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Review

# Environmental toxicogenomics: A paradigm shift in assessing toxicological effects at any stage along the disease continuum

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ABSTRACT

Environmental genomics has revolutionised how researchers can study the molecular basis of adverse effects of environmental toxicants. It is expected that the new discipline will afford efficient and high throughput means to delineate mechanisms of action, risk assessment, identify and understand basic pathogenic mechanisms that are critical to disease progression, predict toxicity of unknown agents and to more precisely phenotype disease subtypes. This might become a crucial tool in biological response marker or biomarker discovery in studying disease continuum. To illustrate how toxicogenomics can be useful, we provide here an overview of some of the past and potential future aspects of environmental genomics. The present article also reviews the principles and technological concerns, and data analysis of toxicogenomics. Applications in Pakistan are also discussed.

## 1. Introduction

Environment has become home to various synthetic and natural chemicals, radiations, insecticides, waste by-products, microbes and other potential toxicants, which produce adverse health effects in majority of the population.

Exposure to toxicants was not well understood previously, as the effects of environmental toxicants were not detected until they accumulated in the human body in sufficient quantities to cause detectable symptoms. Thus, most toxicants were only identified after widespread environmental contamination led to outbreaks of chemical poisonings (Jayapal 2009). Today, it is known that serious health consequences occur not only from heavy exposure, but also from low level chronic exposures to environmental toxicants (Sokol et.al 2002).

Studying the adverse human effects of chronic, low level exposures to environmental toxicants is difficult. There is substantial inter-individual variability in the uptake and effects of toxicants due to genetic susceptibility, metabolic variation, and nutritional status (Jayapal 2009). Environmental toxicants tend to cause nonspecific deficits or alterations. In contrast with clinical therapeutics, the study of environmental toxicants has almost relied entirely on observational studies and experimental animal models. Increasingly, however, researchers are using biological markers to directly measure the actual levels of suspected environmental chemicals in human tissues and fluids, and link these exposures with disability or disease (Jayapal 2009). The ability to dissect the

mechanisms of environmental toxicity that are related to health issues is an important challenge facing scientists. In addition, the problems of identifying environmental factors involved in the aetiology of human disease and of performing safety and risk assessments for drugs and chemicals have long been formidable issues.

Most human diseases result from a combination of environmental exposures and genetic variation. However, it is not fully understood why certain people develop disease when challenged with environmental agents and others remain healthy. Thus, a more complete understanding of how genetic characteristics affect the human response to environmental exposures is needed to improve approaches to the prevention and control of environmentally induced diseases. Genetic alterations in critical regulatory pathways such as genomic maintenance and DNA repair mechanisms, cell-cycle checkpoints, apoptosis and telomere length and control of micro-environmental factors and others may predispose cells to carcinogenesis (Wu et al. 2004).

Recent technological advances in the areas of genomics, proteomics, and metabolomics have provided researchers with new tools for developing biomarkers (biological response markers) specifically indicators that reflect both chemical exposure and the subsequent biological effect. Biomarkers are important in assessing the exposure and for predicting future adverse health outcomes. The development of cellular and molecular biomarkers for disease is an important goal in environmental health research. Such disease surrogates do not need to have extreme sensitivity but must be predictive of a specific disease.

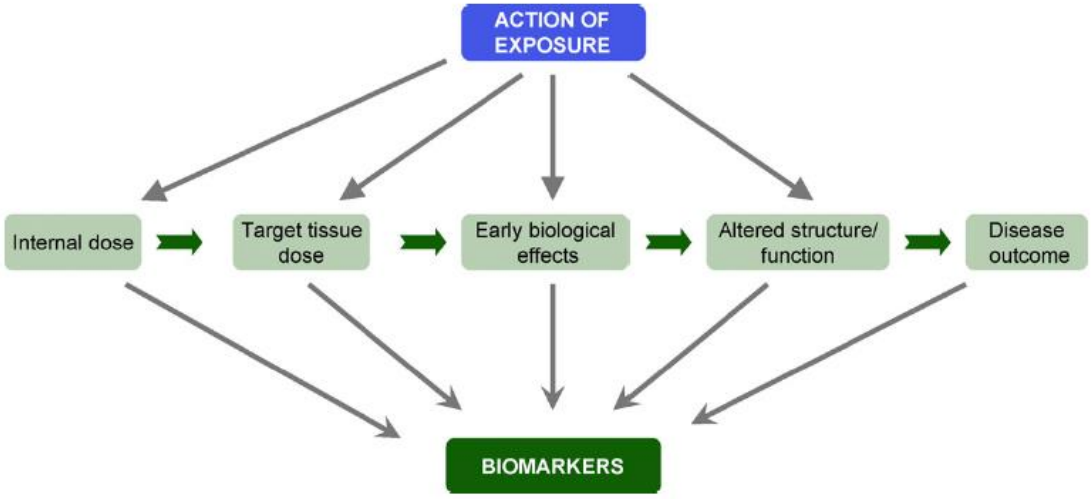
## 2. Foundation of Ecotoxicogenomics

Environmental genomics (Aardema and MacGregor 2002; Irwin et al. 2004; Jayapal 2009) is an emerging discipline of toxicology that enables scientists to identify and characterise the genomic signatures of environmental toxicants, and uses gene expression profiles to study the relationship between exposure and disease outcome and understand gene–environment interactions and their impact on human health. The term 'environmental genomics', at times, is being used interchangeably with the term 'toxicogenomics'. It has evolved from early gene-expression studies which described the response of a biological system to a particular toxicant. The combination of microarray experiments and a classical toxicology study led to the development of a new scientific field in 1999 viewed as toxicogenomics which is the fusion of three scientific fields: toxicology, molecular biology, and bioinformatics (Jayapal 2009). The main objectives of toxicogenomics are: to understand the relationship between gene–environment interaction and human disease susceptibility; to discover useful biological response markers of disease and exposure to toxic substances; and to sort out the molecular mechanisms of toxicity (Jayapal 2009).

Traditional toxicological testing utilizes whole animals to test the potential toxicity of chemicals. These tests are expensive, time consuming, and offer very little information on early, low dose effects in target cells. By monitoring thousands of genes in cells simultaneously, DNA microarray technology provides the opportunities to characterise the gene expression patterns induced by environmental toxicants. It also allows us to understand the biological effects and mechanisms on a genome-wide scale and to make tailored therapeutics to specific pathologies possible (Brown and Botstein 1999). The advances in high-throughput technologies provide an opportunity to identify the early, sensitive genes of cellular toxicity resulting from toxicant exposure (Aardema and MacGregor 2002). This new "omics" discipline offers direct comparison of expression values for a control against an altered condition revealing a set of biomarkers indicative of that altered state. This exposure fingerprint can then be used as a tool for toxicant exposure classification and predicting mode of action (Hamadeh et al. 2002a). The data generated from toxicogenomics will not only save time, cost and animal use relative to conventional methods but also enable us to perform

certain human studies that could not be carried out at overtly toxic exposures (Aardema and MacGregor 2002).

Biomarkers are valuable tools for understanding the nature and extent of human exposure and risk from environmental toxicants. The use of toxicogenomic approaches to biomarker discovery can be widely applied to both environmental and clinical exposure scenarios in response to disease (Benninghoff and Williams, 2007; Brown et al. 2004). They can serve as quantitative measures of toxicant exposures and biologically effective doses, as well as early warning signals of biologic effect.




***Source:*** Singapore Delft Water Alliance and Defence Innovative Research Programme 2011

**Fig. 1** shows sequence of events and parameters between exposure to a toxicant and aetiology of disease in an order. Ideally, biomarkers can be identified at any stage along the disease continuum, from external exposure to the final response of interest or concern. Exposure and disease outcome-specific patterns of gene profiles have been used to identify molecular changes that can be used as biomarkers of toxicity (Hamadeh et al. 2002a) and these profiles can provide insights into mechanisms of toxicity (Fertuck et al. 2003) and disease causation (Hamadeh et al. 2004).

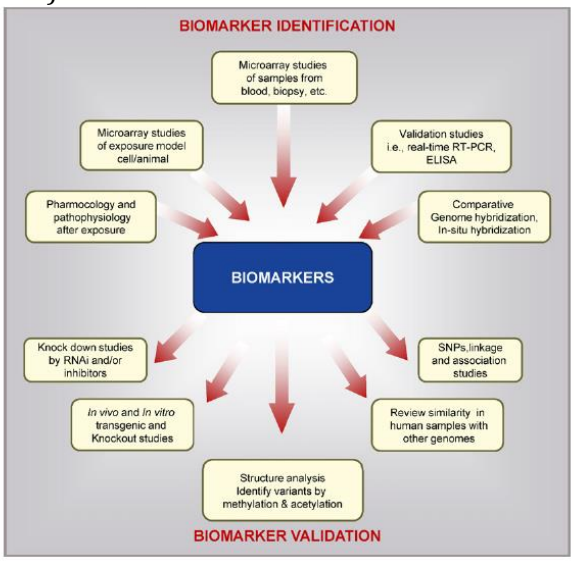
When the body's cells are exposed to a toxicant, they respond by altering the pattern of expression of genes within their chromosomes. The production of protein encoded by a given gene may be over expressed, decreased, or remain unchanged, depending upon the type of exposure. Genetic susceptibility influences all the events and parameters along the process. These events could be identified when chemical is transported and transformed within an organism to produce a dose to a target tissue and the interactions of the chemical at the cellular and molecular levels leading to a toxic endpoint. The ability to capture a snapshot of the transcriptome at any event and parameter along the continuum for a toxicology study of interest progresses our basic knowledge of adverse events and provides the necessary scientific framework for developing new strategies. The data generated will provide information about cellular networks of differentially expressed genes, define important target molecules associated with the toxicity mechanism and biomarkers. The compilation of such experimental data, together with toxicoinformatics tools and computational modelling will be important in deriving a new understanding of toxicant-related disease (Tennant, 2002).

## 3. Biological response markers

The development of '-omics' technology for analysing global expression patterns, where changes in thousands of genes can be investigated simultaneously, is providing unparalleled opportunities for establishing the molecular basis of toxicological and pharmacological responses (Furness 2002; Orphanides 2003).

The early detection of such interactions involving genes, gene products and environmental factors could lead to early detection and development of effective prevention strategies and elucidation of mechanisms of pathogenesis. To elucidate mechanisms of toxicity and pathogenesis of disease, one needs to know about the biomarkers that are involved and have insight about timing of their expression, quantity, activity and flux. Toxicogenomic approaches will lead to the discovery of sensitive and specific biomarkers for a wide range of effects at the molecular and cellular level. Such studies have the potential to transform clinical and toxicological practice and are likely to result in the substitution of classical biomarkers discovery (Aardema and MacGregor 2002; Boorman et al. 2002; Waters et al., 2003b).

Fig. 2 summarises strategies applied for discovering biomarkers. Studies suggest that early interactions between genes, gene-products and environmental factors can be identified and monitored by transcriptomic analysis possibly by using single cell organisms or in vitro tissue culture systems (Afshari 2002; Waters et al. 2003a). Genome-wide expression analysis will allow for the discovery of unique biomarkers and molecular mechanisms characteristic of specific diseases or dysfunctions; and will lead to the development and validation of test systems that are inexpensive, time-consuming and dependent on the use of model organisms. This approach is being exploited for investigating the similarities and differences at the gene expression level between compounds such as acetaminophen, clofibrate, and benzo(a)pyrene (Cunningham and Lehman-McKeeman 2005; Fuhrman et al. 2000). One study has shown that gene expression changes in HepG2 human hepatoma cells could distinguish between two mechanistically unrelated classes of toxicants, providing further confirmation that cells have distinct molecular responses to different toxic stimuli (Burczynski et al. 2000).




***Source***: USDA 2009

**Fig. 2.** Strategies applied in the identification and validation of biological response markers sensitivity to environmental toxicants. (a) Strategies applied for identification of biological response markers; (b) biological validation of potentially identified biological response marker by various schemes. RT PCR, reverse transcription-polymerase chain reaction; SNPs, single nucleotide polymorphism.

## 4. Data Analysis

When toxicogenomics ushered to the forefront as an area of research investigation and possible drug safety application (Jayapal 2009), it was following on the heels of the initial success of large-scale genome initiatives related to areas such as cancer biology, the cell cycle, development, and differentiation. Typical toxicogenomics experiments follow transcript changes across a genome following exposure of cells or tissues to a compound or environmental insult). ''Validation'' of the toxicogenomics hypothesis that these transcript changes lead to an ability to group compounds with similar effects and/or elucidate mechanistic insights previously unknown with the chemical action requires not only technical precision of the cell/organ exposure, sample collection, and processing components of the experiments but also complex computational and bioinformatics approaches and resources.

With the wealth of the genomic data collected from series of microarray experiments, investigators quickly realized that databases and analytical tools were essential in order to effectively manage and condense the data into a more manageable form. Building on the momentum gained from leveraging databases and computational algorithms for genome sequencing efforts, engineers, statisticians, mathematicians, and computer scientists began to develop analytical tools and shared resources for microarray gene expression data. Analysis of toxicogenomics data can follow several different paths including class discovery, comparison, prediction, and mechanistic analysis (Jayapal 2009).

## 5. Applications

Acting in concert with individual susceptibility, environmental factors such as smoking, diet, and pollutants play a role in most human cancer. However, new molecular evidence indicates that specific groups-characterized by predisposing genetic traits or ethnicity, the very young, and women-may have heightened risk from certain exposures. This is illustrated by molecular epidemiologic studies of environmental carcinogens such as polycyclic aromatic hydrocarbons and aromatic amines. Individual genetic screening for rare high-risk traits or for more common, low-penetrant susceptibility genes is problematic and not routinely recommended. However, knowledge of the full spectrum of both genetic and acquired susceptibility in the population will be instrumental in developing health and regulatory policies that increase protection of the more susceptible groups from risks of environmental carcinogens (Burczynski et al. 2000).

The rapid sequencing of the genomes of a number of organisms, including humans, has led to major changes in the drug industry. The abundance of genome data, and the reagents generated from these genomes, have enabled the study of changes in large numbers of genes and proteins in parallel, using methods such as DNA microarrays to examine gene expression changes, or 2D polyacrylamide electrophoresis (2D-PAGE) to observe changes in the expression of proteins. While these techniques have been in use for several years, their application has primarily focused on the target discovery phase, with some early work carried out on drug- or toxin-induced changes in proteins using 2D-PAGE. In the last two years, a slew of publications have appeared on the application of array technologies to the study of toxicology (Burczynski et al. 2000).

Although long-term treatment with low doses of 14-membered macrolides is widely applied in management of patients with chronic inflammatory diseases, e.g., diffuse panbronchiolitis, chronic bronchitis, or chronic lung damage in newborns, the physiological mechanisms underlying the action of macrolides in these conditions are unclear. To clarify the pathological basis of these diseases and also to aid in the design of novel drugs to treat them, molecular target(s) of macrolides were investigated. The experiments involved long-term culture of human small airway epithelial

cells (hSAEC) in media containing 14-membered macrolides erythromycin (EM) or clarithromycin (CAM), or a 16-membered macrolide, josamycin (JM), which lacked clinical anti-inflammatory effects. Then gene expression profiles were analysed in the treated cells using a cDNA microarray consisting of 18,432 genes. Nine genes were identified whose expression was significantly altered during 22 days of culture with EM, and seven that were altered by CAM in that time. Four of those genes revealed similar behavior in cells treated with either of the 14-membered macrolides, but not JM. The products of these four genes may be candidates for mediating the ability of 14-membered macrolides to suppress chronic inflammation.

The polychaete, Perinereisnuntia, has been used as an indicator species to assess the environmental condition of benthic communities in coastal marine environments. Recently, high-throughput sequencing technology has been proven to be a useful method for analyzing expressed sequence tags (ESTs)in non model species. Thus, extensive cDNA information has been obtained by the pyrosequencing method, to utilize the polychaete species as a test organism for sediment quality monitoring studies (Fertuck et al. 2003).

Linkages between the historical and newer toxicological tools are currently being developed in order to predict and assess risk. Being able to classify chemicals and other stressors based on effects they have at the molecular, tissue, and organismal levels helps define a systems biology approach to development of streamlined, cost-effective, and comprehensive testing approaches for evaluating environmental hazards. Corals were exposed to either natural or anthropogenic stressors to elicit the expression of stress genes for isolation and incorporation onto the array. A total of 32 genes involved in protein synthesis, apoptosis, cell signaling, metabolism, cellular defense and inflammation were included on the array. Labeled cDNA from coral *(Montastraea faveolata)* exposed to elevated seawater temperature, salinity and ultraviolet light was tested against the microarray to determine patterns of gene expression associated with each stressor. Carbonic anhydrase, thioredoxin, a urokinase plasminogen activator receptor (uPAR) and three ribosomal genes demonstrated differential expression across all replicates on the array and between replicate colonies. Specific gene expression patterns produced in response to different stressors demonstrate the potential for gene expression profiling in characterizing the coral stress response (Fertuck et al. 2003).

The zebrafish embryo has repeatedly proved to be a useful model for the analysis of effects by environmental toxicants. This proof-of-concept study was performed to investigate if an approach combining mechanism-specific bioassays with microarray techniques can obtain more in-depth insights into the ecotoxicity of complex pollutant mixtures as present, e.g., in sediment extracts. For this end, altered gene expression was compared to data from established bioassays as well as to results from chemical analysis. Mechanism-specific biotests indicated a defined hazard potential of the sediment extracts, and microarray analysis revealed several classes of significantly regulated genes which could be related to the hazard potential.

Engineered nanoparticles (ENPs) have been produced by nano-biotech companies in recent decades to generate innovative goods in various fields, including agriculture, electronics, biomedicine, manufacturing, pharmaceuticals and cosmetics. ENPs are emerging as a new class of pollutants with eco-toxicological impacts on marine ecosystems because the particles can end up in waterways and reach the sea. Recent laboratory studies in invertebrates and fishes suggest that exposure to ENPs could have harmful effects. Because there is not much data available for gauging the effects of ENPs on marine wildlife, the ultimate ecotoxicological impacts of chronic exposure to ENPs should be investigated further using laboratory tests and field studies (Stierum 2005).

The use of model organisms has been proposed to understand the molecular pathways involved in the mechanisms that may be affected by exposure to ENPs. Sensitive and innovative molecular methods will provide information regarding the hazards of ENPs that may exist in the marine environment. Model organisms that have not been conventionally used for risk assessment and the development of eco-toxicogenomic approaches will result in an improved understanding of the mechanistic modes of action of contaminating ENPs in the marine environment.

Toxicogenomics is predominantly applied for elucidation of mechanisms of action and discovery of novel pathway-supported mechanism-based markers of liver toxicity. transcriptome, proteome and metabolome data can be integrated, supported by bioinformatics to develop a systems biology approach for toxicology. Transcriptomics and proteomics studies on bromobenzene-mediated hepatotoxicity were carried in rats to assess the hepatic effects of food additives and chemicals (Stierum 2005).

## 6. Applications in Pakistan

Cytogenetic analysis of Pakistani individuals occupationally exposed to pesticides in a pesticide production industry was carried out by National Institute for Biotechnology and Genetic Engineering (NIBGE) in 2006. It was analysed that there is an increase in ALT, AST and ALP enzymes associated with liver and a decrease in SChE in individuals working in a pesticide manufacturing industry. An increase in BNMN and MNL frequencies indicated the cytogenetic damage in industrial workers. Finally, a decrease in CBPI values in exposed workers again indicated the genotoxic effect of pesticide exposure (Bhalli 2006).

Other projects undertaken by various research facilities in Pakistan:

- Analysis of Genetic Variations in Sorghum Germplasm of Pakistan Assessed by SSRs AND ISSRs
- Detection of DNA damage by the comet assay in MCL-5 cells exposed to extracts of urban air particulate matter
- Rapid Detection of infectious bursal disease virus using One-step RT-PCR in clinical samples in Pakistan
- In vivo genotoxic effects of a synthetic insecticide, cyhalothrin in fish
- In vitro investigation to explore the toxicity of fungicides for plant growth promoting rhizobacteria
- Damage in Pakistani Pesticide Manufacturing Workers Assay Using the Comet Assay Environmental and Molecular Mutagenesis

## 7. Future Prospects

The field of environmental genomics has enormous potential to affect our ability to accurately assess the risk of developing disease, identify and understand basic pathogenic mechanisms that are critical to disease progression. New toxicogenomics methods have the potential to revolutionise toxicology. Ultimately, the aetiology and prevention of human disease can only be established in the context of both genetic susceptibility and environmental factors (Kurreck, 2003; Opalinska and Gewirtz, 2002).

Toxicogenomics data would identify early response gene signatures in the system. However, caution should be exercised in predicting disease outcome. For example, prediction of carcinogenesis by early phase gene expression profiling may be far-fetched as cancer is a multi-step

process and it would take years to develop tumours following exposure to toxicants. Disease prediction based on the expression data for specific genes requires extensive validation in animal models (Ruepp et al. 2005).

Moreover, data on known tumour suppressors or oncogenes would facilitate the identification of susceptibility and risk. Additionally, individual susceptibility may hinder the accurate prediction of stochastic diseases with longer latent periods. In addition, chronic and accumulative exposures of a particular compound might cause further complications in appropriate extrapolation and interpretation of toxicogenomics data.

In the future, biological response markers will offer increasing opportunities to investigate, prevent, diagnose and treat environmentally induced diseases and disabilities. Better biomarkers of biological effect are therefore needed in order to improve our ability for risk estimation of exposure to environmental toxicants. It is anticipated that the existing technology for detecting genome-wide gene expression changes may contribute to this aim. Important discoveries are being made over the last few years and many investigations are underway enabling the simultaneous handling, management and interpretation of large amounts of data.

To date, biological response markers have been used primarily within pre-clinical toxicology studies and during early clinical stages of drug development. It is anticipated that this methodology can be applied to later stages of clinical development and even into clinical medicine on the one hand and into chemical/drug risk delineation and risk assessment on the other. In recent years, toxicogenomics prognostic models for identifying new chemical entities are being developed in the pharmaceutical industry that may have the potential to induce adverse effects, such as hepatotoxicity (Ruepp et al. 2005). The ability to rapidly distinguish potential adverse effects early and identify the most promising compounds based on gene expression profiling has lead to a new set of toxicogenomic approaches for the development of safer drugs. This approach can be exploited for identifying the multitude of adverse effects induced by compounds.

There are also technological innovations that are already in use which permit RNA profiling of formalin-fixed tissues, potentially making archived tissues from generations of toxicological studies accessible to gene expression analysis (Lewis et al., 2001). Moreover, exploiting model systems such as yeast, C. elegans, rodents, Drosophila and zebra fish will speed up our understanding of environmental exposures on human health. Analogous experiments using environmental toxicants should be performed to determine the gene signatures of exposures in mammalian cell systems.

There are other concerns in assessing the toxicogenomics responses to environmental exposures. These are the individual genotype, lifestyle, age and exposure history (Kaput and Rodriguez 2004). Toxicogenomics will help to determine the degree to which these factors influence the balance between normal and disease states. The idea of monitoring human populations for toxin exposures highlights another major problem in toxicology: individuality. In the future, the physician and pathologist will use these different toxicogenomic analyses at many points of disease management. The paradigm shift will directly affect clinical practice by having an impact on early detection of the disease using transcriptomic patterns of samples, diagnosis based on signatures as a real-time assessment of therapeutic efficacy and toxicity. Promising future developments of toxicogenomics include tailor-made genomic chips specifically addressing endpoints and mechanisms of interest.

Methods and assay-kits for medium throughput analysis will increase in affordability that may support a more common use of these tools (SuperArray Bioscience, MD USA). A biological measure of an individual's susceptibility resulting from an environmental toxicant may enable us to discover ways to reduce or prevent disease by fixing biochemical and molecular functions that have been perturbed by environmental chemicals.

Toxicogenomics approaches are also expected to become a routine and widely used tool for disease diagnosis and classification, which anticipates the future availability of home testing kits for diseases associated to environmental toxicants (Jayapal and Melendez, 2006; Stears et al. 2003). Eventually, microarrays could be used as a routine diagnostic tool called 'microarray readers' with which treatments could be tailored for an individual patient (White 2004). DNA microarray technology will undoubtedly become a major tool in environmental medicine, because it will also improve our diagnostic and prognostic capabilities for specific diseases as well as our ability to examine drug interactions, sensitivities and effectiveness. The potential target gene, which, when knocked down
(e.g. by RNAi), destructs only cancer cells could suggest an approach for new cancer therapies (Wheeler et al., 2004). The possibilities of using cell microarrays for large-scale RNAi studies have been suggested by several researchers (Kurreck, 2003; Opalinska and Gewirtz, 2002). Although this technique is still in its infancy, cell microarrays can significantly reduce the effort essential for rapid cell-based RNAi screens (Wheeler et al., 2004).

Toxicogenomics will help to delineate the modes-of-action of various classes of agents and the unique genetic makeup of certain species and population that render them susceptible to toxicants (Waters et al., 2003b). Studies on strains within a species that are sensitive or resistant to the toxicant-induced disease phenotypes will be particularly valuable in providing further comparative insights into genetic susceptibility and probable disease outcomes.

## 8. Conclusions

Genomic analysis in toxicology provides an opportunity to change and improve the way environmental pollutants are currently investigated. Toxicogenomics approach should also lead to the identification of new genes/targets involved in diseases caused by environmental toxicants, including cancer, immune, nervous and pulmonary/respiratory systems. The identification of novel biological response markers through the sensible use of toxicogenomics, promises more accurate diagnosis and risk assessment of various diseases, leading to more precise prognosis and new therapeutic interventions.

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