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ANALYTICAL CHALLENGE: THE QUANTIFICATION OF BACKGROUND LEVELS OF PCDDs, PCDFs, and cPCBs IN THE UNITED STATES GENERAL POPULATION

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Introduction

Aylward and Hays[1] recently compiled from the literature background body burden data on lipid-adjusted levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in populations from the United States, Canada, Germany, and France from studies over the past 30 years. They reported that mean TCDD levels decreased by almost a factor of 10 during this period, with TCDD levels in 2000 about two parts per trillion (ppt or pg/g fat). They further observed that data for other polychlorinated dibenzodioxins (PCDDs), furans (PCDFs), and co-planar biphenyls (cPCBs) congeners have decreased in recent years, with total toxic equivalents (TEQ) lipid levels in 2000 at least fourfold lower than in 1970. Jackson and Mickhalek[2] also observed further evidence for this temporal trend in the decline in body burden in a study that measured TCDD levels in 1987, 1992, and 1997 in a "control" group of U.S. Air Force veterans, in which the mean TCDD of 1419 persons decreased from 4.5 to 2.0 ppt over the 10-year period. Choi et al.[3] reported a similar trend for PCDDs/PCDFs/cPCBs in Japanese adipose tissue collected in 1970-1971, 1994-1996, and 2000, in which the mean TEQ/g fat decreased from 31.5 ppt in 1970-1971 to 11.9 ppt in 2000. These findings are consistent with the assumption that regulatory efforts over the past several decades aimed at reducing human exposure in the environment, the food supply, consumer products, and the workplace are having beneficial effects. In 2000, the U.S. Environmental Protection Agency (EPA) reported that the release of "dioxin-like" compounds into the environment decreased by almost 80% during 1987-1995.[4] Diet (meat, fish, and dairy products) is now considered the primary source of background exposure.[5, 6] Based on known pharmacokinetics in humans, Aylward and Hays[1] predict that mean TCDD levels in the general U.S. population will decrease to 0.5-1 ppt by 2015, which represents a fourfold reduction from current levels, even if intake levels do not decrease further. As body burdens continue to decline, their measurement, will become even more of an analytical challenge. Presented here are observations regarding some of the variables that can influence the quantification of "background" levels of these environmental toxicants in human serum.

Methods and Materials

Sample Preparation

Serum samples were prepared according to the procedure reported by Turner et al.[7] Samples were spiked with $^{13}C_{12}$-labled internal standards followed by C_{18} solid-phase extraction (SPE) and a multicolumn automated cleanup and enrichment procedure using a Fluid Management Systems Power-Prep/6. An analytical run comprised one method blank, nine unknown samples, and two quality control samples. PCDDs/PCDFs/cPCBs were eluted from the AX-21 carbon columns in the reverse direction with toluene. One µL of dodecane "keeper" was added to the eluants and

solvent evaporated to about 350 µL using a Zymark TurboVap II. Residual toluene was transferred to silanized autosampler vials and evaporated to one µL. Before analysis by high-resolution gas chromatography (HRGC) and high-resolution mass spectrometry (HRMS), vials were reconstituted with 5-µL of ^{13}C-labeled external standard in nonane.

Mass Spectrometry

A Leap Technology GC Pal autosampler was used to make 2-µL injections into an Agilent 6890 gas chromatograph (GC). The GC was operated in the splitless injection mode with a flow of 1 mL/min He through a DB-5ms column (30 m x 0.25 mm x 0.25 µm film). Selected congeners were quantified by isotope-dilution MS using selected ion monitoring (SIM) at 10,000 resolving power (10 % valley) on a Thermo Finnigan MAT 95 XP (5kV) magnetic sector field mass spectrometer operated in the electron impact (EI) mode at 40 eV.[8] Two HRMS quantification schemes were employed: one for measuring TCDD only in one multiple-ion detection (MID) group, used for U.S. Air Force Ranch Hand and Seveso, Italy, studies; and another for TCDD in one of six MID groups used for measuring all seventeen 2,3,7,8-substituted PCDDs/PCDFs and four cPCBs . Additional HRGC/HRMS analyses were performed on the MAT 95 XP after the installation of the Thermo Finnigan low-noise ion-detection system or "sensitivity" upgrade (part # 1150760).

Results and Discussion

Each day after conducting operator maintenance and tuning the MAT 95 XP, a sensitivity check is performed by injecting 20 fg of TCDD standard onto the DB-5ms column. To standardize instrument performance from day to day, the observed signal-to-noise (S/N) ratio on raw data for the 319.8965 mass has to be at least 15:1 (one MID group) to continue analyzing standards and samples. After installation of the new low-noise ion-detection system, we observed a fourfold improvement in the daily sensitivity check (S/N ratio > 60:1). This improvement is consistent with the assertions made by Thermo Finnigan (S/N > 400:1 for 100 fg or >40:1 for 10 fg TCDD mass 321.8939; one MID group). We inferred that the fourfold increase observed in S/N ratio was based on the stated reduction of detector or electronic noise.

To evaluate whether the improved sensitivity obtained with standards could be achieved with serum extracts, we reanalyzed a number of 10-g serum extracts after installation of the low-noise ion-detection system. We did not observe any obvious improvement in S/N ratio for TCDD (six MID groups). We concluded that, with our cleanup method, the S/N for samples is affected more by chemical noise and serum matrix effects than by the reduction in electronic noise from the new ion-detection system.

In population studies, background levels often are close to the limit of detection (LODs). LOD is the point at which a measured value becomes larger than the uncertainty associated with it–or the ability to distinguish between "signal" and "noise." We determined the LOD for TCDD on the MAT 95 XP using an extrapolation method proposed by Taylor[9] based on repetitive measurements for one MID group and six MID groups. Extrapolated estimates for one MID group were based on at least 10 repetitive measurements for TCDD using 0.01, 0.02, 0.05, 0.1, 0.2, and 0.5 pg/µL standards and for TCDD with six MID groups using 0.04, 0.1, 0.2, 1, and 2 pg/µL standards. The resulting statistical LODs are actually instrumental detection limits (IDL). To make these IDLs more applicable, we further used IDLs to compute method detection limits (MDL) by converting pg/µL to ppt TCDD on a lipid-adjusted basis (pg/g lipid) for various sample sizes, assuming a average 70% recovery through cleanup and 0.6 % total lipid (Figure 1).

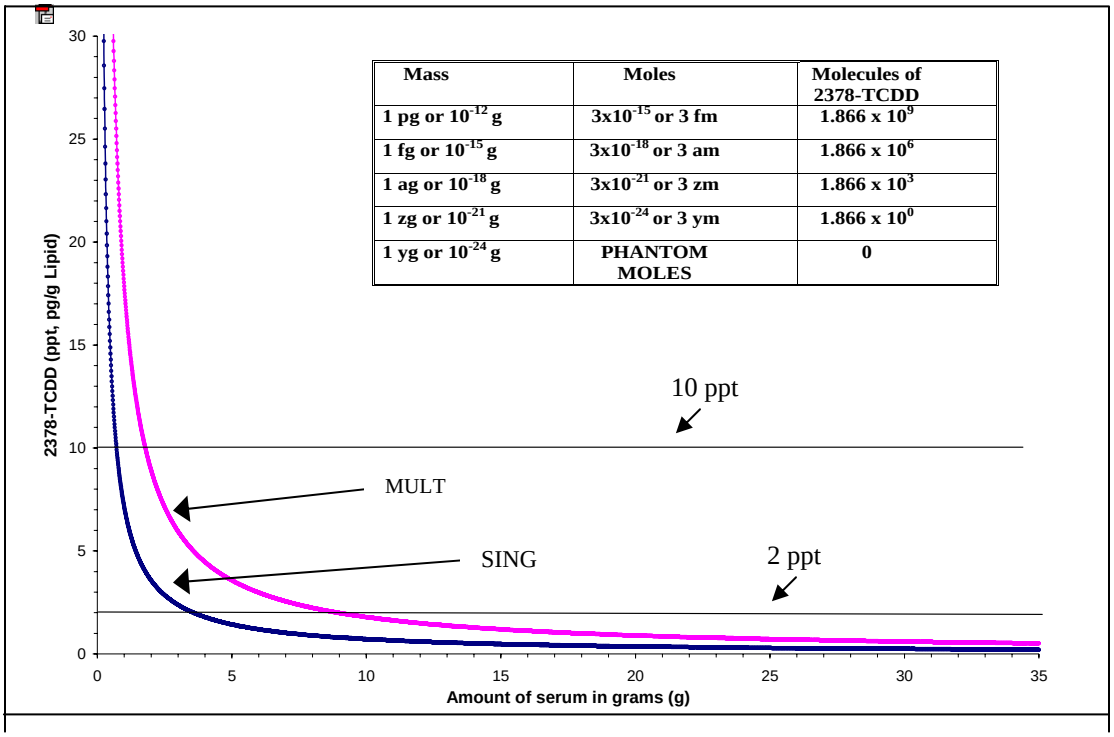

Figure 1: Method detection limits (MLD) for TCDD as function of sample size in grams (assuming 70% recovery and 0.6% total lipid) for one MID group (SING) and six MID groups (MULT).

As mentioned earlier, current mean background levels of TCDD in the United States are about two ppt. Two ppt (pg/g lipid) is equal to 12 fg/g or parts per quadrillion (ppq) on a whole-weight basis (assuming 0.6% total lipid) for a total of 120 fg of TCDD in a 10-g sample. Quantification of 12 ppq is tremendously challenging, considering it is equivalent to measuring 12 seconds in 32,000,000 years. However, during analysis, only 2/5 of the sample or 48 fg (two of five µL in vial) is actually injected into the GC. Regardless of what the frequency distribution of background TCDD values might be in a population (e.g. log normal), a sizeable proportion of the values would be below the mean. Figure 1 shows the amount of serum required for various TCDD MDLs: [9 mL] 2 ppt, [18 mL] 1ppt, [35 mL] 0.5 ppt, and (not shown [70 mL] 0.25 ppt, [90 mL] 0.20 ppt and 180 mL 0.10 ppt) using six MID groups. Figure 1 also shows the amount of serum required for various TCDD MDLs: [9 mL] 0.8 ppt, [18 mL] 0.4 ppt, [35 mL] 0.2 ppt, and (not shown [70 mL] 0.10 ppt, [90 mL] 0.08 ppt and 180 mL 0.04 ppt) using one MID group.

In addition, we frequently have encountered another analytical problem affecting MDLs. As the sample volume available for analysis decreases, method blanks can exceed the MDL for congeners such as 1234678D, 1234678F, and OCDD with OCDF and 3,3',4,4'P (PCB 77) being particularly problematic. MDLs for congeners with positive blanks were calculated as described by Keith.[10] For some studies with small sample volumes, the blank derived MDLs for OCDF and PCB77

often have exceeded the levels in samples, resulting in "nonreportable" data. These method blank problems are consistent with Taylor's prediction that at concentrations of 1 part in 10^{18} almost any substance can be expected to be present in any sample.[9]

Conclusion: In practice, only HRMS instruments have demonstrated the required specificity and sensitivity to measure low femtogram amounts of PCDDs/PCDFs/cPCBs in human samples.[11, 12] Even so, this specificity and sensitivity would not be possible without the application of rigorous sample cleanup (i.e., alumina and AX-21 carbon) and substantial analyte enrichment. Nonetheless, given the significant contribution that matrix effects, chemical noise, or background noise from positive blanks, a fourfold reduction in electronic noise will not predictably improve MDL. Therefore, obtaining an adequate volume of serum becomes obligatory for quantification of background levels. While performing the analyses for a recent study of non occupationally exposed New Zealanders,[13] we quantified TCDD levels below 1 ppt (six MID groups) , achieving MDLs of 0.7 ppt for 25 mL to 0.35 ppt for 50 mL of serum. However, we were unable to determine mean and selected percentile serum concentrations of TCDD for the U.S. population aged 12 years and older for samples from the National Health and Nutrition Examination Survey, 1999-2000[14] because of insufficient sample volume (average MDL was 4.8 ppt with SD 1.8 ppt).

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