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Environ Int. Author manuscript; available in PMC 2012 May 1.

Published in final edited form as:
Environ Int. 2011 May ; 37(4): 709–714. doi:10.1016/j.envint.2011.01.013.

Assaying Organochlorines in archived serum for a large, long-term cohort: Implications of combining assay results from multiple laboratories over time

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Abstract

Conserving irreplaceable, archived serum samples may sometimes conflict with the objective of minimizing measurement error due to laboratory effects. We sought to determine whether we could successfully combine assay results for DDT-related compounds and polychlorinated biphenyls (PCBs) in serum from the same birth cohort obtained from different laboratories over time. Using the Child Health and Development Studies (CHDS) serum archive, we compared variability for assays of a quality control pool to variability for assays of subject serum. The quality control pool was created from native archived serum samples that were pooled, then aliquoted, blinded and inserted pair-wise into assay batches along with the subject serum for 5 studies using CHDS samples conducted over a 13 year period by three different laboratories. We found that the variability between laboratory and over time within laboratory was small relative to inter-individual variability for *p,p′*-DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane), *p,p′*-DDE (1,1′-dichloro-2,2′-bis(*p*-chlorophenyl)ethylene) and *o,p′*-DDT (1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)-ethane). Results were also consistent for most PCB congeners which were detectable in 85% or more of samples. Our results suggest that it is possible to combine assays for DDT and PCB congeners measured at positive levels as they are accumulated for cohort subjects without risking meaningful misclassification due to variation stemming from laboratory or time period. This has significant implications for future study costs, conservation of irreplaceable archived samples and for leveraging past investments for future research. For PCB congeners with very low levels, findings caution against pooling of assays without further exploration.

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Keywords

CV; assay library; organochlorines; measurement error; biomarkers; epidemiology

INTRODUCTION

When managing a valuable biorepository, it is often desirable to combine assay results from different laboratories or across different time periods from the same laboratory. This strategy is particularly important when biomarker assays are expensive, or require relatively large amounts of biospecimen, as is the case for organochlorines such as *p,p′*-DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) and its related compounds, and for polychlorinated biphenyls (PCBs). Moreover, it is desirable to build a library of laboratory results as samples are assayed. An assay library facilitates future studies that may require combining data bases of past investigations.

However, variability across laboratories or over time within the same laboratory could result in misclassification or bias in research studies when results of multiple laboratory assays are combined. In this paper, we report on our experience with building a library of DDT and PCB assay results for the Child Health and Development Studies (CHDS) pregnancy cohort.

The CHDS consists of a 50+ year follow-up of more than 20,000 pregnancies that occurred between 1959 and 1967 near Oakland, California during the time of peak DDT use in the United States. DDT was used during this time period for agricultural and public health purposes until concerns about its toxic effects, environmental persistence, and concentration in the food supply led to a ban in 1972 (Eskenazi, et al. 2009). PCBs were primarily used in the United States as insulating fluids and coolants in electrical equipment and machinery since the 1930s. They remained in use until they were banned by the Environmental Protection Agency in 1979 because of their demonstrated toxicity and environmental persistence (EPA 1979). Exposure continues globally for both DDT and PCBs due to widespread soil contamination and bio-accumulation. In addition, use of equipment containing PCBs is ongoing and DDT is still actively used in some countries (Stockholm Convention 2008). CHDS mothers show some of the highest measured DDT levels in the literature (Cohn, et al. 2007), providing a unique opportunity for epidemiologic study of DDT effects on multiple health outcomes in both mothers and offspring. Current work is being undertaken on PCB levels in the CHDS cohort. Moreover, as new DDT and PCB studies are funded in the CHDS, we are accumulating additional samples and enlarging the assay library, enabling an expansion of the scope of research among not only the first two generations but into subsequent generations as well. Thus the question of whether assay results from multiple laboratories over time can be combined is an important research question that may also apply to other historic cohorts and other chemical compounds.

MATERIALS AND METHODS

We initiated assays of DDT-related compounds and PCBs in archived serum samples from the Child Health and Development Studies Cohort beginning in 1998. These blood samples were collected from pregnant women in each trimester and in the early post-partum period between 1959 and 1967, processed to isolate serum which was generally saved in four aliquots of approximately 3 mL each, and stored since then at −20 degrees centigrade.

DDT-related compounds were determined for a number of different studies. The first two projects investigated 1) the relation of DDT exposure in CHDS mothers and time to pregnancy in their daughters 30 years later (Cohn, et al. 2003) and 2) the relation of

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maternal serum DDT levels and their subsequent risk of breast cancer (Cohn, et al. 2007). The assays for these two studies were performed together between 1998 and 2001 in Laboratory I and comprise assays levels for Study A. At a later date, Laboratory I performed assays for a third study, designated as Study B, of maternal DDT exposure in relation to son's testicular cancer, completed in 2004 (Cohn, et al. 2010).

Concurrently CHDS collaborators conducted studies of maternal serum DDT and cryptorchidism (Bhatia, et al. 2005) and fetal growth (Farhang, et al. 2005). These measurements identified as Study C, were performed in Laboratory III beginning in 1998 (James, et al. 2002).

In the interim, Laboratory I closed and subsequent assays for other CHDS projects, Studies D and E, have been conducted by Laboratory II. Recently completed studies include: 1) an examination of the effects of maternal organochlorines and maternal thyroid levels on child growth and development from infancy to adolescence (Study D); and 2) an examination of the relation of maternal organochlorine exposure and daughter's breast density (Study E). Both of these latter studies are still in the initial stages of analysis. PCB studies in CHDS mothers are in progress and findings are in preparation for publication, but other studies have already reported that adult exposure to PCBs is associated with delayed or accelerated pregnancy (Carpenter 2006; Mendola, et al. 2008).

In studies preceding those reported here, Laboratory I compared its assay results for DDE and selected PCBs with those from three other laboratories and found consistency in the measured levels for all four laboratories (Laden, et al. 2001). Prior to changing laboratories, Laboratory II conducted a small pilot of blind serum samples from both Laboratory I and the Centers for Disease Control laboratory and found comparable results for selected analytes measured by all three laboratories (data not published).

At the time of the first organochlorine assays in 1998, we created a quality control pool of native, archived serum samples from CHDS mothers who had been lost to follow-up, but whose sera had been collected during the same time period (1959–1967) and under the same conditions as study subjects who remained in the study. Approximately 6% to 9% of samples assayed for each CHDS project consisted of these quality control pool samples, inserted in pairs within study batches at random. The order of these quality control pool samples within batches was also randomized and the laboratory was blinded as to the identity of the quality control samples. Herein we report on the DDT and PCB results obtained for the CHDS quality control (QC) pool and study subjects in each laboratory, at each time period.

Assay methods in each laboratory have been described in prior publications (Bhatia, et al. 2005; Brock, et al. 1996; Cohn, et al. 2007; Gammon, et al. 2002; James, et al. 2002; Park, et al. 2009; Petreas, et al. 2008; Rogers, et al. 2004). Briefly, for studies A and B, gas chromatography with electron capture detection (GC/ECD) was used with a capillary column. For studies C, D and E, GC/ECD was used with dual columns. Dual column analysis reduces interference and might result in lower reportable concentrations. Over the time period of study, laboratory personnel changed and instrumentation improved, creating differences in the application of laboratory procedures due to variations in techniques. Thus, inter-laboratory variability could be attributed to differences in procedures, standards, laboratory personnel and equipment. These practical differences make the results of these analyses especially relevant and important.

As for prior publications (Berkowitz, et al. 2003; Cohn, et al. 2010; Cohn, et al. 2003; Cohn, et al. 2007) we used all observed positive values of each analyte in these analyses, even those reported to be below the limit of detection (see Supplemental Table 2). Negative and

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zero values were recoded to the lowest measured value for each analyte. For *op′*-DDT, 2 values for Laboratory I were recoded. For PCB 66, 6 values for Laboratory I and 23 values for Laboratory II were recoded. For PCB 74, 1 value for Laboratory I and 3 values for Laboratory II were recoded. For PCB 99, 2 values for Laboratory I and 1 value for Laboratory II were recoded. For PCB 118, 2 values for Laboratory II were recoded. For PCB 170, 2 values for Laboratory II were recoded. And for PCB 203, 1 value for Laboratory I and 22 values for Laboratory II were recoded (see Supplemental Table 2 for minimum values for each compound).

We considered the following DDT-related compounds: *p,p′*-DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane), *p,p′*-DDE (1,1′-dichloro-2,2′-bis(*p*-chlorophenyl)ethylene), and *o,p′*-DDT (1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)-ethane); and the following PCBs: congeners 66, 74, 99, 118, 138, 153, 170, 180, 187 and 203, selected because they were detectable in greater than 85% of samples. To assess the comparability of the results from the five studies in three laboratories, we used coefficients of variation (CVs), which provide a standard measure for assessing laboratory error (Rosner 1995). The CV is defined as the [standard deviation/mean] expressed as a percentage. CVs were calculated for the quality control pool assays to estimate measurement error (denoted as CV_{qc}) and for assays of the study subjects to measure between-subject or individual variability for each study (denoted as CV_{bs}), The ratio of the two, CV_{bs}/CV_{qc}, (i.e., CV Ratio), indicates the magnitude of the between subject variation to the measurement error. Ratios above 2 represent at least a two-fold higher variation in measurements between subjects than between QCs, thus larger ratios are preferable because they reflect greater variability between individuals relative to measurement error (Hankinson, et al. 1994). Both the CV_{qc} and CV_{bs}, as well as the CV Ratio are measures of the variation within a single study or laboratory, and do not compare the differences in error between different studies or laboratories.

Analysis of variance (ANOVA) calculations were also computed on the QC samples for each study and laboratory to compare the intra- or between-study variation to the inter- or within-study (natural) variation. We performed ANOVA as a formalized test of the differences between labs and studies and as a confirmation of the CV_{bs} and CV_{qc} results. In order to ascertain comparability of the quality control pool over study and time, we calculated the intraclass correlation coefficients (ICCs) for the paired QC samples within each study and laboratory as the ratio of the between-group (lab or study) variance over the total variance. The ICCs and CV_{qc}s provide alternative assessments of the reliability of the estimation of measurement error. Smaller CV_{qc} values indicate lower inherent measurement error and are desirable (Tworoger and Hankinson 2006). A low CV_{qc} is comparable to a high ICC, and both indicate a high level of measurement reliability.

RESULTS

Table 1 shows the means, $CV_{bs,}$ CV_{qc}, ICCs and CV Ratios for the three DDT-related compounds and the ten PCB congeners for each laboratory (I, II and III). Of the 37 CV_{qc}s for measurement error by laboratory, 30 are 30% or less. The CV_{bs}s for the between subject variation in the three laboratories range from 48.5% to 125.7%. Larger CV_{bs}s are expected and preferred because they represent differences in subject exposure. The CV ratios are greater than two in 35 of 37 cases (Figure 1, points above the horizontal line) and greater than or equal to three in 29 of the 37 cases, indicating fairly low levels of measurement error compared to between subject variation (Hankinson, et al. 1994). In general, the compounds that are found at higher mean levels (PCBs marked as “high” have the two highest mean PCB levels) have CV ratios larger than three, while the compounds found at lower mean levels (PCBs marked as “low” have the two lowest mean PCB levels) have lower CV ratios,

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with the exception of PCBs 170 and 203 measured in Laboratory III. We observe similar results for the $CV_{bs,}$ CV_{qc} and CV ratios across studies (Supplemental Table 1). It should be noted that the CV_{qc} and CV_{bs} results for compounds with mean levels close to zero (e.g., PCB 170 and PCB 203) may be somewhat unreliable because small fluctuations in the mean can have a large impact on these measures. However, this effect should be cancelled out in the CV ratio.

The graphs in Figure 2 display the smoothed distributions for the QC samples (dashed lines) and the study subjects (solid lines) for each laboratory for the DDT-related compounds. These distributions illustrate the similarity of the results from the three laboratories for each of the measured DDT-related compounds. Moreover, Figure 2 shows that the variability for the QC samples is considerably lower than the variability for the study subjects, demonstrated by the fact that the dashed curves are taller and narrower than the solid curves.

Similarly, the graphs in Figure 3 display the smoothed distributions for the QC samples (dashed lines) and the study subjects (solid lines) for each laboratory for a selection of the PCBs. The two PCBs examined that were found in the lowest mean concentrations (marked “low”) and the two that were found in the highest mean concentrations (marked “high”) are presented in the graphs. As with the DDT-related compounds, the distributions demonstrate the similarity of results for the three laboratories and that the variability for the QC samples is considerably lower than the variability for the study subjects.

ANOVA F-values and *p*-values were calculated for the QC samples to compare studies and laboratories for the three DDT-related compounds and ten PCB congeners examined (results not shown). The *p*-value is less than 0.05 in all but one case for the laboratory comparison, and in each of these instances the F-value is greater than 1, indicating that most of the variance comes from between-laboratory error, rather than within-laboratory error. Because the QC samples should have the same value for every measurement, the between-laboratory error can be thought of as error introduced by laboratory differences (e.g., from different personnel performing the procedures or different analytical techniques), while the within-laboratory error is the random error that occurs when a measurement is taken multiple times under exactly the same conditions. Hankinson et al. (1994) combine these two types of error under the general term *measurement error*. Although the ANOVA results suggest that measurement error did differ between laboratories, the CV ratios demonstrate that when assays are combined across laboratory, subject error is much larger than measurement error, thus the ANOVA findings do not contradict our results.

ICCs were calculated for QC samples as an alternative to the CV_{qc} to estimate rank order and absolute comparability between laboratories (Table 1) and over time within the same laboratory (Supplemental Table 1). The ICC measures how strongly the pairs of QCs in the same batch resemble each other, compared to all QC samples in a particular lab or study. It can be thought of as the proportion of the total variance for the QC samples that results from between-batch variation. Measurements are considered to be reliable if those within the same batch contribute less to the total variance than those from different batches; this scenario gives a higher ICC (i.e., closer to 1.0). The combination of a low CV_{qc} and high ICC provide the optimal combination for observing an expected association. However, higher CV_{qc} values can be acceptable as long as the ICC is high since this is indicative of a situation where the measurement error is moderately sizeable but is reliably estimated (Tworoger and Hankinson 2006). ICCs greater than 0.6 are often considered to be reflective of moderately good to excellent reliability (Fleiss 1981; Shrout 1998). The ICCs for the three DDT-related compounds and ten PCB congeners were greater than or equal to 0.25 for all 37 of the comparisons by laboratory. Furthermore, many of the ICCs were higher, with 23 of the 37 comparisons by laboratory greater than or equal to 0.6. ICC calculations

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demonstrate reliability in the assay results and are consistent with conclusions based on the CV ratios. Similar results were obtained when ICCs were calculated by study, rather than by laboratory (Supplemental Table 1).

DISCUSSION

Based on our experience, the inclusion of QC samples, blinded to the laboratory, with duplicates within batches and randomized for order within and between batches, is critical for evaluating the impact of combining assay results across laboratories and over time within laboratory. For the three laboratories we worked with, our results for the examined DDT compounds suggest that combining assay results over time and over laboratory is an acceptable analysis strategy.

Our analysis of the PCBs suggests that a more careful approach for pooling assay results is necessary. In general, findings support pooling PCBs measured at higher mean levels across laboratories. However, results caution against pooling assays for PCBs with low mean levels across laboratories. At minimum, stratified analysis by laboratory to check associations prior to pooling should be considered. If pooling seems appropriate, the impact of controlling associations for the laboratory of analysis or the timing of assay should also be examined.

Hankinson et al. (1994) proposed a similar method to the one used here to evaluate laboratory reproducibility of hormone levels for the Nurse's Health Study and reasoned that some laboratory error could be tolerated if there are large between person differences in measurements compared to the laboratory error. This point is essential to our argument because some measurement error is certain to occur and is likely exacerbated by differences in time and place.

The ability to combine assays over time and across laboratories is very important when assays are based on irreplaceable archived samples and where a research program depends on building a library of assays for a large cohort over time. This is particularly critical when assays are costly or the cohort is large. Both of these conditions apply to the study of organochlorine associations in the CHDS cohort, where multiple outcomes are available and funding is obtained incrementally for hypothesis testing.

CONCLUSIONS

We conclude that examination of the error associated with assays of DDT-related compounds and PCBs among individuals compared to that among the quality control data stratified by laboratory and time provides a sound method for the assessment of laboratory differences and laboratory drift over time. The additional precaution of controlling or stratifying for laboratory and time in data analysis should be sufficient when studying exposure to DDT-related compounds and PCBs. This strategy allows conservation of valuable archived serum, and permits building an assay library as new projects are funded.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

ACKNOWLEDGEMENTS AND GRANT INFORMATION

We acknowledge the late Jacob Yerushalmy who designed and initiated the CHDS; Barbara van den Berg, the second Director of the CHDS who conserved the serum archive; Roberta Christianson for her role in coding and documenting the CHDS archive; and Rajiv Bhatia for his study of organochlorines and male genital anomalies

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which provided an essential contribution to the assay library. We are grateful to Susan Hankinson for her timely and crucial recommendation concerning the creation of a quality control pool. This research was supported by the National Institute of Environmental Health Sciences (R01 ES08345; R29 ES09042; R01 ES12460; 1R01 ES017024), the National Cancer Institute (R01 CA72919), the National Institutes of Child Health and Human Development (N01 DK63422; N01 HD63258), and the Lance Armstrong Foundation.

The point of view and conclusions expressed in this paper are those of the authors and do not necessarily represent the official position or policies of the funding agencies named above.

List of all abbreviations and definitions used

QC	quality control sample
***p,p′*-DDT**	(1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane)
***p,p′*-DDE**	(1,1′-dichloro-2,2′-bis(*p*-chlorophenyl)ethylene)
***o,p′*-DDT**	(1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)-ethane)
PCB	polychlorinated biphenyl
LOD	limit of detection
CV	coefficient of variation expressed as a percentage
$\mathbf{CV_{qc}}$	between-QC coefficient of variation expressed as a percentage
$\mathbf{CV_{bs}}$	between-subject coefficient of variation expressed as a percentage
CV Ratio	the ratio of CV_{bs}/CV_{qc}
ICC	intraclass correlation coefficient
CHDS	Child Health and Development Studies

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Research Highlights

- Quality control samples are critical in evaluating assay reliability.
- Findings support a strategy for building an organochlorine assay library over time.
- Pooling assays for biomarkers with low mean levels requires caution.

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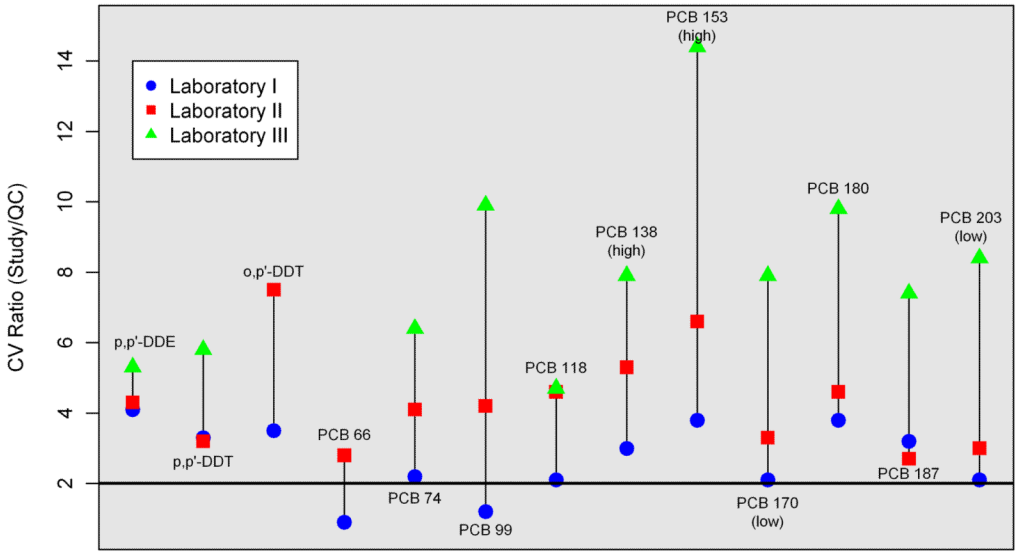

Figure 1.

Comparison of CV Ratios by Laboratory for Each Compound

CV ratios for the three DDT-related compounds and ten PCB congeners examined are shown by laboratory, along with the threshold value of 2. Values above 2 demonstrate that the measurement error is relatively small in comparison to the natural variation in the data. The two PCBs found at the highest mean levels are denoted “high” and the two found at the lowest mean levels are denoted “low.”

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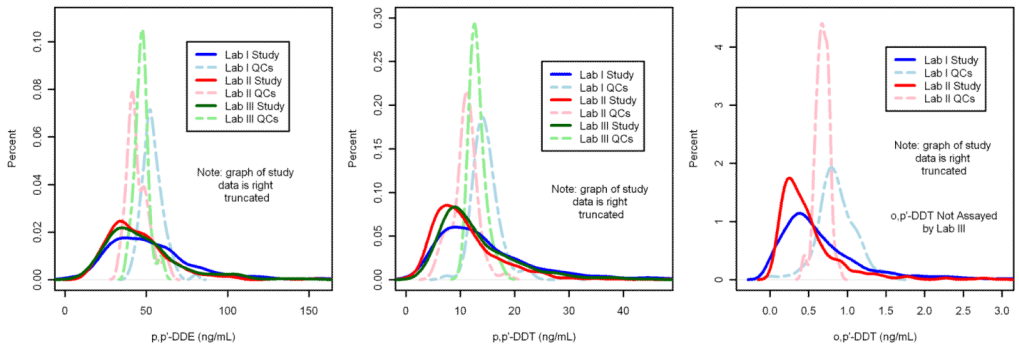

Figure 2.

Smoothed Distributions of *p,p′*-DDE, *p,p′*-DDT, and *o,p′*-DDT According to Assay Laboratory

Comparison of QCs and study data by assay laboratory for (A) *p,p′*-DDE (B) *p,p′*-DDT (C) *o,p′*-DDT

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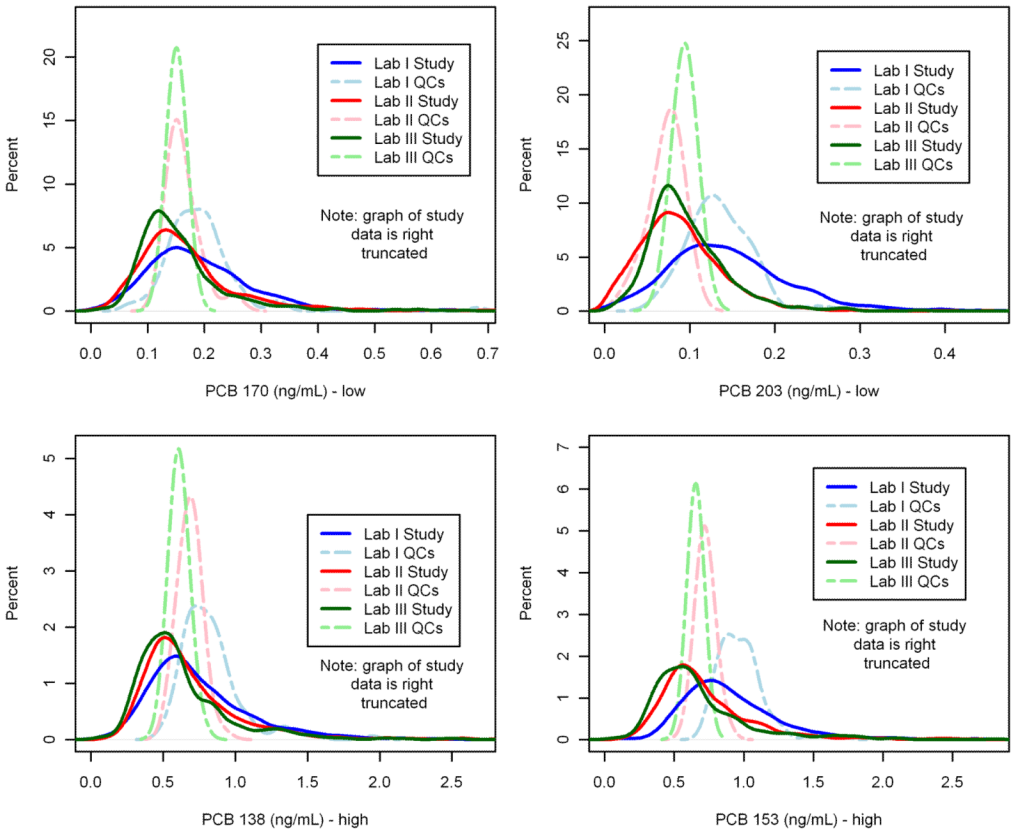

Figure 3.

Smoothed Distributions of High and Low PCBs According to Assay Laboratory Comparison of QCs and study data based on assay laboratory for (A) PCB 170 (low) (B) PCB 203 (low) (C) PCB 138 (high) and (D) PCB 153 (high). “High” and “low” refer to the PCBs found at the two highest and two lowest mean levels.

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TABLE 1

Comparison of Study Subjects and QC samples for DDT-related compounds and PCBs with more than 85% of samples above LOQ, by Laboratory

	Study Subjects			QC samples				CV Ratio
	N	Mean (ng/mL)	CV_{bs}[a] (%)	N	Mean (ng/mL)	CV_{qc}[a] (%)	ICC[b]	(Study/QC)
***p,p′*-DDE**								
Laboratory I	934	51.88	49.1	92	53.78	12.0	0.29	4.1
Laboratory II	900	46.55	65.1	58	44.62	15.2	0.62	4.3
Laboratory III	420	47.54	48.5	20	47.20	9.1	0.79	5.3
***p,p′*-DDT**								
Laboratory I	934	14.50	65.0	92	14.85	19.4	0.63	3.3
Laboratory II	901	11.37	68.0	58	10.89	21.0	0.87	3.2
Laboratory III	420	13.14	57.4	20	13.12	10.0	0.78	5.8
***o,p′*-DDT**								
Laboratory I	892	0.69	100.3	89	0.86	28.9	0.43	3.5
Laboratory II	901	0.52	94.2	58	0.68	12.5	0.83	7.5
Laboratory III	0	---	---	0	---	---	---	---
PCB 66								
Laboratory I	932	0.42	81.9	91	0.44	95.9	0.39	0.9
Laboratory II	909	0.09	87.2	60	0.08	31.0	0.41	2.8
Laboratory III	0	---	---	0	---	---	---	---
PCB 74								
Laboratory I	932	0.26	77.2	91	0.26	35.7	0.40	2.2
Laboratory II	909	0.19	75.7	60	0.19	18.3	0.24	4.1
Laboratory III	420	0.20	59.9	20	0.20	9.4	0.68	6.4
PCB 99								
Laboratory I	932	0.21	84.2	91	0.20	68.0	0.37	1.2
Laboratory II	909	0.22	65.0	60	0.20	15.3	0.79	4.2
Laboratory III	420	0.24	65.8	20	0.22	6.6	0.86	9.9
PCB 118								
Laboratory I	932	0.56	52.8	92	0.56	24.6	0.25	2.1
Laboratory II	909	0.48	58.1	60	0.46	12.7	0.89	4.6

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	Study Subjects			QC samples				CV Ratio
	N	Mean (ng/mL)	CV_{bs}[a] (%)	N	Mean (ng/mL)	CV_{qc}[a] (%)	ICC[b]	(Study/QC)
Laboratory III	420	0.52	71.9	20	0.50	15.4	0.63	4.7
PCB 138 (high)								
Laboratory I	932	0.78	79.7	92	0.83	26.8	0.60	3.0
Laboratory II	909	0.69	54.0	60	0.68	10.2	0.69	5.3
Laboratory III	420	0.64	59.3	20	0.61	7.5	0.80	7.9
PCB 153 (high)								
Laboratory I	933	0.93	65.6	92	0.98	17.1	0.66	3.8
Laboratory II	909	0.73	49.6	60	0.72	7.5	0.56	6.6
Laboratory III	420	0.69	56.8	20	0.65	4.0	0.78	14.4
PCB 170 (low)								
Laboratory I	931	0.20	76.6	91	0.19	37.3	0.45	2.1
Laboratory II	909	0.17	59.6	60	0.16	18.2	0.85	3.3
Laboratory III	420	0.16	55.9	20	0.15	7.1	0.58	7.9
PCB 180								
Laboratory I	931	0.50	70.4	92	0.49	18.3	0.68	3.8
Laboratory II	909	0.50	48.6	60	0.46	10.7	0.83	4.6
Laboratory III	420	0.48	52.7	20	0.46	5.4	0.84	9.8
PCB 187								
Laboratory I	932	0.21	125.7	91	0.22	39.6	0.26	3.2
Laboratory II	909	0.18	59.3	60	0.18	22.1	0.76	2.7
Laboratory III	420	0.16	58.4	20	0.16	7.9	0.73	7.4
PCB 203 (low)								
Laboratory I	930	0.15	67.1	90	0.14	31.6	0.26	2.1
Laboratory II	909	0.09	73.9	60	0.07	24.6	0.82	3.0
Laboratory III	420	0.10	53.6	20	0.09	6.4	0.45	8.4

[a] CV_{bs}=Coefficient of Variation between subjects (individual variablility)

CV_{qc}=Coefficient of Variation within subjects (quality control samples – measurement error)

[b] Intraclass Correlation Coefficient