Data Sources and Data Analysis

The National Health and Nutrition Examination Survey (NHANES)

Biomonitoring measurements for the *Report* were made in samples from participants in NHANES. NHANES is a series of surveys conducted by CDC's National Center for Health Statistics (NCHS) that is designed to collect data on the health and nutritional status of the U.S. population. NHANES collects information about a wide range of health-related behaviors, performs a physical examination and collects samples for laboratory tests. NHANES is unique in its ability to examine public health issues in the U.S. population, such as risk factors for cardiovascular disease. Beginning in 1999, NHANES became a continuous survey, sampling the U.S. population annually and releasing the data in 2-year cycles. The sampling plan follows a complex, stratified, multistage, probability-cluster design to select a representative sample of the civilian, noninstitutionalized population in the United States.

The NHANES protocol includes a home interview followed by a standardized physical examination in a mobile examination center. As part of the examination component, blood is obtained by venipuncture for participants aged 1 year and older, and urine specimens are collected from people aged 6 years and older. Additional detailed information on the design and conduct of the NHANES survey is available at http://www.cdc.gov/nchs/nhanes.htm.

Environmental chemicals were measured in either blood or urine specimens collected as part of the examination component of NHANES. The age range for which a chemical was measured varied by chemical group. Most of the environmental chemicals were measured in randomly selected subsamples within specific age groups. Randomization of subsample selection is built into the NHANES design before sample collection begins. This subsampling was needed to ensure an adequate quantity of sample for analysis and to accommodate the throughput of the mass spectrometry analytical methods.

Age groups and sample sizes for each exposure measurement are provided in each of the tables of results. Blood lead and cadmium levels were measured in all people aged 1 year and older. Serum cotinine was measured in the entire NHANES sample for ages 3 years and older. Total blood mercury was measured in children aged 1-5 years and in women aged 16-49 years. Urine mercury was measured in women aged 16-49 years. Metals, phthalates, polycyclic aromatic hydrocarbons (PAHs), and phytoestrogens were measured in urine from a random one-third subsample of people aged 6 years and older.

Urinary levels of herbicides, selected pesticides, and metabolites of organophosphate pesticides were measured in a random one-half subsample of children aged 6-11 years in 1999 and 2000, a random one-quarter subsample of people aged 12-59 years in 1999, and a random one-third subsample of people aged 12 years and older in 2000. These chemicals also were measured in a random one-third subsample of people aged 6 years and older in 2001 and 2002. Dioxins, furans, polychlorinated biphenyls (PCBs), and organochlorine pesticides were measured in serum from a random one-third subsample of people aged 12 years and older in 1999 and 2000. In 2001 and 2002, dioxins, furans, and coplanar PCBs were measured in a random one-third subsample of people aged 20 years and older and organochlorine pesticides and other PCBs were measured in a random one-third subsample of people aged 12 years and older.

Data Analysis

Because the NHANES sample design is complex, sample weights must be used to adjust for the unequal probability of selection into the survey. Sample weights also are used to adjust for possible bias resulting from nonresponse and are post-stratified to U.S. Census Bureau estimates of the U.S. population. Data were analyzed using the statistical software package Statistical Analysis System (SAS) (SAS Institute Inc., 2002) and the statistical software package SUDAAN (SUDAAN Release 8.0, 2001). SUDAAN uses sample weights and calculates variance estimates that account for the complex survey design.

Guidelines for the analysis of NHANES data are provided by NCHS at http://www.cdc.gov/nchs/data/nhanes/nhanes_general_guidelines_june_04.pdf. These guidelines note that the previous analysis of 1999-2000 data used a jackknife method (available within SUDAAN) for variance estimation that was based on replicate weights. To better address multiple 2-year data sets and combining 2-year data sets into 4-year data sets, NCHS developed a new approach based on masked variance units that uses a Taylor series (linearization) method that is also available in SUDAAN. More details on this approach are provided in the analytical guidelines.

In the *Third Report*, all variance estimates (both 1999-2000 and 2001-2002 data) were calculated using the Taylor series (linearization) method within SUDAAN. In the *Second Report*, 1999-2000 variance estimates were calculated using the jackknife method (See Appendix C for details). The two methods produce very similar, but not identical, variance estimates. Consequently, some confidence intervals for 1999-2000 presented in the *Second Report* will differ slightly from confidence intervals for the same time period presented in the *Third Report*.

Selected percentiles and unadjusted geometric means of analyte concentrations are presented in tables and charts. Percentile estimates were calculated using SAS Proc Univariate using weighted data. Results are shown for the total population and also by age group, gender, and race/ethnicity as defined in NHANES. For these analyses, race/ethnicity is categorized as Mexican American, non-Hispanic black, and non-Hispanic white. Other racial/ethnic groups are sampled, but the proportion of the total population represented by other racial/ethnic groups is not large enough to produce valid estimates. Other racial/ethnic groups are included in estimates that are based on the entire population sample. Age groups are shown for each chemical in the results table. Gender is coded as male or female.

In the text (not in the tables), results are presented of comparisons of geometric mean levels for different demographic groups using analysis of covariance (ANCOVA), which included as covariates age, gender, race/ethnicity, urine creatinine and serum cotinine, as appropriate. ANCOVA allows for comparison of geometric means of two demographic groups after adjusting for these covariates. For example, when comparing geometric mean blood lead levels for adolescents to those for adults, the ANCOVA would first adjust the geometric mean blood lead level for adolescents for gender, race/ethnicity, and serum cotinine and also the geometric mean blood lead level for adults for gender, race/ethnicity, and serum cotinine. The ANCOVA was performed using SUDAAN with a significance level for statistical testing of $\alpha = 0.025$. These analyses were conducted separately for each two year survey period and differences for each survey period were not statistically compared.

Urine creatinine is included as a continuous variable in the ANCOVA for chemicals measured in urine to adjust for urinary dilution. Cotinine is a major metabolite of nicotine and a good indicator of smoking status. Therefore, log cotinine is also included as a continuous variable in ANCOVA analyses of dioxins; furans; PCBs; organochlorine pesiticides; PAHs; and the metals (lead, cadmium, mercury, antimony, barium, molybdenum, thallium) to adjust for known or probable effects of smoking on the levels of these chemicals in blood or urine, including the contribution of chemicals contained in smoke and the effect of chemicals in smoke on the metabolism of other measured chemicals. The decision to adjust for log cotinine was determined by whether log cotinine was a significant predictor of the chemical's concentration and results of research that examined cotinine as a predictive variable.

Concentrations less than the limit of detection (LOD) were assigned a value equal to the LOD divided by the square root of 2 for calculation of geometric means. The LOD is the level at which the measurement has a 95% probability of being greater than zero (Taylor, 1987). Assigning a value of the LOD divided by 2 made little difference in geometric mean estimates. Percentile estimates that are less than the LOD for the chemical analysis are reported as "< LOD." If the proportion of results below the LOD was greater than 40%, geometric means were not calculated. Appendix A contains a table of LOD values for each chemical. For the same chemical, LOD values may change over time as a result of improvements to analytical methods. One possible consequence is that results may be reported as "< LOD" in the 1999-2000 data but be reported as a concentration value above the LOD in 2001-2002 because the analytical method had improved. Thus, for proper interpretation, the LOD values in the tables of descriptive statistics tables should be referenced to the LOD table in Appendix A.

For most chemicals, the LOD is constant for each sample analyzed. For dioxins, furans, PCBs, organochlorine pesticides, and a few other pesticides, each individual sample has its own LOD. These analyses have an individual LOD for each sample, mostly because the sample volume used for analysis differed for each sample. A higher sample volume results in a lower LOD (i.e., a better ability to detect low levels). For these chemicals, the maximum LOD value is provided in the LOD table in Appendix A. The maximum LOD was the highest LOD among all the individual samples analyzed. In general, the mean LOD was about 40-50% of the maximum LOD.

The same procedure for imputing values below the LOD in calculations of geometric means was used for chemicals with individual LODs for each sample. That is, concentrations less than the individual LOD were assigned a value equal to the individual LOD divided by the square root of 2. For chemicals that had individual

sample LODs, a conservative rule was used for reporting percentiles: if any individual sample LOD in the demographic group was above the percentile estimate, the percentile estimate was not reported.

For chemicals measured in urine, separate tables are presented for the chemical concentration expressed per volume of urine (uncorrected table) and the chemical concentration expressed per gram of creatinine (creatinine corrected table). Geometric mean and percentile calculations were performed separately for each of these concentrations. LOD calculations were performed using the chemical concentration expressed per volume of urine, because this concentration determines the analytical sensitivity. For this reason, LOD results for urine measurements in Appendix A are in weight per volume of urine. In the creatinine corrected tables, a result for a geometric mean or percentile was reported as < LOD if the corresponding geometric mean or percentile was < LOD in the uncorrected table. So for example, if the 50th percentile for males was < LOD in the uncorrected table, it would also be < LOD in the creatinine corrected table.

For chemicals measured in serum lipid, separate tables are presented for the chemical concentration expressed per volume of serum (lipid unadjusted table) and the chemical concentration expressed per amount of lipid (lipid adjusted table). Geometric mean and percentile calculations were performed separately for each of these concentrations. LOD calculations were performed using the chemical concentration expressed per amount of lipid, because this concentration determines the analytical sensitivity. For this reason, LOD results for chemicals measured in serum lipid in Appendix A are in weight per amount of lipid. In the lipid unadjusted tables, a result for a geometric mean or percentile was reported as < LOD if the corresponding geometric mean or percentile was < LOD in the lipid adjusted table.