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Review Paper

Molecular markers of heavy metal toxicity – A new paradigm for health risk assessment

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Abstract: *Last decade has witnessed increased interest in studies dealing with molecular markers of health and disease expression of genes. Specific toxicant "signatures" have been detected using genome base technologies such as microarrays. Further, toxins have been classified on the basis of these signatures. Knowledge on these signatures has helped in the identification of novel drug candidates. This review discusses the gene expression studies recently made on arsenic, cadmium, mercury, chromium, lead, copper, nickel, manganese, and other essential elements. Toxicogenomics standards and their organizations have also been briefly described. Although this information can not be considered as complete, recent reports from different laboratories on bacteria, fish, laboratory animals and humans have been summarized. It is expected that toxicogenomics data presented in this review will be helpful in planning and excretion of human health risk assessment programs.*

Key words*: Heavy metals, Molecular markers, Toxicogenomics, Health risk assessment*
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Introduction

Omic technology has seemingly emerged as a final and finite approach to understand the mechanism of a xenobiotic toxicity. "Omics" is a general term used to describe - rapidly growing fields of scientific endeavors, the best known member of which is "genomics"(Waters and Fostel, 2004). Genomics is the study of the genome, the complete genetic complement of an individual or species, rather than the study of single genes. The suffix - omics - refers to the study of a complete set of biological molecules. Just as refers genomics - to the study of genome, proteomics is the study of the entire complement of proteins and metabolomics is the study of complete set of low molecular weight metabolites present in a cell or organism at any one time (de Hoog and Mann, 2004; Whitfield *et al.,* 2004). These "omic" technologies allow the study of a large number of endpoints simultaneously in biological samples. Their application in toxicology and molecular epidemiology holds great promise. However, data generated from them should be treated with great caution. The term "toxicogenomics" was first coined in 1999 to describe the marriage of toxicology and genomics (Nuwaysir, 1999). Since then the field of toxicogenomics has undergone a rapid and uneven surge of growth. Driven by the promise of whole genome gene expression analysis by microarray, toxicogenomics has been advanced as the tool for improved mechanistic toxicology screens, more sensitive and quick toxicity assessment, drug and chemical safety assessment and new drug discovery assays (Hayes and Bradfield, 2005). However, it quickly became evident to toxicologists, making toxicogenomic studies that using enormous data sets from microarray studies would require new tools and new approaches in order to organize and interpret them.

Furthermore, understanding the ultimate toxicological processes with the information provided by the new "omic" technologies seemed unfathomable. The early years of the microarray era were technology driven resulting in homemade and custom arrays with limited usefulness. The latest approach to data analysis of these huge whole genome data sets ushered in the concept of "functional genomics." A long standing definition of functional genomics is the development and application of global (genome-wide or system wide) experimental approaches to gross gene function by making use of the information and reagents provided by structural genomics (construction of high resolution genetic, physical and transcript maps of an organism). It is characterized by high throughput or large scale experimental methodologies combined with statistical and computational analysis of the results. The fundamental strategy has been to expand the scope of biological investigations from studying single genes or proteins to studying all genes or proteins at once in a systemic function (Waters and Fostel, 2004). Thus functional genomics aims to discover the biological function of particular genes and to discover how sets of genes and their products work together in health and disease.

The control of gene expression has been a focus of molecular biology since classic experiments in the 1950s first began to explore how DNA fulfilled its hereditary role. New findings are now demonstrating that low level exposures to a variety of agents, including environmental agents / contaminants can alter gene expression, affecting families of genes that are central to disease resistance, metabolic function, growth and development *etc.*

Considerable attention has been paid within the last decade to monitor changes induced by exogenous agents in the expression

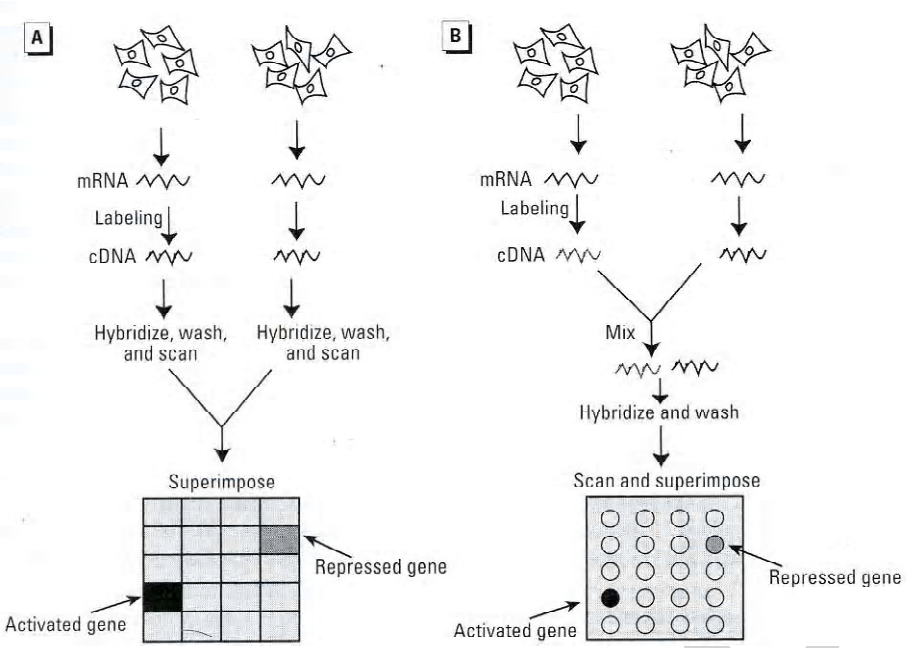

Fig. 1: Gene expression analyses by microarray. (A) One-color expression analysis uses a single fluo-rescent label and two arrays to generate expression profiles for two cell or tissue samples (test and refer-ence samples). Activated and repressed genes are obtained by superimposing images obtained by the two arrays. (B) Two-color expression analysis uses two different fluorescent labels and a single array to generate expression profiles for the test and reference samples. Activated and repressed genes are obtained by superimposing images generated in different channels on a single array. In both cases the monochrome images from the scanner are imported into software in which the images are pseudocolored and merged. Data are viewed as a normalized ratio in which significant deviation from 1 (no change) indi-cates increased (> 1) or decreased (< 1) level of gene expression relative to the reference sample [Source: Teresa Lettieri, *Environ. Hlth. Perspect.*, 114,4-9 (2006)]

of genes. This work has revealed impacts at major control points in the chain of biochemical events that lead to protein synthesis. These impacts can be summarized as follows:

(i) Effect on DNA methylation
(ii) Alteration of hormone concentration
(iii) Up and down regulation of enzymes
(iv) Changes in hormone receptor density
(v) Receptor binding by the exogenous agent
(vi) Alteration in the interaction between bound-ligand receptor complex with transcription factors and the gene promoter site, preventing gene expression
(vii) Activation of hormonally induced transcription factors leading to gene expression.

A central trend in the field of toxicogenomics is that specific toxicant "signatures" can be detected through the use of large scale genome based technologies such as microarrays (Nuwaysir *et al.,* 1999). These signatures consist of the observation of genome wide patterns of gene expression in response of treatment of a specific cell or tissue with a toxicant (Afshari *et al.*, 1999; Rockett and Dix, 1999). One aim of signature identification is the classification of known toxicants into categories based upon specific gene expression patterns in specific tissues/organs. Thus library of signatures could then be used to rapidly and inexpensively screen the drug/chemical toxicity or to search novel drug candidates (Waters and Fostel, 2004).

Overview of gene expression analyses:

The field of DNA microarray has evolved from Ed Southern's key insight (Southern, 1975) twenty five years ago showing that labeled nucleic acid molecules could be used to interrogate nucleic acid molecules attached to a solid support. The resulting Southern blot is considered to be the first DNA array (Southern, 2000). It was only a small step to improve the technique to filter-based screening of clone libraries, which introduced a one-to-one correspondence between clone and hybridization signal (Grunstein and Hogness, 1975). The next advance was the use of gridded libraries stored in micro titer plates and stamped onto filters in fixed positions. With this system, each clone could be uniquely identified and information about it accumulated. Several groups explored expression analysis by hybridizing mRNA to cDNA libraries gridded on nylon filters. The subsequent explosion of array technologies was sparked by two key innovations. The first was the use of nonporous solid support, such as glass, which has facilitated the miniaturization of the array and the development of fluores-cence-hybridization detection (Lockhart *et al*., 1996; Schena *et al.,* 1995, 1998). The second critical innovation was the development of methods for high-density spatial synthesis of oligonucleotides, which allows the analysis of thousands of genes at the same time. Recently, a significant technical achievement was obtained by producing arrays with more than 250,000 oligonucleotides probes or 10,000 different cDNAs per square centimeter (Lipshutz *et al.,* 1999). DNA microarrays

are fabricated by high-speed robots, generally onto glass. Because the DNA cannot bind directly to the glass, the surface is first treated with silane to covalently attach reactive amine, aldehyde, or epoxies groups that allow stable attachment of DNA, proteins and other molecules.

The nucleic acid microarrays use short oligonucleotides [15-25 nucleotides (nt)], long oligonucleotides (50-120 nt), and PCR-amplified cDNAs (100-3,000 bp) as array elements. The short oligonucleotides are used primarily for the detection of single-nucleotide poly-morphisms (SNPs). Because this application requires the discrimination of only one mis-match, the presence of a shorr oligonucleotide maximizes the destabilization caused by mis-pairing (Lockhart *et al.,* 1996). Conversely, the PCR-amplified cDNAs produce strong signals and high specificity (DeRisi *et al.,* 1996). The cDNA elements are readily obtained from cDNA libraries and are typically used for organisms for which only a limited part of the whole genome information is available. The long nucleotides offer strong hybridization signal, good specificity, unambiguous sample identification and affordability (Hughes *et al.,* 2000; Kane *et al.*, 2000; Schena *et al.,* 1998). All these advancements have allowed gene arrays to become a standard tool in molecular toxicology. With this technology, cells or tissues are exposed to toxicants, and then gene expression is measured by collecting mRNA, converting mRNA to labeled cDNA, hybridizing it to the DNA array, staining it with an appropriate dye, and visualizing the hybridized genes using a fluorometer (DeRisi *et al.,* 1996; Lashkari *et al.,* 1997; Schena *et al.,* 1995) (Fig. 1). The raw data are analyzed using bioinformatics software and databases. The aim is to obtain meaningful biological information such as patterns of relative induction/ repression levels of gene expression, participation in biochemical pathways and (in the most favorable cases) genetic signatures.

During last few years, a number of laboratories have identified specific gene expression patterns in specific tissues abused by environmentally significant and toxic metals. This information with special reference to heavy metals has been reviewed in the following paragraphs:

Arsenic: Arsenic is a naturally occurring metalloid. Millions of people are at risk of cancer and other diseases because of chronic arsenic exposure (Flora *et al.,* 2007). General adverse health effects associated with human exposure to arsenicals include cardiovascular diseases, developmental abnormalities, neurologic and neurobehavioural disorders, diabetes, fibrosis of the liver and lung and haematological disorders (Tseng, 2007). Once in the tissue, arsenic exerts its toxic effects through several mechanisms, the most significant of which is the reversible combination with sulfydryl groups. No suitable molecular markers of arsenic toxicity are available at present. Blood/urine concentration of arsenic is currently applied to determine the body/tissue burden of arsenic. Several workers have estimated arsenic concentration in toenails of humans suffering from arseniasis. These observations have been generally considered as biomarkers of exposure.

Arsenic exposure is clearly linked to cancer of skin, lung, bladder, liver and kidney in human (Singh *et al*., 2007; Singh and Rana, 2007). In rodent cells, arsenic has been reported to induce aberrant gene expression including - activation of the proto-oncogene c-myc. Further arsenic is known to exert at least some of its effects through interaction with glutathione (GST). Similarly MT often functions in defense against metal induced injury. Shimizu *et al*. (1998) examined the relationship among GSH, MT gene expression and c-myc expression in cultured rat myoblast (L6) cells. They concluded that cellular levels of GSH, but not MT gene expression play an important role in resistance to arsenic toxicity and aberrant gene expression. Liu *et al.* (2001) defined acute arsenic induced stress related gene expression *in vivo*. The Atlas mouse stress / toxicology array revealed that the expression of genes related to stress, DNA damage and metabolism was altered by acute arsenic treatments. Down regulation of certain cytochrome P450 enzymes occurred with arsenic treatment. Multiprobe RNAse protection assay revealed the activation of the c-Jun/AP-1 transcription complex after arsenic treatments. Implications of arsenic mediated cellular signal transduction, transcription factor activation and aberrant gene expression were studied by Yang and Frankel (2002). They elucidated that arsenic induced signal transduction pathways lead to aberrant gene expression which are involved in the malignant transformation.

These authors concluded that identification of novel molecular targets will help in the treatment of human cancers resulting from arsenic exposure.

Bae *et al.* (2002), identified changes in gene expression that contribute in malignant conversion. c-DNA microarray technology was used. Analysis of multiple human arrays in MNNG - (n-methyl-n-nitro-n-nitrosoguanidine) transformed RHEK-1 cells, designated OM3 and those treated with arsenic or arsenic containing metal mixtures showed unique patterns of gene expression. Genes that were over expressed in OM3 included oncogenes, cell cycle regulators, and those involved in signal transduction. Whereas, genes for DNA repair enzymes and inhibitors of metastasis were suppressed. In arsenic treated rats, multiple DNA repair proteins were over expressed.

A mixture (containing arsenic, cadmium, chromium and lead) treated cells showed increased expression of a variety of genes including metallothionein and integrin 4. The goal of these studies was to identify common battery of genes affected by chemical modulator of the carcinogenic process. In a population based case control study of bladder cancer in new hamsphire, Andrew *et al.* (2003) recorded that intake of arsenic in drinking water at levels > 10 mg/l may decrease nucleotide excision repair gene expression and hypothesized nucleotide excision repair inhibition in arsenic induced carcinogenesis. In a study made by Bae *et al.* (2003) in man, arsenic was reported to interact with several genes *viz* BMP4, CDKN2A, GDF15, KRT18, RAD23AA, XRCC1. Global alteration of

gene expression in human keratinocytes was observed by Rea *et al.* (2003). Yih *et al.* (2002), reported changes in gene expression profiles of human fibroblast in response to sodium arsenite treatment. In a different study made by Liu *et al.* (2004), aberrant gene expression was reported in adult mice exposed *in utero* to inorganic arsenic. Over expression of α-fetoprotein, c-myc, cyclin D1, proliferation associated protein PAG and cytokeratin 80 were more dramatic in arsenic induced hepatocellular carcinoma than spontaneous tumors.

It is important to note that expression changes occurred in adulthood even though arsenic exposure ended during gestation. Sixty genes were differently expressed. In a global gene expression study on the impact of arsenic in *Saccharomyces cerevisae*, Haugen *et al.* (2004) showed, in the regulatory network, several important nodes as centres of arsenic induced activity. The highest scoring proteins included Fh17, Msn2, Msn4, Yap1, Yap2, Pre1, Hsf1 and Met 31. They further confirmed that the transcription factors Yap1, Arr1(Yap 8) and Rpn 4 strongly mediate the cellular adaptation to arsenic induced stress and Cad1 has negligible impact. In a very recent study in arsenic exposed population of Bangladesh made by Argos *et al.* (2006), four hundred and sixty eight genes were differentially expressed in population suffering from arsenic lesions. Their findings show that microarray based gene expression analysis is a powerful method to characterize the molecular profile of arsenic exposure and other arsenic induced diseases. Undoubtedly, gene expression studies have further aided to our understanding on arsenic toxicity.

Cadmiun: Cadmium has many applications to its credit. It is mainly used as a color pigment in paints and plastics, as a cathode for nickel –cadmium batteries and electro plating industry. It is a by-product of zinc and lead mining and smelting which are important sources of environmental pollution. The toxicology of cadmium has been reviewed by Friberg *et al.* (1985), WHO (1992), EPA (1997) and ATSDR (1998). Acute toxicity may result from the ingestion of relatively high concentrations of cadmium through contaminated beverages and food. Long term effects of low-level exposure to cadmium are chronic obstructive pulmonary disease and emphysema and renal tubular disease. There may also be effects on the cardiovascular and skeletal systems. Epidemiologic studies have shown a relationship between occupational exposure to cadmium and lung cancer and possibly prostate cancer. Cadmium has been identified by the International Agency for Research on Cancer as a category 1 human carcinogen (IARC, 1994). Beta-2-microglobulin (B_2M), retinal binding protein (RBP), N-acetyl-β-glucosaminidase (NAG) and metallothionein have been used in conjunction with urinary cadmium levels as markers of cadmium exposure and renal tubular dysfunction. The most important biomarker of cadmium exposure is increased cadmium excretion in urine.

It is also known that Cd affects the transcription of a number of genes. Cd induces those human genes that perform protective functions (Koizumi, 1997), and those coding for metallothioneins (Karin *et al.,* 1984; Schmidt *et al.,* 1985), heat shock proteins (Williams and Morimoto, 1990; Hiranuma *et al.,* 1993) and heme oxysenase 1 (Takeda, *et al.,* 1994). Cd also induces protooncogenes (Jin and Ringertz, 1990).

Development of DNA microarray technology has recently made the comprehensive analysis of gene expression possible using human HeLa S3 cell line. Koizumi and Yamada (2003) could observe 46 up regulated and 10 down regulated genes whose expression levels changed two fold or greater. The expression of protective genes encoding for metallothioneins, antioxidative proteins and heat shock proteins was simultaneously induced.

In addition, altered expression of many genes involved in signaling and metabolism was observed. Lu *et al.* (2001) earlier reported that MT gene expression in peripheral lymphocytes may be a useful biomarker of susceptibility to renal toxicity by cadmium. Genome-wide analysis of temporal gene expression profiles in *E. coli* following exposure to cadmium revealed a shift to anaerobic metabolism and induction of several stress response systems. Wang and Crowley (2005), reported that disruption in the transcription of genes encoding ribosomal proteins and zinc binding proteins may partially explain the molecular mechanisms of cadmium toxicity. Beyersmann (2002) observed the effect of carcinogenic metals on gene expression. Shin *et al.* (2004) using the suppression subtractive hybridization technique identified 29 cadmium inducible genes, primarily involved in inflammation, cell survival and apoptosis. Nur 77 family genes were also identified in cadmium exposed lung. They concluded that Nur 77 plays an important role in cadmium induced apoptosis. Majumder *et al.* (2003) have studied the effect of cadmium on HMOX1, HSP70, MT1A, MT2A and MTFI human genes. Similarly Garrett *et al.* (2002) showed that cadmium results in increased expression of FOS mRNA, JUN mRNA, MT3 mRNA, and MT3 protein in *Homo sapiens*.

Despite large data, overall response of cells to cadmium toxicity at the genome level is not yet completely understood. Further, studies on specific molecular markers are awaited.

Mercury: Mercury demonstrates the diversity of effects by its different chemical species (Goyer, 1996). It is the only metal that exists in a liquid state at room temperature. The vapor is much more hazardous than liquid form. The element exists in three oxidation states. In the zero oxidation state (Hg) mercury exists in metabolic form or as the vapor. The mercurous and mercuric are the higher oxidation states where the mercury has lost one atom (Hg^{+}) and two elections (Hg^{2+}) respectively. In addition mercuric mercury can form a number of stable organic mercury compounds by attaching to one or two carbon compounds. Methyl mercury (CH_3Hg^{+}) is the most important organic form from human health point of view. Different forms of mercury enter into the environment through different pathways. The vapor from metallic mercury is readily absorbed in the lungs. Dissolved form is readily absorbed by the bloodstream and diffuses to all tissues in the body. Gastrointestinal absorption occurs through contaminated

food. Inorganic mercury shows the highest affinity with the kidney whereas organic mercury has greater affinity for brain. Excretion of mercury from the body is by way of urine and feces. All forms of mercury cross the placenta to the fetus as known in experimental animals.

Elemental or metallic mercury is oxidized to divalent mercury after absorption in the body and is probably mediated by catalases. Methyl mercury undergoes biotransformation to divalent mercury compounds in tissues by cleavage of the carbon mercury bond. Within cells, mercury may bind to a variety of enzyme systems including those of microsomes and mitochondria producing non-specific cell injury or cell death. It has a particular affinity for ligands containing sulfydryl groups. Metallic mercury, but not the methyl mercury, induces the synthesis of metallothionein in kidney cells. The recommended standard (time-weight average) for permissible exposure limits for inorganic mercury in air in the workplace is 0.05 mgm^{-3}.

The story of metal-gene expression takes us back to 1993 when O'Halloran (1993), suggested that metalloproteins play a structural and catalytic role in gene expression. The metalloproteins control genes involved in respiration, metabolism and stress response systems *viz*. iron uptake and storage, copper efflux and mercury detoxification. Mercury responsive DNA-distortion mechanism for transcriptional control of detoxification genes was also identified. The relationship of merA gene expression (specifying the enzyme mercuric reductase) to mercury volatilization in aquatic microbial communities was investigated in Reality Lake in Oak Ridge Tennessee (USA).

Level of merA-specific mRNA and Hg(II) volatilization were influenced more by microbial metabolic activity than the concentration of mercury (Nazaret, *et al.,* 1994). Ogunseitan (1998) further suggested a protein method for investigating mercuric reductase gene expression in aquatic environment. Exposure of the mast cell line RBL-2H3 to mercuric chloride in Brown Norway (BN) rat enhanced IL-4 mRNA and its promotor activity. The enhancement of IL-4 gene expression by $HgCl_2$ was significantly reduced by antioxidants. Thus Wu *et al.* (2001), hypothesized that alterations in intracellular reactive oxygen species modulate both IL-4 gene expression and mast cell function. Monetti *et al.* (2002) searched molecular markers for methylmercury exposure. They found that the expression of two genes is completely inhibited by CH_3HgCl. The first one was similar to the human iron-sulfur subunit of succinate dehydrogenate while the other was similar to human homeodomain-interacting protein kinase 3 (HIPK3). Shimada *et al.* (2003) explored the possibility of using metallothionein (MT) mRNA in the larvae of *C. elegans* as a biomarker of short term exposure to heavy metals. They concluded that the induction of MT-II mRNA in the larvae of *C. elegans* can be used as a potential biomarker of exposure. By the time microarray analysis was developed. Pollard *et al.* (2004) used DNA microarray analysis to compare gene expression at the Hmr1 locus in N2B and DBA/2 mice. Within the Hmr1 locus 12 genes had statistically significant differences in expression between the 2 mouse strains after mercury treatment. Of the genes differentially expressed in response to mercury, 8 were increased in expression in the DBA/2 mice compared with the N2B mice. Amongst these, several genes might contribute to resistance to mercury. An analysis of genes in kidney samples from mice exposed to mercury revealed differential expression of seven genes-within the Hmr1 locus. Reports from Comparative Toxicogenomics Database show that mercury results in increased expression of ABCC1 and ABCC2 mRNA and ABCC1 protein (Aleo, 2005). Mercury binds to MT1 and MT2 protein in Altantic white sided dolphin (Das *et al.,* 2002.) It binds to MT1 and MT2 protein in mice also (Shimada *et al.,* 2004). Spatially restricted patterns of gene expression in live embryos became widespread when the green fluorescent protein (GFP) was introduced as a reporter gene (Chalfic *et al.,* 1994). To develop a more sensitive and reliable reporter for gene expression, Raz *et al.* (1998) developed β-lactamase as a marker for gene expression in live zebra fish embryos. Very recently Ayensu and Tchounwou (2006) hypothesized that mercury induced hepatotoxicity is associated with the modulation of specific gene expression in liver cells that can lead to several disease states involving immune system dysfunctions. There was a clear separation in gene expression profiles between controls and mercury treated cells. Hierarchical cluster analysis identified 2211 target genes that were affected. 138 of these genes were up-regulated among which 43 were significantly over expressed ($p>0.001$) with greater than a two fold change and ninety five genes were moderately over-expressed with an increase of more than one fold ($p>0.004$) change. 2023 genes were down regulated with only 45 of them reaching a statistically significant decline ($p>0.05$). These genes were located on all chromosomes except chromosome 22. These genes are responsible for metabolic pathways involving cell cycle, apoptosis, cytokine expression, stress responses, G-protein signal transduction, transcription factors, DNA repair and related genes.

Significant alterations in specific genes provide new directions for deeper mechanistic investigations that would lead to a better understanding of the molecular basis of mercury induced toxicity and human diseases.

Chromium: Chromium occurs in different oxidation states ranging from Cr^{2+} to Cr^{6+} but only trivalent and hexavalent forms are of biological significance. The trivalent is the more common form, however, hexavalent forms such as chromate compounds are of greater industrial importance. Health effects of chromium have been reviewed from time to time (Fishbein, 1981; WHO, 1988; O'Flaherty, 1995). Systemic toxicity occurs from ingestion of high amount of Cr (VI). Low level exposure causes glomerular and tubular damage. Cr (VI) is corrosive and causes chronic ulceration of skin surface. Asthma may be caused by occupational exposure to Cr (Bright *et al.*, 1997). Known toxic effects of chromium in human have been attributed to Cr (VI) where it is reduced to Cr (III) that complexes with intracellular macromolecules. Exposure to chromium particularly in the chrome production and chrome pigment industries is associated

with diseases of the respiratory tract (Langard and Norseth, 1986). The mechanism of Cr (VI) carcinogenicity in the lung is believed to be its reduction to Cr (III) and to generation of reactive intermediates. Cr elicits a variety of effects, (i) at the biochemical level, the formation of coordination covalent interaction of Cr (II) and Cr (III) with DNA and of DNA-DNA and DNA–protein complexes; (ii) at the genomic level, the induction of gene expression (oxidant stress, metallothionein and tumor suppressor genes), gene mutations, DNA lesions, inhibition of protein synthesis and arrest of DNA replication; (iii) at the cellular level- cell cycle arrest, apoptosis and neoplastic transformations (Bridgewater *et al.*, 1998; Singh *et al.,* 1998; Kaltreider *et al.,* 1999; Dubrovskaya and Wetterhahn, 1998; Solis-Heredia *et al.,* 2000). DNA-protein complexes may serve as biomarkers of exposure. There exists conclusive evidence that chromium compounds cause cancer at sites other than the respiratory tract. The effect of DNA damage induced by the carcinogenic Cr (VI) on the function of DNA as a template for transcription of constitutive and inducible genes was examined in chick embryo liver *in vivo* (Hamilton and Wetterhahn, 1989). Cr (VI) had significant effects on the basal and drug inducible expression of 5-aminolevulinate synthase and cytochrome P450. The changes in steady state expression of these two inducible genes were similar to the changes in transcription rate indicating that effects of Cr were principally transcriptional. The basal expression of the genes was increased four to five fold at maximum and the time course of this effect was similar to the time course for Cr (VI) induced DNA damage and repair. Hamilton *et al.* (1998) showed that various genotoxic chemical carcinogens including Cr (VI) principally altered expression of several inducible genes but had little or no effect in constitutive gene expression. As part of the ASTDR mandate to evaluate the toxicity of the mixtures of metals, Tully *et al.* (2000) using Xenometrix system showed that Cr (III) induces CRE, FOS, GADD 153 and XRE promoter at a higher dose of 750 μM. However, Cr (VI) produced significant dose related induction of the p53RE, FOS, NF*k*B RE, XRE, GADD45, HSP70 and CRE promoters at much lower doses in the range of 5-10 μM. Ye and Shi (2001), utilizing the high density oligonucleotide array representing 2400 genes and using RNA of human lung epithelial cells after *in vitro* exposure to Cr (VI) observed that expression of 150 genes was up-regulated and that of 70 genes was down-regulated by Cr (VI). The gene expression profile revealed that Cr may involve redox stress, calcium mobilization, energy metabolism, protein synthesis, cell cycle regulation and carcinogenesis. Izzotti *et al.* (2002), investigated multi gene expression in the liver and lung of rats receiving intra-tracheal instillations of sodium dichromate for three consecutive days. The basal expression of 52 genes out of 216 tested genes was 2.1 to 11.1 times higher in the liver than the lung of control rats. 56 genes were up-regulated (2.1 to 3.0 times) in the lung as an early response to Cr. The altered genes are those involved in the metabolic reduction of Cr (VI) and in a variety of interconnected functions, such as multi drug resistance and stress response, protein and DNA repair mechanisms, signal transduction pathways, apoptosis and cell cycle modulation. Maples and Bain (2004) found 20 differently expressed genes in a mummychogs fish, *Fundulus heteroclitus* after laboratory exposure to Cr. The genes were found to be homologous to known sequences including a fatty acid binding protein, cytochrome P450 2N2 (CYP2N2) and a precursor to the translation initiation factor eIF2B. Fatty acid-binding protein was repressed 3-6 fold in the field-site animals as compared to reference animal. eIF2B was repressed 2-fold and an expressed sequence tag (EST) termed A31 was induced 2.6 fold. The authors speculated the use of A31 as a biomarker of chromium exposure in fish. Now it has been established that chromium exposure alters inducible gene expression, form chromium-DNA adducts and chromium DNA cross links and disrupts transcriptional complexes at the promoter of inducible genes. The inhibitory effect of chromium on B(a)P dependent gene induction was generalized, affecting the induction of over 50 different genes involved in a variety of signal transduction pathways. It has been concluded that chromium inhibits inducible but not constitutive gene expression (Wei *et al.,* 2004). Interaction of Cr(VI)with 1-Cys peroxiredoxin, C3, carboxypeptidase B, Cyp2, EEFIG, EEF2, G6PD, GSTA3, GSTA4, genes in winter flounder *Pseudopleuronectes americanus* has been studied by Chapman *et al.*(2004). Majumder *et al.* (2003) have observed the effects of Cr (VI) on ACTB, GAPDH, HMOX1, HSP70, MT1A, MT2A and MTF1 in *Homo sapiens*. These reports form the basis of molecular toxicity of chromium.

Lead: Lead is a ubiquitous metal. It can be detected in all inert environments and in all biological systems. Lead poisoning predominantly occurs in children. The problem of lead poisoning has been discussed in several reviews (EPA, 1986; Goyer, 1993; NRC, 1993; ATSDR, 1999).The principal routes of exposure in general population are air, water, food, lead glazed pottery, industrial emissions and dust. Other potential sources of exposure to lead are recreational shooting, hand loading of ammunition, soldering, jewelry making, pottery making, gunsmithing, glass polishing, painting and stained glass crafting. Children absorb greater proportion of lead than the adults. More than 90% of the lead in blood is in red blood cells. The largest and kinetically slowest pool is skeleton. The largest soft tissue accumulations of lead occur in liver and kidney but it may be found in most of the tissues of the body. The major route of its excretion is the kidney.

Lead toxicity involves several organ systems. The critical effects in infants and children involve the nervous system (ASTDR, 1999). However, in adult population, it is hypertension. Effects on heme system are treated as biochemical indicators of exposure to lead. Other target organs are gastrointestinal, reproductive and skeletal systems. Lead nephropathy is one of the oldest recognized health effects of lead (Oliver, 1914). Lead is a renal carcinogen in rodents. Microscopical examination of kidney shows dense, homogeneous eosinophilic intranuclear inclusions. There appears to be no specific biomarker for lead induced renal disease. Lead has been found to be immunosuppressive (McCabe and Lawrence, 1991). Gametotoxic effects have been demonstrated in male and female animals (Stowe and Goyer, 1971). Epidemiologic studies suggest a relationship between occupational lead exposure and cancer of the lung and brain.

Organic lead compound, tetraethyl lead (TEL) was used for many years as a gasoline additive. While the production of TEL has been stopped in USA and reduced in many countries, it is still produced and used in some countries. It is metabolized by CYP450 to trimethyl lead (TML).Mechanisms of its toxicity include damage to membranes, disturbances in energy metabolism and direct interference with neurotransmitter synthesis. Symptoms of its toxicity include nausea, vomiting, diarrhea associated with nervous system problem like irritability, headache and restlessness. Chronic heavy sniffing of leaded gasoline results in signs of dementia and encephalopathy, with cerebellar and corticospinal symptoms.

Lead primarily acts by competing with endogenous cations on protein binding sites. In particular, lead can substitute calcium and zinc both in numerous proteins (Goering, 1993; Goldstein, 1993; Simons, 1993; Zawia *et al*., 1998). This substitution can further alter the normal functioning of these proteins and thus can alter the cellular pathways and induce aberrant gene transcription (Zhu and Thiele, 1996; Bouton and Pevsner, 2000). Bouton *et al.* (2001) demonstrated that the expression of numerous genes can be altered by lead. Differential regulation of mRNA expression of N-methyl D-aspartate (NMDA) subunit genes has been observed following lead exposure (Guilarte, 1997). The perturbation of the activity of phosphokinase C (PKC) by lead can cause altered transcriptional regulation of numerous mRNA transcripts regulated by PKC *i.e.* fos and jun (Chakraborti *et al*., 1999). Bouton *et al.* (2001), showed that calcium binding protein annexin A5, is directly bound and activated by nanomolar concentration of lead. Amongst stress-response genes that were up-regulated by lead treatment were GFAP, microsomal glutathione S-transferase, mitochondrial 10KDa heat shock protein, and HSP70 are all involved in general cellular responses to stress. *Daphnia* hemoglobin gene was greatly expressed following lead exposure. He concluded that hemoglobin gene expression may be used as a biomarker of lead toxicity. Significant increases in the level of c-fos, c-jun and egr-1 but not NGF1B mRNA were observed in PC12 cells exposed to lead (Kim *et al*., 2000). It appears that lead induces the expression of immediate early genes by a mechanism that requires protein kinase C. Further studies on lead induced gene expression profile will be important from public health point of view.

Copper: Copper is a nutritionally essential element. In a general population, food, beverages, and drinking water are potential sources of excess exposure. Industrial exposure occurs in miners or through smelting operations, welding and related activities. The metabolism and health effects of copper have been reviewed by WHO (1996), Chan *et al.* (1998)., NRC (2000). Gastrointestinal absorption of copper is normally regulated by homeostatic mechanisms. It is transported through serum initially bound to albumin and later more firmly to ceruloplasmin and transcuprein. The bile is the normal excretory pathway that plays a primary role in copper homeostasis. Copper toxicity occurs in the form of nausea, vomiting and diarrhea (Pizzarro *et al.,* 1999). Ingestion of large amounts of copper salts may produce hepatic necrosis and death. Excessive accumulation of copper in liver, brain, kidneys and cornea manifests into Wilson's disease. This disorder is also called as hepatolenticular degeneration. Genetic studies have identified a linkage between Wilson's disease and chromosome 13. Menke's disease or "Kinky hair syndrome", Indian childhood cirrhosis (ICC) and idiopathic copper toxicosis or non Indian childhood cirrhosis are other disorders caused by copper (Mercer *et al*., 1993; Sethi *et al.,* 1993; Muller *et al.,* 1996).

Molecular mechanisms leading to Wilson disease were paid considerable attention in the past. Bull *et al.* (1993) reported that Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. Earlier a candidate gene for Menke's disease that encodes a potential heavy metal binding protein was identified (Chelly *et al*., 1993). Oh *et al.* (1999) cloned and characterized the promoter region of the Wilson disease gene. Sugimoto *et al.* (1988) synthesized the gene coding for the *Neurospora crassa* copper metallothionein (MT). Gene expression among nine natural isolates of *Sacharomyces cerevisae* grown either in the presence or absence of copper sulfate was compared by Fay *et al.* (2004). They identified 633 genes that showed significant differences in expression among strains. Of these genes, 20 were correlated with resistance to copper sulfate and 24 were correlated with rust coloration. The genes involved in copper metabolism and toxicity were systemically studied by Spee *et al.* (2005). Several proteins in the copper induced Doberman hepatitis (DH) were reduced compared to healthy controls. They included COX17, ATP7A, ATP7B, CP, MT1A and MURR1. However, copper chaperone ATOX1 remained unaffected. Genes for SOD1 and CAT were reduced 7 fold in DH group. Observations on Bcl-2 showed 4-fold reduction in DH group.

Very recently Varela-Nallar *et al.* (2006), showed that copper induces cellular prion-protein genes in neurones. Cellular prion protein (PrP^c) has a normal glycosylation pattern. It is proteinase-K sensitive and reaches the cell surface attached by a glycophosphatidylinositol anchor. They suggested that PrnP (vector driven PrP^c gene) expression is up-regulated by copper in neuronal cells by a MTF-1 independent mechanism.

Nickel: Nickel is ubiquitous in nature. It occurs mainly in the form of sulfide and silicate minerals. Ambient air, as a result of industrial activity, combustion of fossil fuels and waste incineration is known to contain very low level of nickel. Human exposure may occur through inhalation, ingestion and dermal contact. Occupational exposure may be caused by elemental nickel, nickel compounds, complexes and alloy and also the fumes from alloys used in welding and brazing. Food is a major route of exposure for many people. The Environmental Protection Agency estimates that an average adult consumes 100 to 300 μg of nickel per day. Drinking water contains very small amounts of nickel (ATSDR, 1997).

Deposition, absorption and elimination of nickel particles in the respiratory tract largely depend upon the particle size and concentration of nickel. The rate of dermal absorption depends on the rate of penetration in the epidermis, which differs for different forms of nickel. Nickel when administered to animals, is rapidly

distributed to the kidneys, pituitary, lungs, skin, adrenals, ovaries and testes (Sunderman, 1989). Although intracellular ligands for Ni have not been fully characterized, Sunderman (1989) suggested that cysteine, histidine, and aspartic acid may form nickel complexes. It is poor inducer of metallothionein. A nickel binding metalloprotein-nickeloplasmin has been identified in plasma. It is an α-1 glycoprotein complex that plays an important role in extracellular transport, intracellular binding, and urinary and biliary excretion of nickel (Nebor and Hriagu, 1992; Tabata and Sarkar, 1992). Human nutritional requirements for nickel have not been established (WHO, 1996).

Nickel compounds are carcinogenic to human (IARC, 1989). Risks were highest for lung and nasal cancers amongst workers heavily exposed to nickel sulfide, nickel oxide, and to metallic nickel. It has been hypothesized that nickel damages DNA directly through reactive oxygen species (McCoy and Kenney, 1992). This hypothesis is supported by the fact that the antioxidant vitamin E inhibits some chromosomal condensation caused by nickel (Lin *et al.,* 1991). Blood nickel levels provide a guideline as to the severity of exposure. Sodium diethyldithiocarbamate provides protection against clinical effects.

Dr. George Leikauf and colleagues at the University of Cincinnati Medical Center hypothesized that the mouse response to high concentrations of inhaled nickel particles was under genetic control. They performed microarray analysis that indicated that a small fraction (about 200) of more than 8,000 genes examined in lung cells changed their level of expression during exposure to nickel (Prows, 2003). Liang *et al.* (2001) hypothesizes that MTH1 gene (gene that codes for 8-oxo-2'-deoxyguanisine-5'-triphosphate pyrophosphohydrolase) that hydrolyzed 8-oxo-dGTP, a promutagenic product of reactive oxygen species, was affected by carcinogenic nickel. This finding demonstrated that the MTH7 sequence between 5969 and 1331 contained elements responsive to nickel treatment. Their results suggested that up-regulation of murine MTH1 expression by Ni (II) is controlled by the repeat sequences, potential candidates for nickel-response elements. McDowell *et al.* (2003) studied gene expression in mice treated with nickel. The microarray analysis revealed, increased inflammatory mediator, matrix injury repair and hypoxia-induced factor-mediated sequences and decreased lung specific sequences. Miller *et al.* (2004) showed that nickel results in increased expression of Fos mRNA, GSTA7 mRNA, HSP70 mRNA and MT2A mRNA in *Homo sapiens*. Vengellur *et al.* (2005) compared transcriptional responses of a Hep3B cell line exposed to hypoxia, cobalt, nickel and deferoxamine using high density oligonucleotide arrays. They found that cobalt, nickel and deferoxamine influenced transcription of distinct set of genes that were not affected by cellular hypoxia. Mallakin *et al.* (2006) compared the gene expression profiles of murine lung mRNA from control and Mst1R TK-/-(mice deficient in tyrosine kinase domain) mice at baseline and after 24 hr particulate nickel sulfate exposure. Microarray analysis showed that a total 343 transcripts that were significantly changed, either by Ni treatment or between genotypes. Genes responsible for inflammation, edema and lymphocyte function were altered in the Mst1r TK-/- mice. Interestingly the genes for several granzymes were increased in Mst1r TK-/- mice before Ni exposure, compared to controls. This assessment of gene expression indicates the importance of genetic factors in lung injury caused by particulate matter.

Manganese: Manganese is an essential element. It can exist in 11 oxidation states, from -3 to +7. The most common valences are +2, +4, +7. In superoxide dismutase, it exists during oxidative stress. It is cofactor for a number of enzymatic reactions, particularly those involved in phosphorylation, cholesterol and fatty acid synthesis. Manganese concentrates in mitochondria so the tissues rich in these organelles including liver, pancreas, kidneys and intestine have the highest concentration of manganese. It is eliminated through bile and is reabsorbed by the intestine but the principal route of excretion are faeces.

The most common form of manganese toxicity occurs through chronic inhalation of air borne manganese in miners, steel mills, and some chemical industries (ATSDR, 1997). Pathologic changes include epithelial necrosis followed by mononuclear proliferation. Chronic manganese toxicity is called as "Manganism". It is a neuropsychiatic disorder characterized by irritability, difficulty in walking, speech disturbances and compulsive behaviour that may include running, fighting and singing.

Studies on the effect of manganese on gene expression started when Huang *et al.* (1999) observed the decreased expression of manganese superoxide dismutase in SV40 transformed cells in comparison to their normal counterparts. In another study, Huang *et al.* (2001) reported that hypoxia increases Mn-SOD gene expression in cultured glial cells mainly through activation of a protein kinase C (PKC) pathway. Furthermore, Yi *et al.* (2002) examined genes to elucidate HSP67-induced pathways leading to activation of SOD2, the production of tumor necrosis factor alpha (TNF-α) and 1 beta interleukin (IL-1β) in L929 cells. Duan *et al.* (2003) hypothesized that Mn-SOD activity in cancer cells might cause downstream changes in the expression of other tumor suppression genes. They measured maspin mRNA expression in human breast and prostate cancer cells. Maspin mRNA stability was reported to be the major mechanism for maspin up-regulation by Mn-SOD. They recommended the use of Mn-SOD as gene therapy to treat human breast and prostate tumors. Baek *et al.* (2004) using the cDNA array technology discovered 5 genes in the mouse striatum and 9 genes in substantia nigra (SN) changed by more than 50% following Mn exposure. Among them S100β gene was up-regulated following Mn treatment. Furthermore they showed that Mn exposure increased the expression of S100β at protein level in the astrocytes. Dhar *et al.* (2006) demonstrated that specificity protection (SP1) and nucleophosmin (NPM) interact *in vivo* to enhance NF-κB mediated Mn-SOD induction. Interaction between NPM and SP1 or NF-κB at the promoter and enhancer of Mn-SOD gene was verified by these workers. They suggested an intricate relationship between the positive and negative control of Mn-SOD gene expression.

Other essential elements: There is a group of eight metals generally accepted as essential: cobalt, copper, iron, magnesium, manganese, molybdenum, selenium and zinc. We have discussed about copper and manganese in earlier paragraphs. However, a brief account of toxicity of molybdenum, selenium and zinc deserves a separate mention.

Molybdenum (Mo) exists in multiple oxidation states +3, +4, +5, +6, facilitating, electron transfer. In human, chronic exposure to molybdenum is characterized by high uric acid level in serum and urine. It is called as molybdenosis. In India, it is called as "genu valgum". A gout like disease has been observed in inhabitants of a province of former USSR (Chan *et al.* 1998). After repeated oral administration in rats, fatty degeneration of liver and kidney was observed (Nielson, 1996). Cotter and Gunsalus (1989) showed that molybdenum was required both for complete induction of dmsA-lacZ expression during anaerobic growth of *E. coli*. Kolesnikow *et al.* (1992) provided the first evidence that NarL and FNR interact to ensure optional expression of nrK gene. Availability of molybdate and iron is necessary for optimal nrK expression, whereas the availiability of ntrate is not essential.

Zinc is a nutritionally essential metal and a deficiency results in severe health consequences. Excessive exposure to zinc is relatively uncommon and occurs only at very high levels. Seafood, meat, whole grains, dairy products, nuts and legumes are high in zinc. More than 200 metalloenzymes belonging to six major categories *viz.,* oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases require zinc as a cofactor (Cousins, 1996). Zinc induces the synthesis of metallothioneins which is a factor in regulating the metabolism of zinc including absorption and storage (Miles *et al.,* 2000). Zinc is a functional component of several proteins that contribute to gene expression and regulation of genetic activity. Zinc chelates with cysteine and/or histidine in a tetrahedral configuration forming looped structures called "zinc fingers" which bind to specific DNA regions and are bound in various transcription factors such as steroid hormone receptors and polymerase (Wang *et al.*, 1997). It has a role in immune function and the cytokines. Primarily interleukin-1 (IL-1) and IL-6 influence zinc metabolism.

Inhalation of freshly formed fumes of zinc has been associated with metal fume fever. While the mechanisms of zinc ions interaction with immune cells are poorly understood, a striking concurrent effect of zinc is the induction of the biosynthesis of metallothionein (MT), a group of low molecular weight cysteine rich metal binding proteins, believed to play a role in zinc homeostasis. In humans, they are encoded by a family of genes, located at 16q13 containing 10 functional and 7 nonfunctional MT isoforms. Vandeghinste *et al.*(2000) demonstrated using RT-PCR that the MT-2a, MT-1a, MT-1e, MT-1f, MT-1g, MT-1h, and MT-1x genes are expressed in human lymphocytes and these isoforms are further up-regulated by zinc. Brysk *et al.* (1997) compared gene expression levels of zinc-alpha-2-glycoprotein with those of characteristic cytokeratins of stratified epithelia.

Selenium occurs in nature and biological systems as selenite (Se^{6+}), selenite (Se^{4+}), elemental selenium (Se^{0}) and selenide (Se^{22}). Selenium defieciency leads to cardiomyopathy in mammals, including humans (Levander and Burk, 1996). Selenium metabolism is regulated to meet several metabolic needs. The requirement for selenium is related to the degree of oxidant activity and the supply of nutrients such as zinc, copper, manganese, iron, and vitamin E. Keshan disease is caused in human due to selenium deficiency. This is an endemic cardiomyopathy first discovered in Keshan county in the Peoples Republic of China in 1935 (Chen *et al.*, 1980). In livestock and horses, its toxicity is recognized as "alkali disease" characterized by loss of vitality, emaciation, deformity and shedding of hoofs, loss of long hair, and erosion of joints of long bones. Deficiency of vitamin E increases selenium toxicity. It protects cadmium toxicity.

There are quite a few reports available on the regulation of glutathione peroxidase gene expression by selenium. Chada *et al.* (1989) showed that human GPX gene appears to be regulated post transcriptionally, probably cotranslationally, in response to selenium availability. Bermano *et al.* (1996) suggested that organ differences occur during selenoprotein gene expression on selenium repletion in selenium deficient rats. Esworthy *et al.* (1995) suggested that role of GPX in redox drug resistance might account for changes in hgpx2 gene expression. In a significant study made by Calvo *et al.* (2002), cDNA microarray analysis of 8700 features revealed correlation between the tumorigenicity of the C3(1)/Tag-Pr cells and changes in the expression level of genes regulating cell growth, angiogenesis and invasion. They also identified novel genes that may be involved in mechanisms of prostate cancer *eg.* L1-cell adhesion molecule, metastasis associated gene (MTA-21), Rab-25, tumor associated signal transduction-2 (Trop-2), and selenoprotein P- a gene that binds selenium and prevents oxidative stress. This work demonstrated that expression profiling in animal models may lead to the identification of novel genes involved in human prostate cancer biology. Joshi *et al.* (2006) compared gene expression profiles using Affymetrix HU 133A chips in before/after supplementation paired normal esophageal biopsies. They found that the number of differentially expressed genes (n=11) was less than expected by chance (n=18). They concluded that selenomethionine treatment had no measurable effect on gene expression in the normal squamous esophagus of these subjects with dysplasia.

Thus, it is evident that sufficient information on gene expression profiling has been gathered by different laboratories. To systematize the data, toxicogenomic standards have been developed. It will be in the interest of the reader of this article to briefly discuss about these databases.

Toxicogenomic standards and their organizations:

There are a number of technical, interpretation and imple-mentation issues that impede the use of genomic, proteomic, and metabolomic approaches in biomedical research, regula-tory decision-making, and quantitative risk assessment. These include

Table - 1: Toxigenomic standards and their organizations

Standard	Organization
MIAME-Minimum information about [a]Microarray experiment[a]	MGED Society-microarray gene expression Data society
MIAPE-Minimum Information about [a]Proteomics experiment	HUPO PSI-Human proteome organization Proteomics standards initiative
Minimum requirements for designing and recording the results of a metabolic study[b]	SMRS-Standard metabolic reporting Structures working group

[a]Brazma *et al.* (2001), [b]Lindon *et al.* (2003)

the lack of uniform study designs, multiplicity of normalization and analysis strategies (Quackenbush, 2002), questionable reproducibility of microarray data across plat-forms (Mah *et al.,* 2004; Tan *et al.,* 2003; Ulrich *et al.,* 2004; Yauk *et al.,* 2004), the semiquantitative nature of proteomics (Cox *et al.,* 2005; Garbis *et al.,* 2005), limited availability of metabolite annotation to support metabolomics (Kell, 2004), absence of data quality control measures and standards (Shi *et al.,* 2004; Tong *et al.,* 2004) and lack of effective data sharing and reporting standards. Fortunately, several organiza-tions (MGED (Brazma *et al.,* 2001)), and SMRS (Lindon *et al.,* 2003 (Table 1)) have addressed a number of these issues by developing guidelines and standards for the user community. Many journals are now requiring *omics* data to be uploaded into public database repositories that adhere to these standards as a prerequisite for publication in an effort to ensure unhindered public access to the primary data (Ball *et al.,* 2004a, b). However, the availability of published toxicogenomic data, and more specifically microarray and proteomic data, will only be of value if issues regarding cross-platform comparisons and the lack of uniform data quality control measures are resolved.

One of the most challenging aspects of implementing toxicogenomics in risk assessment involves establishing the appropriate supportive infrastructure to facilitate the effective management, integration, interpretation, and sharing of tox-icogenomic data. An effective, flexible, and comprehensive knowledge base is required that is populated with phenotypi-cally anchored toxicogenomic data complemented with ADME, histopathology, clinical chemistry and toxicity data. Currently, several public and commercial toxicogenomic database efforts have been initiated utilizing the Minimum Information about a Microarray Experiment (MIAME) students (Brazma *et al.,* 2001) as a guide. Although commercial databases are highly promoted, there is a lack of peer reviewed publications critically assessing their utility, although these are now starting to emerge (Fletcher *et al.,* 2005; Ganter *et al.,* 2005). Future publications from independent laboratories will further demon-strate their utility and facilitate increased acceptance of toxicoge-nomics in the scientific community. In contrast to the commercial databases, public database efforts are still in development.

Regardless of their origin, it is imperative that these data-bases are able to effectively communicate and share deposited data. Strategies to facilitate electronic data exchange between databases such as microarray gene expression-markup lan-guage (MAGE-ML) (Spellman *et al.,* 2002) and systems biology markup language (SBML) (Hucka *et al.,* 2003) are being developed and wi1l facilitate effective electronic data exchange between compliant repositories. Ideally, these data-bases will provide access to the large, disparate, and robust toxicogenomic data sets required to develop the necessary computational algorithms and models needed to support quantitative risk assessment.

The collective information presented in this review on gene expression profiles of environmentally/nutritionally significant heavy metals should not be considered as complete. However, a variety of results obtained by different authors on bacteria, fish, laboratory animals, and humans have been summarized. As the dose of the drug, organ system and the subject are important determinants, significant gene changes need to be accurately monitored. Cross reference databases, such as National Center for Biotechnology information, Panther, or MAPPF finders are helpful. Gene ontology consortiums standardized annotation terms to advance the use of databases so as to identify biochemical pathways and processes that resulted in the gene changes detected on a microarray. The development of a new program (GO-QUANT) to facilitate the use of toxicogenomics data into health risk assessment is a commendable effort.

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