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Linking Empirical Estimates of Body Burden of Environmental Chemicals and Wellness using NHANES Data

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Abstract

Biomonitoring of industrial chemicals in human tissues and fluids has shown that all people carry a “body burden” of synthetic chemicals. Although measurement of an environmental chemical in a person’s tissues/fluids is an indication of exposure, it does not necessarily mean the exposure concentration is sufficient to cause an adverse effect. Since humans are exposed to multiple chemicals, there may be a combination effect (e.g., additive, synergistic) associated with low-level exposures to multiple classes of contaminants, which may impact a variety of organ systems. The objective of this research is to link measures of body burden of environmental chemicals and a “holistic” measure of wellness. The approach is demonstrated using biomonitoring data from the National Health and Nutrition Examination Surveys (NHANES). Forty-two chemicals were selected for analysis based on their detection levels. Six biological pathway-specific indices were evaluated using groups of chemicals associated with each pathway. Five of the six pathways were negatively associated with wellness. Three non-zero interaction terms were detected which may provide empirical evidence of crosstalk across pathways. The approach identified five of the 42 chemicals from a variety of classes (metals, pesticides, furans, polycyclic aromatic hydrocarbons) as accounting for 71% of the weight linking body burden to wellness. Significant interactions were detected indicating the effect of smoking is exacerbated by body burden of environmental chemicals. Use of a holistic index on both sides of the exposure-health equation is a novel and promising empirical “systems biology” approach to risk evaluation of complex environmental exposures.

Keywords

systems biology; wellness; chemical mixtures; pathways; crosstalk

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INTRODUCTION

Since World War II, synthetic chemical pollutants have accumulated in the environment and food webs on a global basis (Thornton, 2000), and evidence of persistent environmental contaminants has been documented in wildlife, human blood, and breast milk worldwide. (Schafer and Kegley, 2002) These persistent chemicals include heavy metals, OC pesticides, PBDEs, industrial chemicals and byproducts of certain manufacturing processes and waste incineration (e.g., PCBs, dioxins, and furans). (Schafer and Kegley, 2002; Hermanussen *et al.*, 2008) These chemicals persist in the environment for many years (McGinn, 2000; Weber *et al.*, 2008; Zietz *et al.*, 2008); concentrate in fatty tissues and bioaccumulate as they move up the food chain (Czub, *et al.*, 2008); travel long distances in global air and water currents (Jaga and Dharmani, 2003); and have been linked with serious health effects in humans, even at low exposures (Fattore *et al.*, 2002; Schafer and Kegley, 2002). Humans living in industrialized countries are also exposed daily to chemicals that are not internally persistent. Examples of such chemicals are plasticizers (e.g., phthalates), and exposure can occur through food that has been in contact with phthalate containing packaging, as well as direct contact with products that contain phthalates (e.g., plastics, adhesives, detergents, personal-care products such as soap, shampoo, nail polish; EPA, 2008). Many phthalates have been shown to modulate hormonal activity in a dose additive manner (Howdeshell *et al.*, 2008). Other environmentally persistent chemicals with short half lives (i.e., externally/environmentally persistent but short-lived in humans internally) include metabolites of pyrethroid and organophosphate insecticides and polycyclic aromatic hydrocarbons (e.g., benzo(a)pyrene).

Biomonitoring of environmental chemicals in human tissues and fluids has shown that all people, not just those working in or living near major pollution sources, carry a “body burden” of synthetic chemicals in their blood, fat, mother’s milk, semen, urine and breath (Thornton *et al.*, 2002). With more than 83,000 individual industrial chemicals registered with the U.S. EPA for commercial use (EPA, 2011) it is virtually impossible to quantify the exact body burden in any individual – i.e., any measure of body burden is limited by the selection of chemicals analyzed. The 2001–02 set of the National Health and Nutrition Examination Surveys includes biomonitoring of more than 150 chemicals through blood or urine samples (and important for our approach, dozens of biomarkers of effect). These chemicals were selected for inclusion in the surveys based largely on scientific data that suggest exposure in the U.S. population, the seriousness of known or suspected health effects associated with some levels of exposure, the availability and adequacy of analytical methods, and logistical and cost considerations (EPA, 2008; CDC 2010).

Availability of data from national biomonitoring studies such as NHANES provides the opportunity to measure and evaluate body burden in a new way. An important aspect of risk assessment is identifying the ‘bad actors’ in a set of environmental chemicals – i.e., exposure does not necessarily indicate an increase in risk of a health effect. Risk evaluation of environmentally relevant chemical mixtures should account for associations between multiple health endpoints (i.e., health outcomes) and components of complex mixtures from multiple chemical classes. The objective of this paper is to link body burden estimates with a holistic composite index of wellness using human biomonitoring data. The idea is that environmentally relevant mixtures may affect multiple health outcomes. By linking both sides of the exposure-risk equation, the impact of mixtures on multiple outcomes and co-morbidities can be accommodated while relevant human exposure across chemical classes is allowed.

Generally, body burden of environmental chemicals is estimated/evaluated by grouping chemicals with similar modes of action. For example, mixtures of phthalates (Swan *et al.*,

2005) and PCBs (Buck Louis *et al.*, 2005; Cave *et al.*, 2011) were represented by the sum of the components in the set either represented as measured concentrations (Buck Louis *et al.*), quartiles (Swan *et al.*) or ranks of the concentrations above the limits of detection (LOD) of each chemical (Cave *et al.*). Body burden may also be represented by assuming an additivity model with potency extrapolations from animal studies (e.g., Safe, 1998). However, these approaches do not generally accommodate multiple chemical classes, which are more environmentally relevant to human exposure.

Disease-specific composite indices have been accepted as measures of disease severity in a number of conditions: e.g., Crohn's Disease Activity Index; Child-Turcotte-Pugh and Model for End-stage Liver Disease (MELD) for patients with advanced liver disease; and for cardiac disease and stroke (Liao *et al.*, 2005; Rothwell *et al.*, 2005; Horne *et al.*, 2009). The APACHE is used as a hospital mortality assessment in critically ill patients (Zimmerman *et al.*, 2006). The literature on indices of wellness is conflated with quality of life. Because older people often experience a range of comorbidities (Heyrman and Van Hoeck, 1996), a variety of health-related quality of life instruments have been developed that assess multiple concepts of health status and quality of life (Haywood *et al.*, 2005).

Our objective is to use a holistic index of wellness – separate from quality of life – which characterizes multiple organ systems. As such, we have developed the Relative Wellness Index (RWI, Figure 1; extended from Gennings *et al.*, 2010a), that evaluates a subject's overall wellness based on the simultaneous evaluation of the functionality of multiple organ systems (including liver, kidney, vascular, cardiac, and endocrine). The intent of the RWI is to track functions of multiple organ systems as a measure of symptoms of complex underlying conditions. Analogously, a thermometer indicates the presence of a fever; it is a gross diagnostic tool, which detects some underlying condition. The RWI indicates the presence of underlying conditions by measuring the function of one or more organ systems, which are outside the normal (healthy) range. While a thermometer depends on a single input – temperature, the RWI takes into account numerous indicators of the function of multiple organ systems. In this way it is a holistic measure of the degree of wellness or lack of wellness across multiple systems.

The RWI has been shown to be predictive of mortality (i.e., using survival time) in a variety of patient populations. Gennings and colleagues (2010a) developed the RWI (based on a subset of biomarkers) using baseline data from 109 cirrhotic subjects enrolled in the North American Study for the Treatment of Refractory Ascites (NASTRA; Sanyal *et al.*, 2003). The predictability of the index was confirmed (Gennings *et al.*, 2010a) using a separate database obtained from review of records of 1,342 cirrhotic patients referred for consideration of liver transplantation in the Veterans Health System between 1997 and 2008. The RWI using the full set of biomarkers was also significantly associated with mortality in a general population using NHANES III data linked to the vital statistics in the National Death Index (Figure 2; Gennings, 2009). In addition, the RWI is predictive of multiple medical conditions using NHANES (2001–02 data; Figure 3; Gennings, 2009) based on study participants asked if they ever were told by a doctor that they had certain medical conditions. The relative risk was calculated compared to the predicted probability when RWI was 1.0. These results demonstrate that conceptualizing health as a complex uni-dimensional construct, involving dozens of interdependent physiological variables, is predictive of survival time and increased relative risk of multiple medical conditions. In contrast to other health-based or quality-of-life composite indices, the RWI is a physiological composite score based on a wide assortment of biomarkers across organ systems and is not disease-specific; the components of which are available in NHANES (2001–02) laboratory data.

An advantage of using a (somewhat) general measure of wellness is that the impact of multiple pathways on multiple systems may be empirically evaluated. That is, we have grouped 42 environmental chemicals into their primary pathways and calculated an index for each associated with the burden of the chemicals in that pathway. We hypothesize the greater the burden, the greater the impact on wellness. Using such a construction, we test for the significance of each pathway index on RWI and test for interaction between pathways as an empirical measure of crosstalk across pathways. The objective of this paper is twofold: (1) to evaluate the impact pathway-specific indices and an overall measure of body burden (BBI and BBIw) have on a general measure of wellness, the RWI; and (2) to determine whether there is empirical evidence of crosstalk across the selected pathways. Analyses are based on biomonitoring data: NHANES data from 2001–02. The result of the analyses is relative potency estimates for the environmental chemicals evaluated using a holistic measure of wellness and an insight using empirical evidence for crosstalk across primary pathways impacted by exposure to environmental chemicals.

MATERIALS AND METHODS

Data description

The National Health and Nutrition Examination Surveys (NHANES) is a set of studies designed to assess the health and nutritional status of adults and children in the United States. The surveys include personal interviews and physical examinations and have been conducted by the National Center for Health Statistics (NCHS) on a periodic basis from 1971 to 1994. In 1999, the NHANES became a continuous program examining a nationally representative sample of approximately 5,000 persons each year. These persons are located in 15 varying counties across the United States each year. The 2001–2002 NHANES contains data for 11,039 individuals (DSDR 2011).

Selection of Environmental Chemicals included in the Body Burden Index (BBI)

For the current study, we used biomonitoring data from NHANES (2001–02). This version of NHANES collected laboratory samples (urine, serum, blood) from more than 160 environmental chemicals in 1/3 or smaller subsamples. A convenience set of chemicals was selected for study with the inclusion guideline that at least 60% of the samples were greater than the limit of detection (LOD) of the assay thereby allowing for the estimation of the geometric mean (EPA, 2008). The LOD varied from chemical to chemical, and is defined as the level at which the measurement has a 95% probability of being greater than zero. Sets of chemicals considered for evaluation included pesticides/insecticides, phthalates, PCBs, dioxins, furans, polyaromatic hydrocarbons, and some metals (see Table S1 in supplementary materials). In the current analysis, we selected 42 chemicals using this guideline from this set of 131 chemicals with some exceptions: (1) 3 additional OP insecticides were included with more than 52% exceeding the LOD for each; (2) 3-benzo(a) pyrene was included with 52% exceeding the LOD; (3) cadmium and lead were added to the analysis based on results in Cave *et al.*, (2011); urinary cadmium was used instead of blood cadmium with 96% >LOD compared to 50% in blood; and (4) 3-phenoxybenzoic acid, a metabolite of pyrethrins was omitted from analysis as it was distinct from the other sets of chemicals and would thus be in a group of size 1.

A holistic measure of wellness: the RWI

The RWI is comprised of 28 serum biomarkers of effect – all available in NHANES (2001–02). Figure 1 depicts a flow diagram for the construction of the RWI. The biomarkers include: aspartate aminotransferase (AST), creatinine, platelets, white blood cell count, albumin, bilirubin, sodium, hemoglobin, alkaline phosphatase, alanine aminotransferase (ALT), blood urea nitrogen (BUN), calcium, cholesterol, chloride, glucose, globulin, gamma

glutamyl transferase (GGT), hematocrit, lactate dehydrogenase (LDH), phosphorus, potassium, red blood cell count, triglycerides, protein, uric acid, C-reactive protein, thyroxine (T4), and thyroid stimulating hormone (TSH). These biomarkers measure the function of the hematopoietic system, the kidney, and the liver, with some evaluation of the thyroid and cardiovascular system. It does not include direct measures of the nervous system, reproductive system, immune system, or mental health. These biomarkers were selected for the RWI because (1) they are common laboratory markers used by physicians to evaluate the health status of their patients; (2) they are markers for multiple organ systems; and (3) they are measured in NHANES. A broader set of markers could be established for a wellness index but is left for future research.

Pathway-specific groupings

We divided the 42 chemicals into six distinct mechanistic groups, which may affect human health through different pathways, based on the following rationale:

1. The AhR group includes chemicals with intermediate or strong AhR inducing capability such as the dioxins, furans, and coplanar PCBs (Safe 1990; Safe 1998). This receptor is considered to mediate a variety of toxic effects on the hepatic, immune, hematopoietic and other systems by its effects on signaling pathways that determine cell proliferation, differentiation and apoptosis.
2. Agonists of CAR/PXR group include the environmentally persistent non-coplanar PCBs and other non-planar organochlorines (Kopec *et al.*, 2010) and can play a critical role in the disposition and toxicity of environmental chemicals, primarily by altering their metabolism to less toxic or more toxic metabolites (Stanley *et al.*, 2006).
3. The heavy metals, lead and cadmium, are thought to interfere with critical sulfhydryl residues on proteins and thereby exert wide ranging effects on cellular functions as diverse as production of heme, protection against oxidative stress, and production of cellular energy (Nemsadze *et al.*, 2009).
4. The urinary organophosphate metabolites provide a biomarker for exposure to pesticides that inhibit acetylcholinesterase (MacIntosh *et al.*, 1996). While the acute effects of high doses of organophosphate insecticides are well established, the long-term effects of chronic low dose OP exposure are not well understood. Data from animal studies suggest that chronic exposure to low levels of OPs can adversely affect nervous system function including learning and other cognitive effects (Prendergast *et al.*, 1998).
5. The urinary metabolites of polycyclic aromatic hydrocarbons are an indicator of exposure to benzo[a]pyrene, phenanthrene and other polycyclic aromatic hydrocarbons which can undergo metabolic activation to reactive metabolites that can form adducts with DNA and protein leading to altered cell functions (Irigaray and Belpomme, 2010). Polycyclic aromatic hydrocarbons are constituents of second hand cigarette smoke exposure which is linked with cancers in various tissues and other ailments including respiratory diseases (Menzies, 2011).
6. Lastly, the metabolites of phthalates are agonists of peroxisome proliferator activated receptors in addition to androgen receptor and are associated with reproductive toxicity and cancer in rodent studies (Hurst and Waxman, 2003).

Statistical Analysis

Our strategy was to construct pathway-specific indices and a general indicator of body burden allowing for multiple chemicals representing a variety of chemical classes and

exposure routes. Environmental chemicals were measured in serum or urine in generally one-third subsamples in participants 12 years of age or older; all chemicals were never measured in any single participant (e.g., DSDR, 2011). There are at least two difficulties in calculating body burden from NHANES data:

- Combining concentration levels across chemicals measured from blood or urine and in different scales; and
- Accounting for incomplete data for all chemicals. When complete data are not available, one approach is to use multiple imputation methods, which “fill in” missing data using distributional assumptions derived from the available data (Schafer, 1999). However, due to the NHANES sampling design, covariance estimates are not available across many of the chemical classes. For example, no single subject had measurements of both PCBs and phthalates. Thus, standard multiple imputation methods are not informed about inter-class concentration correlations and are limited.

We use a different strategy for estimating body burden based on the following assumptions:

- Chemical concentrations are considered “typical” at the estimated sample means for each chemical in the mixture. Since concentration distributions of individual chemicals are often skewed, a geometric mean is used which is less affected by extreme values. Means are calculated using important covariates (e.g., gender, race, age categories, BMI categories).
- Data are used to provide evidence of concentrations of either “less than typical” or “more than typical” amounts. When data are not available due to sampling design, missing data are imputed with the corresponding mean (i.e., no evidence either way). Imputation was not necessary for the pathway-specific indices – only for the overall body burden index.
- The Body Burden Index (BBI) is defined as the standardized distance between a participant’s concentration levels, x_{ij} (for the j^{th} chemical and the i^{th} subject), and a measure of “center”, $\hat{\mu}_j$ (e.g., geometric mean):

$$BBI_i = \text{sign} \left\{ \sum_{j=1}^c (x_{ij} - \hat{\mu}_j) / \sqrt{v_j} \right\} \sqrt{\sum_{j=1}^c (x_{ij} - \hat{\mu}_j)^2 / v_j} \quad (1)$$

where v_j is a measure of scaling (e.g., standard deviation, SD); in which case, the units of BBI are in terms of number of SDs from the geometric mean across all chemicals. This version of the BBI does not adjust for chemical potency. The same formula was used for the pathway-specific indices.

- A weighted version of BBI is calculated as

$$BBIw_i = \text{sign} \left\{ \sum_{j=1}^c (w_j (x_{ij} - \hat{\mu}_j) / \sqrt{v_j}) \right\} \sqrt{\sum_{j=1}^c (w_j (x_{ij} - \hat{\mu}_j)^2 / v_j)} \quad (2)$$

where w_j is the weight for the j^{th} chemical and the sum of the weights is fixed at the

number of chemicals, i.e., $\sum_{j=1}^c w_j = c$; thus, the unweighted case is comparable where each $w_j = 1$. One of the primary aims of this research is to determine optimal values

for these weights using empirically-determined relative potency values. The *sign* function before the radical in equations (1) and (2) specifies whether a given subject is over- or under-exposed as compared to a “typical” subject.

Following the algorithm described by Gennings and colleagues (Gennings et al 2010b), the calculated weights indicate the relative importance of each chemical within the study mixture in determining the association with a health outcome (here RWI), with larger weights corresponding to chemicals accounting for more of the association than others. For convenience, the weights are expressed in the tables herein in percent of total with higher percentages identifying chemicals with stronger association to the outcome. For example, in a subset of 7 chemicals, when the weights on two chemicals are 90% of the total, these two chemicals are identified as the relative ‘bad actors’. The weights are found using a direct search algorithm that maximizes the association between BBI and RWI.

In the analysis of the full set, starting values for the algorithm were found by evaluating the 42 chemicals in three subsets: those measured in blood/serum, the phosphates and PAHs, and the phthalates and remaining chemicals. With these initial starting weights, the initial step sizes were selected as various multiples of the initial weights. The set of weights with the largest resulting signal to noise ratio (using a Wald-type statistic) was selected. Signal-to-noise ratios (slope estimate divided by its standard error) of more than two are considered evidence of an association between body burden and wellness. Evidence of potential interaction between body burden (BBIw) and smoking status was determined using a subsequent model fit with the specified weights for BBIw and the addition of the cross-product term in the model. In the analysis of the pathway-specific indices, the starting values were set at 1 and initial step sizes were selected over a range. All pairwise combinations of pathway-specific indices where data were available were included in nonlinear models adjusted for other covariates (age, BMI, gender, race, smoking status, activity level and a nutrition index). These models were parameterized to include slope terms for each pathway index (on the scale $\log_{10}(x+10)$) and a cross-product term to allow for interaction across the pathways (i.e., crosstalk).

For comparison to the overall BBIw, a standard stepwise approach was used to determine a subset of the 42 chemicals predictive of changes in RWI. A model building strategy was used as the full set of chemicals conveyed a complex, collinearity structure, which is associated with variance inflation in a full response surface model.

RESULTS

The 42 chemicals or their metabolites included pesticides, phthalates, PAHs, phosphates, and persistent environmental chemicals including dioxins, furans, and PCBs. These chemicals impact six pathways of toxicological interest (Table 1). These include AhR, PXR, AChE and PPAR; we include heavy metals as sulfhydryl agents and PAHs as genotoxic/carcinogenic agents. We construct pathway-specific indices following the BBI formula with the corresponding chemicals – e.g., only the dioxins, furans and co-planar PCBs were used to calculate the AhR index (Table 1).

Correlation estimates were calculated between pathway indices (Table 2). Some correlations could not be calculated in cases where bivariate concentrations were not measured in NHANES, e.g., phthalates in the PPAR index and dioxins in the AhR index. As expected, the correlation between the PXR index (non-coplanar PCBs and OCs) and AhR index (dioxins, furans and co-planar PCBs) was the highest of all bivariate correlation estimates, at 0.56 (Table 2). The correlation between the heavy metals in the sulfhydryl index and the chemicals in the AhR and PXR were the next highest set of correlation estimates at 0.12 and 0.17; all other correlations were not larger than 0.10.

Empirical association of biological pathways and RWI

We hypothesize the greater the concentrations of the chemicals within a pathway, the more the pathway is stressed and the greater the impact on wellness as measured by RWI. Of course, the RWI is not a complete measure of wellness, but it does measure the function of multiple important organ systems. We evaluated the impact of each pathway on RWI by including the index in a nonlinear logistic model of the mean RWI, corrected for other covariate (Table 3). The pathway indices were transformed to the log scale (i.e., $\log_{10}(x + 10)$) to reduce the impact of extreme influential observations. Three of the six indices were negatively associated with RWI (i.e., a decrease in RWI indicates a decrease in wellness): PXR, sulfhydryl, and genotox (diagonal values of Table 3); AhR is negatively associated with RWI when it is not transformed.

Pairwise combinations of these indices were also evaluated in nonlinear logistic models parameterized with slope terms for each index (an additive model; presented below the diagonal in Table 3) and allowing for interaction (presented above the diagonal in Table 3). Of the nine combinations evaluated, in six of the additivity models one of the pathway indices was negatively associated with RWI ($p < 0.05$). These were indices for sulfhydryl, PXR, and genotox. In two of the nine interaction models, there was evidence of interaction (or “crosstalk” between pathways). These were positive interactions between PPAR and the genotox pathways, and between sulfhydryl and AhR pathways. The positive interaction suggests an antagonistic relationship between the pathways where the joint nature of the combination is diminished from that predicted in the no interaction model.

The indices evaluated in Tables 2 and 3 are based on the construction where each component in the index is equally weighted (as in equation (1)). The disadvantage of equally weighted indices is that chemicals in each set which are less potent may dominate the index by including more “noise” than “signal”. Weighted versions of these pathway indices were derived using a search algorithm that finds weights that increase the signal-to-noise ratio of the index in the nonlinear logistic regression model of RWI (Table 4). These weights may be interpreted as empirical potency factors for the chemicals within an index as related to RWI. For example, the unweighted AhR pathway index did not show an important association with RWI. But by increasing the weights on PCB126 (48% of total), two furans: pnCDF (30%) and hxCDF (8%); and one dioxin: OCDD (9%); and down-weighting the other 4 chemicals, the signal-to-noise ratio increased four-fold, from 0.5 to a value greater than 2. Similarly, for the PXR index, two chemicals have greatest potency related to RWI: PCB138 (23% of weight) and trans-nonachlor (67%). Although the search algorithm detected weights that would improve the signal-to-noise ratios for all six pathway indices, the improvement in the AChE index was not strong enough to reach a level of the ratio that would indicate a meaningful relationship (Table 4).

The correlation estimates across the six weighted pathway indices were similar to those in the unweighted indices (Table 2 compared to Table 5). The AhR and PXR indices had the largest correlation estimate (0.59) with next being sulfhydryl with PXR and AhR.

Evaluation of each weighted index in a model of wellness as measured by RWI was repeated (Table 6). Using the weights, five of the six indices were negatively associated with RWI; only the AChE index was not. In additivity models at least one of the indices were negatively associated with RWI in all nine combinations evaluated (Table 6 below the diagonal). There was at least borderline evidence of interaction in 3 of the 9 analyses. The positive interaction terms were estimated between AhR and sulfhydryl indices and between PPAR and the genotox indices. Using the weights, a negative interaction was detected between sulfhydryl and AChE when tested on the original scale (i.e., not log transformed).

Empirical association of bodyburden and RWI

A bodyburden index of all 42 chemicals was estimated using equations (1) and (2) after imputation of missing values with the geometric means from gender, race, age categories and BMI categories. To illustrate, Figure 1 presents the geometric means of the 42 chemicals connected with a solid black line and the 99th percentiles presented with a circle for each chemical using an abbreviation for each chemical selected from the NHANES variable name. The concentration levels for two subjects are presented where the imputation is evident when the concentration level is shown identical to the geometric mean. In Figure 4A, the participant (50 yr old female with BMI of 31) had extreme levels for p,p'-DDE, the dioxins and furans, and relatively high PCBs, oxychlordane and trans-nonachlor. The unweighted BBI was calculated as 20.5 and the weighted BBI (i.e., BBI_w) was 19.2 (SDs above the mean). In comparison, Figure 4B represents the body burden for a 46 yr old female with BMI of 29 who has extreme metabolite concentration levels for both low-molecular-weight and high-molecular-weight phthalates. The BBI was calculated as 30.4 and BBI_w was 8.7 (SDs above the mean).

Of particular interest, is to determine empirical estimates of relative potency of the 42 chemicals included in the analysis. Following the approach used by Gennings *et al* (2010b), using the same algorithm described by Ellis *et al* (2008) and Ellis (2009), empirical relative potency estimates were found using the weighted BBI that increased the ratio of its slope estimate to its standard error (i.e., signal to noise ratio) from 3.9 to 9.3 (Table 4) in the nonlinear logistic model. Five of the 42 chemicals across a variety of chemical groups and biological pathways accounted for 71% of the weight: pnCDF, 9%; PCB126, 5%; trans-nonachlor 13%, lead, 36%, and 3-hydroxyphenanthrene, 8% (Table 4). Summing within each group weights of one or more (accounting for 98% of the total weight), a relative measure of toxicity can be calculated based on the holistic measure of wellness:

- Co-planar PCBs, dioxins, furans (AhR pathway): 17%
- OC pesticides and non co-planar PCBs (PXR pathway): 18%
- Heavy metals (sulfhydryl agents): 40%
- OPs and chlorpyrifos (AChE): 6%
- Phthalates (PPAR pathway): 9%
- PAHs (genotoxic pathway): 8%

It was of interest to test whether the impact of bodyburden of chemical mixtures is enhanced by smoking. An interaction term was added to the model relating the BBI_w and smoking status. When all 42 chemicals were considered, the interaction was negative and significant ($p=0.049$). A negative and significant interaction term for BBI_w and smoking status indicates smoking exacerbates the impact of body burden of chemicals on overall health as measured by RWI.

How does this approach compare to standard stepwise methods?

In the same dataset with means used for imputed values, a stepwise algorithm was used to determine the “worst actors” among the 42 chemicals. Using a 5% significance level to enter and stay in the model, only trans-nonachlor, lead, CPM, and 3-hydroxyphenanthrene were included in the final model with a negative slope and interestingly, 4-phenanthrene was included with a positive slope; demographic variables included gender, age, and BMI. Other chemicals identified using weighted BBI were not selected due in part to correlations with chemicals in the model (e.g., the estimated correlation between trans-nonachlor with oxychlordane was 0.92; with PCB138, 0.64; with pnCDF, 0.58; and with HxCDF, 0.56). In comparison, the weighted index provides relative weights for the components in the model

while accounting for observed concentrations of all 42 chemicals; calculating body burden on a single scale provides easy interpretation of its relationship to a holistic measure of wellness.

DISCUSSION

Like other factors affecting the health of human populations, exposure to environmental contaminants can both cause as well as exacerbate detrimental health effects (EPA, 2008). Of particular concern is that humans are exposed to complex mixtures of these chemicals over a variety of exposure routes, making exposure/risk assessment of actual human body burden difficult to ascertain. On the health effects side, ninety million Americans currently have one or more chronic conditions such as diabetes, hypertension, obesity, or arthritis (Christensen, 2009). Each of these chronic conditions is thought to be associated with one or more different underlying causal mechanisms that “probably involve multiple organ systems through interdependent molecular pathways, complicated by individual genetic differences and environmental factors” (Christensen, 2009). So both the exposure side and the health side of the “equation” are multi-faceted and complex. Current approaches to evaluating the potential health impact from environmental exposures include isolating a health outcome and focusing on a potential set of environmental contaminants that may be related. While this approach may lead to a better understanding of the potential relationship between the set of selected chemicals and the single outcome (e.g., a chronic disease such as diabetes or focus on a single organ such as liver toxicity), it does not account for other exposure sources (i.e., other chemicals) that may also bear on the outcome, nor does it account for the interdependencies and cross-talk between organ systems or the likelihood of co-morbidities.

This study applies a novel approach for the risk evaluation of human environmental chemical exposure and human health. Using holistic indices on both sides of the risk-exposure equation, our approach broadens the vantage point for assessing this risk. One strength of the approach is the ability to group the chemicals based on proposed mechanisms of toxicologic concern and then to use an optimization algorithm to weight the chemicals and thereby determine the “bad actors” in the mixture. Applying this method to the 2001–2002 NHANES dataset, 42 chemicals were selected for the study based on detection levels. Negative associations between the body burden index and RWI were observed for three of the six groups, but this number increased to five of six groups when the BBI_w (i.e., the weighted version of the index) was used. Indeed, weighting of the data significantly increased the signal-to-noise ratio in all six groups, with only the acetylcholinesterase inhibitor group not showing a significant effect. This finding, which needs to be validated using other NHANES datasets, suggests that exposures to one or more chemicals (identified by the weights) in the AhR, CAR/PXR, heavy metal, genotoxicity, and PPAR groups at concentrations that are relevant for exposure in the United States were sufficient to have an adverse effect on the RWI.

Of the five groups, the two with the strongest negative associations (i.e., as exposure concentrations increase, wellness decreases) were the sulfhydryl and PAH groups. In these two groups, the most suspect chemicals were lead and 3-hydroxyphenanthrene, respectively, which accounted for 99% and 40% of the effect on RWI in their subgroups. It is notable that the dominant effects of both of these compounds were evident even without grouping of the chemicals into mechanistic classes (Table 4, right most column, lead 36% and 3-hydroxyphenanthrene 8%). Lead is of interest because it is one of the most prevalent and ongoing environmental toxicants in the United States due to its presence in house paints used before the mid 1970’s when it was banned. Although NHANES data show that blood levels have dropped significantly in Americans since that time, essentially all blood samples tested in NHANES 2001–2002 (>98%) still exhibited measurable levels. Virtually no organ

system is considered immune to chronic effects of lead at blood levels previously thought to be safe ($< 10 \mu\text{g/dL}$), due to the non-specific nature of sulphydryl group binding. Lead has been shown to increase the risk of both cardiovascular disease and cancer, the two major causes of mortality in the United States (Menke *et al.*, 2006). Moreover, a measurable increase in risk of myocardial infarction or stroke was detected at blood levels ($2.0 \mu\text{g/dL}$) lower than the average blood lead level found among Americans ($2.6 \mu\text{g/dL}$) (Menke *et al.*, 2006).

The finding that exposure to a group of chemicals representing the PAHs has an even higher impact on RWI (signal-to-noise ratio of the group BBIw of 8.1 compared to 5.9 for the heavy metals; Table 4, top) supports an important influence of these compounds. The major sources of PAHs are exposure to diesel exhaust and cigarette smoke and may contribute to the effects of passive cigarette smoking. The PAHs from which the hydroxylated compounds measured in urine derived are planar compounds which share in common with dioxins, dibenzofurans and co-planar PCBs the ability to activate the AhR but are distinguished by their genotoxicity, which occurs as a result of metabolic bioactivation into toxic metabolites. The observation that the group of chemicals classified as AhR activators (dioxins,) did not have the same influence may be due to their blood concentrations being too low to significantly activate the AhR. Alternatively, the negative impact of the PAHs may be due to their metabolism to reactive metabolites or to some other mechanism. In addition to the mono-hydroxy metabolites that were measured, PAHs are metabolized to arene oxides, quinones, and dihydrodiols (Shimada, 2006). The quinones and arene oxides are electrophilic metabolites which can react with cellular proteins and nucleic acids to form adducts that initiate various responses, whereas the dihydrodiols are precursors to the highly carcinogenic dihydrodiol epoxides. In addition to cancer, exposures to PAHs are associated with adverse effects on the immune, hematopoietic, reproductive, renal and hepatic systems. It is not clear at present why the negative association with RWI is strongest for selected (not all) metabolites of phenanthrene, pyrene, and fluorene appear, suggesting that their toxicity is associated with specific pathways (and enzymes) of metabolism or that mechanisms for detoxifying or repairing damage due to these specific metabolites is less efficient than for the other metabolites.

Three additional chemicals combine with lead and 3-hydroxyphenanthrene to account for 71% of the weight linking body burden to wellness: PCB126 (3,3',4,4',5-pentachlorobiphenyl), pnCDF (2,3,4,7,8-pentachlorodibenzofuran), and trans-nonachlor. The presence of PCB126 on this short list is interesting because it is considered one of the most toxic PCBs known and accounts for 53% of the toxic equivalency of dioxin-like (co-planar) PCBs in human tissues (Safe, 1993). Previous analyses of NHANES data have linked PCB exposure with increased risk of hypertension and altered thyroid and liver function markers (Meeker *et al.*, 2007; Everett *et al.*, 2008; Cave *et al.*, 2011).

Our approach also makes it possible to test for interactions between groups of chemicals that could provide evidence of “crosstalk” between pathways or mechanisms that alter the risk of toxicity. The negative interaction observed between the sulphydryl-binding agents and the organophosphate group suggests that combined exposure to these two groups together increases the risk of exposure to either group alone, i.e., a greater than additive effect. The findings from both human clinical studies and using animal models support negative effects of lead on cholinergic systems in the body (Nehru and Sidhu, 2001; Saxena and Flora, 2006; Ademuyiwa *et al.*, 2007). Since acetylcholinesterase lacks free sulphydryl groups in its structure, the overall findings suggest that the effect of lead on cholinergic signaling is mediated by indirect mechanisms, e.g., lead-induced oxidative stress (Ademuyiwa *et al.*, 2007).

The positive interaction between the PPAR and genotoxicity groups was highly significant ($p < 0.002$), suggesting that co-exposure mitigates toxicity from one or both groups. Animal toxicology studies suggest that the male reproductive system is a common target of both groups. In agreement with these results, male rat reproductive toxicity resulting from combined treatment with benzo(a)pyrene, a genotoxic polycyclic hydrocarbon, and dibutylphthalate was unexpectedly milder than in rats exposed to BaP alone, an antagonistic interaction (Chen *et al.*, 2011).

An important question raised by our data is what is the specific nature of the effects of the 5 groups of chemicals that results in decreased RWI; that is, which specific organ systems or individual biomarkers are affected by chemicals in these groups? The RWI used in this study consists of 28 different biomarkers, which together provide diagnostic information on multiple organ systems: including biomarkers for the endocrine system (thyroid system and glucose control), inflammation (C-reactive protein), hepatic and kidney function and the cardiovascular systems are included. Many of the 42 chemicals studied here are associated with toxicities affecting functions covered by the RWI. For example, polychlorinated biphenyl exposure is associated with negative effects on function of the hypothalamus-pituitary-thyroid axis (Desaulniers *et al.*, 1999; Meeker *et al.*, 2007). Co-exposure to PCBs and the heavy metal lead is associated with elevated liver aminotransferases, suggestive of toxicity to the liver (Cave *et al.*, 2011). Associations between polycyclic aromatic hydrocarbon exposure and cardiovascular disease have also been reported (Xu *et al.*, 2011).

There are some noteworthy limitations in our approach. The first of these is that some chemicals may by their nature act by more than one of the mechanisms. For example, MEHP is both a PXR agonist as well as a PPAR agonist (Cooper *et al.*, 2008) and the polycyclic aromatic hydrocarbons that give rise to the PAH metabolites measured in NHANES are activators of AhR as well as precursors to genotoxic metabolites. Thus, some of the chemicals can be assigned to more than one mechanistic subclass. In this study we did not attempt to evaluate the impact of assigning these chemicals with dual activities to groups other than the ones shown in Table 4. Another limitation of the approach is the possibility that some of the exposures may be associated, for example, if they originate from a common source of exposure. In Table 1 the strongest correlation in the body burden indices were observed between the AhR and PXR groups ($p < 0.05$), which is not unexpected, since organochlorines comprise the bulk of chemicals in each group and organochlorines often have common sources of exposure. These data suggest that in some cases it may be difficult to differentiate between AhR and PXR in any observed effect on RWI. However, as information on other chemical-gene/protein networks becomes available, it will be possible to group chemicals in other data-driven ways for analysis.

With more than 83,000 individual industrial chemicals registered with the U.S. EPA for commercial use (EPA, 2011), it is virtually impossible to quantify the exact body burden in any individual – i.e., any measure of body burden is limited by the selection of chemicals analyzed. There could be important environmental chemicals that are not measured in NHANES or other biomonitoring studies. Without additional biomonitoring data, our methodology will not capture evidence of important missing components.

RWI is a holistic measure of wellness as defined by the biomarkers that comprise it. It does not provide information on the mechanistic/diagnostic component of patient care. Instead, it provides an indication of underlying health effects. For example, without additional components, it does not diagnose cancer or mental illness. Extensions and further validation of the RWI as a holistic measure of wellness are planned; however, the version of RWI used herein provides evidence that the approach of linking two holistic measures of body burden and wellness informs a relative potency ranking of the chemicals.

The link between body burden and wellness is based on cross-sectional data; longitudinal data are not available in NHANES. The degree of stability of body burden over time has not been quantified using NHANES. If body burden is consistent over time (at least relative to other members of the population) the health consequences may be most predictable; however, through intervention strategies that decrease body burden, the health consequences may be diminished.

In summary, traditional methods of risk assessment utilize a reductionist strategy, which focuses on single chemicals or single chemical groups and single diseases at a time. In reality, the exposure-disease relationship is likely much more complex and multidimensional. The approach we describe addresses these limitations and represents an integrated, holistic strategy for taking a broad scale view of the exposure-disease landscape. The method is novel in that it allows for assessment of multiple chemicals acting by different mechanistic pathways, which may cause more than one problem at a time (i.e., co-morbidities). In this sense, our method can be considered a systems biology approach to the complex problem of risk assessment. Future work will aim to validate this method and focus on identifying the specific diagnostic endpoints affected by the five chemicals most closely associated with the effect of BBIw on RWI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

BBI	body burden index
BBIw	weighted body burden index
BPB	lead, blood
CPM	3,5,6-trichloropyridinol
D03	1,2,3,6,7,8 – hxcdd (hexachlorodibenzo- <i>p</i> -dioxin)
D05	1,2,3,4,6,7,8 – hpcdd (heptachlorodibenzo- <i>p</i> -dioxin)
D07	1,2,3,4,6,7,8,9 – ocdd octachlorodibenzo- <i>p</i> -dioxin)
F03	2,3,4,7,8 – pncdf (pentachlorodibenzofuran)
F04	1,2,3,4,7,8 – hxcdf (hexachlorodibenzofuran)
F05	1,2,3,6,7,8 – hxcdf (hexachlorodibenzofuran)
F08	1,2,3,4,6,7,8 – hpcdf (heptachlorodibenzofuran)
HXC	3,3',4,4',5,5' – hxcb (hexachlorobenzene)
LOD	limit of detection
MC1	mono-(3-carboxypropyl) phthalate
MEP	mono-ethyl phthalate
MHH	mono-(2-ethyl-5-hydroxyhexyl) phthalate
MHP	mono-(2-ethyl-hexyl) phthalate

MIB	mono-isobutyl phthalate
MNM	mono-n-methyl phthalate
MOH	mono-(2-ethyl-5-oxohexyl) phthalate
MZP	mono-benzyl phthalate
NHANES	National Health and Nutrition Examination Survey
OC	organochlorine
OP1	dimethylphosphate
OP2	diethylphosphate
OP3	dimethylthiophosphate
OP4	diethylthiophosphate
OXY	oxychlordane
P01	1-naphthol
P02	2-naphthol
P03	3-hydroxyfluorene
P04	2-hydroxyfluorene
P05	3-hydroxyphenanthrene
P06	1-hydroxyphenanthrene
P07	2-hydroxyphenanthrene
P10	1-hydroxypyrene
P17	9-hydroxyfluorene
P18	9-hydroxyphenanthrene
P19	4-hydroxyphenanthrene
P24	3-hydroxybenzo(a)pyrene
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PDE	p,p'-DDE (dichlorodiphenyldichloroethylene)
PNCB	3,3',4,4',5-pncb (pentachlorobiphenyl)
PBDE	polybrominated diphenylether
ROE	2008 U.S. EPA's Report on the Environment
RWI	Relative Wellness Index
TEF	Toxic Equivalence Factors
TNA	trans-nonachlor
UCD	cadmium, urine

References

- Ademuyiwa OURN, Rotimi SO, Abam E, Okediran BS, Dosumu OA, Onunkwor BO. Erythrocyte acetylcholinesterase activity as a surrogate indicator of lead-induced neurotoxicity in occupational

- lead exposure in Abeokuta, Nigeria. *Environmental Toxicology and Pharmacology*. 2007; 24:183–188. [PubMed: 21783808]
- Buck Louis GM, Weiner JM, Whitcomb BW, Sperrazza R, Schisterman EF, Lobbelt DT, Crickard K, Greizerstein H, Kostyniak PJ. Environmental PCB exposure and risk of endometriosis. *Human Reproduction*. 2005; 20(1):279–285. [PubMed: 15513976]
- Cave MS, Appana S, Patel M, Falkner KC, McClain CJ, Brock G. Polychlorinated biphenyls, lead, and mercury are associated with liver disease in American adults: NHANES 2003–2004. *Environmental Health Perspectives*. 2011; 118(12):1735–42. [PubMed: 21126940]
- CDC. Encyclopedia of Earth. 2010. Fourth National Report on Human Exposure to Environmental Chemicals. Retrieved March 3, 2010
- Chen XH, An H, Ao L, Sun L, Liu W, Zhou Z, Wang Y, Cao J. The combined toxicity of dibutyl phthalate and benzo(a)pyrene on the reproductive system of male Sprague Dawley rats in vivo. *J Hazard Mater*. 2011; 186(1):835–41. [PubMed: 21146287]
- Christensen, CM.; Grossman, JH.; Hwang, J. *The Innovator's Prescription: A Disruptive Solution for Health Care*. The McGraw-Hill Companies; 2009.
- Cooper BW, Cho TM, Thompson PM, Wallace AD. Phthalate induction of CYP3A4 is dependent on glucocorticoid regulation of PXR expression. *Toxicol Sci*. 2008; 103(2):268–77. [PubMed: 18332045]
- Czub G, Wania F, McLachlan MS. Combining long-range transport and bioaccumulation considerations to identify potential Arctic contaminants. *Environ Sci Technol*. 2008; 42(10):3704–9. [PubMed: 18546711]
- Desaulniers D, Leingartner K, Wade M, Fintelman E, Yagminas A, Foster WG. Effects of acute exposure to PCBs 126 and 153 on anterior pituitary and thyroid hormones and FSH isoforms in adult Sprague Dawley male rats. *Toxicol Sci*. 1999; 47(2):158–69. [PubMed: 10220852]
- DSDR. Data Sharing for Demographic Research. 2011. from <http://earlycarerresearch.net/DSDR/studies/25502/detail#bibliographic-description>
- Ellis, R. Dissertation for the Department of Biostatistics. Virginia Commonwealth University; 2009. Deriving Optimal Composite Scores: Relating Observational/longitudinal Data with a Primary Endpoint.
- Ellis R, Gennings C, Benson J, Tibbets B. Validation of a Morbidity Score in a Study of Botulinum Toxin A. *The Toxicologist CD - An official Journal of the Society of Toxicology*. 2008
- EPA. Summary of the Toxic Substances Control Act. 2011. Retrieved March 24, 2011, from <http://www.epa.gov/regulations/laws/tsca.html>
- Everett CJ, Mainous AG 3rd, Frithsen IL, Player MS, Matheson EM. Association of polychlorinated biphenyls with hypertension in the 1999–2002 National Health and Nutrition Examination Survey. *Environ Res*. 2008; 108(1):94–7. [PubMed: 18606400]
- Fattore E, Fanelli RR, LaVecchia C. Persistent organic pollutants in food: public health implications. *J Epidemiol Community Health*. 2002; 56(11):831–2. [PubMed: 12388573]
- Gennings, C. Traditional Primary Endpoints vs “Gestalt Indices” in Clinical Trials. American Association For the Study of Liver Diseases (AASLD) Endpoints Workshop on NAFLD; Chicago, IL. 2009.
- Gennings C, Heuman D, Fulton O, Sanyal AJ. Use of desirability functions to evaluate health status in patients with cirrhosis. *Journal of Hepatology*. 2010a; 52:665–671. [PubMed: 20346534]
- Gennings C, Sabo R, Carney E. An iterative approach to studying the role of complex mixtures in complex diseases: PCBs and endometriosis as a case study. *Epidemiology*. 2010b July 4; 21(Supplement):S1–S8.
- Haywood KL, Garratt AM, Fitzpatrick R. Older people specific health status and quality of life: a structured review of self-assessed instruments. *J Eval Clin Pract*. 2005; 11(4):315–27. [PubMed: 16011644]
- Hermanussen S, Matthews V, Papke O, Limpus CJ, Gaus C. Flame retardants (PBDEs) in marine turtles, dugongs and seafood from Queensland, Australia. *Mar Pollut Bull*. 2008; 57(6–12):409–18. [PubMed: 18313081]

- Heyrman, J.; Van Hoeck, K. Chapter 12: Health outcome measures for older people. In: Hutchinson, A.; McColl, E.; Christie, M.; Recalton, C., editors. *Health Outcome Measures in Primary and Out-Patient Care*. Amsterdam, The Netherlands: Harwood Academic Publishers; 1996. p. 167-175.
- Horne BD, May HT, Muhlestein JB, Ronnow BS, Lappe DL, Renlund DG, Kfoury AG, Carlquist JF, Fisher PW, Pearson RR, Bair TL, Anderson JL. Exceptional mortality prediction by risk scores from common laboratory tests. *Am J Med*. 2009; 122(6):550–8. [PubMed: 19486718]
- Howdeshell KL, Wilson VS, Furr J, Lambright CR, Rider CV, Blystone CR, Hotchkiss AK, Gray LE Jr. A mixture of five phthalate esters inhibits fetal testicular testosterone production in the sprague-dawley rat in a cumulative, dose-additive manner. *Toxicol Sci*. 2008; 105(1):153–65. [PubMed: 18411233]
- Hurst CH, Waxman DJ. Activation of PPARalpha and PPARgamma by environmental phthalate monoesters. *Toxicol Sci*. 2003; 74(2):297–308. [PubMed: 12805656]
- Irigaray P, Belpomme D. Basic properties and molecular mechanisms of exogenous chemical carcinogens. *Carcinogenesis*. 2010; 31(2):135–48. [PubMed: 19858070]
- Jaga K, Dharmani C. Global surveillance of DDT and DDE levels in human tissues. *Int J Occup Med Environ Health*. 2003; 16(1):7–20. [PubMed: 12705713]
- Kopce AK, Burgoon LD, Ibrahim-Aibo D, Mets BD, Tashiro C, Potter D, Sharratt B, Harkema JR, Zacharewski TR. PCB153-elicited hepatic responses in the immature, ovariectomized C57BL/6 mice: comparative toxicogenomic effects of dioxin and non-dioxin-like ligands. *Toxicol Appl Pharmacol*. 2010; 243(3):359–71. [PubMed: 20005886]
- Liao L, Kong DF, Shaw LK, Sketch MH, Milano CA, Lee KL, Mark DB. A new anatomic score for prognosis after cardiac catheterization in patients with previous bypass surgery. *J Am Coll Cardiol*. 2005; 46(9):1684–92. [PubMed: 16256869]
- MacIntosh DL, Spengler JD, Ozkaynak H, Tsai L, Ryan PB. Dietary exposures to selected metals and pesticides. *Environ Health Perspect*. 1996; 104(2):202–9. [PubMed: 8820589]
- McGinn, AP. POPs culture. *World Watch*; 2000. p. 26-36.
- Meeker JD, Altshul L, Hauser R. Serum PCBs, p,p'-DDE and HCB predict thyroid hormone levels in men. *Environ Res*. 2007; 104(2):296–304. [PubMed: 17189629]
- Menke A, Muntner P, Batuman V, Silbergeld EK, Guallar E. Blood lead below 0.48 micromol/L (10 microg/dL) and mortality among US adults. *Circulation*. 2006; 114(13):1388–94. [PubMed: 16982939]
- Menzies D. The case for a worldwide ban on smoking in public places. *Curr Opin Pulm Med*. 2011; 17:116–122. [PubMed: 21365795]
- Nehru B, Sidhu P. Behavior and neurotoxic consequences of lead on rat brain followed by recovery. *Biol Trace Elem Res*. 2001; 84(1–3):113–21. [PubMed: 11817682]
- Nemsadze K, Sanikidze T, Ratian L, Gabunia L, Sharashenidze T. Mechanisms of lead-induced poisoning. *Georgian Med News*. 2009; (172–173):92–6. [PubMed: 19644200]
- Prendergast MA, Terry AV, Buccafusco JJ. Effects of chronic, low-level organophosphate exposure on delayed recall, discrimination, and spatial learning in monkeys and rats. *Neurotoxicol Teratol*. 20:115–122. [PubMed: 9536457]
- Rothwell PM, Giles MF, Flossmann E, Lovelock CE, Redgrave JN, Warlow CP, Mehta Z. A simple score (ABCD) to identify individuals at high early risk of stroke after transient ischaemic attack. *Lancet*. 2005; 366(9479):29–36. [PubMed: 15993230]
- Safe S. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Crit Rev Toxicol*. 1990; 21(1):51–88. [PubMed: 2124811]
- Safe S. Toxicology, structure-function relationship, and human and environmental health impacts of polychlorinated biphenyls: progress and problems. *Environ Health Perspect*. 1993; 100:259–68. [PubMed: 8354174]
- Safe SH. Development validation and problems with the toxic equivalency factor approach for risk assessment of dioxins and related compounds. *J Anim Sci*. 1998; 76(1):134–41. [PubMed: 9464894]
- Sanyal AJ, Gennings C, Reddy KR, Wong F, Kowdley KV, Benner K, McCashland T. North American Study for the Treatment of Refractory Ascites Group . The North American Study for

- the Treatment of Refractory Ascites. *Gastroenterology*. 2003; 124(3):634–41. [PubMed: 12612902]
- Saxena G, Flora SJ. Changes in brain biogenic amines and haem biosynthesis and their response to combined administration of succimers and *Centella asiatica* in lead poisoned rats. *J Pharm Pharmacol*. 2006; 58(4):547–59. [PubMed: 16597373]
- Schafer JL. Multiple imputation: a primer. *Stat Methods Med Res*. 1999; 8(1):3–15. [PubMed: 10347857]
- Schafer KS, Kegley SE. Persistent toxic chemicals in the US food supply. *J Epidemiol Community Health*. 2002; 56(11):813–7. [PubMed: 12388566]
- Shimada T. Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. *Drug Metab Pharmacokinet*. 2006; 21(4):257–76. [PubMed: 16946553]
- Stanley LA, Horsburgh BC, Ross J, Scheer N, Wolf CR. PXR and CAR: nuclear receptors which play a pivotal role in drug disposition and chemical toxicity. *Drug Metab Rev*. 2006; 38(3):515–97. [PubMed: 16877263]
- Swan SH, Main KM, Liu F, Stewart SL, Druse RL, Calafat AM, Sao CS, Redomn JB, Ternand CL, Sullivan S, Teague JL. and the Study for Future Families Research Team . Decrease in Anogenital Distance among Male Infants with Prenatal Phthalate Exposure. *Environmental Health Perspectives*. 2005; 113(8):1056–1061. [PubMed: 16079079]
- Thornton J. Beyond risk: an ecological paradigm to prevent global chemical pollution. *Int J Occup Environ Health*. 2000; 6(4):318–30. [PubMed: 11114125]
- Thornton JW, McCally M, Houlihan J. Biomonitoring of industrial pollutants: health and policy implications of the chemical body burden. *Public Health Rep*. 2002; 117(4):315–23. [PubMed: 12477912]
- U.S. EPA. US EPA's 2008 Report on the Environment (Final Report) EPA/600/R-07/045F (NTIS PB2008-112484). Washington, D.C: 2008.
- Weber R, Gaus C, Tysklind M, Johnston PI, Forter M, Hollert H, Heinisch E, Holoubek I, Lloyd-Smith M, Masunaga S, Moccarelli P, Santillo D, Seike N, Symons R, Torres JP, Verta M, Varbelow G, Fijgen J, Watson A, Costner P, Woelz J, Wycisk P, Zennegg M. Dioxin- and POP-contaminated sites--contemporary and future relevance and challenges: overview on background, aims and scope of the series. *Environ Sci Pollut Res Int*. 2008; 15(5):363–93. [PubMed: 18597132]
- Xu X, Cook RL, et al. Studying associations between urinary metabolites of polycyclic aromatic hydrocarbons (PAHs) and cardiovascular diseases in the United States. *Sci Total Environ*. 2011; 408(21):4943–8. [PubMed: 20701951]
- Zietz BP, Hoopmann M, Funcke M, Huppmann R, Suchenwirth R, Gierden E. Long-term biomonitoring of polychlorinated biphenyls and organochlorine pesticides in human milk from mothers living in northern Germany. *Int J Hyg Environ Health*. 2008; 211(5–6):624–38. [PubMed: 18550430]
- Zimmerman JE, Kramer AA, McNair DS, Malila FM. Acute Physiology and Chronic Health Evaluation (APACHE) IV: hospital mortality assessment for today's critically ill patients. *Crit Care Med*. 2006; 34(5):1297–310. [PubMed: 16540951]

Research Highlights

- Relevant mixtures of environmental chemicals may impact multiple disease pathways.
- Traditional approaches for risk evaluation do not address co-morbidities.
- We estimate a weighted body burden index from biomonitoring data that identifies the ‘bad actors’.
- We evaluate the association of pathway-specific body burden indices on a holistic index of wellness.
- The approach allows for empirical detection of cross-talk between biological pathways.

Figure 1.

Flow diagram that illustrates the work process for the construction of the RWI. A desirability function ranges from 0 to 1 where a value of 0 indicates the least desirable response while a value of 1 is assigned the most desirable response (see Gennings *et al.*, 2010a)

Figure 2. Kaplan-Meier curves for groups defined by RWI using data from NHANES III linked with mortality data from the National Death Index (NDI). All adjacent survival curves are significantly different.

Figure 3.

Relative risk estimates for various medical conditions (angina, cardiac heart disease (CHD), congestive heart failure (CHF), diabetes, failing kidney condition, a liver condition, myocardial infarction (MI), stroke, and a thyroid condition) using the RWI in a logistic regression model parameterized with gender, race, age, and BMI using NHANES (2001–2002) data. Participants were asked if their doctor had ever told them they had each medical condition.

A

B

Figure 4.

Representation of body burden for 42 chemicals for two study participants in NHANES (2001–2002). The solid line connects the geometric mean of each chemical, the circles are the 99th percentile per chemical, and the dashed lines are concentration levels per subject with extreme values of (A) persistent chemicals (e.g., DDE, dioxins, furan) with RWI=0.36; and (B) phthalates with RWI=0.63. The blood levels are lipid adjusted; the urine levels are adjusted by creatinine.

Table 1

Groups of chemicals and selected primary pathways for construction of pathway-specific indices

Chemical Group	Pathway
Dioxins, furans, co-planar PCBs	AhR
Lead, mercury	Sulfhydryl agents
Non-coplanar PCBs, OCs	PXR
OPs	AChE
PAHs	Genotoxic/carcinogenic
Phthalates	PPAR

Table 2

Correlations between bodyburden pathway indices on the log scale without imputed values

Pathway	AhR	PXR	Sulphydryl	AChE	Genotox	PPAR
AhR	1	0.56	0.12	0.03		
PXR	0.56	1	0.17	0.07		
Sulphydryl	0.12	0.17	1	<0.01	0.10	0.03
AChE	0.03	0.07	<0.01	1		
Genotox			0.10		1	0.09
PPAR			0.03		0.09	1

Table 3

Tests for significance in logistic model adjusted for covariates on log scale unless noted otherwise with *. Diagonal elements represent the model with a single pathway index. Results for significance of interaction parameters are above the diagonal; results for significance of slope parameters in an additivity model are below the diagonal

Pathway	AhR	PXR	Sulphydryl	AChE	Genotox	PPAR
AhR	NS	Interaction NS	*Positive Interaction; NS on logscale	Interaction NS		
PXR	Slopes NS	Negative P=0.043	Interaction NS	Interaction NS		
Sulphydryl	Sulphydryl Negative; AhR NS	Sulphydryl Netative; PXR NS	Negative P<0.001	Interaction NS	Interaction NS	Interaction NS
AChE	Slopes NS	PXR Negative; AChE NS	Sulphydryl Negative; AChE NS	NS		
Genotox			Genotox Negative; Sulphydryl NS		Negative P<0.001	Positive Interaction
PPAR			Slopes NS		Genotox Negative; PPAR NS	NS

Table 4

“Signal to noise” ratios (absolute value of the slope estimate divided by its SE) relating BBI (unweighted) and BBIw-type indices with corresponding weights (%) to a holistic measure of wellness, the RWI using NHANES (2001–02). Models include covariates for gender, age, body mass index, activity level, race, smoking status, and a nutrition index.

Pathway or BBI Index		AhR	PXR	Sulphydryl	AChE	PPAR	Genotoxic	BBIw
Signal to Noise Ratio: unweighted		0.5	1.8	3.9	<0.1	0.3	4.2	3.9
Signal to Noise Ratio: weighted		2.2	3.2	5.9	0.4	2.1	8.1	9.3
		Weights (%)						
		Chemical						
AhR	Dioxins *	HxCDD	<1					<1
		HpCDD	<1					<1
		OCDD	9					1
	Furans *	pnCDF	30					9
		HxCDF	3					<1
		HxCDF	8					1
		HpCDF	<1					<1
	Co-planar PCBs *	HxCB	2					1
		PCB126 (3,3',4,4',5-PNCB)	48					5
		PCB138		23				2
PXR	Non-coplanar PCBs *	PCB153	1					<1
		PCB180	<1					<1
		Oxychlordane *	7					3
	OCs	p,p'-DDE *	<1					<1
		Trans-nonachlor *	67					13
		3,5,6-trichloropyridinol #	3					<1
		Lead, blood *		>99				36
	Heavy Metals	Cadmium, urine #		<1				4
		Dimethylphosphate			<1			2
	AChE	Diethylphosphate			>99			3
		Dimethylthiophosphate			<1			<1

	Pathway or BBI Index	AhR	PXR	Sulphydryl	AChE	PPAR	Genotoxic	BBIw
					<1			1
						5		1
						19		3
						16		<1
						42		<1
						<1		1
						<1		1
						<1		1
						18		2
							1	<1
							<1	<1
							<1	<1
							<1	<1
							40	8
							4	<1
							6	<1
							20	<1
							20	<1
							7	<1
							2	<1
							<1	<1

* lipid adjusted;

creatinine corrected

Table 5

Correlations between weighted pathway indices on the log scale without imputed values

Pathway	AhR	PXR	Sulphydryl	AChE	Genotox	PPAR
AhR	1	0.59	0.12	0.04		
PXR	0.59	1	0.14	<0.01		
Sulphydryl	0.12	0.14	1	-0.01	0.09	<0.01
AChE	0.04	<0.01	-0.01	1		
Genotox			0.09		1	0.06
PPAR			<0.01		0.06	1

Table 6

Tests for significance (i.e., NS means $p > 0.05$) in logistic model adjusted for optimized indices on log scale unless indicated by *. Diagonal elements represent the model with a single pathway index. Results for significance of interaction parameters are above the diagonal; results for significance of slope parameters in an additivity model are below the diagonal

Pathway	AhR	PXR	Sulphydryl	AChE	Genotox	PPAR
AhR	Negative P=0.029	Interaction NS	Borderline Positive Interaction (p=0.077)	Interaction NS		
PXR	PXR Negative; AhR NS	Negative P=0.002	Interaction NS	Interaction NS		
Sulphydryl	Sulphydryl Negative; AhR NS	Sulphydryl Negative; PXR Negative	Negative P<0.001	*Negative Interaction (p=0.034); NS on logscale	Interaction NS	Interaction NS
AChE	AChE NS; AhR Negative	AChE NS; PXR Negative	AChE NS; Sulphydryl Negative	NS		
Genotox			Genotox Negative; Sulphydryl NS		Negative P<0.001	Positive Interaction P=0.002
PPAR			*PPAR Negative; Sulphydryl NS		Genotox Negative; PPAR NS	Negative P=0.034