EPHC

Organochlorine Pesticides (OCPs) and Polybrominated Diphenyl Ethers (PBDEs) in the Australian Population: Levels in Human Milk

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- all of the local coordinators who assisted in the recruitment of participants and the subsequent collection and return of samples
- all laboratory staff at National Measurement Institute
- all laboratory staff at State Laboratory of NRW, Münster, Germany.

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EXECUTIVE SUMMARY

This study arose out of the 2002 Review of the PCB Management Plan by the Scheduled Waste Management Network (SWMN) and the National Advisory Body (NAB).

The Review indicated it would be beneficial to obtain some data on the levels of organochlorine pesticides (OCPs) in the Australian population. In 2002, the Environment Protection and Heritage Standing Committee (EPHSC) agreed and noted that the Australian Government Department of the Environment and Heritage (DEH) would commission a study using the same samples from the National Dioxins Program (NDP) breast milk study collected in 2002-03. The study, however, was also broadened to include polybrominated diphenyl ethers (PBDEs).

The OCPs that were analysed included:

hexachlorobenzene	trans-nonachlor
alpha-HCH	p,p'-DDE
beta-HCH	p,p'-DDD
gamma-HCH (lindane)	o,p'-DDT
aldrin	p,p'-DDT
heptachlor	mirex
heptachlor epoxide	oxychlordane
dieldrin	cis- and trans-chlordane

The PBDEs that were analysed included: BDE 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154 and 183.

In total, 173 samples of breast milk 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 collected and analysed as 17 pooled samples covering the following regions:

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

In addition to these samples, a further 24 'historical' samples collected in 1993 by the Key Centre for Applied and Nutritional Toxicology, Melbourne Victoria, were analysed as three pools of eight samples.

In total, 20 pools of breast milk were analysed. All pooled samples were sent to the National Measurement Institute (formally the Australian Government Analytical Laboratories), Sydney, Australia and two duplicate samples were sent to the State Laboratory of NRW, Münster, Germany for inter-laboratory comparison.

OCPs and PBDEs were detected in all pooled samples.

ORGANOCHLORINE PESTICIDES (OCPS)

The overall OCP concentration for all samples was dominated by beta-HCH and p,p'-DDE, a degradation product of DDT. The highest concentrations of OCPs were found in the Sydney pool A and the Melbourne pool A samples. An elevated concentration of HCB was also detected in the sample from rural Queensland.

A comparison of the Melbourne samples collected in 1993 with those collected in 2002/03 showed no significant differences in the concentrations of the OCPs over the ten-year period. However, it should be noted that comparison of the two sample populations is complicated by the fact that details of maternal parity[1] and infant age at the date of collection for the 1993 samples were not available.

Additionally, statistical evaluation of any minor differences observed was complicated by the use of pooled samples and, hence, was not undertaken.

It is noteworthy that a low ratio of DDT to its degradation product DDE was observed in the 1993 samples as well as the 2002/03 samples indicating that exposure to DDT is not recent and is consistent with the use of DDT having been discontinued as an insecticide[2]. Higher ratios of these compounds have been observed particularly in developing countries and are indicative of the continued use of DDT as an insecticide in agricultural and malarial control programs.

Overall, the concentrations of OCPs in the breast milk of these Australian women are low compared to international studies.

POLYBROMINATED BIPHENYLS (PBDES)

For samples collected during 2002/03, the mean concentration of PBDEs for all samples was 11 ng g^{-1} expressed on a lipid basis. The concentration ranged from a minimum of 6.0 ng g^{-1} lipid detected in the Tasmanian sample to a maximum of 18 ng g^{-1} lipid detected in the rural NSW sample. The PBDEs that were found in the highest concentrations were BDEs 47, 99, 100, 153 and 154 contributing on average 50, 17, 12, 10 and 1.3 %, respectively, to the total concentration for each pooled sample.

For the three pooled samples collected in 1993, the mean concentration of PBDEs was 13 ng g^{-1} expressed on a lipid basis. This was higher than that observed in the 2002/03 samples. However, this is not considered significant, as it is the same order of magnitude and there are too many limitations associated with sample size, sampling methodology and analytical uncertainty to draw any firm conclusions on trends. As for the 2002/03 samples, there was a clear dominance of BDE 47, 99,100,153 and 154 with each contributing an average of 42, 26, 12, 12, 8 and 2 %, respectively, to the total concentration for each pooled sample.

The levels reported for the 2002/03 samples are consistent with findings reported internationally. On a worldwide basis, the levels of PBDE compounds detected in breast milk are higher than those levels observed in Europe and Japan but lower than those observed in North America and Canada. It should be noted that much of the data from Japan is based on very low sample numbers and in some cases is the result of only a single analysis. The levels reported for North America and Canada are likely to be related to their high utilisation of

[1] Number of pregnancies a woman has had.

[2] DDT was deregistered for use in Australia in 1987.

products and articles containing penta-BDE. Penta-BDE is one of several compounds in the class of PBDEs and is predominantly used as a flame retardant in polyurethane foam in furniture and electronics.

Little is known about the exact sources and types of PBDE containing products in Australia. From the results of this study, it appears that a significant proportion of PBDE product may be in the form of penta-BDE products. Further investigation of these samples including analysis of individual samples may be warranted in order to determine the exact sources and the levels of these compounds in the Australian population.

It should be noted that it is the advice of the World Health Organization and the National Health and Medical Research Council (NHMRC) in Australia that breast milk is the best food for babies. Breast milk may contain OCPs and PBDEs because of its fat content, but all babies are exposed to these compounds even if they are not breastfed. Alternative foods for babies, such as infant formula, also contain OCPs and PBDEs because they may also contain fat. Several studies around the world in areas where organic pollutant levels are known to be high have still shown that breastfed babies are healthier than those fed infant formula.

GLOSSARY/ABBREVIATIONS

ACT	Australian Capital Territory
analyte	Chemical or substance undergoing analysis
BDE	Brominated diphenyl ether
cohort	Group of sample donors from set geographical location
congener	Closely related chemicals derived from the same parent compound
DEH	Department of the Environment and Heritage
dioxins	Common name for polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and dioxin-like PCBs
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
IQR	Inter-quartile range
IUPAC	International Union of Pure and Applied Chemistry
LCS	Laboratory control sample
LOD	Limit of detection
Melb	Melbourne
MID	Multiple ion detection
ml	Millilitre
NAB	National Advisory Body
nd	Non-detect
ND	Normalised difference
NDP	National Dioxins Program
NRCET	National Research Centre for Environmental Toxicology
NSW	New South Wales
NT	Northern Territory
OCP or OC	Organochlorine pesticide(s)
PBDE	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-*p*-dioxins
PCDFs	Polychlorinated dibenzofurans
ng g^{-1}	Nanogram per gram, 10^{-9} g. Equal to µg per kilogram or ppb
ppb	Parts per billion
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	Regression coefficient
region	Geographical location in Australia
SA	South Australia
SD	Standard deviation
Syd	Sydney
SWMN	Scheduled Waste Management Network
Tas	Tasmania
TEQ	Abbreviation of WHO_{98}-TEQ (this document)

USEPA	United States of America Environmental Protection Agency
UQ	The University of Queensland
Vic	Victoria
WA	Western Australia
WHO	World Health Organization

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1 INTRODUCTION

1.1 OBJECTIVES

The objective of this project was to investigate the levels of organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) in pooled human milk samples in Australia. This project arose out of the 2002 Review of the PCB Management Plan by the Scheduled Waste Management Network (SWMN) and the National Advisory Body (NAB).

The Review indicated it would be beneficial to obtain some data on the levels of organochlorine pesticides (OCPs) in the Australian population. In 2002, the Environment Protection and Heritage Standing Committee (EPHSC) agreed and noted that the Australian Government Department of the Environment and Heritage (DEH) would commission a study using the same samples from the National Dioxins Program (NDP) breast milk study collected in 2002-03. The study, however, was also broadened to include polybrominated diphenyl ethers (PBDEs).

The NDP breast milk study focused on donor cohorts with different potential exposure in Australia (i.e. urban/industrial/rural exposure). The NDP 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 fulfill the selection criteria of the individual cohorts
- collection of samples and completion of the questionnaire with individual donors
- pooling of samples
- analysis in a certified laboratory
- data analysis and interpretation including international comparison
- report writing
- data dissemination for public knowledge and peer review.

1.2 ORGANOCHLORINE PESTICIDES (OCPS)

The term organochlorine pesticide (OCP) refers to a wide range of organic chemicals, which contain chlorine and sometimes several other elements. In the past a range of OCP compounds were used in Australia, including herbicides, insecticides and fungicides. Characteristically, these compounds were very stable. This characteristic is now recognised widely as being a problem because the chemicals can be distributed in the environment where they persist long after their original use. They degrade slowly and being fat-soluble, accumulate in the food chain, eventually ending up in the fat of our bodies.

The use of many OCPs in Australia is now no longer permitted, and a nationwide plan was developed for their overall management. The Organochlorine Pesticides (OCP) Waste Management Plan was prepared between July 1996 and September 1997 as part of the National Strategy for the Management of Scheduled Wastes, and can be found at: http://www.deh.gov.au/industry/chemicals/scheduled-waste/

1.2.1 Background on organochlorine pesticides (OCPs)

Key properties of OCPs which caused concerns were persistence and toxicity. While OCPs were manufactured for their toxicity, the fact that they were also persistent had advantages in that they remained effective against target pests for prolonged periods. Therefore, these chlorinated organic compounds held an important position in pest control in agriculture for a long time, being versatile and, against some pests, very effective.

Since they were first introduced into Australia in the mid-1940s, OCPs have been used in many commercial products, in different forms (e.g. powders and liquids) and in different types and sizes of containers. Originally, OCPs were used widely and commonly to protect crops, livestock, buildings and households from the damaging effects of insects.

Commonly used OCP insecticides included DDT, lindane, chlordane, dieldrin, aldrin and heptachlor. Fungicides included hexachlorobenzene (HCB) and chlorinated phenols such as pentachlorophenol. The herbicide 2,4,5-T was also used.

1.2.2 OCP Uses

DDT came into use in the 1940s and was introduced widely into Australia and New Zealand agriculture in the 1950s. It was the first highly effective broad-spectrum insecticide which gave an extremely high level of control over many important insect pests. It has low acute toxicity to humans, and as such was acclaimed at the time as a wonder chemical. It was also used in large quantities in the control of mosquitoes which caused malaria in tropical countries. DDT was deregistered for use in Australia in 1987.

Chlordane was used to control termites, various types of ants, borers, lawn beetles, curl grubs, cut worms and black beetles.

Dieldrin was used widely against locusts and argentine ants; in the protection of electricity and telephone cable; soil treatment in farm and industrial premises for control of termites; and control of termites in buildings, fences and similar structures.

Aldrin was used as a soil treatment, usually pre-planting, for crops such as sugar cane; it was used in ant control as well as subterranean termite control; the protection of power poles from termites; farm, industrial and domestic control of fleas, flies, lice and mites.

Heptachlor was used similarly to chlordane. It was also commonly used in soil treatment in crops for control of funnel ants and grubs of the grey-black beetle in cane growing areas, and banana beetle borer in banana plantations.

Lindane (gamma-HCH or γ-HCH), one of eight isomers of hexachlorocyclohexane (HCH), is more than 5-20 times more toxic to insects than DDT. It was mainly used against plant eating insects, but had various medical and veterinary applications in treating skin parasites such as lice. Technical-grade HCH was also used as an insecticide and typically contained 10-15% γ-HCH as well as the alpha (α), beta (β), delta (δ), and epsilon (ε) isomers. However, virtually all the insecticidal properties reside in lindane which was deregistered for general use in 1985.

Hexachlorobenzene (HCB) was used as a fungicide or seed disinfectant. It was deregistered for general use between 1985 and 1987.

Chlorinated phenols such as pentachlorophenol (PCP), have been used widely in Australia to protect softwood timber from decay.

2, 4, 5-T was used as a herbicide against broad-leaved woody plants and as a defoliant.

Table 1.1 lists the OCP compounds measured in this study.

Table 1.1 OCP compounds measured in this study.

Compound		CAS Number
HCB	Hexachlorobenzene	118-74-1
α-HCH	alpha hexachlorocyclohexane	319-84-6
β-HCH	beta hexachlorocyclohexane	319-85-7
γ-HCH	gamma hexachlorocyclohexane (lindane)	58-89-9
Aldrin	Aldrin	309-00-2
Heptachlor	Heptachlor	76-44-8
Heptachlor epoxide	Heptachlor epoxide	1024-57-3
Dieldrin	Dieldrin	60-57-1
Oxychlordane	Oxychlordane	27304-13-8
trans-chlordane	trans-chlordane (gamma)	5103-74-2
cis-chlordane	cis-chlordane (alpha)	5103-71-9
trans-nonachlor	trans-nonachlor	39765-80-5
p,p'-DDE	p,p'-dichlorodiphenyldichloroethylene	72-55-9
p,p'-DDD	p,p'-dichlorodiphenyldichloroethane	72-54-8
o,p'-DDT	o,p'-dichlorodiphenyltrichloroethane	789-02-6
p,p'-DDT	p,p'-dichlorodiphenyltrichloroethane	50-29-3
Mirex	Mirex	2385-85-5

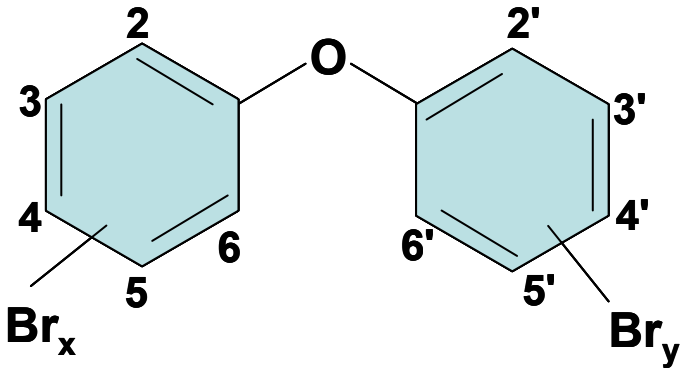
1.3 POLYBROMINATED DIPHENYL ETHERS (PBDES)

Flame retardants are compounds that are used to reduce the flammable nature of a multiplicity of commercial and household items. They are incorporated into plastics, rubbers and textiles and as such are found in electronic equipment, appliances, furniture, construction materials, vehicles and clothing. They are an integral part of modern life and have an important role in reducing the devastating effect of fires both in terms of saving lives and minimising economic cost.

There are two main types of flame retardant compound: reactive and additive flame retardants. Reactive flame retardants form part of the chemical makeup of the polymer and as such are bound to the polymer matrix via covalent bonds. Additive compounds are mixed with polymers during their production and do not form chemical bonds with the polymer. As a consequence, they are able to separate or leach out of the product over time.

Polybrominated diphenyl ethers (PBDEs) (Figure 1.1) belong to the additive group of flame retardants. They are synthesised by brominating diphenyl ether in the presence of a catalyst. There are 10 hydrogen atoms in the diphenyl ether molecule and any of these are able to be exchanged for bromine. Therefore, there are 209 possible PBDE congeners. These are numbered according to the position of the bromine atoms on the ring using the same IUPAC system as that used for numbering polychlorinated biphenyls (PCBs).

Figure 1.1 The structure of polybrominated diphenyl ethers (PBDEs).



x+y = 1-10

As with other organohalogen compounds, PBDEs are very hydrophobic and are very resistant to degradation and, hence, are termed persistent. As a result, they have the potential to bioaccumulate in the environment, animals and humans. PBDEs have been detected in sediments, marine mammals, fish, bird eggs and human milk, serum and adipose tissue (Darnerud et. al, 2001). In contrast to other persistent organohalogens, such as dioxin-like compounds, the levels of PBDEs in some humans and marine mammal populations are reported to be increasing (Hites 2004). The main route of exposure to PBDEs is ingestion via food, mainly fatty fish, meat and dairy products and human breast milk. It is also possible that some exposure occurs via inhalation or dermal absorption, particularly in occupationally exposed cohorts.

While it is clear that PBDEs, like dioxins and PCBs can be transferred to infants during lactation (Mazdai et. al., 2003; Meironyte, et. al, 2003), unlike dioxins and PCBs they do not show a positive association with either age (Petreas et.al., 2003; Darnerud, et.al., 1998; Thomsen et.al., 2002) or length of lactation (Schecter, 2003). Notably, when compared to other organic contaminants such as PCBs and dioxin-like chemicals, a much greater individual variation has been reported in the levels of PBDEs in humans (Schecter, 2003). Hence, it appears that the method of bioaccumulation of PBDEs is different to that of other organic contaminants. The reasons for this and the mechanisms of bioaccumulation of PBDEs are yet to be elucidated. Further research is needed to determine the routes of exposure to PBDEs and the mechanism of bioaccumulation in humans.

Monitoring techniques have revealed that low-level exposure to PBDEs occurs in almost all individuals. However, assessment of health risks associated with such low-level exposure is complicated and to date has not been adequately characterised (MacDonald, 2002). Potential risks associated with exposure to the most bio-active congeners (tri- to octa-BDE) include thyroid hormone disruption, neurodevelopmental deficits and cancer. Further research is needed to elucidate whether a risk is posed and also whether there is a synergistic effect produced from low-level exposure to a range of organic contaminants.

Different PBDE congeners have differing degrees of water solubility, vapor pressure and hydrophobicity. The water solubility and vapour pressure decrease with increasing degree of bromination whereas hydrophobicity increases.

Table 1.2 shows the PBDE congeners that were measured in this study. Note that octabromodiphenylether, nonabromodiphenylether and decabromodiphenylether are not

included in this table because they were not measured by either laboratory. Note that while the octa and deca congeners are commercially produced, the nona congener occurs as an impurity in both products. Historically, the levels of these congeners are not measured in standard analysis of PBDEs in ambient human samples. This is because they are thought to have much lower bio-accumulative properties and also because of the need to use a separate analytical column for detecting these congeners. In contrast BDE 47 has a much higher bioaccumulation potential relative to the amount in the parent penta-product. Human samples including blood, breast milk and adipose tissue are dominated by BDEs 47, 99, 100, 153 and 154.

Table 1.2 PBDE congeners measured in this study.

Congener	Abbreviation
2,2',4-triBDE	BDE 17
2,4,4'-triBDE	BDE 28
2',3,4-triBDE	BDE 33
2,2',4,4'-tetraBDE	BDE 47
2,2',4,5'-tetraBDE	BDE 49
2,3',4,4'-tetraBDE	BDE 66
2,3',4',6-tetraBDE	BDE 71
3,3',4,4'-tetraBDE	BDE 77
2,2',3,4,4'-penta BDE	BDE 85
2,2',4,4'5-pentaBDE	BDE 99
2,2',4,4',6-pentaBDE	BDE 100
2,3',4,4',6-pentaBDE	BDE 119
3,3',4,4',5-pentaBDE	BDE 126
2,2',3,4,4',5'-hexaBDE	BDE 138
2,2',4,4',5,5'-hexaBDE	BDE 153
2,2',4,4',5,6'-hexaBDE	BDE 154
2,3,4,4',5,6-hexaBDE	BDE 166
2,2',3,4,4',5',6'-heptaBDE	BDE 183
2,3,3',4,4',5,6-heptaBDE	BDE 190

Commercially, PBDEs are produced as three products: penta-BDE, octa-BDE and deca-BDE. Mixtures of congeners are formed because they are synthesised using a non-selective bromination process (Alaee et al, 2003). Table 1.3 shows the general compositions of PBDE based commercial products (de Witt 2202). Tables 1.4 and 1.5 show the worldwide market demand for PBDE products in 1999 and 2001, respectively (Bromine, Science and Environmental Forum, Total Market Demand; 2003; available at: www.bsef.com). It is interesting to note that >95 % of the total penta-BDE product was and continues to be utilised by the Americas (Hites, 2004). A voluntary ban on the use of penta-BDE products has been introduced in Europe and in Japan. Australia does not manufacture any PBDE products but the quantity and source of PBDE product in Australia in 1998/99 is shown in Table 1.6 (NICNAS, 2001).

Table 1.3 General composition of PBDE based commercial products

Technical Product	Congener (%)						
	Tetra-BDEs	Penta-BDEs	Hexa-BDEs	Hepta-BDEs	Octa BDEs	Nona-BDEs	Deca-BDEs
Penta-BDE	24-38	50-60	4-8				
Octa-BDE			10-12	44	31-35	10-11	<1
Deca-BDE						<3	97-98

Table 1.4 Total Market Demand in 1999 in Tonnes

	Americas	Europe	Asia	Total
Deca-BDE	24,300	7,500	23,000	54,800
Octa-BDE	1,375	450	2,000	3,825
Penta-BDE	8,290	210	-	8,500
Total	33,965	8,160	25,000	67,125

Table 1.5 Total Market Demand by Region in 2001 in Tonnes

	Americans	Europe	Asia	Total
Deca-BDE	24,500	7,600	24,050	56,150
Octa-BDE	1,500	610	1,680	3,790
Penta-BDE	7,100	150	250	7,500
Total	33,100	8,360	25,980	67,440

Table 1.6 Estimates on the quantities of commercial PBDE products in Australia in Tonnes per year

Substance	CAS number	1998/1999	Future Estimates
Decabromodiphenyl ether	1163-19-5	177	165
Pentabromodiphenyl ether	32534-81-9	72	119
Octabromodiphenyl ether	32536-52-0	47	57
Tetrabromodiphenyl ether	40088-47-9	22	36
Hexabromodiphenyl ether	36483-6-0	10	15
Nonabromodiphenyl ether	63936-56-1	>5	>5
Tribromodiphenyl ether	49690-94-0	4	6

2 PROJECT DESIGN

2.1 SAMPLE COLLECTION

In the 2001 the Australian Government announced the four year National Dioxins Program (NDP) to reduce dioxins and dioxin-like substances in the environment. A priority for the program was to improve our knowledge about dioxin levels in Australia. Studies commenced in 2001 to measure emissions from sources such as bushfires and dioxin levels in the environment, food and population. The findings of these studies were used to determine the risk dioxins pose to our health and the environment. Technical reports on the NDP studies can be found at: http://www.deh.gov.au/industry/chemicals/dioxins/index.html

Technical report No.10 'Dioxins in the Australian Population: Levels in Human Milk' reports on the levels of dioxins in the breast milk of some Australian women. The OCP and PBDE study was carried out as an extension of the NDP study and used the same samples. As the samples were originally collected for analysis of dioxin and dioxin-like compounds, the protocol used was identical to that used by the WHO in their international studies assessing the exposure levels in human breast milk for these chemicals.

In total, 173 samples of breast milk 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 collected and analysed as 17 pooled samples covering the following regions:

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

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 NDP 2002/03 project was also submitted to several ethics committees throughout Australia. Only the original approval is shown in Appendix A. Women who participated in the NDP study signed an additional consent form allowing further analysis and research to be conducted using their samples at any time in the future. Appendix B lists the Committees that the protocol was submitted to and also the approval dates. As requested, gatekeeper approval letters were submitted to the University of Queensland Medical Research Ethics Committee. In the table in Appendix B, 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 led 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 monthly 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 C). Once a potential participant had verbally agreed to participate, they were invited to read an information sheet and to complete a consent form (Appendix D). They were also asked to complete a questionnaire (Appendix E).

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 mls (preferably 150 ml) of expressed milk. This volume was to be collected over the six week period (2-8 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 2 and 8 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 collection of a pool was completed the milk was thawed, thoroughly homogenised and 30 ml from each individual was pooled giving approximately 300 ml of pooled milk sample from each region. The first 10 samples, sample 01-10, in a region were pooled and labeled 'Region A', the next 10 samples collected, sample 11-20, were pooled and labeled 'Region B' and so forth. The pooled samples were then refrozen and transported on ice to Sydney, Australia for analysis and two duplicate samples were sent to Münster, Germany for inter-laboratory comparison.

2.1.3 Sample numbers

The aim of the initial dioxins in breast milk project was to collect samples from 200 women across Australia. At the end of the project the total number of samples collected was 173, of these 16 samples were excluded because they were later found to have violated the inclusion/ exclusion criteria. Collection of samples was slower than anticipated due to the nature of the samples required, the necessity to ensure that all sites had correct ethical clearance and the strict inclusion criteria. This was also experienced in the 2000/01 Australian study which used these same criteria as part of the 3rd round WHO dioxin and dioxin-like compound 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 3-5 days post-delivery and, hence, pressures on post-natal maternal and child health clinics mean that staff have limited time for involvement in and 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, 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.

3 SAMPLE ANALYSIS

Samples were analysed at the National Measurement Institute, Sydney Australia. For inter-laboratory comparative purposes, duplicate pooled samples were analysed at the State Laboratory of NRW in Münster, Germany.

3.1 ORGANOCHLORINE PESTICIDES (OCPS)

The method for determination of persistent OCPs in breast milk matrices was by isotope dilution high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). The analytical methodology for the determination of the OCPs was based on USEPA methods 1613B and 1668A.

Samples were thawed and shaken to produce a homogenous sample. A sub-sample was spiked with a range of isotopically labeled surrogate standards. Proteins were denatured with the addition of potassium oxalate then a liquid-liquid extraction was performed with 2:1 acetone:hexane. Gel permeation chromatography (GPC) was used to remove lipids with clean-up of this extract on a partially deactivated Florisil® column.

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 OCPs were separated by the GC, and then 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.

3.1.1 Organochlorine Analyses

The following standards were all purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and were used for calibration, quantification and determination of recovery of OCPs:

- ES-5019 CS1-8 calibration and verification solutions
- ES-5021 labelled compound surrogate 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). Florisil® 60-80 PR was obtained from Mallinckrodt (Kentucky, USA). The Florisil® was activated by first placing in a muffle furnace overnight at 450 °C and then placed in an oven for 16 hours at 130 °C. Just prior to use the Florisil® was deactivated with the addition 1.2 % w/w Milli-Q water and tumbled for 30 minutes.

3.1.2 Sample preparation

Approximately 100 g of breast milk sample was accurately weighed and spiked with a known amount of the OCP isotopically labeled ^{13}C 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 then 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 subjected to gel permeation chromatography as detailed below.

3.1.3 Gel Permeation Chromatography (GPC) Clean-up

GPC was conducted according to SOP NR104 Rev. 3.0 Preparation/Calibration/Use of GPC Equipment. Briefly, this entails preparation of the size exclusion column using SX-3 Bio Beads, calibration with the first and last eluting OCPs and then injection and collection of the requisite fraction using acetone:hexane (35:65) as the eluent. This fraction was concentrated under vacuum to approximately 1mL of hexane.

3.1.4 Florisil® Column Clean-up

A fritted-glass column was slurry packed with 8 g of deactivated Florisil® using hexane and 1g sodium sulfate added to the top. The concentrated GPC extract was applied in hexane and eluted with 60 ml of DCM:hexane (50:50). This fraction was concentrated under vacuum and the recovery standard was added and then further concentrated using clean dry nitrogen to a final volume of 10 µL prior to High-Resolution Gas Chromatography High-Resolution Mass Spectrometric (HRGC/HRMS) analysis.

3.1.5 High-Resolution Gas Chromatography High-Resolution 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 autosampler. A DB-5 (J & W Scientific, Folsom, CA, USA) capillary column (60m x 0.25mm i.d., film thickness 0.25µm) 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 OCP analysis was: 100 °C (isothermal for 1 min.) then ramp 1 to 200 °C at 40 °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. The temperature program for the OCP analysis was: 110 °C (isothermal for 1 min.) then ramp 1 to 200 °C at 15 °C/min, ramp 2 to 300 °C at 3 °C/min and then ramp 3 to 310 °C (isothermal 8 min) at 15 °C/min. A 1µL splitless injection with an injector temperature of 290 °C for OCP 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 0.7mA 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 are identified using the GC retention time and ion abundance ratios with reference to internal standards. Table 3.1 gives the list of OCPs that were measured. Table 3.2 shows the MID windows for the organochlorines.

Table 3.1 List of OCPs

Compound		CAS Number
HCB	Hexachlorobenzene	118-74-1
α-HCH	alpha hexachlorocyclohexane	319-84-6
β-HCH	beta hexachlorocyclohexane	319-85-7
γ-HCH	gamma hexachlorocyclohexane (lindane)	58-89-9
Aldrin	Aldrin	309-00-2
Heptachlor	Heptachlor	76-44-8
Heptachlor epoxide	Heptachlor epoxide	1024-57-3
Dieldrin	Dieldrin	60-57-1
Oxychlordane	Oxychlordane	27304-13-8
trans-chlordane	trans-chlordane (gamma)	5103-74-2
cis-chlordane	cis-chlordane (alpha)	5103-71-9
trans-nonachlor	trans-nonachlor	39765-80-5
p,p'-DDE	p,p'-dichlorodiphenyldichloroethylene	72-55-9
p,p'-DDD	p,p'-dichlorodiphenyldichloroethane	72-54-8
o,p'-DDT	o,p'-dichlorodiphenyltrichloroethane	789-02-6
p,p'-DDT	p,p'-dichlorodiphenyltrichloroethane	50-29-3
Mirex	Mirex	2385-85-5

Table 3.2 The MID Windows for Organochlorines

Compound Name	Quan. Mass	Ratio Mass 1
HxCB	283.8102	285.8072
HxCB $^{13}C_6$ Surr	289.8303	291.8273
β-HCH	218.9116	220.9086
β-HCH $^{13}C_6$ Surr	226.9283	224.9317
γ-HCH	218.9116	220.9086
γ-HCH $^{13}C_6$ Surr	226.9283	224.9317
Aldrin	262.8570	264.8540
Heptachlor	271.8102	273.8072
Heptachlor Epoxide	352.8440	354.8410
Heptachlor Epoxide $^{13}C_{10}$ Surr	362.8775	364.8745
Dieldrin	262.8570	264.8540
Dieldrin $^{13}C_{12}$ Surr	269.8805	271.8775
Oxychlordane	386.8050	388.8020
Oxychlordane $^{13}C_{10}$ Surr	396.8380	398.8360
trans-chlordane	372.8260	374.8230
cis-chlordane	372.8260	374.8230
Trans-nonachlor	406.7870	408.7840
Trans-nonachlor $^{13}C_{10}$ Surr	416.8205	418.8175
pp-DDE	246.0003	247.9974
pp-DDE $^{13}C_{12}$ Surr	258.0405	260.0376
pp-DDD	235.0081	237.0052
op-DDT	235.0081	237.0052
op-DDT $^{13}C_{12}$ Surr	247.0483	249.0454
pp-DDT	235.0081	237.0052
pp-DDT $^{13}C_{12}$Surr	247.0483	249.0454
Mirex	271.8102	273.8072
Mirex $^{13}C_{10}$ Surr	276.8270	278.8240

3.1.6 Compound identification and quantification criteria

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

- The retention time of the analyte must be within 1 second of the retention time of the corresponding $^{13}C_x$ surrogate standard
- The ion ratio obtained for the analyte must be ±15 % of the theoretical ion ratio
- The signal to noise ratio must be greater than 3:1
- Levels of OCPs 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 %.

3.1.7 Quantification using the Isotope Dilution Technique

The naturally occurring (native) compound was determined by reference to the same compound in which one or more atoms had been isotopically enriched. In this method, all carbon atoms for selected OCP molecules were substituted with carbon-13 to produce $^{13}C_x$-labeled analogs. The $^{13}C_x$-labeled OCPs were spiked into each sample and allowed identification and correction of the concentration of the native compounds in the analytical process. Where $^{13}C_x$-labeled analogs were unavailable the closest structurally similar surrogate was used to calculate relative response factors. 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.

3.1.8 Quality Assurance

- Batch sizes were typically 6-8 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 were established for each MS run.
- The recoveries of all isotopically labelled surrogate standards were calculated and reported.

3.1.9 Data reporting

The basis of reporting for primary and quality control samples is as follows:

- ng g^{-1} on a lipid weight basis
- Data were corrected for recovery of $^{13}C_x$ surrogate standards
- For all samples, data for quantified analytes were reported to 2 or 3 significant figures
- Limit of detection data for non-quantified analytes were reported to 1 significant figure.

3.2 POLYBROMINATED DIPHENYL ETHERS (PBDES)

High resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) was used to determine the levels of PBDEs in breast milk matrices. This method provided data on 16 PBDE congeners determined by the isotope dilution quantification technique. The detection limits and quantification levels in this method were usually dependent on the level of interferences rather than instrumental limitations. The method was 'performance based'. The analytical methodology for the determination of PBDEs was based on USEPA Draft Method 1614.

Samples were thawed, sonicated and shaken to produce a homogenous sample. A sub sample was spiked with a range of isotopically labeled 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 and basic alumina. 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 PBDEs were separated by the GC, and then 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.

3.2.1 PBDE Analyses

The following standards were all purchased from Wellington Laboratories (Ontario, Canada) and were used for calibration, quantification and determination of recovery of PBDEs:

- MBDE-MXC labelled surrogate spiking solution
- MBDE-139-IS internal standard solution
- BDE-CVS-E calibration and verification solutions (CS1-CS5).

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 and basic alumina that is packed in individual Teflon columns and vacuum sealed in aluminium foil packages.

3.2.2 Sample preparation

Approximately 100 g of breast milk sample was accurately weighed and spiked with a known amount of the respective PBDE isotopically labeled $^{13}C_{12}$ 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) in the forward direction at 10 mL/min. The fraction containing the PBDEs is collected from the alumina column during elution using dichloromethane:hexane (50:50). This fraction is concentrated under vacuum and the recovery standard (MBDE-139-IS) are added and then further concentrated using clean dry nitrogen to a final volume of 10 µl prior to High-Resolution Gas Chromatography High-Resolution Mass Spectrometric (HRGC/HRMS) analysis.

3.2.3 High-Resolution Gas Chromatography High-Resolution 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 PBDE analysis was: 110 °C (isothermal for 1 min.) then ramp 1 to 200 °C at 15 °C/min, ramp 2 to 300 °C at 3 °C/min and then ramp 3 to 310 °C (isothermal 8 min) at 15 °C/min. A 1μL splitless injection with an injector temperature of 300 °C for PBDE analysis was employed for all standards and sample extracts. The mass spectrometer operating conditions were: ion source and transfer line temperatures, 240 and 280 °C, respectively; ionisation energy 45 eV, filament current 0.7 mA and electron multiplier voltage set to produce a gain of 106. 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 labelled compounds. Individual congeners are 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. Table 3.3 gives a list of the PBDE congeners. Table 3.4 shows the MID windows for the PBDEs. Table 3.5 shows the theoretical ion abundance rations and QC limits.

Table 3.3 List of PBDEs

PBDE Congener	Abbreviation
2,2′,4′-Tribromodiphenyl ether	BDE-17
2,4,4′-Tribromodiphenyl ether	BDE-28
2,3,4-Tribromodiphenyl ether	BDE-33
2,2′,4,4′-Tetrabromodiphenyl ether	BDE-47
2,2′,4,5′-Tetrabromodiphenyl ether	BDE-49
2,3′,4,4′-Tetrabromodiphenyl ether	BDE-66
2,3′,4′,6-Tetrabromodiphenyl ether	BDE-71
3,3′,4,4′-Tetabromodiphenyl ether	BDE-77
2,2′,3,4,4′-Pentabromodiphenyl ether	BDE-85
2,2′,4,4′,5-Pentabromodiphenyl ether	BDE-99
2,2′,4,4′,6-Pentabromodiphenyl ether	BDE-100
2,3′,4,4′,6-Pentabromodiphenyl ether	BDE-119
3,3′,4,4′,5-Pentabromodiphenyl ether	BDE-126
2,2′,3,4,4′,5-Hexabromodiphenyl ether	BDE-138
2,2′,4,4′,5,5′-Hexabromodiphenyl ether	BDE-153
2,2′,4,4′,5,6′-Hexabromodiphenyl ether	BDE-154
2,3,4,4′,5,6-Hexabromodiphenyl ether	BDE-166
2,2′,3,4,4′,5′,6-Heptabromodiphenyl ether	BDE-183

Table 3.4 The MID Windows for PBDEs

MID Window	Accurate Mass	*Ion Id	Ion Type	PBDE (I= internal standard)
1		Lock		
	247.9837	M	R	MoBDE
	249.9817	M+2	T	MoBDE
	260.0239	M	R	MoBDE (I)
	262.0219	M+2	T	MoBDE (I)
2		Lock		
	327.8922	M+2	R	DiBDE
	329.8903	M+4	T	DiBDE
	339.9324	M+2	R	DiBDE (I)
	341.9305	M+4	T	DiBDE (I)
3		Lock		
	245.968	M-Br_2	R	TriBDE
	247.9661	(M+2)-Br_2	T	TriBDE
	258.0082	M-Br_2	R	TriBDE(I)
	260.0063	(M+2)-Br_2	T	TriBDE(I)
4		Lock		
	323.8785	M-Br_2	R	TeBDE
	325.8765	(M+2)-Br_2	T	TeBDE
	335.9188	M-Br_2	R	TeBDE(I)
	337.9168	(M+2)-Br_2	T	TeBDE(I)
	483.7132	M+2	R	TeBDE
	485.7112	M+4	T	TeBDE
5		Lock		
	403.7870	(M+2)-Br_2	R	PeBDE
	405.7850	(M+4)-Br_2	T	PeBDE
	415.8272	(M+2)-Br_2	R	PeBDE(I)
	417.8252	(M+4)-Br_2	T	PeBDE(I)
	563.6216	M+4	R	PeBDE
	565.6197	M+6	T	PeBDE
6		Lock		
	481.6975	(M+2)-Br_2	R	HxBDE
	483.6955	(M+4)-Br_2	T	HxBDE
	493.7378	(M+2)-Br_2	R	HxBDE(I),(IS)
	495.7357	(M+4)-Br_2	T	HxBDE(I),(IS)
7		Lock		
	561.6060	(M+4)-Br_2	R	HpBDE
	563.6040	(M+6)-Br_2	T	HpBDE
	573.6462	(M+4)-Br_2	R	HpBDE(I)
	575.6442	(M+6)-Br_2	T	HpBDE(I)

❖T=Target Ion=Quantitation Ion; R=Ratio Ion=Qualifier Ion.

Table 3.5 Theoretical Ion Abundance Ratios and QC Limits

No. of Bromine Atoms	m/z's forming the ratio (R/Q)[1]	Theoretical Ratio	QC limits[2]	
			Lower	Upper
1	M/(M+2)	1.03	0.88	1.18
2	(M+2)/(M+4)	0.51	0.43	0.59
2	M/(M+2)	0.43	0.47	0.59
3	M-Br_2/(M+2)-Br_2	1.06	0.82	1.22
3	(M+2)/(M+4)	1.03	0.88	1.18
4	M-Br_2/(M+2)-Br_2	0.53	0.41	0.61
4	(M+2)/(M+4)	0.70	0.60	0.81
4	(M+4)/(M+6)	1.54	1.31	1.77
5	(M+2)-Br_2/(M+4)-Br_2	1.06	0.82	1.22
5	(M+4)/(M+6)	1.03	0.88	1.18
6	(M+2)-Br_2/(M+4)-Br_2	0.71	0.54	0.82
6	(M+4)/(M+6)	0.77	0.65	0.89
6	(M+6)/(M+8)	1.37	1.16	1.58
7	(M+4)-Br_2/(M+6)-Br_2	1.06	0.82	1.22
7	(M+6)/(M+8)	1.03	0.88	1.18
8	(M+6)/(M+8)	0.82	0.70	0.94
9	(M+8)/(M+10)	1.03	0.88	1.18
10	(M+8)/(M+10)	0.73	0.86	0.99

[1] The ratio is defined as the Qualifier ion area (R) divided by the Quantification ion area (T).

[2] QC limits represent ±15 % windows around the theoretical ion abundance ratios.

3.2.4 Compound identification and quantification criteria

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

- The retention time of the analyte must be within 1 second of the retention time of the corresponding $^{13}C_{12}$ surrogate standard
- The ion ratio obtained for the analyte must be ±20 % of the theoretical ion ratio
- The signal to noise ratio must be greater than 3:1
- Levels of PBDE 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 %.

3.2.5 Quantification using the Isotope Dilution Technique

The naturally occurring (native) compound was determined by reference to the same compound in which one or more atoms were isotopically enriched. In this method, all carbon atoms for selected PBDE molecules were substituted with carbon-13 to produce $^{13}C_{12}$-labeled analogs of the brominated diphenyl ethers. The $^{13}C_{12}$-labelled PBDEs were spiked into each sample and allowed identification and correction of the concentration of the native compounds in the analytical process. 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.

3.2.6 Quality Assurance

In order to manage quality assurance batch sizes were typically 6-8 samples. A laboratory blank was analysed with each batch of samples and 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 were established for each MS run and the recoveries of all isotopically labelled surrogate standards were calculated and reported.

3.2.7 Data reporting

The basis of reporting for primary and quality control samples is as follows: ng g^{-1} on a lipid weight basis; PBDEs data were corrected for recovery of $^{13}C_{12}$ surrogate standards; for all samples, data for quantified PBDEs were reported to 2 or 3 significant figures; and limit of detection data for non-quantified PBDEs were reported to one significant figure.

4 OCP AND PBDE LEVELS IN THE BREAST MILK OF AUSTRALIAN WOMEN

4.1 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. The majority of samples were collected during the period 2-8 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 9 weeks. Samples were also accepted from mothers whose babies were one week at the beginning of sampling if sampling continued into the 2-8 week period. The regions were Brisbane, Sydney (2 pools), Melbourne (4 pools), Adelaide (2 pools), Perth, Hobart, rural inland NSW (Dubbo), rural inland Queensland (Dalby), rural Victoria (Bendigo, Ballarat, Lakes Entrance), Newcastle, Wollongong and Darwin. All samples were analysed as pooled samples and there were 17 pooled samples in total. The first 10 samples, sample 01-10, in a region were pooled and labeled 'Region A', the next 10 samples, sample 11-20, collected were labeled 'Region B' and so forth.

All participants were asked to complete a questionnaire (Appendix E) containing questions pertaining to pre and post pregnancy weight, diet, and place of residence as well as information related to their baby. Table F.1 in Appendix F 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. Information regarding diet and occupation was not used for analytical purposes and the data is not presented in this report. It should, however, be noted that no participants reported unusual exposure conditions for OCP contaminants.

A further 24 'historical' samples that had been collected in 1993 were obtained from the Key Centre for Applied and Nutritional Toxicology[3]. They were analysed as three pools of eight samples. No information regarding the demographics of this 'historical' sample population was available, 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 - the current study - and three pools obtained in 1993.

4.2 QUALITY CONTROL AND QUALITY ASSURANCE

Quality control procedures utilised by the National Measurement Institute were presented in Section 3.1.8 for OCPs and 3.2.6 for PBDEs.

4.2.1 Inter-laboratory calibration

For the purposes of inter-laboratory comparison, two samples were analysed at National Measurement Institute, Sydney, Australia and The State Laboratory of NRW, Münster, Germany for OCPs and PBDEs. These were samples duplicate pools from rural NSW and Melbourne B. Normalised differences (ND) are used to compare results from the two laboratories. Box 1 explains normalised difference.

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

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 1. 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 (ng g^{-1} lipid)	Sample B (ng g^{-1} lipid)	ND %
1.0	1.2	18.2
1.0	1.5	40.0
1.0	2.0	66.7
1.0	3.0	100.0
1.0	10.0	163.6
1.0	100.0	196.0

OCP and PBDE compounds were detected in both pooled samples by both laboratories. All concentrations are expressed on a lipid basis. Inter-laboratory comparisons for OCPs are given in Table 4.1 and Figure 4.1 and for PBDEs in Table 4.2 and Figure 4.2.

The list of OCPs in Table 4.1 is different to the list in Table 3.1 as the National Measurement Institute analysed more OCPs than the State Laboratory of NRW. For comparative purposes only those OCPs analysed by both laboratories are listed here. For OCPs there is good agreement between the results obtained by the two laboratories for the two samples, see Table 4.1. The State Laboratory of NRW analysed the samples for nine of the 17 OCPs analysed by the National Measurement Institute. The analysis from the National Measurement Institute detected all of the nine OCPs in both pools, in the Rural NSW and Melbourne B samples, respectively, while 6 out of 9 and 4 out of 9 were detected by the State Laboratory of NRW.

Table 4.1 Inter-laboratory comparisons for OCPs.

Comparison of results obtained from samples analysed at the National Measurement Institute (1) and at the State Laboratory of NRW (2). Normalised differences are also shown. Results are in ng g^{-1} lipid.

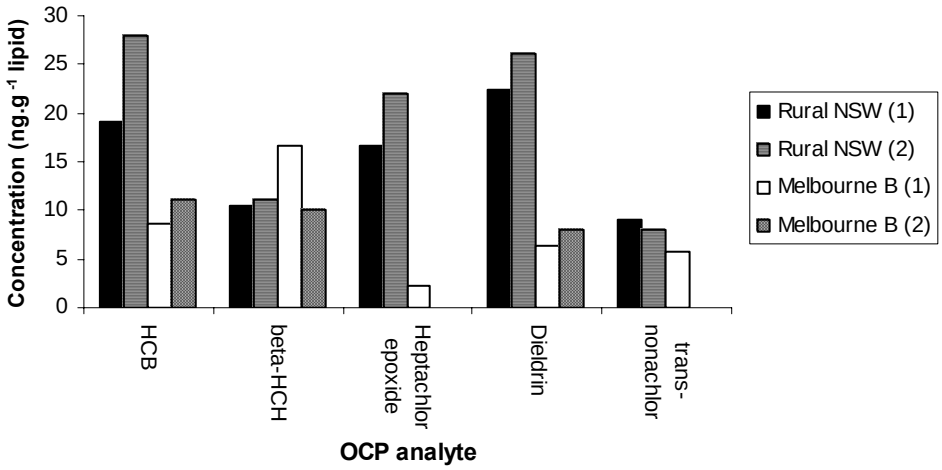
OCP	Rural NSW (1)	Rural NSW (2)	N.D. (%)	Melbourne B (1)	Melbourne B (2)	N.D. (%)
HCB	19.1	28	37.8	8.69	11	23.5
α-HCH	0.026	n.d. (2)	n.c	0.046	n.d.(2)	n.c.
β-HCH	10.4	11	5.6	16.7	10	50.2
γ-HCH	0.15	n.d.(2)	n.c.	0.13	n.d.(2)	n.c.
Heptachlor epoxide	16.7	22	27.4	2.21	n.d.(5)	n.c.
Dieldrin	22.5	26	14.4	6.43	8	21.8
trans-nonachlor	9.02	8	12	5.82	n.d.(5)	n.c.
p,p'-DDE	217	307	34.4	378	367	2.9
p,p'-DDT	5.7	n.d. (5)	n.c.	5.97	n.d.(5)	n.c.

n.c. - not calculated

n.d. ( ) - not detected (limit of detection)

The largest difference between the two laboratories, for OCPs, was observed in the Melbourne B pool for β-HCH with a normalised difference of 50.2 %. For this OCP, the level detected by the National Measurement Institute was higher than that detected by the State Laboratory of NRW. It was not possible to calculate the ND for those OCPs that were not detected in the second laboratory analysis. For both pools these OCPs were α-HCH, γ-HCH and p,p'-DDT. In addition, heptachlor epoxide and transnonachlor were not detected in State Laboratory of NRW analysis of the Melbourne B pool.

Figure 4.1 Comparison of selected results of inter-laboratory OCP analysis.



The numbers 1 and 2 denote the National Measurement Institute and the State Laboratory of NRW, respectively.

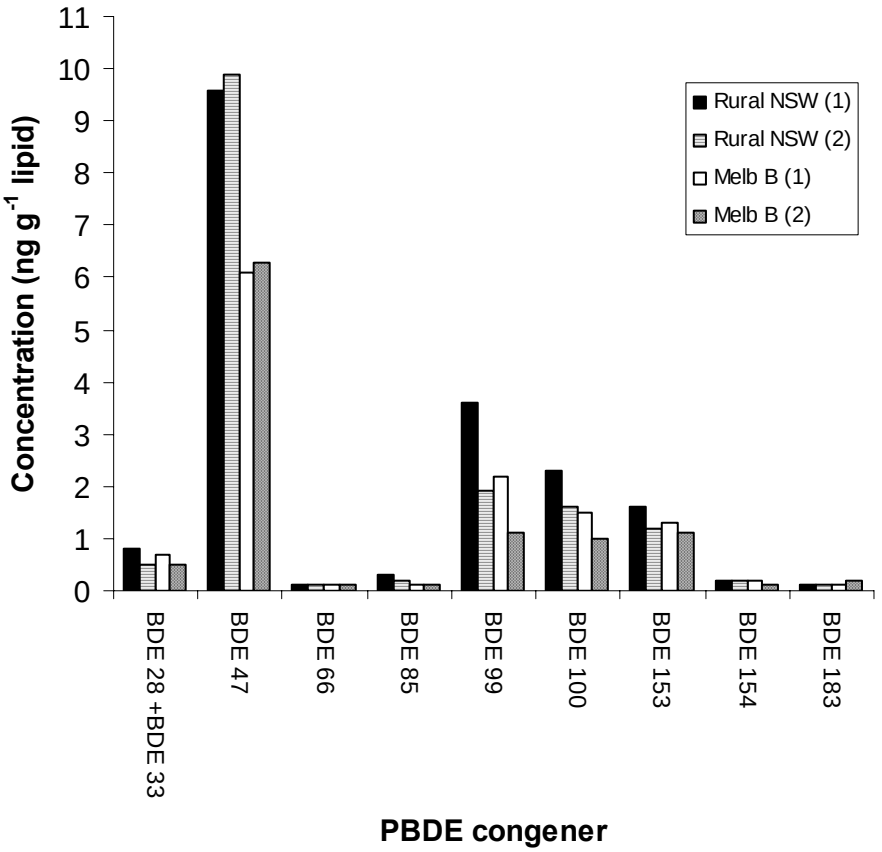
Table 4.2 Inter-laboratory comparisons for PBDEs.

Comparison of results obtained from samples analysed at the National Measurement Institute (1) and at the State Laboratory of NRW (2). Normalised differences are also shown. Results are in ng g^{-1} lipid.

Congener	Rural NSW (1)	Rural NSW (2)	ND (%)	Melbourne B (1)	Melbourne B (2)	ND (%)
BDE 28 + BDE 33	0.8	0.5	34	0.7	0.5	37
BDE 47	9.6	9.9	3	6.1	6.3	3.7
BDE 66	0.1	0.1	32	0.1	0.1	13
BDE 85	0.3	0.2	31	0.1	0.1	33
BDE 99	3.6	1.9	60	2.2	1.1	65
BDE 100	2.3	1.6	37	1.5	1.0	42
BDE 153	1.6	1.2	26	1.3	1.1	14
BDE 154	0.2	0.2	42	0.2	0.1	46
BDE 183	0.1	0.1	18	0.1	0.2	29
Sum Congeners	18.8	15.7		12.4	10.4	

The list of PBDE congeners in Table 4.2 is different to the list in Table 3.3 as the National Measurement Institute analysed more congeners than the State Laboratory of NRW. For comparative purposes only those congeners analysed by both laboratories are listed here. For PBDEs, there is a good agreement between the results obtained by the two laboratories for the two samples. The largest difference between the two laboratories was observed in both the rural NSW and the Melbourne B pools for congener 99 with a normalised difference of 60 and 65 %, respectively. For this congener, the levels detected by the National Measurement Institute were higher than those detected by the State Laboratory of NRW in both pools. For congener 47, the levels detected by both laboratories were remarkably similar in both pools. The normalised differences for BDE 47 for rural NSW and Melbourne B pools were 3 and 3.7 %, respectively. It should be noted that the National Measurement Institute reports PBDE congeners 28 and 33 combined whereas the State Laboratory of NRW reports only BDE 28. It is reported that the two congeners co-elute and so it can be assumed that the results from both laboratories contain mixtures of congeners 28 and 33 (Rayne & Ikonomou, 2003).

Figure 4.2 Comparison of the results of inter-laboratory sample analysis.



The numbers 1 and 2 denote National Measurement Institute and State Laboratory of FWR, respectively.

4.3 LEVELS OF OCPS IN THE BREAST MILK OF AUSTRALIAN WOMEN

4.3.1 Overall evaluation of OCPs detected in pooled breast milk samples collected from Australian women during 2002/03 and from Victorian women during 1993.

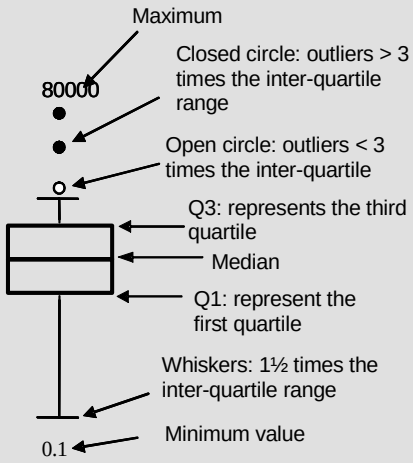
OCP compounds were detected in all pooled breast milk samples. Data sheets showing the concentration of OCPs detected in each pooled sample from 2002/03 are presented in Appendix G, Table 1. The p,p'-DDE and β-HCH were the dominant OCPs detected in all samples. (Figure 4.3, Table 4.3 and Appendix G, Table 1). Sydney A and Melbourne A pooled breast milk samples contained the highest concentration of sum OCPs when compared to other regions. Again the dominant OCPs contributing to these high levels were p,p'-DDE and β-HCH.

Figure 4.4 depicts the concentrations of OCP compounds detected in these samples as a Box and Whisker plot. An explanation of Box and Whisker Plots is given in Box 2.

Box 2: Box and Whisker Plots

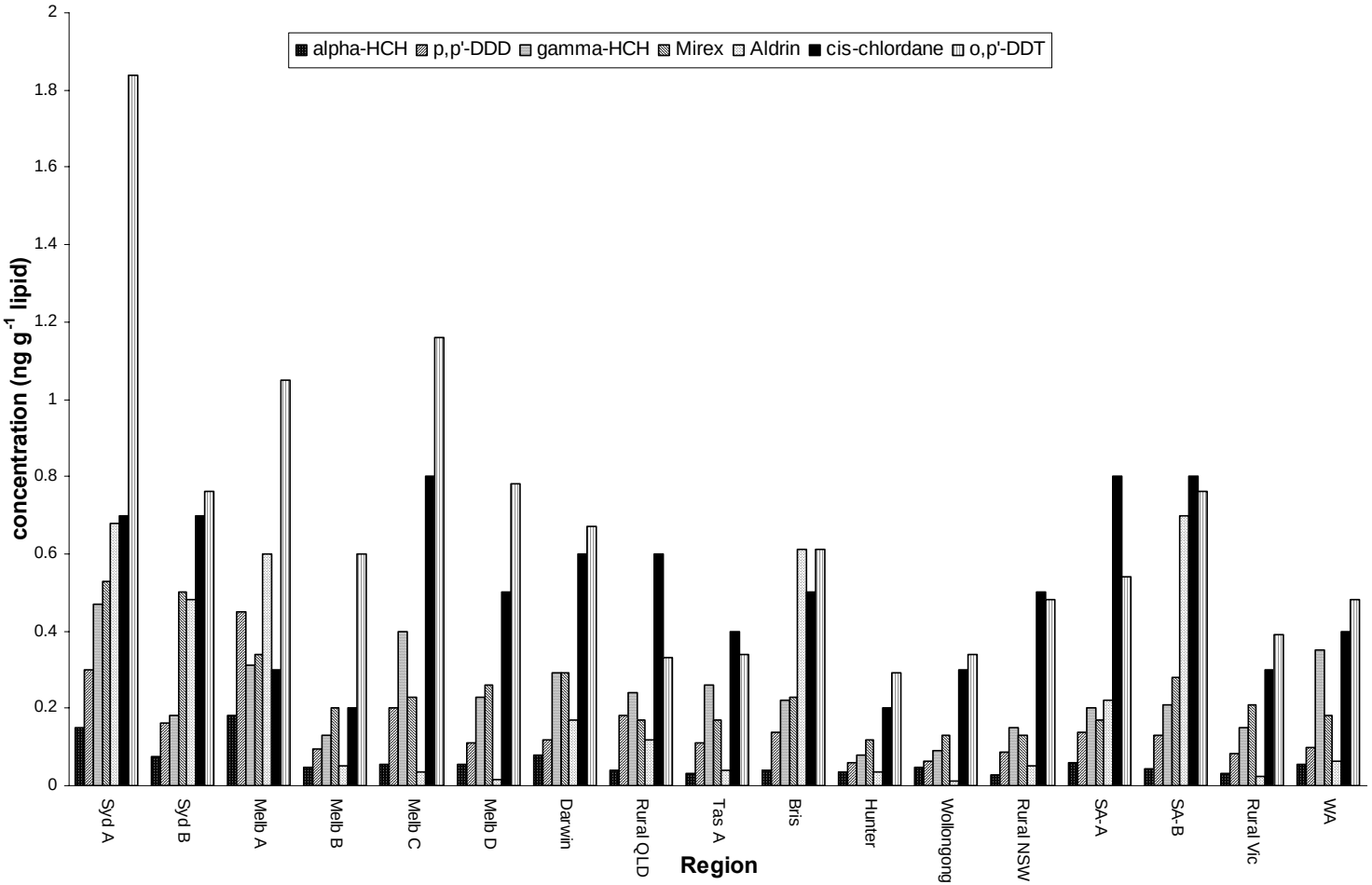
Box and whisker plots are a widely accepted way of presenting environmental data. They show where the data points are concentrated (the box) and the outlying values (the whiskers, open and closed circles). Box plots are often used to compare several sets of data.

Here we use a plot where the boxes represent the 25th and 75th percentiles (1st and 3rd quartiles). The top of the box in these plots is the 75th percentile (75 % of the data fall below this line), while the bottom of the box represents the 25th percentile (25 % of the data fall below this line). The line in the middle of the box represents the median (50 % of the data fall above and 50 % below this number).



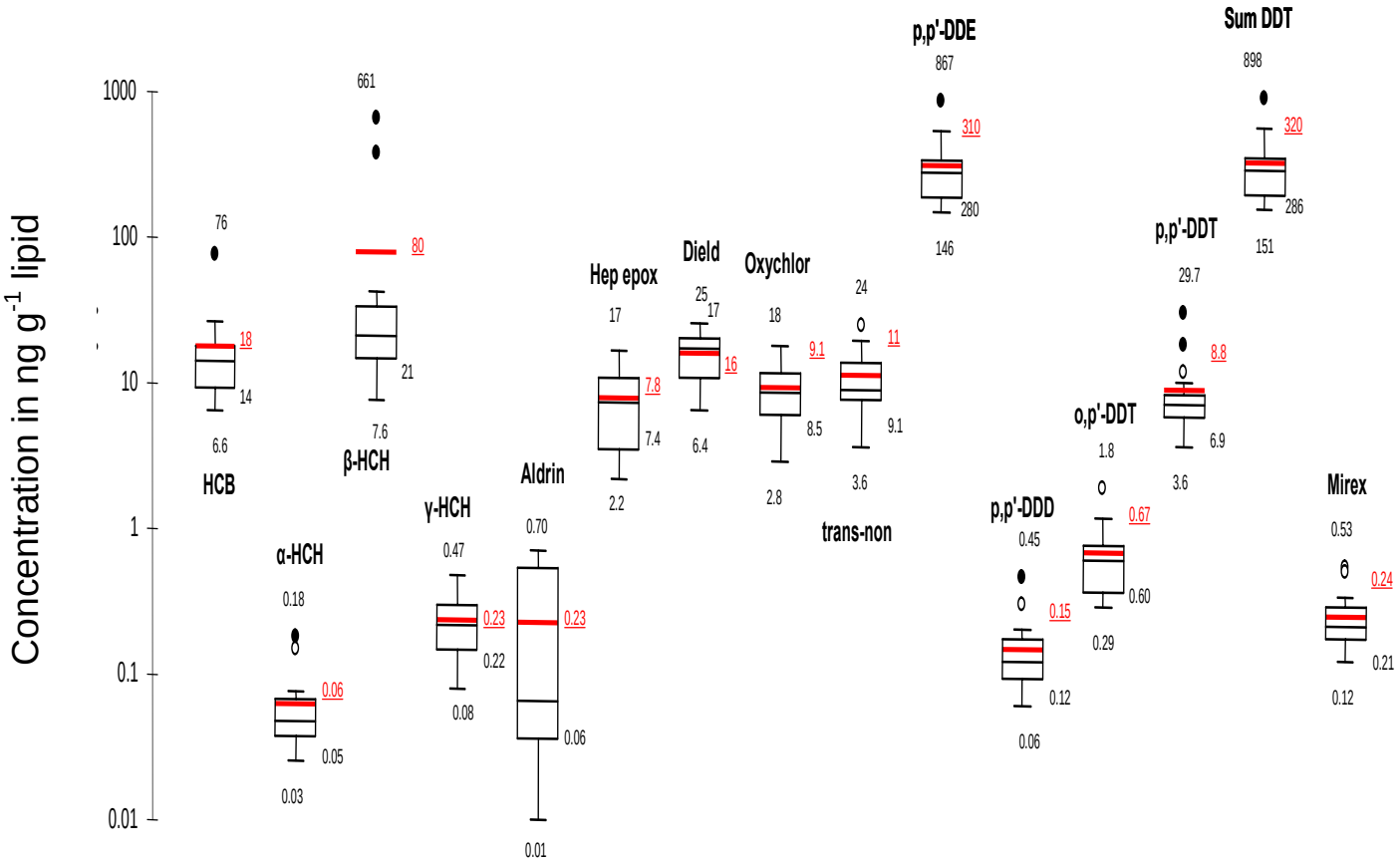
The whiskers on the box extend to data points that are up to 1½ times the Inter Quartile Range (IQR). The IQR is defined as the difference between the 75th and the 25th percentiles, and is equal to the range of about half the data. Outliers which are less than three times the IQR are shown as open circles, while those greater than three times the IQR are shown as closed circles. The statistical and graphical package XLSTAT was used to produce all box plots and calculate percentiles.

Figure 4.3 Concentration of selected OCPs in milk samples collected and pooled for different sampling regions.



Values represent the sum concentrations of OCPs detected in each pooled sample and are expressed on a lipid basis (ng g^{-1} lipid). LOD included.

Figure 4.4 Box and whisker plot of the concentration of OCPs detected in pooled breast milk samples collected from Australian women during 2002/03.



Values represent the median; mean (underlined); maximum; and minimum concentrations of OCPs detected in each pooled sample and are expressed on a lipid basis (ng g^{-1} lipid). LOD included. A description of Box and Whisker Plots is given in Box 2.

From Table 4.3 and Figure 4.4 it is possible to identify outlying points. From the plot it can be seen that the OCP compounds, HCB, α- and β-HCH, p,p'-DDE, p,p'-DDD, p,p'-DDT and o,p'-DDT all show outlying points that are greater than three times the inter-quartile range. With the exception of HCB, all these outliers were detected in either Melbourne A or Sydney A pools. For β-HCH, high levels were recorded from these two pools. The reason for these high levels is not known. Analysis of individual samples would reveal whether this was a pool or individual phenomenon. However, such analysis was beyond the scope of the project and was not possible under the constraints of the Ethical approval. The Box and Whisker plot (Figure 4.4) also indicates pooled samples that contain outlying concentrations that are less than three times the inter-quartile range. These were α-HCH, trans-nonachlor, p,p'-DDD, o,p'-DDT, p,p'-DDT and Mirex. The presence of these outliers means that it is prudent to use the median values in Table 4.3 for comparative purposes.

Table 4.3 Mean, Median, Minimum and Maximum concentrations of OCPs detected in pooled breast milk samples collected from Australian women in 2002/03.

Values represent the concentrations of OCPs detected in each pooled sample and are expressed on a lipid basis (ng g^{-1} lipid). LOD included. The number of compounds in all samples is also shown.

Organochlorine	Mean	Median	Min	Max	No. of positives detected
HCB	18	14	6.6	76	17
α-HCH	0.06	0.05	0.03	0.18	17
β-HCH	80	21	7.6	660	17
γ-HCH	0.23	0.22	0.08	0.47	17
Aldrin	0.23*	0.06*	n.d. (0.05)	0.7	12
Heptachlor	n.c.	n.c.	n.c.	n.c.	0
Heptachlor epoxide	7.8	7.4	2.2	17	17
Dieldrin	16	17	6.4	25	17
Oxychlordane	9.1	8.5	2.8	17	17
trans-chlordane	n.c.	n.c.	n.c.	n.c.	0
cis-chlordane	n.c.	n.c.	n.c.	n.c.	0
trans-nonachlor	11	9.1	3.6	24	17
p,p'-DDE	310	280	150	870	17
p,p'-DDD	0.15	0.12	0.06	0.45	17
o,p'-DDT	0.67	0.6	0.29	1.8	17
p,p'-DDT	8.8	7.0	3.6	30	17
Mirex	0.24	0.21	0.12	0.53	17

n.c - not calculated as all results were non-detects

n.d. ( ) - not detected (limit of detection)

* - including LOD values

The concentrations of heptachlor, trans-chlordane and cis-chlordane were below the limit of detection in all samples. With the exception of aldrin, all other OCPs were detected in all samples.

In addition to the samples collected in 2002/03, twenty-four individual milk samples that had been previously collected from Victorian mothers in 1993 were analysed. These samples are referred to as Melbourne Historical A, B and C and were analysed by the National Measurement Institute as three pools each containing eight samples. The results of this analysis are given in Tables 4.4 and 4.5 and in Figures 4.5-4.8. Note that the DDT compounds

and its metabolites have been depicted on different graphs because of the large differences in concentrations.

Comparison of the Melbourne historical samples (Median Melbourne 1993) and Melbourne pools in 2002/03 (Median Melbourne 2002/03) are depicted in Figures 4.5 to 4.8.

Table 4.4 Concentrations of OCP compounds detected in pooled breast milk samples collected from Victorian women in 1993.

Values represent the mean concentrations of OCPs detected in each pooled sample and are expressed on a lipid basis (ng g^{-1} lipid). LOD included.

Organochlorine	Melbourne Hist A	Melbourne Hist B	Melbourne Hist C
HCB	18	53	18
α-HCH	0.13	0.13	0.28
β-HCH	29	17	27
γ-HCH	0.2	0.2	0.21
Aldrin	0.05	n.d. (0.02)	0.5
Heptachlor	n.d. (0.5)	n.d. (0.5)	n.d. (0.6)
Heptachlor epoxide	4.7	6.0	7.0
Dieldrin	12	22	14
Oxychlordane	5.4	5.1	4.9
trans-chlordane	n.d. (4)	n.d. (3)	n.d. (2)
cis-chlordane	n.d. (1)	n.d. (0.5)	n.d. (0.5)
trans-nonachlor	5.4	5.3	5.7
p,p'-DDE	280	280	360
p,p'-DDD	0.2	0.17	0.18
o,p'-DDT	0.7	0.55	1.3
p,p'-DDT	8.5	12	12
Mirex	0.3	0.18	0.16

n.d. ( ) - not detected (limit of detection)

Figure 4.5 Comparison of the median concentrations of beta-HCH and HCB.

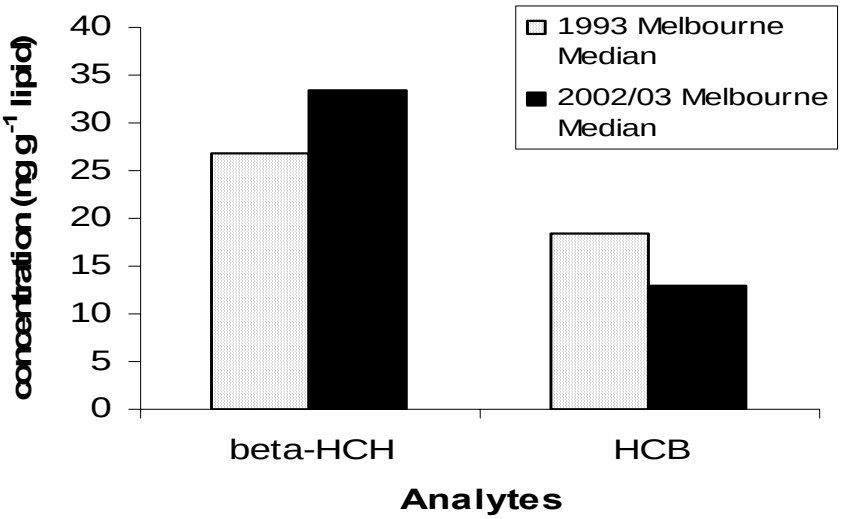


Figure 4.6 Comparison of the median concentrations of OCPs.

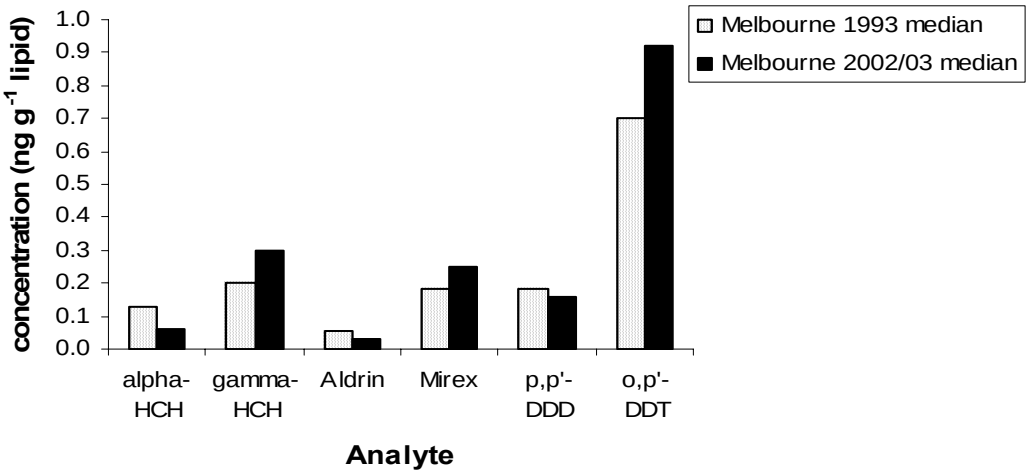


Figure 4.7 Comparison of the median concentrations of OCPs.

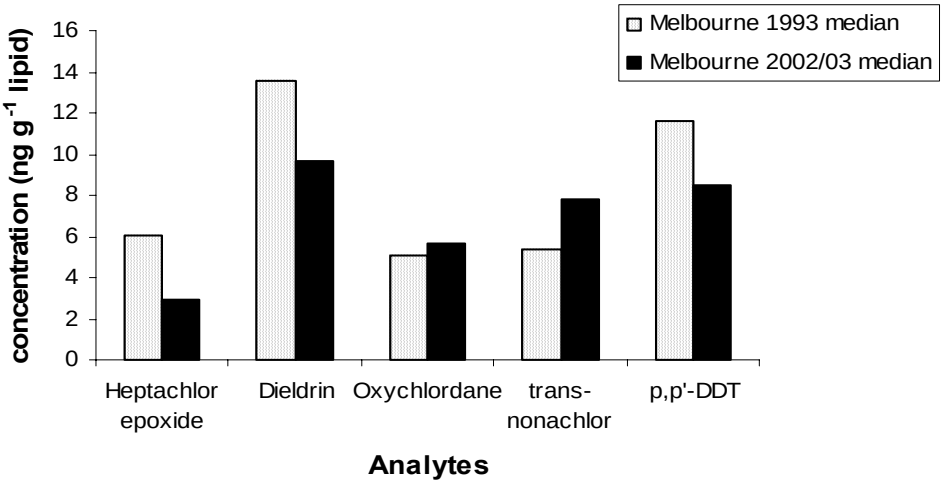
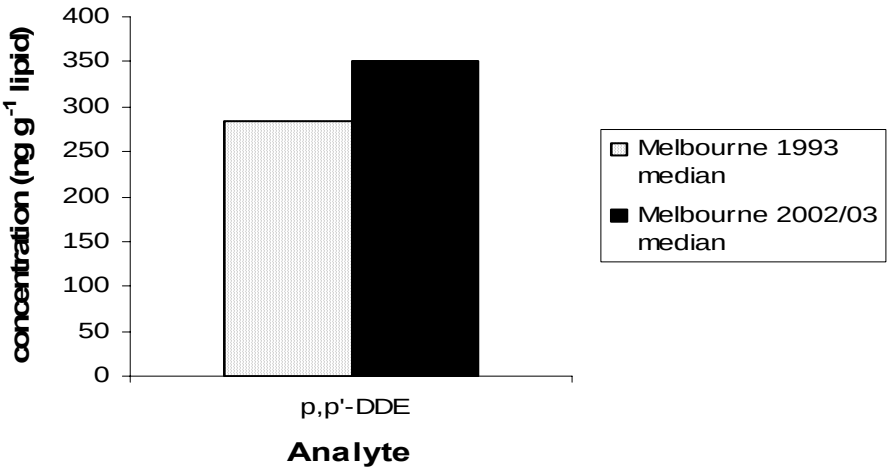


Figure 4.8 Comparison of the median concentrations of p,p'-DDE.



The levels of OCPs detected in the 1993 samples are compared with the samples obtained from Melbourne women in 2002/03. It should be reiterated that demographics and collection criteria are unknown for the 1993 samples and comparisons are made with caution. While there were minimal differences in the concentrations observed in some samples, no systematic trends were found. These are as follows:

- small decreases in concentration for samples collected from 1993 to 2002/03 were observed for HCB, α-HCH, aldrin, p,p'-DDD, heptachlor epoxide and dieldrin
- small increases in concentration for samples collected from 1993 to 2002/03 were observed for β-HCH, γ-HCH, mirex, o,p'-DDT, oxychlordane, transnonachlor and p,p'-DDE.

Statistical evaluation of these differences is complicated by the use of pooled samples and, hence, was not undertaken.

Table 4.5 provides comparative data for all OCPs from Melbourne 1993, Melbourne 2002/03 and all region samples 2002/03 in addition to the mean, median, minimum and maximum values of the Melbourne 1993 samples. With the exception of heptachlor, trans-nonachlor and cis-chlordane, all OCPs were detected. Aldrin was detected in 2 out of 3 samples.

Table 4.5 Mean, Median, Minimum and Maximum concentrations of OCPs detected in pooled breast milk samples collected from Australian women in 1993 & 2002/03.

Organochlorine	Mean 1993	Median 1993	Min 1993	Max 1993	No. of positives detected 1993	All samples 2002/03	Mean Melbourne 2002/03	Median Melbourne 2002/03
HCB	30	19	18	53	3	18	12	13
α-HCH	0.18	0.13	0.13	0.28	3	0.06	0	0
β-HCH	24	27	17	29	3	80	190	33
γ-HCH	0.2	0.2	0.2	0.21	3	0.23	0	0
Aldrin	0.19*	0.05	n.d. (0.02)	0.5	2	0.23*	0	0
Heptachlor	n.c.	n.c.	n.c.	n.c.	0	n.c.	n.c.	n.c.
Heptachlor epoxide	5.9	6	4.7	7	3	7.8	3	3
Dieldrin	15.9	14	12	22	3	16	11	10
Oxychlordane	5.1	5.1	4.9	5.4	3	9	6	6
trans-chlordane	n.c.	n.c.	n.c.	n.c.	0	n.c.	n.c.	n.c.
cis-chlordane	n.c.	n.c.	n.c.	n.c.	0	n.c.	n.c.	n.c.
trans-nonachlor	5.5	5.4	5.3	5.7	3	11	8	8
p.p'-DDE	310	280	280	360	3	310	470	350
p,p'-DDD	0.18	0.18	0.17	0.2	3	0.15	0	0
o,p'-DDT	0.86	0.7	0.55	1.3	3	0.67	1	1
p,p'-DDT	11	12	8.5	12.3	3	8.8	13	8
Mirex	0.21	0.18	0.16	0.3	3	0.24	0	0

The number of compounds detected in all samples is also shown. All values are rounded to two significant figures.

n.c. - not calculated as all were non-detects
* - includes limit of detection values
n.d. ( ) - not detected (limit of detection)

A comparison of the percentage difference in samples collected over the ten year period is given in Table 4.6. It is noteworthy that the low ratio of DDT/DDE in all samples analysed, indicates that the exposure to DDT is not recent and is consistent with DDT having been discontinued as an insecticide (Chikuni et al., 1991, Polder et al., 2003). High ratios of these compounds have been observed particularly in developing countries and are indicative of continued use of DDT as an insecticide in agricultural and malarial control programs.

Overall, the concentrations of OCPs in the breast milk of these Australian women are low when compared on an international basis. β-HCH and p,p'-DDE were the dominant OCPs in all samples. The outliers observed in the Sydney A and Melbourne A samples are difficult to explain without further investigation and perhaps warrant a duplicate analysis to confirm the results. As previously stated, detailed statistical analysis of data obtained from the questionnaires is complicated by pooling of the samples from each region. Despite this, non-statistical evaluation of the data does not indicate the possibility of exposure of any individuals through geographical, dietary or occupational sources.

Table 4.6 Percentage difference in the concentration of OCPs detected in samples collected from Melbourne women in 1993, 2002/03 and all samples collected in 2002/03.

	Mean Melbourne 1993	Mean all samples 2002/03	Mean Melbourne 2002/03	Difference between mean Melbourne 1993 and mean all regions 2002/03 (%)	Difference between Mean Melbourne 1993 and Mean Melbourne 2002/03 (%)
β-HCH	24	20	28	-16	15
β-HCH incl. outliers	24	80	190	226	662
HCB	30	14	12	-53	-59
HCB incl. outliers	30	17	12	-43	-60
heptachlor epoxide	5.9	7.9	3	33	-50
dieldrin	16	16	11	1	-32
oxychlordane	5.1	9	6	78	10
trans-nonachlor	5.5	11	8	102	42
α-HCH	0.2	0.06	0.09	-66	-53
γ-HCH	0.2	0.2	0.3	15	32
aldrin	0.2	0.2	0.2	21	-8
mirex	0.2	0.2	0.3	14	21
p,p'-DDE	310	310	470	1	53
p,p'-DDD	0.2	0.2	0.2	-19	17
o,p'-DDT	0.9	0.7	0.9	-22	4
p,p'-DDT	11	8.8	13	-18	22

*All values are rounded to two significant figures

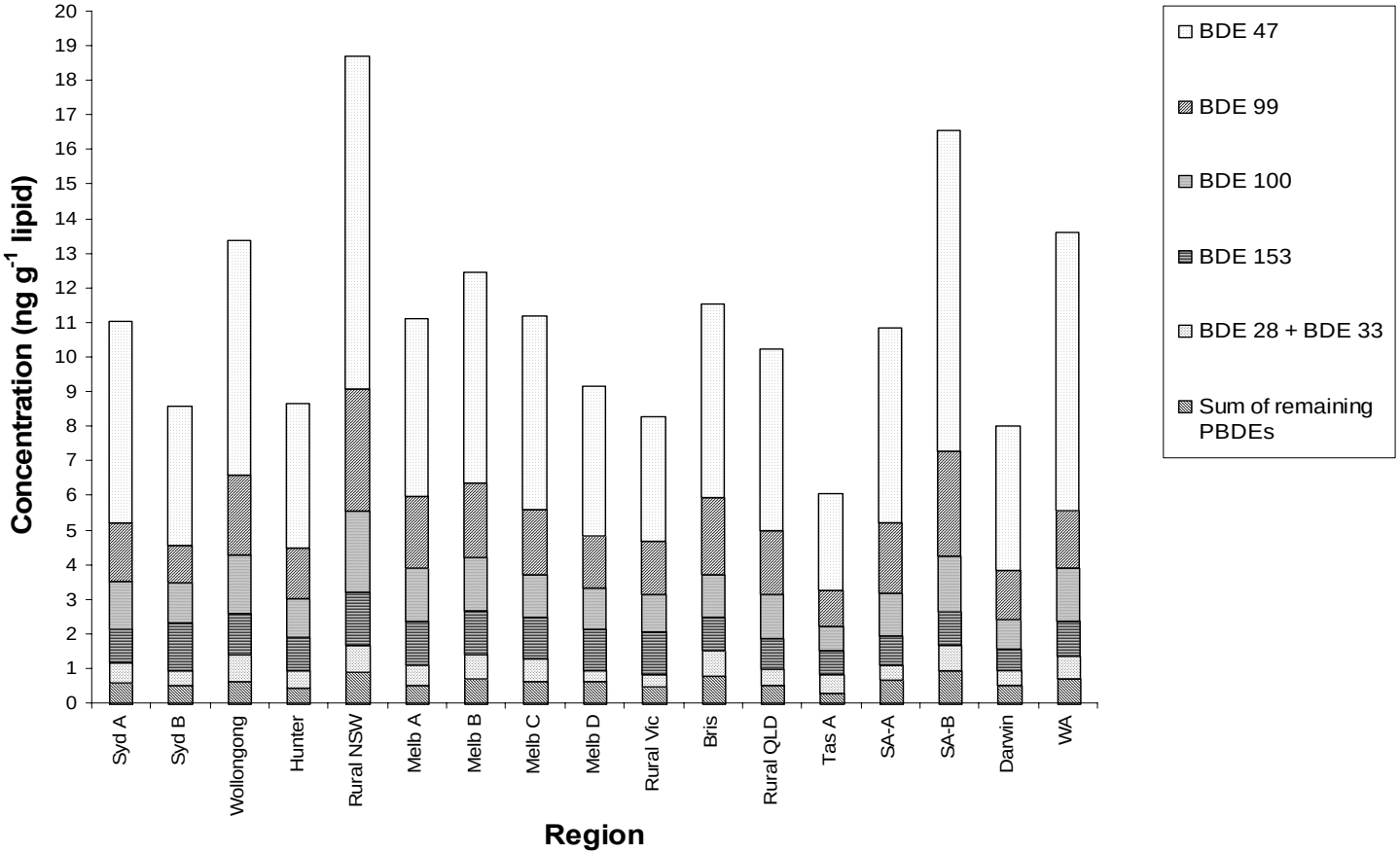
4.4 LEVELS OF PBDES IN THE BREAST MILK OF AUSTRALIAN WOMEN

4.4.1 Overall evaluation of PBDEs in samples collected in 2002/03.

Information regarding PBDE levels in the Australian environment is limited. This study represents the first investigation of the levels of PBDE congeners found in pooled breast milk obtained from Australian women. Data sheets showing the concentration of PBDEs detected in each pooled sample are presented in Appendix G, Table 2. Summary data is presented in Table 4.7. Figure 4.9 shows the total concentration of PBDE congeners detected in each pooled sample collected in 2002/03. Values represent the concentrations of PBDEs detected in each sample and are expressed on a lipid basis (ng compound.g^{-1} lipid). Lipid content was measured in all pooled samples and gave an average concentration of 3.7 ± 0.5 % lipid. PBDE congeners were detected in all samples analysed.

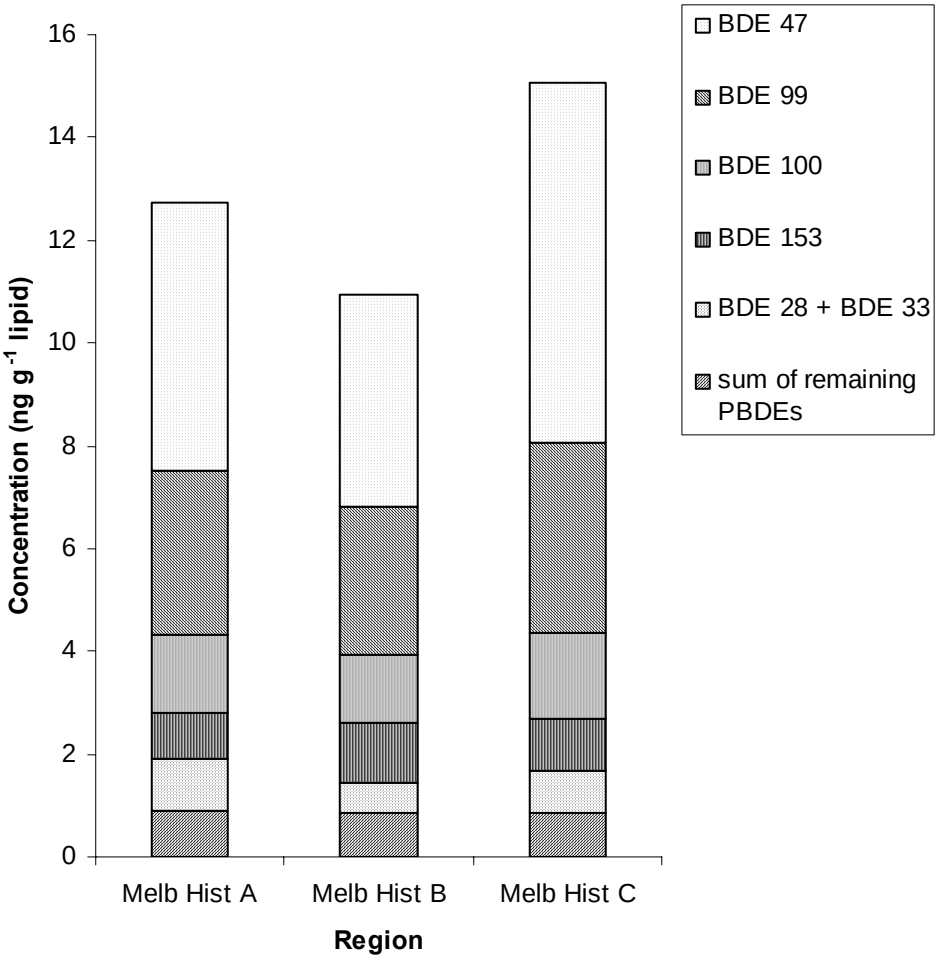
In addition to the samples collected in 2002/03, twenty-four individual milk samples were analysed that had been previously collected from Victorian mothers in 1993. These samples are referred to as Melbourne Historical A, B and C and were analysed by the National Measurement Institute as three pools each containing eight samples. The results of this analysis are given in Table 4.8 and in Figures 4.10-4.12.

Figure 4.9 Concentration of PBDEs in milk samples for different sampling regions.



Values represent the sum concentrations of PBDEs detected in each pooled sample and are expressed on a lipid basis (ng g^{-1} lipid), where a congener was not detected LOD was included.

Figure 4.10 Concentration of PBDEs in milk samples collected in Melbourne in 1993.



Values represent the sum concentrations of PBDEs detected in each pooled sample and are expressed on a lipid basis (ng g^{-1} lipid), where a congener was not detected LOD was included.

The sum PBDE value includes the sum of the 16 congeners listed in Table 4.8 and exclude LOD values for non-detected congeners. BDEs 28 and 33 and BDEs 138 and 166 are reported together. The levels of sum PBDEs varied by a factor of 3.1 from a minimum of 6.0 ng g^{-1} lipid detected in the Tasmanian sample to a maximum of 18.7 ng g^{-1} lipid detected in the rural NSW sample. The lowest sum PBDE concentrations (ng g^{-1} lipid) were found in samples from Tasmania (6.0), Darwin (7.9), rural Victoria (8.2), Hunter (8.6) and Sydney B (8.5). The highest sum PBDE concentrations (ng g^{-1} lipid) were found in samples collected from rural NSW (18.7), South Australia B (16.3), Western Australia (13.4) and Wollongong (13.3). Table 4.7 and Table 4.8 show the mean, minimum, maximum and median and the number of positive detects for PBDE compounds in the pooled breast milk collected during 2002/03 and 1993, respectively.

Table 4.7 Minimum, maximum, mean and median for PBDE compounds detected in pooled samples of breast milk samples collected in 2002/03.

Values given are expressed on a lipid basis (ng g^{-1} lipid).

PBDE	Mean	Min	Max	Median	No. of positive detects
BDE 17	0.01*	n.d. (0.01)	0.02	0.01*	11
BDE 28 + BDE 33	0.56	0.3	0.78	0.54	17
BDE 47	5.6	2.8	9.6	5.6	17
BDE 49	0.12*	n.d. (0.07)	0.2	0.12*	11
BDE 66	0.07	0.03	0.17	0.07	17
BDE 71	n.c.	n.c.	n.c.	n.c.	0
BDE 77	n.c.	n.d. (0.001)	0.002	n.c.	3
BDE 85	0.14	0.05	0.26	0.12	17
BDE 99	1.9	1	3.5	1.8	17
BDE 100	1.3	0.69	2.3	1.2	17
BDE 119	n.c.	n.c.	n.c.	n.c.	1
BDE 126	n.c.	n.c.	n.c.	n.c.	0
BDE 138 + BDE 166	0.02*	n.d. (0.01)	0.04	0.02*	15
BDE 153	1.1	0.59	1.6	1.0	17
BDE 154	0.14	0.09	0.23	0.14	17
BDE 183	0.11*	n.d. (0.05)	0.23	0.1*	16
Sum PBDE	**11**				

* incl. LOD values

n.c. - not calculated as all results (or all but one) were non-detects

n.d. ( ) - not detected (limit of detection)

Table 4.8 Minimum, maximum, mean and median for PBDE compounds detected in pooled samples of breast milk samples collected in 1993.

Values given are expressed on a lipid basis (ng g^{-1} lipid).

PBDE	Mean	Median	Min	Max	No. of positives detected
BDE 17	0.008	0.008	0.006	0.01	3
BDE 28 + BDE 33	0.8	0.8	0.6	1.0	3
BDE 47	5.4	5.2	4.1	7.0	3
BDE 49	n.c.	n.c.	n.d. (0.09)	n.d. (0.2)	0
BDE 66	0.04*	0.04*	n.d. (0.04)	0.06	2
BDE 71	n.c.	n.c.	n.d. (0.006)	n.d. (0.01)	0
BDE 77	n.c.	n.c.	n.d. (0.006)	n.d. (0.008)	0
BDE 85	0.1	0.1	0.1	0.1	3
BDE 99	3.3	3.2	2.9	3.7	3
BDE 100	1.5	1.5	1.3	1.7	3
BDE 119	n.c.	n.c.	n.d. (0.05)	n.d. (0.1)	0
BDE 126	n.c.	n.c.	n.d. (0.03)	n.d. (0.04)	0
BDE 138 + BDE 166	0.04	0.04	0.04	0.04	3
BDE 153	1.0	1	0.9	1.2	3
BDE 154	0.3	0.3	0.3	0.3	3
BDE 183	0.1*	0.2*	n.d. (0.08)	0.2	2
Sum PBDE excl. LOD	**13**				

n.c. - not calculated as all results were non-detects

* - including LOD values

n.d. ( ) - not detected (limit of detection)

A comparison of the percentage difference in samples collected over the ten year period is given in Table 4.9. Overall the levels have remained stable or decreased slightly. BDE 47, 66 and 153 have increased slightly over time.

Table 4.9 Percentage difference in the concentration of PBDE congeners detected in samples collected from Melbourne women in 1993, 2002/03 and all samples collected in 2002/03.

PBDE	Mean Melbourne 1993	Mean all samples 2002/03	Mean Melbourne 2002/03	Difference between mean Melbourne 1993 and mean all regions 2002/03 (%)	Difference between mean Melbourne 1993 and mean Melbourne 2002/03 (%)
BDE 17	0.01	0.01*	0.009*	100	90
BDE 28 + BDE 33	0.8	0.6	0.6	75	75
BDE 47	5.4	5.6	5.3	104	98
BDE 49	n.c.	0.1*	0.1*	n.c.	n.c
BDE 66	0.04*	0.07	0.06	175	150
BDE 71	n.c.	n.c.	n.c.	n.c.	n.c
BDE 77	n.c.	n.c.	n.c.	n.c.	n.c
BDE 85	0.1	0.1	0.1	100	100
BDE 99	3.3	1.9	1.9	58	58
BDE 100	1.5	1.3	1.4	87	93
BDE 119	n.c.	n.c.	n.c.	n.c.	n.c
BDE 126	n.c.	n.c.	n.c.	n.c.	n.c
BDE 138 + BDE 166	0.04	0.02*	0.02	50	50
BDE 153	1.0	1.1	1.2	110	120
BDE 154	0.3	0.1	0.1	33	33
BDE 183	0.1*	0.1*	0.1	n.c.	n.c
Sum PBDE excl. LOD	13	11	11	85	85

* incl. LOD values

n.c. – not calculated as all results were non-detects.

Figures 4.11 and 4.12 depict the mean concentrations of PBDE congeners in Melbourne 2002/03 and 1993.

Figure 4.11 Comparison of the mean concentration of PBDE congeners detected in pooled breast milk obtained from samples collected from Melbourne women in 1993 and 2002/03.

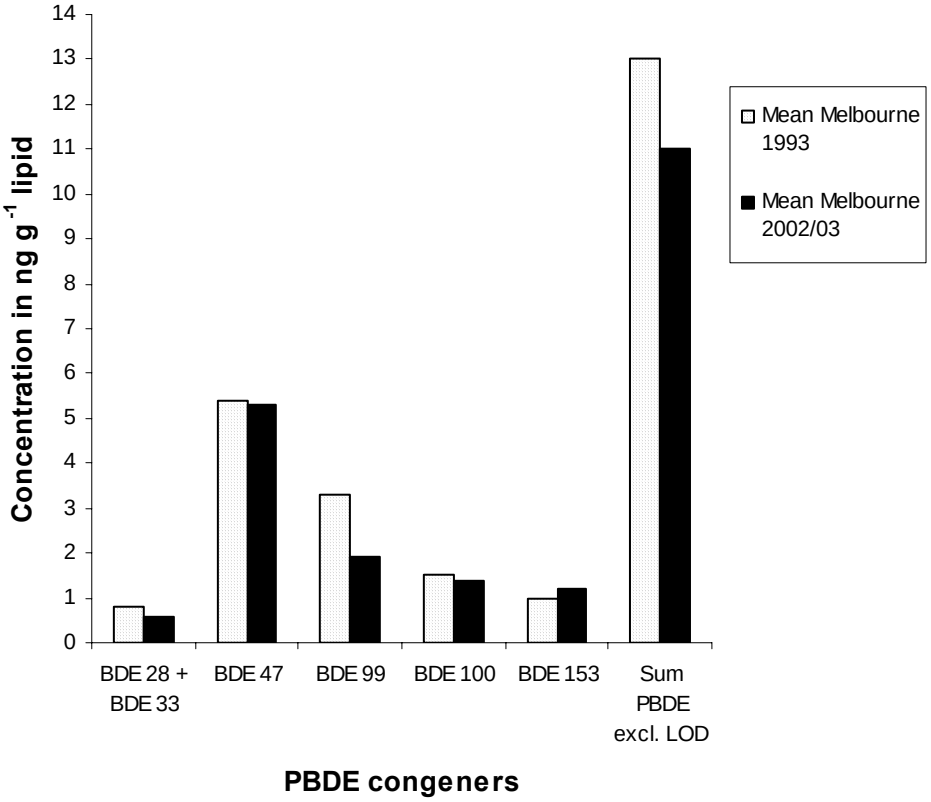
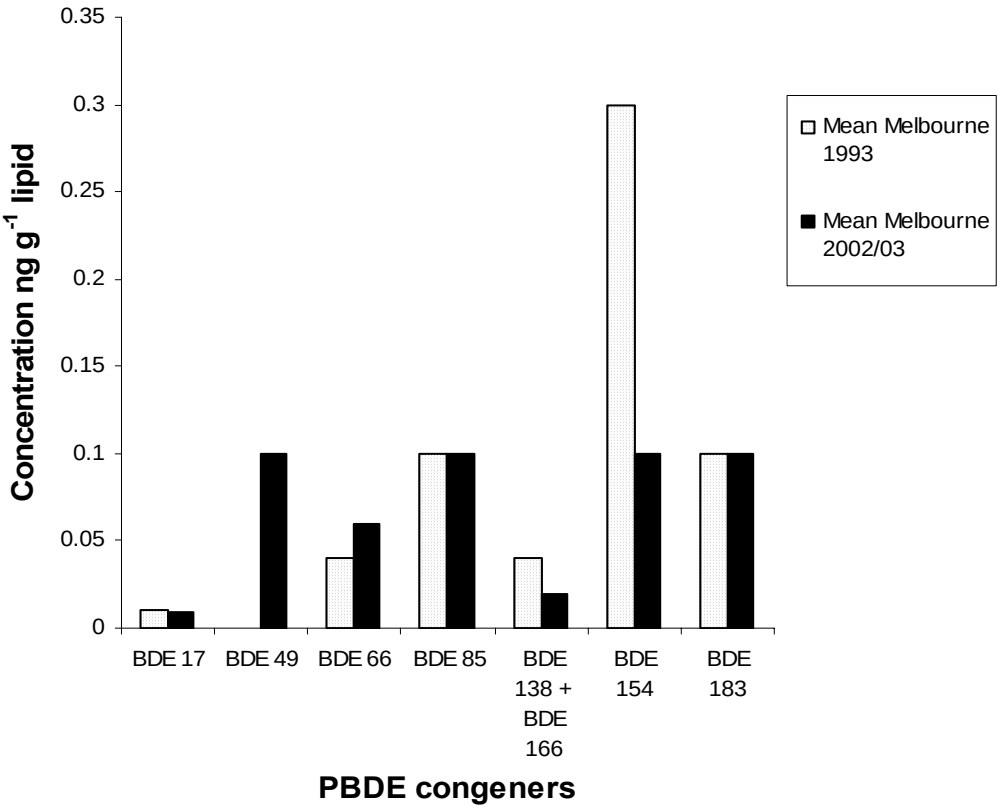
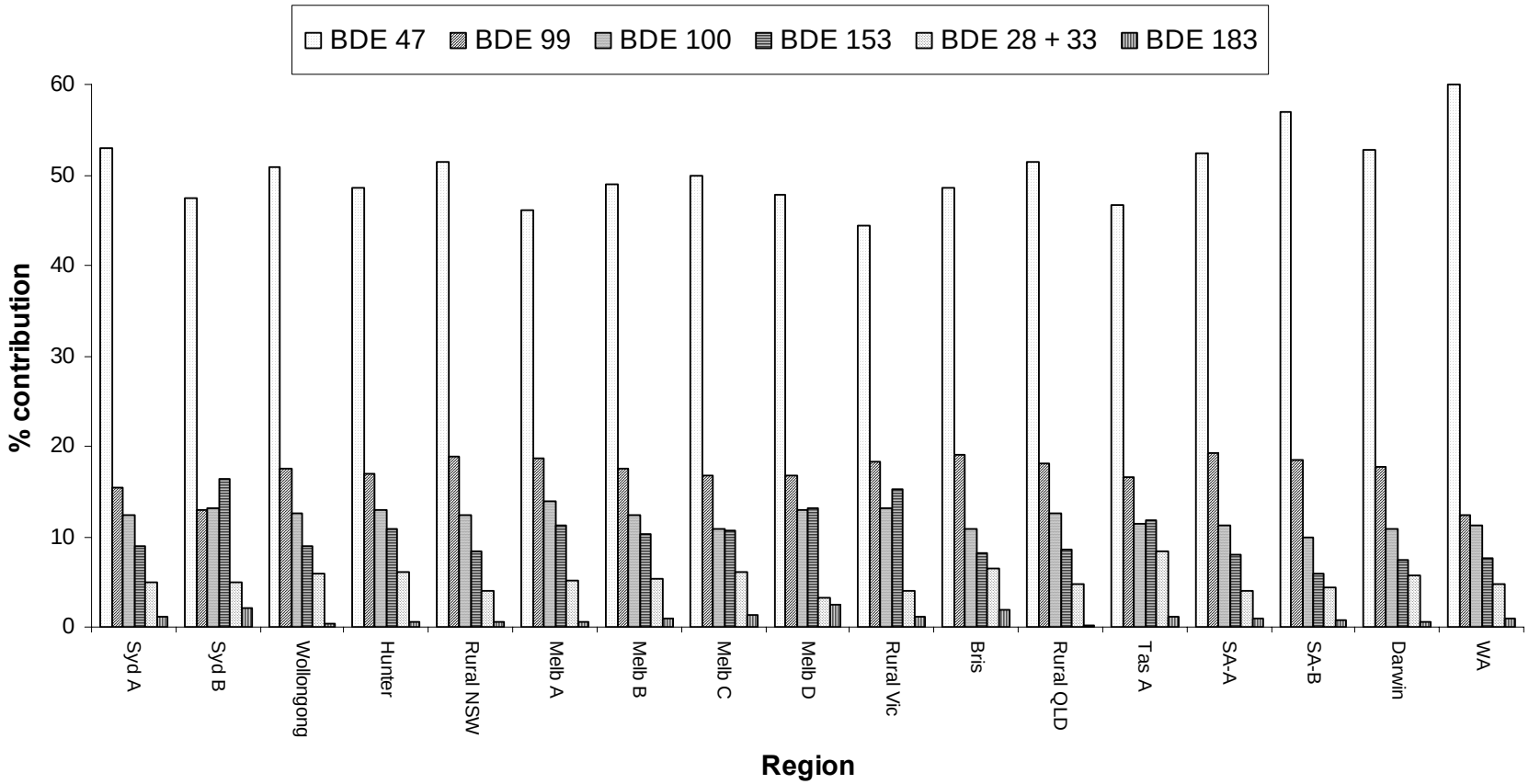


Figure 4.12 Comparison of the mean concentration of PBDE congeners detected in pooled breast milk obtained from samples collected from Melbourne women in 1993 and 2002/03.



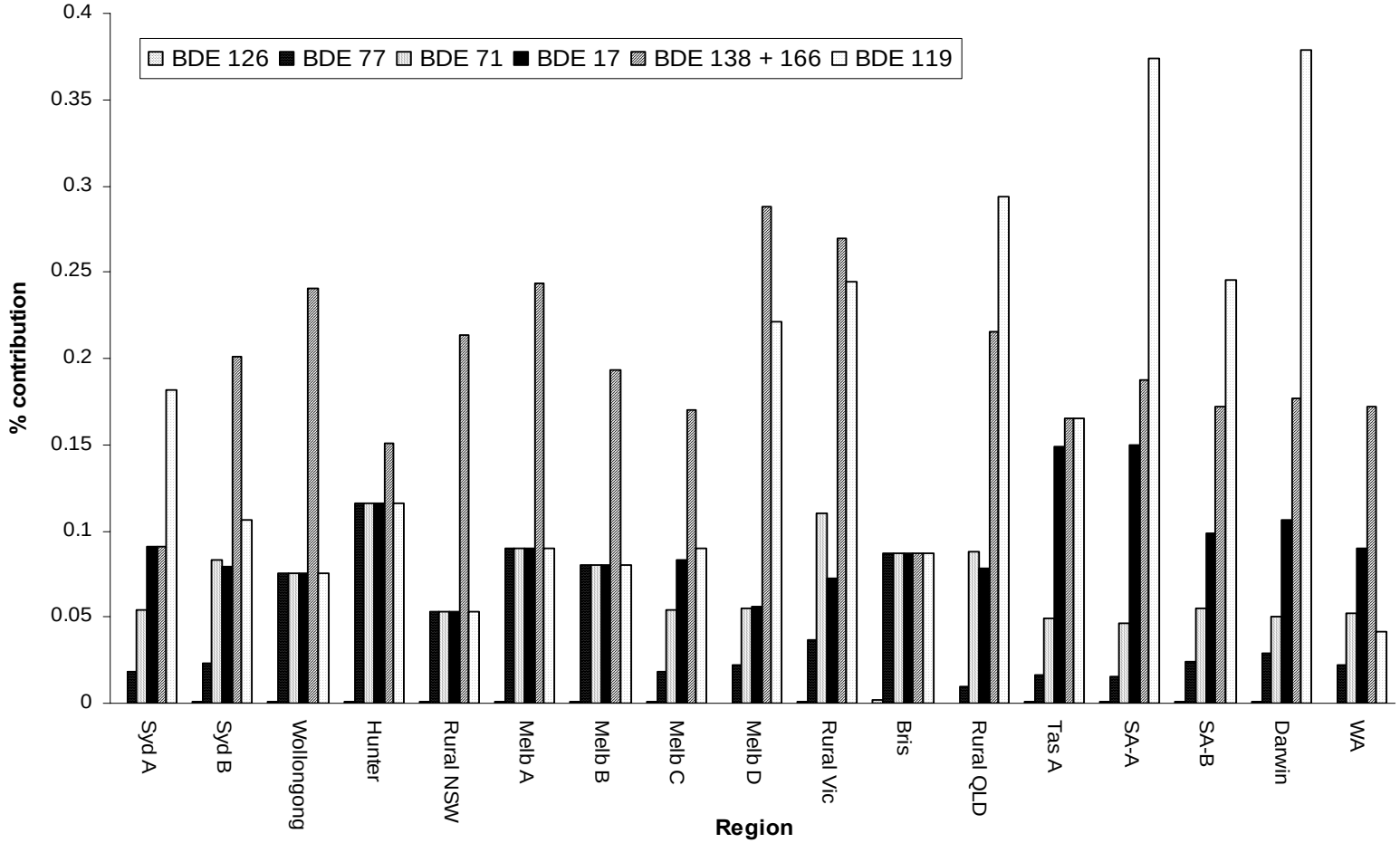
Specific PBDE congeners, BDE 47, BDE 99, BDE 100, BDE 153 and BDE 154, dominate samples obtained from both environmental and human sources (Ryan et al., 2000, Schecter et al., 2003, Lind et al., 2003). Similarly in this study, BDE 47, 99, 100, 153 and 154 were detected in all samples (Figure 4.10) and were the dominant congeners present. In contrast, BDEs 71 and 126 were not detected in any samples while 119 and 77 were detected in 1 and 3 samples, respectively (Figure 4.13-15).

Figure 4.13 Percent contribution of BDE 47, 99, 100, 153, 28 + 33 and 183 to the total sum PBDEs across each sampling region.



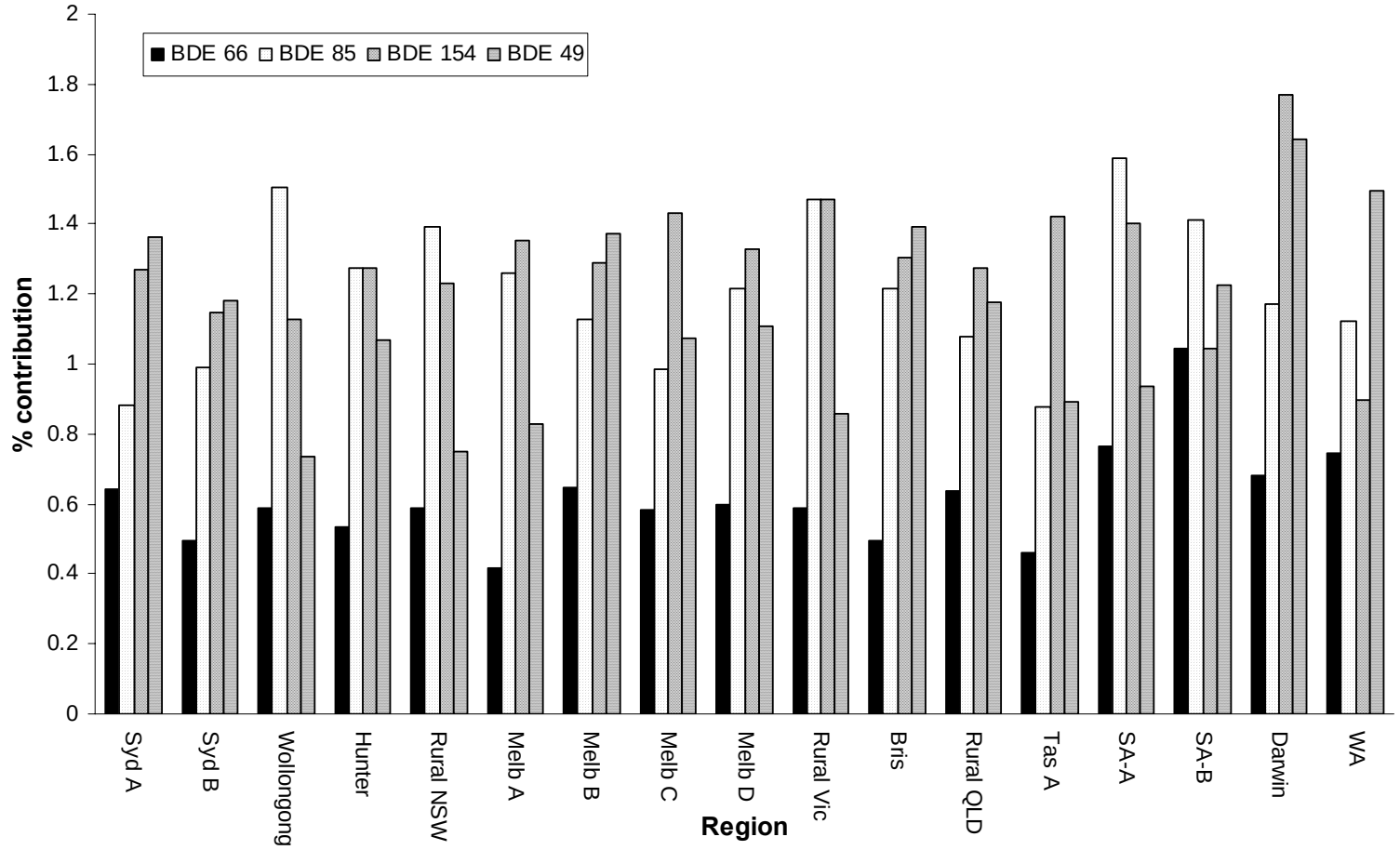
Where analyte not detected, LOD used.

Figure 4.14 Percent contribution of BDE 17, 71, 126, 77, 138 + 166 and 119 to the total sum PBDEs across each sampling region.



Where analyte not detected, LOD used

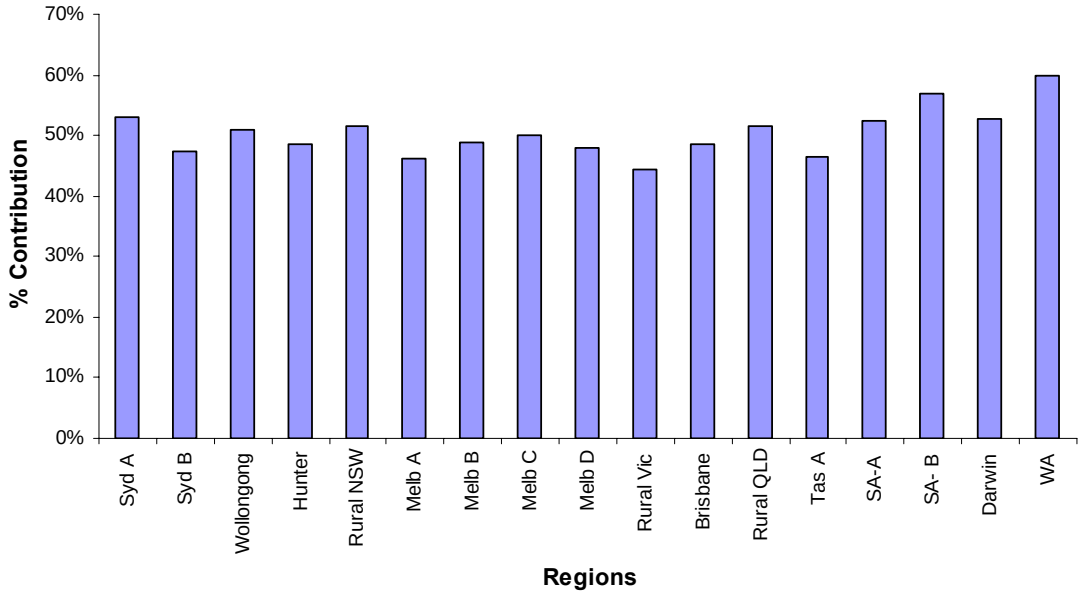
Figure 4.15 Percent contribution of BDE 66, 85, 154 and 49 to the total sum PBDEs across each sampling region.



Where analyte not detected, LOD used

The concentration of BDE 47 varied by a factor of 3.4 from a minimum concentration of 2.8 ng g^{-1} detected in the Tasmanian pool, to a maximum concentration of 9.6 ng g^{-1} detected in the rural NSW sample. For all analysed samples, the greatest contribution to the sum PBDE concentration was from BDE 47 with an average contribution of 50 %.

Figure 4.16 Percentage contribution of BDE 47 to sum PBDE concentration found in pooled breast milk samples obtained from Australian women.



The relative contribution of BDE 47 to the sum PBDE concentration varied from 44 % in the rural Victorian sample to a maximum contribution of 60 % in the Western Australia sample (Figure 4.16). For the 1993 Melbourne samples, BDE 47 contributed an average of 42 % to the sum PBDE concentration. For congeners 99, 100, 153 and 154, the average contribution to the sum concentration was 17, 12, 10 and 1.3 %, respectively, for the 2002/03 samples and 25, 12, 8 and 2 %, respectively, for the 1993 samples. These results are consistent with those reported in other studies (Ryan et al., 2000, Schecter et al., 2003, Lind et al., 2003).

4.4.2 Factors affecting the levels of PBDE compounds in human breast milk.

Factors that affect the levels of PBDEs in human samples such as breast milk and serum are not well understood and are far more variable than for dioxin-like chemicals. PBDEs levels not only vary substantially between populations that live on different continents but also between individuals in a given country/region (Ryan et al., 2002; Schecter et al., 2003). There appears to be no clear relationship between the levels of PBDEs in breast milk and neither maternal age nor length and number of lactation periods (Lind et al., 2003; Darnerud et al., 1998; Schecter et al., 2003). Explanations as to why PBDEs levels in humans are more variable include:

- unclear exposure pathways with more extreme 'point sources' or yet to be identified items that add to contamination
- shorter half-lives (which emphasise more recent exposure)
- shorter historic exposure (we may still be in a period where the human body burden increases for PBDEs whereas we have been decreasing body burdens for dioxins and PCBs over the last two decades).

For this study the determination of a relationship between age and PBDE levels in Australian milk was not possible as pooled samples were used.

Routes of exposure for PBDE compounds have been variously reported to be via dietary exposure, particularly of fish; inhalation of dust containing chemicals that have been mobilised by human intervention; dermal exposure; and occupational exposure (Ohta et al., 2002). Only one case of accidental exposure has been reported in the literature (Sjödin et al., 2003). Thus, occupationally exposed groups may include individuals who work in repair, maintenance or dismantling roles in the electronics or computer industries (Darnerud et. al., 2001; Hovander et al., 2002; Sjödin et al, 1999; Jakobsson et. al., 2002).

Results obtained for the levels of PBDEs in pooled breast milk samples should be treated with caution. High variability both in congener profiles and/or concentration between individual samples has been previously reported and it is recommended that individual samples are analysed (Ryan et al., 2002; Schecter et al., 2003). This level of variability has not been observed in other persistent organic pollutants (POPs) such as PCBs, dioxins and furans (Sjödin et al., 2003). Hence, higher results for a particular region/pool may indicate that an individual within the group has a higher level of PBDE(s) rather than the group as a whole.

Table 4.10 compares the levels of PBDE congeners reported in the present study with those reported from North America, Canada and the European countries, Germany, Finland and Sweden (Hites, 2004). This graph clearly indicates that the concentrations detected in samples obtained from North American and Canadian women are higher than those detected in either Australian or European women. The levels detected in the European countries are lower than those in Australian breast milk.

Table 4.10 PBDE concentrations in ambient human samples in ng g^{-1} lipid

Location	Type	Date	No of replicate samples	47	99	100	153	154	Sum PBDE
Japan	milk	1999	6	0.34	0.1	0.13	0.32	0.03	0.93
Sweden	fetal blood	2000	15	0.98	0.07	0.07	0.17		1.29
Sweden	maternal blood	2000	15	0.83	0.19	0.17	0.56	0.04	1.79
Sweden	milk	2000	15	1.15	0.21	0.14	0.32	0.02	1.84
US	milk	2000	4	126	27	23.5	14.8	1.66	193
Japan	adipose tissue	2000	10	0.46	0.12	0.25	0.38	0.06	1.27
Belgium	adipose tissue	2000	20	1.45	0.28	0.48	2.49		4.7
Czech Rep.	adipose tissue	2000	14	0.4	0.12	0.13	0.41	0.03	1.09
Czech Rep.	adipose tissue	2000	10	1.18	0.34	0.59	0.52	0.06	2.69
Japan	bile	2001	10	0.7	0.14	0.2	1.42	0.07	2.54
Japan	blood	2001	10	1.63	0.26	0.29	1.25	0.09	3.52
Sweden	blood	2001	143	2.77	1.39		1.87		6.03
US	fetal blood	2001	12	25	7.1	4.1	4.4	0.7	41.3
US	maternal blood	2001	12	28	5.7	4.2	2.9	0.3	41.1
Canada	milk	2001	20	13.3	3	2.3	3	0.6	22.2
Japan	liver tissue	2001	10	1.38	0.18	0.22	1.55	0.14	3.48
US	milk	2002	47	18.4	5.7	2.9	2	0.22	29.2
Australia	blood	2003	10	4.7	2.3	0.96	2	0.22	10.9

4.5 A COMPARISON OF OCP AND PBDE LEVELS IN AUSTRALIA WITH OTHER COUNTRIES

4.5.1 Organochlorine compounds

Most of the studies of organochlorine (OCP) compounds in human breast milk did not analyse for dietary and/or lifestyle factors. For those that did, results are included in this section. Studies may report results on a whole milk basis as opposed to a milk lipid basis, therefore, it is difficult to make comparisons between some studies. Results for Australia from 1970 to 2003 are reported here in an effort to demonstrate time trends in concentrations of organochlorine compounds in human breast milk. Literature from various countries is used in order to make comparisons between current Australian levels and current international levels of OCPs in human breast milk. Figures 4.17 and 4.18 show a comparison of the Australian levels with international levels. The results for each year are provided in Appendix H Table H.3.

Australia

Stacey and Thomas (1975) analysed 23 individual milk samples from Perth, Australia, in 1970/71. Samples were analysed for DDT, DDE, dieldrin and HCB. The authors stated that there was no apparent correlation between pesticide residue level in milk samples and body weight of donors or the lipid content of samples. The mean level of DDT, DDE and sum DDT were 10, 61 and 77 ng g^{-1} whole milk, respectively. The mean level of dieldrin and HCB were 5 and 25 ng g^{-1} whole milk, respectively.

Miller and Fox (1973) reported the levels of OCP compounds in individual samples collected in Queensland, Australia. Samples were obtained from an urban area (Brisbane) and a rural area (Mareeba) with 20 samples collected from each area. Samples were collected within 7 days post partum in 1971/72. For Brisbane, the mean levels of HCB, total DDT and dieldrin were 2,200, 8,600 and 930 ng g^{-1} milk lipid, respectively. For Mareeba, the mean levels of HCB, total DDT and dieldrin were 1,200, 17,000 and 810 ng g^{-1} milk lipid, respectively. The authors stated that there was significant difference between the mean total DDT for Brisbane and Mareeba, which may have been due to extensive DDT usage in Mareeba.

Siyali (1973) analysed the milk of 45 women from Sydney, Australia, in 1972. It is not stated whether or not there were criteria for participants. Individual samples were analysed and the mean total DDT (including all isomers and metabolites of DDT) was 64 ng g^{-1}, dieldrin was 5.0 ng g^{-1} and HCB was 16 ng g^{-1} whole milk. Heptachlor epoxide had insufficient positive samples to calculate a significant mean.

Stacey, Perriman and Whitney (1985) analysed 267 breast milk samples, supplied by 140 donors from urban and rural areas of Western Australia, in 1979. It was not stated if criteria were used to obtain participants. There were 130 samples from 45 donors in urban areas and 137 samples from 95 donors in rural areas. Results were provided for samples collected pre- and post-feed as well as samples from random feed times. The results here are for the random samples (urban n=45, rural n=53). For urban areas, the mean levels of HCB, γ-BHC (HCH) and dieldrin were 7, 1 and 7 ng g^{-1} whole milk, respectively. The mean levels of DDE, DDT and total DDT were 29, 10 and 42 ng g^{-1} whole milk, respectively. For rural areas, the mean levels of HCB, γ-BHC (HCH) and dieldrin were 6, 0 and 8 ng g^{-1} whole milk, respectively. The mean levels of DDE, DDT and total DDT were 24, 9 and 36 ng g^{-1} whole milk, respectively.

Stevens et al. (1993) measured the concentration of OCP pesticides in Western Australian nursing mothers in 1990/91. Levels of OCPs were measured in 128 samples of breast milk. Criteria for participants were singleton birth, primiparous, intention to breast feed, a resident of Perth for a minimum of 2 years and Caucasian race. Samples were collected 2-6 weeks post partum. The median levels of DDT, dieldrin, HCB were 800, 50 and 100 ng g^{-1} lipid, respectively. The median levels of heptachlor and chlordane were 20 and 7 ng g^{-1} lipid, respectively.

Quinsey, Donohue and Ahokas (1995) reported the levels of OCPs in breast milk of women in Victoria, Australia in 1993. Sampling was restricted to two locations - an inner urban industrial region and a rural market garden area. There were a total of 60 samples from 23 mothers for OCP analysis. Only primiparous mothers with singleton births were included. All mothers were healthy and breast feeding exclusively. The initial sample was taken on establishment of breast feeding and a second and third sample were taken 1 and 2 months after the initial sample. The mean concentrations of p,p'-DDE and p,p'-DDT were 960 and 230 ng g^{-1} lipid, respectively. The mean concentrations of dieldrin, heptachlor epoxide and HCB were 160, 61 and 411 ng g^{-1} lipid, respectively. The mean concentration of α-, β- and γ-HCH were 71, 350 and 110 ng g^{-1} lipid, respectively. The authors state that OCP concentration was not found to be significantly associated with the residential region of the mother, nor was there any significant association with maternal cigarette usage. No correlation was found between OCP concentration in breast milk and the mother's age or body mass index.

In summary, in 1971/72 an urban area of Queensland had a mean total DDT concentration in breast milk of 8,600 ng g^{-1} lipid, while a rural area had 17,000 ng g^{-1} lipid (Miller et al. 1973). The total DDT concentration of breast milk in Perth, WA, in 1970/71 was 77 ng g^{-1} whole milk

(Stacey et al., 1975). In Sydney, NSW, in 1972, the total DDT level was 64 ng g^{-1} whole milk (Siyali, 1973). In urban and rural WA in 1979 total DDT concentrations in breast milk were 42 and 36 ng g^{-1} whole milk, respectively (Stacey et al., 1985). In 1990/91 (Stevens et al., 1993), sampled breast milk in WA and the mean concentration of DDT (it is not specified if this is p,p'-DDT or other) was 800 ng g^{-1} lipid while in Victoria (Quinsey 1995) the mean concentrations of p,p'-DDT and p,p'-DDE found in breast milk samples collected in 1993, were 230 and 960 ng g^{-1} lipid, respectively. These results can be compared to the mean total DDT found in Melbourne, 1993 of 320 ng g^{-1} and Australia-wide, 2003 of 320 ng g^{-1} lipid. In the current study, the mean levels of p,p'-DDE and p,p'-DDT were 310 and 8.8 ng g^{-1} lipid, respectively.

Sim, Forbes and McNeil (1998) measured the concentration of dieldrin, heptachlor epoxide and oxychlordane in 797 primiparous women during 1991/92. The criteria for participants were: primiparous, singleton birth, totally breast feeding, and 6-12 weeks post partum. Individual samples were analysed and the median levels of dieldrin, heptachlor epoxide and oxychlordane were 39, 7 and 7 ng g^{-1} lipid, respectively. The authors state that increasing maternal age was associated with higher body burden of dieldrin and oxychlordane but no relationship between age and heptachlor epoxide body burden was observed. The mean concentration of dieldrin found in the current study, 16 ng g^{-1} lipid, was half that of the 1991/92 study, while the levels of heptachlor epoxide and oxychlordane were similar at 7.9 and 9.1 ng g^{-1} lipid, respectively.

New Zealand

Bates et al. (1994) analysed individual samples from 38 participants in 1987/88 from Auckland and Christchurch plus two rural areas (Northland and Canterbury). The criteria for participants were: primiparous, aged 20-30 years, normal, healthy pregnancy, mother breast feeding one child only, and a resident of the area for at least 5 years. Samples were collected in the second month post partum. There were no significant differences between urban and rural samples except for lipid content. No association was found between levels of p,p'-DDT and maternal age, although such an association was strong for p,p'-DDE. The results for p,p'-DDT for Auckland, Northland, Christchurch and Canterbury were: 105, 45, 48 and 110 ng g^{-1} (milk lipid), respectively. The results for p,p'-DDE for Auckland, Northland, Christchurch and Canterbury were 1,070, 1040, 2,800 and 3,200 ng g^{-1} lipid, respectively. Results for HCB levels analysed in Auckland, Northland, Christchurch and Canterbury were 20, 21, 30 and 63 ng g^{-1} lipid, respectively. Dieldrin concentrations found in samples from Auckland, Northland, Christchurch and Canterbury were 48, 36, 42 and 67 ng g^{-1} lipid, respectively. For all regions, mean concentrations of β-HCH, HCB and dieldrin were 11, 32 and 47 ng g^{-1} lipid, respectively. The mean concentrations of p,p'-DDE, o,p'-DDT and p,p'-DDT were 1,929, 19.3 and 78 ng g^{-1} lipid, respectively (Bates et al. 2002).

Bates, Thomson and Garrett (2002) analysed 53 individual milk samples collected in 1998/99. Participants were recruited based on the criteria listed in Bates (1994), see above. Samples were collected 5-8 weeks post partum. The mean concentrations of β-HCH, HCB and dieldrin were 16, 11 and 15 ng g^{-1} lipid, respectively, while the mean concentrations of p,p'-DDE, o,p'-DDT and p,p'-DDT were 630, 4.4 and 26 ng g^{-1} lipid, respectively. From the current study, the mean concentrations of β-HCH, HCB, and dieldrin were 80, 18 and 16 ng g^{-1} lipid, respectively, while the mean concentrations of p,p'-DDE, o,p'-DDT and p,p'-DDT were 310, 0.67 and 8.8 ng g^{-1} lipid, respectively.

United Kingdom

Dwarka et al. (1994) reported the levels of OCP compounds in the United Kingdom from 1989 to 1991. 193 samples of breast milk were obtained and samples were either pooled milk from a single donor or in some cases pooled milk from two to six donors. The mean levels of p,p'-DDE and p,p'-DDT were 400 and <20 ng g^{-1} lipid, respectively. The mean levels of β-HCH, γ-HCH, dieldrin and HCB were 80, <20, 30 and 20 ng g^{-1} lipid, respectively. Although collected over 10 years later, the current Australian study found similar mean levels of p,p'-DDE and p,p'-DDT at 310 and 8.8 ng g^{-1} lipid as well as β-HCH, α-HCH, dieldrin and HCB at 80, 0.23, 16 and 18 ng g^{-1} lipid, respectively.

North America

Newsome and Ryan (1999) reported the results of a comparison between levels of OCP compounds collected from 497 donors in Southern Canada in 1992 and 12 in Northern Canada (Keewatin) in 1996/97. The mean number of children breastfed was one for the southern group and two for the northern group. The mean p,p'-DDT, p,p'-DDE and dieldrin levels for southern Canada were 22, 220 and 9.8 ng g^{-1} lipid and in northern Canada, were 24, 440 and 11 ng g^{-1} lipid, respectively. The mean HCB, trans-nonachlor and oxychlordane levels in southern Canada were 15, 18 and 13 ng g^{-1} lipid and in northern Canada, were 43, 78 and 59 ng g^{-1} lipid, respectively. In the current study, the mean p,p'-DDT, p,p'-DDE and dieldrin levels were 8.8, 330 and 16 ng g^{-1} lipid, respectively. The mean levels of HCB, trans-nonachlor and oxychlordane were 18, 11 and 9.1 ng g^{-1} lipid, respectively.

The mean α-, β- and γ-HCH levels for southern Canada were 0.31, 22.6 and 1.03 ng g^{-1} lipid, respectively, and for northern Canada were 4.4, 18 and 0.76 ng g^{-1} lipid, respectively. The mean level of Mirex in southern Canada was 1.9 and in northern Canada 2.3 ng g^{-1} lipid. This is in comparison to the current study where the mean α-, β- and γ-HCH levels were 0.06, 80 and 0.23 ng g^{-1} lipid, respectively. The mean level of Mirex was 0.24 ng g^{-1} lipid.

South America

In a study conducted in 1987/88 in Brazil, Beretta and Dick (1994) measured the levels of OCP compounds in the milk of 30 volunteers who had lived in the urban area of Porto Alegre for at least five years. Individual samples were analysed and 16 out of 30 volunteers were primiparous. The mean levels of p,p'-DDE, p,p'-DDD, p,p'-DDT and o,p'-DDT were 2,500, 30, 120 and 20 ng g^{-1} of lipid, respectively. The mean sum-HCH was 960 ng g^{-1} lipid (α-HCH 40, β-HCH 900 and γ-HCH 20 ng g^{-1} lipid). The mean levels of heptachlor epoxide, dieldrin, HCB and Mirex were 20, 70, 20 and 30 ng g^{-1} lipid, respectively.

Paumgartten et al. (2000) reported the levels of OCP compounds in human milk from Rio de Janeiro, Brazil in 1992. A pooled sample of breast milk from 40 mothers, 83% primiparous, was analysed. Donors were 4-6 weeks post partum and had lived in the urban area of Rio de Janiero for at least 5 years. The concentration of α-HCH was 1 ng g^{-1} lipid, β-HCH 270 ng g^{-1} lipid and γ-HCH 5 ng g^{-1} lipid. The concentrations of HCB, dieldrin and heptachlor epoxide were 12, 23 and 8 ng g^{-1} lipid, respectively. The levels of p,p'-DDT, p,p'-DDE, p,p'-DDD and sum DDT were 180, 1,500, 6 and 1,700 ng g^{-1} lipid, respectively.

Romero et al., (2000) reported the concentrations of OCP pesticides in milk of Nicaraguan mothers. Samples were collected in 1994/95, from 101 mothers at one and two months post partum. Criteria for participants were: one child breast-fed per mother, milk provided 4-8 weeks post partum, pregnancy free of complications, mother and infant healthy, and mother lived for at least 3 years in the area of study. The mean levels of p,p'-DDE and p,p'-DDT were

2,800 and 130 ng g^{-1} milk lipid, respectively. The mean levels of dieldrin, heptachlor epoxide, heptachlor, β-HCH and lindane (γ-HCH) were 18, 6, 1, 6 and 1 ng g^{-1} milk lipid, respectively. There were no measurable concentrations of p,p'-DDD, Aldrin, or α-HCH. The authors state that place of residence (i.e. mothers who live in rural versus urban locations) was associated significantly with DDE and dieldrin levels.

The levels of p,p'-DDE were higher in South American samples (range 1,500-2,800 ng g^{-1} lipid) when compared to the Australian samples (311 ng g^{-1} lipid). The mean level of β-HCH in the current study was 80 ng g^{-1} lipid which is lower than the Brazilian studies but higher than that of the Nicaraguan study.

Continental Europe

In Sweden, Noren and Meironyte (2000) reported the levels of OCP compounds in human breast milk for the periods 1967, 1972-1985, 1988-1994 and 1996-1997. Pooled samples were analysed and 55-65 % of the mothers were primiparous. The mean concentration of p,p'-DDT was 1,300 ng g^{-1} lipid in 1967, 270 ng g^{-1} lipid in 1978 and 14 ng g^{-1} lipid in 1997. The mean concentration of HCB was 120 ng g^{-1} lipid in 1972, 19 ng g^{-1} lipid in 1980 and 12 ng g^{-1} lipid in 1997.

Schade and Heinzow (1998) analysed current extent of contamination and also the time trend from 1986 to 1997 from mothers in northern Germany. To assess the current levels of organochlorines, 246 milk samples, collected from summer 1995 to summer 1997, were analysed. The duration of lactation ranged from 2-156 weeks and 59 % of the mothers were primiparous. The mean levels of β-HCH, DDT and HCB were 40, 240 and 80 ng g^{-1} lipid, respectively.

From the time trend analysis, milk samples were selected from over 3,500 samples collected from 1986 to 1997 in northern Germany (Schade and Heinzow 1998). The following criteria were used: maternal age 27-31 years, primipara, breast feeding for 16-24 weeks, not an immigrant, had been a resident in northern Germany, and had spent less than one year away from Germany. Results for DDT, HCB and β-HCH were presented in a graph, which shows that all levels have decreased during the 12 year period. The median DDT level decreased from 920 ng g^{-1} in 1986 to 230 ng g^{-1} in 1997, the median HCB level from 510 to 60 and the median β-HCH level decreased from 190 to 30 ng g^{-1} lipid. The authors state that maternal age, parity[4], duration of past breast feeding episodes and weight differences had statistically significant relationships with DDT, HCB and β-HCH concentrations.

Schlaud et al. (1995) analysed the breast milk of 156 primiparous German women, who had been born and grew up in Germany. Participants were 4-6 weeks post partum and samples were collected in 1992/93. The median levels of DDT and dieldrin were 380 and 14 ng g^{-1} milk lipid. The median level of heptachlor, γ-HCH, β-HCH and HCB were 22, 16, 45 and 220 ng g^{-1} lipid, respectively.

Johansen et al., (1994) reported on the levels of OCP pesticides in the breast milk of 28 mothers from Oslo, Norway, collected in 1991. They were primiparous and a resident of Oslo for 3 years with a normal and healthy baby. Samples were collected 5 days post partum. The levels of sum DDTs and HCB were 340 and 41 ng g^{-1} lipid weight, respectively. (Sum DDTs refers to the sum of the concentrations of p,p'-DDE, o,p'-DDD, o,p'-DDT, p,p'-DDD and p,p'-DDT.) The

[4] Number of pregnancies a woman has had.

level of oxychlordane, trans-nonachlor and sum HCH were 9.4, 19 and 36 ng g^{-1} lipid, respectively. (Sum HCH refers to the sum of the concentrations of α- HCH, β-HCH and γ-HCH).

Polder et al. (2003) investigated the levels of OCPs in sub-Arctic and Arctic locations in Russia. In total, 140 samples were collected in 1996/97. Samples were collected from four Russian city hospitals from primiparous and multiparous mothers 3 days post partum. The locations were Kargopol, a small inland town, Severodvinsk, a harbour town, Arkhangelsk, an industrial town and Naryan-Mar, a coastal town. Individual samples were analysed. The authors state that of the OCPs studied, HCB and sum-CHLs (three chlordanes) showed positive but weak correlations to age and negative but weak correlations were observed between p,p'-DDE, p,p'-DDT and infant birth weight. Mean concentrations for primiparous mothers (n=98) from all four regions are listed in Appendix H Table H.2. The authors state that the highest mean concentrations of HCB were found in Naryan-Mar (130 ng g^{-1} lipid) and the highest mean concentrations of sum HCHs, sum CHLs and sum DDTs were found in Arkhangelsk (410, 48 and 1,400 ng g^{-1} lipid).

Polder et al. (1998) reported the levels of organochlorine compounds in human milk from the Kola Peninsula, Russia. In 1993, 15 milk samples were collected each from Murmansk and Monchegorsk, both industrial areas. Individual samples were analysed. Samples were collected 14 days post partum and 60 % of mothers were primiparous. The mean levels of OCPs for Murmansk and Monchegorsk are provided in Appendix H, Table H.3. Sum DDT in Murmansk was 1,500 ng g^{-1} milk lipid and in Monchegorsk was 1,100 ng g^{-1} milk lipid. The sum HCH was 860 ng g^{-1} milk lipid in Murmansk and 750 ng g^{-1} milk lipid in Monchegorsk.

Czaja et al. (1998) reported the levels of organochlorine compounds in 462 milk samples from Poland. It is not mentioned if criteria were used to obtain participants. Results are listed for milk collected on '4th day' and 'mature milk'. The mean levels of HCB, α-, β-, and γ-HCH were 1.3, 0.2, 1.4 and 0.2 ng g^{-1} whole milk for 4th day milk and 2, 0.5, 3.3 and 0.4 ng g^{-1} whole milk for mature milk. The mean levels of p,p'-DDT, p,p'-DDD and p,p'-DDE were 5, 0.9 and 21 ng g^{-1} whole milk for 4th day milk and 3.4, 0.4 and 28 ng g^{-1} whole milk for mature milk.

Lutter et al. (1998) reported the levels of organochlorines in breast milk in Kazakhstan where 92 samples were collected from 7 sites. The criteria used to obtain participants were: first lactation, infant 2-8 weeks of age, and mother and infant apparently healthy. Results are presented for total samples (n=101), individual samples (n=77) and pooled samples (n=24). The mean concentration of β-HCH in total samples was 2,200 ng g^{-1} lipid, HCB was 91 ng g^{-1} lipid and DDE was 2,000 ng g^{-1} lipid. Organochlorine compounds were either not detected (heptachlor, heptachlor epoxide, cis-chlordane, trans-chlordane, dieldrin) or detected in limited samples (aldrin, γ-HCH, oxychlordane, mirex and trans-nonachlor). The authors state that the mean concentration of total DDT was 1,700 ng g^{-1} lipid. Hooper et al., (1997) analysed the levels of organochlorine pesticides in Southern Kazakhstan. Criteria for participants were: primiparous, healthy mothers, and sample collected 2-8 weeks post partum. OCP pesticides were analysed in 76 milk samples. The mean concentrations of α- and β-HCH were 78 and 2,200 ng g^{-1} lipid, respectively. The mean concentrations of HCB, DDE and DDT were 91, 2,000 and 300 ng g^{-1} lipid, respectively.

Larsen et al., (1994) analysed 64 human milk samples from four major Italian cities for DDT, DDE, HCB and β-HCH. Samples were collected from Florence, Milan, Pavia and Rome in 1987. Half the samples from Florence and all from Pavia were from rural areas. The authors state that most mothers had not changed residence in the 5 years before pregnancy. Milk sampling

was performed 4-8 weeks post partum and according to the authors, few mothers were primiparous. Individual samples were combined to create pools for the four cities. The mean concentration of DDE, DDT, HCB and β-HCH were 2,200, 150, 180 and 130 ng g^{-1} milk lipid, respectively. The levels of OCP compounds varied within Europe, for example, β-HCH ranged in concentration from 40 ng g^{-1} lipid in Germany (Schade et al. 1998) to 2,000 ng g^{-1} lipid in Kazakstan (Lutter et al. 1998) while the mean concentration found in the current study was 80 ng g^{-1} lipid.

Asia

In South India (Tanabe et al., 1990), 25 milk samples were collected in 1988 from rural, urban and cosmopolitan areas in South India. Samples were collected 3 days to 17 months post partum. The overall mean concentration of sum HCH (α-, β-, γ- and δ-HCH) was 6,200 ng g^{-1} lipid. The mean concentration of sum DDT (sum of p,p'-DDD, p,p'-DDE and p,p'-DDT) was 1,200 ng g^{-1} lipid. Results from the four regions are given in Appendix H Table H.2. This is in contrast to the current Australian concentrations of mean sum HCH and mean sum DDT of 80 and 320 ng g^{-1} lipid, respectively.

Middle East

In Egypt, Dogheim et al. (1996) reported the levels of organochlorine compounds from 31 mothers in Kafr El-Zayat Governorate and 11 from Cairo in 1994. Results were calculated on a whole milk basis. For Kafr El-Zayat, the mean levels of α-, β-, γ-HCH and total HCH were 1.9, 120, 2.6 and 120 ng g^{-1} whole milk, respectively. For Cairo the mean levels of α-, β-, γ-HCH and total HCH were 3.1, 190, <1.0 and 190 ng g^{-1} whole milk, respectively. For Kafr El-Zayat, the mean level of sum-DDT was 140 ng g^{-1} whole milk and for Cairo it was 96 ng g^{-1} whole milk.

Africa

In Zimbabwe, Chikuni et al. (1991) determined the levels of OCPs in human milk of 40 mothers living in the greater Harare area in 1989. Participants were chosen if they had lived in either the high or low density suburbs around the capital city for more than 5 years. Individual samples were analysed and parity ranged from 1 to 3. The mean sum DDT was 6,000 ng g^{-1} lipid and the mean concentration of o,p'-DDT, p,p'-DDT and p,p'-DDE were 340, 2,400 and 2,500 ng g^{-1} lipid, respectively. The mean concentrations of α-, β- and γ-HCH were 30, 840 and 40 ng g^{-1} lipid, respectively. The mean concentration of heptachlor epoxide and dieldrin were 10 and 50 ng g^{-1} lipid, respectively.

In Zimbabwe, Chikuni et al. (1997) assessed the levels of DDT and its metabolites using 175 milk samples, in 1993 to 1995. The criteria for participants were: they had lived in the area for at least 5 years; and healthy and breast feeding their first, second or third child. Around 40 % of mothers were primiparous and were one to four weeks post partum. The mean levels of p, p'-DDE, p, p'-DDT and sum-DDT for Zimbabwe were 4,500, 1,300 and 6,500 ng g^{-1} milk lipid, respectively and for Kariba, Zimbabwe were 14,000, 9,100 and 25,000 ng g^{-1} milk lipid, respectively. The authors state that vector control programmes, agricultural activities and dietary habits were the main contributing factors towards high levels of pesticides. The results from Africa are considerably higher than that of Australia where the mean sum DDT level was 320 ng g^{-1} lipid.

Figure 4.17 Various International levels of p,p'-DDE in human milk

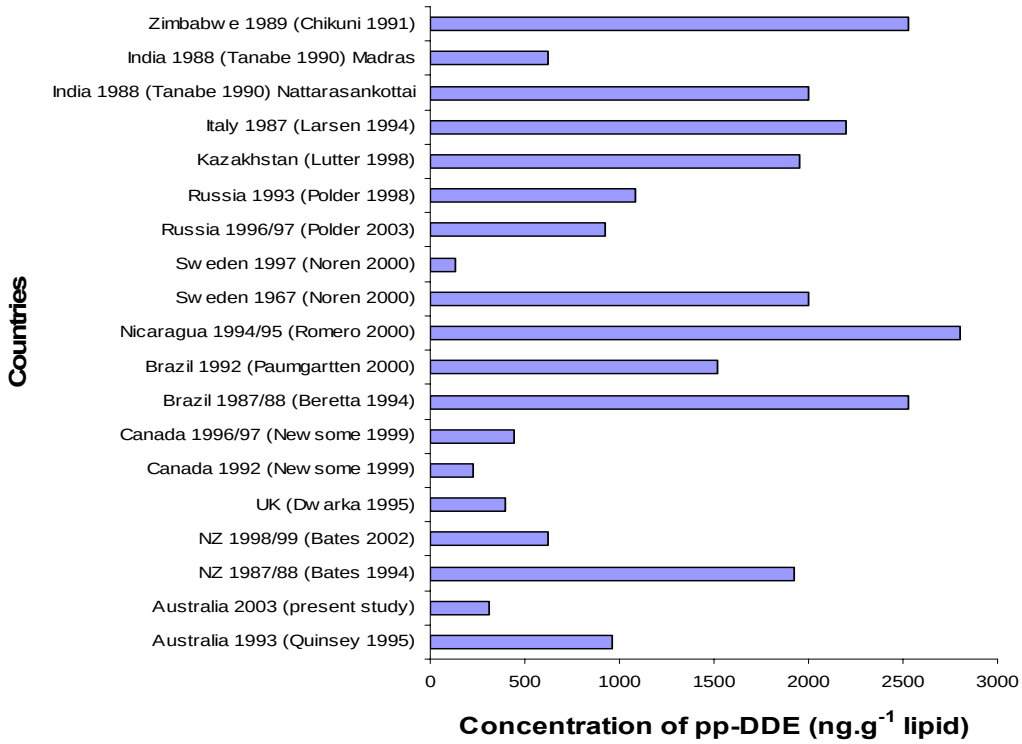
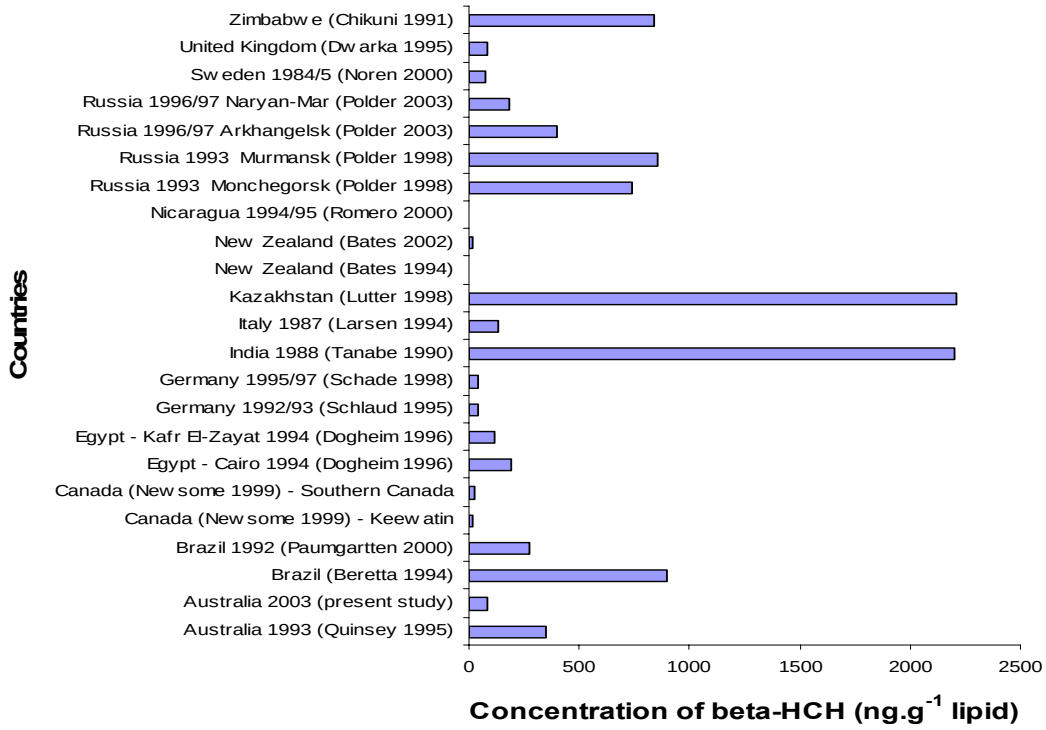


Figure 4.18 International levels of beta-HCH in human milk



4.5.2 PBDEs

There are some issues that must be considered when comparing the results of PBDE levels from various studies. These are noted by LaKind et al (2000) and include:

- various sampling and analysis methodology, for example, pooled samples versus individual samples
- incomplete reporting, for example, reporting demographic information on the sample donors
- non-representative sampling, for example, use of small sample sizes
- duration of sampling, for example, timing of the sample collection
- number and types of chemicals, i.e. different studies include analysis on difference chemicals.

In addition, most of the studies of PBDEs in human breast milk did not compare PBDE results to dietary and/or lifestyle factors. The results are included here if the study did report such comparisons. The value for mean concentration of sum PBDEs for the current study is the mean across all regions of the sum of BDE 17, 28, 33, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 166, 153, 154 and 183. The congeners used to obtain sum PBDE values in the various studies are included in the text if supplied by the authors and concentrations are reported to two significant figures. Figure 4.19 shows a comparison of the Australian levels and international levels.

North America

Ryan et al. (2000) determined levels of BDEs in Canadian human milk. This article reported on ten individual milk samples, five each from Quebec and Ontario, collected in 1992. The pattern of the BDEs showed considerable contributions from congeners 47, 99, 100 and 153 and lesser amounts from 28, 154 and 183. The mean sum BDE that was composed of congeners 28, 47, 99, 100, 153 and 183 was 5.8 ng g^{-1} lipid. The age of the sample donors was not mentioned. Ryan et al. also provided a table of the variation of BDEs in composite samples of Canadian human milk. Milk collected from Canada-wide in 1992 had a sum BDE level of 16 ng g^{-1} lipid whereas milk collected Canada-wide in 1981/82 had a sum BDE of 0.21 ng g^{-1} lipid. The authors state that this difference was of almost two orders of magnitude and that more recent sampling should substantiate this finding.

Ryan et al. (2002) reported on the BDE content of individual human milk samples collected in Vancouver, Canada between 2001 and 2002. The authors compared this to levels found in Vancouver and Canada-wide in 1982, 1986 and 1992. The mean BDE content of human milk in 2001/02 in Vancouver was 43 ng g^{-1} lipid with the range of 0.9-280 ng g^{-1} lipid. BDE 47 dominated the congener pattern with lesser but significant amounts of congeners 99, 153, 100, 28, 154 and 183 in that order. The BDE median values from Vancouver between 1992 and 2002 had increased about fifteen fold. In 1992, the authors found the sum BDE level in Vancouver to be 4.7 ng g^{-1} lipid with a range of 0.9-16 ng g^{-1} lipid and Canada-wide to be 15 ng g^{-1} lipid with a range of 0.5-590 ng g^{-1} lipid. Their results from 1982 and 1986 (2 values) were from pooled samples. The 1986 Vancouver pool had a value of 0.7 ng g^{-1} lipid, the 1986 Canada pool had a value of 0.6 ng g^{-1} lipid, and the 1982 Canada pool had a value of 0.2 ng g^{-1} lipid, note that the authors did not specify if this was a mean or median value.

Schecter et al. (2003) reported the levels of PBDE congeners in individual samples of US mothers' milk. They analysed 47 individual milk samples from nursing mothers' aged 20 to 41 years, measuring up to 13 PBDE congeners. Samples were collected from mothers in Austin and Dallas, Texas in 2002. BDE 47 contributed most to the sum of PBDEs (54 %), followed by BDE 99 (16.8 %), BDE 100 (8.5 %) and BDE 153 (5.9 %). The mean sum BDE was 74 ng g^{-1} lipid and the range was 6.2-420 ng g^{-1} lipid. In this analysis sum PBDE included 10 PBDE congeners for the Austin samples (28, 47, 66, 85, 99, 100, 138, 153, 154, 183) and three additional congeners for the Dallas samples (17, 66, 209). Sum PBDE levels were not correlated with maternal age or

length of time nursing. Parity was not specified, although the authors noted that number of children of the mother did not correlate with the PBDE concentrations. Schecter et al. (2003) stated that the findings showed extremely elevated levels (10 to 100 times) in many participants compared to contemporaneous levels reported in Europe.

The levels of PBDEs in North America range from a mean of 43 ng g^{-1} lipid in Canada in 2001/02 (Ryan 2002) to 74 ng g^{-1} lipid in Texas, US in 2002 (Schecter et al. 2003), these levels are considerably higher than the levels found in the current study (mean sum PBDE 11.1 ng g^{-1} lipid). In the current study PBDE 47 (50 %) contributed the most to the mean sum PBDE concentration, followed by 99 (17 %), 100 (12 %) and 153 (10 %).

Continental Europe

Lind et al. (2003) determined the concentrations of PBDEs in the breast milk of 93 primiparous women. The samples were collected from 1996 to 1999 in Uppsala County, Sweden. The mean age of the participants was 27 years. Dietary and lifestyle factors were also obtained from the participants. The mean PBDE concentration was 4.0 ng g^{-1} lipid with BDE 47 the major congener. The authors found no significant relationship between breast milk concentrations of PBDEs and dietary intake of PBDE, age, body mass index, alcohol consumption or computer usage. A weak but significant association between PBDE concentrations and smoking was observed.

Meironyte and Noren (1999) and, Noren and Meironyte (2000) reported on the analysis of pooled samples of breast milk collected at eight time periods between 1972 and 1997 in Stockholm. The years were 1972, 1976, 1980, 1984/1985, 1990, 1994, 1996 and 1997. In each time period 55-75 % of mothers were primiparous. BDE 47 was the most abundant PBDE congener in all samples followed by BDE 99 and BDE 153. The sum concentration of BDE congeners in human milk increased from 0.07 to 4.0 ng g^{-1} lipid during the 25-year period studied. The year specific concentrations are listed in Appendix H Table H.1. The authors stated that an infant's exposure to PBDEs had increased 60 times between 1972 and 1997.

In Sweden, Meironyte and Guvenius et al. (2001) analysed human milk samples from 1998 to 2000 for PBDEs. Pooled milk samples from 1998, 1999 and 2000 collected in Stockholm were used for analysis. Each pool contained milk from 20 mothers and there were two pools for each year. The average age of the mothers was 30-31 years and 65-75 % were primiparous. BDE 47 was the predominant congener in all samples and constituted about 60 % of the total PBDE amount. Congeners BDE 153, BDE 99 and BDE 100 occurred at the next highest concentrations. The sum PBDE concentrations were 3.9, 3.5 and 2.8 ng g^{-1} lipid in the years 1998, 1999 and 2000, respectively. The authors stated that the levels of PBDEs in human milk had declined during previous years with the highest concentrations of PBDEs found in 1997 and the levels in 2000 similar to those in 1995.

Darnerud et al. (1998) reported on PBDE levels in breast milk from 39 primiparous mothers from Uppsala County, Sweden. The age of the mothers was from 22 to 36 years old. However, the year the study was carried out was not stated. The observed mean value of sum PBDE was 4.4 ng g^{-1} lipid and the median was 3.4 ng g^{-1} lipid. BDE 47 was the major congener in the breast milk found at a mean concentration of
2.5 ng g^{-1} lipid. The authors found that apart from the significant relationships between sum PBDE and body mass index and smoking habits, there were no other significant relationships between PBDE levels and mother's age, computer usage frequency, consumption of fish, consumption of alcohol, place of residence during mothers own childhood and adolescence or

birth weight of the child. The number of observations may have been too small to observe any correlations.

Darnerud et al. (2002) analysed individual samples from 124 primiparous mothers in Uppsala, Sweden for levels of PBDEs. The women were aged from 20 to 35 years old and samples were collected from 1996-2001. The mean and median values for sum PBDE were 3.8 and 3.1 ng g^{-1} lipid. Sum PBDE consisted of five congeners (BDE 47, 99, 100, 153 and 154). BDE 47 was the major congener in the milk samples. The authors note that if the data were separated according to sampling year, a peak in sum PBDE is suggested at around 1998. The mean concentrations of sum PBDE in Sweden over the years 1997-2001, as reported in the aforementioned studies are less than half the mean concentration of sum PBDEs in Australia in 2003 at 11 ng g^{-1} lipid.

In Finland, a study by Strandman et al. (2000) compared levels of PBDEs in placenta with the levels in individual samples of human milk from eleven donors taken from 1994-1998. Parity varied among the donors and age ranged from 25 to 42 years. The concentrations of BDEs 28, 47, 99 and 153, were determined as 0.16, 1.3, 0.39 and 0.39 ng g^{-1} lipid, respectively. BDE 47 was the predominant congener in all samples. The sum PBDE ranged from 0.88 to 5.89 ng g^{1} lipid in human milk and from 1.0 to
4.4 ng g^{-1} lipid in placenta. The authors note that the four highest sum concentrations were from donors of their first childbirth.

Baumann et al. (2003) analysed 103 milk samples for PBDEs from mothers in The Netherlands. As with many of the other studies, BDE 47 was the congener that occurred in the relatively highest concentrations at a mean level of 1.5 ng g^{-1} lipid. The median sum of congeners 47, 99, 100 and 153 was 3.13 ng g^{-1} lipid. The authors note that their results are quite comparable with the results of the Swedish study carried out by Darnerud et al. (2002). Much like the Swedish levels, the Finnish and Dutch levels are around half the mean sum Australian level of 11 ng g^{-1} lipid.

In Germany, Fürst (2001) analysed human milk samples for PBDEs from 1992 and 2000. The 1992 sample constituted approximately 1 kg of remaining milk fat from human milk samples used previously. The data from 2000 constituted the mean concentration from seven donors. The concentrations of all congeners were very similar for the two years with the exception of BDE 153, which was higher in 2000. Fürst concluded that with all precaution, the results do not seem to follow the same trend as reported for human milk from Sweden showing a continuous increase since 1972 with a doubling of the PBDE levels every five years. Results are presented graphically and taking the concentrations from the figure, the level of BDE 47 was approximately
0.73 ng g^{-1} lipid in 1992 and approximately 0.65 ng g^{-1} lipid in 2000. This is in contrast to the current Australian study where in 2003 the mean level of BDE 47, was 5.6 ng g^{-1} lipid.

United Kingdom

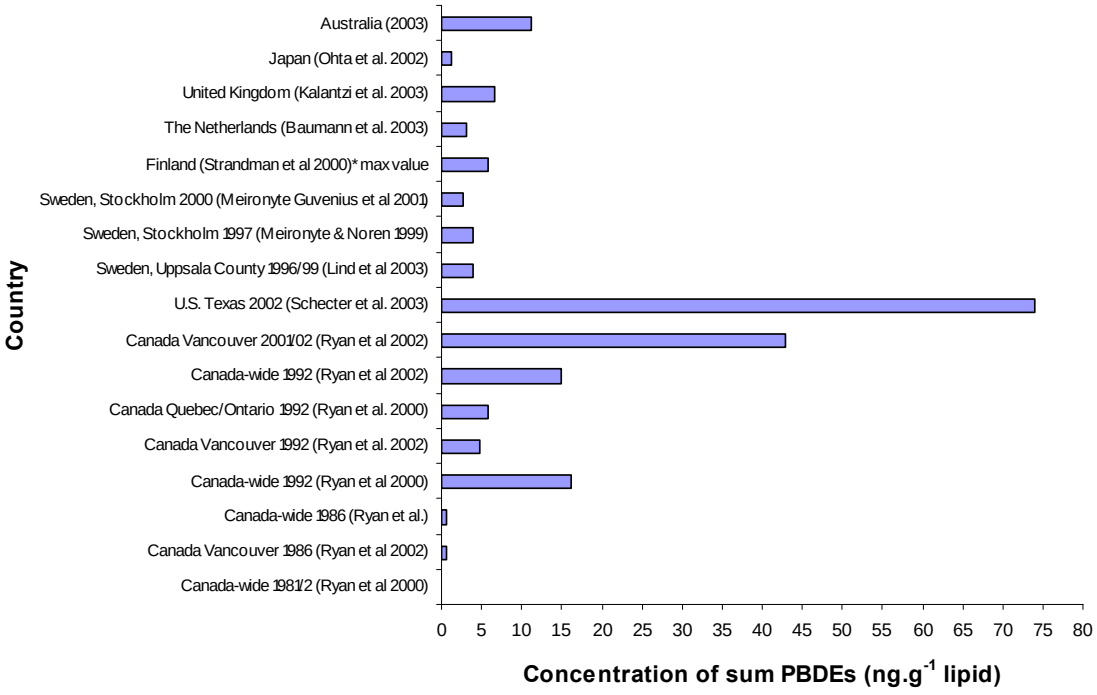
In the United Kingdom, Kalantzi et al. (2003) analysed 52 individual human breast milk samples for PBDEs. Samples were donated anonymously and without criteria between 2001 and 2003. PBDE congener 47 was always found at the highest concentrations followed by BDE 153, 99 and 100. In the current study, BDE 47 was also found in the highest concentrations followed by BDE 99, 100 and 153. The total PBDE concentrations ranged from 0.3 to 69 ng g^{-1} lipid with a geometric mean of 6.6 ng g^{-1} lipid. This level is lower than that found in the current study of 11 ng g^{-1} lipid.

Asia

Akutsu et al. (2003) investigated the time-trend of polybrominated diphenyl ethers in Japanese mother's milk. The investigators used eight pooled samples from Osaka from primiparous mothers aged 25-29 years. The years of collection were 1973, 1978, 1983, 1988, 1993, 1998, 1999 and 2000. Individual samples from Kanagawa (n=10) and Okayama (n=3) were also obtained in 1999 and consequently analysed. The sum of all PBDE congeners, on a concentration basis, continuously increased during the period between 1973 and 1988. After a decrease at the beginning of the 1990s, the concentration of sum PBDEs seemed to increase again and began leveling in 1999. The authors graphed their results of the levels of PBDEs in Japanese mothers' milk for 1999, from the three prefectures areas in Japan. From this figure the sum PBDE (sum of 183, 153, 154, 99, 100, 47 and 28) for Kanagawa was under 2.0 ng g^{-1} lipid; Okayama was over 1.5 ng g^{-1} lipid; and Osaka was under 1.5 ng g^{-1} lipid.

In Japan, Ohta et al. (2002) measured the concentrations of PBDEs in the breast milk of 12 primipara nursing women, one month after delivery. The women were all from the same region of Japan and their ages varied between 24 and 33 years. Sum PBDE concentrations ranged between 0.67 and 2.8 ng g^{-1} lipid with an average concentration of 1.3 ng g^{-1} lipid. The most abundant PBDE congeners were BDE 47 and BDE 153. The authors found a strong positive relationship between total PBDE levels in human milk and the frequency of fish consumption. The levels of PBDEs in Japan are lower than those found in the current study in Australia where the mean concentration was 11 ng g^{-1} lipid.

Figure 4.19 International levels of sum PBDEs (ng g^{-1} lipid)



5 SUMMARY OF FINDINGS

The results of this study provided an indication of the levels of OCPs and PBDEs in human breast milk. OCPs and PBDEs were detected in all pooled samples.

Organochlorine Pesticides (OCPs)

The overall OCP concentration for all samples was dominated by beta (β)-HCH and p,p'-DDE, a degradation product of DDT. The highest concentrations of OCPs were found in the Sydney pool A and the Melbourne pool A samples. An elevated concentration of HCB was also detected in the sample from rural Queensland.

A comparison of the Melbourne samples collected in 1993 with those collected in 2002/03 showed no significant differences in the concentrations of the OCPs over the ten-year period. Small decreases in the concentration from samples collected in 1993 to the samples collected in 2002/03 were observed for HCB, α-HCH, aldrin, p,p'-DDD, heptachlor epoxide and dieldrin. Small increases in concentration from samples collected in 1993 to the samples collected in 2002/03 were observed for β-HCH,
γ-HCH, mirex, o,p'-DDT, oxychlordane, transnonachlor and p,p'-DDE.

However, it should be noted that comparison of the two sample populations is complicated by the fact that details of maternal parity[5] and infant age at the date of collection for the 1993 samples were not available.

Additionally, statistical evaluation of any minor differences observed was complicated by the use of pooled samples and, hence, was not undertaken.

It is noteworthy that a low ratio of DDT to its degradation product DDE was observed in the 1993 samples as well as the 2002/03 samples indicating that exposure to DDT is not recent and is consistent with the use of DDT having been discontinued as an insecticide[6] (Chikuni et al., 1991, Polder et al., 2003). Higher ratios of these compounds have been observed particularly in developing countries and are indicative of the continued use of DDT as an insecticide in agricultural and malarial control programs.

Overall, the concentrations of OCP pesticides in the breast milk of these Australian women are low compared to international studies. β-HCH and p,p'-DDE were the dominant OCPs in all samples. The outliers observed in the Sydney A and Melbourne A samples are difficult to explain without further investigation and perhaps warrant a duplicate analysis to confirm the results. As previously stated, detailed statistical analysis of data obtained from the questionnaires is complicated by pooling of the samples from each region. Despite this, non-statistical evaluation of the data does not indicate the possibility of exposure of any individuals through geographical, dietary or occupational sources.

Polybrominated Biphenyl ethers (PBDEs)

For samples collected during 2002/03, the mean concentration of PBDEs for all samples was 11 ng g^{-1} expressed on a lipid basis. The concentration ranged from a minimum of 6.0 ng g^{-1} lipid detected in the Tasmanian sample to a maximum of 18 ng g^{-1} lipid detected in the rural NSW sample. The PBDEs that were found in the highest concentrations were BDEs 47, 99, 100, 153

[5] Number of pregnancies a woman has had.
[6] DDT was deregistered for use in Australia in 1987.

and 154 contributing on average 50, 17, 12, 10 and 1.3 %, respectively, to the total concentration for each pooled sample.

For the three pooled samples collected in 1993, the mean concentration of PBDEs was 13 ng g^{-1} expressed on a lipid basis. This was higher than that observed in the 2002/03 samples. However, this is not considered significant, as it is the same order of magnitude and there are to many limitations associated with sample size, sampling methodology and analytical uncertainty to draw any firm conclusions on trends. As for the 2002/03 samples, there was a clear dominance of BDE 47, 99,100,153 and 154 with each contributing an average of 42, 26, 12, 12, 8 and 2 %, respectively, to the total concentration for each pooled sample.

The levels reported for the 2002/03 samples are consistent with findings reported internationally. On a worldwide basis, the levels of PBDE compounds detected in breast milk are higher than those levels observed in Europe and Japan but lower than those observed in North America and Canada. It should be noted that much of the data from Japan is based on very low sample numbers and in some cases is the result of only a single analysis. The levels reported for North America and Canada are likely to be related to their high utilisation of products and articles containing penta-BDE. Penta-BDE is one of several compounds in the class of PBDEs and is predominantly used as a flame retardant in polyurethane foam in furniture and electronics. In 2001, the market demand for penta-BDE in North America was 7,100 tonnes representing 95 % of the world market demand. In comparison, the market demand in Europe, Asia and the rest of the world in 2001 was 150 (2 %), 150 (2 %) and 100(1 %) tonne, respectively.

Little is known about the exact sources and types of PBDE containing products in Australia. From the results of this study, it appears that a significant proportion of PBDE product may be in the form of penta-BDE products. Further investigation of these samples including analysis of individual samples may be warranted in order to determine the exact sources and the levels of theses compounds in the Australian population.

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 OCPs and PBDEs because of its fat content, but all babies are exposed to these compounds even if they are not breastfed. Alternative foods for babies, such as infant formula, also contain OCPs and PBDEs because they may also contain fat. Several studies around the world in areas where organic contaminants levels are known to be high have still shown that breastfed babies are healthier than those fed infant formula.

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7 APPENDICES

APPENDIX A ETHICS APPROVAL LETTER

OFFICE OF RESEARCH AND POSTGRADUATE STUDIES

DIRECTOR
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Friday, 6 December 2002

Dr Jochen Mueller
National Research Centre for Environmental Toxicology

Dear Dr Mueller

Concerning:Ethical clearance for project:- ***Dioxinlike Compounds In Human Milk - 25/11/02 - AMENDMENT***

Clearance No: H/308/NRCET/00

The Medical Research Ethics Committee has approved your project.

Please note that:-

(i) The Clearance number should be quoted on the protocol coversheet when applying to a granting agency and in any correspondence relating to ethical clearance;

(ii) Clearance will normally be for the duration of the project unless otherwise stated in the institutional clearance;

(iii) Adverse reaction to treatment by subjects, injury or any other incident affecting the welfare and/or health of subjects attributable to the research should be promptly reported to the Head of Department and the Behavioural and Social Sciences Ethical Review Committee.

(iv) Amendments to any part of the approved protocol, documents or questionnaires attached to this clearance are to be submitted to the Behavioural and Social Sciences Ethical Review Committee for approval.

H/308/NRCET/00

(v) Advisers on 'Integrity in Research'
As part of the University's commitment to the institutional statement, *Code of conduct for the Ethical Practice of Research (1990)*, and the NH&MRC's *National Statement on Ethical Conduct in Research Involving Humans (1999)*, designated positions have been appointed as advisers on integrity in research. The Chairperson of each ethics committee acts in an advisory capacity to provide confidential advice on such matters as misconduct in research, the rights and duties of postgraduate supervisors, and procedures for dealing with allegations on research misconduct within the University. The contact number for the Chairperson of each ethics committee can be obtained from the Ethics Officer.

(vi) The Committee reserves the right to visit the research site and materials at any time during the project.

(vii) It is the Committees expectation whenever possible, this work should result in publication and the Committee would require details to be submitted for our records.

Staff and students are also encouraged to contact either the Ethics Officer (3365 3924), or Chairperson on other issues concerning the conduct of experimentation/research (e.g. involvement of children, informed consent) prior to commencement of the project and throughout the course of the study.

Yours sincerely

Michael Tse
Ethics Officer

Encs.
cc: Head of School, National Research Centre for Environmental Toxicology

Institutional Approval Form For Experiments On Humans Including Behavioural Research

Chief Investigator:	Dr Jochen Mueller
Project Title:	Dioxinlike Compounds In Human Milk - 25/11/02 - AMENDMENT
Supervisor:	None
Co-Investigator(s)	Dr Fiona Harden
Department(s):	National Research Centre for Environmental Toxicology
Project Number:	H/308/NRCET/00
Granting Agency/Degree:	Environment Australia
Duration:	March 2002 – March 2003

Comments:

Name of responsible Committee:-
Medical Research Ethics Committee
This project complies with the provisions contained in the *National Statement on Ethical Conduct in Research Involving Humans* and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:-
Dr Bill Vicenzino
Chairperson
Medical Research Ethics Committee

Date 4-Dec-2002 Signature

APPENDIX B LIST OF SITES

Region	Site name	Agreed to participate	Approval	Recruited participants	Number of participants recruited
Sydney	Westmead Hospital	Yes	Yes	No	0
	Hills Parenting Centre – Castle Hill	Yes	UQ – Gatekeeper Letter	Yes	8
	King George Hospital	Yes	Not submitted	No	0
	South East Sydney Area Health Service	Yes	Yes	No	0
	Ramsgate	Yes	UQ – Gatekeeper Letter	No	0
	Tresillian Family Care Centres	Yes	Yes	Yes	20 (10 used for analysis)
	Sydney	Yes	UQ	Yes	2
	Royal North Shore Hospital	Yes	Not submitted	No	0
Melbourne	Banyule	Yes	UQ – Gatekeeper Letter	Yes	3
	Bayside	No	No	No	0
	Casey	No	No	No	0
	Darebin	No	No	No	0
	Glen Eira	Yes	UQ – Gatekeeper Letter	Yes	5
	Latrobe City Maternal and Child Health	No	No	No	0
	Monash Maternal and Child Health	Yes	UQ – Gatekeeper Letter	Yes	2
	Royal Women's Hospital Melbourne	Yes	Yes	Yes	15
	Stonnington Maternal and Child Health	Yes	UQ – Gatekeeper Letter	Yes	1*
	ISIS Primary Care Brimbank	Yes	UQ – Gatekeeper Letter	Yes	10
	Whitehorse	Yes	UQ – Gatekeeper Letter	Yes	4
Brisbane	Inala Community Health Centre	Yes	UQ – Gatekeeper Letter	Yes	10
	Mater Hospital	Yes	Yes	No	0
	Mater Hospital Redlands	Yes	Yes	Yes	1*
	QEII Hospital	Yes	Yes	No	0
	Mater Private Clinic	Yes	UQ – Gatekeeper Letter	Yes	
Perth	Uni of WA	Yes	Yes	Yes	11
	Woodside Hospital	Yes	?Yes	Yes	0

Region	Site name	Agreed to participate	Approval	Recruited participants	Number of participants recruited
Adelaide	Women's and Children's Hospital	Yes	Yes	Yes	21
Tasmania	Kingston Community Health Centre	Yes	Yes	No	0
	Launceston General Hospital	Yes	Yes	No	0
	Launceston Family & Child Health Clinic	Yes	UQ – Gatekeeper Letter	Yes	10
Canberra	ACT Health	Yes	Yes	No	0
Darwin	Private lactation consultant	Yes	UQ	Yes	4
Wollongong	University of Wollongong	Yes	Yes	Yes	12
Newcastle	Hunter Health Service	Yes	Yes	Yes	9
Gladstone	Maternity Unit	Yes	UQ – Gatekeeper Letter	Yes	3*
Coastal QLD	Innisfail Hospital	Yes	UQ	Yes	2*
	Proserpine Hospital	Yes			0
Coastal NSW	Northern Rivers Area Health - Ballina Community Health Centre	Yes	Yes	Yes	1*
Coastal Vic	Glenelg Shire Community Health Centre	Yes	Yes	No	0
	Lakes Entrance Community Health	Yes	UQ – Gatekeeper Letter	Yes	2
	Bass Coast Shire - Maternal & Child Health Centre	Yes	UQ – Gatekeeper Letter	No	0
	Wonthaggi	Yes	UQ – Gatekeeper Letter	No	0
Inland QLD	Dalby Hospital (QLD Health)	Yes	UQ – Gatekeeper Letter	Yes	8
	Roma Hospital	Yes	UQ	No	0
	St. George Hospital	Yes	UQ	No	0
	Mt Isa Health Services	Yes	Yes	No	0
	Townsville Health Service	Yes	Yes	No	0
Inland NSW	Dubbo (Macquarie and Far-west Area Health Service)	Yes	Yes	Yes	10
Inland Vic	Ararat	No	No	No	0
	Highton	Yes	No	No	0
	Maroondah	No	No	No	0
	Maribrynong	No	No	No	0

Region	Site name	Agreed to participate	Approval	Recruited participants	Number of participants recruited
	Port Phillip	No	No	No	0
	Hamilton				
	Wodonga Maternal and Child Health	Yes	UQ	No	0
	Wangaratta Maternity Unit			No	0
	Manningham	No	No	No	0
	Bendigo	Yes	UQ	Yes	1
	Portland	Yes	UQ	Yes	3*
	Ballarat			Newspaper advertisement	1

*not included in final analysis

APPENDIX C ADVERTISEMENT AND WEBSITE

THE UNIVERSITY OF QUEENSLAND

Call for breast milk study volunteers

Pregnant women and new mothers who are breastfeeding are invited to take part in a national breast milk study being conducted throughout Australia by the University of Queensland.

Volunteers are needed to complete the study. To participate you must be:

- A first-time mother with a baby aged two to eight weeks (IVF babies are fine)
- Exclusively breastfeeding
- Willing to provide 100-150 mls of expressed milk
- Healthy
- A resident of your area for the past five years.

If you would like more information please call 1800 550030 or visit http://www.uq.edu.au/nrcet/BreastMilkStudy.html.

If you are having your First Baby, are Healthy, intend to Breastfeed and are willing to participate in a national research project, then

WE NEED YOU !

Your Breast Milk is Vital.

Breast milk is perfect for your baby. It provides your baby with all of his or her nutritional needs. And for now, it also provides you with the chance to participate in an important study which may help improve the long-term health of all Australians.

Dr Jochen Muller and Dr Fiona Harden, in conjunction with The University of Queensland, is conducting a national research project which will identify typical levels of certain chemicals in breast milk. The results of the research will enable us to compare levels in Australia with other countries, but most importantly, it will help us reduce our exposure to certain chemicals in Australia.

It's Easy

You can collect your sample over a six week period by expressing a small amount of breast milk after feeding your baby. This can be frozen and stored. Once you have expressed 100 – 150mLs of milk, we will arrange for it to be collected.

Would you like to be involved?

To participate you must be:

- A first time Mum.
- Exclusively breastfeeding.
- Willing to provide 100 –150ml of expressed milk.
- Healthy (IVF babies are fine)
- Living in your area for the past 5 years.

150mls of your breast milk could help make a world of difference!!!

If you would like to participate, or would like more information, please telephone the Projector Coordinator, Dr Fiona Harden on 1800 550030 24Hours / 7 Days a Week or e-mail f.harden@uq.edu.au

APPENDIX D PARTICIPANT INFORMATION AND CONSENT FORM

PARTICIPANT INFORMATION AND CONSENT FORM

Evaluation Of The Levels Of Dioxin-Like

Compounds In Breast-Milk In Australia

THE UNIVERSITY OF QUEENSLAND

General Information

We would like to invite you to take part in a research project to be conducted by Dr Jochen Müller and Dr Fiona Harden as part of a national study on dioxin-like chemicals in the environment. The purpose of this study is to find out the typical levels of dioxin-like chemicals in human breast-milk in Australia.

Breast-milk provides perfectly optimized nutrition to infants and there are no equivalent alternatives to breastfeeding. Dioxin-like compounds include some of the most toxic chemicals and they are very long-lived in the environment. Also, since they are far more fat soluble than water soluble they accumulate in fatty tissue. Persistent chemicals such as dioxin-like chemicals enter the human food chain and can be found in breast-milk, infant formulas as well as many other food items including meat and dairy products at low concentrations.

It should be noted that despite the occurrence of elevated levels of dioxins in samples from highly industrialized countries in Europe and North America, The World Health Organization recommends that breastfeeding should continue to be encouraged as the benefits to the overall health and development of infants far outweigh any negative effects of chemical exposure.

The dioxin concentrations in breast-milk are a good indication of the levels of dioxins in our body. By analyzing breast-milk we aim to obtain the first information of the levels of these chemicals in Australian mothers as well as exposure of infants via this route. The results from this study will allow us to assess the exposure of mothers and children in Australia and to compare the levels in Australia with international levels. The results of this study also should help us to identify sources of these compounds in specific areas and therefore allow us to undertake effective steps to reduce exposure of the population.

Approximately 250 breast-feeding mothers around Australia will be taking part in this study.

If you take part

If you agree to take part in this study we will require you to collect 100-150 millitres of breast-milk collected between week 2 and 8 after the birth of your baby. We will supply you with a container to store the sample and will arrange for it to be returned to us. You do not have to collect the sample in a single sitting and so you may collect several small samples to obtain the 100-150 mls. We would suggest that you feed your baby first and then express a small amount of milk into the collection bottle. This can then be placed in the freezer until you are ready to collect another sample. Simply collect subsequent samples in the same jar and refreeze. The sample may be collected by either hand expressing or by using an electric or manual pump. If you require assistance, instructions will be given to you about the sampling. Please feel free to contact Fiona Harden on the number given below. If you collect the sample at home, please ensure that you have understood and followed the instructions on the pump. We request that you store the sample in the freezer until we have arranged for it to be picked up.

The study will also involve the completion of a questionnaire (31 either short answer or multiple choice questions) that will remain strictly confidential. These questions are mainly concerned with your diet and lifestyle. The study will not include any use of medication.

Possible risks

Many mothers routinely perform extraction of breast-milk, and we believe the risk to do so, is minimal. However, please ensure that the sampling equipment (pump) has been sterilized before use according to the procedures outlined with the equipment. Only take the sample if you have excess milk. Please cease collection of milk if you experience any difficulty expressing milk or feel that either you or your child are being compromised by your participation in the study. If you experience any pain or notice any hot or flushed areas around your breast, please consult your usual medical practitioner or clinic sister immediately.

How do you and your child benefit from this study?

The study will mix individual milk samples from participating women in a given area to obtain a pooled sample. These pooled samples will be analysed for levels of dioxin-like compounds and other pesticides. You will be able to obtain the study results for the pooled samples once the study is completed and the results are interpreted. However, since no individual samples will be analysed there will be no immediate benefits to you and your child from this study since the levels of dioxins in breast-milk are the result of a life time accumulation in the mother. The benefits will be long term by identifying current exposure of the population in specific areas of Australia and provide the means to identify and work towards reducing the sources. In countries where similar studies have been carried out, significant reductions (up to 70%) have been achieved in dioxin levels.

Do you need to take part in this study?

You do not need to take part in the study unless you want to do so. However, since we will pool the milk samples (mix milk samples from many women who live in a given area) you cannot discontinue your participation once we have received your sample and it has been added to the pool. However, if you do wish to withdraw your consent we are able to destroy your questionnaire and any milk not pooled. You have the option on the consent form to agree to part of the sample you donate being stored for future research on pollutants. This future research WILL NOT include genetic research. If you do not sign this second consent your remaining sample will be destroyed. You are able to withdraw your consent from this future research and request that your sample be destroyed.

Will the information you give be confidential?

Coded information collected during the study will be stored in a computer. You will not be personally identified in the study. Only the participating organization or your doctor and study staff will know that the information is related to you. The results of the study may be published in the scientific literature but your identity will not be revealed.

All personal information from the consent form will be kept secure and separate from other material including your completed Questionnaire

Please do not write your name on the Questionnaires.

Contacts

This study has been cleared by the Medical Research Ethics Committee at the University of Queensland and will be conducted in accordance with the National Health and Medical Research Council's guidelines. You are free to discuss your participation in this study and any queries you may have with the Project Coordinator: Dr Fiona Harden (Telephone No: 07-3274 9016) or the Secretary at the NRCET: 07-32749003). If you would like to speak to an officer of the University not involved in the study, you may contact the Ethics Officer on 3365 3924.

PARTICIPANTS CONSENT FORM

Dioxins and dioxin-like compounds in breast-milk

THE UNIVERSITY OF QUEENSLAND

Investigator: Dr Jochen Müller

I, the undersigned hereby agree to participate in the above research project.

I have been given clear information, both verbal and written, about this study and understand what has been stated.

I have been informed of any risks to my health or well-being.

I have been given the opportunity to have a member of my family or a friend present while the study was explained to me.

I have been assured that no personal identifiers regarding my questionnaire will be divulged and that the results of any tests will not be published so as to reveal my identity.

I am aware that this study has been approved by one of the Human Ethics Committees of the University of Queensland.

I am aware that I may request further information about the project as it proceeds.

I am aware that I may withdraw my consent at any time but that any milk sample that has been pooled with other samples is unable to be destroyed.

Signed: ..... Date: .....

I agree that a part of the sample I donated for the **Dioxins and dioxin-like compounds in breast-milk** study can be stored and used for future research on pollutants.

Signed: ..... Date: .....

Participant Identification: (please print)

Full Name: _____

Address: _____

_____

Telephone: _____

APPENDIX E QUESTIONNAIRE FOR PARTICIPANTS

Please complete the following questionnaire providing as much detail as possible.

The information provided by your answers will be kept strictly confidential. The questionnaire will be stored in a de-identified state that is it will be coded and your name will be detached from the answers. Only the researchers will have access to your code.

Please print all answers.

Where boxes are provided for alternative answers, please tick those that apply.

Name: .....

Residential Address: .....

.....

.....

.....

.....

Telephone Contact:.....

1. What is your country of birth? .....
2. What is your date of birth? .....
3. Where have you lived (town or closest town and state eg Newcastle, NSW) for:
   a. The last 5 years.....
   b. The previous 5-10 years.....
   c. The previous 10-20 years.....
   d. The previous 20+ years.....
4. What date did you start sample collection? (DD/MM/YY) .....
5. When date did you finish sample collection? (DD/MM/YY) .....
6. What is your height (cm)?: .....
7. What was your weight before you became pregnant? (kg)? .....
8. What was your weight just prior to delivery of your baby? (kg) .....
9. What is your baby's date of birth? .....
10. What is your baby's sex? ☐ Female ☐ Male
11. What was your baby's birth weight (grams)?.....
12. Are you a smoker? ☐ Yes ☐ No
13. If yes, how many per day? .....
14. How long have you smoked for? .....
15. Have you ever smoked? ☐ Yes ☐ No
16. If yes when (approximately) did you have your last cigarette?.....
17. Which diet best describes your dietary habits?
    a. Mixed Diet ☐
    b. Vegetarian but with dairy products and Eggs ☐
    c. Strictly Vegetarian ☐

18. Did your dietary habits change markedly after you became pregnant? If yes please provide details.

.....

.....

.....

.....

19. On average, how often do you eat fish or other seafood?

a. Never ☐
b. Less than once a week ☐
c. Once a week ☐
d. Twice a week ☐
e. More than twice a week ☐

20. If eaten, describe the seafood most often consumed.

.....

.....

21. On average how often do you consume milk and milk products, including cheese?

a. Never ☐
b. Less than once a week ☐
c. Once a week ☐
d. Twice a week ☐
e. More than twice a week ☐

22. On average, how often do you consume meat?

a. Never ☐
b. Less than once a week ☐
c. Once a week ☐
d. Twice a week ☐
e. More than twice a week ☐

23. Have you ever worked in any of the following areas?

a. Pesticide Industry ☐
(including insecticides, herbicides, fungicides and biocides)

b. Forestry Industry ☐
c. Manufacturing of Electrical Transformers ☐

24. If yes, what was your job? .....

25. How long did you work in this industry for? .....

26. Were you an office worker? ☐ Yes ☐ No

27. Have you ever had contact with any of the following chemicals?

    a. Pesticides: ☐
    including insecticides, herbicides, fungicides and biocides

    b. Wood treatment chemicals ☐

28. If so how often

    a. Yearly ☐
    b. Monthly ☐
    c. Weekly ☐
    d. Daily ☐

THANK YOU FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE.

APPENDIX F DEMOGRAPHICS OF PARTICIPANTS

Table F.1 Demographics of Participants.

Region (no. of samples in pool)	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 (gram) (± 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**

APPENDIX G OCP AND PBDE RESULTS

Table G.2 Concentration of OCP compounds detected in pooled breast milk samples collected from Australian women in 2002/03.

Values are expressed on a lipid basis (ng g^{-1} lipid)

Region	Syd A	Syd B	Wollongong	Hunter	Rural NSW	Melb A	Melb B	Melb C	Melb D	Rural Vic	Bris	Rural QLD	Tas	SA-A	SA-B	Darwin	WA
HCB	14	15	9.1	6.6	19	12	8.7	14	14	8.8	27	76	9.7	15	11.7	23	17
α-HCH	0.15	0.07	0.05	0.03	0.03	0.18	0.05	0.06	0.06	0.03	0.04	0.04	0.03	0.06	0.04	0.08	0.06
β-HCH	380	27	15	7.6	10	660	17	29	38	15	26	21	12	14	18	43	21
γ-HCH	0.47	0.18	0.09	0.08	0.15	0.31	0.13	0.4	0.23	0.15	0.22	0.24	0.26	0.2	0.21	0.29	0.35
Aldrin	0.68	0.48	<0.01	0.03	<0.05	<0.6	<0.05	0.04	0.01	0.02	0.61	0.12	0.04	0.22	0.7	0.17	0.06
Heptachlor	<4	<20	<2	<2	<3	<2	<1	<5	<20	<10	<3	<3	<0.8	<20	<24	<6	<10
Heptachlor epoxide	11	16	7.3	8.5	17	2.6	2.2	3.1	3.9	3.9	7.4	8.1	2.4	11	10	5.1	14
Dieldrin	15	19	9.6	17	22	6.8	6.4	18	13	11	20	21	15	20	22.9	11	25
Oxychlordane	15	18	9.9	9.7	15	6.0	5.1	6.2	5.3	6.3	8.3	11	2.8	7.2	12	8.9	8.5
trans-chlordane	<2	<2	<1	<1	<3	<2	<1	<3	<2	<2	<2	<2	<2	<3	<4	<3	<2
cis-chlordane	<0.7	<0.7	<0.3	<0.2	<0.5	<0.3	<0.2	<0.8	<0.5	<0.3	<0.5	<0.6	<0.4	<0.8	<0.8	<0.6	<0.4
trans-nonachlor	24	17	8.8	12	9.0	7.8	5.8	9.7	7.7	8.5	10	19	3.6	12	15	9.1	7.3
p,p'-DDE	540	280	190	150	220	870	380	320	320	180	330	180	150	300	280	350	250
p,p'-DDD	0.3	0.16	0.06	0.06	0.09	0.45	0.10	0.2	0.11	0.08	0.14	0.18	0.11	0.14	0.13	0.12	0.10
o,p'-DDT	1.8	0.76	0.34	0.29	0.48	1.0	0.6	1.2	0.80	0.39	0.61	0.33	0.34	0.54	0.76	0.67	0.48
p,p'-DDT	18	7.0	4.3	3.6	5.7	30	6.0	9.9	7.1	6.0	12	8.1	4.6	7.8	6.3	7.2	7.0
Mirex	0.53	0.5	0.13	0.12	0.13	0.34	0.2	0.23	0.26	0.21	0.23	0.17	0.17	0.17	0.28	0.29	0.18

* Excluding LOD values

Table G.3 The concentrations of PBDE compounds detected in pooled breast milk samples collected from Australian mothers during 2002/03.

Values are expressed on a lipid basis (ng g^{-1} lipid)

Region	Syd A	Syd B	Wollongong	Hunter	Rural NSW	Melb A	Melb B	Melb C	Melb D	Rural Vic	Bris	Rural QLD	Tas A	SA-A	SA-B	Darwin	WA
Lipid Content	**4.30%**	**4.2%**	**3.7%**	**4.3%**	**3.2%**	**3.6%**	**3.1%**	**3.1%**	**4.5%**	**4.1%**	**2.8%**	**3.5%**	**3.8%**	**3.5%**	**3.1%**	**4.2%**	**4.3%**
PBDE 17	0.01	0.0067	<0.01	<0.01	<0.01	<0.01	<0.01	0.009	0.0051	0.0059	<0.01	0.008	0.009	0.016	0.016	0.0084	0.012
PBDE 28 + PBDE 33	0.54	0.42	0.78	0.52	0.75	0.57	0.67	0.68	0.30	0.32	0.74	0.48	0.51	0.43	0.72	0.45	0.64
PBDE 47	5.84	4.01	6.77	4.18	9.63	5.12	6.07	5.59	4.33	3.62	5.59	5.25	2.82	5.60	9.29	4.18	8.03
PBDE 49	0.15	<0.1	0.098	0.092	0.14	0.092	0.17	0.12	<0.1	<0.07	0.16	0.12	0.054	<0.1	<0.2	0.13	<0.2
PBDE 66	0.071	0.042	0.078	0.046	0.11	0.046	0.08	0.065	0.054	0.048	0.057	0.065	0.028	0.082	0.17	0.054	0.1
PBDE 71	<0.006	<0.007	<0.01	<0.01	<0.01	<0.01	<0.01	<0.006	<0.005	<0.009	<0.01	<0.009	<0.003	<0.005	<0.009	<0.004	<0.007
PBDE 77	<0.002	<0.002	<0.01	<0.01	<0.01	<0.01	<0.01	<0.002	<0.002	<0.003	<0.01	0.001	<0.001	0.0017	<0.004	0.0023	<0.003
PBDE 85	0.097	0.084	0.2	0.11	0.26	0.14	0.14	0.11	0.11	0.12	0.14	0.11	0.053	0.17	0.23	0.093	0.15
PBDE 99	1.70	1.10	2.32	1.46	3.52	2.07	2.17	1.88	1.51	1.50	2.20	1.84	1	2.05	3.00	1.4	1.66
PBDE 100	1.36	1.12	1.68	1.11	2.33	1.54	1.53	1.22	1.17	1.07	1.25	1.29	0.69	1.21	1.6	0.86	1.51
PBDE 119	<0.02	<0.009	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.03	<0.01	<0.04	<0.04	<0.03	0.006
PBDE 126	<0.005	<0.007	<0.01	<0.01	<0.01	<0.01	<0.01	<0.006	<0.003	<0.01	<0.02	<0.003	<0.003	<0.01	<0.01	<0.006	<0.006
PBDE 138 + PBDE 166	<0.01	0.017	0.032	0.013	0.04	0.027	0.024	0.019	0.026	0.022	<0.01	0.022	0.01	0.02	0.028	0.014	0.023
PBDE 153	0.98	1.39	1.18	0.93	1.56	1.25	1.27	1.19	1.19	1.25	0.95	0.87	0.72	0.86	0.97	0.59	1.0
PBDE 154	0.14	0.097	0.15	0.11	0.23	0.15	0.16	0.16	0.12	0.12	0.15	0.13	0.086	0.15	0.17	0.14	0.12
PBDE 183	0.13	0.18	0.046	0.046	0.1	0.054	0.12	0.14	0.23	0.094	0.22	0.028	0.07	0.1	0.11	<0.05	0.12

Table G.2 Continued

Region	Syd A	Syd B	Wollongong	Hunter	Rural NSW	Melb A	Melb B	Melb C	Melb D	Rural Vic	Bris	Rural QLD	Tas A	SA-A	SA-B	Darwin	WA
Sum of PBDE congeners*	**11.0**	**8.47**	**13.30**	**8.62**	**18.70**	**11.10**	**12.40**	**11.2**	**9.05**	**8.17**	**11.50**	**10.2**	**6.05**	**10.7**	**16.3**	**7.92**	**13.4**
Mean sum PBDE (ng g^{-1} lipid)			**11.1**														
%contribution of PBDE 47	53.0	47.4	50.9	48.5	51.5	46.1	49.0	50.0	47.9	44.3	48.6	51.4	46.6	52.4	57.0	52.8	60.0
%contribution of PBDE 99	15.4	13.0	17.4	16.9	18.8	18.6	17.5	16.8	16.7	18.4	19.1	18.0	16.5	19.2	18.4	17.7	12.4
%contribution of PBDE 100	12.3	13.2	12.6	12.9	12.5	13.9	12.3	10.9	12.9	13.1	10.9	12.6	11.4	11.3	9.8	10.9	11.3
%contribution of PBDE 153	8.9	16.4	8.9	10.8	8.3	11.3	10.2	10.6	13.2	15.3	8.3	8.5	11.9	8.0	5.9	7.4	7.6
%contribution of PBDE 154	1.3	1.1	1.1	1.3	1.2	1.4	1.3	1.4	1.3	1.5	1.3	1.3	1.4	1.4	1.0	1.8	0.9
%contribution of PBDE 183	1.2	2.1	0.3	0.5	0.5	0.5	1.0	1.3	2.5	1.2	1.9	0.3	1.2	0.9	0.7	0.6	0.9

Table G.4 The concentrations of OCP compounds detected in pooled breast milk samples collected from Australian mothers during 1993.

Values are expressed on a lipid basis (ng g^{-1} lipid)

Organochlorine	Melb Hist A	Melb Hist B	Melb Hist C
HCB	18	53	18
α-HCH	0.13	0.13	0.28
β-HCH	29	17	27
γ-HCH	0.2	0.2	0.21
Aldrin	0.05	n.d. (0.02)	0.5
Heptachlor	n.d. (0.5)	n.d. (0.5)	n.d. (0.6)
Heptachlor epoxide	4.7	6.0	7.0
Dieldrin	12	22	14
Oxychlordane	5.4	5.1	4.9
trans-chlordane	n.d. (4)	n.d. (3)	n.d. (2)
cis-chlordane	n.d. (1)	n.d. (0.5)	n.d. (0.5)
trans-nonachlor	5.4	5.3	5.7
p,p'-DDE	280	280	360
p,p'-DDD	0.2	0.17	0.18
o,p'-DDT	0.7	0.55	1.3
p,p'-DDT	8.5	12	12
Mirex	0.3	0.18	0.16

Table G.5 The concentration of PBDE compounds detected in pooled breast milk samples collected from Australian mothers during 1993.

Values are expressed on a lipid basis (ng g^{-1} lipid)

	Melb Hist A	Melb Hist B	Melb Hist C
BDE 17	0.01	0.06	0.008
BDE 28 + BDE 33	1.0	0.6	0.08
BDE 47	5.2	4.1	7
BDE 49	<0.2	<0.09	<0.1
BDE 66	<0.04	0.037	0.06
BDE 71	<0.01	<0.006	<0.006
BDE 77	<0.008	<0.006	<0.006
BDE 85	0.1	0.11	0.1
BDE 99	3.2	2.9	3.7
BDE 100	1.5	1.3	1.7
BDE 119	<0.1	<0.05	<0.06
BDE 126	<0.04	<0.03	<0.03
BDE 138 + BDE 166	0.04	0.04	0.04
BDE 153	0.9	1.2	1
BDE 154	0.3	0.3	0.3
BDE 183	<0.08	0.2	0.2
Sum PBDE excl. LOD	12	11	15

APPENDIX H INTERNATIONAL DATA

Table H.1 Levels of PBDEs (ng g^{-1}) in breast milk in various countries from 1972 to 2003

Country	Sampling year	No. of samples	Time period after delivery	% Primiparous	Sum PBDE	2,2',4,4'-tetraBDE (BDE-47)	Reference
Canada	1992	10	unknown	unknown	5.79	3.39	Ryan et al (2000)
Canada - Maritimes	1992	20	unknown	unknown	19.08	na	Ryan et al (2000)
Canada - Quebec	1992	20	unknown	unknown	18.75	na	Ryan et al (2000)
Canada - Ontario	1992	20	unknown	unknown	2.57	na	Ryan et al (2000)
Canada - Prairies	1992	20	unknown	unknown	5.7	na	Ryan et al (2000)
Canada - wide	1992	100	unknown	unknown	16.24	na	Ryan et al (2000)
Canada - wide	1981/82	200	unknown	unknown	0.21	na	Ryan et al (2000)
Canada	2001-02	20	unknown	unknown	42.8	18#	Ryan et al (2002)
Canada	1992	9	unknown	unknown	4.7	na	Ryan et al (2002)
Canada	1992	72	unknown	unknown	15	na	Ryan et al (2002)
US	2002	47	2-109 weeks	unknown	73.9	40.8	Schecter et al (2003)
Sweden	1996-99	93	3-4 weeks	100%	4.01	2.35	Lind et al (2003)
Sweden	1972	75	unknown	55-75%	0.07	na	Meironyte et al. (1999)
Sweden	1972-97	102-340	1-90 days	55-75%	Na	0.06-2.28*	Meironyte et al. (1999)
Sweden	1976	78	unknown	55-75%	0.28	na	Meironyte et al. (1999)
Sweden	1980	116	unknown	55-75%	0.48	na	Meironyte et al. (1999)
Sweden	1984/85	102	unknown	55-75%	0.72	na	Meironyte et al. (1999)
Sweden	1990	20	1-90 days	55-75%	1.21	na	Meironyte et al. (1999)
Sweden	1994	20	1-90 days	55-75%	2.15	na	Meironyte et al. (1999)
Sweden	1996	20	1-90 days	55-75%	3.11	na	Meironyte et al. (1999)
Sweden	1997	40	1-90 days	55-75%	4.01	na	Meironyte et al. (1999)

Country	Sampling year	No. of samples	Time period after delivery	% Primiparous	Sum PBDE	2,2',4,4'-tetraBDE (BDE-47)	Reference
Sweden	1998	40	unknown	65-75%	3.88	2.29	Meironyte Guvenius et al (2001)
Sweden	1999	40	unknown	65-75%	3.46	1.97	Meironyte Guvenius et al (2001)
Sweden	2000	40	unknown	65-75%	2.79	1.7	Meironyte Guvenius et al (2001)
Sweden	Published - 1998	39	unknown	100%	4.5	2.52	Darnerud et al (1998)
Sweden	1996-2001	124	3-4 weeks	100%	3.79	2.24	Darnerud et al 2002
Finland	1994-98	11	unknown	54%	na	1.31	Strandman et al (2000)
The Netherlands	1998	103	6-10 days	100%	na	1.53	Baumann et al (2003)
Germany	1992	unknown	unknown	unknown	na	0.73#	Fuerst (2001)
Germany	2000	7	unknown	unknown	na	0.65#	Fuerst (2001)
UK	2001-03	52	unknown	unknown	6.6	3	Kalantzi et al (2003)
Japan	1973	21	30-90 days	100%	na	nd	Akutsu et al (2003)
Japan	1978	21	30-90 days	100%	0.1	0.03	Akutsu et al (2003)
Japan	1983	19	30-90 days	100%	0.59	0.26	Akutsu et al (2003)
Japan	1988	24	30-90 days	100%	1.64	0.67	Akutsu et al (2003)
Japan	1993	30	30-90 days	100%	0.96	0.32	Akutsu et al (2003)
Japan	1998	35	30-90 days	100%	2.31	1.03	Akutsu et al (2003)
Japan	1999	30	30-90 days	100%	1.45	0.62	Akutsu et al (2003)
Japan	2000	27	30-90 days	100%	1.39	0.53	Akutsu et al (2003)
Japan	1999	1	0-30 days	100%	1.57	0.57	Akutsu et al (2003)
Japan	1999	1	0-30 days	0%	1.36	0.37	Akutsu et al (2003)
Japan	1999	1	0-30 days	0%	3.51	2.15	Akutsu et al (2003)
Japan	1999	1	0-30 days	0%	0.78	0.3	Akutsu et al (2003)
Japan	1999	1	0-30 days	0%	1.33	0.52	Akutsu et al (2003)
Japan	1999	1	0-30 days	0%	1.92	0.82	Akutsu et al (2003)
Japan	1999	1	0-30 days	0%	0.56	0.2	Akutsu et al (2003)

Country	Sampling year	No. of samples	Time period after delivery	% Primiparous	Sum PBDE	2,2',4,4'-tetraBDE (BDE-47)	Reference
Japan	1999	1	0-30 days	100%	291	187	Akutsu et al (2003)
Japan	1999	1	0-30 days	0%	3.97	2.25	Akutsu et al (2003)
Japan	1999	1	0-30 days	0%	1.1	0.58	Akutsu et al (2003)
Japan	1999	1	30-90 days	100%	1.95	1.11	Akutsu et al (2003)
Japan	1999	1	30-90 days	100%	2.53	1.06	Akutsu et al (2003)
Japan	1999	1	30-90 days	100%	0.73	0.27	Akutsu et al (2003)
Japan	unknown	12	4 weeks	100%	1.3	na	Ohta et al (2002)

na = not assessed

* ng g^{-1} lipid, range and median

data presented graphically estimate used

Table H.2 Levels of DDT compounds in breast milk in various countries from 1967-2003.

Country	Year	Region	Sample size	*pp*-DDT	sum DDT	DDE	DDD	Reference
Australia	1970/71	Perth	23	10.0#	77.0#	61.0#	na	Stacey et al. (1975)
Australia	1971/72	Brisbane	20	na	8600	na	na	Miller et al. (1973)
Australia	1971/72	Mareeba	20	na	16900	na	na	Miller et al. (1973)
Australia	1972	Sydney	45	na	64.0#	na	na	Siyali (1973)
Australia	1979/80	WA	45	10.0#	42.0#	29.0#	na	Stacey et al (1985)
Australia	1979/80	WA	53	9.0#	36.0#	24.0#	na	Stacey et al (1985)
Australia	1990/91	Perth	128	na	800*	na	na	Stevens et al (1993)
Australia	1993	Victoria	60	225	na	960	na	Quinsey et al. (1995)
Australia	2003	national	17	8.8	320.3	311	0.15	Present study
New Zealand	1987/88	Auckland	11	105	na	1067	na	Bates et al. (1994)
New Zealand	1987/88	Northland	10	45	na	1036	na	Bates et al. (1994)
New Zealand	1987/88	Christchurch	9	48	na	2833	na	Bates et al. (1994)
New Zealand	1987/88	Canterbury	8	112	na	3212	na	Bates et al. (1994)
New Zealand	1987/88	All	38	78.03	na	1928.95	na	Bates et al. (1994)
New Zealand	1998/99	Christchurch, Auckland North Canterbury Northland	53	25.6	na	626	na	Bates et al. (2002)
United Kingdom	1989-1991	Unknown	193	<20.0	na	400	na	Dwarka et al. (1994)
Canada	1992	National	497	22.1	na	222	na	Newsome WH (1999)
Canada	1996/1997	Keewatin	12	24.2	na	441	na	Newsome WH (1999)
Brazil	1987/88	Porto Alegre	30	120	2980	2530	30	Beretta et al. (1994)
Brazil	1992	Rio de Janeiro	40 (1 pool)	180	1700	1520	6	Paumgartten et al. (2000)
Nicaragua	1994-1995	Rio Atoya	101	129	na	2805	n.d.	Romero et al. (2000)
Sweden	1967	Unknown	210	1300	na	2000	na	Noren et al. (2000)
Sweden	1968/69	Unknown	75	1020	na	1700	na	Noren et al. (2000)
Sweden	1972	Unknown	227	710	na	2420	na	Noren et al. (2000)
Sweden	1974	Unknown	250	470	na	1800	na	Noren et al. (2000)
Sweden	1976	Unknown	1500	340	na	1500	na	Noren et al. (2000)

Country	Year	Region	Sample size	*pp*-DDT	sum DDT	DDE	DDD	Reference
Sweden	1978	Unknown	745	270	na	1270	na	Noren et al. (2000)
Sweden	1979	Unknown	805	210	na	1130	na	Noren et al. (2000)
Sweden	1980	Unknown	973	185	na	1055	na	Noren et al. (2000)
Sweden	1984/85	Unknown	102	61	na	500	na	Noren et al. (2000)
Sweden	1988/89	Unknown	140	47	na	480	na	Noren et al. (2000)
Sweden	1990	Unknown	40	42	na	369	na	Noren et al. (2000)
Sweden	1991	Unknown	40	36	na	255	na	Noren et al. (2000)
Sweden	1992	Unknown	20	22	na	227	na	Noren et al. (2000)
Sweden	1994	Unknown	20	12	na	199	na	Noren et al. (2000)
Sweden	1996	Unknown	20	14	na	164	na	Noren et al. (2000)
Sweden	1997	Unknown	40	14	na	129	na	Noren et al. (2000)
Germany	1995-1997	Schleswig-Holstein	246	240	na	na	na	Schade et al. (1998)
Germany	1992-1993	Lower Saxony	156	380.0*	na	na	na	Schlaud et al. (1995)
Norway	1991	Oslo	28		338	na	na	Johansen et al. (1994)
Russia	1996/97	Kargopol	10	108	991	869	6	Polder et al. (2003)
Russia	1996/97	Severodvinsk	37	147	1131	979	6	Polder et al. (2003)
Russia	1996/97	Arkhangelsk	40	194	1392	1192	7	Polder et al. (2003)
Russia	1996/97	Naryan-Mar	11	171	1103	923	9	Polder et al. (2003)
Russia	1993	Monchegorsk	15	145	1055	892	4.1	Polder et al. (1998)
Russia	1993	Murmansk	15	178	1474	1269	8.2	Polder et al. (1998)
Poland	unknown (pub. 1998)	Warsaw and regions of Poland	462	5.0#	na	21.1#	0.9#	Czaja et al. (1998)
Poland	unknown (pub. 1998)	Warsaw and regions of Poland	462	3.4#	na	28.2#	0.4#	Czaja et al. (1998)
Kazakhstan	unknown (pub. 1998)	Central and Southern	101	na	1730	1955	na	Lutter et al. (1998)
Italy	1987	Florence, Milan, Pavia, Rome	64	150	na	2200	na	Larsen et al. (1994)
South India	1988	Chidambaram	11	170	1000	840	2.4	Tanabe et al. (1990)
South India	1988	Chinnoor Parangipettai	5	400	2000	1600	6	Tanabe et al. (1990)
South India	1988	Madras	6	140	760	620	14	Tanabe et al. (1990)
South India	1988	Nattarasankottai	3	290	2300	2000	25	Tanabe et al. (1990)

Country	Year	Region	Sample size	*pp*-DDT	sum DDT	DDE	DDD	Reference
Egypt	1994	Kafr El-Zayat	31	8.52#	143.21#	138.21#	1.38#	Dogheim et al. (1996)
Egypt	1994	Cairo	11	9.33#	96.36#	100.0#	<1.0#	Dogheim et al. (1996)
Zimbabwe	1993-1995	Esigodini	11	250	1607	1176	na	Chikuni et al. (1997)
Zimbabwe	1993-1995	Nyanga	20	2185	10060	6825	na	Chikuni et al. (1997)
Zimbabwe	1993-1995	Kwekwe	28	894	4174	2826	na	Chikuni et al. (1997)
Zimbabwe	1993-1995	Kadoma	24	1254	7047	5049	na	Chikuni et al. (1997)
Zimbabwe	1993-1995	Bulawayo	29	1213	6379	4477	na	Chikuni et al. (1997)
Zimbabwe	1993-1995	Harare	24	1846	7980	5377	na	Chikuni et al. (1997)
Zimbabwe	1993-1995	Kariba	39	9080	25259	13606	na	Chikuni et al. (1997)
Zimbabwe	1989	Harare	40	2390	6000	2530	na	Chikuni et al. (1991)

*median

whole milk basis

Table H.3 Levels of HCH compounds in breast milk in various countries from 1967-2003.

Country	Year	Region	Sample size	α HCH	ß HCH	γ HCH	sum HCH	Reference
Australia	1979/80	Western Australia	45	na	na	1.0#	na	Stacey et al. (1985)
Australia	1979/80	Western Australia	53	na	na	0.0#	na	Stacey et al. (1985)
Australia	1993	Victoria	60 (23 donors)	71	345	108	na	Quinsey et al. (1995)
New Zealand	1987/88	Overall	38	na	11.2	na	na	Bates et al. (1994)
New Zealand	1998/99	Christchurch, Auckland, North Canterbury, Northland	53	0.19	16.3	0.6	na	Bates et al. (2002)
United Kingdom	1989-91	Unknown	193	na	80	<20.0	na	Dwarka et al (1994)
Canada	1992	Southern Canada	54	0.31	22.6	0.76	na	Newsome et al. (1999)
Canada	1996/97	Keewatin	12	4.39	18.2	1.03	na	Newsome et al. (1999)
Canada	1992	national	497	0.31	22.6	1.03	na	Newsome et al. (1999)
Brazil	1987/88	Porto Alegre	30	40	900	20	960	Beretta et al. (1994)
Brazil	1992	Rio de Janeiro	40 (1 pool)	1	270	5	na	Paumgartten et al. (2000)
Nicaragua	1994/95	Rio Atoya	101	n.d.	6	n.d.	na	Romero et al (2000)
Sweden	1972	unknown	227	na	280	na	na	Noren et al. (2000)
Sweden	1974	unknown	250	na	120	na	na	Noren et al. (2000)
Sweden	1976	unknown	1,500	na	130	na	na	Noren et al. (2000)
Sweden	1978	unknown	745	na	120	na	na	Noren et al. (2000)
Sweden	1979	unknown	805	na	110	na	na	Noren et al. (2000)
Sweden	1980	unknown	973	na	96	na	na	Noren et al. (2000)
Sweden	1984/85	unknown	102	na	72	na	na	Noren et al. (2000)
Germany	1992/93	Lower Saxony	156	na	45.0*	16.0*	na	Schlaud et al. (1995)
Germany	1995-97	Schleswig-Holstein	246	na	40	na	na	Schade et al (1998)
Norway	1991	Oslo	28	na	na	na	36	Johansen et al. (1994)
Russia	1996/97	Kargopol	10	4	304	2	296	Polder et al. (2003)
Russia	1996/97	Severodvinsk	37	5	376	1	382	Polder et al. (2003)
Russia	1996/97	Arkhangelsk	40	5	401	2	408	Polder et al. (2003)

Country	Year	Region	Sample size	α HCH	ß HCH	γ HCH	sum HCH	Reference
Russia	1996/97	Naryan-Mar	11	5	183	2	190	Polder et al. (2003)
Russia	1993	Monchegorsk	15	5.6	739	0.7	745	Polder et al. (1998)
Russia	1993	Murmansk	15	4.5	853	0.4	858	Polder et al. (1998)
Poland	(pub. 1998)	Warsaw and regions of Poland	462	0.2#	1.4#	0.2#	na	Czaja et al. (1998)
Poland	(pub. 1998)	Warsaw and regions of Poland	462	0.5#	3.3#	0.4#	na	Czaja et al. (1998)
Kazakhstan	(pub. 1998)	Central and Southern	101	n.d.	2210	n.d.	na	Lutter et al. (1998)
Italy	1987	Florence, Milan, Pavia, Rome	64	na	130	na	na	Larsen et al. (1994)
South India	1988	Chidambaram	11	700	7,900	78	8,800	Tanabe et al. (1990)
South India	1988	Chinnoor Parangipettai	5	170	2,200	24	2,400	Tanabe et al. (1990)
South India	1988	Madras	6	220	2,600	25	2,900	Tanabe et al. (1990)
South India	1988	Nattarasankottai	3	640	9,500	60	10,000	Tanabe et al. (1990)
Zimbabwe	1989	Harare	40	30	840	40	na	Chikuni et al. (1991)
Egypt	1994	Kafr El-Zayat	31	1.85	115	na	15.97	Dogheim et al. (1996)
Egypt	1994	Cairo	11	3.14	191	na	93.44	Dogheim et al. (1996)

* median

\# whole milk basis