



Serum concentrations of TCDD and other dioxin-like compounds in US Air Force veterans of Operation Ranch Hand



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HIGHLIGHTS

- We measured PCDDs, PCDFs and PCBs in serum of Vietnam War veterans (US Air Force).
- Median TCDD levels in 2002 were higher in Ranch Hands than in comparison veterans.
- Levels of other PCDDs, PCDFs and noPCBs did not differ substantially between groups.
- Levels of moPCBs did not differ substantially between Ranch Hands and comparisons.
- We also compared results to those from comparable age groups studied in NHANES.

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ABSTRACT

We measured serum concentrations of seven dibenzo-p-dioxin congeners (PCDDs), ten dibenzofurans (PCDFs), four non-ortho polychlorinated biphenyls (noPCBs) and six mono-ortho polychlorinated biphenyls (moPCBs) in 1950 veterans of the Vietnam War. The veterans were participants in the Air Force Health Study (AFHS) who attended the final medical examination in 2002. Blood samples were collected from 777 Ranch Hands involved in the aerial spraying of herbicides in Vietnam and a comparison group of 1173 veterans ("Comparisons") who served in Southeast Asia during the same time period. Results for moPCBs were based on a random subsample of 800 veterans.

The median 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) concentrations in 2002 were 5.0 pg g⁻¹ lipid in Ranch Hands and 2.2 pg g⁻¹ lipid in Comparisons. No substantial differences were found in measured concentrations of other PCDDs, PCDFs, and noPCBs. Similarly, no substantial differences were found for moPCBs in the subsample. The median total dioxin toxic equivalent (TEQ) in Ranch Hands was 18.7 pg g⁻¹ lipid for PCDDs, 3.4 pg g⁻¹ lipid for PCDFs, and 3.2 pg g⁻¹ lipid for noPCBs. Median TEQs in Comparisons were 14.4 pg g⁻¹ lipid for PCDDs, 3.5 pg g⁻¹ lipid for PCDFs, and 3.3 pg g⁻¹ lipid for noPCBs. These TEQs, with the exception of PCDD TEQ in Ranch Hands (primarily due to elevated TCDD), were similar to or lower than those reported for similar age and gender groups in the 2001–2002 National Health and Nutrition Examination Survey (NHANES). These findings support the assumption that the Ranch Hand veterans were not more highly exposed to dioxin-like compounds other than TCDD than were Comparison veterans or the general US population.

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1. Introduction

The Air Force Health Study (AFHS) was a 20-year prospective study that examined health outcomes in US Air Force veterans of Operation Ranch Hand, the unit responsible for aerial spraying of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-contaminated Agent

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Orange and other herbicides in southern Vietnam from 1962 to 1971. US Air Force veterans in the AFHS comparison group flew or serviced C-130 transport aircraft in Southeast Asia (SEA) during the same time period and did not spray herbicides. They were matched on age, race and military occupation to Ranch Hand veterans and included as a control group. All veterans were male, 94% were White (the distinction between Hispanic and non-Hispanic White was not made) and 6% were African-American (Lathrop et al., 1984; Michalek et al., 1990; Wolfe et al., 1990). Comparisons spent on average less than 30% of their SEA service time in Vietnam

and were mainly stationed in other SEA countries (Pavuk et al., 2005). The study included comprehensive physical examinations and in-person interviews conducted in 1982, 1985, 1987, 1992, 1997, and 2002. Multiple health endpoints were examined over the course of the study in association with the exposure group (Ranch Hand), with serum TCDD measured from the 1987 physical examination forward (Kerger et al., 2012; Lathrop et al., 1984; Michalek et al., 1990; Michalek and Pavuk, 2008; Pavuk et al., 2003; Wolfe et al., 1995).

The herbicide Agent Orange was a 1:1 mixture of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in diesel oil and was contaminated during the production process with TCDD from less than 0.05 to almost 50 ppm (Buckingham, 1982; IOM, 1994). The US military applied herbicides by aerial spraying from C-123 aircraft in Vietnam in order to defoliate the forests to deny cover and food crops to the opposing forces. Between 1965 and 1970, more than 11 million gallons of Agent Orange were sprayed, along with smaller quantities of Agents White (2,4-D; picloram) and Blue (cacodylic acid). Agents Purple (2,4-D; 2,4,5-T), Blue, Pink (2,4,5-T), and Green (2,4,5-T) were used for defoliation from 1962 to 1965, while only Agents White and Blue were used from 1970 to 1971 (Buckingham, 1982; Stellman et al., 2003). The only contaminant of concern identified in Agent Orange and other herbicides containing 2,4,5-T was TCDD; consequently, other dioxins or dioxin-like compounds were not measured in the AFHS cohort originally (Wolfe et al., 1990). Only at the 2002 AFHS physical examination did the participants have blood drawn to test for polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) in addition to TCDD. However, no results for these compounds other than TCDD were included in the final AFHS report in 2006 because funding for the analyses was not available at that time (Michalek et al., 2005).

Kahn et al. (1988) measured other PCDD congeners and PCDFs in Ranch Hands previously and showed that exposed veterans were unlikely to have dioxins other than TCDD elevated in fat or blood lipids; it is often overlooked, though, that this pilot study was conducted with only a small sample of 10 exposed veterans. Later, Kang et al. (1991) analyzed Vietnam War veterans' serum for multiple dioxins and similar compounds, confirming a low likelihood of elevated concentrations of dioxins other than TCDD in veterans that did not routinely handle Agent Orange. Analyses by Schecter et al. (1996) suggest that elevated serum TCDD concentrations would still be detected, decades after initial exposure, in Vietnam War veterans highly exposed to Agent Orange. The AFHS study protocol with the US Air Force concluded in September 2007. Upon the recommendation of a committee of the Institute of Medicine on the disposition of the AFHS (IOM, 2006), Congress passed a law requiring the study datasets, medical records, and blood specimens collected since 1982 be transferred to a new custodian, the Medical Follow-up Agency (MFUA) (Public Law 109-364). After completing a consent process, electronic data and records for 90.2% of the AFHS participants who attended at least one physical examination were transferred to MFUA.

The present study is part of the project begun in 2003 to complete the exposure assessment of this cohort by measuring four main classes of dioxin-like organochlorine compounds: PCDDs, PCDFs, non-ortho-substituted polychlorinated biphenyls (noPCBs), and mono-ortho-substituted polychlorinated biphenyls (moPCBs). The main goal was to determine in the whole Ranch Hand cohort whether exposure to dioxin-like compounds other than TCDD might have occurred in Vietnam or at a later time and may have confounded any of the associations observed with the TCDD in any previous studies of the cohort.

We previously reported results of PCDDs, PCDFs, and PCBs in a non-random sample of 106 veterans who attended the 2002

examination and had no previous TCDD measurements (Pavuk et al., 2007). Those earlier measurements are included in the present report. For the first time, results of analyses of serum PCDDs, PCDFs, and PCBs are presented for the entire sample of 1950 veterans who attended the final 2002 AFHS physical examination.

2. Methods

A detailed description of the AFHS methods are published elsewhere (Wolfe et al., 1990). In the present study, 1174 Comparisons and 777 Ranch Hand veterans who attended the 2002 physical examination at the Scripps Clinic, California, each had 40 ml of blood collected for dioxin measurements. One Comparison veteran's blood sample was damaged in shipping and not subsequently redrawn, leaving 1173 Comparison veterans' blood samples that were analyzed. Participation was voluntary and informed consent was obtained at the examination site. Serum was harvested from blood within 2 h of blood collection, frozen and stored at -20°C . Specimens were later shipped on dry ice to Brooks City-Base, TX, and stored at -70°C . Starting in 2003, samples were sent to the dioxin laboratory at the Centers for Disease Control and Prevention (CDC) for analysis. At CDC, analyses were conducted as technical assistance and in compliance with Research Determination procedure; an exemption from the CDC IRB was obtained. AFHS data collection was approved by the US Air Force IRB.

Seven 2,3,7,8-substituted PCDD congeners, ten 2,3,7,8-substituted PCDFs, and four noPCBs (PCBs 77, 81, 126, and 169) were measured using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Details of the procedures were first described by Patterson et al. (1986, 1987) and modified for current analyses (Turner et al., 1997). Ortho-substituted PCBs were measured in serum by high-resolution gas chromatography isotope-dilution high-resolution mass spectrometry (HRGC/ID-HRMS) as described by Sjödin et al. (2004); results for six moPCBs (PCBs 118, 105, 167, 156, 157, and 189) are presented here.

The concentration of each analyte for both groups was calculated from its linear calibration curve. Study specimens were analyzed in batches of 24 intermixed with quality control ($n = 3$) and method blank samples ($n = 3$) in accordance with previously published procedures (Taylor, 1981). All data were reviewed using comprehensive quality assurance and quality control procedures and the analytical results were reported on both a whole-weight and lipid-adjusted basis. Serum lipids were calculated using an enzymatic summation method (Bernert et al., 2007). Detection limits were reported for each analyte on a whole weight and lipid-adjusted basis and were corrected for sample weight and analyte recovery. Medians and percentiles of distribution were compared among race and age groups by examining whether or not the 95% confidence intervals overlap, which is a conservative method of rejecting the null hypothesis (Schenker et al., 2002). For comparison with other studies, World Health Organization (WHO) Toxic Equivalency Factors (TEFs) from both 1998 and 2005 were used to report toxic equivalents (TEQs) for PCDDs, PCDFs, and dioxin-like PCBs (van den Berg et al., 1998, 2006). For concentrations below the limit of detection, we substituted the limit of detection divided by the square root of 2 (Hornung and Reed, 1990). Technical problems with chromatographic columns from the equipment supplier precluded completion of analyses on 1044 samples for ortho-substituted PCBs in 2006 before the samples had to be returned to the Air Force. Consequently, results presented here for moPCB congeners are based on analyses completed on a random sample of 800 veterans. We compared

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