[Environmental Health and Preventive Medicine 4, 174-183, January, 2000]

Review

The Endocrine Disruptive Effects of Mercury

Xinqiang ZHU*[1,2], Yukinori KUSAKA*[1], Kazuhiro SATO*[1] and Qunwei ZHANG*[1]

*[1] *Department of Environmental Health, School of Medicine, Fukui Medical University, Fukui*
*[2] *Department of Toxicology, School of Public Health, Medical College, Zhejiang University P. R. China*

Abstract

Mercury, identified thousands of years ago is one of the oldest toxicants known. The endocrine disruptive effects of mercury have recently become one of the major public concerns. In this report, the adverse effects of mercury on the hypothalamus, pituitary, thyroid, adrenal gland, and gonads (testis and ovary) in laboratory animals as well as in humans are reviewed. The effects of both environmental and occupational exposures to organic, inorganic, or metallic mercury are explained. There is sufficient evidence from animal studies supporting the disruptive effects of mercurials on the functions of the thyroid, adrenal, ovary, and testis, although several factors make it difficult to extrapolate the animal data to the human situation. However, the human studies performed so far, which focused mainly on serum hormone levels, failed to provide any conclusive data to confirm the findings from the animal studies. Therefore, further well-designed epidemiological studies are urgently needed. The possible mechanisms of the toxic effects are also discussed. The broad enzyme inhibition and the influence on the combining of hormones by their receptors, which seem due to its avid binding to sulphydryl, may account for the primary mechanism. The interference with intracellular calcium metabolism, and peroxidation may also be involved.

Key words: mercury, hormone, pituitary/thyroid/adrenal gland, ovary, testis

Introduction

The endocrine system is one of the three important integrating and regulatory systems in the human body. The other two are the nervous and immune systems. The major endocrine glands include the hypothalamus, pituitary, thyroid, parathyroid, adrenal gland, pancreas, and gonads (ovary and testis). Hormones are natural secretory products of the endocrine glands and travel via the blood to exert their effects at distant target tissues or organs by binding to specific cell surfaces or nuclear receptors. They are responsible for the maintenance of homeostasis, reproduction, development, and behavior.

An endocrine disruptor is broadly defined as an exogenous agent, either synthetic or natural, that interferes with the synthesis, storage, release, transport, metabolism, binding, action, or elimination of natural hormones in the body. Investigators have expressed their concern over the estrogenic effects of environmental chemicals for nearly 30 years[1-4]. In the 1990s, this concern has become intensified, and focused not only on environmental estrogens but also on any agents that pose adverse effects on any aspects of the entire endocrine system[5-8]. There have been hundreds of chemicals proved or suspected of having endocrine disruptive effects. They include natural products (i.e., phytoestrogens), pharmaceuticals (i.e., diethylstilbestrol, ethynyl estradiol), industrial chemicals (i.e., alkylphenols, bisphenol A), and environmental pollutants (i.e., DDT, PCBs, dioxin, and PAHs). It has been reported that the endocrine disruptors may have a variety of adverse biological effects on human health as well as on aquatic life or wildlife. They involve hormone dependent human cancers (breast, testicular and prostate cancers), abnormal sexual development, reduced male fertility, alteration in pituitary and thyroid functions, immune suppression, and neurobehavioral abnormalities. This evidence has drawn great attention from scientists, administrators as well as the public.

Among the numerous endocrine disruptors, the persistent environmental contaminants such as organochlorine pesticides, particularly DDT and its metabolites, polychlorinated biphenyls (PCBs), dioxins, and polyaromatic hydrocarbons have been given preferential concern and have been intensively investigated. More recently, increased attention has been focused on nonyl-phenol, bisphenol A, and phthalates for their wide usage. Heavy metals such as lead, cadmium, and mercury are also reported to have an endocrine disruptive potential. Therefore, they are also on the list of "known & suspected hormone disruptors" published by The

Received Aug. 30 1999/Accepted Nov. 13 1999
Reprint requests to: Xinqiang ZHU
Department of Environmental Health, School of Medicine, Fukui Medical University, Matsuoka-cho, Fukui 910-1193, Japan.
TEL: +81-776-618336 FAX: +81-776-618107
E-mail: Zhux@fmsrsa.fukui-med.ac.jp

World Wildlife Fund[9].

There have been several reviews on the endocrine disruptive effects of lead and cadmium but none on mercury. This review focuses on the reports of the adverse effects of mercury in which the common theme of endocrine disruption has been hypothesized, suspected or demonstrated. Available literature was searched for on Medline with the key word mercury combined with toxicity, hormone, endocrine, hypothalamus, pituitary, thyroid, adrenal, gonad, testis, and ovary, respectively. Other articles presented in the reference lists of those selected papers were also screened and, if they were useful, adopted. Both environmental and occupational exposure to organic or inorganic mercury was included. All the pathological, physiological or biochemical changes caused by mercury in all the endocrine organs are discussed if they were mentioned in the original papers. This report puts forward an overview of the current state of science relative to endocrine disruption in humans, wildlife, and laboratory animals, but is not intended to be an exhaustive review in general.

Mercury identified thousands of years ago is one of the oldest toxicants known. There are three main chemical forms of mercury: (1) organic mercury, used as fungicides, herbicides, and wood preservatives; (2) inorganic mercury, for antiseptic and dermatological preparations; and (3) elemental mercury, used in the production of batteries, thermometers, and fluorescent tubes. The exposure routes of mercury include the ingestion of food such as fish and seafood, dermal absorption, and inhalation. In addition, amalgam tooth fillings were identified as the major source of mercury contributing to the body burden in humans without occupational exposure[10]. It is well known that excessive exposure to mercury results in neurotoxic and nephrotoxic disorders. Moreover, mercury taken into the body is distributed and deposited in the endocrine glands as well as in the liver, kidney, brain, and other tissues of humans and laboratory animals[11-14]. Thus, its latent effects on the endocrine system are also becoming a major concern. However, reports with the topic of endocrine disruptive effects of mercury are much fewer than those dealing with lead and cadmium. Therefore, we had to base our study on these relatively limited data for this review.

The effects on the hypothalamus

The hypothalamus and upper central nervous system are the top controllers and regulators of the hypothalamus-pituitary-target gland (thyroid, adrenal gland, testis, and ovary) axis, and are simultaneously regulated by the lower glands as feedback.

Exposure to mercuric chloride, methylmercuric chloride, or mercury vapor resulted in mercury deposits in the hypothalamus of rats[15, 16]. Methyl mercury was demethylated in the brain especially in the thalamus and pituitary where inorganic mercury increased to a higher extent and was eliminated considerably more slowly than in other brain sites[17]. Nevertheless, there are very few reports concerning the adverse effects of mercury on the hypothalamus in laboratory animals, and no reports concerning the human hypothalamus.

More than twenty years ago, Lamperti and Niewenhuis reported ultrastructural changes found in the neurons of the arcuate nucleus of female hamsters exposed to 1 mg of mercuric chloride daily during one estrous cycle. There was a significantly higher concentration of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the pituitaries of the mercury treated animals during certain periods of the estrous cycle. However the plasma levels of FSH and LH were not interfered[18]. A single injection of mercuric citrate resulted in a change in the circadian rhythm of the cytoplasmic RNA content and caused a general reduction of the RNA content in the hypothalamic neurons of mice[19]. It is well known that the hypothalamic hormones are released in a pulsatile manner, and isolated anterior pituitary target cells respond to pulsatile administration of these hormones better than to continuous exposure. Therefore, the alteration of the circadian rhythm of the cytoplasmic RNA content might imply an impairment of hypothalamic function. In addition, mercury was reported to inhibit the specific binding of luteinizing hormone-releasing hormone (LHRH) to the hypothalamic membrane[20].

Up to the present, no epidemiological studies concerning human hypothalamic endocrine function have been reported.

The effects on pituitary

The anterior pituitary, under the control of hypothalamic hormones, secretes a number of hormones that regulate the growth and function of other endocrine glands. The posterior pituitary hormones regulate water balance and milk ejection from the lactating mammary gland.

Mercury was found to be deposited in the pituitaries of laboratory animals and humans after exposure to mercury by various routes[13, 21-25]. The amount of mercury deposits increased significantly in the pituitaries of adult rats exposed to methyl mercury at 20mg/l in drinking water[22]. Mercury was found to be present throughout the anterior pituitary. The lateral part of the gland contained significantly more mercury than the central part of the gland did. Intracellular accumulation of mercury was ultrastructurally localized in the lysosomes and granules of secretory cells, such as somatotrophs, thyrotrophs, and corticotrophs. While, apart from the vacuolation of lysosomes, no other structural damages were observed in the cells containing mercury[22].

Nylander reported that mercury concentrations in the pituitary glands of three deceased dentists were significantly higher than those in the controls (135-349 Vs 5-97 ng/g wet weight)[13]. However, there have not been any reports of definite pituitary dysfunction related to environmental or occupational exposure to mercury. Langworth et al reported that in a group of dental personnel exposed to mercury vapour, neither basal serum levels of thyroid-stimulating hormone (TSH), prolactin (PRL), thyroxine (T_4), triiodothyronine (T_3), and cortisol, nor the TSH and PRL responses to thyrotrophin-releasing hormone (TRH) were significantly different from those in the control group[27]. In the same year, Erfurth et al got the same result[28]. They investigated a group of workers comprised 11 male workers with a mean exposure time of 4.5 years, a mean urinary mercury concentration of 26 nmol/mmol creatinine, and the blood mercury concentrations ranging from 70-170 nmol/l. Similarly, both the basal serum concentrations of pituitary hormones TSH, PRL, FSH, LH and their response after administration with thyrotrophin and gonadotrophin showed no difference between the exposed group and the control group[28]. These two epidemiological studies have the same limitation in results due to the small size of samples with only 11 exposed workers in each.

Later on, McGregor and Mason carried out a study in a well-characterized population occupationally exposed to mercury vapour[29]. Twenty-eight workers with urine mercury levels greater than 40 nmol/mmol creatinine were selected as an exposure

group. Pituitary hormones of FSH, LH, TSH and PRL were measured as indices for pituitary function. Again, no relationships between any of the above hormones and blood or urine mercury levels, and the length of occupational exposure were found.

More recently, a somewhat larger cross sectional study was carried out by Barregard et al [30]. They examined 41 male chloralkali workers exposed to mercury vapour and the same number of occupationally unexposed age-matched control from the same company. The chloralkali workers had mean blood and urinary mercury concentrations of 46 nmol/l and 15 nmol/mmol creatinine, respectively. The results showed that TSH and PRL concentrations were not significantly associated with the exposure levels. In this study the blood and urinary mercury concentrations reflecting recent and longer-term exposure to mercury, respectively, were not very high. This limitation may make the result fail to be very convictive. However, it should be noted in McGregor and Mason's study, that the body burden of mercury in exposed workers was quite high with a mean blood mercury concentration higher than 130 nmol/l, and a mean urine mercury concentration higher than 60 nmol/mmol creatinine. Therefore, the negative result indicates that the usual environmental or occupational exposure to mercury at present seems to have no evident adverse effect on the pituitary endocrine function.

The effects on thyroid

The thyroid gland produces two iodoamino acid hormones, 3,5,3'-triiodothyronine (T_3) and 3,5,3',5',-tetraiodothyronine (thyroxine, T_4), which have long been recognized for their importance in regulating systemic metabolism, development, and tissue differentiation.

A large amount of mercury was found in the human thyroid and pituitary following occupational exposure [11, 26]. The mercury concentrations in these two endocrine organs were 3-4 times higher than that in the kidney and more than 10 times that in the liver [11]. This preferential deposit may account for the high affinity of mercury to the thyroid gland.

Ghosh and Bhattacharya reported the direct thyrotoxicity of mercury on rabbits [31]. Rabbits were administrated intramuscularly with 20 mg/kg of mercuric chloride. Within 24 hours of the administration, the thyroid peroxidase activity increased significantly over the control group with a concomitant rise in the T_3 level. Simultaneously, there was a remarkable fall in the T_4 level, and thus a high T_3/T_4 ratio. The results suggested that acute mercury lethality would induce immediate hyperthyroidism, and that the T_3-toxicosis may be caused by a preferential synthesis of T_3 and/or preferential deiodination of T_4 to T_3. Goldman and Blackburn also reported that oral administration with 2.5 mg of mercuric chloride to the rats for 40 days resulted in enlargement of the thyroid organ, increased iodide uptake, elevated protein bound iodine, and caused a coupling defect in the synthesis of T_3, but not T_4 [32].

Kawada et al examined the acute toxic effects of methylmercuric chloride and mercuric chloride in mice [33]. Some results in this study contracted those in Goldman and Blackburn's report. Mice injected intraperitonealy with 5 μ g/g body weight of methylmercuric chloride or mercuric chloride for two consecutive days showed a reduced, rather than increased, uptake of ^{131}I by the thyroids. The serum bound ^{131}I-iodide was also reduced in both of the mercury-treated groups. And no increased thyroid weight was found. These differences may be partially ascribed to the exposure periods of mercurials. Animals may have developed a tolerance for mercuric chloride after a long-term exposure in Goldman and Blackburn's study [32]. It is interesting that Kawada et al also found that the mercurials caused a coupling defect in the synthesis of iodothyronines that was consistent with Goldman and Blackburn's finding [32, 33]. Blood thyroxine level was measured by radioimmuno-assay using a RIA-Mat T_4 kit. Both mercurials reduced the serum T_4 levels. The reduction was more significant with the inorganic mercurial than with the organic mercurial [33].

Subsequently, these researchers examined the subacute toxicity of methylmercuric chloride and mercuric chloride on mouse thyroid [34]. Male ddY mice were given 50, 100, and 150 μ g/day of either mercurial in separate groups in drinking water for a month. Body weight and thyroid organ weight were not significantly different in the 50 or 100 μ g/d groups compared to the controls. Whereas, in the 150 μ g/d group, the body weight reduced dramatically after methylmercuric chloride but not after mercuric chloride treatment. It is not clear whether the growth inhibition was due to the general toxicity of organic mercury or an endocrine factor was involved, because the growth hormone was not measured in this study. In the highest exposure group, both treated with organic and inorganic mercurial, thyroid organ weights were also found to be significantly decreased, rather than increased [34]. The distribution measurement of isotopically labelled mercurials showed that the mercurials suppressed the radioiodide incorporation into the iodothyronine fraction, but not into the iodotyrosine fraction, indicating that mercurials inhibited the coupling process but did not interfere with the organification of iodide [34]. Serum T_4 but not T_3 level was affected by mercurials, suggesting that thyroidal secretion of T_4 was inhibited, but the peripheral conversion of T_4 to T_3 might not be affected [34]. However, Chopra previously reported that a higher concentration of mercurial did inhibit extrathyroidal conversion of T_4 to T_3 in vitro [35]. Apparently, it can not be extrapolated to the status of low exposure in vivo.

Sin et al reported that mice were orally treated with mercuric chloride or mercuric sulphide in a dose of 6 μ g Hg^{2+}/g body weight for 10 days. The result showed that both serum T_4 and T_3 decreased significantly in the mercuric chloride treated group. In the mercuric sulphide treated group, however, only serum T_3 decreased, while T_4 remain unchanged [36]. It is interesting that prolonged treatment of the same dose of mercuric sulphide for 4 weeks induced a decrease in T_4 but not in T_3 [37]. This was in contrast to the status of thyroid hormones at the end of the 10-day treatment [36]. The results of these studies revealed that the effects of mercury on the thyroid might vary according to the variation of dose, route, and period of administration as well as the species of laboratory animals and the chemical forms of mercury. A single high dose of parenteral administration of mercurial is not likely to reflect the status of actual human exposure to these chemicals. Thus studies with chronic oral administration at a low dose are more meaningful and believable in determining the thyrotoxicy of mercury.

According to the limited existing epidemiological studies, the present occupational exposure to mercurials seems not to have had an obvious adverse effect on the function of the human thyroid [27-29], though a series of laboratory animal studies did show some morphological and / or functional injuries of the thyroids [31-37]. Exceptionally, in Barregard and colleague's study, a slight, but significant, increase in serum free T_4 concentration

and in the ratio free T_4/free T_3 was found. Also serum free T_3 was inversely associated with a cumulative mercury exposure [30]. The authors suggested that this indicate a possible inhibition of mercury on 5'-deiodinase, which is responsible for the conversion of T_4 to T_3. It is regrettable that the 5'-deiodinase activities were not measured simultaneously. The effects of long-term moderate exposure to mercury on human serum thyroxine waits to be further affirmed.

The effects on adrenal gland

The adrenal gland is comprised of two morphologically and functionally different parts, the cortex and medulla. The adrenal cortex synthesizes dozens of different steroid molecules, but only a few of them have biological activities. These are sorted into three classes of hormones: glucocorticoids, mineralocorticoids, and androgens. These hormones initiate their actions by combining with specific intracellular receptors. The adrenal medulla, under the control of sympathetic and parasympathetic nerves, produces catecholamine hormones: dopamine, norepinephrine, and epinephrine. The functional status of the pituitary-adrenal axis is usually estimated by the combination of several parameters: plasma corticosterone concentrations, adrenal secretory capacity in vitro, and the capacity of the liver to metabolize corticosterone in vitro. The cortisol response to stress or to adrenocorticotropic hormone (ACTH) stimulation is also a useful index.

The adrenal gland contained almost the same concentration of mercury as the pituitary or thyroid did in mice administrated, by gavage, with 1 μ Ci/day/animal of either isotopically labeled methylmercuric chloride or mercuric chloride for 30 days [33]. The localization of mercury in the adrenals of the rats exposed to 20 mg/l methylmercury in drinking water was reported by Rasmussen and Thorlacius-Ussing [38]. Accumulations were often observed in both the zona glomerulosa and reticularis. Both epinephrenic and norepinephrenic cells contained mercury deposits. A slight increase in the amount of deposits in chromaffin cells was observed with increasing exposure time. Ultrastructural deposits were found in the lysosomes of cortical cells and in both the lysosomes and the secretory granules of chromaffin cells [38]. A regionalized deposit pattern was observed in the adrenal [39]. After inhalation, metallic mercury is oxidized by catalase-H_2O_2 complex to the ionic form (Hg^{2+}) and accumulates in a number of endocrine organs such as adrenal cortex, thyroid, corpora lutea of the ovaries, and interstitial tissues of the testes, as well as in the lung, liver, and other organs of rats and monkeys. The liver and adrenal seem to have a reserve capacity to oxidized HgO. It should be noted that ethyl alcohol, which is a known catalase inhibitor, can depress oxidation and retention of metallic mercury in most organs and in the whole body and thus increase the blood concentration of HgO. This then causes an increase in the retention of mercury in the liver and adrenal cells [39].

The effects of mercury on the adrenal gland were demonstrated in several animal models. Thaxton et al [40] reported that mercury administered to young chickens via drinking water caused a depressed growth, an increased mortality rate, and inhibited the normal maturation of the adrenal glands. Deficiencies of cholesterol and corticosterone were found in the adrenal. Also the supplement of exogenous corticosterone alleviated, in part, the toxic effects of mercury [40]. Kirubagaran and Joy reported that the adrenocortical-pituitary activity of catfish was impaired by mercury exposure [41]. The catfish were given mercuric chloride, emisan 6 (a methoxy-ethyl mercury fungicide), or methylmercuric chloride for 45, 90, and 180 days. The adrenalcortical cells were stimulated at 90 days in all the mercurial treated groups. Hyperplasia, lymphocyte infiltration, fibrosis, and necrosis in some areas of adrenal were found after 180 days of treatment [41]. The plasma cortisol level decreased significantly, and the ACTH cells in the pituitary were hypertrophied and degranulated, suggesting increased secretion of ACTH [41].

More studies have been carried out with rodents. In rats receiving methyl mercury in drinking water (20mg/l) for 180 days, the cytoplasm of cells in adrenal zona fasciculata were heavily vacuolated and distinct necrotic cells were observed in other cortical zones [38]. Burton and Wayne examined the adrenal and testicular functions of rats after acute and subchronic exposure to methylmercury [42]. Male Sprague-Dawley rats received a single intraperitoneal injection of 7.0 mg methylmercuric chloride, resulting in death in 50% of them. The swimming fatigue time, measured 6 hours after injection, showed no difference between the methylmercury treated group and the control group. The basal serum corticosterone levels were not significantly different in these groups, though the ACTH stimulated serum corticosterone level was somewhat lower in the methylmercury treated group than in the control group [42]. Male weanling Sprague-Dawley rats were intraperitoneally injected with 0.26mg of methylmercury every other day for 6 weeks. Adrenal weight was more than twice that of the control group. Histologically, marked hyperplasia of the zona fasciculata was observed. The hyperplastic adrenal weight was reduced by approximately 50% after daily treatment of cortisone acetate for 4 days [42]. Worker tolerance, measured as swimming time, decreased dramatically. The mean swim fatigue time was 6.9 ± 2.5 min in the mercury intoxicated group, while it was longer than 180 min in the control group. Corticosterone and cortisone supplement resulted in a moderate increase in swimming time [42]. Basal serum levels of corticosterone were also not significantly different between the mercury treated group and the control group. However, the concentration of corticosterone after ACTH stimulation or ether stress was significantly lower in the mercury treated group than that in the control group, indicating clearly that chronic methylmercury exposure injured the responsibility of the adrenal cortex to ACTH or stress. Impaired cortisol stress response was also found in fish from environments polluted by mercury and other chemicals [43]. Prenatal methylmercury exposure in mice resulted in diminished hepatic metabolism of corticosterone in vitro. However, plasma levels of corticosterone were not affected [44].

Nishiyama et al reported that the ACTH-induced corticosteroid production in cultured rat adrenal cortical cells was not affected by mercury application at 1 μ M [45]. In contrast, when the concentration reached 100 μ M, mercury exerted an adverse effect on the viability of isolated rat adrenal cortical cells [46]. Thus, the concentration of mercury applied to the culture system seems to be critical. There was a reduction in ACTH-stimulated corticosterone production by adrenal decapsular cells as well as LH-stimulated testosterone production by Leydig cells [46]. The impairment of adrenal steroid production and metabolism was also observed in vivo [42, 43, 47]. It is suggested that Hg^{2+} directly caused a defect in adrenal steroid biosynthesis by inhibiting the activity of 21 alpha-hydroxylase [47]. Considering that mercury is a bioplasm toxicant and broad enzyme inhibitor,

it is reasonable to suppose that other steroidogenesis enzyme inhibitions may also be involved in the mercury induced impairment of steroid metabolism. Alteration of steroid biosynthesis in adrenal and gonad glands were also reported in seal models[48, 49].

The effect of mercury on adrenal medulla hormone was studied in vitro[50, 51]. Hart and Borowitz reported that mercury, at concentrations ranging from 10^{-5}-10^{-3}, stimulated the release of catecholamine from isolated perfused bovine adrenals. The duration of adrenal catecholamine release induced by mercury is longer than that by other divalent ions such as cadmium or barium[50]. The mercury-induced catecholamine release declined in a biphasic manner. Different mechanisms for adrenal catecholamine release by mercury may be involved in each phase. In the initial phase mercury may act directly on catecholamine stores since it is independent of extracellular calcium and not blocked by magnesium. The secondary phase of adrenal catecholamine release probably involves alteration of membrane structure caused by mercury[51]. The Mg^{2+}-ATPase and Ca^{2+} -ATPase activities in microsomal fraction from bovine adrenal medulla were inhibited by mercury[51].

The effect of mercury on the human adrenal hormone levels after occupational exposure was mentioned only in three reports, and no significant changes were found[27, 28, 30].

The effects on gonads

The gonads, ovary and testis, are bifunctional organs that produce germ cells and sex hormones. The ovary produces ova and the steroid hormone (estrogen and progesterone); the testis produces spermatozoa and testosterone. These two functions are closely related and approximated, for high local concentrations of the sex hormones are required for germ cell development. The production of these hormones is tightly regulated through a feedback loop that involves the pituitary and the hypothalamus. Certainly, the sex hormones are closely related to sexual behavior, embryo and fetal development, the maintenance and outcomes of pregnancy. Though there was abundant literature concerning the effects of mercuials on these aspects, the effects of mercury on these phases are not discussed in this review, because these effects may result from mercurials through mechanisms other than direct disturbance of the sex hormones. Therefore, only the effects of mercurials on the production of germ cells and sex hormones, and on the menstrual cycle, which is directly dependent on female hormones, are reviewed.

The gonads seem to have a special affinity for mercury as do other endocrine organs. Metallic mercury is oxidized by the catalase-H_2O_2 complex to the ionic form and deposited in these organs[39]. In rat ovaries the deposits of mercury were shown to occur particularly in macrophages of atretic young and mature ovarian follicles and corpora lutea, within granulosa cells of atretic follicles, and in the lutein cells of freshly formed corpora lutea[53]. In the ovary of hamster when injected with 1mg of mercuric chloride daily throughout a four-day estrous cycle, the mercury was also more concentrated in the corpora lutea than in the follicles of interstitium[54]. Rat testes also contain a high concentration of mercury after exposure[55, 56], although the intercellular and intracellular deposits were not clearly elucidated. The testicular toxicities of mercurials, including impaired spermatogenesis and/or steroidogenesis, have been demonstrated in a number of laboratory animal species: fish[49, 57, 58], fowls[59, 60], rodents[42, 46, 55, 56, 61-64], boars[65], and primates[66]. The reproductive impairments in wild animals such as the Florida panther were also suspected to be related to mercury exposure[67].

Sertoli cells from ducks fed with methylmercuric chloride at 5 or 15mg/d for 12 weeks, had dilated smooth endoplasm reticum, increased lysosomes, myelinoid figures, vacuolations, cytoplasmic and nuclear debris, cristolyses of mitochondria, distended Golgi's complexes, reduced smooth endoplasm reticulum and microtubules[59]. Ultrastructural changes were found mainly in primary spermatocytes and spermatids, while spermatogonia seem to be more resistant[59]. Chowdhury and Arora compared the testicular toxicities of mercuric chloride between the rat, mouse, guinea pig, and hamster. The animals were administered with 1, 2, and 5mg mercuric chloride via ip. At the lowest dose testicular degeneration was observed in all of the hamsters, partial degeneration in the rats and mice, and none in the guinea pigs. Leydig cell atrophy and spermatogenic inhibition occurred at the dose of 2 mg/kg. Severe testicular degeneration and cellular deformation were found in both seminiferous tubules and Leydig cells in all of the species[62]. Lee and Dixon evaluated, with isotope labeled base or amino acid incorporation technique in vitro and with serial mating test in vivo, the effects of mercury on spermatogenesis and fertility ability in mice[63]. The results showed that CH_3Hg^+ or Hg^{2+} at concentrations ranging from 10-3 to 10-8 M reduced the incorporation of thymidine by spermatogonia, of uridine by elongated spermatids, and of L-leucine by late elongated spermatids by around 40 percent. In vivo intraperitoneal administration with 1 mg/kg also significantly inhibited the uptake of thymidine, uridine and L-leucine by their respective spermatogenic cells. The results of the serial mating study indicated that the primary affect of the mercurials was on spermatogonial cells, premeiotic spermatocytes and early-elongated spermatids, with no apparent affect on spermatozoa in the testis, epididymis or vas deferentia[63]. These results were somewhat different from those in ducks, in which the spermatogonia were more resistant to mercurials[59].

Impairments of testicular steroidogeneses were observed in vitro after mercury treatment in isolated seal and rat testicular incubate systems[46, 49]. Burton and Wayne reported that in rats receiving methyl mercury at 0.26mg intraperitoneally every 48 hours over 6 weeks, both basal and human chorionic gonadotropin (HCG) stimulated serum testosterone concentrations decreased by 80%[42]. Progressive degeneration of Leydig cells and a decrease in their nuclear diameters and populations were associated with a gradual increase in the deposition of mercury after methlmercuric and mercuric chloride treatment. These Leydig cell structural deformations were correlated with the diminution of 3 beta-hydroxy-delta 5-steroid dehydrogenase and serum testosterone level, indicating steroidogenic impairment by mercurial treatment[55].

Mohamed et al demonstrated the effects of methyl mercury on testicular functions in primate after a subchronic low level exposure, which seems to be more consistent with human occupational exposure[66]. Adult male monkeys were treated orally with methyl mercury at 50 or 70 μ g/kg/day for 20 weeks. Spermatozoal production, motility and morphology, and serum testosterone were determined before, during and after treatment. The results showed that the mercury treatment significantly decreased motile spermatozoa, scores of sperm speed and forward progression, and increased abnormal sperm tail forms. The mercury-induced semen abnormalities were not accompanied by

any obvious changes in the serum testosterone level. Moreover, no consistent histological impairment was detected in testicular biopsies at the end of treatment[66].

The testicular toxic effects of mercury on humans have not been illuminated as clearly as on laboratory animals. Popescu reported that workers occupationally exposed to methyl mercury and ethyl mercury had hypospermia and teratospermia[68]. Only three patients were included in this report. The patients were occupationally exposed to high concentration of mercury over 6 - 8 years. At the time when they were examined, they had not been exposed for 2 - 6 months and their urinary excretion of mercury varied between 340 and 480 μ g/l. The patients complained of decreased libido and impotence as well as other mental symptoms [68]. There is circumstantial evidence to suggest that mercury is the aetiological factor for so-called Young's syndrome characterized by obstructive azoospermia. This syndrome seems to be a late complication of mercury intoxication[69]. And there is increasing evidence supporting the possible association between this syndrome and mercury[70]. However, a series of epidemiological or clinical studies showed that mercury exposure was not associated with human semen parameters, serum sex hormones, or male fertility[28, 29, 71-73]. Barregard and his colleagues found even a positive correlation of cumulative mercury exposure with serum total testosterone but not with free testosterone[30]. The decreasing extent of current occupational exposure, compared to that found decades ago, might be responsible for these negative results.

The effects of mercury on female gonad function were also reported in fish[74-76], mice[77], and hamsters[54, 78, 79]. Fish exposed to mercury-polluted water had reduced gonadal steroidogenesis and pituitary gonadotropin releasing[74], inhibited ovarian maturation [75], decreased ovarian weight, and decreased number and diameter of oocytes[76]. The meiosis of ova was affected by mercury in mice and hamsters[77, 78]. Cyclic female hamsters, given 1 mg of mercuric chloride daily during the 4-day cycle, demonstrated alterations in the histology of the reproductive tract, progesterone levels and cyclicity. On day 3 and day 4 of the estrus cycle, the mercury treated animals had no uterine hypertrophy. The ovaries showed retarded follicular development and morphologically prolonged corpora lutea. Plasma progesterone levels were significantly lower on day 2 of the first cycle and day 1 of the second cycle[79]. When hamsters were given a total of 3 or 4 mg of mercuric chloride during the first estrus cycle, 60% of the animals did not ovulate by day 1 of the third cycle[54].

In human epidemiological studies, no clear relationship between mercury exposure levels and female hormone disorder and fertility was observed. Rowland et al reported that female dental assistants occupationally exposed to mercury vapor were less fertile than unexposed controls. However, women with low exposure were more fertile than unexposed controls[80]. This study had a limitation on exposure estimation, because the information was collected from questionnaires without biological measurement of mercury exposure. Fu investigated the reproductive hazards in 704 female workers exposed to low-level metallic mercury and 583 controls. No significant relationships were found except the incidence and severity of dysmenorrhea[81]. Gerhard and his colleagues recently reported that women with hormone disorders excreted more urinary mercury than those without hormone disorders after oral administration of the chelating agent 2,3-dimercaptopropane-1-sulfonic acid[82]. This result indicates that there is a possible relationship between mercury exposure and female infertility. Gerhard and Runnebaum also reported a similar result that in the so-called chewing-gum test, women with hormone disorders or alopecia had, on the average, the highest mercury excretion during the wash-out test[83]. However, other factors, which were not properly ruled out in these studies, may also be involved in the etiology of infertility. Thus, the exact correlation between mercury exposure and human female infertility is far from confirmed.

The effects on other endocrine tissues and cells

The effect of mercury on endocrine pancreas was studied in vitro. Braaten and his colleagues added phenyl mercuric acetate or phenyl mercuric nitrate at 1.4 μ M/ml to the primary culture cells from fetal or neonatal rat endocrine pancreas. The mercury selectively destroyed the fibroblastoid cells yielding and morphological intact. This action of mercury appeared to be mediated by reversible inhibition of sulfhydryl enzymes, since the cytotoxic effects were completely inhibited by adding glutathione or thioglycolate. After removal of the metal agent from the culture media, many pancreas islets sent out cytoplasmic projection, containing a large number of oriented microtubules, which serve as bridging units to adjacent endocrine cells[84]. The function of the pancreas hormone, insulin, was also affected by mercury. An exposure of isolated fat cells from rat epididymal fat pads to mercury significantly inhibited the stimulation of glucose metabolism by insulin without affecting the antilipolytic action of the hormone[85]. The influence of mercury on the secretion of human chorionic gonadotropin in superfused young placental tissue was reported by Boadiet al[86]. Mercuric chloride at concentrations of 0.75, 3.0, 6.0 and 12.0 μ g/ml in the culture media increased the secretion of hCG in a dose-dependent manner after a 24 hour incubation. It is not known, however, whether mercury stimulates the secretion of hCG by the placenta in pregnant women. There have not been any reports concerning the effect of mercury on the parathyroid hormone.

The possible toxic mechanism of mercury on the endocrine system

The mechanisms of mercury toxicities on the endocrine system are not clearly illuminated. They may be mediated by a series of alternations. The broad enzyme inhibitory property, due to its avid binding to sulphydryl, may account for a majority of the possible mechanisms. Some of the enzyme inhibitory effects were mentioned above. Na^+K^+-ATPase activity in the membraneous preparation from hog thyroid was found to be inhibited by 50% in organic mercurial at a concentration of 4×10^{-5} M and in inorganic mercurial at a concentration of 6×10^{-7} M. The Mg^{2+}-ATPase activity in the preparation was neither inhibited by methylmercuric chloride up to a concentration of 2×10^{-3} M nor by mercuric chloride up to 1×10^{-4} M[33]. However, the Mg^{2+}-ATPase and Ca^{2+}-ATPase in the microsomal fraction from the bovine adrenal medulla were sensitive to mercury[51].

The biosynthesis of steroid hormones in the adrenals or gonads involved a number of enzymes generally called steroidgenese enzymes. 3-beta-hydroxy-Δ^5-steroid dehydrogenase (3β-HSD) which is present in all steroidogenic tissues is one of the key enzymes in adrenal and gonad steroid hormone biosynthesis. Mercurials were found to inhibit testicular 3β-HSD in fish[58] and rats[87]. Catfish exposed to sublethal concentrations of methylmercuric chloride, mercuric chloride, and emisan 6 (0.04, 0.05 and 0.5mg/l, respectively) showed

significantly reduced activity of 3β-HSD. A very weak enzyme activity was observed in the mercuric chloride- and emisan 6-treated groups, while the enzyme activity was completely absent in the methylmercuric chloride group[58]. Immature male rats were administered with mercuric chloride at dosages of 0.05 and 0.1 mg/kg intraperitoneally daily for 90 days, resulting in dramatically decreased enzyme activity[87]. The synthesis of testosterone by Leydig cells is closely associated with the process of spermatogenesis. Thus, the reduction of testosterone synthesis may account for the observed inhibition of spermatogenesis by mercury. On the ovary, however, the effect of mercury may be in contrast to that on the testis or adrenal. Mondal and his colleagues recently reported that mercuric chloride treatment induced the activity of 3β-HSD in the oocyte of fish[88]. Both in vitro and in vivo mercuric chloride treatment demonstrated a remarkably high rate of progesterone synthesis accompanied by a low rate of conversion to 17β estradiol in the oocyte of Channa punctatus. The accumulation of progesterone was positively correlated with a significant increase in 3β-HSD activity in the mercury-treated fish. Thus, it indicated that at an early stage of intoxication mercury plays a role in the induction of 3β-HSD. The authors also demonstrated that the induction of this enzyme was mediated by a specific binding of Hg to the plasma membrane Na^+K^+-ATPase and an increase in the specific messenger RNA translating 3β-HSD[88]. It is not known whether mercury can induce 3β-HSD in the mammalian oocyte. Also, the mechanism of the low rate of conversion of 17β estradiol was not explained by the authors. It is well known that aromatase is the key enzyme in estrogen biosynthesis in the ovary and testis. Thus, a proper examination of this enzyme is meaningful to get a deep insight into the mechanism of the steroidogenesis disturbance caused by mercury.

The influence of mercury on the combining of hormones by their receptors is also involved in the mechanism of mercury endocrine toxicity. Steroid hormone receptors contain a reactive sulfhydryl group or groups required for hormone binding. Using a preparation of chick oviduct progesterone receptor and intestinal vitamin D receptor, Coty demonstrated that the mercurial reagents inhibited hormone binding to the aporeceptors and dissociated hormone-receptor complexes[89]. Organic mercurials mersalyl, p-mercuribenzoate, p-mercuriphenylsulfonate at the same concentration of 1 mM, and inorganic mercurial mercuric chloride at 0.4 mM inhibited the binding of both hormones to their receptors by 97.1-100%, and replaced the hormones from the hormone-receptor complexes by 77.5-94.9%. The effect of mersalyl required an active mercurial function. Prior addition of excess thiol reagent prevented both the inhibition of binding and the replacement of bound hormone. Furthermore, hormone displacement by mersalyl could be reversed by the addition of excess thiol reagent. However, it was supposed by Griest and Coty that the effect of mercurials on hormone binding was more complex than that deduced from studies performed on the receptors in solution. The progesterone receptor may contain a second, low-affinity hormone-binding site that is insensitive to mercurials[90]. Whether mercurials can completely block progesterone binding to its receptor is still unknown. It was also reported that mercuric chloride inhibited the binding of tritiated progesterone, dexamethasone and testosterone to their respective cytoplasmic receptors, prepared from the eggshell gland mucosa of the domestic fowl, while methylmecuric chloride was less potent than mercuric chloride[91]. Mercuric chloride could also inhibit estradiol binding to rat uterine nuclear receptors, while other divalent ions Mn^{2+}, Ba^{2+}, Ca^{2+} and Mg^{2+} enhanced the binding[92].

The interference with intracellular calcium metabolism is one of the important mechanisms of mercury toxicity on the endocrine system as well as on other organs. Mercury and other heavy metals are known to inhibit neurohypophyseal hormone release[93, 94]. The mercuric chloride induced inhibition of hormone release is accompanied by enhanced Ca^{2+} uptake by the neurohypophysis, with a decreased Ca^{2+} accumulation in cytosol and a decreased accumulation in mitochondria, suggesting that mercuric chloride may inhibit vasopressin release through a disruption of the Ca^{2+} pumping mechanism in the neurohypophysis. Clifton et al reported that mercuric chloride at a concentration of 0.5mM reduced $^{45}Ca^{2+}$ binding to calmodulin (CM), purified from bovine neurohypophysis by 20%, and inhibited endogenous CM-stimulated Ca^{2+}, Mg^{2+}-ATPase activity from rat brain mitochondria in a dose-dependent manner[95]. Thus the inhibition of vasopressin release from an intact gland in the presence of mercuric chloride may be associated with a disruption of calcium in the neurohypophysis. Atchison and Hare supposed that at least two mechanisms were involved in the alteration of intracellular Ca^{2+} concentration. Firstly, methylmercury disrupts the regulation of Ca^{2+} from an intracellular Ca^{2+} pool, and secondly, it increases the permeability of plasma membrane to Ca^{2+}. In addition, methylmercury also blocks plasma membrane voltage-dependent Ca^{2+} and Na^+ channels[96].

Lipid peroxidation may be another molecular mechanism for mercury-induced injury to endocrine glands. Huang et al reported that rats administrated with 5mg/kg mercuric chloride subcutaneously had an increased concentration of malondialdehyde (MDA) in the testis, whereas, the concentration of MDA was reduced after pretreatment with antioxidants and chelators to mercuric chloride-treated rats[97]. Methyl mercury-induced neurotoxicity in rat cerebellar granule cells was also demonstrated to be mediated by the oxidative mechanism[98].

Summary

The effects of mercury on the human endocrine system are extremely limited. According to the results of these limited studies, the present environmental and occupational exposures to mercury are shown not to have evident adverse effects on endocrine functions. In modern industrial activities, preventive measures and technological improvement have undoubtedly reduced occupational exposure to and the environmental contamination of mercury. The human body burden of mercury was found to have decreased dramatically during this century[99]. Thus, as for environmental exposure, the present ambient mercury level may not be so hazardous as to result in a disturbance of human endocrine functions.

In laboratory animal studies, however, mercury was found to have the potential to cause a series of impairments of almost all the endocrine glands in various animal models. However, several factors limit the ability to extrapolate the animal data to the human situation. The physiological differences among species make it difficult to compare the findings between different animals, and between animals and humans. Dose-response and exposure evaluations are critical in the risk assessment. It is helpful to compare the mercury contents in the brain and endocrine glands, by biopsy or autopsy, between experimental

animals and human subjects who were occupationally exposed to mercury. The exposure period is also important. The physiological or pathological responses can differ along with the variation of exposure periods, although the organ deposits of the chemical reach the same content. Thus, animal studies with low-level long-term exposure are of great help. Anyway, well-designed laboratory animal studies and epidemiological studies are urgently needed to confirm the endocrine disruptive effects of mercury.

Finally, it should be pointed out that the endocrine disruptive effects should not be evaluated in isolation from those effects induced via different modes/mechanisms. The toxic effects due to endocrine disruption may not always be the most critical effect nor the most sensitive endpoint. Therefore, the mercury-induced endocrine disruptive effect is merely one of the aspects that should be taken into account when comprehensive risk assessment is made.

References

1) Bitman J, Cecil HC. Estrogenic activity of DDT analogs and polychlorinated biphenyls. J Agr Food Chem 1970; 18: 1108-12.
2) Nelson JA, Struck RF, James R. Estrogenic activities of chlorinated hydrocarbons. J Toxicol Environ Health 1978; 4: 325-39.
3) Mclachlan JA. Estrogens in the environment. Amsterdam: Elsevier, 1980.
4) Mclachlan JA. Estrogens in the environment. II: Influence on Development. Amsterdam: Elsevier, 1985.
5) Mclachlan JA, Korach KS. Symposium on estrogens in the environment, II. Environ Health Perspect 1995; 103 (Suppl 7): 3-4.
6) Kavlock RJ, Daston GP, DeRosa C, et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: A report of the U.S. EPA-sponsored workshop. Environ Health Perspect 1996; 104 (Suppl 4): 715-40.
7) Ankley GT, Johnson RD, Detenbeck NE, Bradbery SP, Toth G, Folmer LC. Development of a research strategy for assessing the ecological risk of endocrine disruptors. Rev Toxicol Ser B: Environ Toxicol 1997; 1: 231-67.
8) Crisp TM, Clegg ED, Cooper RL, et al. Environmental endocrine disruption: An effect assessment and analysis. Environ Health Perspect 1998; 106 (Suppl 1): 11-56.
9) Shinsuke Tanabe. Endocrine disrupting chemicals-what is the problem? Iwanami booklet No.456 Tokyo: Iwanamishoten, 1998.
10) World Health Organization. Environmental Health Criteria 118, Inorganic Mercury. Geneva: WHO, 1991.
11) Kosta L, Byrne AR, Zelenko V. Correlation between selenium and mercury in man following exposure to inorganic mercury. Nature 1975; 254: 238-9.
12) Khayat A, Dencker L. Whole body and liver distribution of inhaled mercury in the mouse: Influence of ethanol and aminotriazole pretreatment. J Appl Toxicol 1983; 1: 66-74.
13) Nylander M. Mercury in pituitary glands of dentists. Lancet 1986; I: 442.
14) Danscher G, Horsted-Bindslev P, Rungby J. Traces of mercury in organs from primates with amalgam fillings. Exp Mol Pathol 1990; 52: 291-9.
15) Moller-Madsen B, Danscher G. Localization of mercury in CNS of the rat. IV. The effect of selenium on orally administered organic and inorganic mercury. Toxicol Appl Pharmacol 1991; 108: 457-73.
16) Ernst E, Christensen MK, Poulsen EH. Mercury in the rat hypothalamic arcuate nucleus and median eminence after mercury vapor exposure. Exp Mol Pathol 1993; 58: 205-14.
17) Vahter ME, Mottet NK, Friberg LT, Lind SB, Charleston JS, Burbacher TM. Demethylation of methyl mercury in different brain sites of Macaca fascicularis monkeys during long-term subclinical methyl mercury exposure. Toxicol Appl Pharmacol 1995; 134: 273-84.
18) Lamperti A, Niewenhuis R. The effects of mercury on the structure and function of the hypothalamo-pituitary axis in the hamster. Cell Tissue Res 1976; 170: 315-24.
19) Lach H, Srebro Z, Dziubek K, Krawczyk S, Szaroma W. The influence of mercury and lead compounds on the circadian rhythm of cytoplasmic RNA in hypothalamic neurons of mice. Folia Histochem Cytochem (Krakow) 1983; 21: 3-4.
20) Chan A, Webb RM, Yang CM, Jin CB. The effect of estrogen on luteinizing hormone-releasing hormone binding sites in hypothalamic membranes. Neuropharmacology 1987; 26: 1395-401.
21) Thorlacius-Ussing O, Moller-Madsen B, Danscher G. Intracellular accumulation of mercury in the anterior pituitary of rats exposed to mercury chloride. Exp Mol Pathol 1985; 42: 278-86.
22) Moller-Madsen B, Thorlacius-Ussing O. Accumulation of mercury in the anterior pituitary of rats following oral intraperitoneal administration of methyl mercury. Virchows Arch [Cell Pathol] 1986; 51: 303-11.
23) Nylander M. Relation between mercury and selenium in pituitary glands of dental staff. Bri J Ind Med 1989; 46: 751-2.
24) Danscher GP, Horstedt-Bindslev P, Rungby J. Traces of mercury in organs from primates with amalgam fillings. Exp Mol Pathol 1990; 52: 291-9.
25) Kanabrocki EL, Greco J, Graham LA, et al. Trace elememts in human pituitary. Int J Nucl Med Biol 1976; 3: 73-6.
26) Nylander M, Friberg L, Eggleston D, Bjerkman L. Mercury accumulation in tissues from dental staff and controls in relation to exposure. Swed Dent J 1989; 13: 235-43.
27) Langworth S, Rojmark S, Akesson A. Normal pituitary hormone response to thyrotrophin releasing hormone in dental personnel exposed to mercury. Swed Dent J 1990; 14: 101-3.
28) Erfurth EM, Schutz A, Nilsson A, Barregard L, Skerfving S. Normal pituitary hormone response to thyrotrophin and gonadotrophin releasing hormones in subjects exposed to elemental mercury vapour. Bri J Ind Med 1990; 47: 639-44.
29) McGregor AJ, Mason HI. Occupational mercury vapour exposure and testicular, pituitary and thyroid endocrine function. Hum Exp Toxicol 1991; 10: 199-203.
30) Barregard L, Lindstedt G, Schutz A, Sallsten G. Endocrine function in mercury exposed chloralkai workers. Occup Environ Med 1994; 51: 536-40.
31) Ghosh N, Bhattachara S. Thyrotoxicity of chlorides of

cadmium and mercury in rabbit. Biomed Environ Sci 1992; 5:236-40.
32) Goldman M, Blackburn P. The effect of mercuric chloride on thyroid function in the rat. Toxicol Appl Pharmacol 1979; 48 (1 pt 1): 49-55.
33) Kawada J, Nishida M, Yoshimura Y, Mitani K. Effects of organic and inorganic mercurials on thyroidal functions. J Pharmacobiodyn 1980; 3: 149-59.
34) Nishida M, Yamamoto T, Yoshimura Y, Kawada J. Subacute toxicity of methylmercuric chloride and mercuric chloride on mouse thyroid. J Pharmacobiodyn 1986; 9: 331-8.
35) Chopra IJ. A study of extrathyroidal conversion of thyroxine (T_4) to 3,3′5-triiodothyronine (T_3) in vitro. Endocrinology 1977; 101: 453-63.
36) Sin YM, Teh WF, Wong MK, Reddy PK. Effect of mercury on glutathione and thyrod hormones. Bull Environ Contam Toxicol 1990; 44: 616-22.
37) Sin YM, Teh WF. Effect of long-term uptake of mercuric sulphide on thyrod hormones and glutathione in mice. Bull Environ Contam Toxicol 1992; 49: 847-54.
38) Rasmussen BL, Thorlacius-Ussing O. Ultrastructural localization of mercury in adrenals from rats exposed to methyl mercury. Virchows Arch B Cell Pathol Incl Mol Pathol 1987; 52: 529-38.
39) Khayat A, Dencker L. Organ and cellular distribution of inhaled metallic mercury in the rat and Marmoset monkey (callithrix jacchus): Influence of ethyl alcohol pretreatment. Acta Pharmacol Toxicol (Copenh) 1984; 55: 145-52.
40) Thaxton P, Parkhurst CR, Cogbern LA, Young PS. Adrenal function in chickens experiencing mercury toxicity. Poult Sci 1975; 54: 578-84.
41) Kirubagaran R, Joy KP. Changes in adrenocortical-pituitary activity in the catfish, Clarias batrachus (L.), after mercury treatment. Ecotoxicol Environ Sat 1991; 22: 36-44.
42) Burton GV, Meikle AW. Acute and chronic methyl mercury poisoning impairs rat adrenal and testicular function. J Toxicol Environ Health 1980; 6: 597-606.
43) Hontela A, Rasmussen JB, Audet C, Chevalier G. Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs, and mercury. Arch Environ Contam Toxicol 1992; 22: 278-83.
44) Grady RR, Kitay JI, Spyker JM, Avery DL. Postnatal endocrine dysfunction induced by prenatal methylmercury or cadmium exposure in mice. J Environ Pathol Toxicol 1978; 1: 187-97.
45) Nishiyama S, Nakamura K, Ogawa M. Effects of heavy metals on corticosteroid production in cultured rat adrenolcortical cells. Toxicol Appl Pharmacol 1985; 81: 174-6.
46) Ng TB, Liu WK. Toxic effect of heavy metals on cells isolated from the rat adrenal and testis. In Vitro Cell Biol 1990; 26: 24-8.
47) Veltman JC, Maines MD. Alterations of heme, cytochrome P-450, and steroid metabolism by mercury in rat adrenal. Arch Biochem Biophys 1986; 248: 467-78.
48) Freeman HC, Sangalang G, Uthe JF, Ronal K. Steroidogenesis in vitro in the harp seal (Pagophilus groenlandicus) without and with methyl mercury treatment in vivo. Environ Physiol Biochem 1975; 5: 428-39.
49) Freeman HC, Sangalang G. A study of the effects of methyl mercury, cadmium, arsenic, selenium, and PCB, (Aroclor 1254) on adrenal and testicular steroidogeneses in vitro, by the gray seal Halichoerus grypus. Arch Environ Contam 1977; 5: 369-83.
50) Hart DT, Borowitz JL. Adrenal catecholamine release by divalent mercury and cadmium. Arch Int Pharmacodyn Ther 1974; 209: 94-9.
51) Borowitz JL. Mechanism of adrenal catecholamine release by divalent mercury. Toxicol Appl Pharmacol 1974; 28: 82-7.
52) Yamanaka K, Yamada S, Hayashi S, Hayashi E. Inhibition by chloromazine, metals and l-ascorbic acid of calcium-ATPase and magnesium-ATPase in bovine adrenal medullary microsome. Jpn J Pharmacol 1984; 34: 447-55.
53) Stadnicka A. Localization of mercury in the rat ovary after oral administration of mercuric chloride. Acta Histochem 1980; 67: 227-33.
54) Lamperti AA, Printz RH. Localization, accumulation, and toxic effects of mercuric chloride on the reproductive axis of the female hamster. Biol Reprod 1974; 11: 180-6.
55) Vachhrajani KD, Chowdhury AR. Distribution of mercury and evaluation of testicular steroidogenesis in mercuric chloride and methylmercury administered rats. Indian J Exp Biol 1990; 28: 746-51.
56) Yang JM, Jiang XZ, Chen QY, Li PJ, Zhou YF, Wang YL. The distribution of $HgCl_2$ in rat body and its effects on fetus. Biomed Environ Sci 1996; 9: 437-42.
57) McIntyre JD. Toxicity of mercury for steelhead trout sperm. Bull Environ Contam Toxicol 1973; 9: 98-9.
58) Kirubagaran R, Joy KP. Inhibition of testicular 3β-hydroxy-Δ^5-steroid dehydrogenase (3β-HSD) activity in catfish Clarias batrachus (L.) by mercurials. Indian J Exp Biol 1988; 26: 907-8.
59) McNeil FI, Bhatnagar MK. Ultrastructure of the tesis of Pekin ducks fed methyl mercury chloride: Seminiferous epithelium. Am J Vet Res 1985; 46: 2019-25.
60) Maretta M, Marettova E, Skrobanek P, Ledec M. Effect of mercury on the seminiferous epithelium of the fowl testis. Acta Vet Hung 1995; 43: 153-61.
61) Khera KS. Reproductive capability of male rats and mice treated with methyl mercury. Toxicol Appl Pharmacol 1973; 24: 167-77.
62) Chowdhury AR, Arora U. Toxic effect of mercury on testes in different animal species. Indian J Physiol Pharmacol 1982; 26: 246-9.
63) Lee IP, Dixon RL. Effects of mercury on spermatogenesis studied by velocity sedimentation cell separation and serial mating. J Pharmacol Exp Ther 1975; 194: 171-81.
64) Evenson DP, Jost LK, Bear RK. Effects of methyl methanesulfonate on mouse sperm chromatin structure and testicular cell kinetics. Environ Mol Mutagen 1993; 21: 144-53.
65) Schulz O, Walzel R, Hacker U, Umlauft K. The influence of chemical noxae on the sperm quality of boars used for insemination. 2. Mercury. Arch Exp Veterinarmed 1988; 42: 610-7.
66) Mohamed MK, Burbacher TM, Mottet NK. Effects of methyl mercury on testicular functions in Macaca fascicularis monkeys. Pharmacol Toxicol 1987; 60: 29-36.
67) Facemire CF, Gross TS, Guillette LJ. Reproductive impairment in the Florida panther: Nature or nurture? Environ Health Perspect 1995; 103 (Suppl 4): 79-86.
68) Popescu HI. Poisoning with alkylmercury compounds. Br

Med J 1978; 1: 1347.
69) Hendry WF, A'Hern PR, Cole PJ. Was Young-1s syndrome caused by exposure to mercury in chilhood? Br Med J 1993; 307: 1579-82.
70) Dally A, Hendry B. Declining sperm count. Increasing evidence that Young's syndrome is associated with mercury. Br Med J 1996; 313: 44.
71) Alcer KH, Brix KA, Fine LJ, Kallenbach LR, Wolfe RA. Occupational mercury exposure and male reproductive health. Am J Ind Med 1989; 15: 517-29.
72) Chia SE, Ong CN, Lee ST, Tsakok FH. Blood concentrations of lead, cadmium, mercury, zinc, and copper and human semen parameters. Arch Androl 1992; 29: 177-83.
73) Hanf V, Forstman A, Costea JE, Schieferstein G, Fischer I, Schweinsberg F. Mercury in urine and ejaculate in husbands of barren couples. Toxicol Lett 1996; 88: 227-31.
74) Mukherjee D, Kumar V, Chakraborti P. Effect of mercury chloride and cadmium chloride on gonadal function and its regulation in sexually mature common carp Cyprinus carpio. Biomed Environ Sci 1994; 7: 13-24.
75) Reddy PS, Tuberty SR, Fingerman M. Effects of cadmium and mercury on ovarian maturation in the red swamp crayfish, Procambarus clarkii. Ecotoxicol Environ Safe 1977; 37: 62-5.
76) Dey S, Bhattacharya S. Ovarian damages to Channa punctatus after chronic exposure to low concentrations of Elsan, mercury, and ammonia. Ecotoxicol Environ Sat 1989; 17: 247-57.
77) Jagiello G, Lin JS. An assessment of the effects of mercury on the meiosis of mouse ova. Mutat Res 1973; 17: 93-99.
78) Watanabe T, Shimada T. Endo A. Effects of mercury compounds on ovulation and meiotic and mitotic chromosomes in female hamsters. Teratology 1982; 25: 381-4.
79) Lamperti AA, Printa RH. Effects of mercuric chloride on the reproductive cycle of the female hamster. Biol Reprod 1973; 8:378-87.
80) Rowland AS, Baird DD, Weiberg CR, Shore DL, Shy CM, Wilcox AJ. The effect of occupational exposure to mercury vapour on the fertility of female dental assistants. Occup Environ Med 1994; 51: 28-34.
81) Fu WZ. Effect of mercury exposure on reproduction in female workers. Chung Hua Yu Fang I Hsueh Tsa Chih 1993; 27: 347-9.
82) Gerhard I, Monga B, Waldbrenner A, Runnebaum B. Heavy metals and fertility. J Toxicol Environ Health, Part A 1998; 54: 593-611.
83) Gerhard I, Runnebaum B. The limits of hormone substitution in pollutant exposure and fertility disorders. Zentralbl Gynakol 1992; 114: 593-602.
84) Braaten JT, Jarlfors U, Smith D, Mintz D. Purification of monolayer cell cultures of the endocrine pancreas. Tissue Cell 1975; 7: 747-62.
85) George JM. Effect of mercury on response of isolated fat cells to insulin and lipolytic hormones. Endocrinology 1971; 89: 1489-98.
86) Boadi WY, Shurtz-Swirski R, Barnea ER, Urbach J, Brandes JM, Yannai S. The influence of mercury on the secretion of human chorionic gonadotropin in superfused young placental tissue. Pharmacol Toxicol 1992; 71: 19-23.
87) Chowdhury AR, Vachhrajani KD, Chatterjee BB. Inhibition of 3-bata-hydroxy-Δ^5-steroid dehydrogenase in rat testicular tissue by mercuric chroride. Toxicol Lett 1995; 27: 45-9.
88) Mondal S, Mukhopadhyay B, Bhattacharya S. Inorganic mercury binding to fish oocyte plasma membrane induces steroidogenesis and translatable messenger RNA synthesis. Biometals 1997; 10: 285-90.
89) Coty WA. Reversible dissociation of steroid hormone receptor complexes by mercurial reagent. J Bio Chem 1980; 255: 8035-8037.
90) Griest RE, Coty WA. Binding of the chicken oviduct progesterone receptor to steroid affinity resins: resistance to elution with mercurial reagent. J Steroid Biochem 1984; 21: 29-34.
91) Lundholm CE. Influence of chlorinated hydrocarbons, Hg^{2+} and methyl-H^+ on steroid hormone receptors from eggshell gland mucosa of domestic fowls and ducks. Arch Toxicol 1991; 65: 220-7.
92) Brecher P, Pasquina A, Wotiz HH. Effect of metal ions on edtradiol binding to uterine nuclear receptors. Endocrinology 1969; 85: 612-4.
93) Clifton GG, Pearce C, Elliot K, Wallian JD. Mercuric chloride inhibition of vasopressin release from the isolated neurointermediate lobe of the rat pituitary. Biochim Biophys Acta 1986; 887: 189-95.
94) Dyball REJ, Wright RJ. Inhibition of neurohypophysia hormone release from the isolated rat neural lobe by ferrous chloride in the incubation medium. J Endocrinol 1977; 75:327-8.
95) Clifton GG, Oelsner D, Anderson CR, Pearce CJ, Wallin JD. The effects of mercuric chloride on calmodulin-mediated Ca^{+2} tansport in rat brain. Am J Med Sci 1990; 299: 26-31.
96) Atchison WD, Hare NF. Mechanisms of methylmercury-induced neurotoxicity. FASEB 1994; 8: 622-9.
97) Huang YL, Cheng SL, Lin TH. Lipid peroxidation in rats administrated with mercuric chloride. Biol Trace Elem Res 1996; 52: 193-206.
98) Sarafian T, Verity MA. Oxidative mechanisms underling methyl mercury neurotoxicity. Int J Dev Neurosci 1991; 9: 147-53.
99) Kevorkian J, Cento DP, Hyland JR, Bagozzi WM, Hollebeke EV. Mercury content of human tissues during the twentieth century. Am J Pub Health 1972; 62: 504-13.