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An interlaboratory study of perfluorinated alkyl compound levels in human plasma ☆

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Abstract

We conducted an interlaboratory study which differed from the typical study of this type because of its emphasis on comparing intralaboratory variability in results. We sent specimens to six laboratories experienced in the analysis of perfluorinated alkyl compounds in blood matrices and that use stringent procedures to control and assure accuracy and precision. Each received an identical set of 60 plasma specimens that were analyzed in six completely independent batches. Split specimens were included so that within- and between-batch coefficients of variation could be calculated. All laboratories used liquid chromatography–tandem mass spectrometry (LC–MS/MS). The concentrations of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and perfluorohexanesulfonate (PFHxS) measured in the specimens in general showed a high level of agreement, although in some cases the agreement was only moderate. The average within- and between-batch coefficient of variation for PFOS was 9.1% and 9.3%; for PFOA was 14.5% and 14.5%; and for PFHxS was 14.5% and 17.0%. The recent availability of labeled internal standards, among other advances, has facilitated improvement in the accuracy and precision of the assays. Considering the degree of between-subject variation in levels among people in background-exposed populations, the results indicate that biomarker-based epidemiologic studies of associations with health could have reasonable precision.

Keywords: Perfluorinated alkyl compounds; Interlaboratory study; Persistent organic pollutants; Interdisciplinary studies; Research design; Epidemiologic methods

1. Introduction

The identification of several perfluorinated alkyl compounds at low concentrations in wildlife (Giesy and Kannan, 2002) and human sera from the general population (Hansen et al., 2001) has resulted in a growing interest in biomonitoring (Kannan et al., 2004; Houde et al., 2006;

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Butenhoff et al., 2006; Calafat et al., 2006, 2007) and assessing the possibility that such exposure could affect human health. Epidemiologic studies among populations with such exposure are needed to confirm or assess the safety of current exposures. At the low concentrations in blood matrices from the general population, accurate and precise measurements of these compounds pose analytical challenges. Marked imprecision in measurements, as suggested by a recent interlaboratory study (van Leeuwen et al., 2006), could hobble epidemiologic studies in which concentrations of perfluorinated alkyl compounds in blood matrices are examined in relation to aspects of health.

Perfluorinated alkyl compounds have been used to manufacture many industrial and consumer products such as repellent coatings for paper, fabric, and carpets, in heat resistant or impermeable materials, and insecticide formulations (Kissa, 2001; Lewandowski et al., 2006; Dinglasan-Panlilio and Mabury, 2006). Perfluorooctanoate (PFOA), perfluorooctanesulfonate (PFOS), and perfluorohexanesulfonate (PFHxS) are three representative perfluorinated alkyl compounds that have been recently shown to have relatively long elimination half-lives in humans (Olsen et al., 2005). Resistance to degradation and potential for accumulation have led to assessments of hazard (OECD, 2006). Health risks have been evaluated based on serum levels (Butenhoff et al., 2004; 3M Company, 2003; Swedish Kemikalieinspektionen, 2004). Many of the animal toxicology studies have also measured serum levels, and have shown LOAELs at serum concentrations hundreds to thousands of times higher than the median general population levels; however, for several outcomes no effect levels were not established (Luebker et al., 2005).

The analytical methodology underlying these biomonitoring data has continued to evolve, and the number of laboratories engaged in analyzing samples has continued to increase. Martin et al. (2004) and de Voogt and Saez (2006) have reviewed the challenges in conducting these analyses. These challenges include sample preparation methods (e.g., avoiding coextractants), separation of isomers, lack of pure standards or stable-isotope labeled standards for quantitation, matrix effects and tandem mass spectrometry detection, and contamination of solvents, labware, and matrix. These factors underscore the need for interlaboratory studies aimed at developing a better understanding of the potential uncertainty in analyses based on precision and accuracy of measurements.

One prior interlaboratory study has been reported (van Leeuwen et al., 2006). In that study, among 18 participating laboratories, the interlaboratory coefficients of variations for analysis of plasma unknowns were PFOS, 32%; PFOA, 51%; and PFHxS, 64%. In that study, the procedures used by the laboratories to control and assure accuracy and precision of the assays were not clear, nor were the details of the methods.

The present study was designed to investigate intra- and interlaboratory variability. In an interlaboratory study, or "round robin", a sample of material is divided into identical aliquots and analyzed at different laboratories. The design of such studies depends on whether they address technical issues of primary interest to analytical chemists, or more general aspects of the assay relevant to scientists who must choose a laboratory collaborator or interpret results. While this type of exercise is frequently done as part of routine quality control or study planning, the results can be of wider interest when a particular assay is new or especially challenging.

In some interlaboratory studies one aliquot is sent to each participating laboratory. This allows calculation of the "method variation", which is also called the interlaboratory coefficient of variation (CV). This value gives insight into assay performance, and provides a means to compare the results of the assay with minor variations. However, if multiple aliquots are sent to each laboratory, this can allow, in addition, a standardized characterization of the within- and between-batch coefficients of variation at each laboratory, which are often more crucial to epidemiologic validity than is calibration (Hankinson et al., 1994). By batch, we mean an independent unit of analysis, including a full standard curve, blanks, control samples, and a group of unknowns all run in the same contiguous time frame. Given the unusual degree of difficulty in accurately quantitating perfluorinated alkyl compounds, the authors conducted an interlaboratory study in which the participating laboratories were sent multiple aliquots of multiple specimens comprising both unknowns and quality control samples at a prepared concentration. We included six completely independent laboratories that employ stringent procedures to control and assure the accuracy and precision of their measurements. The laboratories were selected because either they had extensive experience with the assay or their work on other low-level contaminants was well regarded by us or others, and they were able to commit to having the assays completed according to a specified schedule. Other laboratories that were not included also met these criteria. With careful selection of the six laboratories, this number was sufficient for us to achieve an important goal—objective selection of a strong scientific partner. Sending multiple split samples to each laboratory increased the cost per laboratory relative to a traditional interlaboratory study, and these assays are relatively expensive. With just six laboratories we were limited in some ways, e.g., our ability to identify specific reasons for interlaboratory variability in results.

Thus, the present study was done to gain an improved understanding of the range in quality of laboratory assays of perfluorinated alkyl compounds in plasma that were available in 2006—therefore allowing comparison to the true variation in levels among subjects and an assessment of whether biomarker-based epidemiologic