# Uses of NHANES biomarker data for chemical risk assessment: Trends, challenges and opportunities

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## 1. Abstract

**Background.** Each year, the US NHANES measures hundreds of chemical biomarkers in samples from thousands of study participants. These biomarker measurements are used to establish population reference ranges, track exposure trends, identify population subsets with elevated exposures, and prioritize research needs. There is now interest in further utilizing the NHANES data to inform chemical risk assessments.

**Objectives.** This article highlights: 1) the extent to which NHANES chemical biomarker data have been evaluated, 2) groups of chemicals that have been studied, 3) data analysis approaches and challenges, and 4) opportunities for using these data to inform risk assessments.

**Methods.** A literature search (1999-2013) was performed to identify publications in which NHANES data were reported. Manual curation identified only the subset of publications that clearly utilized chemical biomarker data. This subset was evaluated for chemical groupings, data analysis approaches, and overall trends.

**Results.** A small percentage of yearly NHANES-related publications reported on chemical biomarkers (8% yearly average). Of eleven chemical groups, metals/metalloids were most frequently evaluated (49%), followed by pesticides (9%) and environmental phenols (7%). Studies of multiple chemical groups were also common (8%). Publications linking chemical biomarkers to health metrics have increased dramatically in recent years. New studies are addressing challenges related to NHANES data interpretation in health risk contexts.

**Conclusions.** This article demonstrates growing use of NHANES chemical biomarker data in studies that can impact risk assessments. Best practices for analysis and interpretation must be defined and adopted to allow the full potential of the NHANES to be realized.

## 2. Introduction

The US National Health and Nutrition Examination Survey (NHANES) is designed to assess the health and nutritional well-being of children and adults in the US (http://www.cdc.gov/nchs/nhanes/about_nhanes.htm). Participation in the NHANES is voluntary, confidential, and follows a complex, multistage, probability cluster design. As such, weighted NHANES data are considered representative of the entire US (non-institutionalized, civilian) population. Thousands of volunteers are invited each year to participate via interviews, questionnaires, and examinations. "Spot" biological samples (e.g., blood and urine at a single time point) are provided by most participants and analyzed for chemical biomarker levels. These biomarker data are published in the *National Reports on Human Exposure to Environmental Chemicals* (NER) stratified by age group, gender, and race/ethnicity. They are also made publically-available online alongside demographic information, questionnaire responses, medical examination results, and other laboratory data (http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm).

The first NHANES survey (NHANES I) was conducted from 1971 to 1975. (http://www.cdc.gov/nchs/nhanes/prior_nhanes.htm). The NHANES II (1976-1980), Hispanic HANES (1982-1984), and NHANES III (1988-1994) then preceded what is now a continuous survey (1999-present). NHANES II was the first to evaluate biomarkers of environmental chemical exposure; specifically, blood lead levels. Chemical biomonitoring was expanded in NHANES III (1988-1994) to include biomarkers of selected pesticides, phthalates, and volatile organic compounds (VOCs). The number of monitored chemical biomarkers rose from 27 as captured in the first NER (NHANES 1999), to 116 in the second NER (1999-2000), 148 in the third NER (2001-2002), and 212 in the most recent (fourth) NER (2003-2004) (CDC 2001, 2003,

2005, 2009). The July 2014 "Updated Tables" of the fourth NER include additional biomonitoring data from NHANES 2005-2006, 2007-2008, 2009-2010, and 2011-2012, bringing the current total to 298 chemical biomarkers (CDC 2014). This most current suite of biomarkers incorporates analytes from over a dozen chemical groups, including brominated flame retardants (BFRs), dioxins and furans, environmental phenols, fungicides, herbicides, insecticides (e.g., organophosphates [OPs], organochlorines [OCs], pyrethroids, carbamates), metals/metalloids, perfluorinated compounds (PFCs), phthalates, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), VOCs, and others.

In 2009, the US Government Accountability Office (GAO) reported that the US Environmental Protection Agency (EPA) "has made limited use of biomonitoring data in its assessment of risk posed by commercial chemicals" (GAO 2009). The GAO further recommended that EPA develop a strategy to categorize existing biomonitoring data, identify limitations in analytic approaches, and prioritize data gaps. The National Research Council (NRC) of the National Academies has also recommended the increased use of biomarker data to support risk assessment activities (NRC 2006, 2007, 2009). In their 2012 publication, *Exposure Science in the 21st Century*, the NRC reported that "The NHANES data provide a unique and growing potential for evaluating source-exposure and exposure-disease relationships in a national population-based representative sample", and that biomarker data sets "will be essential for evaluating the efficacy of exposure reduction policies, and for prioritizing and assessing chemical risks" (NRC 2012). In response to these reports, this study examines NHANES-related publications over the past fifteen years (1999-2013) for the purpose of highlighting specific uses of the chemical biomarker data. Attention is given to the percentage of NHANES-related publications that have focused on chemical biomarkers, and the chemical groups that have been commonly studied. To identify

opportunities to impact risk assessment activities, publications are examined for their approaches to assessing chemical exposures, and to linking exposures to measures of human health. Consistent with the GAO recommendations, the goals of this study are to highlight the state-of-the-science for interpreting NHANES chemical biomarker data, challenges that can limit the use of these data in risk assessments, and opportunities to enhance data interpretation strategies.

## 3. Methods

Publications that have reported on the US NHANES data were identified using the PubMed advanced search builder. The PubMed search was performed in two steps (specific search strings are given in Supplemental Material, Table S1). For step one, publications were identified between 1999 and 2013 that included query terms for "NHANES" (or "National Health and Nutrition Examination Survey") and "United States" (or "U.S.A", "USA", "U.S.", "US") in the title/abstract. Query terms for Unites States were included because publications based on non-US NHANES data (e.g., Korea NHANES) were identified in preliminary test searches. For step two, additional query terms related to biomarkers (i.e., "biomarker", "biomarkers", "biomonitoring", "urine", "urinary", "blood", or "serum") were added. Search results from steps one and two were separated by publication year using a PubMed filter.

Publications identified in step two of the literature search were manually curated using published titles and abstracts. Publications were selected for additional analysis only if they clearly utilized NHANES chemical biomarker data. For this investigation, "chemical biomarkers" did not include endogenous biomarkers (e.g., hormones, antibodies, and inflammatory markers), tobacco-specific biomarkers (e.g., cotinine), dietary biomarkers (e.g., vitamins/nutrients, essential minerals), or biomarkers of phytoestrogens, isoflavonoids, or aflatoxin. If a study's use of

NHANES chemical biomarker data could not be determined using only the published title and abstract, the full text was obtained and examined to inform the final selection decision.

During the manual curation, it was determined for selected publications which specific chemical biomarkers were studied and which analysis approaches used (Supplemental Material, Table S2). Decisions regarding chemical biomarker groupings and analysis approaches for all publications were made by a single author (JRS) followed by a review of each classification by one of the other co-authors. Specific chemical biomarkers were first organized into chemical groups using guidance from NHANES documents (e.g., http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/environmentalhealth_03.pdf). Certain chemical groups were then combined to allow a streamlined trends analysis. For example, dioxins, furans, and PCBs were considered as a single group, as were insecticides, herbicides, and fungicides (termed "pesticides"). Finally, each publication was assigned to one of the designated chemical groups. Studies that reported on at least two of the defined chemical groups were considered "multi-group".

Selected publications were also assigned to one of two primary data analysis categories, defined here as "exposure assessment" and "health association"; studies in both analysis categories are considered relevant to the risk assessment process. Health association studies examined statistical associations between chemical biomarker levels and health measures (e.g., disease incidence, medical examination results). Exposure assessment studies were broadly defined, and used chemical biomarker data to: 1) establish reference ranges for the US population, 2) evaluate data from other (non-NHANES) studies, 3) track exposure trends over time, 4) evaluate differences in exposure across population subsets, 5) identify important predictors of exposure, or 6) estimate the percentage of the population with exposures that exceed a reference level. Many exposure

assessment studies performed a combination of these analyses. Thus, it was not feasible to partition these studies into smaller categories. Investigations that addressed both exposures and health associations were categorized as health association studies.

Data were analyzed using Microsoft Excel (Office 2013, Microsoft Corporation, Redmond, WA) and SAS statistical software (v. 9.3, SAS Institute, Cary, NC). Figures were prepared using Microsoft Excel, GraphPad Prism (v. 4.03, GraphPad Software, San Diego, CA), and R (v. 3.0.1) (Team 2013).

## 4. Results

### Yearly Publications

Sixty-eight publications from 1999 were identified that contained keywords related to "NHANES" and "United States" (Figure 1). Over 400 publications were identified from 2013 using the same search criteria. These results reflect a six-fold increase over a 15-year span, and a median yearly increase of 13%. Considerably fewer publications were identified after adding additional keywords related to "biomarkers". Only 27 publications from 1999 were identified that contained keywords related to "NHANES", "United States", and "biomarkers". Close to 200 publications from 2013 were identified using the same keywords, indicating an approximate seven-fold increase over the 1999 baseline. Interestingly, the yearly ratios of biomarker-related publications (step two results) to total NHANES-related publications (step one results) were fairly consistent, ranging from 0.36 to 0.47 with a median value of 0.43. Results from a simple regression analysis showed no significant linear trend ($p = 0.7$) in this ratio, suggesting that the proportional use of NHANES biomarker data (not specific to chemical biomarkers) has been stable over the period of time examined in this study.

Only a small percentage of the total NHANES-related publications specifically reported on chemical biomarkers (8% yearly average). The number of identified publications elevated from five in 1999 to 44 in 2013, representing a 9-fold increase over 15 years. The yearly ratios of chemical biomarker-related publications (manual curation results) to total NHANES-related publications (step one results) increased from 0.07 in 1999 to 0.10 in 2013. Simple linear regression results showed a significant positive effect ($p = 0.007$) of publication year on ratio estimates. This result suggests an increase over time in the proportion of NHANES-related studies that focus on chemical biomarker measurements.

**Chemical Groups**

Each publication identified through manual curation was assigned to one of eleven groups based on the chemical biomarkers that were studied (Figure 2). Metals/metalloids were by far the most commonly studied group. Studies of metals/metalloids (particularly lead, cadmium, mercury, and arsenic) comprised nearly half (49%) of the chemical biomarker-related publications. The second most studied chemical group was pesticides (9%), which included OP, OC, and pyrethroid insecticides, as well as herbicides, fungicides, and halogenated phenolic compounds. Environmental phenols (including bisphenol A, triclosan, and parabens) were the third most studied group (7%), followed by phthalates (5%), PFCs (5%), PAHs (4%), dioxins/furans/PCBs (4%), VOCs (3%), and BFRs (2%). Multi-group studies comprised 8% of the chemical-biomarker related publications. The remaining 4% of studies focused on a group defined as "other" chemicals; seven out of the ten publications in this group focused on perchlorate.

**Analysis Categories**

Step one of the PubMed literature search (NHANES + US query) yielded 3224 publications, step two (NHANES + US + biomarkers query) yielded 1382 publications, and manual curation yielded 273 publications (Figure 3a). Out of the 273 studies that focused on chemical biomarkers, 148 (54%) performed an exposure assessment, and 125 (46%) examined health associations. These results suggest that the chemical biomarker-related publications are evenly split between analysis categories over the past fifteen years. Figure 3b shows the number of yearly publications for the two analysis categories. Limited numbers of papers were observed early in the review period, so data across 1999, 2000, and 2001 were combined. Prior to 2008, no trends were observed for either category. However, a sharp rise in exposure assessment studies was observed in 2004, and then again in 2008. These elevations likely reflect releases of the NHANES 1999-2000, 2001-2002, and 2003-2004 datasets (CDC 2003, 2005, 2009). The number of yearly exposure assessment studies remained relatively flat between 2008 and 2013. Health association studies, however, increased dramatically in number over the last five years of the review period. In fact, nearly 70% of the curated 2013 publications focused on health associations. This suggests growing interest in using the NHANES data to link chemical biomarkers and health measures.

**Trends by Group and Category**

The number of yearly chemical biomarker-related publications are shown in Figure 4 after stratification by chemical group and analysis category. Between 1999 and 2003, publications focused almost exclusively on metals/metalloids (28 out of 31); the strong focus on this group continued across all 15 years of the review period. A lack of publications related to other chemical groups prior to 2004 mirrors the public release dates of the NERs; while data on metals and select

231 VOCs, pesticides, and phthalates were available in 1999 (from NHANES III), data on additional
232 chemicals were not available until later years (CDC 2003, 2005, 2009). Indeed, Figure 4 illustrates
233 that initial studies involving PAHs were published in 2004, and those involving PFCs,
234 dioxins/furans/PCBs, environmental phenols, and BFRs between 2006 and 2008.

235 Exposure assessment studies preceded health association studies for most chemical groups
236 (Figure 4). This is not surprising given that many early studies focused on establishing biomarker
237 references ranges for the US population (Barr et al. 2004; Calafat et al. 2008a, b; Grainger et al.
238 2006; Nichols et al. 2007; Silva et al. 2004; Sjodin et al. 2008). For dioxins/furans/PCBs, VOCs,
239 BFRs, pesticides, and "other" chemicals, exposure assessment studies comprised the majority of
240 the group-specific publications (over 70% in each case). The number of publications was more
241 balanced across analysis categories for metals/metalloids, phthalates, PFCs, environmental
242 phenols, and multi-group chemicals. For these groups, between 40% and 60% of the publications
243 focused on exposure assessment. The recent upward trend in health association studies (Figure
244 3b) is reflected most clearly for metals/metalloids, environmental phenols, and multi-group
245 chemicals (Figure 4). An increasing focus on exposure assessments of multi-group chemicals is
246 also evident over recent years (Figure 4).

247

248 **5. Discussion**

249 The NHANES continues to be the largest source of chemical biomarker data in the US.
250 The publications included in this review have collectively reported on tens-of-thousands of
251 measurements from representative samples of the US population. However, it appears that only a
252 small percentage of published studies related to the NHANES have focused on chemical biomarker
253 data. Indeed, for most years in our review period (1999-2013), less than 10% of the total

NHANES-related publications focused on these data. This result highlights an opportunity for exposure scientists, epidemiologists, toxicologists, and risk assessors/managers to make better use of this vast resource. It is expected that the focus on chemical biomarkers will rise as more scientists become aware of potential uses of the NHANES data. Specifically, interest will increase given new methods and applications for interpreting NHANES data in health risk contexts. The following sections discuss key findings of this review, challenges related to these findings that can limit the use NHANES data for chemical risk assessment, and examples of new methods and guidance that will help future studies overcome these challenges.

**Key Findings**

Biomarkers of metals/metalloids have been studied far more frequently than those of other chemical groups (Figures 2 and 4). There are several reasons for this imbalance. First, biomarker levels of select metals have been reported for a broader participant age range (including children younger than six years of age), and over more survey years. For example, blood lead was monitored in NHANES II (1976-1980), whereas biomonitoring for environmental phenols, PFCs, and BFRs began in NHANES 2003-2004. A second reason is that biomarker-based reference levels exist for certain metals (e.g., lead and mercury), allowing direct risk-based analysis of the NHANES measurements. For most chemicals, surrogate biomarker reference levels are not available, thereby limiting the direct use of biomarker data in this context. For these chemicals, models are required to link biomarker measurements to external exposure reference levels (described in detail below), like an EPA reference dose (RfD).

Despite the availability of biomarker data for hundreds of other chemicals, the number of publications on metals/metalloids has actually increased over the past five years (particularly

277 studies of health association [Figure 4]). However, it is also apparent that the focus is beginning
278 to broaden across chemical groups. In particular, there is evidence for increasing attention on both
279 multi-group exposure assessment and health association studies (Figure 4). Studies of this nature
280 will be necessary in order to systematically evaluate impacts of multiple chemical stressors, and
281 non-chemical stressors, on human health. It is important to note, however, that these multi-group
282 studies are restricted to the inventory of chemical biomarkers in the NHANES, and therefore still
283 represent semi-targeted assessments (Pleil and Stiegel 2013). As such, the discovery of broader
284 exposure and health associations will rely on other, less targeted, datasets (Rappaport 2012;
285 Rappaport et al. 2014).

286 Over the entire fifteen-year review period, the number of publications was fairly balanced
287 across analysis categories, with about half focused on exposure assessment, and half on health
288 associations. A surprising result was the recent dramatic increase in published health association
289 studies (Figure 3b). Results of our literature search (Supplemental Material, Table S2) suggest
290 that these types of studies have been performed using biomarkers across nearly all NHANES
291 chemical groups. Furthermore, our results indicate that individual chemical biomarkers have been
292 examined for associations with a variety of health measures. For example, bisphenol A biomarker
293 data has been examined for associations with heart disease, obesity, type-2 diabetes, allergic
294 asthma, metabolic syndrome, peripheral arterial disease, immune dysfunction, and markers of
295 other chronic diseases (Supplemental Material, Table S2). The vast array of potential associations
296 between chemical biomarkers and health measures encourages research of this nature – indeed,
297 multiple health measures (more than 20, in some cases) have been examined for associations with
298 biomarkers in almost all chemical groups (e.g., PAHs, PFCs, phthalates, pesticides, and
299 metals/metalloids) (Supplemental Material, Table S2).

## Challenges and opportunities for health association studies

Interpreting results for thousands of conceivable associations is a daunting task (Greenland 2008; Patel and Ioannidis 2014). Newer studies have therefore begun to simultaneously evaluate relationships between chemical biomarkers and health measures as part of exposure-wide association studies (EWAS) (Patel et al. 2010; Patel et al. 2012; Patel et al. 2013; Patel et al. 2014). These studies better address statistical challenges related to multiple comparisons since more systematic methods are utilized. For NHANES-related health association studies to be considered in a risk assessment context, however, best practices are still needed for interpreting reported associations against the background of all possible associations (real and spurious). One approach is to compare a reported association with the median association amongst related biomarkers in a specific chemical category (Patel and Ioannidis 2014). This comparison indicates whether a reported association is remarkable relative to background, yet is dependent on predefined categories. An alternative approach is to first comprehensively test all possible associations, and then report the strength of a single association relative to all results. Although this approach can be computationally prohibitive depending on model complexity, computationally efficient methods, such as frequent itemset mining, are now being systematically applied to NHANES datasets (Bell and Edwards 2014).

Specific attention has been given to the cross-sectional design of the NHANES as it impacts studies of health association (LaKind et al. 2012). Notably, concurrent measures of biomarkers and health measures from the NHANES are not useful for demonstrating temporality. Therefore, NHANES data alone are not well-suited for evaluating causation (or reverse causation) (Hill 1965), and health association studies often require follow-up targeted analysis. Furthermore,

single spot measurements of chemical biomarkers in the NHANES may not be reliable surrogates of average or peak exposure levels, and may not be relevant to exposures experienced during critical life stages (Aylward et al. 2014; Bradman et al. 2013). Studies have shown that large measurement error associated with spot measures can contribute to exposure misclassification and increase the likelihood for biased statistical associations (Armstrong 1998; Jurek et al. 2006). Short-lived biomarkers in particular are prone to these challenges (Lin et al. 2005; Sobus et al. 2010b). Since some short-lived biomarkers are increasingly a focus of health association studies (e.g., environmental phenols and phthalates [Figure 4]), there is a need for methods that can help minimize measurement error. There is also a need for guidance on interpreting statistical associations between concurrent measures of short-lived biomarkers and chronic disease (LaKind et al. 2012).

Other challenges for health association studies stem not from the NHANES study design, but from the biomarkers themselves, and the methods used for their quantitation. Issues related to chemical specificity, method sensitivity, and biological relevance are well documented and generally agreed upon (NRC 2006; Sobus et al. 2010a; Zelenka et al. 2011). Other issues, however, are still topics of intense debate. For example, a consensus has not been reached on how and when to adjust specific biomarkers for biological matrix effects. Levels of urinary and blood-based biomarkers, in particular, may require adjustment for variable urine output and lipid content, respectively. A recent study found that the direction (+/-), magnitude, and significance of associations between urinary phthalate metabolites and body size (waist circumference and body mass index) can differ depending on adjustments to the biomarkers (e.g., creatinine-adjusted vs. unadjusted concentration) (Christensen et al. 2014). These results highlight a clear need for standardized biomarker adjustment and analysis practices.

Guidance documents exist that can aid the planning, analysis, reporting, and interpretation of health association studies (Rooney et al. 2014; Vandenbroucke et al. 2007). In particular, the Biomonitoring, Environmental Epidemiology, and Short-Lived Chemicals (BEES-C) instrument developed by Lakind and colleagues (2014) targets critical issues that are unique to studies of short-lived chemical biomarkers. This instrument can be used for assessing the quality of health association research based on epidemiological study design and biomarker selection and measurement. It therefore serves as a resource for those planning studies using the NHANES chemical biomarker data, or those looking to evaluate published studies as part of a weight-of-evidence assessment. Discussions and evaluations surrounding the BEES-C instrument and other guidance documents are needed in order to clearly define and communicate best practices for health association studies involving the NHANES data.

**Challenges and opportunities for exposure assessment studies**

Challenges exist for certain exposure assessment studies just as they do for health association studies. For example, measurement error can bias statistical associations between exposure metrics (e.g., dietary information and occupation) and chemical biomarker levels. This bias can impact the identification of important exposure sources and pathways for target chemicals. From a risk assessment standpoint, however, the most important challenges are those faced when linking chemical biomarker measurements to reference exposure levels. Since models are generally required to make these linkages, results are prone to error stemming from both the models themselves and the data inputs. Based on our review, studies that utilized models fell into two general categories: 1) those that reconstructed exposure levels from NHANES biomarker data (reverse modeling) (e.g., (Blount et al. 2007)); and 2) those that compared biomarker

measurements to model-predicted biomarker estimates (forward modeling) (e.g., (LaKind et al. 2009)). Models used for forward (biomarker) and reverse (exposure) predictions varied tremendously in terms of their complexity, ranging from simple analytical models to complex physiologically-based pharmacokinetic (PBPK) models involving Markov Chain Monte Carlo analyses (Allen et al. 2007; Lyons et al. 2008). In each study, the ability to make accurate exposure or biomarker predictions was dependent upon the model applicability (e.g., how well the model described the exposure-biomarker relationship), existing knowledge about the likely exposure scenarios (e.g., frequency of exposure), and measurement error (Tan et al. 2012).

Challenges related to measurement error stem from a lack of repeated chemical biomarker measurements in the NHANES. Spot biomarker distributions may not reflect distributions of average biomarker concentrations, which can only be obtained from repeated measures. For example, distribution tails (e.g., $5^{th}$ and $95^{th}$ percentile) are often wider for spot measurements, particularly when examining short-lived biomarkers (Aylward et al. 2014; Christensen et al. 2012; Koch et al. 2014; Sobus et al. 2011). As such, most NHANES-related studies have compared the median (or other central tendency estimate) of a spot biomarker distribution to a point estimate based on an exposure reference level (Aylward et al. 2013). While this approach informs exposures to the US population as a whole, it does not fully utilize the data in the upper percentiles, where there is increased probability of higher exposures. A recent article addressed this issue by offering a mathematical approach to estimate distributions of average biomarker levels given distributions of spot measurements (Pleil and Sobus 2013). This approach can be used to calculate population exceedance against chronic exposure-based reference levels – that is, the percentage of the US population (or subset) with inferred average exposure in excess of a reference level.

A second issue related to modeling and the lack of repeated measurements in the NHANES is the inability to interpret biomarker results for individual participants. Especially for a short-lived biomarker, a single high measurement may reflect a consistently high exposure, or a single recent elevated exposure. Such a determination generally cannot be made given a lack of supplemental exposure data in the NHANES. As such, individuals' measurements have often been collectively considered to make population inferences (Angerer et al. 2011). As an alternative, a stochastic modeling method was recently published that allows exposure evaluation at the individual participant level (Phillips et al. 2014). This method combines exposure models and PBPK models to predict biomarker distributions that are consistent with a reference exposure level. Measurements from NHANES individuals are then interpreted probabilistically with respect to the reference level. This approach marks a significant advancement towards interpreting participant-level biomarker measurements without collecting additional samples and data.

In this article, significant advancements in the approaches used to interpret NHANES chemical biomarker data have been highlighted. Methods are now systematically evaluating data across chemicals, health measures, and study participants. These methods are facilitating multi-chemical/group assessments that will help prioritize needs for follow-up targeted assessments. Moving forward, it will become increasingly important to articulate and follow best practices for assessing biomarkers of individual chemicals, chemical groups, and the expanding NHANES chemical inventory. This review plays an important role in this process by highlighting trends in recent research, as well as key challenges and research opportunities moving forward.

**Limitations of this study**

A major goal of this study was to evaluate trends in the uses of NHANES chemical biomarker data using a sample of publications. There are some limitations with the methods used for sample selection and analysis. First, all publications evaluated here were identified using the PubMed advanced search builder. Articles not indexed on PubMed were not captured in our search. Second, our search was restricted to publications that explicitly listed the NHANES in the title/abstract. It is likely that there will be some studies that utilized NHANES data without mentioning the survey name in the publication title/abstract. Third, all PubMed searches included query terms related to "US" in the title/abstract. This search criteria guarded against the inclusion of non-NHANES studies, but restricted the number of publications that were curated and included in the trends analysis. Fourth, electronic publications (epubs) for 2013 were included in the results from all PubMed searches and manual curations. The inclusion of these results elevated the number of 2013 publications for each search step. However, only two out of 43 publications in 2013 were included as part of the final manually-curated list, indicating that this should have little or no impact on the trends seen.

The final limitation of this study relates to the binning of publications (second curation) based on chemical group. NHANES biomarkers have been defined with slight differences across survey years. As such, groupings here were based on both recent NHANES documents and empirical evidence from the selected literature (specifically, biomarkers that have been routinely co-examined were grouped together). Using this approach, the number of biomarkers across chemical groups was variable. For example, the group "environmental phenols" included few biomarkers, whereas "pesticides" included many biomarkers from a variety of classes. No attempt was made to weigh groups based on the number of biomarkers. This has implications when designating certain publications as "multi-group". Specifically, some multi-group publications

examined many biomarkers across all chemical groups. Others investigated few biomarkers across only two groups. A few publications examined many biomarkers as part of one large chemical group, and were not considered "multi-group".

Each of the limitations discussed above may have introduced some amount of error or bias into our analysis. The main objective of this investigation, however, was to gain a better understanding of the primary uses of the NHANES data based on a sample of studies from the published literature. The trends observed here do indeed highlight existing research challenges and opportunities to advance the science. Future investigations of NHANES data usage will provide further information regarding the recent trends observed in the present study.

## 6. Conclusions

This article is amongst the first to investigate trends in the uses of NHANES chemical biomarker data. Extrapolating from our results, it is likely that more than 100 articles will be published each year that examine these data. Given this usage, it is likely that NHANES data will impact chemical risk assessment decisions. New methods and guidelines are rapidly emerging to address challenges that face analysis, reporting, and interpretation of the NHANES data. Since exposure assessment and health association studies are moving towards multi-chemical/group assessments (Belova et al. 2013; Patel et al. 2013; Wambaugh et al. 2013), it is increasingly important to define and adopt best research practices. Such measures will allow the full potential of the NHANES to be realized, and defensible decisions based on the data and emerging science to be made.

## Figure legends

Figure 1. Yearly publications (1999-2013) related to the US NHANES (asterisks), biomarkers (squares), and biomarkers of environmental chemicals (circles). PubMed search and selection methods are given in Supplemental Material, Table S1.

Figure 2. Chemical groups studied using NHANES biomarker data.

Figure 3. Tree diagram for publications identified via PubMed searches, selected via manual curation, and categorized by data analysis approach (A). Trends in data analysis approaches from 1999-2013 (B).

Figure 4. Yearly chemical biomarker-related publications stratified by chemical group and analysis category. Darker colors reflect a higher number of publications for a particular chemical group in a particular year. The legend (right) shows the mapping of publication count to color. (E) = exposure assessment; (H) = health association.

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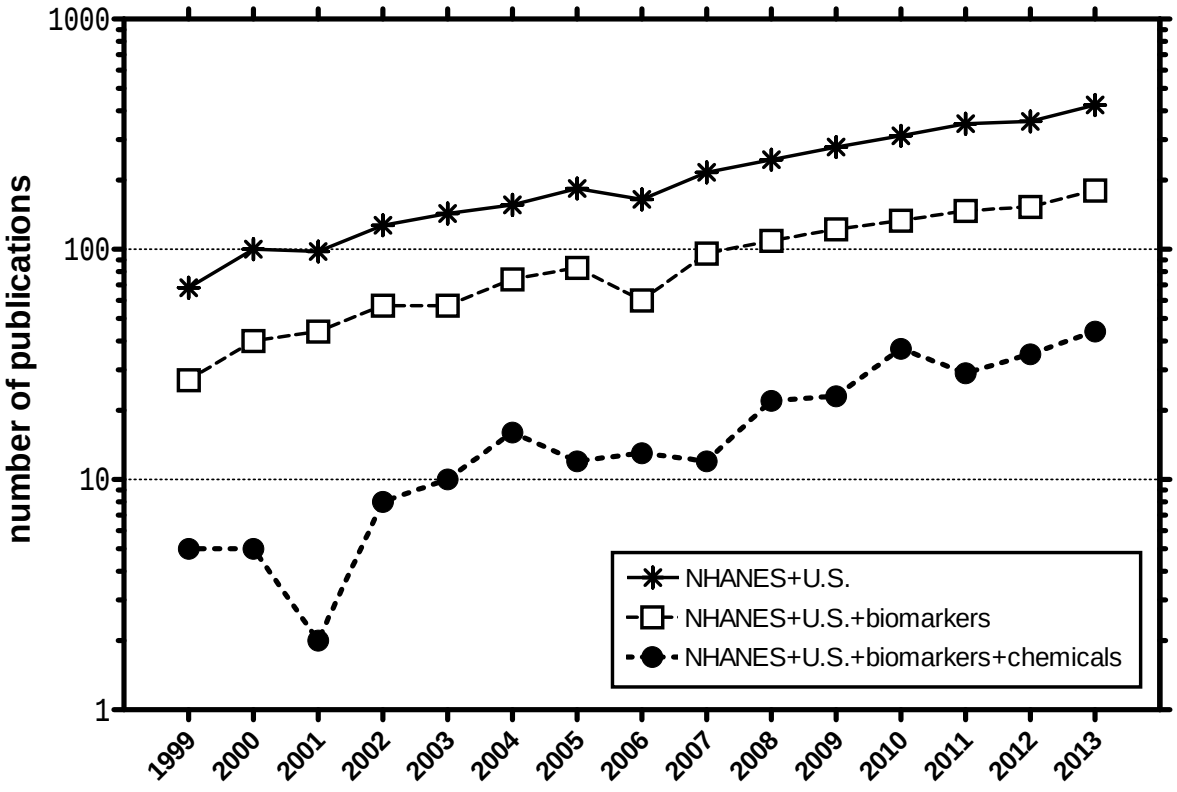
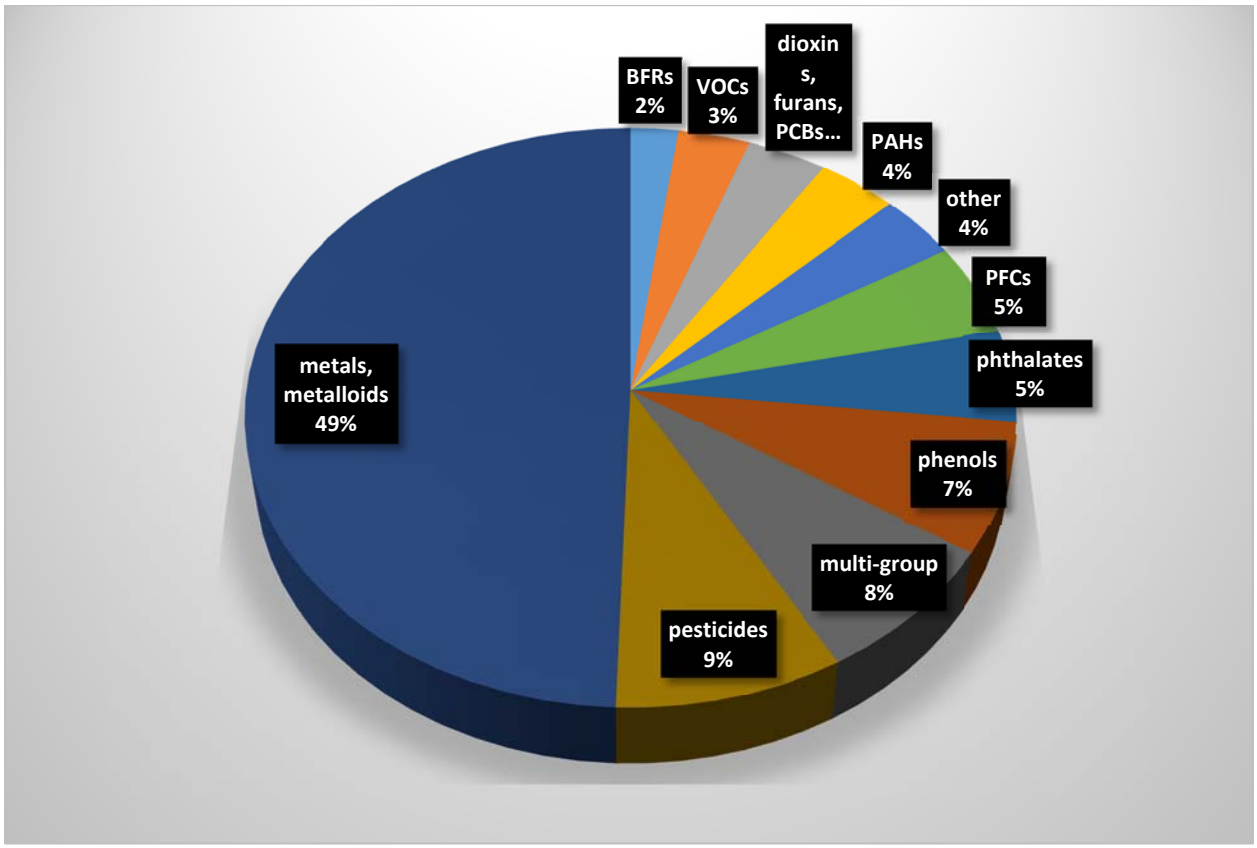
## Figures




620

621 **Figure 1**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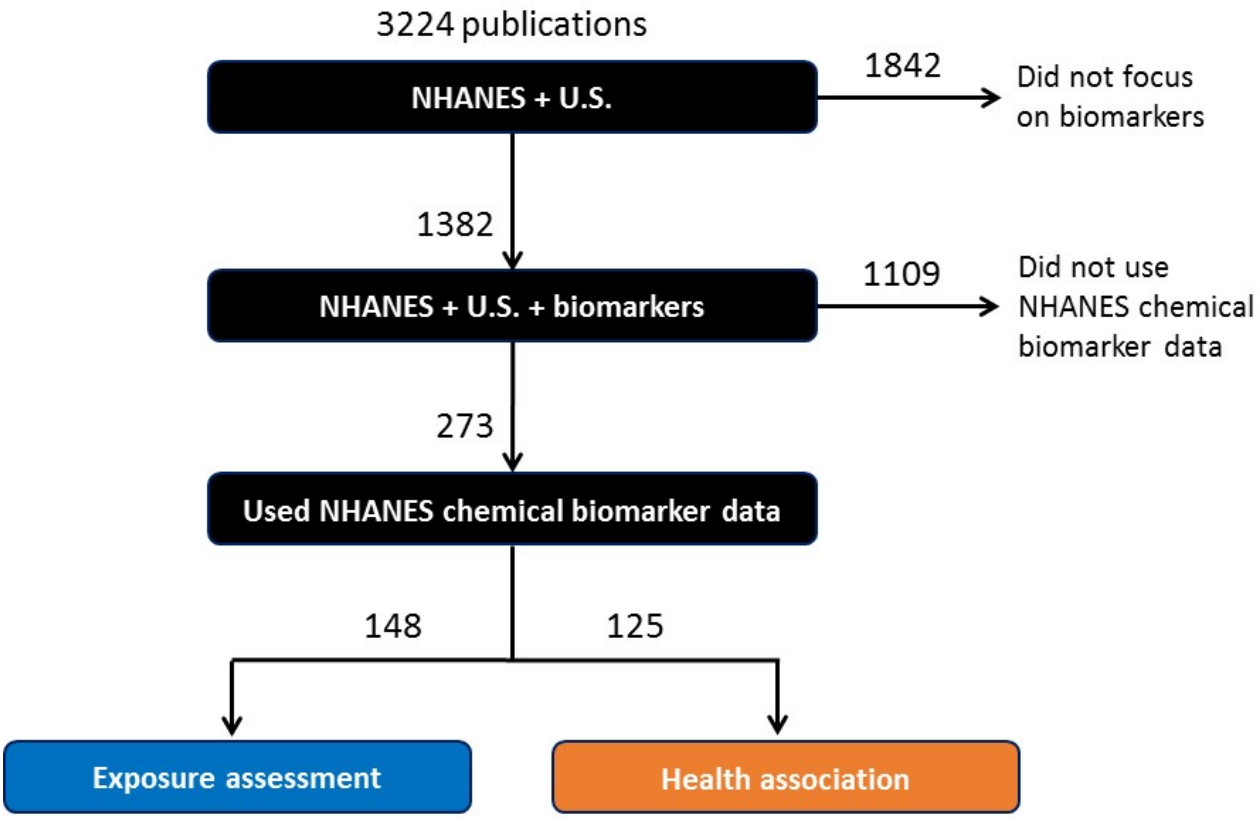
metals, metalloids 49%

**Figure 2**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Did not focus on biomarkers
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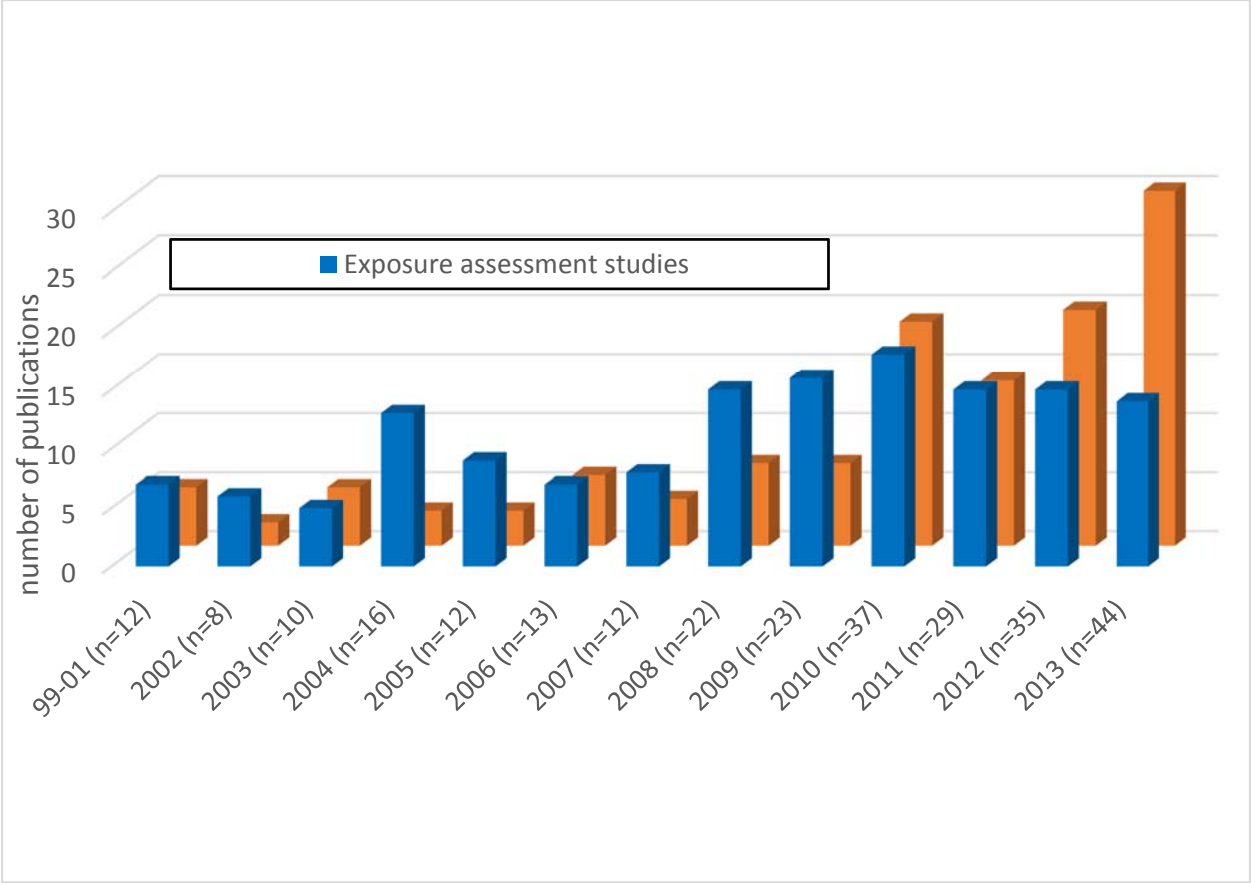
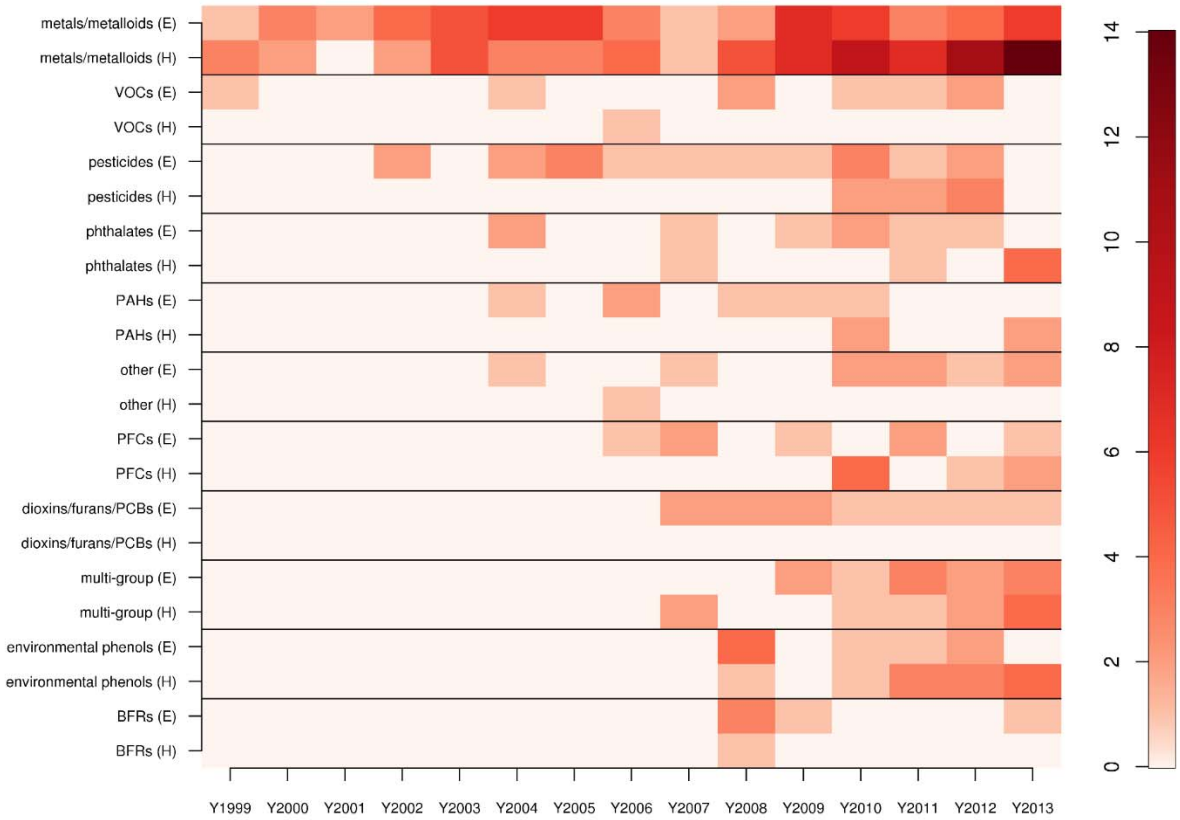
148
125
Exposure assessment
Health association

**Figure 3a**




626

627 **Figure 3b**

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629 **Figure 4**