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Geometric mean estimation from pooled samples

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Abstract

Biomonitoring for environmental chemicals presents various challenges due to the expense of measuring some compounds and the fact that in some samples the levels of many compounds may be below the limit of detection (LOD) of the measuring instrument. Even though various statistical methods have been developed to address issues associated with data being censored because results were below the LOD, the expense of measuring many compounds in large numbers of subjects remains a challenge. One solution to these challenges is to use pooled samples. There are many problems associated with the use of pooled samples as compared with individual samples, but using pooled samples can sometimes reduce the number of analytical measurements needed. Also, because pooled samples often have larger sample volumes, using pooled samples can result in lower LODs and thereby decrease the likelihood that results will be censored. However, many data sets obtained from environmental measurements have been shown to have a log-normal distribution, so using pooled samples presents a new problem: The measured value for a pooled sample is comparable to an arithmetic average of log-normal results and thus represents a biased estimate of the central tendency of the samples making up the pool. In this paper, we present a method for correcting the bias associated with using data from pooled samples with a log-normal distribution. We use simulation experiments to demonstrate how well the bias-correction method performs. We also present estimates for levels of PCB 153 and *p,p′*-DDE using data from pooled samples from the 2001 to 2002 National Health and Nutrition Examination Surveys.
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1. Introduction

One of the problems that arises in trying to characterize environmental exposures is that levels of contaminants in some or many individuals are not detectable by the instrumentation. The reason that levels are not detectable can be due to insufficient matrix or extremely low exposure levels. Such results are said to be below the limit of detection (LOD) as determined by the sampling and analytical method. In spite of continued improvements in the sensitivity of assays to detect lower and lower concentrations, the percentage of results below the LOD is not declining and may actually be increasing concurrent with decreasing exposure levels. Several papers (Persson and Rootzen, 1977; Gleit, 1985; Haas and Scheff, 1990; Helsel, 1990; Hornung and Reed, 1990; Travis and Land, 1990; Huybrechts et al., 2002; Baccarelli et al., 2005) have addressed the problem of estimating the mean or geometric mean of a population subject to results below the LOD.

Another problem that arises in trying to characterize environmental exposures is the expense of measuring levels of some compounds, especially the polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), in hundreds or thousands of subjects. To address both this problem and the problem of results below the LOD, Bates et al. (2004, 2005) pooled samples according to stratification criteria for various demographic variables of interest. By pooling samples they were able to drastically reduce the number of analytical measurements. And because their pooled samples had larger sample volumes, they experienced lower LODs and fewer non-detects.

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Of course, valuable information about the intra-individual variation can be lost when pooled samples are used (Bignert et al., 1993). To deal with this problem Bates et al. (2005) designed their study so that replicate pools were created for each demographic group, where each of r replicate pools was composed of the same number (k) of individual samples and each sample contributed the same volume to a replicate pool. Because with this design the individual samples are assigned at random to the replicate pools, the variance (σ_p^2) among the r replicate pools is comparable to the variance among r means of size k and therefore, the variance (σ_i^2) among the individual samples making up the pools is equal to $k\sigma_p^2$. Thus the pooled-sample design of Bates et al. (2005) allowed them to obtain estimates of the actual variability among the individual samples used to form the pools even though no individual sample measurements were made. But because the number of replicate pools was limited to only 2 or 3, the reliability of their variance estimates was minimal.

Another potential problem with estimates based on pooled samples has to do with the fact that the measured value for a pooled sample is comparable to an arithmetic average of levels in the individual samples making up the pool. Thus using pooled samples can lead to biased estimates of central tendency if the distribution of the underlying data tends to be log-normal.

We present a method for correcting the bias associated with measurements of pooled samples composed of log-normally distributed individual samples. This bias-correction method incorporates a technique for optimizing estimation of the among individual sample variances obtained from the variability among replicate pools within-demographic groups. We then present the results of simulation experiments to demonstrate how well the bias-correction method performs. We also present estimates for levels of PCB 153 and *p,p′*-DDE using pooled samples obtained as part of the 2001–2002 National Health and Nutrition Examination Survey (NHANES).

2. Materials and methods

Serum samples used to prepare the pools analyzed for the study were selected from those obtained by venipuncture from 2150 participants, a random one-third subsample of people 12 years of age and older from NHANES 2001–2002 and representative of the US general population for this age range. NHANES, conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC), is an ongoing survey designed to measure the health and nutrition status of the civilian non-institutionalized US population. The sampling scheme for NHANES 2001–2002, a complex multistage area probability design, included 11 039 persons. Data were collected through household interviews and through standardized physical examinations conducted in mobile examination centers. On the basis of self-reported data, a composite race/ethnicity variable helped define three major racial/ethnic groups: non-Hispanic blacks, non-Hispanic whites, and Mexican Americans. Informed consent was obtained from all study participants.

After collection of samples, serum specimens were divided into aliquots and were stored cold (2–4 °C) or frozen until they were shipped on dry ice to CDC's National Center for Environmental Health (NCEH). Serum samples were stored frozen after receipt at NCEH at −20 °C until needed. The 2150 individual serum samples available were categorized in 24 demographic groups (Table 1), each representing a combination of race/ethnicity, gender, and age (12–19 years, 20–39 years, 40–59 years, and 60 years and older). A total of 1831 individual serum samples were used to prepare the 54 pooled serum samples analyzed for this study. On the basis of the number of individual serum samples per demographic group, multiple pooled samples were available for 14 demographic groups, and one pooled sample was available for 10 demographic groups (Table 1). To ensure that no individual sample overly influenced the pooled results, all serum samples included in any one pool (25.5 ml each) were of equal volume (i.e., each individual sample contributed 750 μl). Most pools included 34 randomly selected individual serum samples. Only 31 individual serum samples were available, however, for the pool representing non-Hispanic black men 60 years of age or older. In addition, each of the two pools representing Mexican American men between 20 and 39 years of age consisted of only 33 individual specimens. After preparation, the serum pools were stored at −20 °C until analysis.

Table 1
Number of pooled serum samples prepared from NHANES 2001–2002 participants per demographic group

Race or ethnicity	Gender	Number of samples[a] per age group			
		12–19 years	20–39 years	40–59 years	60+ years
Non-Hispanic white	Male	3 (105, 102)	3 (112, 102)	3 (125, 102)	4 (154, 136)
	Female	3 (120, 102)	4 (155, 136)	3 (120, 102)	4 (157, 136)
Non-Hispanic black	Male	3 (115, 102)	1 (54, 34)	1 (53, 34)	1 (31, 31)
	Female	3 (123, 102)	1 (63, 34)	1 (45, 34)	1 (44, 34)
Mexican American	Male	3 (108, 102)	2 (67, 66)	1 (49, 34)	1 (36, 34)
	Female	4 (140, 136)	2 (84, 68)	1 (45, 34)	1 (45, 34)

[a] Number of pooled serum samples (number of individual serum samples available, number of individual serum samples used to prepare the pool[s]).