National Dioxins Program

Technical Report No. 10
Dioxins in the Australian Population:
Levels in Human Milk

A consultancy funded by the Australian Government
Department of the Environment and Heritage

Prepared by Dr Fiona Harden, Dr Jochen Müller and Leisa Toms

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3. Inventory of Dioxin emissions in Australia 2004
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7. Dioxins in Fauna in Australia
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Phone: 1800 803 772
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National Dioxins Program
c/- Chemical Policy
Department of the Environment and Heritage
GPO Box 787
CANBERRA ACT 2601
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Foreword

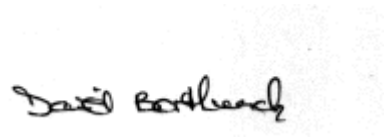
When the Australian Government established the four year National Dioxins Program in 2001, our knowledge about the incidence of dioxins in Australia was very limited.

The aim of the program was to improve this knowledge base so that governments were in a better position to consider appropriate management actions. Starting in mid 2001, a range of studies were undertaken which involved measuring emissions from sources such as bushfires, as well as dioxin levels in the environment, food and population. The findings of these studies were used to shed light on the risk dioxins pose to our health and the environment.

This work has been completed and the findings are now presented in a series of twelve technical reports.

Having good information is essential if there is to be timely and effective action by governments; these studies are a start. Our next step is to foster informed debate on how we should tackle dioxins in Australia, as this is an obligation under the Stockholm Convention on Persistent Organic Pollutants. The Department of the Environment and Heritage will be working closely with other Australian Government, State and Territory agencies to take this step.

Ultimately, the effective management of dioxins will be the shared responsibility of all government jurisdictions with the support of the community and industry.



David Borthwick

Secretary

Department of the Environment and Heritage

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- the project teams from the CSIRO, the National Research Centre for Environmental Toxicology and Pacific Air & Environment who undertook the studies assessing the levels of dioxins in the environment, the population and from emission sources, the overseas experts who provided advice to these organisations, and the many individuals across Australia who collected the samples in the field
- the Department of Agriculture, Fisheries and Forestry, who assessed the levels of dioxins in agricultural commodities
- Food Standards Australia New Zealand and the Department of Health and Ageing and who assessed the levels of dioxins in foods and assessed the health effects of dioxins
- officers of the Chemical Assessment Section in DEH who assessed the ecological effects of dioxins
- members of the National Dioxins Project Team which included representatives from the State and Territory environment protection agencies, the Australian Health Ministers Conference and the Primary Industries Ministers Council
- members of the National Dioxins Consultative Group which included representatives from industry and agricultural sectors, environment and public health groups and research institutions.

The Department would also like to especially thank Dr Simon Buckland (Environment Risk Management Authority, New Zealand) and Dr Joel Michalek (US Air Force) who provided valuable review on an early draft of this report.

Project Team

Fiona Harden (project leader), Jochen Müller, Leisa Toms, Michael Moore - National Research for Environmental Toxicology, Brisbane Qld.

Debbie Burniston, Robert Symons - Australian Government Analytical Laboratories, Pymble NSW.

Jorma Ahokas - Royal Melbourne Institute of Technology, Melbourne Vic.

Peter Fürst - State Laboratory of NRW (North Rhine Westpalia), Münster Germany.

Olaf Päpke - ERGO, Hamburg Germany.

Contributors

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- All of the mothers and babies who donated or attempted to donate their precious time and milk samples for this study
- All of the local coordinators who assisted in the recruitment of participants and the subsequent collection and return of samples
- All laboratory staff at AGAL
- All laboratory staff at State Laboratory of NRW, Münster, Germany.

The National Research Centre for Environmental Toxicology is cofunded by Queensland Health.

Executive Summary

The objective of this project was to investigate the levels of dioxin-like compounds in pooled human milk samples collected throughout Australia. These samples included polychlorinated dibenzo-*p*-dioxins and furans (PCDD/PCDFs) and polychlorinated biphenyls (PCBs). This study was carried out as part of the National Dioxins Program for the Australian Government Department of the Environment and Heritage. The study focused on donor cohorts with different potential exposure to dioxins and dioxin-like compounds in Australia (i.e. urban/industrial/rural exposure). The study was carried out in the following stages:

- obtaining appropriate ethical approval
- selection of the regions
- selection of cohorts suitable to provide information on the levels of dioxins and dioxin-like compounds in breast milk throughout Australia
- contact with local agencies to support the study
- identification of volunteers who fulfil the selection criteria of the individual cohorts
- collection of samples and completion of the questionnaire with individual donors
- pooling of samples
- analysis of dioxins and dioxin-like compounds in a certified laboratory
- data analysis and interpretation
- report writing
- data dissemination for public knowledge and peer review.

The results of this study provide a measure of the levels of polychlorinated PCDDs, PCDFs and PCBs in pooled human breast milk collected throughout Australia in 2002/03. Breast milk samples were collected from primiparae[1] mothers recruited from a variety of sources. In order to allow direct comparison with previous World Health Organization (WHO) studies, volunteering mothers were selected using the following criteria:

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

[1] A woman who is pregnant for the first time, or a woman who has only given birth to one child.

- A resident of the area for the past five years.

In total, 173 samples were collected from 12 regions of Australia during the period March 2002 and September 2003. Of these, 16 were excluded because they were later found to have violated the inclusion/exclusion criteria. The remaining 157 samples were analysed as pooled samples and there were 17 pooled samples in total. These 157 samples covered the following sampling regions:

- Brisbane
- Sydney (two pools)
- Melbourne (four pools)
- Adelaide (two pools)
- Perth
- Hobart
- Rural inland NSW (Dubbo)
- Rural inland Queensland (Dalby)
- Rural Victoria (Bendigo, Ballarat, Lakes Entrance)
- Newcastle
- Wollongong
- Darwin.

In addition to these samples, a further 24 "historical" samples collected in 1993 by the Key Centre for Applied and Nutritional Toxicology[2], were analysed as three pools of eight samples. In total, 20 pools of breast milk were analysed.

All pooled samples were sent to the Australian Government Analytical Laboratories, Sydney, and two duplicate samples (i.e. 50% volume of two of the pools) were sent to the State Laboratory of NRW, Münster, Germany. Both laboratories are accredited for dioxin analysis.

PCDD/PCDFs and PCBs were detected in all pooled samples. For samples collected during 2002/03, the mean and median levels, expressed as TEQ (middle bound), were 9.0 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±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). This pool, however, showed a lower concentration of lipids in the breastmilk and since the concentrations of dioxin-like chemicals are normalised to the lipid content the elevation is likely to be related to an abnormally low lipid concentration in that sample and not an elevated level of contaminants. It is unclear why the lipid content was lower but it may have been due to preparation of the milk prior to analysis or analytical problems with the lipid determination in this sample. Samples obtained from Brisbane/Queensland for

[2] Based at the Royal Melbourne Institute of Technology, Melbourne, Australia.

the WHO breast milk study as well as the results from the SE-Qld region for the blood study have not shown elevated levels of dioxin-like chemicals and, therefore, this result is not thought to reflect higher exposure of Brisbane women to these compounds.

For samples collected in 1993, the mean and median levels, expressed as TEQ (upper bound), were 16 and 16.4 pg TEQ g^{-1} lipid, respectively. Lipid content was measured in all pooled samples and the average concentration was found to be 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 worldwide 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% (Malisch and van Leeuwen, 2003). Similarly, a decline of 70% was observed in a study conducted in New Zealand (Bates et al, 2001).

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 worldwide 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. (Department of the Environment and Heritage fact sheet http://www.deh.gov.au/industry/chemicals/dioxins/factsheet3.html)

Glossary/Abbreviations

ACT	Australian Capital Territory
Australian Lab	Australian Government Analytical Laboratories, Sydney
cohort	group of sample donors from set geographical location
congener	closely related chemicals derived from the same parent compound
Dioxins	common name for polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans.
Dioxin-like Compounds	common name used in this report for polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls together.
EnTox	National Research Centre for Environmental Toxicology
Furan	polychlorinated dibenzofuran.
GC/MS	gas chromatography/ mass spectrometry.
German Lab	State Laboratory of NRW, Münster, Germany.
Homologue	a group of structurally related chemicals that have the same degree of chlorination.
HRGC/HRMS	high resolution gas chromatography/ high resolution mass spectrometry.
IUPAC	International Union of Pure and Applied Chemistry.
LCS	laboratory control sample.
LOD	limit of detection, the lowest level at which a chemical can be measured in a sample by the analytical method used.
Lower bound TEQ	Toxic equivalencies (TEQ) for which concentration of a non-detected congener assumed to be equal zero.
Melb	Melbourne.
mL	milliliter.
Middle bound TEQ	Toxic equivalencies (TEQ) for which concentration of a non-detected congener assumed to be equal to half the non detect value.
Mono-*ortho* PCBs	includes PCB congener numbers 105, 114, 118, 123, 156, 157, 167, 189.
nd	non-detect.
NDP	National Dioxin Program.
Non-*ortho* PCBs	Includes PCB congener numbers 77, 81, 126, 169.
NRCET	National Research Centre for Environmental Toxicology.
NSW	New South Wales.
NT	Northern Territory.
OCDD	Octachlorodibenzo-*p*-dioxin.

OCDF	Octachlorodibenzo-furan.
PCBs	Polychlorinated biphenyls.
PCDDs	Polychlorinated dibenzo-p-dioxins.
PCDFs	Polychlorinated dibenzofurans.
pg g^{-1}	Picogram per gram, 10-12 g. Equal to nanogram per kilogram (ng kg^{-1}).
Pool	Samples collected within each strata.
POPs	Persistent organic pollutants.
QC	Quality control.
QA	Quality assurance.
QLD	Queensland.
QHSS	Queensland Health Scientific Services.
r^2	Coefficient of determination.
Region	Geographical location in Australia.
SA	South Australia.
Tas	Tasmania.
TEQ	Abbreviation of WHO_{98}-TEQ (this document).
TEF	Toxicity equivalency factors.
Upper bound TEQ	Toxic equivalencies (TEQ) for which concentration of a non-detected congener assumed to be equal to the non detect value.
UQ	The University of Queensland.
Vic	Victoria.
WA	Western Australia.
Weighted mean	mean concentrations calculated taking into account the number of individuals in each pool.
WHO	World Health Organization.
WHO_{98}-TEQ	World Health Organization toxic equivalent: the quantified level of each individual congener multiplied by the corresponding TEF. TEQs of each congener are summed to achieve an overall toxic equivalency for a sample (Van den Berg, 1998). In this document WHO_{98}-TEQ is abbreviated to 'TEQ'.

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1. Introduction

1.1. Background

At its meeting in December 2000, the Australian and New Zealand Environment and Conservation Council (ANZECC) requested the development of a discussion paper on dioxins for use in consultation with stakeholders. In April 2001, public meetings were held in several cities across Australia to seek public input into the development of a possible National Dioxins Program. These workshops noted the lack of information on dioxins in Australia and recommended that data be obtained on levels in the environment and the population. Following on from these consultations, a proposal for a National dioxins program was tabled at the meeting of ANZECC in June 2001. At this meeting, Council noted that the Australian Government would fund a National Dioxins Program (NDP) with $5 million over four years and that this program would generate data over the following two years which could be used to determine whether a specific regulatory approach would be required to manage dioxins. The NDP is being implemented by the Australian Government Department of the Environment and Heritage (DEH) in three phases:

- information gathering about the current levels of dioxins in Australia
- risk assessment using the information gathered as a basis to assess the potential risks of dioxins to the environment and human health
- development of measures to reduce, and where feasible, to eliminate the release of dioxins in Australia.

Dioxin is a general term often used to describe a group of compounds that belong to the larger family of persistent organic pollutants (POPs). These include polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs). In this report, these three groups of compounds will be referred to as dioxin-like compounds. POPs include some of the most toxic chemicals with respect to adverse health effects on humans and wildlife. The long-term uptake of POPs can have chronic effects in organisms such as carcinogenesis, endocrine disruptive properties, immune system effects and developmental abnormalities (Van den Berg et al., 1998). Physico-chemical properties of these compounds result in their extreme persistence in the environment, their ubiquitous distribution via long-range atmospheric transport from sources to remote areas and their ability to bioaccumulate and biomagnify in higher trophic organisms. Typically, more than 90% of the POP body burden in humans and other mammals is accumulated via food, in particular seafood, meat and dairy products (U.S. EPA 2000, Liem et al., 2000; Fürst et al., 1992a).

1.1.1. Dioxin and dioxin-like compounds

POPs include chlorinated hydrocarbons such as PCDDs, PCDFs and PCBs. PCDDs and PCDFs are tricyclic aromatic hydrocarbons and belong to the family of chemicals known as the halogenated hydrocarbons. They are comprised of two benzene rings joined via one or two oxygen atoms at adjacent carbons on each of the benzene rings. The base structures of these compounds are shown in Figure 1.1. Numbers 1-9 indicate

the possible positions of the chlorine atoms. They may have up to eight chlorine atoms attached at carbon atoms 1 to 4 and 6 to 9. Each individual compound is referred to as a congener, with 75 possible PCDD congeners and 135 possible PCDF congeners depending on the location and/or the number of attached chlorine atoms. The biological activity and toxicity of an individual congener varies with the number and position of the chlorine atoms. It should be noted that the most toxic effects are produced from congeners that have chlorine atoms in the 2,3,7,8 positions. Table 1.1 shows the homologue names, possible congener structure and the number of possible 2,3,7,8 congeners for the dioxin and furan family of compounds.

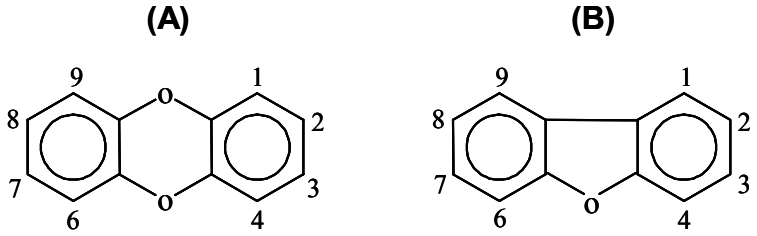

Figure 1.1 The structures of polychlorinated (A) dibenzo-p-dioxins and (B) dibenzofurans.

Table 1.1 Homologues and congeners of PCDDs and PCDFs

Abbreviation	Homologue name	No. of possible congeners	No. of possible 2,3,7,8-chlorinated congeners
MCDD	Monochlorodibenzo-*p*-dioxin	2	0
DiCDD	Dichlorodibenzo-*p*-dioxin	10	0
TrCDD	Trichlorodibenzo-*p*-dioxin	14	0
TCDD	Tetrachlorodibenzo-*p*-dioxin	22	1
PeCDD	Pentachlorodibenzo-*p*-dioxin	14	1
HxCDD	Hexachlorodibenzo-*p*-dioxin	10	3
HpCDD	Heptachlorodibenzo-*p*-dioxin	2	1
OCDD	Octachlorodibenzo-*p*-dioxin	1	1
Total dioxins		**75**	
MCDF	Monochlorodibenzofuran	4	0
DiCDF	Dichlorodibenzofuran	16	0
TrCDF	Trichlorodibenzofuran	28	0
TCDF	Tetrachlorodibenzofuran	38	1
PeCDF	Pentachlorodibenzofuran	28	2
HxCDF	Hexachlorodibenzofuran	16	4
HpCDF	Heptachlorodibenzofuran	4	2
OCDF	Octachlorodibenzofuran	1	1
Total furans		**135**	

1.1.2. Polychlorinated biphenyls

PCBs are compounds that were commercially produced by the chlorination of biphenyl. These compounds were graded and marketed according to their chlorine content. Some exhibit a structural and chemical similarity to the PCDDs and PCDFs. The base structure of PCBs is shown in Figure 1.2. Numbers 2-6 (2'-6') indicate the possible positions of the chlorine atoms at ortho (o), para (p) and meta (m) positions, respectively. As with dioxins, chlorine atoms may attach at any of the positions 2-6 and 2'-6' and there are up to 209 different congeners. The toxicity and the biological activity of PCBs are also dependent on the number and position of the chlorine atoms. Table 1.2 shows the distribution of PCB congeners derived from the basic biphenyl structure.

Table 1.2 Distribution of PCB congeners

No of Cl substituents	Cl_1	Cl_2	Cl_3	Cl_4	Cl_5	Cl_6	Cl_7	Cl_8	Cl_9	Cl_{10}
No of congeners	3	12	24	42	46	42	24	12	3	1

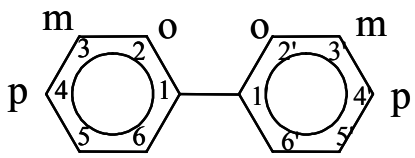

Figure 1.2 The structures of polychlorinated biphenyls (PCBs).

1.1.3. Sources of dioxin-like compounds

PCDD and PCDFs are generally regarded as trace contaminants in a number of chemical products and are formed as by-products during industrial, chemical and combustion processes where carbon containing organic material is incinerated in the presence of chlorine. These include the manufacture of chemicals such as chlorophenols and herbicides including 2,4,5 trichlorophenoxyacetic acid (2,4,5-T), and in combustion or incineration processes such as waste incinerators and fossil fuel power plants. They also enter the environment from non-industrial and natural sources including domestic wood and waste burning, uncontrolled forest fires, vehicle emissions and tobacco smoke (Gaus et al., 2001).

Since dioxins comprise a large group of compounds with different toxicity, the concept of toxic equivalency factors (TEF) has been developed to facilitate risk assessment of exposure to complex mixtures of these compounds and promote international consistency in addressing dioxin contamination (Van den Berg et al., 1998 and 2000). This concept is based on the evidence that dioxin-like compounds share a common mechanism of action - binding to the Ah-receptor. Of these compounds, 29 are considered by the World Health Organization (WHO) to have significant toxicity (Van den Berg et al., 1998). The toxicity of the different compounds relative to that of 2,3,7,8-TCDD is determined on the basis of toxicological data. Each compound is then assigned a TEF relative to that of 2,3,7,8-TCDD. Hence, the closer the TEF is to 1.0, the more toxic the congener. By multiplying the TEF of the individual compounds by their concentration, toxic equivalencies (TEQ) can be calculated and used for risk characterisation and management purposes. Table 1.3 shows the TEF for PCDDs, PCDFs and dioxin-like PCBs for humans and mammals (Van den Berg et al., 1998).

Table 1.3 TEF values for PCDDs, PCDFs and PCBs

Congener	TEF value	Congener	TEF value
Dibenzo-p-dioxins		Non-ortho PCBs	
2,3,7,8-TCDD	1	PCB#77	0.0001
1,2,3,7,8-PnCDD	1	PCB#81	0.0001
1,2,3,4,7,8-HxCDD	0.1	PCB#126	0.1
1,2,3,6,7,8-HxCDD	0.1	PCB#169	0.01
1,2,3,7,8,9-HxCDD	0.1		
1,2,3,4,6,7,8-HpCDD	0.01		
OCDD	0.0001		
Dibenzofurans		Mono-ortho PCBs	
2,3,7,8-TCDF	0.1	PCB#105	0.0001
1,2,3,7,8-PeCDF	0.05	PCB#114	0.0005
2,3,4,7,8-PeCDF	0.5	PCB#118	0.0001
1,2,3,4,7,8-HxCDF	0.1	PCB#123	0.0001
1,2,3,6,7,8-HxCDF	0.1	PCB#156	0.0005
1,2,3,7,8,9-HxCDF	0.1	PCB#157	0.0005
2,3,4,6,7,8-HxCDF	0.1	PCB#167	0.00001
1,2,3,4,6,7,8-HpCDF	0.01	PCB#189	0.0001
1,2,3,4,7,8,9-HpCDF	0.01		
OCDF	0.0001		

1.2. Dioxin-like compounds in breast milk

As previously discussed, 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 thus exposing the foetus in utero. It has been demonstrated that PCDDs occur in breast milk (Langhorst and Shadoff, 1980) and thus the infant is additionally exposed to these compounds during the lactation period. Numerous studies worldwide have demonstrated that human milk is contaminated with dioxin-like compounds. As a consequence, the WHO organised two international studies on dioxin-like compounds in breast milk in 1987/88 and 1992/93. These studies demonstrated that levels of dioxins in breast milk are relatively high in industrialised countries when compared to non-industrialised countries (Liem et al., 2000), PCDD/PCDFs were higher in human milk from mothers with their first child (Fürst et al., 1989), and that the levels decreased over a given lactation period (Fürst et al., 1992). Furthermore, since PCDD/PCDF concentration in blood and human milk of the respective samples 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 (Päpke, 1998).

1.3. Objectives

The objective of this project was to investigate the levels of dioxin-like compounds in pooled human milk samples of cohorts in Australia. The compounds that were analysed were polychlorinated dibenzo-dioxins and furans (PCDD/PCDFs) and polychlorinated biphenyls (PCBs). The study focused on donor cohorts with different potential exposure to dioxins and dioxin-like compounds in Australia (i.e. urban/industrial/rural exposure). The study was carried out in the following stages:

- obtaining appropriate ethical approval
- selection of the regions
- selection of cohorts suitable to provide information on the levels of dioxins and dioxin-like compounds in breast milk throughout Australia
- contact with local agencies to support the study
- identification of volunteers who fulfil the selection criteria of the individual cohorts
- collection of samples and completion of the questionnaire with individual donors
- pooling of samples
- analysis of dioxins and dioxin-like compounds in a certified laboratory
- data analysis and interpretation
- report writing
- data dissemination for public knowledge and peer review.

2. Project Design

2.1. Sample collection

The study was carried out as part of the National Dioxins Program for DEH[3]. In order to allow direct comparison with previous 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.

2.1.1. Ethics approval

The project was originally submitted to the University of Queensland Medical Research Ethics Committee as an amendment to an earlier project conducted for the WHO. Approval for the WHO study was received on 11 January 2001 and for the present project on 6 December 2002. Both projects were allocated Clearance Number H/308/NRCET/00. The 2002/03 project was also submitted to several ethics committees throughout Australia. Appendix B lists the Committees that the protocol was submitted to and also the approval dates. As requested, gatekeeper letters were submitted to the University of Queensland Medical Research Ethics Committee. Only the original approval is shown in Appendix A. In the table in Appendix A, the term "not submitted" is listed in the Approval column if the project was not submitted to the ethics committee due to withdrawal of interest by the site.

It should be noted, that there were some difficulties with regards to the length of time it took to obtain approvals and this lead to delays in undertaking the study. As there is no central ethics committee in Australia, every hospital or health service is governed by its own ethics committee. Some sites were satisfied with using The University of Queensland ethics committee approval and provided a gatekeeper letter confirming consent for their site to participate, however, some sites required the protocol be submitted through their own ethics committee. There were lengthy waiting times between submission of the protocol and return of queries or feedback and then again until approval. Ethics committees meet at most once a month although some meet less frequently and if the deadline for submission is missed then it has to wait until the next meeting. As a result the approval time for some sites was up to six months.

2.1.2. Participant selection and recruitment

Following appropriate ethics committee approval, 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 (Appendix E). Once a potential participant had verbally agreed to participate, they were invited to read an information sheet and to complete a consent form (Appendix C). They were also asked to complete a questionnaire (Appendix D).

[3] Formally Environment Australia.

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 100 ml (preferably 150 ml) of expressed milk. This volume was to be collected over the six week period (two to eight weeks post-partum)
- Healthy pregnancy, mother and child
- A resident of the area for the past five years.

Once lactation was established, about 100 ml of milk was collected by each of the participants during the period between two and eight weeks post partum. 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 the collection of a pool was completed, the milk was thawed, thoroughly homogenised and 30 ml from each individual was separately pooled giving approximately 300 ml of sampled milk from each region. The pooled samples were then refrozen and transported on ice to Sydney and Münster, Germany for testing.

2.1.3. 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 and 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. This was also experienced in the 2000/01 Australian study which used these same criteria as part of the third round WHO exposure studies. The two most difficult criteria were the baby age range and residential status.

For the baby age range criteria, it should be noted that on average, women leave hospital three to five days post-delivery and, hence, pressures on post-natal maternal and child health clinics mean that staff have limited time for involvement in and the recruitment of participants in research projects. In addition, the first three months post-partum can be a difficult time for new mothers and for many women, therefore, providing a breast milk sample for a research project was not possible. The research team was very sensitive to this and no pressure was placed on any individual to participate in the study.

For the residential status criteria, it was more difficult than anticipated to find women who had resided in a given area for five years prior to the birth of their first child.

2.1.4. WHO 2001 Breast Milk study

Prior to participating in the National Dioxins Program, the project team participated in the third WHO Coordinated exposure study that was conducted in 2001. Details of the WHO project are given in Appendix G, and this study will be referred to in this report as the 'WHO study'.

Briefly, breast milk samples were collected, analysed and published as part of the third WHO Coordinated exposure study on the levels of PCDDs, PCDFs and PCBs in human milk. Eighteen countries, including Australia and New Zealand, participated in this study. For Australia, two pooled samples were submitted for analysis. These samples were collected from primipara mothers residing in Brisbane and Wollongong. The protocol used in both studies was identical. The samples collected in the WHO study were analysed at the State Institute for Chemical and Veterinary Analysis of Food, in Frieburg, Germany.

2.2. Sample analysis

Samples were analysed at the Australian Government Analytical Laboratories in Sydney. Duplicate pooled samples for two cohorts were also sent to the State Laboratory of Norhrhein Westfalen (NRW) in Münster, Germany. AGAL participated in the fourth round of the interlaboratory comparison on dioxins in food 2003 run by the Norwegian Institute of Public Health, where lipid measurements were reported.

2.2.1. PCDDs, PCDFs and dioxin-like PCBs

The method for the determination of tetra- through octa-chlorinated PCDD/PCDFs and PCBs in breast milk matrices is through high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS).

This method provides data on all toxic 2,3,7,8-chlorinated PCDD (seven) and PCDF (ten) isomers and the 12 'dioxin-like' PCB congeners, designated as toxic by the WHO. The PCDD/PCDFs and PCBs are determined by the isotope dilution quantitation technique. This technique allows determination of the dioxin toxicity equivalent (TEQ_{DF}) as well as the PCB toxicity equivalent (TEQ_{P}) for the 'dioxin-like' PCBs in a sample using WHO_{98} toxicity equivalency factors (TEFs). The total toxic equivalents (TEQ_{DFP}) are calculated as the sum of $TEQ_{DF} + TEQ_{P}$.

The detection limits and quantification levels in this method are usually dependent on the level of interferences rather than instrumental limitations. The method is 'performance based'. The analytical methodology for the determination of PCDD/PCDFs and PCBs are based on US EPA methods 1613B and 1668A, respectively.

Samples were thawed, sonicated and shaken to produce a homogenous sample. A subsample was spiked with a range of isotopically labelled surrogate standards. Proteins were denatured with the addition of potassium oxalate then a liquid-liquid extraction was performed with 2:1 acetone:hexane. Clean up was effected by partitioning with sulfuric acid then distilled water. Further purification was performed using column chromatography on acid and base modified silica gels, neutral alumina and carbon dispersed on celite. After cleanup, the extract was concentrated to near

dryness. Immediately prior to injection, internal standards were added to each extract, and an aliquot of the extract was injected into the gas chromatograph. The analytes were separated by the GC and detected by a high-resolution (≥10,000) mass spectrometer. The quality of the analysis was assured through reproducible calibration and testing of the extraction, cleanup, and GC/MS systems.

2.2.2. PCDD, PCDF and dioxin-like PCB analyses

The following standards were all purchased from Wellington Laboratories (Ontario, Canada) and were used for calibration, quantification and determination of the recovery of PCDD/PCDF and dioxin-like PCBs.

PCDDs/PCDFs

- EPA-1613-CVS calibration and verification solutions (CS-1 to CS-5)
- EPA-1613-LCS labelled compound surrogate solution
- EPA-1613-ISS-ST internal standard solution.

Dioxin-like PCBs

- WP-CVS calibration and verification solutions (CS-1 to CS-7)
- WP-LCS labelled surrogate spiking solution
- WP-ISS internal standard solution

Acetone, dichloromethane, hexane, and toluene were all OmniSolv® grade sourced from Merck KgaA (Darmstadt, Germany). Ethyl acetate and anhydrous sodium sulfate (granular) were both AR grade sourced from Mallinckrodt (Kentucky, USA). AnalaR® sulfuric acid S.G. was sourced from Merck (Victoria, Australia). All chromatographic columns were purchased from Fluid Management Systems. (Waltham, MA, USA) and were used without any further treatment. They comprised multi-layer (basic/neutral/acidic) silica, basic alumina and PX-21 carbon dispersed on celite which are packed in individual Teflon columns and vacuum sealed in aluminium foil packages.

2.2.3. Sample preparation

Approximately 100 g of breast milk sample was accurately weighed and spiked with a known amount of the respective PCDD/PCDFs and dioxin-like PCB isotopically labelled $^{13}C_{12}$ surrogate spiking solutions. The proteins in the sample were denatured using potassium oxalate added directly to the sample. Liquid-liquid extraction was performed using 2:1 acetone hexane with the aqueous layer back extracted three consecutive times. The combined organic layers were subsequently dried over sodium sulfate and the solvent removed for lipid determination. The lipid was dissolved in hexane and subsequently cleaned up using multiple extractions with concentrated sulfuric acid until the acid layer remained colourless. The hexane extracts were washed several times with water and dried through cleaned anhydrous sodium sulfate. The extracts were then concentrated prior to clean-up on the Power-Prep™ system. Elution through the different columns is computer controlled, and requires applying the hexane extract first onto the multi-layer silica and using hexane at a flow rate of 10 ml/min onto the alumina column. Dichloromethane:hexane (2:98) at 10 ml/min is used initially

and then the solvent strength is modified to dichloromethane:hexane (50:50) and transferred to the carbon column which is eluted with ethyl acetate:toluene (50:50) in the forward direction at 10 ml/min. The flow is then reversed and the carbon column is eluted with toluene at 5 ml/min.

Two fractions are collected. The first fraction is collected from the alumina column during elution using dichloromethane:hexane (50:50) and contains the mono-ortho and di-ortho PCBs. The second fraction containing PCDD/PCDFs and non-ortho PCBs are eluted from the carbon column during the reverse elution with toluene. The two fractions are concentrated separately under vacuum and the respective recovery standards (EPA-1613-ISS-ST & WP-ISS) are added and then further concentrated using clean dry nitrogen to a final volume of 10 µL prior to HRGC/HRMS analysis.

2.2.4. High-Resolution Gas Chromatography and Mass Spectrometric Analysis

All experiments were conducted on a MAT95XL HRMS (ThermoFinnigan MAT GmbH, Bremen, Germany) coupled to an Agilent 6890 GC (Palo Alto, CA, USA) equipped with a CTC A200S auto-sampler. A DB-5 (J & W Scientific, Folsom, CA, USA) capillary column (60 m x 0.25 mm i.d., film thickness 0.25 µm) was used as the primary analytical column with ultra-high purity Helium as the carrier gas. A flow rate of 1.0 ml/min was maintained throughout the chromatographic run. The temperature program for the PCDD/PCDF and PCB analysis was 100 °C (isothermal for 1 min.), then ramp 1 to 200 °C at 40 ^{o}C/min, ramp 2 to 235 °C (isothermal for 10 min) at 3 °C/min and then ramp 3 to 310 °C (isothermal 9 min) at 5 °C/min. A 1 µL splitless injection with an injector temperature of 290 °C for PCDD/PCDF/PCB analysis was employed for all standards and sample extracts. The mass spectrometer operating conditions were ion source and transfer line temperatures, 240 °C and 280 °C, respectively; ionisation energy 45eV, filament current 0.7 mA and electron multiplier voltage set to produce a gain of 10^6. Resolution was maintained at 10,000 (10% valley definition) throughout the sample sequence. Multiple ion detection (MID) experiments were performed in the electron impact mode with monitoring of the exact masses of appropriate ions for native and labeled compounds. Individual congeners were identified using the GC retention time and ion abundance ratios with reference to internal standards. A DB-dioxin (J & W Scientific, Folsom, CA, USA) capillary column (60 m x 0.25 mm i.d., film thickness 0.15 µm) was used for confirmation analysis when necessary.

2.2.5. Analyte identification and quantification criteria

PCDD/PCDFs and 'dioxin-like' PCBs

For positive identification and quantification, the following criteria must be met:

- The retention time of the analyte must be within one second of the retention time of the corresponding $^{13}C_{12}$ surrogate standard
- The ion ratio obtained for the analyte must be ±15% (±20% for PCBs) of the theoretical ion ratio
- The signal to noise ratio must be greater than 3:1
- Levels of PCDD and PCDF congeners in a sample must be greater than 3 times any level found in the corresponding laboratory blank analysed

- Surrogate standard recoveries must be in the range 25-150%.

2.2.6. Quantification using the Isotope Dilution Technique

The naturally occurring (native) compound is determined by reference to the same compound in which one or more atoms has been isotopically enriched. In this method, all carbon atoms for selected PCDD/PCDF and PCB molecules have been substituted with carbon-13 to produce $^{13}C_{12}$-labeled analogs of the chlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls. The $^{13}C_{12}$-labelled PCDD/PCDFs and PCBs are spiked into each sample and allow identification and correction of the concentration of the native compounds in the analytical process. Homologue totals for the tetra to octachloro dibenzo-*p*-dioxins and dibenzofurans are calculated by summing the total areas for each positively identified congener within each group and quantifying these totals using the mean relative response factor (RRF) of the determined RRFs for a homologue series.

The proprietary chromatographic integration package supplied with the Thermo Finnigan instrument, (Xcalibur®), was used to target all monitored compounds and create a text file that was further manipulated in Excel to produce the final certificate of analysis.

2.2.7. Quality Assurance

PCDD/PCDFs and 'dioxin-like' PCBs

- Batch sizes were typically six to eight samples
- A laboratory blank was analysed with each batch of samples
- A suitable laboratory control sample (LCS) was analysed with each batch of samples as a replicate to assess method precision
- The GCMS resolution, performance and sensitivity was established for each MS run
- The recoveries of all isotopically labelled surrogate standards were calculated and reported
- Ten percent of all samples were analysed by an independent crosscheck QC laboratory.

2.2.8. Lipid Determination

The lipid content is determined gravimetrically from the combined solvent extract during the first step of the analysis. The solvent from three extractions is combined into a tared flask and the solvent evaporated under reduced pressure until there is no appreciable change in the weight. This is then determined as the lipid content.

2.2.9. Data reporting

The bases of reporting for primary and quality control samples are given in the Table 2.3.

- PCDD/PCDFs and 'dioxin-like' PCB data were corrected for recovery of ^{13}C surrogate standards
- For all samples, data for quantified analytes were reported to two or three significant figures
- Limit of detection data for non-quantified analytes were reported to one significant figure.
- Total toxic equivalents were calculated using WHO_{98}-TEFs, see Table 2.1 and 2.2 both excluding limit of detection values and at medium bound concentrations using half limit of detection values for PCDD/PCDFs and DL-PCBs. Note that in the results section in Tables 3.4 and 4.5, lower, middle and upper bound values are given. It is worth noting that there is little, if any, difference in TEQ level between upper and lower bound values. This indicates that the results are not markedly impacted by non-detected congeners. For this reason middle bound values are reported but not discussed in the text.

Table 2.1 List of analytes of PCDD/PCDFs and WHO_{98}-TEF

Dioxins	**WHO_{98}-TEF**
2,3,7,8-TetraCDD	1
1,2,3,7,8-PentaCDD	1
1,2,3,4,7,8-HexaCDD	0.1
1,2,3,6,7,8-HexaCDD	0.1
1,2,3,7,8,9-HexaCDD	0.1
1,2,3,4,6,7,8-HeptaCDD	0.01
OctaCDD	0.0001
Furans	
2,3,7,8-TetraCDF	0.1
1,2,3,7,8-PentaCDF	0.05
2,3,4,7,8-PentaCDF	0.5
1,2,3,4,7,8-HexaCDF	0.1
1,2,3,6,7,8-HexaCDF	0.1
1,2,3,7,8,9-HexaCDF	0.1
2,3,4,6,7,8-HexaCDF	0.1
1,2,3,4,6,7,8-HeptaCDF	0.01
1,2,3,4,7,8,9-HeptaCDF	0.01
OctaCDF	0.0001

CDD - chlorinated dibenzo-p-dioxin
CDF - chlorinated dibenzofuran
PCB - polychlorinated biphenyl

Table 2.2 List of analytes PCBs and WHO_{98}-TEF

Congener	IUPAC No.	WHO_{98}-TEF
Non-ortho PCBs		
3,3',4,4'-tetrachlorobiphenyl	PCB#77	0.0001
3,4,4',5-tetrachlorobiphenyl	PCB#81	0.0001
3,3',4,4',5-pentachlorobiphenyl	PCB#126	0.1
3,3',4,4',5,5'-hexachlorobiphenyl	PCB#169	0.01
Mono-ortho PCBs		
2,3,3',4,4'-pentachlorobiphenyl	PCB#105	0.0001
2,3,4,4',5-pentachlorobiphenyl	PCB#114	0.0005
2,3',4,4',5-pentachlorobiphenyl	PCB#118	0.0001
2',3,4,4',5-pentachlorobiphenyl	PCB#123	0.0001
2,3,3',4,4',5-hexachlorobiphenyl	PCB#156	0.0005
2,3,3',4,4',5'-hexachlorobiphenyl	PCB#157	0.0005
2,3',4,4',5,5'-hexachlorobiphenyl	PCB#167	0.00001
2,3,3',4,4',5,5'-heptachlorobiphenyl	PCB#189	0.0001

WHO_{98}TEFs from Van den Berg et al., Environ. Health Perspect. 106(12) pp. 775-792 (1998).

2.2.10. Reporting basis for contaminant concentrations

Table 2.3 documents the reporting basis for the PCDD/PCDFs and dioxin-like PCBs.

Table 2.3 Reporting basis for contaminant concentrations

Contaminant class	Reporting basis
PCDDs and PCDFs	**pg g^{-1} on a lipid weight basis**. Total toxic equivalents for PCDDs/PCDFs (WHO_{98}-TEQ_{DF}) will be calculated using the WHO Toxic Equivalents Factors (WHO_{98}-TEFs).
'dioxin-like' PCBs	**pg g^{-1} on a lipid weight basis.** Total toxic equivalents for 'dioxin-like' PCBs (WHO_{98}-TEQ_P) will be calculated using the WHO Toxic Equivalents Factors (WHO_{98}-TEFs). Total toxic equivalents for PCDDs/PCDFs and 'dioxin-like' PCBs (WHO_{98}-TEQ_{DFP}) will be calculated from the addition of the respective WHO_{98}-TEQ_{DF} and WHO_{98}-TEQ_P values.

Additional analytical methodology information is available in Appendix F.

3. Levels of dioxin-like compounds in the breast milk of Australian women

3.1. Sample Collection

In total, 157 individual breast milk samples were collected between March 2002 and September 2003. One sample was collected in October 2001, however, as the majority of samples were collected in 2002 and 2003, collection time will be referred to as 2002/03. All samples were collected during the period two to eight weeks post partum from primipara mothers from 12 regions throughout Australia. The exceptions were rural inland Queensland, one baby was aged 11 weeks, South Australia-B, one baby was aged 10 weeks and, Tasmania, one baby was aged nine weeks. Samples were also accepted from mothers whose babies were one week at the beginning of sampling and sampling continued into the two to eight week period. The regions were Brisbane, Sydney (two pools), Melbourne (four pools), Adelaide (two pools), Perth, Hobart, rural inland NSW (Dubbo), rural inland Queensland (Dalby), rural Victoria (Bendigo, Ballarat, Lakes Entrance), Newcastle, Wollongong and Darwin. All samples were analysed as pooled samples and there were 17 pooled samples in total.

Table 3.1 shows the number of samples collected from each region and pool, as well as the demographics of the sample population. Information in this table provides the average and range data for maternal age, pre-pregnancy weight, pre-delivery weight, infant age at date of collection, percentage of male and female infants, and infant birth weight for all pooled samples and for the entire sample population.

A further 24 "historical" samples that had been collected in 1993, were obtained from the Key Centre for Applied and Nutritional Toxicology, Melbourne, Australia. They were analysed as three pools of eight samples. No information regarding the demographics of this "historical' sample population was available and, hence, only limited comparison can be made with the well-defined group of recent samples.

For the current study, analysis was carried out on 20 pools of breast milk, 17 pools obtained in 2002/03 from the current study and three pools obtained in 1993.

As previously stated, two pooled samples were collected separately in 2001 as part of the WHO study. These samples were from Brisbane and Wollongong and were made up of 12 and 10 participants, respectively. These samples were analyzed at the Institute of Chemical and Veterinary Analysis of Food, Freiberg, Germany. Table 3.2 gives the demographics of the WHO study participants.

Table 3.1 Demographics of study participants.

Region (n)	Maternal mean age (years) (± SD)	Maternal age range (years)	Mean pre-pregnancy weight (kg) (± SD)	Pre-pregnancy weight range (kg)	Mean pre-delivery weight (kg) (± SD)	Pre-delivery weight range (kg)	Infant mean age at collection (weeks) (± SD)	Infant age at collection range (weeks)	Percentage of female infants	Infant mean birth weight (g) (± SD)	Infant birth weight range (g)
Melb-A (10)	28.7±3.9	24-35	61.6± 9.1	52-80	75.5±10.1	63-99	5.2±2.4	2-8	50% (5/10)	3364 (382)	2794-4004
Melb-B (10)	31.2±3.5	24-36	62.7 ±13.1	47-88	76.3±12	59-96	4.6±2.1	2-8	40% (4/10)	3231 (610)	2034-4065
Melb-C (10)	31.5±3.3	24-37	66.2 ±12.7	52-95	80.1±12.9	65-102	3.8±1.9	1-7	60% (6/10)	3558 (507)	2700-4520
Melb-D (9)	30.9±2.9	27-37	66.8 ±10.5	54-90	83.4±10.8	68-104	3.6±1.9	1.5-6	56% (5/9)	3156 (505)	2219-3705
Syd-A (9)	33.2±3.1	28-37	59.5 ±13	47-90	75.9±16.9	61-110	4.8±2.3	1.1-7	66% (6/9)	3297 (333)	2800-3770
Syd-B (10)	34.0±3.4	31-40	58.2 ±4.8	50-66	71.1±6.4	63-80	6.6±1.9	3-8	60% (6/10)	3422 (311)	2885-3935
Bris-A (10)	32.1±4.0	27-36	70.5 ±12.0	52-94	84.2±9.5	65-98	5.3±1.9	2-7	40% (4/10)	3506 (409)	2960-4200
Hunter (9)	29.6±4.2	25-39	63.7 ±9.7	48-84	80±11.4	63-100	5±1.8	3-8	33% (3/9)	3660 (804)	1900-4410
Woll (12)	28.6±5.8	20-44	65.2 ±10.8	55-90	73.6±8.8	61-88	4.3±1.9	2-8	50% (6/12)	3410(315)	2875-4030
Rural NSW (10)	28.3±3.1	24-34	66.4 ±10.0	48-84	77.1±9.2	59-89	4.2±1.3	2-6	50% (5/10)	3308 (548)	2440-4000
Rural QLD (8)	30.6±4.0	26-37	59.6 ±12.1	47-81	75.0±9.0	64-91	5.1±3.2	2-11	37.5% (3/8)	3143 (516)	2345-3878
Darwin (4)	33.8±6.8	24-39	63 ±4.4	58-68	76.8±4.3	72-82	4.5±1.0	3-5	50% (5/10)	3281 (402)	2690-3550
SA-A (10)	30±6.7	18-40	68.5 ±12.9	53-92	81.9±10.5	70-96	2.7±1.3	1-5	40% (4/10)	3667.(340)	3260-4230
SA-B (11)	32±4.2	25-38	64.3 ±10.7	53-80	80.9±15.3	61-107	3.4±2.7	1-10	9% (1/11)	3611(603)	2670-4550
Rural Victoria (5)	30± 3.5	25-33	64.6 ±10-4	54-80	75.5±8.1	66-86	5±1.6	3-7	60% (3/5)	3156 (435)	2450-3490
WA (11)	32.5 ±3.5	24-36	82.9 ±28.2	54-136	95.9±28.4	67-154	5.8±1.5	3-7	36% (4/11)	3386(592)	2700-4690
Tas-A (9)	26.8 ±5.4	18-34	64.8 ±11.9	54-93	78.7±12.1	66-103	5.9±2.5	2-9	44% (5/9)	3385 (579)	2565-4385
OVERALL	**30.7 ±4.5**	**18-44**	**65.6 ±13.6**	**47-136**	**79.2±13.8**	**59-154**	**4.7±2.2**	**1-11**	**46% (72/157)**	**3398 (499)**	**1900-4690**

Table 3.2 Demographics of participants in the 2001 and 2002/3 WHO studies.

	Industrial Town NSW 2000/01	Industrial Town NSW 20003	Urban Centre Qld 2000/01	Urban Centre Qld 2003
Maternal average age (years)	30.0 (2.6)	28.6 (5.8)	32.3 (5.8)	32.1 (4.0)
Maternal age range (years)	26-34	20-44	24-47	27-36
Average pre-pregnancy weight (kg)	58.3 (5.9)	65.2 (10.8)	66.8 (10.7)	70.5 (12.0)
Pre-pregnancy weight range (kg)	54-72	55-90	53-83	52-94
Average pre-delivery weight (kg)	70.3 (9.1)	73.6 (8.8)	78 (9.9)	84.2 (9.5)
Pre-delivery weight range (kg)	64-93	61-88	63-97	65-98
Infant average age at sample collection (weeks)	4.3 (1.9)	4.3 (1.9)	5.3 (2.1)	5.3 (1.9)
Infant age at sample collection (weeks)	3-7	2-8	2-8	2-7
% of male infants	70 (7/10)	50 (6/12)	50 (6/12)	60 (6/10)
% of female infants	30 (3/10)	50 (6/12)	50 (6/12)	40 (4/10)
Infant average birth weight (g)	3393.7 (219.1)	3409.5 (315.4)	3471.2 (430.8)	3506.4 (409.0)
Infant birth weight range (g)	3001-3735	2875-4030	2650-4200	2960-4200

3.2. Quality Control and Quality Assurance

Quality control procedures utilised by AGAL were presented in Section 2.2.7.

3.3. Inter-laboratory calibration

For the purposes of inter-laboratory comparison, two duplicate samples were analysed by both AGAL and the State Laboratory of NRW, Germany. These samples were from a rural region in western NSW and an urban region from Melbourne, Victoria.

Dioxin-like compounds were detected in all pooled samples. The levels, expressed as TEQs together with normalised differences (see Box 1), obtained from each laboratory for all congeners are shown in Table 3.3. The results obtained for PCDDs are very similar between the two laboratories with the largest normalised difference found for OCDD being only 37%. For the PCDFs, the differences in some of the compounds are greater but this is due to these compounds being close to the limit of detection. Data were similarly treated by both laboratories. Laboratory blanks were not subtracted by either laboratory.

Overall, there is a good agreement between the results from the two laboratories for the two samples. Although, it is noteworthy that the results obtained by AGAL are slightly higher for most congeners as well as the values expressed on a TEQ basis. One of the

highest observed differences was found for the most toxic PCB, PCB 126 in the sample from rural NSW with more than a factor of three difference between the relatively high AGAL results and the results from the State Laboratory of NRW, Germany. Since PCB 126 is a key contributor to the TEQ, this difference between the two results caused much of the difference in the TEQ between the two laboratories.

Box 1. Normalised Differences

In this report, comparisons between replicate samples or replicated analysis have been made using the mean normalised difference. The normalised difference between two samples is mathematically defined as:

$$\text{normalised difference (\%)} = \frac{|\text{value a} - \text{value b}|}{\frac{(\text{value a} + \text{value b})}{2}} \cdot 100$$

Table Below provides a demonstration of the normalised difference (ND) values that would result from a range of differences in sample values.

Examples of normalised differences (ND) that would result from different sample values.

Sample A (pg g^{-1} lipid)	Sample B (pg g^{-1} lipid)	ND %
1.0	1.2	18
1.0	1.5	40
1.0	2.0	67
1.0	3.0	100
1.0	10.0	160
1.0	100.0	200

Table 3.3 Inter-laboratory comparisons.[4]

Region	Rural NSW German laboratory	Rural NSW Australian laboratory	Mean normalised difference - Rural NSW	Melb B German laboratory	Melb B Australian laboratory	Mean normalised difference - Melb B
Lipid Content:	**2.60%**	**3.20%**	**-20.7%**	**2.80%**	**3.10%**	**10.2%**
PCDDs						
2,3,7,8-TCDD	0.8	0.7	*13.5%*	0.96	0.9(nd)	*n.c*
1,2,3,7,8-PeCDD	2.1	2.4	*13.3%*	3.20	3.1	*3.2%*
1,2,3,4,7,8-HxCDD	1.2	1.5	*22.2%*	2.50	1.7	*38.1%*
1,2,3,6,7,8-HxCDD	6.4	7	*9.0%*	8.30	10	*18.6%*
1,2,3,7,8,9-HxCDD	1.9	2(nd)	*n.c*	2.00	1.8	*10.5%*
1,2,3,4,6,7,8-HpCDD	13.2	13	*1.5%*	12.70	14	*9.7%*
OCDD	49	71	*36.7%*	58.70	82	*33.1%*
PCDFs						
2,3,7,8-TCDF	0.23	0.6(nd)	*n.c*	0.29	0.8(nd)	*n.c*
1,2,3,7,8-PeCDF	0.10(nd)	0.7	*n.c*	0.14	0.6	*121.1%*
2,3,4,7,8-PeCDF	1.50	2.2	*37.8%*	3.00	3.8	*23.5%*
1,2,3,4,7,8-HxCDF	0.63	0.8	*23.8%*	0.87	1.2	*31.9%*
1,2,3,6,7,8-HxCDF	0.60	0.9(nd)	*n.c*	0.91	1.2	*27.5%*
2,3,4,6,7,8-HxCDF	0.35	0.6	*51.1%*	0.52	0.6	*17.5%*
1,2,3,7,8,9-HxCDF	0.05(nd)	0.2(nd)	*n.c*	0.05(nd)	0.2(nd)	*n.c*
1,2,3,4,6,7,8-HpCDF	0.85	1.4	*48.9%*	1.20	1.9	*45.2%*
1,2,3,4,7,8,9-HpCDF	0.05(nd)	0.1(nd)	*n.c*	0.10(nd)	0.4	*n.c*
OCDF	0.50(nd)	0.9	*n.c*	0.50(nd)	1.3	*n.c*
Non-ortho PCBs						
PCB 77	n.c	76	*n.a*	n.c	38	*n.a*
PCB 81	n.c	11	*n.a*	n.c	3.6	*n.a*
PCB 126	7.7	28	*113.7%*	17.7	18	*1.7%*
PCB 169	2.8	4(nd)	*n.c*	13.2	10	*27.6%*
Mono-ortho PCBs						
PCB 105	1070	1450	*30.2%*	840	960	*13.3%*
PCB 114	180	220	*20.0%*	260	320	*20.7%*
PCB 118	3200	4190	*26.8%*	3200	4310	*29.6%*
PCB 123	160	90	*56.0%*	150	74	*67.9%*
PCB 156	540	730	*29.9%*	1900	2240	*16.4%*
PCB 157	150	220	*37.8%*	350	490	*33.3%*
PCB 167	180	310	*53.1%*	380	570	*40.0%*
PCB 189	30	41	*31.0%*	120	160	*28.6%*
$TEQ_{DF\&PCBs}$ (incl. LODs)	6.6	9.8	*38.9%*	10.9	11.8	*7.6%*

[4] Mean normalised differences between samples analysed at AGAL and at the State Laboratory of NRW. Note that normalised differences were not calculated if a congener was not detected at either or both laboratories. Not calculated (n.c); not applicable (n.a.). Values are in pg g^{-1} lipid based including LODs.

3.4. Levels of PCDDs/PCDFs and PCBs in the breast milk of Australian women

3.4.1. Overall evaluation of PCDD/PCDFs and PCBs in samples collected in 2002/03.

Data sheets for all samples analysed are presented in Table 3.4 and PCDD/PCDF and PCB TEQ are presented in Figure 3.1. TEQs are expressed as lower, middle and upper bound. Note that in the results section in Tables 3.4 and 3.5, lower, middle and upper bound values are given. An explanation of the meaning of upper, middle and lower bound values is given in Box 2. As previously stated, it is worth noting that there is little if any difference in TEQ level between upper and lower bound values. This indicates that the results are not markedly affected by non-detected congeners. For this reason middle bound values are reported in the data tables but are not discussed in the text.

Dioxin-like chemicals were detectable in all pooled breast milk samples. Lipid content was measured in all pooled samples and gave an average lipid concentration of 3.7±0.5%. The mean and median levels, expressed as PCDD/PCDF & PCB TEQ, for all pooled samples were 9.0 and 8.9 pg TEQ g^{-1} lipid, respectively.

Box 2 Calculation of Upper, Middle and Lower Bound TEQ Values

Congeners that record concentrations below the detection limit may be expressed as either *upper*, *middle* or *lower* bound values and either of the following methods may be used.

***Upper Bound*: The TEQ level of the congener is calculated using the detection limit**

***Middle Bound:* The TEQ level is calculated using half the detection limit**

***Lower Bound*: The TEQ level is calculated using a zero concentration**

The greater the number of non-detectable congeners, the greater the difference in the upper and lower bound values.

Box 3 Calculation of Weighted Means

In order to take into account the number of individuals in each pool, weighted mean concentrations were calculated according to the following formula:

$$\sum_{i=1}^{s} w_i c_i / \sum_{i=1}^{s} w_i$$

where: **s = number of stratum**

c = analyte concentration

w = number of individuals in each pool

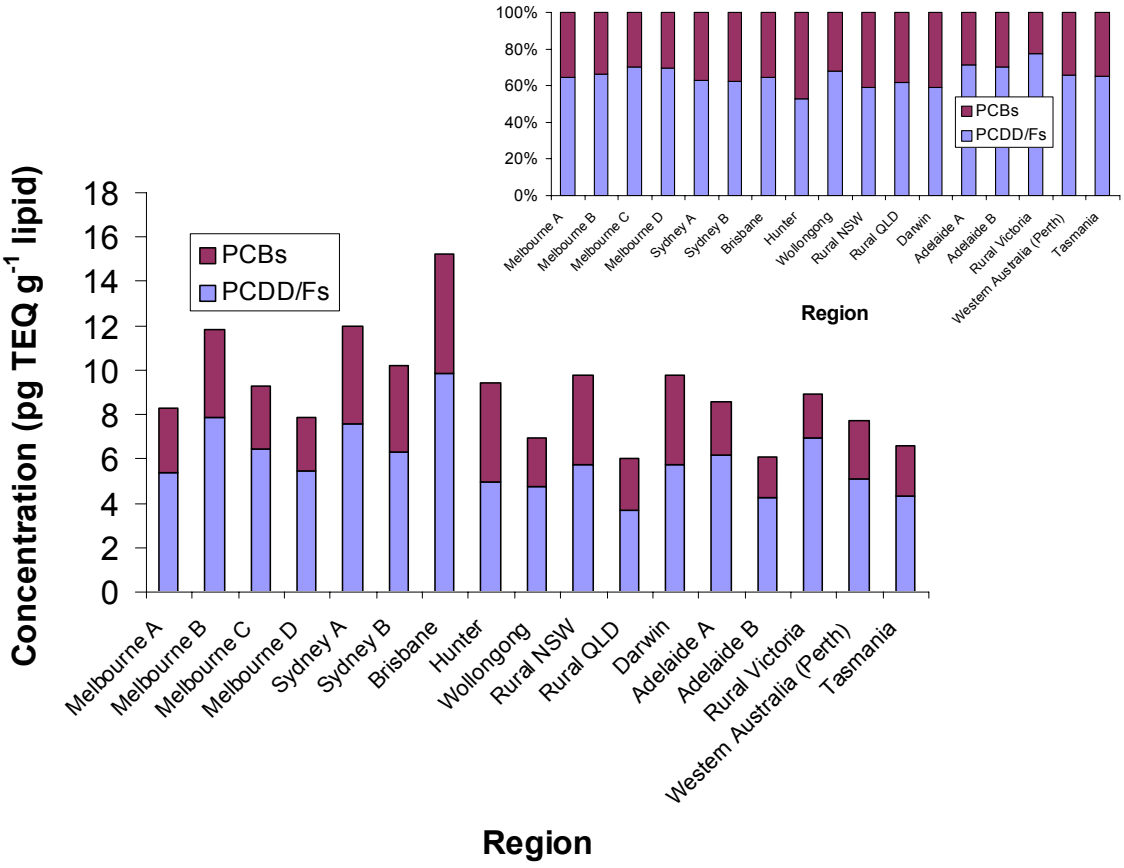

Figure 3.1 Concentration of dioxin-like chemicals in breast milk samples for each sampling region[5].

The levels of dioxin-like chemicals expressed as upper bound TEQ varied by a factor of 2.5 from a minimum value of 6.0 pg TEQ g^{-1} lipid detected in the rural Queensland sample, to a maximum value of 15.2 pg TEQ g^{-1} lipid detected in the Brisbane sample. The lowest concentrations were found in samples from rural Queensland, Tasmania, Wollongong and one of the samples from Adelaide.

Table 3.3 shows the minimum, maximum, weighted mean, median and percentage contribution to the total TEQ for all PCDD/PCDF and PCB congeners. The highest concentrations were found in a sample from Brisbane followed by samples from Sydney and Melbourne.

The highest levels of dioxin-like chemicals (15.2 pg TEQ g^{-1} lipid) were detected in the Brisbane pool. This was surprising since in the WHO study, the levels of dioxin-like chemicals were 8.3 TEQ pg TEQ g^{-1} lipid. Accompanied with the relatively (for this study) high level of dioxin-like chemicals in the Brisbane sample, came an unusually low lipid content with 2.8% compared to 3.8±0.50% in all remaining 16 samples. For results that are expressed on a lipid basis, a reduced lipid content will cause an overestimation of the actual concentration.

[5] Presented are the levels expressed as $TEQ_{PCDD/PCDF}$ and TEQ_{PCB} and the inset provides a direct evaluation of the contribution of the PCBs and PCDD/PCDFs to the overall TEQ in each sample pool.

The relatively low levels of lipid in the Brisbane sample in combination with previous results from the WHO study suggest that the Brisbane result may in part be overestimated. For all other data, there seems to be no trend of increased level of dioxin-like chemicals related to low lipid content of the sample. In fact, if anything there seems to be a slightly positive relationship between lipid content and levels of dioxin-like chemicals. Hence, this artifact, if so, is specific to the Brisbane sample only (Figure 3.2.).

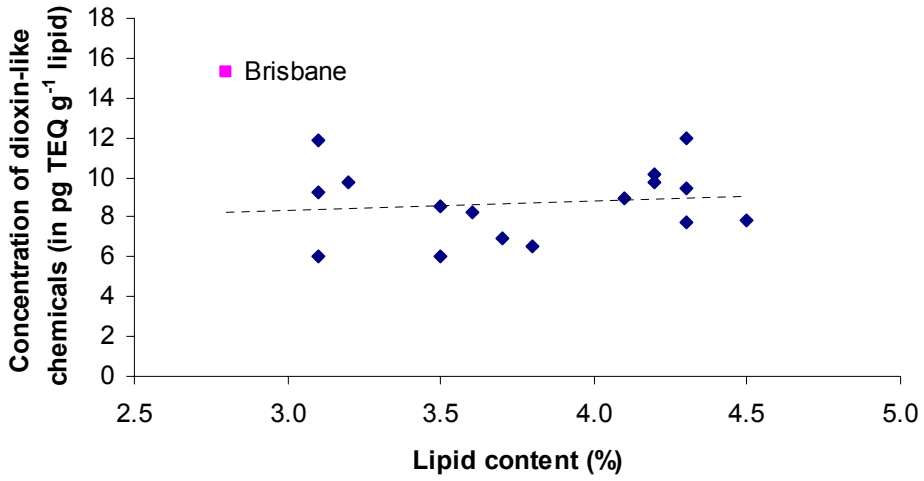

Figure 3.2 Total TEQ of all samples collected in 2002/03 versus the lipid content[6].

[6] The R^2 value was 0.02.

Table 3.4 Minimum, maximum, weighted mean and median of the 2002/03 pooled breast milk samples in pg g^{-1}.

2002/03	Minimum	Maximum	Weighted mean*	Mean	Median	% contribution to TEQ
Lipid Content:	2.8%	4.5%	3.7%	3.5%	3.7%	
PCDDs						
2,3,7,8-TCDD	0.5	1.7	0.8	0.8	0.7	**8.8**
1,2,3,7,8-PeCDD	1.5	3.6	2.3	2.3	2.3	**25.5**
1,2,3,4,7,8-HxCDD	0.9	2.2	1.5	1.5	1.5	**1.6**
1,2,3,6,7,8-HxCDD	4.8	14	8.6	8.8	8.5	**9.6**
1,2,3,7,8,9-HxCDD	0.7	2.4	1.5	1.6	1.8	**1.7**
1,2,3,4,6,7,8-HpCDD	6.4	14	10.6	10.7	11	**1.2**
OCDD	37	92	61.2	61.7	62	**0.07**
PCDFs						
2,3,7,8-TCDF	0.1	2	0.5	0.5	0.4	**0.6**
1,2,3,7,8-PeCDF	0.1	1.2	0.4	0.4	0.3	**0.2**
2,3,4,7,8-PeCDF	0.9	4.1	2.5	2.4	2.4	**13.8**
1,2,3,4,7,8-HxCDF	0.5	2.4	0.8	0.9	0.8	**0.9**
1,2,3,6,7,8-HxCDF	0.5	1.5	0.9	0.9	0.9	**1.0**
2,3,4,6,7,8-HxCDF	0.2	0.7	0.4	0.4	0.4	**0.5**
1,2,3,7,8,9-HxCDF	0.1	0.1	0.2	0.2	0.1	**0.2**
1,2,3,4,6,7,8-HpCDF	0.9	4.6	1.8	1.7	1.9	**0.2**
1,2,3,4,7,8,9-HpCDF	0.0	0.4	0.1	0.1	0.2	**0.01**
OCDF	0.1	2.1	0.8	0.8	0.7	**0.001**
Non-Ortho PCBs						
PCB 77	1.5	76	20.5	19.4	7.1	**0.02**
PCB 81	0.8	11	3.2	3.1	2.4	**0.004**
PCB 126	7.8	30	17	17	14	**18.9**
PCB 169	0	13	7.2	7.1	6.7	**0.8**
Mono-Ortho PCBs						
PCB 105	380	1600	940	930	890	**1.0**
PCB 114	130	350	220	220	190	**1.2**
PCB 118	1500	6500	3400	3300	2730	**3.8**
PCB 123	29	130	67	66	63	**0.07**
PCB 156	670	2900	1300	1200	1070	**7.0**
PCB 157	170	590	300	290	250	**1.7**
PCB 167	170	820	380	380	330	**0.04**
PCB 189	41	210	87	85	79	**0.1**
TEQ PCDD						
Lower Bound	2.4	7.2	4.2	4.3	4.1	
Middle Bound	2.7	7.2	4.3	4.4	4.2	
Upper Bound	3.1	7.2	4.4	4.5	4.3	
TEQ PCDF						
Lower Bound	0.6	2.6	1.5	1.5	1.4	
Middle Bound	0.6	2.6	1.5	1.5	1.4	
Upper Bound	0.6	2.7	1.6	1.5	1.5	
TEQ PCDD/PCDF						
Lower Bound	3.7	9.7	5.7	5.8	5.5	
Middle Bound	3.7	9.8	5.8	5.9	5.6	
Upper Bound	3.7	9.9	5.9	6.0	5.7	
TEQ PCB						
Lower Bound	1.8	5.4	3.1	3.1	2.6	
Middle Bound	1.8	5.4	3.1	3.1	2.6	
Upper Bound	1.8	5.4	3.1	3.1	2.6	
TEQ_{DFP}						
Lower Bound	6.0	15.1	8.8	8.9	8.9	
Middle Bound	6.0	15.2	8.9	9.0	8.9	
Upper Bound	6.0	15.2	9.0	9.1	8.9	

*weighted mean concentrations were calculated to take into account the number of individuals in each pool.

PCDD and PCDF congeners were detected in 93 and 62%, respectively. The levels of TCDD detected in all samples were very low and ranged from <0.6 pg g^{-1} lipid in the Wollongong sample to 1.4 pg g^{-1} lipid detected in the Sydney-A sample. On a concentration pg g^{-1} lipid basis, OCDD was the PCDD/PCDF congener with the highest concentration in all pooled breast milk samples.

The average contribution of TCDD to the total TEQ for all pooled samples was 8.7%. 1,2,3,7,8-PeCDD had the highest mean percentage contribution to the mean TEQ, 25.5%, followed by 2,3,4,7,8-PeCDF at 13.8%, then 1,2,3,6,7,8-HxCDD at 9.6%. This is similar to the blood study where 1,2,3,7,8-PeCDD had the highest percentage contribution at 19%, followed by 1,2,3,6,7,8-HxCDD at 11% and 2,3,4,7,8-PeCDF at 8.1%. For PCDD/PCDFs, the TEQ varied by a factor of 2.7 from a minimum value of 3.7 pg TEQ g^{-1} lipid detected in the rural Queensland sample, to a maximum value of 9.9 pg TEQ g^{-1} lipid detected in Brisbane, Queensland. For the furans, the levels of some congeners were consistently found to be near or below the limit of detection. These were 2,3,7,8-TCDF, 1,2,3,7,8,9-HxCDF and 1,2,3,4,7,8,9-HpCDF.

PCB congeners were detected in all analysed samples. From the non-ortho PCBs, PCB 126 had the highest contribution to the mean total TEQ at 18.9%. From the mono-ortho PCBs, PCB 156 had the highest at 7%. A similar result was observed in the blood serum study where PCB 126 had the highest contribution for the non-ortho PCBs at 18% and for the mono-ortho PCBs, PCB 156 had the highest at 9.2% (Harden et al. 2003).

For PCBs, the TEQ varied by a factor of 3.0 from a minimum value of 1.8 pg TEQ g^{-1} lipid detected in the South Australia-B sample, to a maximum value of 5.4 detected in the Brisbane sample. On average, PCBs contributed around 30% to the total TEQ. The inset of Figure 3.1 shows the percentage contribution of PCDD/PCDFs and PCBs to the total TEQ. Hunter has the highest PCB contribution at 47%. The mean contribution of individual PCB congeners to the total TEQ can be seen in Table 3.3. It is noteworthy that the percentage contribution of PCB TEQ to total TEQ is consistent. This indicates that the sample base is fairly homogenous and that the exposure of individuals within the pools is similar.

Table 3.5 PCDD/PCDF and PCB results (pg g^{-1}) from pooled breast milk samples all regions of Australia in 2002/03

Region	Syd-A	Syd-B	Hunter	Wollongong	Rural NSW	Melb-A	Melb-B	Melb-C	Melb-D	Rural Vic	Darwin	Tas	SA-A	SA-B	WA	Rural QLD	Bris	Overall mean (incl. LODs)
Lipid Content:	**4.30%**	**4.20%**	**4.30%**	**3.70%**	**3.20%**	**3.60%**	**3.10%**	**3.10%**	4.50%	4.10%	**4.20%**	**3.80%**	**3.50%**	**3.10%**	**4.30%**	**3.50%**	**2.80%**	**3.70%**
PCDD/PCDF Congeners																		
2,3,7,8-TCDD	1.4	0.77	0.82	<0.6	0.69	0.65	<0.9	0.85	0.72	0.86	0.98	0.49	0.64	0.5	0.57	0.51	1.7	**0.8**
1,2,3,7,8-PeCDD	2.7	2.4	1.8	1.5	2.4	2.2	3.1	2.6	2.1	2.9	2.9	1.9	2.3	1.7	1.9	1.8	3.6	**2.3**
1,2,3,4,7,8-HxCDD	1.8	1.4	1.3	1.1	1.5	1.5	1.7	2.2	1.6	2.2	2.1	1.3	1.5	0.95	1	0.86	1.8	**1.5**
1,2,3,6,7,8-HxCDD	9.9	8.5	7.5	6.5	7	8.1	10	11	8.7	11	13	6.6	9.9	6	7.7	4.8	14	**8.6**
1,2,3,7,8,9-HxCDD	2.2	1.6	0.72	<1	<2	<0.7	1.8	1.8	1.8	2.4	2.4	1.2	1.8	1.1	1.7	1.5	1.6	**1.5**
1,2,3,4,6,7,8-HpCDD	11	8.5	14	9.5	13	6.4	14	13	7.2	13	11	8.9	14	8.4	9.6	7.8	12	**10.6**
OCDD	62	46	77	57	71	46	82	64	50	68	65	48	92	49	37	57	78	**61.2**
TOTAL PCDDs (excl. LODs)	**91**	**69**	**103**	**76**	**96**	**65**	**113**	**96**	**72**	**100**	**97**	**68**	**122**	**68**	**60**	**74**	**113**	**87**
2,3,7,8-TCDF	<0.3	0.39	<0.3	0.65	<0.6	<0.2	<0.8	<0.2	0.37	<0.2	<0.9	0.19	0.69	0.13	0.33	<0.2	2	**0.5**
1,2,3,7,8-PeCDF	0.28	0.26	0.16	1.1	0.7	0.18	0.57	<0.1	0.21	0.11	<0.4	<0.05	0.41	<0.1	0.22	<0.1	1.2	**0.4**
2,3,4,7,8-PeCDF	3.2	3.2	1.9	2.6	2.2	2.4	3.8	2.2	2.3	2.5	1.3	1.5	2.6	2	2.4	0.86	4.1	**2.5**
1,2,3,4,7,8-HxCDF	1.1	1	<0.4	<0.5	<0.8	<0.6	1.2	0.79	0.65	0.71	2.4	0.49	1.4	0.57	0.75	0.55	<1	**0.8**
1,2,3,6,7,8-HxCDF	1.2	1.1	0.66	1.1	0.92	0.75	1.2	0.87	0.74	0.72	1.2	0.53	1.1	0.64	0.87	0.47	1.5	**0.9**
2,3,4,6,7,8-HxCDF	0.41	0.42	0.37	<0.5	0.59	<0.3	0.62	0.35	0.28	0.37	0.44	0.2	0.6	0.26	0.35	0.24	0.73	**0.4**
1,2,3,7,8,9-HxCDF	<0.05	<0.03	<0.6	<0.2	<0.2	<0.08	<0.2	<0.04	<0.04	0.077	<0.5	<0.05	<0.07	<0.05	<0.05	<0.09	<0.3	**0.2**
1,2,3,4,6,7,8-HpCDF	1.9	2	4.6	<0.2	1.4	0.88	1.9	<0.9	<0.7	1.5	<1	<0.6	1.6	<0.8	2	<1	3.3	**1.8**
1,2,3,4,7,8,9-HpCDF	<0.09	<0.05	<0.07	2.9	<0.1	<0.09	0.39	<0.05	<0.07	<0.07	<0.3	<0.07	<0.1	<0.05	<0.04	<0.2	<0.2	**0.1**
OCDF	0.26	0.46	2.1	1.3	0.94	<0.4	1.3	<0.2	0.14	<0.6	<0.6	0.17	<2	0.15	<0.2	<0.4	1.8	**0.8**
TOTAL PCDFs (excl. LODs)	**8.4**	**8.8**	**9.8**	**9.7**	**6.8**	**4.2**	**11**	**4.2**	**4.7**	**6**	**5.3**	**3.1**	**8.4**	**3.8**	**6.9**	**2.1**	**15**	**8.3**
Sum of PCDD/PCDFs (excl. LODs)	99	78	113	85	102	69	124	100	77	106	103	72	131	72	66	76	127	**94**

Table 3.5 Cont'd PCDD/PCDF and PCB results (pg g^{-1}) from pooled breast milk samples all regions of Australia in 2002/03

Region	Syd-A	Syd-B	Hunter	Wollongong	Rural NSW	Melb-A	Melb-B	Melb-C	Melb-D	Rural Vic	Darwin	Tas	SA-A	SA-B	WA	Rural QLD	Bris	Overall mean (incl. LODs)
PCB Congeners																		
Non-Ortho PCBs																		
PCB 77	5	1.5	36	34	76	26	38	7.1	1.6	1.7	6.5	15	4.1	1.9	2.1	21	52	**20.5**
PCB 81	2.4	1.1	3.6	4.4	11	1.8	3.6	4.1	1.1	0.77	0.92	3	2.3	1	1.3	5.2	5.3	**3.2**
PCB 126	24	21	30	12	28	14	18	14	12	12	11	14	14	7.8	15	14	25	**17**
PCB 169	11	9.1	5	5.3	0	9	10	7.2	6.7	6.6	7.2	3.9	6.2	6.7	6.8	3.7	13	**7.2**
Sum Non-ortho PCBs	**42**	**33**	**75**	**56**	**115**	**51**	**70**	**32**	**21**	**21**	**26**	**36**	**27**	**17**	**25**	**44**	**95**	**46**
Mono-Ortho PCBs																		
PCB 105	1600	950	1600	760	1400	910	960	1000	690	380	750	770	640	410	600	890	1460	**939.9**
PCB 114	320	290	240	160	220	210	320	250	190	150	190	160	160	150	180	130	350	**218.4**
PCB 118	5300	3870	4900	2700	4200	3500	4300	3300	2700	1500	2500	2400	2400	1800	2400	2300	6390	**3390.1**
PCB 123	85	84	100	60	90	63	74	66	44	41	51	48	51	29	44	63	130	**66.9**
PCB 156	1700	1600	980	850	730	1400	2200	1200	1100	700	1300	670	920	1100	1100	740	2870	**1259**
PCB 157	420	390	260	220	220	320	490	290	250	180	320	170	210	240	250	180	590	**297.1**
PCB 167	540	480	420	340	310	400	570	330	300	170	430	210	290	250	300	320	820	**385.3**
PCB 189	93	100	60	62	41	83	160	82	79	53	87	46	68	83	72	71	210	**86.4**
Sum Mono-ortho PCBs	**10000**	**7700**	**8500**	**5200**	**7300**	**6900**	**9100**	**6500**	**5300**	**3200**	**5700**	**4400**	**4800**	**4100**	**4900**	**4600**	**12820**	**6539**
TEQ PCDD/PCDF																		
Lower Bound	7.51	6.33	4.83	3.92	5.38	5.17	6.85	6.39	5.47	6.92	6.8	4.28	6.12	4.25	5.07	3.67	9.73	**5.7**
Middle Bound	7.53	6.34	4.89	4.31	5.58	5.25	7.35	6.39	5.48	6.93	6.89	4.29	6.13	4.26	5.07	3.69	9.8	**5.8**
Upper Bound	7.55	6.34	4.96	4.74	5.74	5.36	7.85	6.47	5.49	6.94	6.98	4.3	6.13	4.27	5.08	3.71	9.87	**5.9**
TEQ PCB																		
Lower Bound	4.46	3.83	4.46	2.24	3.97	2.91	3.99	2.78	2.39	1.98	2.44	2.27	2.43	1.81	2.62	2.3	5.37	**3.1**
Middle Bound	4.46	3.83	4.46	2.24	4.01	2.91	3.99	2.78	2.39	1.98	2.44	2.27	2.43	1.81	2.62	2.3	5.37	**3.1**
Upper Bound	4.46	3.83	4.46	2.24	4.01	2.91	3.99	2.78	2.39	1.98	2.44	2.27	2.43	1.81	2.62	2.3	5.37	**3.1**
TEQ_{DFP}																		
Lower Bound	**12**	**10.2**	**9.3**	**6.2**	**9.4**	**8.1**	**10.8**	**9.2**	**7.9**	**8.9**	**9.2**	**6.6**	**8.6**	**6.1**	**7.7**	**6**	**15.1**	**8.8**
Middle Bound	**12**	**10.2**	**9.4**	**6.6**	**9.6**	**8.2**	**11.3**	**9.2**	**7.9**	**8.9**	**9.3**	**6.6**	**8.6**	**6.1**	**7.7**	**6**	**15.2**	**8.9**
Upper Bound	**12**	**10.2**	**9.4**	**7**	**9.8**	**8.3**	**11.8**	**9.3**	**7.9**	**8.9**	**9.4**	**6.6**	**8.6**	**6.1**	**7.7**	**6**	**15.2**	**9**

Table 3.6 PCDD/PCDF and PCB results (pg g^{-1}) from pooled breast milk samples from Melbourne in 1993, Melbourne 2002/03 and overall mean from all regions 2002/03[7].

Region – Melbourne	1993 A	1993 B	1993 C	Mean 1993	Mean 20002/03 All data	Mean Melb 2002/03
Lipid Content (%)	4.3	3.1	4.2	**3.9**	**3.7**	**3.6**
PCDD/PCDF Congeners						
2,3,7,8-TCDD	<0.8	1.2	1.5	**1.2**	**0.8**	**0.78**
1,2,3,7,8-PeCDD	4.1	4.4	4.5	**4.3**	**2.3**	**2.5**
1,2,3,4,7,8-HxCDD	2.8	4.2	3.7	**3.6**	**1.5**	**1.7**
1,2,3,6,7,8-HxCDD	15	17	18	**17**	**8.6**	**9.4**
1,2,3,7,8,9-HxCDD	2.8	4	6.1	**4.3**	**1.5**	**1.5**
1,2,3,4,6,7,8-HpCDD	<20	<30	<40	**40**	**11**	**10**
OCDD	<85	<70	<200	**200**	**61**	**60**
TOTAL PCDDs (excl. LODs)	25	31	34	30	87	87
2,3,7,8-TCDF	<0.2	<0.4	0.34	**0.3**	**0.5**	**0.39**
1,2,3,7,8-PeCDF	<0.3	0.86	1.7	**1.0**	**0.4**	**0.27**
2,3,4,7,8-PeCDF	4.2	3.9	4.1	**4.1**	**2.5**	**2.7**
1,2,3,4,7,8-HxCDF	<0.5	1.6	2.8	**1.6**	**0.8**	**0.81**
1,2,3,6,7,8-HxCDF	0.99	1.6	2	**1.5**	**0.9**	**0.89**
2,3,4,6,7,8-HxCDF	0.2	0.22	0.3	**0.2**	**0.4**	**0.39**
1,2,3,7,8,9-HxCDF	<0.2	<0.1	<0.1	**0.2**	**0.2**	**0.18**
1,2,3,4,6,7,8-HpCDF	5.7	15	30	**17**	**1.8**	**1.1**
1,2,3,4,7,8,9-HpCDF	<0.2	<0.3	<0.8	**0.4**	**0.1**	**0.15**
OCDF	<0.4	<0.3	<0.3	**0.3**	**0.8**	**0.51**
TOTAL PCDFs (excl. LODs)	**11**	**23**	**41**	**25**	**8.3**	**7.4**
Sum of PCDD/PCDFs (excl. LODs)	36	54	75	**55**	**94**	**92**
PCB Congeners						
Non-Ortho PCBs						
PCB 77	12	6.6	13	**10**	**20**	**18**
PCB 81	1.8	1.9	2.1	**1.9**	**3.2**	**2.6**
PCB 126	23	18	20	**20**	**17**	**14**
PCB 169	20	9.5	11	**13**	**7.2**	**8.2**
Sum Non-ortho PCBs	**57**	**36**	**46**	**46**	**46**	**43**
Mono-Ortho PCBs						
PCB 105	2100	1200	1500	**1600**	**940**	**900**
PCB 114	710	340	280	**440**	**220**	**240**
PCB 118	10000	4200	5600	**6800**	**3400**	**3400**
PCB 123	100	77	120	**99**	**67**	**62**
PCB 156	5400	1700	1900	**3000**	**1300**	**1470**
PCB 157	1000	350	440	**610**	**300**	**340**
PCB 167	1500	520	900	**990**	**390**	**400**
PCB 189	500	130	160	**260**	**86**	**100**
Sum Mono-ortho PCBs	**22000**	**8600**	**11000**	**14000**	**6600**	**7000**

[7] To calculate means, where no congeners were detected, the highest non-detect value was used.

Table 3.6 Cont'd PCDD/PCDF and PCB results (pg g^{-1}) from pooled breast milk samples from Melbourne in 1993, Melbourne 2002/03 and overall mean from all regions 2002/03.

Region	1993 A	1993 B	1993 C	Mean 1993	Mean 2002/03 All data	Mean Melb 2002/03
TEQ PCDD/PCDF						
Lower Bound	8.4	11	12	**10**	**6**	**6**
Middle Bound	9.0	11	12	**11**	**6**	**6**
Upper Bound	9.6	11	12	**11**	**6**	**6**
TEQ PCB						
Lower Bound	7.4	3.7	4	**5**	**3**	**3**
Middle Bound	7.4	3.7	4	**5**	**3**	**3**
Upper Bound	7.4	3.7	4	**5**	**3**	**3**
TEQ_{DFP}						
Lower Bound	16	14	16	**15**	**9**	**9**
Middle Bound	16	14	16	**16**	**9**	**9**
Upper Bound	17	15	16	**16**	**9**	**9**

3.4.2. Overall evaluation of PCDD/PCDFs and PCBs in samples collected in 1993.

Twenty-four individual milk samples were collected from Victorian mothers in 1993. These samples are referred to as Melbourne Historical-A, B and C and were analysed by AGAL as three pools each containing eight samples. Data sheets for these samples are given in Table 3.5. PCDD/PCDFs and PCBs were detected in all pooled samples from 1993. The levels of dioxin-like chemicals expressed as upper bound TEQ varied by a factor of 0.85 and ranged from 14.6 to 17.0 pg TEQ g^{-1} lipid with an average of 16.0. Table 3.6 shows the minimum, maximum, mean, median and percentage contribution to the total TEQ of PCDD/PCDF and PCB congeners.

3.4.3. Levels of dioxin-like chemicals in archived human milk samples from Melbourne

The levels were consistently higher for all congeners in the 1993 samples compared to the levels observed in the 2002/03 samples obtained from the Melbourne pools alone, and data from all pools combined. The total TEQ levels ranged from 14.6-17 pg g^{-1} lipid (upper bound). The mean TEQ PCB was 5.1 pg g^{-1} which contributed 32% to the mean total TEQ. For two of the historical samples, PCB contributed 25% to the total TEQ and for the other it contributed 44%. As with the 2002/03 samples (Melbourne pools alone and data from all pools combined), the PCB with the highest percentage contribution for the1993 samples was PCB 126. For the mono-ortho PCBs, PCB 156 contributed 9.4%. The mean concentration of TCDD in the 1993 sample was 1.2 pg g^{-1} lipid and this is around 50% higher than in the 2002/03 samples where the mean was 0.8 pg g^{-1} lipid. As with the 2002/03 samples (Melbourne pools alone and data from all pools combined), OCDD was the PCDD/PCDF congener with the highest concentration in all samples.

A comparison of samples collected from Melbourne women (Tables 3.6, Figure 3.3, 3.4, 3.5, 3.6) in 1993 with those collected for the present study shows clearly that the levels of these chemicals have 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 were 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 2002/03, from 16±1.4 to 9.1±1.3 pg g^{-1} lipid. Consistently, PCDD/PCDFs as well as PCBs decreased in this period. The percent decrease of PCDD/PCDFs, PCBs and total TEQ were 54, 60 and 56%, respectively. This percent decrease reflects the world-wide trend over recent decades of declining levels of dioxin-like compounds in the environment and humans. This was seen in the third round WHO exposure studies, where on average the decline between the second round in 1993 and the third round in 2003 was about 40% (Malisch and van Leeuwen 2003). However, it is slightly less than the 70% decrease seen in the New Zealand breast milk study (Bates, 2001).

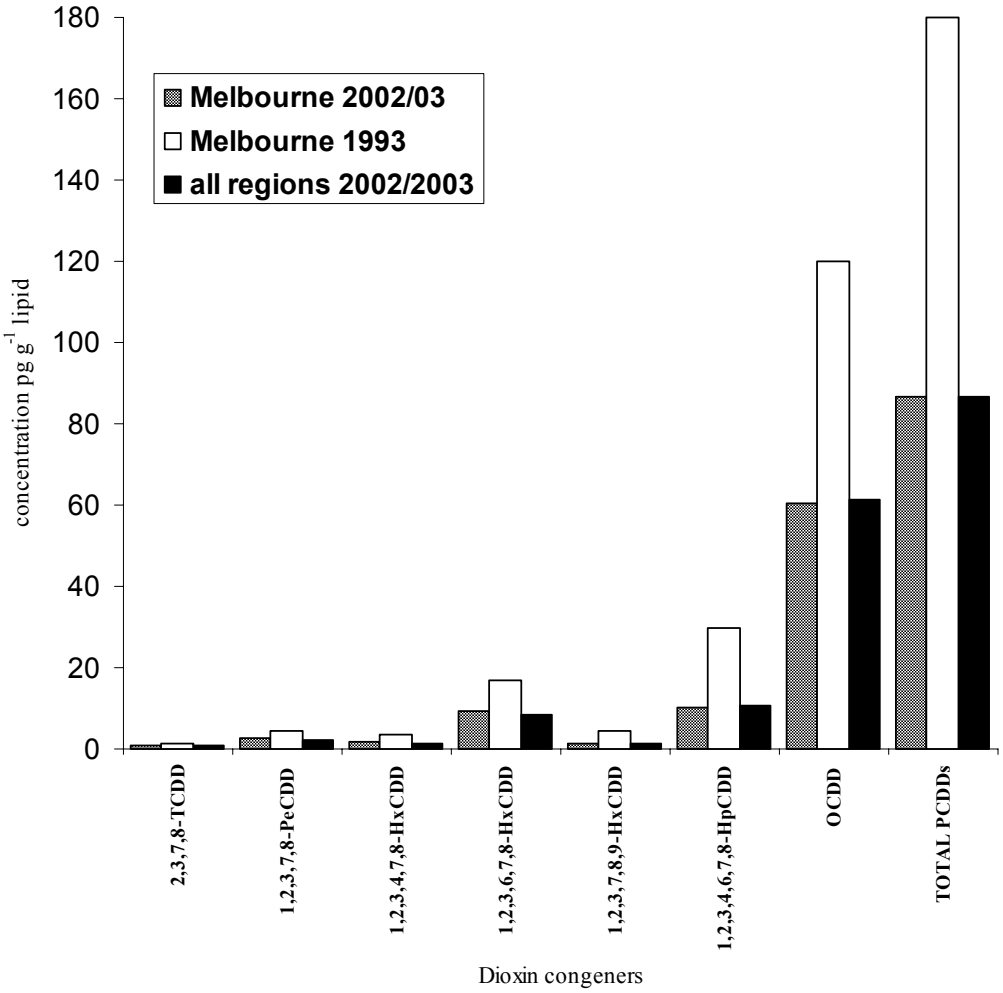

Figure 3.3 Comparison of concentrations of PCDD congeners for Melbourne 2002/03, all pools data 2002/03 and Melbourne pools 1993[8].

[8] Values given are non-weighted means.

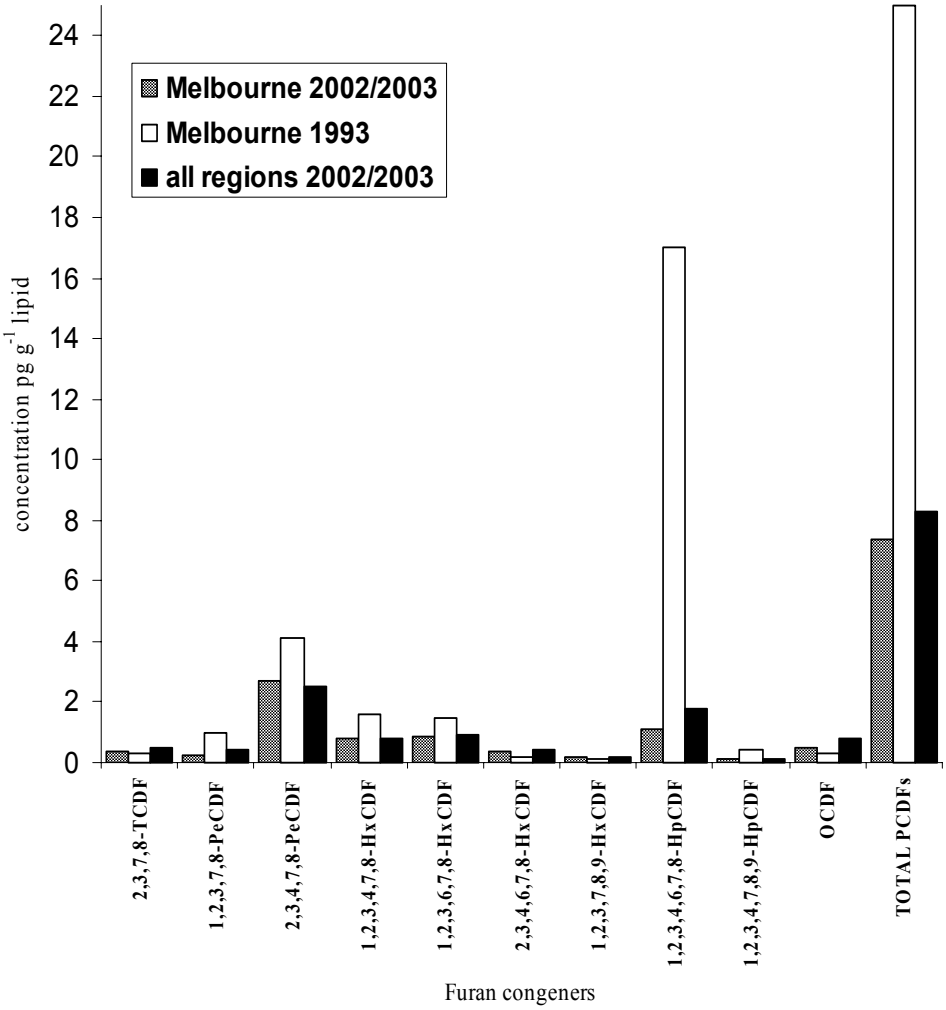

Figure 3.4 Comparison of concentrations of PCDF congeners for Melbourne 2002/03, all pools data 2002/03 and Melbourne pools 1993[9]

[9] Values given are non-weighted means.

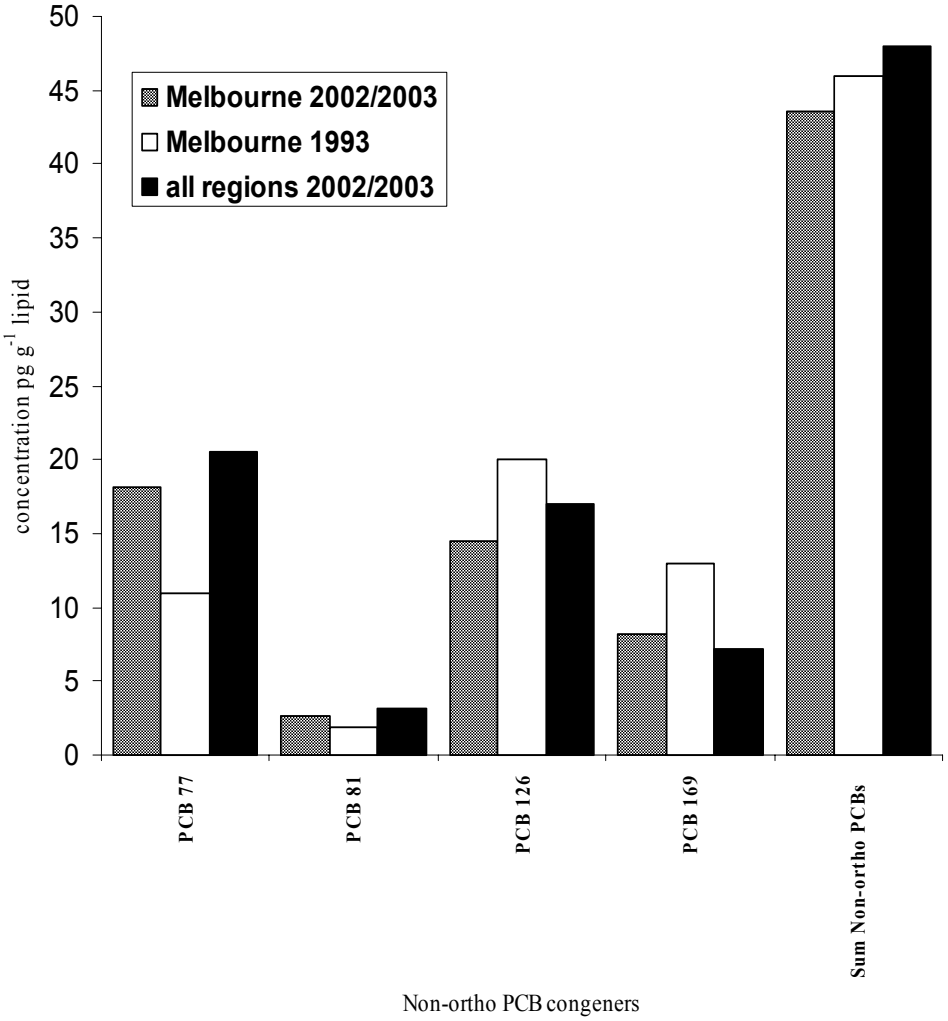

Figure 3.5 Comparison of concentrations of non-ortho PCB congeners for Melbourne 2002/03, all pools data 2002/03 and Melbourne pools 1993[10].

[10] Values given are non-weighted means.

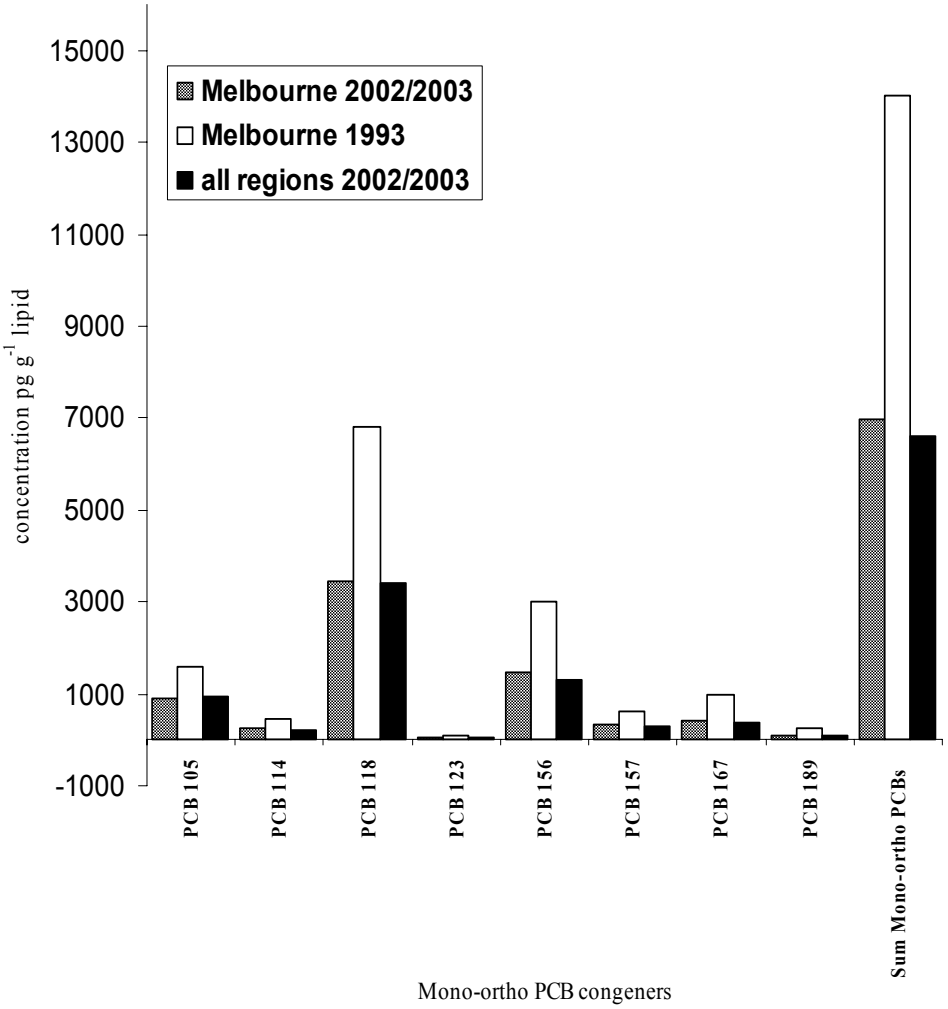

Figure 3.6 Comparison of concentrations of mono-ortho PCB congeners for Melbourne 2002/03, all pools data 2002/03 and Melbourne pools 1993[11].

[11] Values given are non-weighted means.

Table 3.7 Summary of minimum, maximum, weighted mean and median of pooled breast milk in 1993 samples (pg g^{-1})

1993	Minimum	Maximum	Weighted mean	Median	% contribution to TEQ
Lipid Content %	3.1%	4.3%	3.9%	4.2%	
PCDD/PCDF Congeners					
2,3,7,8-TCDD	<0.8	1.5	1.2	1.2	**7.3**
1,2,3,7,8-PeCDD	4.1	4.5	4.3	4.4	**27.1**
1,2,3,4,7,8-HxCDD	2.8	4.2	3.6	3.7	**2.2**
1,2,3,6,7,8-HxCDD	15.0	18.0	16.7	17.0	**10.4**
1,2,3,7,8,9-HxCDD	2.8	6.1	4.3	4.0	**2.7**
1,2,3,4,6,7,8-HpCDD	<20.0	<40.0	30.0	30.0	**1.9**
OCDD	<70.0	<200.0	118.3	85.0	**0.07**
2,3,7,8-TCDF	<0.2	0.3	0.3	0.3	**0.2**
1,2,3,7,8-PeCDF	<0.3	1.7	1.0	0.9	**0.3**
2,3,4,7,8-PeCDF	3.9	4.2	4.1	4.1	**12.7**
1,2,3,4,7,8-HxCDF	<0.5	2.8	1.6	1.6	**1.02**
1,2,3,6,7,8-HxCDF	1.0	2.0	1.5	1.6	**1.0**
2,3,4,6,7,8-HxCDF	0.2	0.3	0.2	0.2	**0.2**
1,2,3,7,8,9-HxCDF	<0.1	<0.2	0.1	0.1	**0.1**
1,2,3,4,6,7,8-HpCDF	5.7	30.0	16.9	15.0	**1.1**
1,2,3,4,7,8,9-HpCDF	<0.2	<0.8	0.4	0.3	**0.03**
OCDF	<0.3	<0.4	0.3	0.3	**0.0002**
PCB Congeners					
Non-Ortho PCBs					
PCB 77	6.6	13.0	10.5	12	**0.01**
PCB 81	1.8	2.1	1.9	1.9	**0.0012**
PCB 126	18.0	23.0	20.3	20	**12.7**
PCB 169	9.5	20.0	13.5	11	**0.8**
Mono-Ortho PCBs					
PCB 105	1230	2140	1626.7	1510	**1.0**
PCB 114	280	710	443.3	340	**1.4**
PCB 118	4220	10500	6780	5620	**4.2**
PCB 123	77	120	99	100	**0.06**
PCB 156	1710	5420	3010	1900	**9.4**
PCB 157	350	1040	610	440	**1.9**
PCB 167	520	1550	990	900	**0.1**
PCB 189	130	500	263.3	160	**0.2**
TEQ PCDD/PCDF					
Lower Bound	8.4	11.8	10.3	10.6	
Middle Bound	9.0	12.0	10.6	10.8	
Upper Bound	9.6	12.2	10.9	11.0	
TEQ PCB					
Lower Bound	3.7	7.4	5.1	4.2	
Middle Bound	3.7	7.4	5.1	4.2	
Upper Bound	3.7	7.4	5.1	4.2	
TEQ_{DFP}					
Lower Bound	14.3	15.9	15.4	15.9	
Middle Bound	14.5	16.4	15.7	16.2	
Upper Bound	14.6	17.0	16.0	16.4	

3.5. Factors affecting the levels of dioxin-like compounds in human breast milk.

The levels of dioxin-like compounds in human breast milk may be influenced by a number of factors. Previous studies conducted in Europe (Fürst et al., 1992) have reported the following as important influences:

- Factors related to the lactation period itself
- Dietary factors
- Living conditions, including the region(s) in which an individual has resided.

3.5.1. Factors related to the maternal characteristics

Besides exposure, the key factors affecting the levels of dioxin-like compounds in human breast milk are the number of children that have been breast fed by an individual, the total length of time for the lactation period and the maternal age (Fürst et al., 1992). For the present study, the first two factors were controlled by the inclusion criteria that limited participants to primipara mothers' breastfeeding an infant that was aged between two to eight weeks post-partum. As previously stated, these criteria were chosen so that the results of this study could be directly compared with those obtained during the WHO exposure studies. A plot of the TEQ versus average age of donor mothers found no significant relationship (i.e. r^2=0.16). Similarly, the data from the New Zealand breast milk study was unable to identify a clear relationship (r^2=0.10) between age and dioxin levels in the study participants' breast milk (Bates et al, 2001). In contrast, other studies have found a positive correlation between increasing maternal age and the levels of dioxin-like compounds (Fürst et al., 1992 and Beck et al., 1989). The lack of correlation between these two factors may be related to the lower levels observed in the Australian and New Zealand populations compared to those in Europe and North America. This relationship would be better assessed by examining individual samples, not pooled samples, as was the case in the New Zealand breast milk and serum studies.

3.5.2. Dietary factors

With the limited data available, no relationship was observed between the type and levels of food consumed by individuals and the levels of dioxin-like compounds in any of the pools. A better measure of the relationship to diet would be to undertake, on an individual basis, a detailed assessment of dietary intake over a long period of time in concert with multiple measurements of the levels of dioxin-like compounds. This was beyond the scope of this project.

3.5.3. Living conditions including regional variation

Table 3.7 shows the results obtained from urban, regional and industrial areas. The levels of dioxin-like chemicals observed in all analysed samples were remarkably similar. No consistent trends were observed between pools obtained from rural, industrial or urban regions of Australia. This is in good agreement with results obtained in other studies including New Zealand (Bates et al., 2001) and Europe (Fürst et al., 1992, Deml et al, 1996), and the recently completed Australian study *Dioxins in the Australian Population: Levels in Blood* (Harden et al., 2003). With regard to living

conditions, no individuals reported any unusual lifestyle or occupational exposure mechanisms.

Table 3.8 Mean PCDD/PCDF and PCB results (pg g^{-1}) from pooled breast milk samples by urban, industrial and rural regions[12].

	Urban regions (n=12) MEAN	Industrial regions (n=2) MEAN	Rural regions (n=3) MEAN
Lipid Content:	3.7%	4.0%	3.6%
PCDD/PCDF Congeners			
2,3,7,8-TCDD	0.8	0.7	0.7
1,2,3,7,8-PeCDD	2.5	1.7	2.4
1,2,3,4,7,8-HxCDD	1.6	1.2	1.5
1,2,3,6,7,8-HxCDD	9.5	7.0	7.6
1,2,3,7,8,9-HxCDD	1.7	0.7	2.0
1,2,3,4,6,7,8-HpCDD	10	12	11
OCDD	60	67	65
TOTAL PCDDs (excl. LODs)	**86**	**89**	**90**
2,3,7,8-TCDF	0.5	0.5	0.3
1,2,3,7,8-PeCDF	0.3	0.6	0.3
2,3,4,7,8-PeCDF	2.6	2.3	1.9
1,2,3,4,7,8-HxCDF	1.0	0.5	0.7
1,2,3,6,7,8-HxCDF	1.0	0.9	0.7
2,3,4,6,7,8-HxCDF	0.4	0.4	0.4
1,2,3,7,8,9-HxCDF	0.1	0.4	0.1
1,2,3,4,6,7,8-HpCDF	1.5	2.4	1.3
1,2,3,4,7,8,9-HpCDF	0.1	1.5	0.1
OCDF	0.6	1.7	0.6
TOTAL PCDFs (excl. LODs)	6.9	9.7	5.0
PCB Congeners			
Non-Ortho PCBs			
PCB 77	13	35	332
PCB 81	2.3	4.0	5.7
PCB 126	16	21	18
PCB 169	8.1	5.2	3.4
Sum Non-ortho PCBs	40	65	60
Mono-Ortho PCBs			
PCB 105	890	1200	910
PCB 114	230	200	170
PCB 118	3400	3800	2700
PCB 123	64	80	65
PCB 156	1400	910	720
PCB 157	330	240	190
PCB 167	410	380	270
PCB 189	97	61	55
Sum Mono-ortho PCBs	6900	6900	5000
Sum of PCDD/ PCDFs (excl. LODs)	93	99	95

[12] Mean values were calculated including the limit of detection

Table 3.8 Cont'd Mean PCDD/PCDF and PCB results (pg g^{-1}) from pooled breast milk samples by urban, industrial and rural regions

	Urban regions (n=12)	Industrial regions (n=2)	Rural regions (n=3)
	MEAN	MEAN	MEAN
TEQ PCDD/PCDF			
Lower Bound	6.2	4.4	5.3
Middle Bound	6.2	4.6	5.4
Upper Bound	6.3	4.9	5.5
TEQ PCB			
Lower Bound	3.1	3.3	2.8
Middle Bound	3.1	3.4	2.8
Upper Bound	3.1	3.4	2.8
TEQ_{DFP}			
Lower Bound	9.3	7.7	8.1
Middle Bound	9.3	8.0	8.2
Upper Bound	9.4	8.2	8.2

3.5.4. Intra-region variability

Intra-regional variability was assessed by examination of the results from the four Melbourne pools. Dioxin-like chemicals were detected in all pools. The levels expressed as TEQs ranging from 7.9-11 pg g^{-1} lipid with a coefficient of variation of about 15%. Hence, the reproducibility of the sampling/pooling at least for the Melbourne samples was not greater than the typical error of the analytical techniques for the analysis of dioxin-like chemicals.

3.6. Comparison of the levels of dioxin-like chemicals in breast milk and blood

Breast milk and blood have both been used to measure the levels of dioxin-like compounds in humans. Results of previous studies (Greizerstein et al., 1999; Deml et al., 1996; Schecter et al., 1991 and 1998) indicate that, when expressed on a lipid basis, lower levels were observed in breast milk than in blood. In contrast, Greizerstein (1996) found higher levels of PCBs in breast milk compared to blood and congener profiles differed between the two media. For the present study, no blood samples were collected from the participants and so the results obtained from the recently completed study *Dioxins in the Australian Population: Levels in Blood*, (Harden et al., 2003) were used for comparative purposes. For the serum study, more than 9,000 de-identified blood samples representing males and females from five different regions and age groups were selected from samples collected for routine pathology testing. It should be noted that comparison of the breast milk and serum levels and congener profiles did not form part of the original study design for either project. Additionally, the participants in the serum study were not selected on the basis of the strict inclusion/exclusion criteria that were applied to the breast milk study and, hence, comparison of the results of the two studies is complicated. Despite these limitations, the results of the breast milk study are compared with those obtained for the serum study for all females in the 16-30 and the 31-45 years age groups.

For the recently completed "Dioxin-like compounds in the blood serum of the Australian population" report, an equation was derived which could be used to estimate the concentrations of these chemicals in an individual on the basis of their age (3.3 exp0.0251age). In Figure 3.7, the measured concentrations from the breast milk study are compared with predicted results on the basis of the average age of the donor mothers in each milk pool and using the regression model from the blood study. In general, the model seems to slightly under predict concentration but overall, there is good agreement between the measured results and the predicted results as for more than 50% of the samples, the predicted results are within 30% of the measured result.

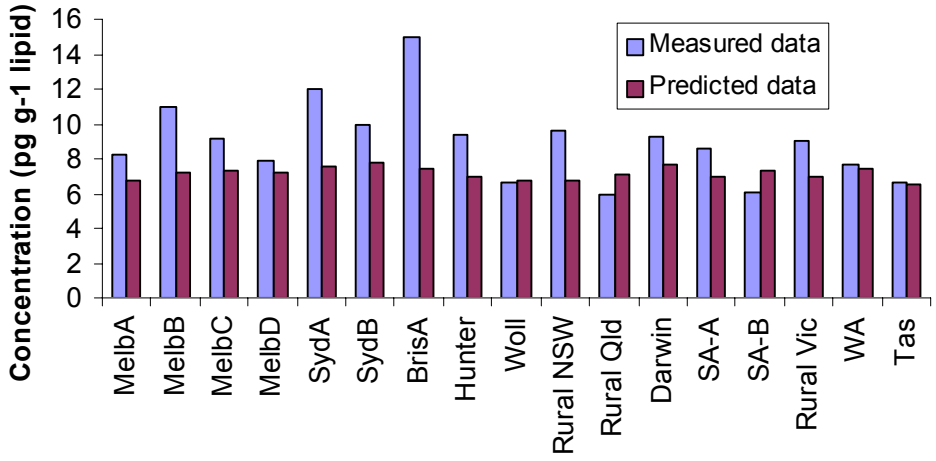

Figure 3.7 Concentrations of breast milk compared with predicted results using regression model from blood study.

As previously stated, the samples included in the breast milk study were obtained from mothers who met clearly defined inclusion criteria as determined by the WHO in their exposure studies. This made comparison with the blood study, where the sample donors were selected on the basis of age and postcode only, complicated because of the possibility of confounders that were not present in the breast milk study population. These include differences in parity, age range, differences in residential history and the possibility, albeit slight, of occupational or geographical contamination although the latter would result in increased, not decreased levels.

Despite the overall good comparability of the data from the blood and the breast milk study it is noteworthy that the PCDD/PCDF and PCB congener profiles in the breast milk samples are slightly different from those observed in the blood study of the same age group. Figure 3.7 depicts the ratio of the mean concentration of each congener obtained from the serum of women aged 16-45 years compared with the mean concentration obtained from women participating in the breast milk study and plotted for each congener. If the value is 100, then the levels in the two media are the same. If the ratio is greater than 100, then the levels in breast milk are higher than those found in blood and vice versa. Note, that for the purposes of this comparison, all chemicals that were detected in less than 30% of breast milk samples were excluded. Specifically, PCBs and lower chlorinated dibenzodioxins occur at similar or even higher concentrations in the breast milk samples compared to the blood samples. However, with increasing level of chlorination (i.e. increasing hydrophobicity) there is a shift

towards higher concentrations of congeners in the blood samples compared to breast milk samples. For example, while the most hydrophobic of all dioxin-like chemicals, octachlorodibenzodioxin is the dominant congener in blood and breast milk samples, its concentration is usually about four times higher in the blood samples than in the breast milk samples. This shift in the congener profile from the blood to breast milk is indicative of a kinetic limitation related to the physico-chemical properties of the individual congeners. This limitation has been observed not only for the transfer from blood to milk but also in the resorption efficiency in the intestine and across the blood/brain barrier (Greizerstein et al., 1999; Deml et al., 1996; Schecter et al., 1991 and 1998).

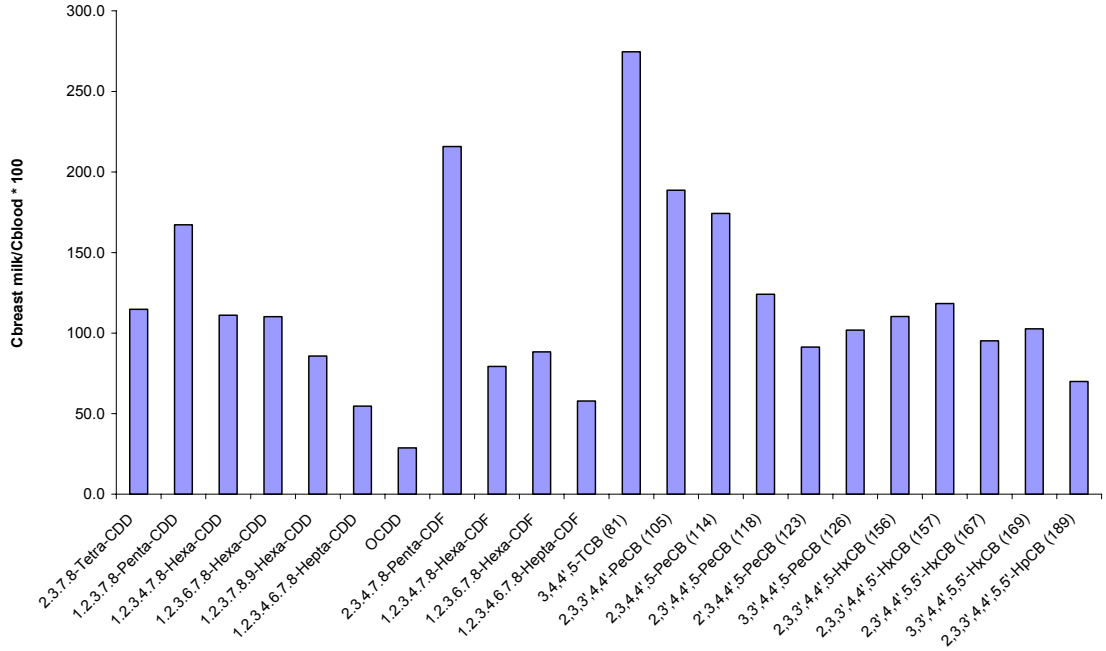

Figure 3.8 Comparison of congener profile from breast milk and blood.

3.7. Limitations of the Study

The main limitation of this study was in the use of pooled samples for analysis. The primary disadvantage with the use of pooled samples is that it limits statistical analysis in the assessment of relationships and trends. Ideally, individual samples would have been analysed but this was impossible to do with the budget allocation and also introduces ethical issues with regard to what should be done if high levels were detected in an individual. Other limitations included variation in the times of collection that is the time of day that the sample was collected and the length of lactation period. The authors believe that collecting serum samples from women at the time they gave their breast milk sample would have strengthened the study. This was difficult to do, particularly with primiparous women who may have many stresses associated with the initial post-partum period and was also impossible to do within the study budget. Overall, despite some limitations, the collection and analysis of pooled samples proved to be a cost-effective method to determine the levels of dioxin-like chemicals in the breast milk of Australians.

3.8. A comparison of levels of dioxin-like compounds with other countries

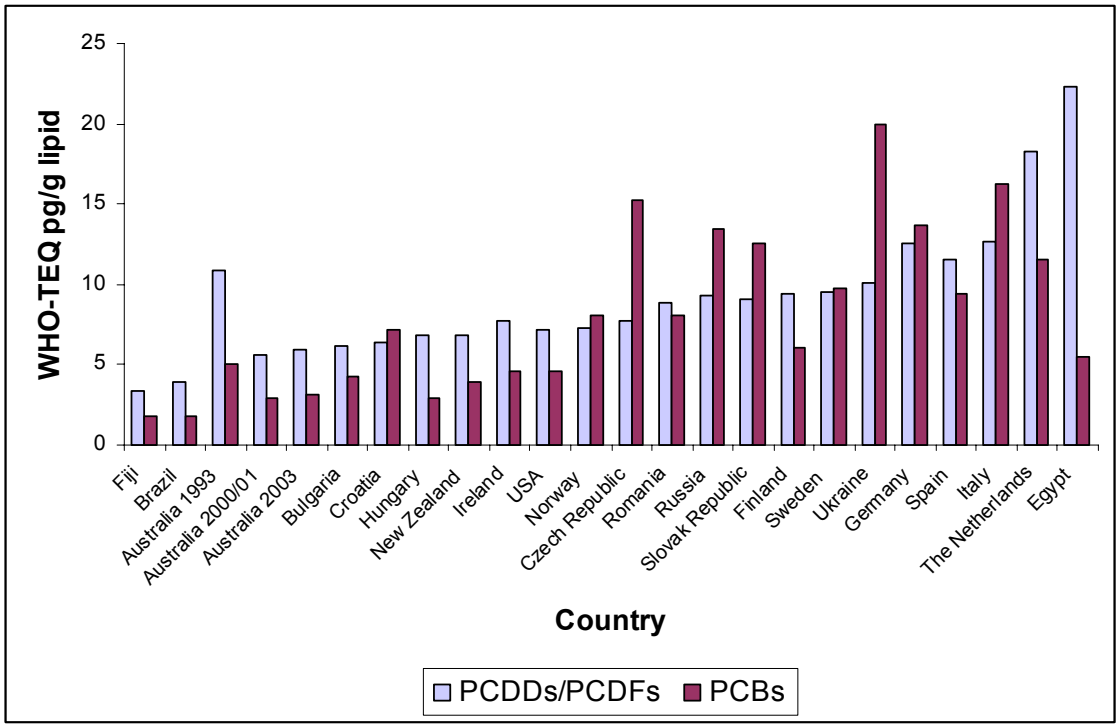

Figure 3.9 International levels of PCDD/PCDFs and PCBs in pooled human milk.

The protocol used in the current study is the same as that used in the WHO studies, therefore, the best international comparisons are made with the results of the third round WHO exposure studies. The mean results are depicted in Figure 3.9.

The lowest levels of PCDD/PCDFs and dioxin-like PCBs were found in countries in the Southern hemisphere (Fiji, Brazil, Australia and New Zealand). It was also found that a number of European countries (Bulgaria, Croatia, Hungary and Ireland) and the USA also had low levels of PCDD/PCDFs and dioxin-like PCBs. High levels were found in a number of Western European countries (Italy, Spain, Germany and The Netherlands) and Egypt had the highest TEQ levels (Malisch and van Leeuwen, 2003).