USE OF SERUM DATA TO EVALUATE EXPOSURE TO POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS: A CASE STUDY OF THE POTENTIAL INFLUENCE OF AGE, DETECTION LIMITS AND LIPID ASSAY METHODS

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Abstract
In 2007, blood levels of the seventeen 2,3,7,8-substituted PCDD/Fs were measured in 11 individuals aged 37-79 years who purportedly lived near an operating wood-treating facility. An initial evaluation of these data concluded that these individuals had clearly been exposed to "potentially unsafe levels of these contaminants;"[1] another analysis by the same author suggested that the community residents should be "evacuated."[2] However, as presented here, there were numerous shortcomings regarding the data analysis and results interpretation. First, the average limits of detection (LODs) for the PCDD/F congeners were 5-27 times higher for the cohort than for the corresponding NHANES population. As a result, fewer congeners were detected in the cohort (average: 3 congeners) relative to NHANES participants (average: 8 congeners). Second, although PCDD/F body burden increases with age, comparison of PCDD/F levels measured in the cohort with the referent NHANES population was not adjusted for the effect of age. Third, the cohort and NHANES lipid-adjusted concentrations were derived using very different lipid analytical techniques. To address these concerns, we reanalyzed both the cohort and NHANES data. We found that the PCDD/F toxic equivalence quotient (TEQ) in each cohort member was well within the TEQ range from the age-matched NHANES referent population.

Introduction
Human exposure to low levels of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) occurs frequently given the ubiquitous presence of these compounds in the environment. PCDD/F exposures are often assessed by measuring these substances in serum and other biological media (i.e., body fat or breast milk). Recently, advances in laboratory techniques have allowed scientists to measure extremely low levels (typically pg/g or parts per trillion) of these chemicals in human tissue samples. Nonetheless, the utility of PCDD/F serum data as an exposure assessment tool can be extremely limited, and even manipulated, if relevant characteristics of the referent and potentially exposed populations are not considered or the analytical methodologies utilized are not consistent between populations.

Accurately measuring small amounts of PCDD/Fs in tissue often depends on the age of the person (i.e. concentrations have been shown to be age-dependent and it is therefore easier to detect concentrations in older individuals[3-5]), the method used to prepare and analyze the sample, instrument performance, and the sample volume available for analysis (i.e., larger sample volumes typically allow for the measurement of smaller concentrations[6]). The LOD, which is typically used to approximate levels when the analytical instrument cannot measure a detectable concentration in a sample, can be influenced by all of these factors, with higher LODs resulting in higher "assumed" concentrations for samples with no "measureable" levels. In addition, accurate measurement of serum lipid content, which is necessary for proper lipid-normalization of PCDD/F concentrations, is also contingent on the analytical method used. Specifically, lipid analyses using enzymatic methods have been shown to produce higher values for serum lipid content than gravimetric methods.[7-11]

Here, we calculated lipid-adjusted serum TEQ concentrations, based on the 17 2,3,7,8-substituted PCDD/F congeners, for 11 individuals who purportedly lived near a wood treatment plant (the "cohort") and compared them with levels estimated for the general U.S. population using the 2003-2004 NHANES data. Using these data we demonstrate the importance of proper analysis of the NHANES data in addition to the consequences of not adjusting for age or considering differences in analytical methodologies.

Materials and Methods

As reported by Tam et al. (2008)[1] the serum of 11 individuals between the ages of 37 and 79 years was collected in May 2007 from the cohort. The samples were analyzed for PCDD/Fs and lipid content by Severn Trent Laboratories in Sacramento, CA. U.S. EPA Method 8290 was used to determine concentrations of PCDD/Fs, and lipid content was measured gravimetrically. The total TEQ for each individual subject was calculated by summing the product of each lipid-adjusted PCDD/F congener concentration and the associated 2005 WHO TEF.[12] Samples with concentrations below the LOD (C_{ND}) were assumed to have a concentration equal to the lipid-adjusted LOD divided by the square root of two ($C_{ND} = LOD/\sqrt{2}$), which is the same proxy value used for non-detected concentrations in the NHANES data. To adjust for the effect of using a gravimetric method to measure lipid content, rather than the enzymatic method utilized by the Centers for Disease Control and Prevention (CDC) for the NHANES surveys, we multiplied total TEQ concentrations by a factor of 0.558.[7]

Reference values for concentrations of PCDD/Fs measured in the general U.S. population were developed using data collected by the CDC and National Center for Health Statistics (NCHS) as a part of the 2003–2004 NHANES survey.[13] Statistical analyses methods have been described previously in detail by Ferriby et al. (2007)[3] and Scott et al. (2008).[4] Briefly, the total TEQ for each participant was calculated by summing the product of each lipid-adjusted PCDD/F congener concentration and the associated 2005 WHO TEF.[12] Further details regarding the NHANES procedures, survey components, questionnaires, and examination are available at the CDC's National Center for Health Statistics website: www.cdc.gov/nchs/nhanes.htm. Weighted TEQ summary statistics (including geometric means and 25th, 50th, 75th, and 95th percentiles) were calculated for non-Hispanic white and black survey participants by age group. Because LODs are not reported in the NHANES data file, they were calculated only for those participants noted to have a concentration below the LOD using the equation $LOD = C_{ND} * \sqrt{2}$. All analyses of the NHANES data were completed using SAS (Cary, NC) and SUDAAN (Research Triangle Park, NC) statistical software packages.

Results and Discussion

As illustrated in Figure 1, the percentage of individuals with PCDD/F concentrations below the LOD varied significantly between the cohort and the 2003-2004 NHANES population for almost all congeners (Figure 1). Indeed, most PCDD/F congeners were not detected in the cohort andonly OCDD was detected in all 11 individuals. With the exception of OCDD, average LODs for the cohort ranged from 5 to 27 times higher than the LODs for the NHANES population (Figure 3). These discrepancies clearly had an impact on the number of congeners detected for each individual of the cohort being well below the average number of congeners detected in the NHANES population (Figure 2).

As a result of the high LODs and wide range of ages in the cohort, total TEQ distributions varied considerably between the cohort and the NHANES population. In particular, the distribution of concentrations for all of the cohort members (i.e. individuals 37+ years of age) was not representative of specific age groups within the cohort, and thus, significantly underestimated measures of central tendency for the older individuals (Figure 4). Although concentrations for the cohort appeared to be elevated relative to NHANES, this difference was solely an artifact of the large percentage of individuals in the cohort with PCDD/F concentrations below the LOD combined with the relatively high analytical LODs in this dataset. These data disparities artificially inflate the estimates of total TEQ in the cohort. When only the detected congeners were considered (i.e. $C_{ND} = 0$), total TEQ concentrations measured in the cohort were well within the ranges of age-specific concentrations for the NHANES population (Figure 5). Moreover, adjustment of total TEQ concentrations for the cohort to reflect the use of gravimetric methods for determining lipid content further reduced total TEQ levels in this group (Figure 6).

These results demonstrate not only the insensitivity of the analytical methods and instruments used by the laboratory that conducted the analyses to detect concentrations of PCDD/Fs in the cohort, but also the effect of these limitations on interpretation of the data and conclusions regarding exposure of this cohort to PCDD/Fs. Specifically, the elevated limits of detection increased the number of individuals with non-detected concentrations, thereby resulting in overestimated total TEQ concentrations. In addition, we have shown the importance of using similar methods for determining serum lipid content when comparing lipid-normalized concentrations of PCDD/Fs in a potentially exposed group with a referent population. Certainly, if different

methods for measuring lipid levels are utilized, the data can be corrected to increase the accuracy of comparisons to NHANES concentrations. Just as notable, to ensure precise assessments of exposure using PCDD/F biomonitoring data, concentrations must be adjusted for age as numerous studies have shown that body burden increases significantly with age.[3-5,14-20] This is particularly important in potentially exposed cohorts that have a wide range of ages.

Figure 1: Percentage of Individuals with PCDD/F Levels below the LOD.

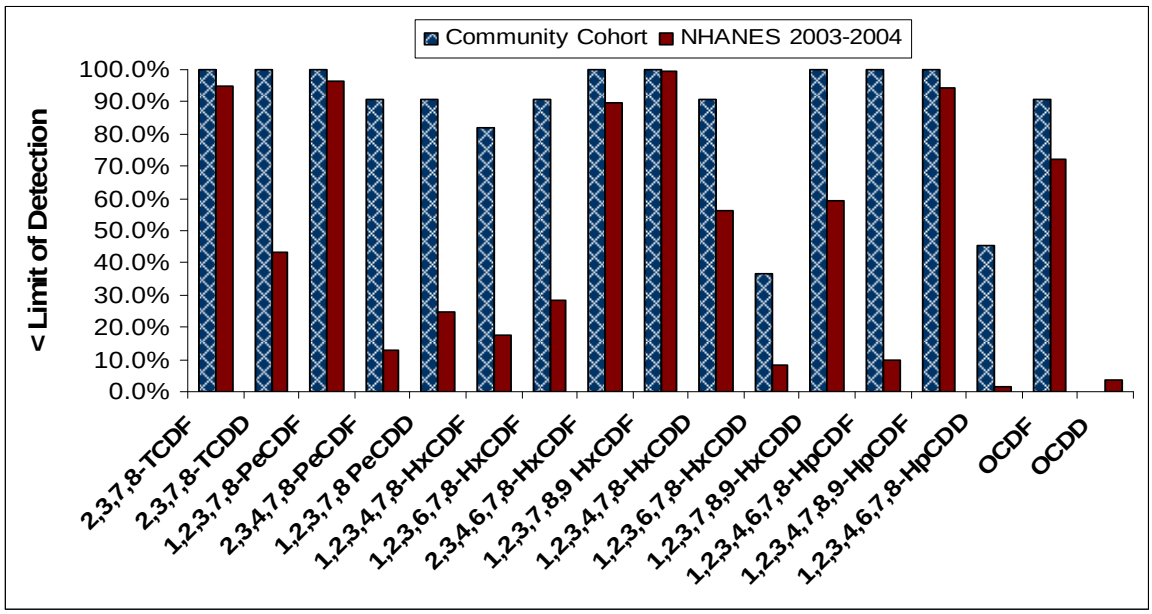


Figure 2: Number of Detected Congeners for Each Participant of the Cohort and Average Number of Detected Congeners for the Cohort and NHANES Population.

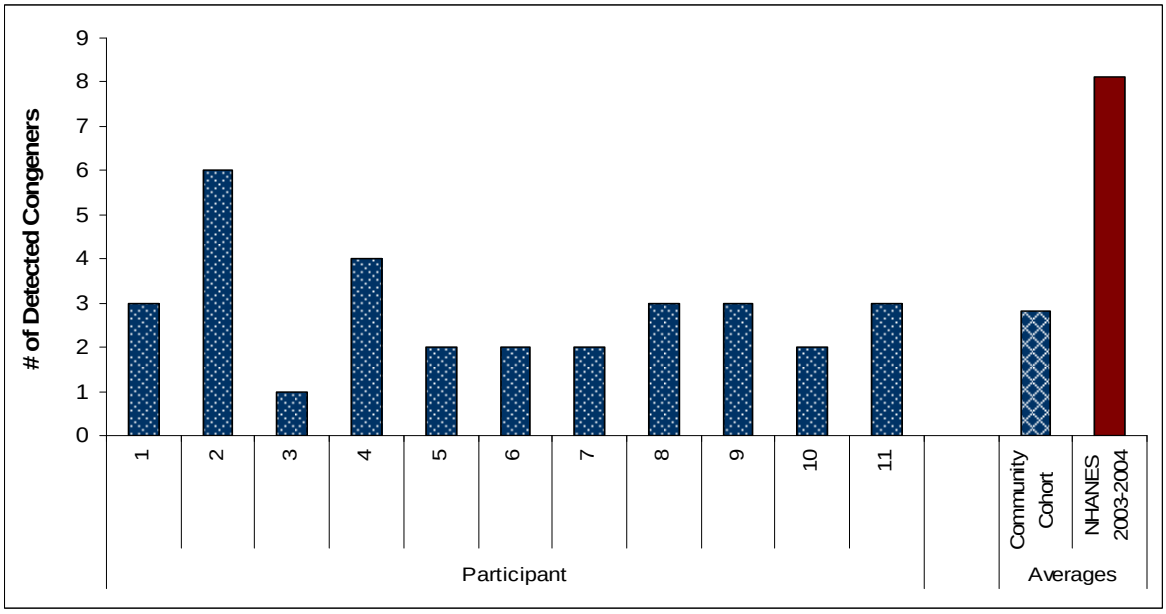
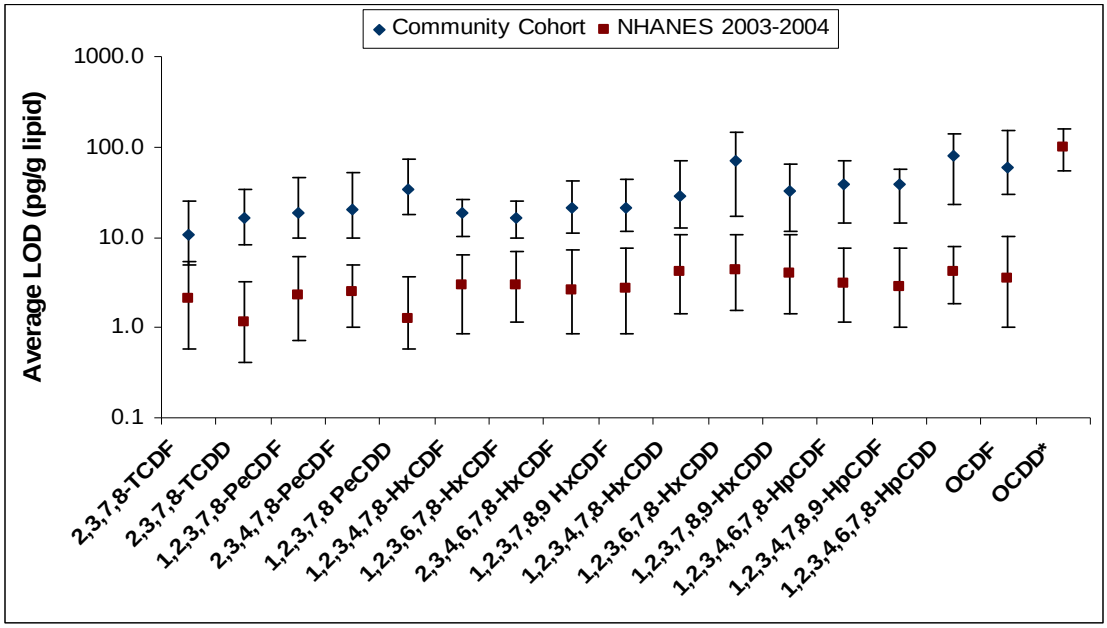
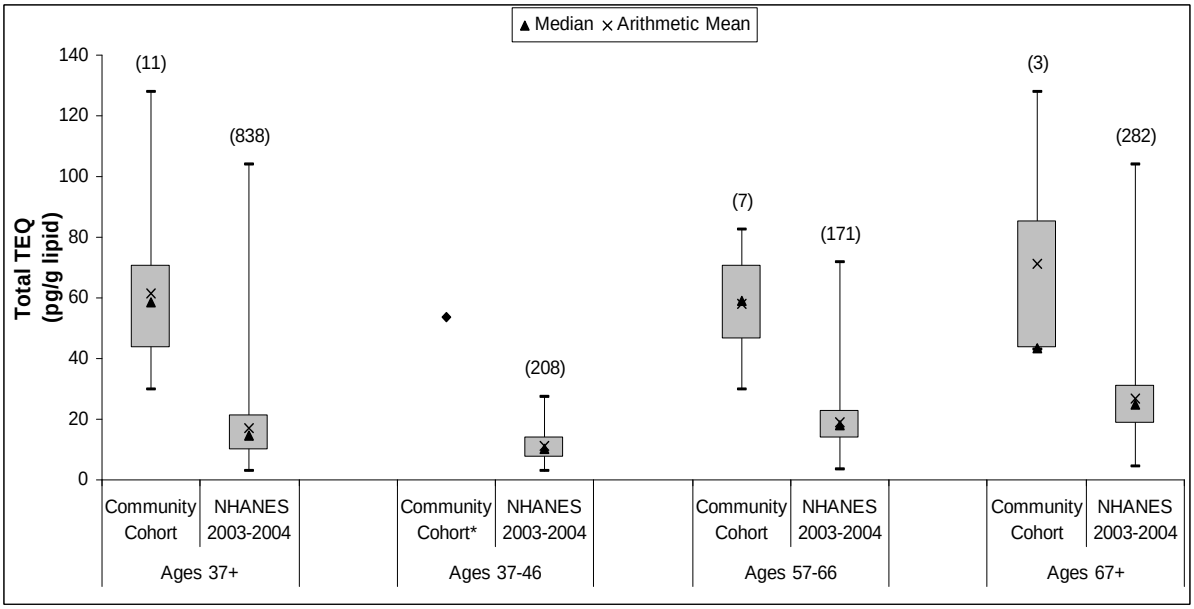


Figure 3: Average, Minimum, and Maximum Limits of Detection by Study Population (pg/g lipid).



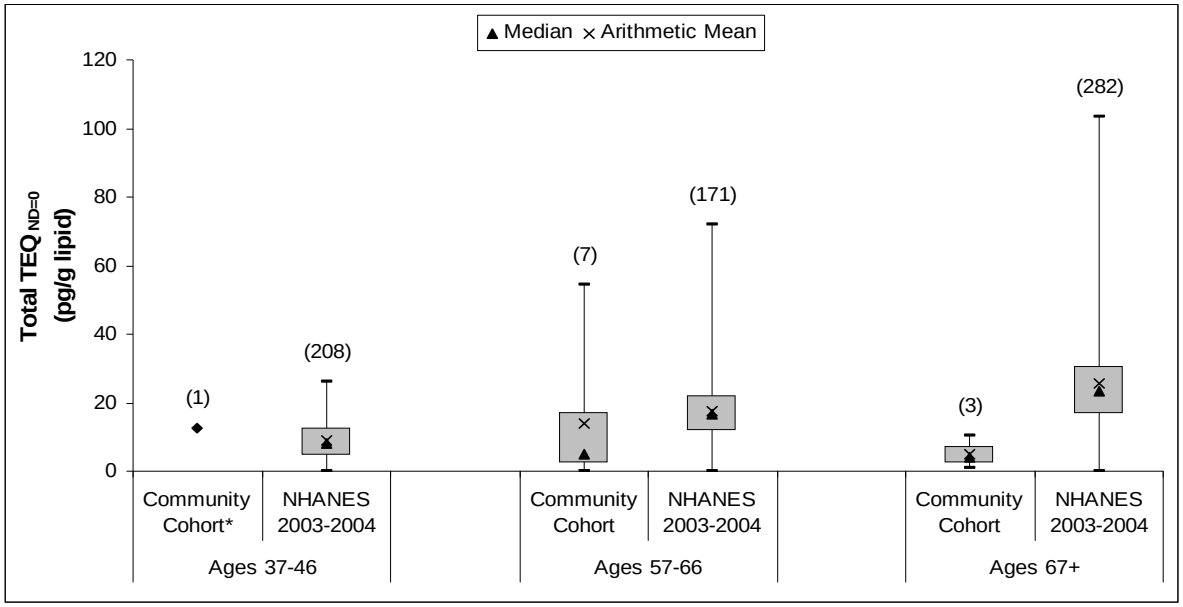
*For the 2003-2004 NHANES participants aged 37+ years, the number of non-detects for OCDD was only 29 out of 838 people. No LOD for the Cohort was reported as all individuals had measurable concentrations.

Figure 4: Distribution of Total TEQ Concentrations by Age Group when Non-detected Values Were Imputed Using LOD/SQRT(2).



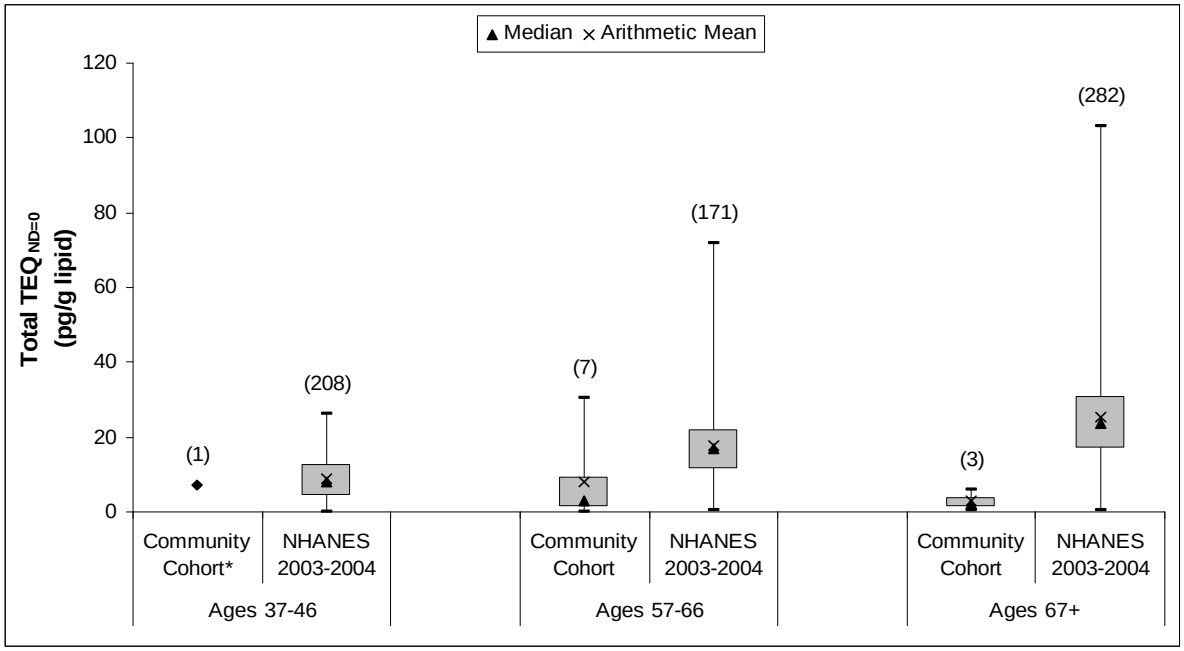
*Data point presented is total TEQ concentration for the single individual in this age group. Sample numbers are provided in parentheses. Statistics presented include minimum; 25th, 50th, and 75th percentiles; arithmetic mean; and maximum.

Figure 5: Distribution of Total TEQ Concentrations by Age Group when Non-detected Concentrations Were Assumed To Equal Zero.



*Data point presented is total TEQ concentration for the single individual in this age group. Sample numbers are provided in parentheses. Statistics presented include minimum; 25th, 50th, and 75th percentiles; arithmetic mean; and maximum.

Figure 6: Distribution of Total TEQ Concentrations by Age Group when Non-detected Concentrations Were Assumed To Equal Zero. Concentration Data for the Cohort Have Been Adjusted for the Use of Gravimetric Methodology to Measure Lipid Content.



*Data point presented is total TEQ concentration for the single individual in this age group. Sample numbers are provided in parentheses. Statistics presented include minimum; 25th, 50th, and 75th percentiles; arithmetic mean; and maximum.

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References
1. Tam L.K., Wu C.D., Clark J.J. and Rosenfeld P.E. *Organohalogen Compounds* 2008;70:2252.
2. SWAPE (Soil Water Air Protection Enterprise).*Expert Report and Affidavit on Environmental Contamination and Community Exposure from the Former Durawood Treating Company Facility.* June 16, 2008.
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.
4. Scott L.L.F., Unice K.M., Scott P., Nguyen L.M., Haws L.C., Harris M. and Paustenbach D. *J Expo Sci Environ Epidemiol* 2008;18:524.
5. Lakind J.S., Hays S.M., Aylward L.L., Naiman DQ. *J Expo Sci Environ Epidemiol* 2009;19:435.
6. CDC (Centers for Disease Control and Prevention). *Third National Report on Human Exposure to Environmental Chemicals.* US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Environmental Health: Atlanta, GA, 2005 NCEH Pub. No. 05-0579.
7.. Mills W., Nienow C., Swetman G., Cox R., Tondeur Y., Webber J. and Leblanc A. *Organohalogen Compounds* 2007; 69:1158.
8. Covaci A., Voorspoels S., Thomsen C., Bavel B.V. and Neels H. *Sci Total Environ* 2006;366:361.
9. Pascal M., Eppe G., Zeghnoun A., Falq G., Focant J., Pauw E.D., Massart A. and Frery N. *Organohalogen Compounds* 2007;69:2284.
10. Akins J.R., Waldrep K. and Bernert J.T. *Clin Chim Acta* 1989;184:219.
11. Grimvall E., Rylander L., Nilsson-Ehle P., Nilsson U., Stromberg U., Hagmar L. and Ostman C. *Arch Environ Contam Toxicol* 32:329.
12. Van den Berg M., Birnbaum L.S., Denison M., DeVito M., Farland W., Freely M., Fiedler H., Hakansson H., Hanberg A., Haws L., Rose M., Safe S., Schrenk D., Tohyama C., Tritscher A., Tuomisto J., Tysklind M., Walker N. and Peterson R.E. *Toxicol Sci* 2006;93:223.
13. NCHS (National Center for Health Statistics). *National Health and Nutrition Examination Survey Data*. US Department of Health and Human Services, Centers for Disease Control and Prevention: Hyattsville, MD, 2005.
14. Bates M.N., Buckland S.J., Garrett N., Ellis H., Needham L.L., Patterson D.G., Turner W.E. and Russell D.G. *Chemosphere* 2004;54:1431.
15. Falk C., Hanrahan L., Anderson H.A., Kanarek M.S., Draheim L., Needham L. and Patterson D. *Environ Res* 1999;80: S19-S25.
16. Kang D., Tepper A. and Patterson D.G. *Chemosphere* 1997;35:503.
17. Kiviranta H., Tuomisto J.T., Tukiainen E. and Vartiainen T. *Chemosphere* 2005;60:854.
18. Orban J.E., Stanley J.S., Schwemberger J.G. and Remmers J.C. *Am J Public Health* 1994;84:439.
19. Patterson D.J., Patterson D., Canady R., Wong L., Lee R., Turner W., Caudill S., Needham L. and Henderson A. *Organohalogen Compounds* 2004;66:2878.
20. Patterson D.J., Turner W., Caudill S.P. and Needham L.L. *Organohalogen Compounds* 2005;67:1577.