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*Research Article*

# Accumulation trends and patterns of some heavy metals in liver of Wistar rats following exposure to contaminated water

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**ABSTRACT**

Heavy metals are known to cause damage through indirect oxidative effects. A comparative study on the bioavailability and liver accumulation of lead (Pb), cadmium (Cd), and mercury (Hg) in rats following continuous exposures to Hg (10 ppm), Cd (200 ppm) and Pb (100 ppm) in drinking water was carried out for six weeks. The accumulations in the liver were determined using AAS. Analysis gave evidence that the rate of change was not the same for all three metals for all three periods of time; there was statistically ($p<0.05$) significant interaction between the types of metal and time in their relationship to levels accumulation of the metals. While Hg and Pb showed peak accumulation at the second week of exposure, there was decrease in accumulation by the fourth and sixth week. Cd on the other hand showed a continuous increase in accumulation over the six weeks of study. Results indicate that the heavy metal concentration in the liver is under some physiological control, which may involve the chemistry of these metals resulting in different uptake, accumulation and elimination rates from the liver.



## INTRODUCTION

Environmental pollution and occupational exposure to heavy metals contribute to major chronic and malignant diseases, with effects seen in all tissues of the body. The effects observed with heavy metal poisoning include carcinogenicity, immunotoxicity and neurotoxicity and are thought to occur through the generation of oxygen radicals leading to oxidative stress and altered physiological and biochemical characteristics (Valko et al., 2005).

Heavy metals (inclusive of mercury, cadmium and lead) are dangerous because they tend to accumulate in a biological organism over time, compared to the chemical's concentration in the environment. Metals accumulate in living things anytime they are taken up and stored faster than they are metabolized or excreted (Klaassen, 1999). In the environment, these metals can remain for decades or centuries, increasing the likelihood of human exposure.

The availability and toxicity of heavy metals on animals is strongly influenced by the biology of the

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organism and its internal concentration as determined by rates of uptake and elimination (Laskowski et al., 2010), nutrients, diet and/or metabolism (Nwokocha et al., 2011) the presence of metal-binding ligands (Leslie et al., 2001). Blood and tissue pharmacokinetics can play major roles in the distribution of heavy metals due to their sensitive to fat and other tissue storage (Andersen et al., 2001). Affinity for proteins and capacity to simulate ion channels (Goyer, 1997) can also affect their distribution. The sex and routes of exposures to these chemicals also affect their bioavailability in some tissues exposed to the heavy metals (Nwokocha et al., 2012), While Wang and Rainbow, (2005) had suggested that the detoxificatory fate and tissue burden of metals in animals are more important in affecting metal accumulation.

Some animals / organisms have developed elimination methods that help to excrete even the assimilated quantities of the heavy metal, thus reducing the transfer of the chemicals from the gut lumen or body wall to body fluids and subsequently their transfer to internal target sites (Neuhauser et al., 1995; Laskowski et al., 2010). Boháč and Pospíŝil (1989) characterized animals into groups based on their metal handling as "deconcentrators", microconcentrators" and "macroconcentrators" as their accumulation and elimination patterns could differ due to their biology. This has led to two-phase toxicokinetics model (Spurgeon and Hopkin, 1999; Sterenborg et al., 2003; Vijver et al., 2006) and a third phase model that allows for different assimilation and/or elimination rates (Laskowski et al., 2010).

Studies on the bioavailability and accumulation of these heavy metals had involved the sampling hair from exposed animals for the assessment of heavy metal bioavailability (McLean et al., 2009), even bones, but storage of heavy metals in organs, such as the liver, could be a good mechanism to cope with toxicants (Shore and Douben, 1994; Damek-Poprawa and Sawicka-Kapustra 2003, 2004). The study was thus to investigate and comparatively analyze the accumulation and bioavailability of heavy metals in the liver following exposures to mercury, lead and cadmium, also an understanding of the time course of heavy metal uptake, accumulation and elimination rates from the mammalian liver.

## MATERIALS AND METHODS

### *Animals and experimental design*

Seven-week-old male Wistar rats weighing 150-180 g were obtained from the Animal house of the Faculty and used for the study. The study was conducted following Ethical approval and "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. The animals were kept at constant room temperature with 12 h of light/dark cycles. All animals received normal rat chow and had access to tap water *ad libitum* during the acclimatization period.

### *Experimental protocol*

Pb-exposed groups received tap water that contained 100 ppm Pb (Lead acetate); Cadmium exposed groups received tap water that contained 200 ppm Cd (cadmium chloride), while mercury exposed groups received tap water that contained 10 ppm Hg (mercury chloride) water directly from the drinking water bottle. Control group was fed with normal rat chow and tap water only. All groups of rats were fed with normal rat chow for the period of study (six (6) weeks) while all administrations were through the oral route.

### *Tissue Preparation and Analysis*

At the end of the experimental period, the rats were sacrificed under chloroform anesthesia. Liver (1 g) was excised and transferred in polypropylene vials for analysis. Before acid digestion, a porcelain mortar was employed to grind and homogenize the dry tissue samples in 5mls of normal saline. After digestion, all samples the concentrations of Lead and cadmium were analyzed for Cd and Pb using flame atomic absorption spectrophotometer (Perkin Elmer A.A. 3030) with D2 background correction device. Cold vapour technique was used for the analysis of Hg. (Nwokocha et al., 2012)

### *Statistical analysis*

The results are expressed as mean ± SEM. Student's t test and Two-way analysis of variance (ANOVA) with Bonferonni's post-test was performed where applicable using Data analysis was done using Stata version 12.0 and Origin™ 5.0 (Microcal Software Inc., Northampton, USA) created graphical displays of the data. Estimates were deemed statistically significant of the associated $p$ value less than 0.05. Scatter plots of the points for each metal were examined and a linear spline with knots at specified points – weeks 2, 4 and 6 – were fitted using the linear regression model approach. This method enabled estimation of the rate of change in accumulation between weeks 0 and 2, 2 and 4, and 4 and 6 and an estimation of whether the rates of change differed with the types of metal by virtue of inclusion of an interaction term.

## RESULTS

### *Pattern of accumulation of heavy metals: Degree of heavy metal accumulation in the liver*

The degrees of Hg, Cd and Pb accumulation in the liver following exposure for six weeks are as shown in Figs 1 to 3. Following continuous exposure of 10 ppm Hg in drinking water, there was an initial rise in liver concentration by the second week, which reduced by the fourth and sixth weeks of study. With exposure to 200 ppm Cd, there was an initial rise by the second week, which remained stable up to the fourth week but shot up again by the sixth week. However with exposure to 100 ppm Pb, we observed an initial rise in the liver accumulation by the second week, which decreased and remain the same by the fourth and sixth weeks of study.

**Fig. 1.**
Fitted regression lines for the relationship between level of metal accumulation and time for the different metals.

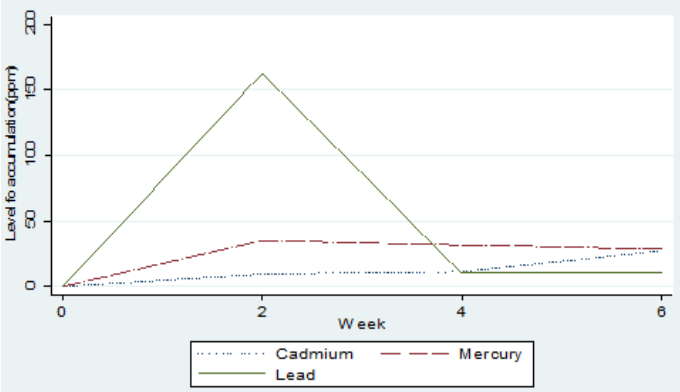


**Table 1:**
Change in accumulation of metal per week for the periods specified for the respective metals.

| Metal | Week 0 – 2 | Week 2-4 | Week 4-6 |
|---|---|---|---|
| Cadmium | 4.7 (-1.901 – 11.301) | 1.0 (-5.601-7.601) | 7.9(1.299-14.501)* |
| Mercury | 17.6 (10.999 - 24.201)** | -1.8 (-8.401-4.801) | -1.4(-8.001 – 5.201) |
| Lead | 81.3 (74.70-87.901)*** | -76.3(-82.901--69.699)*** | 0.2(-6.401 – 6.801) |

*=P<0.05, **=P<0.01, ***=P<0.001 – significant difference relative to week 2.

Table 1 gives the change in accumulation of the respective metals for each one-week increment in time. Analysis gave evidence that the rate of change was not the same for all three metals for all three periods of time. In other words, there was statistically significant interaction between the types of metal and time in their relationship to levels accumulation of the metals. Whereas the rate of change was not significantly different from zero for cadmium for weeks 0 to 2, the rate of change was significantly different from 0 for the other two metals for this same time period. Whereas the rate of change was not significantly different from zero for cadmium and mercury for weeks 2 to 4, the rate of change was significantly different from 0 for lead for this same time period. Whereas the rate of change was not significantly different from zero for lead and mercury for weeks 4 to 6, the rate of change was significantly different from 0 for cadmium for this same time period.

There was generally an increase in the level of the metals from week 0 to week 2 by the rate of change was statistically significant for mercury and lead only. From week 0 to week 2, mercury can be expected to increase by 17.6ppm ($P<0.01$) each week while lead can be expected to increase by 81.3ppm ($P<0.001$) each week. There was negligible positive change in accumulation of cadmium from weeks 2 to 4 and this change was not significantly different from zero. A negative change in the accumulation was observed for the other two metals but this statistically significant for lead only From weeks 2 to 4, lead can be expected to decrease by 76.3ppm per week ($P<0.001$). From weeks 4 to 6, levels of cadmium but not the other metals can be expected to change significantly. For each week the levels of cadmium accumulation can be expected to increase by 7.9ppm ($P<0.05$). Figure 1 show the fitted regression lines for the different time periods for the different metals.

## DISCUSSION

Our results give evidence that the rate of change was not the same for all three metals for all three periods of time. Our observation in this study was that when animals were exposed to mercury, lead and cadmium, there was an initial increase in accumulation and assimilation pattern after two (2) weeks of exposure, which could arise perhaps due to a decrease in the elimination of these metals. This is followed by a decrease in metal accumulation and elimination for mercury and lead but an equilibrium concentration for cadmium after four (4) weeks of exposure following the one compartment model. With further exposures we observed differences in the toxicokinetics and behaviours of the various metals. Pb and Hg continue to decrease in the tissue studied while Cd showed a further increase in accumulation and or reduced elimination.

The availability and toxicity of heavy metals is strongly influenced by the biology of the organism and species (Hopkin et al., 1993), as many organisms have developed some elimination methods that help to

excrete even the assimilated quantities of the heavy metals (Laskowski et al., 2010). Other factors affecting tissue accumulation and toxicity include sex and routes of exposures (Nwokocha et al., 2012), blood and tissue pharmacokinetics (Andersen et al., 2001), trophic status of the exposed species (Walker and Livingstone, 1992), nutrients and nutritional status (Nwokocha *et al*., 2011). The factors that influence uptake and loss have a key role in ecotoxicology, as they can be used to predict the physiological fate of a pollutant (Walker et al., 1996).

Accumulation in the liver is usually in high concentrations, irrespective of the exposure or uptake routes, it could be a good mechanism to cope with toxicants (Damek-Poprawa and Sawicka-Kapustra, 2004). Metal levels in the liver rapidly increase during exposure, and remain high for a long time of depuration, when other organs are already cleared; as such accumulation is a function of uptake and elimination (Jezierska and Witeska, 2006). Different compartment models have been described but these models cannot give a satisfactory explanation to changes found in the accumulation and elimination patterns for various metals (Neuhauser et al., 1995; Spurgeon and Hopkin, 1999; Lagisz et al., 2005), as can be seen with unexpected patterns of metals accumulation in this study. The observed patterns are not due to analytical and experimental errors and may have important physiological basis.

We expected that under continuous exposure, there will be a tendency for total internal body concentrations to increase as long as the animal is exposed to the metal, bearing in mind that these metals bioaccumulate and are not degradable, this was not the case for all the metals studied. According to Laskowski et al., (2010), an animal may show a lag in physiological response that results in a delay in the onset of efficient elimination and/or decrease in metal assimilation following an exposure to high doses of these metals. These results indicate that the heavy metal concentration in the body is under some physiological control, which may involve the chemistry of these metals. The chemistry may involve the different affinities of these metals to the liver tissues, which results in different uptake, accumulation and elimination rates. Future studies will include a look at the decay phenomena to understand the elimination rates and or heavy metal distribution across the tissues/ organs.

## REFERENCES


Andersen ME, Sarangapani R, Reitz RH, Gallavan RH, Dobrev ID, Plotzke KP. (2001). Physiological modeling reveals novel pharmacokinetic behavior for inhaled octamethylcyclotetrasiloxane in rats. *Toxicol. Sci*. **60**: 214-231.

Boháč J, Pospíšil J. (1989). Accumulation of heavy metals in invertebrates and its ecological aspects. In: Vernet J-P, editor. Heavy metals in the environment, 1. Edinburgh: CEP Consultants Ltd.;.p. 354.

Damek-Poprawa M, Sawicka-Kapusta K. (2004). Histopathological changes in the liver, kidneys, and testes of bank voles environmentally exposed to heavy metal emissions from the steelworks and zinc smelter in Poland. *Environ. Res*.; **96**:72-78.

Goyer RA. (1997). Toxic and essential metal interactions. *Annu Rev. Nutr*. **17**: 37-50.

Klaassen CD, Liu J, Choudhuri S. (1999). Metallothionein: an intracellular protein to protect against cadmium toxicity. *Ann. Rev. Pharmacol. Toxicol*. **39**: 267-294.

Lagisz M, Kramarz P, Niklinska M. (2005). Metal kinetics and respiration rates in F1 generation of carabid beetles (*Pterostichus oblongopunctatus* F.) originating from metal contaminated and reference areas. *Arch Environ Contam Toxicol*. **48**: 484-489.

Laskowski R, Bednarska AJ, Spurgeon D, Svendsen C, van Gestel CA. (2010). Three-phase metal kinetics in terrestrial invertebrates exposed to high metal concentrations. *Sci. Total Environ.* **15**: 3794-3802.

Leslie EM, Deeley RG, Cole SP. (2001). Toxicological relevance of the multidrug resistance protein 1, MRP1 (ABCC1) and related transporters. *Toxicology* **167**: 3-23.

McLean CM, Koller CE, Rodger JC, MacFarlane GR. (2009). Mammalian hair as an accumulative bioindicator of metal bioavailability in Australian terrestrial environments. *Sci Total Environ*. **15**: 3588-3596.

Neuhauser EF, Cukik ZV, Malecki MR, Loehr RC, Durkin PR. (1995). Bioconcentration and biokinetics of heavy metals in the earthworm. *Environ Pollut*. **89**:293-301.

Nwokocha CR, Owu DU, Ufearo CS, Iwuala MOE., (2011). Comparative study on the efficacy of *Garcinia kola* in reducing some heavy metal accumulation in liver of Wistar rats. *J. Ethnopharmacol*. **135**: 488-491.

Nwokocha CR, Ufearo CS, Owu DU, Idemudo NC, Ojukwu LC. (2012). In vivo distribution of lead in male and female rats after intraperitoneal and oral administration. *Toxicol. Ind. Health*. **28** : 2131 – 135.

Spurgeon DJ, Hopkin SP. (1999). Comparisons of metal accumulation and excretion kinetics in

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http://www.jaaps.aapsnet.org

earthworms (Eisenia fetida) exposed to contaminated field and laboratory soils. *Appl. Soil Ecol.* **11**: 227-243.

Sterenborg I, Vork NA, Verkade SK, van Gestel CAM, van Straalen NM. (2003). Dietary zinc reduces uptake but not metallothionein binding and elimination of cadmium in the springtail, *Orchesella cincta*. *Environ. Toxicol. Chem*. **22**:1167-1171.

Valko M, Morris H, Cronin MT. (2005). Metals, toxicity and oxidative stress. *Curr. Med. Chem.* **12**: 1161-1208.

Vijver MG, Vink JPM, Jager T, van Straalen NM, Wolterbeek HT, van Gestel CAM. (2006). Kinetics of Zn and Cd accumulation in the isopod *Porcellio scaber* exposed to contaminated soil and/or food. Soil Biol. *Biochem*. **38**:1554-1563.

Wang WX, Rainbow PS. (2005). Influence of metal exposure history on trace metal uptake and accumulation by marine invertebrates. *Ecotoxicol. Environ. Saf*. **61**:145-159.