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COMMENTARY

Adapting concepts from systems biology to develop systems exposure event networks for exposure science research

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Abstract

Systems exposure science has emerged from the traditional environmental exposure assessment framework and incorporates new concepts that link sources of human exposure to internal dose and metabolic processes. Because many human environmental studies are designed for retrospective exposure evaluations they often do not provide practical toxicological outcome parameters. Our goal was to examine concepts from systems biology research and adapt them to a network approach that maps forward to a perturbation event using two hypothetical examples. The article proposes that environmental exposure studies should not only retrospectively document exposure levels, but also measure biological parameters that can be used to inform relevant systemic changes.

Keywords: *Network diagram; biological parameter; protein adducts; cytokine response; variable clusters; mixed effects model; ADME*

Introduction

The study of environmental influences on human disease is composed of two main activities, exposure assessment and adverse health effect observation. These comprise the basis of human epidemiology and are used to decipher the actual causes of apparently random occurrences of disease with the ultimate goal of developing intervention strategies. Understanding the relationship between exposures to environmental stressors is complex for several reasons. Thousands of anthropogenic trace level chemicals in the environment vary spatially and temporally. The resultant disease onset can be subtle and take as long as decades (Karalliedde et al. 2003, Boothe and Shendell 2008; Ritz and Wilhelm 2008; Baas et al. 2009). Furthermore, many chronic and systemic diseases are rare, and so appear to be random in the population presumably because individuals have different chemical exposures and varying responses to external stressors including not only the environmental chemicals, but also diet, lifestyle choices, and various human activities (Perera 1997; Simmons and Portier 2002). Despite this apparent randomness, the importance of identifying environmental chemicals with linkage to adverse health effects has been in the consciousness of the public health community for over 30 years (Ames 1979).

Recent advances in proteomics and genomics, and the decoding of human DNA structures from the Human Genome Project, are now being incorporated into a "systems biology" understanding of life processes and their perturbations (Stark 2008; Aggarwal and Lee 2003; Bruggeman and Westerhoff 2007). Based on systems approaches at the molecular and cellular level, researchers are beginning to interpret the overall toxicological pathways (or networks) that can lead from an environmental trigger to an adverse outcome (Ekins et al. 2005; Smith and Rappaport 2009; Wild 2005). High throughput *in vitro* toxicological screening is also being implemented

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at the cellular level (Rusyn and Datson 2010) but there is concern regarding extrapolation to low dose models (Crump et al. 2010); addressing this issue is one of the goals of this work.

In this article, the underlying concepts of systems biology are extended to and integrated with a systems exposure framework at the human level of organization. We propose that the endpoint for exposure research should be dose at the target and simultaneously serve as a starting point for a toxicological pathway or effects network. Systems exposure explores the relationship between external exposure measurements (concentrations in air, water, food, etc.) and "biologically relevant exposure metrics" (concentrations in blood, breath, urine etc.) that can be related to, or serve as, surrogates for a dose at a specific target. Through such biological metrics, systems can also be used to statistically evaluate the influences on biomarker variability (Birnbaum 2010; Pleil 2009, Sobus et al, 2010). Fundamentally, this is analogous to the systems biology approaches of genomics, proteomics, and metabonomics, etc. that interpret the complex interactions of human biochemistry at the molecular and cellular levels of organization (Edwards and Preston 2008; Sheldon and Cohen-Hubal 2009). We propose that the traditional concept of assessing exposure based on blood-borne exogenous compounds or excreted chemicals in breath and urine (Pleil et al 2007) be expanded into a systems exposure approach using forward mapping to measured biological parameters that can be linked to target dose. Integrating systems approaches for exposure and biology will provide quantitative estimates of the perturbation to the overall systems biology that is caused by the environmental factors.

Systems biology concepts

Systems biology approaches are currently being developed to address quantitative risk assessment with respect to environmental stressors (Hubal 2009). The concepts of individual susceptibility and toxicological thresholds have been explored at the molecular and genetic level (Jenkins et al. 2009; Au 2007; Bonassi et al. 2001; Dorne 2009). In the broadest sense, the systems biology framework of human health has been described as a series of complex networks at various levels of organization such as human disease networks and gene disease networks (Loscalzo et al. 2007; Barabasi and Oltva 2004). Such network concepts have been combined; for example, Edwards and Preston (2009) have proposed a system of three distinct tiered networks arranged as stacked planes representing organism, cellular, and molecular levels of organization; Figure 1 shows an adaptation of these proposed networks (Edwards and Preston 2009). We note that this illustration is meant to show only the parallels among specific research strategies and emphasize that all three tiers are always engaged in the overall systemic processes.

System exposure concepts

In a complementary fashion to systems biology, a systems exposure framework links key events to characterize stressors and the processes that will lead from environmental concentration to dose at the critical target pathways. Environmental stressors are believed to effect system biology networks; Gallagher et al. (2009) have proposed that some form of environmental perturbation is

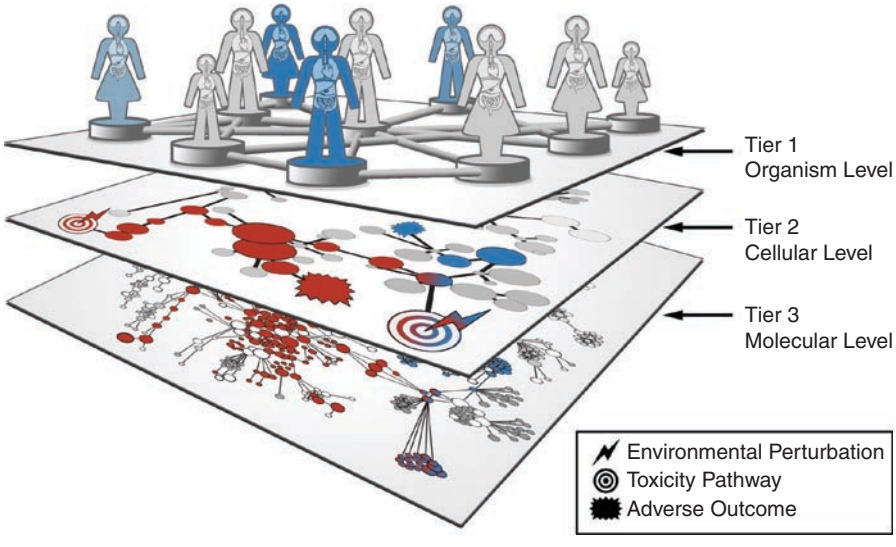

Figure 1. Systems biology framework diagram showing examples of interactions at the organism and population level, at the cellular level, and at the molecular level. These networks are not independent, but constantly interact; typically pre-clinical effects appear at the middle (cellular) tier (adapted from Edwards and Preston 2009).

responsible for initiating the adverse events cascade leading to pathological outcome in the second tier network in Figure 1. To quantify such effects prospectively and to reconstruct the cause of such events retrospectively requires an additional set of linked events: the exposure events network.

Herein, we propose a networked structure diagram analogous to Figure 1 wherein the key events of exposure are connected from the exposure route (dermal, ingestion, inhalation, infection, etc.) through absorption, distribution, metabolism and elimination (ADME) parameters and lead to a biomarker measurement. To fully integrate with systems biology, systems exposure then relies on a final mapping strategy from empirical biomarker to a quantitatively parameterized system perturbation that serves as an endpoint for the systems exposure network, and a beginning point for the key events network shown in Figure 1.

Figure 2 shows such a proposed exposure network diagram in a planar format. The upper edge represents the absorption exposure pathway leading from the skin, stomach, or lung to the central compartment (circulating blood). The center of the diagram shows mechanisms of metabolism that produce a series of short-lived chemicals such as reactive oxygen species (ROS), reactive intermediaries, and electrophiles that can quickly transform to more stable compounds or possibly attack cells, DNA, proteins, etc. (Gracy et al. 1999; Arif and Gupta 1996; van der Vliet et al. 2008). The left edge of the planar diagram lists some possible measurable biomarkers such as exogenous and endogenous metabolites, damage markers, and protein response markers (for example: Anderson and Eling 1976; Farmer 1995; Wessels et al. 2003; Pleil 2008; Needham 2008). Those biomarkers that can be further mapped to the bottom edge of the diagram representing system perturbations or toxicity starting points are designated as measurable biological parameters. The parameters of particular value are continuous variables within in the larger set of the "biologically relevant exposure metrics" that serve as quantitative links between key events in a disease process and individual exposure profiles. We note that this diagram is not meant to be exhaustively detailed, but is instead used to illustrate the potential systems pathways and indicate where empirical measurements can be made. The overall network is always further impacted by individual susceptibility, repair functions, and other host factors that cannot all be diagrammed.

Examples of specific exposure networks

There are undoubtedly many examples of environmental exposures that could result in measurable biological parameters of probative value. For this discussion, we propose two different examples of exposure networks representative of the linkage concepts developed above: blood-borne protein adducts and pulmonary cytokines.

Protein adducts

Electrophilic species from various sources, including environmental contaminant metabolism, are highly likely to form adducts with nucleophilic molecules such as proteins and DNA. Thus, chemical adducts can serve both as an indicator of previous exposure and as an indicator of systemic perturbation of normal large molecule chemistry (Rubino et al. 2009, Swenberg et al.

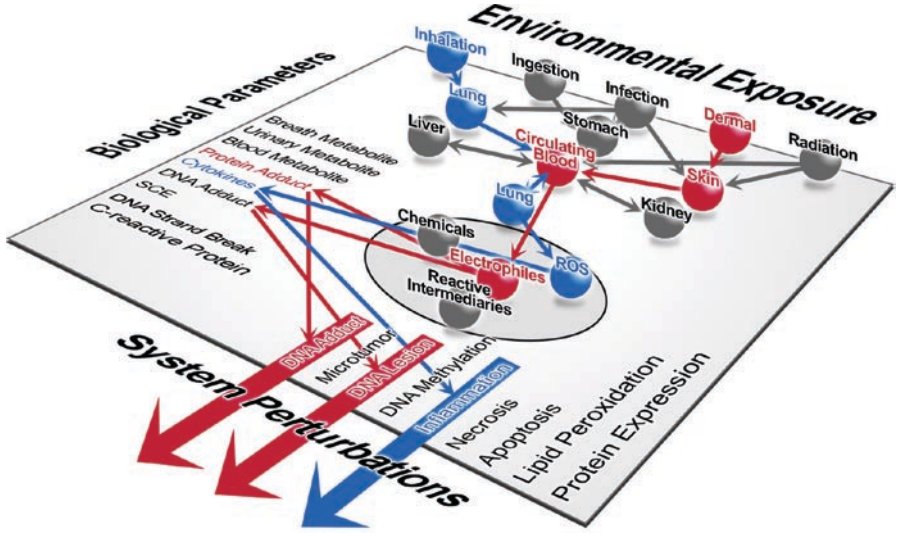

Figure 2: Simplified diagram of the exposure event network plane with two forward mapping examples. 1) blue pathway: diesel exhaust inhalation exposure resulting in production of reactive oxygen species (ROS) that stimulate a cytokine response which can be measured and quantitatively linked to an inflammatory response; and 2) red pathway: dermal exposure to pesticides resulting in electrophilic compounds that generate protein adducts which can be measured and quantitatively linked to DNA damage.

2001, Funk et al. 2010). In general, protein adducts are relatively innocuous and stable, whereas DNA adducts can lead to DNA damage, or can be corrected with various repair mechanisms. As such, a protein adduct has the advantage of serving as a stable surrogate for DNA damage from electrophiles (Meyer and Bechtold 1996). An additional advantage is that proteins are much more abundant than DNA; for example, hemoglobin and albumin are the two main proteins in human blood representing about 150 mg/ml and 40 mg/ml, respectively; in contrast, DNA represents about 0.006 mg/ml (Jansen et al. 2009, Tornqvist et al. 2002).

As a specific example, we use potential hemoglobin and albumin adducts as biologically relevant parameters of pesticides exposure (Angerer et al. 2007; Noort et al. 2008). The hemoglobin proteins are confined to the red blood cells which have an average life-time of 120 days in humans (Krishnan and Dixit 2009). As such, exogenous adducts serve as markers for the mean exposure from the previous three months. This is of particular interest for prenatal development as a newborn baby's adducts represent third trimester in-utero exposures. For adults, the combination of adducts, phase-1 metabolites, and native compounds measurements can provide both an integration of exposure for the previous months and for more recent events on the scale of days and hours (Neri et al. 2006; Coghlin et al. 1991). Similarly, albumin adducts experience exponential decay with a half-life of about 21-25 days and so they serve as a slightly shorter term retrospective exposure marker (Rappaport et al. 2002). As mentioned above, both forms of adducts are considered scalable indicators of DNA adducts and lesions that are initiators of adverse events on the cellular level. The red pathway in Figure 2 shows the route for this scenario. We stress that DNA adducts could serve just as well as biological parameters if appropriate measurements could be made, but that protein adducts were chosen as the example here due to their relative abundance advantage in blood.

Pulmonary cytokines

Inflammation is a systemic response that protects the human body from foreign substances, bacteria, and viruses. Upon a perceived external attack, certain signaling proteins (cytokines), such as interleukins IL-1 and IL-6, and tumor necrosis factor (TNF-α) are released by affected cells. The cytokines serve to recruit neutrophils (a type of white blood cell) to the affected tissues to initiate the protective response (Monton and Torres 1998). This response may also have a negative effect when neutrophils release reactive oxygen species (ROS) and hydrolytic enzymes to perform antibiotic functions as these molecules also attack normal cells (Tao et al. 2003). Chronic exposure leads to chronic inflammation which can aggravate a variety of diseases including cancer, chronic obstructive pulmonary disease (COPD), atherosclerosis, rheumatoid arthritis, cardio pulmonary disease, and inflammatory bowel disease (Coussens and Werb 2002; Hippe et al. 2010; Kim et al. 2008; Sirera et al. 2003; Stenvinkle and Alvestrand 2002; Feldmann and Maini 2001; Nishimura et al. 2009). As such, quantitative measurement of cytokines in biological media are directly linked to inflammatory response and thus to adverse health initiating events.

A variety of airborne contaminants have been linked with pro-inflammatory response in the lungs via cytokine production by airway epithelial cells. As a specific example of environmentally triggered inflammation, we consider inhalation exposure to diesel exhaust (DE) which is composed of fine and ultra-fine particles, inorganic and organic gases, and semi- and non-volatile organic species such as polycyclic aromatic hydrocarbons (PAHs) (Stenfors et al. 2004). The resulting cytokine response to DE is usually measured in bronchial lavage fluid (BLV), but sputum and exhaled breath condensate (EBC) may serve as non-invasive sampling options (Tsiligianni et al. 2005). Cytokines tend to be transient with a time frame on the order of 6 to 24 hrs after exposure events, but random intermittent exposures, as expected from environmental sources, generally present as biologically damped concentration profiles. Therefore, mean levels of cytokines in pulmonary fluids can serve as markers for chronic inflammation levels, which in turn can be quantitatively linked to risk of adverse health effects (Robroeks et al. 2010; Duramad et al. 2007). The blue pathway in Figure 2 shows this example.

Computational applications for exposure science

Mixed model approach

Once the exposure event network is established, objective measurements of the environment and the metadata from the human subjects can be interpreted with multivariate models that use the biological parameters as dependent variables. A standard method for interpreting complex data is based on the linear mixed effects regression model (Rappaport 2008, Singer 1998). The model has the basic format:

$$Y_{hij} = \left\{ \beta_1 X_{1_{hij}} + \beta_2 X_{2_{hij}} \ldots + \beta_p X_{p_{hij}} \right\} + \alpha_h + b_{hi} + \epsilon_{hij}$$

where:

- Y_{hij} is the value of the biological parameter for the j^{th} observation of the i^{th} subject in the h^{th} group;
- $X_{1_{hig}}, X_{2_{hij}}, \ldots X_{p_{hij}}$ are the values of the fixed effect variables such as environmental chemical concentrations (in air, water, food, dust, etc.), and host factors such as age, health state, gender, genetic polymorphisms, ethnicity, etc.;

- p is the total number of fixed effects (note: the host factors may be fixed for all j within a given i);
- β_1, β_2,... β_p, are the corresponding modeled coefficients for the fixed effects and host factors;
- α_h is the random effect for the h^{th} group;
- b_{hi} is the random effect for the i^{th} subject from the h^{th} group;
- ε_{hij} is the residual (unexplained) error for the j^{th} observation of the i^{th} subject from the h^{th} group; and
- q is the total number of random effects.

Software applications for this style approach are commercially available (e.g. proc MIXED, SAS Cary, NC). Upon calculation, the coefficients and their p-values and can be interpreted to determine the effect of including the particular fixed effect or random effect variable in the final model for explaining the variance in the biological parameters' values. This can be done with iterative steps of forward addition or reverse elimination with the eventual objective being a parsimonious model without appreciable loss of modeling power. Once the final model is established, we can observe which exposure parameters and fixed effects are more likely to cause perturbations to the systems biology.

Variable over-modeling and co-linearity

The proper use of the mixed model in the previous section is often not straightforward. A primary consideration is the relative value of subject observations (n) with respect to the number of independent variables represented by the host factors, environmental variables, and random effects ($m = p+q$). This is a reasonable concern because the model's shape loses predictive power if too many parameters can be varied to "hit" the data points. Consider that any two points will determine a straight line with two degrees of freedom, although the true shape may be a curve; any three points will determine a parabola with three degrees of freedom although the true shape may be an exponential curve or a straight line, etc. When n and m are too close, we have a situation of "over-modeling" where different shapes can equally well approximate the same data set; a common rule of thumb is that $n/m \geq 10$ (Pleil and Lorber 2007; Harrell 2002). A second concern regarding the mixed model approach is that of predictor variable co-linearity. As a common example, consider that both subject height and subject weight could be independent variables in the model. In normal human populations, height and weight are collinear to some extent, that is, taller people tend to weigh more than shorter people. Using both parameters in the model, therefore, could lead to unstable parameter estimates.

Both of these issues have been addressed in the literature. A particular approach found to be useful for environmental data employs the concept of "variable clustering" (proc VARCLUS, SAS, Cary, NC) which can be thought of as a variant (or inverse) of principal components analysis (PCA) (Domany 2003; Kettenring 2006). Note that PCA is very different from the VARCLUS approach; PCA groups samples together whereas VARCLUS groups independent variables together. The VARCLUS procedure analyzes the covariance structure of the independent variables and assigns groups with common traits. The results can be visualized in a dendrite style diagram, or dendrogram, as in Figure 3. Here we present hypothetical data wherein increased grouping results in decreased explained variance; for example, forming 8 groups out of 13 variables only reduces the explained variance from 100% to about 93% which may be considered a good trade-off. This approach addresses both the "parsimonious model" objective (minimizing the necessary parameters) in that the n/m ratio increases, and also the co-linearity issue by collapsing co-varying parameters into groups. This has been implemented successfully for assessing compound correlation and source apportionment for sparse environmental dioxin measurements (Pleil and Lorber 2007). There are many different ways of combining independent variables into groups depending on their physical meaning or their relative importance to the anticipated use of the data. For example, one could use previously defined groupings such as body mass index (BMI) as a surrogate for both height and weight, or relative contributions to a total measurement, or simple sums of concentrations, or choosing one parameter of a group to represent the remaining members.

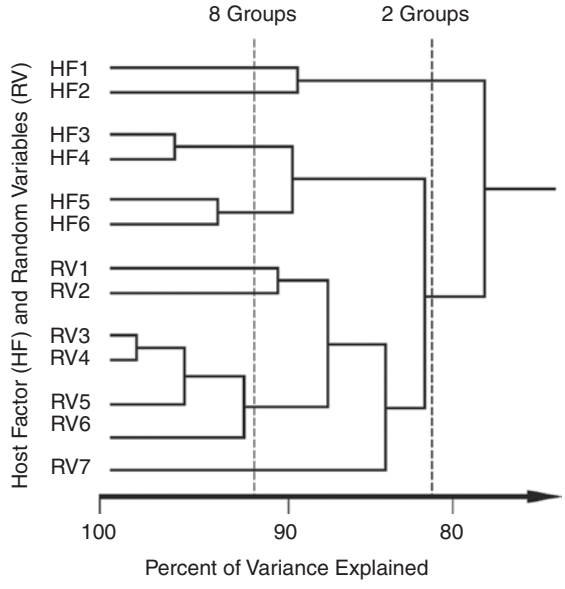

Figure 3. Example dendrogram of variable cluster analysis. Starting with a total of m = 13 independent variables (p = 6 host factors and q = 7 environmental variables), forming 8 clusters (HF1, HF2, HF3+HF4, HF5+HF6, RV1, RV2, RV3+RV4+RV5+RV6, and RV7) results in explained variance of ~93%.

Concluding remarks

The implementation of biological parameters (especially continuous variables) that can be objectively linked to a systems biology perturbation should become a primary design criterion for the development of future environmental exposure studies. The subsequent regression interpretation using such biological parameters as the dependent variable is a powerful tool for assessing the importance of other parameters, especially those reflecting the timing and pathway of the environmental exposures. At the present, many studies rely on environmental measures or simple excreted biomarkers as the dependent variables. Although these approaches are valuable for exposure assessment and for demonstrating that certain sub-populations were indeed exposed, they do not provide a direct link for a perturbation of the normal metabolism or systems biology. Only if there is a measured metabolic systems change that could potentially lead to an adverse effect can we assess the public health risk in a quantitative and empirical manner. Measuring biologically relevant exposure parameters in biological media and establishing a quantitative systems exposure event network are the features that will philosophically transform environmental exposure assessment into a broader environmental systems exposure science. In short, we propose that exposure science measurements should provide some scalable parameter that is relevant to a toxicological starting point.

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Declaratation of interest

The authors declare they have no competing financial interests.

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