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# Sex differences in renal handling of inorganic mercury in aged rats

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ABSTRACT

The sex of an individual/animal has been shown to play an important role in many biological processes. Furthermore, sex may also be a factor in the way environmental toxicants, such as heavy metals, are handled by organisms. However, the effect of sex on the handling and disposition of heavy metals, such as mercury (Hg), has not been shown. Aging has also been shown to be a factor in the accumulation of heavy metals in that older individuals tend to have higher burdens of these metals. Therefore, the purpose of the current study was to evaluate the effect of sex on the accumulation of mercury in aged animals. Aged male and female rats were injected intravenously with 0.5 μmol or 2.0 μmol·kg$^{-1}$ $HgCl_2$ (containing radioactive Hg) and organs were harvested after 24 h. In general, the renal accumulation of Hg was significantly greater in males than in females. Similarly, urinary excretion of Hg was greater in males than in females. There were no significant differences between males and females in the burden of Hg in other organs. Sex differences in the renal accumulation of Hg may be related to differences in the expression of membrane transporters involved in the uptake of mercuric species into tubular epithelial cells. The results of the current study illustrate the need to evaluate both sexes when assessing the renal effects of environmental toxicants.

## 1. Introduction

The sex of an organism has been recognized recently as an important factor in numerous physiological and pathological processes (Clayton, 2018; Li et al., 2018). Indeed, recent studies have identified sex differences in expression of various transport proteins, including glucose transporters (Nagai et al., 2014), organic anion transporting polypeptides (OATP) (Brzica et al., 2018), and organic anion transporters (OAT) 1 and 3 (Breljak et al., 2013). In addition, sex differences in response to exposure to toxicants such as cadmium, lead, and mercury have been reported (Vahter et al., 2007; Hazelhoff et al., 2018).

Mercury (Hg) is a prevalent environmental toxicant to which humans are exposed through occupational, dietary, and environmental routes. The primary route of mercury exposure in most humans is ingestion of methylmercury or inhalation of mercury vapor; however, it is important to consider that once ingested and absorbed, a fraction of the absorbed mercury is biotransformed to inorganic mercury ($Hg^{2+}$) (Norseth and Clarkson, 1970a, 1970b). $Hg^{2+}$ is a major nephrotoxicant in adults, children, and fetuses and chronic exposure can lead to significant accumulation of $Hg^{2+}$ within target organs and tissues (Bridges and Zalups, 2017a; Oliveira et al., 2015). Therefore, it is important to understand the disposition of $Hg^{2+}$ within biological systems. Analyses of the U.S. National Health and Nutrition Examination Survey (NHANES) population indicate that blood and urinary concentrations of mercury and other heavy metals is greater in males than females (Mortensen et al., 2014; Shim et al., 2017). Exposure to mercury has been shown to alter the renal and hepatic expression of membrane transporters differently in males and females but differences in the renal and hepatic burden of Hg between sexes have not been studied (Hazelhoff et al., 2018; Hazelhoff and Torres, 2018).

Accumulation of toxic heavy metals such as Hg, has been shown to be greater in older individuals (Mortensen et al., 2014; Shim et al., 2017; Wang et al., 2014; Wang et al., 2018). These findings may be due to a general decline in renal health, chronic exposure to various forms of mercury, biotransformation to $Hg^{2+}$, and consequent accumulation in target organs. In addition, kidneys of older individuals appear to be more susceptible to toxic effects of these metals (Bridges and Zalups, 2017b). Similar to human studies, published studies in rats have shown that renal accumulation of Hg is greater in older rats (Bridges et al., 2014; Oliveira et al., 2016). Interestingly, sex has been shown to be a significant factor in comorbidities associated with aging (Kiely et al., 2019; Gordon et al., 2017). Therefore, it is important to characterize the effect of sex on the handling of heavy metals in an aged population. The current study was designed to test the hypothesis that sex plays a role in the disposition and accumulation of Hg in aged rats.

## 2. Materials and methods

Adult Wistar rats, approximately 20 months old (600–800 g), were obtained from our breeding colony housed in Mercer University School of Medicine vivarium. Animals used for this study were excess stock from previous experiments and were aged to 20 months for the purpose of this study. Animal health was monitored daily and evaluated based on

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**Table 1**
Body and organ weights for male and female rats.

| | Total renal mass (g) | Cortex (g) | OSOM (g) | ISOM (g) | IM (g) |
|---|---|---|---|---|---|
| Male | 4.13 ± 0.3 | 0.093 ± 0.013 | 0.046 ± 0.006 | 0.021 ± 0.004 | 0.013 ± 0.003 |
| Female | 3.02 ± 0.2* | 0.063 ± 0.006 | 0.040 ± 0.004 | 0.018 ± 0.001 | 0.014 ± 0.001 |
| | Body weight (g) | Liver (g) | Spleen (g) | Heart (g) | Blood (mL) |
| Male | 752.5 ± 41.7 | 17.9 ± 1.0 | 1.25 ± 0.1 | 2.04 ± 0.2 | 45.15 ± 2.5 |
| Female | 679.4 ± 50.2 | 15.3 ± 1.1 | 0.80 ± 0.04* | 1.51 ± 0.1 | 40.76 ± 3.0 |

* Significantly different ($p < 0.05$) from same organ in males.

appearance, appetite, and mobility. Animals that appeared frail or lacked adequate mobility were euthanized and were not included in the study. Throughout the experiment, rats were provided a standard laboratory diet (Tekland 6% rat diet, Envigo Laboratories) and water *ad libitum*. The animal protocol was approved by the Institutional Animal Care and Use Committee under the guidelines of the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

Adult male and female Wistar rats were exposed to either a non-nephrotoxic dose of $HgCl_2$ (0.5 μmol (0.13 mg)·kg$^{-1}$ in 2 mL saline) or a nephrotoxic dose of $HgCl_2$ (2.0 μmol (0.52 mg)·kg$^{-1}$ in 2 mL saline) by intravenous injection into the femoral vein under anesthesia induced by 5% isoflurane (Bridges et al., 2008). Each injection also contained 1 μCi of radioactive mercury ([$^{203}$Hg]). [$^{203}$Hg] was made at the Missouri University Research Reactor as described previously (Bridges et al., 2004). Twenty-four hours after injection with $HgCl_2$, rats were euthanized with an intraperitoneal injection of ketamine and xylazine (70/30 mg kg$^{-1}$). The kidneys, liver, heart, spleen, and blood were collected from each rat for determination of [$^{203}$Hg] content (Bridges et al., 2008). Body and organ weights are included in Table 1. Each kidney was trimmed of fat and fascia, weighed, and cut in half along the mid-transverse plane from the hilum to the lateral margin. A 3-mm transverse slice was obtained from one-half of the left kidney and was used for dissection of renal zones (cortex, outer stripe of the outer medulla (OSOM), inner stripe of the outer medulla (ISOM), and inner medulla (IM)). The remaining half was weighed and placed in a polystyrene tube for determination of Hg content. Both halves of the right kidney were weighed and placed in individual polystyrene tubes for determination of Hg content. Samples were counted for 5 min each in a Wallac Wizard 3 automatic gamma counter (Perkin Elmer, Boston, MA).

The amount of Hg in each sample was calculated using standard computational methods. Six "standards" containing 0.1 mL each of the injection solution (25 nmol or 100 nmol per 0.1 mL) were counted in the gamma counter to obtain counts per minute (cpm). A mean was calculated and using the known concentration of the standard, cpm/nmol was calculated according to the following formula: standard cpm in 0.01 mL ÷ nmol in 0.1 mL. The following formula was used to calculate nmol in each tissue sample: sample cpm ÷ cpm/nmol. All data were analyzed with a one-way ANOVA followed by Tukey's multiple comparison test. A *p*-value of $< 0.05$ was considered to be statistically significant. Each group of animals contained 4 rats. Data are expressed as mean ± SE.

## 3. Results

The amount of Hg detected in each organ and tissue collected is shown in Table 2. The accumulation of Hg in liver, heart, spleen, and blood was not significantly different between males and females exposed to the same dose of Hg.

When rats were injected with a non-nephrotoxic dose of $HgCl_2$ (0.5 μmol·kg$^{-1}$), there was no difference in the amount of Hg in the renal cortex between male and female rats (Fig. 1A). In contrast, when rats were injected with a nephrotoxic dose of $HgCl_2$ (2.0 μmol·kg$^{-1}$), the amount of Hg in the renal cortex of male rats was significantly greater than that of female rats (Fig. 1A). Similarly, the amount of Hg in the outer stripe of the outer medulla (OSOM) was greater in male rats than in corresponding female rats at both doses (Fig. 1B).

Urinary excretion of Hg was also greater in male rats than in females (Fig. 2). The urinary excretion of Hg from rats exposed to 0.5 μmol $HgCl_2$ appeared to be greater in males than females, but the difference was not significantly significant. However, in rats exposed to the 2.0 μmol dose, the urinary excretion of Hg was significantly greater in males than in females.

## 4. Discussion

The current data provide important information regarding the effect of sex on mercury handling in aged rats. These data show that the renal burden of Hg was affected significantly by the sex of the animal. The present findings may be due to sex-related differences in the expression of the organic anion transporters (Oat) 1 and 3, which have been shown to mediate the basolateral uptake of mercuric species into proximal tubular cells (Aslamkhan et al., 2003).

Studies in human kidney have shown that male rats have greater levels of OAT1 expression while OAT 3 expression appears to be greater in females (Buist and Klaassen, 2004). Furthermore, studies in Oat1 knockout rats suggest that Oat 1 plays a larger role in the uptake of Hg than Oat 3 (Torres et al., 2011). Therefore, we suggest that the enhanced expression of Oat 1 in male rats is a major contributor to the enhanced renal

**Table 2**
Amount of Hg detected in each organ or tissue from male and female rats exposed to 0.5 μmol or 2.0·μmol kg$^{-1}$ $HgCl_2$. *, significantly different ($p < 0.05$) than males exposed to same dose.

| | Total renal mass (nmol/g) | Cortex (nmol/g) | OSOM (nmol/g) | ISOM (nmol/g) | IM (nmol/g) |
|---|---|---|---|---|---|
| Male 0.5 μmol | 61.12 ± 7.91 | 66.84 ± 7.9 | 97.25 ± 6.1 | 17.93 ± 1.1 | 0.6 ± 0.1 |
| Female 0.5 μmol | 69.41 ± 4.23 | 86.88 ± 4.7 | 43.37 ± 8.36* | 4.27 ± 1.7* | 0.5 ± 0.06 |
| Male 2.0 μmol | 163.02 ± 16.4 | 197.76 ± 18.1 | 198.06 ± 5.74 | 48.69 ± 21.5 | 1.08 ± 0.2 |
| Female 2.0 μmol | 98.58 ± 4.79* | 127.74 ± 4.8* | 138.37 ± 10.1* | 36.87 ± 2.0 | 0.69 ± 0.05 |
| | Liver (nmol/g) | Spleen (nmol/g) | Heart (nmol/g) | Blood (nmol/g) | Urine (nmol/mL) |
| Male 0.5 μmol | 1.35 ± 0.21 | 1.17 ± 0.2 | 0.18 ± 0.02 | 0.28 ± 0.02 | 0.12 ± 0.05 |
| Female 0.5 μmol | 0.98 ± 0.09 | 0.83 ± 0.15 | 0.16 ± 0.02 | 0.28 ± 0.06 | 0.03 ± 0.01 |
| Male 2.0 μmol | 6.43 ± 0.43 | 7.44 ± 1.54 | 0.91 ± 0.14 | 1.54 ± 0.18 | 0.587 ± 0.01 |
| Female 2.0 μmol | 6.23 ± 0.51 | 7.92 ± 1.51 | 0.75 ± 0.07 | 1.81 ± 0.18 | 0.11 ± 0.03* |

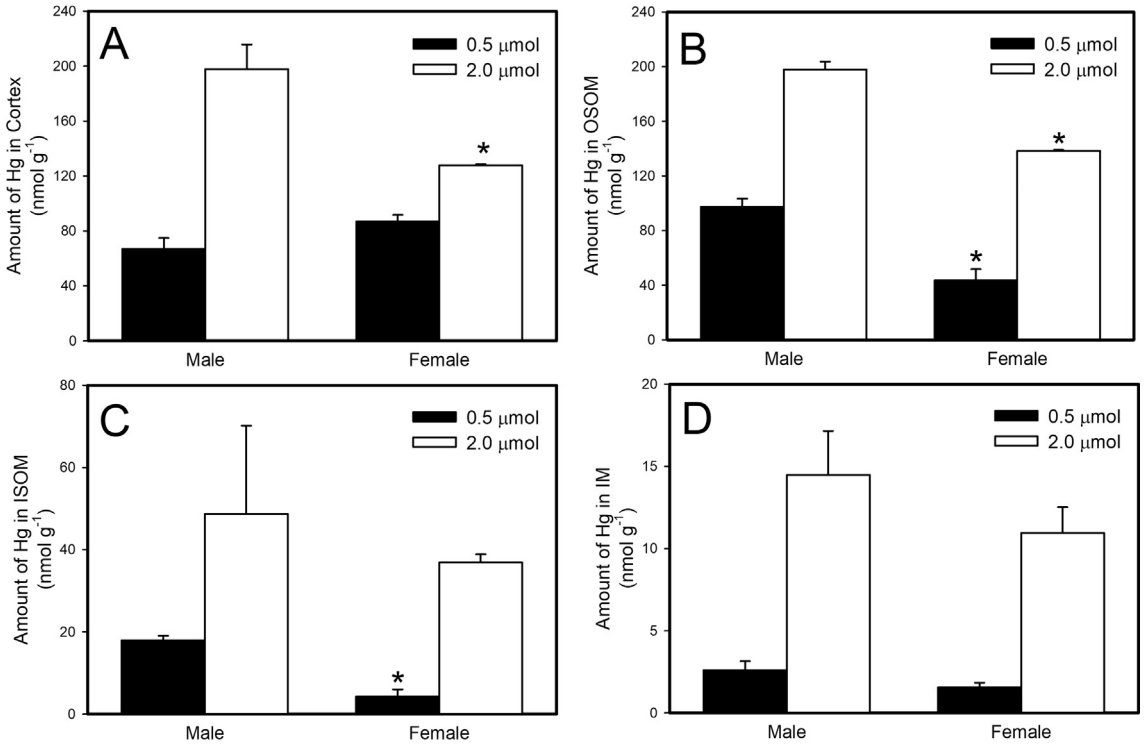


**Fig. 1.** Amount of Hg in zones of the kidney. The amount of Hg in the cortex (A) was significantly lower in female rats than males when rats were exposed to a nephrotoxic dose (2.0 μmol·kg$^{-1}$) of $HgCl_2$ but not when rats were exposed to a non-nephrotoxic dose (0.5 μmol·kg$^{-1}$). In contrast, the amount of Hg in the outer stripe of the outer medulla (OSOM; B) was significantly lower in females exposed to both doses of $HgCl_2$ than in corresponding males. Interestingly, the amount of Hg in the inner stripe of the outer medulla (ISOM; C) was significantly lower in female rats exposed to 0.5 μmol·kg$^{-1}$ dose than in corresponding male rats. There was no difference between sexes in rats exposed to the 2.0 μmol·kg$^{-1}$ dose. The amount of Hg that accumulated in the inner medulla (IM; D) was not significantly different between males and females exposed to either dose. ∗ = significantly different ($p < 0.05$) than mean of corresponding group of male rats. $n = 4$.

accumulation of Hg in males. Exposure of Wistar rats to $HgCl_2$ was found to increase the expression of Oat 1 in male and female rats while expression of Oat 3 was increased only in females (Hazelhoff et al., 2018). The magnitude of the increase was greater in females but this increase was offset by a corresponding increase in the multidrug resistance-associated protein (Mrp) 2 (Hazelhoff et al., 2018), which mediates the export of mercuric ions into the tubular lumen (Bridges et al., 2008). These studies further support the current theory that sex-specific expression of Oat 1 and Oat 3 account for the differences in Hg accumulation between male and female rats.

Oat 1 and Oat 3 are both highly expressed in the renal cortex, which may explain why no differences in cortical Hg accumulation were observed between males and females exposed to the lower dose of Hg (0.5 μmol). However, exposure to the higher dose of Hg (2.0 μmol) may lead to greater sex-related differences in the expression of Oat 1 and Oat 3 and these differences in expression are likely the basis of the sex-related differences in Hg accumulation. In the OSOM, the expression of Oat 1 has been shown to be greater than that of Oat3 (Lungkaphin et al., 2006); which may account for the finding that the accumulation of Hg (at both doses) was greater in the OSOM of male rats compared with that of female rats.

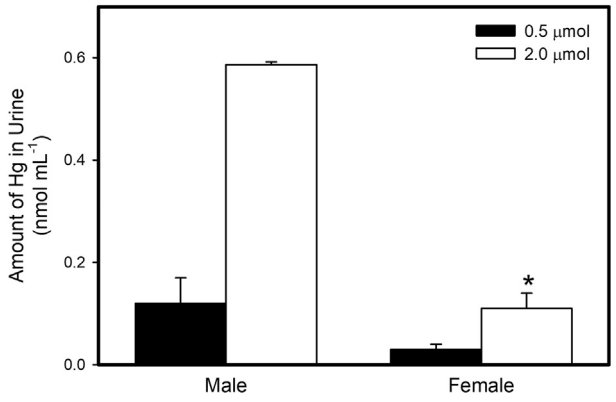


**Fig. 2.** Urinary excretion of Hg. The amount of Hg excreted in urine of female rats exposed to the 2.0 μmol·kg$^{-1}$ dose of $HgCl_2$ was significantly lower than that of corresponding male rats. Interestingly, there was no difference between sexes in urinary excretion of Hg in rats exposed to 0.5 μmol·kg$^{-1}$ $HgCl_2$. ∗ = significantly different ($p < 0.05$) than mean of corresponding group of male rats. n = 4.

The amount of Hg in the inner stripe of the outer medulla (ISOM; Fig. 1C) was significantly greater in males exposed to the 0.5 μmol dose of $HgCl_2$ than in corresponding females. When rats were exposed to the 2.0 μmol dose, the accumulation in the ISOM of male rats tended to be greater than that of females but the difference was not statistically different (Fig. 1C). Accumulation of Hg in the ISOM likely represents the content of Hg in blood of the peritubular capillaries and vasa recta. Similarly, the amount of Hg detected in the inner medulla (IM) (Fig. 1D) is likely representative of the hematologic content of Hg in that zone.

In contrast, the accumulation of Hg in other organs and tissues (liver, heart, spleen, and blood) was not significantly different between male and female rats. This was somewhat surprising since we expected the lower renal content of Hg in females to lead to elevated blood content of Hg in those animals. Furthermore, a recent analysis of human data also reported no significant difference in blood content between older males and females (Valcke et al., 2019). In females, Hg may have been delivered to reproductive organs (uterus, ovaries) and abdominal fat, which is usually greater in females. Accumulation of Hg in these areas should be an area of future study.

In summary, the current study shows significant differences in the renal accumulation of Hg between male and female aged rats. These differences are likely due to sex-specific differences in physiological mechanisms and/or expression of transport proteins such as Oat 1 and Oat 3. The current study is important in that it demonstrates that sex may play a major role in the handling of nephrotoxicants and other xenobiotics.

## CRediT authorship contribution statement

**Elizabeth H. Pittman:**Investigation, Writing - original draft.**Nigel D'Souza:**Investigation.**Taylor N. Mathis:**Investigation.**Lucy Joshee:**Investigation, Supervision, Formal analysis.**Jennifer L. Barkin:**Data curation, Formal analysis, Writing - review & editing.**Christy C. Bridges:**Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing - review & editing.

## Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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## References

Aslamkhan, A.G., Han, Y.H., Yang, X.P., Zalups, R.K., Pritchard, J.B., 2003. Human renal organic anion transporter 1-dependent uptake and toxicity of mercuric-thiol conjugates in Madin-Darby canine kidney cells. Mol. Pharmacol. 63 (3), 590–596.

Breljak, D., Brzica, H., Sweet, D.H., Anzai, N., Sabolic, I., 2013. Sex-dependent expression of Oat3 (Slc22a8) and Oat1 (Slc22a6) proteins in murine kidneys. Am. J. Physiol. Ren. Physiol. 304 (8), F1114–F1126.

Bridges, C.C., Zalups, R.K., 2017a. Mechanisms involved in the transport of mercuric ions in target tissues. Arch. Toxicol. 91 (1), 63–81.

Bridges, C.C., Zalups, R.K., 2017b. The aging kidney and the nephrotoxic effects of mercury. J. Toxicol. Environ. Health B Crit. Rev. 20 (2), 55–80.

Bridges, C.C., Bauch, C., Verrey, F., Zalups, R.K., 2004. Mercuric conjugates of cysteine are transported by the amino acid transporter system b(0,+): implications of molecular mimicry. J. Am. Soc. Nephrol. 15 (3), 663–673.

Bridges, C.C., Joshee, L., Zalups, R.K., 2008. Multidrug resistance proteins and the renal elimination of inorganic mercury mediated by 2,3-dimercaptopropane-1-sulfonic acid and meso-2,3-dimercaptosuccinic acid. J. Pharmacol. Exp. Ther. 324 (1), 383–390.

Bridges, C.C., Joshee, L., Zalups, R.K., 2014. Aging and the disposition and toxicity of mercury in rats. Exp. Gerontol. 53, 31–39.

Brzica, H., Abdullahi, W., Reilly, B.G., Ronaldson, P.T., 2018. Sex-specific differences in organic anion transporting polypeptide 1a4 (Oatp1a4) functional expression at the blood-brain barrier in Sprague-Dawley rats. Fluids Barriers CNS 15 (1), 25.

Buist, S.C., Klaassen, C.D., 2004. Rat and mouse differences in gender-predominant expression of organic anion transporter (Oat1-3; Slc22a6-8) mRNA levels. Drug Metab. Dispos. 32 (6), 620–625.

Clayton, J.A., 2018. Applying the new SABV (sex as a biological variable) policy to research and clinical care. Physiol. Behav. 187, 2–5.

Gordon, E.H., Peel, N.M., Samanta, M., Theou, O., Howlett, S.E., Hubbard, R.E., 2017. Sex differences in frailty: a systematic review and meta-analysis. Exp. Gerontol. 89, 30–40.

Hazelhoff, M.H., Torres, A.M., 2018. Gender differences in mercury-induced hepatotoxicity: potential mechanisms. Chemosphere 202, 330–338.

Hazelhoff, M.H., Bulacio, R.P., Chevalier, A., Torres, A.M., 2018. Renal expression of organic anion transporters is modified after mercuric chloride exposure: gender-related differences. Toxicol. Lett. 295, 390–396.

Kiely, K.M., Brady, B., Byles, J., 2019. Gender, mental health and ageing. Maturitas 129, 76–84.

Li, Q., McDonough, A.A., Layton, H.E., Layton, A.T., 2018. Functional implications of sexual dimorphism of transporter patterns along the rat proximal tubule: modeling and analysis. Am. J. Physiol. Ren. Physiol. 315 (3), F692–F700.

Lungkaphin, A., Lewchalermwongse, B., Chatsudthipong, V., 2006. Relative contribution of OAT1 and OAT3 transport activities in isolated perfused rabbit renal proximal tubules. Biochim. Biophys. Acta 1758 (6), 789–795.

Mortensen, M.E., Caudill, S.P., Caldwell, K.L., Ward, C.D., Jones, R.L., 2014. Total and methyl mercury in whole blood measured for the first time in the U.S. population: NHANES 2011–2012. Environ. Res. 134, 257–264.

Nagai, K., Yoshida, S., Konishi, H., 2014. Gender differences in the gene expression profiles of glucose transporter GLUT class I and SGLT in mouse tissues. Pharmazie 69 (11), 856–859.

Norseth, T., Clarkson, T.W., 1970a. Studies on the biotransformation of 203Hg-labeled methyl mercury chloride in rats. Arch. Environ. Health 21 (6), 717–727.

Norseth, T., Clarkson, T.W., 1970b. Biotransformation of methylmercury salts in the rat studied by specific determination of inorganic mercury. Biochem. Pharmacol. 19 (10), 2775–2783.

Oliveira, C.S., Joshee, L., Zalups, R.K., Pereira, M.E., Bridges, C.C., 2015. Disposition of inorganic mercury in pregnant rats and their offspring. Toxicology 335, 62–71.

Oliveira, C.S., Joshee, L., Zalups, R.K., Bridges, C.C., 2016. Compensatory renal hypertrophy and the handling of an acute nephrotoxicant in a model of aging. Exp. Gerontol. 75, 16–23.

Shim, Y.K., Lewin, M.D., Ruiz, P., Eichner, J.E., Mumtaz, M.M., 2017. Prevalence and associated demographic characteristics of exposure to multiple metals and their species in human populations: the United States NHANES, 2007–2012. J. Toxicol. Environ. Health A 80 (9), 502–512.

Torres, A.M., Dnyanmote, A.V., Bush, K.T., Wu, W., Nigam, S.K., 2011. Deletion of multispecific organic anion transporter Oat1/Slc22a6 protects against mercury-induced kidney injury. J. Biol. Chem. 286 (30), 26391–26395.

Vahter, M., Akesson, A., Liden, C., Ceccatelli, S., Berglund, M., 2007. Gender differences in the disposition and toxicity of metals. Environ. Res. 104 (1), 85–95.

Valcke, M., Ouellet, N., Dube, M., Laouan Sidi, E.A., LeBlanc, A., Normandin, L., Balion, C., Ayotte, P., 2019. Biomarkers of cadmium, lead and mercury exposure in relation with early biomarkers of renal dysfunction and diabetes: results from a pilot study among aging Canadians. Toxicol. Lett. 312, 148–156.

Wang, X., Bonventre, J.V., Parrish, A.R., 2014. The aging kidney: increased susceptibility to nephrotoxicity. Int. J. Mol. Sci. 15 (9), 15358–15376.

Wang, X., Mukherjee, B., Park, S.K., 2018. Associations of cumulative exposure to heavy metal mixtures with obesity and its comorbidities among U.S. adults in NHANES 2003–2014. Environ. Int. 121 (Pt 1), 683–694.