A FRAMEWORK FOR EVALUATING SERUM DIOXIN DATA DERIVED FROM BIOMONITORING STUDIES

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Introduction

The use of human biomonitoring studies as a tool for evaluating exposure to various chemicals is increasing given the advances in technology and statistics. However, in each of its National Reports on Human Exposure to Environmental Chemicals, the Centers for Disease Control and Prevention (CDC) has emphasized that the mere presence of a chemical in a biological specimen provides no evidence that the chemical is responsible for adverse health effects[1]. The Committee on Human Biomonitoring for Environmental Toxicants (CHBET) concurred, recognizing the significant challenges inherent to interpreting these data[2].

Dioxins (PCDDs) and dioxin-like compounds (DLCs), including PCDFs and PCBs, have been the focus of many biomonitoring studies to date. These compounds are notable because of their ability to persist in the environment, to accumulate in biological tissues, and because of their common aryl hydrocarbon receptor-mediated mechanism of toxicity. Many of these compounds have been introduced into the environment through various combustion and manufacturing processes, though the primary route of exposure for most humans is via dietary intake, particularly consumption of animal products. Though measurable concentrations of DLCs are readily observed in human populations, interpretation is difficult given that specific adverse effects have not been observed to coincide with specific concentrations measured in humans (i.e., no dose-response relationship). Moreover, several studies suggest that the dioxin serum levels at which adverse health effects occur is likely several orders of magnitude above the general population exposure level. These facts present a unique challenge for investigators interested in assessing dioxin exposure and health risk through biomonitoring methodology. Given the complexities and uncertainties associated with using biomonitoring data in exposure assessments, this framework provides a useful and comprehensive tool for evaluating such data.

Materials and methods

A transparent, multi-step decision framework was developed for use in determining whether measured DLC levels are similar to or different from a reference population (Figure 1). The initial step (***Step 1. Define Reference Population***) of the framework involves identifying a reference population. In the absence of a study-specific reference population, sampling data from the National Health and Nutrition Examination Surveys (NHANES) can be used to develop background reference values. Studies such as the NHANES are recommended since data are available to characterize demographics (e.g., age, BMI, sex), allowing investigators to select a reference group with demographical characteristics that are similar to the study population[2,3]. In the second step (***Step 2. TEQ calculations***), lipid-adjusted dioxin toxic equivalency serum concentrations (TEQ) are calculated for individuals in both the study population and reference population.

Step three (***Step 3. Individual TEQ Comparison to Benchmark***) involves the comparison of each individual's TEQ to the selected benchmark from the reference population. If individuals are found to have TEQ concentrations above the benchmark, a statistical test of proportions is used to evaluate whether the study population as a whole may have experienced exposures beyond that of the reference population. When evaluating biomonitoring data, the goal is to characterize individuals that are truly elevated relative to the general population. Therefore, comparisons to a mean or upper confidence limit of a mean are of limited use since there is considerable variability among "normal" individuals in biomonitoring data. As such, an upper percentile concentration from the reference population is recommended for the evaluation benchmark. Percentiles are commonly used as benchmarks when evaluating health

related measurements. In particular, the 95th percentile is often used to describe individuals who are elevated for a particular parameter. The 95th percentile, which also has been utilized in previous human biomonitoring studies that focused on dioxins levels[4,5], is chosen because natural variability among individuals for health benchmarks is typically high (i.e., the range of "normal" values is large). For this framework, the 95th percentile TEQ concentration from a demographically similar reference population is recommended.

In step four (***Step 4. Group Comparison***), a statistical test of proportions is conducted to evaluate whether the study group as a whole has serum dioxin TEQ concentrations that are similar to, or different than, the dioxin TEQ levels of the reference population. This test is based on the expectation that a certain number of individuals in any subpopulation will exceed the 95th percentile of the reference population[9]. A group is considered to be above background if there is a statistically significant increase above 5% of the proportion of workers tested that exceed the 95th percentile of the demographically-similar reference group. If the results of the proportions test indicate that there is a significantly greater number of individuals in the study population with TEQ concentrations that exceed the 95th percentile relative to the number expected based on the reference population (i.e., > *p0*), then further assessment of the study population is warranted. If the proportions test indicates that fewer individuals were above the 95th percentile than expected, no further investigation is warranted; proceed to step seven and report study results.

For the individuals with a TEQ concentration above the 95th percentile, exposure questionnaires and congener profiles are evaluated. Exposure questionnaire surveys (***Step 5a. Evaluate Questionnaire Data***) may allow study investigators to better understand the habits and history of each study participant and are capable of identifying sources of exposure unique to the individual. Since participant recall can be a major source of uncertainty in questionnaire data, survey design and presentation is critical; questions must be communicated in a comprehensive yet clear manner. If there is sufficient information to indicate that one or more participants with elevated TEQs were uniquely exposed to alternative sources of dioxin, then these participants are removed from the group of participants with TEQs above the 95th percentile for step six.

Congener fingerprint analysis, a statistical method driven by principle components analysis (PCA), may also be useful for comparing dioxin congener profiles of individuals with elevated TEQs with those profiles typical of the reference population **(Step 5b. Analyze Congener Profiles**). This method has been used to compare individual conger profiles to environmental congener profiles in previous studies[6,7]. In this step, it is very important that the demographic characteristics of the study population and reference populations are similar; for this step, it is particularly informative to evaluate the congener profiles of a study-specific referent population to fully characterize potential regional exposure influences. If the results of the analyses identify one or more individuals with congener profiles that are different from the regional congener profile, then these individuals are removed from the group of participants with TEQs above the 95th percentile for step six. If both a national and regional reference population are utilized, individuals with a profile similar to the regional profile, but different than the national profile, are kept in the elevated TEQ group.

If the analysis of questionnaire data and congener profiles identify any study participants with elevated TEQ levels as candidates for exclusion, re-run the test of proportions (***Step 6. Re-evaluate Group Comparison***) described in step four with these individuals removed from the group of participants with TEQs above the 95th percentile. If the results of Step 6 (or Step 4) demonstrate that the number of individuals in the study population with elevated TEQs is equal to or less than expected, then it can be concluded that the level of dioxin exposure within the study population is no different than background in national or regional populations. On the other hand, if results indicate that the proportion of study population with elevated TEQs is higher than expected, the study population has likely been exposed to DLCs at concentrations which exceed background exposures. This situation calls for further investigations to identify exposure sources and pathways (e.g., comparative congener analysis of environmental media, etc.). As an additional conservative measure, if the blood samples for either the study or reference

populations were during different time periods, the older sample data can be adjusted to account for the declining levels of DLCs in the general population[8].

Results and discussion:

The use of human biomonitoring studies as a tool for characterizing exposure in a study population is greatly dependent upon the study design. Though powerful analytical techniques are generally available for describing chemical body burdens, little can be said about such data in the absence of appropriate controls or the survey data necessary to characterize the study population demographics, life style, and history. The current framework builds on many of the topics identified by the CHBET as integral to the development of a typical biomonitoring study, with a focus mostly on aspects of design and analysis (study conduct, communication, and ethical responsibilities generally are not discussed here)[2]. Given the complexities and uncertainties associated with using biomonitoring data in exposure assessments, this framework provides a useful and comprehensive tool for evaluating serum dioxin data in both study and reference populations.

References:

1. CDC (2005). *NCEH* Pub No. 05-0570.
2. NAS (2006). National Academies Press. ISBN: 0-309-66312-1.
3. Ferriby L.L., Knutsen J.S., Harris M., Unice K.M., Scott P., Nony P., Haws L.C., and Paustenbach D. *J Expo Sci Environ Epidemiol* 2007; 17: 358-371.
4. Orloff K.G., Hewit D., Metcalf S., Kathman S., Lewin M., and Turner W. *Journal of Exposure Analysis and Environmental Epidemiology* 2001; 11: 352-358.
5. Patterson D.G., Patterson D., Canady R., Wong L. Lee R., Turner W., Caudill S., Needham, L., and Henderson A. 2004; *Organohal Comp* 66: 2878-2883
6. Lee C.C., Chen H.L., Su H.J., Guo Y.L., and Liao P.C. 2005; *Chemosphere* 59: 1465-1474
7. Lee C.C., Guo Y.L., Kuei D., Chang H., Hsu J., Wang S., and Liao P. 2006; *Chemosphere* 65: 436-448
8. Lorber M. *Sci Total Environ* 2002; 288: 81-95
9. NIST e-Handbook: http://www.itl.nist.gov/div898/handbook/

Figure 1: Biomonitoring Framework Decision Tree