*BioMetals* **17:** 567–568, 2004.



# Oxidative stress induced by lead, cadmium and arsenic mixtures: 30-day, 90-day, and 180-day drinking water studies in rats: An overview

Bruce A. Fowler, Margaret H. Whittaker, Mike Lipsky, Gensheng Wang & Xue-Qing Chen
*Program in Toxicology, The University of Maryland, Baltimore, U.S.A.*

*Key words:* arsenic, cadmium, lead, oxidative stress

**Abstract**

Humans are frequently exposed to combinations of lead (Pb), cadmium (Cd) and Arsenic (As) but there is a paucity of actual data on the molecular effects of these agents at low dose levels. The present factorial design studies were undertaken in rats to examine the effects of these agents at LOEL dose levels on a number of molecular parameters of oxidative stress in hematopoietic and renal organ systems following oral exposure in drinking water at 30, 90 and 180 day time points. Results of these studies demonstrated dynamic, time-dependent alterations in both molecular targets and inducible oxidative stress protective systems in target cell populations. In general, cellular protective systems, which protected against oxidative damage at the 90 day time point, appeared to be finite such that molecular manifestations of oxidative stress became statistically significant at the 180 day time point for several of the combination exposure groups. These data demonstrate the importance of duration of exposure in assessing the toxic potential of Pb, Cd and As mixtures at low dose levels.

## Introduction

Lead (Pb), arsenic (As) and cadmium (Cd) are ubiquitous air and water pollutants that continue to threaten the quality of public health around the world. These elements are associated with a multitude of adverse health effects, such as cancer and nephrotoxicity (Madden & Fowler 2000). Previous *in vivo* studies in rats (Fowler & Mahaffey 1978; Mahaffey *et al.* 1981) demonstrated a number of additive interactions at elevated 'stressor' dose levels of these elements following subchronic oral exposures. Previous *in vitro* studies (Madden *et al.* 2002) have demonstrated a number of interactions between these elements at the level of the stress protein response in kidney cell cultures. As a result of more recent studies from a number of laboratories, oxidative stress is thought to play a major role in the development of Pb, Cd and As-related adverse health effects. The present studies were undertaken in order to investigate potential mechanisms of Pb, Cd- and As-induced oxidative stress following short-term, sub-chronic and chronic oral exposure to these elements at LOEL dose levels.

## Materials and methods

Weanling, male Sprague-Dawley rats (15/group) were fed a semi-purified diet (Ziegler Brothers) and exposed to deionized drinking water containing Pb (25 mg/l as lead acetate), Cd (10 mg/l as cadmium chloride), As (5 mg/l as sodium arsenite), or as PbxCd, PbxAs, CdxAs or PbxCdxAs mixtures via drinking water for 30-, 90-, or 180-days.

These dose levels were selected from a prior series of dose-response studies to determine the selected LOEL dose levels for the interaction study. A variety of molecular biomarkers such as alterations in the heme pathway, stress protein response and oxidative DNA damage which have been, previously shown to be sensitive to these elements on an individual basis were measured in kidney, blood and urine in order to evaluate interactive effects from concomitant exposures.

## Results

Results of these studies (Whittaker *et al.* 2004, in press; Wang *et al.* 2004, in press) yielded a number of interesting findings regarding the dynamic nature of cellular anti-oxidant and related defense mechanisms following exposure to these toxic elements.

Overall, relative to controls, urinary ALA (mg/day) was significantly increased in The Pb, Cd, As, PbxCd, PbxAs and PbxCdxAs groups after 30-days. These effects were attenuated at the 90- and 180-day timepoints. Oxidative stress levels in the kidney were monitored through the measurement of kidney carbonyls and nonprotein thiols. Kidney carbonyls (nmoles/mg protein) (a marker of protein oxidation) were increased after 30-days of exposure. Following 90-days of exposure, carbonyl levels decreased in the Pb, Cd and As groups, relative to controls, and but remained elevated in the combination groups (relative to controls). Following 180-days of exposure, carbonyl levels were decreased in the Cd, As, and CdxAs groups (relative to controls). Kidney non-protein thiols ($\mu$moles/mg protein) were used to estimate glutathione levels, and increased after 30 days of exposure in most treatment groups. After 90 days of exposure, non-protein thiols were increased in some treatment groups and decreased in others. Non-protein thiols were increased after 180-days of exposure in the various treatment groups relative to controls.

In addition to alterations in the above cellular anti-oxidant mechanisms, a number of other cellular defense mechanisms were found to respond to prolonged exposure to these elements under various mixture conditions.

More recent studies (Wang *et al.* 2004) have demonstrated perturbations of a sensitive molecular systems such as the heme biosynthetic pathway, DNA adduct formation and the stress protein response. Erythrocyte protoporphyrin (ZPP) showed significant increases in all exposure groups at 30 days, no change at 90 days and increases in the As and PbxCdxAs groups at 180 days. Blood ALAD was inhibited in all treatment groups at the 30 and 90 day time points and in only the Pb treatment group at 180 days. Renal ALAD showed a more complex response pattern. The oxidative DNA adduct, 8-hydroxy-2-deoxyguanosine (8OHdG) was found to be increased in only the CdxAs treatment group at 180 days. The 32 kD stress protein (heme oxygenase $-1$) was found to be increased in only the PbxCdxAs treatment group at 180 days. Induction of metallothionein (MT) was observed in a number of the treatment groups including those not receiving cadmium apparently as a result of oxidative stress.

Overall, the results of these studies demonstrate that significant interactions between Pb,Cd, and As do occur at LOEL dose levels. The ability to measure these disturbances in the normal biology of target cell populations such as the renal proximal tubule cells is regulated by a number of inter-related but limited cellular defense mechanisms. These data also demonstrate that short-term exposures to Pb, Cd or As at LOEL dose levels increase concentrations of oxidative stress precursors and oxidative stress levels, while longer-term exposures to Pb, Cd or As tend to result in decreased production of oxidative stress levels, apparently due, in part, to the induction of glutathione or other molecules (such as metallothionein), which attenuate increases in oxidative stress. Despite these compensatory responses to oxidative stress, evidence of alterations in the heme pathway, stress protein responses, DNA oxidative damage was observed in some of the combination exposure groups at the 180 day time point indicating that the finite nature of these protective cellular mechanisms.

## Acknowledgement

Supported in part by USEPA STAR Grant # 827161.

## References

Fowler BA, Mahaffey KR. 1978 Interaction between lead, cadmium and arsenic in relation to porphyrin excretion patterns. *Environ Hlth Perspec* **25**, 87–90.

Madden EF, Fowler BA. 2000 Mechanisms of nephrotoxicity from metal combinations: A review. *Drug Chem Toxicol* **23**, 1–12.

Madden EF, Akkerman M, Fowler BA. 2002 A comparison of 60 kDA, 70 kDA and 90 kDA stress protein expression in normal rat NRK-52 and human HK-2 kidney cell lines following *in vitro* exposure to arsenite and cadmium alone or in combination. *J Biochem Mol Toxicol* **16**, 24–32.

Mahaffey KR, Capar SG, Gladen BC, Fowler BA. 1981 Concurrent exposure to lead cadmium and arsenic: effects on toxicity and tissue metal concentrations in the rat. *J Lab Clin Med* **98**, 463–481.

Wang G, Chen X-Q, Lipsky MM, Whittaker MH, Fowler BA. 2004 (in press), Interactions of lead, cadmium and arsenic in rat kidneys. *The Toxicologist* (Abstract).

Whittaker MH, Lipsky MM, Wang G, Chen X-Q, Fowler BA. 2004 (in press) Oxidative stress induced by lead, cadmium and arsenic mixtures: 180 - day drinking water studies in rats. *The Toxicologist* (Abstract).