Review of Biomonitoring Issues for Consideration by the Scientific Guidance Panel of The California Environmental Contaminant Biomonitoring Program

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Introduction We would like to thank the Scientific Guidance Panel for this opportunity to provide comments on the issue of biomonitoring, with specific emphasis on the upcoming Maternal Infant Environmental Exposure Project (MIEEP). Our organizations at both the Dow Chemical Company and Dow AgroSciences LLC have conducted numerous occupational and non-occupational biomonitoring exposure studies over several decades and feel that our learnings on these projects can be of benefit to researchers in this area.

Background As you know, biomonitoring generally involves the analysis of biological tissue or fluids for levels of endogenous or xenobiotic compounds or their metabolites. These data are generated to evaluate relative exposures across time, geographic locations, or occupational environment. Correlation of a relevant biomarker's levels with chemical exposure levels can also be made, but requires prior knowledge of the pharmacokinetic fate of the molecule in the human body. Most often urine samples are analyzed in these studies, due to the non-invasive nature of sample collection and better enrollment by potential study participants. Blood samples are sometimes taken, especially for short-lived or volatile biomarkers. In all cases, analytical chemistry methods are required with sufficient selectivity and sensitivity to provide high quality data. For materials ubiquitous in the environment, proper techniques for specimen collection and handling to either avoid or account for external trace contamination of specimens with the analyte of interest are critical to valid interpretation of the data generated. Some specific concerns with these study design parameters are discussed in detail below.

Biomonitoring Study Design Issues

Relevant Biomarker Selection For chemicals that are not metabolized in the human body, measurement of the specific chemical in urine or blood represents a means of

evaluating exposure to that compound. However, a wide variety of chemicals are rapidly broken down to metabolites, often as the compound is first absorbed and passes through the liver prior to reaching the general blood circulation. In these cases, one or more of the major metabolites are generally chosen as surrogates to represent exposure to the parent chemical. While this strategy works well, investigators need to be cognizant that the metabolite chosen as a biomarker may also be present in the environment, and as such, biomonitoring results from this type of study would not be specific for the parent compound in question.

An example of this issue is with the organophosphate compound chlorpyrifos (CPF). CPF is hydrolyzed in the environment to 3,5,6-trichlorpyridinol (TCPy). While TCPy measurements in blood or urine may be an appropriate biomarker for occupational-level exposures, several authors have shown that the vast majority of the TCPy present in the general population arises from exposure to TCPy itself in the diet, and not CPF. Barr et al. have estimated that 80-90% of the TCPy in urine samples comes from pre-hydrolyzed CPF (Env. Res., **99**, 314-326, 2005). Based on these data, TCPy measurements from programs such as NHANES need to be interpreted as representing exposure to both the parent compound and/or the hydrolysis product.

Sample Collections Samples collected for biomonitoring analysis are generally collected only once per study subject, due to financial constraints, difficulty with subject compliance for longer periods of specimen collection, or invasiveness of sampling. As a result, it is critical that the sampling times be optimized to reflect the exposure paradigm being measured (i.e., single-exposure, steady-state). For blood samples taken from short-half life compounds, it is important to sample within one elimination half-life of exposure, otherwise later sampling may afford only non-detects that would result in calculations of no exposure to the chemical. For urine samples, spot-samples are often taken only once, often at the morning void. Scher et al. have shown that spot samples may deviate from the mean urinary concentrations by 2-3 fold, compared to 24-hr composite samples, thereby increasing the variability in predictions of exposure levels from this type of biomonitoring data (J. Exp. Sci. Env. Epi., **17**, 350-357, 2007). Specific sampling approaches need to be evaluated for each biomarker, based on the known physical-chemical and pharmacokinetic properties.

Another very important consideration in biomonitoring sample collection is the quality control on sample collection, processing, shipment and storage. When certain xenobiotics are ubiquitous in the environment, the biomarkers for these materials are often present in solvents or materials used in the analyses at trace levels (parts per billion down to parts per quadrillion). As current analytical instrumentation can often quantitate effectively at these concentrations systematic contamination of the samples during collection or thereafter could afford artifactually high results. At the opposite extreme, any chemical instability in the biomarker could result in inaccurate

underestimates of exposure. As a result, it is critical that appropriate fortified control matrix samples be prepared, shipped, stored and processed with the study samples to verify lack of sample contamination or analyte degradation. Specifics for these techniques can be found in numerous regulatory guidelines (i.e., U.S. EPA guidelines for Exposure and Risk Assessment Calculations: Series 875-Occupational and Residential Exposure Test Guidelines, Version 5.4, Working Draft of February 10, 1998, p. C9). Finally, analytical chemistry methods should be well validated in the biological matrix of interest and across the concentration range to be measured in the study samples. A good example of guidelines to follow for method validation has been prepared by the U.S. FDA (Guidance for Industry: Bioanalytical Method Validation, May 2001; www.**fda**.gov/.../**Guidance**ComplianceRegulatoryInformation/**Guidance**s/UCM070107.pdf)

Data Interpretation Once biomarker quantitative data have been generated, there is often a desire to back-calculate what the potential exposure level was to a chemical that puts into context the biomonitoring results obtained in the study participants. Numerous authors have published methods for this evaluation. A recent overview by Hays et al. describes the approaches of forward dosimetry (predicting biomarker levels in humans at a Reference Exposure Value) and reverse dosimetry (back-calculating exposure from a series of modeled biomarker time-course datasets over a range of doses) (Reg. Tox. Pharm., **47**, 96-109, 2007). These methods of extrapolation to exposure concentration require a validated pharmacokinetic (PK) or physiologically-based PK model, which incorporates the known rates and routes of chemical uptake, distribution, metabolism and elimination in the human body. Without accurate assessments of these parameters, the model predictions of exposure from biomarker levels could be in error. Going forward, it will be increasingly important to evaluate and interpret biomonitoring (i.e., exposure) data with internal dosimetry and bioavailability estimates, as this can inform on the potential for resultant toxicity.

Summary Biomonitoring data can provide useful information on the trends and magnitude of exposure to endogenous and xenobiotic chemicals, although utility of the data is ultimately dependent on several factors including the analytical specificity for the metabolite/analyte of interest, how representative a discrete sample (i.e., which provides insight on exposure) is of actual internal dose and/or body burden, and an understanding of the human pharmacokinetics. Looking forward, it will be increasingly helpful to place into context biomonitoring data with reference values to determine whether regulatory and public health initiatives are protective or if additional exposure mitigation efforts are warranted.