LONGITUDINAL CHANGES OF ORGANOCHLORINE PESTICIDE RESIDUES IN BREASTMILK

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Experimental

Breastmilk samples were collected from 36 primiparous women in the UK on day 2 or 4, 7, 14 days and 1, 3 and 6 months post partum (pp). Samples were expressed using Avent ISIS manual breastpumps, mid-morning after the infant had fed and from the breast on which the infant had fed. Sixteen organochlorine pesticide residues were sought; fat was extracted from the samples using acetone/hexane. The extracts were dried and the solvent was evaporated using a Kuderna-Danish distillation apparatus. The fat was re-dissolved in hexane and the resulting solution was divided for separate high performance gel permeation chromatography (HPGPC) clean-up, or subjected to sulfuric acid matrix decomposition (SMD) clean-up. Analysis was by gas chromatography (GC) with electron capture detection (ECD) or electron impact mass spectrometry (MS) with selective ion monitoring (SIM). A limit of quantification of 0.01 mg/kg breastmilk fat was achieved for all samples of $\geq$5g. All statistical analyses were carried out using SPSS for Windows release 9.0.0 (SPSS Inc. Headquarters, 233 S. Wacker Drive, 11th floor, Chicago, Illinois 60606, USA).

Mean recoveries were 77-106%.

Results

Of the 53 women enrolled into the study, 37 provided breastmilk samples. Background information on women is shown in table 2. Two hundred and seventy four samples were collected of which 249 were of sufficient volume for analysis (27 samples <5g, 60 samples 5-9.9g, 178 samples $\geq$ 10g).

Twenty women had not dieted before pregnancy; seventeen women had dieted before pregnancy losing 1-14.5 kg at any one time (mean 3.2 kg).

Residues of ten of the 16 organochlorine pesticides, their impurities or breakdown products were not detected in any sample (α-HCH, heptachlor, heptachlor epoxide, aldrin, endrin, oxychlordane, cis-chlordane, trans-chlordane, *o,p'*-DDT or *p,p'*-TDE). Because of the very small size of some of the samples, it was not always possible to reach a limit of quantification of 0.01 mg/kg breastmilk fat. Residues of 6 pesticides were detected and are summarised in table 2. Only samples collected between 8am and 2pm are included to try to reduce any possible effects that circadian variation (Hytten, 1954) may have on breastmilk fat concentrations.

Ln transformed residues data were analysed by ANOVA test to determine the significance of the changes in residue concentration with time and between women. The Wilcoxon Signed Ranks test was also carried out on concentrations with time to confirm findings of ANOVA. The results are in table 3. Only datasets for HCB, *p,p'*-DDE and β-HCH had a sufficient number of samples with detectable residues to carry out this analysis.

Despite the fact that residue concentrations appear highly variable, they do not reach statistical significance with time (figures 1 and 2).

Multiple linear regression was used to determine whether any of the other studied parameters affected the concentration of HCB, β-HCH or *p,p'*-DDE in breastmilk.

For ln mean *p,p'*-DDE, 28.3% of the variation in residues could be explained by maternal age (p<0.001, B=0.071 (95%CL 0.034-0.109)).

The equation describing the relationship was:

ln mean *p,p'*-DDE = (0.0713 x maternal age) - 4.3584.

For ln mean HCB, 9.7, 15.2and 19.6% of the variation in residues could be explained by maternal body weight pre-pregnancy (p=0.049, B=0.010 (95%CL 0.000-0.020)), pregnancy (p=0.019, B=0.013 (95%CL 0.002-0.024) and 30 days pp (p=0.009, B=0.016 (95%CL 0.004-0.027).

The equation describing the relationship at 30 days pp was:

ln mean HCB = (0.0157 x maternal body weight at 30 days pp) -5.235.

Discussion

This is the first study considering changes to concentrations of organochlorine pesticide residues in breastmilk to have been carried out in the UK. β-HCH, *p,p'*-DDE and HCB were detected in samples from a high percentage of women (>85-96%) but was not always detected in all samples; *p,p'*-DDT, dieldrin and γ-HCH were detected in samples from a much lower percentage of women. Comparing findings in this study to those of the last survey of breastmilk in the UK carried out in 1997-8 (Harris *et al.*, 1999), this study shows a higher percentage of samples containing detectable residues. Samples in this current study were collected exclusively from primiparous women compared to the previous UK survey where parity varied between 1-6; other studies have shown parity to be negatively correlated with concentrations of DDT, DDE, β-HCH, dieldrin, HCB, *trans*- and *cis*-nonachlor or chlordane with increasing parity (summarised in Harris *et al.*, 2001) so, again, this is not an unexpected finding. However, maximum, median and mean concentrations of the six detected organochlorine pesticide residues were all lower in this study compared to the previous UK survey.

In assessing relationships which influence the occurrence of organochlorine pesticide residues in breastmilk fat, maternal age was a significant predictive factor for *p,p'*-DDE. This is consistent with other studies where concentrations of the more biologically persistent organochlorines increased with age (summarised in Harris *et al.*, 2001).

Mobilisation of fat reserves was one of the most commonly cited reasons for the transfer of organochlorines into breastmilk. However, there was very little evidence presented to support these conclusions. A number of studies have examined body mass index (BMI; weight (kg)/height^2 (m^2)) or weight changes during lactation. Generally these studies have concluded that there is little or no correlation with body weight changes or BMI (summarised in Harris *et al.*, 2001). Where concentrations were affected by weight, overweight women were reported to excrete lower concentrations (Polishuk *et al.*, 1977) or concentrations of dieldrin were shown to increase with increasing weight loss (but only after prolonged lactation) (Dagnelie *et al.*, 1992). However, in this study, a significantly positive correlation i.e. heavier women excreted higher residue concentrations of HCB, was seen with maternal body weight at three time periods up to 30 days pp. This lends some support to the conclusions that mobilisation of fat stores can contribute to the occurrence of organochlorine residues in breastmilk.

References

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	mean	range
age (years)	30.7	20-41
height (m)	1.66	1.52-1.77
pre-pregnancy weight (kg)	62.5	45.0-106.2
pregnancy weight (kg)	75.1	55.4-104.0
30 day post-partum weight (kg)	66.4	49.8-97.4
pregnancy weight gain (kg)	13.1	-2.2-24.1
infant birth weight (kg)	3.58	2.66-4.40

Table 1. Summary of women in study

	HCB	γ-HCH	β-HCH	*p,p'*-DDE	dieldrin	*p,p'*-DDT
Minimum	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Mean	0.017	0.028	0.050	0.167	0.027	0.026
Median	0.016	0.018	0.028	0.112	0.013	0.018
Maximum	0.038	0.065	0.523	1.411	0.217	0.084
Standard Error	0.000	0.007	0.006	0.014	0.014	0.003
Standard Deviation	0.005	0.020	0.090	0.219	0.052	0.020
% samples containing detectable residues	59.6	3.6	77.2	96.0	6.0	19.6
% women with detectable residues	86.5	8.1	97.3	100	24.3	32.4

Table 2. Summary of detectable organochlorine pesticide residues in breastmilk of primiparous women

	ANOVA - between women (p)	ANOVA - with time (p)	Variance attributable to women (%)	Wilcoxon Signed Ranks test (p)
HCB	<0.001	0.944	3.3	0.068-0.917
β-HCH	<0.001	0.844	13.9	0.068-0.917
p,p'-DDE	<0.001	0.891	35.1	0.069-0.983

Table 3. Significance of changes in organochlorine pesticides residues (ln transformed) with time and between women donating samples

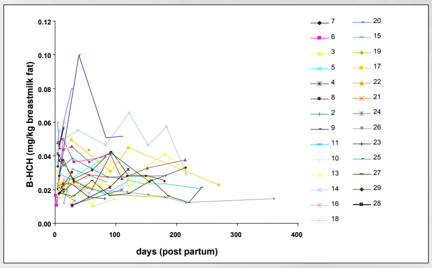

Figure 1. Longitudinal changes of β-HCH in breastmilk fat

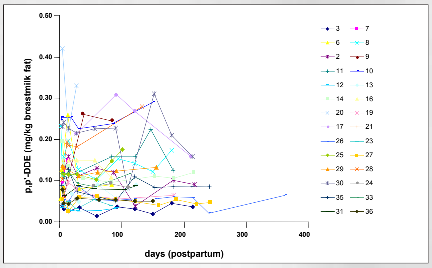

Figure 2. Longitudinal changes of *p,p'*-DDE in breastmilk fat

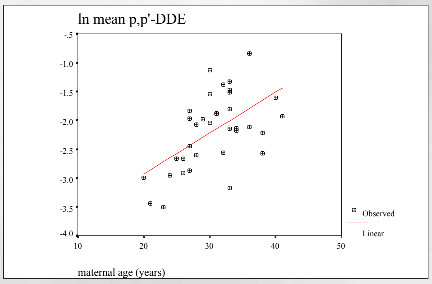

Figure 3. The relationship between maternal age and *p,p'*-DDE concentration in breastmilk

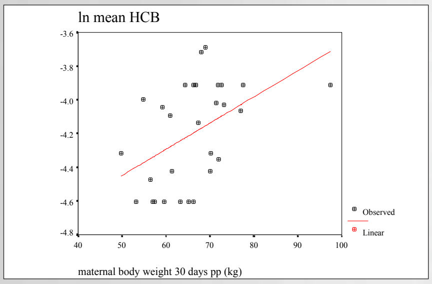

Figure 4. The relationship between maternal body weight at 30 days pp and HCB concentration in breastmilk

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