LABORATORY QUALITY ASSESSMENT PROCEDURES FOR SERUM DIOXIN/FURAN MEASUREMENTS: U.S. AIR FORCE VIETNAM VETERAN RANCH HAND, NIOSH CHEMICAL WORKERS, AND SEVESO, ITALY STUDIES

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ABSTRACT

An overview of the Centers for Disease Control (CDC) Quality Assurance (QA) program, focusing on data quality assessment procedures for serum dioxin/furan measurements is presented.

INTRODUCTION

In 1985, CDC developed the analytical capability to measure body burden levels of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs). Reliable measurements are essential to undertake large-scale epidemological studies, designed to detect possible adverse health effects in populations with alledged excessive exposure, and the data must be supported by unequivocal evidence to prove its credibility. A comprehensive QA program is the vehicle by which these goals are achieved and demonstrated.

In the beginning, we adopted the "Principles of Environmental Analysis", recommended by the American Chemical Society Subcommittee on Environmental Analytical Chemistry (Keith *et al.*, 1983), as a guide in establishing a QA program. These *principles* were later expanded by one of the original authors (Taylor, 1987). Taylor pointed out that the objective of a laboratory QA program is to document that the analytical measurement system is operating in a state of statistical control. The importance of this objective was emphasized by Eisenhart (1969), "... until a measurement operation has attained a state of statistical control, it cannot be regarded in any logical sense as measuring anything at all." Much of the philosophy and many of the procedures in these references have been incorporated into our QA program.

QA has two separate but related activities: (a) quality control -- which includes all standard operating procedures invoked to control laboratory errors; and (b) quality assessment -- which involves all techniques used to evaluate the precision of the measurement process and accuracy of the data. This overview of our QA program focuses on data quality assessment procedures used during the U.S. Air Force Vietnam Veteran Ranch Hand, National Institute for Occupational Safety and Health (NIOSH) Chemical Workers, and Seveso, Italy studies.

EXPERIMENTAL

The CDC analytical method (Patterson *et al.*,1986 and Patterson *et al.*, 1987), prototype automated cleanup apparatus (Lapeza, Patterson, and Liddle, 1986), method modifications for serum 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) by high-resolution gas chromatography/high-resolution isotope-dilution mass spectrometry (Patterson *et al.*, 1989a), and the validation of the method for sixteen other 2,3,7,8-chloro-substituted PCDDs/PCDFs (Patterson *et al.*, 1989b) have been previously reported. An improved automated sample cleanup apparatus is described in an accompanying paper in this issue (Isaacs, Turner and Patterson, 1990). Before analysis, samples are arranged into runs of five (method blank, three serum specimens, and one QA sample of pooled human serum), spiked with the appropriate ^{13}C-labeled internal standard(s), equilibrated, extracted, and processed through the five-column semiautomated cleanup procedure. Prior to mass spectral analysis, the enriched samples are reconstituted with toluene containing $^{13}C_6$-1,2,3,4-TCDD as an external standard. This compound is measured in each sample and used to calculate the percent recovery of the internal standard(s), monitor the chromatographic isomer specificity index, and demonstrate that the resolving power is at least 10,000.

RESULTS AND DISCUSSION

The quality control and data handling components of our QA program have been described (Turner, *et al.*, 1989). Our method has attained a state of statistical control by proper training of laboratory personnel and their strict adherence to all standard operating procedures -- covering every aspect of the laboratory operation, from sample collection to data reporting. The quality assessment component of our QA program is based on the use of Shewhart-type quality control (QC) charts. QC charts are basic tools for QA. They provide a graphical means to demonstrate statistical control, monitor the measurement process, diagnose measurement problems, document measurement uncertainty, and generally aid in methodology development (Taylor, 1987).

Conceptually, a measurement system can be thought to be analogous to that of a "flight envelope" in aeronautics, where each type of engine has an optimum flight regime, depending on variables like airspeed, range, and altitude. Using factors such as these for a given engine, engineers can chart the flight envelope. Flight is possible only within the multi-dimensional boundaries of the envelope. Extending this analogy, critical analytical variables can likewise be charted. Taken collectively, the composite boundaries define the "performance envelope" of the measurement process. If the result of an analytical determination lies within the envelope, the process is "in control", otherwise it is "out of control". The CDC dioxin/furan measurement system is complex, composed of 8-10 analysts [using six automated sample cleanup apparati] and 4-5 operators [using three high-resolution mass spectrometry systems]. The overall system is treated as a single process, operating within a defined "performance envelope".

The numerous criteria for a valid analytical run have been previously given (Patterson *et al.* 1989a). These criteria can be applied to TCDD or any of the other sixteen PCDDs/PCDFs. Using TCDD as an example, some of the QC charts maintained for each run and sample include signal to noise ratio (S/N), the ratio of the intensities of ions 320 to 322 (native) and ions 332 to 334 (^{13}C-label), mass spectrometer resolving power, isomer specificity index (chromatographic overlap), retention time of a peak relative to an analytical standard, percent recovery of the internal standard(s), and calculated value(s) of serum QA samples. These QC charts may be examined by any combination of the variables -- cleanup run date, analyst, apparatus, mass spectrometry run date, mass spectrometer, and mass spectrometer operator. An unknown specimen is quantitated only if the method blank in the run has a S/N < 3, all of the criteria for a valid measurement for the unknown are met and its calculated concentration is greater than the limit of quantification, all of the criteria for the QA sample in the run are met, and the observed concentration of the QA sample is within its 99% confidence limits. Final results for unknown specimens are reported in parts-per-trillion on a lipid-adjusted basis. The total lipid for each specimen is determined by a "summation" method (Akins, Waldrep, and Bernert, 1989); and individual lipid values (free cholesterol, total cholesterol, triglycerides, and phospholipids) must also meet bench and blind QA requirements before a result is reported. The accuracy of a the measurement is ensured when all of these criteria are concurrently met.

TABLE 1. Summary of Method Precesion Data for the Measurement of 2,3,7,8-TCDD in Human Serum Quality Assurance Samples.

Pool ID	Sample Size (g)	n (runs)	Mean[c] (ppq)	SD	RSD
J[a]	100	105	22.1	3.4	15.3
K[b]	100	134	69.1	9.0	13.1
M[a]	100	150	20.8	3.2	15.3
N[b]	100	65	155.4	17.2	11.1
O[a]	100	165	18.8	3.3	17.4
P[b]	100	119	167.5	16.9	10.1
Q[a]	100	162	16.5	2.8	17.1
R[b]	100	66	74.0	5.8	7.9

[a]Pooled Normal Human Serum [b]2,3,7,8-TCDD Spiked Pooled Human Serum [c]Means are expressed on a whole weight basis in parts-per-quadrillion (ppq).

Long-term estimates of method precision are obtained by running a serum QA sample in every run. Table 1 shows the precision of the method over the past three years for TCDD, during which time we analyzed more than 3000 unknown specimens. The estimates of method reproducibility are expressed

as relative standard deviation (RSD). They range from 7.9 to 17.4 percent, depending on concentration level, and reflect "total method variation". The data quality assessment techniqes presented have demonstrated that our measurement system for ultra-trace levels of PCDDs/PCDFs in human serum is indeed operating in a state of statistical control.

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