# MEASUREMENT OF TRICLOSAN AND PENTACHLOROPHENOL IN SERUM FROM THE HISTORIC COHORT OF CALIFORNIA WOMEN (1960s, 1980s, 2000s): A PILOT STUDY

Park J-S[1], Guo W[1], Rhee A[1], Harwani S[1], Wang Y[1], Baek J-S[1], Wang M[1], Cohn B A[2], Cirillo P M, Factor-Litvak P[3], Petreas M[1]

[1]California Department of Toxic Substances Control, California Environmental Protection Agency, Berkeley, CA 94710; [2]Center for Research on Women's and Children's Health, Child Health and Development Studies, Public Health Institute, Berkeley, California 94709, USA; [3]Department of Epidemiology, Columbia University, New York, New York 10027, USA

**Abstract**

We optimized our current serum method to measure triclosan and pentachlorophenol by GC/HRMS and we used it to analyze California human serum collected during three different time periods: 1960s, 1980s and 2000s. Recovery of these compounds from the matrix spike samples prepared with bovine serum was in the reasonable error range (±25%). By using this method, we found that levels of pentachlrorophenol (max=51.9, mean 19.4±10.2 ng/mL wet wt.) in maternal serum from the 1960s (n=18) was among the highest recorded for human blood, while high levels of triclosan (mean 2.6±3.0 ng/mL wet wt.) were measured in contemporary serum (n=13), reflecting the different time periods and patterns of use of these chemicals in California. In addition, since high levels of pentachlorophenol and triclosan can be indicative of possible exposures to PCDD/PCDF, continuous monitoring of these contaminants is warranted to assess health risks and to support measures to reduce exposures to these contaminants.

## Introduction

Triclosan (TCS) and pentachlorophenol (PCP) have been found in human blood[1,2]. TCS is an antibacterial/anti-microbial agent, having been widely used in personal care products (e.g., toothpaste and soap) since 1960s[3,4]. Pentachlorophenol (PCP) has been extensively used as a fungicide for wood treatment since the 1930s and is still being used with increased regulation[5]. Public health concerns for these compounds have increased due to their endocrine disrupting properties such as disruption of thyroid homeostasis, abnormalities in reproduction, immunotoxicity, and cancer risk[5-10]. Despite normal metabolic excretion, some amount of these are still retained in the body[1,2,11], possibly via binding and circulating with blood proteins. TCS and PCP are also of concern because they are potential sources of environmental polychlorinated dioxin/furan (PCDD/PCDF) formed as impurities during the production and/or from heat and ultraviolet irradiation of these compounds[12,13]. Thus, it is possible that exposure to those compounds also result in exposure to PCDD/PCDF. In addition, halogenated phenolic compounds such as PCP and OH-PCBs, and presumably triclosan due to its structural similarities, have been reported to show high placental transfer rate[14], which poses a threat to developmental health.

The primary purpose of this pilot study was to assess whether our current blood analysis method for measuring OH-PCBs and OH-PBDEs could also be used for the measurement of TCS and PCP and to subsequently optimize it to measure those emerging contaminants in our Historic Cohort of California Women, to provide information on chemical trends in California residents.

## Materials and Methods

***Populations:*** Our Historic California Women Cohort study is a compilation of serum, adipose and milk samples collected over the last 40 years (1960s, 1980s, and 2000s) in the course of a number of distinct studies, each with its own design and objectives. All study participants provided written informed consent. After the completion of the original studies, left over samples or extracts can be used to explore further hypotheses. Availability of these archived samples provides an excellent vehicle to study trends of legacy chemicals, as well as to identify new chemicals of concern. For this pilot study we selected serum samples from three time periods:

1960s: Maternal serum samples provided by the Child Health Development Study (CHDS), a longitudinal birth cohort of over 20,000 births among members of the Northern California Kaiser Foundation Health Plan, collected from pregnant women enrolled between 1959 and 1967[15,16].
1980s: Serum samples from cancer-free women (controls) from a case-control breast disease study conducted during 1981-1986 by the University of California- San Francisco.
2000s: Contemporary serum samples collected during 2003-2009 by the Environmental Chemistry Laboratory and from a Sacramento Blood Bank.

***Analytical Method:*** Bovine calf serum (HyClone Logan, Utah, USA) was used for the recovery test experiment. TCS, PCP, and 4'-OH-CB159 (surrogate standard) and $^{13}C_{12}$ PCB-178 ( injection standard) were purchased from Sigma-Aldrich (St. Lois, Missouri, USA), Ultra Scientific (North Kingstown, RI, USA), and Wellington Laboratory (TerraChem Inc., USA), respectively. Diazomethane was synthesized in hexane by using N-nitroso-N-methylurea (Sigma-Aldrich). Other chemicals and solvents used for the analysis include dichloromethane and hexane (trace analysis, Burdick and Jackson), methanol, methyl-tert butyl ether, and water (HPLC grade, Fisher Sci., USA), 2-propanol (99.9%, pesticide grade, Fisher Sci., USA), hydrochloric acid, sulfuric acid (98%), potassium hydroxide, potassium chloride, sodium hydroxide, ethyl alcohol (94-96%, 200 proof) (Fisher Sci., USA), and silica (200-400 mesh) (Sigma-Aldrich, USA).

All analyses were performed in the ultra clean laboratory of the Department of Toxic Substances Control, Berkeley, CA. The blood extraction method used is described elsewhere[2]. In summary, we separated the phenolic fraction by using MTBE:hexane (1:1,v/v), denaturation (6M HCl and 2-propanol), KCl (1%) wash, and KOH (0.5M) phase separation. After acidification and derivatization, the extracts in the phenolic fractions were cleaned up by using concentrated $H_2SO_4$ (98%) and then a Pasteur pipette column packed with acidic silica gel (1:2, w/w) and activated silica gel. The two phenolic compounds of interest were determined as methyl derivatives by using gas chromatography with a high resolution mass spectrometer (ThermoFisher DFS) equipped with a programmable temperature vaporization (PTV) injector and DB-5 capillary column (15m × 0.25 mm i.d., 0.10 μm thickness, J&W Scientific, Folsom, CA). The initial GC temperature was set to 90 $^{o}$C and held for 1.5 min followed by a 15 $^{o}$C/min increase to 250 $^{o}$C, 40 $^{o}$C/min to 300 $^{o}$C and held for 3 min[1]. The molecular ions were monitored for all analytes; [$(M)^+$ and $(M+2)^+$]. We derivatized the external calibration standards simultaneously with the serum sample extracts for accurate quantification. Precision and accuracy from surrogate spike, inner batch duplicate samples, and control samples were within analytical error ranges (±25%). The concentrations are expressed as ng/mL wet wt. since they are preferentially bound to blood protein rather than lipids.

**Results and Discussion**

We validated the method using the liquid/liquid extraction and column clean up described above[2] and further optimization of the GC-HRMS technique. In the method validation experiment, the average recovery of TCS was 86±14%, from the five duplicated bovine serums spiked with TCS (2 ng). The GC/HRMS detected 0.20 pg of both PCP and TCS with S/N ratios > 5.

As part of the Child Health Development Study, we had analyzed 1960s' California serum for PCBs (n=459, average $\Sigma_{11}$PCBs = 3.27±1.53 ng/mL ww) and we also analyzed a subset of this population for OH-PCBs (n=30, average $\Sigma_8$OH-PCBs =0.14±0.08 ng/mL)[17]. Using this modified method we measured PCP and TCS concentrations in 18 samples from the same subset (n=30). For contemporary samples, we used a Blood Bank serum pool (n=3) and 10 individual samples. Figure 1, showing the chromatogram of PCP and TCS seen in 1960s and contemporary serum of California residents, and Figure 2 showing means and standard errors, confirm that the level of PCP was remarkably high in the 1960s, while much lower levels of PCP were found in contemporary samples. In contrast, TCS was prevalent in the contemporary serum while only trace level was measured in the 1960s samples. This is expected since PCP has been used as fungicide for wood treatment since the 1930s and is still being used with limitations in the industry[5], while TCS has been circulating within the market (e.g., personal care products) for shorter time (~45 years)[3,4]. The 1960s serum PCP levels ranged from 4.49 to 51.9 ng/mL wet wt (mean 19.4±10.4), which are among the highest levels ever found in humans or wildlife and even higher than the 4,4'-DDT levels (mean 12.2±6.50) in this population. PCP can be a biotransformation product of hexachlorobenzene. However, (with our limited preliminary samples size) these two compounds didn't correlate (r=0.03), indicating multiple sources for PCP found in blood. Contemporary mean serum TCS levels (2.6 ng/mL wet wt.) are comparable to those found in European populations[1] with mean levels ranging from 0.02-16.0

ng/mL wet wt. In this report we found that TCS is retained in the body similarly to other phenolic compounds (e.g., PCP). In addition, high levels of PCP and TCS measured in serum can be indicative of possible exposures to PCDD/PCDF. Therefore, given the persistence of these contaminants and the widespread and (for TCS) increasing use in everyday products, monitoring PCP and TCS is warranted to support measures to reduce exposures (Green Chemistry). Analyses of additional serum samples from California residents are in progress to examine chemical trends every two decades (1960s, 1980s, 2000s).

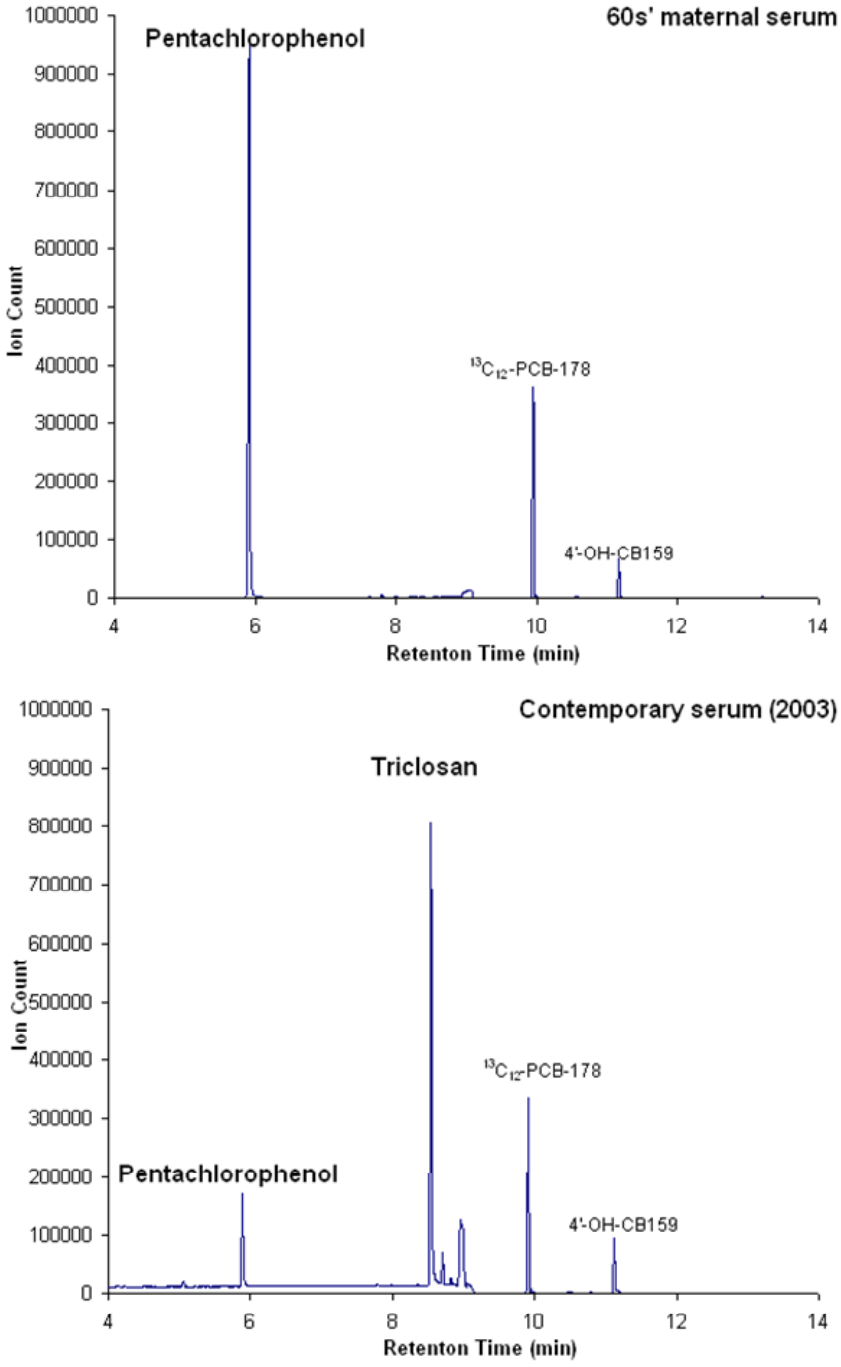


Figure 1. GC-HRMS chromatogram of triclosan and pentachlorophenol in 1960's and contemporary human serum.

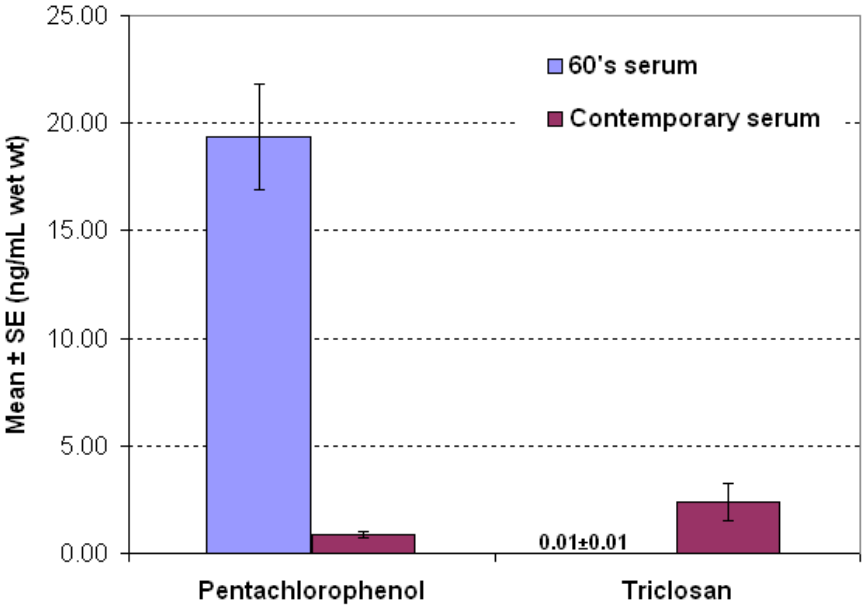


Figure 2. Levels of pentachlorophenol and triclosan found in 1960s (n=18) and contemporary samples (n=13).

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