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New Aspects of Cadmium as Endocrine Disruptor

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Cadmium (Cd) is an industrial and environmental pollutant that exerts adverse effects on a number of organs in humans and animals. Reproductive organs, such as the testis and placenta, are sensitive to the toxic effects of Cd. In animal experiments, high-dose exposure to Cd induced severe testicular interstitial hemorrhage with edema, and increased incidence of fetal death and placental necrosis. Low-dose exposure to Cd affects steroid synthesis in male and female reproductive organs. In 1998, the Ministry of Environment in Japan listed Cd in the strategy plan SPEED98 as one of the chemicals suspected of having possible endocrine disrupting activity. Recently, it has been shown that Cd has potent estrogen- and androgen-like activities *in vivo* and *in vitro*, by directly binding to estrogen and androgen receptors. However, the precise mechanisms underlying the effects of Cd as an endocrine disruptor remain to be elucidated. In this review, we will discuss evidence thus far presented concerning the effects of Cd on the endocrine system.

1. Introduction

Cadmium (Cd) was discovered in 1817, but was not used commercially until the end of the 19th century. Since World War II, Cd has been used in batteries, pigments, stabilizers for plastics, electroplating, coating, and alloys.(1) The industrial use of a large amount of Cd and the disposal of waste containing Cd led to a gradual increase in Cd concentration in water, soil, and food.(2) Cd discharged in water and soil is accumulated in plants, particularly cereals. Because rice is the major staple food in Japan, long-term rice consumption is considered to be a risk factor for the toxic effects of Cd. Cigarette smoking is also a source

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of Cd exposure.[3] Each cigarette contains 1–2 μg of Cd, and 40–60% of Cd in inhaled smoke is absorbed in the pulmonary epithelium into the systemic circulation.[3] In addition, Tsuchiya and Sugita estimated the half-life of Cd in the kidney to be 12–22 years because of its low excretion rate from the body.[4,5] Therefore, it is necessary to clarify the biological effects of long-term exposure to Cd at a low concentration.

Cd has been identified as a human carcinogen by the International Agency for Research on Cancer and the US National Toxicology Program.[1,6,7] However, the precise mechanism of carcinogenesis caused by Cd remains unknown. The following are hypotheses on the carcinogenic mechanism of Cd: inhibition of DNA-repairing activity[8]; and abnormality in DNA methylation by the inhibition of DNA methylation enzymes.[9] In 1998, the Ministry of Environment in Japan listed 67 chemicals in the strategy plan called SPEED98, including three metals, namely, Cd, lead (Pb), and mercury (Hg), that have possible endocrine disrupting activity. Many researchers have investigated chemicals that mimic or block the actions of endogenous steroid hormones through their interaction with hormone receptors.[10,11] Recently, it has been reported that Cd exhibits estrogen- and androgen-like activities *in vivo* and *in vitro*, and binds to estrogen and androgen receptors.[12,13] These observations suggest a direct action of Cd-enhancing cellular responses to estrogen and androgen. Our goal in this review is to discuss evidence of the effects of Cd on the endocrine system. We start with the effects of Cd on general reproductive functions, and then describe the characteristics of Cd that mimic those of estrogen and androgen.

2. Cd Affects Reproductive Organs and Steroid Synthesis

2.1 *Male reproductive organs*

It has been reported that Cd is hazardous to testicular functions.[14–17] The initial lesions associated with Cd exposure in rodent testis include severe interstitial hemorrhage with edema. At high concentrations, Cd causes testicular necrosis. Cd-induced testicular necrosis occurs despite the fact that very little Cd accumulates in the testis.[18] The primary target of Cd in the testis has been hypothesized to be the vasculature, because similar damage can be produced by ligating the blood vessels of the testis.[19] After exposure to Cd for several weeks, testicular weight decreases and the testis becomes atrophic and calcified.[20] Furthermore, a study showed that a single low dose of Cd exposure causes spermiation failure, although no discernable testicular pathological damage is observed.[21] Moreover, Cd disrupts steroid biosynthesis in a variety of cells,[22] including rat Leydig cells.[23] Also, a low dose of Cd exposure that does not induce overt testicular atrophy significantly reduces gonadotropin-stimulated serum testosterone levels.[24] The production of testosterone may be disrupted by Cd without inducing a loss of testosterone-producing cells by necrosis.

Recently, Gunnarsson *et al.*[25] have reported that Cd strongly induces testicular prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) synthesis. In addition, their results indicated that the elevation of $PGF_{2\alpha}$ causes testosterone production inhibition, possibly via reduced expression of steroidogenic acute regulatory protein (StAR). StAR plays a role in the negative regulation of progesterone production in the corpus luteum, through the activation of phospholipase C (PLC)/PKC, which can decrease cholesterol transport from the outer to the inner mitochondrial mem-

brane.[26] Although the inhibitory effect of $PGF_{2\alpha}$ on StAR expression has not yet been established in the testis, $PGF_{2\alpha}$ has been shown to reduce StAR expression in the corpus luteum in rats.[27] Wang *et al*.[28] showed that cyclooxygenase 2 regulates steroid production mainly by influencing StAR activity. More studies are needed to establish the exact mechanism of how $PGF_{2\alpha}$ inhibits testosterone synthesis.

On the contrary, Zeng *et al*.[29] reported that serum testosterone levels were significantly increased by chronic oral Cd exposure (50, 100, 200 ppm) for three months. Although the mechanism underlying the Cd-induced increase in the serum testosterone levels is unclear, Zeng *et al*. speculated that chronic oral Cd exposure might have induced endocrine homeostasis disruption through a mechanism different from that associated with other routes of Cd administration, *e.g*., subcutaneous or intraperitoneal administration used in most studies.

Strain differences in regard to resistance to Cd-induced testicular toxicity have been found in mice. All strains of resistant mice have been shown to descend from the Bagg-albino stock, implying that susceptible strains are of the wild type, and resistant strains are of the mutant type.[30] In a resistant A/J strain, Cd transport was significantly reduced in the testis, compared with a sensitive 129/J strain.[31] The percent concentration of Cd that reached the testis in A/J mice in 60 min was less than one tenth that in 129/J mice. The transport of Cd was competitively inhibited by zinc (Zn), but not by calcium. Studies of isolated tubules and analysis of testicular fluid compartments demonstrated no significant difference in Cd uptake or efflux between the two strains. Therefore, the difference in testicular sensitivity to Cd due to strain difference appears to be related to variations of the transport system for Cd in the testicular vasculature. We reported that pretreatment with the synthetic antiandrogen cyproterone acetate reduced Cd accumulation and induced cellular tolerance to Cd in rat liver cells.[32]

Metallothionein (MT) is often associated with cellular tolerance to Cd.[33, 34] Liu *et al*.[35] studied the role of MT in Cd-induced testicular injury using MT-null and wild-type 129/SvJ mice. MT-null mice were equally as sensitive as wild-type mice to Cd-induced testicular injury as evaluated from photomicrographs of the testis and testicular hemoglobin content. Their results indicated that MT is not associated with sensitivity to Cd-induced testicular injury. In support of this notion, MT transgenic mice with MT overexpression were not refractory to Cd-induced testicular injury.[36]

Strain-dependent resistance to Cd-induced testicular injury is associated with a single major recessive gene, named Cdm, which is localized to chromosome 3.[37] Recently, Dalton *et al*. have identified Cdm as Slc39a8 encoding SLC39A8 (ZIP8), a member of the Zn transporters named ZRT-, IRT-like protein (ZIP).[38] The overexpression of Slc39a8 in cultured mouse fetal fibroblasts leads to more than a 10-fold increase in Cd influx and accumulation and a 30-fold increase in sensitivity to Cd-induced cell death. The nucleotide sequences of Slc39a8 were completely identical in two sensitive and two resistant strains of mice. Using *in situ* hybridization, they found that ZIP8 mRNA is prominently expressed in the vascular endothelial cells of the testis in the sensitive mouse strains but not in the resistant strains. Therefore, Slc39a8 is Cdm, defining sensitivity to Cd toxicity specifically in vascular endothelial cells of the testis. On the other hand, the Himeno's group[39–42]

established a Cd-resistant cell line from immortalized MT-null mouse cells, and found that these Cd-resistant cells exhibited a marked decrease in Cd uptake rate. In addition, the uptake rate of manganese (Mn) was also markedly decreased in these Cd-resistant cells. Subsequent studies of the kinetics of Cd and Mn uptakes by Cd-resistant and parental cells revealed that the Mn transport system with a high affinity for Mn is also used for cellular Cd uptake, and that this pathway is suppressed in Cd-resistant MT-null cells. It is intriguing to examine whether the Cd/Mn transporter found in MT null cells is identical to ZIP8.

2.2 *Female reproductive organs*

The gynogenesis vessel is also a vascular organ sensitive to Cd toxicity. Subcutaneous injections of Cd to pregnant rats produced a high incidence of fetal death and placental necrosis. However, the fetuses that directly received Cd *in utero* were relatively resistant to Cd toxicity, and the surviving fetuses tolerated much higher levels of Cd in tissues compared with the fetuses exposed to Cd via maternal injection. Thus, Cd-induced fetal death may not be caused by the direct effect of Cd on the fetus. It has been proposed that the placental accumulation of Cd results in trophoblastic damage, leading to a decrease in uteroplacental blood flow, and then a decrease in nutrient and oxygen transport to the fetus.[43] In humans, maternal exposure to Cd is associated with low birth weight.[44]

Recently, the effects of Cd on female steroidogenesis have been described, but results vary depending on the experimental model and the dose used. Acute exposure to Cd *in vivo* decreased serum concentrations of progesterone and estradiol depending on the reproductive stage in rats.[45] Cd exposure induced a delay in the enhancement of ovarian progesterone secretion during days 1 through 6 of pregnancy. Blood progesterone level also did not increase until day 4 of pregnancy in Cd-exposed rats.[46] In the placenta of smoking women, an increase in Cd concentration and a decrease in progesterone level were found.[47] In cultured rat granulose, luteal, and ovarian cells, Cd inhibited progesterone synthesis.[48,49] The recent studies conducted using cultured human placental trophoblastic cells suggest that Cd reduces progesterone synthesis by inhibiting the gene expression of the low-density lipoprotein (LDL) receptor, which controls the internalization of cholesterol into steroidogenic cells,[50] cytochrome P450 side chain cleavage (P450scc), which coverts cholesterol to pregnenolone, and 3β-hydroxysteroid dehydrogenase, which coverts pregnenolone to progesterone.[51] On the other hand, some reports have indicated that Cd administered to female rats during estrus and diestrus resulted in increased serum progesterone level[52,53] and stimulated progesterone synthesis in both cultured porcine granulose cells[54] and Jar choriocarcinoma cells, a malignant trophoblast cell line.[55] Henson and Chedrese discussed the dual effects of Cd on progesterone synthesis in their review.[56] They suggested that low concentrations of Cd stimulate P450scc gene transcription resulting in enhancement of the steroidogenic pathway, whereas high concentrations of Cd inhibit P450scc activity resulting in the suppression of progesterone synthesis. The effects of Cd appear to be mediated via a *cis*-acting element located 100 base pair upstream of the transcription start site of the P450scc gene.

3. Effects of Cd on Binding of Steroid Receptor to DNA

The steroid hormone receptor superfamily is a group of cytoplasmic receptors that act as a transcriptional enhancer protein, binding specifically to a hormone response element on DNA.[57] The DNA binding domain is highly conserved among different members of this superfamily. Two Zn fingers, each with a Zn atom coordinated to four cysteine residues, are found in the DNA-binding domain of these proteins. Predki and Sarkar[58] demonstrated that the Zn atom in the Zn fingers of an estrogen receptor can be replaced by several other metals such as copper (Cu), cobalt (Co), nickel (Ni), and Cd. The replacement of Zn by Cd and Co had no apparent effect on the protein binding to DNA, whereas replacement by Ni and Cu inhibited estrogen receptor binding to its response element. Thus, Cd may replace Zn without any noticeable difference in a number of physiological activities.

4. Cd Mimics Steroid Hormone Actions

4.1 *Effects of Cd on estrogen receptor*

Martin and colleagues reported conclusive evidence that Cd has a potent endocrine disrupting activity (that mimics estrogen action) in *in vivo* experiments using female rats.[12] The exposure of ovariectomized rats to Cd increased uterine weight and promoted the growth and development of the mammary glands. In the case of the uterus, the weight increase was accompanied by endometrial proliferation, induction of progesterone receptor, and enhanced expression of the complement component C3 gene. Cd increased epithelium density and induced milk protein formation in the mammary glands. These effects were similarly observed in 17β-estradiol exposure, and the effects of Cd were suppressed in an antiestrogen, ICI-182780. Moreover, *in utero* exposure to Cd affected mammary gland development, induced earlier onset of puberty, and increased the number of terminal end buds in female offsprings, which are typical responses to endocrine disruptors.

Martin and colleagues previously reported that Cd exhibited estrogen activity through an estrogen receptor α (ERα) in MCF-7 breast cancer cells.[59] They performed several experiments to test the hypothesis that Cd may exert its effects through ERα. Cd induced increases in the amount of progesterone receptor (PgR) protein and the expression of pS2, an estrogen-inducible gene, in MCF-7 cells, and these effects were completely blocked by the anti-estrogen. Treatment with Cd of the ERα-negative breast cancer cell line transducted with an ERα gene caused increases in the mRNA levels of PgR and pS2. The same cell line transducted with antisense ER did not show any effects of Cd on the mRNA levels of PgR and pS2. Cd also stimulated the expression of a reporter gene containing a consensus estrogen response element. On the other hand, Cd did not modify the binding of estradiol to ERα. These results suggest that the effects of Cd are mediated directly by ERα and are independent of estradiol.

4.2 *Effects of Cd on androgen receptor*

Martin and colleagues tested whether Cd also mimics androgen by activating the androgen receptor (AR). In LNCap cells, a hormone-dependent human prostate cancer cell line, Cd exhibited androgen-like effects on cell growth and gene expression.[13] Because treatment with an antiandrogen blocked these effects, it is suggested that the effects of Cd are mediated though AR. In addition, it was shown that Cd can bind to a hormone-binding domain of AR with high affinity and inhibit the endogenous hormone binding to the receptor. In castrated rats and mice, Cd showed androgenic effects on the weights of the prostate gland and seminal vesicle complex, and induced the expression of androgen-regulated genes. A single dose of Cd induced 1.6- and 1.4-fold increases in the weights of the prostate gland and seminal vesicle, respectively, whereas two doses of Cd induced 1.97- and 1.65-fold increases, respectively. The *in vivo* effects of Cd were also blocked by an antiandrogen, suggesting that Cd may activate AR in the prostate gland. However, the *in vivo* androgen-like effects of Cd (10 or 20 μg $CdCl_2$/kg body weight) are weak compared with the estrogen-like effects of Cd (0.5 or 5 μg $CdCl_2$/kg body weight).[12] More detailed research is necessary to clarify this difference.

Regarding ERα activation by Cd, Martin and colleagues have shown that Cd binds to ERα with amino acid residues located on helices H4, H8, and H11, and at the interface of the loop and H12,[60] suggesting that Cd may reposition H12 in a manner similar to estradiol. Many of the amino acid residues of ERα identified as important in the interaction with Cd are conserved in the hormone binding domain of AR.[61,62] Therefore, it seems likely that Cd activates AR by a mechanism similar to that for ERα. However, further studies are necessary to define the precise mechanism by which Cd activates ERα and AR. Thus far, only Martin and colleagues have reported that Cd can mimic androgen and estrogen *in vitro* and *in vivo*. To the best of our knowledge, no other scientists have confirmed these findings.

5. Conclusions

Several lines of evidence have indicated that low dose Cd exposure affects the action of steroid hormones in the reproductive organs of both males and females. First, Cd disrupts steroidogenesis including the syntheses of androgen, progesterone and estrogen, leading to the suppression of reproductive functions. Second, Cd exhibits estrogen- or androgen-like activities *in vivo* and *in vitro*, through direct binding to AR and ERα. It is possible that Cd plays different roles in the inhibition and/or stimulation of steroid hormonal activity. The effects of Cd on the reproductive organs may vary depending on the dose and treatment method as well as animal age and status, i.e., embryonic age and pregnancy, respectively. Martin and colleagues[12] demonstrated that Cd is a typical endocrine disruptor. *In utero* exposure to Cd has profound effects on the mammary gland and on the growth and development of infants, suggesting that exposure to Cd may be a potential risk factor for breast cancer.[12] Further studies are required to substantiate these findings in other experimental models, and in humans.

References

1 International Agency for Research on Cancer (1993): Beryllium, Cd, mercury, and exposure in the glass manufacturing industry. In: International Agency for Research on Cancer Monographs on the Evaluation of the Carcinogenic Risks to Humans. Vol. 58, IARC Scientific Publications, Lyon.

2 Landis, W.G. and Yu, M-H. (1999): Routes of exposure and modes of action. In: *Introduction to Environmental Toxicology. Impacts of Chemicals upon Ecological System. 2nd ed.* Lewis Publishers, Boca Raton, F.L., pp.93–130.

3 ATSDR (1999): Toxicological Profile for Cd. Agency for Toxic Substances and Disease Research, Atlanta.

4 Tsuchiya, K. and Sugita, M. (1971): A mathematical model for deriving the biological half-life of a chemical. *Nord. Hyg. Tidskr.* **52**: 105–110.

5 Sugita, M. and Tsuchiya, K. (1995): Estimation of variation among individuals of biological half-time of Cd calculated from accumulation data. *Environ. Res.* **68**: 31–37.

6 National Toxicology Program (2000): Ninth Report on Carcinogens. National Toxicology Program, Research Triangle Park (NC).

7 Waalkes, M.P. (2002): Metal carcinogenesis. In: B. Sarkar Eds.: *Handbook of Heavy Metals in the Environment*. Marcel Dekker, New York, N.Y., pp.121-146.

8 Jin, Y.H., Clark, A.B., Slebos, R.J., Al-Refai, H., Taylor, J.A., Kunkel, T.A., Resnick, M.A. and Gordenin, D.A. (2003): Cd is a mutagen that acts by inhibiting mismatch repair. *Nat. Genet.* **34**: 326–329.

9 Takiguchi, M., Achanzar, W.E., Qu, W., Li, G. and Waalkes, M.P. (2003): Effects of Cd on DNA-(Cytosine-5) methyltransferase activity and DNA methylation status during Cd-induced cellular transformation. Exp. Cell Res. 286:355-365.

10 Borgeest, C., Greenfeld, C., Tomic, D. and Flaws, J.A. (2002): The effects of endocrine disrupting chemicals on the ovary. *Front Biosci.* **7**: 1941–1948.

11 McLachlan, J.A. (2001): Environmental signaling: What embryos and evolution teach us about endocrine disrupting chemicals. *Endocr. Rev.* **22**: 319–341.

12 Johnson, M.D., Kenney, N., Stoica, A., Hilakivi-Clarke, L., Singh, B., Chepko, G., Clarke, R., Sholler, P.F., Lirio, A.A., Foss, C., Reiter, R., Trock, B., Paik, S. and Martin, M.B.(2003): Cd mimics the *in vivo* effects of estrogen in the uterus and mammary gland. *Nat. Med.* **9**: 1081–1084.

13 Martin, M.B., Voeller, H.J., Gelmann, E.P., Lu, J., Stoica, E.G., Hebert, E.J., Reiter, R., Singh, B., Danielsen, M., Pentecost, E. and Stoica, A.(2002): Role of Cd in the regulation of AR gene expression and activity. *Endocrinology* **143**: 263–75.

14 Parizek, J. and Zahor, A. (1965): Effects of Cd salts on testicular tissue. *Nature* **177**: 1036–1037.

15 Kotsonis, F.N. and Klaaseen, C.D. (1977): Toxicity and distribution of Cd administered to rats at sublethal doses. *Toxicol. Appl. Pharmacol.* **41**: 667–680.

16 Laskey, J.W. and Phelps, P.V. (1991): Effect of Cd and other metal cations *in vitro* Leydig cell testosterone production. *Toxicol. Appl. Pharmacol.* **108**: 296–306.

17 Shiraishi, N. and Waalkes, M.P. (1996): Acquired tolerance to Cd-induced toxicity in the rodent testes. *Toxic. Subst. Mech.* **15**: 27–42.

18 Gun, S.A. and Gould, T.C. (1970): Cd and other mineral elements. In: A.D. Johnson *et al.* Eds.: The Testis. Vol. III Influencing Factors. Academic Press, New York, N.Y., pp.377–481.

19 Nolan, C.V. and Shaikh, Z.A. (1986): The vascular endothelium as a target tissue in acute Cd toxicity. *Life Sci.* **39**: 1403–1409.

20 Elindaer C-G. (1986): Other toxic effects. In: L. Friberg *et al.* Eds. : Cd and Health: A Toxicological and Epidemiological Appraisal. Vol. I CRC Press, Boca Raton, F.L., pp. 159-204.

21 Hew, K.W., Ericson, W.A. and Welsh, M.J. (1993): Single low Cd dose causes failure of spermiation in the rat. *Toxicol. Appl. Pharmacol.* **121**: 15–21.
22 Pasky , K., Varga, B. and Lazar, P. (1992): Cd interferes with steroid biosynthesis in rat granulose and luteal cells *in vitro*. *BioMetals* **5**: 245–250.
23 Phelps, P.V. and Laskey, J.W. (1989): Comparison of age-related changes in *in vivo* and *in vitro* measures of testicular steroidogenesis after acute Cd exposure in the Sprague-Dawley rat. J. *Toxicol. Environ. Health* **27**: 95–105.
24 Laskey, J.W., Rehnberg, G.L., Laws, S.C. and Hein, J.F. (1984): Reproductive effects of low acute doses of Cd chloride in adult male rats. *Toxicol. Appl. Pharmacol.* **73**: 250–255.
25 Gunnarsson, D., Svensson, M., Selstam, G. and Nordberg, G. (2004): Pronounced induction of testicular PGF (2 alpha) and suppression of testosterone by Cd-prevention by Zn. Toxicology. **200**: 49–58.
26 Niswender, G.D., Juengel, J.L., Silva, P.J., Rollyson, M.K. and McIntush, E.W. (2000): Mechanisms controlling the function and life span of the corpus luteum. *Physiol. Rev.* **80**: 1–29.
27 Fiedler, E.P., Plouffe, L.Jr., Hales, D.B., Hales, K.H. and Khan, I. (1999): Prostaglandin F (2 alpha) induces a rapid decline in progesterone production and steroidogenic acute regulatory protein expression in isolated rat corpus luteum without altering messenger ribonucleic acid expression. *Biol. Reprod.* **61**:643–650.
28 Wang, X., Dyson, M.T., Jo, Y. and Stocco, D.M. (2003): Inhibition of cyclooxygenase-2 activity enhances steroidogenesis and steroidogenic acute regulatory gene expression in MA-10 mouse Leydig cells. *Endocrinology* **144**: 3368–3375.
29 Zeng, X., Jin, T., Ahou, Y. and Nordberg, G.F. (2003): Changes of serum sex hormone levels and MT mRNA expression in rats orally exposed to Cd. *Toxicology* **186**:109–118.
30 Taylor, B.A., Heiniger, H.J. and Meier, H. (1973): Genetic analysis of resistance to Cd-induced testicular damage in mice. *Proc. Soc. Exp. Biol. Med.* **143**: 629–633.
31 King, L.M., Banks, W.A. and George, W.J. (1999): Differences in Cd transport to the testis, epididymis, and brain in Cd-sensitive and -resistant murine strains 129/J and A/J. *J. Pharmacol. Exp. Ther.* **289**: 825–830.
32 Takiguchi, M., Cherrington, N.J., Hartley, D.P., Klaassen, C.D. and Waalkes, M.P. (2001): Cyproterone acetate induces a cellular tolerance to Cd in rat liver epithelial cells involving reduced Cd accumulation. *Toxicology* **165**: 13–25.
33 Rugstad, N.E. and Norseth, T. (1975): Cd resistance and content of Cd-binding protein in cultured human cells. *Nature* **257**:136–137.
34 Waalkes, M.P. and Goering, P.L. (1990): Metallothionein and other Cd-binding proteins: recent developments. *Chem. Res. Toxicol.* **3**: 281–288.
35 Liu, J., Corton, C., Dix, D.J., Liu, Y., Waalkes, M.P. and Klaassen, C.D. (2001): Genetic background but not metallothionein phenotype dictates sensitivity to Cd-induced testicular injury in mice. *Toxicol. Appl. Pharmacol.* **176**: 1–9.
36 Dalton, T., Fu, K., Enders, G.C., Palmiter, R.D. and Andrews, G.K. (1996): Analysis of the effects of overexpression of metallothionein-I in transgenic mice on the reproductive toxicology of Cd. *Environ. Health Perspect.* **104**: 68–76.
37 Dalton, T.P., Miller, M.L., Wu, X., Menon, A., Cianciolo, E., McKinnon, R.A., Smith, P.W., Robinson, L.J. and Nebert, D.W. (2000): Refining the mouse chromosomal location of Cdm, the major gene associated with susceptibility to Cd-induced testicular necrosis. *Pharmacogenetics* **10**:141–151.
38 Dalton, T.P., He, L., Wang, B., Miller, M.L., Jin, L., Stringer, K.F., Chang, X., Baxter, C.S. and Nebert, D.W. (2005): Identification of mouse SLC39A8 as the transporter responsible for Cd-induced toxicity in the testis. *Proc. Natl. Acad. Sci. U. S. A.* **102**:3401–3406.

39 Yanagiya, T., Imura, N., Kondo, Y. and Himeno, S. (1999): Reduced uptake and enhanced release of Cd in Cd-resistant metallothionein null fibroblasts. *Life Sci*. **65**: PL177–182.
40 Yanagiya, T., Imura, N., Enomoto, S., Kondo, Y. and Himeno, S. (2000): Suppression of a high-affinity transport system for manganese in Cd-resistant metallothionein-null cells. *J. Pharmacol. Exp. Ther*. **292**:1080–1086.
41 Himeno, S., Yanagiya, T., Enomoto, S., Kondo, Y. and Imura, N. (2002): Cellular Cd uptake mediated by the transport system for manganese. *Tohoku J. Exp. Med*. **196**:43–50.
42 Himeno, S. (2002): Application of metallothionein null cells to investigation of Cd transport. *J. Inorg. Biochem*. **88**: 207–212.
43 Levin, A.A., Plautz, J.R., di Sant'Agnese, P.A. and Miller, R.K. (1981): Cd: placental mechanisms of fetal toxicity. *Placenta Suppl*. **3**: 303–318.
44 Frery, N., Nessmann, C., Girard, F., Lafond, J., Moreau, T., Blot, P., Lellouch, J. and Huel, G. (1993): Environmental exposure to Cd and human birthweight. *Toxicology* **179**:109–118.
45 Piasek, M., Schonwald, N., Blanusa, M., Kostial, K.and Laskey, J.W. (1996): Biomarkers of heavy metal reproductive effects and interaction with essential elements in experimental studies on female rats. *Arh. Hig. Rada. Toksikol*. **47**: 245–259.
46 Paksy, K., Varga, B., Naray, M., Olajos, F. and Folly, G. (1992): Altered ovarian progesterone secretion induced by Cd fails to interfere with embryo transport in the oviduct of the rat. Reprod. Toxicol. **6**: 77–83.
47 Piasek, M., Blanusa, M., Kostial, K. and Laskey, J.W. (2001): Placental Cd and progesterone concentrations in cigarette smokers. *Reprod. Toxicol*. **15**: 673–681.
48 Paksy, K., Varga, B. and Lazar, P. (1992): Cd interferes with steroid biosynthesis in rat granulosa and luteal cells *in vitro*. *BioMetals* **5**: 245–250.
49 Piasek, M. and Laskey, J.W. (1999): Effects of *in vitro* Cd exposure on ovarian steroidogenesis in rats. *J. Appl. Toxicol*. **19**: 211–217.
50 Jolibois, L.S.Jr., Burow, M.E., Swan, K.F., George, W.J., Anderson, M.B. and Henson, M.C. (1999): Effects of Cd cell viability, trophoblastic development, and expression of low density lipoprotein receptor transcripts in cultured human placental cells. *Reprod. Toxicol*. **13**: 473–480.
51 Kawai, M., Swan, K.F., Green, A.E., Edwards, D.E., Anderson, M.B. and Henson, M.C. (2002): Placental endocrine disruption induced by Cd: effects on P450 cholesterol side-chain cleavage and 3beta-hydroxysteroid dehydrogenase enzymes in cultured human trophoblasts. *Biol. Reprod*. **67**: 178–183.
52 Piasek, M. and Laskey, J.W. (1994): Acute Cd exposure and ovarian steroidogenesis in cycling and pregnant rats. *Reprod. Toxicol*. **8**: 495–507.
53 Paksy, K., Varga, B. and Lazar P. (1997): Zn protection against Cd-induced infertility in female rats. Effect of Zn and Cd on the progesterone production of cultured granulosa cells. *BioMetals* **10**: 27–35.
54 Varga, B., Zsolnai, B., Paksy, K., Naray, M. and Ungvary, G. (1993): Age dependent accumulation of Cd in the human ovary. *Reprod. Toxicol*. **7**: 225–228.
55 Powlin, S.S., Keng, P.C. and Miller, R.K. (1997): Toxicity of Cd in human trophoblast cells (JAr choriocarcinoma): role of calmodulin and the calmodulin inhibitor, zaldaride maleate. *Toxicol. Appl. Pharmacol*. **144**: 225–234.
56 Henson, M.C. and Chedrese, P.J. (2004): Endocrine disruption by Cd, a common environmental toxicant with paradoxical effects on reproduction. *Exp. Biol. Med. (Maywood)* **229**:383–392.
57 Evans, R.M.(1988): The steroid and thyroid hormone receptor superfamily. *Science* **240**: 889–895.
58 Predki, P.F. and Sarkar, B. (1992): Effect of replacement of "Zn finger" Zn on estrogen receptor DNA interactions. *J. Biol. Chem*. **267**: 5842–5846.

59 Garcia-Morales, P., Saceda, M., Kenney, N., Kim, N., Salomon, D.S., Gottardis, M.M., Solomon, H.B., Sholler, P.F., Jordan, V.C. and Martin, M.B. (1994): Effect of Cd on estrogen receptor levels and estrogen-induced responses in human breast cancer cells. *J. Biol. Chem.* **269**: 16896–16901.

60 Stoica, A., Katzenellenbogen, B.S. and Martin, M.B. (2000): Activation of estrogen receptor-alpha by the heavy metal Cd. *Mol. Endocrinol.* **14**: 545–553.

61 Matias, P.M., Donner, P., Coelho, R., Thomaz, M., Peixoto, C., Macedo, S., Otto, N., Joschko, S., Scholz, P., Wegg, A., Basler, S., Schafer, M., Egner, U. and Carrondo, M.A. (2000): Structural evidence for ligand specificity in the binding domain of the human androgen receptor. Implications for pathogenic gene mutations. *J. Biol. Chem.* **275**: 26164–26171.

62 Poujol, N., Wurtz, J.M., Tahiri, B., Lumbroso, S., Nicolas, J.C., Moras, D. and Sultan, C. (2000): Specific recognition of androgens by their nuclear receptor. A structure-function study. *J. Biol. Chem.* **275**: 24022–24031.