

Storage of Serum in Plastic and Glass Containers May Alter the Serum Concentration of Polychlorinated Biphenyls

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Valid exposure assessment and biomonitoring of toxicants rely on standardized specimen collection, handling, storage, and measurement. In a study designed to determine organochlorine concentrations in blood samples, we recruited participants from registered anglers in Michigan. After participants were interviewed, blood was collected from study subjects, either at home by a phlebotomist or in a commercial blood-draw station. The phlebotomists stored their samples in glass containers, but without our knowledge, the commercial laboratory transferred the specimens to plastic containers for freezing in its central facility. Samples were analyzed in the Analytical Chemistry Section Laboratory of the Michigan Department of Community Health. This laboratory also provided information on storage in glass ($n = 28$) versus plastic containers ($n = 113$). We conducted linear regression analyses to assess factors that may explain the concentrations of polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (DDE), and polybrominated biphenyls (PBBs). Our results indicate that storage of serum in plastic containers altered the total concentrations of PCBs, in particular, the higher chlorinated PCBs (PCB-180 and PCB-199), but not DDE or PBBs. No other characteristics of the samples could explain the higher PCB values (0.75 $\mu\text{g/L}$ vs. 0.45 $\mu\text{g/L}$; $p = 0.025$) of those stored in plastic containers. The proportion of PCB detects in both subsamples did not differ. Some preceding studies have provided information on whether specimens were stored in glass or plastic containers; however, a number of studies have not. We suggest the initiation of a new review process to determine whether these earlier reports were based on unbiased PCB determinations. We recommend standardizing specimen collection, handling, storage, and measurement, which is particularly necessary for newly emerging analytes. **Key words:** DDE, glass, PBB, PCB, plastic, storage. *Environ Health Perspect* 112:643–647 (2004). doi:10.1289/ehp.6768 available via <http://dx.doi.org/> [Online 2 February 2004]

Serum concentrations of polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE) are frequently used as markers of exposure for different health outcomes including cancer, reproductive failures, and metabolic, endocrine, and developmental disorders (Axmon et al. 2001; Laden et al. 2001; Longnecker et al. 2001; Osius et al. 1999; Walkowiak et al. 2001). Because these compounds are persistent and have long half-lives, single serum or blood measurements are used to assess individual exposure. In 1980, the U.S. Environmental Protection Agency (U.S. EPA) established rules for the collection, preservation, and storage of samples (Watts et al. 1980). These rules were established to minimize the introduction of impurities that might interfere with the quantification of specific analytes by gas chromatography (GC). In particular, plastic containers should be strictly avoided when collecting specimens because of the presence of minute traces of certain components present in plastic that are known to play havoc with GC electron capture detectors. Recently, certain high-density polypropylene containers have become available that may not contaminate samples collected to determine organochlorine compounds (OCs) such as PCBs or DDE (Needham LL, personal communication). Because the U.S. EPA rules established in 1980 have not been updated,

environmental studies should adhere to these quality standards. Some studies provide information on whether their study samples were stored in glass containers, as well as how the containers were transported and stored (Longnecker et al. 2000; Nawrot et al. 2002; Walkowiak et al. 2001). However, in a number of publications on OC as markers of exposure, authors omitted information about transportation and storage of samples (e.g., Covaci et al. 2002; Fangstrom et al. 2002; James et al. 2002; Korrick and Altshul 1998; Laden et al. 2001; Moysich et al. 1998; Zheng et al. 2000).

In this article we describe an investigation conducted in Michigan between 1996 and 2000. The objective of the study was to test whether PCB serum concentrations in humans are associated with adverse effect in human reproduction, in particular, male and female hormones. In the course of the study, we learned that commercial blood-draw stations and clinical laboratories stored serum specimens in plastic containers (to the best of our knowledge, high-density polypropylene). Phlebotomists we hired for this study, however, followed the established protocols for the storage of serum specimens in glass containers. In this article, we focus on the question of whether plastic containers may alter the concentration of PCBs, polybrominated biphenyls (PBBs), and DDE in stored serum samples.

Materials and Methods

Population. Participants were recruited from the files of registered anglers in Michigan. According to the established protocols of the Human Subject Committee at Michigan State University, all participants provided written consent. The core study included an interview and collection of blood, either at home by a phlebotomist or in a commercial blood-draw station. Our study design attempted to recruit men and women couples.

Interview and phlebotomy. Individual information (sex, age, height, weight, smoking status) and details of potential exposure to PCBs, PBBs, DDE, and other OCs as a result of fish consumption from the Great Lakes were collected in telephone interviews. In particular, we asked each participant for their lifetime duration of fish consumption in 5-year group intervals and for the number of meals of sport-caught fish they had consumed in the last 12 months. To compare regional differences, we categorized place of residence (the west coast of Michigan representing the shoreline of Lake Michigan; the Saginaw area or Saginaw Bay of Lake Huron; and the Detroit area representing access to Lake St. Claire, and the Detroit River and its tributaries).

Blood was collected at different stations of a commercial laboratory. After centrifugation, the serum sample was divided into separate aliquots for analyses of hormones and OCs. All serum specimens collected at the commercial laboratory were stored in plastic containers at -20°C . Blood was also collected by phlebotomists hired specifically for this study. These phlebotomists visited study participants in their homes. All serum specimens collected by these phlebotomists were stored in glass containers. Finally, serum specimens were sent to the Analytical Chemistry Section Laboratory (ACSL) of the Michigan Department of Community Health for analysis. Laboratory results for each specimen were reported back to the Michigan State

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University Department of Epidemiology. These reports included the type of container used to store each specimen.

Determination of PCBs, PBBs, and DDE. The ACSL performed the laboratory analysis for PCB congeners [International Union of Pure and Applied Chemistry (IUPAC) designations], as well as for PBBs and DDE, in 1999. In brief, OCs were extracted into diethyl ether/hexane (1:1 vol/vol), and the resulting extract was passed over a Florisil column. The 6% (vol/vol) diethyl ether in hexane fraction from the Florisil column was further fractionated into a PCB fraction and a pesticide fraction using a fully activated silica gel 60 column. PCB and DDE analyses were performed by high-resolution GC with electron capture detection (ECD) according to the modifications of the procedure previously reported by Najam et al. (1999) and Mullin et al. (1984). ACSL investigated 88 different PCB congeners, with a limit of detection (LOD) of 0.03 µg/L for the PCB congener giving the highest ECD response. The total PCB concentration was calculated as the sum of the reportable PCB congeners at or above the respective LOD. The PBBs and other organochlorine pesticides were analyzed using the modifications of a procedure by Needham et al. (1981).

The LOD for the PCBs was between 0.03 and 0.8 µg/L, and the LOD for PBBs and

DDE was 1.0 µg/L. Information about the mode of storage (glass vs. plastic containers) was also obtained from the ACSL.

Statistical approach. The body mass index (BMI) for each participant was calculated as [weight (kilograms)] ÷ [height (centimeters)]². Smoking was grouped into three categories: nonsmokers, ex-smokers, and current smokers. Regarding organochlorines, values < LOD were treated in two ways: a) by focusing on observations with detectable concentrations of PCBs, PBBs, and DDE; and a) by imputing half the LOD for all observations < LOD (Finkelstein and Verma 2001; Hornung and Reed 1990).

For descriptive purposes, we used medians along with their corresponding 5th and 95th percentiles. In an attempt to estimate the impact of the plastic and glass containers, we applied linear regression analyses (Kleinbaum et al. 1988). The potential confounding effects of age, sex, BMI, number of meals of sport-caught fish consumed in the last year, total duration of fish consumption in years, and region of residence were controlled for in the regression analyses.

In order to fulfill the requirements of multivariate normal distribution, we used a log transformation and calculated the adjusted geometric mean for the different specimen containers and potential confounders. In separate models, we estimated the effect of the

specimen container for the sum of PBBs, DDE, and sum of PCBs. These analyses assess the extent to which OC concentrations were affected. All analyses were conducted using SAS software, Version 8 (SAS Institute Inc., Cary, NC).

Results

Of the 143 serum specimens collected, 2 contained an insufficient volume for analysis. PCB congeners were detected in 135 samples (96%), PBBs in 59 (42%), and DDE in 73 (52%). For the 141 specimens analyzed by the ACSL, 28 were stored in glass containers and 113 were stored in plastic containers. Table 1 shows that the median concentration of PCBs is significantly higher in specimens stored in plastic containers (0.87 µg/L vs. 0.46 µg/L). The distribution of the total PCB concentration is shifted toward higher values in serum stored in plastic containers (Figure 1).

For DDE and PBBs, we detected only small differences with regard to type of container. Eight PCB congeners (Table 2) are responsible for the vast concentration of the sum of the PCBs (89% in samples from plastic containers and 94% in samples from glass). These eight congeners were detected in 94% of the samples from plastic containers and in 96% of samples that were stored in glass containers. DDE was detected in 53% of the samples stored in plastic (60 of 113; Table 1) and

Table 1. Comparison of PCB, PBB, and DDE concentrations and characteristics of participants for type of storage container.

Variable	Glass				Plastic			
	No. samples	Median	Percentile		No. samples	Median	Percentile	
			5th	95th			5th	95th
Total PCB concentration, without imputed values	27	0.46	0.12	3.15	108	0.87*	0.15	9.33
Total PCB concentration, with imputed values	28	0.48	0.12	3.15	113	0.80*	0.15	9.33
Congeners without imputed values (LOD, µg/L)								
PCB-153 (0.1)	20	0.25	0.11	0.78	93	0.24	0.11	1.43
PCB-180 (0.1)	27	0.23	0.12	0.64	108	0.30*	0.13	1.44
PCB-187 (0.05)	11	0.09	0.05	0.27	43	0.08	0.05	0.38
PCB-194 (0.03)	18	0.05	0.03	0.23	73	0.07	0.03	0.29
PCB-195 (0.03)	1	0.03	0.03	0.03	11	0.04	0.03	0.15
PCB-199 (0.2)	3	0.22	0.21	0.50	19	0.30	0.16	0.87
PCB-138/163 (0.2)	13	0.48	0.21	1.24	76	0.40	0.20	2.07
DDE concentration	13	1.60	1.00	5.60	60	1.50	1.00	6.80
DDE with imputed value	28	0.50	0.50	4.40	113	1.00	0.50	5.70
PBB concentration	10	2.35	1.00	19.40	49	2.10	1.00	6.10
PBB with imputed value	28	0.50	0.50	10.90	113	0.50	0.50	5.40
Age during survey	28	33.50	28.00	41.00	111	33.00	25.00	50.00
Sport-caught fish meals in the last 12 months	27	6.00	0	34.00	110	7.75	0	52.00
Lifetime of fish consumption (years)	26	25.00	15.00	35.00	109	25.00	10.00	35.00
Weight (kg)	26	86.18	54.43	114.31	108	78.25	54.43	113.40
Height (cm)	28	180.34	162.56	187.96	110	172.72	154.94	185.42
BMI (kg/cm ²)	28	27.29	21.11	35.73	108	25.63	20.05	35.90
Female ^a	32.1%				49.6%			
Smoking status ^b								
Nonsmoker	35.7%				54.1%			
Ex-smoker	25.0%				19.8%			
Smoker	39.3%				26.1%			
Place of residence ^a								
Michigan west coast	57.1%				59.3%			
Saginaw area	42.9%				26.6%			
Detroit area	0%				14.2%			

^aValues indicate the percentage of 28 specimens stored in glass and 113 specimens stored in plastic. ^bValues indicate the percentage of 28 specimens stored in glass and 111 specimens stored in plastic. **p* ≤ 0.05, Wilcoxon test.

46% of the samples stored in glass (13 of 28). The respective percentages for PBBs are 43% for plastic and 36% for glass. No statistically significant differences were observed for the proportions of detection for PCBs, DDE, and PBBs with regard to glass or plastic storage containers.

The median age for subjects was comparable for specimens stored in both types of containers: 33.5 years for specimens in glass containers and 33 years for those in plastic (Table 1). The BMI of the participants was higher in the glass-container group. The glass-container group also includes more smokers and fewer women. Participants in the two groups did not differ with regard to lifetime fish consumption or number of sport-caught fish meals consumed in the last 12 months. All participants in the Detroit area went to commercial blood-draw stations.

Total PCB concentrations were comparable in all three regions (Table 2). PBBs were lower in the Detroit region compared with PBBs in serum samples from the west coast of Michigan, which reflects the historic PBB exposure in western Michigan. Also DDE was

higher in the west coast region of Michigan. For only PBBs and DDE with imputed missing values, but not for PCBs, the regional difference gains statistical significance controlling for confounders ($p = 0.0001$ and $p = 0.02$, respectively).

Controlling for potential confounders, we detected a significantly higher PCB concentration in samples that were stored in plastic containers compared with glass containers (Table 3). This difference in the PCB concentrations from samples stored in glass and plastic containers was independent whether values below the LOD were imputed (half the LOD) or treated as missing values. For DDE and PBBs, no statistically significant differences were identified in regard to specimens stored in either glass or plastic containers.

For total PCB concentration, the confounders age, sex, and the number of sport-caught fish meals consumed in the last year gained statistical significance. PCB levels increased with age ($p = 0.002$). Males had higher PCB concentrations ($p = 0.03$) compared with females. Forty-seven percent of the variance of total PCBs was explained by age,

sex, sport-caught fish meals, years of fish consumption, BMI, smoking history, and type of container.

Twenty percent of the PBB variance was explained in the model without imputed values ($n = 58$) and 15% in the model with imputed values ($n = 141$). No predictor was found to be statistically significant. Thirty-seven percent of the variance of DDE was explained in the model without imputed values ($n = 69$) and 41% in the model where 0.5 $\mu\text{g/L}$ was substituted for values < LOD ($n = 132$). Of the predictors, only age gained statistical importance ($p = 0.0005$).

Of the 88 PCB congeners measured by this procedure, the ACSL identified values > LOD of 0.03–0.2 $\mu\text{g/L}$ in 14 congeners (PCB-074, PCB-99, PCB-105, PCB-118, PCB-138/163, PCB-153, PCB-156, PCB-170/190, PCB-180, PCB-187, PCB-193, PCB-194, PCB-195, and PCB-199). Only 7 congeners showed detectable values in more than three participants. Of these, only the congeners PCB-180 and PCB-199, the latter with few observations, differed with regard to being stored in plastic or glass containers (Table 1). Only the concentration of PCB-180 was statistically significantly different ($n = 127$ with detectable PCB-180 concentrations; geometric mean, plastic vs. glass container: 0.29 $\mu\text{g/L}$ vs. 0.22 $\mu\text{g/L}$; $p = 0.035$). Although the PCB-180 concentration was lower in glass containers, PCB-180 explains 52% of the total PCB concentration of samples from glass and 34% from plastic containers. In general, the profile of the congeners is comparable with those found by Humphrey et al. (2000) in a cohort of fish eaters in Michigan.

To investigate whether the total PCB concentration was still different after subtraction of PCB-180 and PCB-199, we reran linear regression analyses for total PCBs without these congeners. However, the results still showed statistically significantly higher concentrations for storage in plastic versus glass containers ($p = 0.03$).

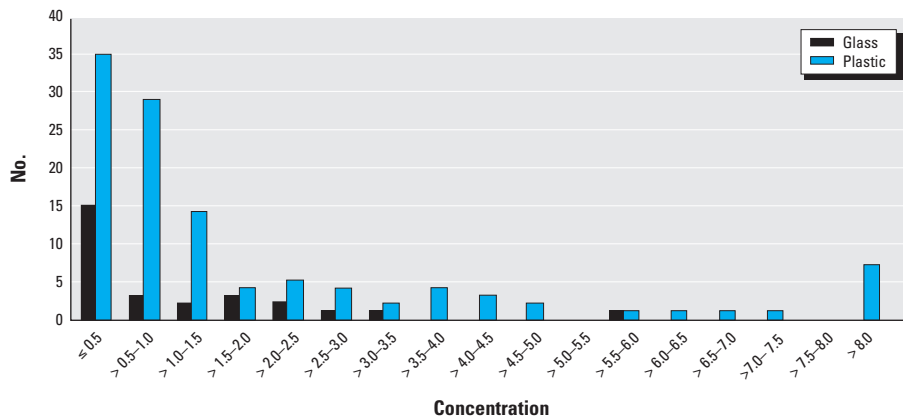


Figure 1. Distribution of the concentration of total PCB concentration ($\mu\text{g/L}$, without imputed values) for type of storage container.

Table 2. Comparison of PCB, PBB, and DDE concentrations for participants in different Michigan areas.

	West coast				Saginaw area				Detroit area			
	No.	Median	Percentile		No.	Median	Percentile		No.	Median	Percentile	
Total PCBs	81	0.75	0.15	7.20	38	0.87	0.12	6.69	16	0.9	0.12	10.5
PBB	50	2.15	1.10	8.70	6	2.10	1.00	5.40	3	1.00	1.00	3.40
DDE	48	1.95	1.00	7.00	16	1.40	1.00	4.40	9	1.50	1.00	2.30

Table 3. Adjusted geometric mean organochlorine concentrations ($\mu\text{g/L}$) for type of storage container.

Variable	Type of container		Probability (<i>F</i> -test) ^a
	Glass	Plastic	
Total PCB concentration	0.44	0.75	0.027
Total PCB concentration, with imputed values	0.45	0.75	0.025
DDE concentration	1.83	1.73	0.79
DDE concentration, with imputed values	0.73	0.80	0.59
PBB concentration	2.83	1.71	0.10
PBB concentration, with imputed values	0.80	0.76	0.79

^aAdjusted for age, sex, BMI, smoking, years of fish consumption, fish meals consumed in the last 12 months, and region (west coast of Michigan, Saginaw area, and Detroit area).

Discussion

Our results indicate that the proportions of detectable halogenated organic compounds are not different in regard to samples kept in glass or plastic containers. However, storage of serum in plastic containers can alter the total concentrations of PCBs, in particular the higher chlorinated PCB congeners (PCB-180 and PCB-199). No other characteristics of these storage subsamples could explain the differences. Thus, indirectly, we must attribute increased PCB concentrations in serum to storage in plastic containers. Surprisingly, the concentrations of DDE and PBBs were not affected.

The high PBB concentrations found in our sample reflects the historic exposure to

PBBs on the western coast of Michigan. The incident occurred in 1973, when PBB, instead of magnesium oxide, was shipped for use in cattle and chicken feed; this error set in motion one of the most notorious environmental disasters on record. In 1974, unexplained deaths of cattle led to the discovery of the contamination of cattle and agricultural products (Landrigan et al. 1979).

At the beginning of the project, we understood that the diagnostic laboratory, which drew and stored 79% of our serum samples in 12 different locations in Michigan, was storing the samples in glass containers. However, after processing and analyzing the samples for reproductive hormones, the laboratory then transferred the specimens to plastic containers for freezing and stored them in its central facility.

Because of unexplained differences and the revelation of critical information on storage in plastic containers, which led to a number of statistical analyses and investigations, the principle investigator (W.K.), who took over the ongoing study when the blood collection was already under way, decided not to use these values as markers of exposure. Our discussion of disclosure of these findings led to disagreements with the former departmental administrator, who interfered and finally divided the project. Because of the risk of disclosure and lack of funding, appropriate reactions were not initiated at the time when the problems were detected. Such measures could have included, for example, chemical analyses of the plastic containers (very likely high-density polypropylene), experimental tests with plastic and glass tubes, and further measurements of potential contaminations.

Thus, a limitation of the analyses is that the inference is only indirect. Because rules for collection, preservation, and storage of samples were established to prevent contamination (Watts et al. 1980) and no other factor could explain the difference, we presume that an alteration of PCB concentration occurred in serum samples stored in some or all of the plastic containers. Siyali and Stricker (1973) attributed high levels of pesticides to plastic containers and stoppers. Burse et al. (1991) reported that Vacutainer tubes and closures for serum storage bottles were suspected to have contaminated serum samples; interferences were detected in 70% of the samples but could not be identified. Our results indicate that PCB concentrations were affected by the type of storage container, but did not indicate alterations in DDE and PBB concentrations in samples stored in plastic containers. Regarding plastic containers, the ACSL has no evidence for the presence of phthalates or other substances used in the production of plastics in the specimens tested. Additional analyses using GC and mass spectrometry in pooled samples from serum stored in glass and

plastic containers did not identify interfering phthalates in the congener peaks. Hence, some unknown substances in the plastic containers that coelute with PCB retention time may have elevated the PCB levels. It is less likely that the glass containers absorbed some organic compounds because storage in glass did not lead to a higher proportion of nondetects. The proportion of nondetects for PCB-180 was 96% for both glass and plastic (Table 1; 27/28 vs. 108/113).

Another limitation of the analyses is that concentrations of the halogenated organic compounds were not corrected for differences in serum lipid concentrations. It is conceivable that higher lipid concentrations of those participants whose serum ended up in plastic containers might explain the higher PCB values. However, this is very unlikely because DDE and PBBs should have also been elevated in individuals with higher lipid serum concentrations.

We are aware that these findings did not result from a well-designed experiment. Nevertheless, our results emphasize the need for further evaluations of specimens stored under controlled conditions (temperature, time) in glass and different plastic container types. In particular, if we move to new emerging analytes, the issue of specimen collection, handling, and storage will require further standardization. However, such investigations have been conducted with food; there is evidence that food products stored in plastic can become contaminated with properties of the packages. For example, plasticizers (phthalates) and naphthalene have been reported to migrate from polyethylene material into food (Ackman and Macpherson 1996; Castle et al. 1989; Lau et al. 1994), as well as vinyl chloride from polyvinyl chloride (Sauvant et al. 1995) and styrene from polystyrene containers (Tawfik and Huyghebaert 1998). However, also the reverse effect, namely, lowering of contaminations because of migration into plastic packaging, has been reported (Simko and Brunckova 1993).

When using organochlorine measurements for the assessment of health effects, contamination due to storage in different types of containers will introduce an information bias. In the best case, the bias is nondifferential, meaning that the type of storage is not related to the health outcomes. In this case, the PCB–health association is likely to be underestimated (or not detected) because of dilution of the true PCB concentration (biased toward the null value). In the worst case, the information bias is differential, implying that the type of storage is directly or indirectly related to health outcomes. In our population, this situation cannot be excluded because the subgroup visited by phlebotomists, who used glass containers, may be different (less mobile or less healthy)

than those who went to the blood-drawing station. Such a scenario could result in a systematic bias of the PCB–health association because the health status is indirectly (via type of phlebotomy) related to storage.

Recommendations

We presume that the identification of this bias has a scientific value and should initiate future quality control. Potentially interfering compounds from storage of biologic specimens in plastic containers need further investigation. The U.S. EPA requests quality assessments for each project. The National Institutes of Health and other agencies do not make this request.

Although analytical methods for the determination of PCBs and other organochlorines were addressed in the Standard Reference Material 1589a document published in August 2000 (National Institute of Standards and Technology 2000), rules for the collection, preservation, and storage of samples have not been updated since 1980. Hence, we may *a)* reinforce the 1980 rules or *b)* provide new guidelines, recommend specific container materials as suggested by Burse et al. (1991), and implement quality control measures for collection and storage material.

We believe that it is necessary for the epidemiologic and toxicologic studies that employed organochlorine concentrations as exposures to disclose how specimens (e.g., serum, human milk) were collected and stored before analyses. No information is available for a large number of studies that had a profound impact on health risk assessments of PCBs. Specifically, we recommend initiating a review process of studies to determine whether results were based on unbiased PCB determinations.

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