Determination of the levels of dioxin and dioxin-like compounds in the Australian population by analysis of pooled human breast milk

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Introduction

Dioxin-like compounds are ubiquitously distributed and humans are exposed to them via various sources but primarily through food. They can be detected in air, water, soil, sediment and biota. These compounds are lipid soluble, poorly eliminated and thus can accumulate in human adipose tissue. They can cross the placenta and are also transferred to breast milk during the lactation process. Therefore infants are exposed ante and postnatally. Since PCDD/PCDF concentration in blood and human milk are very similar when concentrations are expressed on a lipid basis, human milk provides a good monitoring tool of exposure for a given population in a given area[1]. Previously the WHO has co-ordinated international studies on dioxin-like compounds in breast milk. These were conducted in 1987/88, 1992/93 and 2001. In summary, these studies demonstrated that levels of dioxins in breast milk are relatively high in industrialised countries when compared to non-industrialised countries[2]; that PCDD/PCDFs were higher in human milk from mothers with their first child[3]; and that the levels decrease over a given lactation period[4]. The present study aims to examine the levels of these compounds in primiparae women throughout Australia.

Methods

Sample Collection

The study was carried out as part of the National Dioxins Program for the Australian Government Department of Environment and Heritage, then Environment Australia. In order to allow direct comparison with previous World Health Organization (WHO) exposure studies, the protocol used in this study was identical to that used by the WHO in their international studies assessing the exposure levels in human breast milk for dioxin-like compounds and PCBs. Ethical approval was obtained at all appropriate ethics committees prior to the commencement of participant recruitment.

Participant selection and recruitment

Participants who met the required eligibility criteria were recruited from a variety of sources. These included: child health clinics, medical practitioners, lactation consultants, maternity hospitals, ante and postnatal clinics, newspaper and web-based advertising as well as word of mouth. Once a potential participant had verbally agreed to participate, they were invited to read an information sheet and to complete a consent form. They were also asked to complete a questionnaire (not shown here).

Volunteering mothers were selected using the following criteria:

- A primipara (first-time) mother with a baby aged two to eight weeks (mothers of IVF babies were included);
- Exclusively breastfeeding;
- Willing to provide a minimum of 100ml (preferably 150ml) of expressed milk. This volume was to be collected over the six week period (2-8 weeks post-partum);
- Healthy pregnancy, mother and child; and
- A resident of the area for the past five years.

Samples were collected either using a pump or by directly expressing the milk into the glass container that was provided to the volunteering mother by the study team. Samples were stored and shipped frozen to the laboratory at EnTox/QHSS. When collection of a pool was completed the milk was thawed, thoroughly homogenised and 30 ml from each individual was pooled giving approximately 300ml of pooled milk sample from each region. The pooled samples were then refrozen and transported on ice to Sydney, Australia and Münster, Germany.

Sample numbers

The initial aim of the project was to collect samples from 200 women across Australia. At the end of the project the total number of samples collected was 173, of these 16 samples were excluded because they were later found to have violated the inclusion/ exclusion criteria. Collection of samples was slower than anticipated due to the nature of the samples required, the necessity to ensure that all sites had correct ethical clearance and the strict inclusion criteria. The two most difficult criteria were the baby age range and residential status.

Analysis

All pooled samples were sent to the Australian Government Analytical Laboratories, Sydney, Australia and two duplicate samples (i.e. 50 percent volume of 2 of the pools) were sent to the State Laboratory of NRW, Münster, Germany. Both are laboratories accredited for dioxin analysis. Methodologies for analysis of PCDD/Fs in breast milk are described in detail elsewhere[3,4,6]. Briefly, cleanup was performed on all samples, lipid content was determined and samples were analysed using high resolution mass spectrometry.

Results and Discussion

The results of this study provide a measure of the levels of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in pooled human breast milk collected throughout Australia in 2002/03. The study has focused on donor cohorts with different potential exposure to dioxins and dioxin-like compounds in Australia (i.e. urban/industrial/rural exposure).

Samples were collected from 12 regions of Australia (see Figure 1) during the period March 2002 to September 2003. In total, 157 samples were analysed as 17 pooled samples from the following region: Brisbane, Sydney (2 pools), Melbourne (4 pools), Adelaide (2 pools), Perth, Hobart, rural inland NSW (Dubbo), rural inland Queensland (Dalby), rural Victoria (Bendigo, Ballarat, Lakes Entrance), Newcastle, Wollongong and Darwin.

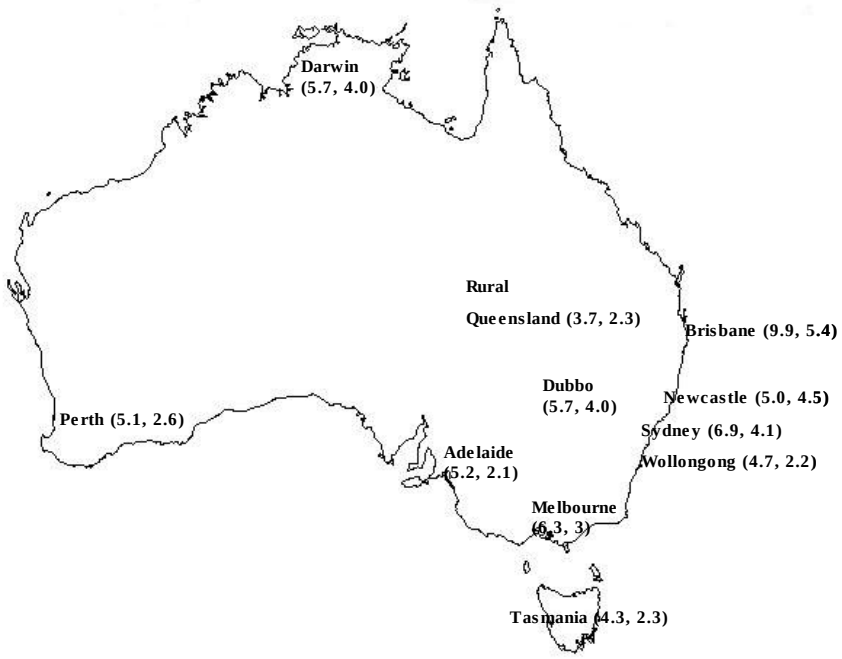

Figure 1. The geographic regions from which samples were collected with concentrations of PCDD/Fs and PCBs in pg WHO-TEQ/g lipid (PCDD/F, PCB).

In addition to these samples, a further 24 "historical" samples collected in 1993 by the Key Centre for Applied and Nutritional Toxicology, Royal Melbourne Institute of Technology, Melbourne, Australia, were analyzed as three pools of eight samples. In total, 20 pools of breast milk were analyzed. PCDD/PCDFs and PCBs were detected in all pooled samples. For samples collected during 2002/03, the mean and median levels, expressed as TEQ were 9.0 pg TEQ g^{-1} lipid and 8.9 pg TEQ g^{-1} lipid, respectively. Lipid content was measured in all pooled samples and gave an average lipid concentration of $3.7 \pm 0.5\%$. No systematic differences were observed in the levels of dioxin-like chemicals in breast milk samples collected from different regions of Australia during 2002/03. A higher level of dioxin-like chemicals was detected in the Brisbane pool (15.2 pg TEQ g^{-1} lipid) but a lower (2.8%) lipid content in this sample suggests that the elevated level may be an

artefact. Figure 2 shows the levels of PCDD/Fs and PCBs detected in samples collected in 2002/03.

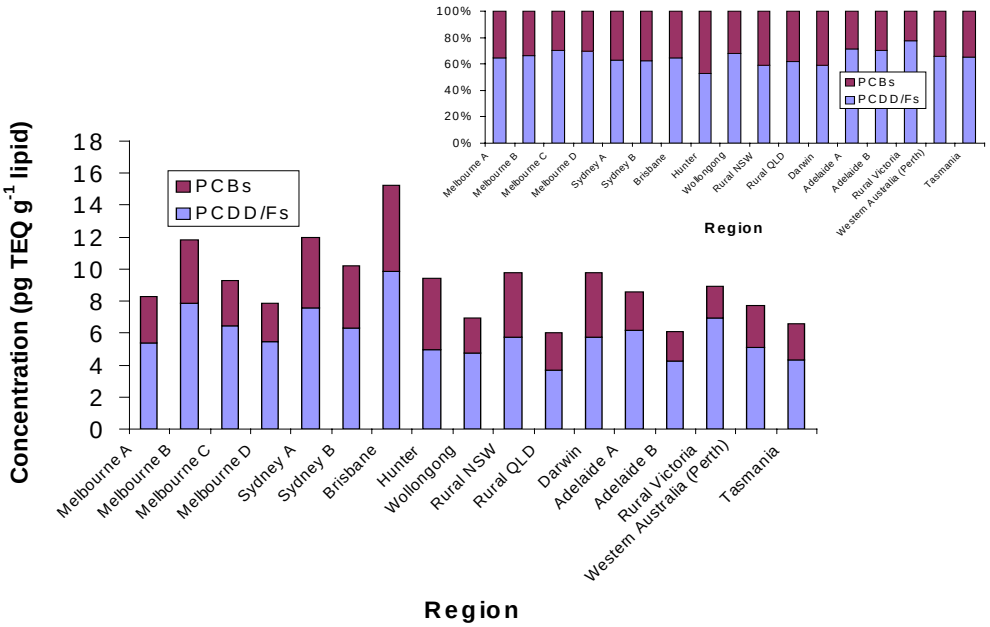

Figure 2. Concentration of dioxin-like chemicals in breast milk samples collected and pooled for different sampling regions. Presented are the levels expressed as $TEQ_{PCDD/PCDF}$ and TEQ_{PCB} and the inset provides a direct evaluation of the percent contribution of the PCBs and PCDD/PCDFs to the overall TEQ in each sample pool.

For samples collected in 1993, the mean and median levels, expressed as TEQ (upper bound) were 16 pg TEQ g^{-1} lipid and 16.4 pg TEQ g^{-1} lipid, respectively. Lipid content was measured in all pooled samples and gave an average lipid concentration of 3.9 ± 0.7%.

A comparison of the samples collected from Melbourne women in 1993 with those collected for the present study showed clearly that the levels of these chemicals decreased over the ten year time period. It should, however, be noted that comparison of the two sample populations is complicated because details of maternal parity and infant age at date of collection was not made available for the older samples. Despite these limitations, a clear decrease in the levels of these compounds over time was observed. The concentration decreased by almost a factor of two from 1993 to 2003, from 16±1.4 to 9.1±1.3 pg g^{-1} lipid. Consistently, PCDD/PCDFs as well as PCBs decreased by about 60% during this period. This reflects the world-wide trend over recent decades of declining levels of dioxin-like compounds in the environment and humans. This was observed in the 3rd round WHO exposure studies, where on average the decline between the 2nd round in 1993 and the

3rd round in 2003 was about 40%[7]. Consistent with this trend a decline of 70% was observed in a study conducted in New Zealand[8].

In summary, the levels of PCDD/PCDFs and PCBs in the breast milk of Australian women are both similar across all regions of Australia and low by international standards. Consistent with world-wide trends, the levels of dioxin-like compounds have decreased over a ten year period from 1993 to 2003 by approximately 60%. It should be noted that it is the advice of the WHO and the National Health and Medical Research Council (NHMRC) in Australia that breast milk is the best food for babies. Breast milk may contain low levels of dioxins because of its fat content, but all babies are exposed to dioxins even if they are not breastfed. Alternative foods for babies, such as infant formula, may also contain dioxins because they may also contain fat. Several studies around the world in areas where dioxin levels are known to be high have still shown that breastfed babies are healthier than those fed infant formula[9].

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