R-357

Categorizing biomarkers of the human exposome and developing metrics for assessing environmental sustainability

Joachim D. Pleil

HEASD/NERL/ORD (D205-05)
U.S. Environmental Protection Agency
Research Triangle Park, NC, USA 27711

pleil.joachim@epa.gov

Abstract:

The concept of maintaining environmental sustainability broadly encompasses all human activities that impact the global environment including the production of energy, use and management of finite resources such as petrochemicals, metals, food production (farmland, fresh and ocean waters), potable water sources (rivers, lakes, aquifers), as well as preserving the diversity of the surrounding ecosystems. The ultimate concern is how one can manage spaceship Earth in the long term to sustain the life, health, and welfare of the human species and the planet's flora and fauna. On a more intimate scale, one needs to consider the human interaction with the environment as expressed in the form of the exposome, which is defined as all exogenous and endogenous exposures from conception onwards including exposures from diet, lifestyle, and internal biology as a quantity of critical interest to disease etiology. Current status and subsequent changes in the measurable components of the exposome, the human biomarkers, could thus conceivably be used to assess the sustainability of the environmental conditions with respect to human health. The basic theory is that a shift away from sustainability will be reflected in outlier measurements of human biomarkers. In this review, the philosophy of long term environmental sustainability is explored in the context of human biomarker measurements and how empirical data can be collected and interpreted to assess if solutions to existing environmental problems might have unintended consequences. The first part discusses 4 conventions in the literature for categorizing environmental biomarkers and how different types of biomarker measurements might fit into the various grouping schemes. The second part lays out a sequence of data management strategies to establish statistics and patterns within the exposome that reflect human homeostasis and how changes or perturbations might be interpreted in light of external environmental stressors. The underlying concept is to identify probative outliers from the "unremarkable exposome" in individuals or sub-populations that could be used for discerning deviations from the healthy environment much like current diagnostic medicine uses batteries of blood and urine tests to screen for pre-clinical disease conditions. Such empirically derived human *in-vivo* data could subsequently be integrated into high-throughput *in-vivo* and *in-silica* testing of environmental and manufactured chemicals to support real world toxicity evaluations.

INTRODUCTION

Human biomarker measurements are used to interpret a variety of conditions including environmental exposures, health state, individual susceptibility, disease mechanisms, clinical interventions, and pharmaceutical dose–response. When taken in aggregate, all biomarker chemicals encompass the human exposome and represent the interactions between the chemical environment and the internal biochemistry of life. In general, exposome research is considered a top-down approach wherein untargeted 'omics measurements are used to interpret changes in the patterns expressed without necessarily identifying each component (Rappaport 2011). As such, the exposome is everything that is not the genome and serves as an umbrella for all of the traditional 'omes such as the metabolome, microbiome, adductome, and proteome, with only the genome left out. Herein, the exposome is defined a bit more restrictively in that only the signal comprised of directly measured or modeled interactions in systems biology are used wherein the analytes are specifically identified or at least known by chemical classification. Patel et al. 2010, have discussed this type of exploratory environment-wide association study (EWAS) for type-2 diabetes mellitus, and Rakyan et al. 2011 have explored environmentally induced epigenetic changes (in this case DNA methylation) for large scale disease associations.

This review describes some common schemes for categorizing biomarkers depending upon ultimate use and proposes an additional application for human exposome measurements as a metric for assessing progress towards the sustainability of the global environment from the standpoint of human health and welfare (Strange et al. 2002, Wilson and Schwarzman 2009, Williams et al. 2009). The ultimate goal is to describe the development of suites of human chemical biomarker measurements that describe the unremarkably affected population (to serve as a statistical baseline), and then compare to measurements in sub-populations (geographic, socio-economic, gender, age group, and ethnicity) thought to have been perturbed by changes in the environment. As such, the review is divided into two distinct parts: 1) *Biomarker Classification Strategies*, and 2) *Biomarker Diagnostic Strategies*. The review is deliberately narrowed in scope to explore the potential effects on the human exposome related to the external environment although this distinction could be considered a moving target, because after all, what is "environment" is subject to interpretation. Finally, the intention is not to predict disease from exposome measurements, but to use deviations from a "normal" pattern in the exposome to indicate a perturbation in the previously sustainable local environment.

Environmental sustainability:

When populations were small, natural resources seemed boundless and "…dilution was the solution to pollution" (popular phrase, origin unknown). Everything people needed was gathered or grown locally, and everything they wanted to get rid of was dumped into the river and swept away by the unending water supply, or was burned and swept away by the wind. However, as small settlements turned into more densely populated towns and cities, people had to travel further and work harder to gather resources (building materials, water, food, and energy supplies)

and the waste disposal from human activities became more complicated. The streets became open sewers running off into the watershed, and the smoke from burning for energy production or for waste disposal accumulated in the air. Our forebears began to realize that easily available resources were becoming scarcer and that contamination could result in adverse health effects. Management, technology, and control were needed so that increasing populations could be sustained (see http://en.wikipedia.org/wiki/History_of_Sustainability and references therein).

The concept of environmental sustainability is not new; it is the embodiment of mass and energy balance applied to the use and reuse of all things in the surrounding world. As a species, only in the past few generations have we experienced that resources are becoming limited. Oil is requiring more remote and deeper drilling sites, aquifers and surface waters are receding, fish populations are decreasing, forests are being cleared for ever increasing agricultural demands, and in general, the global climate and complex biological ecosystems are showing signs of stress. As the increasing global population is using more energy, food, water, consumer products, chemicals, and pharmaceuticals, the remaining resources are experiencing various contaminations and alterations that are building up. This harsh reality even permeated into popular music as:

"...look at Mother Nature on the run in the 1970's..."

(Neil Young, After the Gold Rush, Reprise Records, Warner Bros., Sept. 1970)

The presumed infinite sink of the environment for dilution and natural attenuation of mankind's activities is no longer sufficiently infinite. Therefore, evidence indicates that the solution to maintaining a viable biosphere is to manage resources in a sustainable manner; the challenge is how to measure success, and also how to fix one problem without creating another. In a recent interview, Dr. Paul Anastas, Assistant Administrator for EPA's Office of Research and Development and the Science Advisor to the Agency, summed up this charge as follows (http://www.chemistryviews.org/details/ezine/693111/Building_a_Sustainable_World.html):

> *"...Building a sustainable world - a world where today's society meets its needs while preserving the ability of future generations to meet their own - is the single most important challenge of our time."*

The broad strokes of sustainability revolve around the total ecology of the planet and all of the species of living organisms. For example, the basis for US Government regulations currently in place for eventually achieving environmental sustainability are the Clean Air Act of 1970 (Public Law 91-604, Dec. 31, 1970, listed in: 42 USC §7401), the Federal Water Pollution Control Amendments of 1972 (Public Law 92-500-Oct. 18, 1972 listed in: 33 USC §1151), and the Endangered Species Act of 1973 (Public Law 93-205-Dec. 28, 1973, listed in: 16 USC §1531-1544). As such, the metrics for US progress have been comprised of air and water measurements for criteria pollutants, and the observation and counting of animal populations.

Similarly, other Governments have implemented laws regarding the environment: for example, the European Union has a series of Directives in place regarding Ambient Air Quality (Directive 2004/107/EC, Directive 2008/50/EC), for climate change (Directive 2011/76/EU), water quality (Directive 2008/105/EC), and others. Japan developed a comprehensive "Environment Basic Law" in 1993, which has environmental sustainability at its core; the Japanese are also well known internationally for implementation of the Kyoto Protocol in June 2001. Australia also has central legislation for sustainability called the "Environment Protection and Biodiversity Protection (EPBC) Act" of 1999; which has wide ranging scope including specific regulations for mining, fishing, fire management, wetlands protection, as well as for air, water and wastes. Essentially, all countries have some form of environmental protection regulations in force for maintaining the sustainability of the planet.

Human exposome:

Humans tend to focus on how changes in the environment affect them directly. This is captured by the disciplines of environmental exposure science, environmental toxicology, and environmental epidemiology that describe the physical, chemical, and mathematical links among exposure, dose, and effect in response to environmental stressors (Edwards and Preston 2008, Sobus et al. 2011, Pleil and Sheldon 2010). Recently the concept of gene x environment interaction was added to help explain apparently random disease incidence (Smith and Rappaport 2009). The concept of the human exposome has now been proposed as a counterpart to the human genome. The rationale is that the genome is the unique blueprint of an individual for life processes whereas the exposome is the collection of chemicals in the body representing all exogenous and endogenous exposures from conception onwards including exposures from diet, lifestyle, and internal biology (Wild 2005). Together, the exposome-genome interaction comprises the primary factors of critical interest leading to disease etiology (Wild 2011). This concept has been popularly captured as:

"Genetics loads the gun, but environment pulls the trigger"

and has been attributed in various forms to Elliott Proctor Joslin MD, founder of the Joslin Diabetes Center, Harvard University Medical School (Paul Elliott, http://nas-sites.org/emergingscience/files/2011/12/Paul-Elliott.pdf) and also to Prof. Judith Stern from University of California, Riverside, CA (Suk et al. 2002). This pertinent expression is now shaping the dialog and understanding of environmental exposure science (e.g.) although the reality is undoubtedly much more complicated (Pleil et al. 2011a, Ziech et al. 2010, Pleil 2009, Olden and White 2005).

Like the human genome, the complexity of the human exposome is immense, and most likely varies greatly between and within individuals. As such, the exposome concept is mostly practical for applications using subsets of chemicals that have common modes of action within

human systems biology. In general, chemicals defining subsets of the exposome are measured empirically in human biological media, although animal and computational models are also useful for proposing chemical pathways, concentrations, and reactive intermediaries that can not be realistically (or ethically) measured in humans (Angerer et al. 2007, Au 2007, Cogliano et al. 2004, Smolders et al. 2009).

The interpretation of the constituents of the human exposome is crucial for unraveling the causes of disease, however, it is not a simple task. In an interview on Nov. 10, 2010, Prof. Stephen Rappaport, Director of the Center of Exposure Biology, University of California, Berkeley, (http://www.downtoearth.org.in/node/2264) stated:

> *"...characterizing the exposome... is the same kind of challenge the human genome project faced when DNA sequencing was in its infancy."*

In fact, a review of recent epidemiological studies indicated that about 70 to 90% of chronic disease risks are from "non-genetic", or environmental factors suggesting that quantitative assessment of the internal historic record of exposure is needed to discover the major causes of chronic diseases (Rappaport and Smith 2010).

Human biomarkers:

As discussed above, concentration measurements in environmental media such as soil, water, air, and food can be complemented with empirical measurements of exogenous chemicals in human biological media such as blood, breath and urine and with a variety of chemical metabolites and biologically relevant endogenous compounds. Such measurements allow direct assessment of the penetration (or uptake) of chemical contaminants and, with simultaneous or time dependent analyses, also allow evaluation of removal or detoxification pathways. Biomarker measurements of chemical metabolites, conjugated and adducted chemical species, and internal response chemicals including proteins, reactive oxygen species, and signaling compounds can also be used to assess the metabolism, systemic effects, and intrinsic vulnerability of the biological system to external chemicals. The concept that the incidence of relatively rare and apparently random disease in the general population has a component of "bad luck" (Epstein 1998) is being challenged by more detailed studies of the exposome, its biomarker constituents, and statistics (Link BG 2008, Smith et al. 2011, Funk et al. 2010, Kurdistani 2011, Pleil et al 2011b, Lin et al. 2005). Advances in analytical methods and instrumentation have made the detection and identification of thousands of biological chemicals a reality resulting in an explosion of available data for exposome interpretation (Rhea et al. 2010, Nagaraj and Mann 2011, Seignuric et al. 2010, Hu et al. 2009, Reichenbach et al. 2011).

In a recent address at the National Academy of Sciences (Washington, DC, Feb. 2010), Prof. John Groopman, Chair of Environmental Health Sciences at Johns Hopkins University commented that:

"In the last 10 years, sensitivity of mass spectrometry for human biomarkers (adducts) has increased 1000 fold and the number of non-detectable human samples has dramatically dropped."

and went on to conclude that:

"Complex diseases, e.g. cancers, are all individualized at the molecular level..."

Because ultra-trace level analytical sensitivities for measuring biomarkers are now being approached, one can imagine that detailed measurement of constituents of the exposome may become an indispensible tool for complementing genetic factors in predicting apparently stochastic disease outcome. Furthermore, establishing the current status for tracking time-based changes of human exposomes may become a useful metric for assessing the overall sustainability of the environment and for identifying human sub-populations that are impacted by mesa–scale environmental challenges at the communities level.

BIOMARKER CLASSIFICATION STRATEGIES

This review proposes that, in addition to the traditional measurements of environmental concentration of exogenous chemicals and health assessment of animal species populations, the path towards environmental sustainability could be monitored with a variety of biomarker measurements of the human exposome. The theoretical structure under which biomarkers are incorporated into the exposure to disease progression can be as complicated as one wants to make it; for the purpose of this discussion, consider the conceptual diagram in Figure 1 wherein the measured and modeled parameters are explicitly shown in parallel tracks. This is based on recent work describing the "Biomonitoring Framework" articulated by the U.S. Environmental Protection Agency's National Exposure Research Laboratory (Sobus et al. 2011, Tan et al. 2011). Using this overall scheme, the first step along this path is the adaptation of existing biomarker measurements and recognizing the underlying framework within which the data were collected and how they are categorized. Note that in the following discussion, the exact placement of a particular group of biomarkers within the two Figure 1 biomarker boxes can be somewhat arbitrary.

Categories of biomarkers

Biomarkers may be categorized in different ways in the literature, but the actual chemicals measured, their interpretation, and their ultimate use in deriving conclusions are not unique to any given grouping scheme. There are 4 fundamental methods for articulating distinctions among biomarkers: (1) by origin, (2) by biological function, (3) by kinetic activity, and (4) by biological medium, each with a few major internal divisions as listed below in Table 1. Any

given compound could fall into one or more groups within a category or sub-category, and all can be assigned at least one role in one of the major categories. Certainly, the structure presented here could be redrawn any number of ways and categories could be collapsed into each other or further subdivided. However, as it stands, Table 1 is both inclusive of all major classification schemes, yet parsimonious and balanced enough to be grasped quickly by the reader.

These categories are generally assigned based on the experimental design under which the measurements were made. In addition, there may be further sub-categories used within these basic categories and divisions based on molecular weight or phase (gas, liquid, solid), functional groups (nitrogen, sulfur, oxygen, or halogen), chemical activity (oxidizing, reducing, or inert), composition (metallic, organic, or inorganic) or mode of action (mutagenic, carcinogenic, apoptotic, or innocuous).

Biomarker origin: The first column in Table 1 reflects decisions made when biomarker analytical methods are developed and represent their chemistry or type. For example, a method for non-polar low molecular hydrophobic compounds from fuels exposure will require completely different sampling and analytical strategies than would polar hydrophilic compounds resulting from internal human oxygen metabolism and enzymatic processes. Observable "response" biomarkers may not need any instrumentation at all and could conceivably be detected by odor, color, or temperature. The exogenous sub-category is comprised of compounds that are, or contain, the original chemical of exposure. Blood borne benzene from fuel exposure, benzene oxide and phenol from phase-1 metabolism, benzene hemoglobin adducts, and urinary benzene diols and s-phenylmercapturic acid are all examples of this sub-category (Qu et al. 2003, Waidyanatha et al. 2004a, Funk et al. 2010). Methods that target such "chemical" biomarkers usually are designed to identify the original chemical structure of the compound of exposure as the primary analytical tag. The endogenous compounds, on the other hand, are typically composed of volatile oxygenated species such as alcohols, ketones, aldehydes, and organic acids, and of larger molecules such as heat shock proteins and cytokines (Hubbard et al. 2009, Gupta et al. 2010). Here, there are no unique chemical units to focus on and, in addition, these compounds are always present at significant levels. The analytical strategy is to look at patterns of the endogenous compound classes and determine their relative abundances as the probative signal. The last subcategory, response, is not technically part of the chemical exposome as commonly used, but refers to some form of overt change or condition that might be linked to chemicals or a perturbation in the exposome. Some examples are: cherry red fingernails as a biomarker of CO exposure; blue lips from oxygen deficit or hypothermia; body temperature, heart rate, blood pressure representing current metabolism; breath and urine odor indicating diet or health state; skin lesions or hives marking irritation or allergenic response. Admittedly, this category is one of convenience because all of these observable conditions are indeed produced by chemical constituents of the exposome. However, they serve as biomarkers in the broad definition: consider that medical diagnostics, before the "lab-test", originated with a

touch of the hand to the forehead to detect fever, an observed rash indicating measles, a change in breath odor indicating diabetes, pulmonary or kidney disease, swelling of joints to indicate gout, and other common clinical indicators of human ailments.

Biomarker function: The second column in Table 1 reflects how biomarker measurements are interpreted or used. In this case, the subcategory of exposure could include any of the exogenous chemical compounds of column 1, as well as certain members of the endogenous and response groups. For example, benzene, or chemical biomarkers of benzene, certainly reflect benzene exposure; however, cytokine production or protein expression may mark pulmonary inflammation from inhaled chemicals such as NO_2, or a change in heart rate variability may reflect PM2.5 exposure (Kim et al. 2006, Hesterberg et al. 2009, Liao et al. 2011). The subcategory of susceptibility is generally composed of the "omics" biomarkers; that is, particular sequences in the protein coding DNA can make a subject more or less able to detoxify certain chemicals, relative abundances of certain circulating proteins themselves may indicate quality of repair function, and generic markers of DNA aberrations such as strand breaks and sister chromatid exchange (SCE) are indicative of ongoing damage from exposures (Hunter 2005, Benton et al. 2011, Collins and Azqueta 2011). The sub-category of effect biomarkers overlaps the susceptibility biomarkers to the extent that deviations from expected normal levels may indicate a change such as DNA strand breaks and SCE. In addition, effects biomarkers include certain chemical biomarkers that could be mapped forward to effects; for example, measurements of polycyclic aromatic hydrocarbons (PAH) adducts of DNA, either in adult circulating blood or in umbilical cord blood are indicators of potential adverse health or birth outcome (McClean et al. 2007, Perera et al. 2005), or elevated levels of endogenous compounds such as cytokines signal inflammatory or allergenic response (Monton and Torres 1998). Other effects biomarkers can directly demonstrate an outcome; for example, cell count assays for necrosis and apoptosis indicate increased toxicity and damage (Ortiz et al. 2000), measurement of forced expiratory volume (FEV) shows changes in lung function (FrancoSuglia et al. 2008), and various fine motor function, memory, balance, and perceptual acuity tests are used as measures of neurotoxicity (Smith et al. 1997, Herpin et al. 2008). Again, some of these parameters are not technically part of the exposome, but could be interpreted as reflecting changes in the exposome constituents that could not be directly measured.

Biomarker kinetics: The third column in Table 1 reflects in which manner biomarkers are used to track chemicals entering and moving through the body; this comprises the empirical component of pharmacokinetic modeling (Barton et al. 2006). The subcategories are mostly self-explanatory and the measured compounds are generally exogenous (typically environmental) chemical biomarkers. Absorption is the total systemic uptake and can be further interpreted to reflect exposure pathway (inhalation, ingestion, dermal contact) and environmental medium such as air, water, food, soil or dust. Typically, the biomarker measurements for absorption are the actual native compounds as found in blood or breath. Other meta-data including environmental concentrations, breathing and ingestion rates, and activity patterns are

used to achieve context and quantitation of absorption. For example, the difference between inhaled and exhaled chloroform concentrations serves as an estimate of chloroform absorption through both dermal and inhalation pathways (Pleil and Lindstrom 1997, Lindstrom et al, 1997). Distribution is a more generic form of biomarker application and refers to the empirical partitioning within the body; for example, blood borne benzene and exhaled benzene measurements are a direct measure of the blood/breath partition coefficient (Wallace et al. 1997). Metabolism is estimated via the relative amount of disappearance of a native compound and the appearance (if detectable) of a chemical metabolite. For example, absorbed methyl-tertiary-butyl ether (MTBE) gives rise to the production of the phase-1 metabolite tertiary butyl alcohol (TBA); both can be measured in the blood and breath of a human subject and subsequently provide empirical evidence of metabolism (Pleil et al. 2007). Elimination is often estimated by measurement of compounds in excretion pathways such as breath, urine, and feces. For example, absorption of the pesticide chlorpyrifos can be monitored by the urinary elimination of the metabolite 3,5,6-trichloro-2-pyridinol (Koch et al. 2001).

Biomarker medium: The fourth column represents biomarkers grouping based on available biological media. In medical diagnostics, blood is considered the gold standard as a biological fluid because it represents the central compartment of the body; every living cell interacts with the circulating blood and exchanges nourishment for waste. Essentially all medical and environmental biomarker compounds previously discussed are found at some level in the blood. The caveats are that blood sampling is invasive, the sampling volume and frequency are limited, the levels of many compounds of interest are low, and the blood matrix itself is complex. New research is now ongoing to make blood sampling less onerous to the subject by using minimally invasive dried blood spot collection (Funk et al. 2008). Exhaled breath is an extremely versatile biomarker medium in that its collection is non-invasive, the supply is essentially unlimited, and it serves both as a window into the blood as well as a quantitative elimination pathway. Furthermore, the breath matrix is dilute and lends itself well to analytical methods developed for air. The main disadvantage of breath sampling is that biomarkers are generally limited to gas phase (volatile) compounds. Recent advances in exhaled breath condensate collection now also allow analysis of some semi- and non-volatile compounds (Pleil 2008, Sawyer et al. 2009). Urine is the most commonly used biological medium for human biomarker studies. It is relatively easy to obtain, has general acceptance with the public (in comparison to blood), and serves as a major quantitative elimination pathway for model inputs. The available compounds in urine are primarily water-soluble organics and so generally represent phase-1 or 2 metabolites and other modified, adducted, or conjugated polar chemicals comprising biological waste products (Hecht 2002, Kim et al. 2009, Waidyanatha et al. 2004b). In some cases, the native compounds (e.g. benzene, naphthalene, phenanthrene) in urine can also be used as an indicator of previous exposure (Waidyanatha, et al. 2001, Sobus et al. 2009). One of the main drawbacks is that urinary concentrations are confounded by a variety of parameters including hydration, void volume, current metabolism state, and time since last void. In addition, urine sample availability is not predictable; that is, human subjects can not generally provide samples on command.

BIOMARKER DIAGNOSTIC STRATEGIES

In the following discussion, the assumption is that the general "healthy" population without remarkable environmental or occupational exposures represents a defined sustainable health state and exposome, or at least the acceptable *status quo*. This assumption can not be objectively proven as in today's world it is statistically more likely to live longer and healthier lives than our forebears and thus there is reasonable confidence that the current environment is acceptably sustainable with respect to humans (Goklany 2007).

The next stage along the path to using biomarkers as a diagnostic tool for assessing sustainability is to consider what kinds of future measurements (and how many) need to be made to provide context and continuity for existing available information. As presented in the previous section, the human exposome (through empirically measured biomarkers) includes a broad spectrum of molecules that may not be directly probative for assigning previous exposures, but could indicate a metabolic disturbance relevant for toxicological assessment. In fact, many such compounds may not carry a chemical signature from the environmental exposure at all, but perhaps only reflect slight perturbations in the systems biology in response to an external exposure. This model is implemented regularly in diagnostic medicine, for example, consider standard tests for cholesterol, triglycerides, and liver enzymes in blood as markers for dietary choices and health risk, and glucose and proteins in urine as markers for kidney damage and infection (Pagana and Pagana 2009). The results are only used for further investigations if they fall outside of some pre-determined "normal" range.

If one hopes to eventually use exposome biomarkers as indicators of sustainability, one needs a strategy for accumulating relevant empirical data. Due to the subtlety of the external environment (many chemicals at low levels) coupled with the enormous chemical complexity of normal life processes (energy production, growth and repair, and reproduction), teasing out relevant changes and their respective chemical interactions and patterns is a daunting task. Table 2 shows a progression of necessary steps from a pragmatic perspective for incorporating biomarker data into a sustainability assessment. The columns represent discrete activities that will collectively comprise the information describing the interaction between the environment and the human systems biology as detailed below.

Measuring exposome state: A first step towards understanding the individual exposome and its current state is to develop methodology for measuring as many of the exposome constituents (both environmental and biological) as possible. Some classic examples are derived from traditional exposure assessment studies wherein existing methods provide biomarker data for suites of native environmental chemicals in human exhaled breath, blood, and urine in the general public (Wallace 1987, Crinnion 2010, Egeghy et al. 2005), gasoline and jet fuel evaporation (such as hexane, nonane, decane, benzene, toluene, naphthalene, and MTBE)

(Lindstrom and Pleil 2002, Serdar et al. 2003, Prah et al. 2004), solvents and consumer products usage (including chloroform, bromoform, vinyl chloride, trichloroethylene, tetrachloroethylene, and Freons) (Pleil and Lindstrom 1997, Waksman and Phillips 2004), and products of incomplete combustion such PAHs (Strickland et al. 1996, Pleil et al. 2010). There are also newer methods for determining chemical such as pesticides (including chlorpyrifos and permethrin) metabolites in urine (Bouvier et al. 2005) and normally occurring endogenous compounds such as alcohols and aldehydes in exhaled breath (Ligor et al. 2008). New methods are currently in development for measuring cytokines in blood and urine and for large molecule analysis for hemoglobin and albumin adducts and glucuronide conjugates (Wild 2009). An overall scan of endogenous markers can be achieved in liquid media using nuclear magnetic resonance (NMR) spectrometry (Wishart 2011) or in exhaled breath with new technologies such as proton transfer reaction mass spectrometry (PTR-MS) (Herbig and Amann 2009). Other in vitro methods have also been employed with human cell lines to explore metabolite production in response to external environmental stimuli (Sawyer et al. 2010, Gabelova et al. 2007). All together, a suite of measurement methods needs to be available to gather as much empirical data as possible.

Identifying and interpreting exposome constituents: The second step is to develop an idea of what is normal (or unremarkable) in the exposome for as many chemicals as possible. One needs to strive to establish statistical parameters that describe the prevalence, expected values, and distribution of measureable exposome compounds in the general population. Host factor grouping based on factors such as age, gender, ethnicity and geography can then be used to further partition these statistics. Once summary statistics are established, outlier measurements can be identified that may have probative value for exposure assessment. This concept is not new; the underlying tenet of most common diagnostic medical tests, for example blood pressure and body temperature values, is that the measurements are only of interest if they are above or below certain established normal limits.

An important start for developing normal statistics levels in the environment and the exposome are broad cross-sectional studies like the NHANES survey research program at US Centers for Disease Control and Prevention (National Health and Nutrition Examination Study, http://www.cdc.gov/nchs/nhanes.htm) wherein population based measurements are provided for numerous biomarkers. Other exposure studies, such as EPA's TEAM study (Total Exposure Assessment Methodology Study, Wallace 1987), NHEXAS (National Human Exposure Assessment Survey, http://www.epa.gov/heasd/edrb/nhexas.html), CTEPP (Children's Total Exposure to Persistent Pesticides, http://www.epa.gov/heasd/ctepp/), and other persistent organic pollutant studies, DEARS (Detroit Exposure and Aerosol Research Study, http://www.epa.gov/dears/) have also provided limited statistical results for environmental chemicals and chemical biomarkers in blood, breath, and urine. In addition, Pleil et al. 2009 have recently measured normal ranges of background levels of circulating (blood borne) PAH, endogenous alcohols and aldehydes in breath (Hubbard et al. 2009), and cytokines in urine

(personal communication, Michael Madden, US EPA and Matthew Stiegel, University of North Carolina, Chapel Hill). Research is progressing for identifying background levels of volatile metabolites in urine and blood headspace and are exploring immunochemical tests for large molecule (e.g. protein, DNA, RNA) expression.

Developing exposome correlations: The third step is to make biomarker measurements of individuals or sub-populations with known (or suspected) remarkable exposures to deduce which, if any, constituents of the exposome experience significant changes. This can be done with controlled chamber studies and with observational studies. For example, in a chamber experiment, subjects can be studied longitudinally (pre- and post-exposure) and/or with respect to control (sham) exposures. Differences among exposome constituents can then be interpreted with respect to known (applied) exposure parameters. This has the primary advantages in that the exposure is known and stable; as such, any variance in biomarker response is from the subject's intrinsic response. Furthermore, the exposure can be turned on and off allowing the study of uptake and elimination kinetics. In an observational or panel study, simultaneous environmental and biomarker measurements are made and treated as continuous variables; one can then search for dose-response relationships to identify compounds of interest. In this type of study, one has the advantage of many more subjects, but we can never exactly describe all of the nuances of the exposure variability. In short, different styles of studies provide different insights for exposome correlations to the external environment.

Establishing sustainability metrics of the exposome: The fourth and final step is creating specific statistical parameters for exposome constituents that can be compared with new measurements to assess perturbations. This task begins with accumulating biomarker data from the random testing of individuals or sub-populations for exposome constituents shown to be associated with particular environmental stressors. From step 1, compound groups and analytical methods are identified; from step 2, one observes which values represent an unremarkable distribution; from step 3, one gains some insight as to which constituents of the exposome may be affected by environmental conditions. With this information, it is possible to make measurements and compare analytical results from individuals with unknown exposure status to their respective population statistics. Subjects (or groups of subjects) that display significant statistical differences from expected values can then be further studied to identify potential causes, propose mitigation strategies, and assess risk. One of the most important aspects of this last step is to develop rigorous mathematical and statistical techniques to evaluate changes not only in the individual measured analytes, but also in their patterns within sample and groups. This requires careful attention to the underlying assumptions including independence of samples, uniformity of variance, statistical distribution, and over-modeling issues (e.g. sufficient sample numbers and repeat measures to avoid random associations). The ultimate goal is to have a battery of empirical analytical procedures available that are all linked back to pattern and concentration statistics for the general, unremarkable population, and then deploy these tests in communities that are under some form of environmental stress. If any of the sub-population statistical results

fall significantly outside of the expected normal ranges or response patterns of the human exposome, then the researcher can infer that there is some form of a perturbation occurring and investigate at a more detailed level.

Interpreting toxicity pathways: Although suites of biomarkers that are directly accessible for measurement in living humans may not necessarily be sufficient to prove toxicity in the local environment, they could conceivably be mapped forward to toxicity response in conjunction with *in-vivo* animal data, *in-vitro* cell line data, or *in-silica* quantitative structure-activity relationship (QSAR) or computational chemistry data (Pleil and Sheldon 2010, Moser 2011, Bushnell et al. 2010, Kavlock et al. 2008). Taken all together, the categories in Table 2 serve to illuminate the empirical procedures that can be implemented to identify perturbations in the exposome and make some conjectures of cause based on meta-data analysis and overall pattern shifts. The final step is to use this information in a continuous feedback system to integrate with new high-throughput *in-vitro* and *in-silica* chemical toxicity assessments (Wetmore et al. 2012). The concept of making as many biomarker measurements as possible to establish patterns reflective of sustainability can then serve directly as a guide for interpreting toxicity pathways in the human system. Observable perturbations (as reflected in the human exposome) result from external influences and so the environmental exposure, uptake, dose, and biologically relevant dose information are all critical to assess the toxicity of environmental chemicals to real world health outcomes (Cohen Hubal et al. 2010, Egeghy et al. 2011).

SUMMARY REMARKS

The first part of this review proposed that the nominal categorization of human biomarkers can be based on different criteria, but that it is the chemicals themselves, regardless of origin, function, kinetics, or media that comprise the total human exposome. Overall, specific biomarkers can fall into more than one category, and as analytical procedures become more specific and sensitive, new suites of low levels of bio-chemicals are continually being added to the overall exposome catalog. Herein, the most prevalent groupings of biomarkers have been described with the hope that future advances in biomarker measurements can be consistently interpreted and classified. In this review article, the focus is on the ambient (or chemical) environment as the source for human exposome perturbations. Certainly, one must always be cognizant of broader definitions of "environment" to include factors such as diet, exercise (human activity), current health state, and then consider such meta-data in subsequent interpretations. Finally, for consistent communication and collaboration among researchers, it is important to properly interpret (and thus cross-link) the classifications schemes before assigning probative value to groups of biomarkers.

The second part of the review proposed that environmental sustainability could be assessed by observing changes in the human exposome as reflected in the broad spectrum of biomarker

chemicals. As such, one strives to measure and interpret as many suites of biomarker chemicals as possible so that in the long term, it is possible to see if environmental stressors are perturbing human systems biology from established unremarkable levels and patterns. Such perturbations may not necessarily be irreversible, but will serve as sentinel indicators in the population and alert the observers as to when and where potentially unsustainable environmental conditions begin to appear. This population-based approach is analogous to diagnostic/preventative medicine for individuals wherein specific compounds are measured such as liver enzymes or blood lipids that are then compared to expected, or normal values. In the case of the environmental sustainability metric, it will be important to interpret the patterns of a larger variety of different chemicals simultaneously and have a statistically robust database for discerning what is unremarkable and what is a perturbation in the exposome. Finally, such empirical perturbation observations from human biomarker measurements can be integrated into high-throughput *in-vitro* and *in-silica* testing to help verify system biology level, or "real-world" human toxicity of environmental and manufactured chemicals.

ACKNOWLEDGEMENTS

The author thanks Stephen Rappaport from University of California, Berkeley for many insightful discussions regarding biomarkers and the human exposome; he also thanks Jon Sobus, Linda Sheldon, Tzipporah Kormos, Michael Madden and Myriam Medina-Vera of US EPA, Terrence Risby from Johns Hopkins University, and Matthew Stiegel from University of North Carolina, Chapel Hill for their expert advice. This research has been subjected to (EPA) Agency review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

Angerer J, Ewers U, Wilhelm M, 2007. Human biomonitoring: State of the art. Int J Hyg Environ Health 210(3-4):201-228.

Au WW, 2007. Usefulness of biomarkers in population studies: From exposure to susceptibility and to prediction of cancer. Int J Hyg Environ Health 210:239-246.

Barton HA, Pastoor TP, Baetchke K, Chambers JE, Diliberto J, Doerrer NG, Driver JH, Hastings CE, Ivengar S, Krieger R, Stahl B, Timchalk C, 2006. The acquisition and application of absorption, distribution, metabolism, and excretion (ADME) data in agricultural chemical safety assessments, Crit Rev Tox 36:9-35.

Benton MA, Rager JE, Fry RC, 2011. Comparative genomic analyses identify common molecular pathways modulated upon exposure to low doses of arsenic and cadmium, BMC Genomics 12:173.

Bouvier G, Seta N, Vigouroux-Villard A, Blanchard O, Momas I, 2005. Insecticide urinary metabolites in nonoccupationally exposed populations, J Toxicol Environ Health B 8:485-512.

Bushnell PJ, Kavlock RJ, Crofton KM, Weiss B, Rice DC, 2010. Behavioral toxicology in the 21st century: Challenges and opportunities for behavioral scientists; Summary of a symposium presented at the annual meeting of the Neurobehavioral Teratology Society, June 2009. Neurotoxicol Teratol 32:313-328.

Cogliano VC, Baan RA, Straif K, Grosse Y, Secretan MB, Ghissani FE, Kleihues P, 2004. The science and practice of carcinogen identification and evaluation, Environ. Health Persp 112:1269-1274.

Collins AR, Azqueta A, 2011. DNA repair as a biomarker in human biomonitoring studies; further applications of the comet assay, Mutat Res, doi:10.1016/j.mrfmmm.2011.03.005.

Crinnion WJ, 2010. The CDC fourth national report on human exposure to environmental chemicals: What it tells us about our toxic burden and how it assists environmental medicine physicians. Altern Med Rev. 15:101-109.

Edwards SW, Preston RJ. 2008. Forum: Systems biology and mode of action based risk assessment. Tox Sci 106:312-318.

Egeghy PP, Quackenboss JJ, Catlin S, Ryan PB, 2005. Determinants of temporal variability in NHEXAS-Maryland environmental concentrations, exposures, and biomarkers, J Exp Anal Environ Epidemiol 15:388-397.

Egeghy PP, Judson R, Gangwal S, Mosher S, Smith D, Vail J, Cohen Hubal EA, 2011. The exposure data landscape for manufactured chemicals, Sci Total Environ doi:10.1016/j.scitotenv.2011.10.046.

Epstein RJ, 1998. Bad genes, bad diseases, and bad luck, Quart J Med 91:861-864.

Franco Suglia S, Gryparis A Schwartz J, Wright RJ, 2008. Association between traffic-related black carbon exposure and lung function among urban women, Environ Health Persp 116:1333-1337.

Funk WE, Waidyanatha S, Chaing SH, Rappaport SM, 2008. Hemoglobin adducts of benzene oxide in neonatal and adult dried blood spots, Cancer Epidemiol Biomarkers Prev. 17:1896-1901.

Funk WE, Li H, Iavarone AT, Williams ER, Riby J, Rappaport SM, 2010. Enrichment of cysteinyl adducts of human serum albumin, Anal Biochem 400:61-68.

Gábelová A, Valovicová Z, Bacová G, Lábaj J, Binková B, Topinka J, Sevastyanova O, Srám RJ, Kalina I, Habalová V, Popov TA, Panev T, Farmer PB, 2007. Sensitivity of different endpoints for in vitro measurement of genotoxicity of extractable organic matter associated with ambient airborne particles (PM10), Mutat Res, 620:103-113.

Goklany IM, 2007. The improving state of the world: Why we are living longer, healthier, more comfortable lives on a cleaner planet, Cato Institute, Washington, DC.

Gupta SC, Sharma A, Mishra M, Mishra RK, Chowdhuri DK, 2010. Heat shock proteins in toxicology: How close and how far? Life Sci 86:377-384.

Hecht S, 2002. Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer, Carcinogenesis 23:907-922.

Herbig J, Amann A, 2009. Proton transfer reaction-mass spectrometry applications in medical research, J Breath Res 3:020201.

Herpin G, Gauchard GC, Vouriot A, Hannhart B, Barot A, Mur JM, Zmirou-Navier D, Perrin PP, 2008. Impaired neuromotor functions in hospital workers exposed to low levels of organic solvents, Neurotoxicol Res 13:185-196.

Hesterberg TW, Bunn WB, McClellan RO, Hamade AK, Long CM, Valberg PA, 2009. Critical review of the human data on short-term nitrogen dioxide (NO2) exposures: Evidence for NO_2 no-effect levels. Critical Rev Toxicol 39:743-81.

Hu C, van der Heijden R, Wang M, van der Greef J, Hankemeier T, Xu G, 2009. Analytical strategies in lipidomics and applications in disease biomarker discovery, J Chromat B 877:2836-2846.

Hubbard HF, Sobus JR, Pleil JD, Madden MC, Tabucchi S, 2009. Application of novel method to measure endogenous VOCs in exhaled breath condensate before and after exposure to diesel exhaust, J Chromat B 877:3652-3658.

Hunter DJ, 2005. Gene-environment interactions in human disease, Nat Rev: Genet 6:287-298.

Kavlock RJ, Ankley G, Blancato J, Breen M, Conolly R, Dix D, Houck K, Hubal E, Judson R, Rabinowitz J, Richard A, Setzer RW, Shah I, Villeneuve D, Weber E, 2008. Computational toxicology – A state of the science mini review, Toxicol Sci 103:14-27.

Kim S, Vermeulen R, Waidyanatha S, Johnson BA, Lan Q, Smith MT, Zhang L, Li G, Shen M, Yin S, Rothman N, Rappaport SM, 2006. Modeling human metabolism of benzene following occupational and environmental exposures. Cancer Epidemiol Biomarkers and Prev15:2246-2252.

Kim K, Aronov P, Zakharkin SO, Anderson D, Perroud B, Thompson IM, and Weiss RH, 2009. Urine metabolomics analysis for kidney cancer detection and biomarker discovery, Mol Cell Proteomics 8:558-70.

Koch HM, Hardt J, Angerer J, 2001. Biological monitoring of exposure to the general population to the organophosphorus pesticides chlorpyrifos and chlorpyrifos-methyl by determination of their specific metabolite 3,5,6-trichloro-2-pyridinol, Int J Hyg and Environ Health 204:175-180.

Kurdistani SK, 2011. Histone modification in cancer biology and prognosis, Prog Drug Res 67:91-106.

Liao D, Shaffer ML, He F, Rodriguez-Colon S, Wu R, Whitsel EA, Bixler EO, Cascio WE 2011. Fine particulate air pollution is associated with higher vulnerability to atrial fibrillation - the APACR study, J Toxicol Environ Health A 74:693-705.

Ligor T, Ligor M, Amann A, Ager C, Bachler M, Dzien A, Buszewski B. 2008. The analysis of healthy volunteers' exhaled breath by the use of solid-phase microextraction and GC-MS, J Breath Res. 2:046006.

Lin YS, Kupper LL, Rappaport SM, 2005. Air samples versus biomarkers for epidemiology, Occup Environ Med 62:750-760.

Lindstrom AB, Pleil JD and Berkoff DC, 1997. Alveolar breath sampling and analysis to assess trihalomethane exposures during competitive swimming training, Environ Health Persp 105:636-642.

Lindstrom AB, Pleil JD, 2002. A review of the USEPA's single breath canister (SBC) method for exhaled volatile organic biomarkers, Biomarkers 7:189-208.

Link BG, 2008. Epidemiological sociology and the social shaping of public health, J Health Sociol Behav 49:367-384.

McClean MD, Wiencke JK, Kelsey KT, Varkonyi A, Ngo L, Eisen EA, Herrick RF, 2007. DNA adducts among asphalt paving workers. Ann Occup Hyg 51:27-34.

Montón C, Torres A. 1998. Lung inflammatory response in pneumonia. Monaldi Arch Chest Dis. 53:56–63.

Moser VC, 2011. Functional assays for neurotoxicity testing, Toxicol Patol 39:36-45.

Nagaraj N, Mann M, 2011. Quantitative analysis of the intra- and inter-individual variability of the normal urinary proteome, J Proteomic Res 10:637-645.

Olden K, White SL, 2005. Health related disparities: influence of environmental factors, Med Clin N Am 89:721-738.

Ortiz A, Lorz C, Catalan MP, Justo P, Egido J, 2000. Role and regulation of apoptotic cell death in the kidney: Y2K update, Front Biosci 5:D735-D749.

Pagana KD, Pagana TJ, 2009. Mosby's Manual of Diagnostic and Laboratory Tests, 4th Edition, Elsevier Inc., Philadelphia, PA.

Patel CJ, Bhattacharya J, Butte AJ, 2010. An environment-wide association study (EWAS) on type-2 diabetes mellitus, PLoS One 5:e10746.

Perera FP, Rauh V, Whyatt RM, Tang D, Tsai WY, Bernert JT, Tu YH, Andrews H, Barr DB, Camann DE, Diaz D, Dietrich J, Reyes A, Kinney PL, 2005. A summary of recent findings on birth outcomes and developmental effects of prenatal ETS, PAH, and pesticides exposures, Neurotoxicology 26:573-587.

Pleil JD, Lindstrom AB, 1997. Exhaled human breath measurement method for assessing exposure to halogenated volatile organic compounds, Clin Chem 43:723-730.

Pleil JD, Kim D, Prah J, Ashley DL and Rappaport SM, 2007. Exposure reconstruction for reducing uncertainty in risk assessment: Example using MTBE biomarkers and a simple pharmacokinetic model, Biomarkers 12:331-348.

Pleil JD, 2008. Role of Exhaled Breath Biomarkers in Environmental Health Science, J Toxicol Environ Health B 11:613-626.

Pleil JD, 2009. Influence of systems biology response and environmental exposure level on between-subject variability in breath and blood biomarkers, Biomarkers 14:560–571.

Pleil JD, Stiegel MA, Madden MC, and Sobus JR, 2011a. Heat map visualization of complex environmental and biomarker measurements. Chemosphere 84: 716-723.

Pleil JD, Sobus JR, Sheppard PR, Ridenour G, and Witten ML, 2011b. Strategies for evaluating the environment-public health interaction of long-term latency disease: The quandary of the inconclusive case-control study, Chem-Biol Interact doi:10.1016/j.cbi.2011.02.020.

Pleil JD and Sheldon LS, 2010. Adapting concepts from systems biology to develop systems exposure event networks for exposure science research. Biomarkers 16: 99-105.

Pleil JD, Stiegel MA, Sobus JR, Tabucchi S, Ghio AJ, and Madden MC, 2010. Cumulative exposure assessment for trace-level polycyclic aromatic hydrocarbons (PAHs) using human blood and plasma analysis, J Chromatogr B 878:1753–1760.

Prah J, Ashley D, Blount B, Leavens T, Pleil J, Cardinalli F, 2004. Dermal, oral, and inhalation pharmacokinetics of methyl tertiary butyl ether (MTBE) in human volunteers, Toxicol Sci 77:195-205.

Qu Q, Shore R, Li G, Jin X, Chen LC, Cohen B, Melikian AA, Eastmond D, Rappaport S, Li H, Rupa D, Waidyanatha S, Yin S, Yan H, Meng M, Winnik W, Kwok ES, Li Y, Mu R, Xu B, Zhang X, Li K, 2003. Validation and evaluation of biomarkers in workers exposed to benzene in China, Res Rep Health Eff Inst. 115:1-72.

Rakyan VK, Down TA, Balding TJ, Beck S, 2011. Epigenome-wide association for common human diseases, Nat Rev Genet 12:529-541.

Rappaport SM and Smith MT, 2010. Epidemiology. Environment and disease risk, Science 33:460-461.

Rappaport SM, 2011. Implications of the exposome for exposure science, J Expo Sci Environ Epidemiol 21:5-9.

Reichenbach SE, Tian X, Tao Q, Ledford EB, Wu Z, Fiehn O, 2011. Informatics for cross-sample analysis with comprehensive two-dimensional gas chromatography and high resolution mass spectrometry (GCxGC-HRMS), Talanta 83:1279-1288.

Rhea JM, Diwan CA, Molinaro RJ, 2010. Mass spectrometry-coupled techniques for viral-related disease biomarker identification, Biomark Med 4:859-870.

Sawyer K, Samet JD, Ghio AJ, Pleil JD, and Madden MC, 2009. "Responses measured in the exhaled breath of human volunteers acutely exposed to ozone and diesel exhaust" J Breath Res 2:037019.

Sawyer K, Mundandhara S, Ghio AJ, Madden MC, 2010. The effects of ambient particulate matter on human alveolar macrophage oxidative and inflammatory responses, J Toxicol Environ Health A. 73: 41-57.

Seigneuric R, Markey L, Nuyten DS, Dubernet C, Evelo CT, Finot E, Garrido C, 2010. From nanotechnology to nanomedicine: Applications to cancer research, Curr Mol Med 10:640-652.

Serdar B, Egeghy PP, Waidyanatha S, Gibson R, Rappaport SM, 2003. Urinary biomarkers of exposure to jet fuel, Environ Health Persp 111:1760-1764.

Smith MT, Rappaport SM. 2009. Editorial: Building exposure biology centers to put the E into GxE interaction studies. Environ Health Persp 117: A334-A335.

Smith LB, Bhattacharya A, Lemasters G, Succop P, Puhala E, Medvedovic M, Joyce J, 1997. Effect of chronic low-level exposure to jet fuel on postural balance of US Air Force personnel, J Occupat Environ Med 3:623-632.

Smith MT, Zhang L, McHale CM, Skibola CF, Rappaport SM, 2011. Benzene, the exposome and future investigations of leukemia etiology, Chem-Biol Interact doi:10.1016./j.cbi.2011.02.010.

Smolders R, Schramm KW, Nickmilder M, Schoeters G, 2009. Applicability of non-invasively collected matrices for human biomonitoring, Environ Health 8:8.

Sobus JR, McClean MD, Herrick RF, Waidyanatha S, Nylander-French LA, Kupper LL, Rappaport SM, 2009. Comparing urinary biomarkers of airborne and dermal exposure to polycyclic aromatic compounds in asphalt-exposed workers, Ann Occup Hyg. 53:561-571.

Sobus JR, Tan YM, Pleil JD, and Sheldon LS, 2011. "A tiered approach to biomonitoring for human exposure and health research", Sci Total Environ 409:4875-4884.

Strange EM, Lipton J, Beltman D, Snyder BD, 2002. Scientific and societal considerations in selecting assessment endpoints for environmental decision making, Sci World J 2 (Suppl 1):12-

Strickland P, Kang D, Sithisarankul P, 1996. Polycyclic aromatic hydrocarbon metabolites in urine as biomarkers of exposure and effect, Environ Health Persp 104(Suppl 5):927-932.

Suk WA, Olden K, Yang RSH, 2002. Chemical mixtures research: Significance and future perspectives, Environ Health Persp 110(Suppl 6): 891-892.

Tan YM, Dary CC, Chang DT, Goldsmith MR, Pleil JD, Sobus JR, and Tornero-Velez R, 2011. A detective story: Reconstructing human exposures using biomarkers of exposure and other "clues", J Toxicol Environ Health B 15:22-38.

Waidyanatha S, Rothman N, Fustinoni S, Smith MT, Hayes RB, Bechtold W, Dosemeci M, Guilan L, Yin S, Rappaport SM, 2001. Urinary benzene as a biomarker of exposure among occupationally exposed and unexposed subjects, Carcinogenesis 22:279-286.

Waidyanatha S, Rothman N, Li G, Smith MT, Yin S, Rappaport SM, 2004a. Rapid determination of six urinary benzene metabolites in occupationally exposed and unexposed subjects, Anal Biochem 327:184-199.

Waidyanatha S, Zheng Y, Serdar B, Rappaport SM, 2004b. Albumin adducts of naphthalene metabolites as biomarkers of exposure to polycyclic aromatic hydrocarbons, Cancer Epidemiol Biomarkers Prev. 13:117-124.

Waksman JC, Phillips SD, 2004. Biologic markers of exposure to chlorinated solvents, Clin Occup Environ Med. 4:413-421.

Wallace, LA, 1987. The Total Exposure Assessment Methodology (TEAM) Study, Summary and Analysis, Vol. 1, EPA/600/6-87/002a, June 1987; (http://exposurescience.org/pub/reports/TEAM_Study_book_1987.pdf) accessed 5/13/2011.

Wallace LA, Nelson WC, Pellizzari ED, Raymer JH, 1997. Uptake and decay of volatile organic compounds at environmental concentrations: application of a four compartment model to a chamber study of five human subjects, J Expo Anal Environ Epidemiol 7:141-163.

Wetmore BA, WambaughJF, Fergusen SS, Sochaski MA, Rotroff DM, Freeman K, Clewell HJ, Dix DJ, Andersen ME, Houck KA, Allen B, Judson RS, Singh R, Kavlock RJ, Richard AM, Thomas RS, 2012. Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment, Toxicol Sci 125:157-174.

Wild CP, 2005. Complementing the genome with an "exposome": The outstanding challenge of environmental exposure measurement in molecular epidemiology, Cancer Epidemiol Biomark Prev 14:1847-1850.

Wild CP, 2009. Environmental exposure measurement in cancer epidemiology. Mutagenesis 24:117-125.

Wild CP, 2011. Future research perspectives on environment and health: the requirement for a more expansive concept of translational cancer research, Environ Health 10(Suppl 1):S15.

Williams PR, Inman D, Aden A, Heath GA, 2009. Environmental and sustainability factors associated with next generation biofuels in the U.S.: What do we really know?, Environ SciTechnol 43:4763-4775.

Wilson MP, Schwarzman MR, 2009. Toward a new U.S. chemicals policy: Rebuilding the foundation to advance new science, green chemistry, and environmental health, Environ Health Persp 117:1202-1208.

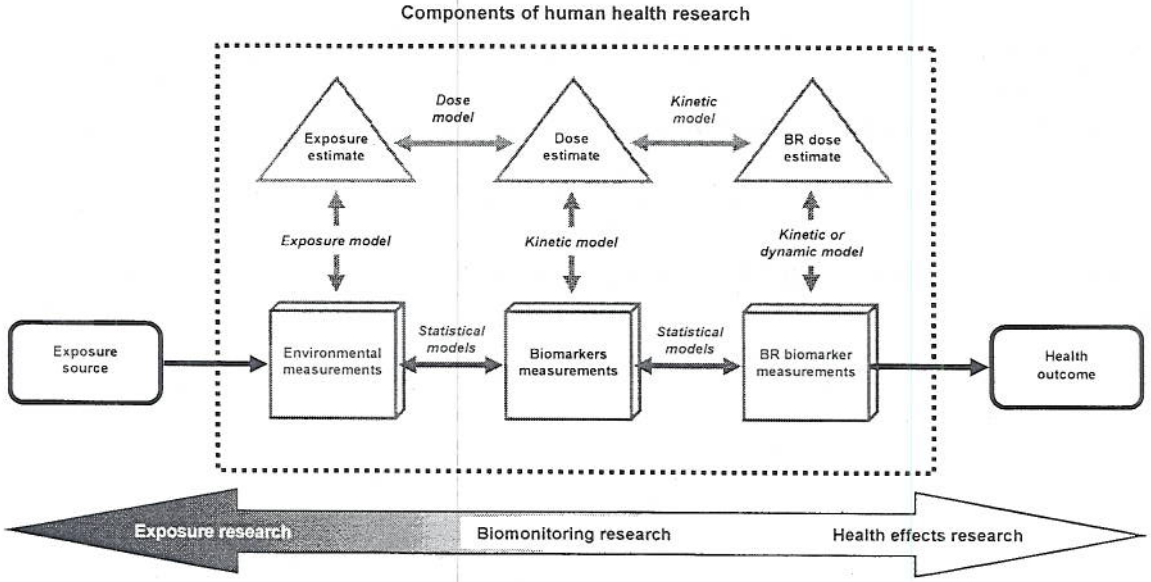
Wishart DS, 2011. Advances in metabolite identification, J Bioanal 3:1769-1782.

Ziech D, Franco R, Georgakilas AG, Georgakila S, Malamou-Mitsi V, Schoneveld O, Pappa A, Panayiotidis MI, 2010. The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development, Chem-Biol Interact 188:334-339.

Figure caption:

Figure 1. Conceptual framework for biomarkers research at U.S. EPA. Blue boxes represent measured data, red triangles represent modeled estimates, and arrows represent the types of models used to link these components together. The human exposome is contained in the "Biomarkers measurements" and "Biologically relevant (BR) biomarker measurements" boxes.

Figure 1.


Components of human health research
Exposure estimate
Dose model
Dose estimate
Kinetic model
BR dose estimate
Exposure model
Kinetic model
Kinetic or dynamic model
Exposure source
Environmental measurements
Statistical models
Biomarkers measurements
Statistical models
BR biomarker measurements
Health outcome
Exposure research
Biomonitoring research
Health effects research

Tables:

Table 1. Biomarker categories and their major subdivisions

Biomarker origin	**Biomarker function**	**Biomarker kinetics**	**Biomarker medium**
exogenous	exposure	absorption	blood
endogenous	susceptibility	distribution	breath
response	effect	metabolism	urine
		elimination	

Table 2. Biomarker data strategies for developing sustainability indicators

Measurement Methodology	**Exposome Constituents**	**Exposure Correlations**	**Sustainability Metrics**
environmental	identification	chamber	mean values
biological	distribution	observational	outliers
'omics'	summary stats.	epidemiological	patterns