Chemosphere xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Pilot study to assess effects of collection tube types and processing delay on measurements of persistent organic pollutants and lipids in human serum

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ARTICLE INFO

Article history:
Received 30 October 2013
Received in revised form 6 April 2014
Accepted 7 April 2014
Available online xxxx

Handling Editor: J. de Boer

Keywords:
Polychlorinated biphenyls (PCBs)
Polybrominated diphenyl ethers (PBDEs)
Organochlorine pesticides (OCPs)
Perfluorinated compounds (PFCs)
Serum Separation Tubes (SSTs)
Red top tubes (RTs)

ABSTRACT

Glass red top tubes (RTs), traditionally used to draw blood for biomonitoring studies, have some limitations during field sampling (e.g., tube breakage, timely processing may be difficult). This pilot study examined whether serum separation tubes (SSTs) with delayed processing time (48 h) can be used instead of red top tubes (RTs) to accommodate field conditions. Using state-of-the-art methodologies, PBDEs, PCBs, OCPs, PFCs, cholesterol and triglycerides were measured to evaluate any differences among 2 test conditions (RTs with 2 h processing time; SSTs with 48 h processing time). Between the 2 test conditions, we observed high rank correlations among the measured compounds and no statistically significant differences in the levels of measured compounds. We conclude that SSTs with delayed processing time (48 h) produce similar results as RTs with short processing time (2 h), suggesting that SSTs could be good substitutes for RTs for new epidemiological and biomonitoring field studies. The use of SSTs offers a tremendous opportunity for the use of samples archived in various SSTs.

1. Introduction

Several types of collection tubes are routinely used in clinical settings to draw blood for medical tests. In all its studies, the California Biomonitoring Program (www.biomonitoring.ca.gov) has followed CDC's guidelines and recommended laboratory procedures (Gunter and Koncikowski, 1996). It has traditionally used glass red top tubes ("RTs") with no coating to collect blood for the analysis of trace levels of perfluorinated compounds (PFCs) and persistent organic pollutants (POPs) such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in human serum to minimize artifact effects (e.g., background levels and interferences) during sample collection, process, and storage (Gunter and Koncikowski, 1996). However, the RTs are fragile (made of glass) and their use requires timely centrifugation to separate, and then transfer the serum into multiple vials in a biosafety or fume hood prior to freezing, shipping or storage. Together these requirements present obstacles for field biomonitoring studies where timely processing may be difficult, if not impossible, and the absence of proper equipment may lead to contamination.

Plastic serum separation tubes (Tiger Top, "SSTs") with clot activator and double gel for transport are also routinely used in clinical laboratories. The SSTs are coated with silicone and contain a clot activator gel for maximum serum separation. SSTs may be more suitable for large field epidemiologic and biomonitoring studies of POPs because they are durable and require only a single step of handling (centrifugation) without the need for transferring the serum before freezing, shipping and storage. However, there is no information on whether these extra material could cause higher background and interference in the trace analysis of POPs in human serum. The objective of this study was to evaluate whether measured values of PFCs and POPs in serum are affected by the choice of tube type and processing delay. Specifically, we performed a series of tests to explore whether we could use SSTs instead of RTs and whether we could process the blood samples within 48 h instead of 2 h after the blood draw to facilitate field studies. We set 48 h as the maximum delayed processing time because, in our current studies, we received all samples within 48 h after collection from the field where sample process cannot be done without proper equipment (e.g., centrifuge). We hypothesized that concentrations of POPs in blood collected in SSTs with delayed process would not be different from those collected in traditional RTs and processed within 2 h.

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http://dx.doi.org/10.1016/j.chemosphere.2014.04.007
0045-6535/

Fig. 1. Two types of blood collection tubes. SSTs are made of plastic and contain separation gel at the bottom of tubes. Red top tubes are made of glass and have no additives and no silicone coating. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Blood collection tubes

2.1.1. Glass red top tubes (RTs)

Red Top, 10 mL BD® collection tube (Becton & Dickinson product number 366441) with no interior coating, non-silicone stopper lubrication and no anticoagulant.

2.1.2. Plastic serum separation tubes (SSTs)

Tiger Top, 10 mL BD® collection tube (Becton & Dickinson product number 367985) with clot activator, double gel for transport, and silicone coated interior. The tubes are shown in Fig. 1.

2.2. Study subjects

Eleven healthy adult volunteers (2 males and 9 females) were enrolled in this pilot study with signed informed consent. A certified phlebotomist drew blood sequentially in SSTs and RTs from each participant (5–8 mL/tube).

2.3. Test conditions for tube types and processing delay

All blood samples collected in RTs were allowed to clot for 2 h at room temperature (RT.2). After clotting, the RTs were centrifuged and all serum transferred to a second set of RTs. The second set of RTs was centrifuged again to ensure that the serum was free of any cells and the serum was then transferred to pre-cleaned amber glass storage vials. The vials were capped and stored upright at −20 °C prior to analysis. This condition is considered the standard procedure and it has been routinely used for POP analysis.

The blood samples collected in SSTs were allowed to clot for 48 h at 4 °C (SST.48). After clotting, SSTs were centrifuged to achieve a good separation of serum – all serum should be isolated above the gel. After serum separation, the SSTs were stored upright at −20 °C prior to analysis.

2.4. Standards and reagents

2.4.1. POP standards and reagents

Nine ^{13}C-labeled PBDEs ($^{13}C_{12}$-BDE-28, -47, -99, -153, -154, -183, -197, -207, and -209) (Wellington Laboratories, Canada) and nine ^{13}C-labeled PCBs and seven ^{13}C-labeled OCPs ($^{13}C_{12}$-PCB-101, -105, 118, -138, -153, -156, -170, -180, -194, $^{13}C_{12}$ 2,4′-DDT, $^{13}C_{12}$-4,4′-DDE, $^{13}C_{12}$-4,4′-DDT, $^{13}C_6$-hexachlorobenzene, $^{13}C_{10}$-oxychlordane, $^{13}C_{10}$-trans-nonachlor, and $^{13}C_6$-β-BHC) (Cambridge

Table 1
Detection frequency, measured compound concentrations (ng mL^{-1}, except for lipids), precision, bias, Spearman rank correlation coefficient and *p*-values for each analyte and tube type (RT.2 and SST.48).

Compound	Name	MDL	Detection Frequency (%)[a]	Analytical Precision (% CV)	SST.48 Concentration		RT.2 Concentration		SST.48/RT.2 Ratios		Spearman Corr Coeff	Bias	Paired *t*-test[b]
					Median	%CV	Median	%CV	Median	%CV		% Change	Adjusted p-Values
Lipids	CHOL (mg dL^{-1})		100	1.7	162	61.54	168	62.04	0.96	0.99	0.99	−2.60	0.90
	TG (mg dL^{-1})		100	1.8	68.0	66.04	68.0	66.35	1.00	1.00	0.97	−2.84	0.90
PBDEs	BDE-47	0.008	100	7.0	0.09	75.70	0.08	74.78	1.07	1.01	1.00	−2.40	0.86
	BDE-99	0.018	45	5.0	0.02	65.45	0.02	65.90	0.88	0.99	0.91	−8.30	0.86
	BDE-100	0.008	73	7.0	0.02	73.32	0.01	77.18	1.28	0.95	0.96	3.72	0.90
	BDE-153	0.016	64	7.0	0.04	70.04	0.03	71.76	1.29	0.98	0.61	−1.40	0.90
Pesticides	4,4′-DDE	0.016	73	16	0.92	80.57	0.46	414.1	1.99	0.19	0.65	−0.37	0.93
	HCB	0.008	100	8.0	0.06	62.99	0.06	63.24	1.15	1.00	0.38	1.39	0.90
	t-Nonachlor	0.008	91	8.0	0.06	74.67	0.06	83.16	1.07	0.90	0.63	−4.02	0.87
PCBs	PCB-74	0.008	55	7.0	0.01	71.34	0.01	76.81	0.78	0.93	0.90	−24.9	0.40
	PCB-99	0.008	45	12	0.01	69.23	0.01	67.45	1.67	1.03	0.85	23.6	0.86
	PCB-138	0.008	64	9.0	0.02	118.8	0.04	83.48	0.45	1.42	0.38	3.34	0.90
	PCB-153	0.008	73	2.0	0.10	108.1	0.07	120.6	1.40	0.90	0.56	−4.08	0.90
	PCB-180	0.008	91	13	0.12	82.77	0.09	91.58	1.37	0.90	0.66	8.43	0.86
PFCs	Me-PFOSA-AcOH	0.014	100	12	0.17	89.51	0.14	78.34	1.21	1.14	0.98	17.4	0.40
	PFHxS	0.018	100	9.0	0.87	82.29	0.81	77.15	1.08	1.07	0.97	2.04	0.40
	PFNA	0.014	100	12	0.64	63.43	0.72	62.92	0.89	1.01	0.48	−5.09	0.86
	PFOA	0.029	73	12	1.34	402.4	1.34	242.0	1.00	1.66	0.87	−0.41	0.93
	PFOS	0.090	100	12	5.67	72.04	5.02	72.37	1.13	1.00	0.95	−1.28	0.90
	PFOSA	0.008	64	12	0.04	112.3	0.04	94.41	0.96	1.19	0.97	−0.46	0.86
	PFUdA	0.026	73	13	0.14	91.76	0.09	97.10	1.54	0.94	0.87	22.3	0.40

[a] Concentrations of both test conditions greater than MDL.
[b] Paired *t*-test used only data where both concentrations were greater than MDL.

Please cite this article in press as: Guo, W., et al. Pilot study to assess effects of collection tube types and processing delay on measurements of persistent organic pollutants and lipids in human serum. Chemosphere (2014), http://dx.doi.org/10.1016/j.chemosphere.2014.04.007