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Concentrations of organochlorine pesticides in pooled human serum by age and gender

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ABSTRACT

Organochlorine pesticides (OCPs) have been used for many decades in Australia with cessation of selected persistent and bioaccumulative OCPs ranging from the 1970 s to as recently as 2007. The specific aims of this study were to use samples representative of an Australian population to assess age and gender differences in the concentration of OCPs in human blood sera and to investigate temporal trends in these chemicals.

Serum was collected from de-identified, surplus pathology samples over five time periods (2002/03, 2006/07, 2008/09, 2010/11 and 2012/13), with 183 serum pools made from 12,175 individual samples; 26 pools in 2002/03, 85 pools in 2006/07 and 24 pools each in 2008/09, 2010/11 and 2012/13. Samples were analyzed for hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), γ -hexachlorocyclohexane (lindane) (γ-HCH), oxy-chlordane, trans-nonachlor, *p,p′*-DDE, o,p′-DDT, *p,p′*-DDT and Mirex. Stratification criteria included gender and age (0–4; 5–15; 16–30; 31–45; 46–60; and > 60 years) with age additionally stratified by adults > 16 years and children 0–4 and 5–15 years.

All pools from all collection periods had detectable concentrations of OCPs with a detection frequency of > 60% for HCB, β-HCH, trans-nonachlor, *p,p′*-DDT and *p,p′*-DDE. The overall OCP concentrations increased with age with the highest concentrations in the > 60 years groups. Females did not have higher mean OCP concentrations than males except for HCB concentrations (p=0.0006).

Temporal trends showed overall decreasing serum concentrations by collection period with the exception of an increase in OCP concentrations between 2006/07 and 2008/09. Excluding this data point, HCB decreased from year to year by 7–76%; β-HCH concentrations decreased by 14 – 38%; trans-nonachlor concentrations decreased by 10 – 65%; *p,p′*-DDE concentrations decreased by 6 – 52%; and *p,p′*-DDT concentrations decreased by 7 – 30%. The results indicate that OCP concentrations have decreased over time as is to be expected following the phase out of these chemicals in Australia.

1. Introduction

Organochlorine pesticides (OCPs) such as dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexanes (HCHs), chlordanes, hexachlorobenzene (HCB) and Mirex are persistent, lipophilic chemicals that are known to accumulate in human tissues (Laug et al., 1951; Egan et al., 1965). They also degrade slowly in humans, animals, air, water and soil and are subject to long-range transport (Shen et al., 2005; Kang et al., 2012; Mrema et al., 2012). Exposure to these chemicals has been linked to reproductive effects (Longnecker et al., 2005; Mahalingaiah et al., 2012), cancer (as reviewed in Mrema et al., 2012), reduced childhood growth in boys (Burns et al., 2012) and development of obesity, dyslipidaemia and insulin resistance (Lee et al., 2011). The major exposure pathways in the general population are diet (U.S. Department of Health and Human services et al., 2002), breastfeeding (Mueller et al., 2008) and placental transfer (Siddiqui et al., 1981; Dewan et al., 2013). These OCPs are covered under the Stockholm Convention on persistent organic pollutants (POPs) to which Australia is a signatory. As such, there is an obligation as part of Article 16 of the Stockholm Convention for Australia to contribute to

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global monitoring (Stockholm Convention on POPs, 2010).

In Australia, DDT was used from the 1940 s as a pesticide. While the Australian phase out began in the 1970 s, its use continued as benefits were considered to outweigh disadvantages (Australian Academy of Science, 1972). By 1975, OCPs were registered for use against a wide range of pests in most Australian crops as well as termiticides in domestic, agricultural and commercial buildings and as insecticides around livestock quarters, sheep yards and food storage structures. The use of OCPs for virtually every agricultural purpose was stopped by 1985, except for Mirex as a termiticide. Mirex was discontinued in 1995 in all Australian states and territories except for the Northern Territory, where its use ceased in 2007 (Australian Pesticides and Veterinary Medicines Authority, 2013).

In a review of OCPs, knowledge gaps, including the interplay of age, gender, collection period, and cohort effects were reported (Porta et al., 2008). The only study on long term trends of OCPs in the Australian population focused on breast milk samples, and compared new data with data from the literature and suggested that OCP concentrations were decreasing (Mueller et al., 2008). Biomonitoring is valuable to assess both continued risk and mode of exposure for OCPs. We developed a human biomonitoring program that used pooled surplus pathology serum specimens stratified on the basis of age and gender. The current study aims to investigate temporal trends of various persistent organic pollutants and to expand our assessment of human body burden of OCPs in the Australian population. The specific objectives of this study were to use samples from an Australian population to: investigate temporal trends by comparing results from samples collected in 2002/03, 2006/07, 2008/09, 2010/11, and 2012/13; and assess differences in OCP concentrations associated with age and gender in sera collected in a given period.

2. Materials and methods

2.1. Sample collection

Pooled samples of human blood serum from males and females were collected in metropolitan South East Queensland, Australia in 2002/03, 2006/07, 2008/09, 2010/11 and 2012/13. These samples were used to provide robust serum data on OCP concentrations for five time points over the past decade. All samples were obtained in collaboration with Sullivan Nicolaides Pathology (SNP) from de-identified surplus pathology samples. Stratification criteria included age (Table 1) and gender.

Table 1
Stratification of age (years) for each collection year.

2002/03	2006/07	2008/09	2010/11	2012/13
–	Umbilical Cord sample	–	–	–
–	0–0.5	–	–	–
–	0.6–1	–	–	–
–	1.1–1.5	–	–	–
–	1.6–2	–	–	–
–	2.1–2.5	–	–	–
–	2.6–3	–	–	–
–	3.1–3.5	–	–	–
–	3.6–4	–	–	–
–	–	0–4	0–4	0–4
–	4.1–6	–	–	–
–	6.1–9	–	–	–
–	9.1–12	–	–	–
–	12.1–15	–	–	–
–	5–15	5–15	5–15	5–15
< 16	–	–	–	–
16–30	16–30	16–30	16–30	16–30
31–45	31–45	31–45	31–45	31–45
46–60	46–60	46–60	46–60	46–60
> 60	> 60	> 60	> 60	> 60

Each serum pool comprised 100 individual de-identified surplus serum samples, except those pools created for the 2006/07 sampling that contained up to 30 samples per pool. A replicate pool was created for each strata (age group, gender and collection period). Specific details on the sampling regime is given in Harden et al. (2007); Toms et al. (2009, 2012).

In total, 12,175 individual samples were used to make 183 pools; 26 pools (n =2555) in 2002/03, 85 pools (n =2420) in 2006/07, and thereafter 24 pools (n =2400) were used (2008/09 until 2012/13). While within a sampling period no donor contributed more than one sample to the study, it was not possible to determine if any one donor contributed to more than one collection period. University of Queensland Medical Research Ethics Committee and the Queensland University of Technology Ethics Committee granted ethics approval for this study.

2.2. Sample analysis

The OCPs investigated were: hexachlorobenzene (HCB), β -hexachlorocyclohexane (β-HCH), γ -hexachlorocyclohexane (lindane) (γ-HCH), oxy-chlordane, trans-nonachlor, *p,p′*-DDE, o,p′-DDT, *p,p′*-DDT and Mirex. The samples were analyzed using the methods that have been described previously (Jones et al., 2012). Briefly, a set of samples was defined as 24 unknown samples with three analytical blanks and three quality control/quality assurance (QC/QA) samples and were processed using a semi-automated sample preparation method. Human sera (2 g) were weighed into test tubes and fortified with internal standards (13C-labeled) using a 215 Liquid Handler (Gilson Inc, Middleton, WI). Then, formic acid and water were added to denature proteins and dilute the samples on the liquid handler. The target analytes were extracted into dichloromethane using the solid phase extraction (SPE) workstation (Rapid Trace®, Zymark, Hopkinton, MA). Clean up was performed on a two layered column. The top layer comprised activated silica and the bottom layer comprised silica gel/sulfuric acid (2:1 by weight). The top layer retained polar lipids such as cholesterol, while the bottom layer degraded the remaining lipids to produce an extract suitable for the measurement of target analytes. This procedure was automated using the modular SPE workstation. Samples were evaporated to 1 mL and transferred to the gas chromatograph vials, which were previously spiked with recovery standards. Samples were further evaporated to 10 μL and analyzed by gas chromatography high resolution mass spectrometry. A DFS (ThermoFinnigan, Bremen, Germany) instrument was used for the analysis. The chromatographic separations were carried out on a 6890 gas chromatograph (Agilent Technologies, Atlanta, GA) fitted with a DB5HT capillary column [(15 m, 0.25 mm inner diameter, and 0.10 μm thickness)]. The results are expressed as ng/g lipid and are reported to two significant figures. Where a chemical was found to be below the limit of detection (LOD) it is reported as < LOD. The LOD was dependent on sample size and blanks. The samples were analyzed in three runs; the 2006/07 samples were analyzed in 2007, the 2002/03 and 2008/09 samples were analyzed in 2009 and the 2010/11 and 2012/13 samples were analyzed in 2013.

2.2.1. Quality Assurance/Quality Control (QA/QC)

Six blind field blanks were included in the analytical runs (two in the 2006/07 analysis; one each in the 2008/09, 2010/11 and 2012/13 analyses). The field blanks were comprised of bovine serum (Sigma Aldrich B8655), expected to have OCP concentrations below the detection limit in the analytical methodology. The blanks used were aliquoted into the collection tubes used by the pathology laboratory, frozen, defrosted and placed in the sampling containers used for human samples. No OCPs were detected in the blanks, indicating that contamination did not occur during the sample collection and pooling process.