinc metalloproteins, as are many other proteins (reviewed in Berg and Shi, 1996). Alterations of Zn metabolism, for example, or substitution of Cd for Zn in zinc metalloproteins, could alter normal physiology, including reproductive function.

4.4 Integrative Evaluation

Animal studies relevant to the female reproductive toxicity of Cd have investigated possible effects on fertility, litter size, ovarian pathology, and the estrus cycle. Epidemiological studies of human populations have considered endpoints such as spontaneous abortion, stillbirth, or preterm labor. Human studies have not been identified which evaluate endpoints of importance in the animal literature on Cd, such as fertility and abnormalities of the female reproductive cycle.

Fertility deficits have been reported in experimental breeding studies where both sexes were exposed to Cd. Studies in which female, but not male, experimental animals were exposed to Cd have not given consistent evidence of adverse effects on fertility. There is some evidence for an adverse effect of cadmium exposure on ovarian weights, but this has not been a consistent finding. Cadmium treatment by the oral or inhalation routes has been associated in rats, but not in mice, with lengthened estrus cycles. In humans, elevated cadmium levels, as measured in maternal blood or infant hair, have been significantly correlated with adverse effects such as preterm labor or a diagnosis of

"small for dates." While interpretation of the human epidemiological studies is complicated by the difficulty of controlling for confounding exposures, particularly to cigarette smoke and other metals such as lead, the available human data do not contradict the evidence available from animal studies.

Adverse effects on the fertility of experimental animals have been reported in several studies following cadmium exposure of both females and males by the oral route. Only one such study reported no effect, but the highest concentration of Cd given in the drinking water was 5 ppm. In all of these studies, both males and females were treated, hence it is not possible to determine whether any observed changes in fertility were due to effects on one or both sexes. Studies in which females, but not males, were exposed to Cd have not given consistent evidence of adverse effects on fertility. In one study involving multiple rounds of mating, pregnancy and lactation, no effect on female fertility was produced by Cd exposure of up to 50 ppm in feed. When Cd exposure was combined with a diet deficient in vitamin and mineral content, there was a significant decrease in female fertility. This decrease was far greater than that resulting from the deficient diet alone. The authors concluded that their finding supported the idea that Cd can exert toxic effects through interference with other trace elements, especially when those elements are in short supply.

No abnormal ovarian pathology has been reported following Cd exposure, even in animals showing adverse effects on other organs. Females seem to be generally less sensitive to Cd-induced toxicity than males, showing lower mortality rates, and less severe organ pathology than males in the same dose-groups in the same studies (see section 3.3.4). Ovarian weights were reported to have been significantly decreased with cadmium exposure in two studies by the same authors, but not in several other studies.

Cadmium-treatment by the oral or inhalation routes has been associated with lengthened estrus cycles in rats, but not in mice. The lengthening is apparently particular to the diestrus phase of the cycle, with some percentage of treated animals entering persistent diestrus. It is expected that prolonged diestrus would result in a lowered lifetime fertility, but no standard continuous-breeding data are available to directly demonstrate such an effect. However, significant changes in estrus cycle length are indicative of female reproductive toxicity (US EPA, 1988; Moore *et al*., 1995), as they are symptomatic of alterations in the intrinsic controlling mechanisms of the female reproductive system (US EPA, 1988).

Elevated Cd levels in humans, as measured in maternal blood or infant hair, have been significantly correlated with adverse effects such as pre-term labor or a low birthweight for gestation age. However, it is very difficult in such studies to isolate any effects of Cd from potentially confounding exposures such as Pb or cigarette smoking. No associations have been found between Cd levels and spontaneous abortion, stillbirth or premature birth. Overall, the available epidemiological data do not contradict the evidence from animal studies concerning the female reproductive toxicity of cadmium.

Table 3. Animal studies of female reproductive effects of Cd by inhalation

Reference	Study Design	Reported effects[(1)]
Baranski, 1984	rat, 5 months plus gestation, 5 hr/d, 5 d/wk 0, 0.02, 0.16 mg/m^3	Fertility and litter size not reduced at any exposure.
Baranski, 1985	rat, 4-5 months plus gestation, 5 hr/d, 5 d/wk 0, 0.02, 0.16, 1.0 mg/m^3	Maternal death increased at 1.0 mg/m^3. Litter size and maternal weight gain not reduced at any exposure.
Baranski and Sitarek, 1987	rat, 20 weeks, 5 hr/d, 5 d/wk 0, 0.02, 0.16, 1.0 mg/m^3	Lengthened estrous cycle at 1.0 mg/m^3. Female weight gain reduced at 1.0 mg/ m^3. Female death increased at 1.0 mg/ m^3.
Kutzman *et al*., 1986	rat, 62 days, 6 hr/d, 5 d/wk, 0, 0.3, 1.0, 2.0 mg/m^3	Maternal weight gain reduced at 1.0 mg/m^3. Maternal death increased at 2.0 mg/m^3 (0% control, 100% treated). Fertility and litter size not reduced at any exposure.
NTP, 1995	mouse, gestation days 4-17, 6 hr/d 0, 0.05, 0.5, 2.0 mg/m^3	Maternal death increased at 2.0 mg/m^3 (0/33 control, 5/33 treated). Maternal weight gain reduced at 2.0 mg/m^3. Reduced ratio of pregnant to sperm positive females at 0.5, 2.0 mg/m^3.
NTP, 1995	mouse, 13 weeks, 6 hr/d, 5 d/wk 0, 0.025, 0.05, 0.1, 0.25, 1.0 mg/m^3	Estrous cycle not altered, female death not increased, female weight gain not reduced at any exposure.
NTP, 1995	rat, gestation days 4-19, 6 hr/d 0, 0.05, 0.5, 2.0 mg/m^3	Maternal weight gain reduced at 2.0 mg/m^3. Maternal deaths, resorptions not increased. Increased ratio of pregnant to sperm positive females at 2.0 mg/m^3.
NTP, 1995	rat, 13 weeks, 6 hr/d, 5 d/wk 0, 0.025, 0.05, 0.1, 0.25, 1.0 mg/m^3	Lengthened estrous cycle at 1.0 mg/m^3. Female weight gain not reduced, female death not increased at any exposure.

[(1)] Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

Table 4. Animal studies of female reproductive effects of Cd by oral routes

Reference	Study Design[(1)]	Reported effects[(1)]
Food		
Loeser and Lorke, 1977	dogs, in feed, 3 months 0, 1, 3, 10, 30 ppm	No effect on organ weights, gross pathology or histopathology of ovaries and uteri at any exposure.
Pond and Walker, 1975	rat, in feed, gestation 0, 200 ppm	Female food consumption and weight gain reduced at 200 ppm. Litter size not reduced at any exposure.
Pond and Yen, 1983	rat, in feed, 7 weeks plus gestation 0, 61.3 ppm	Litter size and adult weight gain not reduced at any exposure.
Whelton *et al*., 1988	mouse, in feed, 252 days (6 rounds of reproduction) 0, 0.25, 5.0, 50.0 ppm	No effects of cadmium alone. Combined with a deficient diet, reduced fertility at 50 ppm.
Wills *et al*., 1981	rat, in feed, 4 generations (male and female exp.) 0.080, 0.100, 0.125 ppm	Fertility decreased at 0.125 ppm.
Gavage		
Baranski *et al*., 1983	rat, gavage, 5 weeks plus mating period (up to 3 weeks) plus gestation, 5d/wk 0, 0.04, 0.4, 4 mg/kg/d	Fertility and litter size not decreased at any exposure.
Baranski, 1985	rat, gavage, days 7-16 gestation 0, 2, 12, 40 mg/kg/d	Retarded intrauterine development at all exposures (retarded ossification of sternum and ribs). Litter size decreased at 40 mg/kg Increased congenital defects at 40 mg/kg
Baranski and Sitarek, 1987	rat, gavage, 14 weeks, 5 d/wk 0, 0.4, 4, 40 mg/kg/d	Female weight gain reduced at 4 mg/kg/d. Female death increased at 40 mg/kg/d. Lengthened estrous cycle at 40 mg/kg/d.
Borzelleca *et al*., 1989	rat, gavage, 1 day or 10 days 0, 25, 51, 107, 225 mg/kg/d ($CdCl_2$)	Ovarian weight not affected.

[(1)] Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

Table 4 (continued). Animal studies of female reproductive effects of Cd by oral routes

Reference	Study Design[(1)]	Reported effects[(1)]
Scharpf *et al*., 1972	rat, gavage, gestation days 6-19 0, 20, 40, 60, 80 mg/kg/d	Viable fetuses/litter decreased at 60, 80 mg/kg/d. Female death increased at 60, 80 mg/kg/d.
Simmons *et al*., 1984	rat, gavage, gestation days 6-18 0, 10, 25, 50 mg/kg/d	Female death increased at 50 mg/kg/d Litter size not decreased at any exposure.
Sutuo *et al*., 1980a, 1980b	rat, gavage, 6-9 wk plus gestation (male and female exposed) 0, 0.1, 1.0, 10 mg/kg/d	Litter size decreased at 10 mg/kg/d. Ovary weight reduced at 0.1 and 10 mg/kg/d. Female weight gain reduced at 10 mg/kg/d.
Water		
Ahokas *et al*., 1980	rat, water, gestation days 1-21 0, 1, 10, 100 ppm	Female weight gain reduced at 10, 100 ppm. Food intake reduced at 10, 100 ppm Water intake reduced at 1, 10, 100 ppm Litter size not decreased at any exposure.
Ali *et al*., 1986	rat, water, gestation 0, 4.2, 8.4 ppm	Litter size not decreased at any exposure. Adult weight gain not decreased at any exposure.
Baranski, 1986	rat, water, gestation days 1-20 0, 60 ppm	Litter size not decreased at any exposure.
Baranski, 1987	rat, water, gestation days 1-20 0, 60, 180 ppm	Female food and water intake and weight gain reduced at 60, 180 ppm. Fertility, litter size not decreased and female death not increased at any exposure.
Borzelleca *et al*., 1989	rat, water, 10 days 0, 15, 138, 287 ppm ($CdCl_2$)	Ovarian weight not affected.
Hastings *et al*., 1978	rat, water, 90 days plus gestation 0, 17.2 ppm	Female weight gain not reduced at any exposure. Litter size not decreased at any exposure.

[(1)] Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

Table 4 (continued). Animal studies of female reproductive effects of Cd by oral routes

Reference	Study Design[(1)]	Reported effects[(1)]
Kelman *et al*., 1978	rat, water, gestation 0, 10, 25 ppm	Litter size and rate of conception not affected at any exposure.
Laskey *et al*., 1980	rat, water, 2 generation (gestation + 100 d + gestation) 0, 0.1, 1, 5 ppm	Fertility, litter size and adult weight gain not decreased at any exposure.
Saxena *et al*., 1986	rat, water, gestation 0, 100 ppm	Litter size not decreased at any exposure.
Schroeder and Mitchner, 1971	mouse, water, 2 generation (male and female exposed) 0, 10 ppm	Fertility and fecundity of breeding pairs decreased in the F2 generation at 10 ppm. Litter size not decreased at any exposure.
Sowa and Steibert, 1985	rat, water, gestation days 1-20 0, 50 ppm	Female water consumption and weight gain reduced at 50 ppm. Litter size not decreased at any exposure.
Sowa *et al*., 1982	rat, water, 30 days; 30days + gestation; gestation 0, 100 ppm	Fertility not decreased at any exposure.
Takizawa *et al*., 1981	rat, water, 180 days >0.05, 50, 200 ppm	Fertility not decreased at any exposure.
Webster, 1978	mouse, water, gestation days 1-19 0, 10, 20, 40 ppm	Litter size not decreased at any exposure.
Webster, 1978	mouse, water, gestation days 1-19 0, 10, 20, 40 ppm	Litter size not decreased at any exposure.
Webster, 1988	mouse, water, 1 mo. plus gestation days 1-18 0, 0.0015, 0.24, 40 ppm	Litter size not decreased at any exposure.

[(1)] Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

5. Male Reproductive Toxicity

5.1 Human Data

Studies of the male reproductive toxicity of cadmium in humans have addressed fertility, hormone levels, and testicular pathology, as well as semen and sperm parameters. In a well-designed cohort study that controlled adequately for confounding by lead exposure, Gennert and coworkers (1992) found no difference in fertility between wives of cadmium-exposed and unexposed workers. Twenty-five percent of the 83 cadmium-exposed workers were considered to be "excessively exposed", as they displayed symptoms of kidney dysfunction. Another group of 77 occupationally exposed men were evaluated for the effects of cadmium on the pituitary-testicular endocrine axis (Mason, 1990). Fertility, per se, was not evaluated. There were no changes in the levels of serum testosterone, luteinizing hormone (LH), or follicle-stimulating hormone (FSH) associated with cadmium exposure. The authors point out that elevated plasma FSH in association with normal LH and testosterone levels is found in patients with severe damage to seminiferous tubules. No evidence was found for this condition in the exposed group, but other types of testicular damage, potentially leading to infertility, were not ruled out by the parameters studied.

An early autopsy case series (Smith *et al*., 1960) looked at six men who had died from chronic occupational exposure to cadmium. The cause of death in these cases was determined to be severe disabling emphysema; their other organs were considered to be pathologically normal. In four cases the testes were examined histologically, and there was found to be profound depression of sperm maturation. The authors suggested that this finding was a secondary effect of the terminal illness. A much more recent study (Oldereid *et al*., 1993) looked at cadmium concentrations in the reproductive organs of 41 men who had died from causes other than cadmium poisoning. Cadmium concentration increased with increasing age, particularly in epididymides and seminal vesicles. The authors did not consider that this age-dependent increase in testicular cadmium was generally a significant factor in infertility, as it did not become apparent until after about age 40.

Cigarette smoking is a significant source of cadmium exposure in humans, and several studies have looked at the relationship between smoking, cadmium levels, and infertility. In a study of men attending an infertility clinic (Saaranen *et al*., 1989), higher cadmium concentrations were found in the seminal plasma of smokers than in non-smokers. However, cadmium concentrations in seminal plasma or serum were not correlated with parameters of semen quality, nor with fertility during the previous 6 months. Cadmium, lead, and zinc levels, as well as sperm parameters, were measured in the semen of 58 smokers and nonsmokers being tested for infertility (Oldereid *et al*., 1994). Cadmium levels in seminal plasma increased with increasing tobacco consumption, but this was statistically significant only with 20 or more cigarettes smoked per day. No changes in

sperm count, quality, motility, or morphology were found with increasing cadmium levels. Zinc levels were significantly decreased, however, even with the lower cigarette consumption of between 1 and 19 per day. As decreasing zinc levels were significantly correlated with increasing cadmium levels, the authors suggest that cadmium hindered the transport of zinc into prostatic fluid. This in turn would reduce the levels of zinc normally available to the spermatozoa, potentially compromising chromatin stability. While a direct association with smoking was not investigated, chromatin zinc deficiency and low chromatin stability have been associated with cases of unexplained infertility.

A study from an infertility clinic in Singapore compared the levels of cadmium and lead in the blood and seminal plasma of smokers and nonsmokers (Chia *et al*., 1994). Significant negative correlations were found between cadmium blood concentration and sperm density or normal sperm morphology. A significant negative correlation was also found between serum cadmium concentration and sperm volume. Cadmium levels in both blood and seminal plasma showed significant positive correlations with "cigarette years". Another study from the same clinic found that blood cadmium levels were negatively correlated ($r=0.23$, $p<0.05$) with sperm density in men with low sperm density, but not in men with high sperm density (Xu *et al*., 1993b). Reduction in sperm density was greatest among men with blood cadmium concentrations greater than 1.5 μg/L. Seminal plasma levels were correlated with sperm volume reduction ($r=0.29$, $p<0.05$). In contrast, in a study of men without occupational exposure to cadmium, no association was found between semen cadmium levels and sperm count, sperm concentration or ejaculate volume (Noack-Fuller *et al*., 1993). A positive correlation was found between semen cadmium levels and sperm motility ($r=0.053$, $p<0.02$), linear velocity ($r=0.757$, $p<0.001$), and curvilinear velocity ($r=0.643$, $p<0.002$). Overall, these men had lower semen cadmium levels than those in the Saaranen and Xu studies.

A study of the effects of cadmium on human sperm *in vitro* (Dwivedi, 1983) demonstrated inhibition of spermatozoan choline acetyl transferase (ChAT) activity. Sperm motility was reduced by 25% in these experiments. Similar results for ChAT activity were found in rats following either acute or chronic cadmium exposure *in vivo*. The chronically-exposed rats also suffered from impaired fertility, leading the author to conclude that cadmium-induced sterility is caused by inhibition of sperm motility. The inhibited motility was due to insufficient availability of acetylcholine, in turn resulting from inactivation of choline acetyltransferase.

5.2 Animal Data

5.2.1 Fertility

Decrements in the fertility of male rats have been demonstrated following oral administration of cadmium as either a single, high dose, or with long-term exposure to lower doses. One fertility study, conducted by the inhalation route, found no adverse effects. Studies which have not identified effects on male fertility have generally used

lower doses, shorter exposure periods, or have evaluated only a single generation of exposed animals.

No effects on fertility were found in male Sprague-Dawley rats given a single oral dose of cadmium at 6.25, 12.5, or 25 mg/kg (Dixon *et al*., 1976). Fertility studies were conducted by serial matings over a 70-day period subsequent to treatment. A single oral dose of 25 or 50 mg/kg ^{109}Cd was similarly ineffective (Kotsonis and Klaassen, 1977). Adverse effects on the fertility of male Sprague-Dawley rats were found, however, at higher doses of 100 or 150 mg ^{109}Cd /kg (Kotsonis and Klaassen, 1977). Fertility was investigated 14 days following dosing, by caging each male with an untreated female for a period of one week. The pregnancy status of females was investigated 21 days after the first day of the mating trial. Male rats given the two highest doses of $CdCl_2$ weighed significantly less than controls on days 2 through 14 following dosing.

No effects on fertility or other reproductive endpoints were found in Fischer 344 rats exposed to cadmium as a $CdCl_2$ aerosol for 62 exposure days (Kutzman *et al*., 1986). A cadmium concentration of 2.0 mg/m^3 was lethal to all animals within 45 exposure days. Six days following the cessation of exposure to 0, 0.3, or 1.0 mg Cd/m^3, treated animals were caged for mating with untreated animals of the opposite sex. Females were sacrificed 19 days after mating, and no changes were found in the numbers of viable embryos, early or late resorptions, or corpora lutea.

No effects on male fertility were found in 3 studies providing cadmium in drinking water for periods of up to 24 weeks (Dixon *et al*., 1976; Kotsonis and Klaassen, 1978; Zenick *et al*., 1982). Dixon and coworkers (1976) gave cadmium in drinking water to male Sprague-Dawley rats at concentrations of 0.001, 0.01, or 0.1 mg/L (calculated by the authors as giving a maximum dose of 14 μg/kg body weight/day), for 90 days. In a study by Zenick and coworkers (1982), no changes in fertility were identified in male rats given cadmium as $CdCl_2$ in drinking water at concentrations of 0, 17.2, 34.4, or 68.8 ppm for 70 to 80 days (Zenick *et al*., 1982). The treatment period was chosen to correspond to the time required for a complete sperm cycle, in order to expose all stages of the cycle. Litters sired by treated males showed no changes from controls in litter size, numbers of implantations, implants per corpora lutea, or pup weights. No changes were found in behavioral assays performed on live-born offspring of treated males at 21 or 90 postnatal days of age. At the end of a 3, 6, 12, or 24-week period of exposure to 10, 30, or 100 ppm Cd in drinking water, male rats were mated with untreated females (Kotsonis and Klaassen, 1978). No differences were found between treated and control groups in the fractions of females impregnated or in the size of litters produced.

In contrast to the results described above, a dose and time-dependent decrease in fertility was found in male Sprague-Dawley rats given 1 or 2 μg Cd/ml drinking water for one to three months (Dwivedi, 1983). After the first four weeks of treatment, each male was caged with three females. After one week of cohabitation, the females were replaced with three new females. This was repeated at weekly intervals for the duration of the treatment period. Pregnancy was evaluated on the 14th day following mating. By the end of the treatment period, fertility of the low-dose males was reduced to 33% of control

values, while high-dose males were completely sterile. In another study (Saygi *et al*., 1991) fertility of male Wistar rats was evaluated following 52 weeks consumption of drinking water which contained cadmium at 10 mg/L. At the end of this time, treatment was discontinued, and males were caged with two untreated females for 30-days. Fertility was determined on the basis of whether births were observed during this time. A significant decrease in male fertility was detected.

Reduced sperm counts in F_1 males, but no decreases in fertility, were observed in a two-generation reproductive toxicity study of Sprague-Dawley rats (Laskey *et al*., 1980). The animals were given cadmium in drinking water at concentrations of 0, 0.1, 1.0, and 5.0 ppm. Cadmium treatment did not produce measurable effects on relative testes weights of F_1 males at 50 or 130 days of postnatal age, or on epididymal sperm counts at 50 days. These animals were mated at approximately 100 days of age, and no adverse effects on fertility were noted. At 130 days of age, serum testosterone levels were no different from controls, but epididymal sperm counts were significantly decreased in the 5.0 ppm group. At this time-point, liver weights were significantly decreased in both the 1.0 and 5.0 ppm groups, and body weight was decreased at all three cadmium concentrations.

In contrast to the results of Laskey and coworkers (1980), adverse effects on fertility have been reported following oral exposure of rats or mice to Cd (Schroeder and Mitchener, 1971; Sutuo *et al*., 1980a, 1980b; Wills *et al*., 1981). Schroeder and Mitchener (1971) gave Cd to male and female Charles River mice at 10 ppm in drinking water for multiple generations. When 3 out of 5 pairs in the F2B generation failed to breed, the experiment was discontinued. Adverse effects on fertility were found in a multigeneration study of cadmium in rats (Wills *et al*., 1981). Cadmium was given in the diet at concentrations of 0.080 (basal diet), 0.100 or 0.125 ppm, estimated to provide doses of approximately 4.4, 5.5, and 6.9 μg/kg body weight/day. The numbers of litters produced per female mated was significantly reduced in the high-dose group. This decrease was progressive in succeeding generations, from the F_2 through the F_4. The decrease in numbers of litters produced, combined with the lack of effect on other pregnancy or fetal parameters, was interpreted as indicating a primary effect of cadmium on fertility. As the study design involved treating both males and females, it is not possible to determine whether one or both sexes were affected.

In the study of Sutuo and coworkers (1980a, 1980b), cadmium was given to male and female Sprague-Dawley rats prior to and during mating, and to females throughout gestation. The route of exposure was stated to be oral, and while the method of administration was not specified, the context suggests that it was gavage. The doses given were 0.1, 1.0, or 10 mg Cd as $CdCl_2$/kg/day. Males and females were caged together for mating 6 days per week, for a maximum of 3 weeks, with a weekly change of partners if necessary. Of the females which successfully mated, all were pregnant. Within this group, however, there were significant decreases in the number of total implants, and in the number of live fetuses per litter. Only 5/13 high-dose group females mated successfully, as evidenced by the presence of sperm in vaginal smears. There was also an increase in the mean length of time to mating from 3.6 days in the controls to 9.0 days in the high-dose group. Among other possibilities, failure to achieve a sperm-

positive mating could have resulted from male infertility, abnormalities of male and/or female mating behavior, or possible alterations in the female estrus cycle.

5.2.2 Organ Pathology

Changes in testes weights and/or testicular pathology have been described after a single, high, oral dose of cadmium (Andersen *et al*., 1988; Bomhard *et al*., 1987; Kotsonis and Klaassen, 1977), or after long-term exposure to lower doses of cadmium (Borzelleca *et al*., 1989; Kutzman *et al*., 1986; Saygi *et al*., 1991; Saxena *et al*., 1989; Zielinska-Psuja *et al*., 1979). Shorter-term exposures and lower doses have not been associated with adverse male reproductive effects (Bomhard *et al*., 1987; Borzelleca *et al*., 1989; Caflisch, 1994; Zenick *et al*., 1982). Testes, prostate, seminal vesicle, and body weights were unchanged from control values in male rats given 0.001, 0.01 or 0.1 mg cadmium/L drinking water for up to 90 days (Dixon *et al*., 1976). Nor were there any significant changes in plasma levels of FSH or LH. A similar lack of effect on body weight, testes weight, or cauda weight was found in male rats given 17.2, 34.4, or 68.8 ppm cadmium in drinking water for 70 to 80 days (Zenick *et al*., 1982). No gross pathological findings were noted in the testes of male Wistar rats given drinking water containing 10 ppm cadmium for 40 weeks (Saygi *et al*., 1991). At the histopathological level, however, there was evidence of necrosis of sperm-cell precursors, as well as damage to the seminiferous tubules and surrounding tissue. This pathology was more severe by the end of 56 weeks. Cadmium could not be detected in the testes by the methods used in this study, but was observed to accumulate in liver and kidney.

The testes of Sprague-Dawley rats given a single oral dose of 100 or 150 mg Cd/kg body weight showed histopathological changes at 2 and 14 days following dosing (Kotsonis and Klaassen, 1977). These effects consisted of focal testicular necrosis and reduced spermatogenesis. Kidney, liver, heart, pancreas, small intestine, spleen and lung were all considered to be within normal limits. Thus, testicular injury was the major toxic effect of an acute, sublethal, oral dose of cadmium. No effects were observed at lower doses of 25 or 50 mg $CdCl_2$/kg. The testicular concentration of cadmium at 2 days following dosing were ~0.3 and 0.4 μg/g tissue at the 2 highest doses, respectively. These levels were far lower than those found in the liver, which held the majority of the cadmium body-burden, kidney and intestine. A similar pattern of relative tissue concentrations was also seen at 10 days following acute oral Cd, at doses ranging from 5 - 790 μmol/kg bw (Anderson *et al*., 1988).

In the studies of Zielinska-Psuja and coworkers (1979), testicular damage was not observed until male rats had been exposed to cadmium chloride daily for at least 6 months. The animals were given $CdCl_2$ in feed pellets, to give doses of 0, 8.8 or 88 mg/kg body weight/day. They were sacrificed for pathological examination at 6, 9, 15, or 18 months of age, following 3, 6, 12, or 16 months of treatment, respectively. Animals in both dose groups failed to gain weight at control levels. Final body weights of the low-dose group were 10-15% lower than those of controls; final weights of the high-dose group were 50-70% lower than controls. Absolute testes weights of the high-dose group

were significantly reduced at all time points. This effect was seen in the low-dose group only after 6 and 12 months of treatment, and had resolved by 15 months of treatment. Relative testes weights were significantly increased over controls at both doses of $CdCl_2$, but only at the 6 months (of treatment) timepoint. Testicular histopathology was normal in both dose groups after 3 and 6 months of treatment. At later timepoints, the high-dose group showed abnormalities, particularly of the central area of the testis. These effects were described as: reduced diameter of seminiferous tubules, thickening of the border tissue, and a proliferation of connective tissue which appeared to have replaced the normal endocrine and germinative elements. After 3 months of treatment, the serum concentrations of luteinizing hormone (LH) was no different between treated and control animals. Serum testosterone of high-dose animals was significantly lower than that of controls at this timepoint. After 6 months of treatment, LH levels were significantly higher than controls in both treated groups. Serum testosterone was also significantly increased, but only in the high-dose group. After 12 months of treatment, serum LH of the high-dose group was significantly lower than in controls, and testosterone levels did not differ between groups. After 15 months of treatment, LH levels in both treated groups were lower than controls, and testosterone was significantly increased over controls only in the low-dose group. Testicular concentrations of cadmium (per 100 g tissue) increased with dose and time of exposure.

Other studies of chronic cadmium exposure have described similar effects. Significant increases in relative testes weights have been noted in several studies (Borzelleca *et al*., 1989; Saxena *et al*., 1989; Kutzman *et al*., 1986). Inhalation of a $CdCl_2$ aerosol containing cadmium at a concentration of 1.0 mg/m^3 was the mode of exposure in the study of Kutzman and coworkers (1986), other studies were conducted by the oral route. Male rats, given 50 ppm cadmium in drinking water for 120 days following weaning, had relative testes weights which were significantly increased over controls (Saxena *et al*., 1989). Their absolute testes weights were increased over controls, and their body weights were lower than controls, but neither of these measures reached statistical significance. At the histological level, there was a significant increase in damage to the seminiferous tubules, and a significant decrease in the diameter of the seminiferous tubules. In contrast, no histopathological changes were noted in the testes of rats given 10, 30, or 100 ppm Cd in drinking water for 3, 6, 12, or 24 weeks (Kotsonis and Klaassen, 1978).

Testes to body weight ratios were increased in male rats given $CdCl_2$ by gavage at doses of 25 and 51 mg/kg/day for 10 days (Borzelleca *et al*., 1989). At a higher dose of 107 mg $CdCl_2$/kg/day, relative testes weights were no different from controls, but the absolute testes weights were significantly reduced. Body weights were also significantly lower than controls, in a dose-dependent manner at all doses of $CdCl_2$, as were the weights of many other organs. Testicular atrophy and necrosis were observed in the 107 mg/kg group, along with atrophy or loss of spermatogenic elements. Mortality was high in this group, with three out of ten animals dying before the end of the study. At the highest dose level of 225 mg $CdCl_2$/kg/day, all ten animals died before the end of the ten-day treatment period. This study also had a drinking water component, with animals given $CdCl_2$ calculated to give doses of 2.5, 25, or 51 mg $CdCl_2$/kg/day for 10 days. There was

a dose-dependent decrease in body weight gain, and a significant increase in the testes to body weight ratio at the high dose. There were no histopathological changes in the testes, and no deaths occurred during the exposure period.

The effects of cadmium exposure on male reproductive organs has also been evaluated in rabbits, beagle dogs and sheep. An increase in the size of lysosomes of the Sertoli cells was the only significant change noted in a study of male rabbits given 20 μg Cd/ml drinking water for 10 months (Boscolo *et al*., 1985). This finding was attributed to the accumulation of metallothionein. Cadmium content of the testes averaged 0.8 μg/g wet weight, a significant increase over controls, while levels of zinc and copper were unchanged.

No pathological changes were found in the prostate, testes, or other organs of beagle dogs given cadmium in their feed for 3 months (Loeser and Lorke, 1977). Cadmium concentrations in the liver and kidneys of treated animals increased with dose (0, 1, 3, 10, and 30 ppm). Weight gain was normal, and there were no clinical signs of toxicity.

Growing sheep were grazed on forage which was grown on soil amended with municipal sewage sludge (Hogue *et al*., 1984). Cadmium levels were higher, but not significantly so, in the tissues of exposed sheep as compared to controls. Body weight gain and carcass quality were unaffected. There were no changes at the ultrastructural level in liver, kidney, muscle, or testes. There were no significant changes in epididymal weights or motility of cauda epididymal sperm. The only significant changes in the exposed group were an increase in blood uric acid levels, and an increase in testes weight relative to body weight.

5.2.3 Sperm and Semen Parameters

Sperm counts and motility have been shown to be affected by cadmium exposure, but only after long-term treatment. Seventy to eighty days exposure to 17.2, 34.4, or 68.8 ppm cadmium in drinking water had no effect on the sperm count and sperm morphology of male rats (Zenick *et al*., 1982). In contrast, after 120 days exposure to 50 ppm cadmium in drinking water, there was a significant increase in the percentage of nonmotile sperm, and a significant decrease in epididymal sperm counts of male rats (Saxena *et al*., 1989). A reduced number of spermatids per testis was observed in rats, but not in mice, exposed to 1 mg cadmium/m^3 by inhalation for 13 weeks, 5 days/week, 6 hours/day (NTP, 1995). There were no concomitant histopathological lesions in the reproductive organs of male rats, suggesting that the effects on spermatid number could be related to factors other than direct cellular damage, such as hormonal changes.

The mechanism by which cadmium affects sperm parameters is unknown, but certain aspects of cadmium's influence on testicular biochemistry have been evaluated. Radioactive cadmium was incorporated into early elongated spermatids, spermatogonia, and early spermatids (Dixon *et al*., 1976). Compared to other spermatogenic cell types, the largest proportion of the dose was incorporated into late elongated spermatids.

Incorporation of thymidine, uridine, and L-leucine by specific spermatogenic cell types was not affected by cadmium either *in vitro* or *in vivo.*

A significant increase was found in the alkalinity of luminal fluid in the seminiferous tubules of Sprague-Dawley rats given 50 or 100 ppm cadmium as $CdCl_2$ in drinking water for 40 days (Caflisch, 1994). No effects were found on the pH of other areas, such as the epididymal duct. Nor were there any effects on testis or epididymal weights, or on plasma testosterone levels. The functional significance of altered pH in the seminiferous tubule fluid is unclear, although alkalinization of seminiferous and epididymal duct fluid has been described as part of the toxic response of the testes to a single sc injection of cadmium (Caflisch and DuBose, 1991).

The activity of spermatozoan choline acetyl transferase (ChAT) was significantly reduced in male rats following either chronic or acute exposure to cadmium (Dwivedi, 1983). A similar result was found for human sperm, following *in vitro* incubation with cadmium, with decrements in motility noted at the same time. The chronically-treated rats also suffered from impaired fertility, leading the author to postulate a causal relationship between ChAT activity, sperm motility, and infertility.

5.3 Other Relevant Data

Cadmium has been measured in the reproductive organs of human males, as well as in human seminal fluid. The concentration of Cd found in testes, epididymides, and seminal vesicles was found to increase with age in a sample of men not known to have had excessive exposures to Cd (Oldereid *et al*., 1993). In general, testes and other reproductive organs accumulate relatively small amounts of Cd compared to kidney and liver. In a sample of 41 men who died suddenly, from causes other than Cd-poisoning, testicular Cd concentrations averaged 13.7% of that in liver and 0.6% of the concentration in kidney (Oldereid *et al*., 1993). For 3 men who died from lung disease attributed to chronic cadmium exposure, testicular Cd levels were highly variable in proportion to Cd accumulated in the liver (Smith *et al*., 1960). In these cases, testicular Cd ranged from 2.0 to 24% of the levels measured in the liver of the same individual. In the case of a suicide who died about 7 days following ingestion of approximately 25 mg Cd/kg of CdI_2, the Cd concentration in the testes was 11% of that found in the liver and kidneys (Wisniewska-Knypl *et al*., 1971).

Studies investigating the concentration of Cd in human seminal fluid have found it to be generally low (less than 1 ppb), and slightly lower than the concentration of Cd in blood. In heavy smokers, seminal Cd levels are elevated about 2-fold (Chia *et al*., 1994; Saaranen *et al*., 1989; Xu *et al*., 1993b, 1994). Dwividi (1983) found motility of human sperm *in vitro* to be inhibited by 25% in the presence of 5×10^{-4} M Cd (56 ppm), a concentration orders of magnitude higher than that reported for human seminal fluid.

In rodents, distribution of orally administered Cd to the testes is influenced by the dose, duration of treatment, and the time between exposure and evaluation. Testicular Cd

concentrations have generally been found to be about 1 to 10% of the concentrations measured in liver or kidneys (Andersen *et al*., 1988; Dixon *et al*., 1976; Kotsonis and Klassen, 1977, 1978; Sabbioni *et al*., 1978; Saygi *et al*., 1991; Waalkes, 1986). These results appear to be consistent with reports of relative testicular Cd levels in humans.

The major low-molecular weight Cd and Zn-binding proteins isolated from testes of mice, rats, monkeys and humans are not MTs (Deagan and Whanger, 1985; Kaur et al., 1993; Ohta *et al*., 1988; Waalkes and Perantoni, 1986; Waalkes *et al*., 1984a, 1984b, 1988a, 1988b). These proteins differ from MT in having a low cysteine content, having aromatic amino acids, and not being inducible by Zn. Other data suggest that there may be some MT, and/or MT mRNA in testes of rats and mice. (Abel *et al*., 1991; De *et al*., 1991; Nishimura *et al*., 1990, Nolan and Shaikh, 1986; Shaikh, 1993; Wahba *et al*., 1994). This difference between testes and other organs may render the testes relatively sensitive to Cd.

The relationships between Cd, other elements, and male reproductive toxicity remain unclear. Exposure of male rats to 25 ppm Cd and 25 ppm Pb in drinking water for 120 days, had more severe effects on the testes than 50 ppm of either metal alone for the same period (Saxena *et al*., 1989). The addition of 50 ppm Zn to water containing the other two metals, showed that zinc had a protective effect against Pb and Cd toxicity. Cadmium accumulation in the liver, kidney, and testes of treated rats was markedly enhanced in rats fed a Zn-deficient diet (Waalkes, 1986). Cadmium treatment of Zn-deficient animals led to far lower Zn levels in testes and kidney than were seen with low dietary Zn alone. On the other hand, addition of up to 200 ppm Cd to an otherwise normal diet, had no effect on testicular Zn levels after 6 weeks of treatment.

5.4 Integrative Evaluation

Studies in humans and experimental animals have addressed fertility, testicular pathology, and sperm and semen parameters. Fertility of human males has not been demonstrated to be compromised by exposure to Cd, but the available studies cannot be considered conclusive. In experimental animals, adverse effects on male fertility have been observed with single oral doses of 100-150 mg/kg, but not at lower doses of up to 50 mg/kg. Cadmium has been shown to affect testes weight, cause testicular lesions at the histological level, result in reduced sperm counts and sperm motility, and adversely affect fertility when given to experimental animals under certain conditions of dose and exposure period. Evidence available from studies in humans does not contradict the experimental results, but the human data alone do not demonstrate a causal relationship between cadmium exposure and male reproductive toxicity.

Pathological findings in the testes of experimental animals exposed to Cd by the oral or inhalation routes have included increased relative testes weights. Significant reductions in absolute and relative testes weights have also been reported, after longer exposures to higher doses. Histopathological changes have been observed in the testes following single oral doses, or with chronic exposure to cadmium. Higher doses and longer

exposure periods were associated with more severe effects. Cd has been found to accumulate in the testes of humans and experimental animals, although at far lower concentrations than found in organs such as the liver and the kidney. In humans, testicular Cd content appears to increase with advancing age. In an autopsy series of men who died from lung-damage caused by cadmium, sperm maturation was found to be profoundly inhibited. It was not clear whether this finding was directly due to cadmium exposure, or was a consequence of the terminal illness.

In some studies of long-term oral exposure to Cd, fertility has not been affected. In other studies of Cd-exposed males, fertility has been impaired. Reduced fertility has generally been found in multigeneration studies when both sexes were Cd exposed.

Cd has been detected in human semen, and Cd levels are positively correlated with heavy use of cigarettes. Significant negative correlations have been found between seminal cadmium levels and variables such as sperm density, normal sperm morphology, sperm volume, and sperm motility. Cadmium inhibits the activity of human spermatozoan choline acetyl transferase (ChAT) *in vitro*; sperm motility was also reduced in these experiments. Inhibition of ChAT activity has also been demonstrated in rats following either chronic or acute exposure to cadmium *in vivo*. Chronically exposed rats in this study also suffered from impaired fertility, suggesting a relationship between ChAT activity, sperm motility, and reduced fertility. Other mechanistic studies in animals have documented localized alterations in testicular pH, and shown that Cd can be incorporated into spermatogenic cells. Sperm counts and motility have been shown to be depressed in Cd-exposed animals, but only after long-term treatment. These changes were not necessarily accompanied by histopathological lesions of the testes, or by measurable changes in fertility.

Table 5. Animal studies of male reproductive effects of Cd by inhalation

Reference	Study Design	Reported effects[(1)]
Kutzman *et al*., 1986	rat, 62 days, 6 hr/d, 5 d/wk 0, 0.3, 1.0, 2.0 mg/m^3. Males mated with unexposed females	Male death increased (control 0/46, 0.3 mg/m^3 0/46, 1.0 mg/m^3 5/46, 2.0 mg/m^3 46/46). Male weight gain reduced at 1.0, 2.0 mg/m^3. Testes weight increased at 1.0 mg/m^3 (no data at 2.0 mg/m^3). No effect on the number of viable embryos, preimplantation loss, resorption frequency, or fetal death at any dose tested.
NTP, 1995	mouse, 13 weeks, 6 hr/d, 5 d/wk, 0, 0.025, 0.05, 0.1, 0.25, 1.0 mg/m^3	No increase in male death, no change in weight gain, testes weights, or sperm count.
NTP, 1995	rat, 13 weeks, 6 hr/d, 5 d/wk 0, 0.025, 0.05, 0.1, 0.25, 1.0 mg/m^3	Reduced sperm count at 1.0 mg/m^3. No increase in male death. No change in body weight gain or testes weight.

[(1)] Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

Table 6. Animal studies of male reproductive effects of Cd by oral route

Reference	Study Design	Reported effects[(1)]
Feed		
Hogue *et al*., 1984	sheep, in feed, 152 days grazed on control pasture, or pasture grown on sewage sludge-amended soils.	Relative testes weight increased in exposed animals. No changes in sperm motility or body weight gain in exposed animals.
Loeser and Lorke, 1977	dog, in feed, 3 months 0, 1, 3, 10, 30 ppm	No increase in deaths, no changes in weight gain or testicular histopathology at any exposure.
Wills *et al*., 1981	rat, in feed, 4 generations (male and female exposed) 0.080, 0.100, 0.125 ppm (0.080 in control diet)	Fertility reduced at 0.125 ppm. No increase in deaths, not changes in weight gain or in testicular histopathology.
Zielinska-Psuja *et al*., 1979	rat, in feed, 16 months 0, 5.4, 54 mg/kg/d (treatments given only as doses, not as concentrations in food)	Weight gain reduced at 5.4, 54 mg/kg/d. Absolute testes weight reduced, histopathological damage to seminiferous tubules found at 54 mg/kg/d. Changes in hormone levels at 5.4, 54 mg/kg.
Gavage		
Andersen *et al*., 1988	mouse, gavage, 1x 0, 0.56, 3.9, 7.8, 16, 30, 59, 88 mg/kg	Dose-related increase in death of treated males, evident at the 3 highest doses: control 0/16; 30 mg/kg, 2/54; 59 mg/kg, 11/60; 80 mg/kg 36/42. Testicular necrosis found at 59, 88 mg/kg.
Bomhard *et al*., 1987	rat, gavage, 1/week 10 weeks 5 mg/kg/d	No increase in deaths, no effects on weight gain or testicular histopathology.
Bomhard *et al*., 1987	rat, gavage, 1x 0, 50, 100, 200 mg/kg	Increased frequency of death at 100 and 200 mg/kg 28/35 and 24/25, respectively. Transient reductions in weight gain at all 3 doses. Abnormal testicular histopathology at 100 and 200 mg/kg.

[(1)] Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

Table 6 (continued). Animal studies of male reproductive effects of Cd by oral route

Reference	Study Design	Reported effects[(1)]
Gavage		
Borzelleca *et al*., 1989	rat, gavage, 10 days 0, 15, 31, 66, 138 mg/kg/d	Dose-dependent increase in mortality of exposed males. 100 % mortality at high dose. Male weight gain reduced at 15, 31, 66 mg/kg/d. Relative testes weight increased at 15 mg/kg/d. Absolute testes weight reduced at 66 mg/kg/d. Testicular necrosis found at 66, 138 mg/kg/d.
Borzelleca *et al*., 1989	rat, gavage, 1x 0, 15, 31, 66, 138 mg/kg	Male death increased in two highest dose groups (control 0/10, 66 mg/kg 3/10, 138 mg/kg 3/10) Male weight gain not reduced, testes weight not altered at any exposure.
Dixon *et al*., 1976	rat, gavage, 1x 0, 6.25, 12.5, 25 mg/kg	Male fertility not decreased at any exposure.
Kotsonis and Klassen, 1977	rat, gavage, 1x 0, 25, 50, 100, 150 mg/kg	Male LD_{50} at 225 mg/kg. Male weight gain reduced, male fertility reduced, spermatogenesis reduced, testicular necrosis found at 100, 150 mg/kg.
Sutuo *et al*., 1980a, 1980b	rat, gavage, 9 weeks (male), 6 weeks plus gestation (female) 0, 0.1, 1.0, 10 mg/kg/d	Male weight gain reduced at 10 mg/kg/d. No change in testes weight at any dose tested. MALE + FEMALE EXPOSURE: Reduced number of sperm positive matings at 10 mg/kg/day. Implants/female reduced, live fetuses/litter reduced at 10 mg/kg. Increase in mean length of time to mating at 10 mg/kg. MALE ONLY EXPOSURE: No evidence of dominant lethal effect.

[(1)] Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

Table 6 (continued). Animal studies of male reproductive effects of Cd by oral route

Reference	Study Design	Reported effects[(1)]
WATER		
Borzelleca *et al*., 1989	rat, water, 10 days 0, 10.4, 96, 187 ppm	Relative testes weight increased at 96, 187 ppm. Male weight gain reduced at 10.4, 96, 187 ppm. No increase in deaths. No changes in testicular histopathology, or changes in absolute testes weights.
Boscolo *et al*., 1985	rabbit, water, 10 months 0, 20 ppm	No changes in weight gain, testes weight, or spermatogenesis. Increased size in lysosomes of the Sertoli cells.
Caflisch, 1994	rat, water, 40 days 0, 50, 100 ppm	No change in testes weight or testicular histopathology. Increased alkalinity of luminal fluid of the seminiferous tubules at both exposures.
Dixon *et al*., 1976	rat, water, 90 days 0, 0.001, 0.01, 0.1 ppm	No change in male fertility or testes weights.
Dwividi, 1983	rat, water, 12 weeks 0, 1, 2 ppm	Male fertility decreased at 1, 2 ppm.
Kotsonis and Klaassen, 1978	rat, water, evaluated at 3, 6, 12, and 24 weeks 0, 10, 30, 100 ppm	Proteinuria and kidney histopathological effects found at 30, 100 ppm. No changes in body weight gain, testes weights or histopathology, fertility, or litter size.
Laskey *et al*., 1980	oral, rat, 2 generation males and females exposed 0, 0.1, 1, 5, ppm	Body weight decreased at all 3 concentrations. Liver weights decreased at 1 and 5 ppm. No changes in relative testes weights or fertility. Decreased epididymal sperm counts at 5 ppm.

[(1)] Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

Table 6 (continued). Animal studies of male reproductive effects of Cd by oral route

Reference	Study Design	Reported effects[(1)]
WATER		
Saxena *et al*., 1989	rat, water, 120 days 0, 50 ppm	No changes in body weights or absolute testes weight. Relative testes weight increased, abnormal testicular histopathology, sperm count and motility decreased at 50 ppm.
Saygi *et al*., 1991	rat, water, 52 weeks 0, 10 ppm	Male fertility reduced . Necrosis of sperm-cell precursors, and damage to seminiferous tubules.
Schroeder and Mitchner, 1971	mouse, water, 3 generation (male and female exposed) 0, 10 ppm	Failure to breed after 2nd generation at 10 ppm.
Zenick *et al*., 1982	rat, water, 70 - 80 days 0, 17.2, 34.4, 68.8 ppm	No change in body weight, testes weight, or weight of cauda epididymis. No evidence for effect on fertility.

[(1)] Effects reported to be statistically significant at $p \leq 0.05$ or, if statistical significance was not reported, incidence data are provided.

Appendix: Developmental and Reproductive Effects of Cadmium by Injection.

A.1. Developmental Effects by Injection

There is a substantial body of information on the effects of injection of cadmium via the intra-peritoneal (ip), sub-cutaneous (sc) and intra-venous (iv) routes on developmental and reproductive parameters in a variety of animal species. Since the body of information on the effects of exposure to cadmium via inhalation and oral exposures is extensive, the data from studies by injection, not typically a route for human exposure, are reviewed in an appendix.

A.1.1 Toxic Effects

The developmental effects in animal models of cadmium injection have been extensively studied, and Appendix Table A1 summarizes studies identified in the literature. In rat, mouse, and hamster, a highly consistent picture of fetal death or resorptions, malformations, and reduced fetal or birth weight has been observed. Fetal death or resorptions and/or malformations have been observed following injection, in rats (iv, ip, or sc; at 1.0 to 5.6 mg/kg/d), mice (ip or sc; at of 0.67 to 6.1 mg/kg), or hamsters (iv; at 1.1 to 2 mg/kg). Doses required to produce effects appears to be dependent on routes, periods, or number of injections. Strain differences in sensitivity, which have been observed in mice and hamsters, may also play a role. In rats, the sc route is usually less effective than the iv or ip routes in eliciting developmental effects. The vast majority of studies were by injection on single days of gestation. A few studies used injections for 3 to 9 days during gestation, but only one reported results (no effect) for the entire length of gestation. As would be expected, the specific malformations observed and the doses required vary by the time of exposure.

Effects on the placenta have been frequently observed. In several studies, degeneration or necrosis of the placenta occur at the same doses as fetal death. More subtle alterations occur at lower doses. This is discussed in greater detail in subsequent sections.

The pre-implantation embryo has been found to be relatively insensitive to maternal Cd injection. However, injection shortly before implantation would take place has been observed to block implantation. This will be discussed in greater detail in the female reproductive effects by injection section.

A.1.1.1 Developmental effects reported

Malformations have been observed in 3 species by all injection routes tested (hamster by iv, mouse by ip and sc, and rat by iv, ip, and sc). Malformations and abnormalities reported include skeletal (skull and jaw, vertebra, tail, ribs, forelimbs and hindlimbs) and soft tissue (eye, ear, heart, kidney, bladder and abdominal wall), as well as hydrocephalus, diaphragmatic and umbilical hernia, and edema (see references in Table A1).

Almost all of the studies which found malformations were by injection on a single day during gestation. In the hamster, malformations have been observed following injection of Cd on gds 8 or 9. In mice, malformations have been observed following single Cd injection on gd 7-12. In rats, malformations have been observed following single Cd injection on gds 8-15. Only 1 report (with 4 sub-studies) using injections on multiple days found malformations. The specific malformations (or abnormalities) observed vary by the day of Cd administration. Several studies have injected Cd into different groups of animals on a series of single days during gestation and reported the frequency of specific malformations observed (Chernoff ,1973; Ferm, 1971; Gale and Ferm, 1973; Holt and Webb, 1987; Murdoch and Cowan, 1981; Padmanabhan and Hameed, 1990; Parzyck *et al*., 1978; Samarawickrama and Webb, 1981; Webster and Messerle, 1980).

Embryonic or fetal death has been another frequently reported developmental toxicity endpoint of Cd injection (see references in Table A1). Many studies have reported the co-occurrence of malformations or abnormalities and embryo/fetal death (e.g., Naruse and Hayashi, 1989; Murdoch and Cowen, 1981; Khera 1991; Ferm, 1971; Gale and Ferm, 1973; Layton and Layton, 1979; Saillenfait *et al*., 1991; Saltzman *et al*., 1991; Daston *et al*., 1991b). In general, there appears to be a tendency for injections shortly after implantation to produce malformations at lower doses than embryonic or fetal death, whereas later in gestation embryonic or fetal death occurs at the same or lower dosages. There also appears to be a tendency for studies with exposure on multiple days to find embryonic or fetal death to be more sensitive than malformations. Saillenfait *et al*. (1991) used 4 injections on gds 8, 10, 12, and 14 and found embryonic or fetal death to be the more sensitive endpoint. Similarly, Daston *et al*. (1991b) used 10 injections on gds 6-15 and found the same result (although there was a slight, but not statistically significant, increase in malformations at lower dosages than embryonic or fetal death). No studies were found where exposures on multiple days produced malformations at lower dosages than embryonic or fetal death.

Some of the results of a typical study of the effects of Cd by injection are presented in Table A2. In this study, mice were injected (ip) with 2.4 mg Cd/kg at various times from gd 7 to gd 10. Several typical characteristics are illustrated by this data. First, the type and frequency of malformations changes depending upon the time of injection, with neural tube defects predominating earlier, and limb defects predominating later. Second, the frequency of fetal death and resorptions also changes. Third, the frequency of effects is high: up to 72% for limb defects and 47% for fetal death and resorptions.

A.1.1.2 Placental damage and other effects

Injection of Cd has been found in several studies to cause adverse effects on the placenta. At higher dosages, it causes hemorrhage and necrosis. At lower dosages, functional effects have been observed, including reduced blood flow and reduced transport of nutrients to the embryo or fetus. The relationship between these effects and embryonic or fetal effects will be discussed further under mechanisms (below).

Malformations and fetal death and/or resorptions in mice injected intraperitoneally with Cd (2.4 mg Cd/kg) at different gestation times (Webster and Messerle, 1980)

Gestation day and hour of injection	Fetal death and/or resorption (%)	Neural tube defects (%)	Limb defects (%)
7-10d (saline control)	5	1	0
7 d, 1 hr	45	67	0
8 d, 1 hr	20	56	4
8 d, 15 hr	14	10	28
9 d, 1 hr	13	0	72
10 d, 1 hr	47	0	67

There are several reports of placental necrosis, hemorrhage, and/or blood clotting in mid and late gestation. These involve rats and mice exposed via iv, ip, and sc routes. Embryonic or fetal death typically occurs at the same dose levels as these placental effects (Chiquoine, 1965; Di Sant'agnese *et al*., 1983; Khera, 1991; Levin and Miller, 1980; Padmanabhan, 1986; Parizek, 1964, 1965; Samarawickrama and Webb, 1981). In studies at gd 10 in rats by iv and ip injection, necrosis of decidua and visceral yolk sac were also observed (Khera, 1991). In the mouse, at various gestation days, necrosis of decidual tissue has also been observed (Chiquoine, 1965).

Other adverse effects on the placenta have also been reported. In the rat, after injection (sc) with Cd on gd 18, at 4.5 mg Cd/kg, it was found that the maternal blood flow to the placenta was reduced by 40% and 73% at 12-16 and 18-24 hours (respectively) after injection. High levels of fetal death also occurred, in parallel with reduced blood flow. Blood flow was reduced to both live and dead fetuses, with a greater reduction to dead than live fetuses (Levin and Miller, 1981). A similar experiment using injection (sc) of 4.5 mg Cd/kg on gd 12 found reduced placental blood flow by 35% at 16-18 hours, which recovered to normal by 24-26 hours. Embryonic or fetal death was not observed at this dosage, but was observed at slightly higher dosages (Saltzman *et al*., 1989). In the

mouse, after injection (sc) on gd 16, at 3.0 mg Cd/kg, reduced placental transfer of Vitamin B12 and Zn to the fetus was reported. No effect on placental transfer of a-aminobutyric acid or deoxyglucose was found (Danielsson and Dencker, 1984). In the rat, injection (iv) of 1.25 mg Cd/kg on gd 12 (a teratogenic dose) resulted in 75% reduction in Zn transport to the embryo 4 hours after injection, which returned to normal by 48 hours. No effect on the transport of deoxyglucose, leucine, or formate was found (Samarawickrama and Webb, 1979; Webb and Samarawickrama, 1981). In another study in which rats were injected with 1.25 mg Cd/kg on gd 12, the uptake of Zn, Fe, and Cu was measured. It was found that the uptake of Zn and Fe to the fetus was not affected 4 hours after treatment, but was reduced at 24 hours. Uptake of Cu to the fetus was reduced at 4 hours after treatment, and reduced, but not statistically significantly, 24 hours after treatment. Uptake to the placenta was similar to uptake to the fetus. Actual tissue levels of these metals were not measured (Holt and Webb, 1986).

Effects on placental weight have been observed in some studies (Hazelhoff Roelfzema *et al*., 1985; Nayak *et al*., 1989; Padmanabhan, 1986; Padmanabhan and Hameed, 1990). Other studies have not found an effect on placental weight (Hazelhoff Roelfzema *et al.,* 1988b; Piasek and Laskey, 1994). There was a report of reduced volume density of fetal blood vessels in the placenta (Copius Peereboom-Stegeman *et al*., 1983), but this was not found in subsequent reports from the same group at the same or higher dosages (Hazelhoff Roelfzema *et al*., 1987, 1988a). Altered glycogen levels and glycogen phosphorylase activity have also been reported (Hazelhoff Roelfzema *et al*., 1987, 1988a).

A.1.2. Tissue Distribution in the pregnant animal

There have been numerous studies of the distribution of injected Cd in the middle and late parts of pregnancy, mainly in rats and mice. The distribution from single injection is complex, with concentrations changing over time, and varying by dose, time, and route of injection. For the first 24 hours after injection, the highest concentrations were found in the maternal liver. The placenta was a major point of initial accumulation. The embryo or fetus accumulates a much lower concentration of Cd than the placenta, typically 1-2%. Over longer periods of time, the maternal kidney concentration increases, and other tissues tend to remain the same or decrease. Cadmium is excreted rather slowly in urine (1% in 7 days) and feces (2% in 7 days), except possibly for a small amount (to 17%) in bile shortly after injection of very high dosages (Cherian and Vostial, 1977; Christley and Webster, 1983; Dencker, 1975; Godowicz, 1986; Hazelhoff Roelfzema *et al.,* 1988b; Levin *et al*., 1987; Lucis *et al*., 1969; Naruse and Hayashi, 1989; Piasek and Laskey, 1994; Saillenfait *et al*., 1992; Saltzman *et al*., 1989; Samarawickrama and Webb, 1981; Sonawane *et al*., 1973; Wolkowski, 1974; Webb and Samarawickrama, 1981).

In a study by Levin *et al*. (1987), rats were injected sc on gd 18 with 4.5 mg Cd/kg. Some of the relevant results are presented in Table A3. From this table, it can be seen that the peak maternal blood level of Cd is achieved at 5 minutes, the earliest time point studied. Thereafter, the blood level drops, reaching a plateau around 1/10th of the peak

value in 2-4 hours. The maternal liver has the highest concentration of Cd at all time points, and increases for the duration of the measurements. The placenta has the next highest concentration, which also increases for the duration of the measurements. The concentration of Cd in the fetus is much lower than the placenta. Pharmacokinetic modeling of the data was conducted. It was found that there were 2 components to absorption: one nearly instantaneous (equivalent to iv), and the other constant over the duration of the experiment (i.e. hours). The best (only acceptable) fit to the data required both exchangeable and non-exchangeable compartments in liver, kidney, and placenta. The exchangeable compartments filled rapidly, whereas the non-exchangeable compartments filled slowly. The authors comment that the non-exchangeable compartments could correspond to metallothionein binding, although other alternatives are possible.

Tissue distribution of Cd in pregnant rats injected subcutaneously on gestation day with 4.5 mg Cd/kg (Levin *et al*., 1987)

Time	Cd Concentration (nmol/g tissue)					
	Blood	**Liver**	**Kidney**	**Heart**	**Placenta**	**Fetus**
5 min.	15.3	39.9	8.2	5.3	21.9	NA
15 min	7.2	100.4	12.8	5.0	46.9	NA
30 min	3.0	109.8	14.7	4.3	46.7	0.05
1 hour	2.0	151.9	17.8	4.6	62.0	0.16
2 hours	1.5	124.4	16.0	5.4	64.2	0.21
4 hours	1.2	136.5	23.4	5.2	68.6	0.40
6 hours	1.3	171.8	25.2	6.6	80.7	0.70
12 hours	1.3	237.0	37.6	8.4	95.9	1.5
18 hours	1.2	311.0	50.0	12.9	94.7	2.67

A somewhat similar study was conducted in rats on gd 20, with iv injection of 1.58 mg Cd/kg (Samarawickrama and Webb, 1981). Tissue Cd concentrations were tested at 7 time points from 5 minutes to 24 hours afterwards. Results were closely similar to those from sc reported by Levin *et al*. (1987), above. As expected, the peak blood level was observed at 5 minutes, the earliest time point tested. Although the iv study used 0.35x the dose of the sc study, the peak blood level in the iv study was 3.6x that in the sc study. Also, the time for blood concentration to drop to a plateau was 30-60 minutes, compared to 2-4 hours in the sc study. Another difference in the iv study was that the placental Cd concentration peaked at the earliest time point, and then dropped by about 40% over the next 24 hours.

It has also been found that the distribution of injected Cd varies somewhat by day of injection and dosage. A study was conducted where rats were injected (iv) with 0.1, 0.4, or 1.6 mg Cd/kg on gd 12, 15, or 20, and the distribution of Cd observed after 24 hours (Sonawane *et al*., 1975). The percentage of Cd/g tissue in placenta increased with dose

on gd 12 and 15, but decreased on gd 20. The concentration in placenta increased with increasing gestational age. The fetal percentage of Cd/g tissue increased with dose and mostly increased with gestational age. The placental:fetal ratio of concentration varied from 618 at 0.1 mg Cd/kg to 11.2 at 1.6 mg Cd/kg after gd 12 injection. For other times and dosages, the ratio varied from 42 to 104. After treatment on gd 20, the fetal liver contained 46-58% of the total fetal Cd. The fetal liver had a Cd concentration of 0.22% to 2.93% that of the maternal liver.

The mechanism(s) by which Cd is transported or not transported to the embryo or fetus are poorly understood. Indeed, there are conflicting results as to the efficiency with which the placenta transports Cd. In experiments with perfused placenta from guinea pig, Cd was found to readily cross from maternal to fetal or fetal to maternal circulation (Kelman and Walter, 1977, 1980). In experiments with perfused placenta from humans, very little Cd was able to pass from the maternal to fetal side (Wier and Miller, 1987). Comparable experiments on the mouse, rat, or hamster do not appear to have been performed.

A.1.3 Mechanisms of developmental toxicity of injected cadmium

A.1.3.1 Placental effects and developmental toxicity

There are data consistent with injection of Cd in late gestation causing fetal death as a result of placental damage. In one study, rats were injected (sc) with 4.5 mg Cd/kg on gd 18. It was found that maternal blood flow to the placenta was decreased by 40% after 12-16 hours, and 73% after 18-24 hours. Morphological changes of the placentas, including necrosis, were also observed. Fetal death paralleled the reduced blood flow (20% after 12-16 hours and 60% after 18-24 hours). It was found that blood flow was reduced to both live and dead fetuses: the magnitude of the reduction was about twice as great for the dead fetuses as for the live fetuses (Levin and Miller, 1981). In a parallel study, rat uteri at gd 18 were exposed and the fetuses were injected directly (ip) with 0, 0.022, 1.7, 3.4, or 6.7 mg Cd/kg. Fetuses injected with Cd alternated with fetuses injected with saline control. Some of the relevant results are presented in Table A4. The fetal body burden of Cd was about 7 times greater from the direct fetal injection at 6.75 mg Cd/kg than from maternal injection at 4.5 mg Cd/kg. However, the incidence of fetal death was lower for direct fetal injection than for maternal injection. The placental Cd burden was much lower from fetal injection than from maternal injection. This has been interpreted as indicating that the fetus is much less sensitive to Cd than is the total maternal-fetal unit (Levin and Miller, 1980).

Comparison of fetal death and fetal and placental Cd concentrations from maternal and fetal injection of Cd (Levin and Miller, 1980).

Treatment	Fetal death	Placental Cd	Fetal Cd

	(%)	(nmol/g)	(nmol/g)
maternal, sc, 4.5 mg Cd/kg [(1)]	74.9	113.1	3.1
fetal, ip, 6.75 mg Cd/kg	11.5	9.5	21.0
fetal, ip, saline control	4.0	(not reported)	(not reported)

[(1)] No data for maternal controls (i.e., maternal injection of saline) were included in this study. However, a similar study by the same group found fetal death in maternal controls to be around 2-3% (Levin and Miller, 1981).

There is parallel evidence for effects on the placenta in mid-gestation. Rats injected (sc) with 4.5 mg Cd/kg on gd 12 were found to have maternal blood flow to the placenta reduced by 35% after 16-18 hours, but recovered to normal after 24-26 hours. This is a smaller magnitude of effect than that observed on gd 18. Embryonic or fetal death was not observed at this dosage, but was increased at a slightly higher dosage (5.6 mg Cd/kg) (Saltzman *et al*., 1989).

In a series of studies, rats were injected via iv, ip, or sc routes on gd 10 at various dosages. Subgroups were examined for placental effects 24 hours after injection and for fetal effects at term. In the ip and iv studies, major adverse effects on the placenta (including hemorrhage and necrosis of the ectoplacental cone) were observed at lower dosages than were malformations and resorptions or dead fetuses. No major effect on the placenta was found by sc, although malformations and resorptions or fetal death were found at the higher dosages used. No attempt was made to examine placental effects such as blood flow (Khera, 1991). Other studies have found that injection of Cd during mid-gestation produces placental effects at the same or lower doses as embryonic or fetal death and/or malformations (Nayak *et al*., 1989, Padmanabhan, 1986).

A.1.3.2 Detoxification and metallothionein

As discussed above and in the general metabolism section (2.4), Cd is excreted very slowly. However, there is considerable evidence that injected Cd exerts toxic effects for a limited period of time: perhaps 1 to 2 days (Feuston and Scott, 1985; Saltzman *et al*., 1989; Tam and Liu, 1985; Webster and Messerle, 1980). There is considerable evidence that the induction of metallothionein serves to detoxify Cd by sequestering it.

Metallothionein (MT) is an unusual protein of 6-7 kD molecular weight, with about 30% cysteine (sulfhydril) amino acids. It is able to very tightly bind up to 7 Cd ions (or other heavy metals) (Kagi and Schaffer, 1988). MT is present in numerous tissues, especially the liver, kidney, and placenta. In responsive cells, exposure to Cd, or several other heavy metals, or several organic compounds, results in increased transcription of the MT genes, increased MT mRNA, and increased MT protein. Recently, experiments involving the disruption of mouse genes for the main isoforms, MT I and MT II, have shown that increased sensitivity to systemic Cd toxicity resulted. Mice with the disrupted genes reproduced normally (Michalska and Choo, 1993; Masters *et al*., 1994).

Protective effects of treatments which result in MT induction have been reported. Pretreatment with Cd at subtoxic doses has been found to be protective. In hamsters, pretreatment with 0.55 mg Cd/kg (sc) or 1.1 mg Cd/kg (ip) on gd 6 or 7 resulted in large reductions in malformations and resorptions resulting from treatment with 1.1 mg Cd/kg (iv) on gd 8. Pretreatment on gd 6 was more effective than on gd 7 (Ferm and Layton, 1979). In mice, pretreatment with Cd at 1.3 mg Cd/kg (ip) up to 2 weeks before mating resulted in reduced malformations and resorptions resulting from treatment with 0.67 mg Cd/kg (ip) on gd 9 (Layton and Ferm, 1980). Pretreatment with Hg (Layton and Ferm, 1980) and Bi (Naruse and Hayashi, 1989) have both been shown to reduce malformations resulting from injection of Cd. Pretreatment with Bi was shown to induce maternal liver and kidney MT protein by 3 and 16 fold, respectively.

MT mRNA and protein are present in normal (untreated) rat maternal liver and kidney, placenta, and fetal liver. The ratio of mRNA to protein is much higher in placenta than in the other 3 tissues, suggesting a substantial reserve of protein synthesis capacity (Huber and Cousins, 1988). Injection of Cd results in increases in MT mRNA in the placenta (measured 4 hours afterwards) (De *et al*., 1989). Injection of Cd results in increased levels of MT protein in maternal liver (Munoz and Deiter, 1990). In contrast, injection of Cd results in little or no change in fetal liver, brain, or forelimb MT protein (Fujita *et al*., 1982; Munoz and Deiter, 1990). Injection of Cd on gd 10 resulted in no change in mouse embryonic MT mRNA at a teratogenic dose, although a large increase was observed at a maternally lethal dose (De *et al*., 1990). Early in development, the decidua and yolk sac also have high levels of MT mRNA (De *et al*., 1989). A study of the time course of MT induction in rat liver following injection of Cd found that MT concentrations were slightly increased at 6 hours, and began a rapid increase at 12 hours, which continued to 72 hours (the end of the observations) (Lehman-McKeeman and Klassen, 1987).

Following injection of Cd, some of the Cd in tissues is bound to MT, but much is bound to other proteins, or possibly other molecules. In mice injected (sc) with Cd on gd 10, 13, or 17 and examined 1-24 hours later, Cd was found in both the cytosol and the insoluble fraction ("pellet", corresponding roughly to the nucleus, cytoskeleton, and membranes) in both the embryo and placenta. Fractionation of cytosol found Cd bound to a protein of the same size as MT and to other, larger proteins (Wolkowksi, 1974). In rats injected (iv) with Cd on gd 20, after 8 or 24 hours some Cd was found to be bound to MT, but most was bound to other cytosol proteins or insoluble cell components (Samarawickrama and Webb, 1981). In non-pregnant rats, injected (iv) with Cd and examined 5 hours later, liver cytosol was fractionated. Some of the Cd was bound to a protein with the same size as MT, but the majority of Cd was bound to proteins of higher molecular weight (Cherian and Vostial, 1977). An *in vitro* experiment used dually perfused human placenta, with Cd added to the maternal perfusate, and examined Cd distribution after 2-6 hours. It was found that almost all Cd was protein bound. In the cytosolic fraction from the placenta, the majority of Cd was bound to proteins of higher molecular weight than Cd (Wier and Miller, 1987).

A.1.3.3 Cd developmental toxicity and altered Zn metabolism

It has been hypothesized that the adverse developmental effects of Cd injection, especially resorptions and malformations, are mediated by altered metabolism of Zn (Daston, 1982; Ferm and Carpenter, 1967; Hartsfield *et al*., 1992; Samarawickrama and Webb, 1979; Sato *et al*., 1985; Webb and Samarawickrama, 1981). This is supported by 4 lines of evidence: Zn is protective against the effects Cd, Zn deprivation can cause resorptions and malformations, marginal Zn deprivation increases the effects of Cd, and Cd injection alters the placental transfer of Zn.

Coinjection of Zn has been found to be protective against developmental toxicity from Cd injection in several studies. In hamsters injected (iv) with Cd and/or Zn on gd 8, the coinjection of Zn reduced or eliminated resorptions and malformations from Cd (Ferm and Carpenter, 1967; Hartsfield *et al*., 1992). In rats injected (sc) with Cd and/or Zn on gd 12-15, Zn virtually eliminated resorptions from Cd (Daston, 1982). In mice injected with Cd and/or Zn on gd 5, Zn prevented reduced litter size (Belmonte *et al*., 1989). In these studies the moles of Zn were similar to the moles of Cd (i.e. molar ratio of Zn:Cd between 1:1 and 2:1).

There is also a study where the prior administration of Zn was found to be protective. In this study, mice were injected with Zn 12 and 7 hours before Cd on gd 14. It was found that Zn was protective against loss of litters (Chiquoine 1965). In this study, however, the protective effects could also have been mediated by induction of MT.

Several studies have shown adverse developmental effects from Zn deprivation (reviewed in Keen and Hurley, 1989, also Keen *et al*., 1993). Severe zinc deprivation (less than or equal to 0.5 ppm in diet) has been shown to cause increases in fetal death, resorptions, and malformations in rats and mice (Hurley *et al*., 1971; Sato *et al*., 1985). Marginal deprivation has been shown to cause increased in malformations in rats (at 7.2 ppm) (Parzyck *et al*., 1978), but not in mice (at 10 ppm) (Sato *et al*., 1985).

Marginal Zn deprivation has been found to increase the adverse developmental effects of injected Cd. A study in rats found that marginal Zn deficiency (7.2 ppm in diet vs. 136 ppm in control diet) increased the frequency of Cd induced malformations and/or resorptions (Parzyck *et al*., 1978). A study in mice found that marginal Zn deficiency (10 ppm in diet vs. 50 ppm in control diet) increased the frequency of Cd induced malformations, but had no effect on litter size (Sato *et al*., 1985). However, pair fed controls were not used in these studies.

Studies of Cd injection have found reduced placental transfer to the fetus of trace radiolabelled Zn in mice (Danielsson and Dencker, 1984) and rats (Holt and Webb, 1986; Samarawickrama and Webb, 1979; Webb and Samarawickrama, 1981). Cd injection has produced reduced fetal concentrations of Zn in the rat (Daston 1982). However, a study in mice found no effect of Cd injection on embryonic Zn concentration (Feuston and Scott, 1985). Due to differences in experimental protocols, the significance of this apparent difference is difficult to evaluate.

Several authors have advanced the hypothesis that injection of Cd produces adverse developmental effects by interfering with the activity of Zn-dependent enzymes, including carbonic anhydrase and thymidine kinase. In principal, this could result from either the reduction of available Zn, or from competition of Cd with Zn for binding sites (Daston, 1982; Ferm and Carpenter, 1967; Hartsfield *et al*., 1992; Samarawickrama and Webb, 1979; Sato *et al*., 1985, Webb and Samarawickrama, 1981). Recently, it has been recognized that Zn is an essential component of proteins other than enzymes, including the Zn-fingers of the steroid/retinoic acid receptor superfamily of transcription factors (reviewed in Berg and Shi, 1996; Falchuk, 1993).

A.1.4. Integrative evaluation

A large body of studies have demonstrated that injection of Cd produces severe developmental toxicity in mouse, rat, and hamster (see Table A1). Specific effects observed include embryonic and fetal death, malformations, and reduced fetal or birth weight. Placental damage, necrosis, and other effects, such as reduced blood flow and nutrient transport have been frequently reported. Generally, these effects co-occur with developmental effects. Under some conditions maternal death has been observed to occur at the same dosages as embryonic or fetal death, or malformations. However, under other circumstances, maternal death was not observed at these dosages.

A very small fraction of injected Cd reaches the fetus, and concentrations in the fetus and fetal organs are quite small compared to the mother. In contrast, the placenta accumulates relatively high levels of Cd. There is considerable evidence that many of the developmental effects of Cd injection are a result of effects upon the placenta. This is particularly true in late gestation, and there is evidence that the same is true in mid-gestation. Although Cd is excreted very slowly, it appears that the period of time over which it exerts pronounced effects is limited. This is likely due to the induction of MT, which serves to sequester and detoxify Cd.

There is also evidence that the developmental effects of Cd injection involve altered Zn metabolism. This could involve interference with Zn-dependent enzymes and/or the steroid/retinoic acid receptor superfamily by either the reduction of available Zn, or the displacement by Cd of Zn from proteins.

A.2. Female reproductive effects by injection.

A.2.1. Introduction

There is relatively little information available on female reproductive effects of Cd given by injection. In general, there is considerable overlap between endpoints reported as

female reproductive toxicity and those reported as developmental toxicity following Cd exposure by injection routes.

A.2.2. Animal toxicity studies

Pregnant mice were given 6.1 mg Cd/kg bw by injection (sc) on one of gds 1-17 (Chiquoine, 1965). No effects were observed from single injections on gds 1-4. Injection on day 5 resulted in failure of 4 out of 5 mice to produce litters. Cd treatment on one of gds 6-17 resulted in complete loss of litters and necrosis of placental or adjacent decidual tissue. In a continuation of this study, females failing to produce litters as a result of Cd injections given on on gd 14 were rebred after a 2 week recovery period. All of these animals subsequently produced litters and raised their young normally.

Pregnant mice were given Cd by injection (ip) on gd 7 or 8 at doses of 3.1-3.7 mg/kg (Tam and Liu, 1985). Litter size was reduced and resorption frequency increased following treatment on either day. The size of the genital ridge was reduced in gd 13 female fetuses, but the number of primordial germ cells was unchanged. On gd 16, in some ovaries, necrosis of germ cells was observed. Among female offspring allowed to reach sexual maturity, no significant change of ovarian weight was observed. When mated, no change was observed in their reproductive capacity (specifically fertility or litter size). In contrast, following prenatal exposure to Cd by injection, adult male offspring had reduced numbers of motile sperm and reduced fertility.

Female rats were given 3 or 5 mg Cd/kg bw by injection (sc) during the diestrus phase of their cycle, or during pregnancy on gd 8 or 17 (Piasek and Laskey, 1994). Ovarian, uterine, and body weights were not affected by this treatment. In the rats treated during diestrus, the estrus cycle proceeded normally, and no ovarian or uterine histopathological changes were observed. Serum progesterone levels were not affected, but serum estradiol was reduced. Similar results were found for rats treated on gd 8 or 17, with the exception that in rats treated on gd 8, there was “minimal to mild” congestion with blood of the thecal layer in ovarian follicles.

Pregnant mice were given Cd by injection (sc) on gd 2 or 4 at doses of 2.8 or 4.2 mg /kg. Injection on gd 2 resulted in reduced number of implantations on gd 5, but no reduction on gd 8. The authors interpreted this finding as a temporary delay in implantation. When evaluated on gd 8, injection on gd 4 resulted in reduced implantations at the lower dose of 2.8 mg Cd/kg and complete failure to implant at the higher dose of 4.2 mg Cd/kg. *In vitro* culture of 8 cell embryos and morulae found that 5.6 mg Cd/L inhibited blastocyst formation (De *et al*., 1993).

A.2.3. Other relevant data

Studies have been conducted on pregnant rats given Cd by injection (sc) on gd 17 or 18. It was observed that the ovaries were not a major site of Cd accumulation. The ovaries were found to have lower Cd concentrations than placenta, or maternal liver or kidney (Levin *et al*., 1987; Piasek and Laskey, 1994).

A.2.4. Integrative evaluation

The ovary does not appear to be a primary target organ for the toxicity of Cd given by injection. Possible effects of prenatal Cd exposure on the genital ridge of female fetuses appeared to have resolved themselves by adulthood, and did not interfere with eventual fertility. Single injections of Cd were not found to interfere with the estrus cycle, although serum estradiol was reduced. When given during postimplantation stages of pregnancy, injections of Cd resulted in embryo or fetal death.

A.3. Male reproductive effects by injection.

A.3.1. Introduction.

The male reproductive effects of Cd given by injection have been studied in several species. Adverse effects have been observed in mouse, rat, vole, hamster, and monkey. No major differences have been described between the iv, ip, and sc routes of injection. Most studies have used only a single injection, although effects have been similar in studies using repeated injections for up to 45 days (see Table A.3.2.A.).

A.3.2. Animal toxicity studies

A consistent pathogenic sequence has been observed following administration of a single Cd injection to sexually mature males (Aoki and Hoffer, 1978; Chiquoine, 1964; Gouveia, 1988; Parizek and Zahor, 1956; Parizek, 1957; Wong and Klaassen, 1980). Alterations of the vascular bed occur within a few hours after injection, allowing leakage of fluids into the interstitium with consequent edema. After several more hours, cell death and disorganization of the germinal epithelium become evident. This is followed by hemorrhage and necrosis at about 8-24 hours post injection. Hormone secretion and sperm production are then reduced or abolished. Over a period of weeks, fibroblasts and blood vessels proliferate and islands of Leydig cells appear. Eventual recovery of hormone production has occurred in some cases, but recovery of spermatogenesis has not been demonstrated. In parallel with the histopathological changes, testicular weight increases over the first 24 hours, and then decreases over the following days to weeks. Reduced sperm concentration or failure of spermatogenesis has occurred in some studies at doses lower than those causing abnormal histopathology or altered testes weight (Hew *et al*., 1993; Lohiya *et al*., 1976; Laskey *et al*., 1984, 1986; Saksena *et al*., 1977).

Complete sterility has been observed in 2 studies which evaluated the effects of Cd injection on male fertility (Saksena *et al*., 1977; Wong and Klaassen, 1980).

Injection of Cd has been reported to produce male reproductive effects in animals at doses ranging from 0.62 to 61 mg Cd/kg b.w./d. Most studies report effects in the range of 1 to 4 mg/kg b.w. (see Table A.3.2.A). Testicular effects occurred at doses considerably lower than those causing death (e.g. Lau *et al*., 1978; Laskey *et al*., 1984; and other references in Table A.3.2.A). Other toxic effects, including reduced body weight gain and liver damage, have sometimes been observed at the same doses as testicular effects (Bomhard *et al*., 1987; Klaassen and Wong, 1982; Laskey *et al*., 1984, 1986; Parizek and Zahor, 1956; Singhal, 1981; Wong and Klaassen, 1980; Wong *et al*., 1980).

Age and strain-related differences in sensitivity to the male reproductive toxicity of injected Cd have been documented. Newborn male animals are resistant to the testicular toxicity of Cd, with sensitivity not becoming apparent until shortly before sexual maturation (Klaassen and Wong, 1982; Laskey *et al*., 1986; Wong and Klaassen, 1980). Additionally, there are marked differences between mouse strains in sensitivity to the testicular effects of Cd given by injection (Abel *et al*., 1991; Hata *et al*., 1980; Maitani and Suzuki, 1986; Nolan and Shaikh, 1986; Shaikh *et al*., 1993, Taylor *et al*., 1973; Waalkes *et al*., 1988b). Resistance to Cd-induced testicular toxicity has reportedly been mapped to a single autosomal recessive gene, cdm (Taylor *et al*., 1973).

A.3.3. Other relevant data

Even at doses which produce testicular toxicity, the testes of mice and rats have not been shown to accumulated high levels of Cd. Twenty-four hours following a single injection, the testicular Cd concentration ranged from 0.7% to 2% of that found in liver, and 2% to 9% of that in kidney (Hata *et al*., 1980; Klaassen and Wong, 1982; Shaikh *et al*., 1993; Shiraishi *et al*., 1994). In rat testes, after 7 injections given on alternate days, the Cd concentration was 1.5% to 3% of that in the kidneys, and 0.6% to 0.9% of that in liver (Wong *et al*., 1980). The peak testicular Cd concentration was reached immediately following injection, and dropped by about 50% over the subsequent 2 days (Klaassen and Wong, 1982). The concentration of Cd in the testes then remained roughly constant from 2 to 21 days. The initial testicular Cd concentration was about 5-fold higher in 4-day old rats than in 70-day old rats (Klaassen and Wong, 1982).

Pretreatment of experimental animals with Zn or calmodulin inhibitors has been shown to reduce or eliminate the testicular toxicity of injected Cd (Niewenhuis and Prozialeck, 1987; Parizek, 1957; Shiraishi *et al*., 1994; Stacey *et al*., 1983; Wahba *et al*., 1994). Injections of Zn, in excess of 10 to 200 times the effective dose of Cd, gave total protection against the testicular toxicity of Cd (Parizek, 1957; Stacey *et al*., 1983; Wahba *et al*., 1994). Injections of Cr had a similar protective effect (Stacey *et al*., 1983). Pretreatment with calmodulin inhibitors was also found to be fully protective in mice,

and partially protective in rats, against Cd-induced testicular toxicity (Niewenhuis and Prozialeck, 1987; Shiraishi *et al*., 1994).

In rats and most strains of mice, injection of Cd or Zn does not increase testicular levels of MT mRNA or of other Cd-binding proteins. In contrast, both Cd and Zn induce MT mRNA and protein in the liver (Abel *et al*., 1991; Klaassen and Wong, 1982; Nolan and Shaikh, 1986; Shiraishi and Waalkes, 1994; Shiraishi *et al*., 1994; Waalkes *et al*., 1988b; Wahba *et al*., 1994). A parallel observation is that calmodulin inhibitors induce MT mRNA and protein in rat liver, but not MT mRNA or Cd-binding protein in testes (Shiraishi and Waalkes, 1994).

The major low-molecular weight Cd and Zn-binding proteins isolated from testes are not identical to the MTs found in liver and kidney. Specifically, they have little or no cysteine, whereas MT has about 30% cysteine, and they have aromatic amino acids, whereas MT does not. These low-molecular weight, Cd-binding proteins have been found in mouse, rat, and monkey (Deagan and Whanger, 1985; Ohta *et al*., 1988; Waalkes and Perantoni, 1986; Waalkes *et al*., 1984a, 1984b, 1988a, 1988b). Other data suggest that there may be some MT, and/or MT mRNA in testes. In mice, immunological techniques have found a protein in testes which binds to anti-MT antibodies (Abel *et al*., 1991; Nishimura *et al*., 1990; Nolan and Shaikh, 1986; Shaikh *et al.*, 1993). However, the question of whether these antibodies cross-react with the Cd-binding proteins which are not MT was not addressed. In rats and mice, mRNA for MT is present in the testes (De *et al*., 1991; Wahba *et al*., 1994). Cadmium distribution and the biochemistry of MT and other Cd-binding proteins has been compared among Cd-resistant and sensitive mouse strains (Abel *et al*., 1991; Hata *et al*., 1980; Maitani and Suzuki ,1986; Nolan and Shaikh, 1986; Waalkes *et al*., 1988b). No consistent relationship has been observed.

A.3.4. Integrative interpretation

Adverse effects on the male reproductive system have been observed following administration of Cd by injection to sexually mature mice, rats, voles, hamsters, and monkeys. Effects reported include hemorrhagic necrosis of the testes, reduced sperm production, sterility, and impaired hormone production. These are relatively sensitive endpoints, compared to non-reproductive endpoints. This relative sensitivity is not associated with high levels of Cd accumulation. Testicular sensitivity to Cd may be related to the predominance of an alternative Cd-binding protein, and the lack of highly inducible MT in testes. On the other hand, pretreatment with Zn, Cr, or calmodulin inhibitors induce MT in liver and other tissues and are protective against Cd-induced testicular toxicity. Parizek (1957) suggested that Zn may directly antagonize the effects of Cd, but conditions of the study did not exclude a protective effect resulting from MT induction. The cdm gene, an autosomal recessive, confers resistance to Cd-induced testicular toxicity in some strains of mice; the mechanism of this resistance is unknown.

A.4. Summary Tables

Appendix Tables 1. Studies of Developmental Effects of Cadmium by Injection

Appendix Table 1A. Studies of developmental effects of Cd by intravenous injection.

Reference	Study Design[1]	Reported effects[2]
Ferm and Carpenter, 1967	hamster, gd 8 0, 1.1 mg/kg	Maternal death not increased Resorptions and malformations increased
Ferm, 1971	hamster, gd 8 (10 AM; 8 PM) and gd 9 (10 AM; 4 PM) 0, 1.1 mg/kg	Resorptions and malformations increased in morning and afternoon/evening Cd treatment groups for both treatment days
Gale and Ferm, 1973	hamster, gd 8 (10 AM; 8 PM) and gd 9 (10 AM; 4 PM) 0, 1.1 mg/kg	Malformations increased in morning and afternoon/evening Cd treatment groups for both treatment days. Resorptions increased for gd 8 but only slightly for gd 9 treatment groups
Gale and Layton, 1980	hamster (LSH, PD4, LVG), gd 8 0, 1.1 mg/kg	Resorptions not increased Malformations increased
	hamster (MHA, LHC, CB), gd 8 0, 1.1 mg/kg	Resorptions and malformations increased
Gale, 1979	hamster, gd 8, doses 0, 1.1 mg/kg	Resorptions and malformations increased
Hartsfield et al., 1992	hamster, gd 8 0, 1.2, 1.8 mg/kg	Maternal weight gain reduced at 1.8 mg/kg Resorptions and malformations increased, fetal weight reduced at 1.2, 1.8 mg/kg.
Hatori et al., 1990	hamster, gd 8 0, 1.2 mg/kg	Resorptions and malformations increased Fetal weight reduced
Holt and Webb, 1987	rat, (1x) gd 8, 10, or 12 0, 1.25, 1.5 mg/kg	Maternal death only at 1.5 mg/kg (all died) Malformations increased in both dose groups
	rat, gd 14 0, 1.25, 1.5 mg/kg	Maternal death (all - 1.5 mg/kg; 25% - 1.25 mg/kg) Fetal death and resorptions at 1.25 mg/kg Malformations at 1.25 mg/kg

Appendix Table 1A (continued). Studies of developmental effects of Cd by intravenous injection.

Reference	Study Design[(1)]	Reported effects[(2)]
Khera, 1991	rat, gd 10 0, 1.6, 2.8 mg/kg	Maternal death increased and maternal weight gain reduced at 2.8 mg/kg. Hydrocephaly malformations and placental necrosis at both dose levels; anophthalmia only at 2.8 mg/kg Resorptions not increased at either dose. Fetal weight reduced at 2.8 mg/kg.
Samarawickrama and Webb, 1981	rat, (1x) gd 4 or 8 0.6, 1.0, 1.58, 2.5, 3.98 mg/kg	Maternal LD_{50} = 2.2 mg/kg.
	rat, (1x) gd 12 or 16 0.6, 1.0, 1.58, 2.5, 3.98 mg/kg	Maternal LD_{50} = 2.5 mg/kg. Placental hemorrhage: ED_{50} = 2.5 mg/kg.
	rat, gd 20 0.6, 1.0, 1.58, 2.5, 3.98 mg/kg	Maternal LD_{50} = 1.1 mg/kg. Placental hemorrhage: ED_{50} =1.1 mg/kg. Maternal death increased at 1.58 and 3.98 mg/kg but not 0.6 or 1.0 mg/kg
	rat, (1x) gd 8-15 0, 1.0, 1.25, 1.58 mg/kg	Maternal death not increased at any dose. Malformations (anophthalmia/microphthalmia and hydrocephalus) increased at 1.25 mg/kg for groups treated on gd 8-13, but not gd 14 and 15. (No data on malformations at 1.0, 1.58 mg/kg) Fetal weight reduced at 1.0, 1.25, 1.58 mg/kg.
Tassinari and Long, 1982	hamster, gd 8 0, 1.2 mg/kg	Malformations increased Resorptions not increased, embryo or fetal weight not reduced

(1) Doses are expressed as Cd (instead of specific chemical form tested, e.g., $CdCl_2$).
(2) Effects indicated were noted as biologically significant or statistically significant at $p < 0.05$ by authors.

Appendix Table 1B. Studies of developmental effects of Cd by intraperitoneal injection.

Reference	Study Design[(1)]	Reported effects[(2)]
Belmonte et al., 1989	mouse, gd 5 0, 1.35 mg/kg	Litter size reduced at 1.35 mg/kg.
Christley and Webster, 1983	mouse, gd 9 0.00066, 0.040, 2.4 mg/kg	Embryo weight reduced at 2.4 mg/kg.
Fujita et al., 1982	rat, gd 14-16 0, 0.56 mg/kg/d	Maternal weight gain not reduced, litter size not reduced, and malformations not increased at dose tested.
Godowicz, 1986	mouse (KP and CBA), gd 17 0, 1.3 mg/kg	Fetal death increased both strains
Holt and Webb, 1987	rat, gd 12 0, 1.0, 1.25, 1.5, 2.0 mg/kg	Maternal death not increased at any dose. Litter size reduced, resorptions and malformations increased, fetal weight reduced at multiple doses.
	rat, (1x) gd 8, 10, 12, or 14 0, 1.25 mg/kg	Maternal death not increased Malformations increased for all dosed gd groups.
Khera, 1991	rat, gd 10 0, 1.8, 2.4, 3.1, 3.4 mg/kg	Maternal death increased at 3.4 mg/kg Maternal weight gain reduced at 3.1, 3.4 mg/kg. Placental necrosis found at each Cd dose level Resorptions and malformations (anophthalmia and hydrocephaly) increased at 3.4 mg/kg Fetal weight reduced at 3.1, 3.4 mg/kg.
Layton and Layton, 1979	mouse (AKR/J, DBA/2J, CBA/J, A/J, C57BL/6J, BALB/cJ), (3x) gd 9, doses 0, 1.3 (total) mg/kg	Resorptions increased in BALB/cJ only Malformations increased all strains
Munoz and Dieter, 1990	mouse, gd 9-11 0, 1.0, 2.0 mg/kg/d	Fetal weight reduced at 2.0 mg/kg/d. Litter size not reduced, resorptions not increased, and malformations not increased at either dose
Naruse and Hayashi, 1989	mouse, gd 7 0, 2.5, 5.0 mg/kg	Malformations increased, fetal weight reduced at 2.5, 5.0 mg/kg. Resorptions and fetal death not increased

Appendix Table 1B (continued). Studies of developmental effects of Cd by intraperitoneal injection.

Reference	Study Design[(1)]	Reported effects[(2)]
Parzyck et al., 1978	rat, (1x) gd 8, 10, 12, 14 0, 1.0, 1.5, 2.0 mg/kg	Resorptions increased with day of gestation treated and dose (e.g, only slight in high dose group gd 8, all dose groups gd 14). Malformations observed for gd 8, 10, 12 but not 14, with highest levels in high dose group for gd 8 Fetal weight reduced on all gds at 2.0 mg/kg.
Rohrer *et al.,* 1979	rat, gd 12, 14, 18, 20 0, 1.0, 2.0 mg/kg	Resorptions or fetal death not increased gd 12, 14, 18, 20, but increased for gd 20 at 2.0 mg/kg. Embryonic or fetal weight not reduced for gd 12, 18, or 20 but was reduced for gd 14.
Saillenfait et al., 1991	rat, 4x: gd 8, 10, 12 and 14. 0, 1.2, 1.5, 1.8, 2.1 mg/kg/injection	Maternal death slightly but non-significantly elevated at 1.8 and 2.1 mg/kg Maternal weight gain reduced at 2.1 mg/kg Gestation lengthened at 1.2, 1.5, 1.8, 2.1 mg/kg. Litter size reduced at 1.8, 2.1 mg/kg. Malformations not increased at any dose Birth weight reduced at 2.1 mg/kg. Postnatal survival reduced at 2.1 mg/kg.
Saillenfait *et al*., 1992	rat, 4x: gd 8, 10, 12 and 14. 0, 1.5 mg/kg/injection	Maternal death not increased; litter size, birth weight, postnatal weight gain not reduced; malformations, postnatal death not increased
Sato *et al*., 1985	mouse, gd 8 0, 1.2 mg/kg	Malformations increased, fetal weight reduced Maternal weight gain not reduced, litter size not reduced
Selypes *et al*., 1986	mouse, gd 5 or 9 0, 2.5 mg/kg	Litter size reduced, fetal weight reduced
	mouse, gd 13 0, 2.5 mg/kg	Birth weight reduced Litter size not reduced

Appendix Table 1B (continued). Studies of developmental effects of Cd by intraperitoneal injection.

Reference	Study Design[(1)]	Reported effects[(2)]
Webster and Messerle, 1980	mouse, (1x) gd 7-10 0, 2.4 mg/kg	Resorptions observed for all gd treatments, neural tube defects for gd 7 and 8, limb defects for gd 8, 9, 10.
Tam and Liu, 1985	mouse, gd 7 or 8 0, 3.1-3.7 mg/kg	Resorptions and malformations increased, fetal and birth weight reduced In female offspring , no alterations in ovary weight, fertility, or litter size observed. In male offspring, litter size, fertility, testes weight, and motile sperm percentage reduced

(1) Doses are expressed as Cd (instead of specific chemical form tested, e.g., $CdCl_2$).
(2) Effects indicated were noted as biologically significant or statistically significant at $p < 0.05$ by authors.

Appendix Table 1C. Studies of developmental effects of Cd by intraperitoneal injection of the fetus.

Reference	Study Design[(1)]	Reported effects[(2)]
Levin and Miller, 1980	rat, fetal gd 18 0, 0.022, 1.7, 3.4, 6.75 mg/kg (Each Cd treatment group with own control group: alternating fetuses injected with saline or Cd.)	Fetal death increased above controls for each treatment group; however, no statistics provided by authors. Placental hemorrhage or necrosis not found at any dose tested.
White *et al*., 1990	rat, fetal gd 19 0, 1.9, 3.7, 6.2 mg/kg (Fetuses in one horn saline, fetuses in other horn Cd.)	Hydrocephalus and brain necrosis found at 6.2 mg/kg (no statistics by authors). Fetal death not increased and fetal weight not reduced at any dose.

(1) Doses are expressed as Cd (instead of specific chemical form tested, e.g., $CdCl_2$).
(2) Effects indicated were noted as biologically significant or statistically significant at $p < 0.05$ by authors.

Appendix Table 1D. Studies of developmental effects of Cd by subcutaneous injection.

Reference	Study Design[(1)]	Reported effects[(2)]
Chernoff, 1973	rat, gd 13-16, 15-18, or 16-19 0, 4.9 mg/kg/d	Resorptions or fetal death increased, malformations increased, fetal weight reduced
	rat, gd 14-17 0, 2.4, 3.7, 4.9, 7.3 mg/kg/d	Resorptions or fetal death increased, malformations increased, fetal weight reduced at 3.7, 4.9, 7.3 mg/kg/d.
Chiquoine, 1965	mouse, (1x) gd 1-17 0, 6.1 mg/kg	Placental hemorrhage and necrosis found gd 14-17. No successful litters gd 6-17. Females (5) injected with Cd on gd 14 failed to litter. After a 2 week isolation, they were rebred, and successfully littered.
Copius Peereboom-Stegeman et al., 1983	rat, gd 1-19 0, 0.12 mg/kg/d	Placental change in volume density of blood vessels
Danielsson and Dencker, 1984	mouse, gd 16 0.5 to 5 mg/kg	Fetal death and placental necrosis in no animals at 2 mg/kg, but all at 3 mg/kg. (Results for other doses not reported, no statistics by authors.)
Daston, 1981	rat, gd 12-15 0, 0.61, 1.2, 2.4, 4.9 mg/kg/d	Resorptions or fetal death increased, fetal weight reduced at 1.2, 2.4, 4.9 mg/kg/d.
Daston, 1982	rat, gd 12-15 0, 4.9 mg/kg/d	Live litter size reduced, resorptions increased, fetal weight reduced
Daston *et al*., 1991	mouse, gd 6-15 0, 0.15, 0.31, 0.61, 1.2, 2.4, 4.9 mg/kg/d	Maternal death increased, live litter size reduced, resorptions or fetal death increased, at 4.9 mg/kg/d. Fetal weight reduced at 1.2, 2.4, 4.9 mg/kg/d. Maternal weight gain, malformations not increased at any dose tested.
De *et al*., 1989	mouse, gd 8, doses 0, 5.6 mg/kg	Malformations increased all treated animals
	mouse, gd 14 0, 2.8, 5.6, 8.4, 11.2 mg/kg	mouse, gd 14 0, 2.8, 5.6, 8.4, 11.2 mg/kg
De *et al*., 1990	mouse, gd 10 0, 2.2, 3.4, 4.5, 5.6, 8.4 mg/kg	Maternal death and embryonic death increased at 8.4 mg/kg; malformation elevated for all but lowest dose group; embryonic weight reduced at 4.5, 5.6 mg/kg. No statistics by authors

Appendix Table 1D (continued). Studies of developmental effects of Cd by subcutaneous injection.

Reference	Study Design[(1)]	Reported effects[(2)]
De *et al*., 1993	mouse, gd 2 0, 2.8, 4.2 mg/kg	Embryonic death (by gd 8) not increased for gd 2 treatment, but in both doses for gd 4 treatment. Implantation delayed at 2.8, 4.2 mg/kg for gd 2..
Di Sant'agnese *et al*., 1983	rat, gd 18 0, 4.5 mg/kg	Placental necrosis found, fetal death increased No numerical data presented by authors.
Hazelhoff-Roelfzema *et al*., 1985	rat, gd 1-19 0, 0.061, 0.12, 0.24, 0.49, 0.73, 1.0 mg/kg/d	Placental weight reduced all doses. Litter size and fetal weight not reduced, placental necrosis not found, malformations not increased.
Hazelhoff-Roelf-zema *et al*., 1988a	rat, gd 1-19 0, 0.49 mg/kg/d	Placental histopathological effects not found
Hazelhoff Roelfzema *et al*., 1988b	rat, gd 1-19 0, 0.061, 0.12, 0.25, 0.50, 1.0 mg/kg/d	Maternal weight gain, placental weight, fetal weight, or litter size not reduced; malformations or resorptions not increased.
Khera, 1991	rat, gd 10 0, 3.4, 7.4, 9.8, 12.2 mg/kg	Maternal death not increased, placental necrosis not found; resorption not increased, litter size not reduced. Small increased incidence in malformations (anophthalmia and hydrocephalus) in some dose groups; statistical analysis not provided by authors. Maternal weight gain reduced at all but lowest dose. Fetal weight reduced at 9.8, 12.2 mg/kg.
Lehotzky et al., 1990	rat, gd 7-15 0, 0.12, 0.40, 1.2 mg/kg/d	Litter size reduced, neurobehavioral performance altered at 0.40, 1.2 mg/kg/d. Maternal death not increased, birth weight not reduced at any dose
Levin and Miller, 1980	rat, (1x) gd 17 or 18 4.5 mg/kg (See entry under fetal ip injection)	Placental hemorrhage and necrosis found (no statistics by authors). Fetal death (75%)
Levin and Miller, 1981	rat, gd 18 0, 4.5 mg/kg	Placental blood flow reduced up to 73%. Placental necrosis, and fetal death
Levin et al., 1987	rat, gd 18, 4.5 mg/kg	Fetal death (70%).

Appendix Table 1D (continued). Studies of developmental effects of Cd by <u>subcutaneous</u> injection.

Reference	Study Design[1]	Reported effects[2]
Murdoch and Cowen, 1981	mouse, (1x), gd 1, 2, 4, 8 0, 2.0, 4.0, 8.0 mg/kg	Malformations increased all doses. Implantations not reduced; resorptions not increased. Fetal weight reduced for gd 8 at 8 mg/kg only.
Nayak et al., 1989	mouse, gd 8-10 0, 1.2, 1.7, 2.3 mg/kg/d	Placental weight reduced, resorptions increased at all doses. Fetal weight reduced at 2.3 mg/kg/d.
Padmanabhan, 1986	mouse, gd 7 0, 2.4, 3.7 mg/kg	At both Cd doses, placental and fetal weight reduced, resorptions and malformations increased, placental histopathological effects found.
Padmanabhan, 1987	mouse, gd 7 0, 2.4, 3.7 mg/kg	At both Cd doses, resorptions and malformations (exencephaly) increased, fetal weight reduced
Padmanabhan and Hameed, 1986	mouse, gd 7 0, 2.4, 3.7 mg/kg	At both Cd doses, resorptions and malformations increased, fetal weight reduced.
Padmanabhan and Hameed, 1990	mouse, (1x) gd 7-12 0, 1.2, 1.8, 2.4, 3.7, 4.9, 6.1, 9.2 mg/kg	Maternal death increased at 9.2 mg/kg for all gds. Placental and fetal weight reduced, resorptions or fetal death and malformations increased for all doses for all gds tested
Parizek, 1964	rat, (1x) gd 17-21 0, 4.5 mg/kg	Placental hemorrhage and necrosis found, resorptions increased
Parizek, 1965	rat, (1x) gd 17-21, dose 2.2 mg/kg	Maternal death
Piasek and Laskey, 1994	rat, gd 7 0, 3.0, 5.0 mg/kg	Maternal weight gain or litter size not reduced at either Cd dose
	rat, gd 16 0, 3.0, 5.0 mg/kg	At either Cd dose, maternal weight gain, litter size, fetal weight not reduced; placental weight not altered; resorptions not increased.
Saltzman *et al.,* 1989	rat, gd 12, 18 0, 4.5, 5.6 mg/kg	Placental blood flow reduced at 4.5 mg/kg for gd 12 (no data at 5.6 mg/kg). Resorptions increased at 5.6 mg/kg for gd 12 and both doses for gd 18. Fetal weight reduced only at 5.6 mg/kg for gd 18. Malformations not increased.

Appendix Table 1D (continued). Studies of developmental effects of Cd by <u>subcutaneous</u> injection.

Wolkowski, 1974	mouse (C57BL/10), (1x) gd 8, 9, 10, 13 or 17, doses 0, 2.2 mg/kg	Embryonic or fetal death increased at 2.2 mg/kg. Malformations not increased
	mouse (NAW), (1x) gd 9 or 10 0, 2.2 mg/kg	Embryonic or fetal death not increased

(1) Doses are expressed as Cd (instead of specific chemical form tested, e.g., $CdCl_2$).
(2) Effects indicated were noted as biologically significant or statistically significant at $p < 0.05$ by authors.

Appendix Tables 2: Studies of male reproductive effects of Cd by injection

Appendix Table 2A. Studies of male reproductive effects of Cd by intravenous injection

Reference	Study Design[1]	Reported effects[2]
Stacey *et al*., 1983	rat. 1x, (A) 0, 1.0 mg/kg, (B) 0, 3.9 mg/kg	(A) Testes weight reduced, testicular histopathological effects found (B) Male death increased
Wong and Klaassen, 1980; Klaassen and Wong, 1982	rat, 1x (70 day old animals), 0, 1.0 mg/kg	Fertility reduced, testes weight increased[3] and then reduced (time course), testicular histopathological effects found. Weight gain reduction not found
	rat, 1x (4 day old animals), 0, 1.0 mg/kg	Fertility reduction, testes weight alterations, or weight gain reduction not found.

[1] Doses are expressed as Cd (instead of specific chemical form tested, e.g., $CdCl_2$).
[2] Effects indicated were noted as biologically significant or statistically significant at $p < 0.05$ by authors.

Appendix Table 2B. Studies of male reproductive effects of Cd by intraperitoneal injection.

Reference	Study Design[1]	Reported effects[2]
Aoki and Hoffer, 1978	rat, 1x, 0, 3.4 mg/kg	Testicular histopathological effects
Chiquoine 1964	mouse, 1x, 0, 61 mg/kg	Testicular histopathological effects
	armadillo, opossum, 1x, 0, 6, 12 mg/kg	Testicular histopathological effects not found
Godowicz and Kakol, 1988	mouse, 1x, 0, 1.3 mg/kg	Testes weight reduced, testicular histopathological effects found, sperm abnormalities increased
Gouveia, 1988	rat, 1x, 0, 2.8 mg/kg	Testicular histopathological effects found. Male death not increased at any dose tested.
Hew *et al*., 1993	rat, 1x, 0, 0.31, 0.62 mg/kg	Failure of spermatogenesis at 0.62 mg/kg.
Nagy, 1985	hamster, 1x, 0, 2.2 mg/kg	Testicular histopathological effects Male death not increased
Singhal, 1981	rat, 45 days, 0, 0.62 mg/kg/d	Testes weight reduced, male weight gain reduced

[1] Doses are expressed as Cd (instead of specific chemical form tested, e.g., $CdCl_2$).
[2] Effects indicated were noted as biologically significant or statistically significant at $p < 0.05$ by authors.

Appendix Table 2C. Studies of male reproductive effects of Cd by subcutaneous injection.

Reference	Study Design[(1)]	Reported effects[(2)]
Abel *et al*., 1991	mouse (sensitive strains DBA/2, BXD8, BXD12), 2 days, 0, 1.0 mg/kg/d	Testicular histopathological effects found
	mouse (resistant strains: C57BL/6, BXD5, BXD16, BXD27), 2 days, 0, 1.0 mg/kg/d	Testicular histopathological effects not found
Bomhard *et al*., 1987	rat, 1x, 0, 2.0 or 0, 2.5 mg/kg	Testicular histopathological effects observed at 2.0, 2.5 mg/kg. Male weight gain reduced at 2.5 mg/kg (results not reported at 2.0 mg/kg). Male death not increased.
	rat, 10x, once per week, 0, 0.25 mg/kg/d	Histopathological effects not found, male death not increased, male weight gain not reduced at any dose tested.
Dryden and Gebczynski, 1979	mouse (common and 3 species of European mouse), 1x, 0, 2.2 mg/kg	Testicular histopathological effects
	vole (2 species of European vole), 1x, 0, 2.2 mg/kg	Testicular histopathological effects
Dutt *et al*., 1978	rat, 1x, 0, 4.5 mg/kg	Testicular histopathological effects
Dwividi *et al*., 1987	rat, 1x, 0, 0.24 mg/kg	Testicular histopathological effects
Hata *et al*., 1980	mouse (sensitive strain: DBA/2), 1x, 0, 3.4 mg/kg	Testicular histopathological effects Male death not increased.
	mouse (resistant strain: C3H), 1x, 0, 3.4 mg/kg.	Testicular histopathological effects not found Male death increased, liver histopathological effects
	mouse (resistant strain: BALB/c), 1x, 0, 3.4 mg/kg	Testicular histopathological effects not found, male death not increased
Laskey *et al*., 1984	rat, 1x (70 days old), 0, 0.18, 0.34, 0.83, 1.8, 3.66, 8.3, 17 mg/kg	Sperm concentration reduced at 0.83, 1.80, 3.66 mg/kg (results not reported at 8.3 or 17 mg/kg). Testes weight reduced, male weight gain reduced at 1.8, 3.66, mg/kg (results not reported at 8.3, 17 mg/kg). Male death increased (control to 3.66 mg/kg 0%, 8.3 mg/kg 10%, 17 mg/kg 60%. No statistics by authors).

Appendix Table 2C (continued). Studies of male reproductive effects of Cd by

subcutaneous injection.

Reference	Study Design[1]	Reported effects[2]
Laskey *et al*., 1986	rat, 1x (30 or 50 days old), 0, 0.62, 1.3, 2.8 mg/kg	Sperm concentration reduced, testes weight reduced, at 2.8 mg/kg. Male weight gain not reduced at any dose tested.
	rat, 1x (70 days old), 0, 0.62, 1.3, 2.8 mg/kg	Testes weight reduced, sperm concentration reduced at 1.3, 2.8 mg/kg. Male weight gain not reduced at any dose tested.
Lau *et al*., 1978	hamster, 1x, 0, 4.4, 22 mg/kg	Testes weight reduced at 4.4 mg/kg. All exposed to 22 mg/kg died.
Lohiya *et al*., 1976	monkey, 1x, 0, 2.4, 7.3 mg/kg	Sperm concentration reduced at 2.4, 7.3 mg/kg. Testes weight reduced, testicular histopathological effects found at 7.3 mg/kg. Male death not increased
Maitani and Suzuki, 1986	mouse (sensitive strains DBA/2, CBA/J), 1x, 0, 3.4 mg/kg	Testes weight increased
	mouse (resistant strain C3H/He), 1x, 0, 3.4 mg/kg	Testes weight not altered Male death increased
	mouse (resistant strains BALB/c, C57BL/6), 1x, 0, 3.4 mg/kg	Testes weight not altered.
McKenna et al., 1996	rat, 1x, 0.45 mg/kg	Testicular histopathological effects not found
Niewenhuis and Prozialeck, 1987	mouse, 1x, 0, 0.70, 1.4, 2.6, 3.7, 5.1 mg/kg	Data from Figure indicates testicular weight increased for all but lowest dose group. Testicular histopathological effects found at 3.7 mg/kg (results not reported for other doses).
Nolan and Shaikh, 1986	mouse (sensitive strains: DBA/2J, 129J), 1x, 0, 3.4 mg/kg	Testicular histopathological effects found
	mouse (resistant strains: AJ, C3H/HEJ), 1x, 0, 3.4 mg/kg	Testicular histopathological effects not found

Appendix Table 2C (continued). Studies of male reproductive of Cd by <u>subcutaneous</u> injection.

Reference	Study Design[(1)]	Reported effects[(2)]
Parizek, 1957	mouse, 1x, 0, 4.5 mg/kg	Testes weight reduced
	rat, 1x, 0, 0.2, 2.2, 4.5 mg/kg	Testes weight reduced, testicular histopathological effects found at 2.2, 4.5 mg/kg.
Parizek and Zahor, 1956	rat, 1x, 0, 11 mg/kg	Testicular as well as liver histopathological effects
Saksena *et al*., 1977	rat, 1x, 0, 1.5, 7.6 mg/kg	Fertility reduced at 7.6 mg/kg. Testes weight, sperm concentration reduced at 1.5, 7.6 mg/kg.
Shaikh *et al*., 1993	mouse (sensitive strains: 129/J, DBA, CD-1), 1x, 0, 0.56, 1.1, 2.2, 2.8, 3.4 mg/kg	Testes weight increased for all Cd dose levels (statistically significant trend).
	mouse (resistant strains: A/J, C3H), 1x, 0, 0.56, 1.1, 2.2, 2.8, 3.4 mg/kg	Testes weight not altered (no statistically significant trend).
Shiraishi *et al*., 1994	rat, 1x, 0, 2.8 mg/kg	Testicular histopathological effects Male death not increased
Taylor *et al*., 1973	mouse (sensitive strains: AKR/J, DBA/2J, C57L/J) 1x, 0, 3.4 mg/kg	Testicular histopathological effects
	mouse (resistant strains: A/J, C57BL/6J, C3H/HeJ, BALB/cJ), 1x, 0, 3.4 mg/kg	Testicular histopathological effects not found at any dose tested.
Waalkes *et al*., 1988b	mouse (sensitive strain - NFS; resistant strain - BALB/c), 1x, 0, 2.2 mg/kg	Testicular histopathological effects found the sensitive but not the resistent strain
Wahba *et al*., 1994	rat, 1x, 0, 2.2 mg/kg	Testicular histopathological effects. Male death not increased.
Wong *et al*., 1980	rat, 7x, every other day (7 weeks old), 0, 2.0, 3.0 mg/kg	Testicular histopathological effects found at 2.0, 3.0 mg/kg.
	rat, 7x, every other day (4 days old), 0, 2.0, 3.0 mg/kg	Testicular histopathological effects not found

(1) Doses are expressed as Cd (instead of specific chemical form tested, e.g., $CdCl_2$).
(2) Effects indicated were noted as biologically significant or statistically significant at $p < 0.05$ by authors.

BIBLIOGRAPHY

1. Abel J, de Ruiter N, Kuhn-Velten WN (1991). Comparative study on metallothionein induction in whole testicular tissue and isolated Leydig cells. *Arch Toxicol* **65**(3):228-34.

2. Adamsson E, Piscator M, Nogawa K (1979). Pulmonary and gastrointestinal exposure to cadmium oxide dust in a battery factory. *Environ Health Perspect* **28**:219-222.

3. Ahokas RA, Dilts PV, LaHaye EB (1980). Cadmium-induced fetal growth retardation: Protective effect of excess dietary zinc. *Am J Obstet Gynecol* **136**:216-221.

4. Alessio L, Dell'Orto A, Calzaferri G, Buscaglia M, Motta G, Rizzo M (1984). Cadmium concentrations in blood and urine of pregnant women at delivery and their offspring. *Sci Total Environ* **34**(3):261-6.

5. Ali MM, Murthy RC, Chandra SV (1986). Developmental and longterm neurobehavioral toxicity of low level in utero cadmium exposure in rats. *Neurobehav Toxicol Teratol* **8**:463-468.

6. Altman J, Sudarshan K, Das GP, McCormick N, Barnes D (1971). The influence of nutrition on neural and behavioral development: III. Development of some motor, particularly locomotor patterns during infancy. *Dev Psychobiol* **4**(2):97-114.

7. Andersen O, Nielsen JB, Svendsen P (1988). Oral cadmium chloride intoxication in mice: Effects of dose on tissue damage, intestinal damage, intestinal absorption, and relative organ distribution. *Toxicology* **48**:225-36.

8. Aoki A, Hoffer AP (1978). Reexamination of the lesions in rat testis caused by cadmium. *Biol Reprod* **18**(4):579-91.

9. ATSDR (1993). Toxicological profile for cadmium: Update 1993. **In:** *Agency for Toxic Substances and Disease Registry* US DHHS, Atlanta, GA.

10. Baranowska I (1995). Lead and cadmium in human placentas and maternal and neonatal blood (in a heavily polluted area) measured by graphite furnace atomic absorption spectrometry. *Occup Environ Med* **52**:229-232.

11. Baranski B (1983). Effect of prenatal exposure to cadmium on avoidance acquisition in rats. *Medycyna Pracy* **34**(5-6):381-383.

12. Baranski B (1984). Behavioral alterations in offspring of female rats repeatedly exposed to cadmium oxide by inhalation. *Toxicol Lett* **22**(1):53-61.

13. Baranski B (1985). Effect of exposure of pregnant rats to cadmium on prenatal and postnatal development of the young. *J Hyg Epidemiol Microbiol Immunol* **29**(3):253-262.

14. Baranski B (1986). Effect of maternal cadmium exposure on postnatal development and tissue cadmium, copper and zinc concentrations in rats. *Arch Toxicol* **58**:255-260.

15. Baranski B (1987). Effect of cadmium on prenatal development and on tissue cadmium, copper, and zinc concentration in rats. *Environ Res* **42**:54-62.

16. Baranski B, Stetkiewicz I, Trzcinka-Ochocka M, Sitarek K, Szymczak W (1982). Teratogenicity, fetal toxicity, and tissue concentration of cadmium administered to female rats during organogenesis. *J Appl Toxicol* **2**(5):255-259.

17. Baranski B, Stetkiewicz I, Sitarek K, Szymczak W (1983). Effects of oral, subchronic cadmium administration on fertility, prenatal and postnatal progeny development in rats. *Arch Toxicol* **54**:297-302.

18. Baranski B, Sitarek K (1987). Effect of oral and inhalation exposure to cadmium on the oestrous cycle in rats. *Toxicol Lett* **36**:267-273.

19. Barlow SM, Sullivan FM (1982). Relevance of animal data for man. *Teratology* **26**(3):A5.

20. Barrett HM, Irwin DA, Semmons E (1947). Studies on the toxicity of inhaled cadmium. I. The acute toxicity of cadmium oxide by inhalation. *J Ind Hyg Toxicol* **29**:279-285.

21. Belmonte NM, Rivera OE, Herkovits J (1989). Zinc protection against cadmium effects on preimplantation mice embryos. *Bull Environ Contam Toxicol* **43**(1):107-10.

22. Berg JM, Shi Y (1996). The galvanization of biology: A growing appreciation for the roles of zinc. *Science* **271**.

23. Berglund M, Akesson A, Nermell B, Vahter M (1994). Intestinal absorption of dietary cadmium in women depends on body iron stores and fiber intake. *Environ Health Perspect* **102**(12):1058-66.

24. Berlin M, Blanks R, Catton M, Kazantzis G, Mottet NK, & Samiullah Y (1992). Birthweight of children and cadmium accumulation in placentas of female

nickel-cadmium (long-life) battery workers. *Cadmium in the Human Environment*. Rpt. ID:118. IARC Sci Pub. , Lyon, France.

25. Bhattacharyya MH (1983). Bioavailability of orally administered cadmium and lead to the mother, fetus, and neonate during pregnancy and lactation: An overview. *Science Total Environ* **28**:327-342.

26. Bhattacharyya MH, Whelton BD, Peterson DP (1981). Gastrointestinal absorption of cadmium in mice during gestation and lactation. I. Short-term exposure studies. *Toxicol Appl Pharmacol* **61**:335-342.

27. Bhattacharyya MH, Whelton BD, Peterson DP (1982). Gastrointestinal absorption of cadmium in mice during gestation and lactation. II. Continuous exposure studies. *Toxicol Appl Pharmacol* **66**(3):368-375.

28. Bhattacharyya MH, Sellers DA, Peterson DP (1986). Postlactational changes in cadmium retention in mice orally exposed to cadmium during pregnancy and lactation. *Env Res* **40**:145-54.

29. Bomhard E, Vogel O, Loser E (1987). Chronic effects of single and multiple oral and subcutaneous cadmium administrations on the testes of Wistar rats. *Cancer Lett* **36**:307-15.

30. Bonithon-Kopp C, Huel G, Moreau T, Wendling R (1986). Prenatal exposure to lead and cadmium and psychomotor development of the child at 6 years. *Neurobehahav Toxicol Teratol* **8**:307-10.

31. Borzelleca JF, Clarke EC, Condie LW (1989). Short-term toxicity (1 and 10 days) of cadmium chloride in male and female rats: Gavage and drinking water. *J Am Coll Toxicol* **8**(2):377-404.

32. Boscolo P, Sacchettoni-Logroscino G, Ranel-Letti FO, Gioia A, Carmignani M (1985). Effects of long-term cadmium exposure on the testis of rabbits: Ultrastructural study. *Toxicol Lett* **24**(2-3):145-149.

33. Bryce-Smith D, Deshpande RR, Hughes J, Waldron H (1977). Lead and cadmium levels in stillbirths. *Lancet* **1**(8022):1159.

34. Buchet JP, Roels H, Hubermont G, Lauwerys R (1978). Placental transfer of lead, mercury, cadmium, and carbon monoxide in women. II. Influence of some epidmiological factors on the frequency distributions of the biological indices in maternal and umbilical cord blood. *Environ Res* **15**:494-503.

35. Caflisch C R, DuBose T D (1991). Cadmium-induced changes in luminal fluid pH in testis and epididymis of the rat in vivo. *J Toxicol Environ Health* **32**:49-57.

36. Caflisch C (1994). Effect of orally administered cadmium on in situ pH, PCO2, and bicarbonate concentration in rat testis and epididymis. *J Toxicol Environ Health* **42**(3):323-330.

37. Cesany P, Sevcikova M, Raska L (1991). The influence of external factors on the development of primary palatal cleft. *Acta Chir Plast* **33**(4):242-252.

38. Chan HM, Cherian MG (1993A). Mobilization of hepatic cadmium in pregnant rats. *Toxicol Appl Pharmacol* **120**(2):308-314.

39. Chan HM, Cherian MG (1993B). Ontogenic changes in hepatic metallothionein isoforms in prenatal and newborn rats. *Biochem Cell Biol* **71**(3-4):133-40.

40. Cherian MG, Vostial JJ (1977). Biliary excretion of cadmium in rat. I. Dose-dependent biliary excretion and the form of cadmium in the bile. *J Toxicol Environ Health* **2**:945-54.

41. Chernoff N (1973). Teratogenic effects of cadmium in rats. *Teratology* **8**:29-32.

42. Chia SE, Xu B, Ong CN, Tsakok FMH, Lee ST (1994). Effect of cadmium and cigarette smoking on human semen quality. *Int J Fertil* **39**(5):292-298.

43. Chiquoine AD (1965). Effects of Cd chloride on the pregnant albino mouse. *J Reprod Fertil* **10**:263-5.

44. Chiquone A (1964). Observations on the early events of cadmium necrosis of the testis. *Anat Rec* **149**:23-35.

45. Christley J, Webster WS (1983). Cadmium uptake and distribution in mouse embryos following maternal exposure during the organogenic period. *Teratology* **27**:305-12.

46. Cooper GP, Choudhury H, Hastings L, Petering HG (1978). Prenatal cadmium exposure: effects on essential trace metals and behavior in rats. Proceedings of *Developmental Toxicology of Energy-Related Pollutants*. Mahlum D, Sikov M, Hackett P, Andrew FD (ed). ;Richland, Washington, 1977. Publ: Technical Information Center; US Department of Energy. pp. 627-637.

47. Copius Peereboom-Stegeman JH, Van der Velde WJ, Dessing JW (1983). Influence of cadmium on placental structure. *Ecotoxicol Environ Safety* **7**(1):79-86.

48. Cornwall G, Carter M, Bradshaw W (1984). The relationship between prenatal lethality or fetal weight and intrauterine position in rats exposed to diethylstilbestrol, zeranol, 3,4,3',4'-tetrachlorobiphenyl, or cadmium. *Teratology* **30**(3):341-349.

49. Cresta L, Perdelli F, Franco Y, Raffo E, Cristina M, Diani F (1989). [Possible correlations between urinary cadmium and fetal growth retardation in pregnant women who smoke]. *Minerva Ginecol* **41**(2):85-88.

50. Danielsson BRG, Dencker L (1984). Effects of cadmium on the placental uptake and transport to the fetus of nutrients. *Biol Res Pregnancy Perinatol* **5**(3):93-101.

51. Daston GP (1981). Toxicity of minimal amounts of cadmium to the developing rat lung and pulmonary surfactant. *Toxicol Lett* **9**(2):125-30.

52. Daston GP (1982). Fetal zinc deficiency as a mechanism for cadmium induced toxicity to the developing rat lung and pulmonary surfactant. *Toxicology* **24**:55-63.

53. Daston GP, Overmann GJ, Taubeneck MW, Lehman-McKeeman LD, Rogers JM, Keen CL (1991A). The role of metallothionein induction and altered zinc status in maternally mediated developmental toxicity: Comparison of the effects of urethane and stryrene in rats. *Toxicol Appl Pharmacol* **110**:450-63.

54. Daston GP, Rogers JM, Versteeg DJ, Sabourin TD, Baines D, Marsh S (1991B). Interspecies comparisons of A/D ratios: A/D ratios are not constant across species. *Fund Appl Toxicol* **17**:696-722.

55. De SK, Paria BC, Dey SK, Andrews GK (1993). Stage-specific effects of cadmium on preimplantation embryo development and implantation in the mouse. *Toxicology* **80**(1):13-25.

56. De SK, Dey SK, Andrews GK (1990). Cadmium teratogenicity and its relationship with metallothionein gene expression in midgestation mouse embryos. *Toxicology* **64**(1):89-104.

57. De SK, Enders GC, Andrews GK (1991). High levels of metallothionein messenger RNAs in male germ cells of the adult mouse. *Molecular Endocrinology* **5**(5):628-36.

58. De SK, McMaster MT, Dey SK, Andrews GK (1989). Cell specific metallothionein gene expression in the mouse decidua and placenta. *Development* **107**:611.

59. Deagan JT, Whanger PD (1985). Properties of cadmium-binding proteins in rat testes. *Biochem J* **231**:279-83.

60. Dencker (1975). Possible mechanisms of cadmium fetotoxicity in golden hamsters and mice. Up take by the embryo, placenta and ovary. *J Reprod Fertil* **44**:461-71.

61. Di Sant'Agnese PA, Jensen KD, Levin A, Miller RK (1983). Placental toxicity of cadmium in the rat: An ultrastuctural study. *Placenta* **4**:149-64.

62. Dixon RL, Lee IP, Sherins RJ (1976). Methods to assess reproductive effects of environmental chemicals: Studies of cadmium and boron administered orally. *Environ Health Perspect* **13**:59-67.

63. Dryden GL, Gebczynski M (1979). Sensitivity of European microtines to injected CdCl2. *Acta Physiologica Academiae Scientiarum Hungaricae* **53**(3):355-62.

64. Dutt NH, Wakabayashi K, Inouye S (1978). Effect of CdCl2 on Serum levels of FSH, LH and androgens in the male rat. *Proc Indian Acad Sci* **87(B)**:161-7.

65. Dwivedi C (1983). Cadmium-induced sterility: Possible involvement of the cholinergic system. *Arch Environ Contam Toxicol* **12**(2):151-156.

66. Dwivedi C, Kumar SS, Walker EM Jr, Jones SG, Jones MM. (1987). Dithiocarbamate inhibition of cadmium-induced changes in spermatozoan acetyltransferases. *Res Comm Chem Pathol Pharmacol* **58**(3):355-70.

67. Elinder CG (1985). Cadmium: Uses, Occurances, and Intake. Friberg et al. (eds.) , (Cadmium and Health: A Toxicological and Epidemiological Appraisal), Vol. 1: Exposure, Dose and Metabolism. CRC Press, Boca Raton, FL.

68. Ellis KJ, Vartsky D, Zanzi I, Cohn SH (1979). Cadmium: In vivo measurement in smokers and non smokers. *Science* **205**:323-5.

69. Engstrom B, Nordberg GF (1979). Dose-dependence of gastrointestinal absorption and bilogical half-time of cadmium in mice. *Toxicology* **13**:215-22.

70. Environment Canada (1994). *Priority substances list assessment report: Cadmium and its compounds*. Rpt. ID:40E.

71. Falchuk KH (1993). Zinc in developmental biology. **In:** *Essential and Toxic Trace Elements in Human Health and Disease: An Update.* Prasad AS (eds.) Wiley-Liss, Inc, New York. pp 91-111.

72. Ferm VH (1971). Developmental malformations induced by cadmium. A study of timed injections during embryogenesis. *Biol Neonate* **19**(1):101-7.

73. Ferm VH, Carpenter SJ (1967). Teratogenic effect of cadmium and its inhibition by zinc. *Nature* **216**:1123.

74. Ferm VH, Layton WM Jr (1979). Reduction in cadmium teratogenesis by prior cadmium exposure. *Environ Res* **18**(2):347-50.

75. Feuston MH, Scott WJ Jr (1985). Cadmium-induced forelimb ectrodactyly: A proposed mechanism of teratogenesis. *Teratology* **32**:407-19.

76. Flanagan PR, McLellan JS, Haist J, Cherian MG, Chamberlain MJ, Valberg LS (1978). Increased dietary cadmium absorption in mice and human subjects with iron deficiency. *Gastroenterology* **74**:841-846.

77. Frery N, Nessman C, Girard F, Lafond J, Moreau T, Blof P, Lellach J, Huel G (1993B). Environmental exposure to cadmium and human birthweight. *Toxicology* **79**:109-118.

78. Frery N, Girard F, Moreau T, Blot P, Sahuquillo J, Hajem S, Orssaud G, Huel G (1993B). Validity of hair cadmium in detecting chronic cadmium exposure in general populations. *Bull Environ Contam Toxicol* **50**(5):736-43.

79. Friberg L, Elinder CG, Kjellstrom T (1992). Cadmium. **In:** *Environmental Health Criteria 134* World Health Organization, Geneva, Switzerland.

80. Fujita M, Takabatake E, Tsuchiya K (1982). Effects of maternal exposure to zinc and cadmium on metallothionein in fetal rat liver. *Arch Environ Contam Toxicol* **11**(5):645-649.

81. Gale TF (1979). Toxic effects of cadmium and amaranth on the developing hamster embryo. *Bull Environ Contam Toxicol* **22**(1-2):175-81.

82. Gale TF, Ferm VH (1973). Skeletal malformations resulting from cadmium treatment in the hamster. *Biol Neonate* **23**:149-60.

83. Gale TF, Layton WM (1980). The susceptibility of inbred strains of hamsters to cadmium-induced embryotoxicity. *Teratology* **21**(2):181-6.

84. Gennart JP, Buchet JP, Roels H, Ghyselen P, Ceulemans E, Lauwerys R (1992). Fertility of male workers exposed to cadmium, lead, or manganese. *Am J Epidemiol* **135**(11):1208-1219.

85. Ghezzi I, Toffoletto F, Sesana G, et al (1985). Behavior of biological indicators of cadmium in relation to occupational exposure. *Int Arch Occ Env Health* **55**:133-40.

86. Giavini E, Prati M, Vismara C (1980). Effects of Cd, Pb, and Cu on rat implantation embryos. *Bull Environ Contam Toxicol* **25**:702.

87. Glaser U, Kloppel H, Hochrainer D (1986). Bioavailability indicators of inhaled cadmium compounds. *Ecotox Env Safety* **11**:261-71.

88. Godowicz B (1986). Cadmium accumulation in tissues, organ weights, and foetal mortality in females from two inbred mice strains treated with cadmium chloride. *Folia Biologica* **34**(3):329-36.

89. Godowicz B, Kakol D (1988). Histopathological effect of cadmium on the testes of mice from the KE inbred strain. *Folia Biol* **36**(3-4):159-65.

90. Goon D, Klassen CD (1989). Dosage-dependent absorption of cadmium in the rat intestine measured in situ. *Toxic Appl Pharm* **100**:41-50.

91. Gouveia MA (1988). The testes in cadmium intoxication: Morphological and vascular aspects. *Andrologia* **20**(3):225-31.

92. Gross SB, Yeager DW, Middendorf MS (1976). Cadmium in liver, kidney, and hair of humans, fetal through old age. *J Tox Env Health* **2**:153-67.

93. Gupta A, Gupta A, Chandra SV (1991). Gestational cadmium exposure and brain development: a biochemical study. *Ind Health* **29**(2):65-71.

94. Hansen JC, Wulf HC, Kromann N et al (1985). Cadmium concentrations in blood samples from an East Grenlandic population. *Dan Med Bull* **32**:277-9.

95. Hartsfield JK Jr, Lee M, Morel JG, Hilbelink DR. (1992). Statistical analysis of the effect of cadmium and zinc on hamster teratogenesis. *Biochem Med Metabol Biol* **48**(2):159-73.

96. Hastings L, Choudhury H, Petering HG, Cooper G P (1978). Behavioral and biochemical effects of low-level prenatal cadmium exposure in rats. *Bull Environ Contam Toxicol* **20**:96-101.

97. Hata A, Tsunoo H, Nakajima H, Shintaku K, Kimura M (1980). Acute cadmium intoxication in inbred mice: A study on strain differences. *Chem Biol Interact* **32**(1-2):29-39.

98. Hatori A, Willhite CC, Jones MM, Sharma RP. (1990). Dithiocarbamates and prevention of cadmium teratogenesis in the hamster. *Teratology* **42**(3):243-51.

99. Hazelhoff Roelfzema WH, Roelofsen AM, Copius Peereboom-Stegeman JH (1985). Light microscopic aspects of the rat placenta after chronic cadmium administration. *Sci Total Environ* **42**(1-2):181-4.

100. Hazelhoff Roelfzema WH, Roelofsen AM, Peereboom-Stegeman JH (1987). Glycogen content of placenta and of fetal and maternal liver in cadmium-exposed rats. I: A descriptive light microscopic study. *Placenta* **8**(1):27-36.

101. Hazelhoff Roelfzema WH, Zahn-Breidenbach U, Copius Peereboom-Stegeman JH (1988A). Light and electron microscopic investigation of the rat placenta after cadmium administration during pregnancy. *Anat Embryol* **178**(4):345-51.

102. Hazelhoff Roelfzema W, Roelofsen AM, Herber RF, Peereboom-Steg JH (1988B). Cadmium and zinc concentrations in fetal and maternal rat tissue after parenteral administration of cadmium during pregnancy. *Arch Toxicol* **62**(4):285-90.

103. Henderson RF, Rebar AH, Pickrell JA, et al (1979). Early damage indicators in the lung. Biochemical and ctyological response of the lung to inhaled metal salts. *Toxic Appl Pharm* **50**:123-36.

104. Hew KW, Ericson WA, Welsh MJ (1993). A single low cadmium dose causes failure of spermiation in the rat. *Toxicol Appl Pharmacol* **121**(1):15-21.

105. Hogue DE, Parrish JJ, Foote RH, Stouffer JR, Anderson JL, Stoewsand GS, Telford JN, Bache CA, Gutenmann WH, Lisk DJ (1984). Toxicologic studies with male sheep grazing on municipal sludge-amended soil. *J Toxicol Environ Health* **14**(2-3):153-161.

106. Holloway WR, Thor DH (1988). Social memory deficits in adult male rats exposed to cadmium in infancy. *Neurotoxicology and Teratology* **10**:193-197.

107. Holt D, Webb M (1986). Comparison of some biochemical effects of teratogenic doses of mercuric merrcury and cadmium in the pregnant rat. *Arch Toxicol* **58**:249-54.

108. Holt, D, Webb (1987). Teratology of ionic cadmium in the Wistar rat. *Arch Toxicol* **59**:443-7.

109. Huber KL, Cousins RJ (1988). Maternal zinc deprivation and interleukin 1 influence metallothionein gene expression and zinc metabolism in rats. *J Nutr* **118**:1570-6.

110. Huel G, Boudene C, Ibrahim MA (1981). Cadmium and lead content of matenal and newborn hair: Relationship to parity, birth weight, and hypertension. *Arch Environ Health* **36**(5):221-227.

111. Huel G, Everson RB, Menger I (1984). Increased hair cadmium in newborns of women occupationally exposed to heavy metals. *Environ Res* **35**:115-121.

112. Hurley LS, Gowan J, Swenerton H (1971). Teratogenic effects of short-term and transitory zinc deficiency in rats. *Teratology* **4**.

113. IARC (1993). *IARC Monographs on the evaluation of carcinogenic risks to humans: beryllium, cadmium, mercury, and exposures in the glass manufacturing industry*. Rpt. ID:58. Vol. 58.

114. Janousek V, Krijt J, Malbohan M, Cibula D, Lukas W, Zejda JE, Lammers W, Huisman M, Boersma E R, van der Paauw CG, Vogelaar EF, Winneke G, Schmidt E, Steingruber HJ (1994). Cord blood levels of potentially neurotoxic pollutants (polychlorinated biphenyls, lead and cadmium) in the areas of Prague (Czech Republic) and Katowice (Poland). Comparison with reference values in The Netherlands. *Cent Eur J Public Health* **2**(2):73-76.

115. Kagi JHR, Schaffer A (1988). Biochemistry of metallothionein. *Biochemistry* **27**(23):8509-15.

116. Kaur G, Nath R, Gupta GS (1993). Purification and Characterization of Low Molecular Weight Metal Binding Protein from Human Testes. *Journal of Trace Elements in Experimental Medicine* **6**(1):1-13.

117. Keen CL, Hurley LS (1989). Zinc and reproduction: Effects of deficiency on foetal and postnatal development. Springer.

118. Keen CL, Taubeneck MW, Daston GP, Rogers JM, Gershwin ME (1993). Primary and secondary zinc deficiency as factors underlying abnormal CNS development. *Ann NY Acad Sci* **678**.

119. Kelman BJ, Walter BK, Jarboe GE, Sasser LB (1978). Effect of dietary cadmium on calcium metabolism in the rat during late gestation. *Proc Soc Exp Biol Med* **158**:614-617.

120. Kelman B, Walter B (1977). Passage of cadmium across the perfused guinea pig placenta. *Proc Soc Exp Biol Med* **156**:68-71.

121. Kelman B, Walter B (1980). Fetal to maternal cadmium movments across the perfused hemochorial placenta of the guinea pig. *Toxicol Appl Pharmacol* **52**:400-6.

122. Khera KS (1991). Chemically induced alterations in maternal homeostasis and histology of conceptus: their etiologic significance in rat fetal anomalies. *Teratology* **44**(3):259-97.

123. Kimura M, Otaki N (1972). Percutaneous absorption of cadmium in rabit and hairless mouse. *Ind Health* **10**:7-10.

124. Kjellstrom T, Borg K, Lind B (1978). Cadmium in feces as an estimator of daily cadmium intake in Sweden. *Environ Res* **15**:242-51.

125. Klaassen CD, Wong KL (1982). Cadmium toxicity in the newborn rat. *Can J Physiol Pharmacol* **60**(7):1027-36.

126. Kostial K (1986). Cadmium. *Trace elements in human and animal nutrition. 5th ed. Ed Mertz W. Acad Press NY* **2**:319-345.

127. Kotsonis FN, Klaassen CD (1977). Toxicity and distribution of cadmium administered to rats at sublethal doses. *Toxicol Appl Pharmacol* **41**:667-680.

128. Kotsonis FN, Klaassen CD (1978). The relationship of metallothionein to the toxicity of cadmium after prolonged oral administration to rats. *Toxicol Appl Pharmacol* **46**(1):39-54.

129. Kovar IZ, Strehlow CD, Richmond J, Thompson MG (1984). Perinatal lead and cadmium burden in a british urban population. *Arch Dis Child* **59**(1):36-39.

130. Kuhnert BR, Kuhnert PM, Debanne S, Williams MS (1987). The relationship between cadmium, zinc, and birth weight in pregnant women who smoke. *Am J Obstet Gynecol* **157**(1247-1251)

131. Kuhnert BR, Kuhnert PM, Zarlingo TJ (1988). Associations between placental cadmium and zinc and age and parity in pregnant women who smoke. *Obstet Gynecol* **71**(1):67-70.

132. Kuhnert BR, Kuhnert PM, Lazebnik N, Erhard P (1993). The relationship between placental cadmium, zinc, and copper. *J Am Coll Nutr* **12**(1):31-35.

133. Kuhnert PM, Kuhnert BR, Bottoms SF, Erhard BS (1982). Cadmium levels in maternal blood, fetal cord blood, and placental tissues of pregnant women who smoke. *Am J Obstet Gynecol* **142**:1021-1025.

134. Kutzman RS, Drew RT, Shiotsuka RN, Cockrell BY (1986). Pulmonary changes resulting from subchronic exposure to cadmium chloride aerosol. *J Toxicol Environ Health* **17**:175-189.

135. Laskey JW, Rehnberg MJ, Cahill DF, Pieirzak-Flis Z (1980). Chronic ingestion of cadmium and/or tritium. II. Effects on growth, development and reproductive function. *Environ Res* **22**:466-475.

136. Laskey JW, Rehnberg GL, Laws SC, Hein JF (1986). Age-related dose response of selected reproductive parameters to acute cadmium chloride exposure in the male Long-Evans rat. *J Toxicol Environ Health* **19**:393-401.

137. Laskey, JW, Rehnberg, GL, Laws, SC, Hein (1984). Reproductive effects of low acute doses of cadmum chloride in adult male rats. *Toxicol Appl Pharmacol* **73**:250-5.

138. Lau IF, Saksena SK, Dahlgren L, Chang MC (1978). Steroids in the blood serum and testes of cadmium chloride treated hamsters. *Biol Reprod* **19**(4):886-9.

139. Laudanski T, Sipowicz M, Modzelewski P, Bolinski J, Szamatowicz J, Razniewska G, Akerlund M (1991). Influence of high lead and cadmium soil content on human reproductive outcome. *Int J Gynecol Obstet* **36**:309-315.

140. Lauwerys R, Buchet JP, Roels H, Hubermont G (1978). Placental transfer of lead, mercury, cadmium, and carbon monoxide in women. I. Comparison of the frequency distributions of the biological indices in maternal and umbilical cord blood. *Environ Res* **15**:278-89.

141. Layton WM Jr, Ferm VH (1980). Protection against cadmium-induced limb malformations by pretreatment with cadmium or mercury. *Teratology* **21**(3):357-60.

142. Layton WM Jr, Layton MW (1979). Cadmium induced limb defects in mice: strain associated differences in sensitivity. *Teratology* **19**(2):229-35.

143. Lehman LD, Klassen CD (1986). Dosage-dependent disposition of camdium administered orally to rats. *Toxic Appl Pharm* **84**:159-67.

144. Lehman-McKeeman LD, Klasssen CD (1987). Induction of metallothionein-I and metallothionein-II in rats by cadmium and zinc. *Toxic Appl Pharm* **88**:195-202.

145. Lehotzky K, Ungvary G, Polinak D, Kiss A (1990). Behavioral deficits due to prenatal exposure to cadmium chloride in CFY rat pups. *Neurotoxicology and Teratology* **12**(2):169-172.

146. Levin AA, Kilpper RW, Miller RK (1987). Fetal toxicity of cadmium chloride: The pharmacokinetics in the pregnant wistar rat. *Teratology* **36**:163-70.

147. Levin AA, Miller RK (1980). Fetal toxicity of cadmium in the rat: maternal vs fetal injections. *Teratology* **22**(1):1-5.

148. Levin AA, Miller RK (1981). Fetal toxicity of cadmium in the rat: decreased utero-placental blood flow. *Toxicol Appl Pharmacol* **58**:297-306.

149. Loeser E, Lorke D (1977). Semichronic oral toxicity of cadmium. 2. Studies on dogs. *Toxicology* **7**:225-232.

150. Lohiya NK, Arya M, Shivapuri VS (1976). The effects of cadmium chloride on testis and epididymis of the Indian Hanuman langur, Presbytis entellus entellus Dufresne. *Acta Eur Fertil* **7**(4):339-48.

151. Loiacono NJ, Graziano JH, Kline JK, Popovac D, Ahmedi X, Gashi E, Mehmeti A, Rajovic B (1992). Placental cadmium and birthweight in women living near a lead smelter. *Arch Environ Health* **47**(4):250-5.

152. Lucis OJ, Lucis R, Shaikh ZA (1972). Cadmium and zinc in pregnancy and lactation. *Arch Environ Health* **25**(1):14-22.

153. Lucis OJ, Lynk ME, Lucis R (1969). Turnover of cadmium 109 in rats. *Arch Env Health* **18**:307-10.

154. Machemer L, Lorke D (1981). Embryotoxic effect of cadmium on rats upon oral administration. *Toxicol Appl Pharmacol* **58**:438-443.

155. Maitani T, Suzuki KT (1986). Effect of cadmium on essential metal concentrations in testis liver and kidney of five inbred strains of mice. *Toxicology* **42**(2-3):121-30.

156. Mason HJ (1990). Occupational cadmium exposure and testicular endocrine function. *Hum Exp Toxicol* **9**:91-94.

157. Masters BA, Kelly EJ, Quaife CJ, Brinster RL, Palmiter RD (1994). Targeted disruption of metallothionein I and II genes increases sensitivity to cadmium. *Proc Natl Acad Sci USA* **91**(2):584-588.

158. McKenna IM, Bare RM, Waalkes MP (1996). Metallothionein gene expression in testicular interstitial cells and liver of rats treated with cadmium. *Toxicology* **107**(2):121-30.

159. McKenzie-Parnell JM, Kjellstronm TE, Sharma RP et al (1988). Unusually high intake and fecal output of camium, and fecal output of other trace elements in New Zealand adults consuming dredge oysters. *Env Res* **46**:1-14.

160. McLellan JS, Flanagan PR, Chamberlain MJ et al (1978). Measurment of dietary cadmiu absorption in humans. *J Tox Env Health* **4**:131-8.

161. Michalska AE, Choo KHA (1993). Targeting and germ-line transmission of a null mutation at the metallothionein I and II loci in mouse. *Proc Natl Acad Sci USA* **90**(17):8088-92.

162. Moore JA, Daston GP, Faustman E, Golub MS, Hart WL, Hughes CJ, Kimmel CA, Lamb JCI, Schwetz BA, Scialli AR (1995). An evaluative process for assessing human reproductive and developmental toxicity of agents. *Reprod Toxicol* **9**:61-95.

163. Moore W, Stara JF, Crocker WC et al (1973). Comparison of 115Cd retention in rats following different routes of exposure. *Env Res* **6**:473-8.

164. Muller L, Abel J, Ohnesorge FK (1986). Absorbtion and distribution of cadmium (Cd), copper and zinc following oral subchronic low level administration to rats of different binding forms of cadmium (Cd-acetate, Cd-metallothionein, Cd-glutathione). *Toxicology* **39**:187-95.

165. Munoz C, Dieter HH (1990). Effect of methoxyethanol, cyclophosphamide and cadmium on metallothionein levels during prenatal development in the mouse. *Toxicology Letters* **50**(2-3):263-74.

166. Murdoch RN, Cowen NL (1981). Foetotoxicity of cadmium in Quackenbush strain mice and the effects of the metal on uterine alkaline phosphatase during pseudopregnancy. *Australian J Biol Sci* **34**(3):305-12.

167. Murthy RC, Mohd.Ali M, Chandra SV (1986). Effects of in utero exposure to cadmium on the brain biogenic amine levels and tissue metal distribution in rats. *Ind Health* **24**:15-21.

168. Nagy F (1985). Cadmium-induced alterations in the siberian hamster epididymis. *Arch Androl* **15**:91-104.

169. Naruse I, Hayashi Y (1989). Amelioration of the teratogenicity of cadmium by the metallothionein induced by bismuth nitrate. *Teratology* **40**(5):459-65.

170. National Insitute for Occupational Safety and Health (1996). Registry of Toxic Effects of Chemical Substances (RTECS): Cadmium. **In:** *Tomes* Micromedex, Inc., Englewood, CO.

171. National Library of Medicine (1996). Hazardous Substance Data Bank (HSDB): Cadmium. **In:** *Tomes* Micromedex, Inc., Englewood, CO.

172. National Toxicology Program (1995). *Cadmium oxide (CAS No. 1306-19-0): Administered by inhalation to F344/N rats and B6C3F1 mice*. Rpt. ID:NIH Publication 95-3388. Toxicity Report Series. Vol. 39. USDHHS, PHS, NIH.

173. Nayak BN, Ray M, Persaud TVN, Nigel M (1989). Embryotoxicity and in vivo cytogenetic changes following maternal exposure to cadmium chloride in mice. *Exp Pathol* **36**:75-80.

174. Newton D, Johnson P, Lally AE et al (1984). The uptake by man of cadmium ingested in crab meat. *Hum Toxicol* **3**:23-8.

175. Niewenhuis RJ, Prozialeck WC (1987). Calmodulin inhibitors protect against cadmium-induced testicular damage in mice. *Biol Reprod* **37**:127-33.

176. Nishimura H, Nishimura N, Tohyama C. (1990). Localization of metallothionein in the genital organs of the male rat. *J Histochem Cytochem* **38**(7):927-33.

177. Noack-Fuller G, De Beer C, Seibert H (1993). Cadmium, lead, selenium, and zinc in semen of occupationally unexposed men. *Andrologia* **25**:7-12.

178. Nolan CV, Shaikh ZA (1986). An evaluation of tissue metallothionein and genetic resistance to cadmium toxicity in mice. *Toxicol Appl Pharmacol* **85**(2):135-44.

179. Nordberg GF, Piscator M, Lund B (1971). Distribution of Cd among protein fractions of mouse liver. *Acta Pharmacol Toxicol* **29**:456-70.

180. Ohta H, Seki Y, Imamiya S (1988). Metallothionein-like cadmium binding protein in rat testes administered with cadmium and selenium. *Bull Environ Contam Toxicol* **41**(2):195-200.

181. Oldereid NB, Thomassen Y, Attramadal A, Olaisen B, Purvis K (1993). Concentrations of lead, cadmium and zinc in the tissues of reproductive organs of men. *J Reprod Fertil* **99**(2):421-425.

182. Oldereid NB, Thomassen Y, Purvis K (1994). Seminal plasma lead, cadmium and zinc in relation to tobacco consumption. *Int J Androl* **17**(1):24-28.

183. Padmanabhan R (1986). The effect of cadmium on placental structure and its relation to fetal malformations in the mouse. *Zeitschrift fur Mikroskopisch-Anatomische Forschung* **100**(3):419-27.

184. Padmanabhan R (1987). Abnormalities of the ear associated with exencephaly in mouse fetuses induced by maternal exposure to cadmium. *Teratology* **35**:9-18.

185. Padmanabhan R, Hameed MS (1986). Exencephaly and axial skeletal dysmorphogenesis induced by maternal expousre to cadmium in the mouse. *J Craniofac Genetr Dev Biol* **6**:245-58.

186. Padmanabhan R, Hameed MS (1990). Characterictics of the limb malformations induced by matenal exposure to cadmium in the mouse. *Reprod Toxicol* **4**:291-304.

187. Parizek J (1957). The destructive effect of cadmium ion on testicular tissue and its prevention by zinc. *J Endocrinol* **15**:56-63.

188. Parizek J (1964). Vascular changes at sites of oestrogen biosynthesis produced by parenteral injection of cadmium salts: the destruction of placenta by cadmium salts. *J Reprod Fertil* **7**:263-5.

189. Parizek J (1965). The peculiar toxicity of Cd during pregnancy: an experimental toxemia of pregnancy induced by Cd salts. *J Reprod Fertil* **9**:111-2.

190. Parizek J, Zahor Z (1956). Effect of cadmium salts on testicular tissue. *Nature* **177**:1036-7.

191. Parzyck DC, Shaw SM, Kessler WV, Vetter RJ, Van Sickle DC, Mayes RA (1978). Fetal effects of cadmium in pregnant rats on normal and zinc deficient diets. *Bull Environ Contam Toxicol* **19**(2):206-14.

192. Piasek M, Laskey JW (1994). Acute cadmium exposure and ovarian steroidogenesis in cycling and pregnant rats. *Reprod Toxiol* **8**(6):495-507.

193. Pihl RO (1979). Recent evidence of physical abnormality in children with learning disabilties. *McGill J Educ* **14**:353-364.

194. Pihl RO, Parkes M (1977). Hair element content in learning disabled children. *Science* **198**:204-206.

195. Pond WG, Walker EF,Jr (1975). Effect of dietary Ca and Cd level of pregnant rats on reproduction and on dam and progeny tissue mineral concentrations. *Proc Soc Exp Biol Med* **148**:665-668.

196. Pond WG, Yen JT (1983). Reproduction and progeny growth in rats fed clinoptilolite in the presence or absence of dietary cadmium. *Bull Environ Contam Toxicol* **31**(6):666-72.

197. Prigge E (1978). Inhalative cadmium effects in pregnant and fetal rats. *Toxicology* **10**:297-309.

198. Probst GS (1979). Cadmium: absorbtion, distribution, and excretion in mammals. *Cadmium toxicity, Mennear JH ed, Marcel Dekker, NY* :29-59.

199. Radisch B, Luck W, Nau H (1987). Cadmium concentrations in milk and blood of smoking mothers. *Tox Lett* **36**:147-52.

200. Rahola T, Aaran R-K, Miettenen JK (1973). Retention and elimination of 115mCd in man. *Health physics problems of internal contamination. Akademia (Budapest)* **Akademia**(Budapest):213-8.

201. Rastogi RB, Merali Z, Singhal RL (1977). Cadmium alters behavior and the biosynthetic capacity for catecholamines and serotonin in neonatal rat brain. *J Neurochem* **28**:789-794.

202. Roels HA, Lauwerys RR, Bushet JP et al (1981). In vivo measurement of liver and kidney cadmium in workers exposed to this metal: its significance with respect to cadmium in blood and urine. *Env Res* **26**:217-40.

203. Roels HA, Lauwerys RR, Buchet JP et al (1989). Health significance of cadmium induced renal dysfunction: A five-year followup. *Br J Ind Med* **46**:755-64.

204. Rohrer S, Shaw S, Lamar C (1979). Cadmium fetotoxicity in rats following prenatal exposure. *Bull Environ Contam Toxicol* **23**:25-29.

205. Ruppert PH, Dean KF, Reither LW (1985). Development of locomotor activity of rat pups exposed to heavy metals. *Toxicology & Applied Pharmacology* **78**:69-77.

206. Rusch GM, O'Grodnick JS, Rinehart WE (1986). Acute inhalation study in the rat of comparative uptake, dsitribution and excretion of different cadmium containing materials. *Am Ind Hyg Assoc J* **47**:754-63.

207. Saaranen M, Kantola M, Saarikoski S, Vanha-Perttula T (1989). Human seminal plasma cadmium: comparison with fertility and smoking habits. *Andrologia* **21**(2):140-145.

208. Sabbioni E, Marafante E, Amantini L, Ubertalli L, Pietra R (1978). Cadmium toxicity studies under long term-low level exposure (LLE) conditions. I. Metabolic patterns in rats exposed to present environmental dietary levels of Cd for two years. *Sci Total Environ* **10**(2):135-61.

209. Saillenfait AM, Payan JP, Brondeau MT, Zissu D, de Ceaurriz J (1991). Changes in urinary proximal tubule parameters in neonatal rats exposed to cadmium chloride during pregnancy. *J Appl Toxicol* **11**(1):23-7.

210. Saillenfait AM, Payan JP, Ban M, de Ceaurriz J (1992). Indirect and lactation-associated changes in renal alkaline phosphatase of newborn rats prenatally exposed to cadmium chloride. *J Appl Toxicol* **12**(3):205-10.

211. Saksena SK, Dahlgren L, Lau IF, Chang MC (1977). Reproductive and endocrinological features of male rats after treatment with cadmium chloride. *Biol Reprod* **16**:609-13.

212. Saltzman RA, Miller RK, diSant'Agnese PA (1989). Cadmium exposure on day 12 of gestation in the Wistar rat: distribution, uteroplacental blood flow, and fetal viability. *Teratology* **39**:19-30.

213. Samarawickrama GP, Webb M (1979). Acute effects of cadmium on the pregnant rat and embryo-fetal development. *Env Health Persp* **28**:245-9.

214. Samarawickrama GP, Webb M (1981). The acute toxicity and teratogenicity of cadmium in the pregnant rat. *J Appl Toxicol* **1**(5):264-9.

215. Sasser LB, Kelman BJ, Levin AA, Miller RK (1985). The influence of maternal cadmium exposure or fetal cadmium injection on hepatic mettalothionein concentrations in the fetal rat. *Toxicol Appl Pharmacol* **80**(2):299-307.

216. Sasser LB, Jarboe GE (1977). Intestinal absorption and retention of cadmium in neonatal rat. *Toxic Appl Pharm* **41**:423-31.

217. Sato F, Watanabe T, Hoshi E, Endo A (1985). Teratogenic effect of maternal zinc deficiency and its co-teratogenic effect with cadmium. *Teratology* **31**(1):13-8.

218. Saxena DK, Murthy RC, Chandra SV (1986). Embryotoxic and teratogenic effects of interaction of cadmium and lindane in rats. *Acta pharmacol et toxicol* **59**:175-178.

219. Saxena DK, Murthy RC, Singh C, Chandra SV (1989). Zinc protects testicular injury induced by concurrent exposure to cadmium and lead in rats. *Res Commun Chem Pathol Pharmacol* **64**(2):317-329.

220. Saygi S, Deniz G, Kutsal O, Nevin V (1991). Chronic effects of cadmium on kidney, liver, testis, and fertility of male rats. *Biol Trace Element Res* **31**(3):209-214.

221. Schafer L, Andersen O, Nielsen JB (1986). Effects of dietary factors on g.i. Cd absorption in mice. *Acta Pharm Tox (Copenh)* **59**(Suppl 7):549-52.

222. Schafer SG, Schwegler U, Schumann K (1990). Retention of cadmium in cadmium-naive normal and iron-deficient rats as well as in cadmium-induced iron-deficient animals. *Ecotox Env Safety* **20**:71-81.

223. Scharpf LG, Hill ID, Wright PL, Plank JB, Keplinger ML, Calandra JC (1972). Effect of sodium nitrilotriacetate on toxicity, teratogenicity, and tissue distribution of cadmium. *Nature* **239**:231-234.

224. Schramel P, Hasse S, Ovcar-Pavlu J (1988). Selenium, cadmium, lead, and mercury concentrations in human breast milk, in placenta, maternal blood, and the blood of the newborn. *Biol Trace Element Res* **15**:111-124.

225. Schroeder HA, Mitchener M (1971). Toxic effects of trace elements on the reproduction of mice and rats. *Arch Environ Health* **23**:102-106.

226. Seidenberg JM, Anderson DG, Becker RA (1986). Validation of an in vivo developmental toxicity screen in the mouse. *Teratogenesis Carcinog Mutagen* **6**:361-374.

227. Selypes A, Lorencz R, Berencsi G, Desi I (1986). The effect of prednisolone on the foetotoxicity of cadmium in pregnant mice. *J Appl Toxicol* **6**(1):47-8.

228. Shaikh ZA, Jordan SA, Tewari PC (1993). Cadmium disposition and metallothionein induction in mice: Strain-, sex-, age- and dose-dependent differences. *Toxicology* **80**(1):51-70.

229. Shiraishi N, Rehm S, Waalkes MP (1994). Effect of chlorpromazine pretreatment on cadmium toxicity in the male Wistar (WF/NCr) rat. *J Toxicol Environ Health* **42**(2):193-208.

230. Shiraishi N, Waalkes MP (1994). Enhancement of metallothionein gene expression in male Wistar (WF/NCr) rats by treatment with calmodulin inhibitors: Potential role of calcium regulatory pathways in metallothionein induction. *Toxicol Appl Pharmacol* **125**(1):97-103.

231. Shizuyo S, Yamamoto K, Sendota H, Tomomatsu K, Shimizu Y, Sugiyama M (1980). Toxicity, fertility, teratogenicity, and dominant lethal tests in rats administered cadmium subchronically. I. Toxicity studies. *Ecotox Environ Safety* **4**:39-50.

232. Sikorski R, Radomanski T, Paszkowski T, Skoda J (1988). Smoking during pregnancy and the perinatal Cd burden. *J Perinat Med* **16**:225-231.

233. Simmons DL, Valentine DM, Bradshaw WS (1984). Different patterns of developmental toxicity in the rat following prenatal administration of structurally diverse chemicals. *J Toxicol Environ Health* **14**:121-136.

234. Singhal RL (1981). Testicular cyclic nucleotide and adrenal catecholamine metabolism following chronic exposure to cadmium. *Environ Health Perspect* **38**:111-7.

235. Skog E, Wahlberg JE (1964). A comparative investigatio of the percutaneous absorption of metal compound in the guinea pig by means of the radioactive isotopes: 51Cr, 58Co, 65Zn, 115mCd, 203Hg. *J Invest Dermatol* **43**:187-73.

236. Smart JL, Dobbing J (1971). Vulnerability of developing brain. VI. Relative effects of foetal and early postnatal undernutrition on reflex ontogeny and development of behavior in the rat. *Brain Res* **33**:303-314.

237. Smart JL, Dobbing J, Adlaird BPF, Lynch A, Sands J (1973). Vulnerability of developing brain: Relative effects of growth restriction during the fetal and suckling periods on behavior and brain composition of adult rats. *J Nutr* **103**:1327-1338.

238. Smith JP, Smith JC, McCall AJ (1960). Chronic poisoning from cadmium fume. *J Pathol Bacteriol* **80**:287-297.

239. Smith MJ, Pihl RO, Garber B (1982). Postnatal cadmium exposure and longterm behavioral changes in the rat. *Neurobehav Toxicol Teratol* **4**(3):283-287.

240. Sonawane BR, Nordberg M, Nordberg GF, Lucier GW (1975). Placental transfer of cadmium in rats: influence of dose and gestational age. *Env Health Persp* **12**:97-102.

241. Sorell TL, Graziano JH (1990). Effect of oral cadmium exposure during pregnancy on maternal and fetal zinc metabolism in the rat. *Toxicol Appl Pharmacol* **102**:537 - 545.

242. Sowa B, Steibert E (1985). Effect of oral cadmium administration to female rats during pregnancy on zinc, copper, and iron content in placenta, foetal liver, kidney, intestine, and brain. *Arch Toxicol* **56**(4):256-262.

243. Sowa B, Steibert E, Gralewska K, Piekarski M (1982). Effect of oral cadmium administration to female rats before and/or during pregnancy on the metallothionein level in the fetal liver. *Toxicol Lett* **11**:233-236.

244. Stacey NH, Wong KL, Klaassen CD (1983). Protective effects of chromium on the toxicity of cadmium in vivo. *Toxicology* **28**(1):147-53.

245. Steibert E, Kaminski M, Kaminska O, Kusz E, Krol B, Sowa B, Gralewska K (1984). Effect of oral cadmium administration to female rats before and during pregnancy on the histoenzymatic activity in maternal and foetal kidneys. *Folia Biol (Krakow)* **32**(4):331-340.

246. Sutou S, Yamamoto K, Sendota H, Tomomatsu K, Shimizu Y, Sugiyama M (1980A). Toxicity, fertility, teratogenicity, and dominant lethal tests in rats administered cadmium subchronically. I. Toxicity studies. *Ecotox Environ Safety* **4**:39-50.

247. Sutou S, Yamamoto K, Sendota H, Sugiyama M (1980B). Toxicity, fertility, teratogenicity, and dominant lethal tests in rats administered cadmium subchronically. II. Fertility, teratogenicity, and dominant lethal tests. *Ecotox Environ Safety* **4**:51-56.

248. Tabacova S, Little RE, Balabaeva L, Pavlova S, Petrov I (1994). Complications of Pregnancy in Relation to Maternal Lipid Peroxides, Glutathione, and Exposure to Metals. *Reprod Toxicol* **8**(3):217-224.

249. Tabacova S, Baird DD, Balabaeva L, Lolova D, Petrov I (1994). Placental arsenic and cadmium in relation to lipid peroxides and glutathione levels in maternal-infant pairs from a copper smelter area. *Placenta* **15**(8):873-81.

250. Takizawa Y, Nakamura I, Kurayama R, Hirasawa F, Kawai K (1981). Effect of pregnancy on cadmium-treated rats. *Ind Health* **19**(3):171-176.

251. Tam PPL, Liu WK (1985). Gonadal development and fertility of mice treated prenatally with cadmium during the early organogenesis stages. *Teratology* **32**:453-62.

252. Tassinari MS, Long SY (1982). Normal and abnormal midfacial development in the cadmium-treated hamster. *Teratology* **25**(1):101-3.

253. Taylor BA, Heiniger HJ, Meier H (1973). Genetic analysis of resistance to cadmium-induced testicular damage in mice. *Proc Soc Exp Biol Med* **143**:629-3.

254. Tsvetkova RP (1970). [Materials on the study of the influence of cadmium compounds on the generative function] (Russian; English translation of abstract only). *Gig Tr Prof Zabol* **14**:31-33.

255. US Environmental Protection Agency (1996). Integrated Risk Information System (IRIS): Cadmium. **In:** *Tomes* Micromedex, Inc., Englewood, CO.

256. Varga B, Zsolnai B, Paksy K, Naray M, Ungvary G (1993). Age dependent accumulation of cadmium in the human ovary. *Reprod Toxicol* **7**(3):225-8.

257. Waalkes MP (1986). Effect of dietary zinc deficiency on the accumulation of cadmium and metallothionein in selected tissues of the rat. *J Toxicol Environ Health* **18**(2):301-313.

258. Waalkes MP, Chernoff SB, Klaasen Cd (1984A). Cadmium-binding proteins of rat testes. *Biochem J* **220**:811-8.

259. Waalkes MP, Chernoff SB, Klaasen Cd (1984B). Cadmium-binding proteins of rat testes. *Biochem J* **220**:819-24.

260. Waalkes MP, Goering PL (1990). Metallothionein and other cadmium-binding proteins: Recent developments. *Chem Res Tox* **3**:281-8.

261. Waalkes MP, Perantoni AP (1986). Isolation of a novel metal-binding protein from rat testes. *J Biol Chem* **261**:13097-13103.

262. Waalkes MP, Perantoni A, Palmer AE (1988A). Isolation and partial characterization of the low-molecular-mass zinc/cadmium-binding protein from the testes of the patas monkey (erythroebus patas). *Biochem J* **256**:131-7.

263. Waalkes MP, Perantoni A, Bhave MR, Rehm S (1988B). Strain dependence in mice of resistance and susceptibility to the testicular effects of cadmium: assessment of the role of testicular cadmium-binding proteins. *Toxicol Appl Pharmacol* **93**(1):47-61.

264. Wahba ZZ, Miller MS, Waalkes MP (1994). Absence of changes in metallothionein RNA in the rat testes made refractory to cadmium toxicity by zinc pretreatment. *Hum Exp Toxicol* **13**(1):65-7.

265. Wardell RE, Seegmiller RE, Bradshaw WS (1982). Induction of prenatal toxicity in the rat by diethylstilbestrol, zeranol, 3,4,3',4'.-tetrachlorobiphenyl, cadmium, and lead. *Teratology* **26**:229-237.

266. Webb (1987). Metallothionein in regenertion, reporduction and development. *Experientia Suppl* **52**:483.

267. Webb M (1987). Toxicological significance of metallothionein. *Experientia Supplementum* **52**:109-34.

268. Webb M, Samarawickrama GP (1981). Placental transport and embryonic utilization of essential metabolites in the rat at the teratogenic dose of cadmium. *J Appl Toxicol* **1**(5).

269. Webster W (1978). Cadmium-induced fetal growth retardation in the mouse. *Arch Environ Health* **33**:36-42.

270. Webster W (1979A). Iron deficiency and its role in cadmium-induced fetal growth retardation. *J Nutr* **109**(9):1640-1645.

271. Webster W (1988). Chronic cadmium exposure during pregnancy in the mouse: Influence of exposure levels on fetal and maternal uptake. *J Toxicol Environ Health* **24**(2):183-192.

272. Webster WS (1979B). Cadmium-induced fetal growth retardation in mice and the effects of dietary supplements of zinc, copper, iron and selenium. *J Nutr* **109**(9):1646-1651.

273. Webster WS, Valois AA (1981). The toxic effects of cadmium on the neonatal mouse CNS. *J Neuropathol Exp Neurol* **40**(3):247-257.

274. Webster WS, Messerle K (1980). Changes in the mouse neuroepithelium associated with cadmium-induced neural tube defects. *Teratology* **21**(1):79-88.

275. Whelton BD, Bhattacharyya MH, Carnes BA, Moretti ES, Peterson DP (1988). Female reproduction and pup survival and growth for mice fed a cadmium-containing purified diet through six consecutive rounds of gestation and lactation. *J Toxicol Environ Hlth* **24**:321-343.

276. Whelton BD, Toomey JM, Bhattacharyya MH (1993D). Cadmium-109 metabolism in mice. IV. Diet versus maternal stores as a source of cadmium transfer to mouse fetuses and pups during gestation an lactation. *J Toxicol Environ Health* **40**:531-546.

277. Whelton BD, Moretti ES, Peterson DP, Bhattacharyya MH (1993A). Cadmium-109 metabolism in mice. I. Organ retention in mice fed a nutritionally sufficient diet during successive rounds of gestation and lactation. *J Toxicol Environ Health* **38**(2):115-29.

278. Whelton BD, Moretti ES, Peterson DP, Bhattacharyya MH (1993B). Cadmium-109 metabolism in mice. II. Organ retention in mice fed a nutritionally deficient diet during successive rounds of gestation and lactation. *J Toxicol Environ Health* **38**(2):131-45.

279. Whelton BD, Peterson DP, Moretti ES, Bhattacharyya MH (1993C). Cadmium-109 metabolism in mice. III. Organ retention in mice ovariectomized after experiencing multiple rounds of gestation and lactation. *J Toxicol Environ Health* **38**(2):147-57.

280. Whelton BD, Toomey JM, Bhattacharyya MH (1993D). Cadmium-109 metabolism in mice. IV. Diet versus maternal stores as a source of cadmium transfer to mouse fetuses and pups during gestation and lactation. *J Toxicol Environ Health* **40**(4):531-46.

281. White TE, Baggs RB, Miller RK (1990). Central nervous system lesions in the Wistar rat fetus following direct fetal injections of cadmium. *Teratology* **42**(1):7-13.

282. Wier PJ, Miller RK, Maulik D, DiSant'Agnese P (1990). Toxicity of cadmium in the perfused human placenta. *Toxicol Appl Pharmacol* **105**:156-171.

283. Wier PJ, Miller RK (1987). The pharmacolkinetics of Cd in the dually perfused human placenta. *Trophoblast Res* **2**:357-66.

284. Wills JH, Groblewski GE, Coulston F (1981). Chronic and multigeneration toxicities of small concentrations of cadmium in the diet of rats. *Ecotox Environ Safety* **5**:452-464.

285. Wisniewska-Knypl JM, Jablonska J, Myslak Z (1971). Binding of cadmium on metallothionein in man: An analysis of a fatal poisoning by cadmium iodide. *Archiv fur Toxikologie* **28**(1):46-55.

286. Wolkowski RM (1974). Differential cadmium-induced embryotoxicity in two inbred mouse strains. I. Analysis of inheritance of the response to. *Teratology* **10**(3).

287. Wong K-L, Cachia R, Klaassen CD (1980). Comparison of the toxicity and tissue distribution of cadmium in newborn and adult rats after repeated administration. *Toxicol Appl Pharmacol* **56**(3):317-25.

288. Wong K-L, Klaassen CD (1980). Age difference in the susceptibility to cadmium-induced testicular damage in rats. *Toxicol Appl Pharmacol* **55**:456-66.

289. Xu B, Chia SE, Tsakok M, Ong CN (1993B). Trace elements in blood and seminal plasma and their relationship to sperm quality. *Reprod Toxicol* **7**(6):613-618.

290. Xu B, Chia S, Ong C (1994). Concentrations of cadmium, lead, selenium, and zinc in human blood and seminal plasma. *Biol Trace Element Res* **40**(1):49-57.

291. Xu B, Jin Y, Feng Z, Xu Z, Matsushita T (1993A). Lipid peroxidation induced by maternal cadmium exposure in mouse pups. *Bull Environ Contam Toxicol* **51**(5):772-779.

292. Yuen M, Huang S, Bronstrup A, Lonnerdal B (1994). Effect of oral exposure of cadmium during gestation and lactation on mammary transfer of iron. *FASEB J* **8**(35160).

293. Zenick H, Hastings L, Goldsmith M, Niewenhuis RJ (1982). Chronic cadmium exposure: Relation to male reproductive toxicity and subsequent fetal outcome. *J Toxicol Environ Health* **9**:377-387.

294. Zielinska-Psuja B, Lukaszyk A, Senczuk W (1979). The anti-reproductive effects of long term oral administration of cadmium on the adult male rat. *Int J Androl* **2**:150-161.