

Letters to the Editor

Correspondence re: S. D. Stellman *et al.*, Relative Abundance of Organochlorine Pesticides and Polychlorinated Biphenyls in Adipose Tissue and Serum of Women in Long Island, New York. *Cancer Epidemiol. Biomark. Prev.*, 7: 489–496, 1998

Letter

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Recent concern about potential adverse health effects of chemicals in the environment has placed great pressure on the analytical chemist to provide instant and unequivocal solutions to problems relating to the detection and determinations of trace levels of environmental chemicals. Within the past few years, chemists have been involved in epidemiological studies that attempt to assess possible environmental chemical associations with adverse human health outcomes. The chemist faces the monumental task of determining how to communicate with the epidemiologists/statisticians with whom they are collaborating. At the heart of this communication is the report of residue values and values at or below the analytical chemistry MDLs.² What is needed at the onset is a clear and consistent definition of terms. Most laypersons and many scientists view analytical chemistry as a discipline in which one can routinely make highly accurate and very precise measurements. These data are expected to be without bias and very reproducible when they are obtained performed by trained chemists using modern, state-of-the-art instrumentation. However, in the area of quantitative trace analysis, the uncertainties become relatively large and, in some cases, are nearly the same size as the concentration of the chemical residues that are being measured. In any given laboratory, there are many factors that could lead to either low or high results of the chemicals of interest. The overall effect in any laboratory analysis is that when quantitating residues at trace levels the coefficient of variation may be very large.

As an excellent example of the difficulties in relating trace residue data to epidemiological/statistical interpretation, we have a few comments and general statements concerning the recent paper by Stellman *et al.* (1), published in the June 1998 issue of *Cancer Epidemiology, Biomarkers & Prevention*. Stellman *et al.* (1) proclaim the virtues of their “ability to detect with confidence very small concentrations of OCP/PCBs [organochlorine pesticide/polychlorinated biphenyls], especially in serum, is attributed in part to exploitation of analytical methods based upon advanced chromatographic technology.” However,

as an analytical technique, the identification of unknown GC peaks having poor resolution or that do not have overlapping peaks on one analytical column, has, for many years, been resolved by identifying and separating these peaks using at least two analytical columns with different polarities (2–4). These columns are selected and used in pairs: if peaks do not resolve on one column (the primary column), the secondary column can resolve the nonseparation of the primary column. It has been a common practice, used by most laboratories, to select at least two GC columns having different separation characteristics and to use them together (5, 6, 7). It is unrealistic to believe that using only one GC column that a laboratory would be able to separate all of the environmental chemicals that have ever been produced. Therefore, it is not “advanced chromatography technology” to use at least two GC columns with different separation characteristics to help identify many chemicals that contaminate the environment today. A sound analytical laboratory quality program would dictate these kinds of procedures and detail procedures that allow for the selection of at least a “working pair” of GC columns, a primary GC column and a secondary GC column. This has been routinely used and continues to be used by most laboratories that practice good laboratory quality control procedures (8).

Another good example of the difficulties that chemists face in communicating trace residue level data to other collaborating scientists is in the area of environmental chemicals and their possible association with breast cancer. Recently, analytical chemists have been asked to respond to epidemiological studies that have attempted to associate residue data with adverse human health outcomes, such as breast cancer. These residue data demand analytical determinations at levels that optimize not only the analytical methodology but also the analytical instrumentation. In the majority of these studies, the residue data are “trace analysis.” Trace analysis differs from conventional analytical procedures because of the requirements imposed by the very small amount of sample for analyses and the extremely small quantities of chemicals being determined. Trace analysis methods require not only special attention to small details, such as special cleaning of glassware and purified reagents and solvents but also very careful attention to the formulation and strict adherence to a sound laboratory quality program (9, 10). These requirements are so exacting that these procedures may be regarded as qualitatively different from the usual type of analysis.

Establishing a continuing program to ensure the reliability and validity of the analytical laboratory data produced is a fundamental responsibility of laboratories that report trace residue data. This quality program should begin at the conception of a project, follow through collection and storage of samples, include all phases of chemical and physical analyses, and ex-

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² The abbreviations used are: MDL, method detection limit; GC, gas chromatography; MQL, method quantitation limit.

tend through interpretation and final publication of results. In current epidemiological studies, the epidemiologist should, at the outset of the formulation of the quality program for the project plan, consult and collaborate with the analytical laboratory. The quality program must involve the planned and systematic actions necessary to provide confidence in each analytical result reported by the analytical laboratory. Careful attention to the fine details could possibly affect and cause considerable differences in the final results of the study. Some of these fine details that need to be considered at the very beginning of the study are: how the samples are collected, how the samples are stored, and how much sample the laboratory needs to analyze for the epidemiological/statistical inferences that are going to be made at the completion of the study. All of these items are critical and make a substantial difference in the final results. The key and fundamental concepts to a reliable quality program are: quality control and quality assessment. The quality program is a broad general "umbrella" term for a system of activities that provides the laboratory and the user of the analytical data the assurance that the data meet defined standards of quality. Quality control is a system of activities that control the quality of the analytical data that meet the needs of the user. This system aims to produce data that is satisfactory, adequate, and dependable. Such things as education, training, and instrument calibration/maintenance should be included in the quality control aspects. Quality assessment is the system of activities that provides assurance that the quality control activities are performed effectively. These provide a continuing evaluation of the analytical system performance and ensure that quality data are produced. Such things as performance evaluation samples, reference materials, and plotting control charts are included in quality assessment. A good quality program should have two simultaneously operating features: the intralaboratory and the interlaboratory procedures.

The quality program should define very important and critical terms that the laboratory will be using to report the data. Such terms as sensitivity, MQLs, and MDLs should be defined, understood, and agreed upon by both the epidemiologists/statisticians and the chemists/toxicologists before any samples are collected. If these terms are defined before the analysis of the data, it will eliminate confusion, save much time at the end of the project, and allow for proper assessment and clear interpretation and reporting of the data. There have been many terms used to designate analytical data and they have been defined in various ways. Most authorities in the field agree that the smallest detectable quantity, by whatever name, is related to the SD of sample analyses at or near zero analyte concentrations. These terms are basic performance characteristics of an analytical method and should be calculated for every analyte of interest. It makes no sense to state a very low analytical limit without showing how the value is calculated and then supporting that value with sound and defensible quality control data.

Archibeque *et al.* (11, 12) have described a quality assessment/quality control program that will produce reliable residue data. Reliable and appropriate surrogate material was prepared and information was described to use in spiking and tracking of routine samples analyzed in a monitoring program. We have defined our analytical operating system as follows:

(a) Sensitivity can be defined as the smallest amount of a pure standard that can be detected by the instrument used, in most cases, the gas chromatograph. The sensitivity value does not have any correlation to sample size.

(b) MQL can be defined as the smallest amount of a chemical that can be consistently quantified after wet weight adjustment. The wet weight adjustment must be made to allow for sample sizes that differ, for example, if the analytical

method is based on analyzing 1.0 g of serum sample and only 0.834 g of serum is provided for the analyses. The MQL takes into account the amount of sample that is weighed out for the extraction procedure and the final volume of the extract before the instrumental determinations are performed.

(c) MDL is the smallest amount of a chemical that can be detected but not consistently quantified after wet weight adjustment. The MDL is basically the sensitivity of the instrument for the individual chemical divided by the amount of sample analyzed.

It is critical that the readers understand the differences in the way that scientists report analytical data. Terms are not consistent between laboratories and studies; therefore, careful interpretation of the terminology is needed. A measured value becomes believable only when it is larger than the uncertainty associated with it. We suggest that the analytical community standardize analytical terms so that residue data can be more easily compared.

We in no way wish to disparage the work of Stellman *et al.* (1). Admittedly, the quality program and analytical methodology that was defined in their publication is not within our laboratory's fundamental operating philosophy. We, as scientists, have an obligation to work collaboratively and, in so doing, should share each others ideas and thoughts. This collaboration is essential and must take place in the very beginning of the project planning before any samples are collected. Only if this collaborative effort takes place can any assessment of possible associations of body burdens of these environmental chemicals can be made.

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Reply

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We apologize for any possible misunderstanding or misreading of the work of Dr. Tessari and his colleagues, who have made substantial contributions to the application of quality assurance/quality control procedures in the analysis of organochlorine compounds (1, 2). The methodological issues addressed by Tessari and Archibeque-Engle (3) will assume increasing importance as environmental levels and body burden of long-banned substances such as DDT² in potentially exposed populations continue to decrease over time (4–6) and as attention focuses on the smaller concentrations of biologically defined groups of PCB congeners rather than total PCBs (7, 8).

Our laboratory subscribes to the principles of quality assurance promulgated by the American Chemical Society (9). We completely agree with the need to use consistent definitions of analytical terminology. Our LODs are patterned on the IUPAC and American Chemical Society definition as the smallest concentration that is statistically different from an analytical blank (10). Our reported LOD are consistent with those reported by Wolff *et al.* (11), who used similar volumes of serum (2–5 ml). Concentrations of 25 ppb refer to the level of the internal standard, γ -chlordane, needed to assess recovery; an analysis was considered acceptable only when recovery exceeded 90%. Calibration curves were established with concentrations of analytical standards of the same order of magnitude as in the human samples.

We agree that a cooperative effort is needed to determine why our respective results do not agree more closely. From a panel of nine PCB congeners, Archibeque-Engle *et al.* (12) reported detectable levels of two congeners (BZ153 and BZ118) in fewer than half of their 36 serum samples; with the exception of DDE in all but one sample and β -hexachlorocyclohexane in a single sample, none of the other six pesticides were quantifiable, so that serum adipose correlations could not be computed for the majority of analytes. In contrast, we reported detectable levels for 6 of 7 pesticides and 10 of 14 PCB congeners, including BZ153 and BZ118, in the majority of our serum samples (13). As reported in Table 6 of our paper (13), statistically significant correlations between serum and adipose were observed for 11 of 14 PCB congeners and for all 7 organochlorine pesticide residues even after excluding observations below LOD.

Many studies besides ours have reported strong correlations between adipose and serum levels of organochlorine compounds of interest as possible carcinogens. Needham *et al.* (14) and Sasaki *et al.* (15) have reported correlations of >0.5 for an array of organochlorine pesticides, which includes β -hexachlorocyclohexane, dieldrin, heptachlor epoxide, hexachloroben-

zene, oxychlordane, DDE, and *trans*-nonachlor; moreover, Wolff *et al.* (16) reported $\rho = 0.70$ for those PCBs that elute on a GC column later than DDE and $\rho = 0.98$ for those eluting before DDE.

Many authors have also reported significant correlations between adipose and serum concentrations of related compounds such as DDT ($\rho = 0.8$, Ref. 17), Mirex ($\rho = 0.8$, Ref. 18), TCDD ($\rho = 0.98$, Ref. 19; $\rho = 0.94$, Ref. 20), and PBBs ($\rho = 0.8$ – 0.9 , Ref. 21). These data are more in agreement with the statement of Jensen (22) that “biological monitoring of these chemicals may be undertaken using different tissues, depending on which is most convenient.”

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² The abbreviations used are: DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; DDE, 1,1-dichloro-2,2-di(4-chlorophenyl)ethylene; PCB, polychlorinated biphenyl; PBB, polybrominated biphenyl; LOD, limit of detection.

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