

Organochlorine Pesticide Residues in Human Breast Milk and Placenta in Tohoku, Japan

Kunihiko Nakai¹, Tomoyuki Nakamura³, Keita Suzuki¹, Tomoko Oka¹, Kunihiro Okamura², Norio Sugawara¹, Yoshinori Saitoh³, Takashi Ohba¹, Satomi Kameo¹, Hiroshi Satoh¹

¹Environmental Health Sciences, Tohoku University Graduate School of Medicine, Sendai

²Department of Obstetrics, Tohoku University Graduate School of Medicine, Sendai

³Miyagi Prefectural Institute of Public Health and Environment

Introduction

Organochlorine pesticides are compounds widespread in the environment due to their persistence and highly lipophilic nature, and they accumulate in biological systems. Newborns are exposed to these organochlorine compounds across the placenta and through breastfeeding. Perinatal exposure to these compounds may induce several adverse effects such as lower birth weight¹, neurodevelopmental delay², and disturbance of thyroid hormone status³. DDT, especially, has been suggested to be a neuroendocrine disruptor as well as a functional teratogen in humans^{4 5}. Other pesticides such as dieldrin and endosulfan were also recognized to have estrogenic hormonal activity in animal studies.

Recently, we have started a birth cohort study to examine the effects of exposure to persistent organochemical pollutants and heavy metals on neurodevelopment in Japanese children, The Tohoku Study of Child Development⁶. In this cohort study, biological samples, including maternal peripheral blood, cord blood, placenta, cord tissue, and breast milk have been collected from more than six hundred mother-infant pairs for chemical determinations. The growth of infants has been monitored using neurodevelopmental tests, including the Brazelton Neonatal Behavioral Assessment Scale, the Bayley Scale of Infant Development, the Kyoto Scale of Psychological Development, and others. Exposures to dioxin and related compounds, polychlorinated biphenyls, methylmercury, and several heavy metals were assessed. Additionally, since perinatal exposure to organochlorine pesticides may affect the neurodevelopment of children, we examined the effects of those pesticides in the cohort study.

In the present study, several organochlorine pesticides were analyzed in human breast milk and placenta from 20 mothers to identify the major pesticide compounds found in the cohort subjects. The relationship between pesticides in breast milk and the placenta was analyzed to examine the utilization of the placenta as the material for exposure assessment. Some information regarding the factors affecting the contamination of breast milk and the placenta with organochlorine pesticides

are also discussed.

Methods and Materials

This study was performed as part of our prospective cohort study ⁶. Healthy pregnant women were recruited with their informed consent at obstetrical wards of two hospitals in Tohoku between January 2001 and September 2003. Twenty subjects were randomly selected from the registered subjects of the cohort study, and pairs of breast milk samples and placenta samples were used. The ages of mothers ranged from 21 to 39. The placenta was taken immediately after the delivery, and divided into 20-30 pieces that were randomly separated into 4 groups. Each bottle contained 50-100 g of tissue. The representative samples were finally prepared by homogenization. The mothers were asked to provide breast milk one month after the delivery. The breast milk sample was taken directly into a clean glass bottle. These samples were frozen at -80°C until analysis. Each mother completed a questionnaire to provide personal information such as the number of births, smoking, alcohol consumption during pregnancy, occupation, educational background, food intake, and place of residence. The study protocol was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine.

The pesticides examined were hexachlorobenzene (HCB), α -hexachlorocyclohexane (HCH), β -HCH, γ -HCH, δ -HCH, cis-chlordane, trans-chlordane, oxy-chlordane, cis-nonachlor, trans-nonachlor, p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD, o,p'-DDD, aldrin, endrin, dieldrin, α -endosulfun, β -endosulfun, heptachlor, heptachlorepoxyde, and methoxychlor. Gas chromatographic determination of these organochlorine pesticides was performed with the collaboration of SRL, Inc. (Tokyo, Japan) for sample extraction and Toray Research Center (Tokyo, Japan) for gas chromatography. Briefly, after the samples were spiked with $^{13}\text{C}_6$ -HCB, $^{13}\text{C}_6$ - β -HCH, $^{13}\text{C}_{12}$ -p,p'-DDT, $^{13}\text{C}_{12}$ -endosulfun, and $^{13}\text{C}_{10}$ -chlordane, they were extracted with ethanol/hexane. The organic extracts were finally purified with the use of a Florisil column, and the eluates were concentrated and spiked with $^{13}\text{C}_{12}$ -pentaPCB(#118). A mass spectrometer (AutoSpec, Micromass) coupled to a Hewlett-Packard model HP6800 capillary gas chromatograph equipped with a capillary column (BPX-35, 0.25 mm ID x 25 m, film thickness 0.33 μm , SGE) was used for determination of pesticides. Residue levels were expressed as ng/g extracted fat.

Results and Discussion

HCB, β -HCH, oxy-chlordane, cis-nonachlor, trans-nonachlor, p,p'-DDT, p,p'-DDE, dieldrin, and heptachlorepoxyde were found from all breast milk samples and placenta samples as shown in Table 1, whereas levels of α -HCH, γ -HCH, δ -HCH, cis-chlordane, trans-chlordane, o,p'-DDT, o,p'-DDE, p,p'-DDD, o,p'-DDD, aldrin, endrin, α -endosulfun, β -endosulfun, heptachlor, and methoxychlor were very low or below the detection limit (data not shown). Since using of these organochlorine compounds had been prohibited in the field in the 1970-1980s in Japan, these results reconfirmed their environmentally persistent nature. In Japan, the concentrations of PCBs, β -HCH, and DDTs in breast milk declined gradually from the peak levels observed at the mid-1970s and almost reached equilibrium states ⁷. However, it remains to be elucidated whether the current low levels of organochlorine pesticides affect the neurodevelopment of children.

The concentration of organochlorine pesticides in breast milk mainly depends on their accumulation in the maternal fatty tissue and their subsequent mobilization. Indeed, numerous studies around the world have used human breast milk samples to determine maternal body burden and lactational transfer of pesticides to infants. Since there were excellent correlations of all major pesticides between breast milk samples and placenta samples (Table 1, and the two typical relationships in Fig. 1), placenta is also suggested to be the useful material to estimate the maternal body burden. In addition, the concentrations of some organochlorine pesticides such as HCB, oxy-chlordane, and trans-nonachlor, in the placenta samples had significant negative correlations with parity (Table 2). This finding clearly shows that the mothers eliminate these pesticides during pregnancy and by breastfeeding them into their children. Considering that the concentration of pesticides in breast milk samples had no significant correlation with parity, monitoring of the placental pesticide concentration may contribute to determining the prenatal exposure of infants to organochlorine pesticides. The placenta is a relatively large organ, and is usually discarded after delivery. Utilization of the placenta is possibly suggested for the purpose of assessment of exposure to chemicals.

Table 1: Organochlorine pesticide concentrations in the human milk samples and placenta samples, and the relationship between the 2 samples.

| Pesticide | Milk (ng/g-fat) | Placenta (ng/g-fat) | Correlation Coefficient Milk x Placenta |
|-------------------|--------------------|------------------------|--|
| Hexachlorobenzene | 17.1±10.1 | 9.9±4.1 | 0.693** |
| β-HCH | 83.4±55.1 | 21.5±12.6 | 0.919** |
| oxy-Chlordane | 7.2±3.4 | 2.3±0.9 | 0.644** |
| cis-Nonachlor | 3.7±1.7 | 0.8±0.4 | 0.589** |
| trans-Nonachlor | 18.8±8.6 | 3.8±2.2 | 0.679** |
| p.p'-DDT | 6.2±3.5 | 1.4±0.6 | 0.746** |
| p.p'-DDE | 142.3±73.5 | 46.0±34.6 | 0.569** |
| Dieldrin | 5.0±3.6 | 1.7±1.1 | 0.808** |
| Heptachlorepoide | 3.7±1.4 | 1.4±0.3 | 0.881** |

Spearman's correlation analysis, ** p<0.01, * p<0.05

Table 2: Correlation coefficient values of organochlorine pesticides with fish intake, maternal age at delivery, and parity.

| Pesticide | Fish consumption | | Maternal age | | Parity | |
|-------------------|------------------|----------|--------------|----------|--------|----------|
| | Milk | Placenta | Milk | Placenta | Milk | Placenta |
| Hexachlorobenzene | 0.023 | -0.127 | -0.421 | -0.223 | -0.429 | -0.625** |
| β-HCH | 0.064 | 0.034 | 0.244 | 0.375 | 0.025 | -0.064 |
| oxy-Chlordane | 0.609** | 0.515* | -0.208 | 0.033 | -0.428 | -0.521* |
| cis-Nonachlor | 0.486* | 0.356 | -0.085 | 0.234 | -0.093 | -0.354 |
| trans-Nonachlor | 0.701** | 0.475* | -0.133 | 0.155 | -0.282 | -0.471* |
| p.p'-DDT | 0.341 | 0.267 | -0.179 | 0.06 | -0.053 | 0.089 |
| p.p'-DDE | 0.054 | 0.412 | -0.165 | 0.169 | -0.174 | -0.131 |
| Dieldrin | 0.463* | 0.518* | -0.109 | 0.004 | 0.12 | 0.033 |
| Heptachlorepoide | 0.566** | 0.711** | -0.185 | -0.169 | -0.054 | -0.235 |

Spearman's correlation analysis, ** p<0.01, * p<0.05

Some organochlorine pesticides have been thought to be introduced to humans partly through the consumption of fish and related products⁸. The concentrations of oxy-chlordane, nonachlors, dieldrin, and heptachlorepoide in breast milk samples and placenta samples were indeed correlated with fish consumption; however, HCB, HCH, and DDE had no association. These results indicated that the contribution of fish consumption to the intake of pesticides was dependent on the kind of pesticide. More information regarding risk analysis of pesticide intake is needed for risk management. Maternal age at the time of delivery and parity have been shown to be important factors affecting the concentration of pesticides in breast milk samples⁸. Although parity was a potent factor in our data (Table 2), maternal age had no significant relationship with the concentrations of pesticides in breast milk samples and placenta samples. However, since parity correlated significantly with maternal age (data not shown), multiple regression analysis should be performed to control for the effects of covariates. These issues, and identification of the factors affecting the contamination levels of organochlorine pesticides in breast milk and placenta will be readdressed when we increase the sample size.

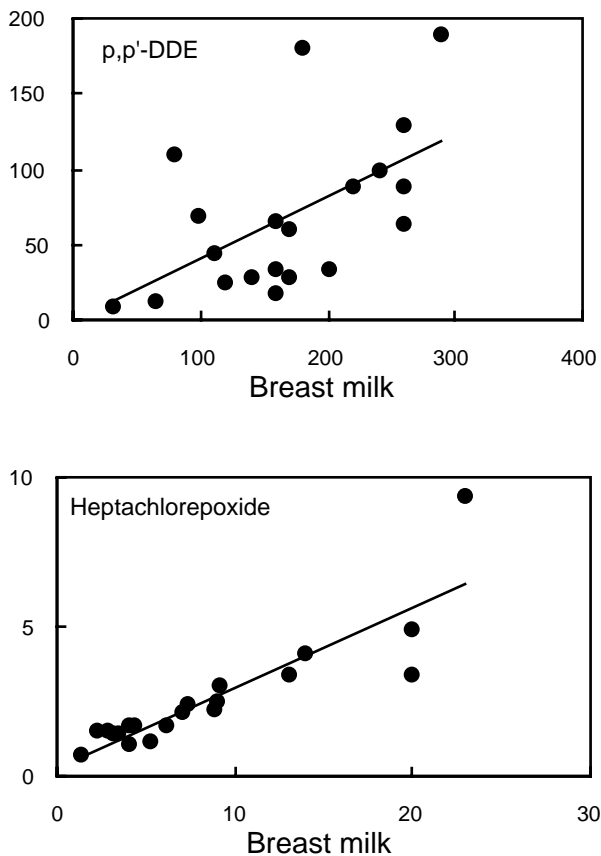


Fig. 1.: Relationship of p,p'-DDE (upper) and heptachlorepoxide (lower) between breast milk and placenta. ng/g-fat, N=20.

Acknowledgment

We thank all families who participated in this study. This research was funded by several grants from the Ministry of Health, Labor and Welfare (Risk Analysis Research on Food and Pharmaceuticals, H15-006), from the Ministry of Education, Culture, Sports, Science and Technology (B, #14370118), and from the Japan Public Health Association (Health Sciences Research Grant on Environmental Health), Japan.

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