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Selenium (Se) as well as mercury (Hg) may influence the methylation and toxicity of inorganic arsenic, but further research is needed with combination of Inorg-arsenic, Se, and Hg

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ABSTRACT

Our studies have indicated that the relative concentration of Se or Hg to As in urine and blood positively correlates with percentage of inorganic arsenic (% Inorg-As) and percentage of monomethlyarsonic acid [% MMA (V)]. We also found a negative correlation with percentage of dimethylarsinic acid [% DMA (V)] and the ratio of % DMA (V) to % MMA (V). In another study, we found that a group of proteins were significantly over expressed and conversely other groups *w*ere under-expressed in tissues in Na-As (III) treated hamsters.

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Introduction.

Inorganic arsenic (Inorg-As) in drinking water.

One of the largest public health problems at present is the drinking of water containing levels of Inorg-As that are known to be carcinogenic. At least 200 million people globally are at risk of dying because of arsenic *(*As) in their drinking water[1-3]. The chronic ingestion of Inorg-As can results in skin cancer, bladder cancer, lung cancer, and cancer of other organs[1-3]. The maximum contamination level (MCL) of U.S. drinking water for arsenic is 10 ug/L. The arsenic related public health problem in the U.S. is not at present anywhere near that of India[4], Bangladesh[4], and other countries[5].

Metabolism and toxicity of Inorg-As and arsenic species.

Inorg-As is metabolized in the body by alternating reduction of pentavalent arsenic to trivalent form by enzymes and addition of a methyl group from S-adenosylmethionine[6, 7]; it is excreted mainly in urine as DMA (V)[8]. Inorganic arsenate [Inorg-As (V)] is biotransformed to Inorg-As (III), MMA (V), MMA (III), DMA (V), and DMA (III)[6] **(Fig. 1)**. Therefore, the study of the toxicology of Inorg-As (V) involves at least these six chemical forms of arsenic. Studies reported the presence of 3+ oxidation state arsenic biotransformants [MMA (III) and DMA (III)] in human urine[9]and in animal tissues[10]. The MMA (III) and DMA (III) are more toxic than other arsenicals[11, 12]. In particular MMA (III) is highly toxic[11, 12]. In increased % MMA in urine has been recognized in arsenic toxicity[13]. In addition, people with a small % MMA in urine show less retention of arsenic[14]. Thus, the higher prevalence of toxic effects with increased % MMA in urine could be attributed to the presence of toxic MMA (III) in the tissue. Previous studies also indicated that males are more susceptible to the As related skin effects than females[13, 15]. A study in the U.S. population reported that females excreted a lower % Inorg-As as well as % MMA, and a higher % DMA than did males[16].

Abbreviation: SAM, *S*-adenosyl-L-methionine; SAHC, *S*-adenosyl-L-homocysteine.

Differences in susceptibility to arsenic toxicity might be manifested by differences in arsenic metabolism among people. Several factors (for examples, genetic factors, sex, duration and dosage of exposure, nutritional and dietary factors, etc.) could be influence for biotransformation of Inorg-As,[6, 17] and other unknown factors may also be involved.

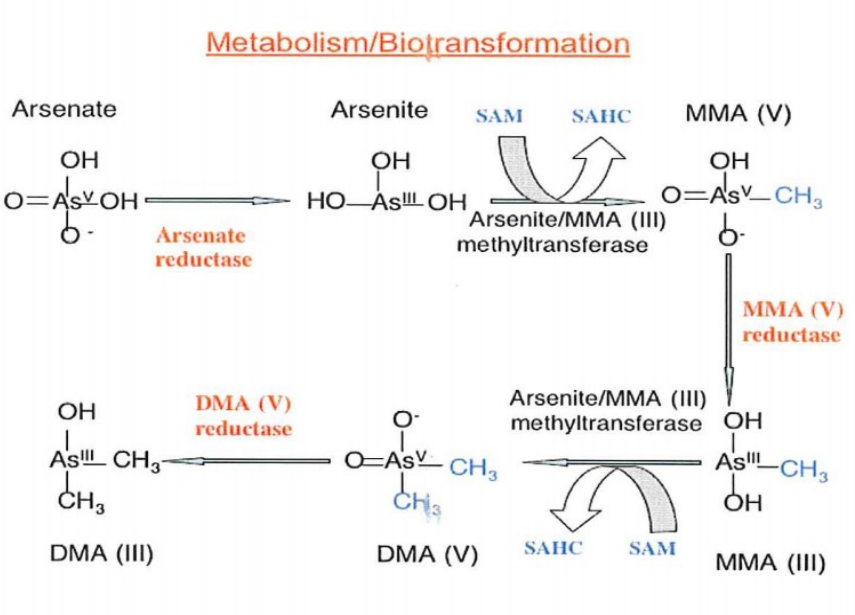

Figure 1. Metabolism of Inorg-As.

The interaction between As, Se, and Hg.

The toxicity of one metal or metalloid can be dramatically modulated by the interaction with other toxic and essential elements[18]. Arsenic and Hg are toxic elements, and Se is required to maintain good health[19]. But Se is also toxic at high levels[20]. Recent reports point out the increased risk of squamous cell carcinoma and non-melanoma skin cancer in those treated with 200 ug/day of selenium (Nutritional Prevention of Cancer Trial in the United States)[21]. However, it is well known that As and Se as well as Se and Hg act as antagonists[22]. It was also reported that Inorg-As (III) influenced the interaction between selenite and methyl mercury[23]. A possible molecular link between As, Se, and Hg has been proposed by Korbas et al. (2008)[24]. The identifying complexes between the interaction of As and Se, Se and Hg as well as As, Se, and Hg in blood of rabbit are shown in **Table 1**.

Table 1. The identifying complexes between the interaction of As and Se, Se and Hg as well as As, Se, and Hg.

	Complex identified
A. Interaction between As(III) and Se(IV) in erythrocytes [22]	$[(GS)_2AsSe]^-$
B. Interaction between Hg^{+2} and Se(IV) In the blood plasma [22]	$(Hg\text{-}Se)_{100}(GS)_5$
C. Interaction between $[(GS)_2AsSe]^-$ and CH_3HgOH in erythrocyte lysate [24]	$[(GS)_2AsSeHgCH_3]$

Influence of Se and Hg on the metabolism of Inorg-As.

The studies have reported that Se supplementation decreased the As-induced toxicity[25,26]. The concentrations of urinary Se expressed as ug/L were negatively correlated with urinary % Inorg-As and positively correlated with % DMA[27]. The study did not address the urinary creatinine adjustment[27]. Other researchers suggested that Se and Hg decreased As methylation[28-31]**(Table 2)**. They also suggested that the synthesis of DMA from MMA might be more susceptible to inhibition by Se (IV)[29] as well as by Hg (II)[30,31] compared to the production of MMA from Inorg-As (III). The inhibitory effects of Se and Hg were concentration dependent[28-31].

Table 2. The impact of Se as well as Hg for As-induced toxicity and metabolism.

		Results/impact
A.	Se supplementation in humans [25; 26]	Arsenic-induced toxicity decreased ↓
B.	The concentration of urinary Se expressed as µg/L [27]	Negatively (-) correlated with % Inorg-As and positively (+) correlated with % DMA i.e., arsenic methylation increased ↑
C.	Mice on the Se-excess diet [28]	Urinary % Inorg-As increased and the ratio of organic arsenic to Inorg-As decreased i.e., arsenic methylation decreased ↓
D.	Rat hepatocytes treated with As (III) and Se (IV) [29]	Significantly increased cellular Inorg-As(III) and the ratios of DMA (V)/MMA decreased ↓
E.	In liver cytosol treated with As (III) & Se (IV) as well as As (III) & Hg (II) [30; 31]	Arsenite methyltransferase, and MMA(III) methyltransferase activities were inhibited, and arsenic methylation decreased ↓

The literature suggests that reduced methylation capacity with increased % MMA (V), decreased % DMA (V), or decreased ratios of % DMA to % MMA in urine is positively associated with various lesions[32]. Lesions include skin cancer and bladder cancer[32]. The results were obtained from inorganic arsenic exposed subjects[32]. Our concern involves the combination of low arsenic (As) and high selenium (Se) ingestion. This can inhibit methylation of arsenic to take it to a toxic level in the tissue.

Dietary sources of Se and Hg.

Global selenium (Se) source are vegetables in the diet. In the United States, meat and bread are the common source. Selenium deficiency in the U.S. is rare. The U.S. Food and Drug Administration (FDA) has found toxic levels of Se in dietary supplements, up to 200 times greater than the amount stated on the label[33]. The samples contained up to 40,800 ug Se per recommended serving.

For the general population, the most important pathway of exposure to mercury (Hg) is ingestion of methyl mercury in foods. Fish (including tuna, a food commonly eaten by children), other seafood, and marine mammals contain the highest concentrations. The FDA has set a maximum permissible level of 1 ppm of methyl mercury in the seafood[34]. The people also exposed mercury via amalgams[35].

Proteomic study of Inorg-As (III) injury.

Proteomics is a powerful tool developed to enhance the study of complex biological system[36]. This technique has been extensively employed to investigate the proteome response of cells to drugs and other diseases[37, 38]. A proteome analysis of the Na-As (III) response in cultured lung cells found *in vitro* oxidative stress-induced apoptosis[39]. However, to our knowledge, no *in vivo* proteomic study of Inorg-As (III) has yet been conducted to improve our understanding of the cellular proteome response to Inorg-As (III) except our preliminary study [40].

Preliminary Studies: Results and Discussion

The existing data **(Fig. 1)** from our laboratory and others show the complex nature of Inorg-As metabolism. For many years, the major way to study, arsenic (As) metabolism was to measure Inorg-As (V), Inorg-As (III), MMA (V), and DMA (V) in urine of people chronically exposed to As in their drinking water. Our investigations demonstrated for the first time that MMA (III) and DMA (III) are found in human urine[9]. Also we have identified MMA (III) and DMA (III) in the tissues of mice and hamsters exposed to sodium arsenate [Na-As (V)][10, 41].

Influence of Se as well as Hg on the As methyltransferase.

We have reported that Se (IV) as well as mercuric chloride ($HgCl_2$) inhibited As (III) methyltransferase and MMA (III) methyltransferase in rabbit liver cytosol. Mercuric chloride was found to be a more potent inhibitor of MMA (III) methyltransferase than As (III) methyltransferase[30]. These results suggested that Se and Hg decreased arsenic methylation. The inhibitory effects of Se and Hg were concentration dependent[30].

Influence of Se and Hg in urine and blood on the percentage of urinary As metabolites.

Our human studies indicated that the ratios of the concentrations of Se or Hg to As in urine and blood were positively correlated with % Inorg-As and % MMA (V). But it negatively correlated with % DMA (V) and the ratios of % DMA (V) to % MMA (V) in urine of both males and females (unpublished data) **(Table 3).** These results confirmed that the inhibitory effects of Se as well as Hg for the methylation of Inorg-As in humans were concentration dependent. We also found that the concentrations of Se and Hg were negatively correlated with % Inorg-As and % MMA (V). Conversely it correlated positively with % DMA (V) and the ratios of % DMA (V) to % MMA (V) in urine of both sexes (unpublished data). These correlations were not statistically significant when urinary concentrations of Se and Hg were adjusted for urinary creatinine **(Table 3)**. Interactions of As, Se, Hg and its relationship with methylation of arsenic are summarized in **Figure 2**.

Table 3: Spearman Correlation Coefficients for (i) arsenic metabolites vs. the ratios of Se or Hg to As, and (ii) arsenic metabolites vs. Se or Hg (Males, n=93 and females, n=98).

	Urine (µg/g creatinine)				Blood (µg/L)		Urine (µg/L)	
	Ratio of Se to As	Se	Ratio of Hg to As	Hg	Ratio of Se to As	Se	Se	Hg
Males:								
% In-As	+0.264[a]	+0.174	+0.471[e]	+0.331[b]	+0.25[illegible][a]	-0.043	-0.346[c]	-0.021
% MMA	+0.100	+0.090	+[illegible].171	+0.151	+0.09[illegible]	-0.018	-0.369[c]	-0.262[a]
% DMA	-0.254[a]	-0.117	-0.441[d]	-0.270[b]	-0.296[b]	+0.017	+0.354[c]	+0.064
%MMA/%In-As	-0.163	-0.080	-0.300[b]	-0.187	-0.197	-0.057	+0.087	-0.133
%DMA/%MMA	-0.145	-0.094	-0.288[b]	-0.233[a]	-0.190	+0.007	+0.446[e]	+0.239[a]
Females:								
% In-As	+0.290[b]	+0.160	+0.413[d]	+0.342[c]	+0.361[c]	+0.111	-0.400[d]	-0.142
% MMA	+0.250[a]	-0.004	+0.204[a]	+0.093	+0.26[illegible][b]	+0.033	-0.322[b]	-0.182
% DMA	-0.337[c]	-0.168	-0.385[d]	-0.332[c]	-0.33[illegible][c]	-0.124	+0.378[c]	+0.136
%MMA/%In-As	-0.026	-0.081	-0.245[a]	-0.277[b]	-0.15[illegible]	-0.007	+0.138	-0.061
%DMA/%MMA	-0.312[b]	-0.038	-0.274[b]	-0.162	-0.316[b]	-0.063	+0.349[c]	+0.175

[a]$p<0.05$; [b]$p<0.01$; [c]$p<0.001$; [d]$p<0.0001$; [e]$p<0.00001$

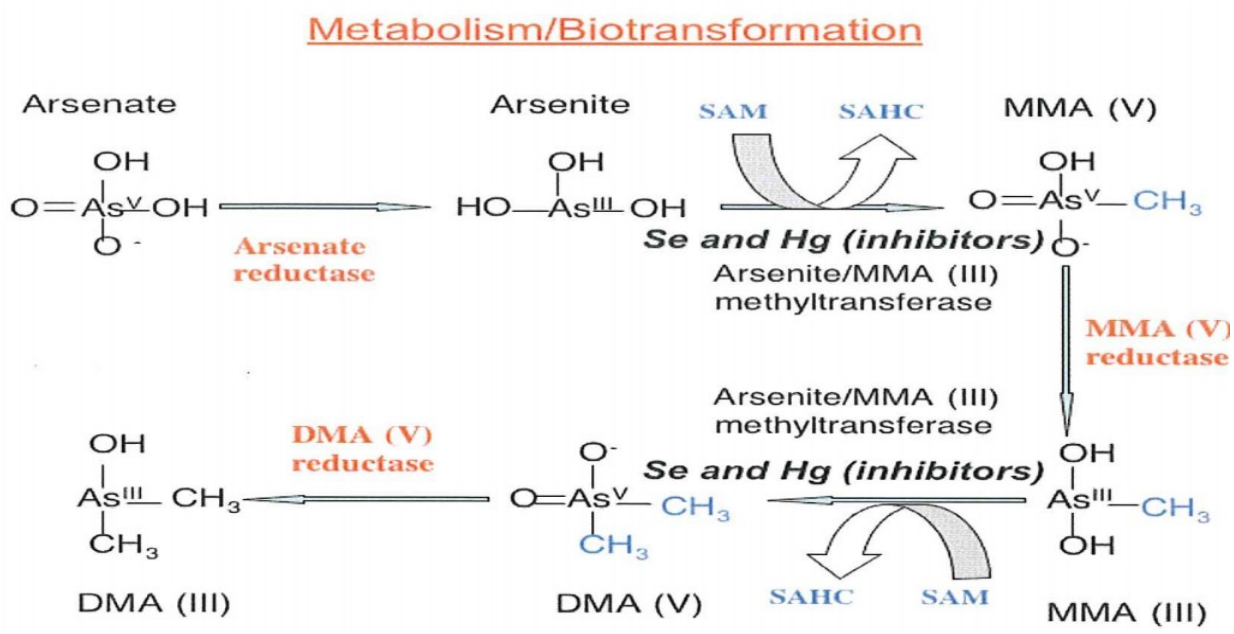

Figure 2. Inhibitory effects of Se and Hg on As metabolism.

Sex difference distribution of arsenic species in urine.

Our results indicate that females have more methylation capacity of arsenic as compared to males. In our human studies (n= 191) in Mexico, we found that females (n= 98) had lower % MMA ($p<0.001$) and higher % DMA ($p=0.006$) when compared to males (n= 93) **(Fig. 3).** The means ratio of % MMA (V) to % Inorg-As and % DMA (V) to %MMA (V) were also lower ($p<0.05$) and higher ($p<0.001$), respectively in females compared to males.

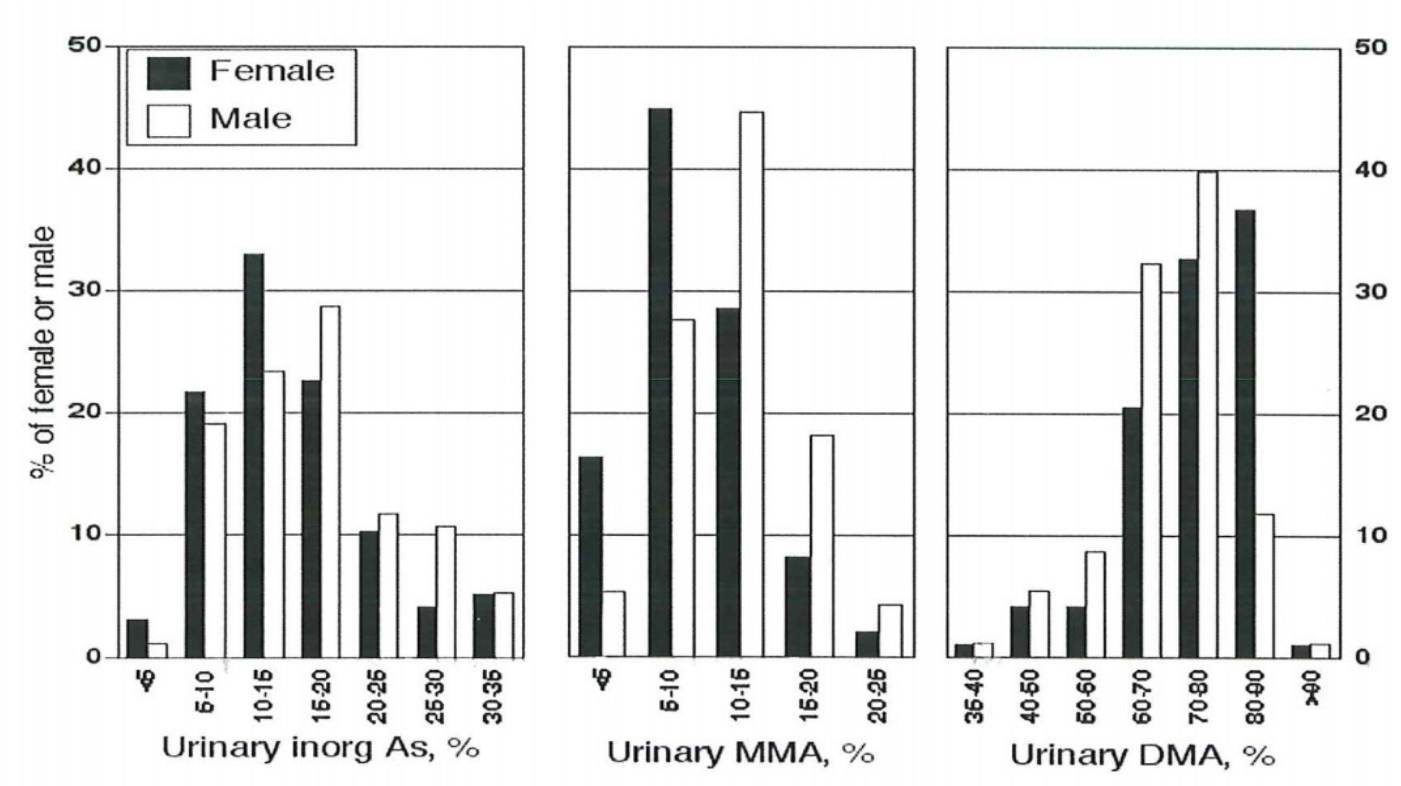

Figure 3. Frequency distribution of As metabolites in urine.

The protein expression profiles in the tissues of hamsters exposed to Na-As (III).

In our preliminary studies[40], hamsters were exposed to Na-As (III) (173 pg/ml as As) in their drinking water for 6 days and control hamsters were given only the water used to make the solutions for the experimental animals. After DIGE (Two-dimensional differential in gel electrophoresis) and analysis by the DeCyder software, several protein spots were found to be over-expressed (red spot) and several were under expressed (green spot) as compared to control **(Figs. 4a-c)**. Three proteins (one was over-expressed and two were under-expressed) of each tissue (liver and urinary bladder) were identified by LC-MS/MS (liquid chromatography-tandem mass spectrometry).DIGE in combination with LC-MS/MS is a powerful tool that may help cancer investigators to understand the molecular mechanisms of cancer progression due to Inorg-As.

Propose a new research

These results suggested that selenium (Se) as well as mercury (Hg) may influence the methylation of Inorg-As and this influence could be dependent on the concentration of Se, Hg and/or the sex of the animal. Our study also suggested that the identification and functional assignment of the expressed proteins in the tissues of Inorg-As (III) exposed animals will be useful for understanding and helping to formulate a theory dealing with the molecular events of arsenic toxicity and carcinogenicity. Therefore, it would be very useful if we could do a research study with combination of Inorg-arsenic, Se, and Hg.

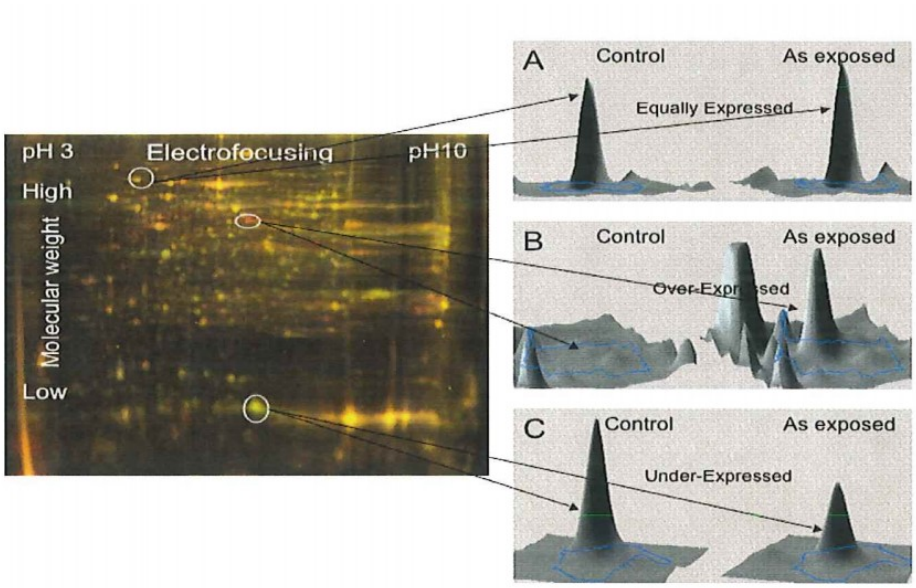

Figure 4a. Three-dimensional stimulation of over-and under expressed protein spots in the liver of hamsters exposed to Na-As (III) using DeCyder software[40].

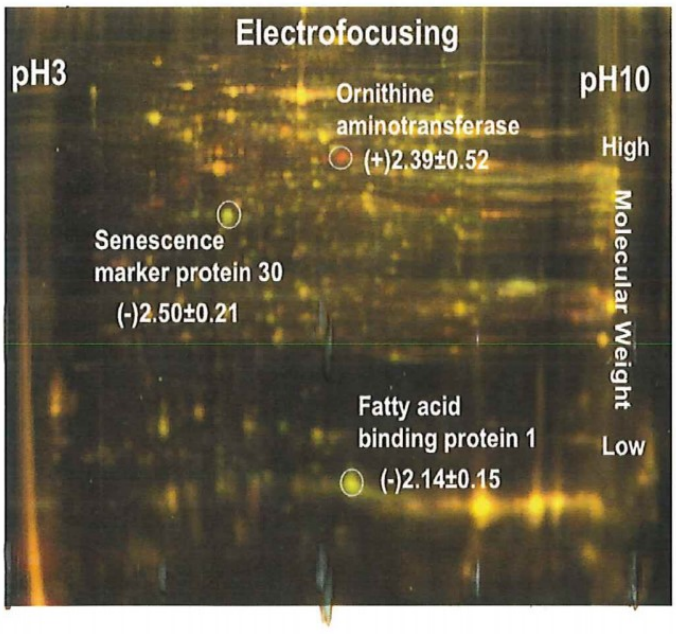

Figure 4b. The protein expression profile in the liver of hamsters exposed to Na-As (III).

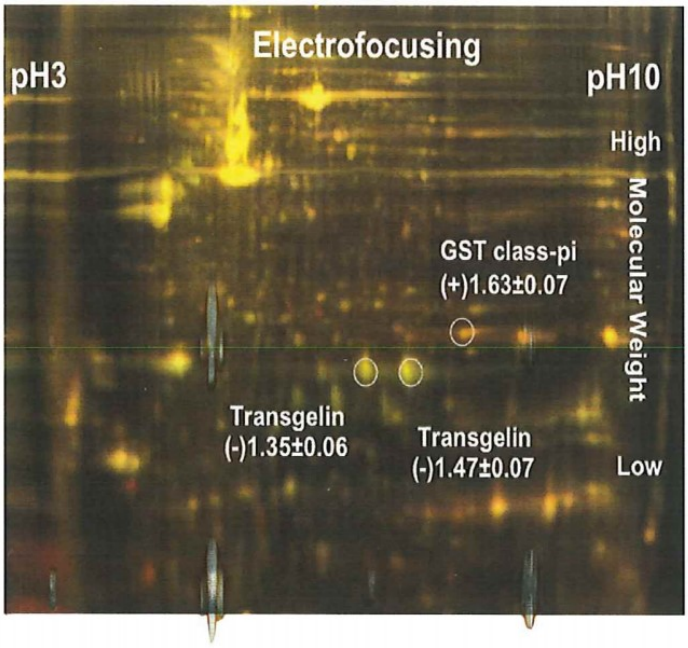

Figure 4c. The protein expression profile in the urinary bladder of hamsters exposed to Na-As (III).

The new research protocol could be the following:

For metabolic processing, hamsters provide a good animal model. For carcinogenesis, mouse model is well accepted. The aims of this project are: 1) To map the differential distributions of arsenic *(*As) metabolites/species in relation to selenium (Se) and mercury (Hg) levels in male and female hamsters and 2) To chart the protein expression profile and identify the defense proteins in mice and hamsters after As injury.

Experimental hamsters (male or female) will include four groups. **The first group** will be treated with Na-As(III), **the second group** with Na-As (III) and Na-selenite [(Na-Se (IV)], **the third group** with Na-As (III) and methyl mercuric chloride (MeHgCl), and **the final group** with Na-As (III), Na-Se (IV), and MeHgCl at different levels. Urine and tissue will be

collected at different time periods and measured for As species using high performance liquid chromatography/inductively coupled plasma-mass spectrometry (HPLC/ICP-MS). For proteomics, mice (male and female) and hamsters (male and female) will be exposed to Na-As (III) at different levels in tap water, and control mice and hamsters will be given only the tap water. Tissue will be harvested at different time periods. Two dimensional differential in gel electrophoresis (2D-DIGE) combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) will be employed to identify the expressed protein.

In summary, we intend to extend our findings to: 1) Differential distribution of As metabolites in kidney, liver, lung, and urinary bladder of male and female hamsters exposed to Na-As (III), and combined with Na-As (III) and Na-Se (IV) and/or MeHgCl at different levels and different time periods, 2) Show the correlation of As species distribution in the tissue and urine for both male and female hamsters treated with and without Na-Se (IV) and/or MeHgCl, and 3) Show protein expression profile and identify the defense proteins in the tissues (liver, lung, and urinary bladder epithelium) in mice after arsenic injury.

***The significance* of this study:**

The results of which have the following significances: *(*A) Since Inorg-As is a human carcinogen, understanding how its metabolism is influenced by environmental factors may help understand its toxicity and carcinogenicity, (B) The interactions between arsenic *(*As), selenium (Se), and mercury (Hg) are of practical significance because populations in various parts of the world are simultaneously exposed to Inorg-As & Se and/or MeHg, (C) These interactions may inhibit the biotransformation of Inorg-As (III) which could increase the amount and toxicity of Inorg-As (III) and MMA (III) in the tissues, (D) Determination of arsenic species profile in the tissues after ingestion of Inorg-As (III), Se (IV), and/or MeHg+ will help understand the tissue specific influence of Se and Hg on Inorg-As (III) metabolism, (E) Correlation of arsenic species between tissue and urine might help to understand the tissue burden of arsenic species when researchers just know the distribution of arsenic species in urine, (F) The identification of the defense proteins (over-expressed and under-expressed) in the tissues of the mouse/hamster may lead to understanding the mechanisms of inorganic arsenic injury in human.

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