

## REGIONAL DIFFERENCES OF BLOOD DIOXIN AND ORGANOCHLORINE PESTICIDES CONCENTRATIONS OF JAPANESE FEMALE FARMERS. - APPLICATION OF CALUX ASSAY FOR EPIDEMIOLOGICAL STUDY -

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### Introduction

We hypothesized that farmers who used pesticides and herbicides frequently may have higher dioxin body burden due to impurities in these chemicals. To elucidate body burden of dioxins and organochlorine pesticides, and relationships between health status of female farmers and environmental contaminants including dioxins, we designed epidemiological studies in 5 distant districts in Japan. In this paper, we demonstrate that CALUX assay for dioxin is applicable in epidemiology and a useful dioxins screening method for identifying higher exposure groups. In addition, the current analysis of dioxin contamination among female farmers in Japan revealed a distinct difference of distribution of blood dioxin concentration in the studied districts in Japan.

### Material and Methods.

Female farmers in five districts scattered in the Japan Archipelago were asked through regional agricultural organizations to cooperate in a nation-wide health survey studying relationships among health, nutrition and contaminants. The Committee on Medical Ethics in Jichi Medical School approved this research protocol and we obtained informed consents from all volunteer examinees. Health examinations included serum chemistry and urinalysis, bone metabolic markers, and wrist bone density measured by Dual Energy X-ray Absorption (DEXA) scanner as well as life, medical and diet history questionnaires double-checked by nurses and nutritionists at interview.

### Result and Discussion

We previously reported a validation study of the CALUX assay in biological samples<sup>1,2</sup>. We collected 70 ml blood samples from volunteers in the medical school. Ten-ml and 50-ml whole blood samples were used for CALUX assay and HRGC-MS measurement, respectively. Results for test samples measure by CALUX bioassay for dioxins at Hiyoshi Corporation were compared in a double blind study to HRGC/MS results in blood measured at Shimazu Techno-Research (Kyoto, Japan). When we started to apply CALUX assay for human biological samples, correlation coefficient (R-values) for PCDD+PCDF+coPCB per gram fat was 0.4758. As the test blood samples were taken from young volunteers with lower dioxin body burden, most of the samples had low dioxin concentrations. In addition, as CALUX assay is slightly less sensitive to coPCB, we excluded coPCB fractions for measurement. The R-values on PCDD+PCDF per gram wet weight and per gram fat were 0.7445 and 0.6417, respectively. When we limit applications of CALUX assay for blood specimens to evaluate

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PCDD+PCDF fraction and set detection limit of 20 pg/g fat, the R-values of PCDD+PCDF per gram wet weight and per gram fat is 0.9161 and 0.8297 respectively. This showed that CALUX assay is practically sensitive enough for screening moderately high exposure groups for dioxins (Table1).

**Table 1.** Correlation coefficients (R) of Blood Dioxin Concentrations between HRGC/MS and CALUX Assay

		pg/g wet weight	pg/g fat
PCDD+PCDF+co-PCB			
All sample	(n=21)	***	0.4758
Samples > 20pgTEQ/gFat	(n=14)	0.8218	0.7305
PCDD+PCDF			
All sample	(n=20)	0.7445	0.6417
Samples > 20pgTEQ/gFat	(n=13)	0.9161	0.8297

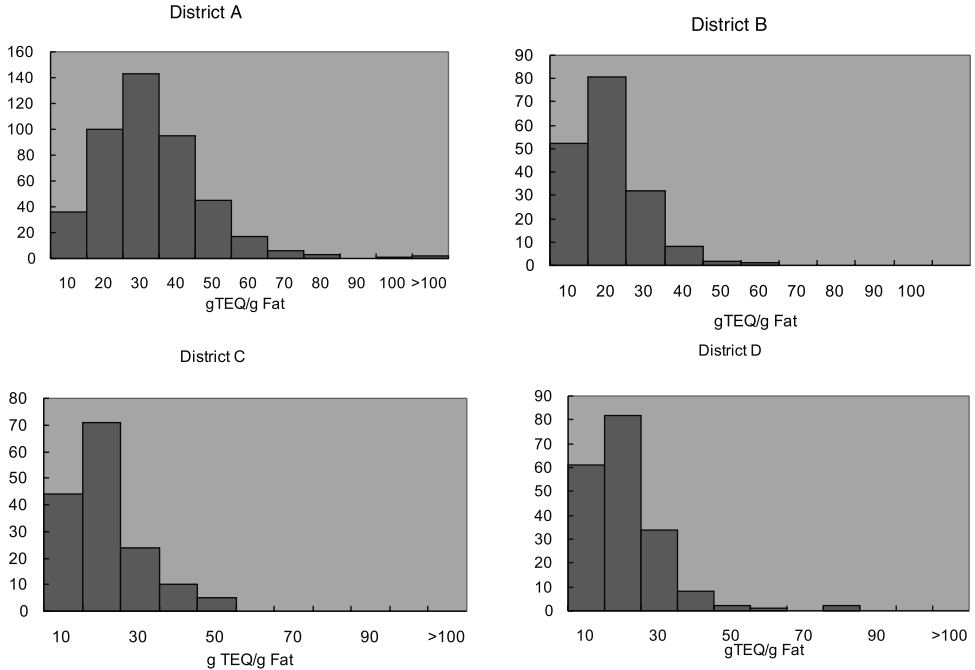
In the current study we measured blood dioxin (PCDD+PCDF) concentrations in 1160 female farmers out of 1407 volunteers in five districts. The average ( $\pm$ SD) of examinees' age was  $55.5 \pm 10.3$ . The average ( $\pm$ SD) blood dioxin concentration was  $32.3 \pm 12.1$  pg TEQ/g fat. When results for these five districts were compared, the average dioxin concentration in District A was distinctively high. Seventy percent of samples were higher than the detection limit (20 pg TEQ/g fat) in District A, while in the other districts approximately 25 % were higher. This indicates that there may be some dioxin contamination in district A, and it should be reexamined by HRGC-MS to determine the isomer pattern. HRGC-MS results may help to identify the character and origin of the contamination in this district. Figure 1 showed distribution of incidence is shifted to the right in District A and there are some individuals whose blood dioxin concentrations are more than 100 pg TEQ/g fat.

**Table 2.** Blood Dioxin Concentrations of Female Farmers in Five Districts of Japan

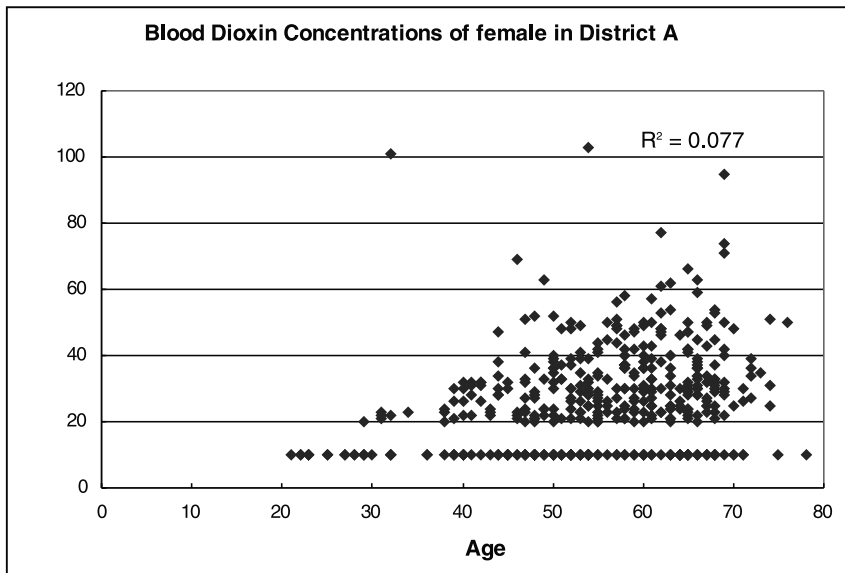
	District A	District B	District C	District D	District E	Total
Total No. of Examinees	596	203	202	204	202	1407
Age of the Examinees	$60.3 \pm 11.4$	$55.5 \pm 9.5$	$56.9 \pm 9.2$	$51.6 \pm 8.0$	$57.8 \pm 9.3$	$55.5 \pm 10.3$
No. of Blood Samples	449	177	155	191	188	1160
No. of Detected	313	43	40	47	38	481
No. of Not Detected	136	134	115	144	150	679
% of >20pgTEQ/g Fat	70 %	24 %	26 %	25 %	20 %	41 %
Range	20.0-102.7	20.0-50.0	20.6-46.7	20.7-71.1	20.3-66.9	20.0-102.7
Median	30.7	25.2	25.6	23.6	25.3	29.4
Average $\pm$ S.D.	$30.0 \pm 12.7$	$27.9 \pm 7.0$	$28.9 \pm 7.6$	$26.6 \pm 11.3$	$31.7 \pm 13.0$	$32.3 \pm 12.1$

Female middle-aged farmers were asked to participate in nation-wide health check regarding osteoporosis, nutrition and contaminants. Dioxins (PCDD+PCDF) in 10 ml of heparinized whole blood were determined by CALUX assay. Detection limit was 20 pg TEQ/g fat.

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**Figure 1.** Regional Difference of Blood Dioxin Concentrations of Female Farmers



**Figure 2.** Data lower than detection limit (20 pg TEQ/g fat) are treated as half of detection limit (10 pg TEQ/g fat) and plotted in the figure.

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Figure 2 shows correlation between age and blood dioxin level. The analysis of dietary history revealed moderate correlation to fish consumption. These data show quite similar results to HRGC-MS measurements recently conducted by Japan's Ministry of Environment.

## Acknowledgement

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## References

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