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Effect of mercury vapor inhalation on rat ovary: Stereology and histopathology

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Abstract

Aim: Mercury, an environmental contaminant, is a risk factor for health in whole living organisms. In this study, we investigated whether mercury vapor (HgO) inhalation has an effect on rat ovary.

Methods: Twelve Wistar albino rats were divided equally into experimental (Hg) and control groups (n = 6). Animals in the Hg group were exposed to HgO for 45 days at a dose 1 mg/m^3/day, after which, histological and stereological assessment were carried out.

Results: Ovaries exposed to HgO had histo-morphometric alterations. HgO inhalation resulted in reduction of the total number of primordial, primary and Graaf follicles. Also, mean volume of ovary, medulla and cortex, corpus luteum (c. luteum) and Graaf follicles was decreased in the Hg group. Moreover, there was a significant increase in total volume of the atretic follicles. On light microscopy, thickening of tunica albuginea, increase of fibrils within the connective tissue, congestion of the capillaries and venous vessels, thinned walls and fibrin deposition in some large blood vessels, and edema were seen. Also, irregular follicle and oocyte borders, and hydropic degeneration in follicular granulosa cells were detected.

Conclusion: Structural alterations could be attributed to the toxic influence of HgO on rat ovary. The use of Hg should therefore be more controlled to minimize its toxic effect.

Key words: histopathology, inhalation, mercury vapor, ovary, rat, stereology.

Introduction

Mercury (Hg) is a critical environmental contaminant that exists in liquid form at room temperature. Having been absorbed, Hg is transported via the bloodstream to the organs, where it accumulates in various tissues.[1,2] Hg accumulation then induces production of reactive oxygen species (ROS) and so generates toxicity in the body.[3–5] Accumulation of Hg can happen in any part of the reproductive system such as the corpora lutea and ovaries, and toxicity may develop in the system.[6–9]

Few epidemiological studies have been done on abnormalities of the reproductive system due to Hg. It has been reported that Hg is associated with stillbirth, congenital malformation, and spontaneous abortion in female dentists.[10] There was a considerable association between Hg-exposed women and the occurrence of disorders of the menstrual cycle and also reduction of fertility.[9–13] In some animal studies, Davis *et al*. noted changes in the estrous cycle, estradiol, and progesterone levels due to HgO inhalation in female rats.[14] Also, inhibition of ovulation was seen in Hg-exposed female golden hamsters.[15] Therefore this phenomenon can affect the

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feedback systems between the hypothalamus, pituitary, and ovary.[13–16]

To date, few studies have been carried out on the effect of HgO inhalation on the female reproductive system, and none of these studies included unbiased stereological analysis or detailed histological evaluation of the ovary following Hg inhalation. In this study, we assessed the effects of HgO inhalation on rat ovary using histopathology and stereology.

Methods

Subjects and animal care

In this study, 12 adult female Wistar albino rats were used, each weighing 200 g, and 8–10 weeks old. Ethics approval for this investigation was granted by the Experimental Research and Application Center of Atatürk University. Subsequently, rats were obtained from the Experimental Animal Research and Application Center of Atatürk University (Erzurum, Turkey). All rats were kept at 22 ± 2°C under a 12:12-h day–night cycle with controlled humidity of 50 ± 5%. Rats were given access to standard rat chow and water ad libitum. The rats were divided into two groups each containing six individuals (n = 6): a control group and an Hg group. During the 45-day study period, HgO-treated rats were housed in a specially designed bulb, and subsequently exposed to HgO inhalation (1 mg/m^3/day).[12,17] The control group rats (untreated subjects) were kept in a plastic cage without exposure to HgO or stress. After completion of the study, rats in both groups were anesthetized using 2–3% sevoflurane (Sevorane® Liquid 250 mL; Abbott, Istanbul, Turkey) in 100% oxygen. All of the rats were then killed, and subsequently perfused, and the ovaries dissected completely out.

Histology

For the fixation procedure, the ovaries were embedded in 10% formaldehyde solution, and tissues were processed using graded alcohols, xylene and paraffin. Then, 5-μm-thick sections were obtained using a rotary microtome from blocked ovary tissue in a transverse plane. All of the sections were mounted onto glass slides, and stained with H&E for light microscopy.[18] After staining, true section thickness was confirmed using Stereoinvestigator version 9.0 (Microbrightfield; Gantenbein; Ankara, Turkey).

Follicle classification

Ovarian follicles contain a single immature oocyte.[19] Histologically, in the ovarian sections, there were three major types of follicles: primordial; growing primary; and mature Graaf. In the primordial follicles, primary oocytes were enclosed within thin and single squamous or cubical epithelial cells, and comprised the majority of the follicles in the ovary.[20] In the primary follicles, the oocytes were covered by cuboidal epithelial cells. Ultimately, Graaf follicles were the most enlarged follicles, and consisted of cumulus oophorus, corona radiata and a large antrum. Also, corpora lutea were defined as developing from the Graaf follicles following ovulation. Moreover, many atretic follicles undergoing degeneration before or after maturity were observed in the ovarian slices.

Morphometry

The quantitative data were assessed using a research microscope (Olympus BX 51 microscope; Olympus Optical, Tokyo, Japan) with DP 71 camera (Olympus, Olympus Optical, Tokyo, Japan). Images of the ovarian sections were obtained using the microscope–camera system and were evaluated for histopathology and stereology using Adobe Photoshop CS3 (San Jose, CA, USA).

Stereology

Ovarian volume estimation

The Cavalieri and point-counting grid method were used to measure the volume of the ovarian structures.[21] To achieve unbiased assessment, tissue blocks must be sectioned consecutively at equal intervals.[22] For this purpose, two different point-counting test grids were utilized on the parallel sections. The point density of the point-counting grid was designed according to pilot studies, to obtain acceptable coefficient of error (CE).[23] The CE and coefficient of variation were estimated according to previously published formulas.[24] Two different point-counting grids were composed of dense and loose points, according to the area of interest. The loose points were used to appraise the volume fraction of the cortex and medulla of the ovary. Dense points were used for estimation of the follicle volume. A test grid was placed on the screen of the PC as a reference section. Subsequently, the point-counting grid was superimposed randomly on the cut surface of images of interest, and the volume of each ovarian structure was calculated using the following formula:[25]

$$\text{volume (V)} = t \times a(p) \times (\Sigma P),$$

where t is the section thickness, a(p) is area of the each point on the grid and ΣP is total number of points hitting the surface of interest in the sections.

Estimation of total follicle number

The physical dissector method was applied to pairs of sample sections, providing valid and unbiased estimation.[26] Consecutive sections were processed using systematic random sampling. One of the two sections was selected as a reference section and the other as a look-up section. To estimate profile of interest in the reference section, light microscopical image of the cut surface was separated into equal parts of areas x and y axes using Stereoinvestigator 9.0 (Microbrightfield; Gantenbein). Photographs were then taken of each previously determined area using a CCD camera. This procedure was then repeated in the look-up section. All of the images related to the dissector pairs were then transferred to another computer, so that the fields could be investigated for profile of interest. An unbiased counting frame was superimposed on the corresponding counting field.[27] Subsequently, the particle profiles were acceptable when they were viewed in the reference section but not in the look-up section, and vice versa.[28] We calculated the numerical density of follicles per unit volume, using the following equation:

$$N = \frac{\Sigma Q^{-}}{\Sigma^{V}\text{Disector},}$$

where N is total number of each follicle type, ΣQ^{-} is the number of counted follicles and ΣV (t × A) is the total volume of the dissectors.

Results

Stereology

In Hg group rats, significant reduction was seen in the mean volume of ovary ($P < 0.01$), medulla ($P < 0.01$), cortex ($P < 0.05$), Graaf follicles ($P < 0.01$) and c. luteum ($P < 0.05$) compared with the controls (Table 1). In contrast, there was significant increase in the mean volume of atretic follicles ($P < 0.05$) compared with the control group (Table 1). Meanwhile, the total number of both the primordial and primary, as well as of the Graaf follicles, was significantly decreased in the Hg group rats compared with the control group ($P < 0.01$; Table 2).

Histopathology

No histopathological changes were noted in the control group ovaries (Fig. 1), but substantial changes were seen

Table 1 Volumetry results (mean ± SEM)

Groups	Ovary volume (mm^3)	Cortex medulla volume (mm^3)	Medulla volume (mm^3)	Corpus luteum volume (mm^3)	Graaf follicle volume (mm^3)	Atretic follicle volume (mm^3)
Control	465.26 ± 21.35	44.51 ± 8.14	509.77 ± 32.43	179.93 ± 7.72	97.14 ± 13.86	33.18 ± 9.67
HgO	290.62 ± 15.42**	39.06 ± 5. 29*	329.69 ± 26.73**	132.04 ± 11.18*	50.78 ± 8.94**	53.13 ± 6.49*

*$P < 0.05$; **$P < 0.01$.

Table 2 Follicle quantification (mean ± SEM)

Groups	Total no. primordial follicles	Total no. primary follicles	Total no. Graaf follicles
Control	1620 ± 65	623 ± 81	6 ± 4
HgO	976 ± 57**	417 ± 19**	3 ± 2**

**$P < 0.01$.

in the Hg group (Fig. 2). The tunica albuginea was thickened in the ovaries of rats exposed to HgO. Under the tunica albuginea inside the ovary, distinctive structural alterations were seen: fibrils within connective tissue were increased, capillaries and blood vessels were congested, the walls of some large and dilated veins were thinned and had fibrin deposition, edema was recognizable in the stroma, and many maldeveloping follicles were identified inside the ovarian stroma (i.e. follicle borders were irregular, and hydropic degeneration was observed in follicular granulosa cells). Oocyte borders within the follicles were irregular.

Discussion

Exposure to HgO used in dental clinics as filling material, in the atmosphere, and in various occupations has an adverse influence on body health.[29–31] It is thought that Hg suppresses the DNA repair mechanisms and molecular mechanisms, resulting in cell damage, increased lipid peroxidation products, and decreased glutathione peroxidase enzyme activity.[32,33] Also, the toxicity of Hg could attenuate the activity of antioxidant enzymes, and impair mitochondrial function.[34,35]

Given the ability of Hg to spread into tissue, the reproductive system in female individuals is susceptible to disorders following exposure to Hg.[30,36] Such factors can directly or indirectly affect the architectures of the ovary and their components, and also cause abnormalities in ovulation. For example, Hg can induce single-strand breaks in DNA and reduction of DNA replication in Chinese hamster ovary cells.[37,38] In the present study, HgO inhalation caused follicular atrophy and degeneration, and significant alterations were noted in HgO-treated ovarian samples. The total number of primordial, primary and Graaf follicles was significantly decreased in the HgO-treated rats. In other studies it was found that Hg could accumulate in mature ovarian follicles, in the c. lutea and within granulosa cells of the atretic follicles.[14,39]

The hypothalamus plays a regulatory role in the reproductive axis involving the hypothalamus, pituitary gland and ovary. After HgO inhalation, Hg can be deposited in the rat hypothalamus and pituitary.[40,41] Thereafter, plasma luteinizing hormone (LH) level can be disturbed as a result of the changes in secretion of LH-releasing hormone into the hypothalamus.[42] Also, it is well known that gonadotropins (LH, follicle-stimulating hormone), the most powerful follicle survival factors, ensure healthy function of the ovary.[43] There is a direct correlation between exposure to Hg and decrease in the production of sex hormones in fish.[44] One study noted that reduction of cholesterol occurred in ovary following exposure to Hg, followed subsequently by reduction of steroidogenesis and steroid hormone synthesis.[45] Hg exposure also inhibits the activity of 3-β-hydroxy-Δ^5-steroid dehydrogenase, which is present in all steroidogenic tissues.[45–47] Thus, progesterone secretion abnormalities in the c. lutea were seen, due to luteal cell membrane damage, therefore, cellular metabolism was disturbed.[45–47] Additionally, the suppression of 17β-estradiol by Hg enhances ovarian follicular apoptosis in fish.[48] Likewise, apoptosis of ovarian follicle cells (i.e. granulosa, theca) due to Hg is associated with oxidative stress and also alterations in follicle survival factors (hormones).[49,50] Hence, Hg can induce severe stress response and oxidative damage in the human ovary, and folliculogenesis can be affected as a result of hormonal irregularity.[50,51]

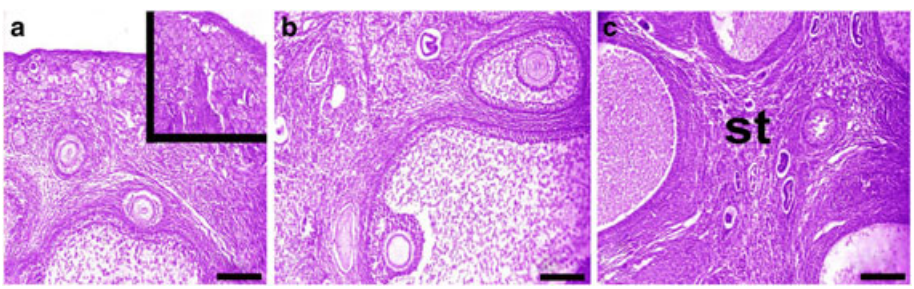

Figure 1 (a–c) Light microscopy images of ovaries in the control group, showing healthy ovarian stroma and follicle structures. st, stroma. Inset, primordial follicles. Scale bars, 60 μm.

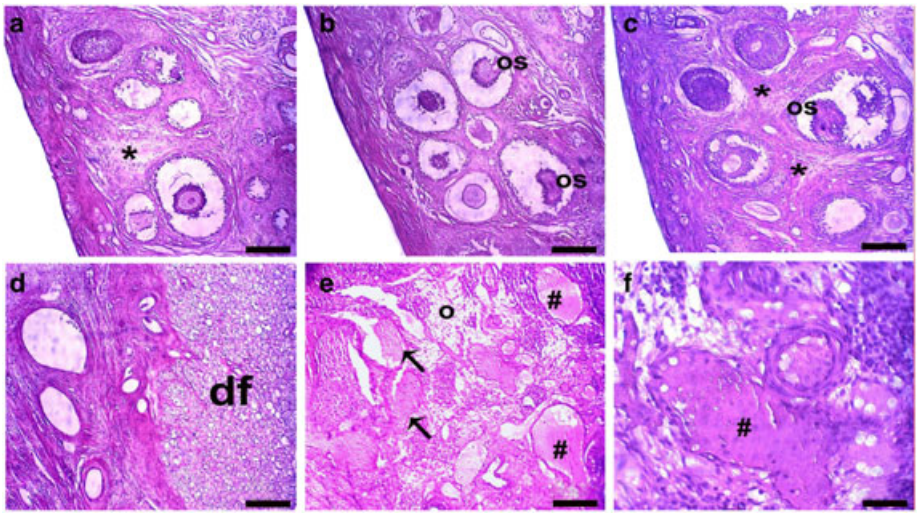

Figure 2 (a–f) Light microscopy images of ovaries in the HgO group. *Increased fibril content in the connective tissue areas; #fibrin content within the blood vessels. df, including follicle cells with hydropic degeneration; o, edema; os, irregular border of oocytes. Arrows, thinned wall structure of blood vessels. Scale bars: a–c, 60 μm; d–f, 40 μm.

The morphometric alterations in the architecture of follicles and ovary observed in the present study are in accordance with previous studies. It has been proved that reduction in gonad size is caused by exposure to Hg in female fish.[51] In the present study, mean volume of whole ovary, cortex, medulla, c. lutea and Graaf follicles were stereologically estimated on prepared sections for both the HgO and control groups. In the HgO group, all volumes were reduced in comparison with controls, indicating that Hg can affect the morphology and maturity of the c. lutea in the rat, and lead to a lack of follicular maturation and decrease in healthy follicles.

On histopathology, prepared slides of ovaries were evaluated to identify possible structural alterations. The results indicated that HgO exposure can affect the female reproductive system, due to the structural alterations caused by the accumulation of Hg in the ovarian tissue in the Hg group. Edema was also recognizable in the ovaries of the Hg group, along with mononuclear cell infiltration into the ovarian stroma and structural alterations of blood vessels. Hg can also affect the cardiovascular system and endothelial function.[52] Namely, Hg reduces endothelial formation, which is responsible for repair, resulting in endothelial dysfunction.[53] An increase in connective tissue fibrils were also found in the ovary of the HgO-treated group; this might be due to the physiological alterations of the fibril-producing cells at the outer part of the ovary. The tunica albuginea was thickened; with regard to blood vessels, capillaries and venous vessels were congested. In the walls of some large vessels, thinning and fibrin deposition were occasionally observed.

It has been reported that exposure to Hg caused changes in the hypothalamo-neurohypophyseal complex and arrest in oocyte development,[54] and apoptosis of ovarian granulosa cells.[55] In the present study, in the Hg group the follicles were not healthy, and had irregular follicle boundaries. Also, granulosa cells around the oocytes had undergone hydropic degeneration, and the boundaries of the oocytes located within the follicles were also irregular.

In conclusion, to our knowledge, this is the first study to estimate follicle number and volumes in rat ovary exposed to Hg, using stereological techniques. In the present study, we investigated the potential impact of HgO inhalation on both numerical and volumetric morphometrics of the ovary and their components, and also on histopathology. We found that female rat ovary was affected by HgO inhalation. Thereby, alterations of ovarian structures were correlated with HgO inhalation. Hence, exposure to HgO might have a toxic effect on the reproductive system in humans. Therefore, public health authorities should make all necessary efforts to limit the use of Hg.

Disclosure

The authors declare no conflicts of interest.

References

1. Yasutake A, Marumoto M, Yoshida M. Neurotoxic action of inorganic mercury injected in the intraventricular space of mouse cerebrum. *J Toxicol Sci* 2010; **35**: 767–771.
2. Iranmanesh M, Fatemi SJ, Golbafan MR, Balooch FD. Treatment of mercury vapor toxicity by combining deferasirox and deferiprone in rats. *Biometals* 2013; **26**: 783–788.
3. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Curr Top Med Chem* 2001; **1**: 529–539.
4. Stacchiotti A, Morandini F, Bettoni F *et al.* Stress proteins and oxidative damage in a renal derived cell line exposed to inorganic mercury and lead. *Toxicology* 2009; **264**: 215–269.
5. Zhao JQ, Wen YF, Bhadauria M *et al.* Protective effects of propolis on inorganic mercury induced oxidative stress in mice. *Indian J Exp Biol* 2009; **47**: 264–269.
6. Khayat A, Dencker L. Fetal uptake and distribution of metallic mercury vapor in the mouse: Influence of ethanol and aminotriazole. *Int J Biol Res Pregnancy* 1982; **3**: 38–46.
7. Saleh I, Coskun S, Mashhour A *et al.* Exposure to heavy metals (lead, cadmium and mercury) and its effect on the outcome of in-vitro fertilization treatment. *Int J Hyg Environ Health* 2008; **211**: 560–579.
8. Clarkson TW. The toxicology of mercury. *Crit Rev Clin Lab Sci* 1997; **34**: 369–403.
9. Schuurs AH. Reproductive toxicity of occupational mercury. A review of the literature. *J Dent* 1999; **27**: 249–256.
10. Sikorski R, Juszkiewicz T, Paszkowski T, Szprengier-Juszkiewicz T. Women in dental surgeries: Reproductive hazards in occupational exposure to metallic mercury. *Int Arch Occup Environ Health* 1987; **59**: 551–557.
11. De Rosis F, Anastasio SP, Selvaggi L, Beltrame A, Moriani G. Female reproductive health in two lamp factories: Effects of exposure to inorganic mercury vapour and stress factors. *Br J Ind Med* 1985; **42**: 488–494.
12. Colquitt PJ. The effect of occupational exposure to mercury vapour on the fertility of female dental assistants. *Occup Environ Med* 1995; **52**: 214.
13. Rowland AS, Baird DD, Weinberg 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.
14. Davis BJ, Price HC, O'Connor RW, Fernando R, Rowland AS, Morgan DL. Mercury vapor and female reproductive toxicity. *Toxicol Sci* 2001; **59**: 291–296.
15. Watanabe T, Shimada T, Endo A. Effects of mercury compounds on ovulation and meiotic and mitotic chromosomes in female golden hamsters. *Teratology* 1982; **25**: 381–384.
16. WHO. *Environmental Health Criteria 118 Inorganic Mercury.* Geneva: World Health Organization, 1991.
17. Morgan DL, Chanda SM, Price HC *et al.* Disposition of inhaled mercury vapor in pregnant rats: Maternal toxicity and effects on developmental outcome. *Toxicol Sci* 2002; **66**: 261–273.
18. Bancroft JD, Cook HC, Stirling RW. *Manual of Histological Techniques and their Diagnostic Applications*. London: Churchill Livingstone, 1994.
19. Iwata H, Hashimoto S, Ohota M, Kimura K, Shibano K, Miyake M. Effects of follicle size and electrolytes and glucose in maturation medium on nuclear maturation and developmental competence of bovine oocytes. *Reproduction* 2004; **127**: 159–164.
20. Mu X, Liao X, Chen X *et al.* DEHP exposure impairs mouse oocyte cyst breakdown and primordial follicle assembly through estrogen receptor-dependent and independent mechanisms. *J Hazard Mater* 2015; **298**: 232–240.
21. Altunkaynak BZ, Önger ME, Altunkaynak ME, Ayrancı E *et al.* Brief introduction to stereology and sampling strategies: Basic concepts of stereology. *NeuroQuantology* 2012; **10**: 31–43.
22. Cruz-Orive LM, Weibel ER. Recent stereological methods for cell biology: A brief survey. *Am J Physiol* 1990; **258**: 148–156.
23. Dursun H, Albayrak F, Uyanik A *et al.* Effects of hypertension and ovariectomy on rat hepatocytes. Are amlodipine and lacidipine protective? (A stereological and histological study). *Turk J Gastroenterol* 2010; **21**: 387–395.
24. Altunkaynak ME, Altunkaynak BZ, Unal D *et al.* A stereological and ultrastructural approach to fetal and newborn rat pancreas. *J Exp Clin Med* 2013; **30**: 147–151.
25. Altunkaynak BZ, Altunkaynak ME. Relationship of body weight and volume of liver. A morphometrical and stereological study. *Saudi Med J* 2007; **28**: 891–895.
26. Gundersen HJ, Bagger P, Bendtsen TF *et al.* The new stereological tools: Disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS* 1988; **96**: 857–881.
27. Unal B, Özbek ME, Aydin MD *et al.* Effect of haloperidol on the numerical density of neurons and nuclear height in the rat hippocampus: A stereological and histopathological study. *Neurosci Res Commun* 2004; **34**: 1–9.
28. Kaplan S, Gökyar A, Unal B, Tunç AT, Bahadir A, Aslan H. A simple technique for localizing consecutive fields for disector pairs in light microscopy: Application to neuron counting in rabbit spinal cord following spinal cord injury. *J Neurosci Methods* 2005; **45**: 277–284.
29. Thygesen LC, Flachs EM, Hanehøj K, Kjuus H, Juel K. Hospital admissions for neurological and renal diseases among dentists and dental assistants occupationally exposed to mercury. *Occup Environ Med* 2011; **68**: 895–901.
30. Jones L, Bunnell J, Stillman J. A 30-year follow-up of residual effects on New Zealand School Dental Nurses, from occupational mercury exposure. *Hum Exp Toxicol* 2007; **26**: 367–374.
31. Hsiao HW, Ullrich SM, Tanton TW. Burdens of mercury in residents of Temirtau, Kazakhstan I: Hair mercury concentrations and factors of elevated hair mercury levels. *Sci Total Environ* 2011; **409**: 2272–2280.
32. Jayaprakash K. Mercury vapor inhalation and its effect on glutathione peroxidase in goldsmiths exposed occupationally. *Toxicol Ind Health* 2009; **25**: 463–465.
33. Huang YL, Cheng SL, Lin TH. Lipid peroxidation in rats administrated with mercuric chloride. *Biol Trace Elem Res* 1996; **52**: 193–206.
34. Pal M, Ghosh M. Studies on comparative efficacy of a-linolenic acid and aeleostearic acid on prevention of organic mercury-induced oxidative stress in kidney and liver of rat. *Food Chem Toxicol* 2012; **50**: 1066–1072.
35. Belyaeva E, Korotkov SM, Saris NE. In vitro modulation of heavy metal induced rat liver mitochondria dysfunction: A comparison of copper and mercury with cadmium. *J Trace Elem Med Biol* 2011; **25S**: 63–73.

36. Hayes AD, Rothstein A. The metabolism of inhaled mercury vapor in the rat studied by isotope techniques. *J Pharmacol Exp Ther* 1962; **138**: 1–10.
37. Christie NT, Cantoni O, Evans M, Meyn RE, Costa M. Use of mammalian DNA repair-deficient mutants to assess the effects of toxic metal compounds on DNA. *Biochem Pharmacol* 1984; **33**: 1661–1670.
38. Cantoni O, Costa M. Correlations of DNA strand breaks and their repair with cell survival following acute exposure to mercury (II) and X-rays. *Mol Pharmacol* 1983; **24**: 84–89.
39. Stadnicka A. Localization of mercury in the rat ovary after oral administration of mercuric chloride. *Acta Histochem* 1980; **67**: 227–233.
40. 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**: 205–214.
41. Nylander M. Relation between mercury and selenium in pituitary glands of dental staff. *Br J Ind Med* 1989; **46**: 751–752.
42. Oliveira FR, Ferreira JR, dos Santos CM *et al*. Estradiol reduces cumulative mercury and associated disturbances in the hypothalamus-pituitary axis of ovariectomized rats. *Ecotoxicol Environ Saf* 2006; **63**: 488–493.
43. Drevnick PE, Sandheinrich MB. Effects of dietary methyl mercury on reproductive endocrinology of fathead minnows. *Environ Sci Technol* 2003; **2003**: 4390–4396.
44. Wood AW, Van Der Kraak G. Inhibition of apoptosis in vitellogenic ovarian follicles of rainbow trout (Oncorhynchus mykiss) by salmon gonadotropin, epidermal growth factor, and 17β-estradiol. *Mol Reprod Dev* 2002; **61**: 511–518.
45. Lamperti AA, Printz RH. Effects of mercuric chloride on the reproductive cycle of the female hamster. *Biol Reprod* 1973; **8**: 378–387.
46. Kirubagaran R, Joy KP. Changes in lipid profiles and 32P-uptake into phosphoprotein (vitellogenin) content of the ovary and liver in the female catfish, Clarias batrachus, exposed to mercury. *Biomed Environ Sci* 1995; **8**: 35–44.
47. Zhu X, Kusaka Y, Sato K, Zhang Q. The endocrine disruptive effects of mercury. *Environ Health Prev Med* 2000; **4**: 174–183.
48. Drevnick PE, Sandheinrich MB, Oris JT. Increased ovarian follicular apoptosis in fathead minnows (Pimephales promelas) exposed to dietary methylmercury. *Aquat Toxicol* 2006; **79**: 49–54.
49. Holden PR. Toxic mechanisms mediated by gene expression. In: Roberts RA (ed). *Apoptosis in Toxicology*. London: Taylor and Francis, 2000; 187–211.
50. Al-Saleh I, Al-Rouqi R, Obsum CA *et al*. Mercury (Hg) and oxidative stress status in healthy mothers and its effect on birth anthropometric measures. *Int J Hyg Environ Health* 2014; **217**: 567–585.
51. Friedmann AS, Watzin MC, Brinck-Johnson T, Leiter JC. Low levels of dietary methyl mercury inhibit growth and gonadal development in juvenile walleye (Stizostedion vitreum). *Aquat Toxicol* 1996; **35**: 265–278.
52. Omanwar S, Fahim M. Mercury exposure and endothelial dysfunction: An interplay between nitric oxide and oxidative stress. *Int J Toxicol* 2015; **34**: 300–307.
53. Kishimoto T, Oguri T, Abe M, Kajitani H, Tada M. Inhibitory effect of methyl mercury on migration and tube formation by cultured human vascular endothelial cells. *Arch Toxicol* 1995; **69**: 357–361.
54. Ram RN, Joy KP. Mercurial induced changes in the hypothalamo-neurohypophysical complex in relation to reproduction in the teleostean fish, Channa punctatus (Bloch). *Bull Environ Contam Toxicol* 1988; **41**: 329–336.
55. Kolesarova A, Roychoudhury S, Slivkova J, Sirotkin A, Capcarova M, Massanyi P. In vitro study on the effects of lead and mercury on porcine ovarian granulosa cells. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 2010; **45**: 320–331.