

# **DIOXIN AND ORGANOCHLORINE PESTICIDE CONCENTRATIONS OF BREAST MILK IN CHINA AND JAPAN.**

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## **Introduction**

Breast milk contains many lipid soluble compounds that are present in mother's adipose tissue. It means that the levels of dioxins and dioxin-like PCBs in breast milk are representing for those in serum lipid and adipose tissue as body burdens. Dioxins and other persistent contaminants in breast milk are apparently a great concern for the health of the neonates. To elucidate body burden of dioxins and organochlorine pesticides in China and Japan, we have collected breast milk specimens in these countries. But the sample volume collected and the funding were limited to measure dioxin concentrations in breast milk by HRGC-MS. For large-scale epidemiological studies, quick and accurate measurements of dioxins are requested. We previously reported a validation study of the CALUX assay in biological samples<sup>1,2,3</sup>. In this paper, we demonstrate that CALUX assay for dioxin was applied for breast milk, and we validated measurement of breast milk by Dioxin and PCB specific (DIPS)-CALUX cell assay in comparison with HRGC/MS method. From our best knowledge, it will be the first report on contaminations of dioxins and organochlorine pesticide in breast milk among Chinese in northern region.

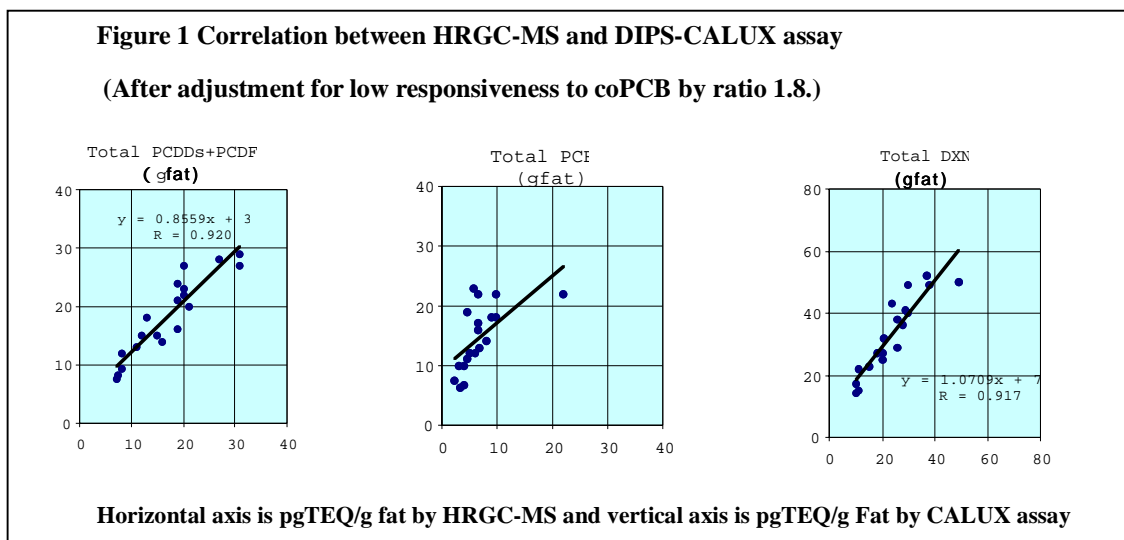
## **Material and Methods.**

We have collected breast milk samples from 47 breast-feeding mothers in Dalian and 32 in Shenyang, Liaoning Province, China, and 20 samples in Japan after collecting informed approval on dioxin measurements from the subjects. In China, the specimens were collected within a week after the

delivery. In Japan, collection time ranged from 3 to 12 weeks after delivery. We measured in total 99 samples by DIPS-CALUX assay at Hiyoshi Corporation. Results for test samples measure by CALUX bioassay for dioxins were compared in a double blind study to HRGC/MS results measured at SRL, Inc.. Persistent organochlorine pesticide concentrations in the Chinese milk were also measured by GC-ECD.

**Results and Discussion:**

Two samples, one from Japan, one from China, with exceptionally low lipid percentage (0.5%, 0.6%) were excluded from the statistical analysis. Correlation coefficient (R) of PCDD+PCDF and PCDD+PCDF+coPCB per g fat were 0.920 and 0.763, respectively. As CALUX assay is slightly less sensitive to coPCB, we adjust low responsiveness by multiplication of 1.8 to the coPCB values, the R value for PCDD+PCDF+coPCB per gram fat was 0.917. We concluded that DIPS-CALUX assay was sensitive enough to apply for human breast milk specimens.



**Talbe 1 GM, median, and range of dioxins concentrations in breast milk in Japan**

	HRGC/MS	CALUX
PCDD+PCDF	0.52, 0.48, (0.25-1.3)	0.52, 0.49, (0.28-1.3) pgTEQ/gwet
coPCB	0.19, 0.18, (0.08-1.10)	0.13, 0.18, (0.05-0.46)
Total	0.72, 0.67, (0.36-2.40)	0.69, 0.66, (0.4-1.4)
PCDD+PCDF	15.5, 19.0, (7-31)	17.0, 18.0, (7.7-29) pgTEQ/gFat
coPCB	5.8, 5.9, (2.2-22)	4.3, 4.5, (1.7-17)
Total	21.6, 24.0, (10-49)	22.5, 20.0, (13-37)

Data analyzed in 19 samples of breast milk from Japanese mothers.

In Table 1, geometric means of dioxin concentrations of Japanese breast milk (n=19) by HRGC/MS were 15.5 pgTEQ/gFat for PCDD+PCDF, 5.8 for coPCB, and those by CALUX were 17.0 for PCDD+PCDF, 4.3 for coPCB, respectively. The values were almost the similar levels of dioxin concentration previously reported in other districts in Japan.

**Table 2 Geometric mean, median and range of dioxins in breast milk**

	Shenyang	Dalian	Japan
n	32	47	19
PCDD+PCDF	8.4, 7.2, (1.6-48.7)	14.5, 14.7, (3.5-148)	17.0, 18.0, (7.7-29)
coPCB	1.8, 1.6, (0.7-11.3)	3.9, 3.5, (1.1-17.0)	4.3, 4.5, (1.7-17)
Total	10.6, 8.8, (3.2-54.1)	19.4, 18.0, (6.1-158)	22.5, 20.0, (13-37)

**The values are measured by CALUX Assay**

The values by CALUX were 10.6 pgTEQ/gFat in Shenyang and 19.4 pgTEQ/gFat in Dalian. Both the values of PCDD+PCDF and coPCB fractions in Dalian were higher than those in Shenyang. It is noteworthy that the levels of the contaminations of Dalian were close to those of Japan. It depicts that dioxin body burden in Dalian may be higher than that in Shenyang. This difference might be due to people in Dalian eat more marine products than those in Shenyang.

**Table 3 Geometric mean, median and range of dioxins in breast milk among age groups**

Age	Shenyang		Dalian		Japan	
	n	pgTEQ/g fat	n	pgTEQ/g fat	n	pgTEQ/g fat
20-25 yo	9	10.8, 9.4, (3.2-54.1)	5	16.0, 17.6, (6.1-71.6)	0	
26-30 yo	16	11.8, 9.2, (4.5-48.4)	36	19.4, 18.5, (7.4-158.9)	4	18.2, 18.5, (16-20)
older than 31	7	8.0, 7.6, (5.1-13.5)	3	20.7, 17.4, (12.6-40.5)	15	25.7, 20.0, (13-78)

**The values are measured by CALUX Assay**

It was also reported previously that dioxin concentrations elevated when mothers are older. But a distinctive difference from Japanese data was that no age-dependent elevations of dioxin concentrations were observed in both cities of China (Table 3). PCB, p,p'DDE,  $\beta$ -hexachlorohexane ( $\beta$ -HCH), and hexachlorobenzene (HCB) were also measured in remaining 20 samples from Dalian and 20 from Shenyang. It revealed that these contaminants were significantly higher in the specimens from Dalian than those from Shenyang (Table 4). There was also no age-dependent elevation of these contaminants in breast milk in China which is also distinct in other developed countries. There might exist heterogeneous sources and routes of the contaminant exposure among these subjects, even if they live in the same city.

**Table 4 Geometric mean, median and range of POPs in Chinese breast milk**

POPs	Shenyang	Dalian
n	20	20
PCB	16.0, 17.6, (6.1-71.6)	36, 39, (11-91)*
pp'DDE	586, 629, (110-3100)	1685,1800, (710-5300)*
β-HCH	396, 370, (110-2200)	961,905 (130-7200)*
HCB	50, 48, (25-120)	71, 78, (31-220)*

The values were expressed by ng/g Fat, \* ; statistically significant (p< 0.05)

### Conclusions

1. DIPS-CALUX assay is suitable tools to assess dioxin concentrations in human breast milk samples in large-scale epidemiological studies.
2. Two cities in Liaoning Province, China showed distinct difference in dioxin concentrations and PCBs and organochlorine pesticides in breast milk. The values of all the contaminants measured were significantly higher in Dalian, a port city in Liaodong Peninsula, than those in Shenyang, an inland capital of the province.
3. There were no age-dependent elevations of the contaminants in Chinese breast milk.

### Acknowledgement

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### References

1. Kayama F., Hamamatsu A., Sagisaka K., Brown D., Clark G., Suzuki T. (2000) The 3<sup>rd</sup> Annual Meeting of Japan Society of Endocrine Disruptors Research.
2. Kayama F., Hamamatsu A., Sagisaka K., Brown D., Clark G., Suzuki T. (2001) Organohalogen Compounds 54: 48-50
3. Fujio Kayama, Hyogo Horiguchi, Etsuko Oguma, Junko Fujino, Hisatoshi Yabushita, David Brown and George Clark. Regional differences of blood dioxin and organochlorine pesticides concentrations of Japanese female farmers. (2002) Organohalogen Compounds 55: 275-278