# SEX DIFFERENCES IN PARAOXONASE ACTIVITY IN SUB-CHRONIC INORGANIC MERCURY EXPOSURE

**Wusu A. D.[1], Ogunrinola O. O.[1], Afolabi O. K.[1]Abam E. O.[1], Babayemi D. O.[1], Balogun E. A.[1],Odukoya O. O.[2] and Ademuyiwa O.[1]**

[1]Biochemistry Department and [2]Chemistry Department, University of Agriculture, Abeokuta, Nigeria

;

(+234)8026585027

Abstract ID 1527
Poster Board : 586

## ABSTRACT

Epidemiological evidences suggest an increased risk of cardiovascular diseases (CAD) as a result of inorganic mercury exposure. The underlying mechanisms underpinning this risk, as well as sex differences in response to mercury exposure, are not yet understood. Paraoxonase (PONase), an enzyme located in the high-density–lipoprotein (HDL) has been shown to protect against CAD. In order to investigate the association between inorganic mercury exposure and CAD, male and female rats were exposed to mercury (0.5, 1.0 and 1.5mg/kg) for 12 weeks. PON activities towards paraoxon (PONase) and phenylacetate (AREase) in plasma, lipoproteins, hepatic and brain microsomal fractions were determined using standard methods. Inhibition of PONase and activation of AREase in plasma and HDL characterised the effects of inorganic mercury in both sexes. Inorganic mercury exposure inhibited PONase by 63% (plasma) and 67% (HDL) respectively in male animals, whereas the female enzyme was inhibited by 80 and 47% respectively. AREase activity was activated by 55 and 53% in male, whereas the activation in female amounted to 25 and 49% respectively. In the VLDL, inorganic mercury inhibited PONase in both sexes whereas AREase was activated in female animals but inhibited in male. In the hepatic microsomal fractions, only the PONase enzyme was inhibited in male animals whereas in female, activation was observed in both enzymes at the highest dose of inorganic mercury. Brain microsomal cholesterol was increased in male but decreased in female by inorganic mercury resulting in altered cholesterol/phospholipid ratios. Our findings indicate that inorganic mercury exposure exerts an inhibitory effect on PONase but activated AREase. Modulation of PON activity may be an early biochemical step in the induction of CAD by mercury. This may also be mediated through changes in membrane fluidity brought about by changes in the concentration of cholesterol in the microsomes.

## INTRODUCTION

Mercury is one of the heavy metals known to be toxic for humans and other organisms (WHO, 1991). Originating from occupational or environmental sources, mercury is present in different industrial settings, in the air, as well as in drinking water and food, resulting in continuous exposure of the whole human population. It is found in both inorganic and organic forms and in different valence or oxidation states in the environment.

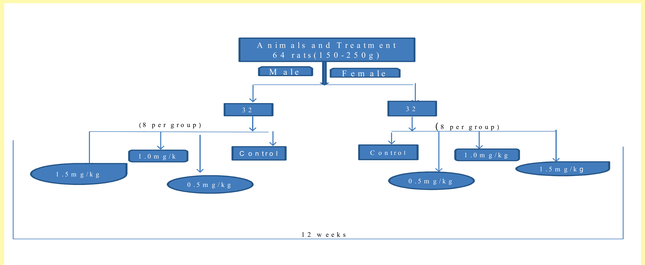
Mercury exposure results in a variety of adverse health effects including neurological, renal, respiratory, immune, dermatological, reproductive and developmental sequela (Risher et al., 2005; Sharma et al., 2007). Recent evidences also show that mercury causes severe oxidative damages (Kim et al., 2005). Mercuric chloride ($HgCl_2$) is a widespread environmental and industrial pollutant, which induces severe alterations in the tissues of both animals and men (Lund et al., 1993; Mahboob et al., 2001). One of the harmful effects of mercury action during its accumulation in a body in a region contaminated by mercury is the excessive release of reactive oxygen species and increased lipid peroxidation in the cells (Lund et al., 1993; Miller et al., 1993).

Paraoxonase (PON; aryldialkylphosphatase E.C. 3.1.8.1) is a Ca2+-dependent liver and plasma esterase that hydrolyses active metabolites of several organophosphorus insecticides (Costa et al., 2005a). It is also present in kidney, heart, brain and the lungs. Its role in these organs remains unknown (Rodrigo et al., 2001), although it has been postulated that it might be acting as an anti-oxidant enzyme in these organs (Rodrigo et al., 2001). PON is transported in plasma as a component of HDL. Recent interest in this enzyme derives from the observation that it might be involved in lipid metabolism by inhibiting oxidative modifications of LDL and HDL, thus protecting against the development of atherosclerosis (Costa et al., 2005ab). PON also metabolises oxidized phospholipids and destroys lipid hydroperoxides, thus functioning as an antioxidant enzyme on HDL (Costa et al.,2005ab).

Long ago, numerous reports have appeared in the literature indicating the involvement of PON in cardiovascular diseases (Jarvik et al., 2000; Mackness et al. 2003; Khan et al., 2009). Given the association between mercury exposure and cardiovascular diseases and PON and cardiovascular diseases, it seems sensible to assume that the association between mercury poisoning and cardiovascular diseases may be mediated at the level of PON. The work reported in this paper explored this hypothesis.

## Materials and methods

**Chemicals**
Mercury chloride was products of Sigma-Aldrich, Missouri, USA.




**Mercury determination**

Whole blood (0.2ml) was digested in nitric and sulphuric acid mixture. Total mercury was determined using Inductively-coupled plasma mass spectrometry (ICP-MS). Results are expressed as µg Hg/ml

**Determination of PON enzyme activities in plasma, HDL and VLDL**

PON enzyme activities were measured against two different substrates (paraoxon and phenylacetate). PON activity towards phenylacetate (AREase) was determined in plasma in 100mM Tris-acetate buffer pH 7.4 containing 10mM calcium chloride as described by Junge and Klees (1984). The rate of phenol generation was monitored at 270nm and 25°C. A molar extinction coefficient of 148 was used to calculate enzyme activity.

HDL and VLDL+LDL were obtained from plasma following precipitation with heparin-MnCl2 solution as described by Gidez et al. (1982). The VLDL+LDL precipitates were then brought to the original plasma volume by addition of 100mM Tris-acetate buffer pH 7.4 after which the PON activity associated with the lipoprotein fractions were determined as described for plasma

PON activity towards paraoxon (PONase) was determined as described by Furlong et al. (1989). Briefly, paraoxon (1.2mM) freshly prepared in 50mM Tris-HCl buffer pH 8.5 containing 1.32mM CaCl2 was incubated at 37°C with appropriate volumes of either plasma, HDL or VLDL. The liberation of p-nitrophenol upon enzymatic hydrolysis of paraoxon was measured spectrophotometrically at 405nm

**Determination of tissue PON activities**

In the tissues, PON is associated with the microsomal fraction . Thus microsomal fractions were obtained from liver and brain as described by Gonzalvo et al. (1997). Microsomal fractions derived from each tissue were then solubilised by adding 0.75% Triton X-100. After vortex-mixing, they were stored at 4°C in an ice bath for 30minutes and thereafter centrifuged at 105000xg for 60minutes at 4°C. The resultant supernatant fraction was used for determination of PON activities towards

**Extraction and determination of lipids in microsomal fractions.**

Lipids were extracted from microsomal fractions using the method of Folch *et al.* (1957) with a slight modification. A 10% microsomal homogenate was prepared in chloroform-isopropanol (7:11, v/v) and vortexes every 5 minutes for 30minutes at room temperature. After centrifugation at 4°C, the supernatant was washed with ice-cold 0.05M KCl. The mixture was centrifuged again and the chloroform layer taken into Eppendorf tubes for lipid analyses. Cholesterol, triglyceride and phospholipids were determined as described by Allain *et al.* (1974), Kriketos *et al.* (2003) and Stewart (1979).

**Statistical analysis**

Results are expressed as mean ± SEM. One way analysis of variance (ANOVA) followed by Tukey's test and t-test was used to analyze the results with $p<0.05$ considered significant. Associations among the parameters and their magnitudes were tested for by using Multiple Linear Regression analysis.

## *RESULTS*

Exposure of the animals to inorganic mercury resulted in an accumulation of mercury in blood, although the accumulation was found to be more in female than in male animals in a dose-dependent manner (Table 1).

Mercury exposure resulted in a significant inhibition ($p < 0.05$) of the activity of PONase in the plasma of female than in male animals and in the HDL, more in male than female (Figure 1). Mercury resulted in activation of AREase activity in plasma and HDL of both sexes but more pronounced in male than in female animals (Figure 2). In all the cases, the inhibition and activation were not dose-dependent.

In the VLDL, the same trend of inhibition and activation was observed with PONase and AREase respectively, more pronounced in male than female animals (Figure 3).

In the brain microsomes, inorganic mercury caused a significant ($p<0.05$) inhibition of female PONase whereas AREase of the same sex was not affected while a significant activation in male PONase and inhibition of AREase in the fraction was observed (Figure 4). In the hepatic microsomes, mercury caused activation of PONase activities in female animals but does not affect that of the male animals (Figure 5). The activation was not dose-dependent.

Since PON is a membrane-bound enzyme and its activity may be affect by the lipid environment, we determined the lipid concentrations of both hepatic and brain microsomes and thereafter calculated the cholesterol/phospholipid ratios (a measure of membrane fluidity) (Figs. 6-9). As indicated in Figs. 6 and 7, mercury exposure resulted in a significant increase ($p < 0.05$) in brain microsomal cholesterol but a decrease in the hepatic microsomes of the male animals and triglyceride concentrations of the two microsomal fractions of male animals were decreased significantly ($p > 0.05$) whereas female animals were not affected.

In the hepatic and brain microsomes of both sexes, phospholipid concentrations were increased significantly ($p > 0.05$) as shown in figure 8 but not dose dependent.

The cholesterol/phospholipid ratios of both the hepatic and brain microsomes as depicted in Figure 9 indicate that inorganic mercury induced decreases in these ratios but more pronounced in female animals.

The associations between blood mercury levels and PON activities in plasma, HDL, VLDL are depicted in Table 2. Blood mercury levels correlated positively with AREases whereas with a few exceptions, blood mercury levels were negatively associated with PONases .

Table 1: Mercury levels in blood of male and female rats after 12 weeks of inorganic mercury exposure

| Mercury dose | Male | Female |
|---|---|---|
| Control | $0.00 \pm 0.00^{a}$ | $0.01 \pm 0.00^{a}$ |
| 0.5mg/kg | $0.04 \pm 0.01^{b}$ | $0.02 \pm 0.00^{a}$ |
| 1.0mg/kg | $0.02 \pm 0.00^{a}$ | $0.04 \pm 0.01^{b}$ |
| 1.5mg/kg | $0.04 \pm 0.01^{b}$ | $0.07 \pm 0.02^{c}$ |

Values for blood are expressed as µgHg/ml. Each value is mean±S.E.M. for 8 rats. Values within a column with different superscripts are significantly different from each other at $p < 0.05$.

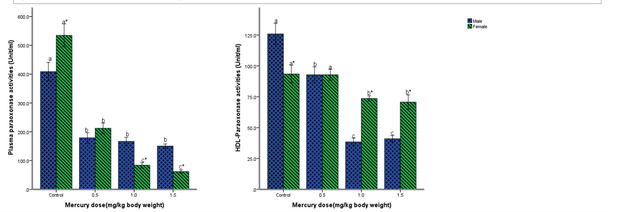



Fig.1: Effects of inorganic mercury on plasma and HDL paraoxonase activity. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

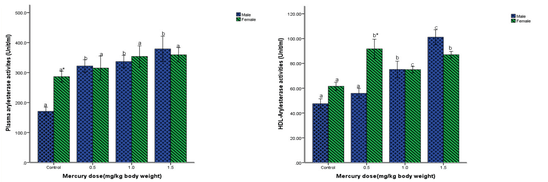



Fig.2: Effects of inorganic mercury on plasma and HDL arylesterase activity. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male

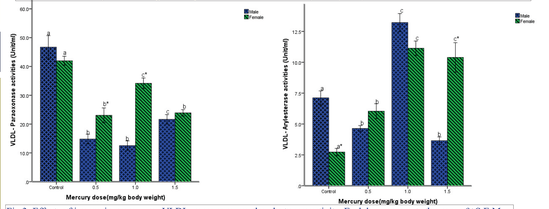



Fig.3: Effects of inorganic mercury on VLDL paraoxonase and arylesterase activity. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

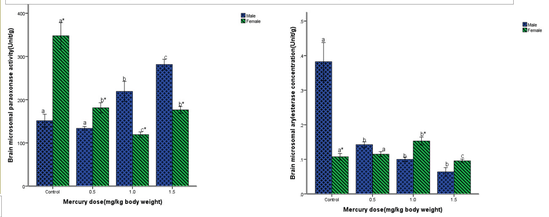



Fig.4: Effects of inorganic mercury on brain microsomal paraoxonase and arylesterase activity. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to

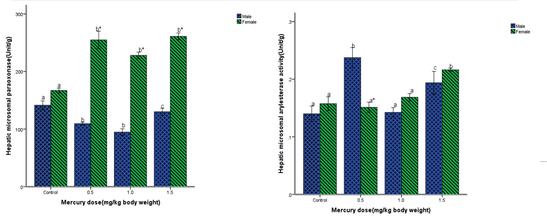



Fig.5: Effects of inorganic mercury on hepatic microsomal paraoxonase and arylesterase activity. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

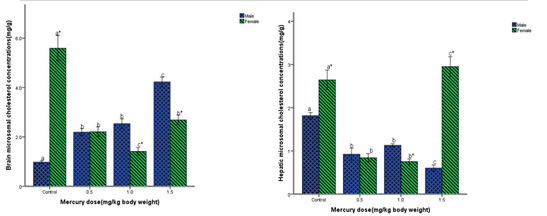



Fig.6: Effects of inorganic mercury on brain and hepatic microsomal cholesterol concentration. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.


Fig.7: Effects of inorganic mercury on brain and hepatic microsomal triglyceride concentrations. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male

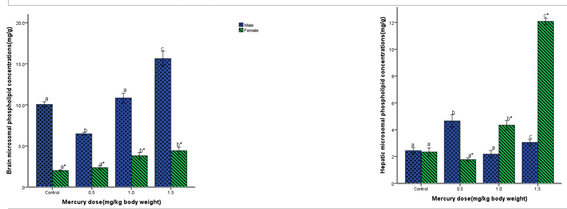



Fig.8: Effects of inorganic mercury on brain and hepatic microsomal phospholipid concentrations. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

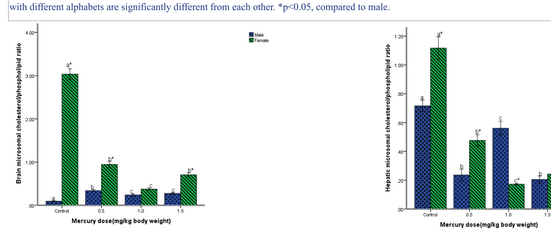



Fig.9: Effects of inorganic mercury on brain and hepatic microsomal cholesterol/ phospholipid ratio. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

Table 2: Association among blood mercury levels, arylesterase and paraoxonase activity (units/ml) in the plasma, HDL and VLDL compartments

| | | Blood Mercury(µgHg) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Plasma | | HDL | | VLDL | |
| | | Male | Female | Male | Female | Male | Female |
| Arylesterase | Correlation coefficient | .474 | .236 | .407 | .246 | -.279 | 454 |
| | P value | .006 | .193 | .021 | .175 | .122 | .009 |
| Paraoxonase | Correlation coefficient | -.427 | -.498 | -.316 | -.515 | -.413 | -.411 |
| | P value | .015 | .004 | .078 | .003 | .019 | .019 |

## CONCLUSIONS

The findings of this study indicate that sub-chronic exposure to inorganic mercury is associated with decreased PONases and increased AREases. This may affect its physiological role and may be an early biochemical event in the cardiovascular effects of mercury. In addition, the inhibition of PON by mercury may be mediated through changes in membrane fluidity brought about by changes in the concentration of cholesterol in the microsomes.

## REFERENCES

Allain CC, Poon LS, Clau CSG, Richmond WY, Fu PD. Enzymatic determination of total serum cholesterol. Clin Chem 1974; 20: 470-478.
Costa LG, Vitalone A, Cole TB, Furlong CE. Modulation of paraoxonase (PON1) activity. Biochem Pharmacol 2005a; 69: 541 – 550.
Costa LG, Cole TB, Furlong CE. Paraoxonase (PON 1): from toxicology to cardiovascular medicine. Acta Biomed. Suppl. 2005b; 2: 50–57.
Folch J, Lees M, Sloane SGH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957; 226: 497 – 509.
Furlong CE, Richter RJ, Seidel SL, Costa LG, Motteblky AG. Spectrophotometric assays for the enzymatic hydrolysis of the active metabolites of chlorpyrifos and parathion by plasma paraoxonase/arylesterase. Anal Biochem 1989; 180:242-247.
Gider LT, Miller GH, Burnstein M, Slagle S, Eder HA. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. J Lipid Res 1982; 23: 1206 – 1223.
Gonzalvo MC, Gil F, Hernández AF, Villanueva E, Pla A. Inhibition of paraoxonase activity in human liver microsomes by exposure to EDTA, metals and mercurials. Chem -Biol. Interact. 1997; 105:169 – 179.
Jarvik, G, Rozek LS, Brophy VH, Hatsukami TS, Richter RJ, Schellenberg GD, . Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is PON1(192) or PON1(55) genotype. Arterioscler. Thromb. Vasc. Biol. 2000; 20: 2441–2447.
Junge W, Klees H. Arylesterase. In: Bergmeyer, H. U. (Ed.). Methods of Enzymatic Analysis, 3rd Ed., Verlag Chemie, Weinheim, Germany. 1984; pp. 8 – 14.
Kim, DS, EH Lee, et al. [Heavy metal as risk factor of cardiovascular disease--an analysis of blood lead and urinary mercury]. J Prev. Med. Public Health 2005; 38: (4), 401-407.
Kirkpatrick AD, Furlet SM, Gan SK, Poynen AM, Chisholm DJ, Campbell IV. Multiple indexes of lipid availability are independently related to whole body insulin action in healthy humans. J Clin Endocr Met 2003; 88 (2):993-998.
Lund, BO, DM Miller, et al. Studies on Hg(II)-induced H2O2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem. Pharmacol. 1993; 45: (10), 2017-2024.
Mackness B, Durrington P, McElduff P, Yarnell J, Azam N, Watt M, Mackness M. Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study. Circulation 2003; 107: 2775 – 2779.
Mahboob, M, KF Shireen, et al. Lipid peroxidation and antioxidant enzyme activity in different organs of mice exposed to low level of mercury. J Environ. Sci. Health. B. 2001; 36: (5), 687-697.
Risher, JF and SN Amler. Mercury exposure: evaluation and intervention the inappropriate use of chelating agents in the diagnosis and treatment of putative mercury poisoning. Neurotoxicology 2005; 26: (4), 691-699.
Rodrigo L, Hernández AF, López-Caballero J, Gil F, Pla A. Immunohistochemical evidence for the expression and induction of paraoxonase in rat liver, kidney, lung and brain tissue: implications for its physiological role. Chem.-Biol. Interact. 2001; 137: 123 – 137.
Sharma, MK, A Sharma, et al. Evaluation of protective efficacy of Spirulina fusiformis against mercury induced nephrotoxicity in Swiss albino mice. Food Chem. Toxicol. 2007;45: (6), 879-887.
Stewart JCM. Colorimetric determination of phospholipids with ammonium ferrothiocyanate. Anal Biochem 1980; 104: 10–14.
WHO. Inorganic Mercury. World Health Organization, Geneva. Environmental Health Criteria 1991; 118