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Chlorinated Organic Contaminants in Breast Milk of New Zealand Women

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Breast milk samples from 38 women in New Zealand were analyzed for organochlorine pesticides, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) as part of a World Health Organization collaborative study of breast-milk contaminants. The women were recuited from two urban areas (Auckland and Christchurch) and two rural areas (Northland and North Canterbury) in the North and South Islands of New Zealand. The best predictor of contaminant concentrations in breast milk was found to be the age of the mother. Regional differences were found for hexachlorobenzene, dieldrin, and *pp*-DDE, reflecting historical use patterns. Urban–rural differences were found for several PCBs, PCDDs, and PCDFs when contaminant concentrations were calculated on a whole-milk basis. However, these differences could be attributed to variation in breast-milk fat concentrations between urban and rural mothers. Urban mothers had about 50% more breast-milk fat than rural mothers. Evidence suggests that breast-milk consumption by babies is regulated by caloric intake. Almost all of the caloric content of milk is in the fat fraction. This suggests that breast-milk contaminant levels calculated on a whole-milk basis do not necessarily reflect the relative levels of exposure of infants to these contaminants. However, the factors that influence breast-milk fat concentration deserve further study.

Introduction

New Zealand is a small, temperate country, more lightly industrialized and more heavily dependent on agriculture and horticulture than most other developed countries. This has led to the use of large amounts of pesticides, particularly the herbicide 2,4,5-T, which contains extremely low concentrations of the highly toxic contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD or dioxin) and was used for controlling woody weeds. This herbicide, which was often applied by aircraft, has been the source of much local concern, particularly in regard to the possibility of toxic effects on the fetuses of pregnant rural women. Organochlorine insecticides have also been used in New Zealand, mainly on pasture for the control of grass grub (*Costelytra zealandica*), although their use was largely phased out in the 1970s. However, the persistence of the organochlorine insecticides ensures that they continue to be detected at low levels in the New Zealand food supply (*1*).

Several studies have previously measured organochlorine pesticides and polychlorinated biphenyls (PCBs) in the body fat of New Zealanders (*2–4*). However, this is the first study that has examined the levels of pesticides and PCBs in the breast milk of New Zealand women and the first study that has measured the levels of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)in any tissues of New Zealanders. Breast milk contains a proportion of fat (usually about 2–5%) in which the substances of interest accumulate. Study of contaminants in breast milk, rather than body fat, has the advantage that the sample can be obtained noninvasively and the data can be used for assessing risks to the breast-feeding infant. In addition, an increasing amount of data on breast milk contaminants from various countries can be used for international comparisons (*5*).

This study was part of an intercountry study on levels of PCBs, PCDDs, and PCDFs in breast milk, coordinated by the World Health Organization's Regional Office for Europe (*5,6*), although the protocol was extended to include organochlorine pesticides. An objective of this particular study was to seek possible urban–rural differences in the exposures of New Zealand women because rural dwellers may have been more heavily exposed to pesticides and their contaminants, and urban populations may have been more exposed to motor vehicle emissions and industrial pollutants.

Methods

Sample Collection

Women were recruited between October 1987 and May 1988 from the prenatal clinics of hospitals serving the two largest cities, Auckland (population 900,000) and Christchurch

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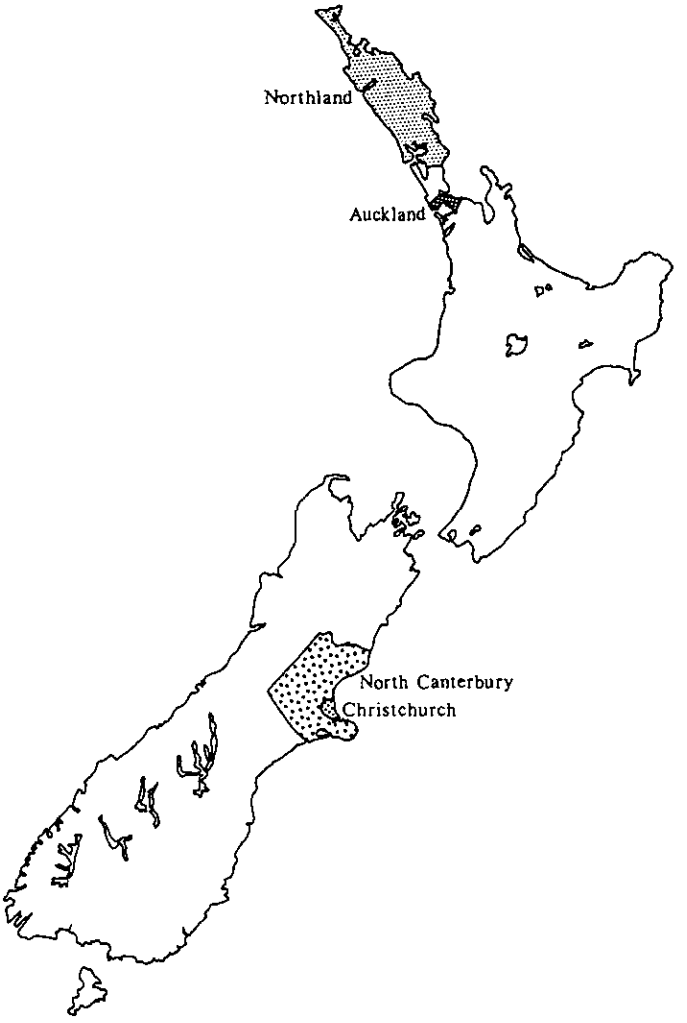

FIGURE 1. Map of New Zealand showing areas where mothers were enrolled.

(population 350,000), in the North and South Islands of New Zealand, plus two rural areas (Northland and North Canterbury), contiguous with the two urban centers (Fig. 1). Selection criteria for participating mothers were based on those recommended for the intercountry collaborative study: *a*) subjects were primiparae between 20 and 30 years of age, *b*) the pregnancy had been normal and both mother and child were healthy, *c*) the mother was breast feeding one child only; *d*) apart from vacations, subjects had lived in the same urban or rural area for at least the last 5 years. " Urban" was defined as living within the boundaries of a city, and "rural" was defined as living more than 3 k from any town with a population of more than 2,500 people.

Collection of samples took place in the second month after birth. Mothers or babies who were ill or not breast feeding were excluded from the study at this stage. Each mother was supplied with a milk collection kit containing specially cleaned containers, plus detailed instructions on the collection procedure. Samples were stored in the home freezer until collection, then stored at -18 °C pending chemical analysis. Each mother completed a questionnaire to provide personal and lifestyle information.

Chemical Analysis

A full description of the analytical methods used in this research will be published separately. However, the methods used are briefly described below.

Organochlorine Pesticides and PCBs. Milk fat was extracted from 25 mL of each breast milk sample, and the fat content was determined (*7*). The extract was cleaned up using Florisil columns, and the organochlorine pesticides and PCBs were analyzed with high-resolution capillary gas chromatography (HRCGC) with electron capture detection. Confirmation was carried out using HRCGC and high-resolution mass spectrometry (HRMS).

PCDDs and PCDFs. About 80–120 mL of each sample was spiked with a range of ^{13}C-labeled PCDD and PCDF standards. Milk fat was extracted from each sample and the fat content determined. The extract was defatted (*8*) and cleaned up (*9,10*) before analysis by HRCGC–HRMS. Quantification was by the isotope dilution method.

Results

A total of 38 samples was collected and analyzed for pesticides and PCBs; 37 had sufficient volume for PCDD/PCDF analysis. The 38 samples came from mothers in Northland (n=10), Auckland (n=11), North Canterbury (n=8), and Christchurch (n = 9). Characteristics of the participating urban and rural mothers and their babies are listed in Table 1.

With the exception of breast-milk fat content, there were no statistically significant differences between the two groups. The urban–rural difference in fat content persisted when areas of residence were separately examined (Table 2). Mothers from both urban areas had a significantly higher fat content than mothers from both rural areas. The possibility that this was an artifact in some way related to the order in which the samples were analyzed for fat content was excluded using Wilcoxon's rank sum test (p=0.91). Fat content was not linearly associated with the age of the mother (r=0.1, p=0.57), although it was associated with body mass index (BMI), which accounted for about 16% of the variation (r=0.40, p=0.01). Urban mothers tended to have slightly higher BMIs.

Analytical data for contaminants measured are presented in the tables, both in terms of milk-fat content and on a whole-milk basis.

Organochlorine Pesticides

Chemical analysis was conducted on 14 organochlorine pesticides (and their metabolites) that had at one time been

Table 1. Characteristics of the mothers and their babies.

	Mean (S. Error)	
Variable	Rural (n=18)	Urban (n=20)
Maternal age, years	25.7 (0.72)	26.7 (0.65)
Maternal height, m	1.60 (0.01)	1.62 (0.02)
Maternal weight, kg	59.6 (1.6)	63.5 (2.7)
Body mass index, kg/m²	22.9 (0.64)	24.2 (0.97)
Fat content of milk, %	2.8 (0.2)	4.1 (0.3)*
Babies' birth weight, kg	3.39 (0.085)	3.43 (0.090)

*P = 0.0005 (t–test).

Table 2. Breast-milk fat content by geographic location of residence.

Area	Type	Mean fat content, %[a]	SE
Christchurch	Urban	4.1	0.53
Canterbury	Rural	2.7	0.22
Auckland	Urban	4.0	0.29
Northland	Rural	2.8	0.24

[a] By analysis of variance both urban areas are significantly different from both rural areas (p=0.01).

registered for use in New Zealand. Only four pesticides or metabolites (hexachlorobenzene [HCB], dieldrin, DDT, and DDE) were found in all samples, and the statistical analyses were confined to those. Limits of detection for pesticides in milk fat ranged from 1 to 23 μg/L.

Table 3 includes the results of analyses of variance for regional differences in pesticide levels detected. When results were expressed in terms of fat content, North Canterbury mothers had the highest pesticide levels detected. However, when calculated on a whole-milk basis, a more complicated pattern emerged, reflecting the higher fat content of the milk of urban mothers.

Also in Table 3 are the results of analyses of covariance for the effects of age on pesticide level while adjusting for region of residence. Both in terms of whole milk or on a fat basis, levels of HCB, dieldrin, and DDE increased linearly with increasing age of the women. Other analyses on a fat content basis found no significant associations between the levels of pesticides detected and BMI, fish intake, smoking status (smoker, nonsmoker or exsmoker), or type of residential water supply (public water supply, roof [rainwater] collection, or private well).

PCBs

Of the 16 PCB congeners and analytically unresolved congener mixtures sought, only 3 were detected in a majority of the samples. Statistical analyses were confined to these three congeners. Table 4 shows an urban–rural comparison, plus the results of linear regressions on age. The different congeners are described according to the nomenclature of Ballschmiter and Zell (*11*).

On a fat basis, no urban–rural or regional differences were found for any of the three PCBs. However, when calculated on a whole-milk basis, the urban level of the hexachloro 153 isomer was significantly higher than the rural level. The urban–rural difference was also somewhat greater for the hexachloro 138 and heptachloro 180 isomers than was the case for results expressed on a fat basis. The age regressions show that the two hexachloro congeners have statistically significant slopes for increasing concentration with age for both fat and whole milk. Other analyses (not shown) found no significant associations between PCB concentrations in fat and BMI, fish consumption, smoking status, or type of water supply.

PCDDs and PCDFs

Of the 15 different PCDD/PCDF isomers and analytically unresolved isomer pairs sought, only 11 were quantitatively detected in a majority of samples. Three were not detected in any samples, and one was found in only eight samples. Statistical analysis was confined to the 11 isomers and isomer pairs detected in most samples. Total toxic equivalency factors (TEFs) have been calculated according to the international system (*12*). Table 5 shows a comparison of PCDD/PCDF levels found in the milk of urban and rural women. No differences were found when results were expressed on a fat basis although, when calculated on a whole-milk basis, three isomers showed significant urban-rural differences. As expected, in each of these cases the concentrations in the milk of urban women were higher than in that of rural women. Table 6 shows the results of linear regressions of contaminant concentrations by age. For both fat and whole

Table 3. Pesticide concentrations in the breast milk of New Zealand women.

	ANOVA by area: mean concentration, μg/kg					ANCOVA (age effect)[b]	
Compound[a]	Auckland (n=11)	Northland (n=10)	Christchurch (n=9)	Canterbury (n=8)	p	Slope, μg/kg/year	p
In milk fat							
HCB	20	21	30	63	0.0001[c]	4.4	0.0001
Dieldrin	48	36	42	67	0.03[d]	3.3	0.01
pp-DDT	105	45	48	112	0.15	-0.8	0.87
pp-DDE	1067	1036	2833	3212	0.0004[e]	212	0.003
In whole milk							
HCB	0.81	0.58	1.27	1.73	0.0075[f]	0.141	0.03
Dieldrin	1.81	0.89	1.66	1.77	0.01[g]	0.98	0.03
pp-DDT	3.86	1.25	2.29	2.86	0.30	-0.011	0.19
pp-DDE	40.2	28.7	119.9	87.0	0.0009[e]	7.29	0.01

Abbreviations: HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; ANOVA, analysis of variance; ANCOVA, analysis of covariance.

[a] Other pesticides and metabolites analyzed (n = no. of positive samples, dl = detection limit in μg/kg of fat): *pp*-TDE (n = 0, dl = 12); *op*-DDT (n=2, dl =20); aldrin (n =0, dl = 2); α-HCH (n = 0, dl = 6); β-HCH (n = 2, dl = 23); γ-HCH (n = 0, dl = 9); heptachlor (n =0, dl = 3); heptachlor epoxide (n = 0, dl = 12); α-chlorodane (n = 0, dl = 6); γ-chlorodane (n = 0, dl = 6).

[b] Model also included terms for area effects.

[c] Canterbury is significantly different from all other areas.

[d] Canterbury is significantly different from Christchurch and Northland.

[e] Canterbury and Christchurch are significantly different from Auckland and Northland.

[f] Canterbury is significantly differnt from Auckland and Northland; Christchurch is significantly different from Northland.

[g] Northland is significantly different from all other areas.

milk, several of the PCDD/PCDF isomer concentrations were strongly associated with age.

Statistical tests sought associations between PCDD/PCDF levels in milk fat and a number of other variables, including BMI, fish consumption, smoking status, and type of residential water supply. With two exceptions, no positive associations were found. The exceptions were 1,2,3,7,8-pentachlorodibenzofuran (1,2,3,7,8-PeCDF), which was associated with BMI (r=0.35, p=0.02), and 2,3,4,6,7,8-hexachlorodibenzofuran (2,3,4,6,7,8-HxCDF), which showed higher levels in those women using roof rainwater collection than in those women receiving water from the public supply (p=0.04). A negative association was found for 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin (1,2,3,4,6,7,8-HpCDD), which was found at higher levels in nonsmokers (p=0.02).

Discussion

This study has been the first to examine levels of organochlorine pesticides, PCBs, PCDDs, and PCDFs in the breast milk of New Zealand women. Our sample of women had two important characteristics: First, the criteria for its selection ensured a high degree of homogeneity in terms of factors known already to influence organochlorine concentrations in breast milk (particularly parity and collection period during lactation). Potentially, this improved the statistical power of our study to detect other factors predictive of contaminant concentrations. Second, half our sample had a stable history of urban residence and half had a stable history of rural residence.

Maternal age was the best predictor we found for contaminant concentrations in breast-milk fat. Increasing age was associated with higher concentrations of pesticides, PCBs, and PCDD/PCDFs (Tables 3, 4, and 6), although some compounds showed no increase with age. An association with increasing age might be expected because these contaminants are all persistent, fat-soluble compounds, known to bioaccumulate in adipose tissue, and breast-milk fat is likely to be in equilibrium with body fat. However, to date, the limited published literature on age-related trends has generally suggested that levels of PCBs in breast milk decrease with maternal age (*13*) and that PCDD/PCDFs in breast milk have no association with maternal age (*14*), although one study (*15*) found increases with age for breast-milk fat concentrations of PCBs and DDE. The rates of increase found were somewhat less marked than those found in this study.

In general, the greater the parity of the mother, the lower the concentration of contaminants in breast milk (*16*). Similarly, concentrations of most contaminants in breast milk decrease over the period of lactation (*13*). Standardizing on these potential confounding factors is likely to facilitate the detection of age-related trends, particularly when the number of subjects is small. In this study, all samples were collected from mothers who were breast feeding their first child and during the second month after birth. With the exception of investigations involved in the current World Health Organization collaborative study, most other studies have obtained breast-milk samples from women of varying parity and at different times during the period of lactation. For example, one study found no association with age for PCDD/PCDF concentrations in breast-milk samples obtained from West German women at various times during their lactation period (*14*). The variation in the period of collection during lactation may have obscured a relationship with maternal age.

What was perhaps unexpected in our results was that, despite the narrow age range of the participating mothers (20–30 years), we still detected marked associations between contaminant concentrations and increasing age. For some compounds the age-regression slopes were remarkably steep, suggesting that New Zealand women less than 20 years old (the lowest age for women to participate) may have particularly low body burdens of organochlorine contaminants. This might be explained by the fact that the use of organochlorine pesticides, PCBs, and compounds containing PCDD/PCDFs has decreased over the last two decades. For example, over the last 20 years, there were several sharp decreases in the concentration of the 2,3,7,8-TCDD contaminant in the herbicide 2,4,5-T, which was widely used in New Zealand. This might explain the particularly steep age-related regression slope for the 2,3,7,8-TCDD isomer. However, such an explanation is unlikely because it would not account for the similarly steep slopes for some of the other PCDD/PCDF isomers, such as 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD (which did not occur in 2,4,5-T).

Our study was designed to detect differences between urban- and rural-living women. Women were selected on the basis of

Table 4. PCB concentrations in the breast milk of New Zealand women.

	Urban–rural comparison, μg/kg, Mean (S. error)		Linear regression on age		
Congener[a]	Urban (n=20)	Rural (n=18)	Intercept, μg/kg	Slope, μg/kg/year	p-Value for slope
In milk fat					
Hexachloro 153	45 (4)	43 (4)	−34	2.9	0.002
Hexachloro 138	26 (2)	28 (3)	−7	1.3	0.01
Heptachloro 180	22 (10)	18 (4)	−32	2.0	0.34
In whole milk					
Hexachloro 153	1.77 (0.17)	1.18 (0.12)*	−1.99	0.13	0.001
Hexachloro 138	1.02 (0.10)	0.79 (0.08)	−0.74	0.06	0.006
Heptachloro 180	0.77 (0.27)	0.56 (0.19)	−1.24	0.07	0.24

[a] Other congeners analyzed (n = no. of positive samples, dl = detection limit in μg/kg of fat): trichloro 28 (n = 3, dl = 6); tetrachloro 74 (n = 0, dl = 9); pentachloro 99 (n = 0, dl = 14); pentachloro 118 (n = 2, dl = 14); hexachloro 146 (n = 0, dl = 12); heptachloro 170 (n = 5, dl = 12); heptachloro 171/174/177 (n = 0, dl = 12); heptachloro 183/185/187 (n = 1, dl = 12); octachloro 194 (n = 1, dl = 3); octachloro 195 (n = 1, dl = 3); octachloro 201 (n = 1, dl = 3); octachloro 202 (n = 1, dl = 3); octachloro 196/203 (n = 1, dl = 3).

* p=0.01 (t-test).

Table 5. Urban–rural differences for polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzofuran isomers in the breast milk of New Zealand women.

Isomer[a]	Mean concentration, ng/kg (SE)							
	In milk fat				In whole milk			
	Urban (n=20)		Rural (n=17)		Urban (n=20)		Rural (n=17)	
Total[b]	16.5	(1.7)	18.1	(1.5)	0.69	(0.11)	0.50	(0.04)
2,3,7,8-TCDF	0.8	(0.07)	1.0	(0.24)	0.03	(0.003)	0.02	(0.007)
2,3,7,8-TCDD	5.1	(0.8)	5.1	(0.5)	0.22	(0.04)	0.14	(0.01)
2,3,4,7,8-PeCDF	5.3	(0.5)	5.6	(0.5)	0.22	(0.02)	0.16	(0.02)
1,2,3,7,8-PeCDD	7.0	(0.8)	7.9	(0.7)	0.30	(0.06)	0.20	(0.02)
1,2,3,4,7,8-HxCDF/ 1,2,3,6,7,8-HxCDF	6.7	(0.8)	8.7	(1.0)	0.30	(0.04)	0.20	(0.03)
2,3,4,6,7,8-HxCDF	0.8	(0.1)	1.0	(0.2)	0.03	(0.004)	0.03	(0.004)
1,2,3,4,7,8-HxCDD/ 1,2,3,6,7,8-HxCDD	30	(3)	38	(5)	1.2	(0.2)	1.1	(0.2)
1,2,3,7,8,9-HxCDD	5.6	(0.6)	6.3	(0.7)	0.2	(0.03)	0.2	(0.02)
1,2,3,4,6,7,8-HpCDF	6.7	(0.5)	7.6	(0.9)	0.3	(0.03)	0.2	(0.02)*
1,2,3,4,6,7,8-HpCDD	52	(4)	52	(5)	2.1	(0.2)	1.4	(0.2)*
OCDD	220	(20)	200	(10)	9.0	(1.0)	5.5	(0.5)⁻

Abbreviations: CDF, chlorinated dibenzofuran; CDD, chlorinated dibenzodioxin, T, tetra; Pe, penta; Hx, hexa, Hp, hepta; O, octa.
[a] Other isomers analyzed (n = no. of positive samples, dl = detection limit in ng/kg of fat): 1,2,3,7,8-PeCDF (n = 8, dl = 0.1); 1,2,3,7,8,9-HxCDF (n = 0, dl = 0.5); 1,2,3,4,7,8,9-HpCDF (n = 0, dl = 0.5); OCDF (n = 0, dl = 5).
[b] Total toxic equivalents (international system).
* $p < 0.05$.
⁻ $p < 0.01$.

Table 6. Results of simple linear regression analyses by age for polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzofuran isomers in the breast milk of New Zealand women.

	Linear regression on age					
	In milk fat			In whole milk		
Isomer	Intercept, ng/kg	Slope, ng/kg/year	p for slope	Intercept, ng/kg	Slope, ng/kg/year	p for slope
Total[a]	-16.8	1.30	0.0004	-1.13	0.07	0.002
2,3,7,8-TCDF	1.21	-0.01	0.77	0.017	0.0004	0.35
2,3,7,8-TCDD	-10.57	0.60	0.0001	-0.54	0.03	0.001
2,3,4,7,8-PeCDF	-5.14	0.40	0.0005	-0.29	0.02	0.0006
1,2,3,7,8-PeCDD	-5.12	0.48	0.006	-0.47	0.03	0.01
1,2,3,4,7,8-HxCDF/ 1,2,3,6,7,8-HxCDF	-0.03	0.3	0.18	-0.03	0.02	0.009
2,3,4,6,7,8-HxCDF	-0.14	0.04	0.31	-0.02	0.002	0.04
1,2,3,4,7,8-HxCDD/ 1,2,3,6,7,8-HxCDD	-9.14	1.65	0.10	-1.44	0.10	0.03
1,2,3,7,8,9-HxCDD	-3.68	0.37	0.03	-0.29	0.02	0.01
1,2,3,4,6,7,8-HpCDF	5.08	0.08	0.65	-0.09	0.01	0.07
1,2,3,4,6,7,8-HpCDD	0.25	2.00	0.08	-0.43	0.09	0.11
OCDD	-2.11	8.15	0.03	-2.83	0.40	0.09

Abbreviations: CDF, chlorinated dibenzofuran; CDD, chlorinated dibenzodioxin; T, tetra; Pe, penta; Hx, hexa; Hp, hepta; O, octa.
[a] Total toxic equivalents (international system).

stable residence in two urban and two rural areas, one of each in both the North and South Islands. Generally, our examination of urban–rural differences was reassuring. For concentrations of PCBs and PCDD/PCDFs in breast-milk fat, we found no significant urban–rural differences or differences between the four areas (Tables 4 and 5). This is particularly noteworthy for 2,3,7,8-TCDD because the detected levels of organochlorine pesticides varied markedly between the areas. In particular, when breast-milk fat concentrations were compared, North Canterbury women had significantly higher levels of HCB, dieldrin, and *pp*-DDE than women from most other areas (Table 3). For *pp*-DDE, women from Christchurch also had high levels. Overall, no urban–rural difference was found, although there was a marked and significant difference between North Island and South Island women for HCB and *pp*-DDE (analysis not shown). In both cases the South Island women had the highest levels. These differences appear to reflect the relative regional levels of use (at least historically) of these pesticides, particularly their use to control grass grub in North Canterbury.

The compound *pp*-DDE is a metabolic product of the persistent insecticide DDT, widely used in New Zealand until the early 1970s. Limited use under permit continues today. Levels of *pp*-DDT itself were not significantly different among the areas (although North Canterbury did have the highest mean level). Also, no association was found between levels of *pp*-DDT and maternal age, although such an association is strong for *pp*-DDE (Table 3). This is probably because the levels of DDT reflected recent exposures to this compound, and *pp*-DDE levels reflected cumulative exposures to DDT over a long period.

The urban–rural comparison of whole-milk levels of the

contaminants produced different results. The relative levels of pesticides in the different areas no longer clearly reflected use patterns (Table 3). Also, the levels of three PCDD/PCDF isomers were significantly elevated in urban women (Table 5), as was one PCB congener (Table 4). Because these substances showed no urban-rural differences in terms of fat concentrations, the whole-milk contaminant pattern can be assumed to reflect differences in the fat content of milk between urban and rural women.

Women from the urban areas had about 50% more fat content in their breast milk than did women from rural areas. This difference was highly statistically significant ($p=0.0005$) and unexpected. The consistency of the difference across urban and rural areas (Table 2) suggests that this is more than a random statistical fluctuation. It is partially accounted for by the higher body mass indexes of the urban women. However, this could explain only a fraction of the difference.

It is known that the fat content of breast milk varies during a feed, the higher concentrations being at the end of the feed. Our instructions to mothers were designed to minimize variation due to this source; instructions specified collection of milk samples at the beginning of the feed. A possible explanation for the urban–rural difference is that urban women tended to disregard these instructions and collected their samples at the end of the feed. However, we have no evidence that this was the case. Moreover, the South Island (Christchurch and North Canterbury) women were all recruited through one maternity hospital and were instructed in the collection of their breast milk samples by the same group of health professionals. This suggests milk collection procedures were likely to be similar for both urban and rural mothers. The mean percentage fat content of the breast milk samples of a large number of published studies ranged from 1.44 to 5.0 (*17*). Both our urban and rural mean levels were well within this range, although the reason for their difference is unknown. At least two other investigations in the same collaborative study have examined an urban–rural difference. However, these studies from Belgium and the Netherlands, did not report any difference in fat content between urban and rural mothers' breast milk (*5*). It is possible that the definitions of "urban" and "rural" used in those studies for selection of mothers were different from the definitions that we used (see Methods).

Whatever the explanation for the urban–rural difference in fat content, there is some evidence that the amount of breast milk consumed by an infant is related to the caloric content of the milk (*18*). Most of the calories in breast milk are in the fat fraction. For that reason, urban infants are likely to consume a lower volume of breast milk than their rural counterparts and would not necessarily be at greater risk from fat-soluble breast milk contaminants. Many studies in the literature present breast-milk contaminants as a proportion of whole milk. However, on the basis of our results it seems that whole milk comparisons, particularly between areas, may sometimes be misleading.

None of the other factors that we assessed, including dietary type, smoking status, type of water supply, and body mass index, showed any convincing association with the levels of the various compounds we detected. In some cases the lack of associations may have been due to limited variation in exposure. For example, virtually all the women in our study reported eating a "mixed diet." Thus, finding a significant difference associated with dietary type would have been practically impossible.

In three cases involving BMI, smoking status, and water supply source, we found statistically significant associations, both positive and negative, for individual PCDD/PCDF isomers (see Results). The likely explanation for these associations, in the absence of other plausible interpretations, is that they arose as a result of our having performed multiple statistical comparisons.

Results of other studies of pesticides, PCBs, and PCDD/PCDFs in breast milk have been summarized (*5,17*). Overall, most of the contaminant levels we found fall in the low to mid-range relative to levels obtained in comparable studies of women in other developed countries. The exception is DDE, levels of which tended to be comparatively high in our study, particularly in North Canterbury women.

In conclusion, we have shown that it is possible to detect regional differences, and possibly other factors, affecting breast-milk contaminant concentrations, even with a small number of participating women. However, to achieve this it may be necessary for the women to be fairly uniform in terms of potential confounding factors known to affect milk contaminant concentrations, particularly parity and period of lactation. We have also shown that, if there are factors that differentially influence breast-milk fat concentrations between areas, comparing contaminant concentrations in terms of whole-milk levels may be misleading. However, little is known about factors that influence breast-milk fat concentrations. These and the implications they have for infant health deserve further investigation.

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